

## Exposure to nicotine during periadolescence or early adulthood alters aversive and physiological effects induced by ethanol

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### ABSTRACT

The majority of smokers begin their habit during adolescence, which often precedes experimentation with alcohol. Interestingly, very little preclinical work has been done examining how exposure to nicotine during periadolescence impacts the affective properties of alcohol in adulthood. Understanding how periadolescent nicotine exposure influences the aversive effects of alcohol might help to explain why it becomes more acceptable to this preexposed population. Thus, Experiment 1 exposed male Sprague Dawley rats to either saline or nicotine (0.4 mg/kg, IP) from postnatal days 34 to 43 (periadolescence) and then examined changes in the aversive effects of alcohol (0, 0.56, 1.0 and 1.8 g/kg, IP) in adulthood using the conditioned taste aversion (CTA) design. Changes in blood alcohol concentration (BAC) as well as alcohol-induced hypothermia and locomotor suppression were also assessed. To determine if changes seen were specific to nicotine exposure during periadolescence, the procedures were replicated in adults (Experiment 2). Preexposure to nicotine during periadolescence attenuated the acquisition of the alcohol-induced CTAs (at 1.0 g/kg) and the hypothermic effects of alcohol (1.0 g/kg). Adult nicotine preexposure produced similar attenuation in alcohol's aversive (at 1.8 g/kg) and hypothermic (1.8 g/kg) effects. Neither adolescent nor adult nicotine preexposure altered BACs or alcohol-induced locomotor suppression. These results suggest that nicotine may alter the aversive and physiological effects of alcohol, regardless of the age at which exposure occurs, possibly increasing its overall reinforcing value and making it more likely to be consumed.

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### 1. Introduction

Use of tobacco is, more often than not, initiated during adolescence, with more than 80% of smokers beginning their habit prior to the age of 18 (CDC, 2008). The implications of early onset of nicotine use are far reaching. It has been reported that subjects who begin smoking during adolescence show increased dependence (Kandel and Chen, 2000) and increased difficulty quitting (Chen and Millar, 1998) compared to adult onset smokers. Additionally, early onset of nicotine use usually precedes the use of other drugs of abuse, particularly alcohol (Nelson et al., 1995), and is seen as a risk factor for and strong predictor of the development of alcohol use and abuse and dependence disorders later in life (DiFranza and Guerrero, 1990; Grant, 1998; Gruzca and Bierut, 2006; Hanna and Grant, 1999; USDHHS, 1994). Nicotine preexposure during periadolescence has

also been shown to enhance the reinforcing and rewarding effects of other drugs of abuse, such as cocaine (McMillen et al., 2005; McQuown et al., 2007) and diazepam (James-Walke et al., 2007).

Although assessments of the changes in the rewarding effects of drugs following nicotine preexposure are important to understanding abuse vulnerability, the overall affective response of (and thus potential to use) a drug is thought to be due to the balance between its rewarding and aversive effects. Such aversive effects likely serve to limit drug self-administration (Brockwell et al., 1991; Simpson and Riley, 2005; Wise et al., 1976). In this context, it is important to assess the impact of adolescent drug exposure on the subsequent aversive effects of drugs of abuse in adulthood. In a recent report, Diaz-Granados and Graham (2007) demonstrated that exposure to alcohol during periadolescence attenuated the aversive effects of alcohol in adulthood in mice as measured by the conditioned taste aversion (CTA) preparation (an index of the aversive effects of drugs; see Garcia and Ervin, 1968; Revusky and Garcia, 1970; Riley and Tuck, 1985; Rozin and Kalat, 1971; [www.CTAlearning.com](http://www.CTAlearning.com)). Similarly, diazepam and alcohol administered in periadolescence attenuated the subsequent acquisition of alcohol and cocaine-induced taste

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aversions, respectively (Graham and Diaz-Granados, 2006; Hutchison et al., 2010). To our knowledge, only a single study has examined the effects of nicotine preexposure during periadolescence on aversion learning in adulthood. In this report, Hutchison and Riley (2008) demonstrated that nicotine during periadolescence had no effect on the acquisition of a conditioned taste aversion induced by a range of cocaine doses, but did appear to slow its extinction.

Given the lack of data on the effects of adolescent nicotine exposure on the aversive effects of alcohol, Experiment 1 examined the impact of nicotine exposure during periadolescence on the ability of alcohol to induce taste aversions. Blood alcohol levels and alcohol-induced changes in core body temperature and locomotor activity were also examined following adolescent nicotine exposure to assess the relationship of alcohol-induced aversions to other alcohol-mediated effects that might impact aversion learning with alcohol, e.g., see Cunningham et al. (1988). In order to determine if any effects seen with adolescent preexposure are specific to the developmental period during which nicotine is administered, the experimental procedures were replicated in adult animals (Experiment 2).

## 2. Experiment 1: Adolescent nicotine preexposure

### 2.1. Materials and methods

#### 2.1.1. Subjects and housing

Subjects were 64 experimentally naïve male Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN). Animals arrived at the laboratory on postnatal day 21 (PND 21) and were allowed to acclimate for approximately one week. They were housed in Plexiglas bins (26×48×21 cm), with four or five animals per bin, and were maintained on a 12:12 light–dark cycle (lights on at 0800 h) and at an ambient temperature of approximately 23 °C. Drug administration, training and testing took place during the light part of the cycle between 0800 and 1500 h. Except where noted, food and water were available ad libitum. Animals were handled daily for a week prior to the start of the experiment to limit the effects of handling stress during preexposure, conditioning and testing (see below). Procedures recommended by the Guide for the Care and Use of Laboratory Animals (1996), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and the Institutional Animal Care and Use Committee at American University were followed at all times.

#### 2.1.2. Drugs and solutions

(–)-Nicotine hydrogen tartrate (Sigma Aldrich Co., St. Louis, MO) was prepared as a 0.5 mg/ml solution dissolved in 0.9% saline. All doses of nicotine are expressed as the base. A 15% (w/v) ethanol (EtOH) solution was prepared from a 95% ethanol stock (Sigma Aldrich, St. Louis, MO) and was diluted with deionized water (dH<sub>2</sub>O). All drugs were administered intraperitoneally (IP). Saccharin (sodium saccharin, Sigma Aldrich, St. Louis, MO) was prepared as a 1 g/l (0.1%) solution in tap water.

#### 2.1.3. Adolescent nicotine preexposure

Beginning on PND 34, animals were divided into two groups and injected with either nicotine (NIC; 0.4 mg/kg IP;  $n=32$ ) or vehicle (VEH; equivolume saline IP;  $n=32$ ). Group assignments were made such that each animal-holding bin had an equal representation of preexposure conditions to avoid bin effects. Injections were given daily for 10 consecutive days from PND 34 to PND 43. This time period is considered mid to late adolescence (Spear, 2000), and the specific time period, dose and route of administration were selected based on prior assessments of nicotine preexposure during periadolescence (specifically, Adriani et al., 2006; James-Walke et al., 2007; Shram et al., 2006). Subsequent to the nicotine preexposure, animals remained on ad libitum food and water and were handled daily for weighing, but were

otherwise left undisturbed to mature until PND 60, at which point they were individually housed in hanging wire mesh cages for the remainder of the study.

### 2.1.4. Conditioned taste aversions

**2.1.4.1. Habituation.** Once the animals reached young adulthood (PND 75), they began a restricted water access regimen. Following 24 h of water deprivation, animals were given 20-min access to tap water daily. Animals were habituated to this restricted access until water consumption stabilized, i.e., animals approached the water bottle within 2 s of its presentation and consumption was within 2 ml of the previous day for a minimum of 4 days with no pattern of steady increase or decrease.

**2.1.4.2. Acquisition.** Once water consumption stabilized, all subjects were given 20-min access to a novel saccharin solution (0.1%) in graduated 50 ml Nalgene tubes (Day 1 of conditioning). Immediately following this initial presentation, animals within each preexposure condition were rank-ordered based on saccharin consumption and assigned to one of four treatment groups, i.e., vehicle (VEH) or ethanol (0.56, 1.0 or 1.8 g/kg; EtOH) such that saccharin consumption was comparable among groups. Within 20 min of group assignment, animals received an IP injection of either VEH (dH<sub>2</sub>O, matched in volume to the 1.8 g/kg dose of EtOH) or one of the three doses of EtOH. This resulted in the following groups: VEH–VEH, NIC–VEH, VEH–0.56, NIC–0.56, VEH–1.0, NIC–1.0, VEH–1.8, and NIC–1.8 (preexposure drug administered in periadolescence is listed first, followed by conditioning drug/dose administered in adulthood). The 3 days following this initial saccharin presentation were water-recovery days during which animals were given 20-min access to water. No injections followed water access on recovery days. This four-day saccharin-drug/water recovery cycle was repeated for a total of 16 days (four complete cycles). On Day 17, all subjects were given 20-min access to both saccharin and water in a final two-bottle aversion test and their relative preference for saccharin was determined ( $[\text{volume of saccharin consumed}/\text{total fluid consumed}] \times 100$ ). Bottle placement was counterbalanced across groups to avoid side preferences, and no injections were given on this day.

**2.1.4.3. Extinction.** The two-bottle test following the acquisition of the CTA functionally served as the first extinction test. Two-bottle extinction tests were continued every day for the next 7 days, for a total of eight extinction tests (including the first two-bottle aversion test; see above). Again, bottle placement was counterbalanced across groups and days, and no injections were given during this time.

