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A clarification regarding this article is appended.

Interoceptive Conditioning with a Nicotine Stimulus Is Susceptible to Reinforcer Devaluation

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Abstract

Pavlovian conditioning processes contribute to the etiology of nicotine dependence. Conditioning involving interoceptive stimuli is increasingly recognized as playing a role in many diseases and psychopathologies, including drug addiction. Previous animal research on diminishing the influence of interoceptive conditioning has been limited to antagonism and nonreinforced exposures to the drug stimulus. The goal of the present research was to determine whether interoceptive conditioning with a nicotine stimulus could be diminished through an unconditioned stimulus (US) devaluation procedure. In two separate experiments, male Sprague–Dawley rats received nicotine injections (0.4 mg base/kg) followed by intermittent sucrose (26%) access in a conditioning chamber. On intermixed saline sessions, sucrose was withheld. Conditioning was demonstrated by a reliable increase in head entries in the dipper receptacle on nicotine versus saline sessions. After conditioning, rats in a devaluation condition were given access to sucrose in their home cages immediately followed by a lithium chloride (LiCl) injection on 3 consecutive days. On subsequent test days, nicotine-evoked conditioned responding was significantly attenuated. Within-subject (Experiment 1) and between-subjects (Experiment 2) controls revealed that the diminished responding was not attributable to mere exposure to the sucrose US in the devaluation phase. Experiment 2 included a LiCl-alone control group. Repeated illness induced by LiCl did not reduce later nicotine-evoked responding. These findings suggest that there is a direct association between the interoceptive stimulus effects of nicotine and the appetitive sucrose US (i.e., stimulus–stimulus) rather than a stimulus–response association.

Keywords: Pavlovian conditioning, interoception, smoking, drug discrimination, stimulus–reinforcer learning

Nicotine dependence is tenacious; 28.6% of adults in the United States are current users of tobacco (Department of Health & Human Services, 2008). Nearly half of tobacco users report a desire to quit (National Institutes of Health, 1998). However, 95% of the individuals who attempt to quit without intervention will relapse within a year (National Institutes of Health, 1998). Numerous cognitive–behavioral and pharmacological strategies for cessation have been developed to aid these individuals (Eisenberg et al., 2008; Gonzales et al., 2006; Hughes et al., 2007; Jorenby et al., 2006). Although pharmacotherapies such as bupropion (Zyban) and varenicline (Chantix) increase long-term abstinence from tobacco when compared with control groups receiving placebo, successful quit rates remain quite low [17% bupropion, average of meta-analysis including 36 clinical trials (Hughes et al., 2007); 26% varenicline, average of meta-analysis including 13 clinical trials (Eisenberg et al., 2008)]. The effectiveness of cognitive–behavioral therapies developed to assist in smoking cessation also remains in question (Conklin & Tiffany, 2002; Niaura et al., 1999). A more thorough understanding of how nicotine acquires control of behavior may provide insight into ways to increase the efficacy of current behav-

ioral and pharmacological approaches to treating chronic tobacco use and nicotine dependence.

One potential factor that likely contributes to the tenacity of the nicotine addiction is interoceptive conditioning involving the nicotine stimulus (Bevins & Murray, 2011). Conceptually, interoceptive conditioning refers to nicotine as a complex perceptible internal conditioned stimulus (CS) that is available for modification through learning if the nicotine CS is reliably paired with either an appetitive or aversive unconditioned stimulus (US). To a smoker, for example, appetitive events or USs may include socialization during a work break, peer acceptance, a filling meal, or other drugs like caffeine or alcohol that reliably co-occur with nicotine (Bevins, 2009; Bevins et al., 2012). Research investigating interoceptive conditioning with the nicotine stimulus is quite limited. One approach to studying the behavioral and neural processes involved in interoceptive conditioning in rats has been the discriminated goal-tracking task (Charntikov et al., 2012; Reichel et al., 2007). In this task, interoceptive conditioning with nicotine as the CS involves intermixed exposure to 2 different types of daily sessions: nicotine and saline. On nicotine days, rats are injected with nicotine and then given in-

termittent access to liquid sucrose in a conditioning chamber. On intermixed days, saline is administered but sucrose is withheld. Nicotine comes to control an increase in approach and head entry into the dipper receptacle [termed goal-tracking; see (Farwell & Ayres, 1979)] compared with saline. One focus of our research on interoceptive conditioning has been to investigate approaches to diminishing nicotine's control over the appetitive approach behavior. Identifying means of reducing the control the nicotine stimulus has on appetitive behavior could provide insight into how interoceptive conditioning may contribute to chronic tobacco use and the associated high relapse rate.

One approach we have taken to decrease nicotine-evoked goal-tracking is pharmacological blockade. This method has also helped provide insight into the neuropharmacological mechanisms mediating the CS effects of nicotine. For example, pretreatment with mecamylamine, a relatively nonselective nicotinic acetylcholine receptor (nAChR) antagonist, reliably reduces nicotine controlled goal-tracking when administered before testing (Besheer et al., 2004; Struthers et al., 2009). A more specific mechanism has been implicated by the demonstration that the nAChR antagonist DHβE, which is selective for α4-containing nAChRs, also blocks nicotine-evoked responding (Struthers et al., 2009). Although pharmacological blockade helps reveal underlying mechanism, this effect is transient and nicotine-evoked behavior returns if nicotine is administered in the absence of the ligand.

