Pharmacology, Biochemistry and Behavior 97 (2010) 370–376

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Exposure to alcohol during adolescence alters the aversive and locomotor-activating effects of cocaine in adult rats

Mary Anne Hutchison *, Daniel L. Albaugh, Anthony L. Riley

Psychopharmacology Laboratory, Department of Psychology, American University, Washington, DC 20016, USA

ARTICLE INFO

Article history:
Received 14 May 2010
Received in revised form 31 August 2010
Accepted 6 September 2010
Available online 15 September 2010

Keywords:
Adolescence
Alcohol
Ethanol
Cocaine
Conditioned taste aversion
Locomotor activity
Rat

ABSTRACT

Objectives: The present study assessed the effect of adolescent alcohol exposure on the later aversive and locomotor-activating effects of cocaine.
Methods: Male rats were exposed to alcohol or vehicle for 10 days [postnatal day (PND) 30–39; 2 g/kg IP]. Taste aversion conditioning began on PND 65. During aversion conditioning, subjects were presented with saccharin followed by cocaine (32 mg/kg; 15, 180 or 300 min post saccharin) or saline. Following each injection, animals were placed in locomotor chambers for 1 h. To determine if any effects seen were specific to the adolescent developmental period, the procedure was replicated in adult animals.
Results: Animals exposed to vehicle during adolescence showed significant aversions at all time delays. Animals exposed to ethanol during adolescence showed a decrease in consumption only at the 15 and 180 min delays. Groups exposed to alcohol during adolescence showed a decrease in gross, and an increase in fine, motor activity in response to cocaine. Animals exposed to alcohol during adulthood also showed attenuated taste aversions.
Conclusions: Exposure to ethanol during adolescence attenuated the aversive effects of cocaine and altered its locomotor-activating effects. Although this effect is not specific to adolescence, this is the time when alcohol use is typically initiated so that such exposure may enhance later abuse liability of cocaine.

1. Introduction

Adolescence in both humans and other animals appears to be a period of heightened vulnerability to high levels of alcohol use (Johnston et al., 2007; McBride et al., 2005). Such use during adolescence can lead to long-term behavioral changes, including alterations in the response to drugs taken later in life. One specific effect that is seen following adolescent alcohol exposure in animal models is a change in the self-administration of alcohol. For example, several studies have found that exposure to alcohol during adolescence increases alcohol consumption later in life (Pascual et al., 2009; Rodd-Henricks et al., 2002; Siciliano and Smith, 2001). In addition, juvenile alcohol exposure leads to resistance to extinction and faster reinstatement of alcohol self-administration (Rodd-Henricks et al., 2002).

Although adolescent alcohol exposure appears to impact its subsequent rewarding effects, little is known about how such exposure may impact its other motivational properties, e.g., any aversive effects. Specifically, drugs of abuse (including alcohol) have been reported to produce both rewarding and aversive effects and the balance of these effects is thought to impact drug taking with the aversive properties of a drug serving as the limiting factor for use (see Brockwell et al., 1991; Simpson and Riley, 2005; Wise et al., 1976). Insight into these effects and how they may be impacted by a variety of factors, e.g., drug history, may be important in understanding and predicting vulnerability to drug use and abuse. In this context, Graham and her colleagues have reported that alcohol exposure during adolescence attenuates the subsequent aversive effects of alcohol (Diaz-Granados and Graham, 2007; Graham and Diaz-Granados, 2006), suggesting that any subsequent changes in alcohol vulnerability may be a function of changes in both its rewarding and aversive effects following adolescent exposure.

Given the traditional progression of drug use, where alcohol often precedes the use of other drugs (Haertzen et al., 1983; Kandel et al., 1992), a better understanding of how adolescent alcohol exposure alters the motivational properties of other drugs may give insight into how such use may affect their abuse liability. One drug of interest in this regard is cocaine, a compound whose use is commonly preceded by alcohol use during adolescence (Haertzen et al., 1983). In adulthood, preexposure to alcohol attenuates cocaine-induced taste aversions (Grakalic and Riley, 2002; Kunin et al., 1999). Such exposure has also been shown to alter other aversive effects of cocaine (as measured by approach–avoidance behaviors for self-administered cocaine; Knackstedt and Ettenberg, 2005), and alcohol exposure may also alter cocaine self-administration (Mierzejewski et al., 2003).

* Corresponding author. Psychopharmacology Laboratory, Department of Psychology, American University, 4400 Massachusetts Avenue, NW, Washington, DC 20016, USA. Tel.: +1 202 885 1731; fax: +1 202 885 1081.
E-mail addresses: hutchison.ma@gmail.com (M.A. Hutchison), alriley@american.edu (A.L. Riley).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.09.006

However, there have been no studies assessing how exposure to alcohol during adolescence may impact subsequent responses to cocaine later in life.

