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The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose

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I. Summary

Stimuli associated with nicotine (NIC) can acquire new meaning via Pavlovian conditioning. If a stimulus is associated with the primary reinforcing effects of NIC, the new conditional properties of the stimulus should make it a more valuable reinforcer (i.e., increase the motivation to obtain the stimulus), and this value should be based, in part, on the strength or intensity of the primary reinforcer (i.e., NIC dose). The purpose of the present study was to investigate whether NIC-conditioned reinforcement increased motivation to obtain non-NIC stimuli, as reflected by performance on a progressive ratio (PR) reinforcement schedule, and whether this increased motivation was systematically related to NIC dose. Two Paired groups were allowed to nose-poke for NIC (0.03 or 0.09 mg/kg/infusion, IV) accompanied by 15-s illumination of a stimulus light (conditional stimulus or CS). Two Unpaired groups (0.03 or 0.09 mg/kg/infusion) could also make a nose-poke response for the CS; however their NIC infusions were controlled by the Paired Group (i.e., yoked design). A fifth group (CS-Only) was allowed to nose-poke for CS-presentations and saline infusions. After 29 conditioning sessions the nose-poke operant was prevented by obscuring the receptacle and the CS (accompanied by saline infusion for all groups), was made contingent upon a novel operant response (lever press). During the acquisition of this novel response, each CS/saline infusion earned increased the number of responses required to earn the next CS/saline infusion. Pairings with the primary reinforcing effects of NIC resulted the acquisition of a novel response for the CS. Motivation to obtain the CS depended on salience (dose) of the primary reinforcement (NIC).

Keywords

Nicotine; Conditioning; Reinforcement; Self-Administration; Pavlovian; Operant

II. Introduction

Nicotine (NIC) is widely regarded as the primary reinforcer in tobacco smoke (Corrigall, 1992; Goldberg et al., 1981; Perkins et al., 1994; Rose and Levin, 1991). According to this view, when NIC reaches the central nervous system it produces a physiological effect that strengthens the preceding behaviors. This definition of primary reinforcement is satisfied by

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the observation that an increase in tobacco use (and thus NIC-intake) typically follows initial exposure to the drug. Accordingly, modeling NIC/tobacco dependence in non-human animals has increasingly relied on paradigms in which the primary reinforcing effects of NIC can be measured (i.e., self-administration).

Despite the face validity of self-administration models, recent advances have suggested that primary reinforcement alone cannot fully capture all the elements of substance dependence (Ahmed and Koob, 1999; Roberts et al., 2007). Recent studies from our laboratory have investigated the interaction between NIC and non-NIC stimuli in rodent self-administration models (Donny et al., 2003). We have demonstrated that NIC interacts with non-NIC stimuli in at least two ways. First, NIC has reinforcement enhancing properties; the effects of NIC can unconditionally potentiate responding for non-drug primary reinforcers (Liu et al., 2007b; Palmatier et al., 2006), conditioned reinforcers (Brunzell et al., 2006) and rewarding electrical brain stimulation (Harrison et al., 2002). This effect of NIC is comparable to other psychomotor stimulants, such as amphetamine, which can increase responding for unconditioned (Glow and Russell, 1973) and conditioned (Robbins, 1976, 1977, 1978) reinforcers. Second, NIC may interact with non-NIC stimuli by associatively altering their status or valence to the subject. An extensive literature describes increases in subjective craving (Tiffany et al., 2000), sympathetic activity (Conklin and Tiffany, 2001), and vigor of smoking behavior (Payne et al., 1991) when abstinent smokers are presented stimuli that are associated with the effects of NIC (e.g., lit cigarette in an ashtray). Complementary pre-clinical studies assert that stimuli associated with NIC self-administration can 'reinstatement' operant responding after extended periods of abstinence imposed by the experimenter (Liu et al., 2006). In both cases, the ability of these stimuli to influence the subject is presumed to stem from prior association with NIC; the new associative properties of the non-NIC stimuli gives them control over a response that they previously did not control.

