Pharmacology, Biochemistry and Behavior 181 (2019) 9–16

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Exploring the interoceptive stimulus effects of nicotine and varenicline

Brady M. Thompson, Scott T. Barrett, Rick A. Bevins*

Department of Psychology, University of Nebraska-Lincoln, Lincoln, NE, USA

ARTICLE INFO

Keywords:
Nicotine
Chantix®
Varenicline
Pavlovian conditioning
Reversal learning
Drug discrimination

ABSTRACT

Learning processes associated with nicotine influence the development of addiction to tobacco products. In the present report, we are interested in the interoceptive stimulus effects of nicotine acquiring control over appetitive behaviors – specifically, reward seeking. Also of interest is the current smoking cessation drug, varenicline (Chantix®). Varenicline, with its nicotine-like stimulus effects, can decrease withdrawal and cravings for a subset of individuals addicted to nicotine, though relapse is still common. We trained rats ($N = 48$) with nicotine (0.4 mg/kg, SC) as an excitatory stimulus (i.e., paired with sucrose) in a drug-discriminated goal-tracking (DGT) task. There was no access to sucrose on interspersed saline days. After acquisition of the initial nicotine-saline discrimination, rats were separated into four groups to test discrimination reversal and drug substitution. The control group maintained nicotine as the excitatory stimulus (NIC+). The substitution group had varenicline (1 mg/kg) replace nicotine as the stimulus paired with sucrose (VAR+). One reversal group had nicotine signal the absence of sucrose (i.e., now available on intermixed saline sessions; NIC−). The last group was similar to the NIC− group except varenicline replaced nicotine on non-reinforced sessions (VAR−). We found that varenicline fully substituted as the training stimulus when the drug-sucrose relation remained in place (VAR+). Both reversal groups acquired the new discrimination, albeit slowly and more variable for the VAR− group in comparison to NIC−. There was an effect of group during substitution testing. Specifically, nicotine fully substituted for varenicline regardless of condition. However, varenicline only partially substituted for the nicotine stimulus. At the start of extinction, responding mimicked that of the rats training condition. However, by extinction session 12, all groups maintained similarly low levels of responding. These findings show nicotine and varenicline share stimulus elements, yet the conclusion of partial to full substitution depends on the nature of the testing protocol.

1. Introduction

Tobacco use is responsible for nearly 6 million deaths per year globally; this rate is projected to reach up to 8 million deaths annually within the next 15 years (CDC, 2014; Faessel et al., 2010). Nicotine appears to be the primary addictive constituent in tobacco that leads to lasting brain and behavior changes, increasing the chances of dependence. Since the 1960s, modifications in policy and increased education on the costs of chronic smoking have helped decrease initiation and smoking rates in the US, dropping from over 45% to around 15% (CDC, 2014; Volkow and Wise, 2005; World Health Organization, 2010). Unfortunately, this means that roughly 50 million people are still at risk for serious health complications associated with chronic tobacco use in the United States alone.

The focus of the present research is on the behavioral and pharmacological processes that contribute to nicotine use, specifically, the interoceptive conditioning processes associated with nicotine-paired rewards. The interoceptive stimulus effects of nicotine have been shown to readily guide and increase appetitive-reward seeking behavior in human and non-human animals (Bevins and Besheer, 2014; Charntikov et al., 2017; Perkins et al., 2016). Our laboratory has used the drug discriminated goal-tracking (DGT) task in rats to study interoceptive conditioning with the nicotine stimulus (Besheer et al., 2004; Palmatier et al., 2004; Pittenger et al., 2015). Briefly, rats receive a daily injection of either nicotine or saline. When given nicotine, rats have access to sucrose in a conditioning chamber via a dipper receptacle. When given saline, rats do not have access to sucrose while in the chamber. Discrimination learning is expressed as the nicotine stimulus evoking an increase in goal-tracking behavior relative to saline. Goal-tracking is defined as an associative learning based search for a reward in the area it was presented in the past (cf. Boakes, 1977; Farwell and Ayres, 1979; in our case, the dipper receptacle). Critically, conditioned responding in this task does not reflect state-dependent learning (for more on this topic see: Bevins et al., 2007; Murray et al., 2011).

* Corresponding author at: Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE, USA.
E-mail address: rbevins1@unl.edu (R.A. Bevins).

https://doi.org/10.1016/j.pbb.2019.04.001
Received 14 January 2019; Received in revised form 1 April 2019; Accepted 2 April 2019
Available online 04 April 2019
0091-3057/