A second approach to decreasing nicotine-evoked goal-tracking is extinction. In extinction, nicotine after training as a CS is repeatedly presented except sucrose is *not* accessible. The goal-tracking conditioned response decreases across the nonreinforced nicotine sessions (Besheer et al., 2004; Murray & Bevins, 2007). A third approach that is a variant of extinction was termed "transfer of extinction learning" by Reichel et al. (2010). In this approach, repeated nonreinforced presentations of a ligand that shares stimulus effects with nicotine (e.g., varenicline or nornicotine) also attenuate subsequent responding evoked by the nicotine stimulus. That is, after training of the nicotine CS, rats are given the alternate ligand in place of nicotine during an extinction phase. If the nicotine stimulus is again tested after extinction, some, but not all, drugs that share stimulus effects with nicotine weaken conditioned responding to the nicotine CS; this weakening tends not to be as complete as when the nicotine stimulus itself is used in the extinction phase (Reichel et al., 2010).

An alternative yet currently unstudied approach to weakening responding evoked by the nicotine CS is devaluation. In a typical devaluation study, a CS such as onset of a tone is repeatedly paired with an appetitive US such as a food pellet. For the devaluation phase, the appetitive US is paired with sickness like that induced by an injection of lithium chloride (LiCl). When the CS is retested, conditioned responding to the CS is reduced (Holland & Straub, 1979; Holland & Rescorla, 1975). Research in this field has focused on diminishing appetitive responding controlled by exteroceptive stimuli such as a brief tone, illumination of a light, or a familiar context. To our knowledge, devaluation of interoceptive conditioning has never been studied. Accordingly, the goal of the present research was to investigate whether interoceptive conditioning and nicotine's control of appetitive behavior was susceptible to US devaluation.

Method

Subjects

Forty experimentally naive male Sprague–Dawley rats ordered at 275–299 g from Harlan (Indianapolis, IN) were housed individually in clear polycarbonate cages (48.3 × 26.7 × 20.3 cm; length × width × height) lined with wood shavings. Rats had ad libitum access to water in home cages, except when noted. After acclimation to the colony, rats were handled for a minimum of 2 min per day for 3 consecutive days before access to food (Harlan Teklad Rodent Diet) was restricted to maintain rats at 85% of their free-feeding body weight. The colony room was temperature and humidity controlled. All experimental sessions were conducted during the light portion of a 12-hour light/dark cycle. Protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Apparatus

Eight conditioning chambers (ENV-008CT; Med Associates, Inc., Georgia, VT) measuring 30.5 × 24.1 × 21.0 (length × width × height) cm were enclosed in sound and light attenuating cubicles fitted with an exhaust fan to provide airflow and mask noise. The front, back, and ceiling of the chambers were clear polycarbonate; side walls were aluminum. A recessed receptacle (5.2 × 5.2 × 3.8 cm; length × width × depth) was on one of the side walls. A dipper arm raised a 0.1-ml cup of sucrose (26% wt/vol) into the receptacle. To record head entries into the dipper, the chambers were equipped with an emitter/detector unit placed 1.2 cm into the recessed receptacle and 3 cm above the rod floor of the chamber. A personal computer with Med Associates interface and software (Med-PC for Windows, version IV) controlled sucrose deliveries and recorded dipper entries.

Drugs

(−)-Nicotine hydrogen tartrate and lithium chloride (LiCl) were purchased from Sigma (St. Louis, MO). Nicotine was dissolved in 0.9% saline and adjusted to a pH of 7.0 ± 0.2 using a dilute NaOH solution. Nicotine injections were given subcutaneously (SC); dose is reported as the base. LiCl was dissolved in distilled water and injected interperitoneally (IP); dose is reported as the salt. All injections were given at a volume of 1 ml/kg.

Experiment 1

Acquisition

To minimize the initial locomotor suppressant effects of nicotine, rats (*n* = 16) received daily injections of 0.4 mg/kg nicotine in their home cages for the 3 days immediately before the start of the experiment (Bevins et al., 2001). Discrimination training consisted of 32 or 44 daily sessions; nicotine sessions and saline sessions were intermixed (see Figure 1 for experimental timeline). The experiment was conducted in two separate replications (replication 1, *n* = 7; replication 2, *n* = 9). Because of experimenter error, rats in the first replication received 44 acquisition sessions, whereas rats in the second replication received 32 sessions. Responding at the end of acquisi-
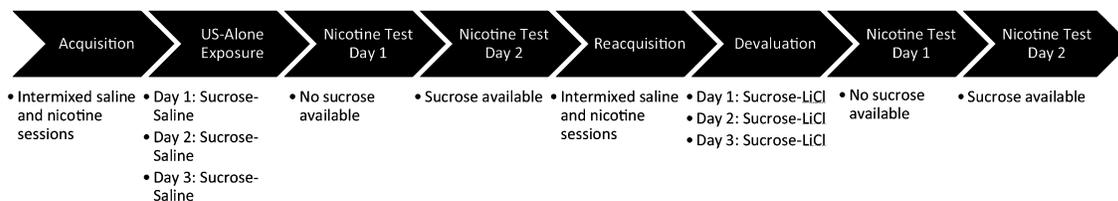
Figure 1. Experimental timeline of Experiment 1.

tion did not differ significantly between replications, $Fs < 2.25$, $p = .156$. Accordingly, the two replications were combined for all analyses. The order of the sessions was pseudorandomly assigned with the stipulation that rats received no more than 2 consecutive days with the same type of session. Nicotine sessions consisted of a 0.4 mg/kg SC nicotine injection 5 min before placement in the chamber for a 20-min session. During each nicotine session, rats had access to 36 deliveries of 26% (wt/vol) sucrose (4 s each). The first sucrose delivery ranged from 124 to 152 s with an average of 137 s from the start of the session; subsequent sucrose deliveries were presented on average every 25 s (range = 4 to 80 s). On saline sessions, rats received a SC saline injection 5 min before placement in the conditioning chamber; sucrose was withheld during saline sessions. Past research has demonstrated that this training protocol would produce a robust discrimination between saline and nicotine as evidenced by increased goal-tracking, before the US is presented, during nicotine compared with saline sessions (e.g., Murray et al., 2007; Wilkinson et al., 2006).

