Psychopharmacology (2014) 231:2261–2271
DOI 10.1007/s00213-013-3375-3

ORIGINAL INVESTIGATION

The effects of nicotine exposure during Pavlovian conditioning in rats on several measures of incentive motivation for a conditioned stimulus paired with water

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Received: 14 February 2013 / Accepted: 24 November 2013 / Published online: 7 December 2013

Abstract
Rationale Nicotine enhances approach toward and operant responding for conditioned stimuli (CSs), but the effect of exposure during different phases of Pavlovian incentive learning on these measures remains to be determined.
Objectives These studies examined the effects of administering nicotine early, late or throughout Pavlovian conditioning trials on discriminated approach behavior, nicotine-enhanced responding for conditioned reinforcement, extinction, and the reinstatement of responding for conditioned reinforcement. We also tested the effect of nicotine on approach to a lever-CS in a Pavlovian autoshaping procedure and for this CS to serve as a conditioned reinforcer.
Methods Thirsty rats were exposed to 13 conditioning sessions where a light/tone CS was paired with the delivery of water. Nicotine was administered either prior to the first or last seven sessions, or throughout the entire conditioning procedure. Responding for conditioned reinforcement, extinction, and the reinstatement of responding by the stimulus and nicotine were compared across exposure groups. Separately, the effects of nicotine on conditioned approach toward a lever-CS during autoshaping, and responding for that CS as a conditioned reinforcer, were examined.
Results Nicotine exposure was necessary for nicotine-enhanced responding for conditioned reinforcement and the ability for nicotine and the stimulus to additively reinstate responding on the reinforced lever. Nicotine increased contacts with a lever-CS during autoshaping, and removal of nicotine abolished this effect. Prior nicotine exposure was necessary for nicotine-enhanced responding reinforced by the lever.
Conclusions Enhancements in the motivating properties of CSs by nicotine occur independently from duration and timing effects of nicotine exposure during conditioning.

Keywords Nicotine · Conditioned reinforcement

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Introduction

Nicotine reinforcement is influenced, in part, by nicotine enhancing the motivational properties of reward-related stimuli (Caggiula et al. 2001; Chaudhri et al. 2006a, b, 2007; Liu et al. 2007; Jones et al. 2010; Palmatier et al. 2007a, b). These conditioned stimuli (CSs) can bias attention, and reinforce continued tobacco consumption in humans; contributing to nicotine dependence and relapse (Franklin et al. 2011; Freeman et al. 2012; Rose et al. 2001). It has been argued (Balfour et al. 2000; Caggiula et al. 2001) that the conditioned reinforcing properties of smoking-associated CSs are at least as important for nicotine reinforcement as the primary reinforcement derived from nicotine itself; an assertion supported by evidence in both human and animal studies of nicotine reinforcement (Balfour et al. 2000; Caggiula et al. 2001; Rose et al. 2001).

The interaction between nicotine and CSs on reinforcement processes can be studied in rats using a behavioral test that measures the acquisition of a new operant response for a conditioned reinforcer (Mackintosh 1974). In this test, during

an initial Pavlovian conditioning phase a CS is associated with a primary reinforcer (i.e., unconditioned stimulus [UCS]), such as water or food. Then, in a second phase, the animal can make a novel operant response for subsequent presentations of the CS, now serving as a conditioned reinforcer. Nicotine has two effects in this test. During the Pavlovian phase, nicotine enhances approach behavior to the location of primary reward delivery in the presence of the CS (Guy and Fletcher 2013; Olausson et al. 2003). Subsequently, during the operant conditioning phase, nicotine enhances responding for that CS as a conditioned reinforcer, an effect that persists over multiple tests (Guy and Fletcher 2013; Olausson et al. 2004a, b).

During the Pavlovian conditioning phase, discriminated approach behavior during CS presentations increases rapidly during the initial trials, and then stabilizes (Guy and Fletcher 2013; Olausson et al. 2003). Presumably this change in rate of approach behavior is a reflection of learning the association between CS and UCS. Olausson et al. (2003) showed that nicotine increased head entries in the reward receptacle in the presence of the CS during the initial conditioning trials. We also found enhanced approach behavior during these early trials, but the effect seemed to persist throughout the conditioning phase. It is possible that the effects of nicotine to enhance approach behavior to a reward delivery receptacle in the presence of a CS may vary depending on whether it is injected during the initial acquisition phase, or once the CS–UCS associations have been formed. Since the ability of the CS to function as conditioned reinforcement is presumably dependent on the nature of the CS–UCS association, the timing of nicotine injections during Pavlovian conditioning may also alter the capacity of the CS to serve as a conditioned reinforcer. To test these possibilities, Experiment 1 compared the effects of nicotine injections administered throughout the Pavlovian conditioning phase with those resulting from nicotine administered in the early conditioning trials, during the acquisition phase, or later, during the maintenance phase. We measured receptacle approach in the presence of the CS during Pavlovian conditioning. Then, in the operant conditioning phase, we examined responding for the conditioned reinforcer and the potentiation of this response after an acute nicotine challenge.

In humans, nicotine associated CSs may enhance subjective "cravings" (Franklin et al. 2011), which in turn can trigger relapse to drug-seeking. In animals, extinguished nicotine self-administration can be reinstated by priming injections of nicotine (Chiamulera et al. 1996) and by response-contingent presentations of nicotine-associated CSs (LeSage et al. 2004; Liu et al. 2006). In the latter case, such CSs may be functioning as conditioned reinforcers. Given the potentially large role of conditioned reinforcing stimuli to maintaining addiction-related behaviors (Balfour et al. 2000; Caggiula et al. 2001; Rose et al. 2001), and the interaction between nicotine and conditioned reinforcers (Olausson et al. 2004a, b; Guy and Fletcher 2013), we measured reinstatement of extinguished operant responding for that reinforcer. Based on previous findings (LeSage et al. 2004; Caggiula et al. 2001), we predicted that reinstatement would be greatest when nicotine was given in conjunction with a conditioned reinforcer. We also examined whether such reinstatement would vary as a function of the timing and duration of nicotine exposure during the initial Pavlovian conditioning phase.

