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Reinstatement of nicotine-seeking behavior by drug-associated stimuli after extinction in rats

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Abstract

Rationale—Smoking-related environmental stimuli have been implicated as an important factor in triggering relapse in abstinent tobacco smokers, and recent evidence indicates that drug-associated stimuli can reinstate nicotine-seeking in rats. However, there is little investigation on the factors that contribute to the latter effect.

Objective—This study examined whether a nicotine-associated visual stimulus (VS) can reinstate nicotine-seeking after extinction in a response-reinstatement model of relapse, and whether the behavioral effects of the VS are sensitive to pharmacological blockade of nicotinic neurotransmission. It also determined whether active lever reassignment after food training influences nicotine self-administration and the VS-induced reinstatement.

Methods—Male Sprague–Dawley rats were trained to self-administer nicotine (0.03 mg/kg/infusion, IV) and associate a VS with each nicotine infusion in 30 daily 1-h sessions. Half of the animals received nicotine infusions for responding at the same lever that previously delivered food; for the other half, infusions resulted from pressing the previously inactive lever during food training. Then, the nicotine-maintained response was extinguished by saline substitution and withholding the VS. One day after rats reached extinction criterion, the reinstatement tests were conducted where the VS was response-contingent represented without further delivery of nicotine. In pharmacological tests, a nicotinic antagonist, mecamylamine, was subcutaneously administered 30 min before reinstatement sessions.

Results—Presentation of the nicotine-associated VS significantly reinstated responding at the previously drug-reinforced lever and pretreatment with mecamylamine effectively attenuated the response-reinstating effect of the VS. Additionally, animals showed similar profiles of nicotine-taking and nicotine-seeking behavior regardless of reassignment of the active lever after food training.

Conclusions—Nicotine self-administration and the VS-induced reinstatement of nicotine-seeking do not result from a lever bias due to prior experience for food reinforcement. Significantly, these results suggest that environmental stimuli associated with nicotine self-administration can effectively elicit nicotine-seeking behavior in abstinent subjects, that this effect is blocked by nicotine antagonism, and that the present procedures may be useful for studying neurobiological mechanisms of nicotine-seeking behavior and relapse.

Keywords

Conditioned stimuli; Extinction; Mecamylamine; Nicotine; Nicotine-seeking behavior; Reinstatement; Self-administration

Introduction

Tobacco use is a chronic relapsing disorder, and recurrent resumption of smoking after abstinence is one of the principal characteristics of nicotine addiction. Although there have been behavioral and pharmacological treatments designed to promote smoking cessation, the great majority of smokers who attempt to quit relapse into tobacco smoking (Balfour and Fagerstrom 1996; Fiore et al. 2000; Shiffman et al. 1998). One factor thought to be important in relapse of drug use, including smoking, is exposure to environmental stimuli previously associated with the drug (Caggiula et al. 2001; Childress et al. 1993; Niaura et al. 1989; O'Brien et al. 1998).

Tobacco smoking may be particularly effective in establishing or magnifying the incentive properties of nicotine-associated environmental stimuli (cues), such as the site, smell and taste of cigarettes, contexts in which smoking occurred, or other behaviors, including other drug-taking, that frequently accompanied smoking (Balfour et al. 2000; Caggiula et al. 2001; Goldberg et al. 1981; Rose and Levin 1991). Clinical studies have demonstrated that smoking cues produce physiological responses (Abrams et al. 1988; Niaura et al. 1989, 1992; Saumet and Dittmar 1985), enhance desire to smoke (Drobes and Tiffany 1997; Droungas et al. 1995; Lazev et al. 1999; McDermut and Haaga 1998; Perkins et al. 1994), and increase the rate, intensity and time of smoking (Mucha et al. 1998; Surawy et al. 1985). Smoking denicotinized cigarettes (i.e., cue alone) produces an equal amount of smoke intake and similar or even higher levels of satisfaction compared to nicotine-containing cigarettes (i.e., cue plus nicotine) (Butschky et al. 1995; Gross et al. 1997; Rose et al. 2000).

