Alcohol 71 (2018) 47–55

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Nicotine affects ethanol-conditioned taste, but not place, aversion in a simultaneous conditioning procedure

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ARTICLE INFO

Article history:
Received 19 December 2017
Received in revised form
21 February 2018
Accepted 26 February 2018

Keywords:
Ethanol
Nicotine
Place aversion
Rat
Taste aversion
Simultaneous conditioning

ABSTRACT

The conditioned taste aversion (CTA) induced by ethanol is a key factor limiting ethanol intake. Nicotine, a drug co-consumed with ethanol, may decrease this aversion by modulating the unconditioned effects of ethanol or by disrupting the association between ethanol and its associated cues. This study analyzed ethanol-induced CTA and conditioned place aversion (CPA) in Long-Evans rats with subchronic exposure to nicotine. The rats were treated with nicotine (0.0 or 0.4 mg/kg) three times before conditioning (on lickometer training sessions 3, 4, and 5) and across conditioning days. During the conditioning the rats were given ethanol (1.3 g/kg) preceded and followed by presentation of a taste (NaCl) and tactile (rod or hole floors) conditioned stimulus (CS+), respectively. On CS– conditioning days, the rats were given vehicle and exposed to alternative stimuli. Three CTA and CPA testing sessions were then conducted. It was found that nicotine reduced ethanol-induced CTA and enhanced locomotor activity, but did not significantly modify the magnitude of ethanol-induced CPA. The effects of nicotine on CTA were observed during both conditioning and testing sessions, and were specific to the NaCl CS+, having no effect on reactivity to water. The dissociation between the effect of nicotine on ethanol-induced CTA and CPA suggests that nicotine does not alter ethanol's motivational properties by generally increasing its positive rewarding effects, nor does it blunt all aversive-like responses to this drug. Instead, nicotine may impede ethanol-induced CTA induced by ethanol by disrupting the neural underpinnings of this specific form of associative learning.

Introduction

Alcohol (ethanol) has reinforcing properties, but these can be mitigated by its aversive motivational effects (e.g., dysphoria, gastrointestinal distress). In preclinical models, these aversive properties are often measured via conditioned taste or place aversion paradigms (CTA and CPA, respectively), during which avoidance of a taste or chamber paired with the effects of ethanol is considered an index of the aversive effects of the drug (Acevedo, Nizhnikov, Spear, Molina, & Pautassi, 2013). These studies also suggest that reduction in the sensitivity to the aversive effects of ethanol modulates the transition to escalated alcohol intake. Adolescent rats, for instance, drink significantly greater amounts of ethanol than adult rats (Doremus, Brunell, Rajendran, & Spear, 2005), a response which is correlated with a significantly reduced CTA induced by ethanol. Further, an exacerbated response to the aversive effects of ethanol is associated with very low levels of ethanol drinking – as shown by rats and humans with deficient breakdown of acetaldehyde (a metabolite of ethanol) (Peana et al., 2017). It is thus important to analyze which factors affect ethanol-induced aversion.

Nicotine, a psychoactive agent widely co-consumed with ethanol (Anthony & Echeagaray-Wagner, 2000; Falk, Yi, & Hiller-Sturmhöfel, 2006), likely diminishes the aversive properties of ethanol. For example, Zarrindast, Meshkani, Rezayof, Beigzadeh, and Rostami (2010) found conditioned place preference after the central co-administration of nicotine and ethanol, but not after the administration of either drug alone. Nicotine administration attenuated (Bienkowski, Piasecki, Koros, Stefanski, & Kostowski,

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https://doi.org/10.1016/j.alcohol.2018.02.005
0741-8329/

1998) or blocked (Kunin, Smith, & Amit, 1999) the acquisition of CTA by ethanol while also reducing the hypothermic effects of ethanol (Rinker et al., 2011), an aversive property necessary for the establishment of ethanol-induced CPA (Dickinson & Cunningham, 1998). In other studies, nicotine heightened the locomotor stimulant effect of ethanol in mice with a genetic propensity for ethanol-induced stimulation (Gubner, McKinnon, Reed, & Phillips, 2013). Additionally, the development, albeit not the expression, of ethanol-induced behavioral sensitization was significantly greater when the drug was co-administered with nicotine (Gubner & Phillips, 2015). These studies (Gubner et al., 2013; Gubner & Phillips, 2015) suggest that nicotine and ethanol act synergistically on common neurobehavioral mechanisms that promote the transition from drinking to AUD (Camarini & Pautassi, 2016).

Nicotine may also affect ethanol-induced aversion by disrupting normal neurotransmission patterns at the basal forebrain, hippocampus, and prefrontal cortex (Placzek & Dani, 2009; Placzek, Zhang, & Dani, 2009). For instance, acquisition of CTA depends on the ability for novel tastes to trigger acetylcholine (ACh) release from the nucleus basalis of Meynert to several neocortical areas critical for acquisition of taste learning integration (particularly the insular cortex; Rodriguez-Garcia & Miranda, 2016), and nicotine significantly modulates these pathways (Arnold, Nelson, Sarter, & Bruno, 2003; Sato, Kawano, Yin, Kato, & Toyoda, 2017). Thus, it is possible that nicotine administration disrupts the acquisition of an association between taste stimuli and their post-ingestive consequences, regardless of any effect on the overall aversive properties of ethanol, and therefore results in the observed attenuation of ethanol-induced CTA (Bienkowski et al., 1998; Kunin et al., 1999; Rinker et al., 2011).

The present study analyzed the effects of subchronic exposure and pre-treatment with nicotine on simultaneously induced ethanol-induced CTA and CPA, in which the conditioned stimulus (CS+) was sodium chloride solution or a tactile floor cue, respectively, and the unconditioned stimulus (US) was the same injection of ethanol (1.3 g/kg). The rats were given nicotine (0.4 mg/kg) three times before commencement of conditioning, and every day – including CS– sessions – during conditioning. We expected this procedure to yield ethanol-induced CTA and CPA. We hypothesized that if nicotine affects ethanol reinforcement by increasing its rewarding properties, then nicotine would reduce CPA. Conversely, if nicotine specifically affects the neural mechanisms responsible for the association of tastes and ethanol aversion, we expected CTA by ethanol to be selectively affected.