To address how early alcohol exposure may alter the motivational properties of cocaine, the present study examined the effects of adolescent alcohol exposure on the aversive effects of cocaine (in adulthood; Experiment 1). This assessment was done using a delayed conditioned taste aversion procedure (Freeman and Riley, 2005; Nachman and Jones, 1974; Revusky and Garica, 1970) in which animals were given access to a novel saccharin solution followed by injections of cocaine after a delay of 15, 180 or 300 min. Although animals display robust taste aversions to solutions paired with an immediate injection of cocaine, these aversions are weakened as the delay between the taste and cocaine increases. Such a procedure allows for an assessment on aversions of varying strengths, providing a baseline amenable to disruption (see Freeman and Riley, 2005). To assess if the long-term effects of alcohol exposure were specific to the aversive properties of cocaine, changes in locomotor activity following cocaine injection were also examined. To determine if any effects reported were a function of the developmental time period in which alcohol was administered, the experimental procedures were replicated in adult animals, i.e., both exposure to alcohol and cocaine aversion conditioning were done in adulthood (Experiment 2).

2. Experiment 1: adolescent ethanol exposure

2.1. Procedure

2.1.1. 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 postnatal day (PND) 20. Animals were housed in Plexiglas cages ($26\times48\times21$ cm) with three or four animals in each cage in a colony room maintained on a 12-h light/dark cycle (lights on at 0800 h) 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 0900 h. 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.

2.1.2. Ethanol administration

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; $n=32$). Group assignments were made such that animals in each cage were administered the same compound. Injections were given daily for 10 consecutive days (PND 30–39). From PND 40 to PND 44, subjects were maintained in their home cages until aversion conditioning. During this time, animals were maintained on *ad libitum* food and water and handled during regular cage maintenance.

2.1.3. Conditioned taste aversions

2.1.3.1. Habituation. On PND 45, animals were individually housed in hanging wire cages ($24.3\times19\times18$ 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.

2.1.3.2. 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 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 and Riley, 2005). Using several time delays produces a graded effect that can be modulated by parametric variations including drug preexposure (Riley et al., 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. 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. 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.

2.1.4. 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 $\times$ 34.5 cm high $\times$ 21 cm deep and was equipped with a 16×4 photobeam array. The walls were clear Plexiglas, and the floor was covered with a single 68.5 cm $\times$ 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. 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 (48×26×21 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).

2.1.5. 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.

2.1.6. Data analysis

Saccharin consumption during acquisition of the taste aversion was analyzed using a 5×2×4 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 4×2×2 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 $p<.05$.

2.2. Results

2.2.1. Conditioned taste aversions

The 5×2×4 repeated measures ANOVA revealed significant effects of Trial [$F(4,232)=68.898$, $p<.001$], Preexposure [$F(1,58)=24.946$, $p<.001$] and Treatment [$F(3,58)=72.998$, $p<.001$], as well as significant Trial×Preexposure [$F(4,232)=14.026$, $p<.001$], Trial×Treatment [$F(12,232)=31.886$, $p<.001$], Preexposure×Treatment [$F(3,58)=4.477$, $p<.01$] and Trial×Preexposure×Treatment [$F(12,232)=1.807$, $p<.05$] 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) ($ps<.05$). Group V-C15 consumed less than both Group V-C180 and Group V-C300 ($p<.05$), and Group V-C180 consumed significantly less than Group V-C300 ($p<.05$). On Trial 3, all cocaine-injected groups again drank significantly less than Group V-V15 ($ps<.05$). 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 ($ps<.05$). Group V-C15 drank significantly less than Group V-C300 ($p<.05$). 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 Fig. 1A.

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) ($ps<.05$). 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 ($ps<.05$). 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 ($p<.05$) (see Fig. 1B).