In animal self-administration experiments, the infusion of nicotine is typically paired with contextual stimuli such as lights (e.g., Caggiula et al. 2001; Corrigall and Coen 1989; Watkins et al. 1999). Importantly, presentation of these contextual stimuli promotes nicotine-taking and nicotine-seeking responses. Goldberg et al. (1981) found a 50% decrease in nicotine self-administration in squirrel monkeys when a brief light stimulus that had been associated with the drug was omitted. Rats spontaneously recovered previously extinguished behavior after re-exposure to the nicotine self-administration context after being maintained in their home cages for 21 days (Shaham et al. 1997). Recently, it has been found that reintroduction of nicotine-paired stimuli after extinction resulted in increased response at the active lever (Caggiula et al. 2001; Cohen et al. 2005; LeSage et al. 2004; Paterson et al. 2005). There have been numerous studies on the reinstatement of cocaine-, alcohol- and heroin-seeking behavior, and these

studies have provided significant information on the underlying mechanisms of relapse (Epstein and Preston 2003; Shaham et al. 2003 for reviews). However, there is little information on the environmental and neurobiological bases of nicotine relapse. Therefore, the present study used a response-reinstatement model of relapse to characterize the significance of a nicotine-associated visual stimulus (VS) in inducing nicotine-seeking behavior after extinction and to examine whether motivational effect of the VS is sensitive to pharmacological blockade of nicotinic neurotransmission by a noncompetitive nicotinic receptor antagonist, mecamylamine.

Drug self-administration studies typically employ food-reinforced training to facilitate learning of the operant response used in subsequent drug self-administration sessions; the food-reinforced lever becomes the drug-reinforced lever (e.g., Caggiula et al. 2002; Cohen et al. 2005; Corrigall and Coen 1989; Paterson et al. 2005). One may speculate that the prior food training produces a bias or residual preference for the active lever that is prolonged and intensified by the general activating effects of a drug like nicotine, and results in high levels of lever pressing that are mistakenly interpreted as evidence for the reinforcing effects of the drug. Thus, another purpose of this study was to examine whether prior lever experience with or without food reinforcement biases acquisition, maintenance, and extinction of nicotine self-administration as well as the VS-induced reinstatement of nicotine-seeking behavior. Specifically, this study included an active lever reassignment design in which the inactive lever during food training was switched to the active lever for nicotine self-administration, whereas the active food lever became the inactive nicotine lever.

Materials and methods

Subjects

Male Sprague–Dawley rats (Charles River), weighing 225–250 g upon arrival, were used. Animals were individually housed in a humidity- and temperature-controlled (21–22°C) vivarium on a 12:12-h light/dark cycle (lights on 7:00 *a.m.* and off 7:00 *p.m.*) with unlimited access to water. After the first week of habituation to the vivarium during which food was freely available, rats were placed on a diet of 20 g of food per day for the remainder of the study. All training and experimental sessions were conducted during the light phase at the same time each day (9:00 *a.m.*–3:00 *p.m.*). All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 86-23, 1996).

Self-administration apparatus

Operant training and reinstatement tests were conducted in operant conditioning chambers located inside sound-attenuating, ventilated cubicles (Med Associates, St. Albans, VT). The chambers were equipped with two response levers on one side panel and with a 28-V white cue light above each lever as well as a white house light on the top of the chambers. Between the two levers was a recessed food receptacle. Intravenous nicotine injections were delivered by a drug delivery system with a syringe pump (Med Associates, model PHM100-10 rpm).

Food training

On days 8–10 after arrival, rats were handled twice per day. Beginning on day 10, animals were placed on the food restriction regimen of 20 g/day, and daily food training sessions began on a continuous reinforcement schedule. The assignment of the active lever was counterbalanced across animals so that half of the rats responded on the right lever and the other half on the left lever for food delivery. Daily sessions lasted 1 h with a maximum delivery of 100 food pellets (45 mg each). After the rats successfully earned 100 food pellets, the reinforcement schedule was increased to FR5 with maximum of 50 food pellets delivered in

each 1-h sessions. Responses at the inactive lever had no consequences. During the food training sessions, there was no presentation of the lever light, and the house light remained on.

