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Nicotine does not produce state-dependent effects on learning in a Pavlovian appetitive goal-tracking task with rats

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Abstract

Past research has shown that when rats received 0.4 mg base/kg nicotine paired reliably with intermittent sucrose delivery that anticipatory sucrose-seeking behavior (i.e., goal tracking) was differentially displayed in the nicotine state relative to intermixed saline sessions in which no sucrose was delivered. The present research extended this observation to a lower dose of nicotine (i.e., 0.2 mg base/kg) and tested a state-dependent learning account of differential conditioned responding. According to this account, the increase in goal tracking on nicotine sessions reflects a chamber-sucrose association that is only recalled when in the nicotine state. We used a 2 × 2 factorial design in which rats received sucrose deliveries in one drug state (nicotine or saline) and were then tested in the same state (Nic→Nic or Sal→Sal) or a different state (Nic→Sal or Sal→Nic) after acquiring the conditioned response. A state-dependency account predicts disruption in conditioned goal tracking for rats that receive a shift in drug state on the test day. This disruption did not occur suggesting that differential control of conditioned responding by nicotine is more likely due to a direct excitatory association between the interoceptive cueing effects of nicotine and the appetitive qualities of sucrose.

Keywords

classical conditioning; interoceptive cue; learning; memory; pharmacological conditioned stimulus; nicotinic acetylcholine; recall; smoking; tobacco

Introduction

Interpretation of behavior in drug conditioning research is sometimes complicated by the observation that performance of an acquired response can be specific to the physiological state (e.g., drug versus no drug) in which conditioning occurred. This phenomenon once referred to as “dissociation” of learning or conditioned responding [16] has come to be more commonly called “state-dependent learning” or “state-dependent recall” [24,37]. An early and impressive example of this phenomenon was reported by Girden and Culler [15]. In one of their dogs, they repeatedly paired a 2-sec bell conditioned stimulus (CS) with a brief shock unconditioned stimulus (US) to the right hind paw. The bell alone acquired the ability to reliably evoke a right hind leg semitendinosus muscle twitch conditioned response (CR). When the dog was subsequently curarized, the bell CS no longer evoked the right muscle twitch CR even though the shock US produced the twitch. In this curare state, the same bell CS was now paired with

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shock to the other (left) hind paw. In the curare state, the bell CS came to evoke a semitendinosus muscle twitch CR in the left leg. Most notably, when the dog recovered from the curare, the bell no longer evoked the most recently trained CR in the left leg. Rather, the bell CS in the no-drug state evoked the earlier trained right muscle twitch CR. Girden and Culler [15] concluded that "the animal under curare is functionally decorticate (cortex depressed); conditioning is therefore subcortical in nature and locus. Accordingly, it is inhibited when the cortex returns to dominance. Conditioning in the normal animal on the contrary is predominately cortical in nature and locus, and therefore disappears under curare since the drug inhibits cortical activity" (pp. 273–274).

This demonstration captures the essence of the state-dependent learning effect; a CR acquired in one physiological state (e.g., curare) is not performed in a different state (e.g., no drug). Additionally, this early exemplar of dissociation of learning also has the essence of a widely used procedural approach for testing state-dependent learning [32]. This approach uses a 2 × 2 factorial design in which training state (drug or no drug) is crossed with testing state (drug or no drug). Thus, the experiment finishes with 4 groups. Two groups have a shift in physiological state from training to testing (drug→no drug; no drug→drug) and two groups remain in the training state during testing (drug→drug; no drug→no drug). A state-dependency account such as that given by Girden and Culler [15] predicts that performance of the acquired behavior will be severely disrupted in groups that receive the shift in drug state (but see [26, 32,33]). Indeed, evidence for state-dependent learning has been reported with such drugs as ethanol, midazolam, mescaline, chlordiazepoxide, pentobarbital, and morphine [11,17,18,19, 24–25,30].

Recently, we have published several examples of a Pavlovian drug discrimination task in rats using the pharmacological effects of nicotine (0.4 mg base/kg) as the interoceptive CS and 4-sec access to liquid sucrose as the US [4,7,36]. In brief, on nicotine sessions rats have intermittent access to sucrose, whereas on saline sessions no sucrose occurs. Rats readily acquire a goal-tracking CR as evidenced by more head entries into the dipper receptacle before the first sucrose delivery on nicotine sessions than in a comparable time period on saline sessions [8,14]. During the peer-review process for previous publications, as well as in conference presentations of this research, several individuals have proposed that this differential control of goal tracking by nicotine might reflect state-dependent learning. Indeed, there is precedent for such an account of discrimination performance in the operant conditioning literature (for a review see [26]). However, discrimination performance based on a drug state is not necessarily the result of state-dependent learning (e.g., [10,23]).