US-Alone Exposure

For the 3 days after acquisition, rats remained in the colony and received exposure to the sucrose US in the home cage. This protocol provided a within-subjects test of whether mere exposure to the sucrose US would decrease later responding to the nicotine CS (Rescorla, 1973). Specifically, water was removed 30 min before the introduction of sucrose. Rats then received 15-min access to 100 ml of 26% sucrose in a standard water bottle. Immediately after sucrose access, rats were injected IP with saline. Sucrose consumption was recorded for each rat. Water was returned 30 min after the sucrose was removed from the home cage.

Testing After US-Alone Exposure

For the 2 days after US-alone exposure, rats were tested for conditioned responding evoked by the nicotine CS. On Day 1 of testing, 0.4 mg/kg nicotine was administered 5 min before placement in the chamber for a 20-min session. During this test session, there was no access to sucrose to assess persistence of responding without the US. On Day 2 of testing, 0.4 mg/kg nicotine was again administered five min before a 20-min session. During this session, sucrose was delivered on a schedule that matched acquisition training. This test allowed us to investigate how conditioned responding was affected when the US was reintroduced.

Reacquisition

Reacquisition commenced 24 h after the 2nd test. Reacquisition was identical to acquisition and consisted of 6 saline and 6 nicotine sessions intermixed as previously described.

Devaluation and Testing

Following 24 h after the last reacquisition session, rats received devaluation training. This phase was similar to the US-alone phase except that immediately after each 15-min access to sucrose, rats were injected IP with 127.2 mg/kg LiCl. Devaluation training occurred for 3 consecutive days. After the last devaluation session, rats were again tested in the chambers for conditioned responding evoked by the nicotine CS. Testing was identical to testing after US-alone training.

Statistical Analyses

Dependent measures: The dependent measure during acquisition and reacquisition training was the rate of dipper entries per second before the first sucrose delivery or an equivalent time from the start of the session if no sucrose was available in the session. Using only dipper entries before any access to sucrose avoids any influence of US exposure on our measure of learning. For test sessions, a percentage-of-baseline-responding was calculated for each rat by dividing the rate of dipper entries before the first sucrose delivery during testing or an equivalent time in a test session without sucrose by the mean response rate for the last 3 nicotine training sessions that preceded testing, Times 100. Sucrose consumption during US-alone exposure and devaluation training was measured in milliliters. Total number of dipper entries during each 5-min bin of the test sessions was also recorded to investigate how goal-tracking varied within test sessions.