Surgery

After food training, the rats were anesthetized with isoflurane and implanted with jugular catheters as described previously (Caggiula et al. 2001). The rats were allowed at least 7 days to recover from surgery. For the first 2 weeks after surgery, the cannulae were flushed twice a day with 0.1 ml of sterile saline containing heparin (20 units/ml), ticarcillin (14 mg/ml), and streptokinase (5 mg/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with the heparinized saline prior to and after the operant conditioning sessions throughout the studies.

Nicotine self-administration

The rats were divided into two groups according to whether the right or left lever was the active lever. One group ($n=7$) was assigned to the Same Lever group for which the active lever for nicotine self-administration was the same as the active lever during food-reinforced training, whereas for the other (Switched Lever) group ($n=8$), the active nicotine lever was the previous inactive lever used during food training. Thus, in both groups, assignment of the active lever was counterbalanced between the right and the left levers across animals. The rats were trained to self-administer nicotine. Daily 1-h sessions were conducted 5 days a week (Monday through Friday). In the training sessions, animals were placed in the operant conditioning chambers and connected to a drug delivery system. Operant conditioning sessions were initiated with illumination of the house light. Once the rats reached the FR requirement at the active lever, an infusion of nicotine (0.03 mg/kg, free base) was dispensed by the drug delivery system in a volume of 0.1 ml in approximately 1 s. For the duration of nicotine infusion, the lever light above the active lever was illuminated, and following the infusion, there was a 20-s time-out during which time all lights were extinguished, and the responding was recorded but not reinforced. The 1-s turn-on of lever light followed by 20-s turn-off of house light was referred to as the nicotine-associated VS. Responses at the inactivate lever were recorded but had no consequence. An FR1 schedule was used for days 1–5, an FR2 for days 6–8, and an FR5 for days 9–30. Experimental events and data collection were controlled by an interfaced computer and software (Med Associates, MED-PC 2.0).

Extinction

After completion of the self-administration phase, the nicotine-reinforced responses were extinguished by withholding nicotine and its associated VS. Specifically, the daily 1-h extinction sessions began with illumination of the house light and responses at the active lever resulted in the delivery of saline rather than nicotine. During the saline infusion, there was no illumination of the lever light. The house light remained on throughout the whole session. Responses at the inactive lever were recorded but had no consequence. The criterion for extinction was that, for 3 consecutive days, the number of responses/session decreased to less than 20% of the number of responses/session that occurred during the last 3 days of nicotine self-administration.

Reinstatement test

One day after the final extinction session, reinstatement tests were conducted during which lever pressing resulted in the VS (1-s turn-on of lever light followed by 20-s turn-off of house light) but saline rather than nicotine infusions. Total lever responses (including time-out responses) were recorded.

Pharmacological test

For 12 rats, reinstatement test sessions were conducted as described above, except that 6 rats received a subcutaneous injection of mecamylamine (2 mg/kg at a volume of 1 ml/kg, dissolved in physiological saline), while the other 6 rats received saline injections 30 min before the session. The 2-mg/kg dose of mecamylamine was selected because it has been found to reliably inhibit nicotine intake in self-administration procedures (e.g., Glick et al. 2002; Watkins et al. 1999).

Statistical analyses

Data were presented as the mean ($\pm$ SEM) number of nicotine infusions and lever responses. Nicotine infusion data were analyzed by using one-factor ANOVA with repeated measures. Lever response data from the self-administration and the extinction phases as well as reinstatement vs extinction were first analyzed by using two-factor ANOVA with repeated measures, and then the active and inactive response data were separately analyzed by using one-factor repeated measures ANOVA. Differences among individual means were verified by subsequent Newman–Keuls post hoc tests. Unpaired t test was used to analyze the effect of Mecamylamine on recovery of lever responses.

