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Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine

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Abstract

Rationale—Nicotine is widely assumed to convey reinforcing properties upon tobacco-related stimuli through associative learning. We have proposed that the reinforcement derived from these conditional stimuli can be inflated by a nonassociative “reinforcement-enhancing” effect of nicotine.

Objectives—Experiment 1 investigated whether nicotine could establish a stimulus as a conditioned reinforcer. Using the same subjects, Experiment 2 examined whether responding for a nicotine-associated stimulus was enhanced by response-independent administration of nicotine.

Materials and methods—Self-administered nicotine (Paired group, 0.03 mg kg¹ infusion^{−1}) or saline (conditional stimulus or CS-Only group) was paired with a stimulus light (CS). An Unpaired group, yoked to the Paired group, received equal exposure to nicotine and the CS, but each event was temporally separated. To test for conditioning, the CS was then made contingent upon a novel lever-pressing response. In Experiment 2, a subset of the paired rats (self-administering) continued to lever press while receiving contingent nicotine and the CS. To determine whether nicotine enhanced responding for the CS, two remaining subsets of the Paired group responded for the CS while receiving nicotine (YNIC) or saline (YSAL) yoked to the self-administering rats. All remaining control groups received response-contingent CS presentations, together with yoked nicotine or saline.

Results—Pairing self-administered nicotine with the CS promoted the acquisition of a novel response for the CS. In Experiment 2, the Paired YNIC group responded at higher rates than control groups receiving YNIC or YSAL.

Conclusions—Nicotine can establish stimuli as conditioned reinforcers for which noncontingent nicotine can enhance responding.

Keywords

Acetylcholine; Learning; Drug abuse; Motivation; Nicotine; Conditioning; Reinforcement

Introduction

Nicotine is the component of tobacco smoke that causes addiction (USDHHS 1988). This statement reflects a consensus that nicotine is a primary source of reinforcement and is fundamental to smoking behavior. However, primary reinforcement by nicotine is not a sufficient explanation for many aspects of tobacco dependence. Recent studies suggest that sensorimotor stimuli associated with tobacco consumption (i.e., smoking) are reinforcing and could be important contributors to dependence. For example, smoking-related stimuli induce cigarette craving and may play a prominent role in relapse (Rose and Levin 1991).

We have recently argued that nicotine administration potentially augments the reinforcement derived from sensory stimuli and that this “reinforcement-enhancing” effect of nicotine may increase smoking behavior in humans (see Chaudhri et al. 2006b). The demonstration that nicotine has a reinforcement-enhancing effect depended on two key findings. First, many studies have shown that sensory events can serve as primary reinforcers—stimuli that are often considered to be “cues” can in fact support operant responding even when they lack an associative relationship with other rewards or punishers (Fowler 1971; Stewart 1960; Stewart and Hurwitz 1958). In our laboratory, we found that a stimulus we regarded as a cue for “time-out” from nicotine reinforcement was also a sensory reinforcer (Donny et al. 2003). Second, nicotine increased responding for sensory reinforcers, and this increase was not based on associative learning (Chaudhri et al. 2007; Donny et al. 2003; Palmatier et al. 2006). This latter effect of nicotine has been confirmed by changing the relationship between nicotine administration and operant behavior/stimulus presentation. Nicotine increased responding for sensory stimuli when it was administered in any of the following ways: self-administered with a separate/concurrent response (Palmatier et al. 2006), administered by separate self-administering subjects (i.e., yoked design; Chaudhri et al. 2007; Donny et al. 2003), continuous intravenous (iv) infusion throughout a 1-h session (Donny et al. 2003), scheduled iv infusions during a 1-h session (Liu et al. 2007), or injected subcutaneously 5 min before a 1-h session (Palmatier et al. 2007b).

