THE EFFECT OF ADOLESCENT ETHANOL EXPOSURE ON COCAINE REWARD, AVERSION AND SELF-ADMINISTRATION IN ADULT RATS

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ABSTRACT

Rationale. Ethanol is the most commonly used drug in adolescence, and ethanol use generally precedes the use of other drugs later in life. The present study explored how adolescent ethanol exposure may alter cocaine reward, aversion and self-administration later in adulthood.

Methods. Male rats were exposed to ethanol (2 g/kg) or vehicle on postnatal days (PND) 30-39. On PND 65, animals began place preference conditioning (Experiment 1), taste aversion conditioning (Experiment 2) or self-administration training for food and cocaine (Experiment 3). In Experiment 1, baseline preferences were determined and then subjects underwent conditioning with 5, 10 or 20 mg/kg cocaine followed by a final preference test. In Experiment 2, subjects were given saccharin followed by injections of cocaine (32 mg/kg) or saline 15, 180 or 300 min later. In Experiment 3, subjects were given operant training sessions for food as well as two sessions on a progressive ratio (PR) schedule. After catheter surgery, subjects were given 10 self-administration training sessions (0.25 or 0.75 mg/kg/infusion) and two PR sessions for cocaine.

Results. In Experiment 1, vehicle-preexposed subjects conditioned with 20 mg/kg cocaine increased time spent on the drug-paired side. Place preferences were evident at 10 mg/kg cocaine in ethanol-preexposed animals. For Experiment 2, animals exposed to vehicle during adolescence displayed a reduction in saccharin consumption at all delays. Animals exposed to ethanol displayed aversions only at the shorter delays (15 or 180 min). In Experiment 3, during the food PR sessions, vehicle-preexposed animals had more responses than animals preexposed to ethanol. Across the cocaine acquisition trials, subjects preexposed to ethanol and given 0.25

mg/kg/infusion had fewer responses and took significantly less drug during the sessions. There were no differences among groups during the cocaine PR sessions.

Conclusions. Adolescent ethanol exposure sensitized the rewarding effects (Experiment 1) and attenuated the aversive effects (Experiment 2) of cocaine. Further, ethanol decreased PR responding for food as well as decreased responding for cocaine (Experiment 3) indicating changes in the sensitivity to food and cocaine. These results suggest that adolescent ethanol preexposure impacts the affective properties of cocaine that may affect later vulnerability to cocaine use.

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CHAPTER 1

GENERAL INTRODUCTION

In 2010, approximately 23 million Americans aged 12 or older reported some form of illicit drug use in the past month (NSDUH, 2011) with approximately 4 million people meeting the criteria for abuse or dependence (NSDUH, 2011). Given the large number of people who use and abuse illicit drugs, it is important to understand the factors that may play a role in both the initiation of drug use as well as the escalation from occasional use to drug abuse or dependence. When looking at human drug use patterns, the majority of people using illicit drugs report a prior history of exposure to licit drugs such as alcohol (specifically ethanol; Degenhardt et al., 2010; Ginzler, Cochran, Domenech-Rodriguez, Cauce, & Whitbeck, 2003; Grant & Dawson, 1998). In these reports, ethanol use is most commonly initiated during adolescence (Degenhardt et al., 2010; Falk, Yi, & Hiller-Sturmhofel, 2006; NSDUH, 2011) and patterns of heavy ethanol use early in life predict later problem drug use (Anthony & Petronis, 1995; Cable & Sacker, 2008; Clark, Kirisci, & Tarter, 1998; Duncan, Alpert, Duncan, & Hops, 1997; Grant & Dawson, 1998). Given that ethanol is one of the most commonly used drugs during adolescence (Johnston, O'Malley, Bachman, & Schulenberg, 2011), it is important to understand how early exposure to ethanol may alter the subsequent abuse liability of other drugs. Despite the number of people who use illicit drugs and the fact that almost all of them had experience with ethanol before moving on to drugs such as cocaine (Degenhardt et al., 2010; Ginzler et al., 2003; Grant & Dawson, 1998), there is very little literature on how ethanol exposure impacts the response to other drugs later in life. Further, given that initial drug use typically begins in adolescence, it is important to understand how early exposure to ethanol may affect the abuse liability of these other drugs.

To help researchers better understand specific factors contributing to drug use and addiction, animal models, mainly with rodents, are often used to observe specific behavioral effects of drug administration. In such models, the likelihood of a drug to be self-administered has been suggested to be a balance between its rewarding and aversive effects (Brockwell, Eikelboom, & Beninger, 1991; Simpson & Riley, 2005; Wise, Yokel, & DeWit, 1976). Specifically, the rewarding effects of a drug would promote its intake, while the aversive effects serve as the limiting factor for self-administration (Stolerman & D'Mello, 1981), and any factor that alters either the rewarding or aversive effects of a drug may also impact its self-administration. By using the conditioned place preference (CPP) and conditioned taste aversion (CTA) procedures to assess drug reward and aversion, respectively, it may be possible to explore how factors such as adolescent drug history may alter the abuse liability of a drug. The present set of experiments aimed to assess this relationship between reward and aversion and self-administration and to see how these measures may be altered by prior drug preexposure. Specifically, in Experiment 1, animals were exposed to ethanol during adolescence and then conditioned with cocaine in the CPP procedure in adulthood. In Experiment 2, the same adolescent preexposure was used, and then the aversive effects of cocaine were evaluated using the CTA procedure. Alterations in locomotor activity were also assessed as another measure of the behavioral effects of cocaine. Finally, in Experiment 3, following the adolescent ethanol exposure, animals were tested for the self-administration of both a natural reinforcer (food) as well as for cocaine self-administration. Taken together, these three experiments provide a picture of how exposure to ethanol early in development alters the rewarding and aversive effects of cocaine, as well as the rate of cocaine self-administration in adulthood.

CHAPTER 2

EXPERIMENT 1: CONDITIONED PLACE PREFERENCE

Introduction

When discussing the abuse potential of a drug, the rewarding effects are often characterized as being a primary motivating factor for its repeated use. In this context, drug reward is differentiated from reinforcement in that reinforcement is typically defined as any operant experimental contingency that increases the probability of a class of behaviors, while reward generally refers to the appetitive nature of a given stimulus (Mackintosh, 1974). The conditioned place preference (CPP) procedure is a commonly-used method of assessing drug reward, while self-administration (described in Experiment 3) is generally considered to be a measure of the overall reinforcing properties of a drug. In place preference conditioning, the subject is introduced to an apparatus containing two distinct chambers. The contextual cues within each chamber generally differ on multiple dimensions, such as having distinct textural as well as visual cues (Roma & Riley, 2005). Conditioning involves injecting the animal with a compound (e.g., cocaine) and then confining it in one of the chambers for a brief period of time. On the following conditioning session, the animal is injected with a neutral substance (such as the drug vehicle) and then placed in the opposite chamber. Following this conditioning procedure, which may be repeated multiple times, the animal is then given unrestricted access to the entire apparatus. If the drug is rewarding, this conditioning procedure generally results in a relative preference for the drug-associated side, as measured by an increase in time spent in this environment. This environmental preference is considered reflective of the drug's motivational (i.e., rewarding) properties (Bardo & Bevins, 2000; Tzschentke, 1998, 2007).

Although the CPP procedure is frequently used to study the rewarding effects of drugs, there has been little work to examine how a history of drug exposure during adolescence may

alter these rewarding effects. The current literature on drug preexposure during adolescence focuses on how stimulants such as methylphenidate (Achat-Mendes, Anderson, & Itzhak, 2003; Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002; Carlezon, Mague, & Andersen, 2003), nicotine (Kelley & Middaugh, 1999; Kelley & Rowan, 2004; McMillen, Davis, Williams, & Soderstrom, 2005; McQuown, Belluzzi, & Leslie, 2007) and cocaine (Schramm-Sapyta, Pratt, & Winder, 2004) may alter the rewarding effects of cocaine in adult animals. Although ethanol is one of the most commonly used drugs during adolescence, the only work examining ethanol preexposure on subsequent cocaine-induced place conditioning has used adult animals (Busse, Lawrence, & Riley, 2005; Le Pen, Duterte-Boucher, Daoust, & Costentin, 1998). In these studies, ethanol was not found to produce alterations in the rewarding effects of cocaine. Given that in humans ethanol use is most likely to start in adolescence (NSDUH, 2011), it is of interest to examine how early ethanol exposure may alter the rewarding effects of other drugs administered later in life. In Experiment 1, this issue was addressed by examining how exposure to ethanol during adolescence alters place preference conditioning in response to cocaine in adult rats.

Procedure

Subjects

Subjects (n = 67) were experimentally naïve male Sprague Dawley rats (Harlan Sprague Dawley Laboratories). The experiment was run in two replicates (n = 35 in the first replicate, n = 32 in the second) under identical parameters, and data were pooled for analysis. All groups were represented in each replicate. Animals arrived in the laboratory on postnatal day (PND) 25 and were housed in Plexiglas bins (26 x 48 x 21 cm) with three or four animals in each bin in a colony room maintained on a 12-h light/dark cycle (lights on at 0800h) and at an ambient

temperature of 23 °C. Training and testing took place during the light part of the cycle, with all procedures beginning at 1200h. Food and water were available *ad libitum* for the entirety of the study. Animals were handled daily for 5 days prior to the start of the experiment to limit the effects of handling stress during the conduct of the research. Procedures recommended by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and the Institutional Animal Care and Use Committee at American University were followed at all times. Food and water consumption and body weight were monitored daily to assess the health of the subjects.

Drugs and Solutions

Ethanol (Sigma Aldrich Co., St. Louis, MO) was prepared as a 15% (v/v) solution in 0.9% saline. Cocaine hydrochloride (generously supplied by NIDA) was prepared as a 10 mg/ml solution in 0.9% saline. Doses of cocaine refer to the weight of the salt. Saline was used for vehicle injections.

Apparatus

All place conditioning procedures were conducted using a 3-chambered automated apparatus (San Diego Instruments Place Preference system, San Diego, CA). The inner dimensions of each main conditioning chamber were 28 cm wide x 21 cm deep x 34.5 cm high; the two chambers were adjoined by a smaller middle chamber measuring 14 cm wide x 21 cm deep x 34.5 cm high. One of the main conditioning chambers featured a white aluminum diamond plate floor with white walls, the other conditioning chambers featured a haircell textured black plastic floor with black walls and the smaller middle chamber was outfitted with a steel rod floor and gray walls. Each individual chamber within each apparatus had two white

LED lights set to minimal brightness within the otherwise unlit room. A total of eight identical apparatuses were used; each apparatus featured a 16 x 4 photobeam array for recording time (sec) spent in each chamber. The room in which the apparatuses were located was illuminated by an 85-watt red light mounted to the ceiling in the center of the room, and background noised was masked by a white noise generator located in the front of the room.

Ethanol Preexposure

On PND 30, animals were divided into two groups and injected intraperitoneally (IP) with either ethanol (Group E; 2.0 g/kg; $n = 35$) or vehicle (Group V; $n = 32$). The time course, dose and route of administration of ethanol were chosen to match previous studies examining the effects of ethanol exposure on cocaine responsivity (Grakalic & Riley, 2002; Hutchison, Albaugh, & Riley, 2010; Slawecki & Betancourt, 2002; Slawecki, Betancourt, Cole, & Ehlers, 2001). Group assignments were made such that animals in each bin (see above) were administered the same compound. Injections were given daily for 10 consecutive days (PND 30 – 39). Body weights were recorded each day to assess the health of the animals. From PND 40 – PND 54, subjects were maintained in their home bins until place conditioning (see below).

Place Preference Conditioning

Baseline test. On PND 55, animals were individually housed in hanging wire cages (24.3 x 19 x 18 cm) and allowed 10 days to acclimate to this environment. This procedure was chosen to match previous work assessing the interaction of ethanol and cocaine (see Busse, Lawrence, & Riley, 2004; Busse et al., 2005; Busse & Riley, 2002; Diaz-Granados & Graham, 2007; Graham & Diaz-Granados, 2006). On PND 65, baseline chamber preferences were determined by placing each animal in the center compartment of the CPP apparatus, then removing the barriers and allowing it free access to the entire apparatus for 15 min. A paired-samples t-test revealed that on

average animals spent significantly less time in the white chamber than the black chamber (268 versus 354 s, $t(66) = -5.1$, $p < 0.001$), indicating a significant apparatus bias (Cunningham et al., 2003; Roma and Riley, 2005); there were no differences between preexposure groups in time spent in either the black or white chambers (ps > .05). Given that the animals showed an initial side preference in the apparatus, a biased conditioning design was used such that all subjects received drug in the white (least-preferred) chamber (see also Kelley & Rowan, 2004; Schramm-Sapyta et al., 2004).