Although many studies have investigated the relevance of NIC-associated 'cues' to reinforced operant behavior in the historical context of reinstatement (Cohen et al., 2005; Liu et al., 2007a), comparably few have investigated how NIC-conditioned reinforcement is acquired (Palmatier et al., 2007c). We have argued that at least two considerations are needed to establish that a NIC-paired stimulus is a conditioned reinforcer (Palmatier et al., 2007c). First, the non-NIC stimulus must support the acquisition of a novel operant response (Mackintosh, 1974). Second, any potential intrinsic reinforcement derived from the stimulus and/or potentiating effects of NIC should be dissociated from conditioned reinforcement using control conditions. Following these guidelines, we recently established that pairings between NIC infusions and a conditioned stimulus (CS, 15-s illumination of a cue-light) established the stimulus as a conditioned reinforcer. The CS supported the acquisition of a novel operant response (lever press) and lever pressing for the CS in the critical group (Paired) occurred with greater frequency relative to groups that had previous experience with the stimulus only (CS-Only) or had received comparable exposure to the CS and NIC infusions in a temporally uncorrelated manner (Unpaired group; Palmatier et al., 2007c).

One aspect of conditioned reinforcement which may be critical for NIC/tobacco dependence is the strength and endurance of responses evoked by non-NIC conditional stimuli (CSs) – stronger associations between the CS (non-NIC stimulus) and unconditional stimulus (US, in this case NIC) are expected to have a deeper influence on behavior. There are a number of factors that may influence the strength of the association between a CS and US, prominent among them is US intensity (also referred to as 'magnitude' or 'salience', see Annau and Kamin, 1961; Martin and Riess, 1969). However, extending the effects of US intensity or salience to drug reinforcers can be complicated by the complex relationship between unit infusion dose and operant behavior. The progressive ratio (PR) schedule has been used to evaluate motivation to obtain the reinforcer in a manner that is minimally confounded with

satiating or rate-limiting effects of reinforcer delivery (Richardson and Roberts, 1996). Using this schedule, there is a positive linear relationship between drug intensity (i.e., unit dose) and self-administration behavior; breaking points increase as dose increases (Richardson and Roberts, 1996) and the efficacy or relative value of various reinforcers can be evaluated directly.

Within limits, a more intense effect of NIC (i.e., dose) should have greater primary reinforcing properties (Chaudhri et al., 2007) and more radically change meaning of a paired CS, resulting in more rapid acquisition and/or greater expression of the new operant response. A CS associated with more intense effects of NIC should have greater efficacy as a conditioned reinforcer. Thus, under the PR schedule the efficacy of a NIC-conditioned reinforcer should depend on the unit NIC dose that accompanied CS presentation (US-intensity or salience). Therefore, the present studies sought to determine whether a CS associated with a more salient US (higher unit NIC dose) would support higher breaking points when the acquisition of a new response occurs on a PR schedule of reinforcement.

III. Method

Subjects

Male Sprague-Dawley rats (174-200 g on arrival, Harlan Farms, IN) were housed individually in hanging wire mesh cages. The light cycle in the temperature- and humidity-controlled colony room was reversed (12:12 h dark:light). Unrestricted access to food and water was allowed for the first 3 days in the colony. Food was subsequently restricted to a diet of 20 g per day allowing growth (approximately 20 g/week) throughout the remainder of the study (Donny et al., 1995). All experiments were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals and all procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Apparatus

Experimental sessions were conducted in 12 operant chambers (BRS/LVE Model RTC-020, MD) measuring $25 \times 31 \times 28$ (w $\times$ l $\times$ h) cm. One wall of each chamber was equipped with two stimulus lights located above two retractable levers. Nose-poke operant receptacles equipped with infrared emitter/detector units were located between the two levers (approximately 3 cm from the floor of the chamber) in each chamber. A house-light fixture containing a red house light was located above the nose-poke receptacle; approximately 2.5 cm from the top of the chamber; illumination and extinction of this house light signaled the beginning and end of each experimental session, respectively. Fifteen second illumination of a white stimulus light served as the CS. Each subject was connected to a drug-delivery system which allowed nearly unrestricted movement in the chamber.

Drugs

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) was dissolved in 0.9% saline and the solution pH was adjusted to 7.0 (± 0.2) with dilute NaOH. Nicotine infusions were delivered at a volume of 0.1 ml/kg/infusion in less than 1 s; infusion doses (0.03 or 0.09 mg/kg/infusion) was calculated from the base form.