Results

Nicotine self-administration

As shown in Fig. 1, after 30 daily 1-h self-administration sessions, rats developed stable levels of operant responding for IV nicotine infusion. The assignment of the active lever had no effect on the number of nicotine infusions with mean ($\pm$ SEM) infusions of 22.5 ± 2.0 in the Switched Lever group and 22.6 ± 2.0 in the Same Lever group, averaged across the last three sessions. A one-factor ANOVA with repeated measures analysis yielded no significant effect of group across the whole self-administration phase [$F(1,13)=0.85$, $p=0.37$]. However, there was a significant interaction between group and session [$F(29,377)=4.17$, $p<0.0001$] and a significant main effect of group across the initial five FR1 sessions [$F(1,13)=45.00$, $p<0.0001$]. Subsequent Newman–Keuls post hoc tests verified that the Same Lever group took more infusions in the first ($p<0.001$) and second ($p<0.05$) sessions (Fig. 1).

As to the number of responses at both the active and inactive levers, an overall repeated measures ANOVA with group as between-subject factor as well as lever and session as within-subject factors yielded significant main effects of lever [$F(1,26)=229.08$, $p<0.0001$] and session [$F(29,754)=20.69$, $p<0.0001$] but not group [$F(1,26)=3.22$, $p=0.08$]. There was significant interaction of group $\times$ session [$F(29,754)=1.51$, $p<0.05$], lever $\times$ session [$F(29,754)=26.54$, $p<0.0001$], and group $\times$ lever $\times$ session [$F(29,754)=3.61$, $p<0.0001$].

The profiles of active lever responses were shown in Fig. 2, top panel. There was no significant effect of group across the whole self-administration phase [one-factor ANOVA with repeated measures: $F(1,13)=0.85$, $p=0.37$]. However, further analysis yielded a significant group effect across the initial five FR1 sessions [$F(1,13)=45.00$, $p<0.001$], and subsequent Newman–Keuls post hoc test verified a significant difference at the first session between the two groups ($p<0.001$).

The patterns of responses at the inactive lever were shown in Fig. 2, bottom panel. One-factor ANOVA with repeated measures analyses yielded significant main effect of group during the initial five FR1, the three FR2 sessions, and the first nine sessions of the FR5 phase (sessions 9 through 17) [$F(1,13)=52.77$, $p<0.0001$; $F(1,13)=14.47$, $p<0.01$; and $F(1,13)=5.57$, $p<0.05$]. However, during the last 13 sessions of the FR5 phase (sessions 18 through 30), there was no significant effect of group [$F(1,13)=0.36$, $p=0.56$].

Extinction

During the extinction phase, saline substitution for nicotine and omission of the nicotine-associated VS extinguished active lever responding (Fig. 3). In the first extinction session, animals emitted a mean ($\pm$ SEM) of 86.3 ± 11.0 (Switched Lever) and 78.0 ± 10.3 (Same Lever) responses at the active lever. During the subsequent extinction sessions, responses gradually decreased. The rats reached the extinction criterion within 12 daily sessions. An overall repeated measures ANOVA yielded significant effects of session [$F(11,286)=18.15, p<0.0001$] and lever [$F(1,1)=44.97, p<0.0001$] but not group [$F(1,26)=0.19, p=0.67$]. There was a significant interaction between lever and session [$F(11,286)=17.62, p<0.0001$]. Further ANOVA analysis of the active lever responses verified the significant change across sessions [$F(11,143)=22.51, p=0.0001$], indicating extinction of responding.

Reinstatement tests

In the reinstatement tests, response-contingent presentation of the nicotine-associated VS elicited significant recovery of responding at the active lever (compared with extinction responses at criterion), and the magnitude of this effect was similar in the Switched Lever and the Same Lever groups (Fig. 4, top). A repeated measures ANOVA analysis with group as between-subject factor, lever, and session (extinction and reinstatement) as within-subject factors revealed significant main effects of session [$F(1,26)=16.90, p<0.001$] and lever [$F(1,26)=20.98, p<0.001$] and a significant interaction between session and lever [$F(1,26)=15.58, p<0.001$]. There was no significant main effect of group [$F(1,26)=0.05, p=0.83$]. Subsequent Newman-Keuls post hoc tests verified the significant ($p<0.01$) difference in the number of response at the active lever between reinstatement vs extinction (averaged across the final three sessions) in both the Switched Lever and the Same Lever groups. However, responses at the inactive lever remained indistinguishable from extinction responses at criterion (Fig. 4, bottom).