### 2.1.5. Ethanol-induced hypothermia

Immediately after the end of extinction, animals were maintained on 20-min daily water access for 2 weeks prior to assessments of changes in body temperature (rectal) in response to a VEH or EtOH injection. Rectal body temperatures were assessed using a digital fast-read thermometer (Model KD-192, BestMed, LLC., Golden, CO) immediately prior to injection of EtOH or VEH (based on the drug/dose given during conditioning) and then again at 15, 60 and 180 min post-injection. During temperature readings, animals were gently held while the lubricated (KY Jelly) tip of the thermometer was inserted approximately 3 cm into the rectum for approximately 10 s and temperatures were recorded manually.

### 2.1.6. Blood alcohol concentrations

Animals were maintained on the restricted water regimen for 2 weeks following the hypothermia assessment at which point blood alcohol concentrations (BAC) were assessed to determine if exposure to nicotine during periadolescence altered the pharmacokinetics of alcohol in adulthood. Animals were given an injection of VEH or EtOH

(again based on their previous group assignments), and then tail blood samples were collected at 15, 60 and 180 min post-injection. Immediately prior to sampling, each animal's tail was soaked in warm tap water for 30–60 s and then wiped dry. Surgical scissors were then used to trim approximately 1 mm off the tip of the tail. For subsequent samplings, tails were re-soaked, but no further incisions were made. Approximately 40–90  $\mu$ l of whole blood was collected at each time point in heparinized capillary tubes (Drummond Scientific, Broomall, PA) and transferred to microcentrifuge tubes. Blood samples were then centrifuged, and the separated plasma was transferred to new vials and frozen at  $-80^{\circ}\text{C}$  until analysis. Approximately 20  $\mu$ l of plasma was diluted with 10  $\mu$ l of isopropanol (internal standard), and samples were analyzed using an Agilent headspace chromatography system (Agilent Technologies, Santa Clara, CA) based on established protocols of the Laboratory of Clinical and Translational Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health.

2.1.7. Locomotor activity

Immediately after blood samples were collected, animals were maintained on ad libitum food and water for 2 weeks, during which time no injections were given. Locomotor activity in response to an acute VEH or EtOH challenge (based on previous group assignments) was then measured in automated locomotor chambers (70 $\times$ 21 $\times$ 34 cm) with gray walls and flooring. Each chamber was illuminated with three white LED lights across the top of the chamber. A total of eight identical chambers were used, each equipped with a 16 $\times$ 4 photobeam array for recording ambulatory and fine motor movements (San Diego Instruments Place Preference System, San Diego, CA). The room in which the chambers were located was illuminated by an 85-Watt red light mounted to the ceiling in the center of the room, and background noise was masked by a white noise generator. For the locomotor assessments, animals were injected with their respective dose of either VEH or EtOH, placed in the locomotor apparatus and left undisturbed for 3 h. Ambulatory (consecutive beam breaks) and fine (repeated breaks of the same beam) locomotor movements were recorded and combined and then analyzed and reported as total locomotor activity per session.

2.1.8. Data analysis

Differences in mean saccharin consumption during the acquisition and extinction of the CTA were each analyzed using a mixed model analysis of variance (ANOVA) with between subjects factors of Preexposure Drug and Conditioning Drug and the within subject factor of Trial. Differences in group means for body temperature and

BAC were each analyzed using a mixed model ANOVA with between subjects factors of Preexposure Drug and Conditioning Drug and the within subject factor of Time. Locomotor activity was analyzed using a one-way ANOVA with between subjects factors of Preexposure Drug and Conditioning Drug. For significant interactions, pairwise comparisons of individual groups were made using Tukey HSD post-hoc tests. Significance levels were set at  $\alpha \leq 0.05$  for all analyses.

2.2. Results

2.2.1. Conditioned taste aversions

2.2.1.1. Acquisition. The 2 (Preexposure Drug) $\times$ 4 (Conditioning Drug) $\times$ 4 (Conditioning Trial) mixed-model ANOVA with repeated measures revealed significant main effects of Conditioning Drug ( $F(3, 56) = 107.643, p = 0.000$ ) and Conditioning Trial ( $F(3, 168) = 10.027, p = 0.000$ ), as well as significant Preexposure Drug $\times$ Conditioning Trial ( $F(3, 168) = 5.620, p = 0.001$ ), Conditioning Drug $\times$ Conditioning Trial ( $F(9, 168) = 58.052, p = 0.000$ ) and Preexposure Drug $\times$ Conditioning Drug $\times$ Conditioning Trial ( $F(9, 168) = 2.930, p = 0.003$ ) interactions.

Tukey HSD post-hoc analyses revealed that among animals preexposed with vehicle at no point did Group VEH-VEH differ from Group VEH-0.56 in saccharin consumption, but on Trials 2 through 4 Group VEH-1.8 drank significantly less saccharin than all other groups (Groups VEH-VEH, VEH-0.56 and VEH-1.0). As well, on Trials 2 through 4 Group VEH-1.0 drank significantly less saccharin than Groups VEH-VEH and VEH-0.56, showing a clear dose-dependent acquisition of the EtOH-induced taste aversion in the animals preexposed with vehicle during periadolescence (see Fig. 1). Similar to the vehicle-preexposed animals, post-hoc analyses revealed that at no point did Group NIC-VEH differ from Group NIC-0.56, but on Trials 2 through 4 Group NIC-1.8 drank significantly less saccharin than all other groups (Groups NIC-VEH, NIC-0.56 and NIC-1.0). Unlike the vehicle-preexposed animals, Group NIC-1.0 did not differ from Group NIC-VEH until Trials 3 and 4 and never differed from Group NIC-0.56, showing a delayed acquisition of the CTA (see Fig. 1). Post-hoc analysis between preexposure conditions at each dose of EtOH revealed that on Trials 3 and 4 animals preexposed with vehicle and conditioned with 1.0 g/kg EtOH (Group VEH-1.0) drank significantly less saccharin than their nicotine-preexposed counterparts (Group NIC-1.0). These differences are indicative of an attenuated aversion to 1.0 g/kg EtOH in the nicotine-preexposed animals.

The 2 (Preexposure Drug) $\times$ 4 (Conditioning Drug) ANOVA performed on the first two-bottle preference test (which also served

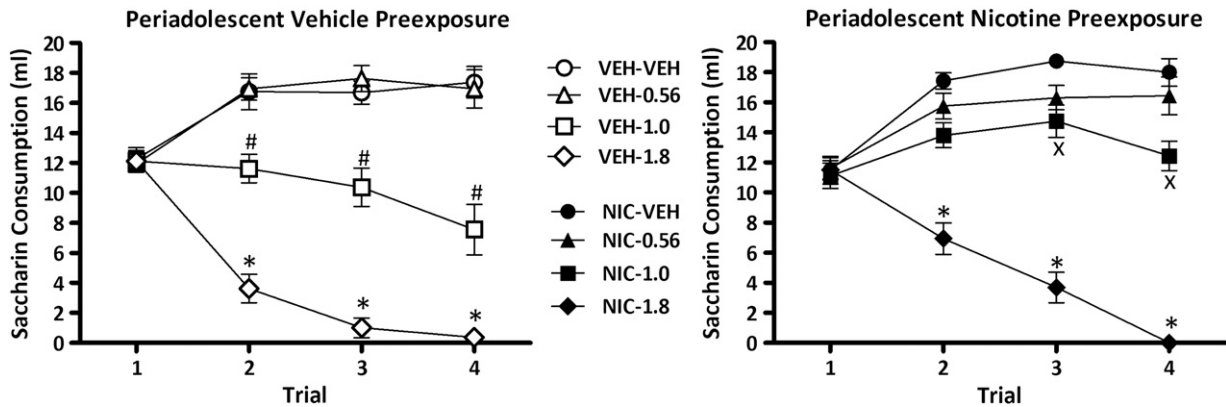


Fig. 1. Mean ( $\pm$ SEM) saccharin consumption (ml) following conditioning with various doses of EtOH in animals preexposed during periadolescence to either VEH (left panel) or NIC (right panel). In the figure legend, preexposure condition is listed first (VEH or NIC) and dose of EtOH is listed second (VEH, 0.56, 1.0 or 1.8 g/kg). Within each preexposure condition, \* indicates that animals conditioned with 1.8 g/kg drank significantly less saccharin than all other doses (VEH, 0.56 and 1.0 g/kg). # indicates that animals conditioned with 1.0 g/kg drank significantly less than those conditioned with VEH and 0.56 g/kg.  $\times$  indicates that animals conditioned with 1.0 g/kg drank significantly less saccharin than those conditioned with VEH.  $p < 0.05$  for all significant differences.

as the first day of extinction) revealed no main effect of Preexposure Drug and no Preexposure Drug  $\times$  Conditioning Drug interaction. There was, however, a main effect of Conditioning Drug ( $F(3, 56) = 64.767, p = 0.000$ ). Post-hoc analysis on Conditioning Drug revealed EtOH dose-dependent differences in saccharin preference, i.e., animals conditioned with EtOH (0.56, 1.0 and 1.8 g/kg) had significantly lower saccharin preferences than animals conditioned with VEH, and animals conditioned with 1.0 and 1.8 g/kg EtOH had significantly lower preferences for saccharin than those conditioned with 0.56 g/kg, but did not differ from each other (see Fig. 2).

**2.2.1.2. Extinction.** The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  8 (Extinction Trial) mixed-model ANOVA with repeated measures revealed significant main effects of Conditioning Drug ( $F(3, 56) = 85.801, p = 0.000$ ) and Extinction Trial ( $F(7, 392) = 21.653, p = 0.000$ ) and a Conditioning Drug  $\times$  Extinction Trial interaction ( $F(7, 392) = 5.071, p = 0.000$ ), but failed to reveal any main or interaction effects of Preexposure Drug, indicating that extinction of the EtOH-induced aversions was dose-dependent, but there were no effects of preexposure (data not shown).

