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Sex differences in α-pyrrolidinopentiophenone (α-PVP)-induced taste avoidance, place preference, hyperthermia and locomotor activity in rats

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Abstract

Rationale: The majority of synthetic cathinone research has used only male subjects, and as a result there are few studies assessing the impact of biological sex on their effects.

Objectives: The current work extends the characterization of the second-generation synthetic cathinone, α-PVP, by investigating how biological sex impacts α-PVP's aversive and rewarding effects important to its use and potential abuse.

Methods: A combined conditioned taste avoidance/conditioned place preference preparation was utilized in which adult male and female Sprague Dawley rats were injected with 1.5, 3 or 6 mg/kg of racemic α-PVP or vehicle (saline) (IP). Following a 24-day washout period, rats were then tested for thermoregulatory effects of α-PVP using subcutaneous microchips to measure body temperature changes over the course of 8 h. This was followed 21 days later by assessments for α-PVP-induced locomotor activity and stereotypies over a 1-h session.

Results: Dose-dependent conditioned taste avoidance was evident in both males and females, although females displayed weaker avoidance at 3 mg/kg compared to males. Males displayed a dose-dependent conditioned place preference, while females did not form a place preference at any dose. α-PVP elicited dose- and time-dependent hyperthermia, with males displaying a faster on-set and delayed off-set compared to females. α-PVP also produced dose- and time-dependent increases in locomotor activity (F>M) and stereotypies (M>F).

Conclusions: As described, males displayed greater rewarding (as indexed by place preference conditioning) and aversive (as indexed by taste avoidance, hyperthermia and stereotypies) effects

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of α-PVP. Although comparisons between males and females in α-PVP self-administration have not been reported, these data suggest that males may be more likely to use the drug. The implications for sex differences in human use of α-PVP were discussed.

Keywords

α-PVP; conditioned taste avoidance; conditioned place preference; sex; locomotor activity; hyperthermia; rats

1. Introduction

Most drugs of abuse have both rewarding and aversive effects whose balance is important in determining their self-administration (Cunningham 1979; Lin et al. 2016; Riley 2011; Stolerman and D'Mello 1981; Verendeev and Riley 2012). Assessing these affective properties of specific drugs and the various experiential (e.g., drug history) and subject (e.g., age, strain) factors that impact these properties (and their relative balance) may be important to the understanding of abuse potential. One drug that has only recently been examined in this context is the second-generation synthetic cathinone or "bath salt", α-pyrrolidinopentiophenone (aka: α-PVP, flakka, gravel). α-PVP is a potent reuptake inhibitor of dopamine (DA) and norepinephrine (NE) with considerably weaker action at the serotonin (5-HT) transporter (Baumann et al. 2016; Glennon and Young 2016; Marusich et al. 2014; Meltzer et al. 2006). Related to these specific neurochemical actions, α-PVP has been reported to support intravenous self-administration in a dose-dependent manner in a variety of species and under a number of experimental procedures (Aarde et al. 2015; Gannon et al. 2018; Huskinson et al. 2017; Javadi-Paydar et al. 2018).

In this context, it is important to note that α-PVP like other drugs of abuse has both rewarding and aversive effects as assessed by place preference conditioning (Gatch et al. 2015; Marusich et al. 2016; Nelson et al. 2017) and conditioned taste avoidance [Nelson et al., 2017, 2018; see Riley et al., 2019]. Although α-PVP is well characterized in relation to these effects, little is known about how such effects are impacted by factors known to affect other drugs of abuse (see Riley, 2011; Riley et al., 2019). One factor that has recently received attention in assessing the abuse potential of other drugs is sex (for reviews, see Becker and Koob, 2016; Becker et al., 2017; Carroll and Lynch, 2016; Riley et al., 2018). For instance, female rats demonstrate greater sensitivity to the rewarding effects of cocaine (as indexed by place preference conditioning; Zakharova et al., 2009), are more likely to acquire cocaine self-administration (Hu et al., 2004; Lynch and Carroll,1999) and show greater escalation of cocaine intake compared to males (Peterson et al., 2014; Roth and Carroll, 2004). The aversive effects of drugs have also been reported to vary with sex, although the direction of the difference appears to be drug dependent (Jones et al., 2006; Sherrill et al., 2011; see Riley et al., 2018). For example, male rats display a greater conditioned taste avoidance with ethanol than do female rats, an indication that male rats are more sensitive to its aversive effects than females (Sherrill et al., 2011). Interestingly, when ethanol and cocaine are co-administered, female Long-Evans rats show greater taste avoidance compared to males (Jones et al., 2006). Such sex-dependent differences have been reported not only in preclinical research but also in clinical populations with both acute and

chronic drug exposure (for a review see Riley et al., 2018). What these differences argue is that sex as a biological variable is critical in understanding potential risk factors involved in drug use and abuse (see Klein et al., 2015; Miller et al., 2017; Wetherington, 2007; 2010).

Although males and females are not often compared in preclinical research on the synthetic cathinones, some work has been done in this area in recent years. For example, Hambuchen and colleagues (2017) reported that there was greater bioavailability and variability in both the S-enantiomer and the racemate of MDPV in male rats compared to females (see also McClenahan et al., 2019). In relation to possible sex differences in affective properties, King et al. (2015) reported that MDPV produced significant conditioned taste avoidance in both male and female Sprague-Dawley rats, although the effect was weaker in females compared to males (MDPV produced comparable conditioned place preferences in males and females; see Daniel and Hughes, 2016 for an assessment of sex differences in anxiety following methylone exposure; M>F).

While this work indicates sex differences with some of the synthetic cathinones (specifically MDPV and methylone), to date, reports examining sex differences with α-PVP are limited (see Marusich et al., 2016 for an assessment of sex differences in the behavioral activating effects of α-PVP). In light of the preclinical work on sex differences associated with cocaine and MDPV as well as their neurochemical similarities with α-PVP in terms of their relative actions on the reuptake transporters for the brain amines (see above), it might be expected that sex differences would be similarly evident with α-PVP. In view of this hypothesis, the goal of the present study was to assess the relative contribution of biological sex to the aversive (taste avoidance conditioning) and rewarding (place preference conditioning) effects of racemic α-PVP. Temperature and locomotor effects were also examined in the induction of hyperthermia and hyperactivity (effects associated with toxic reactions in humans; see Borek et al., 2012; McGraw and McGraw, 2012; Penders et al., 2012; Riley et al., 2019).