Material and methods

Experimental design

A 2 (nicotine treatment: 0.0 or 0.4 mg/kg nicotine) × 2 (ethanol treatment: 0.0 g/kg or 1.3 g/kg on CS+ trials) factorial design was used. The conditioned stimulus (CS+) was sodium chloride solution or a tactile floor cue for CTA and CPA, respectively, and the unconditioned stimulus (US) was the same injection of ethanol (1.3 g/kg) for both CTA and CPA. All rats received vehicle (i.e., 0.0 g/kg ethanol) on CS– trials. The groups that received alternating ethanol/vehicle injections on CS+ and CS– trials will be referred to as *experimental* CTA/CPA groups, whereas those that received vehicle on both trials will be described as *control* CTA/CPA groups. Note that nicotine was given on CS+ *and* CS– days in an attempt to ameliorate the formation of nicotine-CS or nicotine-ethanol associations and to more closely match the experimental procedures employed in similar studies demonstrating nicotine-induced reduction in the strength of CTA conditioned by ethanol (Kunin et al., 1999). Sample sizes were 12–13 rats per group.

Subjects and housing

Forty-nine adult male Long-Evans rats (Envigo, Haslett, Michigan) weighing approximately 350 g upon arrival were individually housed in polycarbonate cages in a temperature- and humidity-controlled environment on a reverse 12:12-h light cycle. Following acclimatization, the rats were weighed and handled daily for three consecutive days prior to commencement of pre-training procedures. All rats were maintained on ~23-h water deprivation schedule throughout training and testing in order to facilitate stimulus consumption. Food was provided *ad libitum* (standard rodent chow, Envigo 2018). Throughout the experiment, the rats were identified through tail marks made by permanent marker. Experimental procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee and complied with the regulations of the Guide for Care and Use of Laboratory Animals (NIH Publications No. 80–23; revised 1996). The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

Drugs

(-)-Nicotine hydrogen tartrate (Sigma Aldrich, St. Louis, Missouri) was dissolved in saline at a final concentration of 0.4 mg/mL (expressed as the free base), and the pH was adjusted to 7.2–7.4 with sodium hydroxide and was injected subcutaneously at a dose of 0.4 mg/kg. Ethanol was the unconditioned stimulus (US) in the CTA and CPA procedures. Two hundred proof ethanol was diluted with saline to a final concentration of 16% v/v and was administered intraperitoneally, at a volume of 10 mL/kg, yielding a dose of 1.3 g/kg. The intraperitoneal injections were performed in less than 10 s and were targeted between the diaphragm and the genitalia. Controls were administered isovolumetric injections of the vehicle solution (0.9% v/v saline). Sodium chloride (NaCl, Sigma Aldrich, St. Louis, Missouri) served as the CS in the CTA procedure, and was dissolved in tap water. A 0.1 M NaCl solution was used during the acquisition of CTA, whereas a range of concentrations (0.01, 0.1, 0.2, 0.3, 0.6, and 1.0 M) was employed during CTA expression sessions.

Conditioned taste and conditioned place aversion procedures

The procedure, depicted in Table 1, included five lickometer training sessions that served to train the rats to drink from a lickometer, a CPA pre-test session, eight simultaneous CTA/CPA conditioning sessions (i.e., four alternating days of CS+ and CS– [conditioning days 2, 4, 6, and 8] presentations for eight total days of conditioning), and three expression sessions. There was no counterbalancing or randomization of the sequence of CS+ and CS– days. All *experimental* rats received pairings of NaCl and a texture with ethanol's effects on conditioning days 1, 3, 5, and 7 (CS+ days) and were given access to just water and exposed to an alternative texture on conditioning days 2, 4, 6, and 8 (CS– days). *Control* rats were administered vehicle across all days.

A Davis rig (Davis MS-160, DiLog Instruments, Tallahassee, Florida) served as the lickometer used to deliver the taste stimuli during the CTA procedures. This apparatus consists of a polycarbonate cage coupled to a motorized table that contains several fluid reservoirs; access to each fluid reservoir is occluded by a computer-controlled shutter. Licks to the various taste stimuli were recorded via a contact lickometer and were compiled using the Davis rig software.

Sixteen conditioned place apparatus (previously described in Cunningham, Tull, Rindal, & Meyer, 2002) were used during CPA conditioning. Two tactile cues were stainless-steel “rod” or perforated metal “hole” interchangeable floor halves that could be

Table 1
Methods for the analysis of simultaneous acquisition of ethanol-induced conditioned taste aversion and conditioned place aversion, in rats pre-treated or not pre-treated with nicotine.

	Lickometer training sessions 1–5	Place conditioning pre-test session	CS+ sessions (conditioning days 1, 3, 5, and 7)	CS− sessions (conditioning days 2, 4, 6, and 8)	Expression (testing) sessions 1, 2, and 3
Nicotine Treatment (0.0 or 0.4 mg/kg)	Only on sessions 3, 4, and 5	NO	YES	YES	NO
Fluid exposure in Davis rig	Water	None	0.1 M NaCl	Water	NaCl (0.01, 0.1, 0.2, 0.3, 0.6, and 1.0 M) or Water
Ethanol Treatment (0.0 or 1.3 g/kg)	NO	All rats received vehicle	YES	All rats received vehicle	Vehicle
Floor texture on place conditioning apparatus	None	Half rod, half hole	CS+ only (rod or hole, counterbalanced)	CS− only (rod or hole, counterbalanced)	Half rod, half hole

The rats were trained in the use of the lickometer across 5 sessions. A place conditioning pre-test session was conducted 72 h after the last pre-conditioning session. They were then given 8 conditioning sessions. Across all conditioning days, the rats were injected with 0.0 or 0.4 mg/kg nicotine, 15 min prior to the commencement of conditioning. In four of the conditioning sessions (CS+ conditioning days), the administration of ethanol or vehicle was preceded and followed by stimulation with taste and tactile conditioned stimuli (CS), respectively. In CS− conditioning days, all of the rats were administered 0.0 g/kg ethanol (i.e., vehicle).

placed below each apparatus. Time spent on the rod and hole floors and distance traveled were acquired by digital cameras and analyzed in real time via Top Scan software (Cleversys Inc., Reston, Virginia).