Effect of mecamlamine on nicotine-seeking

As shown in Fig. 5, pretreatment with mecamlamine significantly attenuated the VS-induced reinstatement of responding at the active lever with a mean ($\pm$ SEM) of 26.0 ± 7.3 responses as compared to the saline control group (54.7 ± 8.8 responses) [$t(10)=2.51, p<0.05$]. Mecamlamine did not change responses at the inactive lever.

Discussion

This study yielded three main findings. First, reintroduction of the VS previously paired with nicotine self-administration reinstated nicotine-seeking behavior after extinction in rats, which suggests that exposure to nicotine-associated environmental stimuli may play an important role in relapse to tobacco smoking in abstinent smokers. Second, animals showed similar profiles of acquisition, maintenance, and extinction of nicotine self-administration and the VS-induced reinstatement of nicotine-seeking behavior regardless of whether or not their prior lever experience had been reinforced with food reward, which rules out a speculation that nicotine self-administration behavior might be due to preexisting lever bias because of prior food training that is typically used in the self-administration paradigms. Third, pretreatment of mecamlamine significantly attenuated the response-reinstating effect of the VS, suggesting the clinical potential of this agent in treatment and prevention of relapse to tobacco smoking in abstinent smokers.

The first objective of the present study was to examine the behavioral significance of a nicotine-associated stimulus in the response-reinstatement model of relapse. The results verified the motivational effects of the nicotine-associated VS. Specifically, response-contingent presentation of the VS significantly reinstated extinguished responding at the active lever

previously reinforced by nicotine. This effect cannot be attributed to the nonspecific arousal effects of reintroduction of the VS because responding at the inactive lever remained at extinction levels. Thus, the reinstatement of responding at the active lever after extinction was selectively controlled by the response-contingent representation of the visual cue that had been associated with delivery and reinforcing actions of nicotine during self-administration training. This finding confirms our previous observation (Caggiula et al. 2001) and is consistent with three recent reports (Cohen et al. 2005; LeSage et al. 2004; Paterson et al. 2005) that showed that reintroduction of nicotine-associated stimuli produced significant recovery of extinguished responding. It also lends support for a previous observation that exposing rats to the self-administration training chambers after 21 drug-free days, during which time the rats remained in home cages, resulted in recovery of lever pressing (Shaham et al. 1997). It is interesting to note that the number of responses in the reinstatement tests was higher than that reported by the other studies (Cohen et al. 2005; LeSage et al. 2004; Paterson et al. 2005). In addition to significant procedural differences such as different cues and reinforcement schedules as well as multiple self-administration, withdrawal, extinction, and behavioral tests, the modest primary reinforcing effect of the VS is likely to be another factor that may have contributed to the higher number of reinstatement responses in the present study, since our studies have demonstrated that the compound VS is capable of maintaining low but sustained levels of responding, indicating its primary reinforcing effect (Caggiula et al. 2002). The relative contributions of intrinsic reinforcing properties of the stimulus and its conditioned reinforcing effects, derived from pairing with nicotine infusions, to stimulus-induced reinstatement of nicotine-seeking behavior are currently being examined.

