Alcoholism: Clinical and Experimental Research

Vol. 43, No. 6
June 2019

Nicotine Produces a High-Approach, Low-Avoidance Phenotype in Response to Alcohol-Associated Cues in Male Rats

Gregory C. Loney, Hailley Angelyn, Liam M. Cleary, and Paul J. Meyer

Background: Nicotine and alcohol use are highly comorbid. Modulation of drug-paired extrinsic and intrinsic cues likely plays a role in this interaction, as cues can acquire motivational properties and augment drug seeking. The motivational properties of cues can be measured through Pavlovian conditioning paradigms, in which cues either elicit approach following pairing with the reinforcing properties of alcohol or elicit avoidance following pairing with the aversive consequences of alcohol. The present experiments tested whether nicotine would enhance the incentive properties of an appetitive ethanol (EtOH) cue and diminish the avoidance of an aversive EtOH cue in Pavlovian paradigms.

Methods: In experiment 1, male Long-Evans rats with or without prior chronic intermittent access to EtOH were administered nicotine or saline injections prior to Pavlovian conditioned approach (PavCA) sessions, during which conditioned approach to the cue ("sign-tracking") or the EtOH delivery location ("goal-tracking") was measured. In experiment 2, male Long-Evans rats were administered nicotine or saline injections prior to pairing a flavor cue with increasing doses of EtOH (*i.p.*) in an adaptation of the conditioned taste avoidance (CTA) paradigm.

Results: Results from PavCA indicate that, regardless of EtOH exposure, nicotine enhanced responding elicited by EtOH-paired cues with no effect on a similar cue not explicitly paired with EtOH. Furthermore, nicotine reduced sensitivity to EtOH-induced CTA, as indicated by a rightward shift in the dose–response curve of passively administered EtOH. The ED_{50}, or the dose of EtOH that produced a 50% reduction in intake relative to baseline, was significantly higher in nicotine-treated rats compared to saline-treated rats.

Conclusions: We conclude that nicotine increases the approach and diminishes the avoidance elicited by Pavlovian cues paired, respectively, with the reinforcing and aversive properties of EtOH consumption in male rats. As such, nicotine may enhance alcoholism liability by engendering an attentional bias toward cues that predict the reinforcing outcomes of drinking.

Key Words: Alcohol, Nicotine, Pavlovian Conditioning, Interoception, Autoshaping, Conditioned Avoidance.

SMOKING AND ALCOHOL drinking are highly comorbid; 43 to 72% of alcohol drinkers regularly smoke tobacco compared to only 15 to 23% of nondrinkers, and smokers are 2 to 4 times more likely to abuse alcohol than nonsmokers (Weinberger et al., 2017a,b). Clinical evidence supports a bidirectional relationship between nicotine and alcohol use, with increasing use of nicotine predicting greater degree of alcohol dependence, and vice versa (Falk et al., 2006). In rodents, nicotine promotes operant ethanol (EtOH) self-administration (Bito-Onon et al., 2011; Doyon et al., 2013; Le et al., 2003), delays extinction of EtOH-seeking behavior (Le et al., 2010), reinstates EtOH seeking (Le et al., 2003), and limits acquisition of EtOH-induced conditioned taste avoidance (CTA; Bienkowski et al., 1998; Kunin et al., 1999; Loney and Meyer, 2019; Rinker et al., 2011). Alcohol- and nicotine-associated stimuli ("cues") likely play an important role in augmenting alcohol seeking. Paired cues can acquire motivational properties and either spur drug seeking and self-administration (Corbit and Janak, 2007; Grusser et al., 2004; Katner et al., 1999; Tomie and Sharma, 2013; Wiers et al., 2009) or elicit avoidance responses when paired with aversive doses of a given drug (Liu et al., 2009; Loney and Meyer, 2019; Loney et al., 2018; Verendeev and Riley, 2011, 2013). Clinical models of cue-induced alcohol craving posit that approach and avoidance inclinations develop, respectively, as a function of the reinforcing and aversive consequences of alcohol use (Breiner et al., 1999; Schlauch et al., 2015). Furthermore, a high-approach/low-avoidance phenotype is associated with a tendency for risky drinking behaviors, an intractable attitude toward substance abuse treatment, and is positively correlated with both cue-elicited craving and alcohol consumption in a clinical setting (Hollett et al., 2017; Schlauch et al., 2015; Stritzke et al.,

From the, Department of Psychology, Center for Ingestive Behavior Research, University at Buffalo, Buffalo, New York .

Received for publication November 21, 2018; accepted April 1, 2019.

Reprint requests: Paul J. Meyer, PhD, Department of Psychology, State University of New York at Buffalo, 204 Park Hall, Buffalo, NY 14260-4110; Tel.: 716-645-0263; Fax: 716-645-3801; E-mail: pmeyer@buffalo.edu

DOI: 10.1111/acer.14043

2004). As such, studies designed to explicitly measure the impact of the subjective aversive and reinforcing properties of commonly abused drugs are critical to understanding underlying neural mechanisms contributing to development of substance use disorders.

In preclinical models, the motivational properties of cues can be inferred using Pavlovian conditioned approach (e.g., PavCA) and avoidance paradigms (e.g., CTA), during which, respectively, an environmental cue predicts the delivery of a reward, such as a food pellet or EtOH solution (Milton and Everitt, 2010; Robinson and Berridge, 1993; Robinson et al., 2014) or a flavor cue predicts passive administration of concentrated EtOH (Bienkowski et al., 1998; Kunin et al., 1999; Liu et al., 2009; Loney and Meyer, 2019; Rinker et al., 2011). In PavCA paradigms, due to the reinforcing value of the stimulus, in response to presentations of a predictive cue, rats generally display a tendency to approach either the cue itself ("sign-tracking") or the reward delivery location ("goal-tracking"), even though no response is required to receive the reward. Such cue reactivity elicited by drug-paired stimuli predicts other measures of incentive value, including conditioned reinforcement, cue-induced reinstatement, and the response to drug cues in multiple other paradigms (Flagel et al., 2009, 2010; Maddux and Chaudhri, 2017; Meyer et al., 2012; Robinson et al., 2014; Saunders and Robinson, 2010; Srey et al., 2015; Versaggi et al., 2016). A number of recent studies have demonstrated the ability for EtOH-paired cues to elicit approach behaviors (Fiorenza et al., 2018; Maddux and Chaudhri, 2017; Srey et al., 2015). However, these studies found opposing effects on cue reactivity, with one demonstrating an increase in sign-tracking over repeated EtOH exposures (Srey et al., 2015) and the other an increase in goal-tracking (Fiorenza et al., 2018). Nicotine has previously been shown to enhance EtOH cue-induced approach (Maddux and Chaudhri, 2017), though the design of that study was unable to differentiate whether nicotine preferentially increased sign- or goal-tracking. Because sign- and goal-tracking are thought to be resultant from differing psychological processes (i.e., Pavlovian mechanisms and cognitive expectation, respectively), these results raise questions about the means by which EtOH cues influence behavior and, more specifically, how prior EtOH experience and nicotine administration interact to affect responding to EtOH cues. Therefore, it is ultimately yet to be determined whether nicotine differentially affects goal- versus sign-tracking to an EtOH cue and whether the tendency for nicotine to enhance conditioned approach is dependent on previous EtOH experience.