Varenicline (Chantix®) is a pharmacotherapy that has increased the likelihood of successful quit attempts for tobacco users, in part, due to its pharmacological similarity to nicotine (Cahill et al., 2010). Tobacco users who wish to maintain abstinence during a serious quit attempt may find that it alleviates craving for nicotine (Cahill et al., 2013; Lange-Asschenfeldt et al., 2016). Varenicline acts as a partial agonist on the α4β2-containing nicotinic acetylcholine receptors (nAChRs) which may account for its increased efficacy when compared to less nicotine-like pharmacotherapies such as bupropion (Zyban®; Potts and Garwood, 2007). Preclinical studies have shown that varenicline has nicotine-like effects. For example, LeSage et al. (2009) found that after training a nicotine (0.4 mg/kg) drug discrimination in a two-lever operant conditioning task, a range of varenicline doses (0.3, 1.0, 3.0 mg/kg) partially substituted for nicotine as evidenced by some responding on the nicotine-associated lever. In contrast, Jutkiewicz et al. (2011) reported that varenicline 1.0 mg/kg fully substituted for nicotine (0.32 mg/kg) in rats trained on a two-nose poke aperture drug-discrimination task. The difference between these two studies suggests that training/testing procedures may influence the substitutability of varenicline for nicotine. Notably, testing with higher doses of varenicline in the nose-poke study, 1.78 mg/kg and 3.2 mg/kg, decreased nicotine-like responding in this study (Jutkiewicz et al., 2011). In the DGT task with a standard substitution protocol, the 1.0 and 3.0 mg/kg varenicline dose fully substituted for a 0.4 mg/kg nicotine stimulus (Reichel et al., 2010). Given these findings, we selected the 1.0 mg/kg varenicline dose for the present study. Although the 3.0 mg/kg dose fully substituted for nicotine in the DGT task, we decided against this dose given the reduced substitution reported by Jutkiewicz et al. (2011) and the non-specific motor impairment reported by us (Reichel et al., 2010) with repeated treatment.

In the present study, two overarching questions guided our experimental design. First, does varenicline substitute as a training drug in the DGT task after rats have learned to discriminate nicotine from saline? Second, how sensitive will the goal-tracking behavior be to a sudden reversal of the discrimination (i.e., reinforcement now given only on saline days)? Reversal learning has been shown using operant-trained behaviors such as key pecks (Ward et al., 2008) and lever presses (Amodeo et al., 2016; McDannald et al., 2015; Ward et al., 2008). Pavlovian-reversal learning studies have also been conducted using exteroceptive stimuli (Bouton and Brooks, 1993; Chudasama and Robbins, 2003) and interoceptive conditioned responses such as heart rate (Tighe et al., 1968). To our knowledge the only example of reversal learning within a discrimination task using an interoceptive nicotine stimulus was reported by Troisi (2013). That study used a reversal of an operant-chain in a discrimination task. While this interesting study is quite different from the one reported herein, it does show that the nicotine stimulus was sensitive to a shift in reinforcers relations; for more detail see Troisi (2013). The present research extends the observation of reversal learning to the DGT task. Further, we determined whether the leading non-nicotine pharmacotherapy for smoking cessation, varenicline, substitutes for the nicotine stimulus in the reversal phase. Adding this drug-substitution component to the reversal learning in the DGT has extended our understanding of the stimulus effects of both nicotine and varenicline.

2. Materials and methods

2.1. Animals

Forty-eight male Sprague-Dawley rats obtained from Envigo (Indianapolis, IN) were individually housed in clear polycarbonate tubs (48.3 × 26.7 × 20.3 cm; l × w × h) lined with Envigo TEK-Fresh shavings in a temperature- (68 ± 5° F) and humidity- (30–70%) controlled room. The rats had free access to water and food (Envigo, Teklad Global Diets®, Indianapolis, IN) for three days after arrival; during this time, they were handled 2 min per day. After the third day, food was restricted to maintain rats at 85% of free-feeding weight (mean ± SD = 316 ± 18.1 g). For each month of the study, we increased each rat's controlled weight (mean ± SD = 269 ± 15.3 g) by 2 g to allow for weight gain while still under the food restriction. The colony was on a 12-h light/dark cycle with experimental sessions conducted during the light period. The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all protocols.

2.2. Apparatus

Med Associates conditioning chambers (ENV-008CT, St. Albans, VT, USA) measuring 30.5 × 24.1 × 21.0 cm (l × w × h) were used. Each chamber had two aluminum sidewalls; the ceiling, front, and back walls were made of clear polycarbonate. Each chamber was equipped with a 0.1-ml cup attached to a dipper arm. The raised dipper arm delivered a 26% sucrose solution (w/v) in a recessed port measuring 5.2 × 5.2 × 3.8 cm (l × w × d) located in the bottom center of the right sidewall. An emitter/detector beam used to measure head entries was located 1.2 cm within the dipper port and 3 cm above the metal rod floor. Each chamber was located in a sound-attenuating cubicle equipped with a fan to diminish outside noise and enhance airflow.

2.3. Drugs

(−)-Nicotine hydrogen tartrate (MP Biomedicals LLC, Santa Ana, CA, USA) and varenicline tartrate (generously provided by NIDA [RTI, Research Triangle Park, NC, USA]) were dissolved in 0.9% saline. Nicotine was brought to a pH of 7.0 ± 0.2 with a dilute sodium hydroxide solution. All solutions were administered subcutaneously (SC) at 1 ml/kg. The varenicline dose is reported by salt weight and nicotine by its base weight. Doses and injection-to-placement-intervals (IPI) were based on our past DGT work (Besheer et al., 2004; Murray et al., 2011; Polewan et al., 2013; Reichel et al., 2010). The nicotine-training dose was 0.4 mg/kg. This dose is a very common dose in the drug discrimination literature (cf. Wooters et al., 2009) and it produced robust discrimination performance in the DGT task (see later). For reasons already described in the Introduction, we selected 1.0 mg/kg varenicline for the nicotine-substitution dose.

