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Reinforcer devaluation as a consequence of acute nicotine exposure and withdrawal

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Abstract

RATIONALE—Nicotine discontinuation produces behaviors in rats that are congruent with anhedonia, and these symptoms may be related to the devaluation of non-nicotine reinforcers.

OBJECTIVE—Four separate experiments were performed to explore the parameters surrounding nicotine-induced reinforcer devaluation.

METHODS—In Experiments 1 and 2, nicotine (0.1 or 0.3 mg/kg) or 0.3 mg/kg nicotine plus 1.0 mg/kg mecamylamine was delivered to rats prior to progressive ratio (PR) schedule sessions in which sucrose was used as a reinforcer. In order to (a) evaluate reinforcer enhancement by nicotine, and (b) reinforcer devaluation in the absence of nicotine, all rats experienced two PR schedule sessions per day for 10 days. Experiment 3 involved nicotine (0.3 mg/kg) and a visual stimulus in place of sucrose reinforcement. In Experiment 4, rats received nicotine (0.3 mg/kg) either before or after a single PR-schedule session for 10 days.

RESULTS—Experiments 1 and 2 demonstrate that reinforcer devaluation is related to the occupation of nicotinic-acetylcholine receptors. Results from Experiment 3 provide some evidence that devaluation occurs with either sucrose or visual-stimulus reinforcement. Experiment 4 demonstrates that a necessary condition for reinforcer devaluation to occur is the concurrent exposure to the reinforcer and nicotine.

CONCLUSIONS—Reinforcer devaluation in rats emerges rapidly in a progressive, orderly fashion that coincides with accumulated exposure to nicotine. These results suggest that reinforcer devaluation may be a feature of nicotine that contributes to the abuse liability of tobacco products.

Introduction

Tobacco use is one of the leading causes of preventable death in the United States (CDC, 2008; Mokdad, Marks, Stroup, & Gerberding, 2004) and the annual economic burden associated with the public-health ramifications of tobacco use is extreme (CDC, 2008).

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Cessation of tobacco smoking can greatly abate both the risk for disease and premature death, yet the vast majority of smokers report that they wish to quit but cannot (Henningfield, Shiffman, Ferguson & Gritz, 2009; Hughes, 2007; Hughes & Hatsukami, 1986; 1992). Tobacco withdrawal is suggested to play a prominent role in maintenance of smoking behavior through the mechanism of negative reinforcement (Allen et al. 2008; Baker, Brandon, & Chassin, 2004; Baker et al. 2004; Henningfield et al. 2009; Kenny & Markou, 2001; Koob, 1996; Piaseki, Fiore, & Baker, 1998; Watkins, Koob, & Markou, 2000). Symptoms of tobacco withdrawal can be divided between the general categories of emotional and somatic responses, and these symptoms are likely attributable to nicotine deprivation (Barr, Boyda, & Procyshyn, 2013; Henningfield et al. 2009; Hughes, 2007; Kosten, Markou, & Koob, 1998, Levinthal et al. 2013; Stolerman & Jarvis, 1997). Somatic responses, such as bradycardia and increased appetite, are reliable reactions to initial abstinence and are therefore clinically significant, but somatic symptoms may be less important targets of research than emotional symptoms. Clinical prospective investigations have reported that the severity of negative affect, in particular, is predictive of tobacco-use relapse (Carton, Le Houezec, Lagrue, & Jouvent, 2002; Cook, Spring, McChargue, & Doran, 2010; Hughes, 2007; Japuntich et al. 2007; Levinthal et al. 2008; 2013; Niara et al. 2001).

The change in affect during tobacco withdrawal can be conceptualized as a syndrome of anhedonia (Baker et al. 2004) and numerous preclinical investigations with rodents have demonstrated that discontinuation of nicotine administration produces behavioral phenomena that are congruent with negative affect (Bruijnzeel et al. 2007; 2009; 2012; Harris et al. 2013; Lesage, Burrows, & Pentel, 2006; Weaver et al. 2012). Several researchers have demonstrated that nicotine withdrawal increases intracranial self-stimulation (ICSS) thresholds (Bruijnzeel et al. 2007; 2009; 2012; Brunzell & Picciotto, 2012; Cryan et al. 2003; Epping-Jordan et al. 1998; Kenny & Markou, 2005; Koob & LeMoal, 2005; Marcinkiewicz et al. 2009; Johnson, Hollander, & Kenny, 2008; Vlachou & Markou, 2013 for a review). The results of these studies are conclusive in that brain-reward mechanisms are altered by prolonged exposure to nicotine, and suggest that the decrease in sensitivity to reward (e.g. Pergadia et al. 2014) resulting from nicotine exposure may be an important aspect of negative reinforcement that maintains tobacco use in humans.

Increased ICSS thresholds may speak to both the maintenance of tobacco use and the escalation from use to dependence. Recent evidence demonstrates that antagonist-precipitated withdrawal from a single dose of nicotine produces a significant increase in ICSS thresholds (Harris et al. 2013). Given that the mechanism underlying the escalation of tobacco use may not be fully described by nicotine's primary-reinforcing characteristics (Chaudhri et al. 2006; Dar & Frenk, 2004), a decrease in reward sensitivity after initial nicotine exposure may help to answer this unresolved issue.

