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Age-Dependent MDPV-Induced Taste Aversions and Thermoregulation: A Behavioral and Physiological Assessment of "Bath Salts"

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AGE-DEPENDENT MDPV-INDUCED TASTE AVERSIONS AND THERMOREGULATION: A BEHAVIORAL AND PHYSIOLOGICAL ASSESSMENT OF "BATH SALTS"

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ABSTRACT

As with all drugs of abuse, MDPV, a primary constituent in "bath salts," is rewarding in rats. However, little is known of its aversive effects, those which might limit drug intake. Recent reports have shown that adolescents are generally less sensitive to the aversive properties of drugs, which may contribute to an increased vulnerability to use and abuse in this population. The present study used the conditioned taste aversion procedure to determine if MDPV induces aversions in rats and if those aversions are age-dependent. Specifically, subjects of both ages were given access to a novel saccharin solution followed by various doses (0, 1.0, 1.8, and 3.2 mg/kg) of MDPV. This procedure was repeated for a total of four conditioning trials followed by a two-bottle test of the aversion. As similar drugs induce thermoregulatory changes in rats, temperature was recorded following MDPV administration to assess if thermoregulatory changes were related to taste aversion conditioning. Adolescent rats displayed less robust MDPV-induced taste aversions than adult rats during acquisition and on the two-bottle assessment. Body temperature measurements revealed that adults exhibited hyperthermia, while adolescents exhibited hypothermia following acute exposure to MDPV. Given that drug use and abuse is a function of the relative balance between the drug's rewarding and aversive effects, the fact that the aversive effects are weaker in adolescents suggests that this population may be more vulnerable to MDPV use and abuse

1. INTRODUCTION

Use and abuse of "bath salts," a new group of designer drugs composed primarily of synthetic cathinones, has been increasing in recent years (Bronstein, Spyker, Cantilena, Green, Rumack, & Dart, 2011). Poison control centers across the United States have reported a dramatic increase in the number of calls relating to these substances, with 0 calls in 2009 to 302 in 2010 and 2,237 in 2011 (NDIC, 2011). Further, anecdotal reports describe symptoms of paranoid psychotic behavior, agitation, hallucinations and delirium following use of "bath salts" (Penders, 2012). It is interesting to note that while the synthetic compounds found in "bath salts" are routinely changing in an effort to circumvent laws on banned substances (USDEA, 2011), there seem to be several common components, e.g., mephedrone, methylone and 3,4-methylenedioxypyrovalerone (MDPV), the latter of which has been a popular constituent (Airuehia, 2012). MDPV is a potent dopamine (DA) and norepinephrine (NE) reuptake inhibitor that increases extracellular concentrations of these neurotransmitters in the mesolimbic pathway and has increased potency and selectivity for the catecholamines as compared with cocaine (Baumann, Partilla, Lehner, Thorndike, Hoffman, & Holy, 2012). By these mechanisms, MDPV induces its euphoric and hallucinogenic effects (Ross, Reisfield, Watson, Chronister, & Goldberger, 2012).

Although there have been many anecdotal reports documenting both the physical and behavioral effects of MDPV (McClean, Anspikian, & Tsuang, 2012; Mugele, Nañagas, & Tormoehlen, 2012; Airuehia, 2012), as well as papers describing patterns and trends in its use (Olives, Orozco, & Stellpflug, 2012), there are relatively few controlled studies examining the drug in rodents (or in humans). In one of the first reports documenting the behavioral effects of MDPV, adult Sprague-Dawley rats were able to acquire and maintain intravenous self-

administration (SA) of 0.05, 0.1 or 0.2 mg/kg per infusion MDPV, doses which were also successful in lowering intracranial self -stimulation (ICSS) thresholds (Watterson, Kufahl, Nemirovsky, Sewalia, Grabenauer, Thomas, Marusich, Wegner, & Olive, 2012). In an assessment of its discriminative stimulus properties in rats, MDPV has been reported to substitute for cocaine and methamphetamine (Forster, Taylor, & Gatch, 2012; for related work in mice, see Fantegrossi, Gannon, Zimmerman, & Rice, 2012). These data, in conjunction with anecdotal reports of human abuse, demonstrate that MDPV has rewarding properties and interoceptive effects similar to a variety of other CNS stimulants.

Although reports on the vulnerability to drugs of abuse commonly focus on reward, there is also another affective property of the drug, i.e., its aversive effect, which has been shown to impact drug taking and may also contribute to abuse vulnerability (Riley, 2011). Specifically, drug use is thought to be a function of the relative balance between its rewarding and aversive effects, with the aversive effects serving to limit drug intake (Riley, 2011). Conditioned taste aversion (CTA) learning is one procedure that has been used to assess the aversive effects of a number of drugs of abuse (Freeman & Riley, 2008), including morphine (Sherman, Pickman, Rice, Liebeskind, & Holman, 1980), cocaine (Goudie, Dickins, & Thornton, 1978), THC (Edwin, 1975) and ethanol (Eckardt, 1976). Notably, MDPV has not been examined for its ability to induce taste aversions, despite the upsurge of use and reports of negative side effects in recent years, as well as the fact that aversions are induced by compounds with similar pharmacological mechanisms (for cocaine see Goudie et al., 1978; for MDMA and *d*-amphetamine see Lin, Atrens, Christie, Jackson, & McGregor, 1993; for methamphetamine see Martin & Ellinwood, 1973). Accordingly, in the present study, MDPV was tested for its ability to induce aversions. This assessment was made in both adult (Experiment 1) and adolescent (Experiment 2) rats given