2. General Methods

2.1. Subjects

The subjects were 96 experimentally naïve male (n = 48) and female (n = 48) Sprague-Dawley rats. Subjects were run in two replicates, and each replicate contained 24 animals of each sex. Animals were bred within the American University animal research facility and were allowed to mature undisturbed until the start of testing. Beginning on post-natal day (PND) 74, all animals were weighed daily to index health status and to reduce handling stress during experimental procedures which began between PNDs 106 – 126 at which point subjects weighed between 317 and 465 (males) and 198 and 265 (females) grams. 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 (National Research Council, 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 and generously provided by the Drug Design and Synthesis Section, MTMDB, NIDA and NIAAA) was dissolved in isotonic saline (0.9%) and injected intraperitoneally (IP) at 1.5, 3 or 6 mg/kg. Isotonic saline (vehicle), equivolume to the highest dose of α-PVP, was administered to controls. Each drug (and vehicle) solution was prepared daily and passed through a 0.2-um filter prior to injection to remove any potential particulates. Saccharin (Sodium Saccharin, Acros Organics) was prepared as a 1 g/l (0.1%) solution in tap water.

2.3. Apparatus

Same sex subjects were socially housed three per home-cage in OptiRat Plus cages (38.9 × 56.9 × 26.2 cm; 1181 cm2). The room in which the cages were located was maintained on a 12-h light/dark cycle (lights on between 0800 – 2000h) at 23 °C. Training and testing took place during the lights-on phase of the light cycle. Unless otherwise stated, food and water were available ad libitum. During taste avoidance conditioning, animals were transferred to a separate testing room and placed in individual hanging, stainless-steel wire-mesh test cages (24.3 × 19 × 18 cm) on the front of which graduated Nalgene tubes could be placed for fluid presentation. For place preference assessments, animals were placed in one of eight identical testing apparatuses. These measured 68.5 × 21 × 34.5 cm (San Diego Instruments Place Preference System, San Diego, CA) and were divided into three distinct areas each equipped with a photo beam array to record location and time spent in specific locations in the apparatus. The left side chamber of the apparatus (28 × 21 × 34.5 cm) had white walls and white metal diamond-plate flooring, the right side (28 × 21 × 34.5 cm) had black walls and black plastic haircell textured flooring and the middle connecting chamber (14 × 21 × 34.5 cm), which was not used for conditioning, had grey walls and metal grid flooring consisting of stainless-steel rods spaced approximately 1 cm apart. The place preference chambers (as well as the room in which the chambers were located) were unlit, and a white noise generator was used to dampen background noise.

For subsequent activity assessments, animals were placed in the same testing apparatuses as during conditioned place preference assessments, but each apparatus was converted so that they no longer had the appearance of clearly demarcated areas (see Hutchison et al., 2010). Each apparatus had three white LED lights set to maximum brightness within the otherwise unlit room and a photo beam array near the floor to track both ambulation (consecutive beam breaks) as well as stereotypies (repeated breaking of the same beam). A white noise generator was again used to mask background noise.

2.4. Procedure

2.4.1. Combined Conditioned Taste Avoidance Conditioned Place Preference

2.4.1.1. Habituation—At the beginning of experimental procedures, subjects were deprived of water and 24 hours later were given 20-min access to tap water in the hanging test cages. Following this access, the animals were returned to their home cages. This limited access procedure was repeated for 12–13 days to allow water consumption to stabilize (approaching the drinking tube within 2 seconds with the average volume of water

consumed not increasing or decreasing by more than 2 ml for 3 consecutive days). Water was presented in graduated 50-ml Nalgene tubes, and intake was evaluated by the difference between pre- and post-consumption volumes.

2.4.1.2. Pre-Test—Following stable water consumption, subjects were given 20-min access to water in the test cages before being placed into the middle grey chamber of the place preference apparatus and allowed to explore all chambers for 15 min. Following this access, animals were returned to their home cages. Each apparatus was wiped down with a cleaning solution (Sani-Plex 128M, one-step disinfectant germicidal detergent) between animals. For each replicate, a paired sample t-test of absolute time spent on the white side vs. absolute time spent on the black side during the 15-min pre-test indicated an unbiased apparatus (both ps > 0.05). Time spent in the middle chamber was not used for conditioning or used in the calculation of side preferences.

2.4.1.3. Conditioning—On Day 1 of this phase, all subjects were placed in their test cages and given 20-min access to a novel saccharin solution. Subjects were run in different groups based on sex and matched on saccharin consumption within each group. Males and females were then assigned to one of four drug groups and injected (IP) with either the saline vehicle or 1.5, 3 or 6 mg/kg of racemic α-PVP. These doses were based on previous work in our laboratory in which racemic α-PVP at 3 mg/kg induced a significant, but intermediate, suppression of saccharin consumption and 6 mg/kg produced almost complete suppression (Nelson et al., 2017; 2018). This resulted in a total of eight groups, i.e., Groups F0, F1.5, F3, F6, M0, M1.5, M3 and M6 where the letter indicates female or male and the number indicates drug dose (n = 12 per group). Following the injections, subjects were taken to an adjacent room and placed on one side of the place preference apparatus in a counterbalanced fashion such that half the animals in a given group were placed on their preferred side (defined as the side each on which each rat spent the most time during the Pre-Test) and the remaining half was placed on their non-preferred side for 30 min. Subjects were then returned to their home cages, and the testing chambers were sanitized prior to the next set of animals. On the next day (Day 2), they were given 20-min access to water in the test cages, injected with vehicle and placed on the opposite side of the place preference chamber for 30 min. This two-day cycle was repeated for a total of four cycles.

2.4.1.4. Place Preference and Taste Avoidance Test—On Day 9, animals were given 20-min access to tap water, placed in the center gray middle area of the place preference apparatus and once again given 15 min to explore all three chambers without restrictions (Post-Test). Time spent in each area was recorded and the time spent in the two conditioning chambers was evaluated for any changes in side preference relative to the amount of time spent in the chambers on the Pre-Test. On the day following place preference testing, animals were placed in the hanging test cages and given 20-min access to both saccharin and tap water in a two-bottle avoidance test with no subsequent injections. On this test, one bottle was offered (saccharin or water) on either the left or the right front of the test cage. Immediately after the first bottle was sampled, it was removed and the second bottle was presented on the opposite side. After that bottle was sampled, it was removed and both bottles were then placed simultaneously on their respective sides of the front of the cage.

The order of presentation and side placement were counterbalanced across animals, and consumption of both saccharin and water was recorded after 20 min had elapsed. Animals were then returned to their home cages with ad libitum water access.

