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ORIGINAL INVESTIGATION

Nicotine as a signal for the presence or absence of sucrose reward: Pavlovian drug appetitive conditioning preparation in rats

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Abstract: *Rationale*: In Pavlovian conditioning research, nicotine is typically conceptualized as the unconditioned stimulus (US) that becomes associated with an exteroceptive conditioned stimulus (CS). This research has not explored the possibility that nicotine can also function as a CS. *Objectives*: The present research examined whether nicotine served as a CS for the presence (CS+) or absence (CS–) of sucrose and started defining its specificity. *Methods and results:* Rats trained in the CS+ condition had nicotine (0.4 mg/kg, base) paired intermittently with brief access to sucrose. Intermixed were saline sessions without sucrose. Nicotine acquired the ability to evoke goal tracking. This conditioned response (CR) decreased across extinction sessions. The CR was sensitive to nicotine dose (ED_{50}=0.113 mg/kg) and administration to testing interval; 0-min and 100-min delays produced no CR. The CS properties were specific to nicotine in that amphetamine and bupropion substitution was incomplete. Rats in the CS– condition received similar discrimination training except that sucrose was paired with saline. Nicotine also served as a CS–; the saline state CS+ acquired control of goal tracking. Mecamylamine, but not hexamethonium, blocked nicotine's ability to serve as a CS+ and CS–, indicating a role for central nicotinic acetylcholine receptors. *Conclusions:* Nicotine served as a signal for the presence or absence of sucrose. The extinction, CS–, and substitution results eliminated a psycho motor stimulant account. The conceptualization of nicotine as a CS suggests novel empirical research in which a drug acquires additional inhibitory and/or excitatory value based on other outcomes present during its effects.

Keywords: Amphetamine, Bupropion, Dopamine, Drug discrimination, Nicotinic acetylcholine receptors, Smoking, Tobacco

Introduction

Nicotine is the primary addictive compound within tobacco products. Of particular interest in the present report is the role that learned associative processes involving nicotine might have in tobacco dependence (Carmody 1990; Rose and Levin 1991; Henningfield et al. 1995, 1996; Lazev et al. 1999; Parrott 1999; Geier et al. 2000). Pavlovian (classical) conditioning, one source of these learned drug associations, typically consists of presenting a relatively neutral stimulus (conditioned stimulus; CS) in close temporal proximity to a more biologically relevant stimulus (unconditioned stimulus; US). Conditioning is evidenced when responding to the CS is modified relative to a control value (Pavlov 1927; Wasserman and Miller 1997). Translated for a typical smoker, the US is presumably the widespread stimulus conditions produced by nicotine (Eikelboom and Stewart 1982). The CS might include throat irritation, taste and odor of cigarettes, or cigarette pack, as well as situational cues such as drinking at a nightclub (Rose and Levin 1991; Rose et al. 1993; Pritchard et al. 1996; Geier et al. 2000).

Animal models have served to elucidate factors involved in acquisition and expression of Pavlovian conditioned associations with nicotine. Such models include conditioned taste avoidance, conditioned tolerance, place conditioning, and locomotor conditioning (Table 1). As summarized in Table 1, associative models and—by extension—tobacco addiction theories place nicotine in the role of the US. For example, in the research by Walter and Kuschinsky (1989), rats had a distinct environment (CS) repeatedly paired with nicotine (US). Controls received similar exposure to the CS and US in a temporally separated fashion. On the test day, both sets of rats were exposed to the CS without nicotine.

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Table 1 Animal models of Pavlovian conditioned associations with nicotine

	Conditioned stimulus	Unconditioned stimulus	Conditioned response	References
Taste conditioning	Novel taste (saccharin, sucrose)	Nicotine effects	Decreased intake of taste, increased aversive reaction (chin rubs, gapes)	Iwamoto and Williamson 1984; Parker 1995; Palmatier and Bevins 2001
Conditioned tolerance	Environment (context)	Nicotine effects	Decreased tail flick, paw lick, heart rate, corticosterone, and increased milk intake	Epstein et al. 1989; Caggiula et al. 1991; Cepeda-Benito et al. 1998
Place conditioning	Environment (context)	Nicotine effects	Increased time in paired environment; decreased time in environment	Fudala and Iwamoto 1986; Shoaib et al. 1994
Locomotor conditioning	Environment (context)	Nicotine effects	Increased activity, sniffing, rearing, and extracellular dopamine	Walter and Kuschinsky 1989; Reid et al. 1996; Bevins et al. 2001

Rats that had the context CS paired with nicotine displayed an increase in activity, sniffing, and rearing relative to controls. These differences were taken as evidence that the environment CS entered into a learned association with the psycho motor effects of nicotine (Bevins et al. 2001).