that for a variety of drugs of abuse aversions are dependent upon age with adolescent animals generally displaying weaker aversions than adults (for reviews, see Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Spear, 2013). Assessing such age differences in aversion learning with MDPV will provide information regarding abuse liability in a population that may be more vulnerable to use and abuse due to a reduction in aversive protectant effects. It is interesting in this context that the recent increase in use of "bath salts" is among teenagers (NDIC, 2011).

In the present series of experiments, body temperature was also assessed to further characterize MDPV's effects in adolescent and adult rats and to determine if temperature changes were related to taste aversion conditioning. Changes in body temperature are common with psychostimulant administration (for MDMA see Dafters & Lynch, 1998; for methamphetamine see Fukumura, Cappon, Pu, Broening, & Vorhees, 1998; for cocaine see Cappon, Morford, & Vorhees, 1998) and have recently been reported for MDPV (see Fantegrossi et. al., 2012). Fantegrossi and colleagues reported that ambient temperature differentially impacted thermoregulatory responses in mice treated with 3, 10 or 30 mg/kg. Specifically, hyperthermic effects outside of the normal circadian range were observed in mice maintained at an ambient temperature of 28°C, but not at a temperature of 22°C (though no significant effects were reported given the large variability of the measure and small group sizes). Given the reported temperature effects following acute MDPV administration in adult mice, and the suggestion that thermoregulatory effects may underlie the degree of taste aversions to ethanol (Cunningham, Niehus, & Bachtold, 2006), the present series of experiments examined thermoregulation and taste aversions following MDPV administration in adolescent and adult rats.

2. EXPERIMENT 1: ADULTS: METHODS

2.1 Subjects

Thirty-three experimentally naïve male Sprague-Dawley rats (Harlan Laboratories; Indianapolis, IN) arrived at the facility on postnatal day (PND) 21 weighing approximately 40 g. Food and water was available *ad libitum* unless noted otherwise. Procedures recommended by the National Research Council (1996), the Committee on Guidelines for the Care and Use of Animals in Neuroscience and Behavioral Research (2003) and the Institutional Animal Care and Use Committee at American University were followed at all times.

2.2 Apparatus

Upon arrival to the animal colony, subjects were initially handled and then group-housed (3 rats per bin) in polycarbonate bins (23 x 44 x 21 cm) with maple woodchip bedding. All subjects were maintained on a 12:12 light-dark cycle (lights on at 0800h) and at an ambient temperature of 22.5°C ± 1.5. All conditioning and testing occurred during the light phase of the light-dark cycle. During adaptation and conditioning, animals were transferred to individual hanging wire-mesh (24.3 x 19 x 18 cm) test cages located in another room, but were returned to their group-housing bins afterwards (see details below).

2.3 Drugs and Solutions

3,4-Methylenedioxypyrovalerone hydrochloride (synthesized at the Chemical Biology Research Branch of the National Institute on Drug Abuse) was dissolved in sterile isotonic saline (0.9%) at a concentration of 1 mg/ml and was subsequently filtered through a 0.2 μm filter to remove any contaminants before being administered intraperitoneally (IP) at a dose of 1.0, 1.8 or 3.2 mg/kg. Sterile isotonic saline was also filtered before being administered to vehicle controls equivolume to the highest dose of MDPV administered (3.2 mg/kg). Volume of the injection was

manipulated in favor of concentration given the influence concentration has on the absorption/distribution of the drug. Sodium saccharin (0.1%; Sigma) was prepared daily as a 1g/l solution in tap water.

2.4 Procedure

2.4.1 Conditioned Taste Aversion

Phase I: Adaptation. Subjects were brought into the laboratory on PND 21 and were maintained on *ad libitum* food and water until PND 77. On this day, subjects were handled, weighed and temperature probes were implanted (see below). For the next 7 days (PND 77-83) *ad libitum* water consumption was recorded. On the following day, the animals' available water was reduced to 50% of the previous day's measurement to encourage consumption of water in the individual test cages to take place on the subsequent day. On this day, subjects were removed from their group-housed bins, scanned for body temperature, weighed and placed into individual test cages. Once completed, the test cages were wheeled to the designated testing room where they were given 45min access to tap water in graduated 50-ml Nalgene tubes. After 45 min, the bottles were removed, consumption was recorded and subjects remained in the hanging cages for an additional 20 min before being returned to their group-housed bin and given *ad libitum* water for the next 22.5 h. On the next day, the amount of water available for each bin was again reduced to 50% (as described above) with the exception that individual test cage consumption was also factored into the previous 22.5 h of consumption. On the subsequent day, subjects were again scanned, weighed and placed into the test cages and given 45 min access to tap water before being returned to their group-housed home cages with *ad libitum* water for the next 22.5 h. This two-day cycle (50% on day one, test cage-access followed by *ad libitum* access on day two) was repeated a total of four times such that consumption was stable in all subjects.

