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The Importance of Acquisition Learning on Nicotine and Varenicline Drug Substitution in a Drug-Discriminated Goal-Tracking Task

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Abstract

Nicotine and varenicline (Chantix®; the leading non-nicotine cessation pharmacotherapy) can come to control appetitive behaviors such as goal-tracking. We tested rats (N = 48) in a drug-discriminated goal-tracking (DGT) task where each rat received daily subcutaneous injections of either nicotine (0.4 mg/kg) or saline (0.9% [w/v]) interspersed across the acquisition phase (Phase 1). On saline days, sucrose was intermittently available. On nicotine days, sucrose was withheld. All rats acquired the discrimination with increased goal-tracking rates on saline days relative to nicotine days. Following acquisition, rats were separated into four groups to assess drug-substitution and discrimination reversal in Phase 2. The first group maintained the stimulus-reinforcer relation from acquisition (NIC−). The reversal group was now given access to sucrose on nicotine days (NIC+). The substitution group replaced nicotine with varenicline (1 mg/kg) while maintaining the acquisition stimulus-reinforcer relation (VAR−). The substitution and reversal group had nicotine replaced by varenicline and the stimulus-reinforcer relation reversed (VAR+). Rats in all groups learned or maintained their Phase 1 discriminations. For Phase 2, the reversal groups (+ conditions) acquired their discriminations within 10 sessions. The VAR−group displayed a pattern of disrupted discrimination at the outset of Phase 2 but was reestablished after continued training. In substitution testing, VAR groups received nicotine and NIC groups received varenicline. The NIC−and VAR−groups displayed full substitution of the test stimulus whereas the NIC+ and VAR+ groups displayed partial substitution of the test stimulus. Rats underwent nicotine extinction in Phase 3. Initial responding for each group mimicked Phase 2 training (i.e., higher responding by the reversal groups). All rats maintained similarly low levels of responding after six sessions. In conclusion, initial learning history with nicotine (i.e., + or −) influenced drug-stimulus substitution and the rate at which new learning (e.g., reversal) occurs with the varenicline and nicotine interoceptive stimuli.

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Introduction

Tobacco-related illness remains the leading cause of preventable death worldwide with roughly 7 million deaths occurring annually and no signs of decreasing (General & Services, 2004; Kaisar, Prasad, Liles, & Cucullo, 2016; World Health Organization, 2014). Such abuse liability would suggest that nicotine is a potent primary reinforcer. However, nicotine as a primary reinforcer has been shown to be relatively weak in both humans and non-human animals (Caggiula et al., 2009; Chaudhri et al., 2007; Le Foll & Goldberg, 2006). If so, then other factors likely influence nicotine use and addiction. For example, nicotine has interoceptive stimulus effects that can come to control appetitive behaviors in rats when it is reliably paired with sucrose (Bevins et al., 2012; Bevins & Besheer, 2014; Thompson, Barrett, & Bevins, 2019). This observation indicates that the experience one has while under the subjective effects of a drug can alter the later behavior evoked by that drug. Given the potential importance of interoceptive conditioning to understanding nicotine use, basic research designed to further understand factors influencing interoceptive conditioning and drug stimuli is important to setting a strong foundation for future inquiry on novel prevention and intervention approaches (cf. Bevins & Besheer, 2014).

Varenicline (Chantix™) is the leading non-nicotine smoking cessation drug approved by the US Food and Drug Administration (Cahill et al., 2013; Le Foll & Goldberg, 2006). Varenicline has discriminative stimulus-effects that are nicotine-like in rodents (Thompson et al., 2019; Wooters, Bevins, & Bardo, 2009). For example, in a two-operandi (e.g., lever) drug-discrimination task, responding on one of the two levers is reinforced when nicotine is administered; responding on the other lever is not reinforced (i.e., extinction). On intermixed saline sessions, the other response-reinforcer contingency was in place. Following development of a stable discrimination, when varenicline replaced nicotine on a test day, the varenicline stimulus evoked nicotine-appropriate responding that was comparable to nicotine (referred to as full substitution; Smith et al., 2007) or below that of nicotine but still greater than saline (termed partial substitution; LeSage, Shelley, Ross, Carroll, & Corrigall, 2009). Similarly, the drug discriminated goal-tracking (DGT) task allows for comparisons to be made on drug-evoked conditioned responding. Typically, in this task, nicotine is paired with intermittent access to sucrose in a dipper receptacle. On the interspersed saline days, sucrose is withheld. Here, nicotine comes to differentially control appetitive reward-seeking or, more specifically, a conditioned response oriented towards the stimuli paired previously with this reinforcer (i.e., the dipper receptacle in our case; Charntikov, Falco, Fink, Dwoskin, & Bevins, 2017; Wooters et al., 2009). This form of conditioned response is referred to as goal-tracking (Boakes, 1977; Farwell & Ayres, 1979). In the DGT task, varenicline at 0.3, 1, and 3 mg/kg evokes goal-tracking comparable to nicotine in brief extinction tests (Reichel, Murray, Barr, & Bevins, 2010).

In a recent DGT study, we trained the discrimination just described with 0.4 mg/kg nicotine. After goal-tracking evoked by the nicotine stimulus stabilized, we replaced nicotine with 1 mg/kg varenicline in the reinforced (positive) sessions; sucrose remained unavailable on saline sessions. In this condition, varenicline fully substituted for nicotine as demonstrated by uninterrupted stable discrimination performance [see Figure 3B of Thompson et al. (2019)]. From the perspective of this report, there were two specific groups of interest in this

earlier study. The first group had the reinforcer relation reversed from the acquisition phase such that saline now predicted access to sucrose; on nicotine days sucrose was withheld (so called NIC−group). Rats in the other group received varenicline substitution in addition to the reversal of the stimulus-reinforcer relation. That is, 1 mg/kg varenicline now signaled the absence of sucrose (VAR−). Regardless of drug stimulus, discrimination performance was disrupted, and an extensive amount of training was necessary before the discrimination between saline and drug was reestablished (i.e., 46 or more training sessions).