Data Analyses

In acquisition and reacquisition, dipper entries were analyzed with a two-way within-subjects repeated measure analysis of variance (ANOVA) with Drug (nicotine vs. saline) as one factor and Session as the repeated measure. Significant interactions were followed by paired t tests with Bonferonni correction to analyze differences between saline and nicotine sessions. Test sessions were analyzed using one-sample t tests that compared percentage of responding to a hypothetical mean of 100%, the value expected if there was no effect of the manipulation. Repeated measures ANOVAs were used to analyze difference in sucrose consumption during home cage US-alone as well as devaluation training. To analyze how goal-tracking varied within test sessions, we used a two-way within-subjects repeated measure ANOVA with Condition (US-alone vs. devalue training) as one factor and 5-min Bin as the repeated measure. Paired t tests with Bonferonni correction were used for post hoc comparisons when prompted by a significant interaction. Statistical significance was declared using $p < .05$ for all analyses.
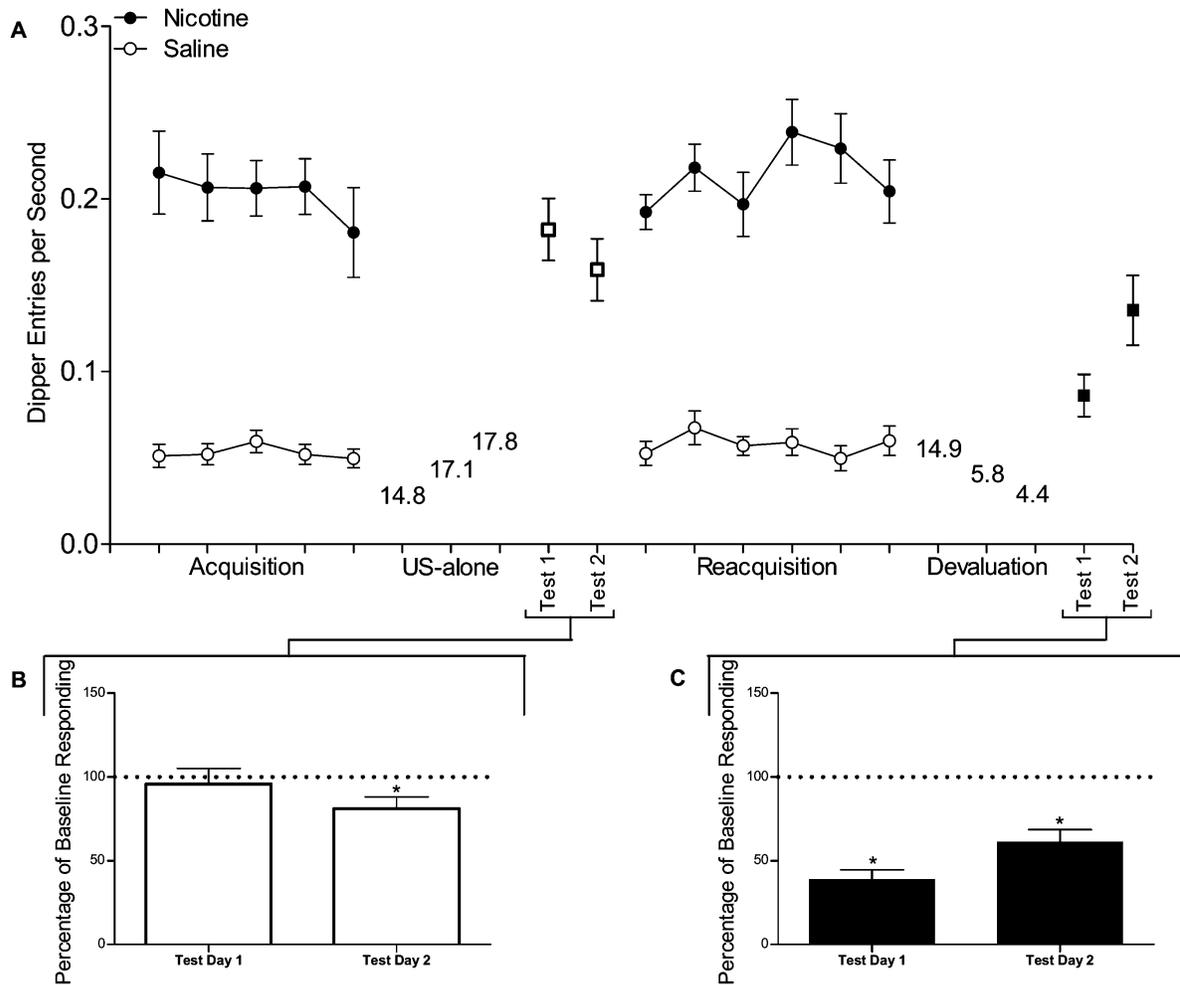
Figure 2. A) Dipper entries per second (± SEM) before the first sucrose delivery (nicotine sessions) or during a comparable time (saline sessions) for the last 5 nicotine and saline acquisition sessions, US-Alone test days (nicotine sessions only), reacquisition, and devaluation test days (nicotine sessions only). Sucrose consumption (ml) during US-alone and devaluation training is also reported in A. **B)** Percentage of baseline responding (± SEM) on the test days after US-alone training. * denotes significant differences (*p* < 0.05) from baseline (100%; dotted line). **C)** Percentage of baseline responding (± SEM) on the test days after devaluation training. * denotes significant differences (*p* < 0.05) from baseline (100%; dotted line).

Results

Acquisition

By the end of acquisition, rats discriminated between saline and nicotine sessions (last five sessions are shown in Figure 2A). Dipper entries were higher on nicotine than on saline sessions, $F(1, 15) = 108.6$, $p < .001$. Neither the main effect of Session nor the Drug × Session interaction was significant, $Fs < 1$.

US-Alone Exposure and Testing

The mean sucrose consumption for the 3 days of US-alone exposure is shown in Figure 2A. There was a trend for an increase in sucrose intake across the 3 days. However, the main effect of Day did not meet the criterion for statistical significance, $F(2, 15) = 3.3$, $p = .051$. On the first test of the nicotine stimulus after US-alone training (see Figure 2B), rats did not

significantly differ in responding compared with their acquisition baseline, $t < 1$. On Day 2 of testing, conditioned responding was significantly reduced at the start of the session compared with baseline levels, $t(15) = 2.69$, $p < .05$, reflecting some extinction of conditioned responding.

Reacquisition

Rats continued to discriminate between saline and nicotine sessions (Figure 2A). Responding on nicotine sessions was significantly higher than on saline sessions, $F(1, 5) = 178.1$, $p < .001$. Neither the main effect of Session nor the Session x Drug interaction was significant, $Fs \leq 1.58$, $p \geq 0.169$.

Devaluation Training and Testing

Sucrose consumption decreased significantly across days (Figure 2A), $F(2, 15) = 55.23$, $p < .001$. Consumption on Day 2 and Day 3 were significantly lower than Day 1. There was no dif-
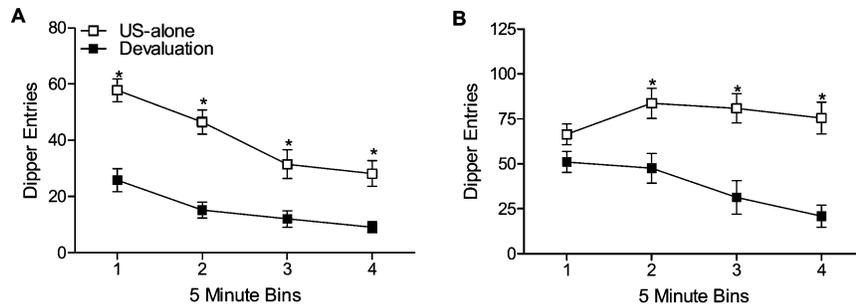
Figure 3. A) Total dipper entries (± SEM) in 5 minute bins during Day 1 of testing (sucrose unavailable) after US-alone and devaluation training. * denotes significant differences (*p* < .05) between responding during US-alone and devaluation testing. **B)** Total dipper entries (± SEM) in 5 minute bins during Day 2 of testing (sucrose available) after US-alone and devaluation training. * denotes significant differences (*p* < .05) between responding during US-alone and devaluation testing.