Phase II: Conditioning. Following the final adaptation cycle, animals were given access to water for 1.5 h and bottles were then removed from the bins completely for 21 h before undergoing saccharin conditioning in the test cages. On this conditioning trial, subjects were scanned and weighed as previously described and given 45 min access to a novel saccharin solution (1 g/l) in the test cages after which they remained for an additional 20 min. At this point, subjects (independent of their group-housed bin) were assigned to one of four groups such that saccharin intake was comparable among groups. Based on these group assignments, and after body temperature was scanned, subjects were then injected with either MDPV (1.0, 1.8 or 3.2 mg/kg IP) or vehicle and then returned to their group-housed bins and given *ad libitum* water for the next 22.5 h. This procedure yielded Groups 0 (n = 9), 1.0 (n = 8), 1.8 (n = 8) and 3.2 (n = 8) for which the number indicates the dose of MDPV administered. On the next day, subjects in each bin were completely water deprived and this two-day cycle (deprivation on day 1 and saccharin access followed by 22.5 h ad libitum recovery on day two) was repeated four times.

Phase III: Two-bottle test. On the day following the final conditioning cycle, subjects were again transferred to test cages where two 50-ml Nalgene tubes (one containing tap water; the other containing the 0.1% sodium saccharin solution) were affixed to the cage for 45 min and consumption of both solutions was recorded. Placement of the bottle was counterbalanced across subjects to prevent positioning effects. After the 45 min access, bottles were removed, consumption recorded and animals were left in the cages for an additional 60 min.

2.4.2 Temperature Assessment

On PND 77, the injection site was aseptically cleaned with alcohol and the temperature transponders (Bio Medic Data Systems, Seaford Delaware; Model # IPTT-300) were rapidly inserted subcutaneously into each animal's left flank with a hypodermic needle. Once inserted,

the transponders were checked daily to assess their placement by palpating the injection site and for proper function by attempting to record the temperatures. On conditioning days (see above), animals' temperatures were recorded during weighing and handling (1000h), immediately before each injection (1145h) and at 30, 60, 90 and 120 min post-injection. The initial scan during handling was to ensure that the probe was functioning and this data was not considered in any statistical analyses. This time-course was also used during water adaptation although no injection was given. For each temperature recording, the probe was scanned twice and the two measurements averaged, with the two measurements never differing by more than 0.9°C. After scanning all the animals, the temperature data were uploaded to a spreadsheet from the Bio Medic Data Systems scanner.

2.4.3 Statistical Analyses

Saccharin consumption (ml) on the four conditioning trials was analyzed using a 4 (Dose) x 4 (Trial) mixed model ANOVA. In the presence of significant interactions, one-way ANOVAs with Tukey's HSD *post hocs* were used to assess differences between dose groups on each trial. One-way ANOVAs with Tukey's HSD *post hoc* analyses were employed to evaluate differences in the percent saccharin consumed and total fluid consumed between the different dose groups on the two-bottle test. Statistical analyses of body temperature were based on the mean of two serial scans per animal. A 4 (Dose) x 5 (Days) x 5 (Interval) mixed model ANOVA was used to evaluate differences in temperature between dose groups across intervals and days (adaptation and four conditioning trials). In the presence of significant three-way interactions, a 4 (Dose) x 5 (Interval) ANOVA was used to assess differences in temperature between dose groups across intervals for each day. In the presence of significant interactions, one-way ANOVAs with

Tukey's HSD *post hoc* analyses were used to evaluate differences between dose groups at each interval. Unless noted, all statistical analyses were based on significance level of $\alpha = 0.05$.

2. EXPERIMENT 2: ADOLESCENTS: METHODS

All procedures were matched to Experiment 1 with the following exceptions; 33 experimentally naïve animals arrived at the laboratory on PND 21 and had the temperature transponders implanted upon arrival; adaptation began on PND 28; only 2 days of water access in the test cages were employed prior to conditioning that began on PND 32; prior to water and/or saccharin access during adaptation, conditioning, and the two-bottle test, subjects were deprived of water for a full 24 h to ensure drinking. The distribution of animals between drug groups was identical to Experiment 1 (n=8 for all drug groups, n=9 for vehicle) as was the ambient room temperature.

3. RESULTS

3.1 Experiment 1: Adults

3.1.1 Acquisition

The 4 x 4 mixed-model ANOVA on saccharin consumption (ml) during conditioning revealed significant effects of Dose [$F(3, 29) = 14.263$, $p < 0.05$] and Trial [$F(3, 87) = 4.937$, $p < 0.05$] as well as a significant Dose x Trial interaction [$F(9, 87) = 11.850$, $p < 0.05$] (see Figure 1A). A subsequent one-way ANOVA indicated significant differences in consumption between groups on Trials 2 [$F(3, 32) = 9.231$, $p < 0.05$], 3 [$F(3, 32) = 18.748$, $p < 0.05$] and 4 [$F(3, 32) = 25.383$, $p < 0.05$]. Tukey's *post hoc* analysis revealed that on Trials 2 and 3 Groups 1.8 and 3.2 drank significantly less saccharin than Groups 0 and 1.0. By Trial 4, all MDPV-treated subjects drank significantly less saccharin than Group 0, and Groups 1.8 and 3.2 drank significantly less saccharin than Group 1.0.