Our account of differential control of goal tracking in the nicotine state has assumed that the pharmacological effects of nicotine serve as an interoceptive cue that enters into an association with the sucrose US. This acquired appetitive property of the nicotine cue comes to evoke an approach CR (goal tracking) to an area where sucrose has occurred in the past [7]. A state-dependent learning account, in contrast, assumes the excitatory association is between the exteroceptive cues that compose the conditioning chamber (e.g., odors, rod floor, fan sound, etc.) and the sucrose US. Differential expression of goal tracking presumably reflects either the lack of recall of the chamber CS-sucrose US association when not in the nicotine state (storage/retrieval mechanism) or the change in the nature of how the chamber stimuli are processed in the absence of the drug (stimulus perception mechanism; cf. [37]). Regardless of the mechanism, this state-dependency account makes a very clear prediction. If a goal tracking CR was trained in the nicotine state, this CR would be lost or significantly impaired when tested without nicotine (i.e., saline). Conversely, a goal tracking CR acquired in a no-drug state (i.e., saline) should be significantly attenuated when tested in the nicotine state. Accordingly, we used the 2 × 2 factorial design described earlier to test this prediction. Before describing that study, however, we describe a separate experiment using our standard Pavlovian drug

discrimination procedures with 0.2 mg base/kg nicotine as the interoceptive cue. We report this experiment because this dose of nicotine is becoming a more widely used training dose in our laboratory, the state-dependent learning experiment was conducted with this dose of nicotine, and, finally, we have not published an example of discrimination performance with this dose.

Methods

Subjects

Fifty-four male Sprague-Dawley rats from Harlan (Indianapolis, IN) were kept in a temperature and humidity controlled colony. Each rat was housed individually in clear polycarbonate tubs lined with wood shavings. Chow access was restricted so that each rat was maintained at 85% of its free-feeding body weight (293±24 g). For rats in Experiment 1, we increased their 85% weight by 2 g after 30 days to approximate 'typical' weight increase in unrestricted conditions. Water was continuously available in the home cage. All experimental sessions were conducted during the light portion of a 12 h light:dark cycle. Experimental protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee and followed the "Guide for the Care and Use of Laboratory Animals" [22].

Apparatus

Eight Med-Associates conditioning chambers (ENV-008CT; Georgia, VT USA) with the inside dimensions of 30.5×24.1×21 cm (l×w×h) were used in these studies. The ceiling and front and back walls were clear polycarbonate. The sidewalls were aluminum and the floor was constructed of stainless steel rods. A recessed liquid dipper well (5.2×5.2×3.8 cm; l×w×d) was located in the lower center of one aluminum wall. When the dipper arm was raised, the rat had access to 0.1 ml of sucrose (26% w/v) in the receptacle. An infrared emitter/detector unit located 3 cm off the floor and 1.2 cm within the receptacle monitored head entries. A second infrared unit mounted 4 cm off the floor and 14.5 cm from the dipper receptacle bisected the chamber and provided a measure of general activity. Each chamber was enclosed in a sound and light attenuating cubicle fitted with an exhaust fan that provided airflow and masked noise. A personal computer with Med-Associates interface and software (Med-PC for Windows, version 4) controlled sucrose deliveries and recorded infrared beam breaks in the dipper and chamber.

Drugs

(-)-Nicotine hydrogen tartrate purchased from Sigma (St. Louis, MO USA) was dissolved in physiological saline and then adjusted to a pH of 7.0±0.2 with a dilute NaOH solution. Nicotine and saline were injected subcutaneously (SC) at 1 ml/kg.

