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AGE DIFFERENCES IN (±)3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED CONDITIONED TASTE AVERSIONS AND MONOAMINERGIC LEVELS

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Abstract

Preclinical work indicates that adolescent rats appear more sensitive to the rewarding effects and less sensitive to the aversive effects of abused drugs. The present investigation utilized the conditioned taste aversion (CTA) design to measure the relative aversive effects of (±)3,4-methylenedioxymethamphetamine (MDMA; 0, 1.0, 1.8 or 3.2 mg/kg) in adolescent and adult Sprague-Dawley rats. After behavioral testing was complete, monoamine and associated metabolite levels in discrete brain regions were quantified using high-performance liquid chromatography coupled to electrochemical detection (HPLC-ECD) to determine if adolescent animals displayed a different neurochemical profile than do adult animals after being exposed to subcutaneous low doses of MDMA. Adolescent rats displayed less robust MDMA-induced taste aversions than adults during acquisition and on a final two-bottle aversion test. MDMA at these doses had no consistent effect on monoamine levels in either age group, although levels did vary with age. The relative insensitivity of adolescents to MDMA's aversive effects may engender an increased vulnerability to MDMA abuse in this specific population.

Keywords

Adolescent; Adult; Sprague-Dawley Rats; MDMA; CTA; HPLC; Monoamine

Given that the initiation of drug use in human populations generally occurs in adolescence (Johnston, O'Malley, Bachman, & Schulenberg, 2012), this is a period of great importance in determining the factors that play a role in the transition from initial drug use to abuse.

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Additionally, adolescence is a life stage characterized by many developmental changes that may interact with the effects of drug administration (Arnett, 1992; Chambers, Taylor, & Potenza, 2003), resulting in differential vulnerability to drug abuse. Preclinical work indicates that adolescent populations are more sensitive to the rewarding effects of abused drugs, a sensitivity that may increase the likelihood of their use and escalation (for a review see Carroll, Anker, & Perry, 2009). For example, adolescent rats self-administer more ethanol (Brunell & Spear, 2005) and nicotine (Levin, Lawrence, Petro, Horton, Rezvani, Seidler, & Slotkin, 2007), exhibit stronger nicotine-induced conditioned place preferences (CPP; Beluzzi, Lee, Oliff, & Leslie, 2004; Brielmaier, McDonald, & Smith, 2007; Shram, Funk, Li, & Lê, 2006; Vastola, Douglas, Varlinskaya, & Spear, 2002) and display greater cocaine-induced locomotor sensitization following repeated administration (Caster, Walker, & Kuhn, 2005) than their adult counterparts, all effects consistent with the hypothesis that adolescent rats find these compounds more rewarding.

Although assessments of drug reward in adolescents are important, drug use and abuse are due to the balance between the rewarding and aversive effects of a given compound (Davis & Riley, 2010; Riley, 2011; Spear & Varlinskaya, 2010; Wise, Yokel, & DeWit, 1976), and an understanding of both affective properties is critical in understanding abuse vulnerability. In this context, adolescent rats appear relatively insensitive to the aversive effects of a number of drugs of abuse, including amphetamine (Infurna & Spear, 1979), cocaine (Schramm-Sapota, Morris, & Kuhn, 2006), THC (Schramm-Sapota, Cha, Chaudhry, Wilson, Swartzwelder, & Kuhn, 2007) ethanol (Anderson, Varlinskaya, & Spear, 2010; Vetter-O'Hagen, Varlinskaya, & Spear, 2009) nicotine (Shram et al., 2006) and morphine (Hurwitz, Merluzzi, & Riley, 2012). In one of the first assessments of age differences in the aversive effects of drugs (as indexed by taste aversion learning), Infurna and Spear (1979) exposed preweanling, periadolescent and adult rats to a sucrose solution paired with one of three doses (1, 4 or 8 mg/kg) of amphetamine. Aversions were weakest in the periadolescent rats compared to preweanlings and adults, indicative of their blunted aversive response to amphetamine administration. Such differential reactivity has now been reported for a wide variety of drugs. Blunted aversive responses in adolescents are reported for the same drugs for which adolescents display an increased reward sensitivity, suggesting that this population is especially vulnerable to the use and abuse of drugs.

A drug that has been popular among adolescent human populations and has received considerable attention since being categorized as a Schedule I controlled substance by the United States Drug Enforcement Administration in 1985 (Martinez-Price, Krebs-Thomson, & Geyer, 2002) is (±)3,4-methylenedioxymethamphetamine (MDMA). Although the initiation of MDMA use in 18–50 year olds has been on the decline since 2002, lifetime usage rates of MDMA for 12th grade students have significantly increased between 2009 and 2011 (Johnston et al., 2012). Use rates in these populations is lower than the peak usage reported in 2001, but the 2011 data depict much higher use than between 2003 and 2009 (Johnston et al., 2012). Coupled with this, there has been a general decrease in reported “perceived risk” of MDMA use for 8th, 10th and 12th graders since 2004 (Johnston et al., 2012), a concerning trend.