One limitation of ICSS investigations is that they do not necessarily show how rodents respond to other available reinforcers during a period of withdrawal (Vlachou & Markou, 2013). One could conceptualize nicotine-withdrawal-induced anhedonia as a problem of *reinforcer devaluation*; activities that once sustained a high rate of responding and enjoyment may fail to act as potent reinforcers during withdrawal. Lesage, Burrows and

Pentel (2006) found that after 8 days of osmotic nicotine infusion, the termination of nicotine administration impaired performance on a progressive ratio (PR) schedule of reinforcement maintained by sucrose. The results from Lesage et al. (2006) suggest that experience with nicotine dramatically decreases the reinforcing value of non-pharmacological incentives. After 16 days of osmotic infusion of nicotine, Weaver et al. (2012) showed that mecamylamine-precipitated withdrawal in rats also produces a diminished efficacy of reinforcers that once sustained a higher rate of responding. Whether reinforcer devaluation is a reliable consequence of nicotine exposure is the focus of the current series of experiments. Furthermore, the present studies have been designed to evaluate the pace at which reinforcer devaluation emerges in relation to accumulated nicotine exposure in a manner that could not be accomplished in the previous investigations of withdrawal-induced motivational decrements (Lesage et al. 2006; Weaver et al. 2012).

Materials and Methods

Subjects

The following experiments involved male Sprague Dawley rats (*Rattus Norvegicus*, obtained from Charles River Laboratories, Montreal, Quebec, Canada), pair-housed (OptiRAT plus animal housing systems, Centennial, Colorado, USA) and maintained at or above their initial free-feeding body weight upon delivery at 65 days old. The rats experienced a 12/12 hr day/night light cycle, and were provided water on an ad-lib basis in their home cages. The animal-holding room was temperature and humidity-controlled, and all rats received 15 g of food per day. All animal-care protocols were approved by the Saint Michael's College IACUC, and all procedures followed the ILAR guidelines for humane animal care in experimentation.

Apparatus

All behavioral investigation utilized standard rat operant chambers (ENV-008, Med-Associates, Saint Albans, Vermont). Each chamber included two nose-poke operant devices located along the same wall, and equidistant from a center liquid-dipper well. The rats were reinforced with sucrose solution (8%, delivered in 0.03ml per reinforcing event) or visual-stimulus presentation (Experiment 3, only). The operant chambers were monitored by a PC for operation and data collection using the MED-PC IV platform.

Drug dosing

Saline (0.9% solution) was administered as a control in 1 ml/kg doses, and also used as the vehicle for the nicotine and mecamylamine. 0.1 and 0.3 mg/kg doses of nicotine were delivered as a base from nicotine ditartrate dihydrate in concentrations that yielded injections of approximately 1 ml/kg (162.21g per mole). In Experiment 2, 1.0 mg/kg mecamylamine hydrochloride was dissolved as a base (167.29 g per mole), and the rats received the administration of mecamylamine 30 minutes prior to the delivery of 0.3 mg/kg nicotine. All drugs were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, Missouri, USA) and administered subcutaneously.

Operant Shaping

Rats were shaped to respond for sucrose solution. The shaping process included two consecutive days consisting of a 15 min session of variable-time (VT), with 30s presentations of sucrose to construct the association between the sound of the liquid-dipper delivery device and the presentation and availability of sucrose. The VT sessions occurred in the dark, in the absence of any visual cues. Photobeam sensors in the liquid-dipper well detected consumption responses, and sucrose solution remained available for 5s following the detection of a head entry.

Fixed-ratio (FR) schedule shaping sessions followed the two VT 30s sessions. During all operant-response sessions (FR or PR), the illumination of the right nose-poke device (LEDg, for a green LED) signaled the initiation of a trial. Upon the completion of the response requirement (e.g. 3 responses under the FR3 requirement), LEDg was terminated, and LEDr (red LED) illuminated synchronously with the lifting of the liquid dipper to signal reinforcer availability. Rats were allowed 5s to consume the sucrose-liquid reward once a head-entry was detected in the dipper well by a photobeam interruption. LEDr and the availability of the liquid dipper terminated synchronously, followed immediately by the illumination of LEDg to signal that responses would result in reinforcement. All rats were placed on a FR1 schedule that terminated after the delivery of 50 reinforcers. The response requirement was increased on a per-subject basis daily to reach a maximum of FR3 within 3 consecutive days of FR shaping. All subjects then experienced 5 once-daily sessions of the FR3 schedule maintained by sucrose.

Progressive Ratio Schedule

The *progressive ratio (PR) schedule* of reinforcement is a sequentially increasing series of response requirements that escalate from trial to trial, such that after a reinforcer is delivered at the termination of one trial (e.g. after one response), then the responses required to obtain reinforcement increase on the next trial (e.g. after 3 responses). The reinforcer magnitude is held constant across trials within an experimental session, so the functional utility of the reinforcer diminishes as the response requirement increases (1, 3, 6, 12, etc.). The PR schedule has been utilized as an assay of motivation among rats (Donny et al. 1999; Lesage et al. 2006; Palmatier et al. 2008). The progressive ratio schedule used in the present experiment escalated in the same fashion as in Arnold and Roberts (1996) and the MED-PC software program was adopted from M.I. Palmatier (Palmatier et al. 2008) and modified.

The PR schedule was introduced on day 6 of operant-schedule shaping (with the exception of Experiment 3 in which rats were maintained on a FR3 schedule). The PR-schedule sessions involved the same stimulus conditions as the FR3 schedule, but expired after 40 min or when a rat's inter-response times or post-reinforcement pauses exceeded 2.5 min. The 2.5 min threshold was a conservative criterion because pilot investigation demonstrated mean inter-response times < 2 s, mean post-reinforcement pauses < 4 s, and 90% of inter-response times and post-reinforcement times < 18 s. Thus, the primary measure of motivation on the PR schedule used was *session duration*. Although it is common for investigators to utilize *breakpoint* which can be defined as the number of ratio-response requirements met before a session expires, session duration is the primary dependent

variable used in the present series of experiments for several reasons. First, given that Exp 3 involves a FR3 rather than PR schedule, a comparison across experiments would be impossible using the breakpoint variable. Although there is some unanimity in the use of breakpoint as the primary dependent variable when drugs are used as a reinforcer, there is variability in what defines a breakpoint (Stafford, Lesage & Glowa, 1998). When food reinforcers are used with PR schedules, there is less consistency across studies (for example, see Weatherly, King & Uran, 2003; Wojnicki, Babbs & Corwin, 2010). For the purposes of the current set of investigations involving the PR schedule, session durations and breakpoints are both derivative of the timeout procedures described above, but the degree to which the two measures concur was evaluated by using breakpoint as a secondary dependent variable. Rowlett et al. (1996) compared breakpoints to session durations and found that the two measures provided congruent information pertaining to reinforcer efficacy.