Experiment 1: Discrimination training with 0.2 mg/kg nicotine For 3 days before the start of discrimination training, all rats were injected with nicotine (0.2 mg base/kg, SC) in the home cage to reduce the initial locomotor suppressant effects of nicotine (cf. [6]). For 40 consecutive days, rats (n=6) received intermixed nicotine and saline sessions. Rats were injected with 0.9% saline or nicotine (0.2 mg/kg, SC) 5 min before placement in the conditioning chamber for 20 min. On nicotine sessions, rats were given 4-sec access to 26% sucrose (w/v) on 36 separate occasions. To prevent timing of sucrose deliveries, four computer programs were generated to vary the interval between deliveries. The average interval between sucrose deliveries was 25 sec (range 4–80 sec); the average interval before the first sucrose delivery was 137 sec (range 124–152 sec). During saline sessions, no sucrose was delivered. However, four saline programs were generated to simulate the nicotine programs; the programs had 4-sec "empty" intervals in place of sucrose deliveries (see Dependent Measure section). Each rat received the four nicotine and four saline programs in random order without

replacement; the only restriction was that no more than two of one session (drug) type occurred consecutively. Training included a total of 20 nicotine and 20 saline sessions.

Experiment 2: Test for state-dependent learning Rats were randomly assigned to two training conditions: 0.2 mg/kg nicotine or saline (n=16 per condition). Training was similar to the discrimination training just described, except each rat received only its assigned solution throughout training and sucrose was delivered in each session (i.e., no negative sessions and no experience with the opposite solution in the chamber). Saline-trained rats received exposure to nicotine (0.2 mg/kg) in the home cage about 1.5 h after their training session. This controlled for non-specific effects of nicotine and reduced the possibility of alterations of motor ability affecting behavior on the test day. Such a modification is recommended by Overton [26] to avoid the asymmetrical data pattern in the 2 × 2 state dependency design associated with motor alterations from initial drug exposure in the no-drug (saline) condition. To control for number of injections, the nicotine-trained rats received a saline injection in the home cage about 1.5 h after completion of the training session. Acquisition training continued for 15 days with sessions being conducted Monday to Friday. The test for state-dependent learning occurred 24 h after the last training session. During the test, half of the rats in each condition (nicotine- or saline-trained) received a shift in training state (Nic→Sal or Sal→Nic). The remaining rats received the training state on the test day (Nic→Nic or Sal→Sal). Within a training condition, we assigned rats to groups (n=8) with the restriction that dipper entries during training did not statistically differ. For testing, rats were injected 5 min before placement in the chambers for 20 min (same as training), but no sucrose was available.

We have never trained rats in this goal-tracking task using a single drug state (i.e., all previous research used discrimination procedures). Thus, we needed controls to provide a benchmark for the level of dipper entries when chamber and drug-state exposure was equated, but sucrose was withheld. Accordingly, during the training phase, control rats received an injection of 0.2 mg/kg nicotine (n=8) or saline (n=8) 5 min before each placement in the chambers, but no sucrose was available. These rats also received home cage injections as described earlier. For the test session, half of the rats in the control condition received a shift in state; the remaining animals received the training state.

Dependent measures

The primary dependent measure in acquisition was number of dipper entries per second before the first sucrose delivery. The rate of entries per second was used because time to first sucrose delivery varied across sessions. Further, this measure avoids including dipper entries induced by sucrose delivery in our main index of learning. For discrimination training, a comparable time was used from the saline sessions (cf. empty intervals). For the state-dependent learning test, we assessed this early and presumably most sensitive measure of performance (i.e., first 2 min), as well as examined how dipper entries changed across the entire 20-min test session. General activity defined as beam breaks recorded from the infrared unit that bisected the chamber was treated the same as dipper entries.

Data Analyses

For discrimination training separate two-way (Condition × Session) repeated-measures analyses of variance (ANOVA) were used to analyze dipper entries and chamber activity. For acquisition in the state-dependency experiment, separate two-way (Condition × Session) mixed ANOVAs were used. Significant Condition × Session interactions prompted pair-wise comparisons for each session using Fisher's LSD tests that control for Type I error rate. Dipper entries and chamber activity of control rats were compared on the last day of acquisition using an unpaired *t*-test. The data for the state-dependency test were analyzed by separate two-way between subjects ANOVAs (Training State × Test State). An ensuing analysis divided total

dipper entries and general chamber activity across the session into 1-min intervals. Separate two-way mixed ANOVAs (Group × Minute) were used to analyze dipper entries and chamber activity. Pair-wise comparisons for each session were conducted with Fisher's LSD tests. For all analyses, statistical significance was declared using a two-tailed rejection region of 0.05.