Fig. 1A and B 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

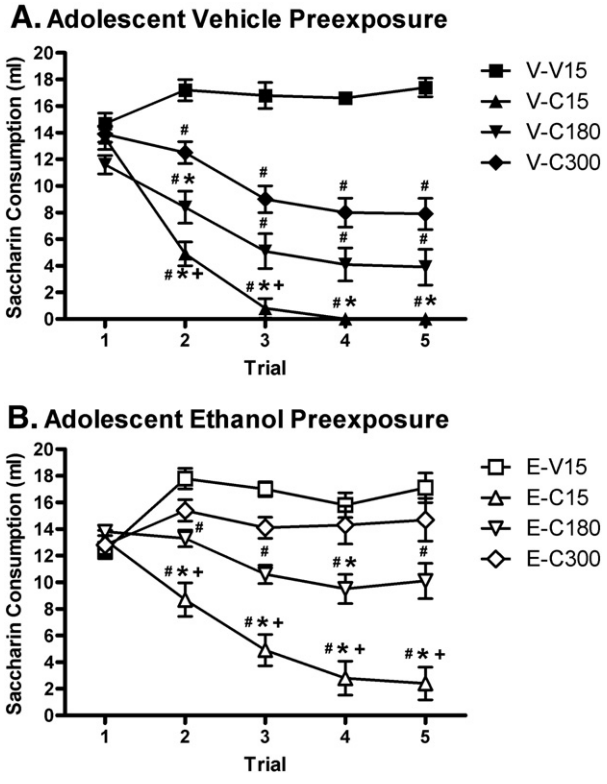

Fig. 1. Mean (±SEM) saccharin consumption across CTA acquisition trials by preexposure condition. A) Groups exposed to vehicle during adolescence (Groups V-V15, V-C15, V-C180 and V-C300; $n=8$ per group). #Significantly different than control (Group V-V15). *Significantly different than Group V-C300. +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.

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 ($ps<.05$), 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 ($ps<.05$). 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 ($ps<.05$). 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 ($ps>.30$).

2.2.2. Locomotor activity

For gross motor activity, the $4\times2\times2$ repeated measures ANOVA revealed a significant effect of Session [$F(3,177)=5.430$, $p<.01$] and Treatment [$F(1,59)=84.364$, $p<.001$], as well as a significant Preexposure × Treatment interaction [$F(1,59)=6.114$, $p<.05$]. 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 ($p<.05$). Further, groups conditioned with cocaine showed significantly higher levels of activity than those conditioned with saline ($ps<.05$). To examine the Preexposure × 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; $p<.05$). 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 Fig. 2A.

The $4\times2\times2$ repeated measures ANOVA for fine locomotor activity revealed a significant effect of Preexposure [$F(1,59)=6.101$, $p<.05$] and Treatment [$F(1,59)=13.444$, $p<.01$], as well as a significant Preexposure × Treatment interaction [$F(1,59)=7.556$, $p<.01$]. None of the other terms containing Preexposure or Session reached significance. In relation to the Preexposure × Treatment interaction, Group EC displayed significantly higher levels of fine locomotor activity than both Group VC ($p<.05$) and Group EV ($p<.05$). 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 Fig. 2B.

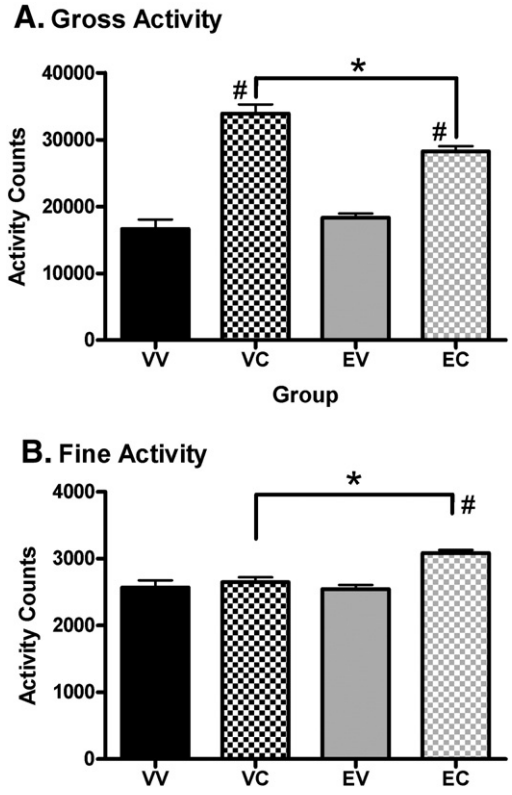

Fig. 2. Locomotor activity counts (±SEM) over 1-h sessions for Groups VV ($n=7$), VC ($n=24$), EV ($n=8$) and EC ($n=24$). A) Gross locomotor activity summed over sessions. #Significantly different than control. *Group VC significantly different than Group EC. B) Fine locomotor activity summed over sessions. #Significantly different than control. *Group VC significantly different than Group EC.