Twin-session method and general procedure

The twin-session method (Experiments 1–3) involved two operant sessions each day, and there were three phases of the experiment. All rats experienced a 5 min blackout period in the operant chambers after the subcutaneous injections and before the first trial of each experimental session. There were 10 *baseline* sessions in which saline was administered prior to both sessions (i.e. SAL/SAL), and at least two hours separated the conclusion of the first operant session and the commencement of the next session. During the *dosing* phase, one session was conducted after the administration of 0.3 mg/kg nicotine and the other was conducted after the administration of saline (i.e. NIC/SAL). The two sessions were again separated by a minimum of two hours to allow for the elimination of nicotine, and the order of nicotine/saline dosing within each day was counterbalanced across days and pseudo-randomized. The sequence of the (a) nicotine and (b) saline doses was randomized each day using a coin toss, such that on some days of dosing, the rats received nicotine (NIC) in the morning and saline (SAL) in the afternoon and the reverse sequence would occur on other days. The purpose of the randomization was so that the time of day could not serve as a contextual cue and allow the rats to anticipate the delivery of nicotine. The *dosing* phase lasted for 10 consecutive days. The *recovery* phase involved returning to baseline conditions, in which both sessions were preceded by the administration of saline (SAL/SAL). The recovery phase also lasted for 10 consecutive days, so there were 30 days total in experimentation beginning with the first day of *baseline* to the final day of *recovery*. For each experiment, the two sessions were separated by a minimum of two hours, and sessions began between 9–10 am each day.

The twin-session design allowed for the within-subject assessment of nicotine-induced reinforcer enhancement following the acute administration of nicotine during the *dosing* phase. The saline sessions during the *dosing* phase provided an opportunity to witness the emergence of reinforcer devaluation in the absence of nicotine. During the *recovery* phase, the abatement of nicotine-induced reinforcer devaluation could be observed as the subjects returned to pre-nicotine levels of operant-schedule performance.

Experiment 1: the effects of 0.1 and 0.3 mg/kg nicotine on PR schedule responding for a sucrose reinforcer—The first experiment was a basic evaluation of

motivation using the PR schedule. All subjects ($N = 11$) experienced the twin sessions across the three phases of the experiment. The rats in each group received either 0.1 mg/kg ($n = 6$) or 0.3 mg/kg ($n = 5$) nicotine prior to one session each day during the *dosing* phase. During *baseline* and *recovery* phases, each rat received saline doses prior to both sessions.

Experiment 2: the effects of 1 mg/kg mecamylamine and 0.3 mg/kg nicotine on PR schedule responding for a sucrose reinforcer—

The purpose of this experiment was to determine whether motivational alterations due to nicotine administration in Experiment 1 were due to activation of nicotinic-acetylcholine receptors. This was accomplished by using the non-competitive nicotinic antagonist mecamylamine in combination with nicotine during the dosing phase. Theoretically, if motivational enhancement by nicotine is prevented with the antagonist, the motivational impairment produced by the absence of nicotine would not emerge. All rats ($N = 18$) experienced the twin sessions following saline administration during *baseline* and *recovery* phases in a manner that was identical to Experiment 1 (i.e. SAL/SAL). During the *dosing* phase, one group of rats in Experiment 2 received (a) 1.0 mg/kg mecamylamine plus 0.3 mg/kg nicotine or (b) saline (SAL) prior to each PR schedule session ($n = 6$). A positive control group was included in the experiment, which was a replication of the 0.3 mg/kg nicotine group from Experiment 1 ($n = 6$). A negative control group experienced only saline throughout all conditions was included ($n = 6$) for comparison.

Experiment 3: the effects of 0.3 mg/kg nicotine on FR3 schedule responding for a visual reinforcer—

The evidence of reinforcer devaluation seen in Experiments 1 and 2 could be specific to sucrose reinforcement or attributed to possible post-ingestive effects of the combination of sucrose and nicotine withdrawal. Therefore, a visual stimulus was used in place of sucrose to determine whether devaluation was a phenomenon that could generalize across reinforcers. All rats ($N = 6$) in Experiment 3 experienced the twin sessions in a manner that followed Experiment 1. All rats received SAL/SAL doses in *baseline* and *recovery*, and NIC/SAL doses (randomized) during the *dosing* phase and 0.3 mg/kg nicotine was used. Whether the visual stimulus would serve as an adequate reinforcer for the PR-schedule across 30 days of experimentation was unclear. Other investigators have used a visual-stimulus reinforcer, repeated nicotine dosing, and a FR3 schedule with success (Chaudri et al. 2006; Donny et al. 2003; Lui et al. 2007; Weaver et al. 2012); therefore, Experiment 3 was a departure from the other experiments in that the FR3 schedule and a visual stimulus were used in place of a PR schedule and a sucrose reinforcer.