Acquisition and final test. The CPP acquisition phase began on PND 66. Subjects in each of the two preexposure conditions, i.e., Groups V and E, were randomly assigned a conditioning dose of cocaine (5, 10 or 20 mg/kg) or vehicle (matched in volume to the 20 mg/kg dose of cocaine). This treatment resulted in eight groups designated as follows: V-0 ($n = 8$), V-5 ($n = 8$), V-10 ($n = 8$), V-20 ($n = 8$), E-0 ($n = 8$), E-5 ($n = 9$), E-10 ($n = 9$) and E-20 ($n = 9$). The letter stands for the preexposure condition (ethanol or vehicle), and the number stands for the conditioning injection (vehicle or 5, 10 or 20 mg/kg cocaine). On Day 1 of the conditioning cycle, half of the animals were administered their conditioning injection (vehicle, 5, 10 or 20 mg/kg cocaine; IP) and confined to the white chamber for 30 min. The remaining animals received a saline injection and were confined to the black chamber for 30 min. On Day 2, animals experienced injections and chamber confinement opposite to those of Day 1. All groups were counterbalanced across conditioning days. This 2-day sequence constituted one conditioning cycle, and the acquisition phase consisted of four such cycles culminating in a final CPP test on PND 74. During the final CPP test, animals were placed in the center compartment and then allowed access to the entire apparatus for 15 min.

Data Analysis

To assess health of the subjects, body weights were analyzed during ethanol preexposure and at the beginning of place preference conditioning. For the preexposure phase, body weights were analyzed using a 10 (Day) x 2 (Preexposure) repeated-measures ANOVA. To determine if ethanol had long-lasting effects on body weight, an independent-samples t-test was used to compare the body weights of subjects in both preexposure groups on the day of the baseline test. To assess the effects of ethanol preexposure on place preference conditioning, a 2 x 2 x 4 repeated-measures ANOVA with the within-subjects factor of Trial (baseline or final CPP test) and the between-groups factors of Preexposure (vehicle or ethanol) and Dose (0, 5, 10 or 20 mg/kg cocaine) was performed with seconds spent in the drug-paired (white) chamber as the dependent variable. Following significant main effects, differences between time spent in the drug-paired chamber during baseline and final CPP tests were analyzed with one-way ANOVAs followed by Tukey's post-hoc tests. To specifically examine changes in preference for the white (drug-paired) chamber between the baseline and final CPP tests for each group, paired-samples t-tests were used. All determinations of statistical significance were set at $p \leq 0.05$.

Results

Body Weights

The 10 (Day) x 2 (Preexposure) repeated-measures ANOVA on body weight during the preexposure phase revealed significant effects of both Day [$F(9, 585) = 2894.604$; $p < 0.001$] and Preexposure [$F(1, 65) = 20.630$; $p < 0.001$] as well as a significant Day x Preexposure interaction [$F(9, 585) = 66.988$; $p < 0.001$]. Independent-samples t-tests on each day showed that on Days 3-10, animals receiving ethanol weighed significantly less than those receiving saline during preexposure (Figure 1). In the assessment of the long-term effects of ethanol on body weight, the

independent-samples t-test on body weights on the day of the baseline test revealed that the significant differences between groups persisted into adulthood ($p < 0.001$). The average weight for Group V was 0.326 kg and the average weight for subjects in Group E was 0.302 kg.

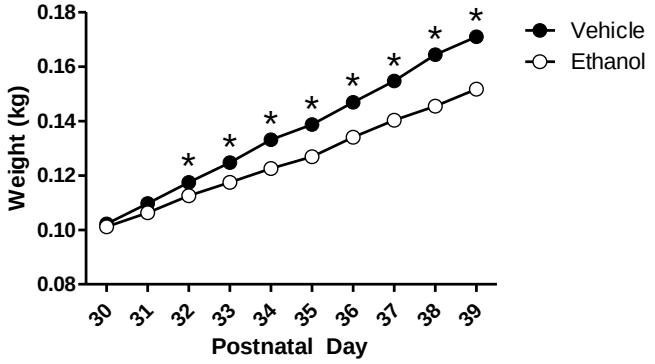

Figure 1. Body Weights During Ethanol Preexposure Phase in Experiment 1. Mean body weights (± SEM) for groups exposed to Vehicle (n = 32) or Ethanol (n = 35). *Significant difference between preexposure conditions.

Place Preference Conditioning.

The 2 (Trial) x 2 (Preexposure) x 4 (Dose) repeated-measures ANOVA on time in the drug-paired chamber revealed significant effects of Trial [$F(1, 59) = 9.892$, $p < 0.01$], Preexposure [$F(1, 59) = 5.100$, $p < 0.05$] and Dose [$F(3, 59) = 5.388$, $p < 0.01$], as well as significant Preexposure x Dose [$F(3, 59) = 4.452$, $p < 0.01$] and Trial x Preexposure x Dose [$F(3, 59) = 3.605$, $p < 0.05$] interactions. Given the significant interaction of all three factors, further analyses were conducted to identify specific dose-response relationships within each preexposure condition on the baseline and final CPP test.

One-way ANOVAs on time in the drug-paired chamber followed by Tukey's post-hoc tests were performed on each trial (baseline and final CPP test) to determine if there were any differences in time spent on the white (drug-paired) side between groups. Analysis of the baseline test (Figure 2A) revealed significant differences between groups ($p < 0.05$).

Specifically, Group V-10 spent significantly more time in the white chamber on this day than Group V-5. There were no other significant differences between groups on this trial. During the final CPP test (Figure 2B), additional significant group differences within each preexposure condition emerged. For the vehicle-preexposed animals, the group exposed to the highest dose of cocaine (Group V-20) spent significantly more time in the drug-paired chamber than any of the other groups (*p*s < 0.05). For animals exposed to ethanol during adolescence, subjects conditioned with 10 mg/kg cocaine spent significantly more time in the drug-paired chamber than their controls (Group E0; $p < 0.05$). None of the other groups (vehicle- or ethanol-preexposed) differed from controls. In addition, there were no significant dose-dependent preexposure differences on either trial.

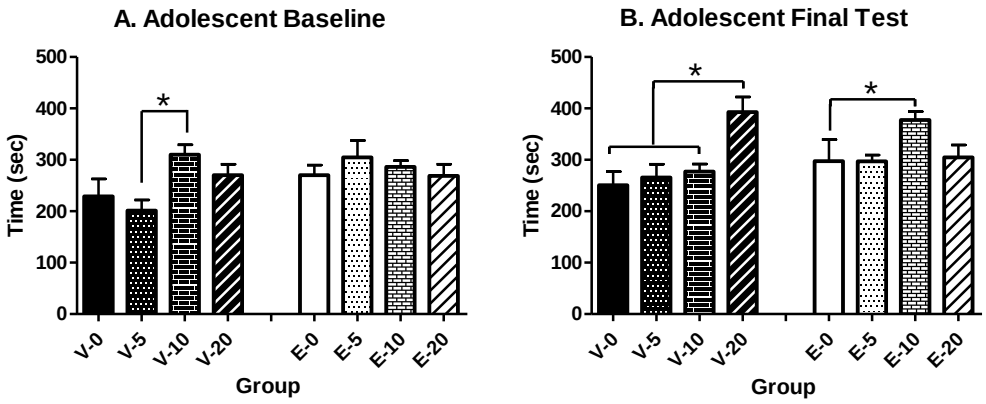

Figure 2. CPP Between Groups Comparison. Time spent in white chamber (+ SEM) during (A) baseline and (B) final CPP test by dose (Groups V-0, V-5, V-10, V-20 and E-0, n = 8 per group; Groups E-5, E-10 and E-20, n = 9 per group). * indicates significant difference between groups.

To more specifically assess the development of a preference for the drug-paired chamber, times spent in this compartment during the baseline and final CPP tests were compared using paired-samples t-tests for each group. A significant increase in time spent in the drug-paired chamber during the final test would indicate a significant preference for that compartment. For animals preexposed to saline (Figure 3A), Group V20 showed a significant increase in time spent

on the drug-paired chamber from baseline to the final test ($p < 0.01$). No other vehicle-preexposed subjects showed a significant increase in time in the drug-paired chamber. In subjects preexposed to ethanol during adolescence (Figure 3B), a significant increase in time spent in the drug-paired chamber was seen for the intermediate dose, Group E10 ($p < 0.01$). None of the other ethanol-exposed groups showed a change in time spent in the drug-paired chamber over trials.

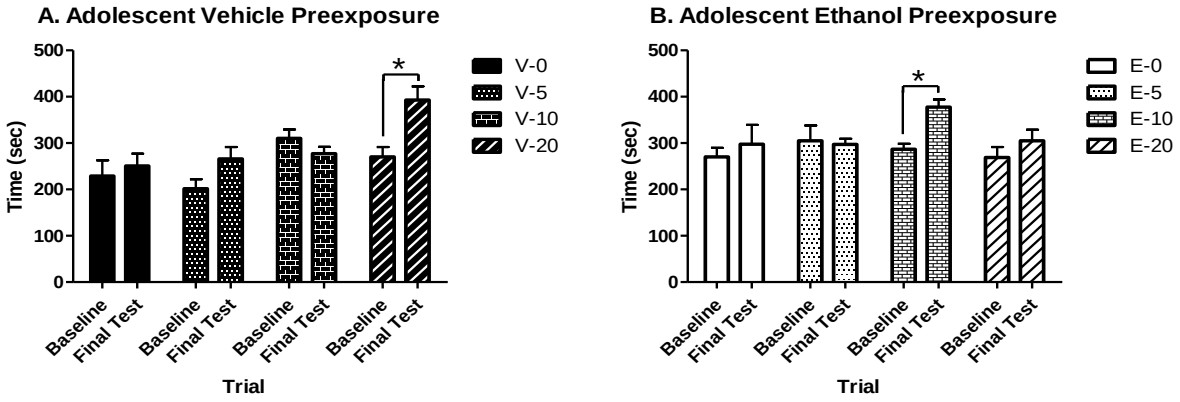

Figure 3. CPP Change From Baseline. Time spent in white chamber (+ SEM) during baseline and final CPP test by trial. (A) Subjects exposed to vehicle during adolescence [Groups V-0 (n = 8), V-5 (n = 8), V-10 (n = 8) and V-20 (n = 8)]; (B) subjects exposed to ethanol during adolescence [Groups V-0 (n = 8), V-5 (n = 9), V-10 (n = 9) and V-20 (n = 9)]. * indicates significant difference between baseline and final CPP tests for Groups V20 and E10.

Discussion

Ethanol is one of the most commonly used drugs during adolescence (Johnston et al., 2011) and commonly precedes the use of other drugs such as cocaine later in life (Degenhardt *et al.*, 2010, Ginzler *et al.*, 2003, Grant and Dawson, 1998). Therefore, it is of interest to see how early exposure to ethanol may alter the abuse liability of a drug such as cocaine later in life. Experiment 1 was designed to assess the effects of ethanol exposure during adolescence on the rewarding effects of cocaine administered in adulthood. As described, a history of ethanol exposure produced a leftward shift of the cocaine dose-response curve. Specifically, animals

preexposed to saline and conditioned with 20 mg/kg cocaine increased the time spent in the drug-paired chamber from baseline to the final test and spent more time in the drug-paired chamber following conditioning. These same effects were evident in the ethanol-preexposed subjects when conditioned at 10 mg/kg cocaine. These results suggest that ethanol preexposure during adolescence sensitizes the rewarding effects of cocaine.

When assessing the abuse liability of a drug, focus is often placed on its rewarding effects, which are commonly measured with the CPP procedure. While the development of place preferences, as well as how they may be modulated by variables such as prior drug history, has been thoroughly examined in adult rats (for a review, see Tzschentke, 2007), little work has been done to explore how reward is changed following adolescent drug exposure. The studies that have assessed the long-term effects of adolescent drug exposure on place preference conditioning later in life have primarily focused on early exposure to nicotine. In studies with mice, it was found that adolescent nicotine exposure decreases the subsequent rewarding effects of both nicotine (Adriani, Deroche-Gamonet, Le Moal, Laviola, & Piazza, 2006) and cocaine (Kelley & Middaugh, 1999; Kelley & Rowan, 2004). Interestingly, when rats are used in these studies of the long-term effects of nicotine, an early history of drug exposure produces a sensitization to the rewarding effects of diazepam (James-Walke, Williams, Taylor, & McMillen, 2007) and cocaine (McMillen et al., 2005; McQuown et al., 2007) in adulthood.