### 2.2.2. EtOH-induced hypothermia

Core body temperatures ( $^{\circ}\text{C}$ ) were analyzed as a change from baseline for each animal. The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  4 (Time) mixed-model ANOVA with repeated measures revealed main effects of Preexposure Drug ( $F(1, 56) = 4.575, p = 0.037$ ), Conditioning Drug ( $F(3, 56) = 30.040, p = 0.000$ ) and Time ( $F(3, 168) = 8.327, p = 0.000$ ). Additionally, it revealed significant Preexposure Drug  $\times$  Conditioning Drug ( $F(3, 56) = 3.318, p = 0.026$ ) and Conditioning Drug  $\times$  Time ( $F(9, 168) = 18.933, p = 0.000$ ) interactions. There was no 3-way interaction among Preexposure Drug, Conditioning Drug and Time. Tukey HSD post-hoc analysis of the Preexposure Drug  $\times$  Conditioning Drug interaction (collapsed across Time) revealed that among animals preexposed with vehicle, the rectal temperatures of those injected with 1.0 g/kg and 1.8 g/kg EtOH (Groups VEH-1.0 and VEH-1.8) showed a significantly greater decrease from baseline temperature overall compared to both VEH injected controls and animals injected with 0.56 g/kg EtOH (Groups VEH-VEH and VEH-0.56). In animals preexposed with nicotine, only the animals injected with 1.8 g/kg EtOH (Group NIC-1.8) showed significant decreases in rectal temperatures compared to all other nicotine-preexposed animals (Groups NIC-VEH, NIC-0.56 and NIC-1.0), while animals injected with 1.0 g/kg EtOH (Group NIC-1.0) did not differ from either Group

NIC-VEH or NIC-0.56. Additionally, animals in Group VEH-1.0 had significantly greater decreases in rectal temperatures than animals in Group NIC-1.0 (see Fig. 3).

### 2.2.3. Blood alcohol concentrations

The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  3 (Time) mixed-model ANOVA with repeated measures revealed main effects of Conditioning Drug ( $F(3, 54) = 111.159, p = 0.000$ ) and Time ( $F(2, 108) = 31.151, p = 0.000$ ) and a Conditioning Drug  $\times$  Time interaction ( $F(6, 108) = 4.953, p = 0.000$ ), but did not reveal any effects of Preexposure Drug. To explore the Conditioning Drug  $\times$  Time interaction, data were collapsed such that Preexposure Drug was removed as a factor. Post-hoc analyses of these data revealed that at 15 min post-injection, animals injected with 1.8 g/kg EtOH achieved BACs significantly higher than all other groups and animals injected with 1.0 g/kg EtOH achieved BACs significantly higher than animals injected with either 0.56 g/kg or VEH. Sixty min after injections, animals injected with 1.8 g/kg EtOH still had BACs higher than all other groups and animals injected with 1.0 g/kg EtOH still had BACs higher than those injected with 0.56 g/kg and VEH. By 180 min after injections, only the animals injected with 1.8 g/kg EtOH still had significantly elevated BACs compared to all other groups (see Fig. 4).

### 2.2.4. Locomotor activity

The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug) ANOVA revealed main effects of both Preexposure Drug ( $F(1, 56) = 5.131, p = 0.027$ ) and Conditioning Drug ( $F(3, 56) = 68.726, p = 0.000$ ), but failed to reveal an interaction between the two ( $F(3, 56) = 1.398, p = 0.253$ ). Thus, nicotine preexposure during periadolescence did not appear to impact the locomotor-suppressing effects of EtOH in adulthood. When collapsed across doses of EtOH, nicotine preexposure greater than 6 weeks prior to locomotor assessment increased locomotor activity compared to controls and there was an EtOH dose-dependent decrease in locomotor activity when data were collapsed across preexposure condition (data not shown).

## 3. Experiment 2: Adult nicotine preexposure

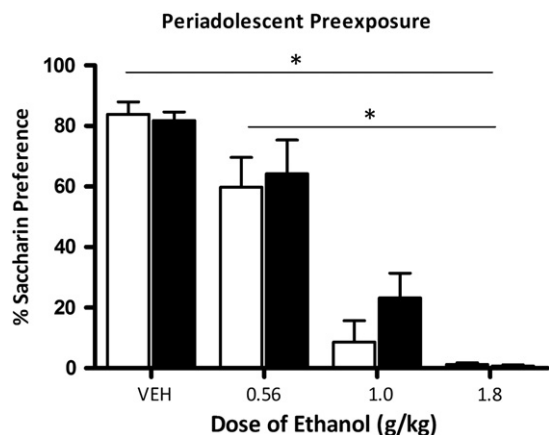
### 3.1. Materials and methods

The procedures for assessing the effects of nicotine preexposure during early adulthood were identical to the procedures outlined in Experiment 1 with the following exceptions. Specifically, on PND 70 (early adulthood) animals were divided into two groups and received once daily IP injections of either 0.4 mg/kg NIC ( $n = 32$ ) or equivolume VEH ( $n = 32$ ) for 10 consecutive days (PND 70–79). In order to match the timing and housing conditions used with the periadolescent subjects, subsequent to the preexposure phase animals in Experiment 2 remained on ad libitum food and water in their group housing conditions until PND 96 at which point they were individually housed in hanging wire mesh cages and allowed to acclimate to these conditions for two weeks. For Experiment 2, habituation began on PND 110 and animals were habituated to the restricted water access regimen until consumption stabilized, at which point aversion conditioning began. The number of acquisition and extinction trials during taste aversion conditioning, as well as the timing between the end of extinction and the hypothermia, blood alcohol and locomotor assessments, were identical to those in Experiment 1.

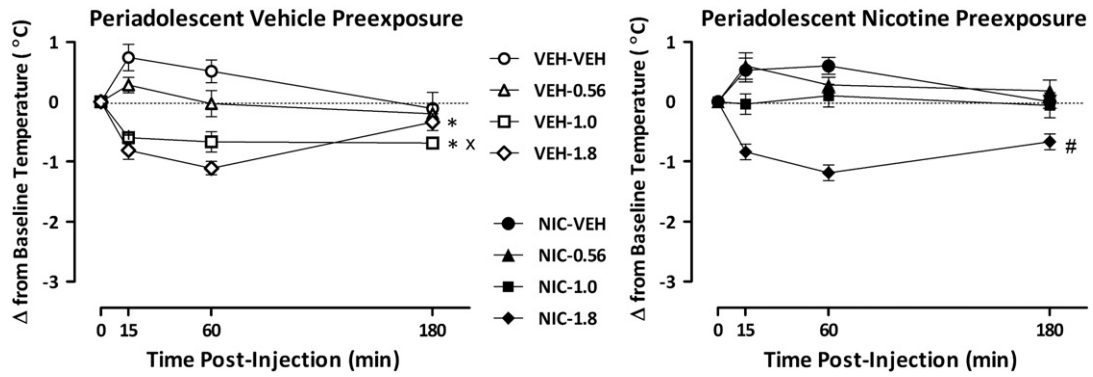
### 3.2. Results

#### 3.2.1. Conditioned Taste Aversions

**3.2.1.1. Acquisition.** The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  4 (Conditioning Trial) mixed-model ANOVA with repeated



**Fig. 2.** Mean ( $\pm$  SEM) percent saccharin preference on the first two-bottle test following conditioning with various doses of EtOH (VEH, 0.56, 1.0 or 1.8 g/kg) in animals preexposed during periadolescence to either VEH (white) or NIC (black). There were no effects of preexposure on the two-bottle test, but saccharin preferences dose-dependently decreased as the dose of EtOH increases. \* indicates significant differences,  $p < 0.05$ .



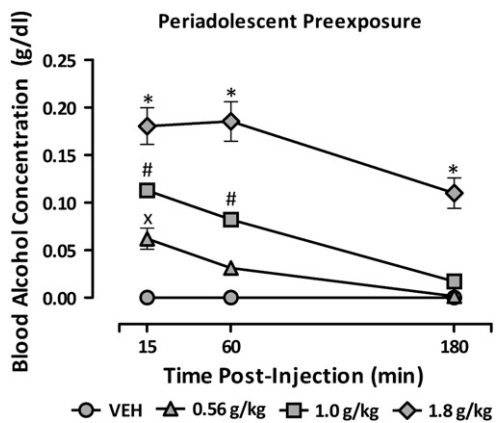
**Fig. 3.** Mean ( $\pm$ SEM) change from baseline temperatures across time for animals preexposed during periadolescence to either vehicle (white) or nicotine (black) and injected with various doses of EtOH (VEH, 0.56, 1.0 or 1.8 g/kg). There were no significant interactions with Time, thus all differences indicated on the graph are between groups when averaged across time points. \* indicates that animals in Group VEH-1.0 and VEH-1.8 had significantly greater changes in temperature compared to animals in Groups VEH-VEH and VEH-0.56. # indicates that animals in Group NIC-1.8 had significantly greater changes in temperature compared to animals in all other NIC preexposed groups.  $\times$  indicates a significant difference between animals in Groups NIC-1.0 and VEH-1.0.  $p < 0.05$  for all significant differences.

measures revealed significant main effects of Preexposure Drug ( $F(1, 56) = 9.478, p = 0.003$ ), Conditioning Drug ( $F(3, 56) = 54.155, p = 0.000$ ) and Conditioning Trial ( $F(3, 168) = 2.712, p = 0.047$ ) as well as significant Preexposure Drug  $\times$  Conditioning Trial ( $F(3, 168) = 8.520, p = 0.000$ ), Conditioning Drug  $\times$  Conditioning Trial ( $F(9, 168) = 37.705, p = 0.000$ ), Preexposure Drug  $\times$  Conditioning Drug ( $F(3, 56) = 5.467, p = 0.002$ ) and Preexposure Drug  $\times$  Conditioning Drug  $\times$  Conditioning Trial ( $F(9, 168) = 2.339, p = 0.017$ ) interactions.