The FR3 sessions for this experiment followed the parameters of the PR-schedule experiments in that the sessions expired after 40 min or when a rat's inter-response times or post-reinforcement pauses exceeded 5 min. The 5 min threshold was used in place of the 2.5 min threshold (as in Exp 1 & 2) because pilot data demonstrated that mean inter-response times and post-reinforcement pauses for the visual stimulus were nearly twice the duration of those of sucrose-solution reinforcement. The presentation of the stimulus events during the sessions were similar to those described in the "operant shaping" section, with a few minor exceptions. The illumination of the right nose-poke device (LEDg) and a houselight signaled the initiation of a trial, and upon the completion of the FR3 requirement, LEDg and

housetlight were terminated. A stimulus light located above the liquid-dipper well was illuminated in conjunction with LEDr, and both terminated synchronously after 1s. A 10-s inter-stimulus blackout was used to mirror procedures reported elsewhere (Donny et al. 2003), followed by the illumination of LEDg and the houselight to signal the start of the next trial.

Experiment 4: reinforcer devaluation for non-nicotine reinforcers as a product of nicotine withdrawal, negative contrast, or incentive learning—

Decrements in motivation were reliably produced after the SAL injections during the *dosing* phase in Experiments 1–3. If the results of Experiments 1–3 were due to negative contrast or some other ‘comparator’ hypothesis, then PR-schedule disruptions evidenced in the previous experiments may be an artifact of the twin-session technique that was used to assess anhedonia. Negative contrast can be defined as response-rate decrease resulting from a downshift in reinforcement contingency (e.g. Flaherty, 1997). Incentive learning theory (Balleine, 1992, 2001; Dickenson & Balleine, 1994; Lopez, Balleine, & Dickinson, 1992) suggests that operant behavior is determined by the interaction of the organism’s motivational state and the first experience of reinforcement. For instance, the reinforcer efficacy of food pellets is determined by a rat’s history with food-pellet reinforcement and their state of hunger (e.g. Balleine, 1992). If rats first experience food-pellet reinforcement when food-satiated, the magnitude of the responses emitted for those food pellets in the future is inhibited compared to rats that have learned about food-pellets when hungry. The twin session method and the PR schedule used in Experiments 1 and 2 required subjects to experience sucrose while (a) in a nicotine state, and (b) in a non-nicotine state (SAL). Thus, the rats learned something about the sucrose in the nicotine state that enhanced its efficacy. The possibility exists that reinforcer devaluation would not occur when rats do not experience sucrose in the nicotine state, but still experience nicotine in their home cages.

Three groups of rats experienced only one session daily ($N = 18$). The PR schedule sessions were the same as those used in Experiments 1 and 2, and sucrose was used to reinforce behavior. One group of rats (the “post-session” group: $n = 6$) were administered 0.3 mg/kg nicotine 1 hour after their PR-schedule sessions during the dosing phase, and another group (the “pre-session” group: $n = 6$) experienced 0.3 mg/kg nicotine just prior to their PR-schedule sessions. Both groups of rats were tested after ten days of once-daily s.c. injection of 0.3 mg/kg nicotine. Therefore, on Day 11 (what would be considered day 1 of recovery in Experiments 1–3), both groups were tested following a saline injection. A third group ($n = 6$) served as a saline-control, and these rats received only saline injections for the same period as the pre and post-session groups.

Results

Experiment 1

Figure 1 depicts the emergence of (a) nicotine-enhanced and (b) gradually diminished PR-schedule performance during the *dosing* phase. Paired-samples *t*-tests were performed to compare the *baseline* endpoint performance measure of session duration between session 1 vs. session 2 for both the 0.1 and 0.3 mg/kg groups, independently; all t ’s < 1.8 , all P ’s $>$

0.05 (degrees of freedom = 4 & 5, respectively). Furthermore, paired-samples t-tests were also used to compare these same measures at the final day of the *recovery* phase; all t 's < 1.2, all P 's > 0.05. Thus, for the *baseline* and *recovery* endpoints, session 1 and session 2 were collapsed for the remaining data analyses, but the data from the 0.1 and 0.3 groups remained separated.

The remaining analyses were performed using mean session durations from the collapsed *baseline* and *recovery* endpoints, and the data obtained from the final day of the *dosing* phase for both SAL and NIC sessions. Therefore, a 2(groups: 0.1 vs. 0.3 mg/kg) x 4(condition/phase: collapsed baseline endpoints, dose 10 saline, dose 10 nicotine, and collapsed recovery endpoints) repeated-measures ANOVA was performed. A significant main effect of group was not discovered, likely because group differences were not apparent at *baseline* and *recovery* endpoints; $F(1,9) = 1.08$, $P > 0.05$. A main effect of condition/phase was found; $F(3,27) = 17.05$, $P < 0.05$, partial $\eta^2 = 0.66$. A significant group x phase interaction was also discovered; $F(3,27) = 5.08$, $P < 0.05$, partial $\eta^2 = 0.39$. Post-hoc multiple comparison (LSD) revealed that the collapsed *baseline* and *recovery* endpoints did not differ from one another (P 's > 0.05) suggesting that session times recovered to baseline levels. Both sessions for the final day of the *dosing* phase (dosing-day 10) differed significantly from both *baseline* and *recovery* (P 's < 0.05). Therefore, 10 sessions of nicotine dosing resulted in significantly increased session times following the administration of nicotine, and significantly lower session times following the administration of saline, regardless of group (0.1 vs. 0.3 mg/kg). Also, session times for the final day of the *dosing* phase differed between the nicotine and saline sessions ($P < 0.05$, NIC > SAL). The results reveal that nicotine dosing yields enhanced motivation for sucrose, as measured by PR-schedule session durations. A secondary consequence of nicotine administration is decreased motivation in the absence of nicotine during the saline sessions. Figure 1 illustrates that the session times decrease in a gradual pattern across the ten days of the *dosing* phase. Furthermore, session times gradually return to baseline levels across the ten days of SAL/SAL sessions in the *recovery* phase.