Lickometer training sessions

Taste conditioning acquisition and testing sessions took place in the automated Davis rig lickometer (Table 1). Prior to the conditioning sessions, the rats were acclimated to, and trained to consume fluid from, the lickometer across five sessions (one per day, total session time: 30 min), as described previously (Loney, Blonde, Eckel, & Spector, 2012). Briefly, on the first training day the rats consumed water *ad libitum* from a stationary fluid reservoir for 30 min. On the second day, the rats were acclimated to the opening and closing of the shutter in which the water reservoir was available for 30-sec periods followed by a 10-sec intertrial interval. On the third day, the water reservoir was available for 30 s, followed by a 1-sec presentation of another water reservoir, which would serve as the water rinse during the expression tests (see below), and followed again by a 10-sec intertrial interval. On the fourth and fifth days of acclimation, the water reservoir access time was shortened to 10 s, followed by 1-sec access to another water reservoir and, finally, a 10-sec intertrial interval. Rats could initiate as many trials as possible during each of the 30-min training days.

Nicotine treatment

The aim of the present work was specifically to assess how subchronic exposure to nicotine affects the ability of ethanol to serve as an US during simultaneous taste and place aversion conditioning (Table 1). The chronic nicotine pre-exposure and pre-treatment was as follows: a) on lickometer training sessions days 3, 4, and 5, the rats were injected with their assigned nicotine treatment (0.0 or 0.4 mg/kg nicotine, 15 min prior to placement in the Davis rig), b) during conditioning days 1–8, the rats were injected with their assigned nicotine treatment (0.0 or 0.4 mg/kg nicotine) 15 min prior to the commencement of the conditioning procedures. The dose of nicotine was selected based on recent studies that assessed the effects of nicotine pre-exposure (Madayag, Czarnecki, Wangler, & Robinson, 2017) or pre-treatment (Maddux & Chaudhri, 2017) upon ethanol seeking in rats. This regimen of nicotine dosing was not only chosen to measure the chronic effects of nicotine treatment but also served to ensure that nicotine did not serve as an US in the CTA and CPA conditioning, as animals received substantial non-contingent exposure to nicotine outside of conditioning trials.

Place conditioning pre-test and CTA/CPA conditioning procedures

Seventy-two hours after the last lickometer Training session, a place conditioning pre-test session was conducted to determine any unconditioned preference for the hole or rod stimulus. During this session, the rats were injected with saline (1.0 mL/kg) and then were gently placed in the place conditioning apparatus for 30 min (Table 1). During this session, half of the floor of the apparatus was covered with the rod surface, whereas the other half featured the hole floor.

Across all 8 conditioning days, following nicotine administration, the rats were placed in the Davis rig and allowed to freely consume 0.1 M NaCl (CS+ days) or water (CS− days) for 30 min. They were then injected with their assigned US (0.0 or 1.3 g/kg ethanol) and immediately transferred to the place conditioning apparatus for another 30 min. Rats in the *experimental* group were given alternate administrations of 1.3 g/kg ethanol or vehicle, in that they were given ethanol on CS+ days (conditioning days 1, 3, 5, and 7) and vehicle on CS− days (conditioning days 2, 4, 6, and 8). Rats from the *control* group were given vehicle across all eight sessions.

During the place conditioning sessions, the floor of the place conditioning apparatus was homogeneously covered with either the rod or hole floor. Tactile stimuli were counter-balanced across all experimental groups, by half of the *experimental* rats receiving ethanol in the hole floor and vehicle in the rod floor, whereas half received ethanol in the rod floor and vehicle in the hole floor. Subsequently, we will refer to the compartments paired with ethanol and vehicle administration as the CS+ and CS−, respectively. Following the 30-min taste access and subsequent US injection, the rats were then immediately transferred to the place conditioning chambers for 30 min.

CTA and CPA expression sessions

Three expression sessions were conducted (Table 1). The first began 24 h after termination of conditioning. Rats were first tested for responsiveness toward various NaCl concentrations, and then they were immediately tested for preference/aversion (i.e., time spent) toward the rod and hole floors in the place conditioning apparatus (test length: 30 min).

To test the expression of CTA, rats were given access to randomized concentrations of NaCl (0.01, 0.1, 0.2, 0.3, 0.6, and 1.0 M) or water for 30 min in the Davis rig. Expression testing procedures were identical to the final pre-conditioning training sessions, in that rats were tasked with licking to a concentration of NaCl for 10 s, followed by a 1-sec water rinse, and then a 10-sec intertrial

interval before a new, random concentration of NaCl was presented. Rats were free to initiate as many trials as possible during the 30-min test session. Immediately after termination of the CTA test, the rats were administered with vehicle and placed into the place conditioning apparatus. Half of the floor of the apparatus was covered with the grid floor and the other half was covered with the rod floor.

Data analysis

CTA acquisition data (i.e., conditioning days 1–8) are expressed as the total licks emitted in response to the taste stimuli (CS+ or CS−) during each 30-min session. To make direct comparisons in the strength of conditioning between nicotine- and saline-treated rats, CS+ and CS− intake data of conditioned rats were transformed into a relative intake score (i.e., licks emitted in response to the stimuli relative to the licks emitted on that same conditioning day by that group's respective unconditioned control group). Specifically, the intake (i.e., number of licks to the CS+ or CS−) from each individual rat conditioned with ethanol was divided by the average intake of its pertinent (nicotine- or saline-treated) control (i.e., treated with saline) group. The rationale was to control for the significant reduction in baseline fluid intake induced by the nicotine treatment.

CTA expression data (i.e., scores on expression days 1, 2, and 3) were collapsed across sessions and transformed to a lick ratio (average licks to a given concentration of NaCl divided by that same rat's average licks to water) to control for differences in local lick rate across the brief-access trials, as well as individual differences in the motivation to rehydrate. Conditioned place aversion scores were expressed as total time (sec) spent on the ethanol-paired floor (CS+), and as pre/post-test difference scores (i.e., time spent on CS+ during post-test minus time spent on CS+ during the pre-test). As such, place aversion scores reflected time spent on the ethanol-paired floor tactile stimulus.