Tukey HSD post-hoc analyses revealed that at no point did Group VEH-VEH differ from Group VEH-0.56; however, vehicle-preexposed animals conditioned with 1.0 and 1.8 g/kg EtOH (Groups VEH-1.0 and VEH-1.8) developed significant aversions compared to controls conditioned with VEH by Trial 2. Additionally on Trial 2, animals in Group VEH-1.8 drank significantly less saccharin than animals in Groups VEH-0.56 and VEH-1.0. By Trials 3 and 4, Groups VEH-1.0 and VEH-1.8 consumed significantly less saccharin than Groups VEH-VEH and VEH-0.56. Further, Group VEH-1.8 drank significantly less saccharin than VEH-1.0 (see Fig. 5). Post-hoc analyses revealed that among animals preexposed to nicotine during adulthood, those conditioned with either 0.56 or 1.0 g/kg (Groups NIC-0.56 and NIC-1.0) never differed from the VEH-conditioned controls (Group NIC-VEH). The only group of nicotine-preexposed animals to develop significant aversions to the EtOH-associated saccharin solution was Group NIC-1.8 (on Trials 2 through 4). Specifically, Group NIC-1.8

drank significantly less saccharin than all other groups (Groups NIC-0.0, NIC-0.56 and NIC-1.0) on those trials (see Fig. 5). Comparisons between preexposure conditions at each dose of EtOH revealed significant differences at the highest dose of EtOH (1.8 g/kg). Specifically, on Trials 2, 3 and 4 animals preexposed with vehicle and conditioned with 1.8 g/kg EtOH (Group VEH-1.8) drank significantly less saccharin than those preexposed with nicotine (Group NIC-1.8). These differences are indicative of an attenuated aversion to 1.8 g/kg EtOH in the NIC preexposed animals.

The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug) ANOVA performed on the first two-bottle preference test (first day of extinction) revealed no main effect of Preexposure Drug and no Preexposure Drug  $\times$  Conditioning Drug interaction. There was, however, a significant main effect of Conditioning Drug ( $F(3, 56) = 64.767, p = 0.000$ ), indicative of dose-dependent differences in saccharin preference. Specifically, animals conditioned with 0.56 g/kg EtOH did not differ from VEH-injected animals in their preference for saccharin, but animals conditioned with 1.0 and 1.8 g/kg EtOH differed from one another and both had significantly lower preferences for saccharin than the VEH and 0.56 g/kg EtOH-injected animals (see Fig. 6).

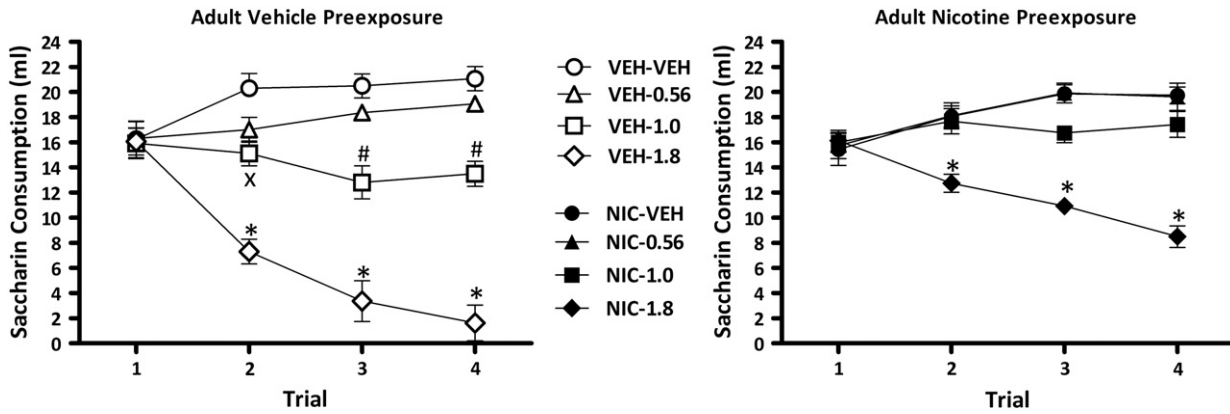


**Fig. 4.** Mean ( $\pm$ SEM) blood alcohol concentration (g/dl) at various time points post-EtOH (VEH, 0.56, 1.0 or 1.8 g/kg). Since there was no effect of preexposure drug, data were collapsed across preexposure condition and reflect the average BAC for all animals at each dose of the conditioning drug (EtOH). \* indicates that 1.8 g/kg is significantly different from all other doses. # indicates that 1.0 g/kg is significantly different from all other doses.  $\times$  indicates that 0.56 g/kg is significantly different from all other doses.  $p < 0.05$  for all significant differences.

**3.2.1.2. Extinction.** One animal was lost during extinction conditioning, and its data was removed from further analysis. The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  8 (Extinction Trial) mixed-model ANOVA with repeated measures revealed significant main effects of Preexposure Drug ( $F(1, 55) = 4.332, p = 0.042$ ), Conditioning Drug ( $F(3, 55) = 99.420, p = 0.000$ ) and Extinction Trial ( $F(7, 385) = 8.506, p = 0.000$ ) as well as significant Conditioning Drug  $\times$  Extinction Trial ( $F(21, 385) = 2.008, p = 0.006$ ) and Preexposure Drug  $\times$  Conditioning Drug ( $F(3, 55) = 5.187, p = 0.003$ ) interactions. There was no Preexposure Drug  $\times$  Conditioning Drug  $\times$  Extinction Trial interaction ( $F(21, 385) = 0.526, p = 0.960$ ). The Conditioning Drug  $\times$  Extinction Trial interaction revealed that, when collapsed across preexposure condition, animals conditioned with EtOH extinguished the conditioned aversion in a dose-dependent manner (i.e., as the dose of EtOH increased, the rate of extinction slowed). Examining the Preexposure Drug  $\times$  Conditioning Drug interaction revealed that, collapsed across trials, animals preexposed with nicotine in early adulthood and conditioned with 1.0 g/kg EtOH had an overall faster rate of extinction of the aversion to the saccharin solution (data not shown).

**3.2.2. EtOH-induced hypothermia**

Core body temperatures ( $^{\circ}\text{C}$ ) were analyzed as a change from baseline for each animal. The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  4 (Time) mixed-model ANOVA with repeated measures revealed main effects of Preexposure Drug ( $F(1, 55) = 17.679, p = 0.000$ ), Conditioning Drug ( $F(3, 55) = 30.294, p = 0.000$ ) and



**Fig. 5.** Mean ( $\pm$ SEM) saccharin consumption (ml) following conditioning with various doses of EtOH in animals preexposed during early adulthood to either VEH (left panel) or NIC (right panel). In the figure legend, preexposure condition is listed first (VEH or NIC) and dose of EtOH is listed second (VEH, 0.56, 1.0 or 1.8 g/kg). Within each preexposure condition, \* indicates that animals conditioned with 1.8 g/kg drank significantly less saccharin than all other doses (VEH, 0.56 and 1.0 g/kg). # indicates that animals conditioned with 1.0 g/kg drank significantly less saccharin than those conditioned with VEH. # indicates that animals conditioned with 1.0 g/kg drank significantly less than those conditioned with VEH and 0.56 g/kg.  $p < 0.05$  for all significant differences.

Time ( $F(3, 165) = 85.855, p = 0.000$ ). Unlike the periadolescent preexposed animals, there were significant Preexposure Drug  $\times$  Time ( $F(3, 165) = 13.128, p = 0.000$ ), Conditioning Drug  $\times$  Time ( $F(9, 165) = 18.610, p = 0.000$ ), Preexposure Drug  $\times$  Conditioning Drug ( $F(3, 55) = 3.325, p = 0.026$ ) and Preexposure Drug  $\times$  Conditioning Drug  $\times$  Time ( $F(9, 165) = 3.343, p = 0.001$ ) interactions with adult preexposure.

Post-hoc analyses revealed that among animals that were preexposed with vehicle, those injected with VEH and 0.56 g/kg EtOH (Groups VEH-VEH and VEH-0.56) never differed from each other. However, those injected with 1.8 g/kg EtOH (Group VEH-1.8) had significantly greater decreases in rectal temperatures than all other vehicle-preexposed groups at 15, 60 and 180 min post-injection. As well, Group VEH-1.0 showed significantly greater decreases in rectal temperature than Group VEH-VEH at 15 min, but those differences resolved by 60 and 180 min post-injection. Among animals preexposed with nicotine, those injected with VEH, 0.56 and 1.0 g/kg (Groups NIC-VEH, NIC-0.56 and NIC-1.0) never differed from each other at any time point, and only those injected with 1.8 g/kg (Group NIC-1.8) ever had significantly decreased rectal body temperatures. Specifically, at 15 min post-injection Group NIC-1.8

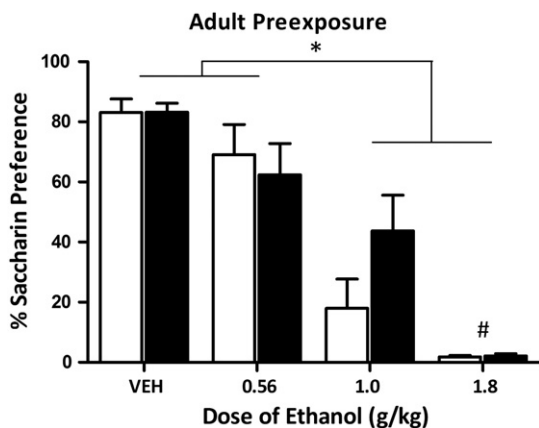
showed significant decreases in rectal body temperature than both the VEH and 0.56 g/kg EtOH-injected animals (Groups NIC-VEH and NIC-0.56). By 60 min post-injection, Group NIC-1.8 had significantly greater decreases in rectal body temperatures compared to all other groups (Groups NIC-VEH, NIC-0.56, and NIC-1.0). By 180 min post-injection, all temperature differences had resolved and all nicotine-preexposed groups were functionally equivalent (see Fig. 7). Direct comparisons between preexposure conditions revealed differences only among those animals injected with 1.8 g/kg EtOH. Specifically, at 60 and 180 min post-injection animals preexposed with vehicle had a greater hyperthermic response to EtOH than those preexposed with nicotine.