2.4. Acquisition

Rats were given a SC injection of nicotine (0.4 mg/kg) in their home cage once a day for 3 days before the start of the experiment to diminish the initial locomotor suppressant effects of nicotine (Bevins et al., 2001; Charntikov et al., 2017). All rats received 32 consecutive days of discrimination training. Nicotine or saline was injected 5 min before a 20-min session. On nicotine sessions, rats had intermittent access to 36 sucrose reinforcers, each presented for 4 s. Four separate programs controlled the time of the first sucrose delivery which occurred on average 140 s after the start of the program (range: 124–152 s). The average time between all subsequent sucrose deliveries was 25 s (range: 4–80 s). Sucrose was withheld on saline sessions. No more than two of the same session type (nicotine vs. saline) occurred on consecutive days. Each rat received a unique order of nicotine and saline sessions.

2.5. Second training phase

For the second training phase, rats were assigned pseudo-randomly to one of four groups and then a two-way repeated measures analysis of variance (ANOVA) on Group and Session was conducted to ensure that there were no group differences in responding during acquisition training ($ps \geq 0.05$). Group NIC+ ($n = 12$) followed the same protocol as the acquisition phase. NIC− ($n = 12$) underwent reversal learning in which interspersed sucrose was now available on saline sessions and withheld on nicotine sessions. VAR+ ($n = 12$) was similar to the initial acquisition phase except varenicline was administered in place of

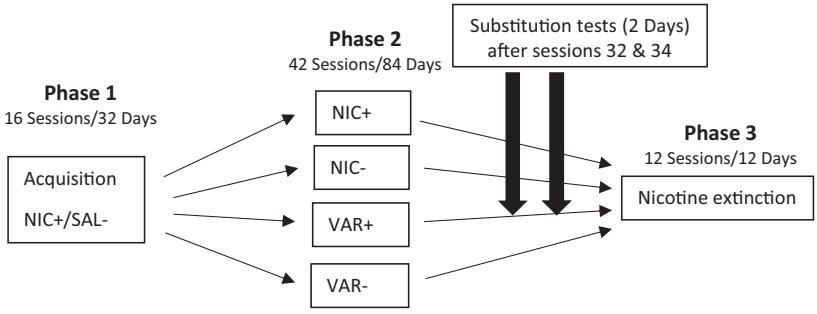

Fig. 1. The flow of the experimental protocol. Phase 1 represents the acquisition of nicotine (0.4 mg/kg) as an excitatory interoceptive stimulus. Phase 2 includes the group maintaining the sucrose-reinforcer relation from Phase 1 (NIC+), the reversal group (NIC−), varenicline-substitution (1.0 mg/kg) group (VAR+), and the reversal and drug-substitution group (VAR−). Regardless of group, all rats underwent two stimulus-substitution tests with the drug not given during Phase 2. After which, all rats underwent Phase 2 retraining before Phase 3 extinction with nicotine (0.4 mg/kg).

nicotine. VAR− (n = 12) was similar to the NIC− group in which sucrose was available on saline sessions but withheld on varenicline sessions. The IPI for varenicline was 15 min, while nicotine injections remained at an IPI of 5 min. In order to control for differences in IPIs, we injected saline at the IPI time of the drug not given during the second training phase. For example, a rat in the VAR+ group received two saline injections on a saline test day, the first given 15 min before chamber placement and the second 5 min before placement. On varenicline sessions, the first injection was varenicline and the second was saline. Interspersed session types and sucrose presentations were as described in the acquisition phase. Testing began after 32 sessions of each type (drug vs. saline). Retraining occurred between test days and before the extinction phase (see Fig. 1 for a methods flow chart indicating session and days per each phase).

2.6. Substitution test days

To determine if there were similarities in the two drug stimuli regardless of learning history, we tested rats twice with the drug that was not given during the second training phase. The first of the two test days occurred 24 h after session 32 of the second training phase (see Fig. 1). Rats that received nicotine during the second training phase were tested in a 140-s substitution test with varenicline, or vice versa. The two-injection protocol from the second training phase was followed here. Sucrose was in the dipper wells but was never available. After the first test, re-training occurred to ensure responding remained stable. The second test occurred 24 h after session 34. We conducted two tests to determine if the substitution pattern was replicable. We declared full substitution if responding was statistically similar between the drugs, but different from saline. Partial substitution was declared when responding evoked by the drugs differed from each other and saline.

2.7. Nicotine extinction

Following the tests, rats underwent retraining so that responding reached pre-test baseline levels. They then started the extinction phase. All rats received nicotine 5 min before the start of a 20-min extinction session. In order to keep contextual cues the same, sucrose was in the dipper well but was never available across the 12 extinction sections.

2.8. Dependent measures

The primary dependent measure for acquisition and the second training phase was dipper entries per sec (Figs. 2A and 3). The per sec measure was calculated by taking the total head entries made before the first sucrose delivery and dividing by the time in seconds before that delivery occurred. Using the dipper entries before the first delivery avoids the influence of sucrose on this measure of learning. Sucrose-free sessions used a matched time bin for calculating head entries per sec. For comparison purposes, we used the per-second measure across the 140-s substitution tests even though sucrose was not available. As detailed later, we also collected and analyzed total dipper entries in the 20-min sessions (see Fig. 4).