Conceptualizing nicotine as a potent US has allowed for important discoveries into basic mechanisms of nicotine dependence and contributed to advances in intervention strategies (e.g., extinction and counter-conditioning; see Rose and Levin 1991). In contrast, the possibility that the pharmacological effects of nicotine might serve as a CS that acquires new excitatory properties [i.e., evoke a conditioned response (CR)] has not been explored. This is not because nicotine lacks cueing properties. The substantial operant drug-discrimination literature leaves little doubt that the interoceptive effects of nicotine can guide reinforced responding. For example, the stimulus effects of nicotine can serve as a cue in rats for responding on one of two levers in an operant conditioning chamber. That is, if pretreated with nicotine (0.4 mg/kg, base) then responding ten consecutive times on the right lever (fixed ratio; FR10) is reinforced with food delivery. In this situation, nicotine is referred to as a discriminative stimulus or S^D. The opposite response-outcome relationship, left lever responding for food, is cued by administration of vehicle (Stolerman 1989).

Additionally, drug–drug conditioning research indicates that one drug can serve as a cue for response-independent delivery of another drug. For example, Revusky et al. (1989) gave rats repeated exposure to pentobarbital (32 mg/kg) 30 min before d-amphetamine (24 mg/kg). Pentobarbital was conceptualized as the CS and amphetamine as the US. Relative to drug-equated controls, the pentobarbital CS evoked an increase in heart rate. A variant of this drug–drug conditioning preparation assumes that the early pharmacological effects of a drug serve as a CS for the subsequent and typically more profound effects of the same drug (US). In an early demonstration with rats, Greeley et al. (1984) found that a low dose of ethanol (0.8 g/kg) reliably paired with a later higher dose of ethanol (2.5 g/kg) came to control an increase in body temperature (i.e., a compensatory hyperthermic CR). For a more recent example, see the research of Siegel and colleagues with morphine (Kim et al. 1999).

In contrast to the drug–drug conditioning situation, we were interested in associative processes involving a non-drug US (i.e., access to sucrose). To this end, we took advantage of a rat's tendency to search in a location where appetitive outcomes have reliably occurred in the past (i.e., goal tracking; Boakes 1977; Farwell and Ayres 1979). There were two main reasons for selecting goal tracking. First, it is an approach behavior directed to a discrete location (e.g., liquid dipper or pellet cup). This feature allows for a clear operational definition of the CR that is reliably observed in many laboratories despite differences in apparatus and protocols. Second, there is a substantial literature showing the utility of goal tracking for studying Pavlovian conditioning processes using more typical CSs (Davey and Cleland 1982; Delamater 1995; Lattal and Nakajima 1998; Rescorla 1999; Bouton and Sunsay 2003). With this in mind, the present research examined the ability of the pharmacological effects of nicotine to serve as a CS+ (signal for intermittent access to sucrose) and CS– (signal the absence of the US). As detailed below, we also investigated the specificity of the conditional stimulus effects of nicotine.

Materials and methods

Animals

Male Sprague Dawley rats from Harlan (Indianapolis, IN) were housed individually in plastic tubs lined with aspen shavings. Water was available in the home cage; access to food was restricted such that each rat was kept at 85% of its free feeding weight (374 ± 58 g). About every 30 days this 85% weight was increased by 2 g to accommodate a typical growth curve. The colony was maintained on a 12-h/12-h light/dark cycle, and all sessions occurred in the light cycle. Experimental protocols were approved by the University of Nebraska–Lincoln IACUC and followed the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

Apparatus

Seven operant conditioning chambers (ENV-018, Med Associates, VT) measuring 30.5×24.1×21 cm (l×w×h) were used. Each chamber had aluminum sidewalls; the ceiling and front and back walls were clear poly carbonate. On the bottom center of one sidewall was a 5.2×5.2-cm (l×w) opening to a recessed dipper receptacle. The dipper arm had a 0.1-ml cup that allowed delivery of a 32% sucrose solution (w/v). An emitter/detector unit, located 1.2 cm within the receptacle and 3 cm from the floor, was used to record head entries. Each chamber was housed in a sound-attenuating cubicle that had a fan providing airflow and masking noise. A personal computer with Med Associates interface and software-timed sessions recorded dipper entries and presented the sucrose.

Drugs

(–)-Nicotine hydrogen tartrate, mecamylamine hydrochloride, hexamethonium bromide, bupropion hydrochloride, and d-amphetamine sulfate (Sigma, St. Louis, MO) were dissolved in saline (1 mg/ml). Nicotine was brought to a pH of 7.0 ± 0.1 with a dilute NaOH solution. Nicotine, hexamethonium, and mecamylamine were injected subcutaneously (s.c.); Amphetamine and bupropion were injected intraperitoneally (i.p.). All injections were at a volume of 1 mg/ml. Nicotine doses are expressed as the base form; all other drug doses are expressed as the salt form.

Nic+ groups (nicotine as a CS+)

Preliminary training

On the first day, rats were trained to access the sucrose solution from anywhere in the chamber within 4 s. The cubicle door was then closed and rats received a 25-min automated session in which the probability of receiving 4-s access to sucrose in a 4-s interval was 0.1333 (two sucrose deliveries per minute). On the second day, rats received a 50-min automated session in which the probability of receiving 4-s access to sucrose in a 4-s interval started at 0.1333 and was decreased across the session to 0.05 (three sucrose deliveries per 4 min). The chamber was dark during dipper training and for all subsequent sessions.