Of particular significance is that the present study determined whether lever experience with food reinforcement during prior food training produces a response bias, which is then nonspecifically sustained by nicotine and can account for the subsequent acquisition and maintenance of nicotine self-administration as well as reinstatement of nicotine-seeking behavior induced by environmental cues. To address this issue, in the present study, the active and inactive levers were switched after completion of food training. In this active lever reassignment procedure, during nicotine self-administration sessions, the previous food-reinforced, active lever was made inactive, while the inactive lever in food training phase was made active, at which responses were reinforced by nicotine infusion. As such, the rats in the Switched Lever group had no prior experience of food reinforcement at the active lever. Overall, these rats showed profiles of nicotine self-administration similar to that of rats of the Same Lever assignment group, where responses at the same active lever were reinforced first with food pellets and in the subsequent drug self-administration training with nicotine infusion. This observation indicates that animals developed stable level of nicotine self-administration regardless of whether or not their prior lever experience had been rewarded by delivery of food pellets, and that nicotine self-administration does not result from a preexisting bias for the active lever due to previous food reinforcement.

An interesting phenomenon is that the inactive lever responses (previously food-reinforced lever) in the Switch Lever group remained higher until session 17 (9th session of the FR5 phase) as compared to that in the Same Lever group. Since operant food-seeking responding was found to be successfully extinguished in seven daily 45-min sessions (Ahmed and Koob 1997), it seemed that in the present procedure where rats received nicotine infusions by responding at the other (active) lever, the food-seeking responses at the inactive lever remained more resistant to extinction. There are two possible factors that might contribute to the extended existence of food-seeking responses. First, nicotine was found to increase the inactive lever response rate in an early study at the same dose as the one used in the present study (Cox et al. 1984). Second, nicotine was found to support lever responding for reinforcing, nonpharmacological stimuli (Caggiula et al. 2002; Donny et al. 2003). The rats of the Switched Lever group had lever experience for food reinforcement in the prior food training phase at the

later-on inactive lever during nicotine self-administration, so that the lever would still bear a reinforcing value (or served as a conditioned reinforcer) due to its previous association with food delivery. Responding at this lever would be supported/sustained by noncontingent nicotine exposure (Chaudhri et al. 2005; Donny et al. 2003). This finding may suggest that the lever switch paradigm after food training is necessary when nicotine self-administration is studied in a short time frame.

The similarity in nicotine self-administration profiles at the final stage of the self-administration phase between the two groups ruled out the possible residual effect of food reinforcement-induced response bias on subsequent lever responding for nicotine self-administration. Moreover, the indistinguishable patterns of extinction and the VS-induced reinstatement of nicotine-seeking behavior further negate influence of this factor. In the present response-reinstatement procedure, the ultimate dependent variable is the stimulus-induced recovery of extinguished lever responding in the reinstatement tests. Since the VS was never associated with food reinforcement in food training sessions, there was no residual influence of previous food training on the reinstatement of nicotine-seeking behavior.

The present data, together with others (Caggiula et al. 2001; Cohen et al. 2005; LeSage et al. 2004; Paterson et al. 2005; Shaham et al. 1997), indicate that the environmental stimuli associated with availability and subjective actions of nicotine may play an important role in relapse to tobacco smoking in abstinent smokers. Thus, these animal studies lend support for clinical observations that smoking-related cues enhance desire to smoke (Drobes and Tiffany 1997; Droungas et al. 1995; Lazev et al. 1999; McDermut and Haaga 1998; Perkins et al. 1994). It has been hypothesized that when compared to other drugs of abuse, environmental stimuli associated with nicotine intake play a more important role in maintaining nicotine self-administration and tobacco smoking (Balfour et al. 2000). Consistent with this view, tobacco smoking or nicotine self-administration is particularly effective in establishing or magnifying the incentive properties of accompanying environmental stimuli (Balfour et al. 2000; Caggiula et al. 2001; Goldberg et al. 1981; Rose and Levin 1991). In light of the fact that the reinstatement tests dissociate the motivation to engage in nicotine-seeking behavior from the direct reinforcing properties of nicotine, the procedures used in the present study may be useful for understanding the factors involved in nicotine relapse and in the neurobiological mechanisms involved in nicotine-seeking behavior and relapse to tobacco smoking.