The ANOVA results reported above demonstrate a significant dose X phase interaction, but whether each dose magnitude (0.1 or 0.3 mg/kg) independently results in greater or lesser session durations after 10 days of dosing requires further examination. To determine whether the sessions of dosing-day 10 (SAL and NIC) differed from baseline, a 3 (condition/phase: collapsed baseline endpoints, dose 10 saline, dose 10 nicotine) repeated-measures ANOVA was performed for each group independently. A marginally significant effect was found for the 0.1 mg/kg group; $F(2,10) = 3.12$, $P = 0.06$, partial $\eta^2 = 0.33$. LSD post-hoc analysis revealed that the saline session (and not the nicotine session) from the final day of the *dosing* phase resulted in significant differences from the *baseline* phase ($P < 0.05$). A significant main effect was found for the 0.3 mg/kg group; $F(2,8) = 25.76$, $P < 0.05$, partial $\eta^2 = 0.87$. Post-hoc analysis performed on the 0.3 mg/kg group revealed that the saline and nicotine sessions from the *dosing* phase both resulted in significant differences from *baseline* ($P < 0.05$). Therefore, only the larger of the two doses yielded significant increases in session durations post-nicotine delivery. Both groups demonstrated lesser session durations in the saline conditions at the end of the *dosing* phase compared to *baseline*, and

an unpaired t-test performed to compare the two groups at the end of the *dosing* phase failed to show that the 0.3 mg/kg dose yielded significantly lower session duration compared to the 0.1 mg/kg dose; $t(9) = 1.11$, $P > 0.05$. The results from these analyses provide some evidence that the effects of nicotine to induce motivational changes are dependent upon dose.

Linear regression analysis was performed using GraphPad Prism 5TM on the saline conditions during the *dosing* phase to statistically verify that session durations degraded over the course of nicotine dosing. The analysis included the sessions durations for each rat during the 10 days of *dosing*, and the analysis showed a significant difference from no slope for both the 0.1 and 0.3 mg/kg nicotine groups; $F(1,9) = 53.05$ & 14.36 , P 's < 0.05 , r^2 's = 0.86 & 0.62 , respectively. A runs test of linearity failed to demonstrate deviations, thus demonstrating that the degradation in PR performance was linear for both groups (P 's > 0.05). To determine when saline sessions during *dosing* resulted in significant differences from *baseline*, repeated measures ANOVA's were conducted on each group's session durations. For the 0.1 mg/kg group, LSD post-hoc multiple comparisons demonstrated that the fourth day of dosing was the first occasion when session times significantly dipped below *baseline*, and *dosing* days 4,5,9, and 10 all differed from *baseline* (P 's < 0.05). For the 0.3 mg/kg group, days 4–10 all differed significantly from *baseline* (P 's < 0.05); see Figure 1. These results suggest that there was a linear reduction in motivation that corresponded to accumulated nicotine exposure for both groups, and that degradation in session durations can be witnessed after 3–4 injections of nicotine.

Breakpoints were defined as the number of ratio requirements completed before the sessions timed out. Breakpoints were examined as a secondary but complementary analysis to the session-duration analysis described above, and the 0.1 and 0.3 mg/kg groups were assessed independently to simplify the analyses. Repeated measures ANOVAs were performed across the 6 conditions/phases (2 baseline endpoints, dose 10 saline, dose 10 nicotine, and 2 recovery endpoints). For the 0.1 mg/kg group, significant differences were discovered; $F(5,25) = 2.43$, $P = 0.06$, partial $\eta^2 = 0.33$. LSD post-hoc multiple comparisons demonstrate that the nicotine condition at the end of the *dosing* phase differed from the saline session, and from both *baseline* endpoints (P 's < 0.05). Significant differences were also revealed for the 0.3 mg/kg group; $F(5,20) = 7.83$, $P < 0.05$, partial $\eta^2 = 0.66$. LSD post-hoc multiple comparisons showed that the nicotine session at the end of *dosing* differed from all other conditions (P 's < 0.05). The saline session at the end of the *dosing* phase differed from one *baseline* and one *recovery* endpoint (P 's < 0.05). Baseline and recovery endpoints failed to differ for both groups (P 's > 0.05). The results from the breakpoint analysis seem to parallel the results from the session duration analysis, and breakpoint values are listed in Table 1.

Experiment 2

Given that more robust effects were found for 0.3 mg/kg than 0.1 mg/kg nicotine in Experiment 1, only 0.3 mg/kg nicotine was used in Experiment 2. One group of rats received the combination dose of 0.3 mg/kg nicotine and 1.0 mg/kg mecamylamine during the *dosing* phase. A 3(groups: saline control, 0.3 mg/kg nicotine, and 1.0 mg/kg mecamylamine plus 0.3 mg/kg nicotine) X 2 (*dosing*-phase endpoint: saline vs nicotine)

ANOVA was performed on both session durations and breakpoints. For session durations, a significant main effect of *dosing*-phase endpoint was discovered ($F(1,15) = 9.5$, $P < 0.05$, partial $\eta^2 = 0.39$) but no group main effect ($F(2,15) = 1.23$, $P > 0.05$). A significant group X *dosing*-phase endpoint was revealed ($F(2,15) = 6.68$, $P < 0.05$, partial $\eta^2 = 0.47$). Breakpoint analyses produced a similar pattern: a significant main effect of *dosing*-phase endpoint was discovered ($F(1,15) = 9.84$, $P < 0.05$, partial $\eta^2 = 0.40$) but no group main effect ($F(2,15) = 0.09$, $P > 0.05$), but a significant group X *dosing*-phase interaction was revealed ($F(2,15) = 5.42$, $P < 0.05$, partial $\eta^2 = 0.42$).