Acquisition

For 40 consecutive days, rats (n=21) received nicotine (Nic+) and saline (Sal–) sessions intermixed. Before each Nic+ session, rats were injected s.c. with nicotine (0.4 mg/kg) 5 min before placement in the chamber. A session lasted 20 min and 32 s, during which there were eight sucrose presentations (4 s each). To prevent rats from timing sucrose delivery, there were four nicotine session programs that varied when sucrose was presented. The average interval between sucrose deliveries was 141 s (range 90–210 s); the average interval before the first sucrose delivery was 120 s (range 90–150 s). Rats were injected s.c. with saline 5 min before the start of a Sal– session. No sucrose was delivered in these sessions. However, four saline programs were generated that had 4-s empty intervals that matched the nicotine programs for location of sucrose deliveries making session length identical for Nic+ and Sal– sessions. Each rat was given the four nicotine and four saline programs in random order without replacement in eight-session cycles with the restriction that no more than two of one program type occurred consecutively. After acquisition training, rats were separated into three groups.

Group 1: extinction, reacquisition, and generalization

Rats (n=7) began extinction training the day after the last acquisition session. During the 14 days of extinction, nicotine was administered as before, but sucrose was not presented. Reacquisition training began the day after extinction. This training was identical to initial acquisition and continued for 20 days: ten nicotine and ten saline sessions. Rats began generalization testing immediately following reacquisition. Generalization testing was conducted in 5-day cycles. Within a cycle, rats experienced two Nic+ and two Sal– sessions in random order. Thus, it took two testing cycles to use the eight programs from acquisition training. Day 5 of each cycle was a 4-min test in which a rat was injected s.c. with its assigned nicotine dose (0.025, 0.05, 0.1, 0.2, 0.4, or 0.6 mg/ kg) 5 min before the session—no sucrose was delivered. A rat was only tested if it met the criterion for that cycle (see Dependent measures). Rats that did not meet the criterion remained in the home cage. Each rat was tested twice on each nicotine dose. That is, once the rat completed its assigned testing order, testing on a new randomly selected order began.

Group 2: injection to testing interval

The day following the final acquisition session, rats (n=7) began the 5-day testing cycle described for group 1. On day 5 of each cycle was a temporal delay test in which rats were injected with the training dose of nicotine, 0, 5, 25, or 50 min before placement in the chambers for the 4-min test. At the 0-min delay, a rat was injected with nicotine and immediately placed in the chamber. Rats were tested twice at each value.

Group 3: nAChR antagonism

Rats (n=7) assigned to this group began the 5-day testing cycles the day after the final acquisition session. On the fifth day of the cycle, each rat was pretreated s.c. with saline, the central and peripheral nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine [(0.5 or 1.0 mg/kg); see Martin et al. 1989], or the peripheral nAChR antagonist hexamethonium [(2.5 mg/kg or 5.0 mg/kg); see Asghar and Roth 1971] 15 min before nicotine (i.e., 20 min before start of the test session). Rats were tested twice at each condition and nicotine (0.4 mg/kg) was administered 5 min before placement in the chambers (cf. training).

Follow-up conditions

As a rat in group 1–3 completed its assigned test values, training on the 5-day cycles continued. If the discrimination criterion was met, then an amphetamine substitution test was conducted on the fifth day to determine the specificity of the Pavlovian discrimination. For this substitution test, a rat was injected i.p. with saline or its assigned dose of amphetamine (0.0625, 0.125, 0.25, 0.5, or 1.0 mg/ kg) and placed in the chamber 15 min later (Bevins et al. 1997). Rats (n=18) were tested once on each dose. A subset of rats (n=8) that completed the amphetamine substitution tests continued training and were tested on a dose of bupropion (10, 20, or 40 mg/ kg). This was of interest given its use as a pharmacotherapy (Zyban) and recent research showing its pharmacological effects substituted for a nicotine S^D (Young and Glennon 2002). Not all rats were tested on each dose (Table 2). Bupropion was injected i.p. 15 min before placement in the chambers (Munzar and Goldberg 2000). Following the bupropion test for some rats and the amphetamine test for other rats, an additional temporal delay test was conducted. This test was identical to the delay test described for group 2 except the values tested were 5 min and 100 min. Rats (n=11) were tested once at each delay.

