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

By

Jennifer L. Cobuzzi

Submitted to the

Faculty of the College of Arts and Sciences

of American University

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

In

Behavior, Cognition and Neuroscience

Chair:

Anthony L. Riley, Ph.D.

Michael H. Baumann, Ph.D.

Terry L. Davidson, Ph.D.

David N. Kearns, Ph.D.

Dean of the College of Arts and Sciences

April 12, 2013

Date

2013

American University

Washington, D.C. 20016

AGE DIFFERENCES IN (±)3,4 METHYLENEDIOXYMETHAMPHETAMINE

(MDMA)-INDUCED CONDITIONED TASTE AVERSIONS

AND MONOAMINE LEVELS

BY

Jennifer L. Cobuzzi

ABSTRACT

Adolescence is a life stage characterized by developmental changes (both biological and behavioral) that may interact with the effects of drug administration. This was assessed in the present experiments in which adolescent and adult rats were compared in their ability to acquire taste aversions induced by (±)3,4-methylenedioxymethamphetamine (MDMA; 0, 1.0, 1.8 or 3.2 mg/kg) utilizing doses that are commonly self-administered by human users. Further, monoamine and metabolite levels in discrete brain regions were quantified using HPLC-ECD in order 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, and age was the predominant factor in predicting relative levels of monoamines and their metabolites (adolescent < adult). Given that drug abuse vulnerability is thought to be a function of the balance between the drug's rewarding and aversive effects, the relative insensitivity of adolescents to MDMA's aversive effects may be important to understanding abuse potential in this specific population.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Mellon Foundation to Anthony L. Riley. A portion of this work was supported by funds from the American University College of Arts and Sciences Doctoral Dissertation Fellowship to Jennifer L. Cobuzzi. A portion of this work was supported by intramural funds from the National Institute on Drug Abuse/National Institutes of Health/Public Health Service/US Department of Health and Human Services to Michael H. Baumann. The author would also like to thank Kayla A. Siletti, Zachary E. Hurwitz and Bradley Wetzell for research assistance and technical support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

# CHAPTER 1

## GENERAL INTRODUCTION

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. 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 et al., 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-Sapyta, Morris, & Kuhn, 2006), THC (Schramm-Sapyta et al., 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 12$^{th}$ 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 8$^{th}$, 10$^{th}$ and 12$^{th}$ 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 (Braida, Iosuè, Pegorini, & Sala, 2005; Marona-Lewicka, Rhee, Sprague, & Nichols, 1996) and adolescent (Catlow et al., 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, Mechan, 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.

# CHAPTER 2

# EXPERIMENT 1 METHODS

## Subjects

The subjects were 33 male Sprague-Dawley, experimentally naïve rats (Harlan Sprague-Dawley, Indianapolis, IN) on postnatal day (PND) 21 weighing an average of 41 g. All procedures were in compliance with the National Research Council Guide for the Care and Use of Laboratory Animals (1996) and approved by the Institutional Animal Care and Use Committee at American University (Protocol #111104).

## Apparatus

Upon arrival to the laboratory, animals were handled and weighed, group-housed in clear polycarbonate (23 x 44 x 21 cm) bins (*n*=3 per bin) with maple woodchip bedding 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 x 19 x 18 cm) test cages for 65 min per day but were subsequently returned to their group-housed bins following each daily session.

## Drugs and Solutions

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 µm 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

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 individual 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 following saccharin consumption and then returned to their home cage and given *ad libitum* water access 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 45-min, the bottles were removed, consumption was recorded and subjects were returned to their home cages where water was available *ad libitum*.

Phase IV: Monoamine/metabolite analysis. Following completion of the two-bottle aversion test of Phase III, animals were decapitated and brain tissue was removed for monoamine analysis via HPLC-ED. Areas of the CTX, DSTR and VSTR were dissected for analysis as previously described (Heffner, Hartman, & Seiden, 1980). Specifically, following live decapitation brain tissue was removed and placed on its dorsal surface in a large rat coronal 1-mm stainless steel brain matrix on wet ice. Two ice-cold razorblades were utilized to remove a 2-mm coronal section, located rostral to the optic chiasm. The brain section was placed on a stainless steel cold plate over dry ice, and razor blades were employed to dissect bilateral regions of the CTX, DSTR and VSTR. Brain tissue sections were each stored in 2 ml capacity cryovials at -80°C until tissue processing was performed.

Following weighing, tissue samples were diluted in 200 µl (CTX) or 1,000 µl (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 in the supernatant using HPLC-ECD. Specifically, 20 µl aliquots of supernatant were injected into a SunFire™ reversed-phase C-18 column (5 µm spheres, 150 x 4.6 mm) that was tethered to a coulometric electrochemical detection system (Thermo Fisher Scientific, Chelmsford, MA). Mobile phase consisting of 50 mM sodium phosphate monobasic (pH=2.75), 250 µM $Na_2$ EDTA, 0.025% sodium octane sulfonic acid and 25% methanol was recirculated at a flow rate of 0.9 ml/min. Chromatographic data were exported to the Empower2 software system (Waters Associates, Milford, MA) for quantification and analysis. Peak heights of unknown samples were compared with peak heights of standards of DA, 5-HT, NE, DOPAC, HVA and 5-HIAA and quantified as pg/mg of tissue (see Baumann, Clark, Franken, Rutter, & Rothman, 2008).

## Statistical Analysis

A 4 (Dose) x 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 paired 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 $\alpha \leq 0.05$, unless otherwise indicated.

CHAPTER 3

EXPERIMENT 1 RESULTS

Phase II: Taste aversion conditioning

The 4 x 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 x 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). Tukey's HSD post hoc analysis revealed 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 1).