Conversely, nicotine interferes with the degree of avoidance of an EtOH-paired cue in CTA paradigms (Bienkowski et al., 1998; Kunin et al., 1999; Loney and Meyer, 2019; Rinker et al., 2011). These CTA paradigms rely on the aversive interoceptive properties of a drug stimulus in order to modulate the motivational properties of a highly palatable stimulus (Loney and Meyer, 2019; Rinker et al., 2011). Thus far, the mechanisms underlying the ability for nicotine to interfere with EtOH-induced CTA are unknown. It could be that nicotine interferes with the cellular mechanisms underlying the acquisition of a CTA. Another possibility is that nicotine specifically reduces the salience of the aversive stimulus properties of EtOH (Rinker et al., 2011). In this case, nicotine-treated rats would be capable of acquiring a sufficient avoidance response relative to a saline-treated rat; they would simply require a higher dose in order to do so. The lack of an effect of nicotine on LiCl-induced CTA (Loney and Meyer, 2019) suggests this to be the case, but studies specifically testing the sensitivity of nicotine-treated, relative to saline-treated, rats to avoidance conditioned by a given dose of EtOH have yet to be conducted.

The present studies were designed to determine the impact of nicotine on sensitivity to the reinforcing properties of EtOH in male rats, as a function of previous EtOH drinking history, by examining its effects on approach to EtOH-paired cues in a discriminated PavCA paradigm wherein a cue explicitly paired with EtOH and a separate cue not paired with EtOH were presented within the same session. Furthermore, we also examined the impact of nicotine on the aversive properties of passively administered EtOH in an adaptation of the CTA paradigm where a flavor cue was paired with increasing doses of EtOH. We hypothesized that, regardless of previous EtOH experience, nicotine would facilitate approach to EtOH-associated cues with no impact on nonassociated cues and that nicotine may interact with previous EtOH history to further spur EtOH-seeking behaviors. We also hypothesized that nicotine would reduce the sensitivity to the aversive consequences of EtOH administration as evidenced by a lateral shift in the $ED_{50,}$ indicating a general reduction in sensitivity for a given dose of EtOH to induce avoidance of a highly palatable saccharin solution. In summary, we show that nicotine administration in male rats produces a high-approach, low-avoidance phenotype toward EtOH-associated cues regardless of previous drinking experience.

MATERIALS AND METHODS

Subjects and Housing

A total of 59 male Long-Evans rats (Envigo, Indianapolis, IN; ~250 to 300 g on arrival) were used across 2 separate experiments (Exp. 1: $n = 34$; Exp. 2: $n = 25$). Sample sizes were chosen based on effect sizes from previous studies (e.g., Loney and Meyer, 2019; Loney et al., 2018). Given that male and female rats differ in their sensitivity to the effects of nicotine (Chaudhri et al., 2005; Loney and Meyer, 2019, we focused on male rats for these initial phenomenological experiments. Rats were housed singly in polycarbonate cages (45 cm length × 24 cm width × 20 cm height) in a temperature- and humidity-controlled room (22 ± 1°C) and maintained on a reverse 12-hour light/dark cycle. Rats were handled daily for 3 days before testing began. Food and water were available ad libitum for the duration of experiment 1, and rats were maintained on 23.5-hour water deprivation during experiment 2. All procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee.

Experiment 1: Effect of Nicotine on EtOH Cue-Elicited Approach During a Discriminated Pavlovian (CS+ and CS−) Approach Paradigm. Apparatus—Pavlovian and operant conditioning occurred in Med Associates chambers (St-Albans, VT; 30.5 × 24.1 cm floor area, 29.2 cm high) inside individual sound- and light-attenuating cabinets (A & B Displays, Bay City, MI) equipped with fans for ventilation and noise masking. A red house light was located in the center panel of the left wall of the chamber for all experiments (27 cm above floor).

A retractable sipper bottle (Med Associates, Model ENV-252M) containing undenatured 200 proof EtOH diluted with tap water to a concentration of 20% (v/v; Decon Labs) was located opposite the house light, in the center panel of the right wall (6 cm above floor). Two retractable, illuminated levers (6 cm above floor) were positioned on either side of the bottle delivery port.

A contact detection system (Med Associates, Model ENV-005-QD) was used to measure contacts with the levers and the faceplates of the lever casing (lever faceplate 7.6 cm W × 8.3 cm H), and contacts with the sipper bottle and its faceplate (sipper bottle faceplate 7.6 cm W × 16.5 cm H). In addition, levers were calibrated such that they were deflected by 15 to 20 g of pressure, and these deflections were included in lever contacts. All data were collected using MED-PC IV.2 software (Med Associates).

Chronic Intermittent Access to EtOH—One week after arrival, rats were assigned to either EtOH-exposed or EtOH-unexposed (water) groups. Exposed rats (n = 17) were tested in a chronic intermittent access to EtOH paradigm (CIA; Loney and Meyer, 2018; Simms et al., 2008), during which rats were given access to 2 bottles containing either EtOH (20% v/v) or tap water for three 24-hour sessions per week (Mon/Wed/Fri). EtOH bottles were presented on alternating sides of the home cage to prevent the development of a side preference. On the intervening days, both bottles contained water. Unexposed rats (n = 17) were given 2 sipper bottles containing water on all days. The procedure lasted 31 days, for a total of fourteen 24-hour EtOH drinking sessions. Changes in water and EtOH bottle weights were recorded to determine total fluid intake, total EtOH intake (g/kg/24 h; grams of EtOH consumed per kilogram of body weight over each 24-hour session), and EtOH preference (%; ratio of grams of EtOH consumed to total grams of fluid consumed over each 24-hour session). To account for fluid loss due to evaporation or spillage, the data for each rat were adjusted by subtracting the average difference in bottle weight from pairs of control EtOH and water bottles placed onto 2 empty cages. Fluid intake was not recorded on Saturday/Sunday.

EtOH Pavlovian Conditioned Approach—Following CIA, rats were trained in a Pavlovian conditioned approach (PavCA) paradigm (Fig. 1*A*). During PavCA testing, rats were assigned to receive either a nicotine (Glentham; 0.4 mg/kg, s.c., dose expressed as free-base, pH was adjusted to ~7.2 to 7.4) or saline injection 15 minutes prior to testing. These nicotine- and saline-treated groups were matched based on EtOH preference during CIA to EtOH. Rats received 1 injection of their assigned drug the day before the first testing session to habituate them to the injection procedure. Testing occurred daily on Monday through Friday of each week for a total of 40 sessions which occurred over 8 weeks. During weeks 1 to 7, rats received the assigned nicotine or saline injection. In order to determine whether nicotine's effects were acute or chronic, during week 8 all rats received saline treatments. Thus, week 8 is referred to as a nicotine removal condition.

On the morning of each session, rats were weighed, injected with nicotine or saline, placed into plastic transfer containers (43.5 cm L × 20.3 cm W × 17.8 cm H) on a transfer cart for 15 minutes, and then moved to the testing room and placed into conditioning chambers. After a 2-minute delay, the illumination of the house light signaled the start of the session. For this experiment, the conditioned stimulus (CS+) was an 8-second extension of 1 retractable lever, which was followed by delivery of the unconditioned stimulus (US), a sipper bottle containing 20% EtOH. The sipper bottle was deployed into the chamber for 30 seconds, and rats were allowed to freely drink from the sipper bottle for the entire 30 seconds before it was retracted. The extension of the other lever (CS−) was not followed by a deployment of the sipper bottle into the chamber. The CS+ and CS− levers were counterbalanced between subjects to either the left or right side of the chamber. Lever CS+ and lever CS− presentations occurred randomly on a VI 105 (60 to 150 seconds) schedule with 15 presentations per lever for a total of 30 presentations. Sessions lasted on average 54.5 minutes.