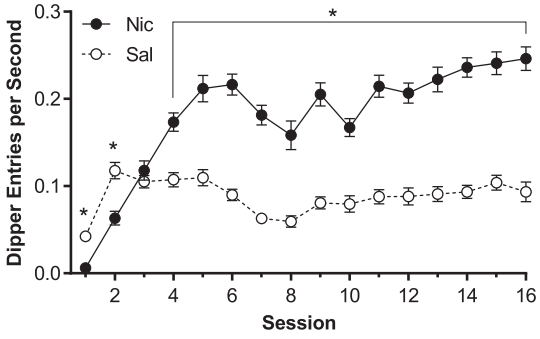

Fig. 2. The acquisition data for all 48 rats combined. The saline stimulus evoked responding significantly higher than that of nicotine for sessions 1 and 2. Rats acquired the nicotine-stimulus discrimination by session 4 and persisted through session 16. Asterisks indicate $p < 0.05$.

2.9. Data analysis

For analyses, we conducted two-way repeated measures ANOVAs using within-subjects factors of Drug (nicotine, varenicline, and saline) and Session. Additional post-hoc pairwise comparisons were conducted when significant interactions occurred; significance was set at $p < 0.05$. For statistical analyses, we used R version 3.3.2 (R Development Core Team, 2016).

3. Results

3.1. Acquisition

Fig. 2 shows the results for the acquisition phase in which all rats had nicotine as the interoceptive stimulus paired with sucrose (NIC+). For the two-way repeated measures ANOVA, there were main effects of Drug [$F(1,47) = 315$, $p < 0.01$], Session [$F(15,705) = 31.3$; $p < 0.01$], and a significant Drug * Session interaction [$F(15,705) = 23.0$, $p < 0.01$]. Nicotine suppressed responding in the first and second sessions compared to saline ($ps < 0.02$). By the fourth session, all rats acquired the discrimination, showing significantly higher rates of dipper entries on nicotine days compared to saline days ($ps < 0.01$). For total dipper entry data in acquisition, see Supplemental Fig. 1.

3.2. Second training phase

Fig. 3A shows dipper entries per sec for the NIC+ group during the second training phase. There were main effects of Drug [$F(1,11) = 85.6$, $p < 0.01$] and Session [$F(41,451) = 3.07$, $p < 0.01$], and a significant Drug * Session interaction [$F(41,451) = 1.64$, $p < 0.01$]. Dipper entries on nicotine days were higher than saline days for all sessions ($ps < 0.01$). Fig. 3B shows dipper entries per sec for the VAR+ group. There were main effects of Drug [$F(1,11) = 50.8$, $p < 0.01$] and Session [$F(41,451) = 1.72$, $p < 0.01$], and a significant

