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Nicotine-evoked conditioned responding is dependent on concentration of sucrose unconditioned stimulus

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Abstract

Previous studies have shown that the interoceptive nicotine conditional stimulus (CS) functions similarly to exteroceptive CSs such as lights or environments. For instance, the appetitive conditioned response (CR) evoked when nicotine is repeatedly paired with sucrose presentations (the unconditioned stimulus; US) is sensitive to changes in training dose (CS salience) and the contiguity between the CS effects and sucrose. The current study was conducted to extend this research by examining the possible role of US intensity in CR acquisition and maintenance. Rats were trained using one of four sucrose concentrations: 0, 4, 16, or 32% (w/v). On nicotine sessions (0.4 mg base/kg), rats received 36 deliveries (4 sec each) of their assigned concentration intermittently throughout the session; sucrose was withheld on saline sessions. In all groups, an appetitive goal-tracking CR was acquired at a similar rate. However, the asymptotic CR level varied with sucrose concentration. The magnitude of the CR was increased in rats trained with higher sucrose US concentrations. These findings are consistent with previous Pavlovian conditioning research, and extend the conditions under which the nicotine state functions as an interoceptive conditional stimulus.

Keywords

appetitive reinforcement; drug discrimination; Pavlovian conditioning; response magnitude; tobacco; stimulus salience

1. Introduction

The consensus among the scientific community is that nicotine and its complex biological and behavioral effects are responsible for chronic tobacco use (World Health Organization, 2008). Among these effects is that the nicotine drug state is perceptible and functions as an interoceptive stimulus in a variety of behavioral tasks with human and nonhuman animals. Of interest in the present report is the ability of the nicotine state to serve as a conditional stimulus (CS) in an appetitive Pavlovian conditioning task (e.g., Besheer et al., 2004). In this task, an injection of nicotine or saline was given before placement in a conditioning chamber. On nicotine sessions, liquid sucrose (i.e., unconditioned stimulus; US) was delivered intermittently. On intermixed saline sessions, sucrose was not available. Using head entries into the sucrose receptacle before the first sucrose delivery as a measure of conditioning (i.e., goal tracking; Boakes, 1977; Farwell and Ayres, 1979), nicotine served as a CS as evidenced by increased dipper entries on nicotine compared to saline sessions.

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Previous research has verified that this interoceptive nicotine stimulus functions in the manner anticipated by research with more "typical" exteroceptive CSs. For instance, the magnitude of the conditioned response (CR) increased with number of conditioning trials (Wilkinson et al., 2006). The nicotine-evoked CR decreased when the sucrose US was withheld (i.e., extinction), and extinction proceeded slower with increased number of training trials and with increased training doses (i.e., stimulus intensity) of nicotine (Besheer et al., 2004; Murray and Bevins, 2007b, in press; Wilkinson et al., 2006). Finally, nicotine-evoked responding cannot be explained by a state-dependent learning account (Bevins et al., 2007). That is, rats that acquired an appetitive dipper entry response in either a nicotine or saline state did not show disrupted responding when tested in the alternate state.

To date, no one has assessed whether conditioned responding to the nicotine CS will vary as a function of US salience/intensity. All of the published research with the nicotine CS from our laboratory has rats on a food restricted diet and either 26% or 32% (w/v) sucrose as the US [see Troisi (2006) for use of food pellets as the US in the Pavlovian conditioning phase of a instrumental-Pavlovian transfer study with nicotine]. Some Pavlovian conditioning theories would predict that the strength of the CR will be proportional to the salience of the US (e.g., Rescorla and Wagner, 1972). Indeed, research investigating aversive conditioning (e.g., Annau and Kamin, 1961; Bevins et al., 1997; Holland, 1979; Kamin and Brimer, 1963; Pavlov, 1927) and appetitive conditioning (e.g., Bevins, 2005; Holland, 1979; Morris and Bouton, 2006; Pavlov, 1927; van den Bos et al., 2004) have shown that increases in the US salience (e.g., foot-shock mA or amount of food per delivery) increase the magnitude of the CR. The goal of the present study was to determine whether conditioned responding to the interoceptive contextual stimuli provided by the nicotine state will vary as a function of US concentration.