Human smokers are exposed to the pharmacological effects of nicotine in the presence of numerous discrete and contextual sensory stimuli. The hypothesis that these stimuli can acquire reinforcing properties (i.e., sustain operant responding) and promote human smoking is widely endorsed by theoretical models (e.g., Caggiula et al. 2001; Chiamulera 2005; Conklin and Tiffany 2002; Rose and Levin 1991) and has generally been supported by empirical studies of human smoking (Lazev et al. 1999; Payne et al. 1991). For example, stimuli with no historical affiliation to smoking can acquire conditional stimulus properties in the laboratory when they are paired with a period of free access to smoking (Lazev et al. 1999). Moreover, exposure to historical “smoking cues” (cigarette butts in ashtrays, etc.) can alter operant behaviors related to smoking, such as decreasing latency to smoke the first cigarette and increasing cigarette puff duration (Payne et al. 1991). Finally, when smokers are allowed to ‘self-administer’ stimuli in the absence of primary reinforcement (i.e., denicotinized cigarettes), the smoking operant declines in a manner that is consistent with ‘extinction’ of conditioned reinforcement (Donny et al. 2007).

The conventional assumption that “smoking cues” acquire conditioned reinforcing properties via pairing with the primary reinforcing effects of nicotine (e.g., Rose and Levin 1991) has not been validated empirically. Some nonhuman studies have reported that stimuli paired with nicotine self-administration can serve as conditioned reinforcers (Cohen et al. 2005; Goldberg et al. 1981). These studies used second-order schedules of reinforcement (Goldberg et al. 1981) or cue-induced reinstatement tests (Cohen et al. 2005) to describe the “acquired” reinforcing properties of the stimuli. However, there are at least two limitations of these data as empirical evidence for nicotine-conditioned reinforcement. First, they do not meet the

criteria established by Mackintosh (1974) for demonstrating conditioned reinforcement; specifically, that the conditional stimulus (CS) must support a novel operant response that has not previously been associated with primary reinforcement. Second, as previously stated, nonhuman subjects find many sensory events to be reinforcing (see Butler and Harlow 1957 for an example with primates), and we have found that nicotine can nonassociatively increase responding for these sensory reinforcers (e.g., Donny et al. 2003). Another criterion of conditioned reinforcement is the demonstration that responding for the CS was a result of prior pairings with the primary reinforcer (in this case, nicotine; Mackintosh 1974). As a result, nicotine-conditioned reinforcement should be dissociated from unconditional sensory reinforcement and the potentiating effects of drug administration.

A demonstration of conditioned reinforcement with nicotine could inform the role of both primary reinforcement and reinforcement-enhancing effects of nicotine in tobacco dependence. For example, we have previously increased the reinforcement-enhancing effect of nicotine by associatively increasing the value of a sensory stimulus (i.e., pairing a stimulus with food delivery, Chaudhri et al. 2006a). By extension, the reinforcement-enhancing effects of nicotine could have a more substantial impact on the incentive value of “neutral” sensory stimuli if the primary reinforcing effects of nicotine have established them as conditioned reinforcers. Therefore, the present study sought to establish nicotine-conditioned reinforcement using a novel and well-defined operant response. A second goal was to determine whether the nonassociative enhancing effects of nicotine could increase responding for a nicotine-paired conditioned reinforcer. To accomplish both goals, we first established a relatively neutral stimulus as a conditioned reinforcer via pairings with nicotine. Conditioned reinforcement was verified with the acquisition of a new response test and inclusion of groups that controlled for learning history and unconditional sensory reinforcement. We then investigated whether self- and experimenter-administered nicotine could enhance responding for that nicotine-conditioned reinforcer.

Materials and methods

Subjects

Male Sprague Dawley rats (Harlan Farms, IN) weighing 174–200 g on arrival were housed individually in hanging wire mesh cages. Rats were housed in a temperature- and humidity-controlled colony room on a reverse 12:12 h light/dark cycle. Unrestricted access to food and water was allowed for 3 days after their arrival. Food access was subsequently restricted to 20 g per day, allowing limited growth (approximately 20 g/week) throughout the remainder of the study (Donny et al. 1995).