Although long-term effects of several drugs have been shown to alter the rewarding effects of cocaine in adulthood, no prior work has employed this assessment to examine an adolescent ethanol exposure paradigm. While the long-term effects of adolescent ethanol exposure had not been previously explored in this context, the interaction between a history of ethanol exposure and subsequent cocaine-induced place preferences in adult rats has been

addressed in previous studies (Busse et al., 2005; Le Pen et al., 1998). Interestingly, in those reports adult ethanol exposure did not produce a sensitization to the rewarding effects of cocaine. Differences between the results reported here (Experiment 1) and this prior work may be due to a number of parametric variations. In terms of the ethanol preexposure procedure, one of the previous studies (Busse et al., 2005) used a series of five spaced injections of 1.5 g/kg ethanol, while the other study (Le Pen et al., 1998) administered ethanol over 14 days in a free-drinking preparation. In contrast, animals in Experiment 1 were exposed to 10 consecutive days of 2 g/kg ethanol injections, which aimed to mimic ethanol exposure across the course of the adolescent developmental period. Given that adolescents tend to have higher levels of ethanol consumption than older adults (Substance Abuse and Mental Health Services Administration, 2010), it may be that the adolescent exposure procedure employed in the present series of experiments caused sensitization that would not be seen with a spaced preexposure regiment. One additional parameter that varied between prior work and Experiment 1 was the conditioning dose of cocaine. The present study used a dose-response approach to the place conditioning procedure and found a preexposure effect at the intermediate (10 mg/kg) dose of cocaine. In contrast, the previous studies each only used one dose of cocaine, which may not have detected all potential changes in the sensitivity to cocaine.

The fact that cocaine-induced place preferences were evident at lower doses in the ethanol-preexposed animals can be explained by a shift in the sensitivity to the rewarding effects of cocaine. An interesting feature of Experiment 1, however, is that ethanol-preexposed animals no longer displayed preferences at the 20 mg/kg dose of cocaine (whereas vehicle-exposed controls did). It is not clear why preferences were no longer evident at the higher dose in the ethanol-preexposed subjects. In studies using animals without a history of ethanol preexposure,

place preferences have also been seen with 20 mg/kg cocaine (Busse et al., 2004; Durazzo, Gauvin, Goulden, Briscoe, & Holloway, 1994). When ethanol and cocaine are given in combination during place preference conditioning, an attenuated preference is seen relative to subjects receiving cocaine alone (Busse et al., 2004; Busse & Riley, 2002), suggestive of the possibility that ethanol enhanced cocaine's aversive effects. In the present study a similar effect may have occurred, with the ethanol preexposure history enhancing the aversive effects of the highest dose of cocaine. Alternatively, given that animals in Experiment 1were individually housed during CPP conditioning (see Busse et al., 2004, 2005; Busse & Riley, 2002; Diaz-Granados & Graham, 2007; Graham & Diaz-Granados, 2006), isolation stress may have affected place preference conditioning, interacting with cocaine to affect its rewarding properties at the higher dose in the ethanol-preexposed subjects (though see Gehrke, Cass, & Bardo, 2006; Smith et al., 2009; Solinas, Chauvet, Thiriet, El Rawas, & Jaber, 2008).

Given that the rewarding effects of a drug play a role in promoting drug-taking behaviors (Bardo & Bevins, 2000), manipulations such as exposure to ethanol during adolescence that result in an increase in the rewarding effects of cocaine may indicate an increased likelihood of later cocaine abuse. However, some caution should be given to this interpretation due to the fact that the CPP procedure only looks at one of the multiple motivational aspects of a drug. In Experiment 1 it was shown that early ethanol exposure causes an increase in cocaine reward, but this does not take into account other properties of the drug that may also influence abuse liability. Since the likelihood to take a drug can be seen as a balance between its rewarding and aversive effects, an assessment of how adolescent ethanol exposure alters the negative motivational effects of cocaine will give a more complete understanding of how the overall abuse liability of cocaine may be changed.

CHAPTER 3

EXPERIMENT 2: CONDITIONED TASTE AVERSION

Introduction

When discussing the abuse liability of a drug, focus is often placed on its rewarding effects as they are viewed as a primary factor in promoting drug taking behaviors. In Experiment 1, it was shown that exposure to ethanol during the adolescent developmental period resulted in a sensitization to the rewarding effects of cocaine, which can be interpreted as a potential increase in cocaine's abuse liability. However, recreational drugs have other motivational properties that contribute to the likelihood that the drug will be abused. For example, while the rewarding effects of a drug may promote its use, negative or aversive effects may serve as a limiting factor for such use. Therefore, in addition to studying the rewarding effects of a drug, including an assessment of how manipulations such as drug history may change its aversive effects will give a more complete picture of how the overall abuse liability may change. The taste aversion procedure is a popular method for assessing the aversive effects of various physical and pharmacological manipulations (Klosterhalfen & Klosterhalfen, 1985; Masaki & Nakajima, 2006; Riley & Freeman, 2004, 2006; Riley & Tuck, 1985; for an alternative explanation, see Grigson, 1997). For example, if a rat is given a novel saccharin solution to drink and is then exposed to radiation, its subsequent consumption of the saccharin solution will decrease (Garcia, Kimeldorf, & Koelling, 1955). This phenomenon is known as a conditioned taste aversion (CTA) and is thought to be an adaptive association between the novel flavor and the aversive effects of the radiation (Garcia & Kimeldorf, 1960; Garcia et al., 1955; for a review, see Freeman & Riley, 2009). Since the initial work by Garcia and his colleagues, other studies have demonstrated that taste aversions can be produced by a variety of compounds (Riley & Tuck, 1985), most commonly emetics such as lithium chloride (Nachman & Ashe, 1973). Interestingly, aversions

are also induced by a number of self-administered drugs (Berger, 1972; Cappell, LeBlanc, & Endrenyi, 1973; Hunt & Amit, 1987) and is thought to be a measure of the negative effects of drugs that may serve as a limiting factor for their use. Although robust and induced by a wide range of compounds, CTAs are affected by a variety of parametric variations (Riley & Freeman, 2004), e.g., route of administration (Ferrari, O'Connor, & Riley, 1991), dose (Nachman & Ashe, 1973), sex (Randall-Thompson & Riley, 2003), strain (Pescatore, Glowa, & Riley, 2005) and age (Schramm-Sapyta, Morris, & Kuhn, 2006; Shram, Funk, Li, & Le, 2006).

One factor that has received considerable attention in terms of impacting aversion learning is drug history, i.e., experience with the conditioning drug prior to aversion conditioning. Studies assessing the effects of drug history on taste aversion learning have been designed such that the preexposure to the drug is given at some point along a continuum of time before conditioning. These studies have shown that aversions are often attenuated following preexposure, with the degree of attenuation dependent on preexposure dose and preexposure interval (Best & Domjan, 1979; Domjan, 1978). Although the effects of drug history on CTA have been well documented, it is surprising that little has been examined regarding the effects of such exposure during adolescence. Previous work in this area has shown that early exposure to ethanol attenuates subsequent ethanol-induced taste aversions (Diaz-Granados & Graham, 2007; Graham & Diaz-Granados, 2006). However, no prior work has been done to examine how early ethanol exposure may alter the aversive effects of other drugs in adulthood. Therefore, in Experiment 2, the effects of ethanol exposure during adolescence on later cocaine-induced CTAs were assessed. A concurrent assessment of ethanol preexposure on cocaine-induced locomotor activity was also performed as another measure of how early drug exposure may alter the behavioral effects of cocaine.

Procedure

Subjects

Subjects (n = 67) were experimentally naïve male Sprague Dawley rats. They were ordered from Harlan Laboratories such that they arrived in the laboratory on approximately PND 20. Animals were housed in Plexiglas cages (26 x 48 x 21 cm) with three or four animals in each cage in a colony room maintained on a 12-h light/dark cycle (lights on at 0800h) and at an ambient temperature of 23 °C. Training and testing took place during the light part of the cycle, with all procedures beginning at 0900h. Food and water, unless otherwise noted, were available *ad libitum*. Animals were handled daily for 5 days prior to the start of the experiment to limit the effects of handling stress during the procedure. Procedures recommended by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and the Institutional Animal Care and Use Committee at American University were followed at all times. Food and water consumption were monitored daily to assess the health of the subjects.

Drugs and Solutions

Ethanol (generously provided by the Department of Chemistry, American University) was prepared as a 15% (v/v) solution in 0.9% saline. Cocaine hydrochloride (generously supplied by NIDA) was prepared as a 10 mg/ml solution in 0.9% saline. Doses of cocaine refer to the weight of the salt. Vehicle injections were saline and were matched in volume to the injections of the corresponding drug. Saccharin (0.1% sodium saccharin) was prepared as a 1 g/l solution in tap water.

Ethanol Preexposure

On PND 30, animals were divided into two groups and injected IP with either ethanol (Group E; 2.0 g/kg; *n* = 35) or vehicle (Group V; *n* = 32). Group assignments were made such that animals in each cage (see above) were administered the same compound. Injections were given daily for 10 consecutive days (PND 30 – 39). Body weights were recorded each day to assess the health of the animals. From PND 40 – PND 44, subjects were maintained in their home cages until aversion conditioning (see below). During this time, animals were maintained on *ad libitum* food and water and handled during regular cage maintenance.

Taste Aversion Conditioning

Habituation. On PND 45, animals were individually housed in hanging wire cages (24.3 x 19 x 18 cm) and allowed 5 days to acclimate to this environment. As above, they were maintained on *ad libitum* food and water during this adaptation period. On PND 50, subjects were water deprived for $23^{2/3}$ h. Beginning on PND 51, subjects were given 20-min access to water (presented in graduated 50-ml Nalgene tubes). This procedure was repeated daily until all animals were approaching and drinking from the tube within 2 s of its presentation.

Conditioning. Water consumption was recorded and averaged for each animal over the last 3 days of habituation. Subjects in each of the two preexposure conditions, i.e., Groups E and V, were then ranked on consumption and assigned to one of four cocaine treatment conditions (see below) so that mean water consumption was similar among groups. Once group assignments were made, subjects in each group were randomly assigned to one of two replicates. The replicates were conditioned on consecutive days, with one beginning the procedure on PND 64 and the other beginning on PND 65.

On the first conditioning day, subjects received 20-min access to a novel saccharin solution. Following saccharin access, subjects received a subcutaneous (SC) injection of 32 mg/kg cocaine at the following temporal delays: 15, 180 or 300 min. A control group from each preexposure condition was given an equivolume SC injection of the drug vehicle (saline) 15 min after saccharin access. These time delays were chosen because previous research has shown that aversion conditioning is a function of the delay interval, i.e., aversions weaken with increasing delays (Freeman & Riley, 2005). Using several time delays produces a graded effect that can be modulated by parametric variations including drug preexposure (Riley, Dacanay, & Mastropaolo, 1984). Saccharin access for the 15-min delay groups (both control and cocaine-treated) was staggered so that half the subjects in each group received saccharin during the normal fluid-access period and half received it 1 h later. Access was staggered to allow for the measurement of all subjects' locomotor response immediately after injection (see below). All other groups received saccharin during the normal fluid-access period. This treatment resulted in eight groups designated as follows: E-V15 (n = 8), E-C15 (n = 9), E-C180 (n = 9), E-C300 (n = 9), V-V15 (n = 8), V-C15 (n = 8), V-C180 (n = 8) and V-C300 (n = 8). The first letter stands for the preexposure condition (ethanol or vehicle); the second letter and number stand for the treatment drug and the delay between saccharin and injection. After the first conditioning day, one subject from Group V-V15 was removed from the study due to failure to maintain body weight, leaving n = 7 animals in the group. This subject's data were excluded from all analyses, including for body weight during the ethanol preexposure phase. On the following 3 days, all animals were given 20-min access to water during the fluid-access period. No injections followed this access. This 4-day cycle of conditioning and water recovery was repeated until all animals received four complete cycles. On the day following the final water-recovery session, all

animals were given 20-min access to saccharin in a one-bottle test of the aversion to saccharin (Final Aversion Test). No injections were given following the test. Fluid consumption was recorded on all saccharin and water-recovery sessions.

Locomotor Activity

Immediately following injection with cocaine or vehicle on each conditioning trial, subjects were placed in locomotor chambers where fine and gross activity levels were recorded for 60 min. To measure locomotor activity in response to cocaine, a modified place conditioning apparatus (San Diego Instruments, San Diego, CA) was used. Each apparatus was 68.5 cm wide x 34.5 cm high x 21 cm deep and was equipped with a 16 x 4 photobeam array. The walls were clear Plexiglas, and the floor was covered with a single 68.5 cm x 21 cm sheet of haircell textured gray Kydex plastic. Each apparatus had four white LED lights set to maximum brightness within the otherwise unlit room. Counts of gross locomotor activity (consecutive beam breaks) and fine motor activity (repeated breaks of the same beam) were recorded for each animal over the 60-min session (see below). Between sessions on all experimental days, chambers were cleaned with soap and water to remove odor cues. Eight activity chambers were used, and each subject was placed in the same chamber on each session. Three subjects (one each from Groups E-C15, E-C180 and E-C300) did not undergo locomotor assessment due to the limited number of chambers. Following injection with cocaine, those subjects were placed in Plexiglas cages (48 x 26 x 21 cm) for 60 min and left in the same room as the locomotor chambers to control for the effects of a novel environment on taste aversion conditioning. The locomotor assessment resulted in four groups, VV (n = 7), VC (n = 24), EV (n = 8) and EC (n = 24), where the first letter designates preexposure condition (ethanol or vehicle) and the second letter refers to treatment (cocaine or vehicle).