Experiment 1 demonstrated a role for nicotine exposure in the expression of nicotine-induced increases in responding for a conditioned reinforcer, and reinstatement of that response after it had been extinguished. However, nicotine did not enhance discriminated approach in the reward receptacle in the presence of the CS during the Pavlovian conditioning phase. In this procedure, the only behavior measured during CS presentations was approach to the location of the primary reward. It is possible that nicotine may have enhanced incentive learning in these animals, but that this effect may not have been apparent in this measure. In fact, Pavlovian-conditioning based incentive learning could be expressed via a number of different behaviors (Flagel et al. 2007; Silva et al. 1998). For example, Silva et al. (1998) showed that animals may engage with reward-predictive stimuli as part of a "generalized search" response to the CS. Other studies have shown that individual animals differ in their conditioned approach behavior; some preferentially approach the CS itself (sign-tracking), while others approach the reward location (goal-tracking) during CS presentations (Flagel et al. 2007, 2010, 2011). Therefore, in a second study we measured the effect of nicotine on approach behaviors to both the CS itself (henceforth referred to as sign-tracking behavior) and to the water receptacle during CS presentations (goal-tracking behavior), using a Pavlovian autoshaping procedure (Flagel et al. 2007) adapted for use in water-deprived animals. Similar to Experiment 1, we varied the timing and duration of nicotine exposure during the Pavlovian autoshaping phase, and subsequently tested the ability of the CS used during autoshaping to serve as a conditioned reinforcer, as well as the effect of acute nicotine on this response. Together, these studies provide a characterization of the effect of nicotine exposure on Pavlovian incentive learning in two different behavioral tests, and whether any such effects translate to differences in nicotine-enhanced motivation for a conditioned reinforcer.

Materials and methods

Animals

Male Long–Evans rats (Charles River, Quebec, Canada) weighing 225–250 g upon arrival were singly housed in a temperature- (~22 °C) and humidity-controlled (~50–60 %)

vivarium on a 12-h light/dark cycle (lights on 0700 h–off 1900 h). Food was available at all times, but water access was restricted as described below. All procedures were approved by the Centre for Addiction and Mental Heath Animal Care Committee and adhered to Canadian Tricouncil guidelines for the humane treatment of experimental animals.

Experiment 1A: effects of nicotine administered during different phases of Pavlovian conditioning on approach behavior and on responding for a conditioned reinforcer

Pavlovian conditioning

Testing occurred in sound-attenuated operant conditioning chambers (Med Associates, St. Albans, VT, USA) containing two retractable levers located 6.5 cm on either side of a recessed water delivery receptacle positioned 3 cm from the floor of the chamber. An infrared photocell detector in the receptacle recorded head entries. A stimulus light was located above each response lever. The day prior to beginning Pavlovian conditioning sessions, animals were restricted to 1 h of water access per day and remained water-restricted throughout conditioning and testing procedures. Each conditioning session consisted of 30 pairings of a 5-s CS followed immediately by the presentation of 0.05 ml of tap water (UCS) on a random time (RT) 60 s schedule of reinforcement. Sessions lasted on average for 30 min. The CS was a 5-s illumination of the two red stimulus lights with the houselight off and a 2.9-kHz, 85-dB tone stimulus presented during the last 0.5 s of the light presentation. Rats were randomly assigned to one of four groups. Group 1 (Saline Controls, $n=10$) was administered saline injections prior to each Pavlovian conditioning session. Group 2 (Nicotine Throughout, $n=9$) received nicotine injections (0.4 mg/kg, SC) just prior to each Pavlovian training session. Group 3 (Nicotine Early, $n=10$) received nicotine injections prior to the first seven Pavlovian conditioning sessions and saline for the remaining sessions. Group 4 (Nicotine Late, $n=10$) received saline injections on sessions 1–7 and nicotine injections prior to sessions 8–13.

Responding for a conditioned reinforcer

During tests of responding for a conditioned reinforcer, two levers were inserted into the chambers. Responding on one lever (CR lever) resulted in presentation of the CS, in the absence of the water reward, on a RR2 schedule of reinforcement so that each response had a 0.5 probability of reinforcement. Responses on the other lever, NCR had no programmed consequences. All rats underwent two counterbalanced test sessions spaced 72 h apart; one session was preceded by a saline injection and one was preceded by a nicotine (0.4 mg/kg, SC) injection.

Responding during extinction conditions and reinstatement tests

Seven 40-min daily extinction sessions were conducted in which responses on both levers had no consequences. Next, rats were injected with saline or nicotine and placed in the chambers where lever responses were recorded, but not reinforced. The order of saline and nicotine treatment was counterbalanced with 72 h between tests. Responding was extinguished in between these two test sessions. After four further daily extinction sessions, a second set of reinstatement tests, administered 72 h apart, was given following injections with saline or nicotine (0.4 mg/kg). This time, responses on the CR lever were paired with conditioned reinforcer presentations.

Experiment 1B: effect of nicotine on responding for a stimulus explicitly unpaired with water

This experiment examined whether responding for the light/tone stimulus in Experiment 1A, and its potentiation by nicotine, was due to the fact that the stimulus acquired conditioned reinforcing properties through pairings with the water UCS.

Unpaired training

Rats were exposed to 13 daily sessions consisting of 30 presentations of the light/tone stimulus used as the CS in experiment 1A and thirty 0.05-ml water deliveries. Both stimuli were presented pseudo-randomly, and were explicitly unpaired. Six rats received saline and six rats received nicotine (0.4 mg/kg) injections prior to these sessions.

Responding for the light/tone stimulus

Two tests of operant responding for the light/tone stimulus, spaced 72 h apart, were conducted as described for Experiment 1A. Tests were preceded by saline or nicotine (0.4 mg/kg) injections.

Experiment 2: effects of nicotine on goal-tracking, sign-tracking and responding for a conditioned reinforcer

Autoshaping

The purpose of this experiment was to determine if nicotine altered approach specifically to a CS, measured over six daily autoshaping sessions. To match the number of injections administered to the Nicotine Throughout and Saline groups

from Experiment 1, rats (n = 40) were divided into two groups and received one injection daily for 7 days prior to behavioral testing. One group received nicotine (0.4 mg/kg, SC; n = 20), and the second group received saline injections (SC; n = 20). Then, water was restricted as described in Experiment 1A.

Training took place in the same chambers as Experiment 1, but with a different configuration. A red houselight was switched on throughout the session. During each session, 25 CS–UCS pairings were delivered on a variable time (VT)-90 s schedule of reinforcement. The CS consisted of the insertion of the left lever into the chamber, backlight illuminated by a flush-mounted 0.6-cm high output LED light. After 8 s, the lever was retracted and 0.05 ml of tap water (the UCS) was delivered to the central water receptacle. Sessions took place at the same time each day and lasted on average 45 min. In all sessions, contacts with the lever-CS (sign-tracking behavior) and head entries into the water receptacle during CS presentations (goal-tracking behavior) were recorded. A lever contact was measured by closure of a microswitch, adjusted to approximately 15 g of tension. Head entries in the water receptacle in the absence of the CS were recorded separately.