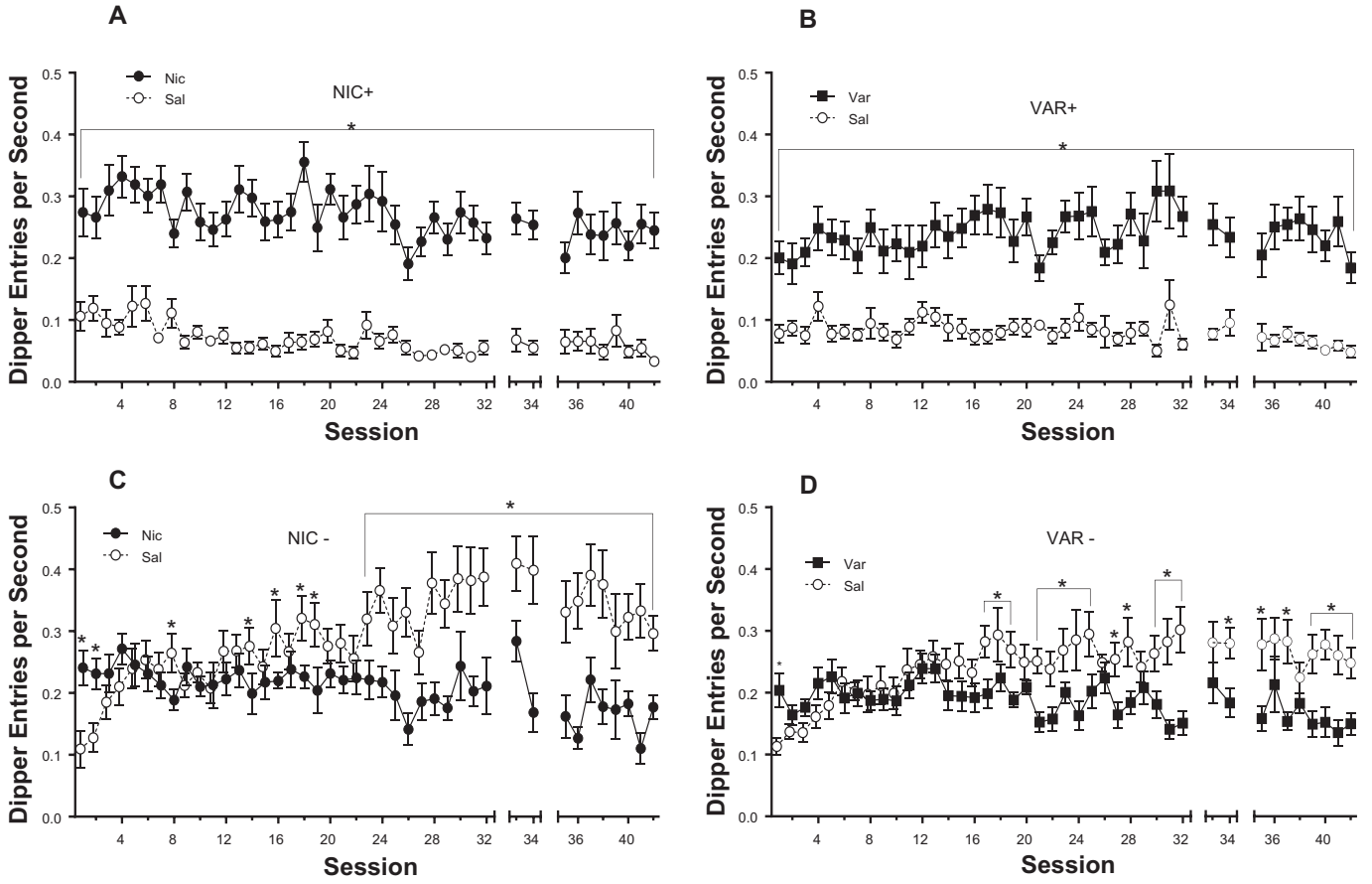

Fig. 3. Panels A and B, display the dipper entry per sec responding for both the NIC+ and VAR+ groups, respectively, in which the drug days continued to be reinforced. Panel C and D display dipper entries for the NIC− and VAR− groups, respectively, in which the drug-reinforcer relation was reversed such that sucrose was now available on saline sessions. Asterisks indicate $p < 0.05$.

interaction [$F(41,451) = 1.45$, $p = 0.04$]. For the VAR+ group, dipper entry rates on varenicline days were higher than saline days for all sessions ($ps \leq 0.02$). Fig. 3C shows the NIC− group. There were main effects of Drug [$F(1,11) = 26.4$, $p < 0.01$] and Session [$F(41,451) = 2.87$, $p < 0.01$], and a significant interaction [$F(41,451) = 7.36$, $p < 0.01$]. As can be seen, reversal of the discrimination took time to develop. Dipper entries were higher on nicotine days than saline days for session 1 and 2 ($ps < 0.01$). There was no difference between saline and nicotine for sessions 3–7, 9–13, 15, 17, and 20–22 ($ps > 0.09$). On saline injection days, rats in the NIC− group increased rates of head entries into the dipper receptacle compared to nicotine on sessions 8, 14, 16, 18, 19, and 23–42 ($ps < 0.04$). This pattern indicates that the reversal was eventually acquired. Fig. 3D shows the VAR− group. There were main effects of Drug [$F(1,11) = 9.47$, $p < 0.02$], Session [$F(41,451) = 2.32$, $p < 0.01$], and a significant interaction [$F(41,451) = 4.45$, $p < 0.01$]. Reversal learning in the VAR− group also took time to develop and appeared less robust than that of the NIC− group. Dipper entries on session 1 were higher when given varenicline than saline ($p < 0.01$), but no differences between varenicline or saline injections for sessions 2–16, 20, 26, 29, and 38 ($ps \geq 0.08$). Dipper entries were greater on saline than on varenicline days for session 17–19, 21–25, 27, 28, 30–37, and 39–42 ($ps < 0.05$).

We examined total dipper entries in the 20-min session to determine whether rats were sensitive to the presentation of sucrose, especially, in the reversal groups (see Fig. 4). For the NIC+ group (Fig. 4A), there were main effects of Drug [$F(1,11) = 137$, $p < 0.01$] and Session [$F(41,451) = 1.48$, $p < 0.04$], but no interaction was found ($F < 1$). Dipper entries were higher on all nicotine sessions compared to saline ($ps < 0.01$). For the VAR+ group (Fig. 4B), there was a main effect of Drug [$F(1,11) = 45.9$, $p < 0.01$] but there was neither an effect of Session [$F(41,451) = 1.03$, $p = 0.41$] nor an interaction ($F < 1$). Dipper entries were higher on all varenicline sessions compared to saline ($ps < 0.01$). For the NIC− group (Fig. 4C), there were main effects of Drug [$F(1,11) = 59.9$, $p < 0.01$] and Session [$F(41,451) = 5.45$, $p < 0.01$], and a Drug * Session interaction [$F(41,451) = 5.95$, $p < 0.01$]. No difference in dipper entries was observed between nicotine and saline for session 1 ($p > 0.06$). However, more dipper entries occurred on saline than on nicotine days for sessions 2–42 ($ps \leq 0.04$). For the VAR− group (Fig. 4D), there were main effects of Drug [$F(1,11) = 39.2$, $p < 0.01$] and Session [$F(41,451) = 1.97$, $p < 0.01$], and a Drug * Session interaction [$F(41,451) = 3.81$, $p < 0.01$]. Dipper entries were higher on saline days than on varenicline days for session 1–42 ($ps < 0.05$). This data pattern for the rats in the NIC− and VAR− groups indicates that they were sensitive to the delivery of the reinforcer very early in reversal training, despite not displaying an early reversal of discrimination indexed by our measurement of interoceptive conditioning (i.e., dipper entries before the first sucrose delivery of each session).