3. Experiment 2: adult ethanol exposure

3.1. Procedure

The procedures described for the assessment of the effects of ethanol exposure in adults were identical to those described for the adolescent subjects with the following exceptions. Specifically, on PND 70 animals were divided into two groups and injected IP with either ethanol (Group E; 2.0 g/kg; $n=32$) or vehicle (Group V; $n=32$). Injections were given daily for 10 consecutive days (PND 70–79). From PND 80 to PND 84, subjects were maintained in their home cages until aversion conditioning. During this time, animals were maintained on *ad libitum* food and water and handled during regular cage maintenance. For Experiment 2, the taste aversion habituation procedure began on PND 85 and aversion conditioning began on PND 105 and PND 106. The locomotor assessment was initiated immediately following each taste aversion conditioning trial as well as the Final Aversion Test.

3.2. Results

3.2.1. Conditioned taste aversions

The $5\times2\times4$ repeated measures ANOVA revealed significant effects of Trial [$F(4,224)=33.555$, $p<.001$], Preexposure [$F(1,56)=78.116$, $p<.001$] and Treatment [$F(3,56)=49.166$, $p<.001$], as well as significant Trial × Preexposure [$F(4,224)=20.486$, $p<.001$], Trial × Treatment [$F(12,224)=21.695$, $p<.001$], Preexposure × Treatment [$F(3,56)=8.866$, $p<.001$] and Trial × Preexposure × Treatment [$F(12,224)=5.354$, $p<.001$] interactions.

One-way ANOVAs followed by Tukey's post-hoc tests revealed significant Treatment effects for the animals exposed to drug vehicle during the preexposure phase (Fig. 3A). Overall, all vehicle-pre-exposed subjects injected with cocaine decreased saccharin consumption with no clear difference among groups. This was supported statistically in that although there were no significant differences among any of the vehicle-preexposed groups on Trial 1, on Trials 2–4 and on the Final Aversion Test all vehicle-preexposed, cocaine-injected groups (Groups V-C15, V-C180 and V-C300) consumed significantly less saccharin than the control group (Group V-V15) ($ps<.05$). There were no differences between the groups injected with cocaine on any trial. One-way ANOVAs also revealed significant treatment effects for subjects exposed to ethanol (Fig. 3B). In general, the subjects conditioned at the shortest delay interval (Group E-C15) developed a significant saccharin aversion across conditioning trial. However, the other cocaine-exposed groups did not show a significant difference in saccharin consumption from controls, with the exception of Group E-C180 which consumed significantly less saccharin than controls on the Final Aversion Test (Trial 5). 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 Group E-C15 consumed significantly less saccharin than controls

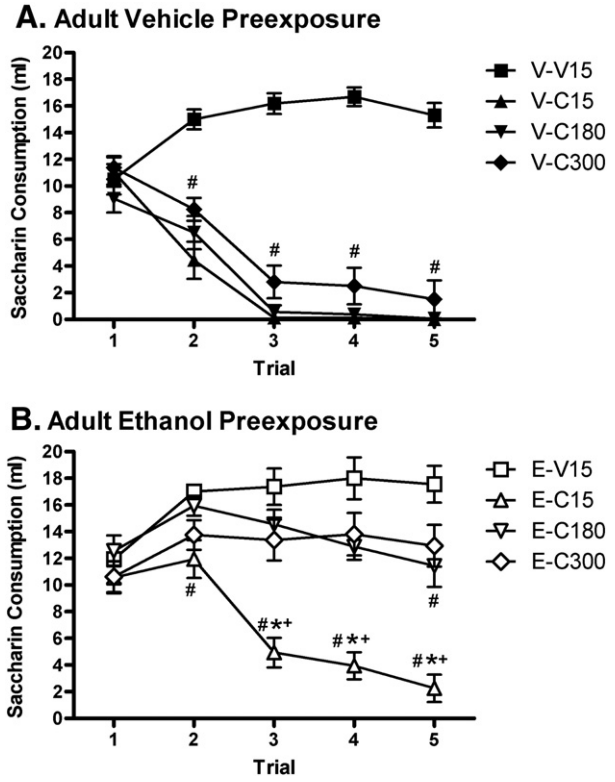

Fig. 3. Mean (±SEM) saccharin consumption across CTA acquisition trials by preexposure condition. A) Groups exposed to vehicle during adulthood (Groups V-V15, V-C15, V-C180 and V-C300; $n=8$ per group). #All cocaine-conditioned groups significantly different than control (Group V-V15). B) Groups exposed to ethanol during adulthood (Groups E-V15, E-C15, E-C180 and E-C300; $n=8$ per group). #Significantly different than control (Group E-V15). *Significantly different than Group E-C300. +Significantly different than Group E-C180.