2.4.2. Temperature—Following an approximately 24-day wash-out period subsequent to combined conditioned taste avoidance and conditioned place preference testing (see above), temperature probes (Bio Medic Data Systems, Seaford, DE; Model #IPTT-300) were implanted subcutaneously between the shoulder blades of each animal. This procedure took place while the animals were under inhaled isoflurane anesthesia, and the surgical site was first sterilized with 70% ethanol before implantation occurred. Subjects were allowed to recover undisturbed for 1 day, and on the following 3 days they were weighed to check for health status and the temperature transponders were scanned to check for proper function. After these preliminary checks, all subjects were again scanned for temperature, weighed and then injected (IP) with saline for 2 days. The initial scans taken on these days were not used in any statistical assessments as they were only for habituating the animals to the procedure and confirming the proper functioning of the equipment. For baseline measures, rats were weighed, scanned and injected with 0.3 ml saline and then additional temperature scans were taken 30 min, 1-h and again every hour up until 8-h post injection. The final procedure was similar to the day before with the exception that subjects were randomly injected with either saline or 1.5, 3 or 6 mg/kg racemic α-PVP, resulting in Groups F0, F1.5, F3, F6, M0, M1.5, M3 and M6 (n = 12 per group). For each temperature recording, the probe was scanned three times and the three measurements averaged. All temperature assessments took place in the animal colony during the lights-on phase. Rats remained in their home cages except for when they were removed for weighing, injections and scanning.

2.4.3. Activity and Stereotypies—Following an additional 21-day wash-out period, animals were weighed and handled for 3 consecutive days. Following this handling period, they were weighed and injected with 0.3 ml saline (vehicle) (IP). Immediately following the injection, animals were taken to an adjacent room and placed into the activity testing apparatuses. Counts of gross locomotor activity and stereotypies were recorded for each animal over a 1-h session that was divided into 12 5-min bins. The next day of testing was similar with the exception that subjects were randomly injected with either saline or 1.5, 3 or 6 mg/kg racemic α-PVP, again resulting in Groups F0, F1.5, F3, F6, M0, M1.5, M3 and M6 (n = 12 per group). Eight activity chambers were used, and each animal was placed in the same chamber on each testing day. Chambers were cleaned between animals.

2.5. Statistical Analysis

Given that the baselines between males and females were statistically different on several assessments, all data were analyzed without the factor of Sex, i.e., assessments with male and female subjects were made in separate analyses. Data from each behavioral assessment were analyzed using separate mixed model ANOVAs where the between subject factor in each was Dose [0 (vehicle), 1.5, 3, and 6 mg/kg] and the within subjects factor varied depending on the assessment (for taste avoidance [Trial 1–4]; for place preference [Pre-test and Post-test]; for temperature [Time (pre-injection, 30 min and 1–8 hours post-injection)]; for motor activity and stereotypies [Time (5-min intervals over the span of 1 h)]. In the

instance of a significant two-way interaction, simple effects of Dose at each within-subjects factor was assessed (multivariate analysis) followed by Bonferroni-adjusted multiple comparisons as warranted. Following these separate analyses, males and females were compared in terms of the specific patterns in main effects, interactions and post-hoc comparisons for each assessment.

Statistical significance was set to $p \leq 0.05$.

3. Results

The outcomes of the analysis of each assessment are presented in Tables 1–4 and in Figures 1–4. Results of each analysis are briefly described below.

3.1 Conditioned Taste Avoidance

Although both female and male rats displayed significant dose-dependent taste avoidance (see Table 1 and Figure 1), the dose-response patterns varied with sex. Specifically, throughout conditioning, male subjects injected with 3 or 6 mg/kg drank significantly less saccharin than those injected with vehicle or 1.5 mg/kg, indicating significant taste avoidance at the two high dose groups. On the other hand, female subjects did not consistently display this same dose-response relationship. At several points, female subjects injected with the intermediate dose of a-PVP (3 mg/kg) consumed more than the high dose condition (6 mg/kg; Trial 2) and did not differ in consumption from subjects injected with the low dose (1.5 mg/kg; Trial 3). On the final trial (Trial 4), the pattern for males and females was identical, i.e., avoidance at the 3 and 6 mg/kg doses with no avoidance at the low dose.

3.2 Conditioned Place Preference

Although the apparatus was unbiased, several individual animals spent more than 65% of the 15-min testing time on one side of the testing apparatus during the Pre-Test (indicative of a strong natural bias for one side of the testing chamber) and were excluded from subsequent statistical analysis (although still run in all behavioral assessments). The resulting group sizes for the place preference analysis (Groups M0: $n = 9$, M1.5: $n = 11$, M3: $n = 11$, M6: $n = 10$, F0: $n = 10$, F1.5: $n = 10$, F3: $n = 10$ and F6: $n = 8$) reflects this exclusion. Males injected with 3 and 6 mg/kg α-PVP displayed conditioned place preferences, spending significantly more time on the drug-paired side on the Post-Test than on the Pre-Test. Females did not display significant changes from Pre- to Post-Test at any dose of α-PVP (see Table 2 and Figure 2).

3.3 Temperature

Both male and female rats treated with α-PVP show significant dose- and time-dependent hyperthermia, although these effects were different between the sexes. For example, male subjects displayed a faster onset of hyperthermia as compared to females, with males showing a significant increase in temperature compared to vehicle-treated subjects at 30-min post injection (compared to 1 hour in females; see Figure 3). Further, this faster onset was evident at lower doses (3 mg/kg) in males. Although both males and females eventually

displayed significant elevations of temperature compared to their respective vehicle groups at all three doses of α-PVP, the duration of hyperthermia at the two highest doses was prolonged in males relative to females (for specific statistical differences, see Table 3 and Figure 3.

3.4 Motor Activity and Stereotypies

Although α-PVP induced significant motor activity (relative to controls) in males and females that was both dose- and time-dependent, the pattern of responding varied with sex. For females, activity throughout the full 1-hr assessment period was greater for the two higher doses of α-PVP compared to 1.5 mg/kg and vehicle. For males, the dose-response function was not linear in that the greatest effect of α-PVP was at the intermediate dose (3 mg/kg) at which activity was greater relative to 1.5 mg/kg, while the highest dose (6 mg/kg) was significantly different from 1.5 mg/kg only at two time points. Control subjects (both males and females) decreased consecutive beam breaks over the session (see Table 4 and Figure 4).

α-PVP induced stereotypies in both males and females that increased or remained stable over the assessment period. The only differences between males and females occurred at 6 mg/kg of α-PVP at which males displayed greater stereotypies than at the low dose (1.5 mg/kg) at several time points, whereas females did not differ between doses at any point in the assessment. Control subjects (both males and females) decreased repeated beam breaks over the session (see Table 5 and Figure 5).

4. Discussion

Given the importance of exploring subject factors like sex in drug effects, combined with the general lack of research regarding sex differences with the synthetic cathinones, the goal of the present study was to determine the effect of biological sex on α-PVP's ability to induce conditioned place preferences and conditioned taste avoidance as well as to impact thermoregulation and activity.