Nicotine exposed animals were administered nicotine, and saline exposed rats received saline 5 min prior to the six Pavlovian autoshaping sessions. Over these sessions, nicotine enhanced approach to the CS (sign-tracking), but not the reward receptacle (goal-tracking). Given these results, we decided to extend the experiment and determine if this approach behavior could be modified by the removal or addition of nicotine administration in six additional autoshaping sessions, resembling the exposure regimen used in Experiment 1. Thus, ten of the nicotine-exposed animals and ten of the saline-exposed animals were switched to saline or nicotine injections prior to a further six autoshaping sessions. For comparison with Experiment 1, these groups were named Nicotine Early and Nicotine Late, respectively. The remaining animals continued receiving saline (Saline group) or nicotine (Nicotine Throughout group) as before.

Responding for a conditioned reinforcer

After 12 Pavlovian autoshaping sessions, all animals underwent tests of responding for the lever-CS as a conditioned reinforcer. The lever-CS was moved to the center panel of the chamber in place of the water receptacle. Two nosepoke ports were placed either side of the lever. Nosepoke responses into the reinforced (CR) port resulted in a 2-s presentation of the illuminated lever. Nosepokes into the other port (NCR) were recorded, but had no programmed consequences. Responses on the lever-CS during conditioned reinforcer presentations were also recorded. Responding for a conditioned reinforcer was measured in 40 min sessions on 2 consecutive days. Then, subjects were given 2 test days, separated by 48 h, that were preceded by counterbalanced saline or nicotine (0.4 mg/kg, SC) injections 5 min prior to placement in the operant conditioning chambers.

Data analyses

Statistical analyses were conducted using SPSS version 15.0. For the Pavlovian phase of Experiment 1, head entry responses into the reward delivery receptacle made during the 5-s CS periods and the 5-s pre-CS periods were expressed as a proportion of the total number of responses per session, as in previous reports (Burton et al. 2010; Guy and Fletcher 2013). These data were analyzed using a three-way, mixed-model analysis of variance (ANOVA) with Session number and Response type (CS/pre-CS) as within-subjects factors and Group (Nicotine Throughout, Nicotine Early, Nicotine Late, or Saline) as the between-subjects factor. Tests of responding for a conditioned reinforcer used a three-way, mixed-model ANOVA with Lever (CR/NCR) and Treatment (Nicotine/Saline) as within-subjects factors and Group as the between-subjects factor. Responding during extinction was examined with a three-way ANOVA with Lever and Extinction Day as within-subjects factors and Group as the between subjects factor. Analyses of reactivation data used a four-way ANOVA with Lever, Treatment, and Reinforcer (conditioned reinforcer present/conditioned reinforcer absent) as within-subjects factors and Group as the between subjects factor.

For Experiment 2, Pavlovian autoshaping data were analyzed using separate ANOVAs for the two Response Types (goal-tracking/sign-tracking). Data from the two phases (sessions 1–6 vs. sessions 7–12) were analyzed separately. Session served as the within-subjects factor and Autoshaping Group (Nicotine/Saline for the ANOVA for phase 1; Nicotine/Nicotine Early/Nicotine Late/Saline for the ANOVA for phase 2) served as the between-subjects factors.

Tests of responding for a conditioned reinforcer in Experiment 2 also used a mixed-model ANOVA with Response Type (CR port/NCR port) and Treatment (Nicotine/Saline) as within-subjects factors and Autoshaping Group (Nicotine/Nicotine Early/Nicotine Late/Saline) as the between-subjects factor. Responses on the lever itself when it was presented as a conditioned reinforcer were analyzed with a two-way ANOVA where Treatment (Saline/Nicotine) was the within-subjects factor and Autoshaping Group was the between-subjects factor.

Violations of sphericity were corrected for using a Greenhouse–Geisser correction for appropriate degrees of freedom. Pairwise comparisons utilized Tukey's HSD or Games–Howell procedures for unequal variance, where appropriate, to fix family-wise error rates at α = 0.05.

Results

Experiment 1A: effects of nicotine administered during different phases of Pavlovian conditioning on approach behavior and on responding for a conditioned reinforcer

Pavlovian approach

Figure 1 shows that all groups developed discriminated approach behavior to the water receptacle (main effect of Response type; $F(1, 35)=177.87$, $p<.001$) with animals responding in the water receptacle more during the 5 s CS periods compared to the 5 s period prior to the onset of the CS. This pattern of discriminated approach increased across sessions (Response type×session interaction; $F(12, 420)=28.89$, $p<0.001$). The overall pattern of behavior did not differ between nicotine administration groups ($p>0.05$).

Test of conditioned reinforcement

Figure 2a shows the mean (±SEM) number of responses on the CR and NCR levers. All groups preferentially responded on the CR lever (main effect of Lever; $F(1, 35)=154.09$, $p<0.001$) and nicotine generally enhanced responding for a conditioned reinforcer (main effect of Treatment; $F(1, 35)=16.684$, $p<0.001$; Treatment×Lever interaction; $F(1, 35)=18.86$, $p<0.001$). Although, the Lever×Treatment×Group interaction was not significant ($p>0.05$), we had a priori hypotheses that a history of nicotine exposure would enhance responding for a conditioned reinforcer. Pairwise comparisons of responding on the CR lever indicated that nicotine enhanced responding for a conditioned reinforcer compared to saline ($p<0.05$), but only in those animals that experienced nicotine during the Pavlovian conditioning phase.

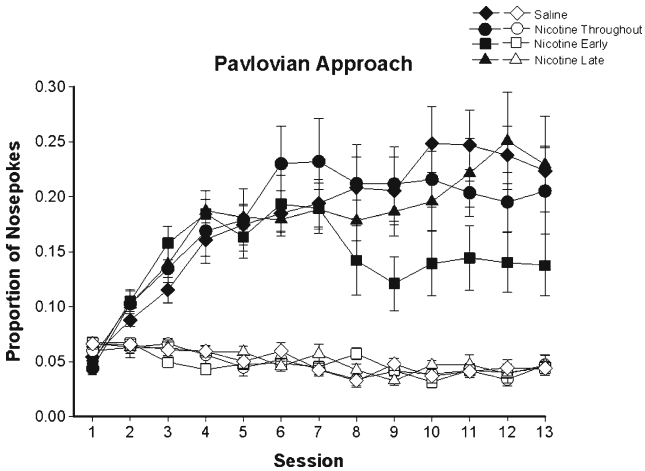

Fig. 1 Pattern of approach behavior in response to presentations of a CS paired with water compared to the 5 s prior to CS presentations (pre-CS) in groups of rats treated with nicotine throughout conditioning (Nicotine Throughout; 0.4 mg/kg; days 1–13; *circles*), nicotine early in conditioning (Nicotine Early; days 1–7, *squares*), nicotine late in conditioning (Nicotine Late; days 6–13, *triangles*), or saline (Saline; days 1–13; *diamonds*). Head entries into the water delivery receptacle were measured during the entire session, during each 5-s period of CS presentations, and during the 5 s immediately preceding each CS. Data points represent the mean (±SEM) proportion of total head entry activity during periods when the CS was presented (*filled symbols*) compared to the 5-s before the onset of the CS (pre-CS; *empty symbols*)

Extinction of responding on the reinforced lever

Following removal of the conditioned reinforcer, responding diminished across days (main effect of Extinction Day; $F(5, 175)=4.223$, $p=0.001$, data not shown). The pattern of responding declined similarly for all groups ($p>0.05$). The number of extinction responses, averaged over the last 3 days for each group, is shown in the first pair of bars on each panel of Fig. 3.