Insert Figure 1 Approximately Here

3.1.2 Two-Bottle Test

A one-way ANOVA on the percent saccharin consumed on the two-bottle test revealed significant differences among groups [$F (3, 32) = 42.607$, $p < 0.05$] (see Figure 1B). Specifically, all MDPV-treated subjects drank a significantly lower percent of saccharin than Group 0, and Groups 1.8 and 3.2 drank a significantly lower percent of saccharin than Group 1.0. Additionally, a one-way ANOVA on total fluid consumed (data not shown) revealed significant differences among groups [$F (3, 32) = 3.675$, $p < 0.05$] such that Group 3.2 drank significantly less than Group 0.

3.1.3 Temperature Assessment

The 4 x 5 x 5 mixed model ANOVA on body temperature revealed significant effects of Day [$F (4, 580) = 19.765$, $p < 0.05$], Dose [$F (3, 145) = 16.542$, $p < 0.05$] and Interval [$F (4, 145) = 30.856$, $p < 0.05$] as well as significant Day x Dose [$F (12, 580) = 6.241$, $p < 0.05$], Day x Interval [$F (16, 580) = 7.10$, $p < 0.05$], Dose x Interval [$F (12, 145) = 2.485$, $p < 0.05$] and Day x Dose x Interval [$F (48, 580) = 1.417$, $p < 0.05$] interactions.

Insert Figure 2 Approximately Here

In relation to the significant three-way interaction, a 4 x 5 mixed model ANOVA on the last day of water adaptation indicated a significant effect of Interval [$F (4, 116) = 28.134$, $p < 0.05$] (data not shown). On the initial conditioning trial (Figure 2A), there was a significant effect

of Interval [$F (4, 116) = 15.161$, $p < 0.05$]. On the second conditioning trial (Figure 2B), there was significant main effect of Interval [$F (4, 116) = 33.083$, $p < 0.05$] and Dose [$F (3, 29) = 6.229$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F (12, 116) = 4.040$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that Groups 1.0 and 1.8 had higher temperatures than Group 0 at 60 and 90 min post-injection, with the additional effect that all drug-treated groups had higher body temperatures than Group 0 at 120 min post-injection. On the third conditioning trial (Figure 2C), there were significant main effects of Interval [$F (4, 116) = 38.791$, $p < 0.05$] and Dose [$F (3, 29) = 6.286$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F (12, 116) = 4.455$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that Groups 1.0 and 1.8 had higher temperatures than Group 0, with Group 1.0 exhibiting higher temperatures compared to Group 3.2 at 90 min post-injection. At 120 min post-injection, all drug-treated groups had higher body temperatures than Group 0. On the final trial (Figure 2D), there were significant main effects of Interval [$F (4, 116) = 32.233$, $p < 0.05$] and Dose [$F (3, 29) = 6.951$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F (12, 116) = 4.203$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that Groups 1.0 and 1.8 exhibited higher temperatures than Group 0 at 60 min, while at 90 and 120 min post-injection all drug-treated groups had increased body temperatures relative to Group 0.

3.2 Experiment 2: Adolescents

3.2.1 Acquisition

The 4 x 4 mixed model ANOVA on saccharin consumption (ml) during conditioning revealed significant effects of Dose [$F (3, 29) = 7.160$, $p < 0.05$] and Trial [$F (3, 87) = 11.216$, $p < 0.05$] as well as a significant Dose x Trial interaction [$F (9, 87) = 3.514$, $p < 0.05$] (Figure 3A).

A subsequent one-way ANOVA indicated significant differences in consumption between the groups on Trials 3 [$F(3, 32) = 10.202$, $p < 0.05$] and 4 [$F(3, 32) = 6.794$, $p < 0.05$]. Tukey's *post hoc* analysis revealed that on Trials 3 and 4 all MDPV-treated groups differed from vehicle.

Insert Figure 3 Approximately Here

3.2.2 Two-Bottle Test

A one-way ANOVA on the percent saccharin consumed on the two-bottle test revealed significant differences among groups [$F(3, 32) = 9.432$, $p < 0.05$] (Figure 3B). Specifically, Groups 1.8 and 3.2 drank a significantly lower percentage of saccharin than Group 0 with Group 1.8 drinking less than Group 1.0. Additionally, a one-way ANOVA on total fluid consumed revealed significant differences among groups [$F(3, 32) = 4.208$, $p < 0.05$] with Groups 1.0 and 3.2 drinking significantly less than Group 0.

3.2.3 Temperature Assessment

The temperature probe of one subject in Group 0 failed to function after the first conditioning day. All data from this one subject were removed from temperature assessments leaving an $n = 8$. The 4 x 5 x 5 mixed model ANOVA on body temperature yielded significant effects of Day [$F(4, 560) = 6.367$, $p < 0.05$], Dose [$F(3, 140) = 11.351$, $p < 0.05$] and Interval [$F(4, 140) = 12.712$, $p < 0.05$] as well as significant Day x Interval [$F(16, 560) = 11.962$, $p < 0.05$], Dose x Interval [$F(12, 140) = 5.753$, $p < 0.05$] and Day x Dose x Interval [$F(48, 560) = 1.774$, $p < 0.05$] interactions.