(Group E-V15) ($p<.05$). There were no other significant differences between any groups on this trial. On Trial 3, Group E-C15 consumed significantly less saccharin than both the controls and the other cocaine-injected groups, i.e., Groups E-C180 and E-C300 ($ps<.05$). This pattern continued through Trial 4. On the Final Aversion Test, Group E-C15 continued to differ from all other groups ($ps<.05$). On this trial, Group E-C180 also consumed significantly less saccharin than controls ($p<.05$).

As in Experiment 1, ethanol preexposure effects were assessed both indirectly (Fig. 3A and B), as well as directly, following vehicle injection and at each of the three delay intervals. 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 Trial 2, Group V-C15 consumed significantly less saccharin than Group E-C15 ($p<.05$). However, these two groups did not differ on Trial 3 or 4 or on the Final Aversion Test. Group V-C180 drank significantly less saccharin than Group E-C180 on Trials 2–4 and on the Final Aversion Test ($ps<.05$). Similarly, Group V-C300 consumed significantly less saccharin than Group E-C300 on Trials 2–4 and on the Final Aversion Test ($ps<.05$). There were no significant differences between the groups injected with saline (Groups V-V15 and E-V15) on any trial or on the Final Aversion Test ($ps>.80$).

3.2.2. Locomotor activity

For gross locomotor activity, the $4\times2\times2$ repeated measures ANOVA revealed a significant effect of Session [$F(3,180)=4.568$, $p<.05$] and Treatment [$F(1,60)=47.509$, $p<.001$]. However, none of the terms containing Preexposure reached significance. Subsequent analyses revealed a decrease in ambulation across sessions, with activity levels on Session 4 significantly lower than those on all other trials ($ps<.05$). There were no other differences between sessions. Subjects injected with cocaine showed significantly higher levels of activity than the groups injected with saline ($p<.05$).

The $4\times2\times2$ repeated measures ANOVA for fine locomotor activity revealed a significant effect of Treatment [$F(1,60)=30.697$, $p<.001$] and a significant Session×Treatment interaction [$F(3,180)=9.749$, $p<.001$]. However, none of the terms containing Preexposure reached significance. Subsequent analyses revealed that on Sessions 1 and 2, there were no significant differences between any of the groups. On Sessions 3 and 4, the subjects injected with cocaine showed significantly increased fine activity levels compared to animals injected with saline ($ps<.05$).

4. Discussion

The present study was designed to assess the effect of adolescent alcohol exposure on the acquisition of a cocaine-induced taste aversion (and locomotor activity) later in life. 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 and Riley, 2005). Adolescent alcohol exposure attenuated this effect, such that aversions were evident only at the two shortest delay periods. In addition, adolescent alcohol exposure altered cocaine-induced locomotor activity by decreasing gross activity and increasing fine activity.

In the context of accounting for the attenuating effects of alcohol 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 and 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 and Cannon, 1974; Cappell and LeBlanc, 1977; Dacanay and Riley, 1982; Randich and LoLordo, 1979; Riley and Diamond, 1998; Riley and 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 and 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 alcohol and cocaine. In this case, preexposure to ethanol attenuates subsequent cocaine-induced taste aversions when preexposure and conditioning are given during adulthood (Grakalic and Riley, 2002; Kunin et al., 1999). Given that the specific dose of alcohol used during preexposure has been shown to condition taste aversions (Berman and Cannon, 1974; Risinger and Cunningham, 1995; Vetter-O'Hagen et al., 2009) and to produce tolerance in other preparations (see Chotro et al., 2009; Driscoll et al., 1985; Sircar and Sircar, 2005, 2006; Vetter-O'Hagen et al., 2009), it may be the case that the present results are an extension of the cross-drug preexposure effect.

Although the cross-drug preexposure effect is one possible explanation for the attenuated aversions, 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 and LoLordo, 1979; Riley and Simpson, 2001), while in the present assessment 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 and Simpson, 2001), one of which is the interval between preexposure and conditioning (Best and Domjan, 1979; Domjan, 1978). In general, the longer the interval, the weaker the attenuation (Barker and Johns, 1978; Cannon et al., 1975; Misanin et al., 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 and Johns, 1978; Cappell and Le Blanc, 1975, 1977). 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 and LeBlanc, 1977). Such parallels suggest that processes underlying the effects reported in the general assessments with US preexposure may be mediating those reported here.