### 3.2.3. Blood alcohol concentrations

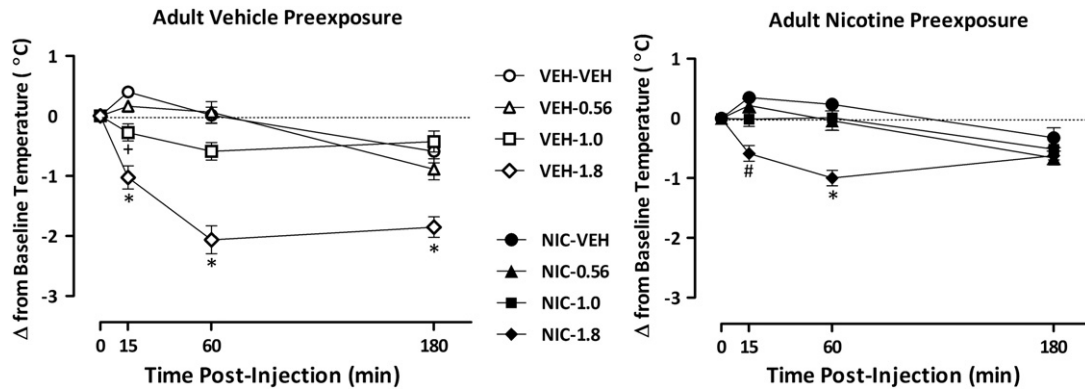
During the processing of the plasma samples for BAC analysis, 4 samples were unable to be processed (either due to contamination or not enough sample volume), and thus excluded from analysis. Similar to the periadolescent preexposed animals, the 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug)  $\times$  3 (Time) mixed-model ANOVA with repeated measures performed on adult preexposed animals revealed main effects of Conditioning Drug ( $F(3, 51) = 109.948, p = 0.000$ ) and Time ( $F(2, 102) = 55.197, p = 0.000$ ) and a Conditioning Drug  $\times$  Time interaction ( $F(6, 102) = 9.301, p = 0.000$ ), but did not reveal any main or interaction effects of Preexposure Drug. To explore the Conditioning Drug  $\times$  Time interaction, data were collapsed such that Preexposure Drug was removed as a factor. Post-hoc analyses of these data revealed that at 15 min post-injection, all animals injected with 1.8 g/kg EtOH achieved BACs significantly higher than all other groups and animals injected with 1.0 g/kg EtOH achieved BACs significantly higher than animals injected with either 0.56 g/kg or VEH. Sixty min after injections, animals injected with 1.8 g/kg EtOH still had BACs higher than all other groups and animals injected with 1.0 g/kg EtOH still had BACs higher than those injected with 0.56 g/kg and VEH. By 180 min after injections, only the animals injected with 1.8 g/kg EtOH still had significantly elevated BACs compared to all other groups (see Fig. 8).

### 3.2.4. Locomotor activity

The 2 (Preexposure Drug)  $\times$  4 (Conditioning Drug) ANOVA revealed main effects of both Preexposure Drug ( $F(1, 55) = 5.607, p = 0.021$ ) and Conditioning Drug ( $F(3, 55) = 25.414, p = 0.000$ ), but failed to reveal an interaction between the two ( $F(3, 55) = 0.859, p = 0.468$ ). Thus, similar to adolescent preexposed animals, preexposure with nicotine in early adulthood increased locomotor activity compared to vehicle-preexposed animals when collapsed across



**Fig. 6.** Mean ( $\pm$ SEM) percent saccharin preference on the first two-bottle test following conditioning with various doses of EtOH (VEH, 0.56, 1.0 or 1.8 g/kg) in animals preexposed during early adulthood to either VEH (white) or NIC (black). There were no effects of preexposure on the two-bottle test, but there was a dose-dependent decrease in saccharin preference. \* indicates the animals conditioned with both 1.0 and 1.8 g/kg had significantly lower preferences for saccharin than those conditioned with both VEH and 0.56 g/kg. # indicates that animals conditioned with 1.8 g/kg had a significantly lower preference than those conditioned with 1.0 g/kg.  $p < 0.05$  for all significant differences.



**Fig. 7.** Mean ( $\pm$ SEM) change from baseline temperature across time for animals preexposed during early adulthood to either vehicle (left panel) or nicotine (right panel) and injected with various doses of EtOH (VEH, 0.56, 1.0 or 1.8 g/kg). In the figure legend, preexposure condition is listed first (VEH or NIC) and dose of EtOH is listed second. Within each preexposure condition, \* indicates significant differences compared to all other groups. † indicates significant difference compared to VEH controls. # indicates significant differences compared to VEH controls and animals injected with 0.56 g/kg.  $p < 0.05$  for all significant differences.

doses of EtOH. As well, EtOH injections produced dose-dependent decreases in locomotor activity independent of preexposure condition. Specifically, animals injected with 1.8 g/kg showed greater locomotor suppression than those injected at all other doses, and animals injected with 0.56 or 1.0 g/kg both showed greater suppression of locomotor activity compared to VEH, but these doses did not differ from each other (data not shown).

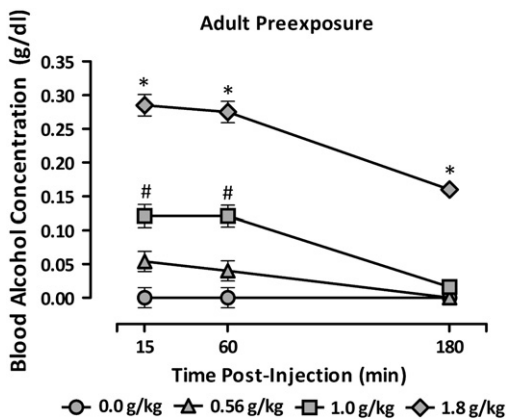
**4. Discussion**

The present experiments were conducted to determine whether nicotine exposure during periadolescence could impact the aversive effects of alcohol later in adulthood. The ability of nicotine exposure during this developmentally sensitive period to alter the aversive effects of alcohol could have important implications for the overall acceptability (i.e., abuse potential) of alcohol in adulthood, given that the overall affective value of a drug is thought to be limited by its aversive effects (Brockwell et al., 1991; Davis and Riley, 2010; Riley and Simpson, 2001; Stolerman and D’Mello, 1981; Wise et al., 1976). Indeed, preexposure to nicotine during periadolescence attenuated the acquisition of taste aversions induced by alcohol in adulthood (compared to vehicle-preexposed controls), i.e., animals preexposed with nicotine and conditioned with 1.0 g/kg drank significantly more saccharin solution than their vehicle-preexposed counterparts (Group NIC-1.0 vs. VEH-1.0). In addition, periadolescent animals preexposed

with nicotine and conditioned with 1.0 g/kg EtOH did not acquire a significant taste aversion (i.e., did not differ from their nicotine-preexposed VEH-conditioned controls) until Trial 3, whereas periadolescent vehicle-preexposed animals showed clear reductions in saccharin consumption compared to their controls by Trial 2. These findings are consistent with several other assessments reporting the attenuating effects of drug exposure during periadolescence on aversion learning in adulthood (see Diaz-Granados and Graham, 2007; Graham and Diaz-Granados, 2006; Hutchison et al., 2010). Although nicotine preexposure was sufficient to attenuate the acquisition of the aversion to alcohol, it did not impact the overall preference for saccharin in the two-bottle test nor did it impact the extinction (data not shown) of the EtOH-induced aversions.

Interestingly, the attenuation of the acquisition of the EtOH-induced CTA by nicotine preexposure does not appear to be unique to the developmental period during which nicotine is administered, as animals preexposed with nicotine as adults also displayed attenuated EtOH-induced CTAs (1.8 g/kg dose; see Experiment 2). Similar to the assessment in periadolescent preexposed animals, animals preexposed to nicotine as adults showed attenuated aversions to alcohol later in adulthood. Specifically, adult nicotine-preexposed animals conditioned with 1.8 g/kg EtOH consumed more saccharin when compared to the adult vehicle-preexposed animals conditioned with 1.8 g/kg EtOH (Group NIC-1.8 vs. VEH-1.8), indicative of an attenuated aversion. Additionally, of the adult nicotine-preexposed animals, only those conditioned with 1.8 g/kg EtOH (NIC-1.8) ever developed significant aversions (differed from their nicotine-preexposed VEH-conditioned controls). Conversely, among adult animals preexposed with vehicle those conditioned with 1.0 and 1.8 g/kg EtOH (Groups VEH-1.0 and VEH-1.8) both showed significant reductions in saccharin consumption compared to their vehicle-preexposed VEH-conditioned controls (Group VEH-VEH) on Trials 2 through 4. Thus, aversions were acquired at a faster rate and at more doses of EtOH in vehicle-preexposed animals than in nicotine-preexposed animals.