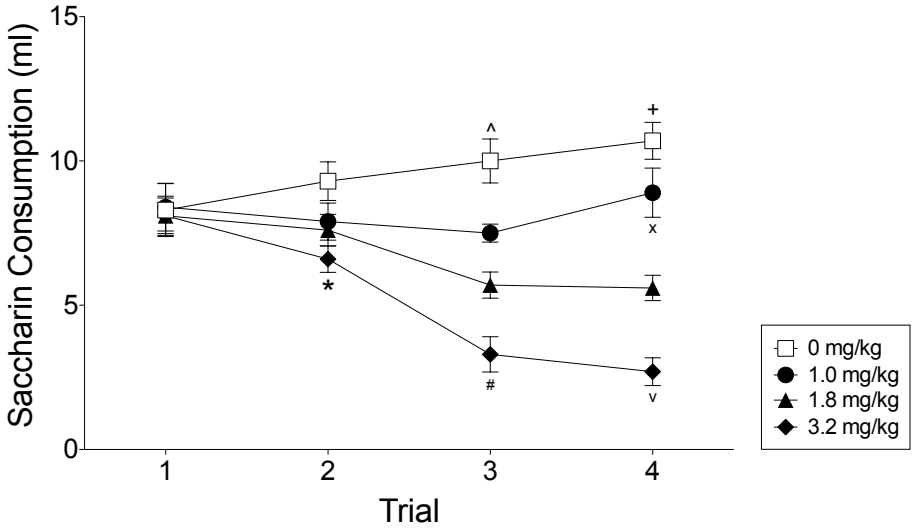


Figure 1. Mean (± SEM) Saccharin Consumption for Adolescent Animals Throughout Phase II: Conditioning. *Group 3.2 consumed significantly less saccharin than Group 0. ^Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0. $^{\#}$Group 3.2 consumed significantly less saccharin than Groups 1.0 and 1.8. $^{+}$Groups 1.8 and 3.2 consumed significantly less saccharin than Group 0. $^{x}$Groups 1.8 and 3.2 consumed significantly less saccharin than Group 1.0. $^{v}$Group 3.2 consumed significantly less saccharin than Group 1.8.

Bonferroni-corrected paired 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].

Phase III: 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 [$F(3,32)=10.469, p<0.05$]. Tukey's HSD post hoc analysis revealed that 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 ($p<0.05$; see Figure 2).

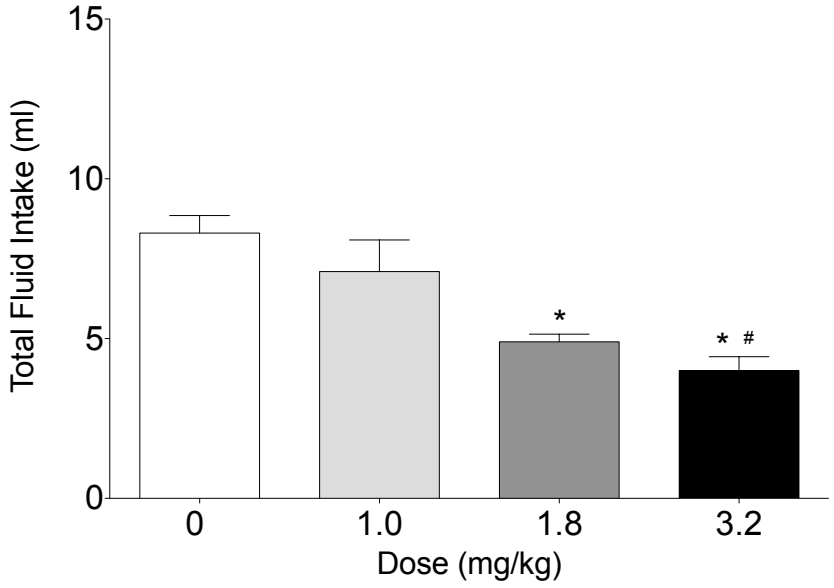


Figure 2. Mean (± SEM) Total Fluid Consumption for Adolescent Animals Throughout Phase III: Two-Bottle Aversion Test. *Groups 1.8 and 3.2 consumed significantly less fluid than Group 0. #Group 3.2 consumed significantly less fluid than Group 1.0.

Given that dose groups consumed different overall levels of fluid, 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 [$F(3,32)=16.168, p<0.05$]. Tukey's HSD post hoc analysis revealed 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 3).

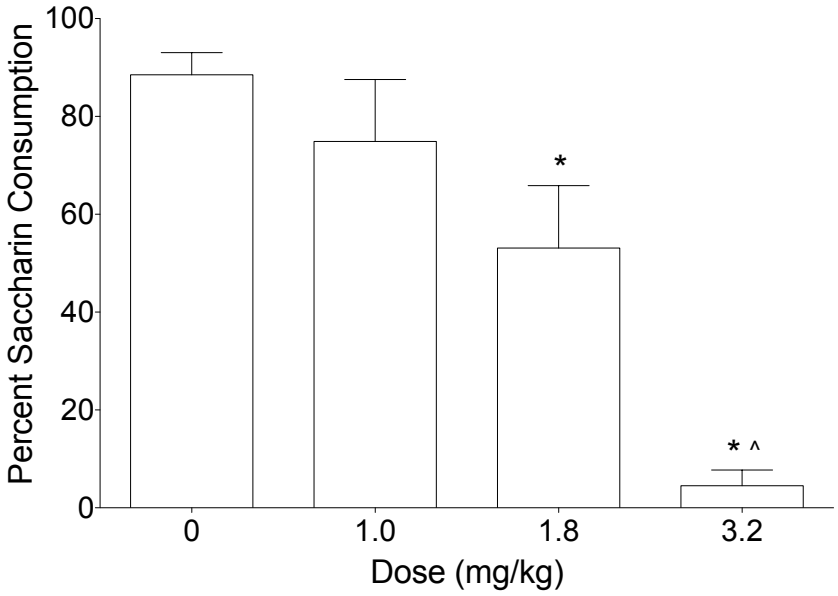


Figure 3. Mean (± SEM) Percent Saccharin Consumption for Adolescent Animals Throughout Phase III: Two-Bottle Aversion Test. *Groups 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0. ^Group 3.2 consumed a significantly smaller percentage of saccharin than Groups 1.0 and 1.8.

Phase IV: 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 (Group 1.8=2); Adolescent DSTR (Group 1.0=1 and Group 1.8=1); Adolescent VSTR (Group 1.8=1). 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; see Table 1). The one-way ANOVAs on monoamine/metabolite levels in the VSTR revealed a significant effect of Dose for 5-HT [$F(3,30)=3.188, p<0.05$]. Tukey's HSD post hoc analysis revealed that samples from Group 1.8 containing significantly lower levels of 5-HT in the VSTR than samples from Group 1.0 ($p<0.05$; see Table 1).