As described, α-PVP induced dose-dependent taste avoidance in both sexes although this effect was weaker in females at the intermediate dose (3 mg/kg) compared to male subjects. These results support our hypothesis that α-PVP would be less aversive in females and are consistent with prior work examining sex-dependent aversive effects of the related synthetic cathinone MDPV (as assessed through a combined taste avoidance/place preference procedure) in which Sprague Dawley females also displayed weaker taste avoidance compared to males (King et al., 2015). As with the data reported here, the differences between males and females were only evident at low (1 mg/kg) and intermediate (1.8 mg/kg) doses of MDPV and on specific trials. The present findings also parallel those in taste avoidance learning with cocaine. For instance, Busse et al. (2005) reported that male Sprague Dawley rats displayed stronger cocaine-induced taste avoidance compared to females at 20 mg/kg (at 30 mg/kg cocaine, males and females did not differ; see also Foltin and Schuster, 1982). Such parallels between drugs are consistent with the fact that α-PVP, MDPV and cocaine share a common mechanism of action in that they block the reuptake of the monoamines at their respective transporters. Their different dose-response functions

likely reflect their different affinities for (and selectivity at) the dopamine, norepinephrine and serotonin transporters (Baumann et al., 2013; Eshleman et al., 2017; Kolanos et al., 2015; Marusich et al., 2014; Simmler et al., 2013; for a discussion, see Nelson et al., 2017), although such neurochemical assessments have not been directly compared in males and females.

Sex differences were also evident in place preference conditioning as only males acquired a preference for the compartment paired with α-PVP (at 3 and 6 mg/kg). Evidence of a place preference was based on a comparison of time spent in the α-PVP-associated compartment on the Pre- and Post-Tests for drug- and vehicle-injected groups. For male subjects injected with the two highest doses of α-PVP, time in the drug-paired chamber during the Post-Test was significantly greater than that at the Pre-Test baseline, indicating a conditioned preference for that chamber (for males injected with the 1.5 mg/kg dose of α-PVP as well as those injected with vehicle, the changes were not significant). While significant at the higher doses, the difference between Pre- and Post-Test was relatively small, i.e., increases from Pre- to Post-Test of 25–27%. In prior work with the synthetic cathinones α-PVP and MDPV, similar increases have been reported (Gatch et al., 2015; King et al., 2015; Nelson et al., 2017; for comparable levels with cocaine, see Pomfrey et al., 2015), although direct comparisons with other assessments are difficult given that the presence and strength of conditioned place preferences are a function of many experimental factors, including drug, dose, strain and apparatus (biased/unbiased; see Cunningham et al., 2003; Roma et al., 2005; Tzschentke, 2007). While significant place preferences were acquired, it is important to note that the nature of the rewarding effects mediating such preferences induced by α-PVP is not known, i.e., whether it reflects a positive effect or a reduction in some aversive component.

Although females failed to display a significant place preference at any dose of α-PVP, they displayed similar increases from Pre- to Post-Test as males. Interestingly, while there was no significant effect of Test for the females, a post-hoc analyses for place conditioning for this group was significant at the highest dose (6 mg/kg; $p = 0.047$). It is certainly possible that with higher doses, females would acquire a preference with α-PVP. Under such conditions, sex differences would still be evident as males would have acquired the preferences at lower doses. Sex difference assessments with other synthetic cathinones and related psychostimulants such as MDPV or cocaine are relatively limited, but generally preferences are displayed by both sexes. For example, MDPV induced place preferences in males and females with no differences between the two (King et al., 2015; see also Hilderbrand and Lasek, 2014 for similar assessments with cocaine in male and female mice). Others report sex-dependent preferences induced by cocaine. For example, Zakharova and colleagues (2009) found that female Sprague Dawley rats treated with cocaine developed place preferences at lower doses than males (for similar results, see Russo et al., 2003 in which females showed preferences not only at lower doses but also with fewer training days compared to males). Further work with α-PVP in females under other conditions, e.g., higher doses, extended training, is important to determine if α-PVP has rewarding effects in both sexes.

Females had a higher baseline body temperature compared to males (see Alsufyani and Docherty, 2017 for similar effects in Wistar rats; see also Sanchez-Alavez et al., 2011; Yang

et al., 2007 for similar results in mice). Despite these different baselines, both sexes displayed significant hyperthermia compared to their respective controls with males having a faster onset and a delayed offset of temperature increases compared to females. Assessments of sex differences with the synthetic cathinones are limited, precluding comparisons of the present work with other assessments. Although there are no studies that assess the impact of sex on α-PVP-induced changes in temperature, Javadi-Paydar et al. (2018) examined pentedrone, pentylone and methylone in male and female Wistar rats and found that there was no effect on body temperature in females in any assessment and only modest effects in males (see also Alsufyani and Docherty, 2017 for comparisons of sex-dependent temperature effects in response to cathinone; F>M). Moreover, papers reporting on the effect of sex on body temperature changes induced by traditional psychostimulants are also limited (see Fukumura et al., 1998 for no effects of sex with methamphetamine; see Wyeth et al., 2009 for sex-dependent effects with MDMA, M>F; see Halladay et al., 2009 for greater temperature effects in males following methylphenidate pre-exposure). In light of the lack of published research of the effect of biological sex on temperature changes with synthetic cathinones and the variability of sex-dependent temperature effects in the literature with other psychostimulants, further research in this area is clearly warranted.

In the current study, both sexes displayed dose- and time-dependent increases in general locomotor activity and stereotypies following α-PVP. The patterns of general locomotor activity were different between the sexes as females appeared more sensitive to the locomotor effects produced by the 6 mg/kg dose of α-PVP (for no sex effect in activity in mice at 3 mg/kg α-PVP, see Marusich et al., 2016). Sex differences in activity following administration of other synthetic cathinones are somewhat mixed and dependent upon the specific drug administered, e.g., while Alsufyani and Docherty (2017) reported that female rats treated with cathinone increased locomotor effects compared to males, Javadi-Paydar et al. (2018) found that pentylone, pentedrone, and methylone increased locomotor activity comparably in both male and female rats (see also Daniel and Hughes, 2016 who found no sex differences in ambulation in response to methylone). In studies with cocaine, female rodents significantly increase their ambulatory activity in comparison to males (Cailhol and Mormede, 1999; Chin et al., 2002; Walker et al., 2001). The differences in stereotypies between males and females were slight and only evident at the highest dose of α-PVP at which males displayed greater stereotypies at several time points. It is interesting that at this same dose males displayed less overall motor activity (see above) suggestive that the increase in stereotypies for males may have masked general ambulation.