In the present study, we extended the work by Thompson et al. (2019) by investigating whether the patterns of substitution and reversal learning were similar when the initial discrimination training started with nicotine signaling the *absence* of the sucrose; intermittent access to sucrose occurred on saline sessions (cf. NIC−group described earlier). As in the Thompson et al. study, once discrimination performance stabilized, rats were separated into four groups. One group continued with NIC−training while another group had 1 mg/kg varenicline replace the nicotine as the drug stimulus (VAR−). The two remaining groups paralleled these groups except the stimulus-reinforcer relation was switched (i.e., NIC+ or VAR+).

Materials and Methods

Animals

Adult male Sprague-Dawley rats ($N = 48$) weighing between 277–306 g upon arrival from Envigo (Indianapolis, IN) were individually housed in polycarbonate tubs (48.3 × 26.7 × 20.3 cm; l × w × h) lined with wood-pulp bedding (Envigo, TEK-Fresh®). The colony was in a temperature- and humidity-controlled room maintained on a 12-hour light/dark cycle (on at 6 AM). All procedures were conducted during the light cycle. Rats were handled daily for 2 min 3 days after arrival, during which time food was available *ad libitum* (Envigo, Teklad Global Diets®). On the fourth day, the food was removed and the rats were maintained at 85% their free-feed weights via post-session feeding. The rats' weights were increased by 2 g each month to account for natural weight gain. All protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Apparatus

All sessions were conducted in a single testing room with ten conditioning chambers (ENV-008CT, Med Associates, Inc., St. Albans, VT, USA; 30.5 × 24.1 × 21.0 cm; l × w × h). Each chamber was housed separately inside a sound- and light-attenuating cubicle. The cubicle was equipped with a fan to increase air circulation and further decrease external noise. Each chamber had two aluminum side walls, polycarbonate ceiling, front and back walls, and metal rod flooring. Liquid trays containing 26% sucrose (w/v) solution were placed beneath an automated dipper arm on the right side-wall of the chamber. The arm was programmed to raise and lower a 0.1-ml cup that was accessible in the recessed port (5.2 × 5.2 × 3.8 cm; l × w × d). An emitter/detector beam fixed 1.2 cm within the recessed port and 3 cm above the metal rod floor recorded head entries.

Drugs

(−)−Nicotine hydrogen tartrate salt (0.4 mg/mL; MP Biomedicals LLC, Santa Ana, CA, USA) was dissolved in 0.9% saline and brought up to a pH of 7.0 ± 0.2 using NaOH (dose expressed as base). Varenicline tartrate (1 mg/mL; generously provided by NIDA [RTI, Research Triangle Park, NC, USA]) was dissolved in 0.9% saline (dose expressed as salt). All solutions were subcutaneously injected at 1 ml/kg. The injection-to-placement intervals (IPIs) were 5 min for nicotine and saline, and 15 min for varenicline. Doses and IPIs used in the present study were based on our previously published work (Barrett, Geary, Steiner, & Bevins, 2018; Bevins et al., 2012; Pittenger, Zeplin, Dwoskin, & Bevins, 2015; Thompson et al., 2019).

Acquisition

Figure 1 provides a graphical representation of the timeline of all experimental protocols. Before the acquisition phase, all rats received daily nicotine injections for three days to diminish the early aversive/suppressant effects of nicotine (cf. Bevins, Besheer, & Pickett, 2001; Charntikov et al., 2017). On day four, rats began training in a drug-discriminated goal-tracking (DGT) task. For this training, the rats received either an injection of nicotine (0.4 mg/kg) or saline (0.9% NaCl [w/v]). A unique order of injections was given to each rat with the restriction that no more than two nicotine or two saline sessions occurred on consecutive days. On saline days, 36 response-independent presentations of sucrose (4-sec limited hold) were available intermittently across the 20-min session. Four different programs determined the timing for access to sucrose deliveries, with the first delivery varying between 124 to 152 sec after session initiation, with an average of 25 sec (range = 4 to 80 sec) between following sucrose presentations. On nicotine days, sucrose presentations were withheld. Acquisition training lasted for 16 nicotine and 16 saline sessions (i.e., 32 days).

Second Training Phase

On day 33, rats were randomly assigned to one of four groups with each group trained on a different stimulus-reinforcer relation. The NIC−group (n = 12) continued to receive sucrose only on saline days. The NIC+ group (n = 13) now received sucrose only on nicotine days and never on saline days (i.e., reversal). In the VAR−group (n = 12), varenicline was substituted for nicotine but the stimulus-reinforcer relation from acquisition was maintained; sucrose was given on saline days and was withheld on varenicline days. Likewise, in the VAR+ group (n = 11), varenicline replaced nicotine but the stimulus-reinforcer relation was reversed such that sucrose was now available only on varenicline days. To control for the difference in nicotine and varenicline IPIs, each rat received two injections daily before session initiation: one at the 15-min IPI and another at the 5-min IPI. Rats in the NIC−and NIC+ groups always received saline at the 15-min IPI, and either nicotine or saline at the 5-min IPI. Likewise, rats in the VAR−and VAR+ groups always received saline at the 5-min IPI and received either varenicline or saline at the 15-min IPI. An error in injection protocol and labelling was discovered by a laboratory technician after this phase had begun. This error resulted in one rat receiving nicotine instead of varenicline from the outset of this phase on the drug-injection days. That rat was reassigned to the NIC+ group resulting in uneven sample sizes. A mixed-measures analysis of variance (ANOVA;

Group*Drug*Session) was conducted on dipper entry rates to ensure that randomized group assignment did not unintentionally produce group differences at the start of this phase (see Results). Training in this phase lasted for 70 days not including intervening stimulus substitution testing days (i.e., the 2 brief test days detailed in the following section).