MDMA has been demonstrated to be both rewarding and aversive in animal models of drug abuse. MDMA is self-administered in rodents (de la Garza, Fabrizio, & Gupta, 2007; Schenk, Gittings, Johnstone, & Daniela, 2003), dose-dependently lowers intracranial self-stimulation thresholds (Lin, Jackson, Atrens, Christie, & McGregor, 1997; Reid, Hubbell, Tsai, Fishkin, & Amendola, 1996) and produces dose-dependent CPP in both adult (Brida, Iosue, Pegorini, & Sala, 2005; Marona-Lewicka, Rhee, Sprague, & Nichols, 1996) and adolescent (Catlow, Badanich, Sponaugle, Rowe, Song, Rafalovich, Sava, Kirstein, & Sanchez-Ramos, 2010) rats, all measures indicative of MDMA's rewarding properties. Conversely, MDMA produces taste aversions to solutions associated with its administration in adult Wistar (Lin, Atrens, Christie, Jackson, & McGregor, 1993; Lin, McGregor, Atrens, Christie, & Jackson, 1994) and Sprague-Dawley (Albaugh, Rinker, Baumann, Sink, & Riley, 2011) rats, although no assessments have examined MDMA-induced taste aversions in adolescent rats of either strain. Accordingly, in the present series of studies MDMA-induced taste aversions were assessed in both adolescent (Experiment 1) and adult (Experiment 2) male Sprague-Dawley rats. Specifically, subjects of both ages were injected subcutaneously with one of three doses of MDMA (1.0, 1.8 or 3.2 mg/kg) or saline vehicle following access to a novel saccharin solution and then tested for their subsequent aversions. The resulting acquisition and expression of a CTA provides information regarding age-dependent aversive effects of MDMA administration. MDMA has been demonstrated to produce profound neurochemical changes to the monoaminergic system (see Baumann, Wang, & Rothman, 2007; Baumann, Zolkowska, Kim, Scheidweiler, Rothman, & Huestis, 2009; Colado, O'Shea, & Green, 2004; Green, Mehan, Elliott, O'Shea, & Colado, 2003; Sprague & Nichols, 2006), and little is known of the neurochemical effects of MDMA at these doses, by this route of administration and in either adolescent and adult rats (see Broening, Bacon, & Slikker, 1994; Finnegan, Ricautre, Ritchie, Irwin, Peroutka, & Langston, 1988; Ricautre, DeLanney, Irwin, & Langston, 1988). As such, upon completion of behavioral testing in each assessment, brain tissue samples from the frontal cortex (CTX) and dorsal (DSTR) and ventral (VSTR) striatum were collected and analyzed via high-performance liquid chromatography coupled to electrochemical detection (HPLC-ECD) for potential age differences in monoamine and metabolite levels.

Methods

Subjects and Apparatus

Sixty-six male Sprague-Dawley, experimentally naïve rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) on postnatal day (PND) 21 and served as the subjects (33 in each experiment). Upon arrival to the laboratory, animals were handled and weighed, group-housed in clear polycarbonate (23 × 44 × 21 cm) bins ($n=3$ per bin) and maintained on a 12:12 h light/dark cycle (lights on at 0800h) at an ambient temperature of 23°C. Food was provided *ad libitum* throughout all phases of the experiment. During adaptation, conditioning and aversion testing (see below), animals were transferred to individual hanging wire-mesh (24.3 × 19 × 18 cm) test cages for 65 min per day but were subsequently returned to their group-housed bins following each daily session. All procedures were in compliance with the National Research Council guidelines for the care and use of laboratory

animals (1996) and approved by the Institutional Animal Care and Use Committee at American University.

Drugs

MDMA (generously supplied by the National Institute on Drug Abuse) was dissolved in sterile isotonic saline (Sigma) at a concentration of 2 mg/ml and was filtered through a 0.2 μ m syringe filter to remove any possible contaminants before being administered subcutaneously at a dose of 1.0, 1.8 or 3.2 mg/kg (see Albaugh et al., 2011; Lin et al., 1993). Sterile isotonic saline was also filtered prior to being administered to vehicle control animals at a volume equal to the highest dose of MDMA administered (3.2 mg/kg). Sodium saccharin (0.1%; Sigma) was prepared daily as a 1g/l solution in tap water.

Procedure

Experiment 1: Adolescent Assessment

Phase I: Adaptation: Subjects were brought into the laboratory on PND 21. During PND 21–25, subjects were maintained on *ad libitum* food and water and weighed and handled daily. Over the next 2 days (PND 26 and 27), daily water consumption for each group-housed bin was recorded to the nearest 0.1 ml. On PND 28, the amount of water available for each bin was reduced to 50% (plus an additional 5 ml to account for inaccessible water) of the average of the previous 2 days' drinking levels to encourage consumption of water that was presented in the test cages on the next day. Specifically, on PND 29 subjects were removed from their group-housed bin, weighed and placed into the test cages where they were given 45-min access to tap water in graduated 50-ml Nalgene tubes affixed to the front of the cage. After this access, the bottles were removed, consumption was recorded to the nearest 0.5 ml 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 PND 30, the amount of water available for each bin was again reduced (as described above) with the exception that individual test cage consumption was also factored into the amount consumed in the previous 22.5 h. On PND 31, subjects were again weighed and handled, placed into the test cages and given 45-min access to tap water. After an additional 20 min, they were returned to their group-housed bin with *ad libitum* water for the next 22.5 h. On PND 32, water available to subjects was again reduced (as described above) before undergoing taste aversion conditioning in the test cages (see below).