The pattern of results obtained here suggests that males were more sensitive to both the rewarding and aversive effects of α-PVP. The fact that males (but not females) displayed α-PVP-induced conditioned place preferences is consistent with the fact that in human settings the typical synthetic cathinone user is male (Carhart-Harris et al., 2011; Johnson and Johnson, 2014; Matthews et al., 2017; Orsolini et al., 2015; Winstock et al., 2010). Male subjects in the present assessment also displayed greater aversive effects as indexed by stronger taste avoidance and by faster onset and delayed offset of hyperthermia (for a discussion of hyperthermia as a key component of synthetic cathinone and general psychostimulant overdose with humans, see German et al., 2014; for reviews of excited delirium, see Mash 2016; Penders et al., 2012; Takeuchi et al., 2010; Vilke et al., 2012; see

also Levine et al., 2013; Pearson et al., 2012; Prosser and Nelson, 2012; Young et al., 2013 for descriptions of hyperthermia in patient case studies following the use of synthetic cathinones). Given that hyperactivity also is a prominent symptom of excited delirium syndrome (described in the reviews Mash, 2016; Penders, 2012; Takeuchi et al., 2010; Vilke et al., 2012), it was surprising that in instances in which differences in hyperactivity were noted in the present study, females displayed greater effects relative to males at the intermediate dose of α-PVP. However, given that there were greater stereotypies in males that could mask changes in overall locomotion might be an indication that males may have been more sensitive to the activating effects of α-PVP than females. The analysis of sex differences and their implications for human drug use must be cautiously made in that in addition to the factors such as age, genetics, history, etc. discussed previously that impact drug reward and aversion (and self-administration), many other issues affect human drug use. Specifically, cultural, social and economic factors (among others) that vary with sex (and gender) complicate the identification and characterization of sex diff erences in the effects of drugs and their potential for use and abuse (for a full discussion, see Greenfield et al., 2007; Riley et al., 2018).

The logic for assessing both the rewarding and aversive effects of α-PVP was that their balance may be important in predicting abuse liability (see above). This prediction is based on the fact that the drug's rewarding effects initiate and maintain its use whereas the drug's aversive effects limit it. Given the generally higher use of the synthetic cathinones (including α-PVP) in humans is primarily by males, it might be expected that in the present pre-clinical assessment that males would display greater rewarding, but fewer aversive, effects of α-PVP. As noted, male rats appeared more sensitive to both general effects. It is important to note that these properties appear independent of each other in that they can be differentially impacted by a host of factors, e.g., pharmacological and physiological manipulations, drug history, genetics, age, e.g., see King and Riley, 2016; Turenne et al., 1996; Verendeev and Riley, 2011. Further, changes in either behavioral index of these rewarding and aversive effects, e.g., place preference and taste avoidance conditioning, should not be seen as comparable changes in affect that necessarily counter the other. Again, it is the relative balance in these affective properties that mediate drug intake (see Cunningham et al. 2009; Ettenberg et al., 2015; Hunt and Amit, 1978; Verendeev and Riley, 2012). Given that this balance can be further impacted by a host of experiential and subject factors that may interact with sex (see Schramm-Sapyta et al., 2014; Riley et al., 2018) to increase or decrease abuse vulnerability, it is important to examine the rewarding and aversive effects of drugs of abuse and their relative contribution to the overall affective response to the drug and how these effects may vary with such experiential and subject factors. A better understanding of how these factors interact may help inform effective treatment and prevention strategies for synthetic cathinone use and abuse.

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Highlights

- Drug use and abuse is a function of the balance between its aversive and rewarding effects with factors such as sex impacting this balance.
- Males displayed greater α-PVP induced taste avoidance than females, although dose-dependent taste avoidance was evident in both sexes.
- Significant differences emerged between males and females in α-PVP's rewarding effects as only males displayed conditioned place preferences.
- Males and females differed in α-PVP-induced hyperthermia with males displaying a faster on-set and delayed off-set compared to females.
- Females displayed greater locomotor activity following α-PVP with increased stereotypies in males compared to females.

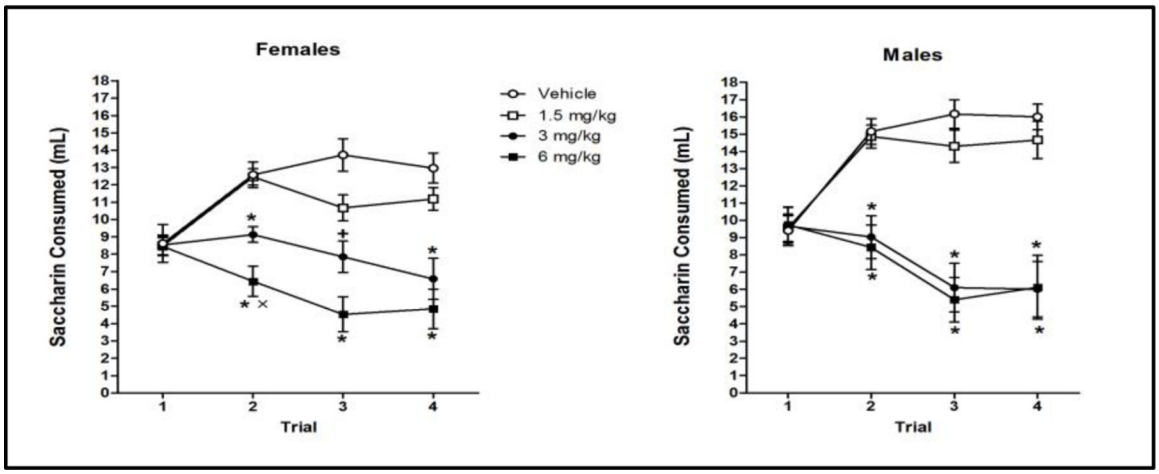

Figure 1.

Mean (+/− SEM) saccharin consumption (ml) over Trials 1–4 for groups injected with α-PVP at 0 (vehicle), 1.5 mg/kg, 3 mg/kg, or 6 mg/kg in female (left panel) and male (right panel) subjects. *significantly different from vehicle and 1.5 mg/kg. $^{+}$significantly different from vehicle. x significantly different from 3 mg/kg.