Measures—The number of lever/faceplate contacts, lever presses, and sipper bottle/faceplate contacts was recorded during lever and sipper bottle presentations for each session. *Sign-tracking* was defined as lever contacts or presses during lever CS+ extensions, while *goal-tracking* was measured by sipper bottle faceplate contacts during lever CS+ extensions. Note that, since the sipper itself was not extended until *after* the CS+, goal-tracking reflects only the faceplate contacts.

Experiment 2: Effect of Nicotine on Sensitivity to the Aversive Properties of EtOH in an Augmented CTA Paradigm. Training—Following acclimatization to the animal holding facility, rats were maintained on 23.5-hour water deprivation. Rats were initially trained to consume their daily allotment of water in 30-minute sessions in their home cage 30 minutes following a saline injection (1 ml/kg, s.c.). Once stable baseline intakes were reached, rats were assigned to 1 of 4 groups in a weight- and intake-balanced fashion: Sal-Sal (n = 5); Nic-Sal (n = 5); Sal-EtOH (n = 7); and Nic-EtOH

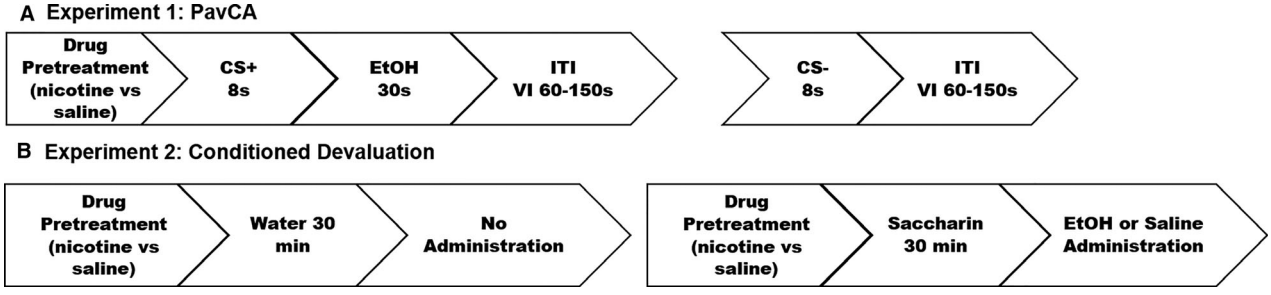

Fig. 1. Diagram of the experimental design for experiments 1 and 2. (**A**). In experiment 1, Pavlovian conditioning sessions consisted of an 8-second extension of either the lever CS+ or CS−. The CS+ was followed by a 30-second extension of the ethanol (EtOH) US via a sipper bottle, while the CS− was not. An intervariable interval between 60 and 150 seconds preceded the start of the next trial. There were 15 lever extensions per lever for a total of 30 trials per session. (**B**) In experiment 2, rats were administered nicotine or saline 30 minutes prior to testing. On nonconditioning days, water was available for 30 minutes, followed by nothing. On conditioning days, 0.1% saccharin was available for 30 minutes, immediately followed by i.p. injection of EtOH. This 2-day pattern was repeated for 14 total days allowing for testing with 7 concentrations of EtOH in ascending order.

($n = 8$) defined as receiving pretreatment with either nicotine or saline and then receiving either an EtOH or saline injection (unconditioned stimulus; US) paired with the flavor cue on conditioning days. Training continued for 3 more days during which rats in the 2 nicotine groups received nicotine injections (0.4 mg/kg, s.c.) in lieu of saline injections.

Testing—Immediately following training, rats were tested in an adaptation of the EtOH-induced CTA paradigm (Fig. 1*B*). Briefly, on the first day of testing, rats were injected with their assigned drug stimulus (saline or nicotine) 30 minutes prior to access to a 0.1% saccharin solution (conditioned stimulus; CS+) for 30 minutes in the home cage. At the end of saccharin drinking, rats were immediately injected *i.p.* with either EtOH or equivolume saline. The subsequent day of testing was identical with 2 critical exceptions: Rats drank water (CS−) in the home cage following which all rats were injected with saline. This 2-day pattern was repeated for 14 days allowing for testing with 7 doses of EtOH (16% v/v delivered at doses of 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, and 2.4 g/kg). EtOH concentrations were tested in ascending order in all rats, and saline injections in the unconditioned controls were matched for volume.

Measures—Saccharin and water intakes were assessed by weighing the individual bottles prior, and subsequent, to home cage drinking sessions. In order to determine the efficacy for a given dose of EtOH to condition avoidance of subsequent saccharin drinking sessions, these data were converted to a devaluation score by dividing intake on a particular conditioning day by the initial, baseline intake. Curves were fit to these suppression scores, and the ED_{50} was calculated using a 3-parameter logistical function:

$$\frac{a}{\left(1+\left(10^{((\log \text{conc}-c)*b)}\right)\right)}.$$

where a is equal to asymptotic consumption, b is equal to the slope of the curve, and c is the ED_{50}, or the concentration at which one-half asymptotic consumption was seen. Curve fitting was conducted with Systat 12.

Data Analyses.—Chronic Intermittent Access to EtOH—For the CIA data, repeated-measures analysis of variance (ANOVA) was conducted on intake data that had been corrected for fluid loss. Differences in total fluid intake were analyzed with *Exposure* (EtOH, water) as the between-groups factor and *EtOH Session* (i.e., 1 to 14) as the within-groups factors. We also examined EtOH intake (g/kg/24 h; grams of EtOH consumed per kilogram of body weight over each 24-hour *EtOH Session*) and EtOH preference (%; ratio of grams of EtOH solution consumed to total grams of fluid consumed over each 24-hour *EtOH Session*) in EtOH-exposed rats across *EtOH Session*. Significance was determined as $p < 0.05$ for this and all measures.

EtOH Pavlovian Conditioned Approach—For Exp. 1, all variables were calculated by taking the average of the data across each 5-session week. Data from PavCA sessions were analyzed using repeated-measures ANOVA with *Week* (1 to 7) and *CS* (CS+, CS−) as within-groups factors, and *Exposure* (EtOH-exposed, unexposed) and *Drug* (nicotine, saline) as the between-groups factors. Fisher's LSD post hoc tests were used to further examine significant main effects and interactions.

EtOH Pavlovian Conditioned Avoidance—Intake suppression scores were analyzed with a repeated-measures ANOVA where *Drug* (nicotine or saline) and *US Treatment* (EtOH or saline) were between-subjects factors and *Day* (1 to 14) and *CS* (saccharin or water) were the within-subjects factors. The ED_{50} concentration in animals receiving EtOH as US was analyzed between nicotine- and saline-treated rats with an independent samples *t*-test. Significance was determined as $p < 0.05$ for this and all other measures. Planned comparisons between nicotine- and saline-treated rats were conducted independently for the EtOH-treated experimental group and saline-treated unconditioned control group.