Similar to the adolescent preexposed animals, preexposure effects were not evident on the two-bottle test in animals preexposed during adulthood. The two-bottle test is thought to be a more sensitive measure of a CTA, allowing for the detection of delayed acquisition of conditioned effects not evident in one-bottle tests (Grote and Brown, 1971). It was utilized in the present set of experiments to determine if there were any preexposure effects at the lowest dose of EtOH that might not have been detected by the one-bottle tests in acquisition. The absence of preexposure effects in the two-bottle preference test is not surprising given this greater sensitivity, i.e., such tests detect any degree of aversions and group differences are often not seen as all subjects display significant aversions (see Batsell and Best, 1993; for



**Fig. 8.** Mean ( $\pm$ SEM) blood alcohol concentration (g/dl) at various time points post-EtOH (VEH, 0.56, 1.0 or 1.8 g/kg). Since there was no effect of preexposure drug, data were collapsed and reflect the average BAC for all animals at each dose of the conditioning drug (EtOH). \* indicates that 1.8 g/kg is significantly different from all other doses. # indicates that 1.0 g/kg is significantly different from all other doses.  $p < 0.05$  for all significant differences.

such effects in other preexposure CTA preparations, see Kunin et al., 2000; Palmatier and Bevins, 2001).

The specific mechanism by which nicotine exposure, in periadolescence or early adulthood, attenuated the aversive effects of alcohol is not known, although several possibilities exist. One possible account of the attenuated aversions reported here is that nicotine exposure altered the pharmacokinetics of alcohol in adulthood, e.g., exposure to nicotine may have increased hepatic enzyme levels or increased the distribution volume for alcohol, resulting in decreases in the blood alcohol concentration (BAC) which might reduce its overall aversive effect. In this context, it has been demonstrated that concurrent *intra-gastric* (but not *intra-peritoneal*) administration of nicotine and alcohol can result in decreased BACs (Parnell et al., 2006). Similarly, the present study found no impact of nicotine preexposure on BACs when alcohol was administered by IP injection, suggesting that changes in BACs unlikely mediated the weaker alcohol-induced aversions reported in the present study. Changes in metabolic rates and volume distribution, however, were not directly assessed and, thus, cannot be ruled out. Furthermore, blood alcohol levels do not necessarily correspond to, and are generally lower than, brain alcohol levels (Crippens et al., 1999; Robinson et al., 2002; Smolen and Smolen, 1989). Additionally, nicotine pretreatment is capable of reducing brain and cerebrospinal fluid concentrations of alcohol which could potentially reduce the aversive effects of alcohol (Hisaoka and Levy, 1985). It is important to note in this context, however, that any differences in brain concentrations would not likely impact the differences reported here given that only peripheral, and not central, administration of alcohol has been shown capable of inducing a CTA (Amit et al., 1977; Brown et al., 1978).

It is clear that the ability of nicotine preexposure to alter the aversive effects of alcohol did not extend to other behavioral effects, specifically locomotor activity. As described, nicotine preexposure during either periadolescence or early adulthood did not alter the locomotor suppression induced by alcohol administration compared to the vehicle-preexposed controls. It has been shown that in acute preparations nicotine is capable of preventing decreased locomotor activity in response to oral alcohol administration in adolescent and adult rats (Lallemant et al., 2009); however, no such effects were seen in the current study with the long delay between nicotine administration and the locomotor assessment with alcohol. Thus, the long-term changes in ethanol-induced taste aversions after nicotine preexposure reported here appear to be specific to aversion learning, at least under the current parameters, and not likely a function of a generalized deficit in behavioral expression.

It is possible that preexposure to nicotine had a general debilitating effect on learning and not an effect specific to aversion conditioning with alcohol. Other preparations, e.g., cued fear conditioning, which relies on the learned association between a conditioned stimulus (CS) and an unconditioned stimulus (US) utilizing processes presumably similar to those involved in taste aversion learning, have been shown to be unaffected by nicotine administration, whether acute, chronic or during withdrawal (Davis et al., 2005). On the other hand, nicotine has been shown to enhance associative learning processes (as evidenced by increased freezing behavior) in contextual fear conditioning models, while acute withdrawal from chronic nicotine has been shown to impair this learning, an effect that is lost after 2 weeks of abstinence from nicotine (Andre et al., 2008; Davis et al., 2005; Kenney and Gould, 2008b; Portugal et al., 2008; Tian et al., 2008). Thus, nicotine's impact on contextual conditioning appears to be an acute effect limited to instances when nicotine is on board or when the animal is going through withdrawal during training (Kenney and Gould, 2008a). In this context, nicotine's limited ability to impact associative learning processes does not likely account for the changes seen in the present study, given that greater than 6 weeks elapsed between nicotine exposure and taste aversion conditioning with alcohol, i.e., a time period in which nicotine would no longer be on

board and past the period expected to be associated with nicotine withdrawal (Davis et al., 2005). Further, if there was a general impairment of nicotine on associative learning processes in taste aversion conditioning, then one would expect nicotine preexposure to impact the acquisition of taste aversion learning universally, particularly under similar parametric conditions (i.e., chronic nicotine preexposure preceding conditioning by greater than 6 weeks). As discussed previously, however, this is not the case (see Hutchison and Riley, 2008 for a description of the failure of nicotine preexposure to attenuate the acquisition of cocaine-induced aversions).

The fact that a history with nicotine attenuated ethanol-induced taste aversions is consistent with a large literature examining the effects of drug preexposure on taste aversion learning (Barker and Johns, 1978; Berman and Cannon, 1974; Cannon et al., 1975; Dacanay et al., 1984; Davis and Riley, 2007; Iwamoto and Williamson, 1984; for a review see Riley and Simpson, 2001). Although generally reported when the preexposed and conditioning drug are the same, such attenuating effects of drug preexposure have also been reported when the drugs are different, i.e., the cross-drug preexposure effect (Aragon et al., 1986; Berendsen and Broekkamp, 1994; Gommans et al., 1998; Palmatier and Bevins, 2001). Although the mechanism underlying the US preexposure effect (either with the same or different drugs) is not fully characterized, for some drugs, e.g., alcohol, nicotine and morphine, it is thought that changes to the drug during preexposure impact the effects of that (or a different drug) at conditioning, weakening its ability to induce aversions (Barker and Johns, 1978; Berman and Cannon, 1974; Hunt et al., 1985; Iwamoto and Williamson, 1984; Simpson and Riley, 2005). The impact of preexposure in the cross-drug design is generally thought to be the result of the development of tolerance to common aversive effects shared by the drugs, presumably due to some common mechanism of action (Riley and Simpson, 2001; Serafine and Riley, 2009, 2010). It is interesting to note that the cross-drug preexposure effect has been previously reported with nicotine and alcohol (Kunin et al., 1999). Specifically, proximal (3 days immediately preceding taste aversion conditioning) nicotine preexposure during adulthood was shown to be capable of abolishing alcohol-induced taste aversions. While the present data further extend this phenomenon to include adolescent nicotine exposure, the present study is fundamentally different in that the delay between preexposure and conditioning far exceeds that which is typically used in such assessments (Barker and Johns, 1978; Cappell and LeBlanc, 1977; Cappell et al., 1975).

Although the present data are consistent with other reports examining the effects of nicotine history on alcohol aversion learning (see above), it is not clear how nicotine is producing this attenuating effect. This is due in part to the fact that there is no clear consensus on the nature of aversion learning in general [e.g., novelty (Domjan and Gillan, 1976; Kalat, 1974; Hunt and Amit, 1987), toxicity (Riley and Tuck, 1985), reward (Grigson, 1997)] or aversion learning specifically with alcohol. In relation to alcohol-induced aversions, several mechanisms have been proposed (see Baker and Cannon, 1982; Elkins et al., 2000; Orr et al., 1993), but the one that has received the most attention is alcohol-induced hypothermia (see Cunningham et al., 1988). Specifically, Cunningham and his colleagues have reported a direct relationship between the strength of aversions induced by alcohol and the degree of alcohol-induced hypothermia, i.e., the greater the alcohol-induced decrease in body temperature, the greater the strength of the aversion induced by alcohol. Consistent with this idea, the concurrent administration of nicotine and alcohol to naïve animals results in a significant decrease in rectal temperature and an increase in alcohol-induced taste aversions (Rinker et al., 2008). Although the present findings show that a history of nicotine during both periadolescence and early adulthood attenuates both alcohol-induced hypothermia and alcohol-induced taste aversions, the relationship was not absolute. Specifically, adolescent VEH-1.0 and VEH-1.8 animals showed dose-dependent differences in the degree of the CTA induced by EtOH, but



had similar overall reductions in body temperature. Similarly, adult VEH–1.0 animals developed significant aversions compared to VEH–VEH controls, but did not display consistently reduced body temperatures to the same dose of EtOH in the hypothermia assessment (see also Roma et al., 2006 who reported a lack of a role of hypothermia in alcohol's aversive effects in inbred LEW and F344 rats). Thus, although hypothermia has been presented as a possible basis for alcohol-induced aversion learning, its general role in aversion learning with alcohol, as well as its mediation of the changes reported here, remains unclear.