Stimulus Substitution Testing

Nicotine or varenicline substitution tests were conducted following sessions 24 and 26 — representing two determinations of substitution responding — separated by four re-training days that included: two drug and two saline sessions. On test days, rats received an injection of the drug that was not experienced during the second phase protocol. Specifically, varenicline for NIC groups and nicotine for VAR groups. Testing was conducted using a brief 140-sec session in which sucrose was not available. Test sessions were kept short to reduce the likelihood of learning about non-reinforcement. The aforementioned two-injection protocol (15 and 5 min IPI) also applied to test days. Training data from sessions 24 and 26 were combined and used as the baseline level of goal-tracking against which to compare responding in the substitution tests. Following the last test, rats continued the second phase training procedures for 9 additional sessions to ensure stable performance before moving to the nicotine extinction phase.

Nicotine Extinction Phase

In extinction, all rats, regardless of group, received a single injection of nicotine 5 min before placement in the chamber. Sucrose was in the dipper trough, but it was never available. Extinction training was conducted daily for fifteen 20-min sessions.

Dependent Measures & Data Analyses

The primary dependent measure of conditioned responding in each phase was dipper entries per second before the first sucrose delivery. This measure was calculated as total head entries made into the dipper receptacle before the initial sucrose delivery divided by the time in sec before that delivery or a matched time bin for non-reinforced sessions. This measure avoids the influence that sucrose presentations have on responding later in the session and thus serves as a conservative measure of learning regarding the drug stimulus. We also used the dipper entry rate measure for substitution testing (dividing the total number of head entries into the dipper receptacle by the 140-sec session time) and extinction (here we divide total head entries by the matched time bins from the acquisition and training phases [i.e., the time before the first sucrose delivery on a given plus session; these variable times average 140-sec across all sessions]). We used R version 3.6.0 (R Development Core Team, 2018) to conduct mixed-measures ANOVAs using within-subject factors of Drug and Session and the between-subject factor of Group. Pairwise comparisons on significant main effects and interactions were conducted using the Holm method for correcting multiple comparisons (Holm, 1979). Significance for all analyses was set at $p < 0.05$.

Results

Acquisition

Figure 2 shows the acquisition of the saline versus nicotine discrimination, where sucrose was available on saline days but withheld on interspersed nicotine days (NIC−). For the three-way mixed-measures ANOVA, there were main effects of Drug (i.e., nicotine or saline; [$F(1,44) = 130$, $p < 0.01$], and Session [$F(15,660) = 33.8$, $p < 0.01$] as well as a Drug*Session interaction [$F(15,660) = 15.8$, $p < 0.01$]. All groups responded at similar rates in acquisition. There was no effect of Group ($F < 1$, $p = 0.73$), nor Group*Session [$F(3,44) = 1.13$, $p = 0.27$], Group*Drug ($F < 1$, $p = 0.80$), and Group*Drug*Session ($F < 1$, $p = 0.58$) interactions. In the post-hoc analyses for the Drug*Session interaction, there were no differences in responding for sessions 1 to 3 ($ps \geq 0.20$) but for sessions 4 to 16, rats responded higher on saline days than on nicotine days ($ps < 0.01$). In brief, rats discriminated the nicotine stimulus from saline by session 4 and the discrimination was maintained through session 16.

Second Training Phase

Figure 3A shows responding for the NIC−group. There was a main effect of Drug [$F(1,11) = 18.6$, $p < 0.01$] but not of Session ($F < 1$) nor an interaction [$F(34,374) = 1.06$, $p = 0.38$]. This pattern of responding was not surprising as the stimulus-reinforcer relation was the same from acquisition. No differences in responding were seen for sessions 2, 4, 7, and 13 ($ps \geq 0.09$), saline responding remained higher than that of nicotine after session 13 through the remainder of the phase.

Figure 3B shows responding for the NIC+ group. Recall that sucrose was now available on days when rats received nicotine (i.e., reversal learning). There were main effects of Drug [$F(1,12) = 40.9$, $p < 0.01$] and Session [$F(34,408) = 3.05$, $p < 0.01$] as well as an interaction [$F(34,408) = 8.35$, $p < 0.01$]. Early responding mirrored that of the NIC−discrimination as saline responding was higher than nicotine for session 1 ($p < 0.01$); no differences in responding for sessions 2 to 4 ($p \geq 0.09$), and reversed by session 5 and remained higher throughout this phase ($ps < 0.05$).

Figure 3C shows responding for the VAR−group. This group maintained the stimulus-reinforcer relation from acquisition but varenicline replaced nicotine in the non-reinforced sessions. There was a main effect of Drug (varenicline or saline; [$F(1,11) = 39.3$, $p < 0.01$] and a Drug*Session interaction [$F(34,374) = 4.00$, $p < 0.01$); there was no effect of Session ($F < 1$, $p = 0.73$). There were no differences in responding for sessions 1 to 5 ($ps \geq 0.07$) and session 10 ($p = 0.16$). Saline responding was higher than varenicline responding for all other sessions ($ps < 0.05$).

Figure 3D shows responding for the VAR+ group. This group had the stimulus-reinforcer relation reversed from acquisition and varenicline replaced nicotine as the drug stimulus. There was a main effect of Drug [$F(1,10) = 25.9$, $p < 0.01$], no effect of Session [$F(34,340) = 1.45$, $p = 0.06$], yet a Drug*Session interaction [$F(34,340) = 6.78$, $p < 0.01$]. There were no differences in responding for sessions 1 to 6, 8, and 9 ($ps \geq 0.06$). Varenicline evoked

responding was higher than saline by session 7 ($p = 0.02$) and the discrimination continued from sessions 10 to 35.