Data Analysis

To assess health of the subjects, body weights were analyzed during ethanol preexposure and at the beginning of taste aversion conditioning. For the preexposure phase, body weights were analyzed using a 10 (Day) x 2 (Preexposure) repeated-measures ANOVAs. Body weights prior to the start of water habituation were assessed on PND 50 using an independent-samples t-test to compare the two preexposure groups. Saccharin consumption during acquisition of the taste aversion (see below) was analyzed using a 5 x 2 x 4 repeated measures ANOVA with the within-subjects factor of Trial (Trials 1-4 and Final Aversion Test) and between-subjects factors of Preexposure (ethanol or vehicle) and Treatment (vehicle or cocaine at a 15-, 180- or 300-min delay). One-way ANOVAs followed by Tukey's post-hoc tests were performed on each trial to analyze differences in saccharin consumption between groups. Locomotor activity counts were summed over the 60-min session and then were analyzed using a 4 x 2 x 2 repeated measures ANOVA with the within-subjects factor of Sessions (1-4) and between-subjects factors of Preexposure (ethanol or vehicle) and Treatment (vehicle or cocaine) for both fine and gross activity. One-way ANOVAs followed by Tukey's post-hoc tests were performed on each session to analyze differences in activity levels between groups. All determinations of statistical significance were set at $p \leq 0.05$.

Results

Body Weights

The 10 (Day) x 2 (Preexposure) repeated-measures ANOVA on body weights revealed a significant effect of Day [$F(9, 756) = 1468.721$; $p < 0.001$], as subjects continued to gain weight over the course of the preexposure phase. None of the terms involving Preexposure reached significance (Figure 4). The independent-samples t-test on PND 50 revealed that there was no

significant effect of preexposure on body weight prior to the start of the water habituation phase of CTA conditioning ($p > 0.05$). The average body weight of animals in Group V was 0.240 kg, and the average weight for Group E was 0.236 kg on this day.

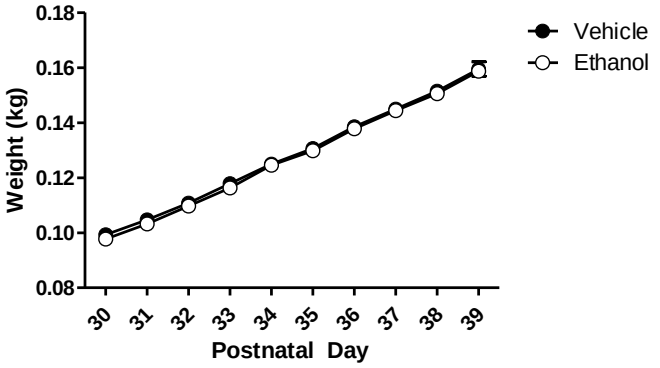

Figure 4. Body Weights During Ethanol Preexposure Phase in Experiment 2. Mean body weights (± SEM) for groups exposed to Vehicle (n = 31) or Ethanol (n = 35). No significant differences between preexposure groups.

Taste Aversion Conditioning

The 5 x 2 x 4 repeated measures ANOVA revealed significant effects of Trial [$F(4, 232) = 68.898$; $p < 0.001$], Preexposure [$F(1, 58) = 24.946$; $p < 0.001$] and Treatment [$F(3, 58) = 72.998$; $p < 0.001$], as well as significant Trial x Preexposure [$F(4, 232) = 14.026$; $p < 0.001$], Trial x Treatment [$F(12, 232) = 31.886$; $p < 0.001$], Preexposure x Treatment [$F(3, 58) = 4.477$; $p < 0.01$] and Trial x Preexposure x Treatment [$F(12, 232) = 1.807$; $p < 0.05$] interactions. Given the significant three-way interaction, one-way ANOVAs followed by Tukey's post-hoc tests were used to assess both treatment effects within each preexposure condition as well as differences between preexposed groups at each time delay interval.

The analysis of subjects exposed to vehicle during adolescence revealed that the conditioning procedure resulted in graded aversions, with the animals injected with cocaine at the shortest interval (15 min) showing the strongest aversions and the animals injected at the

longest interval (300 min) showing the weakest (but still significant) aversions. The group injected with cocaine at the 180-min delay showed an intermediate aversion compared to the other two cocaine-injected groups. These statements were supported statistically. Specifically, although there were no significant differences among any of the vehicle-preexposed groups on Trial 1, on Trial 2 all vehicle-preexposed, cocaine-injected groups consumed significantly less than controls (Group V-V15) (ps < 0.05). Group V-C15 consumed less than both Group V-C180 and Group V-C300 ($p < 0.05$), and Group V-C180 consumed significantly less than Group V-C300 ($p < 0.05$). On Trial 3, all cocaine-injected groups again drank significantly less than Group V-V15 (ps < 0.05). Group V-C15 drank significantly less than Groups V-C180 and V-C300, which did not differ. On Trial 4 and on the Final Aversion Test, all cocaine-injected groups drank less than Group V-V15 (ps < 0.05). Group V-C15 drank significantly less than Group V-C300 ($p < 0.05$). No other groups were different on Trial 4 and on the Final Aversion Test. Saccharin consumption for all groups exposed to vehicle during adolescence is shown in Figure 5A.

Similar analyses revealed that animals exposed to ethanol during adolescence showed significant aversions at the two shortest delay intervals, with the 15 min interval producing stronger aversions than the 180 min interval. However, ethanol-preexposed animals injected at the 300 min interval did not differ significantly in saccharin consumption on any trial compared to their controls. In terms of the statistical analysis, although there were no significant differences among any of the ethanol-preexposed groups on Trial 1, on Trial 2 Groups E-C15 and E-C180 drank significantly less saccharin than the control group (Group E-V15; ps < 0.05). There were no differences between Groups E-V15 and E-C300. Group E-C15 drank significantly less saccharin than either Group E-C180 or E-C300 (ps < 0.05). Groups E-C180 and E-C300 did

not differ on this trial. These patterns were maintained on Trials 3 and 4 and on the Final Aversion Test with the single exception that on Trial 4 Group E-C180 drank significantly less than Group E-C300 ($p < 0.05$) (see Figure 5B).

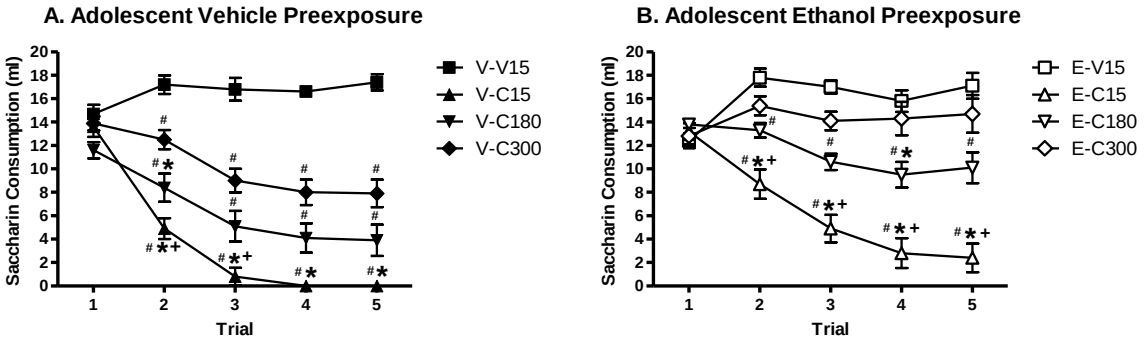

Figure 5. Mean (± SEM) Saccharin Consumption Across CTA Acquisition Trials by Preexposure Condition. A) Groups exposed to vehicle during adolescence (Groups V- 15, V-C15, V-C180 and V-C300; n = 8 per group). #Significantly different than control (Group V-V15). *Significantly different than Group V-C300. +Significantly different than Group V-C180. B) Mean (± SEM) saccharin consumption across CTA acquisition trials for groups exposed to ethanol during adolescence [Groups E-V15 (n = 8), E-C15 (n = 9), E-C180 (n = 9) and E-C300 (n = 9)]. #Significantly different than control (Group E-V15). *Significantly different than Group E-C300. +Significantly different than Group E- C180.

Figures 5A and 5B allow an indirect assessment of the effects of ethanol preexposure on cocaine-induced aversions over varying delays. To more directly assess the effects of ethanol preexposure, animals in the two preexposure conditions were compared following vehicle injection and at the three temporal delays. One-way ANOVAs followed by Tukey's post-hoc tests at each trial revealed that although there were no differences among any groups on the initial exposure to saccharin, ethanol preexposed groups displayed attenuated cocaine-induced taste aversions at all delay intervals over conditioning. Specifically, on Trials 2 and 3 Groups V-C15 drank significantly less than Group E-C15 (ps < 0.05), although they no longer differed on Trial 4 and on the Final Aversion Test. Groups V-C180 drank significantly less than Group E-C180 on Trials 2-4 and on the Final Aversion Test (ps < 0.05). Finally, Group V-C300 consumed

significantly less than Group E-C300 on Trials 3 - 4 (but not on Trial 2) and on the Final Aversion Test (*p*s < 0.05). There were no significant differences between the two preexposure groups conditioned with saline (Group V-V15 and Group E-V15) on any trial or on the Final Aversion Test.

Locomotor Activity

For gross motor activity, the 4 x 2 x 2 repeated measures ANOVA revealed a significant effect of Session [$F(3, 177) = 5.430$, $p < 0.01$] and Treatment [$F(1, 59) = 84.364$, $p < 0.001$], as well as a significant Preexposure x Treatment interaction [$F(1, 59) = 6.114$, $p < 0.05$]. None of the other terms containing Preexposure or Session reached significance. Subsequent analyses revealed that gross locomotor activity decreased across sessions, with subjects showing significantly lower activity counts on Session 4 compared to Session 1 ($p < 0.05$). Further, groups conditioned with cocaine showed significantly higher levels of activity than those conditioned with saline (*p*s < 0.05). To examine the Preexposure x Treatment interaction, gross locomotor activity counts were averaged for each subject across sessions and one-way ANOVAs followed by Tukey's post hoc-tests were performed. Subjects exposed to vehicle during adolescence (Group VC) showed significantly higher gross activity levels than did subjects preexposed to ethanol (Group EC; $p < 0.05$). There was no significant difference between the groups conditioned with vehicle, i.e., Groups VV and EV. Gross locomotor activity counts for each session are shown in Figure 6A.

The 4 x 2 x 2 repeated-measures ANOVA for fine locomotor activity revealed a significant effect of Preexposure [$F(1, 59) = 6.101$, $p < 0.05$] and Treatment [$F(1, 59) = 13.444$, $p < 0.01$], as well as a significant Preexposure x Treatment interaction [$F(1, 59) = 7.556$, $p < 0.01$]. None of the other terms containing Preexposure or Session reached significance. In

relation to the Preexposure x Treatment interaction, Group EC displayed significantly higher levels of fine locomotor activity than both Group VC ($p < 0.05$) and Group EV ($p < 0.05$). There were no significant differences between Groups VV and VC or Groups VV and EV. Fine locomotor activity for all groups on all sessions is shown in Figure 6B.

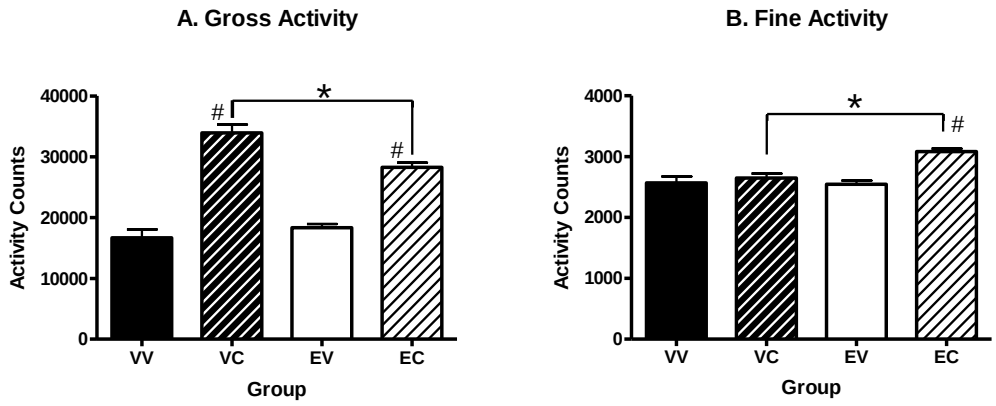

Figure 6. Locomotor Activity Counts (± SEM). A) Gross locomotor activity averaged over 1-h sessions for Groups VV (n = 7), VC (n = 24), EV (n = 8) and EC (n = 24). #Significantly different than control. *Group VC significantly different than Group EC. B) Fine locomotor activity averaged over sessions. #Significantly different than control. *Group VC significantly different than Group EC.