Table 2 Results from substitution tests

Dose (mg/kg)	Mean dipper entries per second (±1 SEM)	*n*
Amphetamine substitution		
Saline	0.038 (±0.005)	18
0.0625	0.036 (±0.005)	18
0.125	0.044 (±0.005)	18
0.25	0.040 (±0.006)	18
0.5	0.055 (±0.007)*	18
1.0	0.068 (±0.011)*	18

*$P<0.05$ as compared with saline

Dose (mg/kg)	Mean dipper entries per second (±1 SEM)	*n*
Bupropion substitution		
Saline	0.035 (±0.004)	5
10	0.055 (±0.012)	5
Saline	0.035 (±0.010)	8
20	0.074 (±0.017)*	8
Saline	0.033 (±0.003)	5
40	0.053 (±0.012)	5

*$P<0.05$ compared with respective saline value

Nic-group (nicotine as a CS–)

Preliminary and acquisition training

Following dipper training, rats (n=7) began acquisition training similar to that of the Nic+ groups (e.g., nicotine dose, injection protocol, programs, etc.) except that sucrose was delivered during saline sessions (Sal+) and not during nicotine sessions (Nic–). Acquisition training continued for 64 days [32 sessions of each type (Sal+ and Nic–)].

nAChR antagonism

On the day following the final training session, rats began the 5-day testing cycles as previously described. On the fifth day of each cycle, rats that met criterion were injected s.c. with an assigned solution (saline, 0.5 or 1.0 mg/kg mecamylamine, or 2.5 or 5.0 mg/kg hexamethonium) 15 min before administration of nicotine. Rats were tested twice with each solution.

Dependent measure, criterion, and data analyses

The main dependent measure was the number of dipper entries per second before sucrose was delivered. A per second measure was used because time between the beginning of the session and the first sucrose delivery varied across sessions. Dipper entries before the first sucrose delivery were used to avoid including dipper entries induced by sucrose. To equate for time, the programs used for sessions in which sucrose was not delivered (i.e., Sal– and Nic–) included comparable 4-s intervals. This procedural maneuver provides a per second measure of dipper entries for analysis using identical intervals. To meet criterion in the 5-day testing cycles, rats had to have more dipper entries per second during each sucrose session relative to both non-reinforced sessions of that cycle. Omnibus tests were one-or two-way repeated-measures analysis of variance (ANOVA). Post-hoc comparisons used paired t-tests. When a rat was tested twice on the same variable (e.g., drug dose or time delay), a single value for analysis was obtained by taking an average of the two values for each rat. For all tests, the dipper entries per second were from the first 2 min of the test. We used the first 2 min because it was comparable to the duration before first sucrose delivery during training and was the duration for the testing criterion. Statistical significance was declared using a two-tailed rejection region of 0.05.

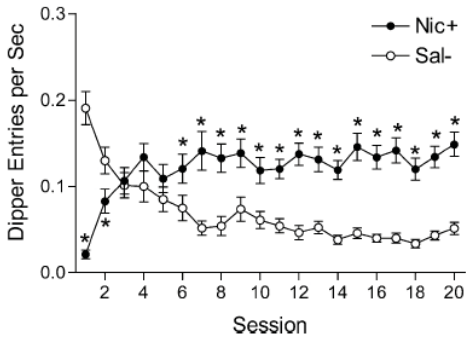

Fig. 1 Mean number of dipper entries per second (±1 SEM) before the first delivery of sucrose during nicotine (Nic+) and saline (Sal–) sessions for all rats (n=21) in the acquisition phase. *Asterisks* (*) denote significant difference ($P<0.05$) from the comparable Sal– session

Results

Nic+ groups

Acquisition. Figure 1 shows the results of the acquisition phase for rats that were trained with nicotine as a CS+. Given that training was identical for all groups, the data were pooled. The two-way, repeated-measures ANOVA revealed a main effect of session ($F_{19,380}$=1.781, P=0.023), of condition (Nic+ vs Sal–; $F_{1,20}$=48.273, $P<0.001$), and a significant session×condition interaction ($F_{19,380}$=18.438, $P<0.001$). On the first and second sessions, nicotine suppressed dipper entries (P values ≤ 0.007). As training continued, nicotine acquired control over goal tracking. From the sixth session on, there were more dipper entries on Nic+ sessions than Sal– sessions (values ≤ 0.024).

Group 1

Extinction. Figure 2A shows the results from the extinction phase. For comparison, the solid line illustrates the average dipper entries per second for the two saline sessions immediately before extinction; dashed lines represent the SEM. There was a significant main effect of session($F_{13,78}$=6.994, $P<0.0001$), indicating that dipper entries decreased across sessions. To examine whether dipper entries returned to levels maintained in the absence of sucrose (complete extinction), goal tracking in each extinction session was compared with saline. Goal tracking was significantly greater than that maintained in the Sal– condition on extinction sessions 1–4, and 9 (P values<0.05)