Stimulus Substitution Tests

Figure 4 displays the findings from substitution testing. To compare responding from the drug stimulus not experienced in the second phase training (i.e., varenicline for NIC groups and nicotine for VAR groups) to the training stimulus, we pooled second phase training data from the two sessions preceding test days one and two. A repeated-measures ANOVA was conducted on Drug to compare the varenicline-, nicotine-, and saline-evoked responding for each group, followed by pairwise comparisons to assess differences between the three stimuli. For the NIC− group, we found an effect of Drug [$F(2,58) = 10.8$, $p < 0.01$]. In this group, nicotine and varenicline evoked less responding than saline ($ps < 0.01$) and nicotine did not differ from varenicline ($p = 0.09$). This pattern of responding indicates that varenicline fully substitutes for the nicotine stimulus for this group. For the NIC+ group, there was an effect of Drug [$F(2,63) = 52.0$, $p < 0.01$]. Varenicline-evoked responding was lower than nicotine ($p < 0.01$) and higher than saline ($p < 0.01$). Further, nicotine-controlled responding was higher than saline ($p < 0.01$) indicating that varenicline only partially substitutes for nicotine in the NIC+ group. For the VAR-group, there was a main effect of Drug [$F(2,58) = 53.0$, $p < 0.01$] such that saline-evoked responding was higher than both varenicline and nicotine ($ps < 0.01$) but the two drug stimuli did not differ ($p = 0.70$), denoting full substitution of varenicline by nicotine. For the VAR+ group, there was a main effect of Drug [$F(2,53) = 30.3$, $p < 0.01$]. Varenicline responding for this group was the highest and differed from both nicotine and saline ($ps < 0.01$); nicotine also differed from saline ($p < 0.01$) denoting partial substitution of nicotine for the varenicline stimulus.

In sum, the two minus groups, NIC−and VAR-, showed full substitution of the training stimulus by the drug not experienced during the second phase; nicotine- and varenicline-evoked responding did not differ from each other and saline-evoked responding was higher than both drug stimuli. The positive groups showed partial substitution of the training stimulus. Nicotine- and varenicline-evoked responding differed from each other while both were higher than that of saline.

Nicotine Extinction

Rats in all four groups underwent extinction with nicotine (see Figure 5). We compared the dipper entries per sec from session 1 to 15 using a repeated-measures ANOVA with Session as a factor. For the NIC−group, there was no effect of Session ($F[14, 154] = 1.38$, $p = 0.17$) as expected given that this is a continuation of the nicotine training from the previous phase. For the NIC+ group, there was an effect of Session ($F[14,168] = 5.62$, $p < 0.01$) with pairwise comparisons indicating that session 1 was higher than sessions 2 and 3 ($ps \leq 0.05$). Session 4 responding did not quite meet significance criterion ($p = 0.07$) but lower responding for sessions 5 to 15 ($ps < 0.01$). The remaining sessions did not differ from each other after the first session. For the VAR−group there was no effect of Session ($F < 1$). There was an effect of Session for the VAR+ group ($F[14,140] = 2.46$, $p < 0.01$) with pairwise comparisons indicating session 1 responding was higher than sessions 3, 7, and 15 ($ps \leq 0.05$). The remaining sessions were not statistically different.

Discussion

Drug discrimination tasks allow *in vivo* comparisons of interoceptive drug effects and are designed to improve our understanding of the similarity or dissimilarity of these subjective effects to other drugs and/or doses (Bevins & Murray, 2011; de Moura & McMahon, 2017; Rosecrans, 1989). In the DGT task used here, the 0.4-mg/kg nicotine dose serves as an interoceptive stimulus when paired with access to sucrose. This effect is evidenced by nicotine-evoked conditioned responding (i.e., dipper entries) relative to the non-reinforced saline sessions (e.g. Charntikov et al., 2017; Charntikov, Tracy, Zhao, Li, & Bevins, 2012; Thompson et al., 2019). As shown in the present study, as well as in previously published studies (addressed below), the 0.4 mg/kg nicotine dose can also signal the absence of the reinforcer; saline (not nicotine) sessions indicate sucrose availability.

To our knowledge, acquisition with the NIC−discrimination in the DGT task is limited to only two other studies. The first demonstration was by Besheer et al. (2004). Similar to the present study, that work paired saline with sucrose in acquisition and withheld sucrose when rats were given 0.4 mg/kg nicotine. One notable difference in the training protocol was the number of sucrose deliveries per positive (SAL+) session; 36 in the present study versus 8 in Besheer et al. (2004). Regardless, this leaner schedule of sucrose delivery resulted in a discrimination with lower rates of nicotine-evoked responding relative to saline-evoked responding (see Figure 5A in Besheer et al., 2004). Of note, Besheer et al. (2004) took the withholding of dipper entries while on nicotine as strong evidence against any psychomotor stimulant account of the discrimination when sucrose was paired with nicotine (NIC+). We echo this conclusion and also highlight their finding that mecamylamine, a nicotinic acetylcholine receptor (nAChR) antagonist, blocked the effects of nicotine in NIC−trained rats (i.e., conditioned responding increased to saline levels). The second study by Murray et al. (2011) used the same training protocol as the present study. In this work, the NIC −training protocol was used to test a state-dependency account regarding the impact of MK-801, an *N*-methyl-*D*-aspartate (NMDA) receptor antagonist, on nicotine- versus saline-controlled responding. The present work replicates and extends this previous research by examining reversal learning as well as substitution of varenicline in the NIC-condition.

Multiple factors affect varenicline substitution for nicotine including learning histories, training drug and dose, and training protocol (LeSage et al., 2009; Levin et al., 2012; Matta et al., 2007; Reichel et al., 2010; Thompson et al., 2019). Keeping this point in mind, we chose the 1.0 mg/kg varenicline stimulus in this study so that we could compare the outcomes here with the reversal training and substitution testing of Thompson et al. (2019). Thompson et al. (2019) found that this dose is readily discriminated from saline when the training drug in the positive sessions was switched from 0.4 mg/kg nicotine to 1.0 mg/kg varenicline. Further, as we were preparing this manuscript for submission, we finished a discriminated goal tracking study that used for the first time 1 mg/kg varenicline as the initial training stimulus. Our typical training dose of nicotine 0.4 mg/kg substituted fully for varenicline; mean total varenicline dipper entries = 70.2 (±12.36 SEM) versus nicotine = 54.3 (±10.78). A paired t-test indicates no difference in total dipper entry during the tests [$t(9) = 0.918$; $p = 0.383$].