Results

Experiment 1: Discrimination training with 0.2 mg/kg nicotine Figure 1a shows the results of discrimination training for rats that were trained with nicotine as a CS. For dipper entries, the two-way repeated-measures ANOVA revealed a significant main effect of Condition [$F(1, 5)=233.64$, $P<0.001$], of Session [$F(19, 95)=3.73$, $P<0.001$], and a significant Condition × Session interaction [$F(19, 95)=2.97$, $P<0.001$]. Relative to saline sessions, dipper entries were higher on nicotine sessions 4, 6, 7, and 9 to 20 ($LSD_{\text{minimum mean difference (mmd)}}=0.063$). For chamber activity, there was a significant main effect of Session [$F(19, 95)=1.96$, $P=0.006$] indicating an increase in activity across sessions (Figure 1b). Chamber activity did not vary by Condition, nor was there a significant Condition × Session interaction [Fs ≤1.29, Ps≥0.21].

Experiment 2: Test for state-dependent learning Figure 2a shows dipper entry scores during the acquisition phase for rats trained in either the nicotine or saline state. A mixed ANOVA revealed significant main effects of Condition [$F(1, 30)=5.94$, $P=0.02$] and Session [$F(14, 420)=14.00$, $P<0.001$], but no significant Condition × Session interaction [$F<1$]. The main effect of Session indicates that a goal tracking CR was acquired across sessions. The main effect of Condition suggests that saline rats had a tendency to respond higher than nicotine rats, but the lack of an interaction indicates that the groups did not differentially acquire the CR. Figure 2b shows chamber activity for the acquisition phase. There was a significant main effect of Session [$F(14, 420)=14.11$, $P<0.001$] and a significant Condition × Session interaction [$F(14, 420)=5.92$, $P<0.001$], but no main effect of Condition [$F=2.49$, $P=0.13$]. Follow-up analyses using Fisher's LSD tests compared chamber activity in the nicotine and saline conditions. Nicotine's locomotor suppressant effects were evident during the initial three sessions; activity was also lower for nicotine-treated rats on session 11 ($LSD_{mmd} = 0.061$). Rats in the control condition did not differ on dipper entries [$t(14)=1.14$, P=0.275] (see Table 1). Control rats trained with nicotine were more active than rats trained with saline [$t(14)=2.49$, $P=0.026$].

Figure 3a shows dipper entries in the first 2 min of the test session (cf. measure for acquisition training). There was no main effect of Training State or Test State [Fs≤1.06, Ps≥0.31] and no Training State × Test State interaction [$F(1,28)=1.16$, $P=0.29$]. This outcome is counter to a state-dependent learning account of the Pavlovian drug discrimination result. Although using dipper entries from the first 2 min of the test session matches the measure from acquisition training and is presumably a conservative test for state dependency because little opportunity for alternative learning has occurred, one might argue that this temporal window was not sufficient for statistical differences to be observed. Accordingly, we conducted identical 2-way ANOVAs on the average dipper entries for the first 5 min and the first 10 min of the test session. Similar to the analysis of the first 2 min data, the Training State × Test State interaction was not significant for either the 5- or 10-min intervals [Fs≤1.59, Ps≥0.32]. Figure 3b shows chamber activity in the first 2 min of the test session. There was no main effect of Training State or Test State [Fs≤1.05, Ps≥0.32] and no Training State × Test State interaction [$F<1$]. We conducted a similar analysis on the dipper entries and chamber activity for control rats (see Table 1). There were no main effects or significant interactions for either dependent measure [Fs≤2.14, Ps≥0.17].

Figure 4a and 4b show total dipper entries per minute across the 20-min test session for the Nic→Nic and Nic→Sal rats and the Sal→Sal and Sal→Nic rats, respectively. A mixed ANOVA including all 4 groups revealed a significant main effect of Minute [$F(19,532)=11.17$, $P<0.001$] and a significant Group × Minute interaction [$F(57, 532)=1.37$, $P=0.04$]; the main effect of Group was not significant [$F=2.50$, $P=0.08$]. To better characterize the interaction, separate two-way mixed ANOVAs were conducted with each training state (i.e., Nic→Nic and Nic→Sal together; Sal→Sal and Sal→Nic together). There was a significant Group × Minute interaction [$F(19, 266)=1.69$, $P<0.04$] only for the nicotine-trained rats (Figure 4a). Nic→Nic rats had significantly higher entry rates on minutes 7, 8, 10, 18, and 19 compared to Nic→Sal rats ($LSD_{mmd}=4.196$). Figure 4c and 4d show general chamber activity for testing in 1-min intervals. A mixed ANOVA including all 4 groups revealed a significant main effect of Minute [$F(19, 532)=17.81$, $P<0.001$], but no effect of Group or Group × Minute interaction [Fs<1]. The main effect of Minute reflects an overall decrease in activity across the session.