3.3. Varenicline and nicotine tests

Recall that we tested rats in the VAR+ and VAR− groups with nicotine on two separate occasions whereas the NIC+ and NIC− groups were tested with varenicline. Responding across the two tests did not differ as the Group * Test Session interaction was not significant ($F < 1$). Accordingly, we averaged the data from the two tests for each rat. This average was used for graphs (see Fig. 5) and the two-way

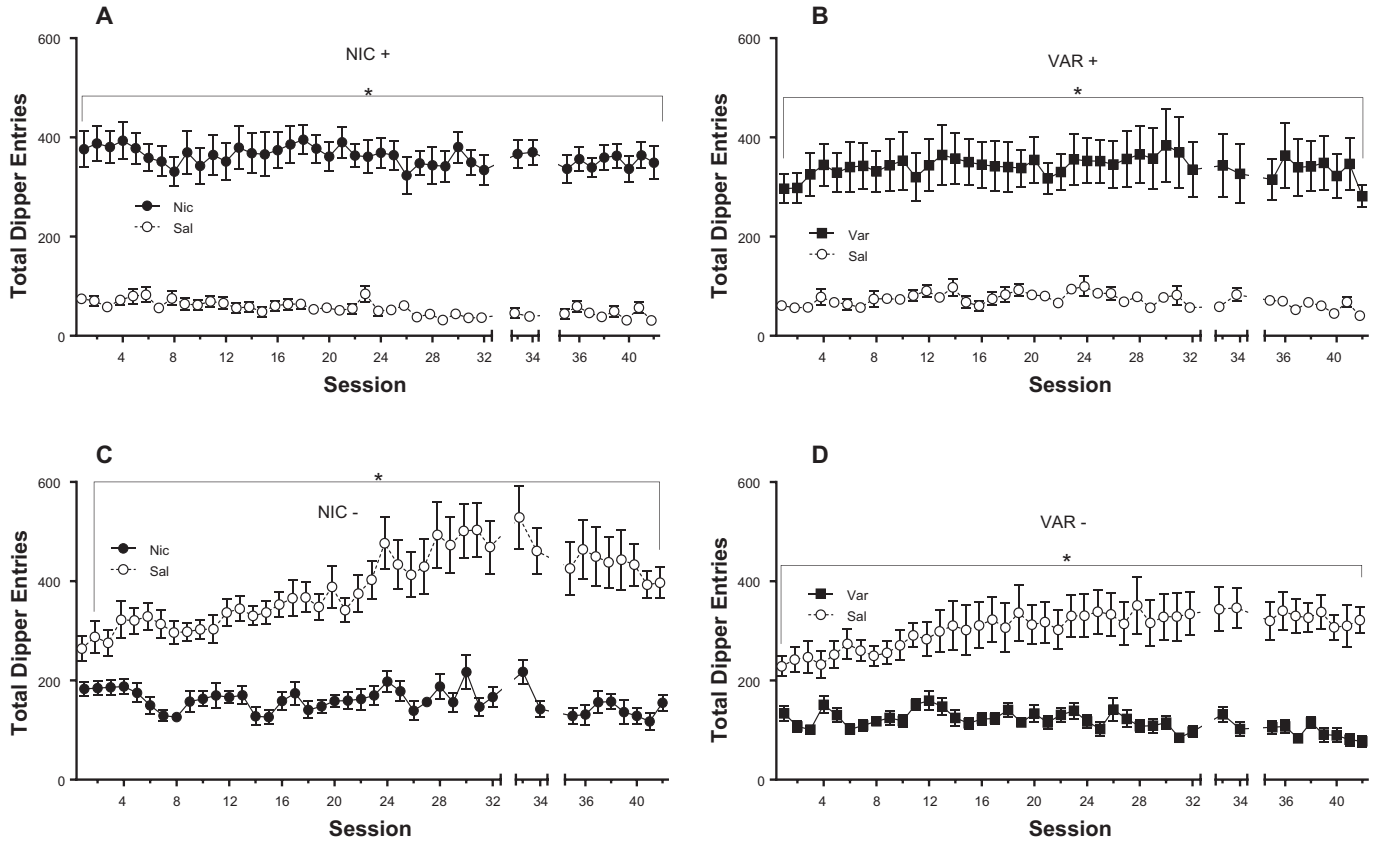

Fig. 4. Panels A and B, display the total dipper entries for the NIC+ and VAR+ groups, respectively, in which drug days were reinforced. Panels C and D, display total dipper entries for the NIC− and VAR− respectively. Each graph shows the rats were sensitive to the US presentation (excluding session 1 for the NIC− group [$p = 0.06$]). Asterisks indicate $p < 0.05$.