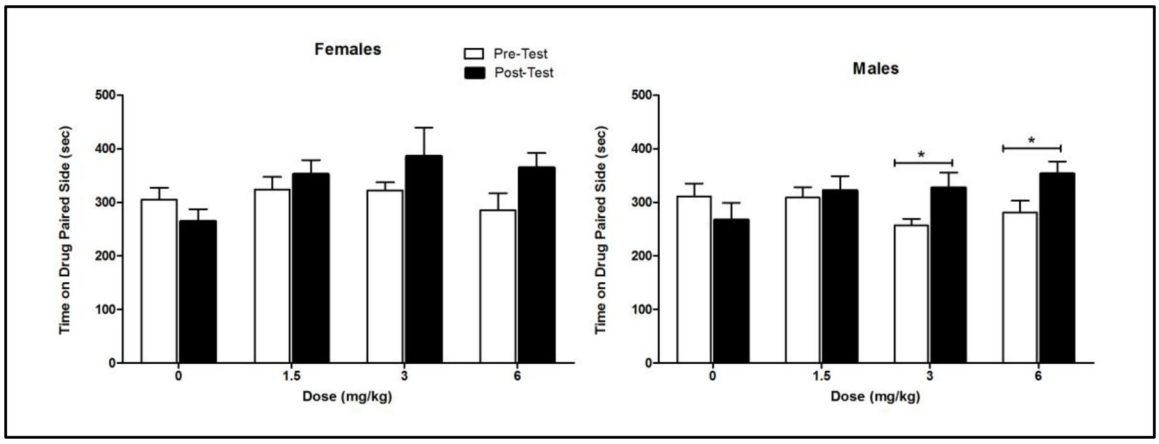

Figure 2.

Mean (+/− SEM) time spent on the drug-paired side for each group at Pretest (white bars) and Post-test (black bars) for groups injected with α-PVP at 0 (vehicle), 1.5 mg/kg, 3 mg/kg or 6 mg/kg in female (left panel) and male (right panel) subjects. *significant difference between Pre-test to Post-test.

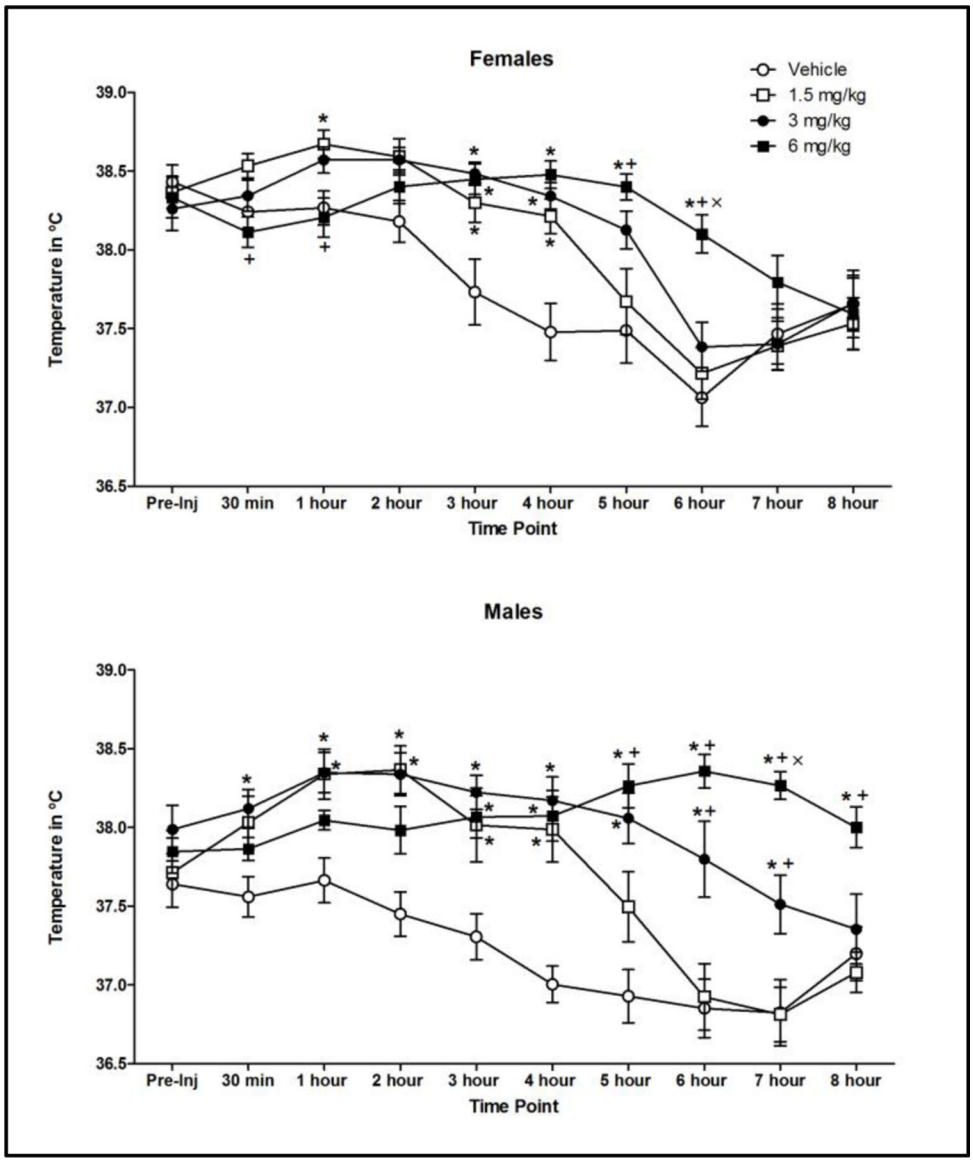

Figure 3.

Mean (+/−SEM) temperature in °C for groups injected with α-PVP at 0 (vehicle), 1.5 mg/kg, 3 mg/kg or 6 mg/kg in female (top panel) and male (bottom panel) subjects at pre-injection, and 30 minutes, 1 hour and every hour up until 8 hours post-injection. *significantly different from vehicle. $^{+}$significantly different from 1.5 mg/kg. ^{x}significantly different from 3 mg/kg.