The effect of reintroducing nicotine or nicotine and a conditioned reinforcer on responding on the CR lever

Figure 3 shows that responding on the CR lever increased when nicotine, the conditioned reinforcer, or nicotine and the conditioned reinforcer were reintroduced on test sessions. As shown by the overall four way interaction, these effects of nicotine challenge and reinforcer availability differed between the Pavlovian training groups (Lever × Reinforcement × Treatment × Group interaction; $F(3, 35)=5.14$, $p=0.005$). This interaction was accounted for by differential three-way interactions between Lever×Reinforcement×Treatment across the four groups. Thus, the three groups exposed to nicotine during Pavlovian conditioning showed a significant three-way interaction between the Lever×Reinforcement×Treatment (Nicotine Throughout, $F(1, 8)=9.64$, $p=0.02$; Nicotine Early, $F(1, 8)=18.99$, $p=0.002$; Nicotine Late, $F(1, 8)=17.96$, $p=0.002$). However, the saline exposed animals did not show this interaction ($p>0.05$). Further decomposition of the three-way interactions indicated that when the conditioned reinforcer and nicotine were both present, responding on the CR lever increased for each of the nicotine-exposed groups compared to responding when just the conditioned reinforcer was made available (Reinforcement×Nicotine interactions; all F values >8, $p<0.03$), but not the Saline group ($p>0.05$). Examining the main effects for each of the four groups revealed that when conditioned reinforcement again was made available, responding in general increased (all F values >17, $p<0.003$). Further analyses indicated that the reintroduction of conditioned reinforcement enhanced responding on the CR lever for all groups compared to saline responding in the absence of reinforcement. However, statistical significance was observed only in the Saline and

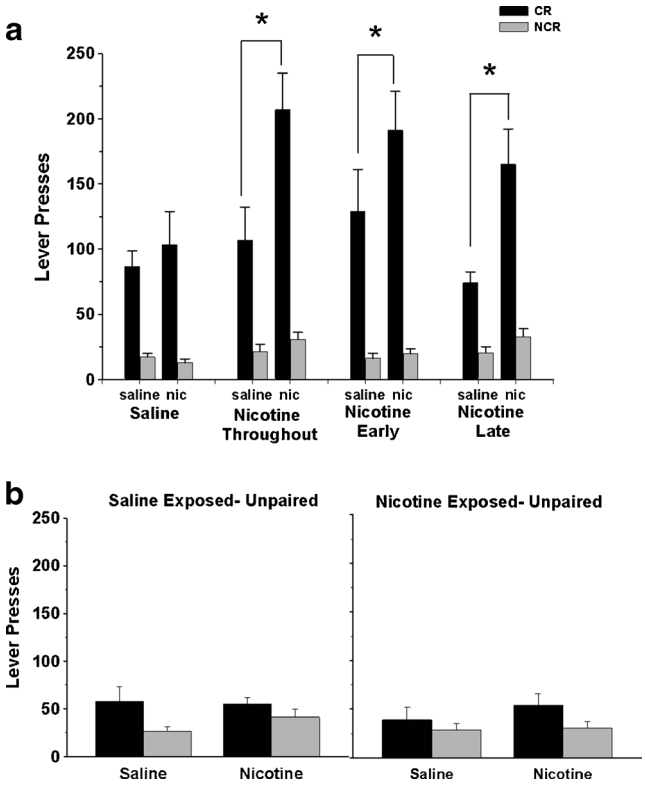

Fig. 2 **a** Effects of nicotine on operant responding on the CR and NCR levers when the light/tone CS had been paired with water during the Pavlovian conditioning phase. Bars depict the mean (±SEM) number of responses on the lever that delivered conditioned reinforcement (CR, *dark bars*) and on the lever with no programmed consequences (NCR, *grey bars*). *$p < 0.05$ compared to corresponding saline treatment. **b** Effects of acute injection with nicotine or saline on responding on the CR and NCR levers when the light/tone CS had been explicitly unpaired with water. Bars represent the mean (±SEM) level of responding on CR (*dark bars*) and NCR levers (*grey bars*). Separate groups of rats had previously been treated with saline (saline exposed) or nicotine (nicotine-exposed) during the unpaired conditioning phase

Nicotine Early groups. Nicotine enhanced overall responding in all the nicotine-exposed groups (all F values >20, $p < 0.002$), but not the Saline group ($p > 0.05$). Nicotine itself enhanced responding on the CR lever in all groups, but significance ($p < 0.05$) was observed only for the Nicotine Throughout and Nicotine Late groups.

Experiment 1B: the effect of nicotine on responding for a stimulus explicitly unpaired with water

As depicted in Fig. 2b, the only statistically significant effect was for the main effect of lever ($F(1, 10)=7.25$, $p < 0.02$). Overall, responding was higher on the CR vs. NCR lever. However, responding was not altered by nicotine exposure during conditioning, or acute nicotine during tests of responding conditioned reinforcement ($p > 0.05$).

Experiment 2: effects of nicotine on goal-tracking, sign-tracking and responding for a conditioned reinforcer

Pavlovian autoshaping phase 1

As shown in Fig. 4a, head entries in the water receptacle (goal-tracking) during CS presentations showed a slight, but significant increase over time (main effect of Session; $F(5, 175)=6.41$, $p = 0.001$). This effect did not differ between nicotine or saline exposed groups ($p > 0.05$). There was also a trend for responding on the lever CS (sign-tracking) to increase across sessions (main effect of Session; $F(5, 175)=2.58$, $p = 0.03$); responding on the lever CS (sign-tracking) was significantly higher for the nicotine exposed animals (Fig. 4c; main effect of Autoshaping Group; $F(1, 35)=9.86$, $p = 0.003$), but the overall pattern of sign-tracking behavior did not differ between groups ($p > 0.05$).