In the present study, the first session of the second phase for the VAR−group can be considered a varenicline substitution test for the nicotine stimulus because the reinforcer-relation parallels acquisition. Here, the varenicline stimulus did not substitute for nicotine as varenicline-evoked responding did not differ from saline on Session 1 (see Figure 3C). Rats in the NIC−group did show the continued discrimination on Session 1. However, there was some variability in Sessions 2, 4, and 8 that weakened discrimination performance. The VAR−group, in contrast, did not display a discrimination until Session 7. Although the cause of variability in the NIC−group is unclear, the group assignment from the acquisition phase is an unlikely reason given that there were no statistical differences between the groups ($F < 1$, $p = 0.73$) entering this second phase training. One possible source of variability may be the change from one-daily to two-daily injections. Recall, one saline and one drug or saline injection were required to control for the different drug IPIs during the second phase. With that noted, the introduction of the two-injection protocol did not seem to disrupt performance in the Phase 2 switch of Thompson et al. (2019) and the full substitution of varenicline in the NIC+ group of that study replicates the full substitution seen in a comparable test using a single-injection protocol by Reichel et al. (2010).

Interestingly, the lack of early substitution by varenicline in the present study is in contrast to the Thompson et al. (2019) study. In that study, varenicline substituted for the nicotine stimulus in the first session of the second phase of training when varenicline replaced nicotine in the NIC+ condition(i.e., VAR+ Figure 3B in Thompson et al., 2019). Perhaps the reward-enhancement effects of nicotine and varenicline (cf. Barrett et al., 2018; Schassburger et al., 2015) biased substitution towards reinforcer-paired stimuli that share stimulus effects. Additionally, both sign- and goal-tracking is enhanced by exposure to nicotine as seen by the enhancement of Pavlovian conditioned approach in a study by Stringfield, Madayag, Boettiger, and Robinson (2019). This enhancement of Pavlovian conditioned approach with sucrose associated stimuli (cf. dipper receptacle in the present study) by nicotine help explain, in part, why shifting the contingency for the drug-reinforcer relation to the plus conditions (VAR+ and NIC+ in the present paper) acquired the reversed discrimination in fewer sessions, and appear more stable, than their counterparts NIC−and VAR−(i.e., reversal to the minus condition) in Thompson et al. (2019).

Recall that the reversal groups in this study went from having saline (no drug) sessions paired with sucrose to the drug session now signaling the availability of sucrose (NIC+ and VAR+; see Figure 3B and D). This is the first time such a reversal in stimulus-sucrose relation has been examined in the DGT task, though we refer the reader to Troisi (2013) which used nicotine- and saline-discriminative stimuli to signal an operant reward sequence which was later reversed. Surprisingly, rats in the reversal groups in the present report learned the discrimination within 10 days of training. The reason for the surprise is that the rapidity of the reversal is in stark contrast to the reversal learning work we recently reported in the Thompson et al. (2019) study. In that earlier study, we started DGT training with the usual protocol of nicotine paired with sucrose and saline indicating no sucrose. Thus, the reversal was to the VAR−and NIC−conditions. When the reversal was in this direction, rats that shifted from NIC+ to NIC−required 46 days of training before the discrimination remerged and stabilized. Further, the rats that shifted to the VAR−condition learned the

discrimination even slower, taking roughly 70 days of training (Figure 3D of Thompson et al. [2019]).

In Thompson et al. (2019), the reversal of the discrimination seemed to be driven as much by conditioned responding (i.e., goal-tracking) gradually increasing in the later saline sessions rather than responding extinguishing or decreasing in the now non-reinforced drug sessions. The pattern in the present study was quite different. Responding evoked on saline sessions quickly diminished as responding evoked by nicotine and varenicline quickly increased. We take this pattern to indicate that as reversal training continued, the interoceptive drug stimulus acquired an association with sucrose (cf. Pittenger & Bevins, 2013) whereas the control of responding by the context (i.e., chamber stimuli) decreased as a competing chamber – no sucrose association was acquired (i.e., extinction; Bouton & Brooks, 1993). The salience of the drug stimulus may increase the rate at which these reward-based associations are learned as outlined below.

Varenicline treatment in humans eases nicotine-withdrawal symptoms and craving (Coe et al., 2005; Garrison & Dugan, 2009). The efficacy of varenicline as a smoking cessation aid has been attributed to the similar pharmacological action on the α4β2 nAChRs (Cahill et al., 2013). Drugs with agonist action on the α4β2 receptors generalize to the nicotine discriminative stimulus better than other heteromeric receptor subtypes such as the α7 and α3β4 receptor subtypes (Smith et al., 2007; Reichel et al., 2010). As Thompson et al. (2019) pointed out, varenicline as a partial agonist may be a weaker, less-salient stimulus than nicotine given varenicline has a decreased dopaminergic effect compared to nicotine (Coe et al., 2005). Perhaps the 1 mg/kg varenicline stimulus overlaps with some, but not all, of the neuropharmacological elements that compose the 0.4 mg/kg interoceptive nicotine stimulus. This effect may explain why more training sessions were needed in the varenicline groups compared to the nicotine counterparts before the discrimination occurred as well as the partial substitution found in the NIC+ and VAR+ groups here. One area of future studies using varenicline and nicotine in the DGT task should focus on differences in training and reversal doses to determine the generality of the finding and their associated conclusions. The difference in substitution patterns between the present study and Thompson et al. (2019) indicate that both varenicline and nicotine are multidimensional stimuli. Both of which have interoceptive stimulus effects that are altered by previously learned associations.