Apparatus

Experimental sessions were conducted in 16 operant conditioning chambers (BRS/LVE Model RTC-020, MD; see Donny et al. 1995 for further details) measuring 25×31×28 (w×l×h) cm. One wall of each chamber was equipped with two retractable levers and stimulus lights located above each lever. In 12 of the chambers, a nose-poke operant receptacle with an infrared emitter/detector unit was located between the two levers (approximately 3 cm from the floor of the chamber). In the remaining four chambers, an aluminum plate replaced the nose-poke operant. A house-light fixture was located above the nose-poke/metal plate, approximately 2.5 cm from the top of the chamber. A red house light was illuminated at the beginning and extinguished at the end of each session. The CS consisted of 15-s illumination of a white cue light located directly above a randomly designated lever. During the acquisition of a new response test (see later), this lever would serve as the “active” lever such that CS presentations were localized near the manipulandum. Rats were connected to a swivel system that delivered intravenous infusions while allowing 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 $0.1 \text{ ml kg}^{-1} \text{ infusion}^{-1}$ in less than 1 s; the unit infusion dose ($0.03 \text{ mg kg}^{-1} \text{ infusion}^{-1}$) was calculated from the base form.

Surgery

After 6 days of habituation to the colony room, rats were implanted with chronic indwelling jugular catheters after lever training. A detailed description of catheter construction and surgical procedures was provided previously (Donny et al. 1998). After surgery, catheters were irrigated daily by infusing 0.1 ml of the antibiotic ticarcillin plus clavulanate (Timentin[®], to reduce postsurgical infections) in a heparinized sterile saline vehicle.

Procedure

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

Experiment 1: nicotine-conditioned reinforcement

Conditioning phase (Sessions 1–20): After 10–14 days of recovery from the surgical procedure, rats were randomly assigned to one of three conditions: Paired ($n=27$), Unpaired ($n=13$), and CS-Only ($n=13$). Conditioning sessions took place over 20 days (5 days per week); each session was scheduled to last 60 min (see later). Rats were placed in the experimental chambers and connected to the drug-delivery system. Illumination of the house light signaled the start of the session; levers remained retracted throughout the session. Depending on group, rats were either given access to the nose-poke receptacle or no manipulandum. Paired rats were given access to the nose-poke, and responses resulted in delivery of a nicotine infusion (0.03 mg/kg) and the 15-s CS. Each nicotine infusion/CS presentation was followed by a 45-s time-out. 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. The reinforcement schedule, a fixed ratio 1 with a 60-s time-out (15-s stimulus+45-s unsignaled, FR1/TO 60 s), was based on our previous studies (e.g., Palmatier et al. 2007a).

Unpaired rats were tested in the chambers with aluminum plates (i.e., no nose-poke receptacle) to reduce the potential for adventitious associations between manipulanda/responses and the two stimulus events. For these rats, CS presentations and NIC infusions were equated to the Paired group with a modified yoking procedure. Each time the Paired group earned an infusion and CS presentation, a CS presentation counter and an NIC infusion counter were each incremented by 1 for the Unpaired group. NIC infusions and CS presentations were then passively administered to the Unpaired group with the constraint that each event was separated by a minimum of 70 s (range 70–110 s, mean 90 s). The order of event presentations was randomly determined to further decrease the contiguity between the CS presentation and NIC infusions. Note that this procedure only equates exposure, not NIC or CS delivery in real time. Because a temporal dissociation between events was in force, some conditioning sessions exceeded 60 min in this group (mean 72 min, range 60–110 min).

Acquisition of a new response tests (Sessions 21–27): Acquisition of a new response tests began on session 21 and were carried out in seven 1-h sessions. For rats in the Paired and CS-Only groups, nose-poke manipulanda were replaced by aluminum plates. Levers were extended into the chamber 30 s after the start of the session. For all groups, 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 still in force; however, the ratio schedule was adjusted to a variable ratio 3 (VR3) with the constraint that, in each session, the first three reinforcers were earned for making a single response (FR1). After the seventh testing session, catheter patency was determined with a 200 mg/kg infusion of sterilized chloral hydrate. Rats that did not lose muscle tone within 1-min of the infusion were excluded from analyses and further experimental testing.