Discussion

Previous research from our laboratory has shown that 0.4 mg/kg of nicotine paired reliably with sucrose acquires the ability to control a goal-tracking CR [4,36]. Further, a 0.2 mg/kg dose of nicotine also served as an interoceptive CS using a fading procedure [7]. In that study, rats were first trained to discriminate 0.4 mg/kg nicotine from saline. Once conditioned goal tracking stabilized, the dose of nicotine was decreased to 0.2 mg/kg. The initial decrease in dose disrupted conditioned responding. However, with continued training conditioned responding stabilized with this lower dose of nicotine. Experiment 1 of the present report extends this research by showing that a fading procedure is not required for a 0.2 mg/kg dose of nicotine to function as interoceptive CS. Although one must be cautious when comparing across studies, there does not appear to be any obvious difference in acquisition of the discrimination between the present study and previous research using 0.4 mg/kg nicotine [36]. Further research, however, is needed to directly assess whether this dose of nicotine affects acquisition and/or maintenance of conditioned responding.

As noted in the Introduction, some have suggested that discrimination performance may be controlled by state-dependent learning/recall of a context CS-sucrose US association rather than a direct conditioned association between the pharmacological effects of nicotine and sucrose. We found little support for this state-dependency account in Experiment 2. For example, in the first 2 min of the test session—the interval used in the training phase to assess acquisition—rats' goal-tracking behavior was not affected by a shift in drug state (groups Nic→Sal and Sal→Nic). Using the average of the first 5 or 10 min of the test session did not change this result. Further, shifting the drug state from training to testing did not alter the pattern of goal tracking across the 20-min extinction test for group Sal→Nic. The removal of nicotine (group Nic→Sal), however, decreased conditioned responding relative to the non-shifted Nic→Nic group late in the test session. Notably, differential withdrawal from nicotine seems an unlikely explanation of this effect in that Sal→Sal rats received a comparable amount of home cage exposure to nicotine, yet their extinction pattern was not decreased relative to the Sal→Nic rats that had nicotine during extinction testing.

A strong proponent of state-dependency theory might assert that this late test session difference between the Nic→Nic and Nic→Sal group provides support for this explanation. Such an assertion, however, is strained in at least 3 ways. First, a state-dependency account is obligated to explain why the group difference did not emerge until 7 min into the test session. The start of the sessions would seem like the most sensitive period to detect a state-dependent learning/recall effect. That is, the state is shifted and there has been little to no chance for new learning in the shifted state early in the test session. Second, the group that had nicotine added to the training context during testing (Sal→Nic) did not show a significant disruption of conditioned

responding throughout the session. This result is contrary to most theoretical formulations of state-dependency that predict a symmetrical effect of shifting drug states. Some researchers have reported asymmetrical patterns in which only the shift from drug to no drug produces the deficit (e.g., [2,27]). Unfortunately, these asymmetrical state dependency accounts get theoretically burdensome (see [32] for a similar conclusion). Third, there is a more parsimonious explanation that relies on stimulus sampling and stimulus element learning models [5,12,13] that readily accounts for the data pattern.

According to a stimulus element account, the CS for rats receiving saline before each training session (Sal→Sal and Sal→Nic) is a multimodal stimulus that includes olfactory, tactile, visual, gustatory, auditory, and temporal elements. During training, the particular elements being sampled in close temporal proximity to sucrose delivery will become increasingly excitatory with each pairing. The acquisition and maintenance pattern of conditioned responding reflects this increase in the associative strength of each element, as well as the increasing number of elements entering into an association with sucrose. According to a stimulus element account not all stimulus elements are necessarily perceived (experienced) at any given moment even after extensive training. Thus, asymptotic conditioned responding at some time sample reflects only a subset of the total associative strength actually conditioned during training. Notably, the rats in the nicotine-training condition (Nic→Nic and Nic→Sal) have the interoceptive cueing effects of nicotine as an additional set of stimulus elements present during training. Thus, on the test day the Nic→Sal group has some set of stimulus elements missing—the interoceptive nicotine cue. Early conditioned responding is not disrupted because nicotine provides only a sub-set of many stimulus elements that compose the context. However, as extinction weakens conditioned responding to the sampled elements, the difference in the number of available stimulus elements between Nic→Nic and Nic→Sal emerges. In contrast, the Sal→Nic rats do not have any stimulus elements missing during the test. Rather, this group had a stimulus element added to the test. If anything, according to a stimulus element account the addition of a new stimulus during testing should produce disruption in conditioned responding early in the session (cf. external inhibition; [28]). Indeed, the mean dipper entries tended to be lower in the Sal→Nic than in the Nic→Sal group in the first few minutes of the session. This trend was not significant and the impact of external inhibition was likely minimized by home cage exposure to nicotine.