CTA conditioning licks were analyzed with a four-factor mixed ANOVA. Nicotine treatment (0.0 or 0.4 mg/kg nicotine) and ethanol treatment (1.3 or 0.0 g/kg ethanol) were the between-group factors, whereas CS (0.1 M NaCl or water) and day served as within-factors. The relative intake scores were analyzed with a three-factor mixed ANOVA. Nicotine treatment was the between-group factor, and CS and conditioning day were the within-subjects factors. CTA expression data were analyzed by an independent factorial ANOVA (between groups: nicotine treatment and ethanol treatment). The dependent variable was licking response to the NaCl concentration employed during the CS+ conditioning session (i.e., the training 0.1 M concentration). A three-way mixed ANOVA analyzed the lick ratios for the different NaCl concentration employed during expression sessions. Nicotine treatment and ethanol treatment served as the between-subjects factors, and NaCl concentration was the within-subjects factor.

Distance traveled in the place conditioning chambers during the pre-test was analyzed via an independent *t* test (grouping factor: nicotine treatment), whereas distance traveled during CS+ and CS− sessions was independently analyzed via repeated-measures ANOVA. Nicotine treatment and ethanol treatment served as between-factors, whereas sessions (CS+ session 1, 2, 3, and 4; or CS− session 1, 2, 3, and 4) were the repeated-measures. Total time (sec) spent on the floor stimulus paired with ethanol (CS+) and pre-test difference scores, across the three CPA expression sessions, were assessed through independent ANOVAs. Each analysis considered nicotine treatment and ethanol treatment as between-factors, whereas expression sessions (tests 1, 2, and 3) were the repeated-measures. The alpha level was 0.05 and the loci of the significant main effects or significant interactions were analyzed via Tukey's or Bonferroni corrected *t* test *post hoc* comparisons where appropriate.

Results

CTA scores on conditioning sessions

Pre-treatment with nicotine interfered with acquisition of an ethanol-induced CTA. The ANOVA revealed main effects of nicotine treatment ($F_{(1,45)} = 5.28$, $p < 0.05$; CS $F_{(1,45)} = 97.05$, $p < 0.0001$) and day ($F_{(3,135)} = 21.96$, $p < 0.0001$), and a significant nicotine treatment × CS × ethanol treatment interaction ($F_{(1,45)} = 5.84$, $p < 0.05$). As shown in Fig. 1A, and revealed by the *post hoc* tests, among nicotine-treated rats, those conditioned with ethanol did not differ from their unconditioned controls in the consumption of the 0.1 M NaCl CS+, whereas vehicle-treated conditioned rats consumed significantly less of the CS+ than their unconditioned controls. *Post hoc* analyses revealed no significant differences in CS+ intake between nicotine- and vehicle-treated rats conditioned with ethanol. Neither nicotine- nor vehicle-treated rats conditioned with ethanol differed from their unconditioned controls in the consumption of the water CS− (Fig. 1B). Planned comparisons revealed that nicotine-treated unconditioned control rats did not decrease their intake from day 1 to day 4 ($F_{(1,45)} = 0.03$, $p = 0.86$), indicating that nicotine alone, when administered prior to testing, was not conditioning any aversion to the CS+ that affected its intake.

The analysis of the relative intake scores in ethanol-treated animals revealed a similar pattern of results. The ANOVA yielded a significant nicotine treatment × CS × day interaction ($F_{(3,66)} = 5.92$, $p < 0.01$). The Bonferroni corrected *post hoc* analyses indicated that nicotine-treated rats drank significantly more of the CS+ on conditioning day 2 than saline-treated rats (Fig. 2), with a trend for significant differences on day 3, which ultimately did not survive correction for multiple comparisons ($p = 0.017$). The relative intake of the CS− was similar across days and between nicotine- and vehicle-treated groups.

CTA scores on expression sessions

The ANOVA conducted on licking responses to the conditioned concentration (0.1 M NaCl) yielded a significant nicotine treatment × ethanol treatment interaction ($F_{(1,45)} = 4.44$, $p < 0.05$, Fig. 3, right panel). The *post hoc* analyses revealed similar licking to the CS+ in nicotine-treated conditioned rats and in their unconditioned controls. On the other hand, vehicle-treated rats conditioned with ethanol licked significantly less to the training CS+ concentration than their unconditioned controls ($p < 0.01$) and significantly less than the nicotine-treated unconditioned controls ($p < 0.05$). The difference in licking between nicotine- and vehicle-treated ethanol conditioned rats at the 0.1 M concentration was not significant (i.e., did not survive correction for multiple comparisons).

Examining the licking responses to multiple NaCl following conditioning revealed that prior conditioning with ethanol suppressed licking to NaCl as a function of its concentration. The mixed ANOVA revealed a main effect of ethanol treatment ($F_{(1,45)} = 30.64$, $p < 0.001$) and a significant ethanol treatment × CS concentration interaction ($F_{(5,225)} = 7.90$, $p < 0.0001$, Fig. 3, left panel).

Locomotion scores during CPA pre-test, conditioning, and testing sessions

Locomotion scores (distance traveled, mm) during the pre-test session were similar ($t_{47} = -1.13$, $p > 0.20$) in rats given nicotine