Specifically, in the present study, we found that learning history altered the substitution-testing patterns of nicotine and varenicline. Recall the after Phase 2 training, varenicline fully substituted for nicotine in the NIC−group but only partially substituted for nicotine in the NIC+ group. During the second phase, the NIC−group required no new learning as the stimulus-reinforcer relation never changed. The NIC+ group, in contrast, had the reinforcer-relation switched. The same effect was found in the VAR−and VAR+ groups (see Figure 4). The VAR−group showed full substitution by nicotine for the varenicline stimulus. This substitution was only partial in the VAR+ group where the reinforcer-relation was reversed. If drug salience was the only factor under consideration for the substitution profile at hand, nicotine would have fully substitute for varenicline, and not vice versa. As described earlier, this pattern was, however, found by Thompson et al. (2019). That is, nicotine fully substituted for both varenicline groups, but varenicline only partially substituted for the

nicotine groups. Such a pattern led them to lean heavily on a stimulus/drug salience account for their substitution findings.

The present work highlights the importance of learning history as well in the substitution profiles. Future research on what associative conditioning factors affect the generalization pattern within a drug and substitution patterns across drugs will be of much interest (cf. Bevins & Palmatier, 2004; Bevins et al., 2012; Palmatier & Bevins, 2008; Reichel et al., 2010). Future work on interoceptive conditioning and the stimulus effects of nicotine, varenicline, and other drug stimuli should also assess sex as a biological variable. Nicotine has been found to increase Pavlovian conditioned approach in female rats more so than male rats (Stringfield et al., 2019) and menthol decreases sensitivity/perceptibility of the stimulus effects of nicotine in female rats using a Pavlovian positive occasion setting task with discriminated goal-tracking as the conditioned response (Huynh et al., 2020).

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Highlights

- Nicotine and varenicline stimuli guide rat's behavior in the DGT task
- The acquisition reinforcer-relation alters drug learning and substitution
- 1.0 mg/kg varenicline fully substitutes for NIC−trained rats
- 0.4 mg/kg nicotine fully substitutes for VAR−trained rats
- Reversal groups (NIC+ & VAR+) showed partial substitution of the testing stimulus

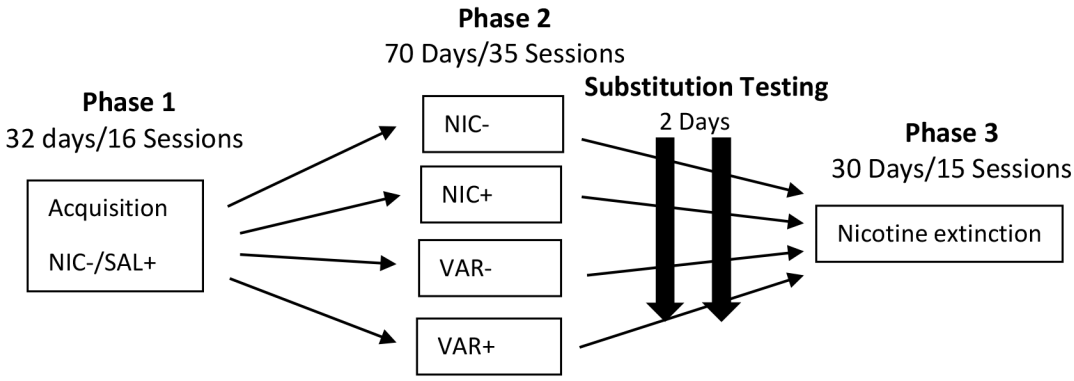

Figure 1.

Figure 1 shows the flow of the methods. In Phase 1, acquisition, nicotine (0.4 mg/kg) serves as an inhibitory interoceptive stimulus (NIC−). In Phase 2 the rats are separated into four groups. NIC−maintains the stimulus-reinforcer relation from Phase 1, NIC+ is the reversal group where nicotine is now an excitatory interoceptive stimulus, the varenicline-substitution group (VAR−) maintains the same stimulus-reinforcer relation from acquisition but varenicline (1 mg/kg) now indicates no sucrose, and VAR+ is the varenicline-substitution and reversal group. Substitution tests were conducted twice. Rats were given the drug that was not experienced during Phase 2 once after session 24 and again after session 26 followed by retraining until Phase 3 nicotine (0.4 mg/kg) extinction (figure adapted from Thompson, Barrett, & Bevins, 2019).