Table 1. Mean (± 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 of Adolescent Animals. Data are expressed as picograms (pg) of analyte per milligram (mg) of tissue. *Group 1.8 displayed significantly lower levels of the analyte than Group 1.0.

| **CTX Analyte (pg/mg)** | **Adol 0 (*n*=9)** | **Adol 1.0 (*n*=8)** | **Adol 1.8 (*n*=6)** | **Adol 3.2 (*n*=8)** |
|---|---|---|---|---|
| **DA** | 23.2 (1.9) | 2.6 (2.8) | 28.0 (4.5) | 18.6 (1.6) |
| **DOPAC** | 8.5 (0.6) | 10.7(1.4) | 10.4 (18) | 7.4 (0.7) |
| **HVA** | 12.0 (0.9) | 15.7 (2.1) | 13.8 (2.7) | 11.2 (1.5) |
| **5-HT** | 158.1 (8.5) | 159.1 (12.1) | 157.0 (11.4) | 164.5 (13.4) |
| **5-HIAA** | 112.3 (4.2) | 119.3 (7.4) | 114.0 (7.2) | 118.5 (4.3) |
| **NE** | 221.7 (7.6) | 234.5 (12.7) | 229.1 (16.7) | 224.4 (14.2) |

| **DSTR Analyte (pg/mg)** | **Adol 0 (*n*=8)** | **Adol 1.0 (*n*=7)** | **Adol 1.8 (*n*=7)** | **Adol 3.2 (*n*=8)** |
|---|---|---|---|---|
| **DA** | 7086.0 (701.2) | 7002.3 (776.4) | 4328.5 (873.9) | 5163.8 (991.0) |
| **DOPAC** | 1023.4 (108.9) | 962.3 (103.0) | 600.1 (103.1) | 73.5.2 (143.6) |
| **HVA** | 546.8 (64.2) | 683.8 (99.6) | 371.8 (55.7) | 458.2 (98.9) |
| **5-HT** | 477.8 (38.0) | 496.2 (29.5) | 485.9 (64.0) | 538.3 (45.6) |
| **5-HIAA** | 558.1 (23.1) | 590.4 (55.3) | 493.9 (49.3) | 559.1 (33.1) |
| **NE** | 471.4 (60.6) | 426.9 (63.7) | 481.1 (94.1) | 459.3 (51.3) |

| **VSTR Analyte (pg/mg)** | **Adol 0 (*n*=8)** | **Adol 1.0 (*n*=8)** | **Adol 1.8 (*n*=7)** | **Adol 3.2 (*n*=8)** |
|---|---|---|---|---|
| **DA** | 3894.7 (278.7) | 3184.0 (541.1) | 2073.7 (692.0) | 2708.3 (426.3) |
| **DOPAC** | 741.0 (50.4) | 627.2 (95.6) | 417.9 (138.2) | 556.5 (87.0) |
| **HVA** | 254.7 (32.0) | 226.9 (24.6) | 167.2 (45.6) | 202.9 (31.4) |
| **5-HT** | 870.9 (36.2) | 950.7 (92.1) | 679.3 (79.8)* | 779.9 (31.0) |
| **5-HIAA** | 504.3 (17.5) | 567.8 (46.9) | 452.2 (44.0) | 512.5 (17.4) |
| **NE** | 909.6 (177.4) | 1232.7 (228.0) | 748.8 (165.6) | 826.7 (94.5) |

# CHAPTER 4

# EXPERIMENT 2 METHODS

## Subjects

The subjects were 33 male Sprague-Dawley, experimentally naïve rats (Harlan Sprague-Dawley, Indianapolis, IN) on postnatal day (PND) 77 weighing an average of 367 g. All procedures were in compliance with the National Research Council Guide for the Care and Use of Laboratory Animals (1996) and approved by the Institutional Animal Care and Use Committee at American University (Protocol #111104).

## Apparatus

Upon arrival to the laboratory, animals were handled and weighed, group-housed in clear polycarbonate (23 x 44 x 21 cm) bins (*n*=3 per bin) with maple woodchip bedding 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 x 19 x 18 cm) test cages for 65 min per day but were subsequently returned to their group-housed bins following each daily session.

## Drugs and Solutions

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 µm 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

Phase I: Adaptation. Subjects were brought into the laboratory on PND 21 and maintained on *ad libitum* food and water while they reached adulthood. During PND 78-81, subjects were maintained on *ad libitum* food and water and weighed and handled daily. Over the next 2 days (PND 82 and 83), daily water consumption for each group-housed bin was recorded to the nearest 0.1 ml. On PND 84, 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 85 subjects were removed from their group-housed bin, weighed and placed into the individual 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 86, 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 87, 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 88, 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 89, 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 following saccharin consumption and then returned to their home cage and given *ad libitum* water access 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 90, 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 89-96.

Phase III: Two-bottle aversion test. On PND 97, 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, the bottles were removed, consumption was recorded and subjects were returned to their home cages where water was available *ad libitum*.

Phase IV: Monoamine/metabolite analysis. Following completion of the two-bottle aversion test of Phase III, animals were decapitated and brain tissue was removed for monoamine analysis via HPLC-ED. Areas of the CTX, DSTR and VSTR were dissected for analysis as previously described (Heffner, Hartman, & Seiden, 1980). Specifically, following live decapitation brain tissue was removed and placed on its dorsal surface in a large rat coronal 1-mm stainless steel brain matrix on wet ice. Two ice-cold razorblades were utilized to remove a 2-mm coronal section, located rostral to the optic chiasm. The brain section was placed on a stainless steel cold plate over dry ice, and razor blades were employed to dissect bilateral regions of the CTX, DSTR and VSTR. Brain tissue sections were each stored in 2 ml capacity cryovials at -80°C until tissue processing was performed.

Following weighing, tissue samples were diluted in 200 µl (CTX) or 1,000 µl (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 in the supernatant using HPLC-ECD. Specifically, 20 µl aliquots of supernatant were injected into a SunFire$^{TM}$ reversed-phase C-18 column (5 µm spheres, 150 x 4.6 mm) that was tethered to a coulometric electrochemical detection system (Thermo Fisher Scientific, Chelmsford, MA). Mobile phase consisting of 50 mM sodium phosphate monobasic (pH=2.75), 250 µM $Na_2$ EDTA, 0.025% sodium octane sulfonic acid and 25% methanol was recirculated at a flow rate of 0.9 ml/min. Chromatographic data were exported to the Empower2 software system (Waters Associates, Milford, MA) for quantification and analysis. Peak heights of unknown samples were compared with peak heights of standards of DA, 5-HT, NE, DOPAC, HVA and 5-HIAA and quantified as pg/mg of tissue (see Baumann, Clark, Franken, Rutter, & Rothman, 2008).

## Statistical Analysis

A 4 (Dose) x 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 paired 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 $\alpha \leq 0.05$, unless otherwise indicated.

CHAPTER 5

EXPERIMENT 2 RESULTS

Phase II: Taste aversion conditioning

The 4 x 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 x 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). ]. Tukey's HSD post hoc analysis revealed 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 4).