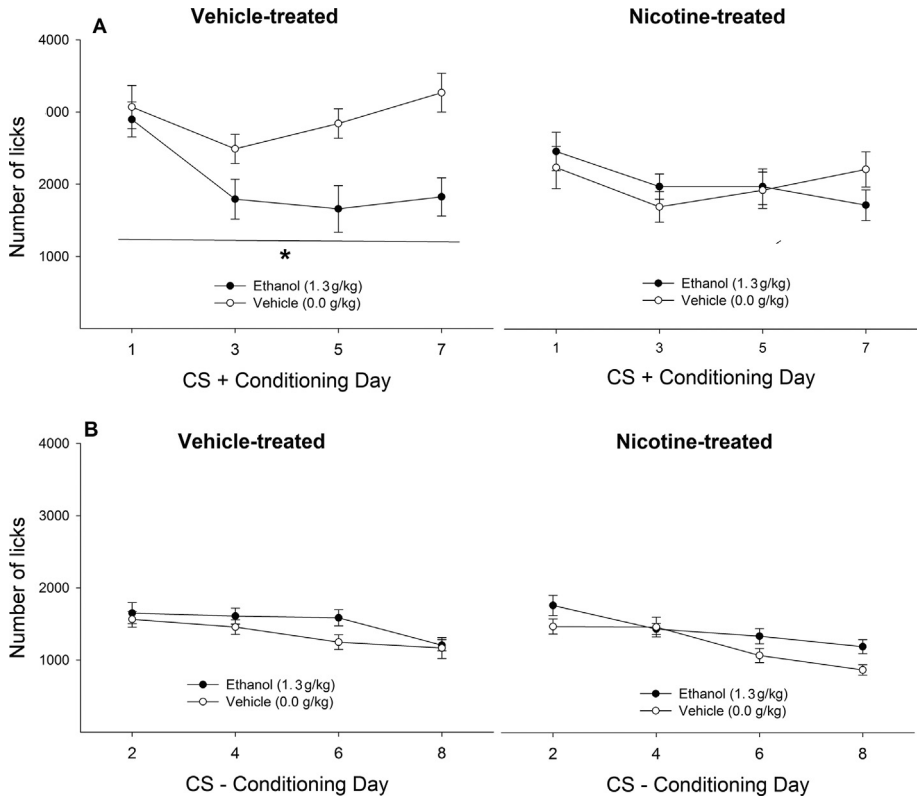

Fig. 1. Pre-treatment with nicotine interfered with acquisition of an ethanol-induced CTA. Number of licks to the NaCl solution associated with ethanol's effects (conditioned stimulus [CS+], conditioning days 1, 3, 5, and 7, Panel A), and number of licks emitted to the alternative stimulus (Water) used in CS− conditioning days (days 2, 4, 6, and 8; Panel B), as a function of nicotine treatment (0.0 [vehicle] or 0.4 mg/kg) and ethanol treatment (0.0 or 1.3 g/kg) during CS+ conditioning days. The asterisk indicates that, among vehicle-treated rats, the conditioned subjects consumed significantly less of the CS+ than their unconditioned controls. Please refer to the text for a full account of the statistical analyses. Values express mean ± SEM.

(M = 25,368.49 ± 1334.54) or vehicle (M = 28,345.83 ± 2219.77) during the pre-conditioning procedures.

Fig. 4 depicts locomotion in the compartments during the CS+ and CS− conditioning sessions. The ANOVA for CS+ scores revealed independent, significant main effects of nicotine treatment ($F_{(1,45)} = 6.13$, $p < 0.05$) and ethanol treatment ($F_{(1,45)} = 14.26$, $p < 0.001$), as well as a significant nicotine treatment × conditioning session interaction ($F_{(3,135)} = 3.48$, $p < 0.05$). Rats treated with nicotine exhibited, according to the *post hoc* tests, significantly greater distance traveled than vehicle-treated counterparts in CS+ sessions 2, 3, and 4, whereas rats given ethanol had significantly lower distance traveled, across trials, than those administered vehicle. The interaction between nicotine and ethanol treatment was not significant and the depressing effect of ethanol was similar across CS+ trials 1–4. Locomotion during CS− trials 2, 3, and 4 was significantly higher in nicotine-treated rats than in counterparts given vehicle (significant main effect of nicotine treatment and significant interaction between nicotine treatment and conditioning session: $F_{(1,45)} = 2.11$, $p < 0.0001$ and $F_{(1,135)} = 3.60$, $p < 0.05$, respectively), a pattern that was not affected by ethanol treatment.

The ANOVA for distance traveled at CPA expression testing sessions 1, 2, and 3 did not yield significant main effects nor significant interactions (descriptive data not shown).

CPA scores during testing sessions 1–3

The analysis of total time (sec) spent on the floor stimulus paired with ethanol (CS+) and pre-test difference scores yielded very similar results. Both analyses revealed that rats given CS+ -ethanol pairings spent less time in the CS+ than controls given vehicle (significant main effect of ethanol treatment, $F_{(1,45)} = 8.15$ and $F_{(1,45)} = 5.43$, respectively; $p < 0.05$). These results, indicative of CPA by ethanol, were similar in rats kept under nicotine treatment and control conditions, and were similarly expressed in testing sessions 1, 2, and 3. The data for CPA scores during tests 1, 2, and 3 can be observed in Fig. 5. No significant main effect of nicotine treatment or session, or significant interactions involving these factors was found.

Discussion

Altered sensitivity to the appetitive and aversive effects of ethanol is a significant predictor of liability for AUD. Subjects at risk for AUD due to familial history of alcoholism are more sensitive to the positive, reinforcing effects of ethanol that take place during the initial, rising limb of the blood ethanol curve (Caneto, Pautassi, & Pilatti, 2018). On the other hand, greater sensitivity to ethanol-induced aversion has been associated with reduced propensity for alcohol drinking (Schramm-Sapyta et al., 2010). In the present study we analyzed how nicotine, which is often consumed in conjunction with ethanol and sometimes preceding it in terms of first age of use, affects subsequent taste and place learning mediated by the aversive effects of ethanol.

The main result of the present work is that nicotine administration reduced ethanol-induced conditioned taste aversion, but did not significantly modify the magnitude of ethanol-induced place aversion. The effects of nicotine on CTA induced by ethanol were observed during acquisition and expression of the taste aversion learning, and were specific to the NaCl CS+, having no effect on responsivity to water. It could be proposed that nicotine