Surgery

After habituation to the colony room, rats were implanted with chronic indwelling jugular vein catheters. A previous study provided detailed description of catheter construction and surgical procedures (Donny et al., 1998). Catheters were irrigated daily with heparinized sterile saline

vehicle (0.1 ml) that included the antibiotic ticarcillin plus clavulanate (Timentin®) to reduce post surgical infections.

Procedure—A schematic representation of experimental procedures, dose conditions, and the number of subjects in each condition is presented in Table 1.

Conditioning Phase (Sessions 1-29)

Following recovery from surgery, rats were randomly assigned to one of three groups: Paired (n=24), Unpaired (n=24) and CS-Only (n=12). Rats in the Paired and Unpaired groups were further assigned to one of two dose conditions (0.03 or 0.09 mg/kg/infusion of NIC, n=12/dose/group); the doses were based on findings from previous studies using the PR schedule of reinforcement (Chaudhri et al., 2007). Conditioning sessions were conducted as previously described (Palmatier et al., 2007a). Briefly, levers remained retracted throughout the session to prevent adventitious pairings between this operant and CS and/or US (NIC) delivery. All rats were given access to the nose-poke receptacle. For Paired rats, meeting the operant schedule resulted in delivery of the assigned NIC infusion (0.03 or 0.09 mg/kg) and the 15-s CS. Each NIC/CS presentation was followed by a 45 s time-out, during which nose pokes had no consequences. The CS-Only group had a similar contingency except that nose-poke responses resulted in CS presentation and a saline infusion. For Paired and CS-Only groups, infusions were delivered during the first second of the 15-s CS. Unpaired rats were allowed to nose-poke for the CS, but NIC infusions were controlled by the Paired group in order to equate US (NIC) exposure. NIC infusions were passively administered to the Unpaired group with the constraint that each infusion was separated by a minimum of 60 s from the subjects last CS presentation or operant response. This procedure only equates NIC exposure, not CS exposure. This procedure was used because passive CS-presentation cannot detect NIC's reinforcement enhancing properties (Caggiula et al., 2002). To evaluate whether responding for the CS is potentiated by NIC, the CS must be response-contingent.

During conditioning an FR schedule was in force so that the number drug-CS pairings was comparable across dose conditions; low fixed ratio schedules tend to produce relatively flat dose-response curves for NIC (Donny et al., 1999; Donny et al., 1998). Initially, a fixed ratio 1/60-s time-out (FR1/TO 60 s) was in force (Palmatier et al., 2007c). During conditioning we observed a potentiating effect of NIC on responding for the CS in the Unpaired group; by the 8th conditioning session, response rates in this group were comparable to Paired rats. In order to better determine whether CS presentations were equally reinforcing in both groups, the schedule was increased to an FR5/TO 60-s for the remainder of the conditioning phase (sessions 9-29).

Acquisition of a New Response Tests (Sessions 30-34)

Acquisition of a new response tests were carried out under a PR schedule, thus the length of each testing session was increased to 2-h. All nose-poke receptacles were sealed by aluminum plates to prevent this response option from interfering with lever pressing. Two levers were extended into the chamber 30 s after the start of the session. Contingencies were identical for all groups and dose conditions, responses on the randomly designated 'active' lever resulted in presentation of the CS accompanied by a saline infusion. The 45 s time-out after each CS was omitted because of the increasing operant schedule (PR); CS-presentation depended on completing the current ratio in force and was unaffected by delay from the last reinforcement earned. After the fifth testing session, catheter patency was determined with a 200 mg/kg infusion of sterilized chloral hydrate. Six rats did not lose muscle tone within 1-min of the infusion were excluded from analyses.

Due to the complex nature of acquisition of a new response tests for conditioned reinforcement (see Discussion); several considerations were made in the schedule and test parameters. The first 4 tests (sessions 30-33) were conceptualized as 'Acquisition Tests'; a relatively slowly accelerating PR schedule was in force. The schedule during these tests was adapted from the formula $5 * (EXP(R * 0.08)) - 5$, where R is equal to the number of reinforcements already earned plus 1 (i.e., next reinforcer; Richardson and Roberts, 1996); the actual number of responses required to earn each reinforcer followed this order: 1,1,2,2,3,3,3,4,4,7,10,12,14,16,19,22. During these tests, only a few subjects reached the breaking point before the 2-hr session ended. Thus, the final test (session 34) was conceptualized as a 'PR Challenge Test'; a steeper PR schedule, adapted from the formula $5 * (EXP(R * 0.12)) - 5$, was in force. The order of ratios was 2,5,7,10,13,16,20,25,31,38,47,57,69,84,102,123,148; all rats reached the breaking point under this steep PR schedule.