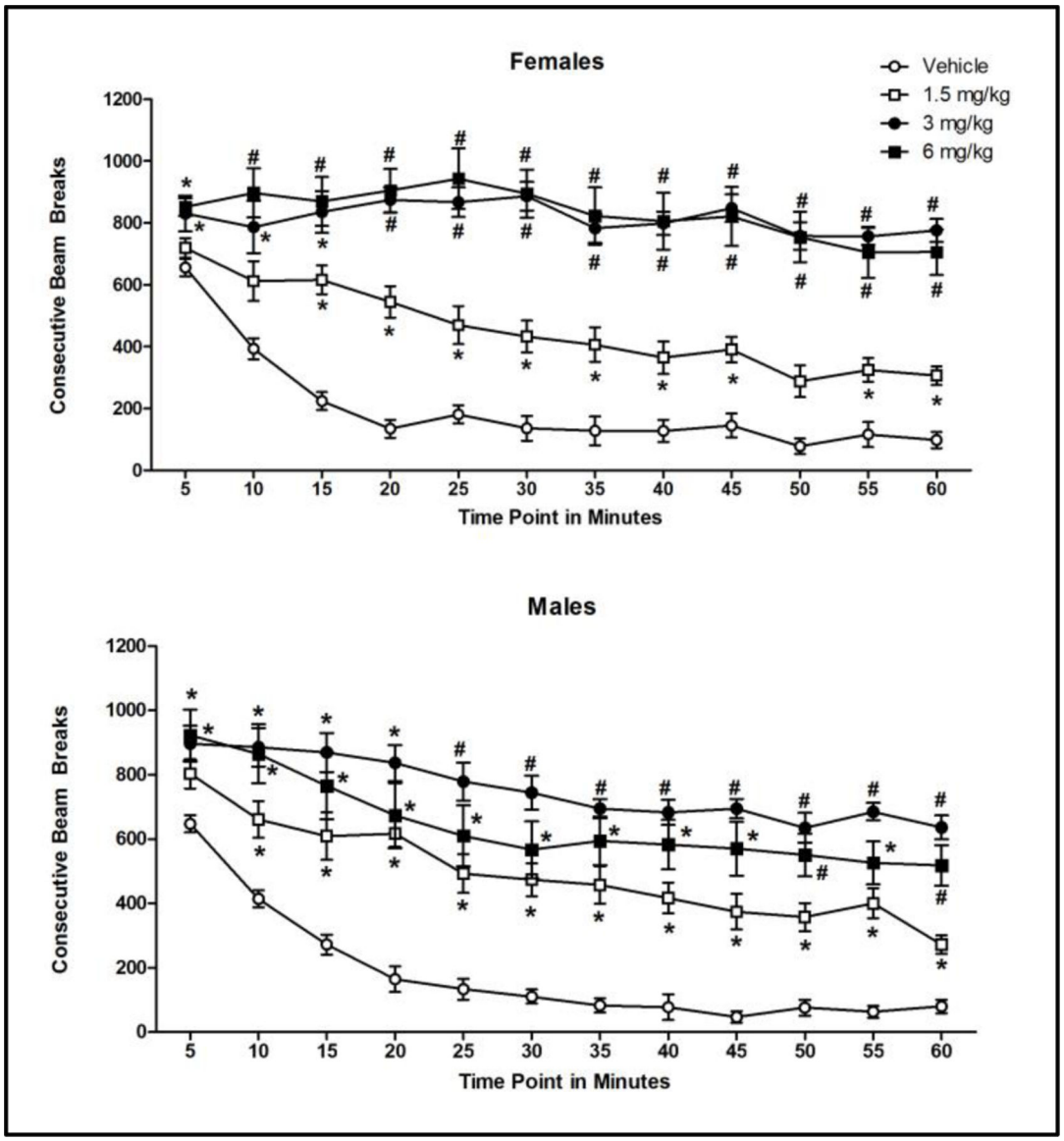

Figure 4.

Mean (+/−SEM) consecutive beam breaks for groups injected with α-PVP at 0 (vehicle), 1.5 mg/kg, 3 mg/kg or 6 mg/kg in female (top panel) and male (bottom panel) subjects in 5-min intervals for 1 hour. #significantly different from 1.5 mg/kg and vehicle; *significantly different from vehicle.

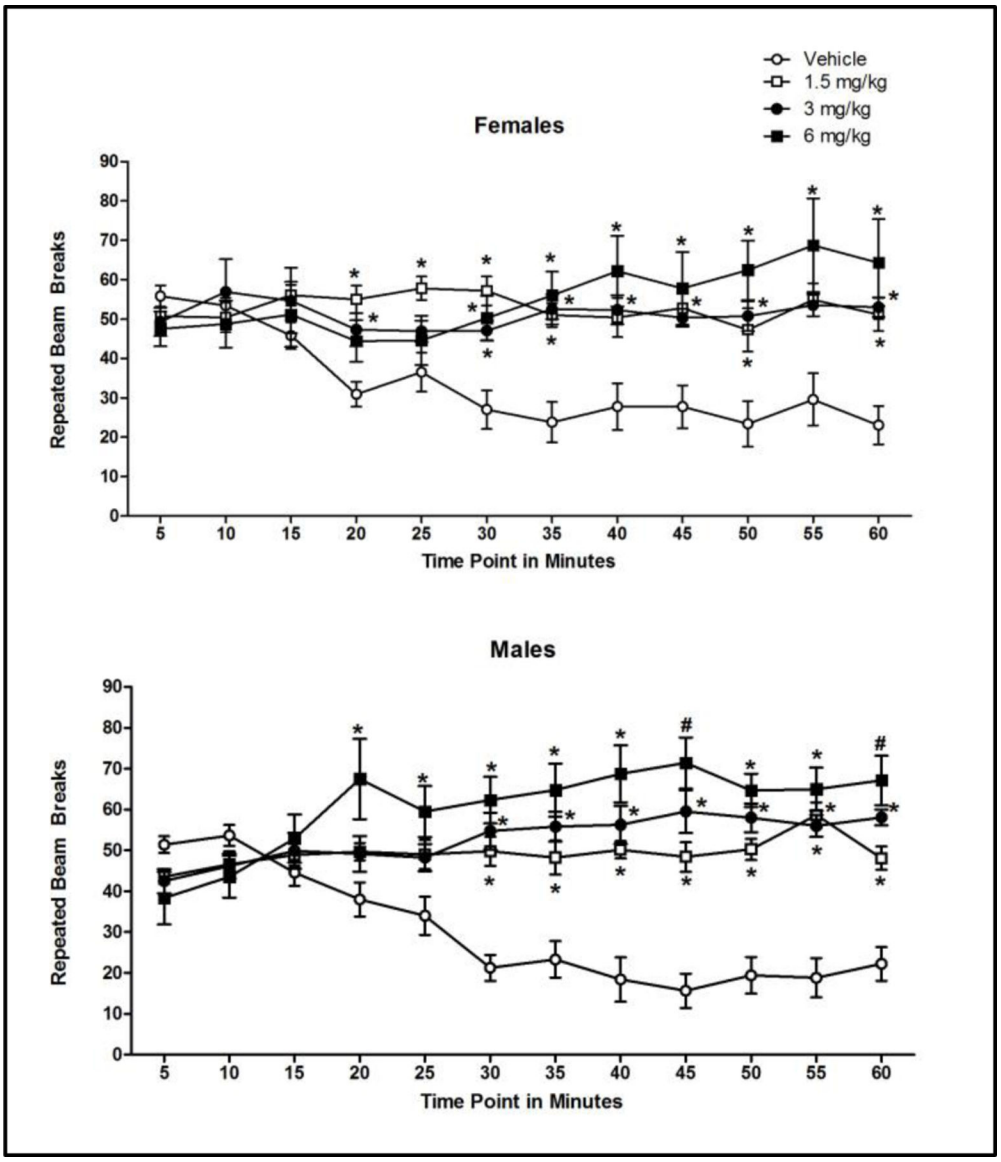

Figure 5.