RESULTS

Experiment 1

Chronic Intermittent Access to EtOH. While total fluid intake decreased among all rats across EtOH drinking sessions, EtOH-exposed rats consumed more total fluid (water + EtOH) than unexposed rats (water only), main effects of *Exposure*, $F(1, 32) = 11.10$, $p < 0.01$, and *EtOH Session*, $F(13, 416) = 20.17$, $p < 0.001$. In addition, EtOH-exposed rats increased their EtOH intake and EtOH preference across EtOH drinking sessions, Fig. 2*A,B*; main effects of *EtOH Session* on EtOH intake (g/kg/24 h), $F(13, 208) = 5.81$, $p < 0.001$, and EtOH preference (%), $F(13, 208) = 8.79$, $p < 0.001$. Post hoc analyses indicated that EtOH intake ($p < 0.001$) and EtOH preference ($p < 0.001$ to 0.02) were significantly higher on sessions 3 to 14 relative to the first EtOH drinking session.

EtOH Pavlovian Conditioned Approach. Sipper Bottle Faceplate Contacts ("Goal-Tracking")—All rats goal-tracked more to the CS+ than the CS−, Fig. 3: main effects of *CS*, $F(1, 30) = 4.58$, $p < 0.001$, *Week*, $F(6, 180) = 3.92$, $p = 0.001$, and *CS* × *Week* interaction, $F(6, 180) = 4.70$, $p < 0.001$, but the number of goal-tracking responses was significantly enhanced by nicotine treatment, as measured by sipper bottle faceplate contacts elicited by the CS+ and CS−, main effect of *Drug*, $F(1, 30) = 13.30$, $p < 0.001$ as well as *CS* × *Drug*, $F(1, 30) = 7.01$, $p < 0.05$, and *CS* × *Drug* × *Week*, $F(6, 180) = 2.33$, $p < 0.05$, interactions. No main effect or interaction with *Exposure* was found. Post hoc analyses of the *CS* × *Drug* × *Week* interaction indicated that nicotine-treated rats made significantly more sipper bottle faceplate contacts than saline-treated rats, regardless of previous EtOH exposure, during lever CS+ extensions across weeks 2 to 6 (Fig. 3*A,B*: $p \leq 0.01$). Additionally, nicotine-treated rats discriminated between the CS+ and CS− across weeks 2 to 7, while saline-treated rats only did so during weeks 5 and 7 (Fig. 3*A*). Thus, nicotine-treated rats discriminated more consistently between the CS+ and CS−, and nicotine specifically enhanced the goal-tracking response to the CS+ more than the goal-tracking response to the CS−. To ensure that the heightened goal-tracking response in nicotine-treated rats was not necessarily due to differences in rate of acquisition, we ran a separate ANOVA comparing the goal-tracking response between nicotine- and saline-treated rats during weeks 5 to 7, a time in which saline-treated rats had demonstrated statistically significant discrimination between the CS+ and

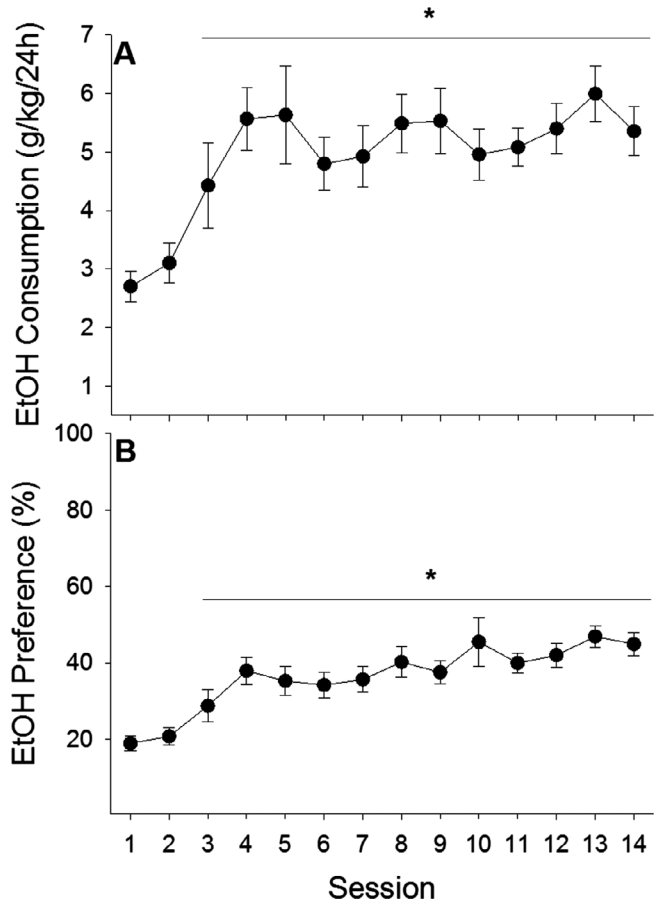

Fig. 2. In experiment 1, ethanol (EtOH)-exposed male rats consumed more total fluid than EtOH-unexposed male rats and demonstrated increased EtOH intake and preference across EtOH sessions. EtOH-exposed rats with access to 2 bottles separately containing water and EtOH (20% v/v) demonstrated increased EtOH intake (**A**) and preference (**B**) across EtOH sessions. Intakes and preferences observed across EtOH sessions 3 to 12 were significantly greater than those on session 1. Asterisk (*) designates significant post hoc analyses ($ps < 0.05$).

CS−. This ANOVA revealed a significant main effect of *Drug*, $F(1, 30) = 6.90$, $p < 0.05$) and a *Drug* × *Week* interaction, $F(2, 60) = 6.19$, $p < 0.01$). Thus, once all rats had effectively acquired the CS+ association, nicotine-treated rats displayed higher goal-tracking responses relative to saline-treated rats.

Next, sipper bottle faceplate contacts elicited by the CS+ and CS− were analyzed separately. For contacts elicited by the CS+, nicotine enhanced goal-tracking relative to saline, Fig. 3*A,B:* main effects of *Drug*, $F(1, 30) = 11.92$, $p < 0.01$, *Week*, $F(6, 180) = 4.85$, $p < 0.001$, and a significant *Drug* × *Week* interaction, $F(6, 180) = 2.42$, $p < 0.05$. No main effect or interaction with *Exposure* was found. Post hoc analyses of the *Drug* × *Week* interaction indicated that nicotine treatment enhanced sipper bottle faceplate contacts elicited by the CS+ relative to saline treatment across weeks 2 to 6 ($p < 0.01$). Furthermore, nicotine enhanced sipper bottle faceplate contacts across weeks 3 to 6 relative to week 1 ($p < 0.05$), while saline did not enhance sipper bottle faceplate contacts across any week relative to week 1, albeit there was a trend for an enhancement when comparing week 7 to week 1 ($p = 0.06$). For contacts elicited by the CS−, we again found no main or interactive effects of *Exposure* but there was a main effect of *Drug* that did not interact with *Week* indicating that nicotine tended to increase responding during the CS− period in general. To ensure that the effects of nicotine were greater for responding during the CS+ period, as was suggested by the significant *CS* × *Drug* × *Week* interaction from the omnibus ANOVA, we next subtracted the CS− responding from the CS+ responding and examined this difference score in a separate ANOVA. Here, we again found a significant main effects of *Drug*, $F(1, 30) = 7.01$, $p < 0.05$, and *Week*, $F(6, 180) = 4.70$, $p < 0.001$, a significant *Drug* × *Week*, $F(6, 180) = 2.33$, $p < 0.05$, interaction. Thus, nicotine treatment, relative to saline treatment, enhanced goal-tracking elicited by the CS+ more than to the CS−.