Discussion

Although the abuse liability of a drug is typically discussed in relation to its rewarding effects, to fully understand the abuse potential of a drug it is important to look at its multiple motivational properties. Given that drugs of abuse are proposed to have rewarding effects that promote their use as well as aversive effects that limit their use, manipulations that alter one or both of these features may lead to an altered abuse liability of the drug. Therefore, examination of how a factor such as early exposure to ethanol may alter both the rewarding and aversive effects of cocaine in adult rats provides a more complete assessment how a change in the abuse liability of cocaine may occur. In Experiment 1, it was found that ethanol exposure during adolescence produced a sensitization to the rewarding effects of cocaine in adult rats. Experiment

2 was designed to assess the effect of adolescent ethanol exposure on cocaine's aversive effects. As described, animals exposed to vehicle during adolescence showed a graded acquisition of the cocaine-induced taste aversion, with longer delays producing a weaker (but still significant) aversion across all trials (see Freeman & Riley, 2005). Adolescent ethanol exposure attenuated this effect, such that aversions were evident only at the two shortest delay periods. Since the aversive effects of a drug play a role in limiting its abuse potential, an attenuation of these aversive effects may indicate an increase in the abuse potential of the drug.

In the context of accounting for the attenuating effects of ethanol preexposure in adolescence on cocaine-induced taste aversions, it is interesting to note the parallels between the results from the present experiment and those assessing the effects of drug preexposure on taste aversion learning in general (Riley & Simpson, 2001). Specifically, preexposure to a drug typically attenuates subsequent aversion learning, a phenomenon known as the US preexposure effect. This attenuation is reported with a variety of drugs and is often attributed to the development of tolerance to the drug's aversive effects (Berman & Cannon, 1974; Cappell & LeBlanc, 1977; Dacanay & Riley, 1982; Randich & LoLordo, 1979; Riley & Diamond, 1998; Riley & Simpson, 2001). Although much of the work on US preexposure involves preexposure and conditioning to the same drug, such effects have also been reported when the preexposure and conditioning drugs are not the same, i.e., the cross-drug preexposure effect (Cappell & Poulos, 1979). This effect is generally explained as the development of cross-tolerance to the aversive effects of the two drugs (Clark et al., 1998). Interestingly, one drug combination for which this has been reported is ethanol and cocaine. In this case, preexposure to ethanol attenuates subsequent cocaine-induced taste aversions when preexposure and conditioning are given during adulthood (Grakalic & Riley, 2002; Kunin, Smith, & Amit, 1999). With this in

mind, it may be the case that the present results are an extension of the well documented cross-drug preexposure effect.

Although it is possible that the results of the present study are due to the cross-drug preexposure effect, it should be noted that there is one major procedural difference between the present study and prior work with the US preexposure effect, specifically, the time between preexposure and conditioning. In general, prior work on the US preexposure effect has used delays between preexposure and conditioning of only a few days (see Randich & LoLordo, 1979; Riley & Simpson, 2001), while in the present assessments the interval was approximately 26 days. The degree to which the US preexposure effect is reported is dependent on a number of variables (see Riley & Simpson, 2001), one of which is the interval between preexposure and conditioning (Best & Domjan, 1979; Domjan, 1978). In general, the longer the interval, the weaker the attenuation (Barker & Johns, 1978; Cannon, Berman, Baker, & Atkins, 1975; Misanin, Hoefel, Riedy, & Hinderliter, 1997), with no US preexposure effect often reported following intervals longer than 96 hours (Cannon et al., 1975). However, there are exceptions to these temporal limitations (Barker & Johns, 1978; Cappell & Le Blanc, 1975; Cappell & LeBlanc, 1977; Diaz-Granados & Graham, 2007; Graham & Diaz-Granados, 2006). For example, Cappell and LeBlanc (1977) reported significant attenuating effects of preexposure to morphine on morphine-induced taste aversions even with intervals up to 28 days (such effects were not evident with amphetamine when the preexposure and conditioning delay extended beyond 7 days; see Cappell & LeBlanc, 1977). Such parallels suggest that processes underlying the effects reported in the general assessments with US preexposure may be mediating those reported here.

The abovementioned account of the effects of drug preexposure focuses on changes in the aversive effects of a specific drug. There are other interpretations of the attenuating effects of ethanol on cocaine-induced aversions, however, that do not assume any changes in such effects. Specifically, it is possible that ethanol preexposure impacts learning and memory, processes on which taste aversion learning is based. This is especially relevant to the present study which used the long-delay conditioning preparation to assess aversion learning. In the present study, animals were given access to saccharin and then injected with cocaine at varying times following saccharin access. It is possible that ethanol preexposure affected (or decreased) the ability of animals to retain memory of the saccharin stimulus over the saccharin-cocaine delay intervals. Such an effect on memory would account for the weaker aversions seen in animals as the delay between saccharin and cocaine increased. The disruptive effects of ethanol during adolescence on learning and memory in adulthood are well documented (Acheson, Stein, & Swartzwelder, 1998; Brown, Tapert, Granholm, & Delis, 2000; Markwiese, Acheson, Levin, Wilson, & Swartzwelder, 1998; Siciliano & Smith, 2001; Swartzwelder, Wilson, & Tayyeb, 1995a, 1995b; White, Ghia, Levin, & Swartzwelder, 2000; White & Swartzwelder, 2004), although much of this work has only found long-lasting deficits in spatial memory. In Experiment 1, animals were able to form an association between the cocaine injection and the drug-paired compartment of the place conditioning apparatus, indicating that learning is still intact. This may suggest that an alteration of mechanisms other than memory underlie the ability to develop a CTA in ethanol-exposed subjects. However, given that most studies on the effects of ethanol on memory do not use an associative design, the possibility of memory disruption contributing to the attenuation of cocaine-induced taste aversions cannot be ruled out.

In addition to altering the aversive effects of cocaine, following adolescent exposure to ethanol animals showed a decrease in gross locomotor activity and an increase in fine locomotor activity. In contrast, other studies examining cocaine-induced locomotor activity often show an increase in gross motor activity over repeated sessions, indicating a sensitization to the motor-activating effects of the drug (e.g. Hope, Simmons, Mitchell, Kreuter, & Mattson, 2006; Manley & Little, 1997; Post, Weiss, Fontana, & Pert, 1992; Sabeti, Gerhardt, & Zahniser, 2003). This difference may be accounted for by parametric variations between studies. Specifically, the dose of cocaine used in the present study, 32 mg/kg, is higher than doses used in studies that primarily focus on locomotor activity (10-20 mg/kg; Hope et al., 2006; Manley & Little, 1997; Sabeti et al., 2003). Given that high doses of cocaine have been shown to produce stereotyped behaviors such as sniffing, rearing and head bobbing (Barr et al., 1983; Canales & Graybiel, 2000; Estevez, Ho, & Englert, 1979), the changes seen in the present study may indicate that ethanol exposure during the adolescent developmental phase can lead to an enhancement of stereotyped behaviors in response to cocaine, as shown by the increase in fine motor activity. Since the psychomotor stimulating properties of drugs of abuse have been associated with their abuse potential (Wise & Bozarth, 1987), the alteration of cocaine's locomotor-activating effects following adolescent ethanol exposure may be another indicator of an alteration of the abuse liability of cocaine.

As previously noted, the abuse potential of a drug can be seen as a balance between its rewarding and aversive effects (Brockwell et al., 1991; Simpson & Riley, 2005; Wise et al., 1976) and alterations to either of these properties may impact the likelihood that the drug will be used. Experiment 2 assessed if exposure to ethanol during adolescence would alter the aversive and locomotor-activating effects of cocaine later in life. The fact that ethanol exposure attenuated cocaine-induced taste aversions indicates that a history of ethanol may lead to an increased

likelihood of subsequent cocaine use. If early ethanol exposure increases the rewarding effects of cocaine (Experiment 1) while simultaneously attenuating its aversive effects (Experiment 2), it would be expected that adolescent ethanol exposure would affect cocaine self-administration later in life. This prediction was tested in Experiment 3 in which the effect of adolescent ethanol exposure on cocaine self-administration was examined.

CHAPTER 4

EXPERIMENT 3: SELF-ADMINISTRATION

Introduction

In order to understand the abuse liability of a drug, it is important to look at factors contributing to drug use as well as at drug-taking behaviors themselves. In the previous experiments, it was shown that exposure to ethanol during adolescence caused a sensitization to the rewarding effects of cocaine (Experiment 1) and an attenuation of cocaine's aversive effects (Experiment 2). Since the rewarding effects of a drug promote, while the negative effects limit, its use, this increase in reward and decrease in aversiveness would suggest that a history of ethanol early in life may cause an increase in the abuse liability of cocaine in adulthood. However, since the previous experiments assessed the rewarding and aversive effects of cocaine independently, it is unclear exactly how this shift in the motivational properties may affect actual drug use. To assess how early life ethanol exposure alters the abuse potential of cocaine in adulthood, the drug self-administration procedure may be used to see how early life drug exposure alters voluntary cocaine administration. This procedure is based on operant principles of reinforcement, where a positive reinforcer is used to increase the probability of a specific behavior. The self-administration preparation often involves the behavior of pressing a lever followed by the delivery of either a natural reinforcer such as food or by an infusion of a drug (Poling & Bryceland, 1979; Schuster & Thompson, 1969; Weeks, 1962). This procedure can be manipulated by changing the strength of the reinforcer (e.g., administering different drug doses) or by altering schedules of reinforcement (e.g., assessing how hard the animal will work to earn the reinforcer; Richardson & Roberts, 1996; Spealman & Goldberg, 1978). Similar to the previous work seen with place preference and taste aversion conditioning, additional procedural manipulations such as drug history can also alter self-administration behaviors (e.g. Horger,

Shelton, & Schenk, 1990; Panlilio, Solinas, Matthews, & Goldberg, 2007; Schenk & Davidson, 1998).

Much of the work with adolescent drug exposure and subsequent self-administration has focused on the effect of exposure to ethanol early in life on subsequent ethanol-drinking behaviors in adulthood. In these studies, it has been found that early exposure to ethanol generally increases subsequent ethanol consumption (Pascual, Boix, Felipo, & Guerri, 2009; Rodd-Henricks et al., 2002; however, see Slawecki & Betancourt, 2002). However, few cross-drug studies, i.e., where different drugs are administered in adolescence and adulthood, have been performed with the self-administration procedure. In these studies, the drugs used are most commonly stimulants. For example, preexposure to amphetamine enhances cocaine self-administration, with the strongest effects being seen at low doses of cocaine (Ferrario & Robinson, 2007; Horger, Giles, & Schenk, 1992; Valadez & Schenk, 1994). Interestingly, no work has been done to examine how early ethanol preexposure may alter cocaine self-administration. Given that ethanol use typically begins during adolescence while cocaine use is typically initiated during early adulthood (NSDUH, 2011), Experiment 3 explored how ethanol exposure during adolescence alters cocaine self-administration in adult rats. To see how such early drug exposure may alter the response to different types of stimuli, animals were allowed to respond for both a natural reinforcer (food) as well as an infusion of cocaine. To see how early exposure may change the acquisition rates as well as the motivation to respond for food or cocaine, two schedules of reinforcement were used. In the first, a fixed-ratio 1 schedule was used where each lever press resulted in the administration of the reinforcer. Additionally, the animals were tested on a progressive ratio schedule of reinforcement, where each subsequent reinforcer required a greater number of lever presses. Given that adolescent ethanol exposure sensitized the

rewarding effects of cocaine (Experiment 1) and attenuated cocaine's aversive effects (Experiment 2), it was predicted that a change in the reinforcing effects of cocaine would be seen in the self-administration procedure.

Procedure

Subjects

Subjects (n = 50) were experimentally naïve male Sprague Dawley rats (Harlan Sprague Dawley Laboratories). The experiment was run in two replicates (n = 20 in the first replicate, n = 30 in the second) under identical parameters, and data were pooled for analysis. All groups were represented in each replicate. Animals arrived in the laboratory on PND 25 and were housed in Plexiglas cages (26 x 48 x 21 cm) with three or four animals in each cage in a colony room maintained on a 12-h light/dark cycle (lights on at 1000h) and at an ambient temperature of 23 °C. Training and testing took place during the light part of the cycle with all procedures beginning at 1400h. Food and water were available *ad libitum* for the entirety of the study. Animals were handled daily for 5 days prior to the start of the experiment to limit the effects of handling stress during the conduct of the research. Procedures recommended by the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and the Institutional Animal Care and Use Committee at American University were followed at all times. Food and water consumption were monitored daily to assess the health of the subjects.

Drugs and Solutions

Ethanol (Sigma Aldrich Co., St. Louis, MO) was prepared as a 15% (v/v) solution in 0.9% saline. Vehicle (saline) injections were matched in volume to the ethanol injections.