Mean (+/−SEM) repeated beam breaks for groups injected with α-PVP at 0 (vehicle), 1.5 mg/kg, 3 mg/kg or 6 mg/kg in female (top panel) and male (bottom panel) subjects in 5-min intervals for 1 hour. #significantly different from 1.5 mg/kg and vehicle; *significantly different from vehicle.

Table 1.

Results of statistical analyses used to analyze saccharin consumption between male and female animals injected with 0, 1.5, 3, or 6 mg/kg of α-PVP.

In females (left), the 4 × 4 mixed model ANOVA on saccharin consumption showed a significant main effect of Trial [$F(3, 132)= 4.110$, $p= 0.008$], a significant main effect of Dose [$F(3, 44)=21.346$, $p < 0.000$], and a significant interaction of Trial x Dose [$F(9, 132)= 7.32$, $p < 0.000$]. In males (right), the 4 × 4 mixed model ANOVA on saccharin consumption revealed a significant main effect of Trial [$F(3, 132) = 4.687$, $p = 0.004$], a significant main effect of Dose [$F(3,44) = 17.460$, $p < 0.000$] as well as a significant interaction of Trial x Dose [$F(9, 132)= 9.362$, $p < 0.000$].

Female Saccharin Consumption				Male Saccharin Consumption			
Factor	**DF**	**F Statistic**	**p**	**Factor**	**DF**	**F Statistic**	**p**
Trial	3, 132	4.110	0.008	Trial	3, 132	4.687	0.004
Dose	3, 44	21.346	< 0.000	Dose	3, 44	17.460	< 0.000
Trial x Dose	9, 132	7.321	< 0.000	Trial x Dose	9, 132	9.362	< 0.000

Table 2.

Results of statistical analyses used to analyze conditioned place preferences between male and female animals injected with 0, 1.5, 3, or 6 mg/kg of α-PVP.

The 4 × 2 mixed model ANOVA in female rats (left) showed no main effect of Test [F(1,34)=3.410, p= 0.074], no main effect of Dose [F(3,34)= 1.594, p=0.209], and no interaction of Test x Dose [F(3,34)= 2.265, p= 0.099]. The 4 × 2 mixed model ANOVA in male subjects (right) revealed no main effect of Test [F(1,37)=3.697, p = 0.062] and no main effect of Dose [F(3, 37)=0.625, p = 0.604], but there was a significant interaction of Test x Dose [F(3,37)= 3.409, p = 0.027].

Female Conditioned Place Preference				Male Conditioned Place Preference			
Factor	**DF**	**F Statistic**	**p**	**Factor**	**DF**	**F Statistic**	**p**
Test	1, 34	3.410	0.074	Test	1, 37	3.697	0.062
Dose	3, 34	1.594	0.209	Dose	3, 37	0.625	0.604
Test x Dose	3, 34	2.265	0.099	Test x Dose	3, 37	3.409	0.027

Table 3.

Results of statistical analyses used to analyze thermoregulatory effects between male and female animals injected with 0, 1.5, 3, or 6 mg/kg of α-PVP.

The 4 × 10 mixed model ANOVA in male rats (right) showed a significant main effect of Time [F(9,396)= 22.074, p <0.000] and a significant main effect of Dose [F(3,44)=9.527, p <0.000] as well as a significant interaction of Time x Dose [F(27, 396)= 8.593, p < 0.000]. The 4 × 10 mixed model ANOVA in female subjects (left) also showed a significant main effect of Time [F(9,396)= 48.674, p < 0.000], a significant main effect of Dose [F(3,44)= 3.208, p = 0.032] and a significant interaction of Time x Dose [F(27, 396)=5.264, p < 0.000].

Female Temperature Effects				Male Temperature Effects			
Factor	**DF**	**F Statistic**	**p**	**Factor**	**DF**	**F Statistic**	**p**
Time	9, 396	48.674	< 0.000	Time	9, 396	22.074	< 0.000
Dose	3, 44	3.208	0.032	Dose	3, 44	9.527	< 0.000
Time x Dose	27, 396	5.264	< 0.000	Time x Dose	27, 396	8.593	< 0.000

Table 4.

Results of statistical analyses used to analyze locomotor effects (distance) between male and female animals injected with 0, 1.5, 3, or 6 mg/kg of α-PVP.

The 4 × 12 mixed model ANOVA in male subjects (right) on locomotor activity showed a significant main effects of Time [F(11, 484)=66.707, p <0.000] and Dose [F(3,44)=28.779, p < 0.000] as well as a significant interaction of Time x Dose [F(33, 484)= 2.629, p < 0.000]. Similarly, the 4 × 12 mixed model ANOVA in female subjects (left) on locomotor activity showed significant main effects of Time [F(11,484)=19.524, p < 0.000] and Dose [F(3,44)= 55.668, p < 0.000] as well as a significant interaction of Time x Dose [F(33,484)= 4.499, p <0.000].

Female Locomotor Effects				Male Locomotor Effects			
Factor	**DF**	**F Statistic**	**p**	**Factor**	**DF**	**F Statistic**	**p**
Time	11, 484	19.524	< 0.000	Time	11, 484	66.707	< 0.000
Dose	3, 44	55.668	< 0.000	Dose	3, 44	28.779	< 0.000
Time x Dose	33, 484	4.499	< 0.000	Time x Dose	33, 484	2.629	< 0.000

Table 5.

Results of statistical analyses used to analyze stereotypic effects (repetitive fine motor movements) between male and female animals injected with 0, 1.5, 3, or 6 mg/kg of α-PVP.

The 4 × 12 mixed model ANOVA in male rats (right) on stereotyped behaviors revealed no significant main effect of Time [F(11,484)= 0.921, p = 0.520] but a significant main effect of Dose [F(3,44)= 19.803, p < 0.000] and a significant interaction of Time x Dose [F(33,484)= 8.560, p < 0.000]. In contrast, the 4 × 12 mixed model ANOVA in female subjects (left) showed significant main effects of Time [F(11,484)= 1.810, p = 0.050], Dose [F(3, 44)= 6.873, p = 0.001], and a significant interaction of Time x Dose [F(33,484)=3.626, p < 0.000].

Female Stereotypic Effects				Male Stereotypic Effects			
Factor	**DF**	**F Statistic**	**p**	**Factor**	**DF**	**F Statistic**	**p**
Time	11, 484	1.810	0.050	Time	11, 484	0.921	0.520
Dose	3, 44	6.873	0.001	Dose	3, 44	19.803	< 0.000
Time x Dose	33, 484	3.626	< 0.000	Time x Dose	33, 484	8.560	< 0.000