Phase II: Taste aversion conditioning: On PND 33, all subjects were weighed and handled and given 45-min access to a novel sodium saccharin solution in the test cages. Immediately following saccharin access, subjects were assigned to one of four groups such that saccharin consumption was comparable among groups. Based on these group assignments, subjects were given a subcutaneous injection of 1.0, 1.8 or 3.2 mg/kg MDMA or saline vehicle 20-min later and then returned to their home cage 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 ($n=8$) where the number indicates the dose of MDMA administered. On PND 34, subjects in each bin had their fluid consumption reduced (as described above) before the subsequent conditioning

day. This procedure (saccharin-24 h recovery-50% deprivation) was repeated four times from PND 33–40.

Phase III: Two-bottle aversion test: On PND 41, subjects were transferred to the 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 front of the cage for 45 min. Placement of the bottles was counterbalanced (left vs. right side) to control for positioning effects. After the 45-min test period, the bottles were removed, consumption was recorded to the nearest 0.5 ml and subjects were returned to their home cages where water was made available *ad libitum*.

Phase IV: Monoamine/metabolite analysis: Immediately following completion of the two-bottle aversion test of Phase III, animals were decapitated and brain tissue was removed for monoamine analysis via HPLC-ECD. Areas of the CTX, DSTR and VSTR were dissected for analysis as previously described (Heffner, Hartman, & Seiden, 1980). Following weighing, tissue samples were diluted in 200 μ l (CTX) or 1,000 μ l (DSTR; VSTR) ice cold 0.1 N perchloric acid, homogenized and centrifuged at 4°C at 15,000 rpm for 15 min. The concentrations of dopamine (DA), serotonin (5-HT), norepinephrine (NE) and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were quantified as picograms (pg) per milligram (mg) of tissue in the supernatant using HPLC-ECD as previously described (see Baumann, Clark, Franken, Rutter, & Rothman, 2008).

Statistical Analysis: A 4 (Dose) $\times$ 4 (Trial) mixed model ANOVA was utilized to assess differences in saccharin consumption (ml) over the four conditioning trials. Where appropriate, subsequent one-way ANOVAs and Tukey's HSD post-hoc analyses were employed to evaluate group differences. Bonferroni-corrected independent samples t-tests were utilized to compare saccharin consumption (ml) between Trials 1 and 4. One-way ANOVAs with Tukey's HSD post-hoc analyses were utilized to assess differences in both total fluid (ml) and percent saccharin consumed between dose groups during the two-bottle aversion test. Prior to the analysis of monoamine/metabolite levels, all neurochemical data were examined for the presence of outliers indicative of a dissection error. Specifically, if the value for one analyte was found to be greater than three standard deviations from the mean, it was excluded from analysis. Further, if data from any individual subject were excluded for two brain regions, it was assumed that there was a general dissection error and all the neurochemical data from that subject was removed from the analysis. One-way ANOVAs with Tukey's HSD post-hoc analyses were utilized to assess group differences in monoamine/metabolite levels for each brain area assayed. Significance was assessed at α 0.05, unless otherwise indicated.

Experiment 2: Adult Assessment

Procedure and Statistical Analysis: The procedure and statistical analyses for Experiment 2 were identical to that for Experiment 1 with the following exceptions to the procedure: Adaptation (Phase I) proceeded from PND 78–88, Taste aversion conditioning (Phase II) proceeded from PND 89–96, the Two-bottle aversion test (Phase III) was administered on

PND 97 and brain tissue for the Monoamine/metabolite analysis (Phase IV) was collected the same day. Group *n*'s were identical to those utilized in Experiment 1.

Results

Experiment 1: Adolescent Assessment

Taste aversion conditioning—The 4×4 mixed model ANOVA on saccharin consumption (ml) over the four conditioning trials revealed significant effects of Trial [$F(3,87)=8.147, p<0.05$] and Dose [$F(3,29)=17.283, p<0.05$] as well as a significant Trial $\times$ Dose [$F(9,87)=9.293, p<0.05$] interaction. Subsequent one-way ANOVAs on individual trials revealed significant differences between dose groups on Trials 2–4 ($p's<0.05$) such that on Trial 2, Group 3.2 consumed significantly less saccharin than Group 0 ($p<0.05$). On Trial 3, Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0 ($p's<0.05$). Additionally, Group 3.2 consumed significantly less saccharin than Groups 1.0 and 1.8 ($p's<0.05$). On Trial 4, Groups 1.8 and 3.2 consumed significantly less saccharin than Groups 0 or 1.0 ($p's<0.05$). Further, Group 3.2 consumed significantly less saccharin than Group 1.8 ($p<0.05$; see Figure 1A).