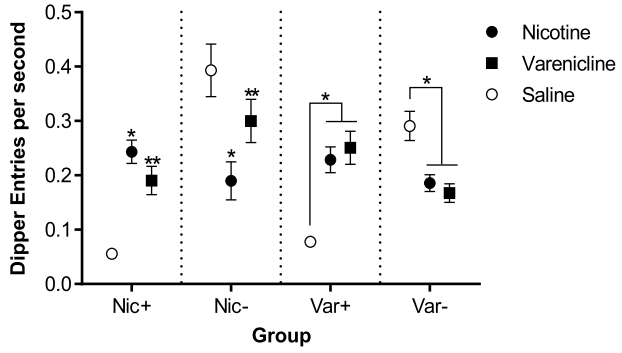

Fig. 5. The results from the substitution tests following sessions 32 and 34 of the second phase. Data were averaged together from the two tests. Varenicline tests in both nicotine groups display partial substitution for the nicotine stimulus. Whereas, nicotine tests in both the varenicline groups show full substitution. A single asterisk indicates significant differences from saline $p < 0.05$. The double asterisk indicates significant difference from both nicotine and saline $p < 0.05$ (i.e., partial substitution).

repeated measures ANOVA with Group (NIC+, NIC−, VAR+, VAR−) and Drug (saline, varenicline, or nicotine) as factors. There was a main effect of Group [$F(3, 44) = 5.91$; $p < 0.01$] and a significant Group * Drug interaction [$F(6, 232) = 46.9$; $p < 0.01$], but no main effect of Drug [$F(2,232) = 2.32$, $p = 0.10$]. For the two NIC groups, varenicline-evoked responding that differed from saline ($ps < 0.01$) and nicotine ($ps < 0.02$). This data pattern suggests partial substitution of varenicline for the nicotine stimulus regardless of training history. In both VAR groups, saline responding was different from that of the nicotine stimulus ($ps < 0.01$). In the same two groups, the nicotine stimulus evoked goal-tracking at rates similar to the varenicline stimulus ($ps \geq 0.30$). This outcome indicates full substitution of the nicotine stimulus for varenicline regardless of whether varenicline signaled the presence (VAR+) or absence (VAR−) of the sucrose reinforcer.

3.4. Nicotine extinction

Fig. 6 displays the extinction phase results. For the two-way repeated measures ANOVA, there was a main effect of Session [$F(11, 484) = 20.9$; $p < 0.01$], a significant Group * Session interaction [$F(33, 484) = 1.91$; $p < 0.01$], but no effect of Group [$F(3, 44) = 2.10$; $p = 0.11$]. Dipper entries here mirrored responding from the second training phase for each group, with higher rates for the NIC+ and VAR + groups relative to the NIC− and VAR− groups ($ps < 0.01$; post-hoc comparisons of plus vs. minus conditions). When comparing within drug condition, the NIC+ group responded at a higher rate than the NIC− group ($p < 0.01$), and VAR+ higher than VAR− ($p < 0.01$). Responding by all four groups reached similarly low levels by session 12 ($ps \geq 0.99$). A significant decrease in responding was observed for the NIC+ and the VAR+ groups from extinction session 1 to extinction session 12 ($ps < 0.01$). For the NIC− and VAR− groups, they maintained low levels of responding across extinction sessions 1–12 ($ps \geq 0.99$). Total dipper entries for all groups reached similarly low levels by the end of the extinction phase (see Supplemental Fig. 2).

4. Discussion

In our laboratory with the DGT task, the interoceptive stimulus effects from nicotine can be discriminated from saline at doses ranging

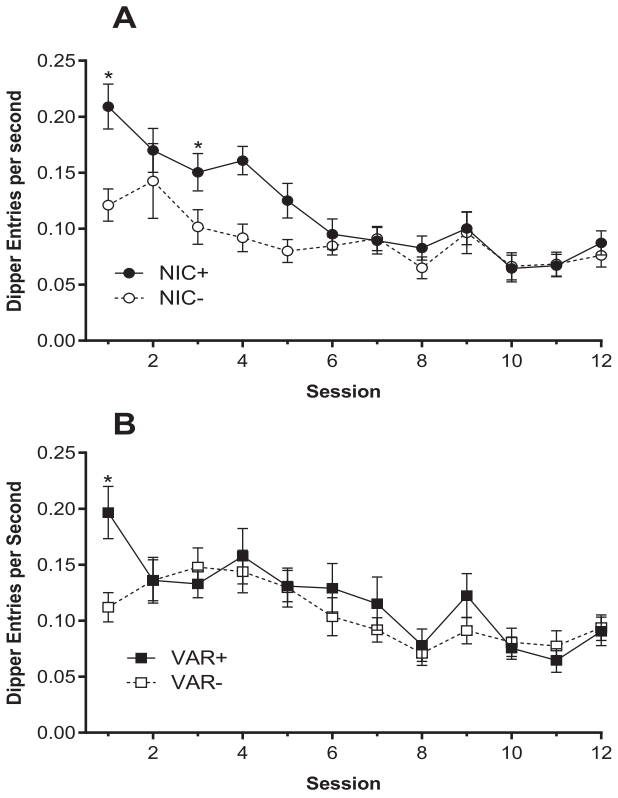

Fig. 6. Panels A and B, display dipper entries per sec for nicotine extinction sessions 1–12 for the NIC+ and NIC− groups and the VAR+ and VAR− groups, respectively. All groups reached similarly low levels of responding by session 12. An asterisk denotes significance set at $p < 0.05$.