Follow-up Study

Unpaired presentations of NIC appeared to increase responding for the stimulus during conditioning (when NIC was administered by the experimenter), but not during testing sessions (when NIC had been withdrawn, see Results). One explanation for this finding is that the CS had weak reinforcing properties and NIC potentiated responding for a sensory reinforcer (Palmatier et al., 2007d). Since the CS supported some operant behavior in the control group (CS-Only), it is possible that this stimulus has some intrinsic reinforcing property, and this property was enhanced by NIC. Thus, the CS (CS + Saline infusions or CS-Only group) used in the present studies should be more reinforcing than no consequence (Saline infusions alone). In order to make this determination we conducted a small follow-up study contrasting nose-poking for the CS and saline infusions (i.e., CS-Only group, n=8) with responding for saline infusions only (SAL-Only group, n=8). All other procedures were identical to the first experiment with one exception. The number of CS presentations in the CS-Only group earned on Session 8 (last day of FR1 testing in the original study) decreased statistically from Session 7 (see Figure 4A, $p < 0.05$). In order to prevent changing the schedule before stable behavior was established, additional FR1 testing sessions were added. The schedule was increased to an FR5 when there was no visual or statistical trend toward increases or decreases in CS presentations/infusions earned across 3 consecutive sessions (Sessions 10-12).

Data Analyses

Analyses included response rates and number of reinforcers earned in each phase. Because of the high correspondence between these two measures, only response rate data are illustrated. For the PR Challenge test, breaking point (final ratio completed) was analyzed using the linear scale (number of reinforcement earned). Each phase was analyzed independently with 2-way ANOVAs contrasting Groups across Sessions. Since NIC Dose was not an orthogonal factor – the 0 dose was a control for both Paired and Unpaired conditions (CS-Only group) – separate ANOVAs contrasted this factor within Paired and Unpaired groups (e.g., CS-Only vs. 0.03 Paired vs. 0.09 Paired). Post-hoc comparisons used t-tests with Bonferroni's correction (session-by-session) to evaluate significant main effects or interactions where appropriate. An a priori alpha criterion was set at $p \leq 0.05$ for all comparisons and multiple p values are reported as 'ps'.

IV. Results

Conditioning Phase

Pairings of NIC and the CS (Paired group) resulted in rapid acquisition of the nose-poke operant. This was confirmed with 2-way ANOVAs evaluating FR1 and FR5 testing in each of the Dose conditions (the CS-Only group was included in each set of analyses). For rats exposed to 0.03 mg/kg/infusion of NIC (Figure 1A), analyses of reinforcers earned revealed significant

main effects of Group and Session, and significant interactions during FR1 (smallest F: Group (2,210)=3.84, $p<0.05$), and FR5 (smallest F: Session (20,600)=8.6, $p<0.001$) testing sessions. The interactions were driven by rats in the Paired group, which earned more CS presentations than CS-Only controls on Sessions 6-8 and 10-29 ($ps<0.05$). The number of reinforcers earned in the Unpaired group did not differ from the CS-Only controls during conditioning (with this lower dose of NIC), and were significantly lower than those earned by the Paired group on sessions 10-15 and 17-29 ($ps<0.05$).

For rats exposed to the 0.09 mg/kg unit NIC dose (Figure 1B), ANOVA revealed only a significant Group $\times$ Session interaction ($F(14,210)=3.43$, $p<0.001$) during FR1 testing. This was probably due to a motor suppressant effect of NIC in the Unpaired group, which earned fewer CS presentations than the CS-Only group on Session 1 ($p<0.05$). During FR5 testing sessions there were significant main effects of Group and Session, as well as a significant Group $\times$ Session interaction (smallest F: interaction (40,600)=2.33, $p<0.001$). The Paired group earned more CS presentations than CS-Only controls on Sessions 11 and 13-29 ($ps<0.05$). The Unpaired group only earned more CS presentations than the CS-Only group on Session 26 ($p<0.05$). Notably, the Paired and Unpaired groups earned a statistically similar number of CS-presentations on all but 4 of the FR5 testing sessions (22, 24, 28, and 29, $ps<0.05$).