Bonferroni-corrected independent samples *t*-tests on saccharin consumption (ml) between Trials 1 and 4 indicated that Groups 1.8 and 3.2 significantly decreased their saccharin consumption over trials [$t(7)=4.556, p<0.0125$ and $t(7)=6.262, p<0.0125$, respectively], while Groups 0 and 1.0 did not significantly alter their saccharin consumption [$t(8)=-2.268, p>0.0125$ and $t(7)=-0.479, p>0.0125$, respectively].

Two-bottle aversion test—A one-way ANOVA on total fluid consumption (saccharin plus water) on the two-bottle test indicated significant differences between dose groups [$F(3,32)=10.469, p<0.05$]. Specifically, Groups 1.8 and 3.2 consumed significantly less fluid than Group 0 ($p's<0.05$) and Group 3.2 consumed significantly less fluid than Group 1.0 ($p<0.05$). Given this, saccharin consumption during the two-bottle test was transformed and analyzed as percent saccharin of total fluid consumed. A one-way ANOVA on percent saccharin consumption revealed significant differences between dose groups [$F(3,32)=16.168, p<0.05$] such that Groups 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0 ($p's<0.05$) and Group 3.2 consumed a significantly smaller percentage of saccharin than Groups 1.0 and 1.8 ($p's<0.05$; see Figure 2A).

Monoamine/metabolite analysis—There was an error in brain extraction precluding the analysis of data from the DSTR and VSTR for one subject in Group 0. Other data were removed from the monoamine/metabolite analysis due to the presence of outliers (see above); the number of subjects removed from each brain region and dose are as follows: Adolescent CTX (2 Group 1.8); Adolescent DSTR (1 Group 1.0 and 1 Group 1.8); Adolescent VSTR (1 Group 1.8). This resulted in the following group sizes for each brain region examined: Adolescent CTX (Group 0, $n=9$; Group 1.0, $n=8$; Group 1.8, $n=6$; Group 3.2, $n=8$); Adolescent DSTR (Group 0, $n=8$; Group 1.0, $n=7$; Group 1.8, $n=7$; Group 3.2, $n=8$); Adolescent VSTR (Group 0, $n=8$; Group 1.0, $n=8$; Group 1.8, $n=7$; Group 3.2, $n=8$). The one-way ANOVAs on monoamine/metabolite levels in the CTX and DSTR revealed no significant effect of Dose for any analyte examined ($p's>0.05$). The one-way ANOVAs on

monoamine/metabolite levels in the VSTR revealed a significant effect of Dose for 5-HT [$F(3,30)=3.188, p<0.05$] with samples from Group 1.8 containing significantly lower levels of 5-HT in the VSTR than samples from Group 1.0 ($p<0.05$).

Experiment 2: Adult Assessment

Taste aversion conditioning—The 4×4 mixed model ANOVA on saccharin consumption (ml) over the four conditioning trials revealed significant effects of Trial [$F(3,87)=42.864, p<0.05$] and Dose [$F(3,29)=56.962, p<0.05$] as well as a significant Trial $\times$ Dose [$F(9,87)=23.070, p<0.05$] interaction. Subsequent one-way ANOVAs revealed significant differences between dose groups on Trials 2–4 ($p's<0.05$) such that on Trial 2, Groups 1.8 and 3.2 consumed significantly less saccharin than Groups 0 and 1.0 ($p's<0.05$). On both Trials 3 and 4, Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0 ($p's<0.05$). Additionally, Groups 1.8 and 3.2 consumed significantly less saccharin than Group 1.0 ($p's<0.05$; see Figure 1B).

Bonferroni-corrected independent samples t-tests on saccharin consumption (ml) between Trials 1 and 4 indicated that Groups 1.0, 1.8 and 3.2 significantly decreased their saccharin consumption over trials [$t(7)=4.456, p<0.0125$; $t(7)=11.212, p<0.0125$; $t(7)=10.764, p<0.0125$, respectively], while Group 0 significantly increased their saccharin consumption [$t(8)=-4.127, p<0.0125$].

Two-bottle aversion test—A one-way ANOVA on total fluid consumption on the two-bottle test indicated significant differences between dose groups on overall fluid consumption [$F(3,32)=11.861, p<0.05$]. Specifically, Groups 1.0, 1.8 and 3.2 consumed significantly less fluid than Group 0 ($p's<0.05$). Given this, saccharin consumption during the two-bottle test was transformed and analyzed as percent saccharin of total fluid consumed. A one-way ANOVA on percent saccharin consumption revealed significant differences between dose groups [$F(3,32)=179.745, p<0.05$] such that Groups 1.0, 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0 ($p's<0.05$; see Figure 2B).