Pavlovian autoshaping phase 2

Head entries into the water receptacle slightly increased over time (main effect of Session; $F(5, 180)=2.91$, $p = 0.04$); however, as depicted in Fig. 4b, this trend did not differ between the four groups ($p > 0.05$). In contrast, the four groups did differ in their overall level of sign-tracking as measured by lever responses (Fig. 4d; main effect of Autoshaping Group; $F(3, 36)=4.46$, $p = 0.01$). Tukey's post-hoc analyses indicated that the animals that were maintained on nicotine (Nicotine Throughout) showed higher levels of lever responding compared to the nicotine-exposed animals switched to saline (Nicotine Early) and the Saline exposed animals ($p < 0.05$). The pattern of sign-tracking behavior remained stable across sessions ($p > 0.05$).

Tests of conditioned reinforcement

All groups responded more in the reinforced aperture (CR) than in the unreinforced (NCR) response aperture (Fig. 5; main effect of Response Type; $F(3, 36)=51.11$, $p < 0.001$). Overall responding was lower on day 2 than day 1, but more so for the CR aperture (main effect of Day; $F(1, 36)=26.26$, $p < 0.001$; Response Type × Day interaction; $F(1, 36)=17.80$, $p < 0.001$). Responding did not differ between the four groups on either test day ($p > 0.05$).

The administration of nicotine prior to conditioned reinforcement testing resulted in increased responding in the CR aperture (Fig. 6; Response Type × Treatment interaction; $F(1, 36)=10.39$, $p = 0.003$). Post-hoc analyses indicated that nicotine enhanced responding for a conditioned reinforcer in those animals with a history of nicotine administration during autoshaping ($p < 0.05$). The animals that received saline throughout the autoshaping phase did not show this effect ($p > 0.05$).

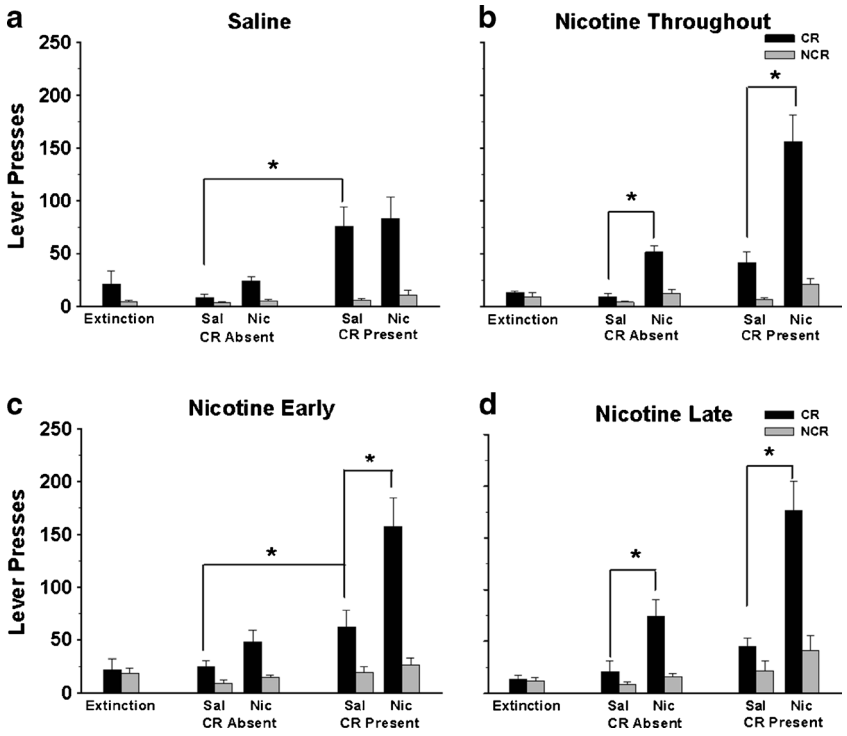

Fig. 3 The effects of nicotine history on the reinstatement of responding for conditioned reinforcement induced by nicotine, the CS, or the combination of nicotine and the CS. Bars show the mean (±SEM) number of responses on the reinforced (CR) lever (*dark bars*) and non-reinforced (NCR) lever (*grey bars*) for the Saline group (**a**), the Nicotine Throughout group (**b**), the Nicotine Early group (**c**), and the Nicotine Late group (**d**). Within each group, responding on the two levers was measured after injection with saline or nicotine, and with or without response-contingent conditioned reinforcer presentations. Average responding over the last 3 extinction days is shown for comparison. *Significant enhancements in responding on the CR lever ($p < 0.05$)

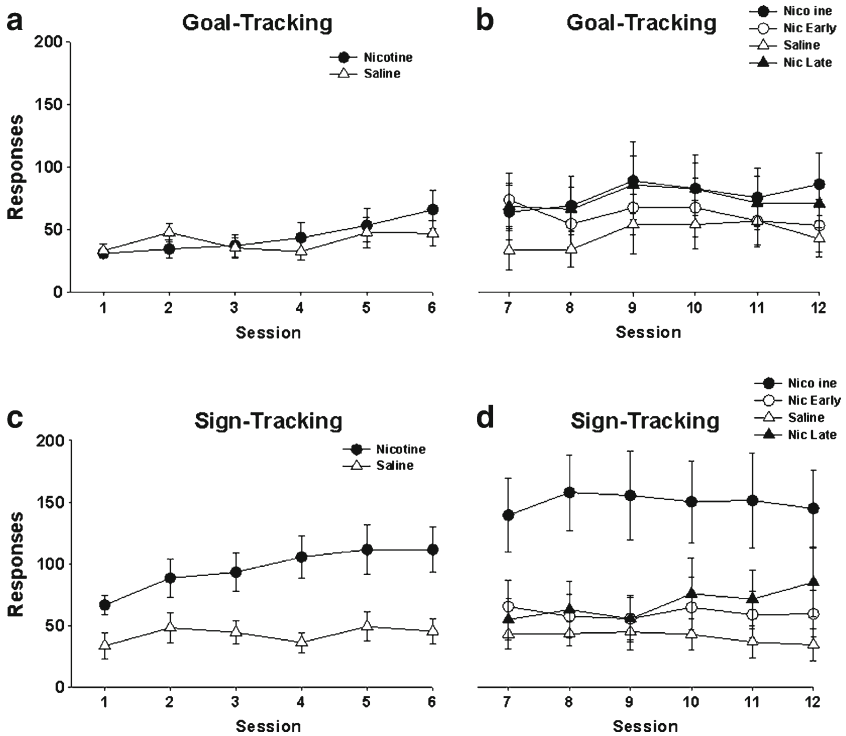

Fig. 4 The effects of nicotine on goal and sign tracking in an autosphaing task. Goal-tracking behavior was measured as the mean (±SEM) number of head entries into the water delivery receptacle during the 8-s lever-CS presentations. **a** Data for the Nicotine (*filled circles*) and Saline groups (*open circles*) for the first six autoshaping sessions. **b** Receptacle entries for the final six sessions, where a subset of Nicotine and Saline-exposed animals were switched to pretreatments with saline (Nicotine Early — *open circles*) or nicotine (Nicotine Late — *filled triangles*). The effects of nicotine exposure on sign-tracking behavior are displayed in **c** and **d** as the mean (±SEM) number of contacts with the lever-CS upon 8-s presentations. **c** Data for Nicotine (*filled circles*) and Saline groups (*open circles*) for the first six autoshaping sessions. **d** Sign tracking for the final six sessions where a subset of Nicotine and Saline-exposed animals were switched to pretreatments with saline (Nicotine Early — *open circles*) or nicotine (Nicotine Late — *filled triangles*)