The issue now becomes by what mechanism nicotine induces such a change in the aversive effects of alcohol. Although the present study did not address this issue, long lasting changes in the nicotinic cholinergic receptor (nAChR) system might explain the decreased ability of alcohol to condition a taste aversion in the present study. A number of studies have demonstrated, *in vitro and in vivo*, that some of the physiological and behavioral effects of alcohol are modulated by nAChRs. For example, alcohol in concentrations of 100 mM or less (concentrations with corresponding levels considered legally intoxicating) potentiate endogenous acetylcholine activity at a number of nicotinic receptor subtypes, including  $\alpha_4\beta_2$ ,  $\alpha_2\beta_4$ ,  $\alpha_4\beta_4$ , and  $\alpha_2\beta_2$  (Harris, 1999). Additionally, nAChRs have been shown to mediate (in part) alcohol-induced dopamine overflow in the nucleus accumbens and have been implicated in the initial reinforcing effects of alcohol. The nonspecific nicotinic antagonist, mecamylamine, infused into the ventral tegmental area (VTA) blocks systemic alcohol-induced increases in accumbal dopamine and attenuates alcohol drinking in rats (Blomqvist et al., 1997; Ericson et al., 1998; Soderpalm et al., 2000). Thus, nicotinic receptors are involved in a number of alcohol's effects, including some of its affective properties. How it might be involved in changes to its aversive effects (via hypothermia or some other system) remains to be determined and suggests that assessing areas of the brain involved in the aversive effects of alcohol, e.g., area postrema, nucleus tractus solitarius, may provide insight into such mechanisms (see Bermúdez-Rattoni and Yamamoto, 1998; Grabus et al., 2004; Reilly, 2009; Sakai and Yamamoto, 1997).

Independent of the basis for the attenuating effects of nicotine exposure during periadolescence (and early adulthood) on alcohol-induced taste aversions, such attenuation could have important implications for the initiation and escalation of alcohol use. Specifically, nicotine exposure could reduce alcohol's aversive effects and influence its overall affective value, increasing its abuse potential. Although possible, there are several caveats to this position. First, the literature examining the effects of nicotine history on alcohol self-administration is inconsistent. For example, Tsui et al. (2001) demonstrated that nicotine during adolescence, using a similar preexposure preparation as the present study, enhanced the acquisition of alcohol consumption in adulthood. However, other studies have demonstrated that continuous nicotine delivered via mini-osmotic pumps during periadolescence does not alter voluntary alcohol consumption immediately subsequent to nicotine exposure (Smith et al., 2002), nor does nicotine preexposure during periadolescence alter alcohol consumption in alcohol-preferring (AA) rats (Kemppainen et al., 2009). Parametric differences (e.g., length of nicotine preexposure, dose of nicotine, route of administration, and strain of rat) preclude any conclusions regarding the influence of alterations in the aversive effects of alcohol after periadolescent nicotine preexposure on alcohol consumption. It is clear that until this relationship is explored under similar parametric conditions to the present study, the effects of nicotine exposure on alcohol self-administration remain unknown. A second caveat is related to the specificity of the effects of nicotine preexposure. As described, both adolescent and adult history with nicotine impacted alcohol-induced aversions. While questioning the specificity of such effects (in terms of the timing of nicotine preexposure), it should be noted that because the majority of smokers initiate their habit during adolescence (CDC, 2008) and rodent models of reward show that adolescents find nicotine more rewarding (Belluzzi et al., 2004; Brielmaier et al., 2007; Chen et al., 2007;

Levin et al., 2007) and less aversive (Shram et al., 2006), adolescents may be the more vulnerable population in initiating use of nicotine which would increase the likelihood of it impacting subsequent alcohol use. Finally, the present work was limited to male subjects. It is important that the effects of nicotine history be examined in females to assess whether such effects are limited to males or generalize to female subjects (for a discussion of this issue, see Wetherington, 2010). How such exposure impacts the aversive and rewarding effects in both sexes is important to understanding any general changes in abuse vulnerability.

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## References

- Adriani W, Deroche-Gamonet V, Le Moal M, Laviola G, Piazza PV. Preexposure during or following adolescence differently affects nicotine-rewarding properties in adult rats. *Psychopharmacology (Berl)* 2006;184:382–90.
- Amit Z, Levitan DE, Brown ZW, Rogan F. Possible involvement of central factors in the mediation of conditioned taste aversion. *Neuropharmacology* 1977;16:121–4.
- Andre JM, Gulick D, Portugal GS, Gould TJ. Nicotine withdrawal disrupts both foreground and background contextual fear conditioning but not pre-pulse inhibition of the acoustic startle response in C57BL/6 mice. *Behav Brain Res* 2008;190:174–81.
- Aragon CM, Abitbol M, Amit Z. Acetaldehyde may mediate reinforcement and aversion produced by ethanol. An examination using a conditioned taste-aversion paradigm. *Neuropharmacology* 1986;25:79–83.
- Baker TB, Cannon DS. Alcohol and taste-mediated learning. *Addict Behav* 1982;7:211–30.
- Barker LM, Johns T. Effect of ethanol preexposure on ethanol-induced conditioned taste aversion. *J Stud Alcohol* 1978;39:39–46.
- Batsell WR, Best MR. One bottle too many—method of testing determines the detection of overshadowing and retention of taste-aversions. *Anim Learn Behav* 1993;21:154–8.
- Belluzzi JD, Lee AG, Oliff HS, Leslie FM. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology (Berl)* 2004;174:389–95.
- Berendsen HH, Broekkamp CL. Comparison of stimulus properties of fluoxetine and 5-HT receptor agonists in a conditioned taste aversion procedure. *Eur J Pharmacol* 1994;253:83–9.
- Berman RF, Cannon DS. The effect of prior ethanol experience on ethanol-induced saccharin aversions. *Physiol Behav* 1974;12:1041–4.
- Bermúdez-Rattoni F, Yamamoto T. Neuroanatomy of conditioned taste aversion. Lesion studies. In: Bureš J, Bermúdez-Rattoni F, Yamamoto T, editors. *Conditioned taste aversion: memory of a special kind*. Oxford, UK: Oxford University Press; 1998.
- Blomqvist O, Ericson M, Engel JA, Soderpalm B. Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. *Eur J Pharmacol* 1997;334:149–56.
- 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;29:74–80.
- Brockwell NT, Eikelboom R, Beninger RJ. Caffeine-induced place and taste conditioning: production of dose-dependent preference and aversion. *Pharmacol Biochem Behav* 1991;38:513–7.
- Brown ZW, Amit Z, Smith B, Rockman GE. Differential effects on conditioned taste aversion learning with peripherally and centrally administered acetaldehyde. *Neuropharmacology* 1978;17:931–5.
- 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;1:270–84.
- Cappell H, LeBlanc AE. Parametric investigations of the effects of prior exposure to amphetamine and morphine on conditioned gustatory aversion. *Psychopharmacology (Berl)* 1977;51:265–71.
- Cappell H, LeBlanc AE, Herling S. Modification of the punishing effects of psychoactive drugs in rats by previous drug experience. *J Comp Physiol Psychol* 1975;89:347–56.
- CDC. Preventing tobacco use. Preventing chronic diseases: investing wisely in health; 2008. Atlanta, GA.
- Chen J, Millar WJ. Age of smoking initiation: implications for quitting. *Health Rep* 1998;9:39–46 (Eng); 39–48 (Fre).
- Chen H, Matta SG, Sharp BM. Acquisition of nicotine self-administration in adolescent rats given prolonged access to the drug. *Neuropsychopharmacology* 2007;32:700–9.