Monoamine/metabolite analysis—There was an error in brain extraction precluding the analysis of data from the CTX, DSTR and VSTR in one subject in Group 1.8. Other data for some animals were removed from the monoamine/metabolite analysis due to the presence of outliers (see above); the number of subjects removed from each brain region and dose are as follows: Adult CTX (1 Group 1.0, 2 Group 1.8 and 1 Group 3.2); Adult DSTR (2 Group 1.0); Adult VSTR (1 Group 1.8). This resulted in the following group sizes for each brain region examined: Adult CTX (Group 0, $n=9$; Group 1.0, $n=7$; Group 1.8, $n=5$; Group 3.2, $n=7$); Adult DSTR (Group 0, $n=9$; Group 1.0, $n=6$; Group 1.8, $n=7$; Group 3.2, $n=8$); Adult VSTR (Group 0, $n=9$; Group 1.0, $n=8$; Group 1.8, $n=6$; Group 3.2, $n=8$). The one-way ANOVAs on monoamine/metabolite levels in the CTX revealed no significant effect of Dose for any analyte examined ($p's>0.05$). The one-way ANOVAs on monoamine/metabolite levels in the DSTR revealed a significant effect of Dose for NE [$F(3,29)=3.319, p<0.05$] with samples from Group 0 containing significantly lower levels of NE in the DSTR than samples from Group 3.2 ($p<0.05$). The one-way ANOVAs on

monoamine/metabolite levels in the VSTR revealed no significant effect of Dose for any analyte examined ($p>0.05$).

Adolescent-Adult Comparisons

Although the two age groups were run as two separate experiments, an exploratory statistical analysis was conducted to examine age-related effects. It should be noted that the animals in Experiments 1 and 2 were matched in every way except for their age and the date on which the experimental procedures were carried out.

Taste aversion conditioning—A 2 (Age) x 4 (Dose) x 4 (Trial) mixed model ANOVA on saccharin consumption (ml) over the four conditioning trials revealed significant effects of Trial [$F(3,174)=41.278, p<0.05$], Dose [$F(3,58)=66.729, p<0.05$] and Age [$F(1,58)=64.310, p<0.05$] as well as significant Dose x Age [$F(3,58)=7.745, p<0.05$], Trial x Dose [$F(9,174)=26.223, p<0.05$], Trial x Age [$F(3,174)=9.339, p<0.05$] and Trial x Dose x Age [$F(9,174)=5.983, p<0.05$] interactions. A subsequent one-way ANOVA revealed significant differences between age and dose groups on Trials 2–4 ($p<0.05$) such that adult Groups 1.8 and 3.2 drank significantly less saccharin relative to adolescent Groups 1.8 and 3.2, respectively ($p<0.05$). Further, on Trials 3 and 4 adult Group 1.0 consumed significantly less saccharin relative to adolescent Group 1.0 ($p<0.05$).

Two-bottle aversion test—Bonferroni-corrected independent samples t-tests used to examine age differences in saccharin preference during the two-bottle test revealed that adult Group 1.0 [$t(14)=5.516, p<0.0125$] and Group 1.8 [$t(14)=4.166, p<0.0125$] consumed a significantly smaller percentage of saccharin relative to adolescents, with no difference between age groups for Group 0 [$t(16)=0.901, p>0.0125$] and Group 3.2 [$t(14)=1.396, p>0.0125$].

Monoamine/metabolite analysis—A 2 (Age) x 4 (Dose) univariate ANOVA was performed for each major monoamine and metabolite examined and for each of three brain regions. For samples from the CTX, a significant main effect of Age was found for DA [$F(1,51)=7.180, p<0.05$], DOPAC [$F(1,51)=7.140, p<0.05$], 5-HT [$F(1,51)=80.841, p<0.05$], 5-HIAA [$F(1,51)=14.339, p<0.05$] and NE [$F(1,51)=168.541, p<0.05$], with adolescent samples containing significantly lower levels of the respective monoamine/metabolite relative to adults (see Table 1). For samples from the DSTR, a significant main effect of Age was found for DA [$F(1,52)=7.977, p<0.05$], DOPAC [$F(1,52)=13.069, p<0.05$], 5-HT [$F(1,52)=49.056, p<0.05$], and NE [$F(1,52)=12.503, p<0.05$] with adolescent samples containing significantly lower levels of the respective monoamine/metabolite relative to adults (see Table 1). For samples from the VSTR, a significant effect of Age was found for 5-HT [$F(1,54)=6.749, p<0.05$] with adolescent samples containing significantly lower levels of 5-HT relative to adults (see Table 1).

Discussion

The experiments described here are the first to report age differences in the aversive effects of MDMA. In particular, MDMA induced dose-dependent taste aversions in both adolescent and adult animals (see also Albaugh et al., 2011; Lin et al., 1993; Lin et al., 1994), but

aversions were significantly weaker in the adolescent subjects. Blunted taste aversions were evident in the doses at which the aversions were acquired, the rate at which the aversions were first evident and the degree of suppression (in both the one- and two-bottle assessments) displayed by the animals. These data with MDMA are consistent with several recent assessments reporting weaker taste aversions in adolescent animals when tested with a variety of drugs of abuse (see Introduction). Although MDMA altered the levels of several monoamines (5-HT in the VSTR and NE in the DSTR) in adolescent and adult animals, when age was added as a factor in the exploratory analysis focusing on age comparisons, no drug-induced effects emerged.