Experiment 2: effects of self- and experimenter-administered nicotine

Contingent/noncontingent nicotine testing (Sessions 28–37): During this experiment, subjects from Experiment 1 with patent catheters were allowed to respond for the CS on an FR3 schedule. Paired rats were assigned to one of three CS self-administration conditions, Paired NIC+CS ($n=9$), Paired YNIC ($n=9$), and Paired YSAL ($n=7$). For rats in the Paired NIC+CS group, CS presentations were accompanied by 0.03 mg/kg NIC infusions. For the latter two groups, nicotine or saline infusions were yoked to the Paired NIC+CS group. The CS-Only ($n=9$) and Unpaired ($n=11$) groups also received nicotine infusions yoked to the NIC+CS group (CS-Only YNIC and Unpaired YNIC, respectively) in this experiment. Although four rats from the CS-Only group failed the patency test after Experiment 1 (session 27), these subjects had never been exposed to nicotine. Therefore, they were retained as a control group and were assigned noncontingent saline infusions during Experiment 2 (CS-Only YSAL). Final group numbers in this phase were determined by a second catheter patency test after session 37 (after the last testing session); rats that were assigned saline infusions during Experiment 2 (Paired YSAL, CS-Only YSAL) were not subjected to the exclusion criteria.

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. Each phase was analyzed independently. Omnibus two-way analyses of variance (ANOVAs) for CS- or nicotine-seeking measures included Group as a between subjects factor and Session as the within subjects factor. In Experiment 2, there were four groups receiving noncontingent infusions of nicotine, two groups receiving noncontingent infusions of saline, and one group that self-administered nicotine (i.e., contingent infusions). Therefore, the omnibus ANOVA included three factors: Conditioning Group (Paired, Unpaired, or CS-Only), Drug (nicotine or saline), and Session. To evaluate nonspecific/reinforcement-enhancing effects of nicotine, subsequent ANOVA contrasted the Drug factor across sessions for the CS-Only groups. Similar follow-up ANOVA's investigated the effects of Drug and Contingency across sessions for Paired groups. Post-hoc comparisons used Tukey's HSD (collapsed across sessions) or 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; multiple p values are reported as " ps ."

Results

Experiment 1: nicotine-conditioned reinforcement

Conditioning phase (Sessions 1–20)—During this phase, Unpaired rats did not have any response option available, and no responses were recorded; response rates and reinforcers earned were contrasted for Paired and CS-Only rats. During conditioning, Paired rats made more nose-poke responses and received more reinforcements than CS-Only controls ($ps < 0.01$), and the two groups diverged over sessions on these measures (Group $\times$ Session interaction, $ps < 0.01$). Follow-up analyses showed that Paired rats responded at a higher rate (Fig. 1) and earned more reinforcers than CS-Only rats on Sessions 7–20 ($ps < 0.0025$).

Acquisition of a new response tests (Sessions 21–27)—When rats were tested on the novel operant (lever press), Paired rats made more active-lever responses and earned more

reinforcers relative to Unpaired and CS-Only groups (main effect of Group, $p \leq 0.01$; Tukey HSD, $ps < 0.05$; Fig. 2b). Notably, the CS-Only and Unpaired groups did not differ from each other on either measure ($ps \geq 0.98$). Although group differences appeared to emerge over sessions (see Fig. 2a), the Group $\times$ Session interactions were not significant ($ps \geq 0.24$).

Response rates on the active and inactive levers were contrasted for each group (Fig. 3a–c). For the CS-Only group (Fig. 3a), responding on the active lever was higher, relative to the inactive lever throughout the testing phase (main effect of Lever, $p < 0.01$). The Paired group (Fig. 3b) also responded at a higher rate on the active lever relative to the inactive lever ($p < 0.01$); however, responding on the two levers diverged over testing (Lever $\times$ Session interaction, $p < 0.01$). For the Unpaired group (Fig. 3c), responding on the active and inactive levers did not differ ($p = 0.37$).