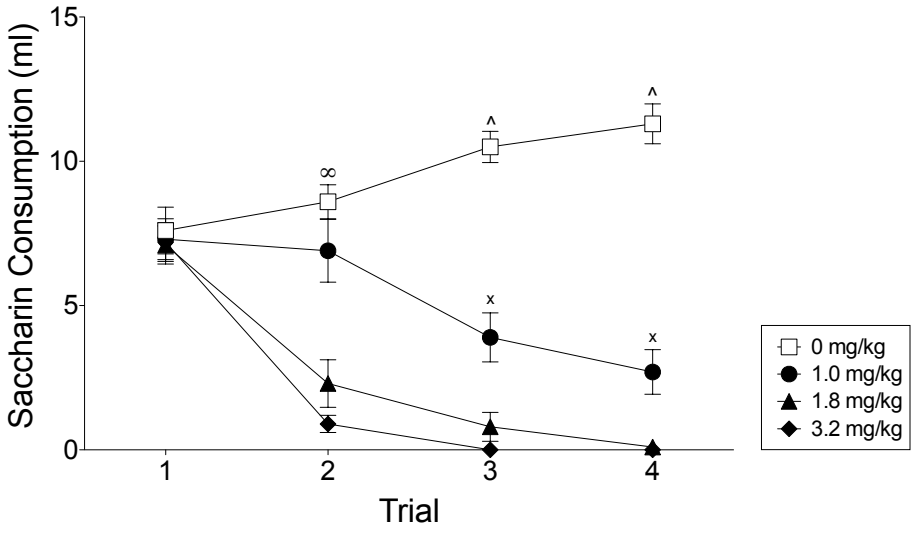


Figure 4. Mean (± SEM) Saccharin Consumption for Adult Animals Throughout Phase II: Conditioning. $^{\infty}$Groups 1.8 and 3.2 consumed significantly less saccharin than Groups 0 and 1.0. ^Groups 1.0, 1.8 and 3.2 consumed significantly less saccharin than Group 0. $^{x}$Groups 1.8 and 3.2 consumed significantly less saccharin than Group 1.0.

Bonferroni-corrected paired 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 [$t(8)=-4.127, p<0.0125$].

Phase III: 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 [$F(3,32)=11.861, p<0.05$]. ]. Tukey's HSD post hoc analysis revealed that Groups 1.0, 1.8 and 3.2 consumed significantly less fluid than Group 0 ($p's<0.05$; see Figure 5).

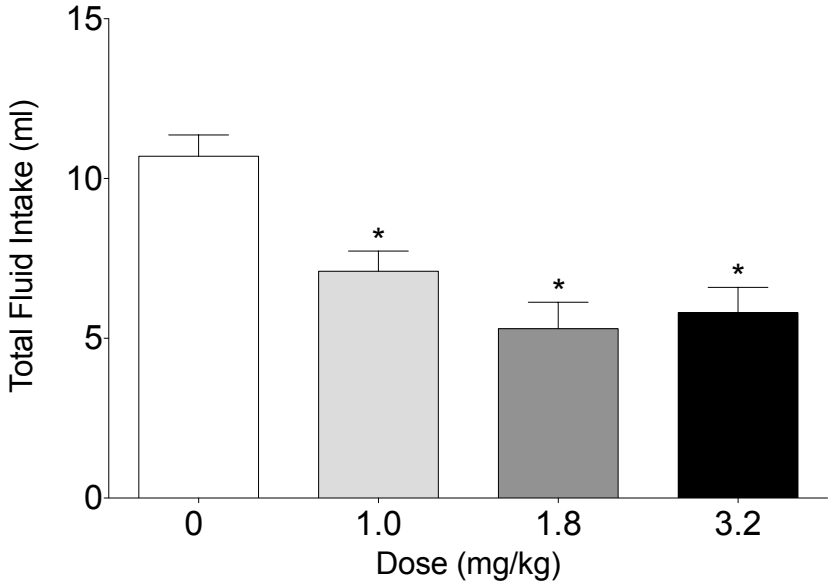


Figure 5. Mean (± SEM) Total Fluid Consumption for Adult Animals Throughout Phase III: Two-Bottle Aversion Test. *Groups 1.0, 1.8 and 3.2 consumed significantly less fluid than Group 0.

Given that dose groups consumed different overall levels of fluid, 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 [$F(3,32)=179.745, p<0.05$]. ]. Tukey's HSD post hoc analysis revealed 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 6).

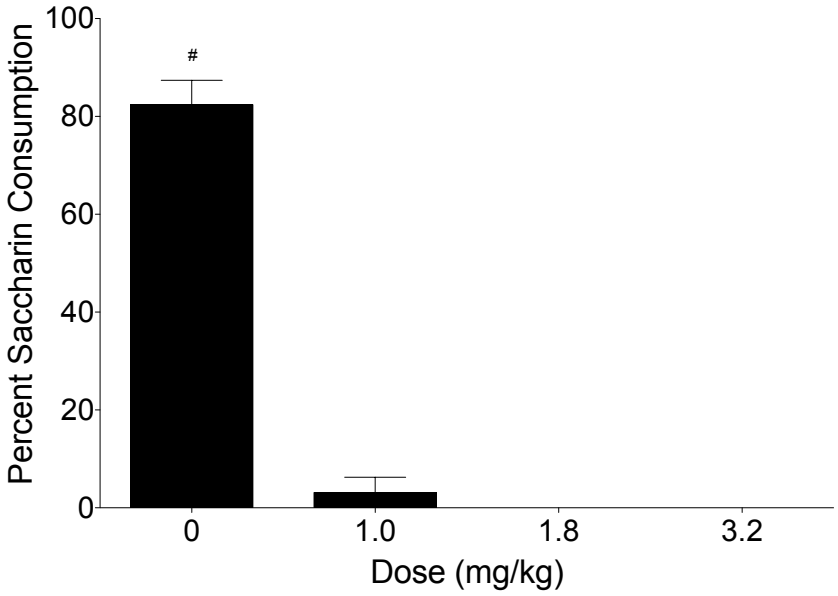


Figure 6. Mean (± SEM) Percent Saccharin Consumption for Adult Animals Throughout Phase III: Two-Bottle Aversion Test. *Groups 1.0, 1.8 and 3.2 consumed a significantly smaller percentage of saccharin than Group 0.