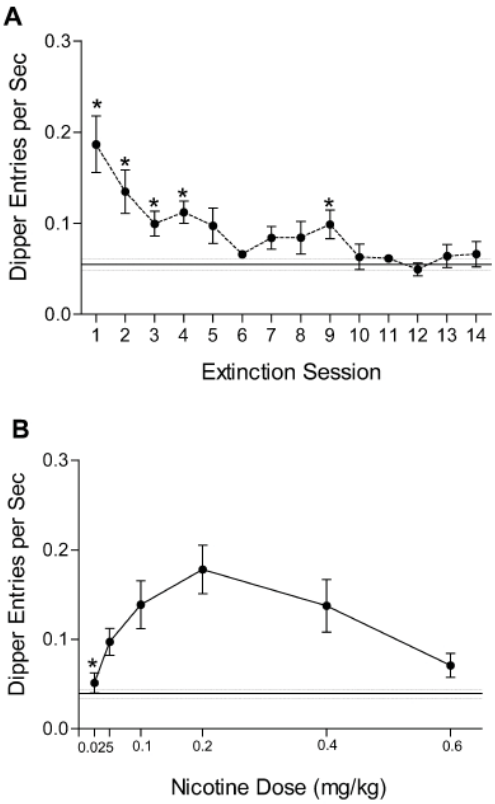

Fig.2 **A** Mean dipper entries per second (± 1 SEM) for the first 2 min of each extinction session for rats in group 1 (*n*=7). The *solid line* represents the average of the first 2 min for the three saline sessions before extinction. *Dashed lines* represent the SEM. *Asterisks* denote significant difference from the saline session average ($P<0.05$). **B** Average dipper entries per second for the first 2 min of the nicotine generalization tests for the same rats after reacquisition training. The *solid line* reflects the average of the first 2 min of the two saline sessions that preceded testing on the 0.4mg/kg dose. *Dashed lines* represent the SEM. The *asterisk* denotes significant difference from the training dose of nicotine (0.4 mg/kg; $P<0.05$).

Nicotine generalization test. As a no-drug baseline, we calculated the average dipper entries per second for the first 2 min of the two saline sessions that preceded testing on the training dose (solid line in Fig. 2B). Because rats were tested twice on each dose, we used the saline sessions before the first test of the training dose (0.4 mg/ kg) for four rats; the value from the second test was used for the remaining three rats. The one-way ANOVA revealed a significant effect of nicotine dose ($F_{5,30}$=8.654, $P<0.0001$). Post-hoc comparisons tested whether each dose was different from the training dose. Goal tracking was significantly lower at the 0.025-mg/kg dose of nicotine ($P<0.05$). There was a tendency for a difference at the 0.6-mg/kg dose (P=0.052). Using the linear portion of the dose–effect curve (0.025–0.2 mg/kg), the median effective dose (ED_{50}) for the nicotine CS+ was 0.113 mg/kg.

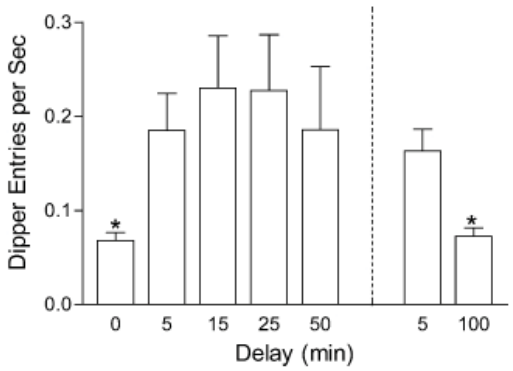

Fig.3 Mean number of dipper entries per second (± 1 SEM) for the first 2 min of the temporal delay tests for rats in group 2 (*n*=7). The two *right-most bars* are from a separate follow-up test of the administration to testing interval (*n*=11). *Asterisks* denote significant difference from the training delay (5 min; $P<0.05$).

Group 2

Injection to testing interval. Changing the time of the nicotine injection before placement in the chamber significantly affected goal-tracking behavior ($F_{4,24}$= 5.094, P=0.004; left portion of Fig. 3). Specifically, nicotine administered immediately before placement in the chamber significantly decreased dipper entries when compared with the 5-min training delay. Continued goal-tracking at the 50-min delay prompted the follow-up condition in which a 5-min and 100-min delay were assessed (right-most bars of Fig. 3). Relative to the 5-min delay, extending the delay to 100 min significantly reduced dipper entries (t_{10}=5.026, P=0.0005).

Group 3

nAChR antagonism. Figure 4 shows the results from the antagonism tests. For these tests, a separate repeated-measures ANOVA was conducted for each antagonist; saline values were used for both ANOVAs. Hexamethonium pretreatment had no effect on dipper entries ($F_{2,12}$=1.487). In contrast, mecamylamine significantly reduced dipper entries($F_{2,12}$=30.42, $P<0.0001$). Relative to saline, this reduction in goal tracking was evident at both mecamylamine doses (P values <0.002), and suggests that the conditional stimulus properties of nicotine are mediated by centrally located nAChRs.

Follow-up conditions

Amphetamine substitution. The results from the amphetamine substitution tests are shown in the top portion of Table 2. The overall ANOVA was significant ($F_{5,85}$=4.905, P=0.0005). Subsequent post-hoc tests compared each amphetamine dose with saline. The two highest amphetamine doses (0.5 mg/kg and 1.0 mg/kg) increased the number of dipper entries relative to saline