Ketamine (50 mg/ml) and xylazine (3.85 mg/ml) were prepared in 0.9% saline. Cocaine hydrochloride (generously supplied by NIDA) was prepared as a 5 mg/ml solution in 0.9% saline. The concentration used for intravenous (IV) administration was 5.0 mg/ml. Doses of cocaine refer to the weight of the salt.

Apparatus

Ten standard (29.0 cm wide x 29.0 cm long x 19.0 cm high) operant chambers were used for all behavioral training and testing. Each chamber was equipped with a non-retractable lever positioned 3.2 cm above the grid floor and 5 cm to the right of a centered food cup. Pellet dispensers were located outside of the chambers. A swivel was located above the center of each operant chamber from which a spring-arm leash was suspended. The terminal end of the leash was tipped with a nylon wing-nut that screwed onto a threaded nylon post embedded in a dental acrylic plate on the animal's skull. This permitted the animals to move about the chamber freely without putting strain on the polyethylene (PE) drug-delivery tubing. For drug delivery, the terminal end of the PE tubing was connected to the external portion of the animal's catheter tubing where it exited between the scapulae. Outside the operant box, the swivel was connected with PE tubing (Plastics One; 0.044 mm ID, 0.814 mm OD) to a 10-ml syringe containing the cocaine solution. The syringe was seated in a syringe pump (Med Associates Inc, St. Albans, VT). All behavioral testing equipment and data acquisition were controlled by a desktop personal computer running Med Associates software (MED-PC for Windows v.1.1).

Ethanol Preexposure

On PND 30, animals were randomly divided into two groups and injected IP with either ethanol (Group E; 2.0 mg/kg; n = 25) or vehicle (Group V; saline; n = 25). Group assignments were made such that animals in each cage were injected with the same compound. Injections

were given daily for 10 consecutive days (PND 30-39). Body weights were recorded each day to assess the health of the animals. From PND 40 - PND 54, subjects were maintained in their home bins until self-administration (see below).

Self-Administration

Operant training. On PND 55, animals were transferred to individual hanging wire cages (24.3 x 19 x 18 cm) and allowed 5 days to acclimate to this environment. On PND 60, animals were gradually food-restricted to 90% of their free-feeding weight. Age and weight charts provided by Harlan Laboratories were used to ensure that animals maintained a proper growth curve. On PND 65, operant conditioning began. Rats were given 2-hr sessions on a fixed-ratio 1 (FR1) schedule during which each lever press was followed by a food pellet. Rats were trained on this FR1 procedure until they earned 100 or more food pellets within a session for three consecutive sessions.

Food PR. Once criterion was met under the FR1 schedule, contingencies were changed to a progressive ratio (PR) schedule of reinforcement to determine how hard subjects were willing to work for food. Food delivery was dependent upon completion of an increasing number of responses within the session (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc.; see Richardson & Roberts, 1996). Sessions concluded after 2 hours. Rats were given two sessions of this PR schedule to assess motivation to work for a food reinforcer.

Surgery. Following the second PR trial, animals were returned to ad lib food and water for at least 48 hours before undergoing surgery to implant an indwelling intravenous catheter. Aseptic surgical techniques were used for all surgical procedures. Rats were anesthetized with 75 mg/kg ketamine and 8 mg/kg xylazine, and a catheter of approximately 3 cm of Silastic tubing

(0.044 mm i.d., 0.814 mm o.d.) was inserted into the right jugular vein. This Silastic tubing was connected to 5 cm of vinyl tubing (Dural Plastics; 0.5mm i.d., 1.0mm o.d.) that was passed under the skin around the shoulder and exited the back at the level of the shoulder blades. The vinyl tubing was threaded through a 10-mm section of Tygon tubing that served as a subcutaneous anchor. Stainless-steel wound clips were used to close the incision. Animals were also fitted with a 20-mm plastic screw head-mount attached to the skull with six jewelers' screws and covered with dental cement (Stoelting). This cranial headmount allowed attachment to a stainless steel spring tether which housed the Tygon tubing connecting the syringe pump to the catheter. The spring tether served to protect the Tygon tubing from being chewed through by the rat. All animals were allowed to recover for a minimum of 7 days before access to cocaine. Catheters were flushed daily with a heparin/gentamycin solution (0.1 ml/infusion; 55 g/ml gentamycin and 2.0 USP/ml heparin) during the recovery period as well as before and after each self-administration session. The patency of the catheters was periodically checked by administering an infusion of 0.05 ml of a 50 mg/ml ketamine solution. A catheter was judged to be patent if ataxia was observed within 10 seconds of infusion. Non-patent catheters were clipped from the exit point on the back, and the animal was re-catheterized in the opposite jugular vein and allowed at least 3 days of recovery before returning to experimental procedures. Three subjects died during surgery (one from Group V and two from Group E). Data from these subjects were included in the analysis of food PR responding.

Cocaine acquisition. After recovery from surgery, cocaine self-administration training began. Animals from each preexposure condition (ethanol or vehicle) were randomly assigned to receive one of two doses of cocaine: 0.25 or 0.75 mg/kg/infusion. This assignment resulted in four conditioning groups, V-0.25, V-0.75, E-0.25 and E-0.75, where the letter refers to the

preexposure condition and the number refers to dose of cocaine administered in the self-administration sessions. Acquisition of cocaine self-administration was assessed in 2-h training sessions for 10 consecutive days. Training was conducted on an FR1 schedule. Following each infusion, house lights inside the self-administration chamber were turned off to signal a 20-s post-infusion time-out period during which lever presses were recorded but did not produce a drug delivery. One subject from Group V-0.75 died during the acquisition phase and one subject from Group V-0.25 was dropped from the study due to a failure to maintain body weight. Cocaine acquisition data from these two subjects were not included in the data analysis, resulting in the following number of subjects in each group: V-0.25, $n = 12$; V-0.75, $n = 10$; E-0.25, $n = 12$; E-0.75, $n = 11$.

Cocaine PR. Following the 10-day acquisition phase, rats were given two sessions of cocaine under the PR schedule. These cocaine PR sessions were identical to those given for food (see above) except that cocaine infusions replaced the delivery of food pellets. For each subject, the dose of cocaine administered during the PR sessions was identical to the dose administered during the acquisition phase. Sessions were concluded after 2 hours.

Data Analysis

To assess the health of the subjects, body weights were monitored over the course of the study. Body weight differences in response to ethanol administration during the preexposure phase were assessed with a 10 (Day) x 2 (Preexposure) repeated-measures ANOVA. Body weights prior to the start of the operant training phase were assessed on PND 60 prior to food restriction with an independent-samples t-test to compare the long-term weight differences between the two preexposure conditions. Number of lever presses and number of rewards earned during PR responding for food were analyzed with a 2 (Trial) x 2 (Preexposure) repeated-

measures ANOVA for both number of lever presses and number of reinforcers earned. Cocaine self-administration was assessed with a 10 (Trial) x 2 (Preexposure) x 2 (Dose) repeated-measures ANOVA. This analysis was done for both number of infusions earned as well as cumulative dose of cocaine administered across the 2-h session. Number of lever presses and number of infusions earned during cocaine PR sessions were assessed with a 2 (Trial) x 2 (Preexposure) x 2 (Dose) repeated-measures ANOVA for both number of lever presses and infusions earned. Significant effects from all analyses were further explored with either t-tests or one-way ANOVAs followed by Tukey's post-hoc tests. All determinations of statistical significance were set at $p \leq 0.05$.

Results

Body Weight

The 10 (Day) x 2 (Preexposure) repeated-measures ANOVA on body weights during the preexposure phase revealed significant main effects of Day [$F(9, 450) = 1957.531$; $p < 0.001$) and Preexposure [$F(1, 50) = 12.659$; $p < 0.01$], as well as a significant Day x Preexposure interaction [$F(9, 450) = 47.373$; $p < 0.001$]. To explore the Day x Preexposure interaction, independent-samples t-tests for body weight differences between the two preexposure groups were run on each day. This revealed that the ethanol-preexposed animals weighed significantly less than the vehicle-treated controls on Days 4 – 10 of the preexposure phase (Figure 7). On PND 60, prior to the start of food restriction, the independent-samples t-test revealed that the difference between groups did not persist through adulthood. Animals that had been injected with vehicle during adolescence weighed an average of 0.284 kg on PND 60, and animals preexposed to ethanol weighed an average of 0.282 kg.

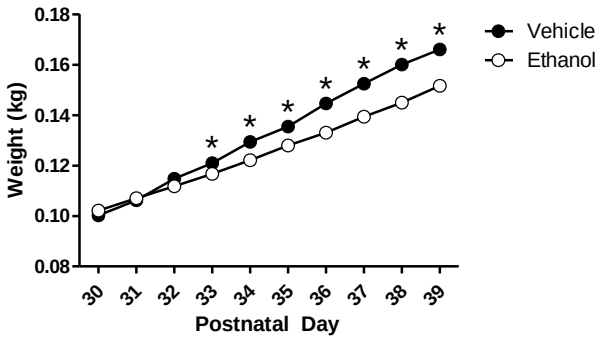

Figure 7. Body Weights During Ethanol Preexposure Phase in Experiment 3. Mean body weights (± SEM) for groups exposed to Vehicle (n = 25) or Ethanol (n = 25). *Significant difference between preexposure conditions.

Self-Administration

Food PR. All rats completed the acquisition criteria for operant responding for food within 8 days (average of 3.8 days for Group V and 4.0 days for Group E). The 2 (Trial) x 2 (Preexposure) repeated-measures ANOVA on the number of lever presses during the two PR trials for food revealed significant main effects of Trial [$F(1, 48) = 15.341$; $p < 0.001$] and Preexposure [$F(1, 48) = 4.541$; $p < 0.05$], although the interaction did not reach significance. To explore the significant effect of Trial, a paired-samples t-test revealed that animals had significantly more lever presses on the second PR trial than on the first ($p < 0.001$; Figure 8A). An independent-samples t-test comparing the two preexposure groups revealed that animals with a history of ethanol exposure had significantly fewer lever presses than the animals exposed to vehicle ($p < 0.01$; Figure 8B). The 2 (Trial) x 2 (Preexposure) repeated-measures ANOVA on number of food reinforcers earned also revealed significant main effects of Trial [$F(1, 48) = 11.215$; $p < 0.01$] and Preexposure [$F(1, 48) = 7.430$; $p < 0.01$]. A paired-samples t-test on Trial showed that animals earned significantly more reinforcers on the second PR trial ($p < 0.001$; Figure 8C). An independent-samples t-test on Preexposure revealed that animals exposed to

vehicle during adolescence earned more reinforcers than those with a history of ethanol exposure ($p < 0.01$; Figure 8D).

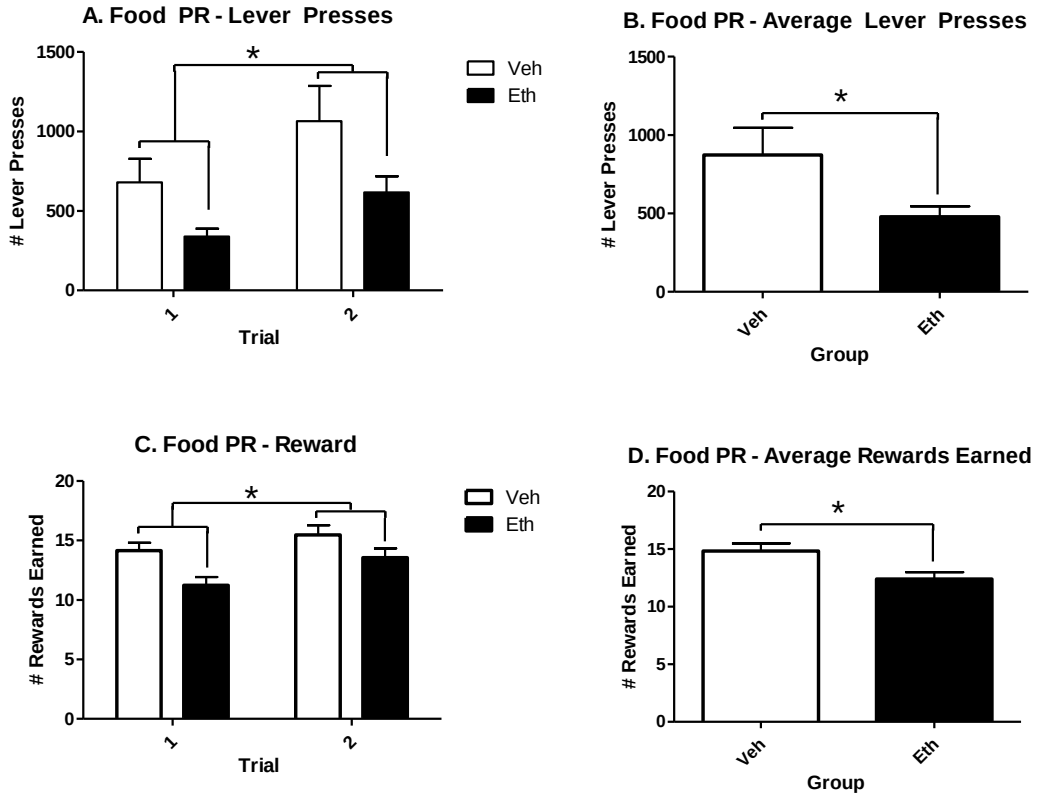

Figure 8. PR Responding for Food (+SEM). A. Number of lever presses during the two PR trials for subjects exposed to Vehicle (n = 25) or Ethanol (n = 25) during adolescence; *significant difference between trials. B. Number of lever presses averaged across the two food PR sessions; *significant difference between preexposure groups. C. Number of reinforcers earned during the two PR trials; *significant difference between trials. D. Number of reinforcers earned averaged across the two food PR sessions; *significant difference between preexposure groups.