Phase IV: 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 (Group 1.0=1, Group 1.8=2 and Group 3.2=1); Adult DSTR (Group 1.0=2); Adult VSTR (Group 1.8=1). 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; see Table 1). The one-way ANOVAs on monoamine/metabolite levels in the DSTR revealed a significant effect of Dose for NE [$F(3,29)=3.319, p<0.05$]. ]. Tukey's HSD post hoc analysis revealed that 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's>0.05; see Table 2).

Table 2. Mean (± 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 of Adult Animals. Data are expressed as picograms (pg) of analyte per milligram (mg) of tissue. *Group 0 displayed significantly lower levels of the analyte than Group 3.2.

| **CTX Analyte (pg/mg)** | **Adult 0 (*n*=9)** | **Adult 1.0 (*n*=7)** | **Adult 1.8 (*n*=5)** | **Adult 3.2 (*n*=7)** |
|---|---|---|---|---|
| **DA** | 28.3 (2.2) | 34.0 (1.7) | 28.6 (7.9) | 24.7 (2.6) |
| **DOPAC** | 11.3 (1.3) | 13.9 (1.6) | 11.5 (3.6) | 11.5 (1.0) |
| **HVA** | 10.4 (3.2) | 11.2 (3.2) | 9.0 (4.2) | 10.0 (2.0) |
| **5-HT** | 290.7 (13.7) | 279.5 (25.8) | 295.5 (34.0) | 242.1 (27.4) |
| **5-HIAA** | 138.2 (7.3) | 147.1 (5.6) | 139.6 (21.1) | 124.6 (7.0) |
| **NE** | 362.8 (14.6) | 365.5 (11.6) | 345.8 (23.7) | 368.7 (10.6) |

| **DSTR Analyte (pg/mg)** | **Adult 0 (*n*=9)** | **Adult 1.0 (*n*=6)** | **Adult 1.8 (*n*=7)** | **Adult 3.2 (*n*=8)** |
|---|---|---|---|---|
| **DA** | 8344.0 (925.1) | 7751.6 (913.4) | 7771.9 (787.5) | 6998.5 (1095.9) |
| **DOPAC** | 1163.8 (115.2) | 1138.1 (100.9) | 1120.7 (68.5) | 1048.1 (111.4) |
| **HVA** | 576.4 (75.5) | 507.8 (39.5) | 538.7 (25.0) | 540.6 (55.2) |
| **5-HT** | 679.4 (44.9) | 824.8 (78.8) | 738.5 (54.8) | 798.0 (60.2) |
| **5-HIAA** | 554.5 (29.5) | 583.6 (25.0) | 568.2 (44.3) | 615.5 (33.3) |
| **NE** | 470.6 (76.0)* | 702.6 (55.5) | 653.3 (146.1) | 926.5 (128.0) |

| **VSTR Analyte (pg/mg)** | **Adult 0 (*n*=9)** | **Adult 1.0 (*n*=8)** | **Adult 1.8 (*n*=6)** | **Adult 3.2 (*n*=8)** |
|---|---|---|---|---|
| **DA** | 3987.7 (435.3) | 2993.9 (467.3) | 3147.8 (468.3) | 4026.4 (794.6) |
| **DOPAC** | 599.3 (72.7) | 469.5 (76.3) | 1020.5 (95.1) | 676.0 (129.2) |
| **HVA** | 235.7 (23.6) | 220.5 (41.0) | 184.3 (20.3) | 258.2 (49.2) |
| **5-HT** | 1004.7 (76.3) | 847.2 (71.6) | 1020.5 (95.1) | 985.1 (111.8) |
| **5-HIAA** | 511.1 (32.5) | 487.0 (28.2) | 542.0 (27.3) | 530.3 (45.4) |
| **NE** | 784.0 (114.5) | 650.9 (98.4) | 936.7 (120.7) | 883.4 (192.3) |

# CHAPTER 6

## ADOLESCENT AND 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.

### Phase II: 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 ($p$'s$<0.05$). Tukey's HSD post-hoc analysis revealed that on Trials 2-4, adult Groups 1.8 and 3.2 drank significantly less saccharin relative to adolescent Groups 1.8 and 3.2, respectively ($p$'s$<0.05$). Further, on Trials 3 and 4 adult Group 1.0 consumed significantly less saccharin relative to adolescent Group 1.0 ($p$'s$<0.05$).

### Phase III: Two-bottle aversion test

Bonferroni-corrected paired samples t-tests used to examine age differences in saccharin preference during the two-bottle aversion 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 [$t(14)=1.396, p>0.0125$].

### Phase IV: Monoamine/metabolite analysis

A 2 (Age) x 4 (Dose) univariate ANOVA was performed for each major monoamine (DA; 5-HT; NE) and metabolite (DOPAC; HVA; 5-HIAA) examined and for each of three brain regions (CTX; DSTR; VSTR). 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 3).

The ratios of DOPAC/DA and 5-HIAA/5-HT were computed as estimates of DA and 5-HT turnover rates, respectively (see Bekris, Antoniou, Daskas, & Papadopoulou-Daifoti, 2005; Fedorova, Hussein, Baumann, Di Martino, & Salem Jr., 2009; Rosen, Finklestein, Stoll, Yutzey, & Denenberg, 1984). Two 2 (Age) x 4 (Dose) univariate ANOVAs were performed for the rates of DA and 5-HT turnover in each of the three brain regions (CTX; DSTR; VSTR) examined. A significant main effect of Age was found for 5-HT turnover in the CTX [F(1,51)=51.831,p<0.05] and DSTR [F(1,50)=26.071,p<0.05] with adult samples displaying lower 5-HT turnover relative to adolescents (see Table 1) in these brain regions. A significant main effect of Age was found for DA turnover in the VSTR [F(1,50)=15.518,p<0.05] with adult samples displaying lower DA turnover relative to adolescents (see Table 3).