In the present study, animals were given access to saccharin and then injected with cocaine at varying times following saccharin access. The use of such a procedure was based on the fact that prior work has reported graded (and weaker) aversions as the delay between saccharin and cocaine injections increased (Freeman and Riley, 2005). If alcohol preexposure impacted the aversive effects of cocaine, it would be predicted that aversions at longer delays would likely not be acquired (with weakening of the aversive effects of cocaine). As described, aversions at the longer delays were, in fact, attenuated by alcohol preexposure. However, there are other interpretations of the attenuating effects of alcohol on cocaine-induced aversions that do not assume any changes in the cocaine's aversive effects. Specifically, it is possible that alcohol preexposure affected the animal's ability to retain the memory of the saccharin taste over the delay interval. Such an effect on memory would account for the weaker aversions seen in alcohol-preexposed animals as the delay between saccharin and cocaine increased. The disruptive effects of alcohol during adolescence on learning and memory in adulthood are well documented (Acheson et al., 1998; Brown et al., 2000; Markwiese et al., 1998; Siciliano and Smith, 2001; Swartzwelder et al., 1995a,b; White et al., 2000; White and Swartzwelder, 2004); however, given that most studies on the effects of ethanol on memory do not use an associative design, it remains unknown if, and to what extent, these effects may contribute to the attenuation of cocaine-induced taste aversions (for a discussion of such issues in the interpretation of taste aversion learning, see Cunningham et al., 2009; Reilly, 2009).

In addition to attenuating cocaine-induced conditioned taste aversions, adolescent exposure to alcohol also had long-lasting effects on the locomotor-activating effects of cocaine. Specifically, following adolescent exposure to ethanol, animals showed a decrease in gross locomotor activity and an increase in fine locomotor activity. These changes may indicate that ethanol exposure during the adolescent developmental phase can lead to an enhancement of stereotyped behaviors in response to cocaine, an effect consistent with previous research showing that adolescent drug exposure alters the locomotor effects of drugs administered later in life (Brielmaier et al., 2007; Torres-Reveron and Dow-Edwards, 2005). These findings also indicate that adolescent exposure to alcohol alters multiple stimulus properties of cocaine, not just its aversive effects.

Although adolescent alcohol exposure clearly altered the aversive effects of cocaine later in life, such attenuation was not limited to exposure during this developmental period. As described, similar exposure during adulthood produced parallel effects on cocaine-induced aversions (Experiment 2), although rats exposed during adulthood did not display graded aversions with increases in the delay interval (unlike the adolescent exposed subjects; for comparisons see Freeman and Riley, 2005) nor any effects on cocaine-induced locomotion. However, given that the two groups were not run at the same time, i.e., the adolescent assessment preceded that of the adults, it is difficult to make formal comparisons between the two groups or to speculate whether similar mechanisms mediate the reported effects of alcohol. This limitation argues that such assessments and comparisons require a design, e.g., side-by-side or split-litter, in which both groups were run under identical conditions and at the same time. Despite this limitation for direct comparisons, it is clear that cocaine-induced aversions (and presumably cocaine's aversive effects) are affected by alcohol exposure in both adolescents and adults.

Given that the abuse potential of a drug can be seen as a balance between its rewarding and aversive effects (Brockwell et al., 1991; Simpson and Riley, 2005; Wise et al., 1976), such results suggest that adolescent ethanol exposure may lead to an increased likelihood of subsequent cocaine use (in adulthood). Although similar effects were seen following alcohol exposure in adulthood, the adolescent developmental period is the time when alcohol use is typically initiated (Kandel et al., 1992) and thus is a critical period for prevention of later drug use (Brown and Tapert, 2004; Chambers et al., 2003; Crews et al., 2007; McBride et al., 2005). The fact that adolescent ethanol exposure produced an alteration in cocaine's locomotor-activating effects shows that there are several stimulus properties of cocaine that are differentially affected by prior exposure to alcohol. To further clarify the mechanisms underlying the multiple effects of alcohol on the behavioral response to cocaine later in life, it will be of interest to further examine the neural mechanisms affected by alcohol and how these changes may differ following adolescent and adult exposure. In addition, further study of how alcohol affects the balance between the rewarding and aversive effects of cocaine will help in understanding how experience with alcohol at different stages of life may alter the abuse liability of cocaine.

References

Acheson SK, Stein RM, Swartzwelder HS. Impairment of semantic and figural memory by acute ethanol: age-dependent effects. Alcohol Clin Exp Res 1998;22(7):1437–42.

Barker LM, Johns T. Effect of ethanol preexposure on ethanol-induced conditioned taste aversion. J Stud Alcohol 1978;39(1):39–46.

Berman RF, Cannon DS. The effect of prior ethanol experience on ethanol-induced saccharin aversions. Physiol Behav 1974;12(6):1041–4.

Best MR, Domjan M. Characteristics of the lithium-mediated proximal US-preexposure effect in flavor-aversion conditioning. Anim Learn Behav 1979;7(4):433–40.