Cocaine Acquisition. The 10 (Trial) x 2 (Preexposure) x 2 (Dose) repeated-measures ANOVA on the number of infusions earned during the acquisition phase of cocaine self-administration revealed significant main effects of Trial [$F(9, 369) = 3.152$; $p < 0.01$], Preexposure [$F(1, 41) = 10.965$; $p < 0.01$] and Dose [$F(1, 41) = 27.354$; $p < 0.001$], as well as a significant Preexposure x Dose interaction [$F(1, 41) = 11.488$; $p < 0.01$]. A Bonferroni pairwise comparison test revealed no additional significant differences between individual trials (Figure

9A). To explore the Preexposure x Dose interaction, the number of infusions earned per session was averaged across trials and between-groups differences were analyzed with a one-way ANOVA followed by Tukey's post-hoc tests. This revealed that subjects exposed to vehicle during adolescence and receiving the low dose of cocaine (Group V-0.25) during self-administration earned significantly more infusions than vehicle-exposed subjects receiving the high dose of cocaine (Group V-0.75). For animals exposed to ethanol during adolescence, this dose-response effect was not seen (Group E-0.25 did not differ from Group E-0.75). When looking at the effect of preexposure on number infusions, there was no effect of adolescent drug history in the two groups given the highest dose of cocaine, Groups V-0.75 and E-0.75. However, at the low dose of cocaine, subjects exposed to ethanol during adolescence (Group E-0.25) earned significantly fewer infusions than those exposed to vehicle (Group V-0.25) (see Figure 9B).

In addition to looking at the number of infusions earned during each acquisition session, for each subject the number of infusions in each session was multiplied by the dose of cocaine administered (0.25 or 0.75 mg/kg/infusion) to get the total drug intake (in mg/kg) over each 2-h session. The cumulative drug intake data were then analyzed with a 10 (Trial) x 2 (Preexposure) x 2 (Dose) repeated-measures ANOVA. This revealed significant main effects of Trial [$F(9, 369) = 3.792$; $p < 0.001$], Preexposure [$F(1, 41) = 6.156$; $p < 0.05$] and Dose [$F(1, 41) = 7.981$; $p < 0.01$], as well as a significant Preexposure x Dose interaction [$F(1, 41) = 7.028$; $p < 0.01$]. The main effect of Trial was assessed with a Bonferroni pairwise comparison test on the cumulative dose data for all subjects. This analysis revealed a significant difference in average drug intake between Trial 1 and Trial 10, with animals taking more drug on the last trial than on the first. No other differences between trials were significant (Figure 9C). To explore the Preexposure x Dose

interaction, the cumulative drug intake data were then averaged across trials and analyzed with a one-way ANOVA followed by Tukey's post-hoc tests. For subjects exposed to vehicle during adolescence, the total amount of cocaine taken over the 2-h session did not differ between Group V-0.25 and Group V-0.75. In contrast, for animals with a history of ethanol exposure, subjects in Group E-0.25 administered significantly less cocaine than those in Group E-0.75. In a comparison of preexposure effects, the two groups receiving the highest dose of cocaine, Group V-0.75 and Group E-0.75, did not differ in the amount of cocaine administered over the sessions. In contrast, subjects in Group E-0.25 administered significantly less cocaine than their counterparts in Group V-0.25 (Figure 9D).

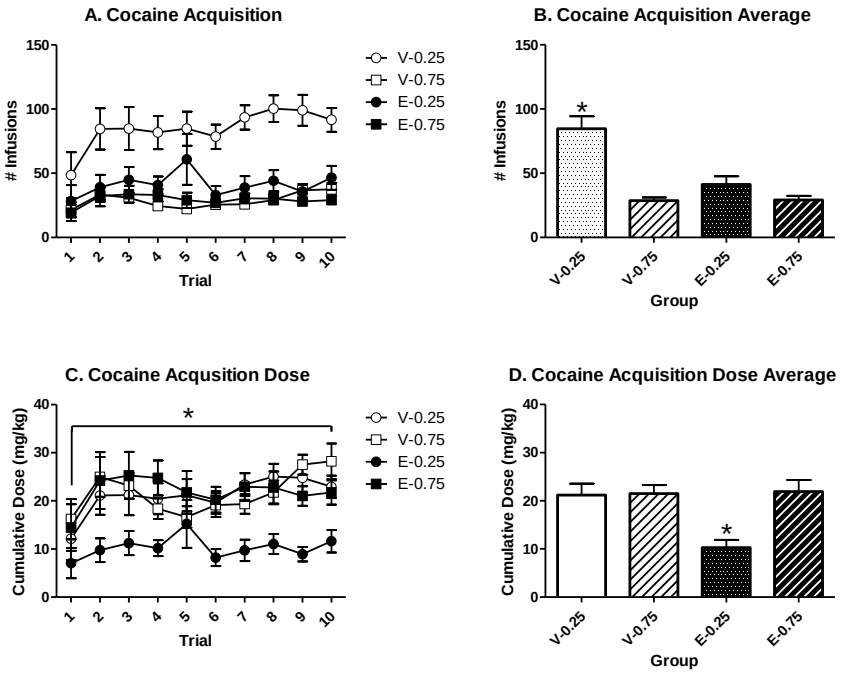

Figure 9. Number of Infusions Earned and Cumulative Dose of Cocaine Administered During Cocaine Self-administration Acquisition. A. Number of infusions earned (±SEM) over ten cocaine acquisition trials (V-0.25, n = 12; V-0.75, n = 10; E-0.25, n = 12; E-0.75, n = 11). B. Number of infusions earned (+SEM) averaged across trials; *significant difference between Group V-0.25 and all other groups. C. Cumulative dose of cocaine (±SEM) taken each session over ten cocaine acquisition trials; *significant difference between Trial 1 and Trial 10. D. Cumulative dose of cocaine taken (+SEM) averaged across acquisition trials; *significant difference between Group E-0.25 and all other groups.

Cocaine PR. The 2 (Trial) x 2 (Preexposure) x 4 (Dose) repeated-measures ANOVA on number of lever presses during the two cocaine PR sessions revealed that there were no significant differences between groups (Figure 10A). Similarly, the 2 (Trial) x 2 (Preexposure) x 4 (Dose) repeated-measures ANOVA on number of cocaine infusions earned revealed no significant differences (Figure 10B).

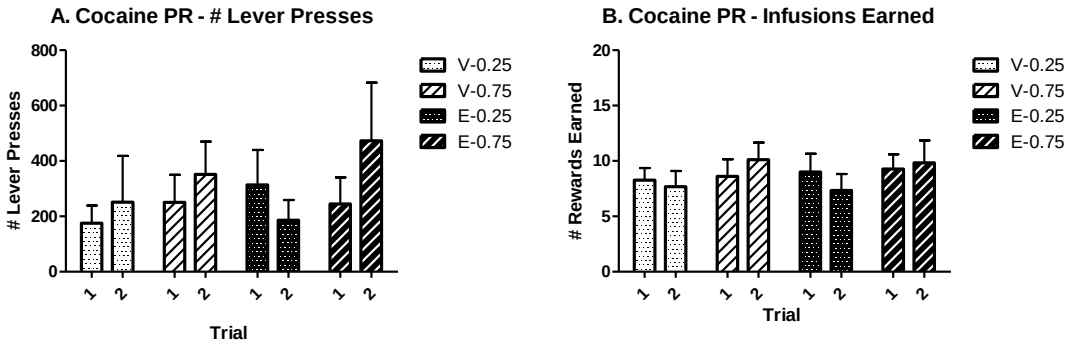

Figure 10. PR Responding for Cocaine (+SEM). A. Number of lever presses for each group (V-0.25, n = 12; V-0.75, n = 10; E-0.25, n = 12; E-0.75, n = 11) during the two PR sessions. No significant differences between groups or trials. B. Number of cocaine infusions earned for each group during the two PR sessions. No significant differences between groups or trials.

Discussion

In Experiment 3 animals were exposed to ethanol (or saline) during adolescence and then were evaluated for the self-administration of food and cocaine in adulthood. Animals exposed to ethanol displayed a decrease in PR responding, performing fewer responses and earning fewer rewards than their saline-preexposed counterparts. Although no preexposure effects were seen at the highest dose of cocaine, at the lowest dose of 0.25 mg/kg/infusion, animals with a history of ethanol exposure during adolescence showed both a lower number of responses as well as decreased total drug intake over the 2-h sessions compared to their vehicle-pretreated controls. No preexposure effects were seen for PR responding for cocaine.

To understand how any manipulation might impact drug intake, it is important to assess the nature of drug self-administration and its display with various doses. Operant responding for cocaine generally follows an inverted u-shaped dose-response curve (Carroll & Lac, 1997; Schenk & Partridge, 1997). The most common interpretation for the shape of this curve is that very low doses are not sufficiently rewarding to produce reliable responding and as the unit dose (and associated reward) increases so does the response rate. Since the rewarding effects of cocaine are thought to be mediated by dopamine release in the nucleus accumbens (Berridge & Robinson, 1998; Di Chiara, 2002; Robinson & Berridge, 1993), once the dose of self-administered cocaine is high enough to produce saturation of the dopamine receptors, the rate of responding will reach a maximum and then begin to decrease as the dose of cocaine continues to increase. This decrease may be due to the fact that dopamine receptors will become more rapidly saturated as the unit dose of cocaine is increased. In Experiment 3, this typical dose-response pattern can be seen in the subjects exposed to saline during adolescence, such that animals receiving the low dose of cocaine, Group V-0.25, had a higher level of responding than those receiving the high dose, Group V-0.75. Although the number of lever presses was different between these two groups, they did not differ on cumulative amount of cocaine, indicating that subjects in both groups reached a point of satiation following intake of similar absolute quantities of cocaine.

The primary goal of Experiment 3 was to see if exposure to ethanol during adolescence would affect the rewarding value of cocaine, i.e., produce a shift in this curve. For subjects exposed to ethanol and then conditioned with the highest dose of cocaine, Group E-0.75, the rate of responding and amount of cocaine administered never differed from their vehicle-pretreated controls (Group V-0.75). This pattern has also been seen in previous studies where drug

pretreatment manipulations that alter responding for lower doses of cocaine do not have an effect on the response to higher doses (Crawford et al., 2011). Doses of cocaine similar to the higher dose included in the present study are likely on the descending limb of the dose-response curve and are often used to establish robust responding while lower doses are used to examine shifts in the dose-response curve (Horger et al., 1992; Horger et al., 1990; Liu, Roberts, & Morgan, 2005). The lack of effect at these higher doses seen in the present and previous studies may be due to a floor effect in response rates (Carroll & Lac, 1997; Schenk & Partridge, 1997) or a result of the stronger rewarding effects of the higher dose of the drug outweighing the subtler effects produced by the pretreatment manipulation (Liu et al., 2005; Pettit & Justice, 1991).