ference between Day 2 and 3. Figure 2C shows conditioned responding to nicotine during devaluation testing as a percentage of baseline responding from the reacquisition phase. After devaluation training, appetitive goal-tracking was reduced from baseline levels on both test days, *t*s(15) ≥ 5.40, *p*s < 0.001.

Within-Test Session Responding

Figure 3A shows total dipper entries in 5-min bins for the first test of nicotine-evoked responding following US-alone and devaluation training. Recall that sucrose was not available during these 20-min tests so as to allow for measurement of conditioned responding without the sucrose US itself affecting responding. There were significant main effects of Condition, *F*(1, 15) = 35.39, *p* < .001, and of Bin, *F*(3, 15) = 28.74, *p* < .001, as well as a Condition × Bin interaction, *F*(3, 90) = 3.39, *p* < .05. Conditioned responding on the first test after devaluation was lower than after US alone throughout the session. Figure 3B shows total dipper entries in 5-min bins for the second test of nicotine-evoked responding after US-alone and devaluation training, sucrose was available on the second test days. There were significant main effects of Condition, *F*(1, 15) = 14.74, *p* < .001, and of Bin, *F*(3, 15) = 10.52, *p* < .001, as well as a Condition × Bin interaction, *F*(3, 90) = 15.50, *p* < .001. Post hoc tests revealed no difference in the first 5 min of testing. However, dipper entries did differ for the remainder of the test session; responding following devaluation was lower than responding following US-alone conditioning. This data pattern suggests that once the sucrose was accessed later goal-tracking was deterred.

Experiment 2

Experiment 1 used a within-subjects design to investigate the effect of devaluation of an appetitive US on interoceptive conditioning with the nicotine stimulus. We found that the nicotine-evoked conditioned response was weakened when the sucrose US was paired repeatedly with LiCl in the home cage. We saw no effect on the appetitive goal-tracking response if rats were merely exposed to sucrose without illness (US-alone phase). These findings suggest that the goal-tracking behavior

controlled by the nicotine CS reflects an excitatory association between the nicotine stimulus and the sucrose US (Holland et al., 1979; Holland et al., 1975). However, before this conclusion can be accepted, an alternative account must be evaluated. There is the possibility that 3 consecutive days of illness induced by the LiCl, and not a conditioned aversion to the sucrose, reduced conditioned responding on subsequent test days. The goal of Experiment 2 was to test this alternative hypothesis. To do so, we used a between-subjects design with 3 groups. One group received sucrose devaluation, whereas the other two groups controlled for sucrose exposure and LiCl exposure (i.e., repeated illness). If attenuation of nicotine-evoked goal tracking in Experiment 1 was due to a devaluation of the sucrose, then only the devaluation group will show a reduction in dipper entries on the test days.

Acquisition

Acquisition training in Experiment 2 (*n* = 24) was identical to Experiment 1 (see Figure 4 for experimental timeline).

Devaluation

After acquisition training, rats were randomly assigned to one of three groups (sucrose-LiCl; sucrose-saline; water-LiCl), with the stipulation that the groups did not significantly differ in responding at the end of acquisition. The three groups were designed to test the effects of sucrose devaluation (sucrose-LiCl), access to the US alone (sucrose-saline), or illness alone (water-LiCl). For three consecutive days, rats received 15-min access to 100 ml of their designated solution (sucrose or tap water) in the home cage, immediately followed by an injection of LiCl (sucrose-LiCl and water-LiCl groups) or saline (sucrose-saline group). As in the earlier experiment, water bottles were removed 30 min before training and then returned 30 min after the injection.

Testing

After the devaluation phase, nicotine's control of conditioned responding was assessed on two separate days. In each test, 0.4 mg/kg nicotine was administered 5 min before placement in the chamber for a 20-min session. On Day 1 of testing, sucrose deliveries were withheld. On the second test day, su-
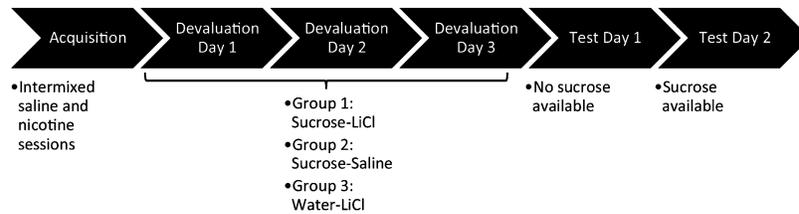
Figure 4. Experimental timeline of Experiment 2.

crose was delivered on a schedule identical to that during acquisition. This testing protocol matches that of devaluation testing in Experiment 1.

Statistical Analyses

The analyses of Experiment 2 were identical to Experiment 1 with 2 exceptions. First, a repeated measures ANOVA was used to analyze difference in sucrose consumption between groups during devaluation training. Second, a two-way (between subjects) repeated measure ANOVAs with Group (sucrose-LiCl; sucrose-saline; water-LiCl) as one factor and Bin as the repeated measure was used to analyze group differences in within session responding during Test 1 and Test 2.