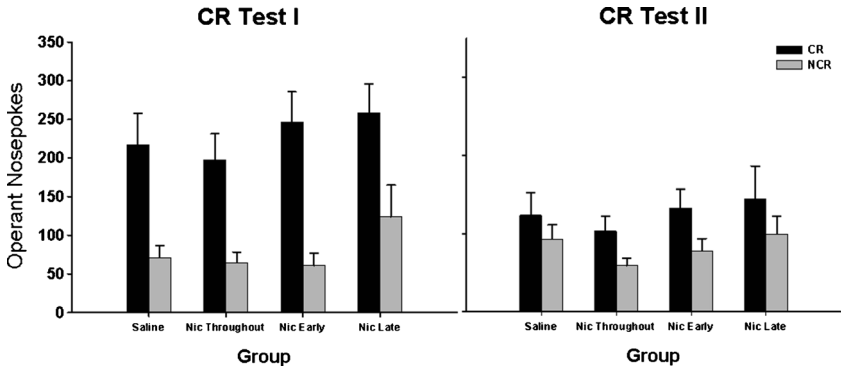

Fig. 5 Animals were tested on two occasions to determine whether the lever CS functioned as a conditioned reinforcer. In all groups, rats preferred responding in the reinforced (CR) operant nosepoke aperture (*dark bars*) to the unreinforced (NCR) aperture (*grey bars*), but this effect was not altered by nicotine exposure during the Pavlovian autosphaping phase

Nicotine also increased contacts with the lever during the test for conditioned reinforcement (Fig. 7; main effect of Nicotine, $F(1, 36)=24.92$, $p< 0.001$), but only for animals with a history of nicotine exposure ($p<0.05$). Further examination of this effect indicated that animals that received nicotine during the early autoshaping trials (Nic Early) or throughout autoshaping (Nic Throughout) exhibited significantly higher lever contacts ($p< 0.05$) than animals that received saline injections (Saline) or nicotine injections in the later trials (Nic Late).

Discussion

These studies generated four main findings. First, in contrast to previous findings (Guy and Fletcher 2013; Olausson et al. 2003), nicotine administered during the Pavlovian phase of a conditioned reinforcement procedure did not significantly enhance CS-elicited approach behavior to the reward-delivery receptacle. However, in a second experiment based on an autoshaping procedure, nicotine strongly enhanced contact with the CS itself during Pavlovian conditioning. Second, the CS that predicted water delivery acquired conditioned reinforcing properties in both the conditioned reinforcement and autoshaping tasks. Responding for the conditioned reinforcer was enhanced by acute injections of nicotine in nicotine-exposed animals, but this effect did not depend on the specific schedule of nicotine exposure. Third, extinguished responding was reinstated by response-contingent presentations of the conditioned reinforcer, and by priming injections with nicotine. These stimuli appeared to have at least an additive effect on reinstating responding.

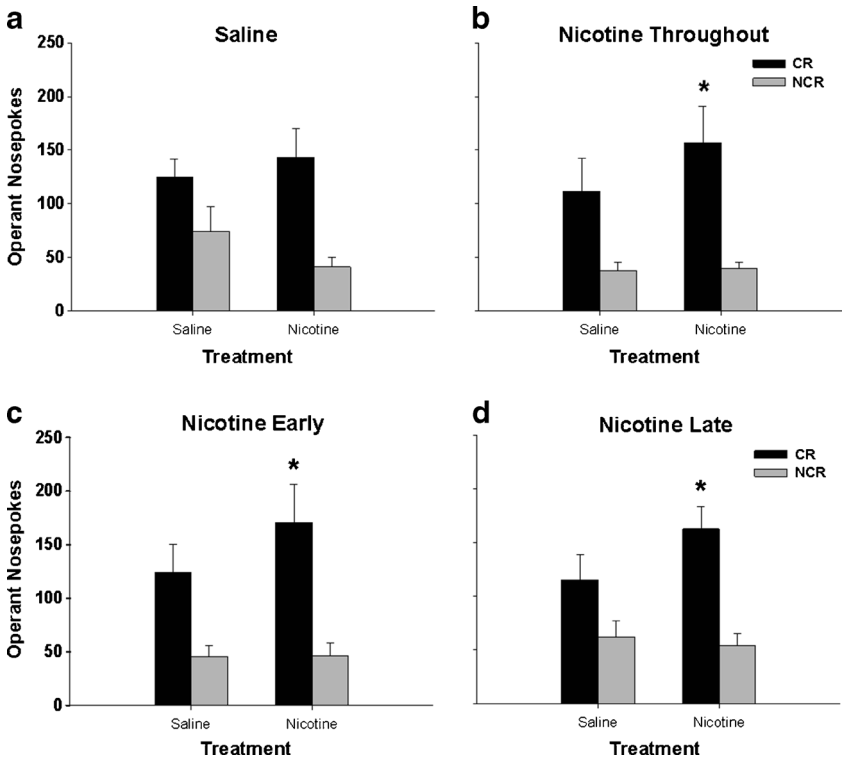

Fig. 6 The ability for nicotine to enhance responding for the lever-CS as a conditioned reinforcer depended on prior exposure to nicotine. Bars represent the mean (±SEM) level of nosepoke operant responding in the reinforced aperture (CR) compared to the aperture with no programmed consequences (NCR) for each of the four training groups. *A significant enhancement in responding in the reinforced nosepoke aperture on nicotine test sessions compared to saline ($p<0.05$)

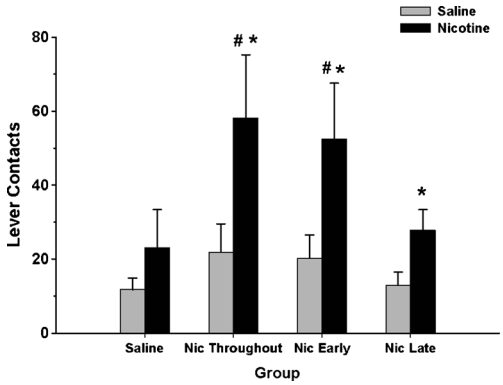

Fig. 7 The schedule of nicotine exposure during Pavlovian autoshaping affects nicotine-enhanced approach toward the CS when it serves as a conditioned reinforcer. Bars depict mean (±SEM) engagement with the lever-CS under the influence of saline (*grey bars*) or nicotine (*dark bars*) for the four different autoshaping groups. *A significant enhancement in lever contacts by nicotine ($p<0.05$). #Significantly higher lever contacts in the nicotine conditioned compared to the Nicotine Late and Saline groups ($p<0.05$)