- Crippens D, White ML, George MA, Jaworski JN, Brunner LJ, Lancaster FE, et al. Gender differences in blood levels, but not brain levels, of ethanol in rats. *Alcohol Clin Exp Res* 1999;23:414–20.
- Cunningham CL, Hawks DM, Niehus DR. Role of hypothermia in ethanol-induced conditioned taste aversion. *Psychopharmacology (Berl)* 1988;95:318–22.
- Dacanay RJ, Mastropaolo JP, Olin DA, Riley AL. Sex differences in taste aversion learning: an analysis of the minimal effective dose. *Neurobehav Toxicol Teratol* 1984;6:9–11.
- Davis CM, Riley AL. The effects of cocaine preexposure on cocaine-induced taste aversion learning in Fischer and Lewis rat strains. *Pharmacol Biochem Behav* 2007;87:198–202.
- Davis CM, Riley AL. Conditioned taste aversion learning: implications for animal models of drug abuse. *Ann NY Acad Sci* 2010;1187:247–75.
- Davis JA, James JR, Siegel SJ, Gould TJ. Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. *J Neurosci* 2005;25:8708–13.
- 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;31:2020–7.
- DiFranza JR, Guerreria MP. Alcoholism and smoking. *J Stud Alcohol* 1990;51:130–5.
- Domjan M, Gillan D. Role of novelty in the aversion for increasingly concentrated saccharin solutions. *Physiol Behav* 1976;16:537–42.
- Elkins RL, Orr TE, Li JQ, Walters PA, Whitford JL, Carl GF, et al. Serotonin reuptake is less efficient in taste aversion resistant than in taste aversion-prone rats. *Pharmacol Biochem Behav* 2000;66:609–14.
- Ericson M, Blomqvist O, Engel JA, Soderpalm B. Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur J Pharmacol* 1998;358:189–96.
- Garcia J, Ervin FR. Appetites, aversions, and additions: a model for visceral memory. *Recent Adv Biol Psychiatry* 1968;10:284–93.
- Gommans J, Bouwknecht JA, Hijzen TH, Berendsen HH, Broekkamp CL, Maes RA, et al. Stimulus properties of fluvoxamine in a conditioned taste aversion procedure. *Psychopharmacology (Berl)* 1998;140:496–502.
- Grabus SD, Glowa JR, Riley AL. Morphine- and cocaine-induced c-Fos levels in Lewis and Fischer rat strains. *Brain Res* 2004;998:20–8.
- Graham DL, Diaz-Granados JL. Periadolescent exposure to ethanol and diazepam alters the aversive properties of ethanol in adult mice. *Pharmacol Biochem Behav* 2006;84:406–14.
- Grant BF. Age at smoking onset and its association with alcohol consumption and DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse* 1998;10:59–73.
- Grisson PS. Conditioned taste aversions and drugs of abuse: a reinterpretation. *Behav Neurosci* 1997;111:129–36.
- Grote FW, Brown RT. Conditioned taste aversions: two-stimulus tests are more sensitive than one-stimulus tests. *Behav Res Meth Instrum* 1971;3:311–2.
- Gruzca RA, Bierut LJ. Cigarette smoking and the risk for alcohol use disorders among adolescent drinkers. *Alcohol Clin Exp Res* 2006;30:2046–54.
- Hanna EZ, Grant BF. Parallels to early onset alcohol use in the relationship of early onset smoking with drug use and DSM-IV drug and depressive disorders: findings from the National Longitudinal Epidemiologic Survey. *Alcohol Clin Exp Res* 1999;23:513–22.
- Harris RA. Ethanol actions on multiple ion channels: which are important? *Alcohol Clin Exp Res* 1999;23:1563–70.
- Hisaoka M, Levy G. Kinetics of drug action in disease states XI: effect of nicotine on the pharmacodynamics and pharmacokinetics of phenobarbital and ethanol in rats. *J Pharm Sci* 1985;74:412–5.
- Hunt T, Amit Z. Conditioned taste aversion induced by self-administered drugs: paradox revisited. *Neurosci Biobehav Rev* 1987;11:107–30.
- Hunt T, Spivak K, Amit Z. Aversive stimulus properties of morphine: evaluation using the drug preexposure conditioned taste aversion paradigm. *Behav Neural Biol* 1985;44:60–73.
- Hutchison MA, Riley AL. Adolescent exposure to nicotine alters the aversive effects of cocaine in adult rats. *Neurotoxicol Teratol* 2008;30:404–11.
- Hutchison MA, Albaugh DL, Riley AL. Exposure to alcohol during adolescence alters the aversive and locomotor-activating effects of cocaine in adult rats. *Pharmacol Biochem Behav* 2010;97:370–6.
- Iwamoto ET, Williamson EC. Nicotine-induced taste aversion: characterization and preexposure effects in rats. *Pharmacol Biochem Behav* 1984;21:527–32.
- James-Walke NL, Williams HL, Taylor DA, McMillen BA. Periadolescent nicotine exposure produces sensitization to reinforcement by diazepam in the rat. *Neurotoxicol Teratol* 2007;29:31–6.
- Kalat JW. Taste salience depends on novelty, not concentration, in taste-aversion learning in the rat. *J Comp Physiol Psychol* 1974;86:47–50.
- Kandel DB, Chen K. Extent of smoking and nicotine dependence in the United States: 1991–1993. *Nicotine Tob Res* 2000;2:263–74.
- Kempainen H, Hyttia P, Kianmaa K. Behavioral consequences of repeated nicotine during adolescence in alcohol-preferring AA and alcohol-avoiding ANA rats. *Alcohol Clin Exp Res* 2009;33:340–9.
- Kenney JW, Gould TJ. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol Neurobiol* 2008a;38:101–21.
- Kenney JW, Gould TJ. Nicotine enhances context learning but not context-shock associative learning. *Behav Neurosci* 2008b;122:1158–65.
- Kunin D, Smith BR, Amit Z. Nicotine and ethanol interaction on conditioned taste aversions induced by both drugs. *Pharmacol Biochem Behav* 1999;62:215–21.
- Kunin D, Latendresse MW, Gaskin S, Smith BR, Amit Z. Preexposure effects of nicotine and acetaldehyde on conditioned taste aversion induced by both drugs. *Pharmacol Biochem Behav* 2000;66:695–9.
- Lallemant F, Ward RJ, De Witte P. The influence of chronic nicotine administration on behavioural and neurochemical parameters in male and female rats after repeated binge drinking exposure. *Alcohol Alcohol* 2009;44:535–46.
- Levin ED, Lawrence SS, Petro A, Horton K, Rezvani AH, Seidler FJ, et al. Adolescent vs. adult-onset nicotine self-administration in male rats: duration of effect and differential nicotinic receptor correlates. *Neurotoxicol Teratol* 2007;29:458–65.
- McMillen BA, Davis BJ, Williams HL, Soderstrom K. Periadolescent nicotine exposure causes heterologous sensitization to cocaine reinforcement. *Eur J Pharmacol* 2005;509:161–4.
- McQuown SC, Belluzzi JD, Leslie FM. Low dose nicotine treatment during early adolescence increases subsequent cocaine reward. *Neurotoxicol Teratol* 2007;29:66–73.
- Nelson DE, Giovino GA, Shopland DR, Mowery PD, Mills SL, Eriksen MP. Trends in cigarette smoking among US adolescents, 1974 through 1991. *Am J Public Health* 1995;85:34–40.
- Orr TE, Walters PA, Carl GF, Elkins RL. Brain levels of amines and amino acids in taste aversion-prone and -resistant rats. *Physiol Behav* 1993;53:495–500.
- Palmatier MI, Bevins RA. Chronic caffeine exposure in rats blocks a subsequent nicotine-conditioned taste avoidance in a one-bottle, but not a two-bottle test. *Pharmacol Biochem Behav* 2001;70:279–89.
- Parnell SE, West JR, Chen WJ. Nicotine decreases blood alcohol concentrations in adult rats: a phenomenon potentially related to gastric function. *Alcohol Clin Exp Res* 2006;30:1408–13.
- Portugal GS, Kenney JW, Gould TJ. Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiol Learn Mem* 2008;89:106–13.
- Reilly S. Central gustatory system lesions and conditioned taste aversion. In: Reilly S, Schachtman TR, editors. *Conditioned taste aversion: behavioral and neural processes*. New York, NY: Oxford University Press; 2009.
- Revusky S, Garcia J. Learned associations over long delays. In: Bower G, Spence J, editors. *Psychology of learning and motivation: advances in research and theory*. New York: Academic Press; 1970. p. 1–84.
- Riley AL, Simpson GR. The attenuating effects of drug preexposure on taste aversion conditioning: generality, experimental parameters, underlying mechanisms, and implications for drug use and abuse. In: Mowrer RR, Klein SB, editors. *Handbook of contemporary learning theories*. Mahwah, NJ: Lawrence Erlbaum; 2001. p. 505–59.
- Riley AL, Tuck DL. Conditioned taste aversions: a behavioral index of toxicity. *Ann NY Acad Sci* 1985;443:272–92.
- Rinker JA, Busse GD, Roma PG, Chen SA, Barr CS, Riley AL. The effects of nicotine on ethanol-induced conditioned taste aversions in Long-Evans rats. *Psychopharmacology (Berl)* 2008;197:409–19.
- Robinson DL, Brunner LJ, Gonzales RA. Effect of gender and estrous cycle on the pharmacokinetics of ethanol in the rat brain. *Alcohol Clin Exp Res* 2002;26:165–72.
- Roma PG, Flint WW, Higley JD, Riley AL. Assessment of the aversive and rewarding effects of alcohol in Fischer and Lewis rats. *Psychopharmacology (Berl)* 2006;189:187–99.
- Rozin P, Kalat JW. Specific hungers and poison avoidance as adaptive specializations of learning. *Psychol Rev* 1971;78:459–86.
- Sakai N, Yamamoto T. Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various USs. *Neuroreport* 1997;8:2215–20.
- Serafine KM, Riley AL. Possible role of norepinephrine in cocaine-induced conditioned taste aversions. *Pharmacol Biochem Behav* 2009;92:111–6.
- Serafine KM, Riley AL. Preexposure to cocaine attenuates aversions induced by both cocaine and fluoxetine: Implications for the basis of cocaine-induced conditioned taste aversions. *Pharmacol Biochem Behav* 2010;95:230–4.
- Shram MJ, Funk D, Li Z, Le AD. Periadolescent and adult rats respond differently in tests measuring the rewarding and aversive effects of nicotine. *Psychopharmacology (Berl)* 2006;186:201–8.
- Simpson GR, Riley AL. Morphine preexposure facilitates morphine place preference and attenuates morphine taste aversion. *Pharmacol Biochem Behav* 2005;80:471–9.
- Smith AM, Kelly RB, Chen WJ. Chronic continuous nicotine exposure during periadolescence does not increase ethanol intake during adulthood in rats. *Alcohol Clin Exp Res* 2002;26:976–9.
- Smolen TN, Smolen A. Blood and brain ethanol concentrations during absorption and distribution in long-sleep and short-sleep mice. *Alcohol* 1989;6:33–8.
- Soderpalm B, Ericson M, Olausson P, Blomqvist O, Engel JA. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav Brain Res* 2000;113:85–96.
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 2000;24:417–63.
- Stolerman IP, D'Mello GD. Oral self-administration and the relevance of conditioned taste aversions. In: Thompson T, Dewes PB, McKim WA, editors. *Advances in behavioral pharmacology*. New York: Academic Press; 1981. p. 169–214.
- Tian S, Gao J, Han L, Fu J, Li C, Li Z. Prior chronic nicotine impairs cued fear extinction but enhances contextual fear conditioning in rats. *Neuroscience* 2008;153:935–43.
- Tsui T, Le AD, Wang AJ, Juzysch W, Harding S. Exposure to nicotine but not alcohol during adolescence enhances the acquisition of alcohol consumption in adulthood (abstract). *Alcohol Clin Exp Res* 2001;25:14.
- USDHHS. Preventing tobacco use among young people: a report of the surgeon general. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 1994.
- Wetherington CL. Sex differences and gonadal hormone influences in drug addiction and sexual behavior: progress and possibilities. *Horm Behav* 2010;58:2–7.
- Wise RA, Yokel RA, DeWit H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science* 1976;191:1273–5.