Table 3. Mean (± SEM) Levels of Monoamines [Dopamine (DA); Serotonin (5-HT); Norepinephrine (NE)], their Metabolites [3,4-dihydroxyphenylacetic Acid (DOPAC); Homovanillic Acid (HVA); 5-hydroxyindoleacetic Acid (5-HIAA)] and DA and 5-HT Turnover Ratios 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. ^Adult animals displayed significantly lower turnover of the analyte than adolescent animals.

| | CTX | | DSTR | | VSTR | |
|---|---|---|---|---|---|---|
| **Analyte (pg/mg)** | **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) |
| **DA Turnover** | 0.42 (0.02) | 0.42 (0.02) | 0.14 (0.00) | 0.15 (0.00) | 0.21 (0.01) | 0.16 (0.01)^ |
| **5-HT Turnover** | 0.74 (0.02) | 0.51 (0.02)^ | 1.15 (0.05) | 0.80 (0.03)^ | 0.64 (0.02) | 0.56 (0.02) |

# CHAPTER 7

## GENERAL 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, respectively, 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 paired 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, & Hinderliter, 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. There are two fundamental ways to perform developmental comparison assessments (in the absence of an in-house breeding protocol). It is first possible to bring in adolescent and adult animals concurrently to the vivarium (to assure that they will be tested with the same time frame relative to shipping) and subsequently compare the ages at their two developmental time points. Alternatively, it is possible to bring in a single supply of adolescent animals and test one group (to assess adolescent reactivity) and then test the second group when it reaches adulthood. There are certainly advantages and disadvantages to each procedure. Animals in the current experiments arrived at the same time and were tested independently at the appropriate age in order to increase the likelihood that there may be more similar genetic backgrounds in animals drawn from concurrent breeding pairs and to have complete control of the adults' developmental histories in the vivarium (not knowing such histories in the suppliers facility). 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-Sapyta 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, Rüther, & 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.

Despite having lower levels of monoamines and their metabolites, adolescents had significantly greater 5-HT turnover in the cortex and dorsal striatum when compared to adults. These results show that adolescents have heightened basal 5-HT transmission in brain regions implicated in modulation of reward circuitry. Under these circumstances, adolescents might have less post-synaptic 5-HT receptors or blunted receptor signaling (i.e., receptor desensitization), thereby rendering them less sensitive to 5-HT-releasing effects of MDMA.

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.

REFERENCES

Abercrombie, E. D., Keefe, K. A., Di Frischia, D. F., & Zigmond, M. J. (1989). Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens and medial frontal cortex. Journal of Neurochemistry, 52(5), 1655-1658.

Albaugh, D. L., Rinker, J. A., Baumann, M. H., Sink, J. R., & Riley A. L. (2011). Rats preexposed to MDMA display attenuated responses to its aversive effects in the absence of persistent monoamine depletions. Psychopharmacology, 216(3), 441-449.

Anderson, R. I., Varlinskaya, E. I., & Spear, L. P. (2010). Ethanol-induced conditioned taste aversion in male Sprague-Dawley rats: Impact of age and stress. Alcoholism: Clinical and Experimental Research, 34(12), 1-10.

Arnett, J. (1992). Reckless behavior in adolescence: A developmental perspective. Developmental Review, 12(4), 339-373.

Baumann, M. H., Clark, R. D., Franken, F. H., Rutter, J. J., & Rothman, R. B. (2008). Tolerance to 3,4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. Neuroscience, 152(3), 773-784.

Baumann, M. H., Wang, X., & Rothman, R. B. (2007). 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: A reappraisal of past and present findings. Psychopharmacology, 189(4), 407-424.

Baumann, M. H., Zolkowska, D., Kim, I., Scheidweiler, K. B., Rothman, R. B., & Huestis, M. A. (2009). Effects of dose and route of administration on pharmacokinetics of (±)-3,4-methylenedioxymethamphetamine in the rat. Drug Metabolism and Distribution, 37(11), 2163-2170.

Bekris, S., Antoniou, K., Daskas, S., & Papadopoulou-Daifoti, Z. (2005). Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behavioural Brain Research. 161(1), 45-59.

Beluzzi, J. D., Lee, A. G., Oliff, H. S., & Leslie, F. M. (2004). Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. Psychopharmacology, 174(3), 389-395.

Berger, B. D. (1973). Conditioning of food aversions by injections of psychoactive drugs. Journal of Comparative and Physiological Psychology, 81(1), 21-26.

Bilsky, E. J., Hui, Y., Hubbell, C. L., & Reid, L. D. (1990). Methylenedioxymethamphetamine's capacity to establish place preference and modify intake of an alcoholic beverage. Pharmacology Biochemistry Behavior, 37(4), 633-638.

Bowers, W. J., Gingras, M. A., & Amit, Z. (1996). Time-dependent exacerbation of amphetamine-induced taste aversions following exposure to footshock. Psychopharmacology, 125(1), 43-49.

Braida, D., Iosuè, S., Pegorini, S., & Sala, M. (2005), 3,4 Methylenedioxymethamphetamine-induced conditioned place preference (CPP) is mediated by endocannabinoid system. Pharmacological Research, 51(2),177-182.

Brenhouse, H. C., & Andersen, S. L. (2008). Delayed extinction and stronger reinstatement of cocaine conditioned place preference in adolescent rats, compared to adults. Behavioral Neuroscience, 122(2), 460-465.

Brielmaier, J. M., McDonald, C. G., & Smith, R. F. (2007). Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats. Neurotoxicology and Teratology, 29(1), 74-80.

Broening, H. W., Bacon, L., & Slikker Jr., W. (1994). Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-Methylenedioxymethamphetamine. The Journal of Pharmacology and Experimental Therapeutics, 271(1), 285-293.

Brunell, S. C., & Spear, L. P. (2005). Effect of stress on the voluntary intake of a sweetened ethanol solution in pair-housed adolescent and adult rats. Alcoholism: Clinical and Experimental Research, 29(9),1641-1653.

Byrne, T., Baker, L. E., & Poling, A. (2000). MDMA and learning: Effects of acute and neurotoxic exposure in the rat. Pharmacology Biochemistry and Behavior, 66(3), 501-508.

Campbell, J. O., Wood, R. D., & Spear, L. P. (2000). Cocaine and morphine-induced place conditioning in adolescent and adult rats. Physiology & Behavior, 68(4), 487-493.

Cappell, H., & Le Blanc, A. E. (1977). Parametric investigations of the effects of prior exposure to amphetamine and morphine on conditioned gustatory aversion. Psychopharmacology, 51(3), 265-271.

Carroll, M. E., Anker, J. J., & Perry, J. L. (2009). Modeling risk factors for nicotine and other drug abuse in the preclinical laboratory. Drug and Alcohol Dependence, 104S(S1), S70-S78.

Caster, J. M., Walker, Q. D., & Kuhn, C. M. (2005). Enhanced behavioral response to repeated-dose cocaine in adolescent rats. Psychopharmacology, 183(2), 218-225.

Catlow, B. J., Badanich, K. A., Sponaugle, A. E., Rowe, A. R., Song, S., Rafalovich, I., et al. (2010). Effects of MDMA (“ecstasy”) during adolescence on place conditioning and hippocampal neurogenesis. European Journal of Pharmacology, 628(1), 96-103.

Chambers, R. A., Taylor, J. R., & Potenza, M. N. (2003). Developmental neurocircuitry of motivation in adolescence: A critical period of addiction vulnerability. American Journal of Psychiatry, 160(6), 1041-1052.