2. Materials and Methods

2.1. Subjects

Thirty-two male Sprague-Dawley rats weighing 408±3 grams before the start of the experiment were obtained from Harlan (Indianapolis, IN, USA). Rats were housed individually in clear polycarbonate tubs lined with wood shavings in a temperature- and humidity-controlled colony. Water was continuously available in the home cage. Daily access to chow (Harlan Teklad Rodent Diet) was restricted such that rats were maintained at 85% of free-feeding body weights. All sessions were conducted during the light portion of a 12 hr light:dark cycle. Protocols were approved by the University of Nebraska-Lincoln Animal Care and Use Committee and followed the `Guide for the Care and Use of Laboratory Animals' (National Research Council, 1996).

2.2. Apparatus

Eight conditioning chambers (ENV-008CT; Med Associates, Inc., St. Albans, VT, USA) measuring 30.5 × 24.1 × 21.0 cm (l × w × h) were used in this study. Sidewalls were aluminum; the ceiling and front and back walls were clear polycarbonate. Each chamber was equipped with a recessed receptacle (5.2 × 5.2 × 3.8 cm; l × w × d) on one sidewall. A dipper arm raised a 0.1-ml cup of solution into the receptacle. An infrared emitter/detector unit, 1.2 cm into the receptacle and 3 cm from the chamber floor, monitored head entries into the dipper. Each chamber was enclosed in a light- and sound-attenuating cubicle fitted with a fan to provide airflow and mask noise. A personal computer with Med Associates interface and software (Med-PC for Windows, version IV) timed sessions, controlled sucrose deliveries, and recorded dipper entries.

2.3. Drug

(−)-Nicotine hydrogen tartrate was purchased from Sigma (St. Louis, MO, USA) and dissolved in 0.9% saline. The pH was adjusted to 7.0±0.2 with a dilute NaOH solution. The training dose of nicotine was 0.4 mg/kg injected subcutaneously (SC) at a volume of 1 ml/kg. Dose is reported in the base form.

2.4. Procedure

Rats were handled for at least 3 min per day for 3 days. Before training started, each rat was given an injection of 0.4 mg/kg nicotine once per day for 3 days in its home cage to minimize the initial locomotor suppressant effects of nicotine (cf. Bevins et al., 2001; Besheer et al., 2004). Rats were randomly assigned to one of four concentrations of the sucrose US: 0 (i.e., tap water), 4, 16, or 32% (w/v). Daily training sessions began the day following the last home cage injection of nicotine. Rats received either 0.4 mg/kg nicotine or saline SC 5 min before placement in the chambers for a 20-min session. During nicotine sessions, there were 36 deliveries of the assigned sucrose solution. Four different programs that varied when sucrose was delivered were used to discourage timing of deliveries. Care was taken to prevent cross-contamination of sucrose concentration by cleaning dippers and using separate dipper wells for each concentration. The average time before the first sucrose delivery across programs was 137 s with a range of 124–152 s. The average time between sucrose deliveries within the sessions was 25 s with a range of 4–80 s. Saline sessions were similar except no sucrose was delivered. Session types and programs were randomly assigned for an individual rat with the restriction that no more than 2 nicotine or 2 saline sessions occurred in a row. Training continued for 22 nicotine and 22 saline sessions.

2.4. Dependent Measure and Data Analyses

The primary dependent measure was rate of dipper entries per second before the first sucrose delivery. To allow for comparable measurement between nicotine (i.e., sucrose) and saline (i.e., no sucrose) sessions, the program types were matched for timing of the intervals from which dipper entries were taken. Acquisition of the CR for each measure was analyzed first using a 3-way mixed measures analysis of variance (ANOVA) with Group (0, 4, 16, or 32%) as the between-subjects factor and Drug (nicotine versus saline) and Session (1 to 22) as within-subject factors. In order to better examine the contribution of each factor, a significant 3-way interaction was followed with 2-way mixed measures ANOVAs examining Drug and Session as the factors for each sucrose concentration. Additionally, 2-way ANOVAs examining Group and Session as the factors for responding on nicotine and on saline were conducted separately. Significant effects in the 2-way ANOVAs were followed with pairwise comparisons using Fisher's least significant difference (LSD) tests. Statistical significance was declared at $p < .05$ for all tests.