Results

Acquisition

Responding during the last five sessions of acquisition is shown in Figure 5A. Rats readily discriminated between saline and nicotine sessions displaying more responding during nicotine sessions, $F(1, 23) = 79.45$, $p < .001$. Although there was a main effect of Session, $F(4, 184) = 4.13$, $p < .01$, the Drug × Session interaction was not significant $F < 1$.

Devaluation Training

Mean liquid consumption on each test day is shown in Figure 5B (inset into Figure 5A). There was a main effect of Group, $F(2, 42) = 153.0$, $p < .001$, a main effect of Day, $F(2, 42) = 9.80$, $p < .001$, and a Group × Day interaction, $F(4, 42) = 5.06$, $p < .01$. Intake was significantly lower on all days in the water-LiCl group compared with the sucrose-saline group and lower than the sucrose-LiCl group on Day 1 and 2. The sucrose-LiCl and sucrose-saline group had similar levels of consumption on Day 1. However, consumption in the sucrose-LiCl group was reduced in comparison to the both sucrose-saline group on Day 2 and 3, as well as their own consumption level on Day 1.

Devaluation Testing

Nicotine-evoked goal-tracking behavior after devaluation is shown in Figure 5C. On Day 1, the group that had the US devalued (sucrose-LiCl) displayed significantly lower levels of responding early in the session compared to their nicotine baseline levels from the end of acquisition, $t(7) = 11.20$, $p < .001$. In contrast, the US-alone group (sucrose-saline), $t(7) = 1.86$, $p = .106$, and the illness only group (water-LiCl), $t(7)1.54$, $p = .168$, did not differ significantly from their respective baselines. A similar pattern was seen on day two of testing (Figure

5D). The sucrose-LiCl group continued to display attenuated levels of responding compared to baseline, $t(7) = 2.50$, $p = .04$. The sucrose-saline and water-LiCl groups did not differ from their baseline, ts < 1.

Within-Test Session Responding

Analysis of dipper entries across the Day 1 test session (Figure 6A), with no sucrose available, revealed that responding decreased across the 5 min bins, $F(3, 63) = 4.95$, $p < .001$, indicating sensitivity to removal of the US. Although responding was lower in sucrose-LiCl group, especially in the first 2 bins, there was no main effect of Group, $F(2, 63) = 2.49$, $p = .107$, and no Group × Bin interaction, $F(6, 63) = 1.24$, $p = .297$. Analysis of dipper entries across the Day 2 test session, with sucrose available, (Figure 6B) revealed a main effect of Bin, $F(3, 63) = 4.95$, $p < .01$, but no main effect of Group, F<1, or a Group × Bin interaction, $F(6, 63) = 1.69$, $p = .138$. For Experiment 2, the devaluation effects appeared to be strongest early in the session (i.e., with the first few minutes; recall Figure 5C and 5D). This pattern differed from Experiment 1 where the effect was longer lasting whether sucrose was or was not available during the test session. One possible explanation for these differences is the within- vs. between-subjects design of Experiment 1 and Experiment 2, respectively. Perhaps experiencing sucrose alone in the home cage across several days has a long lasting effect when it is later devalued in the same cage. Of course, to ascertain the nature of this would require further experimentation on an issue that is tangential to the primary goal of the present studies—determining whether interoceptive conditioning with a nicotine stimulus is susceptible to devaluation.