Coil, J. D., Hankins, W. G., Jenden, D. J., & Garcia, J. (1978). The attenuation of a specific cue-to-consequence association by antiemetic agents. Psychopharmacology, 56(1), 21-25.

Colado, M. I., O’Shea, E., & Green, A. R. (2004). Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. Psychopharmacology, 173(3-4), 249-263.

Colado, M. I., Williams, J. L., & Green, A. R. (1995). The hyperthermic and neurotoxic effects of ‘Ecstasy’ (MDMA) and 3,4 methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. British Journal of Pharmacology, 115(7), 1281-1289.

Connor, T. J., McNamara, M. G., Kelly, J. P., & Leonard, B. E. (1999). 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) administration produces dose-dependent neurochemical, endocrine and immune changes in the rat. Human Psychopharmacology, 14, 95-104.

Cuadra, G., Zurita, A., Gioino, G., & Molina, V. (2001). Influence of different antidepressant drugs on the effect of chronic variable stress on restraint-induced dopamine release in frontal cortex. Neuropsychopharmacology, 25(3), 384-394.

Cunningham, C. L., Gremel, C. M., & Groblewski, P. A. (2009). Genetic influences on conditioned taste aversion. In S. Reilly & T. R. Schachtman (Eds.), Conditioned taste aversion: Neural and behavioral processes (pp. 387-421). New York: Oxford University Press.

Davis, C. M., & Riley, A. L. (2010). Conditioned taste aversion learning: Implications for animal models of drug abuse. Annals of the New York Academy of Sciences, 1187, 247-275.

de la Garza II, R., Fabrizio, K. R., & Gupta, A. (2007). Relevance of rodent models of intravenous MDMA self-administration to human MDMA consumption patterns. Psychopharmacology, 189(4), 425-434.

Fedorova, I., Hussein, N., Baumann, M. H., Di Martino, C., & Salem Jr., N. (2009). An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze. Behavioral Neuroscience, 123(1), 196-205.

Finnegan, K. T., Ricaurte, G. A., Ritchie, L. D., Irwin, I., Peroutka, S. J., & Langston, J. W. (1988). Orally administered MDMA causes a long-term depletion of serotonin in rat brain. Brain Research, 477(1), 141-144.

Freeman K. B., & Riley A. L. (2009). The origins of conditioned taste aversion learning: A historical analysis. In S. Reilly & T. R. Schachtman (Eds.), Conditioned taste aversion: Neural and behavioral processes (pp. 9-36). New York: Oxford University Press.

Gamaro, G. D., Manoli, L. P., Torres, I. L. S., Silveira, R., & Dalmaz, C. (2003). Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochemistry International, 42(2), 107-114.

Goudie, A. J., Stolerman, I. P., Demellweek, C., & D'Mello, G. D. (1982). Does conditioned nausea mediate drug-induced conditioned taste aversion? Psychopharmacology, 78(3), 277-281.

Green, A. R., Mechan, A. O., Elliot, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4 Methylenedioxymethamphetamine (MDMA, "Ecstasy"). Pharmacological Reviews, 55(3), 463-508.

Grote Jr, F. W., & Brown, R. T. (1971). Conditioned taste aversions: Two-stimulus tests are more sensitive than one-stimulus tests. Behavioral Research Methods & Instrumentation, 3(6), 311-312.

Heffner, T. G., Hartman, J. A., & Seiden, L. S. (1980). A rapid method for the regional dissection of the rat brain. Pharmacology Biochemistry and Behavior, 13(3), 453-456.

Herregodts, P., Velkeniers, B., Ebinger, G., Michotte, Y., Vanhaelst, L., & Hooghe-Peters, E. (1990). Development of monoaminergic neurotransmitters in fetal and postnatal rat brain: Analysis by HPLC with electrochemical detection. Journal of Neurochemistry, 55(3), 744-779.

Holder, M. D., Yirmiya, R., Garcia, J., & Raizer, J. (1989). Conditioned taste aversions are not readily disrupted by external excitation. Behavioral Neuroscience, 103(3), 605-611.

Hunt, T., & Amit, Z. (1987). Conditioned taste aversions induced by self-administered drugs: Paradox revisited. Neuroscience & Biobehavioral Reviews, 11, 107-130.

Hurwitz, Z. E., Merluzzi, A. P., & Riley, A. L. (2012). Age-dependent differences in morphine-induced taste aversions. Developmental Psychobiology, doi: 10.1002/dev.21046.

Infurna, R. N., & Spear, L. P. (1979). Developmental changes in amphetamine-induced taste aversions. Pharmacology Biochemistry and Behavior, 11(1), 31-35.

Johnson, B. N., & Yamamoto, B. K. (2009). Chronic unpredictable stress augments +3,4-methylenedioxymethamphetamine-induced monoamine depletions: The role of corticosterone. Neuroscience, 159(4), 1233-1243.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2010). Monitoring the Future national results on adolescent drug use: Overview of key findings 2011. Ann Arbor: Institute for Social Research, The University of Michigan.

Kalsbeek, A., Voorn, P., Buijs, R. M., Pool, C. W., & Ulyings, H. B. M. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. The Journal of Comparative Neurology, 269(1), 58-72.

Klein, S. B., Domato, G. C., & Hallstead, C. (1975). Acquisition of a conditioned aversion as a function of age and measurement technique. Physiological Psychology, 3(4), 379-384.

Klein, S. B., Mikulka, P. J., Domato, G. C., & Hallstead, G. C. (1977). Retention of internal experiences in juvenile and adult rats. Physiological Psychology, 5(1), 63-66.

Lasiter, P. S., & Braun, J. J. (1981). Shock facilitation of taste aversion learning. Behavioral and Neural Biology, 32(3), 277-281.

Levin, E. D., Lawrence, S. S., Petro, A., Horton, K., Rezvani, A. H., Seidler, F. J., et al. (2007). Adolescent vs. adult-onset nicotine self-administration in male rats: Duration of effect and differential nicotinic receptor correlates. Neurotoxicology and Toxicology, 29(4), 458-465.

Lin, H. Q., Atrens, D. M., Christie, M. J., Jackson, D. M., & McGregor, I. S. (1993). Comparison of conditioned taste aversions produced by MDMA and *d*-Amphetamine. Pharmacology Biochemistry and Behavior, 46(1), 153-156.

Lin, H. Q., Jackson, D. M., Atrens, D. M., Christie, M. J., & McGregor, I. S. (1997). Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. Brain Research, 744(2), 351-357.