3. Results and Discussion

Rats learned the Pavlovian drug discrimination regardless of sucrose concentrations. However, asymptotic performance appeared to vary as a function of sucrose concentration (see Figure 1). This impression was supported by the significant Group × Drug × Session interaction, $F(63, 588) = 1.87$, $p < .001$. Further, the follow-up 2-way ANOVAs for each sucrose concentration revealed main effects of Drug, Fs ≥ 31.0, ps ≤ .001, Session, Fs ≥ 1.83, ps ≤ .02, and Drug × Session interactions, Fs ≥ 2.45, ps ≤ .001. The 0% group (Figure 1A) showed higher conditioned responding on nicotine than on saline for sessions 8, 10–12, 14, and 16–22, $LSD_{\text{minimum mean difference (mmd)}} = 0.031$. The 4% group (Figure 1B) developed a more consistent CR than the 0% group with higher conditioned responding on nicotine than saline sessions 8–19, and 21, $LSD_{\text{mmd}} = 0.044$. The 16% group (Figure 1C) showed relatively quick acquisition of conditioned responding that remained stable

throughout training; dipper entries were higher on nicotine sessions 6–22, LSD_{mmd} = 0.044. Given the non-specific increase in dipper entries in the early sessions, some weak motor suppressant effects of nicotine were revealed on sessions 1 and 3. Discrimination performance was similar in the 32% group (Figure 1D) with decreased responding on nicotine sessions 1–3 and higher responding on nicotine sessions 7–8 and 10–22, LSD_{mmd} = 0.062.

The increased dipper entries on nicotine sessions in the 0% group is somewhat surprising given that the rats had free access to water in the colony. One possibility is that the locomotor stimulant effects of repeated nicotine exposure increased dipper entries in a non-associative manner. For two reasons, we believe that this increase in dipper entries in the 0% group reflects appetitive conditioning to nicotine and not a locomotor stimulant effect. First, we assessed whether an infrared beam break occurred in the dipper well during US deliveries. For each rat in each group we determined the mean number of deliveries that were accessed during the 7th and 8th nicotine training sessions (denoted 7/8) and during the 21st and 22nd nicotine training sessions (denoted 21/22). Those time points were chosen because that was when the nicotine-saline discrimination was first evident across groups and when training was complete, respectively. At the 7/8 time point, the mean number of deliveries accessed ± the standard deviation was as follows: 17.9±10.3 for 0%, 25.5±10.2 for 4%, 34.9±1.3 for 16%, and 35.56±0.5 for 32%. At the 21/22 time point, the number of deliveries accessed was 25.3±6.1 for 0%, 34.9±1.1 for 4%, 35.9±0.3 for 16%, and 35.3±2.5 for 32% groups. Although the 0% group did not reach the same level as the other groups, by the end of training they were still accessing 70% of US deliveries. Further, there was a significant increase from the 7/8 to the 21/22 time point in the 0% group [Group x Time Point interaction, $F(3, 60) = 9.83$, $p < .001$, LSD_{mmd} = 3.01]. The second reason is provided by previously published research (Wilkinson et al., 2006) that used intermixed nicotine and saline sessions as in the present study. In that research, there was a control condition that did not receive any sucrose deliveries, regardless of session type, throughout training. There was no difference in dipper entries on nicotine versus saline sessions, indicating that nicotine exposure alone was not sufficient to increase dipper entries above saline levels (see Figure 1, Group 0:0 of Wilkinson et al., 2006). This lack of an effect is inconsistent with a locomotor stimulant account of increased dipper entries in the 0% group of the present study. Overall, the pattern of the dipper access in the current study combined with the earlier findings of Wilkinson et al. (2006) supports a conditioning and not a stimulant account of increased dipper entries on nicotine compared saline sessions in the 0% group. Interestingly, at both time points the 4% group accessed significantly more US deliveries than the 0% group. This result suggests that the 4% sucrose concentration was somewhat more appetitive than the 0% even though there was no difference in CR magnitude.