Insert Figure 4 Approximately Here

The 4 x 5 ANOVA on the last day of water adaptation indicated a significant effect of Interval [$F(4, 112) = 75.350$, $p < 0.05$] (data not shown). On the initial conditioning trial (Figure 4A), there was a significant effect of Interval [$F(4, 112) = 12.226$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F(12, 112) = 6.843$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that Group 1.0 exhibited higher body temperatures than Group 0 at the 0 min interval. At 30 min post-injection, Groups 1.8 and 3.2 had significantly lower temperatures than Group 0 with the added effect that Group 1.8 exhibited a significantly lower temperature than Group 1.0. At 60 min post-injection, Group 3.2 displayed lower temperatures than Group 1.0. On the second conditioning trial (Figure 4B), there was a significant effect of Interval [$F(4, 112) = 8.311$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F(12, 112) = 7.421$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that at the 0 min interval, Group 1.0 exhibited higher body temperatures than Group 0. At 30 min post-injection, Group 1.8 had a significantly lower temperature than Group 0. At 60 min post-injection, Groups 1.8 and 3.2 had significantly lower body temperatures compared to Group 1.0. At 90 min post-injection, Groups 1.0 and 3.2 had significantly higher body temperatures than Group 0. Finally, at 120 min post-injection, Group 3.2 exhibited significantly higher temperatures compared to Group 0. On the third conditioning trial (Figure 4C), there were significant effects of Interval [$F(4, 112) = 13.912$, $p < 0.05$] and Dose [$F(3, 28) = 3.217$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F(12, 112) = 5.588$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses revealed that at the 30 min interval, Groups 1.8 and 3.2 had significantly lower temperatures than Group 0 with the

added effect that Group 1.8 exhibited a significantly lower temperature than Group 1.0. At 60 min post-injection Group 3.2 had significantly lower body temperatures compared to group 1.0, while at 120 min post-injection Group 3.2 had significantly higher body temperatures compared to Group 0. Additionally, at 90 min post-injection Groups 1.0 and 3.2 had significantly higher temperatures than Group 0. On the final conditioning trial, there was a significant effect of Interval [$F(4, 112) = 10.718$, $p < 0.05$] as well as a significant Interval x Dose interaction [$F(12, 112) = 3.009$, $p < 0.05$]. A one-way ANOVA on Interval with Tukey's *post hoc* analyses indicated that at 30 and 60 min post-injection Group 3.2 exhibited significantly lower temperatures compared to Groups 0 and 1.0.

4. AGE COMPARISONS

To provide an analysis of the differences between adult and adolescent subjects, direct age comparisons were made on the acquisition of MDPV-induced taste aversions but direct comparisons on body temperature were not performed given that the effects between adults and adolescents were in opposite directions (i.e., increased and decreased, respectively).

4.1 CTA Comparisons

Consumption for the drug-treated groups was transformed to a percent of the average consumption of Group 0 for each age group across each of the conditioning trials. For each trial, consumption in each age and dose group was calculated as a percent of the average absolute consumption of the vehicle-treated controls (Group 0) on that session. A 2 (Age) x 3 (Dose) x 4 (Trial) mixed-model ANOVA revealed significant main effects of Age [$F(1, 42) = 27.055$, $p < 0.05$], Dose [$F(2, 42) = 6.683$. $p < 0.05$] and Trial [$F(3, 126) = 70.582$, $p < 0.05$] as well as significant Trial x Age [$F(3, 126) = 10.923$, $p < 0.05$], Trial x Dose [$F(6, 126) = 3.885$, $p < 0.05$], Age x Dose [$F(2, 42) = 5.856$, $p < 0.05$] and a Trial x Age x Dose [$F(6, 126) = 3.035$, $p <$

0.05] interactions. Subsequent one-way ANOVAs used to compare Age x Dose differences across conditioning trials revealed significant differences on Trials 2-4 with Tukey's *post hoc* analyses indicating that adolescents consumed a significantly higher percentage of saccharin than their adult counterparts at the doses of 1.8 and 3.2 mg/kg, reflective of the acquisition of weaker aversions in the adolescents at the two highest doses tested.

Additionally, Bonferroni-corrected independent sample t-tests used to examine age differences in saccharin preference on the two-bottle test (Figure 5B) revealed that adolescents consumed a significantly higher percentage of saccharin relative to adults at 1.0 mg/kg [$t(14) = 3.459$, $p < 0.0125$], 1.8 mg/kg [$t(14) = 3.955$, $p < 0.0125$] and 3.2 mg/kg [$t(14) = 2.906$, $p < 0.0125$] with no differences at 0 mg/kg [$t(14) = 4.909$, $p > 0.0125$].

5. DISCUSSION

The present experiments sought to assess whether MDPV is capable of conditioning taste aversions and if such effects differ between adolescent and adult rats. As described, MDPV did induce taste aversions in both age groups, with adolescent subjects acquiring the aversions at a slower rate and to a lesser degree than their adult counterparts. Body temperatures revealed dose-dependent changes that were also age-dependent. Specifically, adults exhibited increased body temperatures while adolescents exhibited decreases compared to their own controls following acute exposure to MDPV, an effect not related to ambient temperature.