Lin, H. Q., McGregor, I. S., Atrens, D. M., Christie, M. J., & Jackson, D. M. (1994). Contrasting effects of dopaminergic blockade on MDMA and *d*-Amphetamine conditioned taste aversions. Pharmacology Biochemistry and Behavior, 47(2), 369-374.

Marona-Lewicka, D., Rhee, G., Sprague, J. E., & Nichols, D. E. (1996). Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacology Biochemistry and Behavior, 53(1), 99-105.

Martinez-Price, D. L., Krebs-Thompson, K., & Geyer, M. A. (2002). Behavioral psychopharmacology of MDMA and MDMA-like drugs: A review of human and animal studies. Addiction Research & Theory, 10(1), 43-67.

McNamara, M. G., Kelly, J. P., & Leonard, B. E. (1995). The effect of acute MDMA administration on body temperature, serum corticosterone and neurotransmitter concentrations in male and female rats. Human Psychopharmacology, 10, 373-383.

Mikulka, P. J., Krone, P. D., Rapisardi, P. L., & Kirby, R. H. (1975). Discrimination between deionized water and D2O in a runway using olfaction in the rat. Physiological Psychology, 3(1), 92-94.

Misanin, J. R., Guanowsky, V., & Riccio, D. C. (1983). The effect of CS-preexposure on conditioned taste aversion in young and adult rats. Physiology & Behavior, 30(6), 859-862,

Misanin, J. R., Anderson, M. J., & Hinderliter, C. F. (2009). Conditioned taste aversion across the life span from prenascence to senescence. In S. Reilly & T. R. Schachtman (Eds.), Conditioned taste aversion: Neural and behavioral processes (pp. 281-308). New York: Oxford University Press.

Moll, G. H, Mehnert, C., Wicker, M., Bock, N., Rothenberger, A., Rüther, E., & Huether, G. (2000). Age-associated changed in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood. Developmental Brain Research, 119(2), 251-257.

National Research Council (1996). Guidelines for the care and use of laboratory animals. Washington, DC: National Academy.

O'Hearn, E., Battaglia, G., De Souza, E. B., Kuhar, M. J., & Molliver, M. E. (1988). Methylenedioxyamphetamine (MDA) and Methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence for neurotoxicity. The Journal of Neuroscience, 8(8), 2788-2803.

Parker, L. A., Limbeer, C. L., & Rana, S. A. (2009). Conditioned disgust, but not conditioned taste avoidance, may reflect conditioned nausea in rats. In S. Reilly & T. R. Schachtman (Eds.), Conditioned taste aversion: Neural and behavioral processes (pp. 92-113). New York: Oxford University Press.

Reid, L. D., Hubbell, C. L., Tsai, J., Fishkin, M. D., & Amendola, C. A. (1996). Naltrindole a δ-opioid antagonist, blocks MDMA's ability to enhance pressing for rewarding brain stimulation. Pharmacology Biochemistry and Behavior, 53(2), 477-480.

Ricaurte, G. A., DeLanney, L. E., Irwin, I., & Langston, J. W. (1988). Toxic effects of MDMA on central serotonergic neurons in the primate: Importance of route and frequency of drug administration. Brain Research, 446(1), 165-168.

Riley, A. L. (2011). The paradox of drug taking: The role of the aversive effects of drugs. Physiology & Behavior, 103(1), 69-78.

Riley, A. L., Davis, C. M., & Roma, P. G. (2009). Strain differences in taste aversion learning: Implications for animal models of drug abuse. In S. Reilly & T. R. Schachtman (Eds.), Conditioned taste aversion: Neural and behavioral processes (pp. 226-261). New York: Oxford University Press.

Riley, A. L., & Mastropaolo, J. P. (1989). Long-delay taste aversion learning: Effects of repeated trials and two-bottle testing conditions. Bulletin of the Psychonomic Society, 27(2), 145-148.

Roma, P. G., Davis, C. M., Kohut, S. J., Huntsberry, M. E., & Riley, A. L. (2008). Early maternal separation and sex differences in the aversive effects of amphetamine in adult rats. Physiology & Behavior, 93(4-5), 897-904.

Rosen, G. D., Finklestein, S., Stoll, A. L., Yutzey, D. A., & Denenberg, V. H. (1984). Neurochemical asymmetries in the albino rat's cortex, striatum, and nucleus accumbens. Life Sciences, 34(12), 1143-1148.

Schenk, S., Gittings, D., Johnstone, M., & Daniela, E. (2003). Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. Psychopharmacology, 169(1), 21-27.

Schramm-Sapyta, N. L., Cha, Y. M., Chaudhry, S., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. Psychopharmacology, 4(4), 867-877.

Schramm-Sapyta, N. L., Morris, R. W., & Kuhn, C. M. (2006). Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacology Biochemistry and Behavior, 84(2), 344-352.

Sengstake, C. B., & Chambers, K. C. (1979). Differential effects of fluid deprivation on the acquisition and extinction phase of a conditioned taste aversion. Bulletin of the Psychonomic Society, 14(2), 85-87.

Shram, M. J., Funk, D., Li, Z., & Lê, A. (2006). Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. Psychopharmacology, 186(2), 201-208.

Spear, L. P., & Varlinskaya, E. I. (2010). Sensitivity to ethanol and other hedonic stimuli in an animal model of adolescence: Implications for prevention science? Developmental Psychobiology, 52(3), 236-243.

Sprague, J. E., & Nichols, D. E. (2006). Neurotoxicity of MDMA (ecstasy): Beyond metabolism. Trends in Pharmacological Sciences, 26(2), 59-60.

Thierry, A. M., Tassin, J. P., Blanc, G., & Glowinski, J. (1976). Selective activation of the mesocortical DA system by stress. Nature, 263, 242-244.

Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. Addiction Biology, 12(3-4), 227-462.

Vastola, B. J., Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2002). Nicotine-induced conditioned place preference in adolescent and adult rats. Physiology & Behavior, 77(1), 107-114.

Verendeev, A., & Riley, A. L. (2012). Conditioned taste aversion and drugs of abuse: History and interpretation. Neuroscience & Biobehavioral Reviews, 36(10), 2193-2205.

Vetter-O'Hagen, C., Varlinskaya, E. I., & Spear, L. P. (2010). Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. Alcohol and Alcoholism, 44(6), 547-554.

Wise, R. A., Yokel, R. A., & DeWit, H. (1976). Both positive reinforcement and conditioned aversion from apomorphine in rats. Science ,191(4233), 1273-1274.