NIC increased the number of CS presentations earned when both NIC infusions and the CS were contingent upon the nose-poke response (Paired groups), and moderately increased CS presentations when NIC was administered non-contingently (Unpaired groups). This was confirmed by 1-Way ANOVAs of steady-state behavior (average CS presentations earned across last 3 conditioning sessions) as a function of Group, within each Dose (Figure 2). For rats in both Dose conditions there were significant main effects of Group ($F_s \geq 18.35$, $ps<0.001$). Follow-up analyses for the 0.03 mg/kg dose condition revealed that only rats in the Paired group earned more CS presentations than the CS-Only control group ($p<0.05$). In contrast, both Paired and Unpaired groups exposed to 0.09 mg/kg NIC infusions earned more CS presentations than the CS-Only group ($ps<0.05$).

Acquisition of a New Response Tests

Both Paired groups (0.03 and 0.09 mg/kg/infusion) acquired a novel response for the CS, relative to the CS-Only control groups. The pattern of responding during the Acquisition Tests suggested that this was somewhat more robust and reliable for rats in Paired group receiving 0.09 mg/kg NIC infusions. Two-way ANOVAs of the Acquisition Tests (Sessions 30-33) included Session as a factor (data not shown). For rats previously exposed to 0.03 mg/kg NIC infusions, there was a significant main effect of Group and a Group $\times$ Session interaction [smallest F: interaction (6,90)=2.49, $p<0.05$]. The Paired group earned more CS presentations than the CS-Only group on sessions 31-33 ($ps<0.05$). CS presentations for the Unpaired group did not differ from CS-Only controls during these sessions ($ps>0.05$), and only differed from the Paired group on Session 31 ($p<0.05$). For rats previously exposed to 0.09 mg/kg NIC infusions, there was a significant main effect of Group and a Group $\times$ Session interaction [smallest F: interaction (6,90)=4.40, $p<0.001$]. The Paired group earned more CS presentations than both CS-Only and Unpaired groups on Sessions 31-33 ($ps<0.05$). CS presentations in the two control groups (Unpaired and CS-Only) did not differ from each other ($ps>0.05$).

The salience of the primary reinforcer (NIC) increased responding for the paired CS (Paired group) during Acquisition Tests (Figure 3A). One-way ANOVAs evaluated Dose using steady state behavior (average CS presentations earned on sessions 32-33) within each Group (Paired or Unpaired; the CS-Only served as the 0 mg/kg control in each ANOVA). For Paired rats, there was a significant main effect of Dose [$F(2,33)=9.49$, $p<0.001$]. In contrast, the Dose factor was not significant for Unpaired groups [$F(2,31)=0.5$, $p=0.61$]. Follow-ups determined that the main effect of Dose for the Paired rats was driven by CS presentations earned in the 0

dose (CS-Only) condition. Rats in both Paired groups (0.03 & 0.09 NIC) earned more CS presentations than CS-Only controls ($p < 0.01$), and did not differ from each other ($p > 0.05$).

The motivation to obtain the CS during the PR Challenge Test was higher for rats that had this stimulus Paired with a more salient NIC reinforcer (0.09 mg/kg, Figure 3B). Oneway ANOVAs revealed a significant effect Dose for Paired rats [$F(2,31)=7.08$, $p < 0.01$], but not Unpaired rats [$F(2,29)=3.83$, $p=0.08$]. Follow-up analyses confirmed that only Paired rats who had self-administered 0.09 mg/kg NIC infusions with the CS earned more CS presentations during the PR Challenge Test ($p < 0.05$).

Follow-Up Study

The CS served as a primary reinforcer under conditions that replicated the conditioning phase of the original study (Figure 4A). Co-presentation of the CS with saline infusions (CS-Only group) increased the number of reinforcements earned relative to saline infusions alone (SAL-Only group) during both FR 1 (main effect of Group, $F(1,154)=6.79$, $p < 0.05$) and FR 5 (main effect of Group, $F(1,224)=17.78$, $p < 0.001$) testing sessions.