Discussion

The role of interoceptive conditioning in drug addiction more generally, and nicotine dependence more specifically, remains a major theoretical and empirical area requiring much more inquiry (Bevins et al., 2012; Verdejo-Garcia et al., 2012). Diminishing control of interoceptive conditioning over acquired appetitive behaviors seems of particular importance to advancing our understanding of the behavioral and neural processes involved in interoceptive drug conditioning. Previous research has demonstrated attenuation of nicotine-controlled behavior using a variety of tactics that include extinction (Besheer et al., 2004), pharmacological blockade (Besheer et al., 2004; Murray et al., 2007; Struthers et al., 2009), and transfer of extinction learning (Bevins et al., 2012; Reichel et al., 2010). An alternative behavioral approach to attenuating nicotine's control over acquired behavior examined in the present report was US devaluation. We found in two separate experiments that when
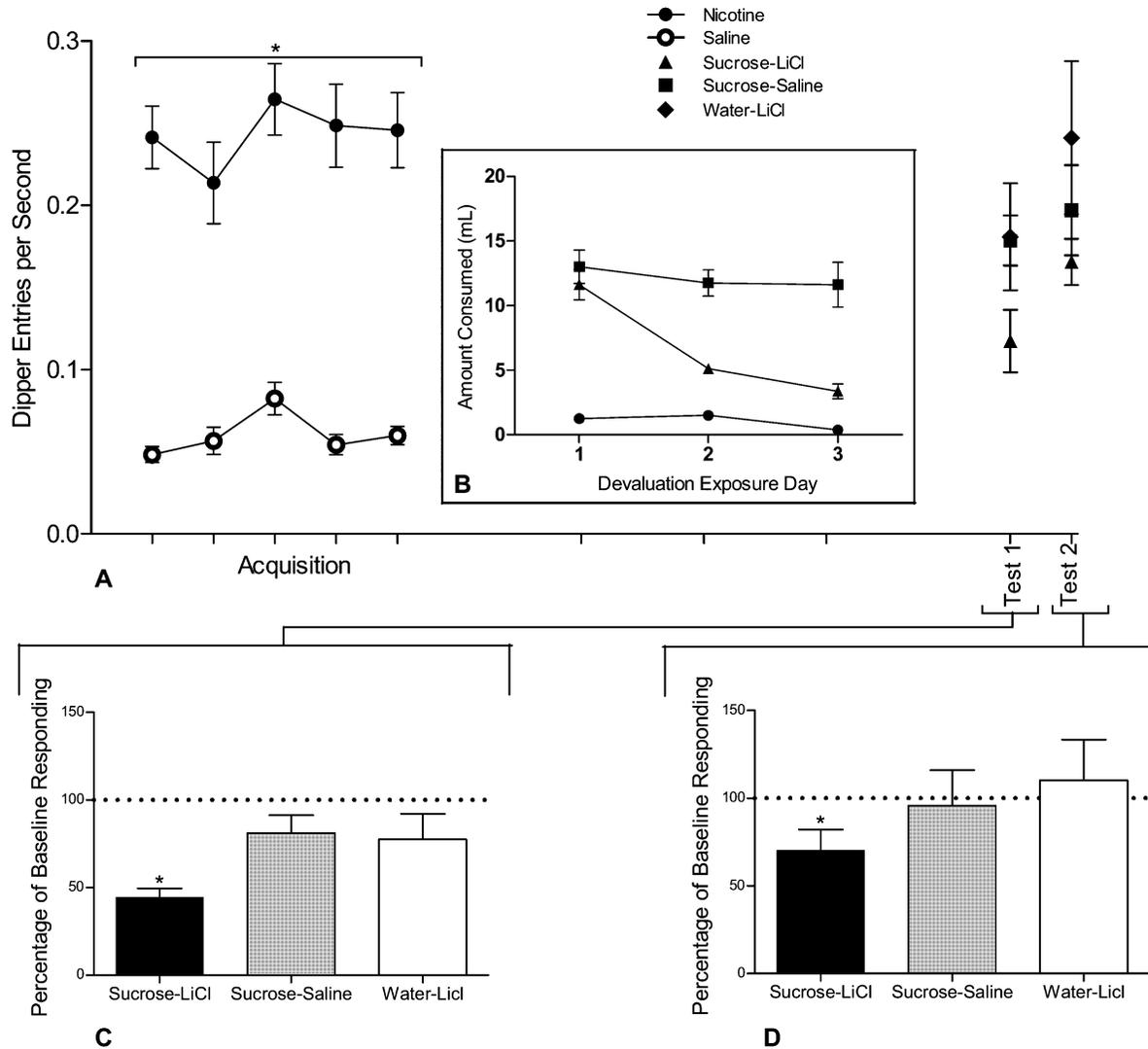
Figure 5. A) Dipper entries per second (± SEM) before the first sucrose delivery (nicotine sessions) or during a comparable time (saline sessions) for the last 5 nicotine and 5 saline acquisition sessions and for testing after devaluation training. **B)** Liquid consumption (ml) during the 3 devaluation exposure days. **C)** Percentage of baseline responding (± SEM) on Test Day 1 after devaluation training. * denotes significant differences (*p* < .05) from baseline (100%; dotted line). **D)** Percentage of baseline responding (± SEM) on Test Day 2 after devaluation training. * denotes significant differences (*p* < .05) from baseline (100%; dotted line).

the sucrose US was repeatedly paired with LiCl-induced illness that the subsequent control of conditioned responding by the nicotine stimulus was attenuated. To our knowledge, this set of experiments is the first demonstration of alteration of conditioned responding to an interoceptive stimulus using a US devaluation approach.

These findings suggest that the goal-tracking behavior evoked by the nicotine CS reflects an excitatory association between the nicotine stimulus and the sucrose US. This supports the notion that the interoceptive Pavlovian association is a stimulus–stimulus association, and not a stimulus–response association (see discussion below). Before we accept this conclusion, however, several alternative accounts should be considered. The first alternative account for a reduction in conditioned responding is that attenuation may be produced by extensive and repeated exposure to the sucrose [that is,

US habituation; compare (Rescorla, 1973)]. This alternate account seems unlikely given that nicotine-controlled behavior was not reduced on Day 1 of testing after US-alone exposure in a between or within-subjects design, but was attenuated after devaluation. A second alternate account is that illness on 3 consecutive days affected subsequent goal-tracking during test sessions independent of a conditioned aversion to the sucrose US. To test this account, Experiment 2 included a control group that received LiCl alone (i.e., the water-LiCl group) across 3 consecutive days. Nicotine-controlled behavior was not reduced in the LiCl alone group, providing evidence that the reduction in goal-tracking behavior early in the test sessions for the devalued condition was not from sickness alone.