The 2-way ANOVA that examined responding only on nicotine sessions (Figure 2A) revealed significant main effects of Group and Session, Fs ≥ 11.92, ps < .001; the interaction was not significant, $F = 1.12$, $p = .27$. An examination of the marginal means showed overall dipper entry rate was similar between rats trained with 16 and 32% sucrose (Ms = 0.149 ± 0.011 and 0.161 ± 0.011, respectively), and between rats trained with 0 and 4% sucrose (Ms = 0.080 ± 0.011 and 0.098 ± 0.011), LSD_{mmd} = 0.032. However, rats trained on 16 or 32% sucrose had higher overall responding than rats trained on 0 or 4% sucrose. Interestingly, the pattern of dipper entries on saline sessions also varied as a function of sucrose concentration (Figure 2B). This impression was supported by a main effect of Group and Session, Fs ≥ 11.47, ps < .001. Subsequent comparisons on the marginal means revealed that the 16 and 32% groups (Ms = 0.079 ± 0.005 and 0.090 ± 0.005, respectively) were higher than the 0 and 4% groups (Ms = 0.047 ± 0.005 and 0.049 ± 0.005, respectively), LSD_{mmd} = 0.015. The significant Group x Session interaction, $F = 2.68$, $p < .001$, denotes that rats in the 16 and 32% groups increased responding early in the session

and then decreased as the discrimination developed, $LSD_{mmd} = 0.03$. A similar data pattern was observed by Murray and Bevins (2007a, 2007b) regardless of whether 0.1, 0.2, or 0.4 mg/kg nicotine functioned as the interoceptive stimulus for a 26% sucrose solution. This early conditioned responding suggests that the chamber stimuli acquired some initial excitation in early training sessions at the higher sucrose concentrations. Presumably the CR controlled by the exteroceptive situational stimuli extinguished as the saline sessions proceeded without sucrose. The lack of context conditioning in the 0 and 4% groups is reflective of the relative weakness of these USs.

Although the goal-tracking response is widely used to study Pavlovian conditioning processes, research suggests that goal tracking is sensitive to both the instrumental and Pavlovian relations (e.g., Boakes, 1977; Farwell and Ayres, 1979). Thus, one could refer to nicotine as the discriminative stimulus that occasions sessions in which head entries will be reinforced. We do not want our use of Pavlovian conditioning terminology to leave the reader with the wrong impression. Instrumental contingencies will likely be revealed as important in the present situation. Further, past research using these techniques and theories to study the discriminative stimulus effects of nicotine have lead to important insights and advances into our understanding of nicotine and its role in chronic tobacco use (see Stolerman & Jarvis, 1995). Further, this research has informed, and continues to inform our research. However, drug discrimination research has primarily been driven by the instrumental framework. We believe that thinking about drug states and drug discrimination from a more associative or Pavlovian conditioning perspective will add to the richness of our understanding of interoceptive stimulus properties by prompting new questions and research directions (cf. Bevins, 2008; Bevins & Palmatier, 2004). As such, we continue to use the Pavlovian conditioning language and theories to describe and guide our research.

Use of the Pavlovian conditioning perspective has resulted in research showing that this goal-tracking CR was susceptible to manipulations such as CS salience (Murray and Bevins, 2007a, 2007b), CS-US contiguity (Wilkinson et al., 2006), number of conditioning trials (Wilkinson et al., 2006), and extinction (Besheer et al., 2004; Murray and Bevins, 2007b, in press; Wilkinson et al., 2006). The findings of the current experiment extended this list to US intensity. Other Pavlovian conditioning research has shown that maintenance of conditioned responding is dependent on the salience of the US (e.g., Annau and Kamin, 1961; Bevins et al., 1997; Kamin and Brimer, 1963; Morris and Bouton, 2006; Pavlov, 1927) and that reinforcing efficacy of sucrose increases with concentration (e.g., Sclafani & Ackroff, 2003). Combined, these data have provided converging support for the utility of this approach and the notion that the nicotine state functions as an interoceptive CS.