The CS did not support the acquisition of a new lever pressing response in a manner that was dissociable from non-specific responding during the testing phase (Figure 4B). The number of CS presentations earned (CS-Only group) during testing sessions was comparable to the number of saline infusions earned by the SAL-Only group ($p \geq 0.17$).

V. Discussion

This study replicated our original finding that a CS paired with the primary reinforcing effects of NIC could acquire appetitive or reinforcing properties by an associative process. This new, reinforcing meaning of the CS endowed it with greater reinforcing efficacy and increased the motivation to earn CS presentations under a PR schedule. Under testing conditions that reliably established breaking points (when the effort required to obtain the CS outweighed the motivation to do so), responding for the CS was only increased after pairing with the highest dose of NIC (0.09 mg/kg/infusion) relative to a CS paired with saline (CS-Only group). This latter finding suggests that pairing the CS with a higher dose of NIC made it a more salient conditioned reinforcer.

The finding that a higher unit NIC dose produced greater conditioned reinforcement is not surprising. Elemental conditioning models suggest that the gain in excitatory strength of a CS depends, in part, on the salience or biological relevance of the US (Rescorla and Wagner, 1972). In studies of conditioned reinforcement, manipulating primary reinforcement by increasing its intensity or changing its relationship with the CS in terms of contiguity (shorter delay) and contingency (higher probability) can increase the expression of conditioned reinforcement (Cheal and Spott, 1968; Mazur, 1997). The trend for the primary reinforcing properties of 0.09 mg/kg NIC to decrease responding during the conditioning phase (Figure 1) was probably due to the use of an FR schedule of reinforcement (Chaudhri et al., 2007; Palmatier et al., 2007b). The more robust behavior observed for the CS paired with a higher unit dose of NIC under PR tests suggests that the conditional reinforcing properties acquired by the CS are a direct function of the intensity of associated NIC infusions.

An important aspect of the acquisition of a new response tests is the complexity of processes that underlie them. Subjects are asked to make an association between a new operant (lever press) and presentation of the CS. While this association is being acquired, the CS is being presented without the primary reinforcer (NIC) which should reduce its excitatory strength (i.e., extinction). The nature of these tests leaves open two possible alternative explanations for our findings. First, the higher unit dose of NIC (0.09 mg/kg/infusion) may have only delayed

extinction of conditioned reinforcement. This account does not alter our conclusions; more potent conditioned reinforcing properties should take longer to extinguish. Second, the non-specific reinforcement enhancing effects of NIC may have carried over to the acquisition of a new response test (Olausson et al., 2004) and been more potent for the 0.09 Paired group. Previous studies have demonstrated that prior exposure to NIC can non-specifically increase responding for a water-paired CS on acquisition of a new response tests (Olausson et al., 2004). However, these effects were not observed in Unpaired groups; 0.03 and 0.09 Unpaired rats responded at rates comparable to the CS-Only group during testing. In fact, the enhancing effects of prior NIC exposure may not be dose-dependent (Olausson et al., 2003). For example, 15 days of prior NIC exposure increased an appetitive conditioned response (CS-evoked approach to a water cup) to the same extent as concurrent NIC exposure (NIC injected only after testing sessions began). The lack of specificity observed in prior exposure studies (Olausson et al., 2003, 2004) makes this account less viable as an explanation for the dose-related changes in conditioned reinforcement observed here.

During the conditioning phase the reinforcement enhancing effects of NIC increased responding for the CS, despite the fact that this stimulus was a very weak reinforcer. A similar pattern was observed in the follow-up study. However, in the latter study, making the CS contingent upon a lever press with an alternative response option eliminated evidence of primary reinforcement and abolished the enhancing effect of NIC. One reason for this finding may be that the lever represents a higher cost response to the subject. In addition, the inactive lever represents a novel stimulus and response option, which could interfere with observing primary reinforcement by evoking competing behaviors. Thus, the reinforcing properties of the CS appear to be so weak that they can only be observed under the most sensitive experimental circumstances; those in which only a single, low-cost response option is available.