As noted earlier, devaluation research over the past 40 years has focused on exteroceptive stimuli. Take the sem-
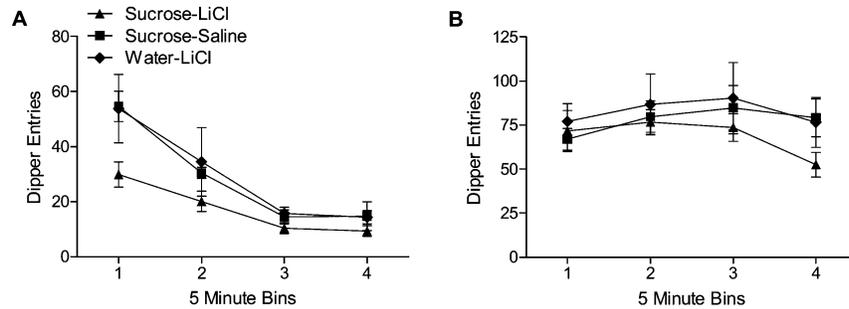
Figure 6. A) Total dipper entries by group (± SEM) in 5-minute bins during Day 1 of testing (sucrose unavailable) after devaluation training. **B)** Total dipper entries by group (± SEM) in 5-minute bins during Day 2 of testing (sucrose available) after devaluation training.

inal work by Holland and Rescorla (1975) as an example. In this research, rats had an exteroceptive CS (tone) repeatedly paired with an appetitive US (sucrose) during initial training. This excitatory conditioning was followed by pairing the sucrose US with high speed rotation (illness) in a separate context from the training environment. Subsequent tests revealed that sucrose-illness pairings reduced conditioned response controlled by the tone CS. The authors concluded that the CS elicits a "representation" of the US which can be devalued by subsequent pairings with sickness. It is the devaluation of this "representation" that produces diminished responding to the CS (cf. Holland & Rescorla, 1975; Ostlund & Balleine, 2007; Reichelt et al., 2011).

Notably, the present set of experiments extends the utility of devaluation procedure to an interoceptive stimulus. The US devaluation effect, seen across both studies, suggests that interoceptive conditioning involving the nicotine CS in the discriminated goal-tracking task reflects a nicotine stimulus-sucrose reinforcer (US) association. The current experiments focused on attenuation of nicotine-controlled behavior by pairing sucrose with illness. This is just one approach to US devaluation. Future research could examine whether a US satiation procedure also diminishes the acquired appetitive behavior controlled by the nicotine stimulus; this could be achieved by giving prolonged sucrose access immediately before CS testing. Future research could also investigate the possibility of revaluation to increase the value of the US by pairing it with a second reinforcer after CS–US training (Rescorla, 1974). If successful, goal-tracking behavior controlled by the nicotine CS would be potentiated. Also of interest in future studies is the underlying neural mechanisms involved with devaluation of a US associated with an interoceptive CS. Previous research with exteroceptive stimuli indicates a role for the orbitofrontal cortex and the basolateral amygdala in the alteration of incentive value of stimuli (Gallagher et al., 1999; Hatfield et al., 1996; Pickens et al., 2003). Whether similar or distinct neural processes also play a role when the CS is an interoceptive stimulus has yet to be determined.

The experiments reported here have advanced our understanding of interoceptive conditioning involving the nicotine stimulus. Namely, the present research provides the best evidence to date that nicotine has acquired appetitive effects that reflect a direct association between the nicotine stimulus and the appetitive sucrose US. We do not necessarily believe that practitioners should directly translate this devaluation protocol and apply it to individuals trying to quit their tobacco use habit. Rather, these finding should prompt awareness of the potential role interoceptive conditioning may have in the addiction process, as well as reflection on how cognitive–behavioral and pharmacological strategies (Conklin et al., 2002; Eisenberg et al., 2008; Gonzales et al., 2006; Hughes et al., 2007; Jorenby et al., 2006; Niaura et al., 1999) could be improved in light of what we know about interoceptive conditioning (Bevins & Murray, 2011).

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Clarification to Pittenger and Bevins (2013)

In the article "Interoceptive Conditioning With a Nicotine Stimulus Is Susceptible to Reinforcer Devaluation," by Steven T. Pittenger and Rick A. Bevins (*Behavioral Neuroscience*, 2013, Vol. 127, No. 3, pp. 465–473. doi: 10.1037/a0032691), the authors were investigating the impact of devaluing a sucrose reinforcer with illness (i.e., lithium chloride administrations) on a conditioned goal-tracking response controlled by a nicotine stimulus. Drugs of abuse generate interoceptive stimuli that are available for conditioning and come to control behavior. Joseph Troisi II and his colleagues have been studying interoceptive drug stimuli and learning processes for many years (Troisi, 2003a; Troisi 2003b; Troisi, 2006; Troisi 2011; Troisi & Akins, 2004; Troisi et al., 2010; Troisi et al., 2012; Troisi et al., 2013). At the time of submission, the authors were unaware of the article published by Joseph Troisi II and collaborators in *The Psychological Record* (Troisi et al., 2012). The authors thank Joseph Troisi II for bringing this article to their attention. In that article, discriminative control of a nose-poking response maintained by a variable interval 30-s schedule of reinforcement was established using nicotine to prompt pellet reinforced responding in one group and to prompt non-reinforcement in a separate group of food restricted rats (i.e., 80% of free-feeding weight). When these rats were placed on free-feed (i.e., devaluation of the reinforcer by satiation), the response rate controlled by the nicotine stimulus, or the absence of that stimulus, was decreased. This demonstration of reinforcer devaluation by satiety in a single-operandum operant drug discrimination procedure precedes the demonstration of the illness-induced devaluation in the Pavlovian discriminated goal-tracking task in Pittenger and Bevins (2013). The authors retract statements to the effect that they were the first to study devaluation of interoceptive conditioning. Further, the authors suggested that "Future research could examine whether a US [*unconditioned stimulus*] satiation procedure also diminishes the acquired appetitive behavior controlled by the nicotine stimulus" (p. 472; italics added for clarification). The findings by Troisi et al. (2012) suggest that satiation will reduce responding controlled by nicotine in the discriminated goal-tracking task.

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