As with many drugs of abuse, aversions induced by MDPV were rapidly acquired (within several conditioning trials) and dose-dependent (for reviews, see Gamzu, Vincent, & Boff, 1985; Hunt & Amit, 1987; Verendeev & Riley, 2012), suggesting that, as indexed by the CTA procedure, MDPV has aversive effects. Interestingly, these aversive effects were found in both adolescents and adults in the absence of any changes in the levels of monoamines (and their

metabolites as measured via HPLC-ECD) induced by the drug (data not shown), suggesting that MDPV's ability to condition aversions occurs at doses and a frequency of administration that do not induce monoaminergic depletions. Like many other drugs of abuse, MDPV also has rewarding effects as measured in SA and ICSS designs. For example, rats self-administer MDPV (0.05, 0.1 and 0.2 mg/kg per infusion) and comparable doses of MDPV also lower ICSS thresholds (Watterson et al., 2012). Interestingly, MDPV (at a dose effective in inducing aversions in the present assessment, i.e., 1 mg/kg) substitutes for cocaine and methamphetamine in a drug discrimination procedure (Forster et al., 2012). Additionally, methamphetamine and MDMA substitute with greater than 80% drug-appropriate responding in adult mice trained to discriminate 0.3 mg/kg MDPV from saline, suggesting similar stimulus effects (Fantegrossi et al., 2012).

Given that drug use and abuse is a function of the relative balance of reward and aversion, characterizing these properties may be important to understanding their relative balance and MDPV's abuse potential. In this context, it is interesting that while MDPV induced aversions, adolescent subjects displayed significantly weaker aversions than adults. These results parallel a growing literature showing similar age-dependent aversive effects of a wide variety of drugs of abuse (for reviews, see Doremus-Fitzwater et al., 2010; Spear, 2013). In such assessments, taste aversions are generally induced at lower doses and are acquired faster and to a greater degree in adult rats compared to their adolescent counterparts. Although little is known about the age differences in the rewarding effects of MDPV, the fact that it is less aversive in adolescent subjects suggests that this population may be vulnerable to its use and abuse.

Although the age differences in MDPV-induced aversions are clear, the basis for these differences remains unknown. It is possible that age-dependent differences in taste processing,

learning, retention and stress reactivity could account for the differences in MDPV-induced aversions, although these factors have been shown to have little contribution (if any) in other assessments of aversion learning between adolescent and adults (see Anderson, Varlinskaya, & Spear, 2010 and Hurwitz, Merluzzi, & Riley, 2013 for a discussion of these issues). The present data, along with the aforementioned reports on adolescent/adult CTA comparisons, implicate instead some developmental phenomenon that generalizes across many drugs and possibly reflects an overall insensitivity to the aversive properties of drugs for adolescents.

A major difficulty with such a conclusion is the fact that little is known about the specific nature of the aversive effects of drugs in general, much less that of MDPV, which has only recently been investigated for its affective properties (Fantegrossi et al., 2012; Forster et al., 2012; Watterson et al., 2012). Although much has been speculated regarding the aversive properties of a host of compounds, including drugs of abuse, there is no consensus (for a recent review, see Verendeev & Riley, 2012). One suggested mechanism proposed for ethanol-induced aversions is hypothermia (see Cunningham et al., 2006), i.e., subjects who exhibit the strongest alcohol-induced aversions are those which have alcohol-induced decreases in core body temperature. Given that MDPV has been reported to affect body temperature (see Fantegrossi et al., 2012), the present experiment assessed if MDPV induced temperature changes, if these changes differed in the two age groups, and if these changes might be related to aversion learning. In the present study, both age groups displayed significant MDPV-induced changes in temperature. Further, these effects differed such that adolescents displayed dose-dependent decreases in body temperature and adults displayed increases. The fact that both groups acquired aversions (albeit to different degrees) yet differed directionally in terms of the effects of MDPV on temperature argues that if temperature changes are related to MDPV-induced aversions this

relationship is complicated and age-dependent. Importantly, within each age group there was no clear dose-dependent relationship between the degree of body temperature changes and strength of taste aversions. It is certainly possible that under different ambient temperatures, MDPV may have induced aversions that were more directly related to its effects on body temperature. As noted by Fantegrossi and colleagues (2012), ambient temperature has been shown to influence the degree of hyperthermia found in adult mice. Specifically, adult mice treated with 3, 10 and 30 mg/kg MDPV exhibited hyperthermia in a warm (28°C) but not cool (22°C) environment. Future research should assess how these two modulating factors interact by examining MDPV's thermoregulatory effects in adolescent and adult rats at varying ambient temperatures and establish if these effects are associated with strength of aversion conditioning.