To determine whether the saline condition at the end of the *dosing* phase produced significantly lesser session durations and breakpoints across groups, a one-way ANOVA revealed significant differences for session durations ($F(2,17) = 4.9$, $P < 0.05$) and breakpoints ($F(2,17) = 4.29$, $P < 0.05$). LSD multiple post-hoc between-groups comparison on session duration shows that the 0.3 mg/kg nicotine group differed from the saline control group ($P < 0.05$) but all other comparisons were non-significant. For a visual comparison of session durations across groups, see Figure 2. Table 1 provides information on breakpoints; LSD multiple comparisons performed across groups on breakpoints shows that the 0.3 mg/kg nicotine groups differed from both other groups (P 's < 0.05), but that the saline versus mecamylamine + nicotine group failed to differ ($P > 0.05$). Taken together, the results from session durations and breakpoints show that 1.0 mg/kg mecamylamine effectively attenuated the effect of 0.3 mg/kg nicotine.

Experiment 3

The effect of 0.3 mg/kg nicotine on FR3 schedule performance maintained by the presentation of a VS was assessed in Experiment 3. The general pattern of session-durations mirrored what was witnessed in Experiment 1 when PR schedule responding was maintained by sucrose (Figure 3); (a) session durations increased following 0.3 mg/kg s.c. injections of nicotine, and (b) decreased during the saline sessions. A 6 (condition/phase: baseline endpoints 1 & 2, saline-dose day 10, nicotine-dose day 10, recovery endpoints 1 & 2) repeated-measures ANOVA was performed on the session duration. A significant main effect was discovered; $F(5,25) = 10.85$, $P < 0.05$, partial $\eta^2 = 0.69$. LSD multiple-comparison post-hoc analyses demonstrated that baseline and recovery endpoints failed to differ (P 's > 0.05); however, nicotine dose-day 10 differed significantly from saline dose-day 10, baseline endpoint 1, and both recovery endpoints (P 's < 0.05). Saline dose-day 10 differed significantly from all other conditions (P 's < 0.05). Therefore, the results of Experiment 3 replicate the findings with PR-schedule performance maintained by sucrose reinforcement.

In a statistical approach similar to Experiment 1, linear regression analysis was performed on the saline sessions during the *dosing* phase to verify that session durations degraded over the course of nicotine dosing. The analysis included the sessions durations for each rat during the 10 days of dosing, revealing a significant difference from no slope; $F(1,9) = 13.5$, $P = 0.7$, $r^2 = 0.28$. The low r^2 value represents a failure of the linear regression to fit the slope, likely due to day-to-day variability in session durations (as depicted in Fig 3) despite the fact that a runs test failed to demonstrate deviations from linearity, P 's > 0.05 . To

determine when saline sessions resulted in significant differences from *baseline*, repeated measures ANOVA's were conducted. LSD post-hoc multiple comparisons demonstrated that the days 6,9 and 10 differed significantly from *baseline* phase (P 's < 0.05). The repeated-measures analysis shows that accumulated experience with nicotine is necessary to produce disruptions in session duration when the visual-stimulus reinforcer and a FR3 schedule are used.

Experiment 4

Experiment 4 involved three groups of rats that experienced single (not twin) sessions throughout the duration of the experiment. These groups received either 0.3 mg/kg nicotine before a daily PR-schedule session (i.e. "pre-session" group) or 0.3 mg/kg nicotine 1 hour after their session ("post-session" group), or no nicotine ("saline control"). The statistical analysis of these groups focused on nicotine-induced reinforcer devaluation, so nicotine enhancement of PR-schedule behavior in the pre-session group was not examined. Therefore, a 3 (groups) X 2 (phase/condition: baseline endpoint, recovery day 1) repeated-measures ANOVA was performed on the session duration and breakpoint variables. The reason why *recovery* day 1 was used instead of dosing-day 10 in this analysis was to equate the groups by condition; on *recovery* day 1, all groups would be tested in the absence of the acute effects of nicotine which would not be the case if the groups were compared on dosing day 10. For session duration, a significant main effect of phase/condition was discovered ($F(1,15) = 25.88$, $P < 0.05$, partial $\eta^2 = 0.63$) as well as a significant group x phase/condition interaction ($F(2,15) = 6.32$, $P < 0.05$, partial $\eta^2 = 0.46$). A main effect of group was significant also; $F(1,15) = 10.49$, $P < 0.05$, partial $\eta^2 = 0.58$, see the interaction in Figure 4. Analysis of breakpoints revealed dose X phase/condition interaction that approached significance ($F(2,15) = 2.72$, $P = 0.09$, partial $\eta^2 = 0.26$), but no significant main effects.

To determine whether the three groups differed on the test day after nicotine dosing terminated, a one-way between groups ANOVA was performed on the two aforementioned dependent variables. Significant differences were discovered for session durations ($F(2,17) = 16.07$, $P < 0.05$) and breakpoints ($F(2,17) = 4.02$, $P < 0.05$). LSD multiple comparisons across groups for both session durations and breakpoints were uniform, such that the pre-session nicotine group differed from both other groups (P 's < 0.05), but the post-session nicotine group and the saline group failed to differ (P 's > 0.05). Therefore, in order for nicotine to produce significant disruptions in sucrose-reinforced behavior, the sucrose reinforcer must be experienced in the presence of nicotine.

Discussion

The series of experiments demonstrate that repeated once-daily administration of nicotine results in extended operant-response session durations and breakpoints, which can be interpreted as nicotine-enhanced motivation. Enhanced motivation was apparent regardless of whether behavior is maintained by sucrose or visual-stimulus presentation; this finding is not novel and is congruent with other research conducted on the rate-enhancing effects of nicotine on behavior (see Perkins, 1999 for a review of rate-enhancing effects of nicotine).

The relatively new discovery is that in a background in which nicotine enhances motivation, the absence of nicotine appears to produce degradation in reinforcer value. The finding that reinforcer devaluation develops rapidly after a few, once-daily doses is congruent with other reports of an immediate increase in ICSS thresholds following withdrawal of a single nicotine dose (Harris et al. 2013).