Brielmaier JM, McDonald CG, Smith RF. Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats. Neurotoxicol Teratol 2007;29(1):74–80.

Brockwell NT, Eikelboom R, Beninger RJ. Caffeine-induced place and taste conditioning: production of dose-dependent preference and aversion. Pharmacol Biochem Behav 1991;38(3):513–7.

Brown SA, Tapert SF. Adolescence and the trajectory of alcohol use: basic to clinical studies. Ann NY Acad Sci 2004;1021:234–44.

Brown SA, Tapert SF, Granholm E, Delis DC. Neurocognitive functioning of adolescents: effects of protracted alcohol use. Alcohol Clin Exp Res 2000;24(2):164–71.

Cannon DS, Berman RF, Baker TB, Atkins CA. Effect of preconditioning unconditioned stimulus experience on learned taste aversions. J Exp Psychol Anim Behav Process 1975;1(3):270–84.

Cappell H, Le Blanc AE. Conditioned aversion by amphetamine: rates of acquisition and loss of the attenuating effects of prior exposure. Psychopharmacologia 1975;43(2):157–62.

Cappell H, LeBlanc AE. Parametric investigations of the effects of prior exposure to amphetamine and morphine on conditioned gustatory aversion. Psychopharmacology (Berl) 1977;51(3):265–71.

Cappell H, Poulos CX. Associative factors in drug pretreatment effects on gustatory conditioning: cross-drug effects. Psychopharmacology (Berl) 1979;64(2):209–13.

Chambers RA, Taylor JR, Potenza MN. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. Am J Psychiatry 2003;160(6):1041–52.

Chotro MG, Arias C, Spear NE. Binge ethanol exposure in late gestation induces ethanol aversion in the dam but enhances ethanol intake in the offspring and affects their postnatal learning about ethanol. Alcohol 2009;43(6):453–63.

Clark DB, Kirisci L, Tarter RE. Adolescent versus adult onset and the development of substance use disorders in males. Drug Alcohol Depend 1998;49(2):115–21.

Crews F, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol Biochem Behav 2007;86(2):189–99.

Cunningham CL, Gremel CM, Groblewski PA. Genetic influences on conditioned taste aversion. In: Reilly S, Schachtman TR, editors. Conditioned taste aversion: behavioral and nerual processes. New York: Oxford University Press; 2009.

Dacanay RJ, Riley AL. The UCS preexposure effect in taste aversion learning: tolerance and blocking are drug specific. Anim Learn Behav 1982;10(1):91–6.