The present experiments sought to determine whether MDPV, the primary constituent of "bath salts," could induce conditioned taste aversions and whether these aversions varied by age. Given that the balance between the rewarding and aversive effects of a drug is thought to influence its abuse liability, the fact that adolescents exhibited weaker MDPV-induced aversions relative to adults suggests this population may be more vulnerable to its use and abuse (see Infurna & Spear, 1979; Schramm-Sapyta, Morris, & Kuhn, 2006; Schramm-Sapyta, Cha, Chaudhry, Wilson, Swartzwelder, & Kuhn, 2007; Anderson et al., 2010; Vetter-O'Hagen, Varlinskaya, & Spear, 2009; Shram, Funk, Li, & Lê, 2006 and Hurwitz et al., 2013 for similar findings with other drugs of abuse). This is especially concerning because MDPV use among teenagers has increased in recent years (NDIC, 2011). Although the age dependency in MDPV-induced aversions is clear, the specific mechanism underlying these effects remains unknown. It is increasingly important to investigate both the physiological and neurochemical mechanisms underlying reward and aversion to better understand use and abuse of MDPV and other drugs.

NOTES

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Figure Captions

Figure 1: Mean (± SEM) saccharin consumption (ml) by adults during acquisition (A) and mean (± SEM) percent saccharin consumed on the two-bottle test (B). $^{+}$denotes a significant difference between Group 0 and Groups 1.8 and 3.2. $^{\%}$denotes a significant difference between Group 1.0 and Groups 1.8 and 3.2. *denotes a significant difference between Group 0 and all drug treated-groups.

Figure 2: Mean (± SEM) body temperature (°C) of adults across the five intervals (0, 30, 60, 90, 120) over the four conditioning trials (Panels A-D). ^denotes a significant difference between Groups 1.0 and 1.8 and Group 0. *denotes a significant difference between all drug-treated groups and Group 0. $^{\#}$denotes a significant difference between Groups 1.0 and 3.2.

Figure 3: Mean (± SEM) saccharin consumption (ml) by adolescents during acquisition (A) and mean (± SEM) percent saccharin consumed on the two-bottle test (B). *denotes a significant difference between Group 0 and all drug treated-groups. $^{+}$denotes a significant difference between Group 0 and Groups 1.8 and 3.2. $^{\Omega}$denotes a significant difference between Groups 1.0 and 1.8.

Figure 4: Mean (± SEM) body temperature (°C) of adolescents across the five intervals (0, 30, 60, 90, 120) over the four conditioning trials (Panels A-D). $^{\Delta}$denotes a significant difference between Groups 0 and 1.0. $^{+}$denotes a significant difference between Group 0 and Groups 1.8 and 3.2. $^{\Omega}$denotes a significant difference between Groups 1.0 and 1.8. $^{\#}$denotes a significant difference between Groups 1.0 and 3.2. $^{\lambda}$denotes a significant difference between Groups 0 and 1.8. $^{\%}$denotes a significant difference between Group 1.0 and Groups 1.8 and 3.2. $^{\beta}$denotes a significant difference between Group 0 and Groups 1.0 and 3.2. $^{\Sigma}$denotes a

significant difference between Group 0 and Group 3.2. [X]denotes a significant difference between Groups 0 and 1.0 and Group 3.2.