Although the basis for the reported age difference in the aversive effects of MDMA is unknown, several possibilities exist. Given that the taste aversion preparation is dependent upon associative learning, it is possible that the age difference in MDMA-induced aversions could reflect a general deficit in learning in adolescent animals relative to adults (for a discussion of this issue in such age and strain comparisons, see Cunningham, Gremel, & Groblewski, 2009; Riley, Davis, & Roma, 2009). While possible, there is a host of work utilizing the CPP procedure which suggests that adolescent animals do not have such a general associative learning deficit. For example, adolescent rats have been reported to display significantly greater nicotine-induced CPP than adults (Beluzzi et al., 2004; Brielmaier et al., 2007; Shram et al., 2006; Vastola et al., 2002). Further, Brenhouse and Andersen (2008) reported greater CPP in adolescent rats to cocaine at 10 mg/kg, with adolescents requiring 75% more extinction trials to extinguish the preference, suggesting that the adolescent population may be especially resistant to extinction of the association (though see Campbell, Wood, & Spear, 2000 for a report of similar expression of CPP to cocaine and morphine in adolescent and adult rats). MDMA-induced CPP has not been assessed concurrently in adolescent and adult animals (see Tzschentke, 2007 for a thorough review of CPP literature), but independent reports show adolescents and adults acquire preferences at comparable doses (see Bilsky, Hui, Hubbell, & Reid, 1990; Catlow et al., 2010; Marona-Lewicka et al., 1996). It is clear from CPP investigations that adolescent animals do not display any general learning deficit that might impact taste aversion conditioning.

It is possible that adolescent animals have some sort of memory deficit, which could affect their ability to retain and express CTAs relative to their adult counterparts. In this context, investigations have demonstrated no age difference in cyclophosphamide- (a chemotherapeutic compound; Misanin, Anderson, & Hinderlitter, 2009) and lithium chloride- (LiCl; Misanin, Guanowski, & Riccio, 1983) induced CTAs when the aversions are tested shortly after conditioning, e.g., 1 day. Age differences can be evident with longer testing delays, e.g., 28, 30 and 60 days post-training (though see Klein, Mikulka, Domato, & Hallstead, 1977 for similar LiCl-induced CTAs in adolescents and adults after either 1- and 28-day testing intervals). Age differences have been reported in LiCl-induced CTAs in two-bottle, but not one-bottle, aversion tests (Klein, Domato, Hallstead, Stephens, & Mikulka, 1975; Mikulka, Krone, Rapisardi, & Kirby, 1975). Indeed, the two-bottle assessment may be more sensitive in detecting group differences (Grote & Brown, 1971; Klein et al., 1975; Riley & Mastropaolo, 1989) than the one-bottle procedure. Although such age differences in taste aversion learning do appear under a variety of conditions, it is important to note that

the parametric conditions reported here, e.g., immediate test and one-bottle assessment, are those under which age differences to classical emetics are not reported, suggesting that the differences in MDMA-induced aversions are unlikely a function of a memory deficit in adolescent subjects.

The effect of fluid deprivation employed in the current procedure may have played a role in the behavioral effects observed. At the end of each assessment, adolescent and adult animals weighed 87.2% and 97.6%, respectively, of age-, housing- and strain-matched animals allowed to grow up in our laboratory for baseline body weight data (data not shown). Given that the adolescent animals in Experiment 1 displayed a greater percentage decrease in body weight relative to animals maintained under *ad libitum* water access than did adults in Experiment 2, it is possible that the fluid deprivation procedure differentially affected the age groups. If this were the case, the weaker aversions in adolescent animals could possibly be due to the fact that these animals were more motivated to consume fluid, regardless of its prior association with MDMA administration. Thus, the blunted aversive response in adolescent animals may not be reflective of affective processing, but differential motivation. Although possible, a recent assessment from our laboratory compared the ability of adolescent and adult rats to acquire taste aversions to morphine following high- and low-fluid deprivation procedures wherein animals were either restricted to 20-min per day of fluid access or the deprivation procedure utilized in the current assessments, respectively. In both of these assessments, there was no difference in the overall pattern of responding between the deprivation conditions with adolescent animals displaying attenuated aversions in comparison to the adults (Hurwitz et al., 2012). Further, the age difference in the aversive effects of MDMA was still evident in the two-bottle test, an assessment that is less influenced by fluid deprivation given that it does not require animals to consume saccharin when the water choice is freely available (Grote & Brown, 1971; Sengstake & Chambers, 1978).