The conclusion that nicotine does not produce state-dependent control over goal tracking in the Pavlovian drug discrimination task is consistent with past research. Although there is a very rich theoretical and empirical literature on state-dependent learning with such drugs as ethanol, chlordiazepoxide, pentobarbital, and midazolam [11,17,18,19,24–27,30,37], such an extant literature does not exist for nicotine. In fact, the only replicated demonstration that nicotine might have a state-dependent effect used human smokers and recall of word lists [21,29,35]. Like other performance tasks using smokers that abstain from nicotine, whether these effects are attributable to nicotine withdrawal (or relief from withdrawal) or to state dependency is unclear. Further, there is a vast literature showing that acute and chronic treatment with nicotine and related nicotinic agonists enhance learning in a wide range of tasks even when nicotine is no longer administered (for reviews see [3,20]). Improvement in performance when the nicotine state is removed from a learning situation is counter to a state-dependent learning/recall account.

General activity in the chambers was measured throughout both experiments. The most notable observation from this data is that a difference in one measure was not necessarily paralleled in the other measure. For example, as the discrimination was acquired in Experiment 1, dipper entries were substantially higher in the nicotine than the saline sessions. In contrast, general activity was identical regardless of drug state. Similarly, the differences in later dipper entries between group Nic→Nic and group Nic→Sal in the extinction test of Experiment 2 were not

seen in the activity data. This dissociation is methodologically important because it indicates that our measure of conditioned responding (i.e., goal tracking) has the potential to be isolated from general activating or suppressing effect an experimental manipulation including drug administration.

In sum, rats readily discriminate 0.2 mg/kg of nicotine from saline as evidenced by increased goal tracking in the nicotine state. The present research suggests that this differential control of conditioned responding is more likely due to a direct excitatory association between the interoceptive cueing effects of nicotine and the appetitive qualities of sucrose rather than a state-dependent recall of some other association (e.g., chamber-sucrose association). Some researchers may prefer to highlight the possibility that the goal-tracking behavior used as our measure of conditioned responding is maintained by adventitious reinforcement of head entries into the dipper. Indeed, the sucrose cannot be obtained without a head poke into the dipper receptacle. The lack of evidence for a state-dependent learning effect equally applies to this operant conditioning account. As we have noted in an earlier publication [3], the research attempting to disentangle the role of stimulus-stimulus versus response-stimulus relations for goal tracking has been mixed (e.g., [8,14]). Until unambiguous evidence demonstrating the exclusive role of operant contingencies is available, we prefer to pursue this avenue of research from a Pavlovian conditioning framework for the mere utilitarian reason that this approach leads to novel and potentially important experiments that could elucidate factors contributing to drug addiction. That is, perhaps the abuse liability of nicotine-containing products could be affected by conditioned associations in which nicotine is an interoceptive cue for other appetitive USs [7,9,34]. Such evidence has been found in humans using diazepam [1] and ethanol [31] as the interoceptive cue.

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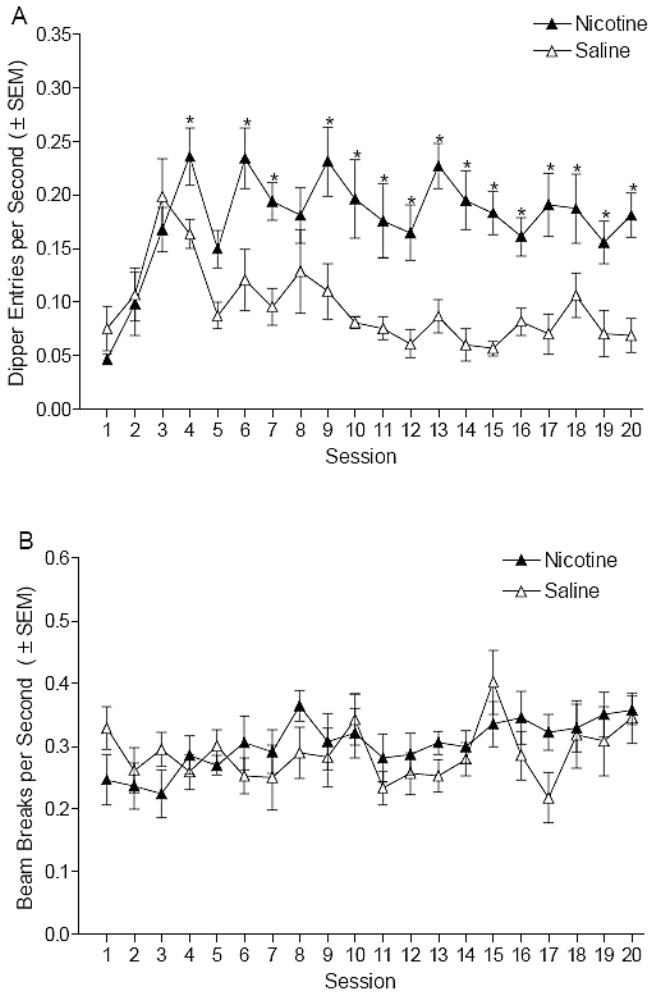