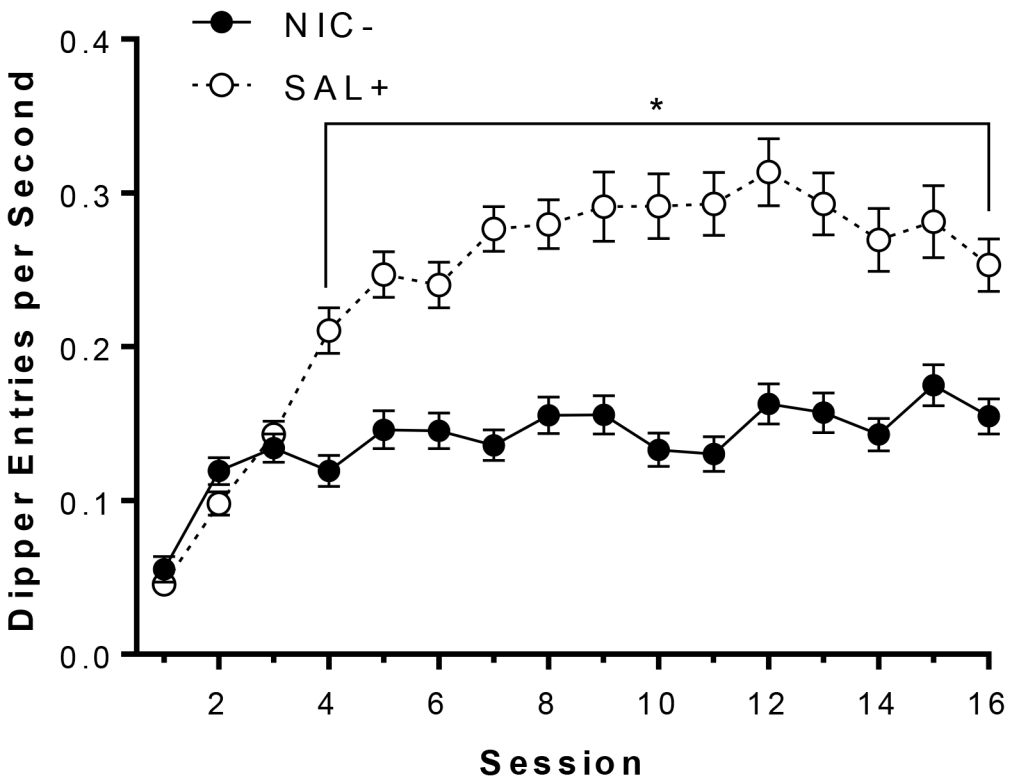

Figure 2.

Figure 2 displays data from the acquisition phase for nicotine versus saline evoked mean responding (+/−SEM). All 48 rats were trained with nicotine associated with the absence of the sucrose reinforcer. Discrimination between nicotine and saline began by session 4 and continued to session 16. Significance was set at $p < 0.05$.

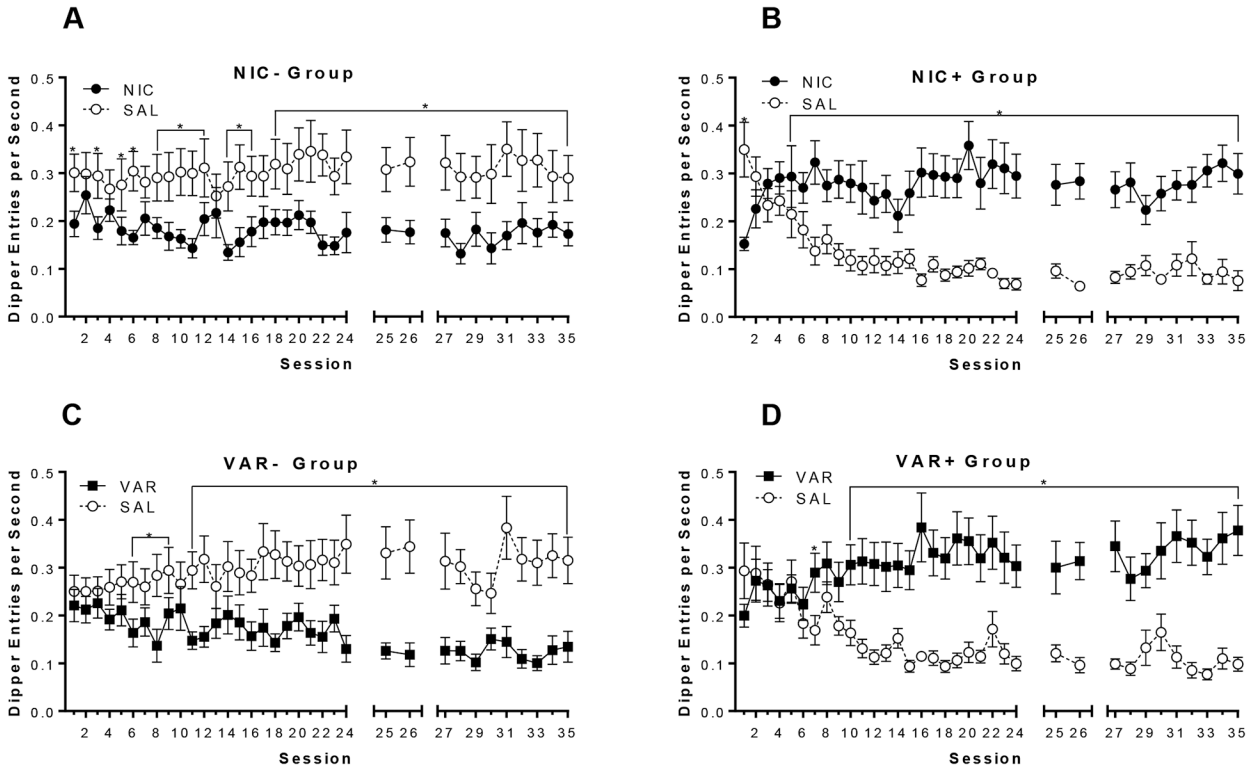

Figure 3.

Figure 3 displays the dipper entries per sec mean responding (+/−SEM) during the second phase training. Panels A and C display data from the NIC−and VAR−groups respectively, in which both groups maintained the stimulus-reinforcer relation from acquisition. Panels B and D display the reversal groups NIC+ and VAR+, respectively, in which rats now received access to sucrose on drug days. Gaps in the x-axis represent the two intervening substitution test days. Significance was set at $p < 0.05$.