Experiment 2: effects of self- and experimenter-administered nicotine

Contingent/noncontingent nicotine testing (Sessions 28–37)—Nicotine increased active-lever responding and the number of CS-presentations earned for rats with a history of pairings between nicotine and the CS (Paired NIC+CS and Paired YNIC, main effects of Group and Drug, $ps \leq 0.03$). Paired rats with access to self-administered nicotine (Paired NIC+CS) responded at the highest rate and earned more reinforcements than all other groups (Fig. 4, $ps < 0.001$). In addition, Paired rats that received yoked nicotine infusions (Paired YNIC) responded at a higher rate than Paired rats receiving yoked saline, Unpaired, and CS-Only groups ($ps \leq 0.01$). Notably, responding and reinforcements earned in the control groups did not differ (Unpaired vs CS-Only, $ps \geq 0.4$), and responding and reinforcements earned in the CS-Only condition did not differ as a function of drug exposure [yoked nicotine (YNIC) vs yoked saline (YSAL), $ps \geq 0.57$].

Responding for the nicotine-paired conditioned reinforcer underwent extinction for rats in the Paired YSAL group (Fig. 4). The difference in active-lever responding and reinforcements earned between Paired subjects receiving contingent or noncontingent infusions increased over testing sessions (Session $\times$ Contingency interactions, $ps < 0.001$) due to increased responding in the Paired NIC+CS group and a corresponding decrease in the Paired YSAL group (i.e., extinction, see Paired groups in Fig. 4). Subsequent contrasts revealed that Paired rats receiving contingent (NIC+CS) and yoked (YNIC) nicotine infusions responded at higher rates and earned more reinforcers than the saline control (Paired YSAL) on sessions 30–37 and 35–37, respectively ($ps \leq 0.004$). Extinction in the Paired YSAL group was confirmed in a subsequent comparison to the CS-Only YSAL control (Session $\times$ Pairing interaction, $ps \leq 0.03$); follow-up tests confirmed that response rates and reinforcers earned by these two groups did not differ from sessions 32–37 ($ps \geq 0.15$). The Paired YSAL group decreased responding in a manner consistent with “extinction” (main effect of Phase, $p = 0.04$) when peak performance in the new response tests (Sessions 23–26, end of “acquisition”) was contrasted with the final 4 days of testing in Experiment 2 (Sessions 34–37, extinction).

Discussion

Stimuli associated with the effects of nicotine are considered to be important factors in maintenance of tobacco dependence and relapse (Caggiula et al. 2001; Rose 2006). We have previously outlined at least two ways that stimuli associated with nicotine could increase the probability of future smoking behavior (Chaudhri et al. 2006b). First, nicotine can increase the responding for reinforcing non-pharmacological stimuli (Donny et al. 2003; Palmatier et al. 2006, 2007a). That is, nicotine increases the incentive value or salience of stimuli that are available in the “nicotine-state.” A number of manipulations have suggested that this change in stimulus value does not depend on associative learning (e.g., see Palmatier et al. 2007a). Second, nicotine may impart conditional reinforcing value upon nonpharmacological stimuli

as the result of associative learning. The present study attempted to demonstrate that a discrete stimulus could acquire reinforcing value when it was paired with the effects of nicotine. Moreover, we sought to determine whether responding for a nicotine-conditioned stimulus could be further increased nonassociatively by the reinforcement-enhancing effects of nicotine. As a result, two new findings emerged. First, a discrete stimulus repeatedly paired with the effects of nicotine can acquire conditioned reinforcing value, as evidenced by acquisition of a novel operant response. Second, the reinforcement-enhancing (nonassociative) effects of nicotine can prolong responding and mask or delay extinction of that conditioned reinforcer.