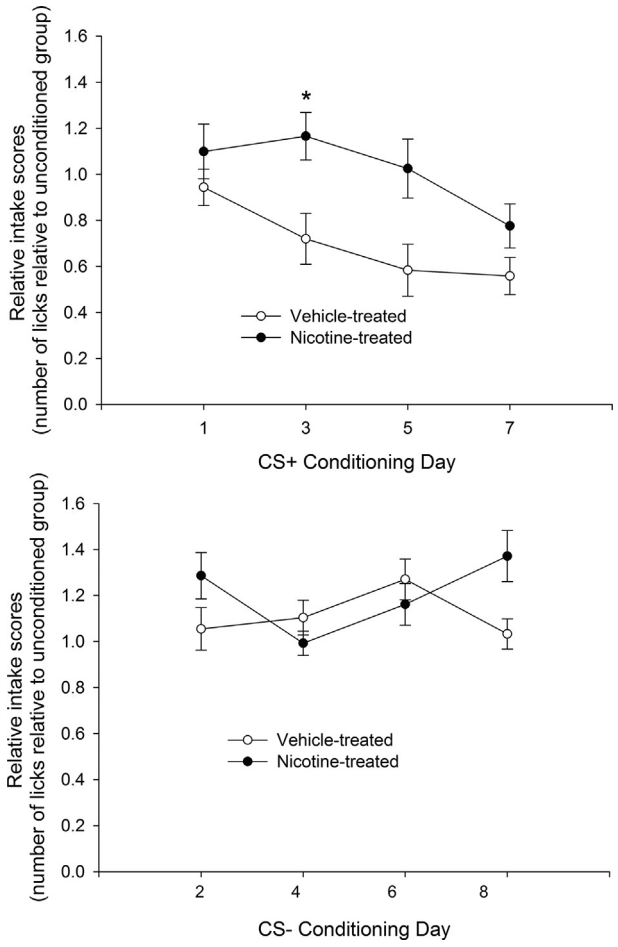

Fig. 2. Pre-treatment with nicotine interfered with acquisition of an ethanol-induced CTA. Relative intake scores (i.e., licks emitted in response to the stimuli relative to the licks emitted on that same conditioning day by that group's respective unconditioned control group) to the NaCl solution associated with ethanol's effects (conditioned stimulus [CS+], conditioning days 1, 3, 5, and 7), and number of licks emitted to the alternative stimulus (Water) used in CS− conditioning days (days 2, 4, 6, and 8; Panel B), as function of nicotine treatment (0.0 [vehicle] or 0.4 mg/kg) during CS+ conditioning days, in conditioned (i.e., ethanol-treated) rats. The asterisk indicates that, on conditioning day 2, the relative intake CS+ scores of nicotine-treated rats were significantly greater than were those of saline-treated rats. There was a trend for group differences on day 3, but this effect did not survive correction for multiple comparisons. Please refer to the text for a full account of the statistical analyses. Values express mean ± SEM.

exerted aversive unconditional effects that resulted in the development of nicotine-induced CTA in nicotine-treated vehicle rats. Nicotine treatment, however, did not significantly alter the amount of either CS+ or CS− consumed across conditioning days in unconditioned control rats, indicating that the effects of nicotine on ethanol-induced CTA were likely not the result of a competing conditioned avoidance response resulting from this level of nicotine pre-treatment.

The dissociation between the effect of nicotine on CTA and CPA by ethanol suggests that nicotine does not simply alter ethanol's motivational properties by producing a general increase in its positive rewarding effects. Nor does it globally reduce the deleterious properties of ethanol in a way that blunts all aversive-like responding to ethanol. Instead, the specificity of the interference indicates that nicotine may impede CTA induced by ethanol by disrupting the neural underpinnings of this specific form of associative learning. For instance, nicotine disrupts the formation of long-term potentiation at insular cortex pyramidal neurons (Sato et al., 2017), and this form of synaptic plasticity could be critical for the acquisition of CTA.

Our interpretation of the effects of nicotine, however, is based on the assumption that the aversive post-ingestive effects of ethanol that condition a taste aversion are identical to those that condition a place aversion. There is a dissociation in the stimuli that will condition viable taste and place aversions. Specifically, exteroceptive stimuli, such as foot shock, are capable of conditioning place aversions while having little to no impact on taste aversions (Holder, Yirmiya, Garcia, & Raizer, 1989; Rusiniak, Palmerino, Rice, Forthman, & Garcia, 1982; Schafe, Fitts, Thiele, LeDoux, & Bernstein, 2000). For instance, it could be that nicotine treatment affects the processing of the interoceptive aversive cues associated with administration of ethanol, while preserving peripheral pain associated with injections of relatively highly concentrated ethanol solutions, therefore interfering with CTA but not CPA. With this in mind, we chose a dose of ethanol believed to condition both CTA and CPA due to its aversive qualities, as opposed to its reinforcing qualities, as suggested with CTA induced by other drugs of abuse, such as cocaine (Liu, Showalter, & Grigson, 2009).

Another potential caveat inherent to our experimental design is the difference in delay between nicotine treatment and exposure to the two conditioned stimuli (i.e., taste, followed by tactile, stimuli). It is possible that nicotine may have interfered with acquisition of CPA if administered more proximal to CPA conditioning. We were explicitly interested in testing acquisition of CTA and CPA in the same rats and conditioning trial. The nature of these two conditioning procedures precludes the ability to counter-balance order of conditioning. Specifically, CTA optimally involves trace

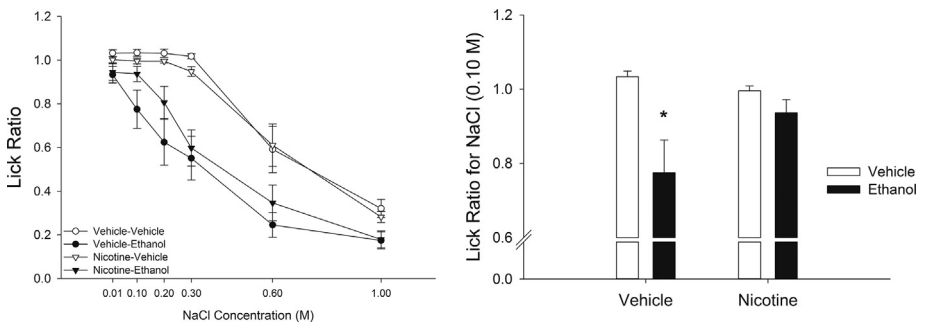

Fig. 3. Nicotine blocked the expression of ethanol conditioned taste aversion to the training concentration of NaCl. Left panel: lick ratio (average licks to a given concentration of NaCl divided by that same rat's average licks to water, collapsed across testing sessions 1–3), as a function of nicotine treatment (0.0 [vehicle] or 0.4 mg/kg) and ethanol treatment (0.0 or 1.3 g/kg) during CS+ conditioning days. Right panel: licking response to the 0.1 M NaCl concentration, which was the concentration paired with the aversive effects of ethanol during conditioning. The asterisk indicates a significant difference ($p < 0.05$) between Vehicle-Ethanol and Vehicle-Vehicle rats. Values express mean ± SEM.