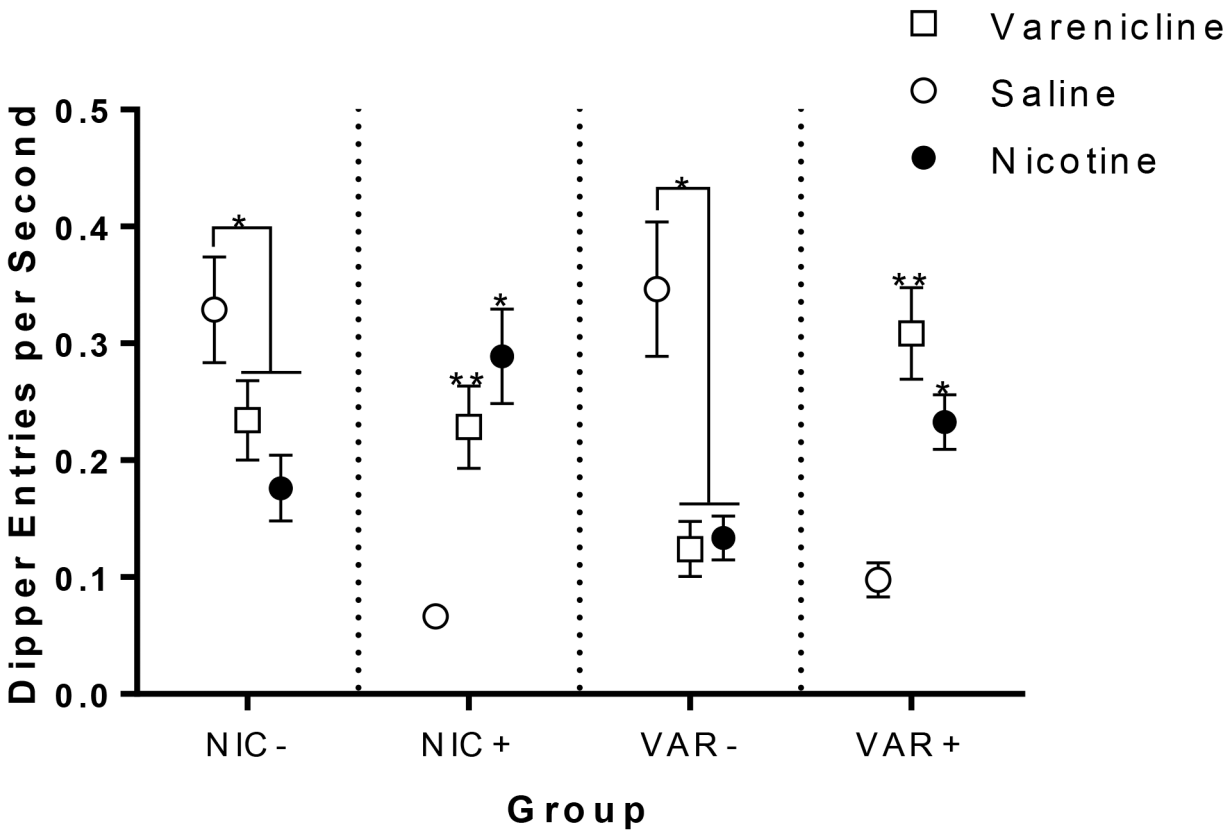

Figure 4.

Figure 4 shows the substitution test data representing mean responding (+/−SEM) for each drug. The rats in the NIC−group showed full substitution of the nicotine stimulus by varenicline. The VAR−group showed full substitution of varenicline by the nicotine stimulus. Rats in the NIC+ group only showed partial substitution of nicotine by the varenicline stimulus. Similarly, the VAR+ group showed partial substitution of varenicline by the nicotine stimulus. Significance was set at $p < 0.05$. An asterisk signifies a difference from saline, a double asterisk signifies differences from both saline and nicotine.

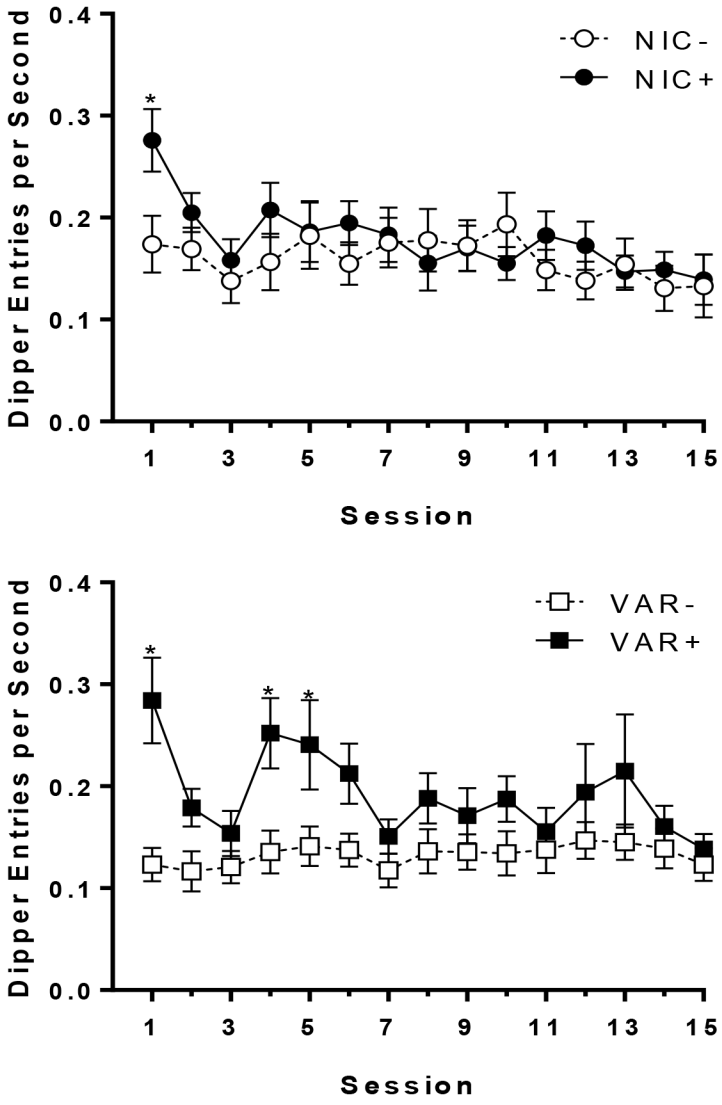

Figure 5.

Figure 5 displays the extinction phase mean responding (+/−SEM). Dipper entry per sec rates by session are shown for the NIC−and NIC+ groups in panel A and the VAR−and VAR + groups in panel B. Significant differences in responding by the plus groups early in this phase mimic that of the second phase training; by the end of this phase no differences remained. Significance was set at $p < 0.05$.