There is a widespread assumption that nicotine endows associated stimuli with conditional value. For example, most bio-behavioral models of tobacco dependence have some associative gain in the value of tobacco-related stimuli at their core (e.g., Balfour 2002; Chiamulera 2005; Di Chiara 2000; Rose and Levin 1991). However, before the present studies, evidence for nicotine-conditioned reinforcement was oblique. Conditioned reinforcement was invoked to explain behavior emitted under second-order schedules (Goldberg et al. 1981) or findings of “cue-induced reinstatement” (Cohen et al. 2005); but no published study had demonstrated the acquisition of a new operant response reinforced by a stimulus, the reinforcing value of which was dependent on prior, specific association with nicotine. The only well-controlled demonstrations of nicotine-conditioned reinforcement are currently limited to the place-conditioning paradigm (e.g., Le Foll and Goldberg 2005; Shoaib et al. 1994). In these studies, multisensory contexts paired with the effects of nicotine can subsequently evoke approach responses. However, there is considerable uncertainty about the circumstances under which nicotine-paired contexts can evoke approach responses (see Le Foll and Goldberg 2005 for review); uncontrolled factors such as stress are often needed to account for discrepant and unexpected findings such as place aversions. This may be due to the dependent measure (i.e., time spent in paired vs unpaired contexts) which has been described as an insensitive and poorly defined operant (Bardo and Bevins 2000; Bevins 2005).

In the present study, we attempted to separate associative and nonassociative changes in behavior by choosing a weak sensory reinforcer. However, the stimulus alone still supported low rates of nose-poke responding (Fig. 1) and greater responding on the active-lever relative to the inactive-lever during testing (Fig. 3a). Therefore, the associative gain evidenced in the test phase of Experiment 1 (Fig. 2) may have been contaminated by a reinforcement-enhancing effect of nicotine. However, some of the characteristics of the enhancing effect of nicotine argue strongly against this interpretation. For example, the enhancing effects of nicotine do not influence responding for weak reinforcers (Palmatier et al. 2007b); suggesting that the acquisition of a new response observed in the Paired group was based on associative learning. Additional support for this conclusion comes from the CS-Only group, which received yoked nicotine infusions in Experiment 2. Without contiguous pairings, nicotine did not alter responding for the CS. Although this group was “pre-exposed” to the stimulus, we have found that such pre-exposure does not retard expression of nicotine’s reinforcement-enhancing effects (Palmatier et al. 2007b). Therefore, even if the enhancing effects of nicotine somehow contaminated the acquisition of a new response, the increase in Paired rats *must* have had an associative basis. This argument is further strengthened by the finding that the newly acquired response underwent a graduated extinction (Paired YSAL; Fig. 4), which suggests that the CS acquired a second associative meaning when it was repeatedly paired with saline.

Although the associative and nonassociative effects of nicotine on sensory reinforcement are complex and difficult to isolate, the confluence of these two phenomena is a very compelling argument for the robustness and intransigence of tobacco dependence. The stimuli associated with smoking presumably vary with regard to their initial primary reinforcing effect. When someone initiates smoking, access to novel peer-groups, novel contexts, eye-pleasing packaging/advertisements for tobacco products, the novel response of manipulating a cigarette

etc. may be moderately reinforcing. In contrast, other stimuli like the taste and smell of tobacco are probably more neutral or even aversive. Accordingly, nicotine would be expected to associatively establish these latter stimuli as reinforcers, and could subsequently nonassociatively potentiate their value. However, a number of additional tests are needed to support this conclusion. For example, the conditioning evidenced in the present study was relatively weak. This is consistent with other findings that have demonstrated weak primary reinforcement by nicotine (Donny et al. 2003) and weak nicotine-conditioned place preferences (Le Foll and Goldberg 2005). A weak primary reinforcer would not be expected to imbue a “neutral” CS with strong reinforcing properties based on associative learning alone. However, changes in the reinforcing effects of nicotine and the strength of associative learning could conceivably increase with more time and additional pairings. Indeed, the increased responding observed in the Paired NIC+CS group of Experiment 2 (Fig. 4) suggests that some additional acquisition/reacquisition was taking place. Thus, an important extension of these findings will be to determine whether more exposure and pairing result in more robust conditioned reinforcement.