Although there were no preexposure effects at the high dose of cocaine, at the low dose, 0.25 mg/kg/infusion, such effects emerged. At this dose, animals with a history of ethanol exposure during adolescence showed a decrease in both number of responses and in the cumulative dose of cocaine administered. Interestingly, this shift was in the opposite direction of what is normally seen when cocaine self-administration follows the preexposure to either cocaine or amphetamine. Specifically, other studies on such preexposure often result in an increased responding for cocaine (Ferrario & Robinson, 2007; Horger et al., 1992; Horger, Wellman, Morien, Davies, & Schenk, 1991; Piazza, Deminiere, Lemoal, & Simon, 1990; Valadez & Schenk, 1994). This increase in responding for cocaine generally follows one of two patterns: a faster acquisition of the operant behavior or an increased responding for a sub-threshold dose of cocaine. In the case of faster acquisition, animals that were preexposed to a drug such as cocaine or amphetamine will reach asymptotic levels of cocaine responding sooner than non-preexposed subjects (Ferrario & Robinson, 2007; Horger et al., 1992; Piazza et al., 1990; Valadez & Schenk, 1994). One contrast between these studies and the present one is that in these previous studies

animals are not trained for food responding prior to starting cocaine self-administration. In the current study, animals were already trained on the operant procedure before beginning responding for cocaine. Since all animals from both preexposure conditions had previously learned to respond for a reinforcer, no differences in rate of acquisition could be seen. Had animals not been trained on the operant task prior to the cocaine self-administration phase, it is possible that different rates of acquisition may have emerged. The other pattern seen following stimulant preexposure is that preexposed animals may acquire responding for a sub-threshold dose of cocaine. As described above, cocaine self-administration follows an inverted-u shaped dose-response curve. Very low doses of cocaine will not support the acquisition of responding, but as the dose increases animals will quickly reach near maximal levels of responding (Carroll & Lac, 1997; Schenk & Partridge, 1997). These low doses are considered to be sub-threshold since they are not sufficient to support responding. Animals that have been preexposed to drugs such as cocaine or amphetamine will respond for these lower doses, indicating that the preexposure resulted in a sensitization to the sub-threshold doses of cocaine (Carroll & Lac, 1997; Horger et al., 1990; Schenk & Partridge, 1997). In the present study, animals in the vehicle-preexposed group responding for 0.25 mg/kg/infusion readily acquired stable responding for cocaine, indicating that this dose was not sub-threshold. Additional factors such as preexposure drug (ethanol instead of stimulants used in these previous studies) may also play a role in the differences seen between the present studies and previous ones. Therefore, while prior studies have shown that drug preexposure may result in an increase in cocaine self-administration, the different effects seen in the previous study may be due to parametric variations which can cause a differential shift in the rewarding effects of cocaine.

One potential explanation for why ethanol-preexposed subjects showed a decrease in responding for the low dose of cocaine is that the pretreatment manipulation caused a decrease in the reinforcing value of cocaine. Previous research has revealed that disruption of the mesolimbic dopamine system by 6-hydroxydopamine lesions (Pettit, Ettenberg, Bloom, & Koob, 1984; Roberts & Koob, 1982) or pharmacological manipulations (Cappendijk & Dzoljic, 1993) also leads to a decrease in cocaine self-administration. Since this circuit plays a critical role in cocaine's rewarding effects (Carlezon & Thomas, 2009; Chen et al., 2006; Robinson & Berridge, 2000; Wise, 2006), the decrease in cocaine self-administration following these manipulations is attributed to a decrease in the rewarding effects of the drug. Therefore, it may be that the alterations caused by early exposure to ethanol may result in a disruption to the reward processing which subsequently interfered with the rewarding effects of cocaine. However, while a decrease in the reinforcing value of cocaine may be the most intuitive explanation for the decrease in responding for the low dose of cocaine, it does not take into account several factors that can influence this interpretation. For one, the previous studies used either methods that directly disrupt the dopamine system, such as 6-hydroxydopamine, or drugs that are not recreationally used or abused, unlike ethanol in the present study. Given the intrinsic differences between ethanol and the compounds used in these previous studies, although the effects are in the same direction it is probable that they are mediated by different mechanisms. In addition, the interpretation of decreased cocaine reward is inconsistent with the results of Experiment 1 and Experiment 2. If adolescent ethanol preexposure decreased the reinforcing efficacy of cocaine, the sensitization seen in Experiment 1 likely would not have occurred. When the results of Experiments 1 and 2 are taken together, it would be expected that an increase in reward and a decrease in aversion would result in an overall increase in the reinforcing efficacy of cocaine.

Therefore, while a decrease in reinforcement cannot be ruled out, it is important to look at other explanations for the results of Experiment 3.

As described above, cocaine self-administration follows an inverted U-shaped dose-response curve and shifts in this pattern may indicate either an increase or decrease in the rewarding value of the drug. In the present study, the decrease in responding observed at the lower dose of cocaine, 0.25 mg/kg/infusion, may be the result of a *leftward* shift of the dose-response curve, a shift generally interpreted as increasing the sensitivity to the drug. At this low dose of cocaine, all subjects from both preexposure conditions responded reliably for the drug, indicating that it was above the level necessary to support operant responding. Therefore, the decrease in responding may indicate that the 0.25 mg/kg/infusion dose is on the descending limb of the dose-response curve, and that the ethanol preexposure shifted the subjective effect of cocaine so that animals were responding as though they were receiving a higher unit dose of the drug. When the average cumulative dose administered over the 2-h sessions was assessed, animals in Group E-0.25 took significantly less drug than any of the other groups while none of the other groups differed from each other. This finding can also be explained by an increase in unit dose of cocaine produced by the early life ethanol exposure for Group E-0.25. Specifically, if animals were responding as though they were receiving a higher dose of cocaine, they would need less total drug to achieve the same effect. A similar change in responding was seen previously when subjects were pretreated with a dopamine agonist, 7-OH-DPAT (Caine & Koob, 1995). In this study, the pretreatment manipulation resulted in a decrease in responding for 0.25 mg/kg/infusion of cocaine and subsequent testing with additional doses indicated that this was due to a leftward shift in the dose-response curve, as seen by an increase in responding for doses on the ascending limb and a decrease in responding for doses of cocaine on the descending limb

of the curve. Similarly, in the present study the change in sensitivity to the low dose of cocaine may indicate that the ethanol-preexposed animals did not need to take as much of the drug to experience rewarding effects that are sufficient to support operant responding. As mentioned above, it is interesting to note that this change was seen only at the lower dose of cocaine. This may be due the fact that higher doses of cocaine are associated with both an increased neurochemical response (Pettit & Justice, 1991) and decreased rates of operant responding (Carroll & Lac, 1997; Schenk & Partridge, 1997), so any changes caused by the ethanol exposure may be masked by floor effects at the higher dose of cocaine.

The changes in self-administration of the lower dose of cocaine caused by adolescent ethanol exposure have important implications for the abuse liability of cocaine. An increase in the reinforcing value of a low dose of cocaine may result in an increased likelihood that the drug will be abused since initial and experimental use of cocaine typically involves low doses of the drug (Kandel & Davies, 1997; Paquette, Roy, Petit, & Boivin, 2010; Tapia-Conyer, Cravioto, De la Rosa, Galvan, & Medina-Mora, 2003). Given that the initial subjective response to a drug is a predictive factor for later continued use (Fergusson, Horwood, Lynskey, & Madden, 2003; Haertzen, Kocher, & Miyasato, 1983), if a person initially uses a low dose of cocaine and has an increased rewarding response, such as what may be seen in the present study for subjects preexposed to ethanol, they may be more likely to continue using the drug and at risk for developing patterns of abuse or dependence. This interpretation is also in accordance with the findings of Experiments 1 and 2, since a sensitization of the rewarding effects and an attenuation of the aversive effects of a drug indicate that its abuse liability will be increased.

While a shift in responding due to ethanol preexposure was seen for FR1 responding for cocaine, no significant effects were seen for PR responding. A similar pattern has been seen in

previous work on the effects of pretreatment with dopamine agonists on cocaine self-administration (Caine & Koob, 1995). It may be that although these manipulations shift the dose-response curve for cocaine on the FR1 schedule, they do not alter the willingness to work for cocaine. When discussing the reinforcing efficacy of a drug, it is important to note that FR and PR contingencies are thought to measure two different aspects of drug reward (Arnold & Roberts, 1997). Responding on an FR schedule will give information on learning and levels of consumption, while PR responding is thought to be a measure of motivation to work for the drug. Therefore, while both are related and play a role in the assessment of the abuse liability of a drug, various manipulations will not always affect them in the same way. It may be that in the present study, there was a shift in the rewarding efficacy of the low dose of cocaine following early ethanol exposure, but no change in the animals' willingness to work for the drug. It should be noted that, in the present study, the lack of a preexposure effect for PR responding for cocaine is in contrast with the results of the PR sessions for food, which found that early ethanol exposure decreased PR responding for the natural reinforcer. However, the reinforcing effects of food and cocaine are thought to be mediated by different circuits (Carelli, Ijames, & Crumling, 2000; Carelli & Wondolowski, 2003), it may be that ethanol differentially affects the response to the different stimuli. Another possibility is that, if cocaine decreases willingness to work for a reward but also increases the reinforcing value of the low dose of cocaine, it may be that these changes in opposite directions may add together and cancel each other out, resulting in no net shift in PR responding for the 0.25 mg/kg/infusion dose of cocaine. It is not entirely clear exactly what is causing the effects on the PR responding for food and cocaine in the present study, or how they may tie in with the results of Experiments 1 and 2. Additional PR assessments, including the use of additional does, may help to elucidate these effects. In addition, a more

thorough examination of the specific parameters used in the present study may help clarify the differences from earlier work.

One pattern that has been seen in prior studies on PR responding for cocaine is that the rate of PR responding will increase as the dose of cocaine increases (Richardson & Roberts, 1996). However, this trend was not seen in the present study, given that there were no differences between the two saline-preexposed groups. It is not entirely clear why this did not occur. Given that dose-dependent effects are commonly observed in PR studies (Richardson & Roberts, 1996; Stafford, LeSage, & Glowa, 1998), it may be that the specific parameters used in the present study influenced these findings. One limitation to the PR procedure used in the present study is that it was done under time-constrained (2 h), rather than open-ended, conditions. The time-constrained procedure can be used to assess effort to work for a reinforcer and generally produces reliable rates of responding (Aberman, Ward, & Salamone, 1998; Hamill, Trevitt, Nowend, Carlson, & Salamone, 1999). However, since some animals were still responding at the end of the 2-h session, true PR breakpoints, which are interpreted as a measure of drug reinforcement (Arnold & Roberts, 1997), could not be assessed. In addition, PR responding generally stabilizes over several trials, so it may be that the two trials used in the present study were not sufficient to attain true levels of PR responding. Therefore, although it appears that primary effort to attain the drug reinforcer did not differ between groups, an additional assessment of cocaine breakpoints would reveal if this was due to a change in the reinforcing value of cocaine.

While the present experiment provides a broad look at how early exposure to ethanol may alter the abuse liability of cocaine, there are several potential future considerations that would help explain why these changes occur. The further assessment of additional doses of cocaine

would give a more complete picture of the cocaine dose-response curve, as well as further information on how it may shift following early ethanol exposure. As mentioned above, using open-ended PR sessions for both food and cocaine would allow for analysis of breakpoints for both these reinforcers. Additionally, the present experiment focused solely on the behavioral response to cocaine. Previous work has examined long-term neurochemical changes due to adolescent ethanol exposure and found that such exposure results in a decrease in DRD_2 levels in the nucleus accumbens (Pascual et al., 2009). Given that the mesolimbic dopamine system mediates the reinforcing effects of both food and cocaine, it is likely that these neurochemical changes mediate the preexposure effects seen in the present study. Therefore, an additional examination of these changes as well as how they may interact with later cocaine exposure would give a more complete picture of how the neurological changes caused by adolescent ethanol exposure play a role in the future abuse liability of cocaine. By continuing to extend this research, a more thorough understanding of how and why early ethanol exposure changes cocaine-taking behaviors in adult rats may be achieved.

CHAPTER 5

SUMMARY

Ethanol is one of the most commonly used drugs during adolescence (Johnston et al., 2011) and commonly precedes the use of other drugs such as cocaine later in life (Degenhardt *et al.*, 2010, Ginzler *et al.*, 2003, Grant and Dawson, 1998). Therefore, the present series of experiments was designed see how early exposure to ethanol may alter the abuse liability of cocaine later in life. To fully understand the abuse potential of a drug, it is important to look at its multiple motivational properties as well as drug-taking behaviors themselves. In Experiment 1, it was found that ethanol exposure during adolescence produced a sensitization in the rewarding effects of cocaine in adult rats, while in Experiment 2 it was shown that early ethanol exposure reduces the subsequent aversive effects of cocaine. In Experiment 3, adolescent ethanol exposure resulted in a decrease in PR responding for food as well as a decrease in responding for a low dose of cocaine. Taken together, these three experiments indicate that exposure to ethanol during adolescence may increase the abuse liability of cocaine administered later in life. However, it should be noted that, as described above, parametric variations including the ethanol preexposure regimen and time between preexposure and conditioning may impact the results and should be considered when making comparisons with previous research. Also, each of the three experiments used different doses and routes of administration of cocaine, and the interpretation of the results assumes that the effects of cocaine can be generalized across these factors. While the three studies should be examined together rather than interpreted individually due to the fact that they all assess different aspects of the response to the same drug, the strength of the effects in each study should not be directly compared due to these procedural differences. In addition, while each of the procedures in the three experiments can be considered relatively pure measures of reward, aversion and overall reinforcement, respectively, all of cocaine's motivational factors

may have some influence on the outcome of each study. Further assessments of these parameters as well as of the neurological mechanisms underlying each of these behaviors may give a more complete view of why these changes occur. These findings help to explain how early ethanol use by humans may lead to an increase in the abuse liability of cocaine later in life. A continued exploration of the interaction between early drug exposure and subsequent drug-taking behaviors will lead to a greater understanding of factors involved in drug abuse and addiction and hopefully provide information for the development of prevention and treatment strategies.

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