Experiments 1 and 2 provide some evidence that reinforcer enhancement and devaluation are tied to the occupation of nicotinic-acetylcholine receptors (nAChRs). There was a dose-related effect of nicotine such that reinforcer devaluation appeared more quickly and was marginally greater in the larger 0.3 mg/kg nicotine dose compared to 0.1 mg/kg. Furthermore, mecamylamine (1.0 mg/kg) co-administration prevented changes in motivation resulting from 0.3 mg/kg nicotine exposure. Therefore, activation of nAChRs are necessary to produce nicotine-induced reinforcer devaluation. The extent to which reinforcer devaluation by nicotine is related to anhedonia or a decrease in reward responsiveness (Pergadia et al. 2014) during tobacco withdrawal is unclear. The present results are consistent with a wide body of literature suggesting that continued stimulation of nAChRs with a nicotine-replacement therapy during tobacco-use cessation serves to attenuate negative affect (e.g. Piper et al. 2011). Nicotine replacement is an effective means of managing withdrawal symptoms, and although purely speculation at this time, nicotine-replacement therapy may serve to attenuate a crisis of reinforcer devaluation.

The twin-session technique used in Experiments 1–3 involved a randomized sequence of nicotine-dose delivery during the *dosing* phase, such that over the course of 10 dosing days, any given saline session could occur either 2 or 20 hours after the last nicotine dose. Nicotine may not be completely eliminated after 2 hours, and for this reason, one might see evidence of more substantial reinforcer devaluation when the saline sessions occurred 20 hours after the last nicotine dose. However, operant-schedule performance was not dependent on the order of dosing (see “N” vs “S” in Figures 1 and 3) and reinforcer devaluation in the saline conditions during the *dosing* phase was evident even when nicotine had been delivered 2 hrs prior.

Experiment 3 was conducted with the visual stimulus to determine whether reinforcer devaluation was an artifact related to food reinforcers, exclusively, and whether the devaluation effect transcended reinforcer type. Conclusions based upon the results of Experiment 3 need to be made cautiously because it involved a FR3 and not the PR schedule that was used in the other experiments. Using the dependent variable of session duration as a common means of comparison, reinforcer devaluation appeared under a similar time-frame (10 days) in all three experiments regardless of whether sucrose or a visual-stimulus reinforcer was used. If the diminished efficacy of sucrose reinforcement in Experiments 1–3 were related to a withdrawal-induced gastrointestinal state incompatible with foraging, then one might expect that a visual stimulus used in place of sucrose would not produce a similar pattern of reinforcer devaluation. Therefore, the finding that operant responding lessened during the non-nicotine sessions when a visual stimulus was used supports the notion that nicotine exposure causes changes to general motivational processes underlying behavior. Of course, sucrose was used initially in Experiment 3 to shape operant responses; therefore, the visual stimulus was a sucrose-conditioned reinforcer. The possibility still exists that

gastrointestinal distress may impair conditioned reinforcement by the devaluation of the originally-trained reinforcer. In cases where reinforcer devaluation impairs operant responding, lithium chloride has been used to interfere with the motivational value of food (Parades-Olay & Lopez, 2002). However, this possibility that nicotine withdrawal interferes with operant responding via digestive impairment is unlikely given that nicotine withdrawal increases, rather than decreases, food consumption in rodents (Levin, Morgan, Galvez, & Ellison, 1987).

Decreased reinforcer efficacy due to nicotine withdrawal have been precipitated by the termination of osmotic nicotine administration (e.g. Lesage et al. 2006) or by the delivery of an antagonist (Weaver et al. 2012), and the results of these previous studies are relevant to nicotine withdrawal. However, the timeframe regarding the emergence of reinforcer devaluation has not been previously evaluated as it may relate to the transition from recreational use of nicotine to dependence. The major benefit of the twin-session design was that it provided the opportunity to witness the emergence of nicotine-induced reinforcer devaluation in a time-sequenced manner. Furthermore, the twin-sessions demonstrated that devaluation may be relative to a dose-dependent reinforcer-enhancing effect. The gradual onset of nicotine-induced reinforcer devaluation and the sequential abatement of reinforcer devaluation are suggestive that learning mechanisms may be at play. Therefore, the return-to-baseline levels of operant performance in the *recovery* phase of Experiments 1 and 3 may be analogous to an extinction event (e.g. Kirshenbaum, Bickel, & Olsen, 2009). The possibility that recovery from nicotine exposure is subject to the mechanism of extinction learning was not explored in the present series of experiments, but it may be a worthwhile analysis for future experimentation.

Experiment 4 was performed to assess whether the properties of incentive learning could be operating to produce nicotine-induced reinforcer devaluation. The experiment was simple in that it assessed PR schedule responding for sucrose after 10 daily doses of nicotine (0.3 mg/kg). The results demonstrate that in order for reinforcer devaluation to emerge within 10 days, sucrose reinforcement must be experienced in the presence of nicotine. Theoretically, the learning about sucrose as a reinforcer was modified by the presence of nicotine, and the basic incentive value of sucrose diminished as a result of that pairing. In terms of the cross-species application of the findings from Experiment 4, the experience of reinforcers in the presence of nicotine (e.g. listening to music while smoking) may undermine the ability of those same reinforcers to maintain responding in the absence of nicotine. Given that smokers tend to experience the world of available reinforcers in the presence of nicotine, reinforcer devaluation and generalized anhedonia may be interrelated processes that contribute to the escalation and maintenance of tobacco use.