In summary, the present studies have established that nicotine-paired stimuli can serve as conditioned reinforcers. In doing so, an important consideration was the use of self-administered nicotine during the conditioning phase. Conditioned reinforcement is typically established by passive experience; normally, the subject is not in control of stimulus/reinforcement delivery (Kruzich et al. 2001; Robbins 1976; Taylor and Robbins 1984). However, there are important pharmacological differences between the effects of self- vs experimenter-administered nicotine (Donny et al. 2000, 2003), which may extend to the reward/reinforcement engendered by the drug. Without the benefit of some ongoing measure of motivation, the experimenter is blind to the valence of a passively received drug. With passive delivery of food and fluid (e.g., Taylor and Jentsch 2001), consummatory responses are specific, easily measured, and provide an ongoing reference for motivational effect. Given the complex effects of experimenter-administered nicotine (Le Foll and Goldberg 2005; Shoaib et al. 1994), we used a self-administration task to establish conditioned reinforcement. Although this created individual variance in the number of pairings received, such a procedure has previously been employed to study drug-conditioned reinforcement (Di Ciano and Everitt 2004) and provides a useful measure of nicotine’s motivational effects. Presumably, the drug was only consumed when it was “wanted,” and consumption could be discontinued when the drug was no longer “wanted.” An important topic of future study will be to investigate whether nicotine-conditioned reinforcement depends on “controllability” of drug/stimulus administration.

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Fig. 1. Mean (+SEM) nose-poke responses for the Paired and CS-Only groups during the 1-h conditioning sessions. *Asterisk* indicates significantly more nose-poke responding in the Paired group, relative to the CS-Only controls ($p < 0.05$)

Fig. 2.

a illustrates mean ($\pm$ SEM) active-lever responses for Paired, Unpaired, and CS-Only groups during the seven acquisition of a new response test sessions. **b** illustrates mean ($\pm$ SEM) responding on the same tests, collapsed across sessions. *Asterisk* indicates significantly more responding in the Paired group, relative to controls ($p < 0.05$)

Fig. 3. **a–c** illustrate mean (+SEM) responses on the active (*filled circle*) and inactive (*open circle*) levers for Paired, Unpaired, and CS-Only groups during the seven acquisition of a new response test sessions

Fig. 4.

Mean ($\pm$ SEM) active-lever responses for all groups in Experiment 2. Subjects from Experiment 1 with patent catheters were used in Experiment 2. Group nomenclatures *before the dashes* (–) represent conditioning treatment in Experiment 1 (Paired, Unpaired, or CS-Only).

Nomenclatures *after the dashes* denote whether nicotine (NIC) or saline (SAL) infusions were given during Experiment 2 and whether the drug infusions were response contingent and paired with the CS (NIC+CS) or response independent (e.g., YNIC or YSAL). Rats in the CS-Only–YSAL group failed the catheter patency check conducted after Experiment 1

Table 1

Operant responses and available reinforcers for groups in each experiment

Experiment 1			Experiment 2	
Group	Conditioning (Sessions 1–20)	Testing (Sessions 21–27)	Group	Active-lever outcome (yoked infusion; Sessions 28–37)
Paired (<i>n</i> =27)	Nose-poke: CS+NIC	Active lever: CS+ SAL	Paired NIC+CS (<i>n</i> =9)	CS+NIC
			Paired YNIC (<i>n</i> =9)	CS [NIC]
			Paired YSAL (<i>n</i> =7)	CS [SAL]
CS-Only (<i>n</i> =13)	Nose-poke: CS+SAL		CS-Only YNIC (<i>n</i> =9)	CS [NIC]
			CS-Only YSAL (<i>n</i> =4)	CS [SAL]
Unpaired (<i>n</i> =13)	YCS//YNIC		Unpaired YNIC (<i>n</i> =11)	CS [NIC]

Rats that passed the catheter patency test in Experiment 1 advanced to Experiment 2. Final subject numbers in each experiment were determined by catheter patency tests (200 mg/kg iv sterile chloral hydrate) after the last session of each experiment.

CS Conditional stimulus (15-s illumination of a white stimulus light); NIC 0.03 mg/kg nicotine infusion; SAL 0.9% saline infusion; YNIC/YSAL/YCS infusions of the indicated solution and/or CS presentations were yoked to the Paired group (Experiment 1) or the Paired NIC+CS group (Experiment 2)