Figure 1.

Panel A displays the mean dipper entries per second across the course of discrimination training for the 0.2 mg/kg nicotine sessions and the saline sessions for Experiment 1. For nicotine sessions, the dipper entries come from the time before the first sucrose delivery; a comparable time was used for saline sessions. Panel B shows general chamber activity (beam breaks per second derived from the same interval as dipper entries) across discrimination training for nicotine and saline sessions.

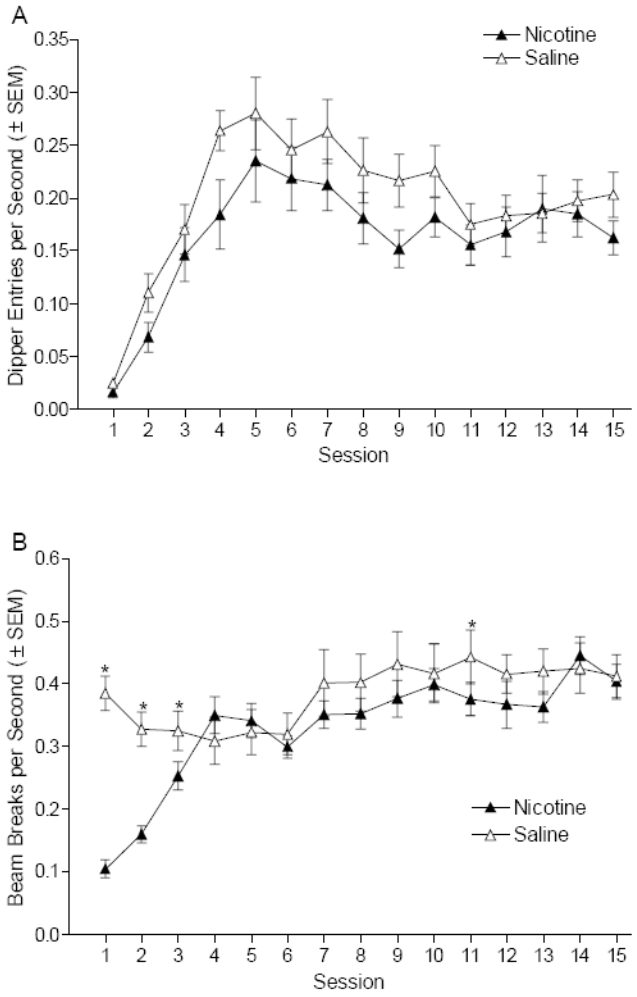

Figure 2.

Panel A displays the mean dipper entries per second across acquisition training for nicotine- and saline-trained rats in the state-dependency experiment. Panel B displays general chamber activity (beam breaks per second) for the same rats.