Although motivation to drink may not have been a contributing factor, it is nonetheless possible that the fluid deprivation schedule employed was more stressful in the adolescent subjects relative to their adult counterparts. Further, it is possible that adolescent animals experienced more stress given that they were given a shorter time to acclimate to the vivarium prior to the initiation of experimental procedures than their adult counterparts. Interestingly, the effects of stress on the development and expression of CTAs are mixed, with reports of stress potentiating CTAs (Bowers, Gingras, & Amit, 1996; Lasiter & Braun, 1981) and in some cases, stress having no effect (Bowers et al., 1996; Holder, Yirmiya, Garcia, & Raizer, 1989; Roma, Davis, Kohut, Huntsberry, & Riley, 2008). If the adolescent animals in the present assessment were under more stress, it might be expected that they would show stronger MDMA-induced aversions. Of note, a recent investigation of the effect of stress on the formation of CTAs induced by ethanol in adolescent animals reported that neither restraint stress nor isolate housing influenced the magnitude of the aversion (Anderson et al., 2010). In the absence of a direct measure of stress in the current assessment, however, differential effects of stress remain a possibility for the behavioral findings reported here.

Perhaps the simplest explanation for the present data is that adolescent rats are less sensitive to the aversive properties of MDMA when compared to adults. This position is consistent with the interpretation of many preclinical investigations of the aversive effects of abused drugs in adolescents (see above). Many investigators have attempted to characterize the underpinnings of aversive effects of toxins such as LiCl and abused drugs such as cocaine (see Freeman & Riley, 2009; Parker, Limebeer, & Rana, 2009). These assessments have provided discussions of possible mediation by nausea (Coil, Hankins, Jenden, & Garcia, 1978) and anxiogenesis (Schramm-Sapota et al., 2006), respectively. However, compounds that diminish nausea and those that reduce anxiety (see Berger, 1972 for a description of aversions induced by the antiemetic, scopolamine and the anxiolytic, lorazepam) also reliably induce taste aversions, suggesting that the nature of aversion learning is complex (Cappell & LeBlanc, 1977; Goudie, Stolerman, Demellweek, & D'Mello, 1982; Hunt & Amit, 1987; for a recent review of this issue, see Verendeev & Riley, 2012). Thus, speculating that there might be differences in this aversive effect in various age groups must be made cautiously. This is especially the case for compounds such as MDMA for which the characterization of its ability to induce aversions is relatively limited (see Albaugh et al., 2011; Lin et al., 1993; Lin et al., 1994).

It is known that relatively high doses of MDMA lead to persistent reductions in brain amines, specifically 5-HT, in adult rats (Baumann et al., 2008; Byrne, Baker, & Poling, 2000; Colado, Williams, & Green, 1995; Connor, McNamara, Kelly, & Leonard, 1999; McNamara, Kelly, & Leonard, 1995; O'Hearn, Battaglia, De Souza, Kuhar, & Molliver, 1988). These investigations that do report depletion have utilized doses of MDMA ranging from 7.5 mg/kg to 40 mg/kg, doses much higher than those used here and those reported in human anecdotal reports (Baumann et al., 2009; Green et al., 2003; Sprague & Nichols, 2006; see Baumann et al., 2007 for a thorough discussion of interspecies scaling). In this context, little is known about the relative reactivity of the adolescent monoamine system (both acute and long-term) to MDMA administration. Of interest, Broening et al. (1994) exposed neonatal (PND 10), adolescent (PND 40) and adult (PND 70) rats to high doses of MDMA (10–40mg/kg) administered orally (po) and reported significant depletion of 5-HT in the CTX and caudate putamen in adolescent and adult rats (though not in the neonatal rats) at 20 and 40 mg/kg. It should be noted that Broening and his colleagues administered MDMA orally, replicating the route of administration utilized by humans, while the present series of assessments administered MDMA subcutaneously. It is, therefore, possible that the route of administration utilized (in addition to the dosing regimen) might affect any MDMA-induced neurochemical changes. In support of this, 5 mg/kg MDMA administered orally in the squirrel monkey is less effective at inducing neurochemical changes than the subcutaneous route (Ricaurte et al., 1988), although it produces similar neurochemical profiles in adult Sprague-Dawley rats (Finnegan et al., 1988) at 7.5 to 30 mg/kg. Given that assessments with the subcutaneous route have not been performed in adolescent animals of either species, it is unknown what effect, if any, MDMA might have on monoamine levels. Therefore, it was of interest to assess whether exposure to MDMA produced a different neurochemical profile in the adolescent age group, especially in comparison to the adults.

As described, there was no consistent effect of MDMA administration on the levels of monoamines or metabolites in the brain regions examined in either adolescents or adults.