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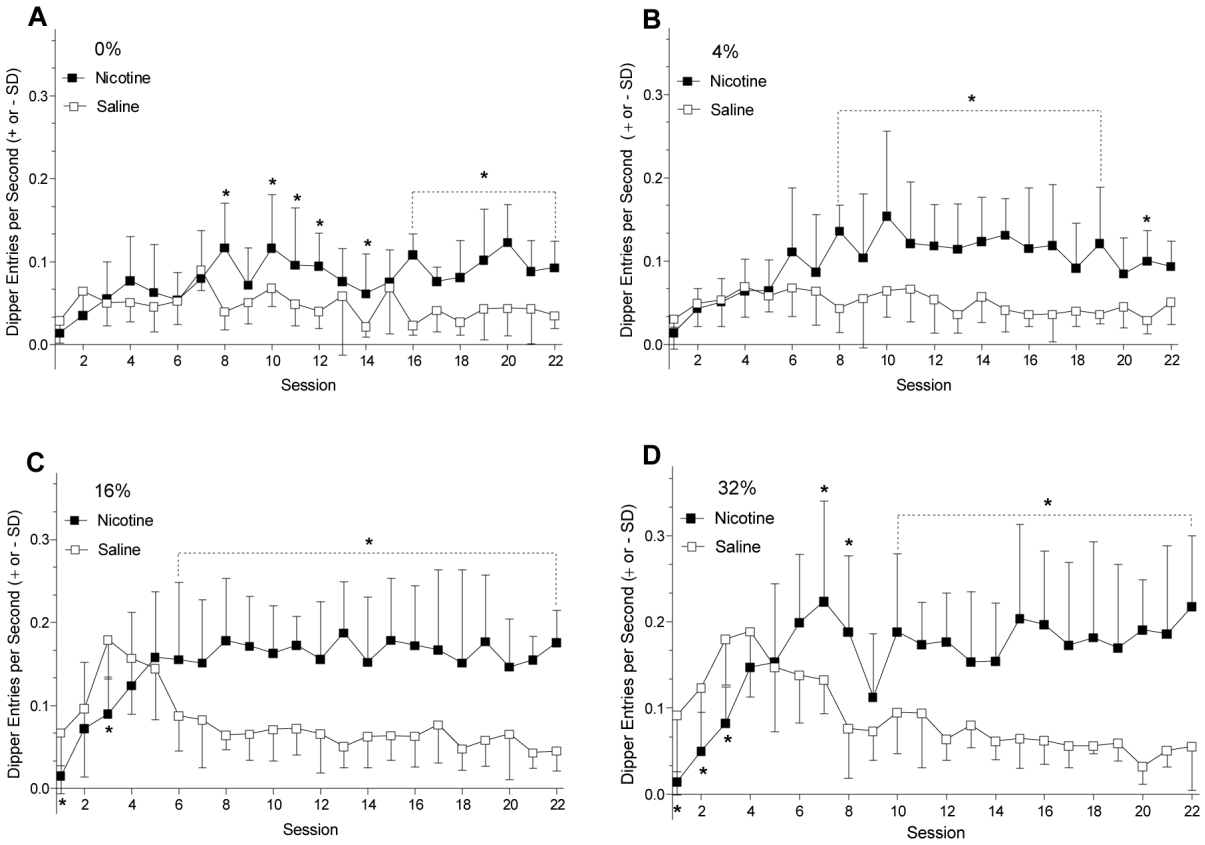

Figure 1.

Acquisition of the nicotine discrimination in dipper entries per second (+ or − SD) for each of the four training groups across training sessions is shown. Panels A–D shows the 0, 4, 16, and 32% sucrose groups, respectively. * denotes significant difference ($p < .05$) from comparable saline session.