Diaz-Granados JL, Graham DL. The effects of continuous and intermittent ethanol exposure in adolescence on the aversive properties of ethanol during adulthood. Alcohol Clin Exp Res 2007;31(12):2020–7.
Domjan M. Effects of proximal unconditioned stimulus preexposure on ingestional aversions learned as a result of taste presentation following drug treatment. Anim Learn Behav 1978;6(2):133–42.
Driscoll CD, Riley EP, Meyer LS. Delayed taste aversion learning in preweanling rats exposed to alcohol prenatally. Alcohol 1985;2(2):277–80.
Freeman KB, Riley AL. Cocaine-induced conditioned taste avoidance over extended conditioned stimulus-unconditioned stimulus intervals. Behav Pharmacol 2005;16 (7):591–5.
Graham DL, Diaz-Granados JL. Periadolescent exposure to ethanol and diazepam alters the aversive properties of ethanol in adult mice. Pharmacol Biochem Behav 2006;84(3):406–14.
Grakalic I, Riley AL. Asymmetric serial interactions between ethanol and cocaine in taste aversion learning. Pharmacol Biochem Behav 2002;73(4):787–95.
Haertzen CA, Kocher TR, Miyasato K. Reinforcements from the first drug experience can predict later drug habits and/or addiction: results with coffee, cigarettes, alcohol, barbiturates, minor and major tranquilizers, stimulants, marijuana, hallucinogens, heroin, opiates and cocaine. Drug Alcohol Depend 1983;11(2):147–65.
Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the future: national results on adolescent drug use, 2006; 2007. Bethesda, MD.
Kandel DB, Yamaguchi K, Chen K. Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory. J Stud Alcohol 1992;53(5):447–57.
Knackstedt LA, Ettenberg A. Ethanol consumption reduces the adverse consequences of self-administered intravenous cocaine in rats. Psychopharmacology (Berl) 2005;178 (2–3):143–50.
Kunin D, Smith BR, Amit Z. Cocaine and ethanol interaction in the conditioned taste aversion paradigm. Physiol Behav 1999;67(4):627–30.
Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS. Differential effects of ethanol on memory in adolescent and adult rats. Alcohol Clin Exp Res 1998;22 (2):416–21.
McBride WJ, Bell RL, Rodd ZA, Strother WN, Murphy JM. Adolescent alcohol drinking and its long-range consequences. Studies with animal models. Recent Dev Alcohol 2005;17:123–42.
Mierzejewski P, Rogowski A, Stefanski R, Goldberg S, Kostowski W, Bienkowski P. Ethanol-reinforced behaviour predicts acquisition but not extinction of cocaine self-administration in the rat. Alcohol Alcohol 2003;38(6):543–9.
Misanin JR, Hoefel TD, Riedy CA, Hinderliter CF. Remote and proximal US preexposure and aging effects in taste aversion learning in rats. Physiol Behav 1997;61(2):221–4.
Nachman M, Jones DR. Learned taste aversions over long delays in rats: the role of learned safety. J Comp Physiol Psychol 1974;86(5):949–56.
National Research Council. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 1996.
National Research Council. Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington, DC: National Academy Press; 2003.
Pascual M, Boix J, Felipo V, Guerri C. Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. J Neurochem 2009;108(4):920–31.
Randich A, LoLordo VM. Associative and nonassociative theories of the UCS preexposure phenomenon: implications for Pavlovian conditioning. Psychol Bull 1979;86(3): 523–48.
Reilly S. Central gustatory system lesions and conditioned taste aversions. In: Reilly S, Schachtman TR, editors. Conditioned taste aversion: behavioral and neural processes. New York: Oxford University Press; 2009.
Revusky S, Garica J. Learned associations over long delays. In: Bowrer G, Spence J, editors. Psychology of learning and motivation: advances in research and theory. New York: Academic Press; 1970.
Riley AL, Diamond HF. The effects of cocaine preexposure on the acquisition of cocaine-induced taste aversions. Pharmacol Biochem Behav 1998;60(3):739–45.
Riley AL, Simpson GR. The attenuating effects of drug preexposure on taste aversion conditioning. In: Mowrer RR, Klein SB, editors. Contemporary learning theory. Hillsdale, NJ: Lawrence Erlbaum Associates; 2001.
Riley AL, Dacanay RJ, Mastropaolo JP. The effects of trimethyltin chloride on the acquisition of long delay conditioned taste aversion learning in the rat. Neurotoxicology 1984;5 (2):291–5.
Risinger FO, Cunningham CL. Genetic differences in ethanol-induced conditioned taste aversion after ethanol preexposure. Alcohol 1995;12(6):535–9.
Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, et al. Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: I. Periadolescent exposure. Alcohol Clin Exp Res 2002;26(11):1632–41.
Siciliano D, Smith RF. Periadolescent alcohol alters adult behavioral characteristics in the rat. Physiol Behav 2001;74(4–5):637–43.
Simpson GR, Riley AL. Morphine preexposure facilitates morphine place preference and attenuates morphine taste aversion. Pharmacol Biochem Behav 2005;80(3):471–9.
Sircar R, Sircar D. Adolescent rats exposed to repeated ethanol treatment show lingering behavioral impairments. Alcohol Clin Exp Res 2005;29(8):1402–10.
Sircar R, Sircar D. Repeated ethanol treatment in adolescent rats alters cortical NMDA receptor. Alcohol 2006;39(1):51–8.
Swartzwelder HS, Wilson WA, Tayyeb MI. Age-dependent inhibition of long-term potentiation by ethanol in immature versus mature hippocampus. Alcohol Clin Exp Res 1995a;19(6):1480–5.
Swartzwelder HS, Wilson WA, Tayyeb MI. Differential sensitivity of NMDA receptor-mediated synaptic potentials to ethanol in immature versus mature hippocampus. Alcohol Clin Exp Res 1995b;19(2):320–3.
Torres-Reveron A, Dow-Edwards DL. Repeated administration of methylphenidate in young, adolescent, and mature rats affects the response to cocaine later in adulthood. Psychopharmacology (Berl) 2005;181(1):38–47.
Vetter-O'Hagen C, Varlinskaya E, Spear L. Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. Alcohol Alcohol 2009;44(6): 547–54.
White AM, Swartzwelder HS. Hippocampal function during adolescence: a unique target of ethanol effects. Ann NY Acad Sci 2004;1021:206–20.
White AM, Ghia AJ, Levin ED, Swartzwelder HS. Binge pattern ethanol exposure in adolescent and adult rats: differential impact on subsequent responsiveness to ethanol. Alcohol Clin Exp Res 2000;24(8):1251–6.
Wise RA, Yokel RA, DeWit H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science 1976;191(4233):1273–5.