The predominant finding with respect to monoamine/metabolite levels was that adolescents uniformly showed lower concentrations than adults. These age differences in monoamine concentrations are consistent with the limited number of developmental assessments of monoamine levels in Wistar rat brain tissue. Specifically, during development overall levels of DA fibers increase until PND 60 (Kalsbeek, Voorn, Buijs, Pool, & Ulyings, 1988), levels of 5-HT increase until PND 70 (though DA and NE appear to level off by PND 26; Herregodts, Velkeniers, Ebinger, Michotte, Vanhaelst, & Hooghe-Peters, 1990) and monoamine transporter levels increase well into adulthood (Moll, Mehnert, Wicker, Bock, Rothenberger, Rther, & Huether, 2000). Although suggestive of age-dependent differences in monoamine levels, it is possible that the differences in monoamine levels reported here might be a function of a differential level of stress between the cohorts (adolescents > adults; see above). While possible, investigations utilizing adult rats have reported that chronic unpredictable stress has no effect on levels of 5-HT and DA in the CTX (Gamaro, Manoli, Torres, Silveria, & Dalmaz, 2003; Johnson & Yamamoto, 2009) and striatum (Johnson & Yamamoto, 2009), with stressed animals displaying comparable levels to non-stressed controls (though see Cuadra, Zurita, Gioino, & Molina, 2001 for data relaying increased levels of DA in the CTX in response to chronic unpredictable stress). Interestingly, foot-shock increases DA activation in the mesocortical system (Thierry, Tassin, Blanc, & Glowinski, 1976) and tail-shock potentiates DA levels 95% above control animal values in the CTX (Abercrombie, Keefe, Di Frischia, & Zigmond, 1989). If adolescent rats were more stressed in the present experiment, it might be expected that they would display increases in monoamines levels in these brain regions. In the absence of a direct measure of stress in the current assessments, it remains unknown what effect, if any, stress had on the neurochemical measures performed.

The present assessments provide further evidence of adolescent insensitivity to the aversive effects of drugs of abuse, in this case, MDMA. This blunted sensitivity suggests adolescent populations may be more vulnerable to drug use and abuse, making them particularly at-risk for the development of dependence. Continued investigations into the relative sensitivity of adolescents to both the aversive and reinforcing effects of drugs may provide insight in understanding drug use and addiction.

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Figure 1.

Panel A: Mean ($\pm$ SEM) saccharin consumption for adolescent animals throughout Phase II: Conditioning. *Group 3.2 consumed significantly less saccharin than Group 0. ^Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0. #Group 3.2 consumed significantly less saccharin than Groups 1.0 and 1.8. +Groups 1.8 and 3.2 consumed significantly less saccharin than Group 0. xGroups 1.8 and 3.2 consumed significantly less saccharin than Group 1.0. vGroup 3.2 consumed significantly less saccharin than Group 1.8. Panel B: Mean ($\pm$ SEM) saccharin consumption for adult animals throughout Phase II: Conditioning. ∞ Groups 1.8 and 3.2 consumed significantly less saccharin than Groups 0 and 1.0. ^Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0. xGroups 1.8 and 3.2 consumed significantly less saccharin than Group 1.0.

Figure 2.

Panel A: Percent ($\pm$ SEM) saccharin consumption for adolescent animals throughout Phase III: Two-Bottle Aversion Test. *Groups 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0. ^Group 3.2 consumed a significantly smaller percentage of saccharin than Groups 1.0 and 1.8. Panel B: Percent ($\pm$ SEM) saccharin consumption for adult animals throughout Phase III: Two-Bottle Aversion Test. #Groups 1.0, 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0.

Table 1

Analyte (pg/mg)	CTX		DSTR		VSTR	
	Adolescent (n=31)	Adult (n=28)	Adolescent (n=30)	Adult (n=30)	Adolescent (n=31)	Adult (n=31)
DA	22.8 (1.4)*	28.9 (1.7)	5910.4 (456.5)*	7733.2 (464.6)	2993.9 (263.1)	3578.68 (283.5)
DOPAC	9.2 (0.6)*	12.0 (0.9)	833.5 (64.1)*	1117.8 (50.4)	593.6 (49.6)	567.2 (46.2)
HVA	13.1 (0.9)	10.2 (1.5)	519.1 (44.4)	544.4 (27.8)	214.4 (16.9)	227.6 (18.0)
5-HT	159.8 (5.4)*	276.6 (12.0)	500.1 (22.0)*	753.9 (29.5)	824.7 (35.6)*	962.1 (44.3)
5-HIAA	116.1 (2.8)*	137.2 (4.9)	550.9 (20.2)	579.8 (16.7)	511.1 (17.6)	515.8 (17.1)
NE	227.1 (6.0)*	361.9 (7.7)	460.1 (32.3)*	681.2 (60.7)	935.3 (89.1)	804.9 (68.3)

Mean ($\pm$ SEM) levels of monoamines [Dopamine (DA); Serotonin (5-HT); Norepinephrine (NE)] and their metabolites [3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid (HVA); 5-hydroxyindoleacetic acid (5-HIAA)] in the cortex (CTX), dorsal (DSTR) and ventral (VSTR) striatum, collapsed across dose of MDMA administered. Data are expressed as picograms (pg) of analyte per milligram (mg) of tissue.

* Adolescent animals displayed significantly lower levels of the analyte than adult animals.