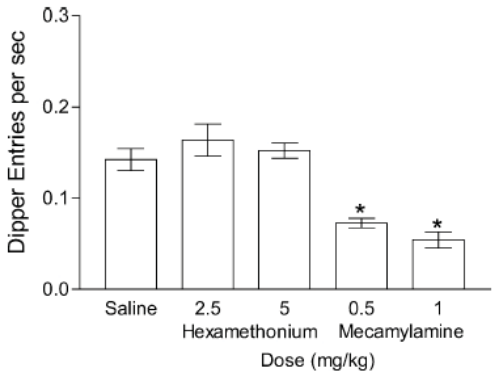

Fig.4 The *left-most set of bars* reflect the mean number of dipper entries per second (± 1 SEM) for the first 2 min of the antagonism tests for rats in group 3 (n=7). Hexamethonium and mecamylamine were administered 20 min before the start of the test (i.e., 15 min before nicotine). *Asterisks* denote significant difference from saline (P<0.05)

(P values<0.02). To determine whether this increase was complete substitution for the nicotine CS, goal tracking evoked by these two doses of amphetamine was compared with goal tracking in the comparable time period of the nicotine training session that immediately preceded testing of the 1.0-mg/kg amphetamine dose. Nicotine controlled significantly more goal tracking (0.163 ± 0.023 dipper entries per second) than either amphetamine doses (0.5 and 1.0 mg/kg; P values<0.0001).

Bupropion substitution. Given the identical injection protocols, each bupropion dose was compared with the saline data collected during the amphetamine substitution phase. Not all rats were tested on each bupropion dose, thus each paired t-test used saline data only from rats that were tested with the bupropion dose being analyzed (lower portion of Table 2). Only the 20-mg/kg dose bupropion increased dipper entries relative to saline (t_7 =2.847, P=0.025). The extent of goal tracking to the 20-mg/kg bupropion dose was significantly less than that seen in the first 2 min of the nicotine session that preceded testing (0.094 ± 0.017 dipper entries per second; P=0.023).

Nic– group

Acquisition. Figure 5A shows the results of the acquisition phase for rats that were trained with nicotine as a CS–. The ANOVA revealed a main effect of condition ($F_{1,6}$= 18.398, P=0.005), of session ($F_{31,186}$=1.644, P=0.024), and a significant session×condition interaction ($F_{31,186}$= 1.822, P=0.008). The variability in this group measure highlights two important results. First, it suggests that only a subset of rats acquired the discrimination by the end of this phase (Fig. 5B). Indeed, four of the seven rats had all positive difference scores (saline per second value minus nicotine value) in the last cycle of this phase indicating consistently more dipper entries in Sal+

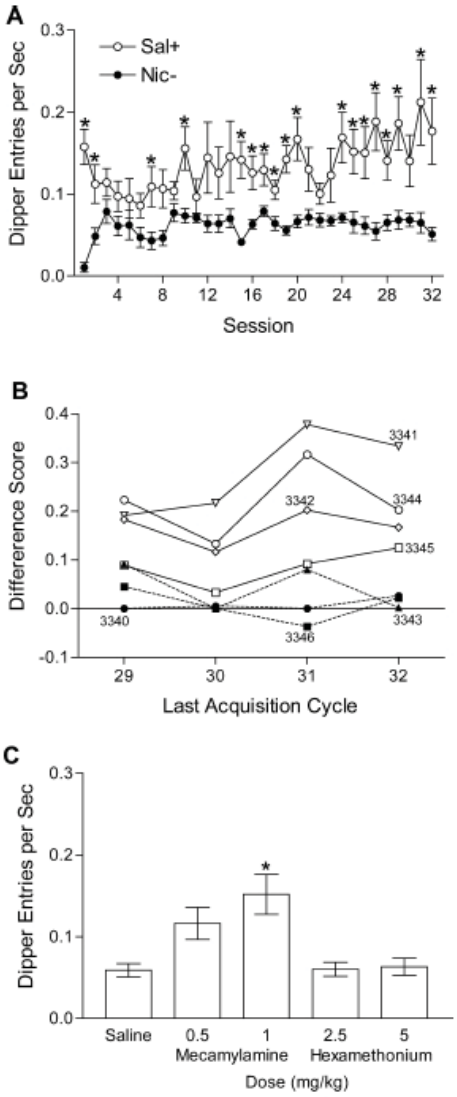

Fig.5 A Mean number of dipper entries per second (± 1 SEM) before the first delivery of sucrose during saline (Sal+) and nicotine (Nic–) sessions for rats in the nicotine CS– condition. *Asterisks* denote significant difference (P<0.05) from the comparable nicotine session. **B** Difference score for each rat during sessions 29 through 32. The *open symbols* represent rats with consistently positive difference scores. The *filled symbols* with *dashed lines* represent rats that did not acquire the discrimination by the last cycle of the acquisition phase. **C** Mean dipper entries per second for the first 2 min of the antagonism tests. Hexamethonium and mecamylamine were administered 20 min before the start of the test (i.e., 15 min before nicotine). Asterisk denotes significant difference from saline (P<0.05).

sessions. Second, the source of the variability was not in withholding responding during nicotine CS– sessions. Rather, the variability was in the use of the non-drug state (operant chamber cues) as the cue for access to the US.

nAChR antagonism

A separate ANOVA was conducted for each antagonist using the same saline values. Pretreatment with mecamylamine increased dipper entries($F_{2,12}$=8.106, P=0.006; Fig. 5C). Relative to saline, this recovery of goal tracking with mecamylamine (cf. Sal+) was significant only at the 1.0-mg/kg dose (P<0.018). In contrast, hexamethonium pretreatment had no effect on dipper entries (F<1), indicating that central nAChRs likely mediate the ability of nicotine to serve as a CS–.