Lever Contacts ("Sign-Tracking")—All rats sign-tracked more during the CS+ than the CS−, as measured by lever contacts elicited by the CS+ and CS−, Fig. 4: main effects of *CS*, $F(1, 30) = 12.75$ $p = 0.001$, *Week*, $F(6, 180) = 6.43$ $p < 0.001$, and a significant *CS* × *Week* interaction, $F(6, 180) = 3.17$ $p = 0.01$. Post hoc analyses of the *CS* × *Week* interaction indicated that the CS+ elicited more lever contacts than the CS− across all weeks following week 1 ($p < 0.001$). No significant main effect or interactions with *Exposure* or *Drug* were found (Fig. 3*B,C*). Thus, all rats were able to discriminate which lever was predictive of the reward and, consequently, the CS+ elicited more sign-tracking than the CS−.

When lever contacts elicited by the CS+ were analyzed separately (Fig. 4*A,B*), nicotine had no significant effect on sign-tracking conditioned responding relative to saline. Repeated-measures ANOVA examining the effects of *Exposure* and *Drug* on CS+ contacts during CS+ extensions across weeks 1 to 7 yielded no significant main effects or interactions with *Exposure*, *Drug*, or *Week*.

EtOH Consumption During Conditioning. Examining the average total licks (Table 1) elicited to the 20% EtOH solution across the 7-week conditioning paradigm revealed a main effect of *Week*, $F(6, 180) = 4.49$, $p < 0.001$, and *Week* × *Drug*, $F(6, 180) = 2.69$, $p < 0.05$, and *Week* × *Exposure*, $F(6, 180) = 4.58$, $p < 0.001$, interactions. Post hoc analyses of the 2 interactions indicated that, as a whole, saline-treated rats consumed significantly more EtOH across weeks 4 to 7, relative to their own consumption on week 1, while nicotine-treated rats tended to start consumption higher and remained relatively stable across testing. For the *Week* × *Exposure* interaction, nonexposed rats, as a whole, consumed significantly more EtOH across weeks 2 to 7, relative to their own consumption on week 1. EtOH-exposed rats did not significantly alter their intake across the 7-week paradigm.

Nicotine Removal. Goal-Tracking—Removing nicotine treatment did not specifically alter goal-tracking elicited by

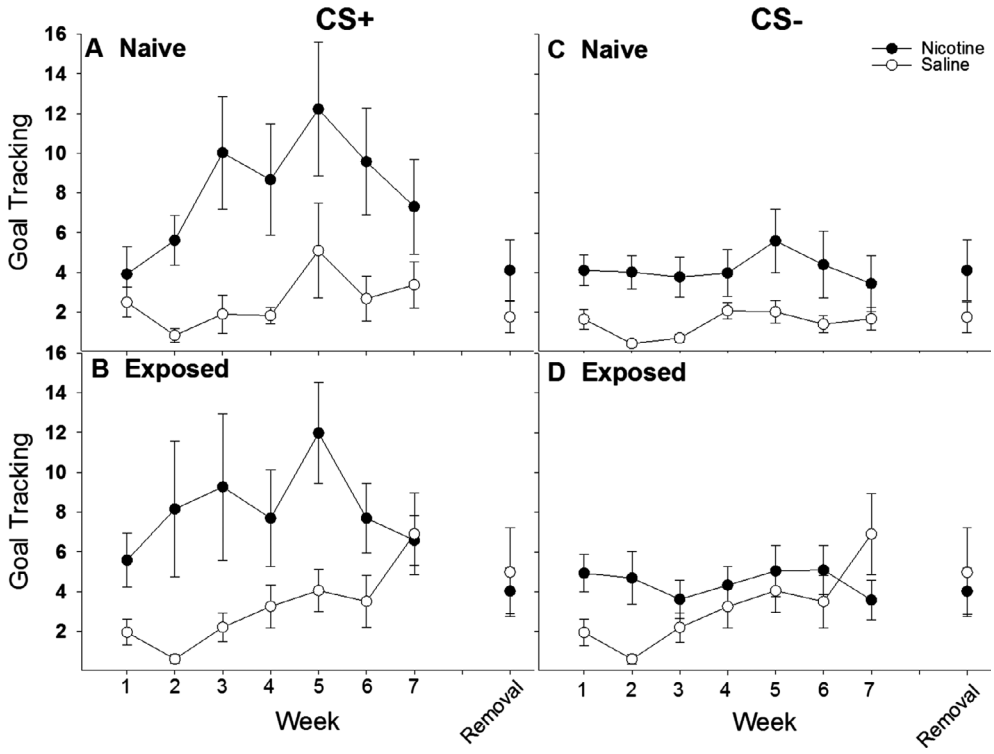

Fig. 3. In experiment 1, nicotine significantly enhanced goal-tracking in response to an ethanol (EtOH)-associated cue relative to saline-treated male rats. Regardless of previous history with EtOH (naive [**A**] vs. exposed [**B**]), nicotine enhanced the tendency to approach and interact with the reward delivery apparatus ("goal-tracking") during an 8-second lever extension (CS+) that immediately preceded delivery of a retractable bottle that contained 20% EtOH. This enhancement of goal-tracking was largest in response to the CS+, though nicotine-treated rats approached the reward receptacle more than saline-treated rats during the CS− as well (**C** and **D**). The enhancement of goal-tracking was lost when nicotine was removed, indicating that the behavioral effects were likely due to acute administration of nicotine.

the CS+ (Fig. 3*A,B*). While a repeated-measures ANOVA on sipper bottle faceplate contacts during the lever CS+ revealed a significant main effect of *Week*, $F(1, 30) = 11.52$, $p < 0.01$, such that sipper bottle faceplate contacts in week 8 were significantly reduced relative to week 7 ($p < 0.002$; Fig. 4*D*), there were no significant main effects or interactions with *Exposure* or *Drug*. Thus, while nicotine treatment did significantly increase goal-tracking across weeks 1 to 7, removing nicotine during week 8 did not result in significantly reduced goal-tracking relative to week 7. This is lack of an effect could be driven by the already evident trend toward decreasing goal-tracking observed starting at week 6 in both naive and EtOH-exposed nicotine-treated rats (Fig. 4*A,B*, respectively). Nevertheless, there were no significant differences among groups in goal-tracking once nicotine was removed, suggesting that the significant differences in responding were due to acute actions of nicotine.

Sign-Tracking—Sign-tracking during nicotine removal (week 8) was compared to the last week of PavCA testing (week 7). In contrast to goal-tracking, removing nicotine treatment decreased sign-tracking elicited by the CS+, Fig. 4*A,B:* interaction of *Drug* × *Week*, $F(1, 30) = 4.41$, $p < 0.05$, and trends for a main effect of *Week*, $F(1, 30) = 3.85$, $p = 0.06$, and an *Exposure* × *Week* interaction, $F(1, 30) = 3.17$, $p = 0.085$. No main effect of *Exposure* was found. Post hoc analysis revealed that previously nicotine-treated rats significantly reduced CS+ contacts during week 8 relative to week 7 ($p < 0.01$; Fig. 4*A*), while saline-treated rats had no significant change in contacts across weeks. Thus, while nicotine treatment did not significantly increase sign-tracking across weeks 1 to 7, removing nicotine during week 8 resulted in reduced sign-tracking relative to week 7.