The effect was also dependent upon a prior history of nicotine exposure during conditioning, but again not schedule-dependent. Finally, the timing of nicotine exposure during Pavlovian autoshaping did appear to affect attraction to the CS itself during the test for conditioned reinforcement following acute nicotine injections (see Fig. 7). Animals that received nicotine during the initial autoshaping trials (Nicotine Early and Nicotine Throughout) displayed higher levels of lever contacts during the test of conditioned reinforcement. Overall, these results replicate and extend reports showing that nicotine interacts with reward-predictive cues to enhance processes related to incentive motivation.

Previously, nicotine administered prior to or throughout the entire Pavlovian conditioning phase enhanced approach to the reward-delivery receptacle when the CS was present (Guy and Fletcher 2013; Olausson et al. 2003). In Experiment 1, we determined whether the timing of nicotine administration was critical to this effect by comparing animals receiving nicotine before each conditioning trial with those receiving nicotine before the first or last 7 days of conditioning. Unlike previous results (Guy and Fletcher 2013; Olausson et al. 2003), nicotine administered before each conditioning session did not enhance approach during CS presentations. However, rats that received nicotine over the first seven conditioning sessions did seem to show a reduction in the amount of discriminated approach behaviour once nicotine injections were discontinued, indicating some influence of nicotine over this response. In Experiment 2, using an autoshaping procedure in which approach to both the CS and the reinforcer location were monitored (Flagel et al. 2007), nicotine selectively enhanced engagement with the reward-predictive illuminated lever CS without significantly altering approach to the reward-receptacle. Such sign-tracking behavior was enhanced in animals that were exposed to nicotine during the first six Pavlovian autoshaping trials, and the removal of nicotine resulted in a decrease in this response. However, animals that received nicotine beginning on the seventh trial (i.e., the Nicotine Late autoshaping group) did not demonstrate enhanced sign-tracking behavior. This implies that the effect of nicotine to enhance sign-tracking requires rats to experience the initial CS–UCS contingencies while under the influence of nicotine.

These latter results complement those of Palmatier et al. (2012a) in showing a selective effect of nicotine on sign-tracking behavior. However, we did not see a long-lasting effect of elevated responding directed toward the CS when nicotine was discontinued, but instead observed decreased sign-tracking behavior. This is likely due to differences in the measures of sign-tracking behavior, the type of reinforcer used (sucrose vs. water), or a combination of both factors. Our measure of sign-tracking behavior was engagement with an illuminated lever-CS (Flagel et al. 2007), rather than head entries into a receptacle located just below the CS (Palmatier et al. 2012a). Perhaps the increased physical effort of engaging in a lever response (Nicola et al. 2005), compared to nosepoke responses, shows differences in sensitivity to nicotine discontinuation. In a different study, where lever responses were recorded as a measure of the reinforcing properties of a visual stimulus, discontinuing nicotine injections resulted in a similar reduction in operant responding (Palmatier et al. 2007a, b). Regarding the type of reinforcer used, evidence from other studies indicates that nicotine is more effective in enhancing approach responses when primary reinforcement with a higher intrinsic reward value is used in conditioning procedures (Chaudhri et al. 2006a, b; Palmatier et al. 2007a, b, 2012a, b). Thus, our use of a water reinforcer, instead of sucrose (Palmatier et al. 2012a), may have resulted in the drop off in sign-tracking behavior when nicotine injections were discontinued. Despite these inconsistencies, results from a number of different procedures show that nicotine can enhance Pavlovian approach behavior, but that the expression of the response may differ based on several procedural variables.

Following completion of the Pavlovian conditioning phases in both test procedures, injections of nicotine enhanced responding for the CS as a conditioned reinforcer only in animals that were exposed to nicotine during Pavlovian conditioning or autoshaping. There were no differences between the Nicotine Early, Late, or Throughout groups in this regard. In a control experiment where the CS and water reinforcer were explicitly unpaired, animals showed a weak preference for the lever delivering the CS, and nicotine had no effect on responding for this stimulus in any group (Fig. 2b). These findings imply that the effects of nicotine observed in the operant conditioning phase of Experiment 1, and reported previously (Guy and Fletcher 2013; Olausson et al. 2004b),

reflect an enhancement by nicotine of the conditioned rewarding properties of the stimulus previously associated with water, rather than a simple nicotine-induced increase in responding for a neutral sensory stimulus (e.g., Chaudhri et al. 2006a, b).

While the effect of nicotine to enhance responding for conditioned reinforcement was not dependent on the precise schedule of prior nicotine administration, the responses on the lever during the test for conditioned reinforcement in Experiment 2 (Fig. 7) indicated some differences between exposure groups in the attribution of salience to the CS. This response appeared to be potentiated by having received nicotine prior to the initial autoshaping sessions (i.e., sessions 1–6). It is possible that sensitization to nicotine (Vezina et al. 2007), regardless of when it is administered during the Pavlovian conditioning phase, may affect the ability of nicotine to subsequently potentiate responding for a conditioned reinforcer. In contrast, approach to the lever-CS while under the influence of nicotine during the test for conditioned reinforcement may reflect differences in learned conditioned responses to presentations of the CS.

In Experiment 1, removal of the conditioned reinforcer from the test context extinguished responding on the CR lever for all groups at a similar rate. The reintroduction of both nicotine and the conditioned reinforcer enhanced responding on the CR lever over extinction levels. However, only the previously nicotine-exposed animals showed an additive enhancement of responding for conditioned reinforcement following the reintroduction of nicotine and the reinforcement after extinction (see Fig. 3). This parallels findings that reacquisition of nicotine-seeking behavior in rodents is stronger when both nicotine-associated CSs and priming injections are used (Caggiula et al. 2001; LeSage et al. 2004). Reinstatement of extinguished responding for the conditioned reinforcer did not differ between the various pre-exposure groups, indicating that the timing of prior nicotine exposure in relation to CS–UCS pairings, or the number of nicotine injections, were not critical factors in determining reinstatement of responding. Again, one implication of this is that effect of nicotine to enhance reinforcement-seeking behavior in the presence of a CS may be a sensitization effect that is not influenced by the schedule of nicotine administration during Pavlovian conditioning.