Another purpose of this study was to examine sensitivity of the response-reinstating effect of nicotine-associated stimulus to pharmacological blockade of nicotinic neurotransmission. The results showed that pretreatment of mecamylamine significantly attenuated reinstatement of active lever responses elicited by response-contingent representation of the VS. The effect of mecamylamine could not be readily attributed to its nonspecific impairment of general locomotor activity since it only inhibited responding at the active lever, while inactive lever responding remained unchanged (slightly but not significantly higher than saline group), and previous observations have shown that this agent did not influence operant responding for food reinforcement at the dose used in the present study (Mansbach et al. 2000). In light of the fact that mecamylamine has been found in animal studies to completely inhibit the discriminative stimulus effects of nicotine (Mansbach et al. 2000; Varvel et al. 1999) and decrease nicotine intake in the nicotine self-administration paradigms (e.g., Corrigall and Coen 1989; Donny et al. 1999; Glick et al. 2002; Watkins et al. 1999) and in human behavioral pharmacological tests to reduce the desire to smoke (Rose et al. 1989) and satisfaction derived from smoking (Lundahl et al. 2000; Nemeth-Coslett et al. 1986), the present finding may suggest clinical potential of this agent for, in addition to its smoking-attenuating effect, treatment and prevention of relapse to smoking in abstinent smokers. However, since nicotinic neurotransmission has been implicated in mediating processes of cognitive attention, associative learning, and memory (Blokland 1995; Olausson et al. 2003; Rezvani and Levin 2001), it is possible that the

reinstatement-attenuation by mecamylamine can be due to its general inhibitory effect on the conditioned goal-directed responses rather than a specific action on cue-induced nicotine-seeking. This issue, along with other characteristics of the effect of this agent such as dose-dependency, is currently under investigation in our lab.

In conclusion, animals showed similar patterns of nicotine-taking and nicotine-seeking behavior regardless of reassignment of the active lever after food training, indicating that nicotine self-administration and reinstatement of nicotine-seeking behavior does not result from a lever bias due to prior experience for food reinforcement. The finding that the VS-elicited reinstatement of nicotine-seeking was blocked by mecamylamine suggests implication of nicotinic neurotransmission in mediating conditioned responses to the nicotine-associated environmental stimuli. These results also suggest that the present procedures may be useful for investigating neurobiological mechanisms of nicotine-seeking and relapse.

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Fig. 1.

Nicotine infusions earned in the self-administration training sessions. The daily 1-h sessions were initiated with the illumination of a house light. Once the rats reached the FR requirement at the active lever, an IV nicotine infusion was delivered. Each nicotine infusion was paired with presentation of a compound visual stimulus (1-s lever light on followed by 20-s house light off). The number of nicotine infusions was presented as the mean. For the purpose of clarity, the standard error of means was not shown. * $p < 0.05$; ** $p < 0.001$ different from Switched Lever group

Fig. 2.

Lever responses made at the active (top) and the inactive (bottom) levers during nicotine self-administration training phase. Responses at the active lever were rewarded with delivery of nicotine infusion and presentation of its associated visual stimulus on the indicated FR schedules, while responses at the inactive lever had no consequence. The number of responses was presented as the mean. For the purpose of clarity, the standard error of means was not shown. * $p < 0.05$; ** $p < 0.01$ (bottom) or 0.001 (top); *** $p < 0.0001$ different from the other group

Fig. 3.

Lever responses made in extinction sessions where saline was substituted for nicotine and the visual stimulus was withheld. The number of responses was presented as the mean. For the purpose of clarity, the standard error of means was not shown

Fig. 4.

Lever responses made at the active (top) and the inactive (bottom) levers during the reinstatement test. Responses at the active lever resulted in presentation of the visual cue (1 s on of lever light followed by 20 s off of house light) and saline rather than nicotine infusion. Responses at the inactive lever had no consequence. The number of responses was presented as the mean $\pm$ SEM. * p <0.01 different from extinction

Fig. 5. Effect of mecamylamine on response-reinstatement induced by a nicotine-associated visual cue. Mecamylamine was administered subcutaneously 30 min before the reinstatement test. The number of responses was presented as the mean $\pm$ SEM. * p <0.05 different from vehicle