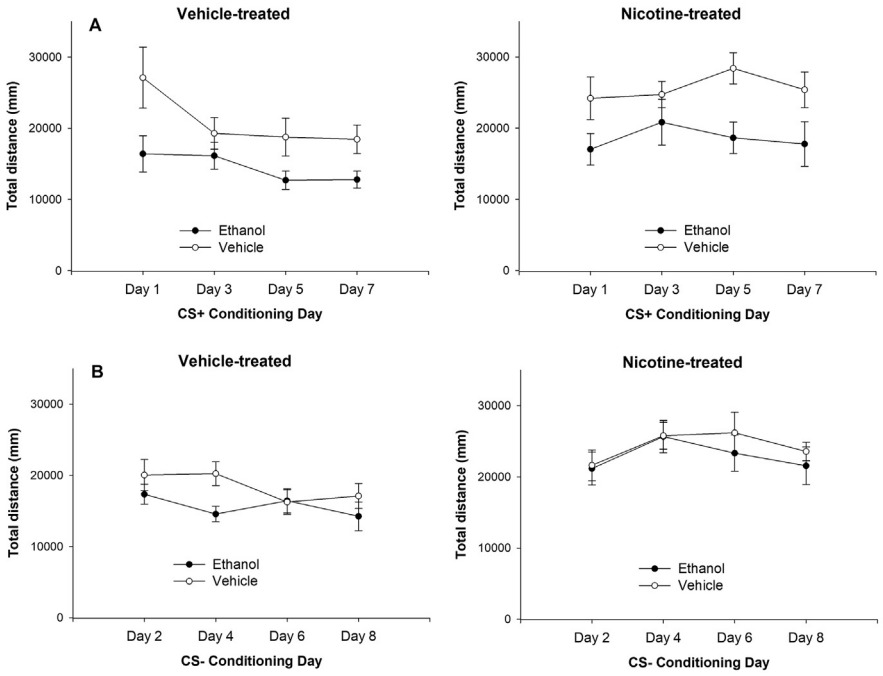

Fig. 4. Nicotine similarly increases saline- and ethanol-induced locomotion. Distance traveled (mm) during conditioning days 1, 3, 5, and 7 (CS+ days, panel A) and conditioning days 2, 4, 6, and 8 (CS− days, Panel B), as function of nicotine treatment (0.0 [vehicle] or 0.4 mg/kg) and ethanol treatment (0.0 or 1.3 g/kg) during CS+ conditioning days. Please refer to the text for an account of the statistical analyses. Values express mean ± SEM.

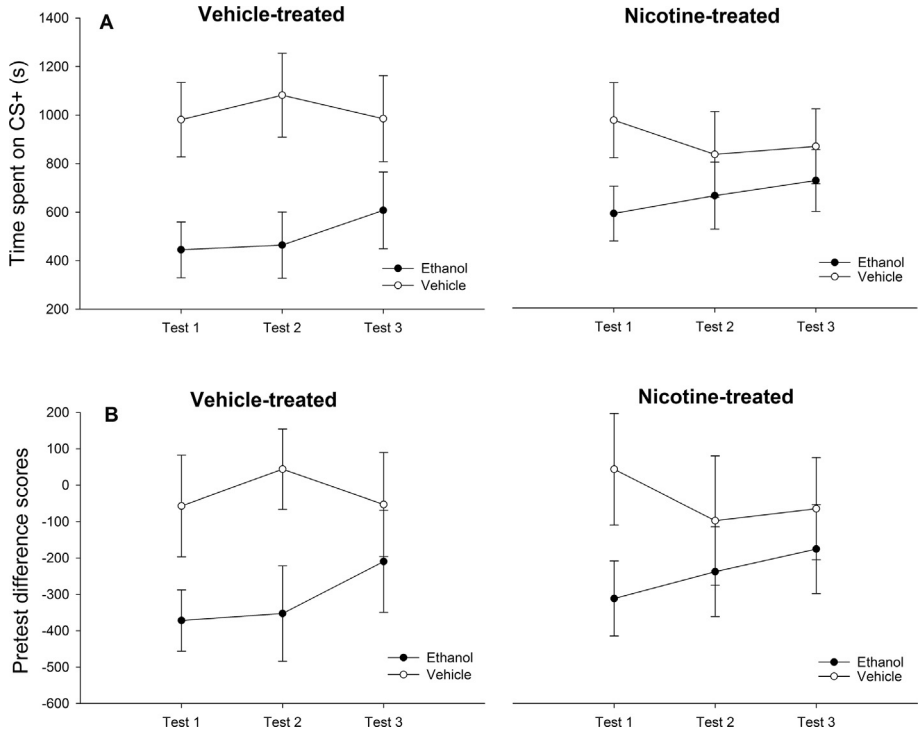

Fig. 5. Nicotine did not significantly affect ethanol-induced place aversion (CPA). Time spent (in sec, panel A) in the chamber associated with ethanol's effects (conditioned stimulus [CS+]) and pre-test difference scores (panel B), during CPA test sessions 1, 2, and 3, as a function of nicotine treatment (0.0 [vehicle] or 0.4 mg/kg) and ethanol treatment (0.0 or 1.3 g/kg) during CS+ conditioning days. Please refer to the text for an account of the statistical analyses. Values express mean ± SEM.

conditioning while CPA/CPP requires simultaneous conditioning (Shetty, Rutledge, & Forster, 2017). Previous research (Kunin et al., 1999) demonstrated that nicotine, administered 60 min prior to CS access (80 min prior to ethanol administration), interfered with ethanol-induced CTA. Given the half-life of nicotine, we chose to administer nicotine 15 min prior to CS access (45 min prior to ethanol administration). As such, we feel confident that our design fell within the time-course of the physiological effects of nicotine,

although this is an important potential caveat that is worth mentioning.