Concluding remarks

These results add to a growing body of evidence indicating that nicotine interacts with reward-associated CSs to alter behavior. The results from the autoshaping procedure suggest that exposure to nicotine early during incentive learning may also enhance attraction toward those reward stimuli, potentially reflecting a form of attention bias. However, results from the operant conditioning phases of these experiments suggest that a probable sensitization to the invigorating effects of nicotine enhances the conditioned reinforcing properties of reward-associated stimuli. This implies that any interactions between nicotine and the CSs during Pavlovian approach behavior may be dissociable from the ability for nicotine to enhance the reinforcing properties of these CSs in the acquisition of a new response. These results have implications for tobacco use and addiction; suggesting that a reinforcing property of nicotine, to enhance the motivational properties of reward-related stimuli (Chaudhri et al. 2006a, b, 2007; Donny et al. 2003; Horger et al. 1992; Liu et al. 2007), can occur regardless of whether the motivational significance of such stimuli was acquired under the influence of nicotine.

Acknowledgments This research was funded by the Canadian Institutes of Health Research (CIHR). The authors thank Christie L. Burton, Ph.D., for her technical assistance, and Eve De Rosa, Ph.D., and Suzanne Erb, Ph.D., for their valuable input to the design of some of these experiments.

References

Balfour DJ, Wright E, Benwell ME, Birrell CE (2000) The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 113:73–83

Burton CL, Nobrega JN, Fletcher PJ (2010) The effects of adolescent methylphenidate self-administration on responding for a conditioned reward, amphetamine-induced locomotor activity, and neuronal activation. Psychopharmacology 208:455–468

Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA, Sved AF (2001) Cue dependency of nicotine self-administration and smoking. Pharmacol Biochem Behav 70(4):515–530

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, Palmatier MI et al (2006a) Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement-enhancing effects of nicotine. Psychopharmacology 189(1):27–36

Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, Sved AF (2006b) Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. Psychopharmacology 184(1):353–366

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, Palmatier MI et al (2007) Self-administered and noncontingent nicotine enhance reinforced operant responding in rats: impact of nicotine dose and reinforcement schedule. Psychopharmacology 190(3):353–362

Chiamulera C, Borgo C, Falchetto S, Valerio E, Tessari M (1996) Nicotine reinstatement of nicotine self-administration after long-term extinction. Psychopharmacology 127:102–107

Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, Clements LA et al (2003) Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacology 169(1):68–76

Flagel SB, Watson SJ, Robinson TE, Akil H (2007) Individual differences in the propensity to approach signals vs goals promote

different adaptations in the dopamine system of rats. Psychopharmacology 191(3):599–607
Flagel SB, Akil H, Robinson TE (2010) Salience to reward-related cues: implications for addiction. J Neuropharmacol 56:139–148
Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA et al (2011) A selective role for dopamine in stimulus-reward learning. Nature 469(7328):53–57
Franklin T, Wang Z, Suh JJ, Hazan R, Cruz J, Li Y, Goldman M, Detre JA, O'Brien CP, Childress AR (2011) Effects of varenicline on smoking cue-triggered neural and craving responses. Arch Gen Psychiatry 68:516–526
Freeman TP, Morgan CJA, Beesley T, Curran HV (2012) Drug cue induced overshadowing: selective disruption of natural reward processing by cigarette cues amongst abstinent but not satiated smokers. Psych Med 42:161–171
Guy EG, Fletcher PJ (2013) Nicotine-induced enhancement of responding for conditioned reinforcement in rats: role of prior nicotine exposure and α4β2 nicotinic receptors. Psychopharmacology 225(2):429–440
Horger BA, Giles MK, Schenk S (1992) Pre-exposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology 107(2–3):271–276
Jones J, Raiff BR, Dallery J (2010) Nicotine's enhancing effects on responding maintained by conditioned reinforcers are reduced by pretreatment with mecamylamine, but not hexamethonium, in rats. Exp Clin Psychopharmacol 18(4):350–358
LeSage MG, Burroughs D, Dufek M, Keyler DE, Pentel PR (2004) Reinstatement of nicotine self-administration in rats by presentation of nicotine-paired stimuli, but not nicotine priming. Pharmacol Biochem Behav 79(3):507–513
Liu X, Caggiula AR, Yee SK, Nobuta H, Poland RE, Pechnick RN (2006) Reinstatement of nicotine-seeking behavior by drug-associated stimuli after extinction in rats. Psychopharmacology 184:417–425
Liu X, Palmatier MI, Caggiula AR, Donny EC (2007) Reinforcement enhancing effect of nicotine and its attenuation by nicotinic antagonists in rats. Psychopharmacology 194:463–473
Mackintosh NJ (1974) The psychology of animal learning. Academic Press, New York
Nicola SM, Taha SA, Kim SW, Fields HL (2005) Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. Neurosci 135:1025–1033
Olausson P, Jentsch JD, Taylor JR (2003) Repeated nicotine exposure enhances reward-related learning in the rat. Neuropsychopharmacology 28(7):1264–1271
Olausson P, Jentsch JD, Taylor JR (2004a) Nicotine enhances responding with conditioned reinforcement. Psychopharmacology 171(2):173–178
Olausson P, Jentsch JD, Taylor JR (2004b) Repeated nicotine exposure enhances responding with conditioned reinforcement. Psychopharmacology 173(1–2):98–104
Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, Sved AF (2007a) Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. Psychopharmacology 195(2):235–243
Palmatier MI, Matteson GL, Black JJ, Liu X, Anthony R, Craven L, Donny EC et al (2007b) The reinforcement enhancing effects of nicotine depend on the incentive value of non-drug reinforcers and increase with repeated drug injections. Drug Alcohol Depend 89(1): 52–59
Palmatier MI, Marks KR, Jones SA, Freeman KS, Wissman KM, Sheppard AB (2012a) The effect of nicotine on sign-tracking and goal-tracking in a Pavlovian conditioned approach paradigm in rats. Psychopharmacology (in press)
Palmatier MI, O'Brien LC, Hall MJ (2012b) The role of conditioning history and reinforcer strength in the reinforcement enhancing effects of nicotine in rats. Psychopharmacology 219: 1119–1131
Rose JE, Behm FM, Westman EC, Johnson M (2001) Dissociating nicotine and nonnicotine components of cigarette smoking. Pharmacol Biochem Behav 67:71–81
Silva FJ, Timberlake W, Gont RS (1998) Spatiotemporal characteristics of serial CSs and their relation to search modes and response form. Anim Learn Behav 26(3):299–312
Vezina P, McGehee DS, Green WN (2007) Exposure to nicotine and sensitization of nicotine-induced behaviors. Prog Neuropsychopharmacol Biol Psychiatry 31:1625–1638