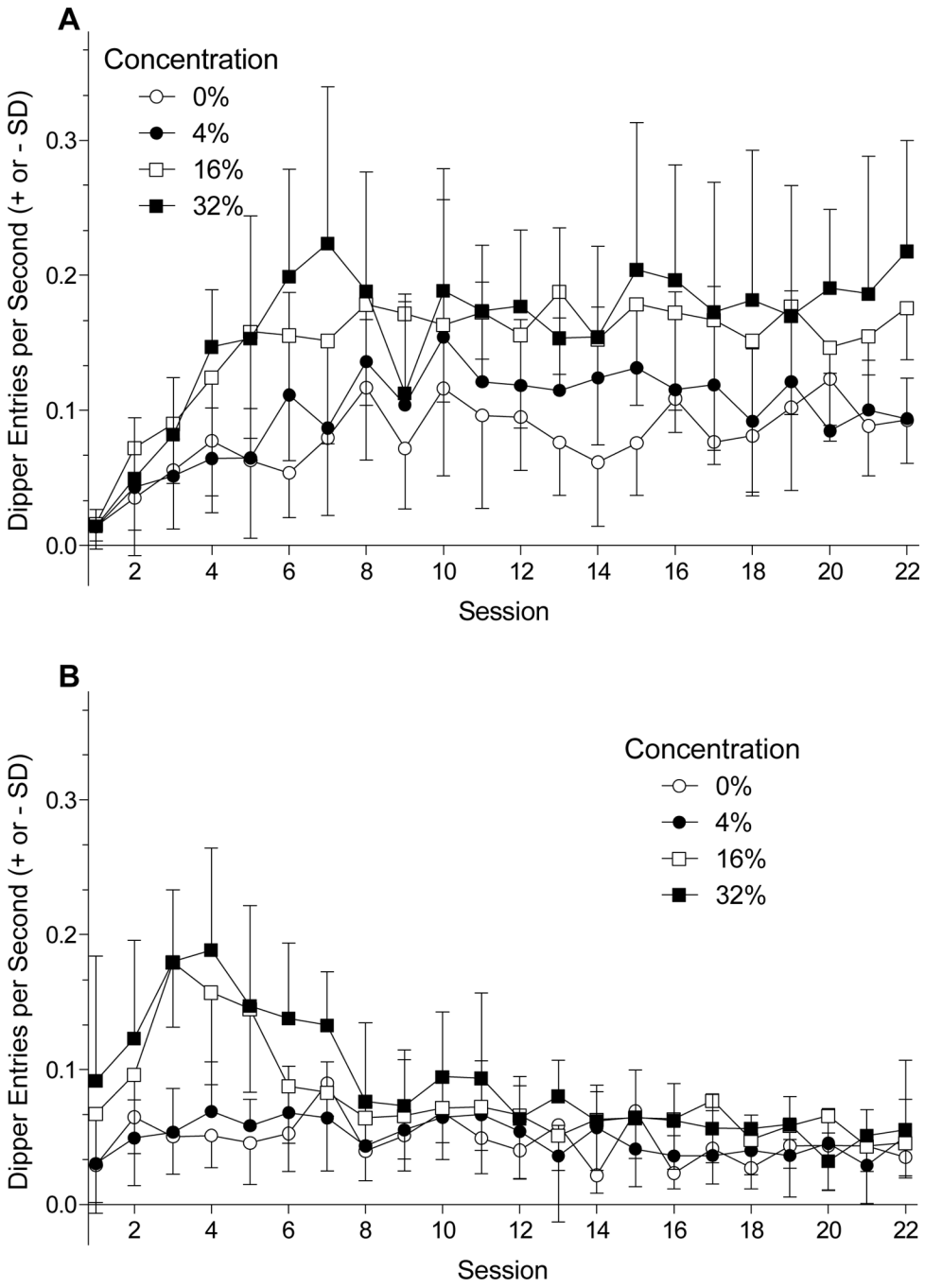

Figure 2.

Panel A shows dipper entries per second (+ or − SD) on nicotine sessions for each of the four sucrose concentrations. Panel B shows dipper entries per second (+ or − SD) on saline sessions for each of the four sucrose concentrations. Significant effects are described in the text.