It can also be argued that the dissociation of the effect of nicotine on CTA and CPA is due to different magnitudes in the strength of the associative memories. That is, CTA may have been weaker than CPA and thus more amenable to disruption by nicotine. This is unlikely because aversive unconditional stimuli, particularly those acting on the internal milieu, have an innate predisposition to pairing with gustatory stimulation, and ethanol-induced CTA has been observed even after a single trial (Verendeev & Riley, 2012). Place conditioning by ethanol in rats, on the other hand, is notoriously more variable (Tzschentke, 1998) and subject to inference by drug and extinction procedures (Bormann & Cunningham, 1997; Font, Houck, & Cunningham, 2017). Ultimately, all of these potential caveats are testable hypotheses and further experimental work is needed to confirm whether, in conditions similar to those of the present study, nicotine specifically disrupts the integration of orosensory and post-ingestive stimuli.

In the present study, nicotine was administered before (i.e., nicotine pre-exposure) and during (i.e., nicotine pre-treatment) conditioning with ethanol, and in CS− (i.e., no ethanol) days. This subchronic administration procedure was found to alleviate the general malaise induced by nicotine administration, therefore resulting in slightly more comparable levels of fluid intake, while still approximating the procedures used by other studies examining the effects of nicotine on ethanol-induced CTA (Kunin et al., 1999). It could be argued that this administration procedure precludes detecting if it is the remote or proximal effects of nicotine that affect ethanol's sensitivity and that perhaps only a pre-treatment (i.e., ethanol and nicotine co-administered in a same injection) would have been preferred. Ultimately, we were interested in assessing the aversive effects of ethanol in a brain that probably experienced plastic changes due to the chronic, on-going, nicotine exposure. Furthermore, administering nicotine on each conditioning day ensured that the interoceptive context induced by nicotine did not acquire associative strength with the forthcoming administration of ethanol.

The present results are consistent with several previous reports. Rinker et al. (2011) found that a chronic 10-day nicotine (0.4 mg/kg) pre-treatment regimen reduced ethanol-induced CTA but did not affect ethanol-induced motor suppression (ethanol dose: 1 g/kg, species: Sprague-Dawley rats). Another study, conducted in mice, reported that 1.0 mg/kg nicotine enhanced locomotion but did not alter the place conditioning effects of 1.0 g/kg ethanol (Gubner, Cunningham, & Phillips, 2015). Inhibition of ethanol- or acetaldehyde-induced CTA by acute nicotine treatment has also been reported (Kunin, Latendresse, Gaskin, Smith, & Amit, 2000; Kunin et al., 1999). There is a remarkable similarity between the results obtained in the present study (also in others; see Kunin et al., 1999), and those obtained in studies that used a more chronic nicotine treatment followed by conditioning in the absence of nicotine's acute effects (Rinker et al., 2011). This suggests that the mechanism responsible for the observed effects is similarly affected by both acute and chronic nicotine. For instance, activation of ß2 NAChrs in the insular cortex, a structure heavily implicated in acquisition of CTA, results in a depression of long-term potentiation (Sato et al., 2017), which may interfere with normal CTA learning. Chronic nicotine treatment results in upregulation of ß2 NAChrs (Besson et al., 2007; Buisson & Bertrand, 2002), which may influence the balance of activation resulting from endogenous ACh release to favor ß2-mediated processes (Besson et al., 2007), thus accounting for similar effects observed in the absence of ongoing nicotine treatment (Rinker et al., 2011).

The present study had the important limitation of using only a single dose of nicotine or ethanol. It is unknown whether similar results would have been reported had other doses been employed. Yet, an important feature (previously employed, with variations, by Cunningham, 1979; Turenne, Miles, Parker, & Siegel, 1996; Verendeev & Riley, 2011, among others), however, was the simultaneous taste/place conditioning procedure that was employed. The pairing of taste and place cues during the course of each conditioning trial is an economic design that also helps dispel historical effects associated with the use of different cohorts, or obvious ethanol pre-exposure effects resulting from conditioning with taste and exteroceptive cues separated by days or weeks. It is noteworthy that a previous study that conditioned the effects of amphetamine to both taste and place cues, in a single individual, reported a promotion of the positive rewarding effects of the drug. Specifically, Lett (1988) found that CTA by amphetamine facilitated the subsequent place preference learning mediated by amphetamine. Apparently, taste conditioning in that study blocked the association between amphetamine-induced aversion and other sensory stimuli, thus enhancing the association between place cues and the appetitive effects of amphetamine. Based on this background, it would not have been a surprise if the conditioning procedures employed in the present study had favored the emergence of the appetitive effects of ethanol. This, however, was not corroborated. As mentioned, ethanol induced a place aversion across testing trials, equally in nicotine- and vehicle-treated groups. Moreover, nicotine did not promote the emergence of ethanol-induced behavioral sensitization, despite having an independent, enhancing effect upon overall levels of motor activity. Ethanol induced a subtle, yet significant, suppression of motor activity, which did not show signs of tolerance across trials, nor was this ethanol-induced suppression of motor activity altered by nicotine.

In summary, the present study supports the notion that nicotine may facilitate engagement in ethanol seeking by selectively inhibiting taste aversive learning involving gustatory cues present during acute intoxication. A regimen of nicotine treatment that is sufficient to interfere with acquisition of ethanol-induced CTA across a range of ethanol doses (Rinker et al., 2011) failed to affect ethanol-induced CPA while significantly reducing ethanol-induced CTA in the same animals. Thus, one potential factor contributing to alcohol and nicotine comorbidity is that nicotine may block the associations between the aversive post-ingestive consequences of overconsumption of alcohol and its taste cues. This may lead to escalation of binge drinking and result in perseverative behaviors and learning deficits that may instigate further escalation of problematic drinking (Obernier, White, Swartzwelder, & Crews, 2002). Specifically, smokers may be more likely to initiate future alcohol drinking sessions following alcohol-induced illness relative to non-smokers, thus contributing to the development of problematic drinking habits.

Conflicts of interest

None.

Acknowledgments and funding sources

This work was a collaborative project between the State University of New York at Buffalo (United States) and Instituto M. y M. Ferreyra (Argentina), and was supported by PICT 2015-0325 to RMP and by R01AA024112 to PJM. The authors would like to thank Amanda Nilsen for the technical help provided.

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