Experiment 2

Regardless of drug treatment (nicotine or saline), rats receiving the flavor cue paired with increasing saline volume displayed a tendency to increase intake of saccharin across testing sessions while rats receiving the same flavor cue paired with increasing EtOH dose decreased their intake of the saccharin solution across days, Fig. 5*A,B:* main effects of *Day*, $F(6, 126) = 12.36$, $p < 0.001$, and *US*, $F(1, 21) = 37.90$, $p < 0.001$, and *Day* × *US* interaction, $F(6, 126) = 21.53$, $p < 0.001$, with no substantial change in the unpaired fluid consumption (i.e., water, *data not shown*; *CS* × *Day* × *US* interaction, $F(6, 126) = 38.94$, $p < 0.001$. Importantly, there was an effect of nicotine to reduce the devaluation conditioned by a given dose of EtOH, Fig. 5*B:* main effect of *Drug*, $F(1, 21) = 4.33$, $p = 0.05$, and *Drug* × *US* interaction, $F(1, 21) = 6.50$, $p < 0.05$, with no effect on saccharin intake when paired with saline (Fig. 5*A*). Planned comparisons

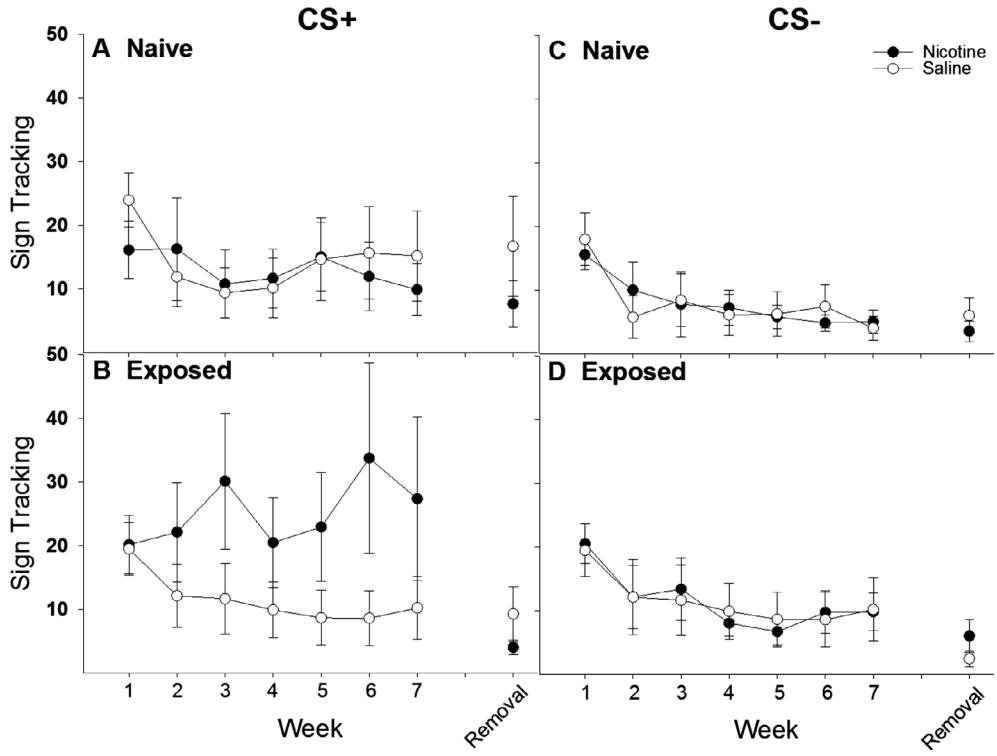

Fig. 4. Nicotine did not significantly affect sign-tracking to an ethanol (EtOH)-associated cue in male rats. There was a trend for nicotine to enhance sign-tracking to the CS+ (**A** and **B**), but only in the rats previously exposed to EtOH in the CIA paradigm, although this effect failed to reach statistical significance. Further support for this trend is provided by the complete loss of a sign-tracking response upon the removal of acute nicotine administration (**B**). Neither nicotine nor EtOH exposure affected responding to the CS− (**C** and **D**).

Table 1. Average Licks to 20% EtOH Across the 7-week Discriminated Approach Paradigm

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
EtOH exposed—nicotine	206 ± 35.2	240 ± 75.0	131.7 ± 21.8	123.7 ± 32.1	122.7 ± 26.4	189.7 ± 41.8	161.9 ± 40.4
EtOH exposed—saline	68.9 ± 9.7	118.1 ± 53.2	118.0 ± 60.0	161.2 ± 55.0	147.4 ± 52.8	114.6 ± 37.7	161.8 ± 61.5
Nonexposed—nicotine	112.6 ± 6.9	201.0 ± 74.1	211.5 ± 65.2	211.5 ± 58.7	259.9 ± 90.3	316.4 ± 109.3	200.0 ± 60.9
Nonexposed—saline	59.1 ± 13.3	95.6 ± 38.8	118.3 ± 38.4	200.1 ± 57.0	261.5 ± 71.2	241.5 ± 93.0	197.0 ± 48.2

between saline- and nicotine-treated rats receiving the flavor cue paired with EtOH revealed that nicotine-treated rats had significantly lower levels of devaluation following pairing with 0.9, 1.8, and 2.1 g/kg EtOH ($ps < 0.05$), with a trend for a difference at the 1.5 g/kg dose ($p = 0.053$), while saline- and nicotine-treated rats receiving the flavor cue paired with equivolume saline did not significantly differ at any point. Finally, when comparing the ED_{50}, it was revealed that nicotine administration shifted the dose–response curve to the right (Fig. 6: $t_{13} = 2.28$, $p < 0.05$) indicating that nicotine-treated rats were less sensitive to the aversive conditioning properties of EtOH relative to saline-treated rats. The dose of EtOH that produced a 50% decrement in intake in nicotine-treated rats was ~2.0 g/kg, while the dose producing an equivalent degree of devaluation in saline-treated rats was ~1.5 g/kg.

DISCUSSION

Summary

In these studies, we show that nicotine significantly impacts two contrasting EtOH-motivated behaviors in male rats. In a Pavlovian conditioned approach paradigm, nicotine facilitated approach elicited by an EtOH cue. However, this approach was directed toward the location of the EtOH reward ("goal-tracking"), rather than toward the EtOH cue ("sign-tracking"), and, further, we did not observe an obvious "switch" from goal-tracking to sign-tracking over extended conditioning as reported by Srey et al. (2015). In addition, nicotine reduced the avoidance of a flavor cue paired with the aversive consequence of acute EtOH intoxication in a Pavlovian conditioned avoidance paradigm. More specifically, nicotine treatment resulted in a significant