Figure 1

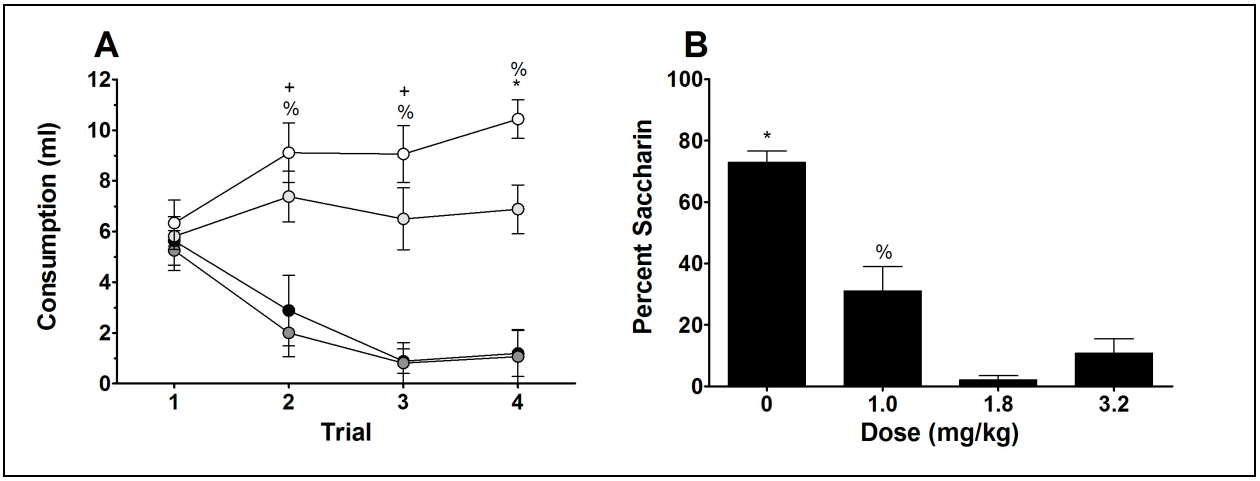

Figure 2

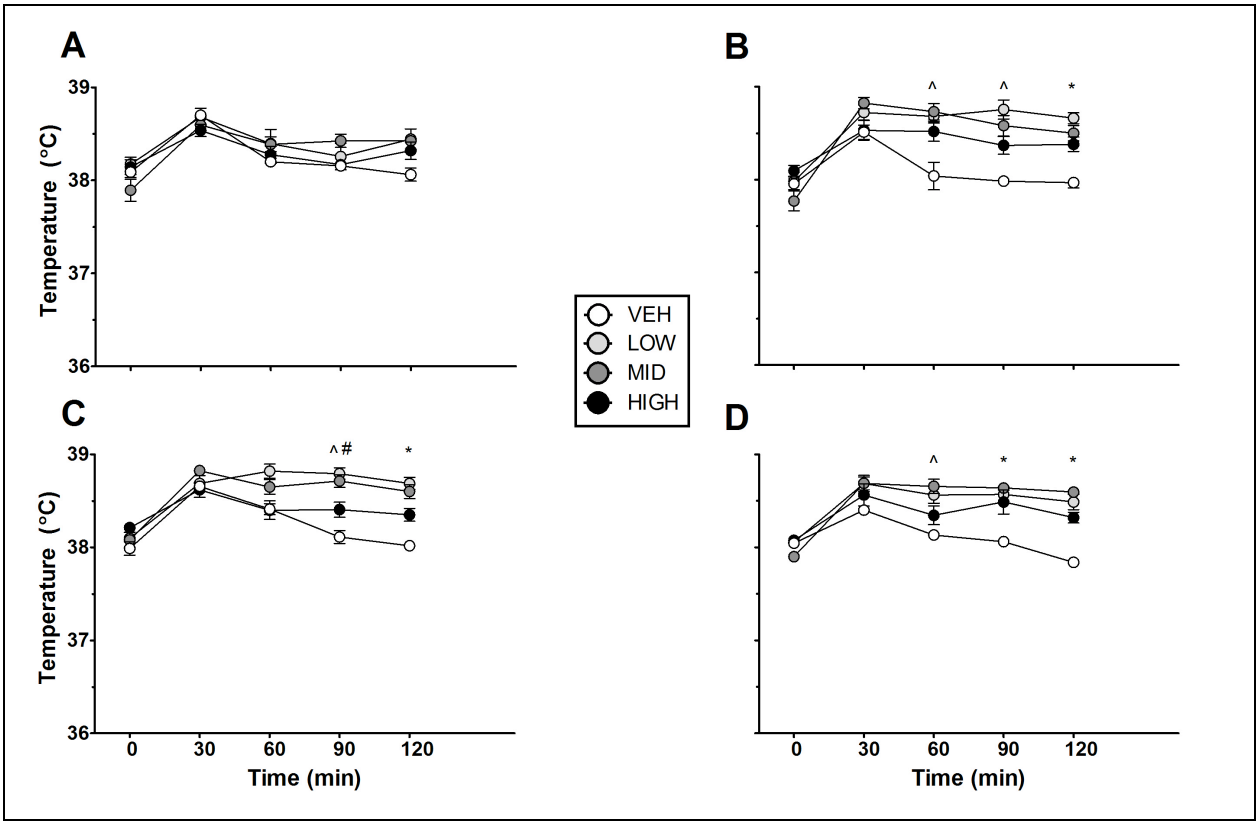

Figure 3

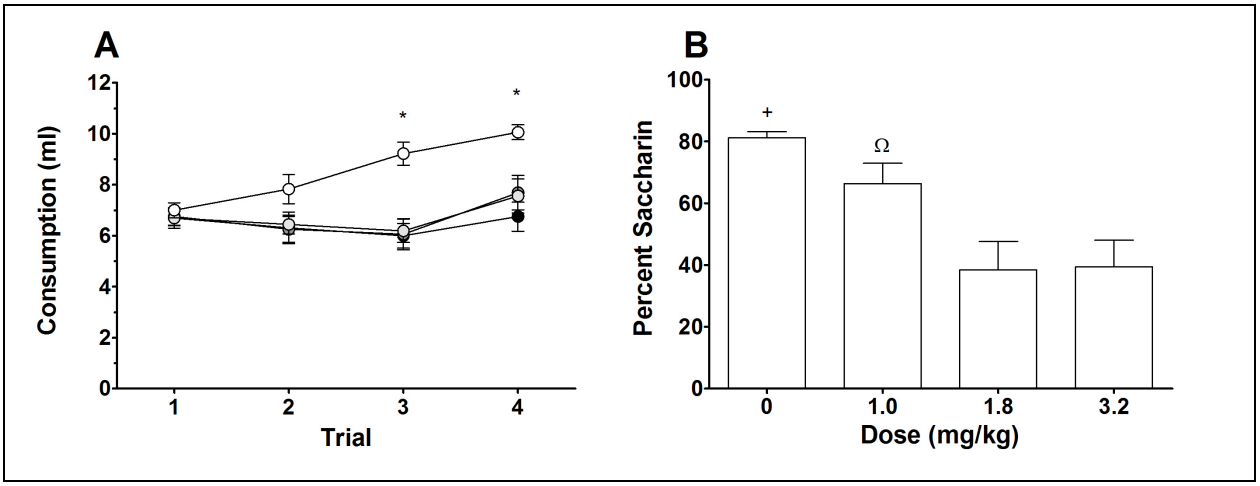

Figure 4