The ‘incentive-learning’ effect may be more pertinent to the transition from recreational use to dependence rather than nicotine withdrawal. Recently Harris et al. (2013) demonstrated that a single dose of 0.5 mg/kg nicotine followed by mecamylamine produced predicted changes in ICSS thresholds in rats. Harris et al. (2013) have speculated that anhedonia produced by an initial dose of nicotine may be a factor that speeds the transition from recreational use to dependence, and the presently reported results serve to support that hypothesis. Although ICSS threshold increases appear to coincide with the emergence of

somatic withdrawal (e.g. Epping Jordan et al. 1999; Harris et al. 2013), unclear at this time is whether somatic symptoms of nicotine withdrawal (e.g. Small et al. 2009) would coincide with the emergence of reinforcer devaluation. Future research may help to resolve this question. In Experiment 4, it is feasible that extended exposure to nicotine in the post-session nicotine group would eventually result in reinforcer devaluation. Clearly, the duration of drug exposure has a profound impact on how the mechanisms of learning are engaged during substance abuse (see Hogarth et al. 2013), and 10 once-daily exposures of 0.3 mg/kg nicotine are not a sufficient analogy to the chronic nicotine exposure which occurs in habitual tobacco use.

The results from Experiment 4 provide some assurance that the reinforcer devaluation produced by nicotine was not a pure pharmacological result of nicotine withdrawal, nor were the results of Experiments 1–3 a product of a negative-contrast effect created by the twin-session technique. Incentive-learning theory predicts that the coincidence of nicotine and sucrose promotes inevitable alterations to the fundamental efficacy of sucrose reinforcement, and this prediction runs in unison with at least two other learning theories such as the *opponent-process model* (Solomon & Corbit, 1974; see also Barr and Markou, 2005; Koob, 1997, LeMoal & Koob, 2007) and the *dual-reinforcement hypothesis* (e.g. Caggiula et al. 2009). The opponent-process model of motivation suggests that unconditioned stimuli that shift central-nervous system processes away from homeostasis automatically elicit endogenous compensatory/opponent reactions that return the nervous system back to baseline, and opponent processes are elicited by conditioned stimuli. Nicotine induces a hedonic state (e.g. Xue & Domino, 2009) so it would follow this general precept that non-nicotine may produce anhedonia when the organism is in an environment associated with nicotine. The dual-reinforcement hypothesis (Donny et al. 2003; Chaudhri et al. 2006, Lui et al. 2007; Palmatier et al. 2008; Weaver et al. 2012) posits that nicotine is a primary reinforcer and it enhances the positive-reinforcing properties of non-pharmacological events (also see Olausson, Jentsch, & Taylor, 2004). This property goes beyond nicotine's ability to establish conditioned reinforcement, and may be better described as an 'incentive amplifying' effect (Palmatier et al. 2013). A logical extension of the dual-reinforcement hypothesis is that in a background in which nicotine enhances reinforcement, the absence of nicotine may reliably produce reinforcer decrement (Weaver et al. 2012). Thus, the experience of sucrose reinforcement while in a nicotine-induced state is a necessary component to produce sucrose devaluation.

Although the pharmacological aspects of nicotine withdrawal have received much deserved attention because of the potential for medical assistance during tobacco-use cessation (Dwoskin et al. 2009; Kenny & Markou, 2001), the learning principles that contribute to the development of various features withdrawal have received less experimental consideration. The degree to which reinforcer devaluation has relevance to cessation of tobacco use, or the abuse liability of tobacco products, remains unclear. Recently in humans, Perkins and Karelitz (2013) demonstrated that nicotine enhances the reinforcer value of sensory stimuli in a manner that coincides with the dual-reinforcement hypothesis. Although their finding remains to be replicated, Perkins and Karelitz (2013) taken in combination with Weaver et al. (2012) and the present report, may provide some indication that nicotine exposure serves

to enhance, and then devalue, non-pharmacological reinforcers. The diminished efficacy of reinforcers in the absence of nicotine during initial tobacco-use cessation may add to affect dysregulation and further complicate sustained abstinence.

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Fig. 1. Session durations (min) for each group (0.3 and 0.1 mg/kg) for the twin sessions throughout the duration of *dosing* and *recovery*, N and S represent whether either nicotine or saline was delivered in the first of the twin sessions during dosing; * $P < 0.05$ compared baseline

Fig. 2. Dosing-day 10 session durations for Exp 2, $*P < 0.05$ in a comparison of groups and conditions

Fig. 3.

VS-group (0.3 mg/kg nicotine) twin-session results throughout the duration of *dosing* and *recovery* and N and S refer to whether nicotine or saline were delivered in the first of the twin sessions for any given day, * $P < 0.05$ compared to baseline

Fig. 4.

Dose x group interaction demonstrating the influence of pre-session nicotine exposure on PR- schedule performance, * $P < 0.05$ compared across groups

A comparison of breakpoint data (number of response requirements met) from Experiments 1, 2 & 4; all doses in mg/kg. The information below presents the means (and standard errors) of the groups and conditions that were used in the statistical analyses on breakpoints.

Table 1

Experiment 1	Condition Endpoints					
	Baseline 1	Baseline 2	Dosing: Saline	Dosing: Nicotine	Recovery 1	Recovery 2
0.1 nicotine	12.67 (1.23)	12.50 (0.61)	10.67 (0.91)	15.33 (1.02)	12.20 (1.31)	12.33 (1.36)
0.3 nicotine	12.00 (1.51)	13.40 (1.53)	9.40 (0.97)	18.00 (0.71)	12.50 (1.01)	13.00 (1.34)
Experiment 2	Dosing: Saline	Dosing: Drug				
Saline	11.67 (0.67)	13.83 (0.75)				
0.3 nicotine (replication)	8.67 (1.20)	16.61 (1.83)				
1.0 mecamylamine + 0.3 nicotine	12.33 (0.88)	12.00 (1.87)				
Experiment 4	Baseline	Post-dosing test				
Saline	12.50 (1.12)	11.91 (0.96)				
Pre-session 0.3 nicotine	13.17 (0.65)	9.05 (0.71)				
Post-session 0.3 nicotine	12.33 (0.67)	13.33 (0.81)				