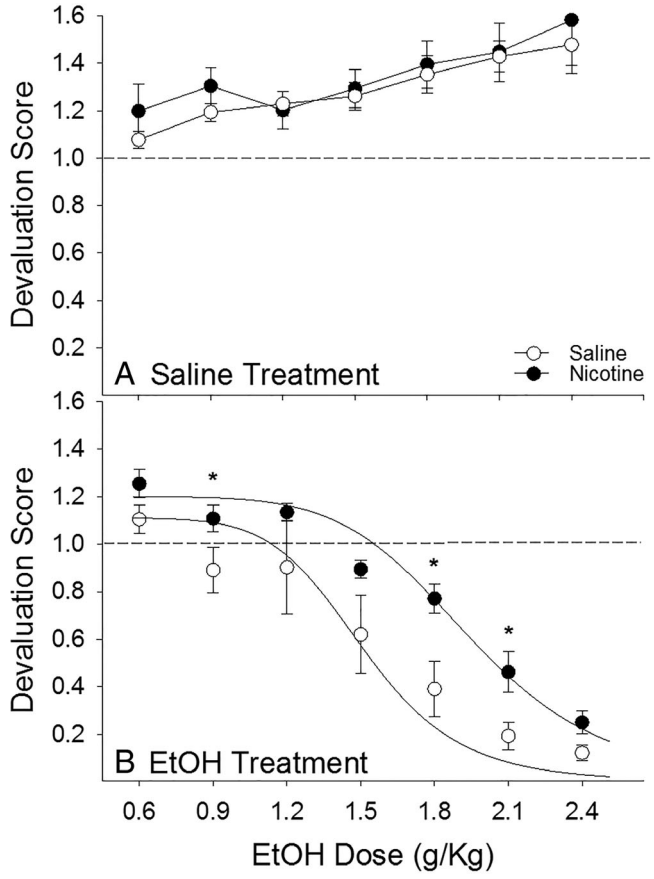

Fig. 5. Nicotine reduced sensitivity to the devaluation induced passive administration of ethanol (EtOH) in male rats. A devaluation score of 1.0 indicates no change from baseline consumption of saccharin, any score above 1.0 indicates an increase in consumption, while any score below 1.0 indicates a decrease. Nicotine administration had no effect on saccharin intake in saline-treated unconditioned controls (**A**). Conversely, nicotine diminished the degree of devaluation induced by increasing doses of EtOH (**B**). Nicotine-treated, relative to saline-treated, rats were significantly more accepting of saccharin following conditioning with 0.9, 1.8, and 2.1 g/kg EtOH, with a nonsignificant trend for increased acceptance at the 1.5 g/kg dose. In general, nicotine-treated rats were less sensitive to the devaluation induced by acute EtOH intoxication. * designates significant group difference ($ps < 0.05$).

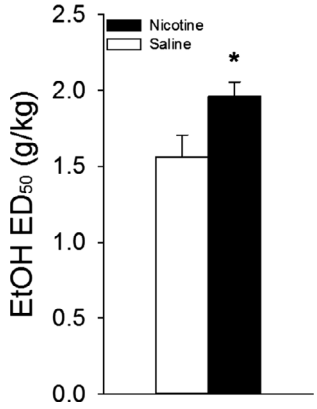

Fig. 6. Nicotine produced a significant rightward shift in the dose–response curve of ethanol (EtOH)-induced devaluation. Calculation of the ED_{50} of the curve from Fig. 5*B* revealed that nicotine pretreatment resulted in a significant rightward shift in the dose–response curve indicating that nicotine-treated rats were less sensitive to the stimulus properties of EtOH that produce stimulus devaluation. The dose that produced a 50% decrement in saccharin intake was ~1.5 g/kg in saline-treated rats compared to ~2.0 g/kg in nicotine-treated rats. * designates significant group difference ($p < 0.05$)

rightward shift in the dose–response curve indicating that acute nicotine administration reduced the sensitivity to the aversive physiological properties of concentrated EtOH, similar to what we have previously shown with morphine (Loney and Meyer, 2019).

Nicotine Increased Goal-Tracking in Response to an EtOH Cue

Regardless of previous history of EtOH drinking, nicotine facilitated goal-tracking toward the EtOH reward following presentation of a lever cue explicitly paired with EtOH (Fig. 3*A,B*). There was a trend for enhancement of sign-tracking, but only in rats that were experienced with EtOH. These data indicate that nicotine affects goal-tracking conditioned approach to an EtOH cue more strongly than sign-tracking, which is in contrast with studies of nicotine's effects on cues that predict nondrug reinforcers (but see Stringfield et al., 2017). We had originally hypothesized that nicotine would enhance sign-tracking to an EtOH cue because nicotine generally increases conditioned approach elicited by a combined auditory and visual Pavlovian EtOH cue (Maddux and Chaudhri, 2017) and specifically increases sign-tracking to other nondrug reinforcers (Palmatier et al., 2013; Versaggi et al., 2016). Furthermore, EtOH cues have previously been shown to elicit sign-tracking after extended conditioning (Srey et al., 2015). However, in our hands, nicotine did not significantly enhance sign-tracking in male rats and instead only affected goal-tracking. This may have been due to the technical features of our design. First, the mechanical noise emitted during the bottle insertion may have served as a CS that was more proximal to EtOH delivery than the lever CS. Indeed, previous research has shown that cues more proximal to reward delivery serve as better conditioned reinforcers than distal cues (Holland, 1980; Meyer et al., 2014; Tindell et al., 2005). This may have promoted conditioned responding to the bottle insertion at the expense of responding to the lever insertion. Second, while rats have been shown to discriminate between EtOH-predictive (CS+) and nonpredictive stimuli (Krank et al., 2008), it is possible that the task was too difficult for all rats to acquire the CS+ lever-EtOH association. Supporting this idea, only nicotine-treated rats consistently discriminated between extensions of the CS+ and CS− as measured by bottle contacts during lever extensions (Fig. 3*A,B*). This is in line with prior research demonstrating that nicotine most consistently increases task

performance in cognitively unimpaired populations during challenging tasks (Hahn et al., 2002; Mirza and Stolerman, 1998; Stolerman et al., 2000), and thus, nicotine may have aided task acquisition here. Analyzing goal-tracking during weeks 5 to 7, once all groups had acquired discrimination between the CS+ and CS−, continued to reveal significantly heightened responding in the nicotine-treated rats relative to saline-treated rats. Similarly, removal of nicotine resulted in similar responding among the groups, albeit goal-tracking was beginning to decrease in the nicotine-treated rats. Unfortunately, our experimental design does not allow us to explicitly dissociate effects that nicotine may be having on acquisition of conditioning responding as nicotine was administered from the start of the test.

Nicotine had Marginal Effects on Sign-Tracking

Nicotine treatment tended to promote sign-tracking to the CS+ more than the CS−, although this effect was transient and not statistically significant (Fig. 4). Therefore, it may have been that nicotine's effects on sign-tracking were obscured by the difficulty of the task. While we note that nicotine preferentially enhanced goal-tracking, relative to sign-tracking, in this study, there was some limited evidence for an effect of nicotine on sign-tracking in rats with a history of EtOH exposure (Fig. 4*B*). This coincides with the significant reduction in sign-tracking that occurred upon nicotine removal during week 8 (Fig. 4*B*). Ultimately, we did not find that EtOH cues uniformly promoted sign-tracking over extended conditioning as has been shown previously (Srey et al., 2015), although we did see a trend toward a decrease in goal-tracking that began around week 6. Interestingly, one other study looking at the effects of nicotine on PavCA elicited by a nondrug cue has also reported substantial increases in goal-tracking responses (Stringfield et al., 2017). Thus, nicotine may affect conditioned responding to both drug and nondrug cues by increasing approach toward an individual's prepotent cue (either the cue or the goal), as has been shown with opioid drugs (DiFeliceantonio and Berridge, 2012), and therefore, this form of conditioning may be highly susceptible to individual variation. Furthermore, we estimate that the rats were consuming ~0.5 to 0.6 g/kg of EtOH during the 1-hour conditioning sessions. As we did not measure BECs, this is an estimation based on the recorded number of licks (Table 1) elicited during the US period. These are relatively low doses, and so it is possible that other factors besides the pharmacological properties of EtOH may have contributed to PavCA. Moreover, the relatively low consumption of EtOH during conditioning, particularly in the naïve animals, may partially explain the low levels of sign-tracking behaviors. The development of sign-tracking toward EtOH-predictive cues may require higher doses of EtOH consumption, a conclusion supported by the finding that the only group that demonstrated notable sign-tracking responses was the nicotine-treated exposed rats which consumed the largest quantities of EtOH during the initial weeks of conditioning (Table 1).