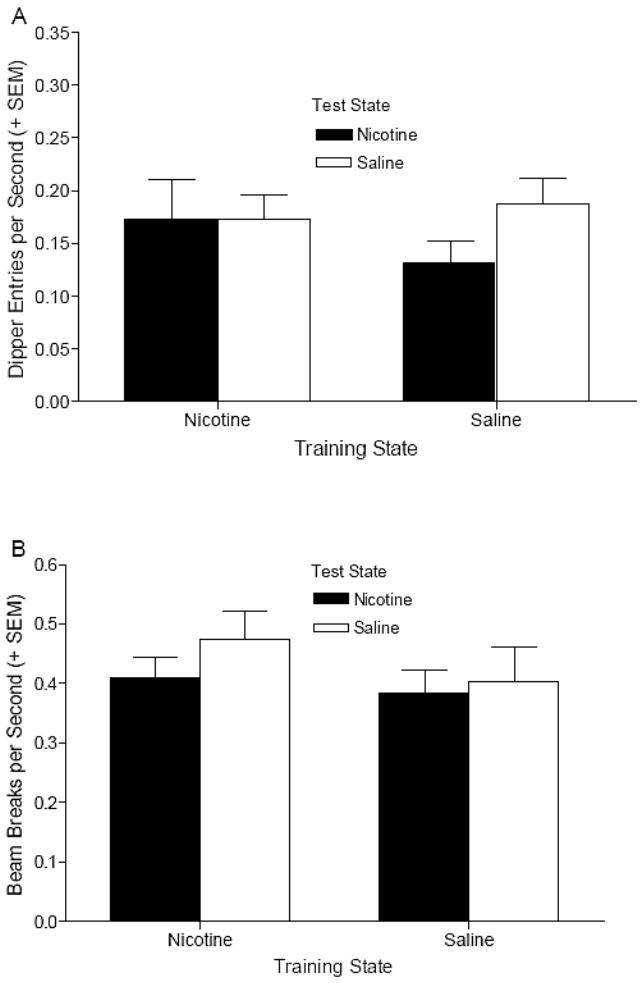

Figure 3.

Panel A displays dipper entries per second during the first two minutes of the test session for Nicotine→Nicotine, Nicotine→Saline, Saline→Saline, and Saline→Nicotine groups of Experiment 2. Panel B displays general chamber activity (beam breaks per second) during the first two minutes of the test session for Nicotine→Nicotine, Nicotine→Saline, Saline→Saline, and Saline→Nicotine groups.

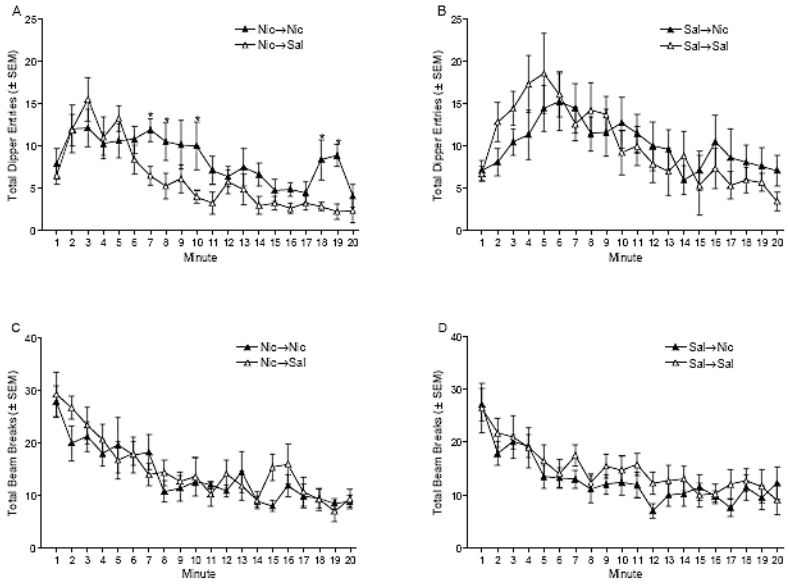

Figure 4.

Panel A displays dipper entries per minute across the test session for Nicotine→Nicotine and Nicotine→Saline rats of Experiment 2. Panel B displays dipper entries per minute across the test session for Saline→Saline and Saline→Nicotine rats. Panel C displays general chamber activity across the test session for Nicotine→Nicotine and Nicotine→Saline rats. Panel D displays activity per minute across the test session for Saline→Saline and Saline→Nicotine rats.

Table 1

Means ± SEM of Experiment 2 controls for dipper entries and activity during training and testing

	Training			Testing	
	Dipper Entries	**Chamber Activity**		**Dipper Entries**	**Chamber Activity**
Nicotine	0.052 ± 0.007	0.378 ± 0.023*	Nic→Nic	0.066 ± 0.007	0.347 ± 0.033
			Nic→Sal	0.060 ± 0.012	0.391 ± 0.042
Saline	0.041 ± 0.006	0.303 ± 0.020	Sal→Sal	0.051 ± 0.013	0.357 ± 0.029
			Sal→Nic	0.044 ± 0.009	0.347 ± 0.046

* Significantly different from rats trained with saline ($p<0.05$).