Discussion

In the early nicotine CS+ acquisition sessions, nicotine suppressed dipper entries below the level seen in early saline sessions. This suppression likely reflects motor ataxia typically seen with higher nicotine doses (Stolerman et al. 1973; Bevins et al. 2001). This suppression dissipated across sessions, and nicotine came to control more dipper entries before first sucrose delivery than saline. We interpret this pattern as reflecting tolerance to the locomotor suppressant effects of nicotine (Stolerman et al. 1973; Clarke and Kumar 1983) and acquisition of an appetitive conditioned association between nicotine and sucrose such that the nicotine CS evokes goal tracking (Farwell and Ayres 1979).

Chronic exposure to nicotine induces locomotor stimulation in rats (Clarke and Kumar 1983; Bevins et al. 2001). An alternative to this conditioning account suggests that the activating effects of nicotine increase the rate of dipper entries. The results from the extinction manipulation do not support this stimulant account. That account predicts no change in the level of dipper entries when the sucrose US is withheld in the extinction phase because nicotine was still present to activate dipper entries. This did not occur. Rather, nicotine-evoked goal tracking decreased systematically across repeated extinction sessions. This sensitivity to removal of the US is an important feature of Pavlovian conditioned associations (Pavlov 1927; Wasserman and Miller 1997) and suggests that the pharmacological effects of nicotine entered into an excitatory association with sucrose.

A variant of the stimulant account suggests that availability of sucrose during acquisition alters the probability of particular behaviors. For example, the chamber for each rat was paired with sucrose 50% of the time. Perhaps intermittent pairings make the exteroceptive cues that compose the chamber mildly excitatory. This weak conditioned excitation might be enhanced by a psycho motor stimulant with appetitive properties such as nicotine. The extinction phase changed the probability of chamber-sucrose pairings to 0. This non-reinforcement would have a cumulative effect on the frequency of different behaviors including dipper entries. This modified stimulant account predicts that administration of another psycho motor stimulant with appetitive effects should similarly increase dipper entries as long as the chamber cues were not extinguished. This did not occur. Amphetamine (0.5 mg/kg and 1 mg/kg) did not even increase dipper entries to half that controlled by nicotine even though these doses of amphetamine readily stimulate a wide range of behaviors (Garrett and Holtzman 1996; Antoniou et al. 1998; Badiani et al. 2000). Indeed, in our laboratory, these doses of amphetamine are more potent at activating locomotor behavior than nicotine (Palmatier et al. 2003).

In two ways, a conditioning interpretation is further supported by the finding that nicotine can serve as a signal for the absence of sucrose (CS–). First, there is a substantial Pavlovian conditioning literature showing that exteroceptive stimuli can readily signal the absence of an US (Pavlov 1927; Bouton and Brooks 1993; Tinsley et al. 2002). The present results suggest that nicotine also has this ability to function as a CS–. An interesting possibility is that the nicotine CS– has become a conditioned inhibitor. Procedurally it is plausible because the nicotine cue occurs in the presence of an excitatory cue (i.e., the chamber). However, establishing whether the nicotine CS– has acquired inhibitory properties will require additional research using specifically developed procedures (Pavlov 1927; Rescorla 1969; Wasserman at al. 1974). Second, nicotine's ability to function as a CS– is inconsistent with either variant of the stimulant account of the CS+ data. That is, appetitive food-seeking behaviors were readily withheld during the nicotine CS–, a result more consistent with a conditioning interpretation.

Conditional control of goal tracking was dose dependent. From a conditioning perspective, this result is important because it demonstrates that changes in the salience of the training CS result in alterations in the CR. Similar results have been reported in a wide range of Pavlovian conditioning preparations (Rohrbaugh et al. 1971; Scavio and Gormezano 1974; Brennan 1975; Czaplicki et al. 1976). The ability to generate an orderly dose–effect function suggests that this preparation might be utilized to study the neuropharmacological processes mediatingthe ability of a drug to serve as a CS in much the same fashion that drug discrimination is used to study processes mediating a drug's ability to serve as a S^D. The generalization function described for the nicotine CS+ is similar to that reported in the drug-discrimination literature (Chance et al. 1977; Pratt et al. 1983; Stolerman et al. 1984; Shoaib et al. 1997). Also, the ED_{50} for the conditional stimulus effects of nicotine using goal tracking (0.113 mg/kg) was in the same range as previous drug-discrimination research using lever pressing [e.g., Chance et al. 1977 (ED_{50} =0.087 mg/kg); Pratt et al. 1983 (ED_{50} =0.14 mg/kg)].