Nicotine Reduced Sensitivity to Devaluation Induced by Acute EtOH Intoxication

We and others have previously shown that preexposure to nicotine blocks acquisition of EtOH-induced conditioned taste avoidance (Bienkowski et al., 1998; Kunin et al., 1999; Loney and Meyer, 2019; Rinker et al., 2011). Here, we demonstrate that nicotine is not simply blocking the ability to learn a CTA to EtOH-paired flavors, but rather that nicotine, relative to saline, reduces the sensitivity to a given dose of EtOH thus resulting in a significant rightward shift in the degree of devaluation conditioned by EtOH (Figs 5*B* and 6). It should be noted that acquisition of CTA occurs over repeated conditioning trials and that increasing doses of EtOH overlap with repeated pairings in our experimental design. Regardless, these data are in line with previous reports that nicotine reduces the aversive physiological consequences of acute EtOH intoxication in rodents (Rinker et al., 2011; Taslim et al., 2011) as well as reports that smokers feel less intoxicated by a given dose of EtOH relative to nonsmokers (Madden et al., 2000). Nicotine had no effect on the consumption of saccharin in our unconditioned control rats (Fig. 5A) and, furthermore, our previous data revealed that nicotine had no effect on a LiCl-induced CTA (Loney and Meyer, 2019) indicating that the effects of nicotine were specific to the EtOH dosing rather than a general effect on either taste processes or the ability to express a CTA. Lower doses of drugs that are readily self-administered by animals can condition an avoidance response to slightly less reinforcing stimuli, such as saccharin, when the two stimuli are presented in sequence. This form of successive contrast has been referred to as the reward-comparison hypothesis (Grigson, 2008). Given that our design employs saccharin as the conditioned stimulus, we cannot entirely rule out that the observed avoidance is, at least in part, mediated by the reinforcing properties of EtOH as opposed to its aversive qualities. Also, identical results were observed when sodium chloride (Loney et al., 2018) served as the conditioned stimulus, as well as with unsweetened Kool-Aid® flavors (unpublished observations) that have no inherent reinforcing value to laboratory rats, therefore the avoidance is likely generated by the aversive properties of EtOH as opposed to its reinforcing qualities. Furthermore, low doses of EtOH (<0.75 g/kg) have been shown to produce avoidance responses indicative of successive contrast while higher doses (~1.5 g/kg) produce avoidance responses that are more in line with aversion (Liu et al., 2009). Here, we report the ED_{50} in saline-treated rats as 1.5 g/kg while the ED_{50} in nicotine-treated rats is 2.0 g/kg, doses that would objectively be on the higher end of the dose–response curve. We have previously demonstrated that our nicotine treatment regimen obscures the interoceptive cues resulting from passive administration of drugs of abuse (Loney and Meyer, 2019). Drug taking is regulated by

interoceptive effects and facilitated by drug-associated external cues. Any reduction in the sensitivity to these interoceptive cues may enhance the reliance on external cues to spur drug seeking similar to the way nicotine self-administration is dependent on predictive external cues (Caggiula et al., 2001). This is consistent with our current finding that nicotine enhances goal-directed alcohol seeking regardless of previous drinking history, while simultaneously diminishing the impact of the aversive postingestive qualities of EtOH.

A reduction in the impact of the aversive consequences of acute EtOH intoxication following nicotine administration may be sufficient to explain the enhancement of the conditioned approach toward stimuli predictive of EtOH delivery by nicotine, regardless of previous experience with EtOH, that we report here (Figs 3 and 4). Specifically, by diminishing the impact of any aversive consequence of EtOH consumption, nicotine could be indirectly enhancing the reinforcing properties of EtOH consumption and thus engendering heightened approach to EtOH-predictive cues. These putative indirect effects would be in addition to the direct effects that nicotine has on the dopaminergic response to EtOH (Doyon et al., 2013), which likely independently drives increased motivational value of EtOH. One potentially important consideration is that we did not measure blood EtOH concentration (BEC) following *i.p.* injection of EtOH as a function of nicotine treatment, although a previous study has demonstrated that nicotine does not impact BEC following *i.p.* EtOH administration, rather only during intragastric administration of EtOH (Parnell et al., 2006). Regardless of mechanism or order of effects, drinking despite adverse consequences is a hallmark of alcohol-use disorders (AUDs; Tiffany and Conklin, 2000; Naqvi and Bechara, 2010; Seif et al., 2013) and we show that nicotine diminishes the aversive consequences of EtOH consumption as evidenced by a significant reduction in the devaluation induced by acute EtOH intoxication. Additional studies are currently being conducted to elucidate the parameters of this effect within this paradigm and other Pavlovian conditioning paradigms aimed at examining the aversive interoceptive qualities of EtOH including, but not limited to, conditioned place avoidance (CPA). We have previously demonstrated a lack of an effect of nicotine on EtOH-induced CPA (Loney et al., 2018), though within that study the time course from nicotine administration to place conditioning was much longer and therefore nicotine may have been less effective. In addition, it is currently unknown what, if any, affect that the modulation of cue reactivity by nicotine as reported here has on operant EtOH self-administration, particularly the development of habitual alcohol seeking that becomes resistant to devaluation (Corbit et al., 2012; Seif et al., 2013).

Nicotine Simultaneously Produces a High-Approach, Low-Avoidance Phenotype

Extrinsic and intrinsic cues paired with the reinforcing properties of alcohol can acquire motivational relevance and drive alcohol seeking, thus increasing the likelihood of relapse (Cannady et al., 2013; Hay et al., 2013; Jupp et al., 2011; Katner et al., 1999). Conversely, cues paired with the aversive physiological properties of alcohol can also acquire motivational relevance and drive active avoidance of alcohol consumption (Kiefer and Morrow, 1991; Liu et al., 2009; Seif et al., 2013; Sheth et al., 2017; Verendeev and Riley, 2013). Clinical models of cue-induced alcohol craving demonstrate that reactivity to reinforcing and aversive EtOH cues independently contribute to the motivational drive to consume EtOH (Breiner et al., 1999; Schlauch et al., 2015). Furthermore, a high-approach, low-avoidance phenotype is associated with heightened EtOH consumption and an intractable attitude toward AUD treatment (Hollett et al., 2017; Schlauch et al., 2015; Sharbanee et al., 2014; Stritzke et al., 2004). Here, we demonstrate that acute nicotine treatment, prior to EtOH administration, produces a high-approach, low-avoidance phenotype even in EtOH - naïve male subjects (Figs 3, 4 and 5*B*). As result, nicotine may be enhancing development and maintenance of, and subsequent relapse to, AUDs through enhancing the motivational drive triggered by alcohol-associated cues and simultaneously diminishing the impact of the aversive physiological properties of overconsumption. We, and others, have previously demonstrated that female rats are more sensitive to both the reinforcing properties of nicotine (Chaudhri et al., 2005) and the diminished avoidance of EtOH-paired cues induced by acute nicotine administration (Loney and Meyer, 2019). Therefore, future studies specifically designed to elucidate mechanisms contributing to enhanced sensitivity to nicotine in females and what contribution this may have on alcoholism liability in women are called for as these studies were not explicitly designed, nor powered to elucidate the effects of sex on responding to nicotine. Regardless, these data provide further support for smoking cessation as an important first step in successful treatment of AUDs.

FUNDING

This research was supported by a grant from the Office of the Director, National Institutes of Health, and the National Institute on Alcohol Abuse and Alcoholism (R01AA024112).

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