Both human and pre-clinical research paradigms have emphasized a role for non-NIC stimuli in human smoking and tobacco dependence (Bevins and Palmatier, 2004; Caggiula et al., 2001; Rose, 2006). The reinforcement enhancing effect can make self-administration of the drug and associated stimuli more robust (Palmatier et al., 2007b). In addition, the primary reinforcing effects of NIC can change the meaning of non-NIC stimuli, such that they acquire conditional reinforcing properties (present study, Palmatier et al., 2007c). The present study established that this latter effect is systematically related to NIC dose, a finding which makes several predictions about conditioned reinforcers in human smoking. First, stimulus control of tobacco seeking behavior should be most potent, and by extension, most resistant to change in subjects that smoke cigarettes with a higher NIC yield. Moreover, other manipulations that increase the relevance of conditional stimuli should make stimulus control over behavior more robust, including number of pairings (history of CS-NIC association), contiguity (how close in time and space the CS and NIC occur) and contingency (how closely correlated the CS is with NIC). Thorough descriptions of these phenomena and their interaction with the reinforcement enhancing effects of NIC will aid in the treatment and prevention of tobacco use.

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Figure 1.

This figure illustrates average (± 1 SEM) CS presentations earned in each group for making nose-poke responses during the conditioning phase. For the Paired group, a 0.03 mg/kg (Panel A) or 0.09 mg/kg (Panel B) nicotine (NIC) infusion accompanied each CS presentation. The Unpaired group was also exposed to NIC; however their infusions were controlled by the Paired group in a manner that explicitly dissociated them from nose-poke responses and CS-presentations. For the CS-Only group, infusions of physiological saline accompanied CS presentations. The nose-poke operant schedule was shifted from a fixed-ratio 1/time-out 60 s (FR1/TO60s) to an FR5/TO60s after Session 8.

Figure 2.

This figure illustrates steady-state behavior from the end of the conditioning phase (average CS presentations earned on sessions 27-29). The CS-Only group is included twice because they served as a non-NIC exposed control for each dose condition. Both Paired groups earned more CS presentations than the CS-Only controls. Rats in the Unpaired group exposed to 0.09 mg/kg NIC infusions also earned more CS presentations than the CS-Only control group, suggesting that moderately reinforcing properties of the CS were enhanced by NIC. * signifies statistical group difference from CS-Only control, $p < 0.05$.

Figure 3.

Panel A illustrates steady-state behavior from the last two acquisition of a new response tests (average CS presentations earned on Sessions 32-33). Analyses of acquisition tests confirmed that Paired rats earned more CS presentations than the CS-Only group. *Panel B* illustrates the number of CS presentations earned on the PR Challenge Test (Session 34). This test was carried out under a steeper PR schedule in which all subjects met the break point criterion (30 min without earning a CS presentation). On the PR Challenge Test, the Paired group exposed to 0.09 mg/kg NIC infusions displayed an increased motivation to obtain the CS, relative to CS-Only controls. * signifies that the Paired group differs statistically from CS-Only control group, $p < 0.05$.

Figure 4.

Panel A illustrates average (± 1 SEM) infusions or CS presentations earned in each group for making nose-poke responses during the conditioning phase of the **Follow-up Study**. For the CS-Only group, infusions of physiological saline accompanied CS presentations. The SAL-Only group served as a non-reinforcement control; only physiological saline infusions were contingent upon nose-poke responses. Analyses confirmed that the CS served as a reinforcer under conditions that replicated the conditioning phase of the original study. *Panel B* illustrates average infusions or CS presentations earned by both groups during the acquisition of a new response tests. Under these conditions the CS did not systematically increase operant behavior.

Table 1

Experimental procedures, dose conditions, and the number of subjects in each condition after catheter patency verification.

Group	NIC Dose (mg/kg/infusion)	Conditioning (sessions 1-29)	Acquisition Tests (sessions 30-33)	PR Challenge (session 34)
Paired	0.03 (n=10)	nose-poke: NIC+CS	lever-press: SAL+CS (light PR schedule)	lever-press: SAL+CS (steep PR schedule)
	0.09 (n=12)			
Unpaired	0.03 (n=11)	nose-poke: CS (yoked NIC infusion)		
	0.09 (n=9)			
CS-Only	0 (n=12)	nose-poke: SAL+CS		