Nicotine's ability to serve as a CS+ or CS– appears to be mediated by centrally located nAChRs. For the CS+, goal tracking was blocked by pretreatment with mecamylamine (0.5 mg/kg and 1 mg/kg) but not by hexamethonium. Similar results have been reported for the discriminative stimulus effects of nicotine (Morrison and Stephenson 1969; Stolerman et al. 1984). For the nicotine CS–, goal tracking increased to saline levels (i.e., the

CS+) with pretreatment of mecamylamine (1 mg/kg), but not hexamethonium. This latter result is notable because antagonism is evidenced as an increase in dipper entries. This feature eliminates any motor impairment account of mecamylamine blockade of goal tracking to the nicotine CS+.

The nature of the nicotine CS+ varied with time since administration. Goal tracking was significantly reduced when nicotine was injected immediately or 100 min before testing. Given that CS-elicited goal tracking is mediated by central nAChRs, this time-dependent data pattern is likely due to changes in brain concentrations of nicotine. For example, Ghosheh et al. (1999) measured brain concentrations of nicotine in rats at different time points after a s.c. injection of 0.8 mg/kg nicotine (base). Ghosheh et al.'s (1999) temporal-effect curve for brain levels of nicotine nicely parallels our temporal-effect function for goal tracking with concentrations of nicotine peaking at 5 min after the injection and, thereafter, declined resulting in a half-life of 52 min. Thus, the loss of goal tracking at the 100-min interval likely reflects a significant decrease in brain levels of nicotine. A reduction in goal tracking when the nicotine CS+ was administered immediately before the test suggests that the brain concentration of nicotine had not reached sufficient levels (Pratt et al. 1983). Similar temporal-effect functions have been reported in the operant drug-discrimination literature (Hirschhorn and Rosecrans 1974; Chance et al. 1977; Pratt et al. 1983; Schechter and Meehan 1992).

The ability of nicotine to serve as a CS+ was not based on a drug versus no-drug discrimination. If this had occurred, amphetamine and bupropion would have fully substituted for the nicotine CS. Instead, amphetamine and bupropion only partially substituted for nicotine indicating stimulus-specific control of goal tracking by nicotine. Incomplete substitution by amphetamine further suggests that the appetitive discrimination was not based on dopaminergic processes or stimulant properties shared by nicotine and amphetamine. This outcome and conclusion is consistent with the operant drug-discrimination literature. That research, using different training and testing procedures consistently reports an inability of amphetamine to completely substitute for a nicotine S^D (Morrison and Stephenson 1969; Schechter and Rosecrans 1972; Stolerman et al. 1984; Mansbach et al. 1998).

Bupropion substitution for the nicotine CS+ was also incomplete suggesting that any similar effect by nicotine and bupriopion on dopamine-containing neurons (Ferris et al. 1983; Ascher et al. 1995; Seppä and Ahtee 2000; Yin and French 2000), at least alone, is not responsible for nicotine's ability to serve as a CS+. The operant drug-discrimination literature is mixed on bupropion substitution for nicotine (full substitution: Wiley et al. 2002; Young and Glennon 2002; no substitution: Shoaib et al. 2003). Further research will be required to determine the procedural details that affect bupropion's ability to evoke nicotine-like responding in both preparations.

One might suggest that differential control of goal tracking reflects state-dependent learning (Overton 1964; Cunningham 1979). With the nicotine CS+ procedure, this would mean that goal tracking is controlled by the chamber cues. However, this chamber-sucrose association is more readily recalled under the effects of nicotine. For the CS– condition, nicotine decreases recall of the chamber-sucrose association learned in a saline state. To our knowledge, the only repeatable demonstration of state-dependent learning with nicotine uses human smokers and recall of word lists (Peters and McGee 1982; Warburton et al. 1986). Like other tasks using abstaining smokers, it is unclear whether effects are attributable to nicotine withdrawal or state dependency. This, and the fact that there have been numerous demonstrations that nicotine typically enhances performance on a learned task even when nicotine is not administered during testing (Levin and Simon 1998), decreases our enthusiasm for a state-dependency account.

In the present research, sucrose was delivered independent of the rat's behavior. From a procedural perspective, this is a Pavlovian procedure in which a stimulus (nicotine) is reliably paired with another stimulus (sucrose). According to this perspective, nicotine has acquired appetitive-motivational value. Because rats have evolutionarily pre-disposed approach tendencies to stimuli that have appetitive qualities (Bolles 1970; Ikemoto and Panksepp 1999), stimuli associated with these appetitive effects also come to control approach behaviors (i.e., goal tracking). Notably, the rat must insert its head into the recessed dipper to access the sucrose. Thus, embedded within the experimental protocol is a response-outcome relationship. If one views the adventitious reinforcement of dipper entries as the controlling variable, then nicotine may be conceptualized as an S^D.The present research was not designed to assess the relative contribution of stimulus-outcome and response-outcome contingencies. However, research that has tried with discrete exteroceptive stimuli has been mixed suggesting that both contingencies might be important (Boakes 1977; Farwell and Ayres 1979). For now, we prefer to use the descriptive and theoretical language provided by the Pavlovian conditioning perspective if for no other reason than it suggests novel empirical research in which drugs serve as CSs acquiring inhibitory and/or excitatory value.

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