from 0.05 to 0.6 mg/kg (Bevins et al., 2007; Charntikov et al., 2017; Charntikov et al., 2012; Polewan et al., 2013; Reichel et al., 2010). We replicated this past work showing that 0.4 mg/kg nicotine quickly acquired control over goal tracking. Interestingly, we found that the stimulus-sucrose relation used in discrimination training with a 0.4 mg/kg nicotine versus saline stimulus was reversible as evidenced by the acquisition of the discrimination in the NIC− group. There is a dearth of literature on reversal learning using drug discrimination and only one known to us for a discrimination involving the nicotine stimulus (see Troisi, 2013). Interestingly, we found that a 1 mg/kg varenicline stimulus fully substituted for the 0.4 mg/kg nicotine stimulus when drug sessions continued to be reinforced (i.e., VAR+). Further, when the substitution drug in the reversal condition was varenicline, this substitution was more tenuous. That is, discrimination reversal in the VAR− group eventually occurred but with much more variability than that displayed by the NIC− group.

This study was the first to have the varenicline stimulus replace nicotine and continue the stimulus-sucrose relation (VAR+) and the reversal (VAR−) in the DGT task. In contrast to nicotine, varenicline is a partial agonist for the α4β2-containing nAChRs and also displays agonist activity for nAChRs containing α3β4 and α7 subunits (Coe et al., 2005; Smith et al., 2007). The α4β2-containing nAChRs in humans has been associated with attention (Roh et al., 2014), learning and memory (Kenney and Gould, 2008), reward valuation (De Biasi and Dani, 2011), cholinergic transmission (Hasselmo and Giocomo, 2006; Prado et al., 2017), and psychiatric disorders (Eaton et al., 2003). This agonist profile of varenicline likely accounts for its effectiveness as a smoking cessation aid by alleviating withdrawal and cravings for some nicotine-dependent smokers (Garrison & Dugan, 2009). The blunted dopaminergic effect compared to nicotine alone may decrease its abuse potential (Coe et al., 2005). These neuropharmacological differences from nicotine could make the varenicline stimulus sufficiently different and/or less salient than the nicotine stimulus. Indeed, our findings suggest that such differences may be important for drug substitution in the DGT task only when new learning is required. Namely, differences in performance only emerged when the drug stimulus-sucrose relation was reversed (cf. VAR− and NIC−).

In future work, it will be interesting to examine the effects of higher and lower doses on learning and performance in the varenicline groups. As detailed earlier, the primary factors driving our decision to use 1.0 mg/kg varenicline were full substitution for nicotine in the DGT task (Reichel et al., 2010), full substitution in a two-nose poke aperture drug-discrimination task (Jutkiewicz et al., 2011), and no evidence for non-specific motor impairment effects with repeated treatment (Reichel et al., 2010). However, an effective substitution dose in the DGT may differ from an effective dose for reversal learning [see Levin et al. (2012) for such differences when studying the reinforcer-enhancement effects of nicotine]. Perhaps reversal learning would be faster and/or less variable with a higher dose (e.g., 3.0 mg/kg) if the motor impairment effects seen with repeated treatment were transient (cf. Reichel et al., 2010). Of course, future research could also explore the impact of the nicotine-training dose on substitution and reversal.

An elemental view of the drug stimulus (cf. Bevins and Besheer, 2014) suggests that the varenicline stimulus lacks one or a number of elements that make up the nicotine stimulus. The finding that the varenicline stimulus only partially prompted nicotine-like responding during substitution testing in both the NIC+ and NIC− groups (see Fig. 5) supports this view. The opposite was not the case. Nicotine fully substituted for the varenicline stimulus in the VAR+ and VAR− groups. This data pattern suggests that nicotine includes all the stimulus elements of varenicline (full substitution) but varenicline lacks some of the stimulus elements of nicotine, hence the partial substitution pattern. Related, Reichel et al. (2010) trained nicotine as an excitatory interoceptive stimulus in the DGT task. The 0.3, 1, and 3 mg/kg varenicline doses fully substituted for the 0.4 mg/kg nicotine-training stimulus when tested in brief 4-min substitution tests. In that same study (see Experiment 5), however, one group of rats received varenicline substitution during repeated 20-min non-reinforced (i.e., extinction) sessions. Varenicline only partially substituted for the nicotine stimulus in these longer repeated sessions that provided an opportunity for the rat to learn about non-reinforcement. The work by Reichel et al. (2010), combined with the present research, indicates that substitution of varenicline for the nicotine stimulus is dependent on the testing protocol. Further, approaches that require or permit the opportunity for new learning such as extinction or reversal learning may be more sensitive at detecting a different set of stimulus elements (cf. Bevins et al., 2012; Reichel et al., 2010).

Reversal learning in discrimination tasks has been considered a form of cognitive flexibility (Prado et al., 2017). The inability to alter a response to a new stimulus-reinforcer relation is often referred to as perseverative errors or perseverative responding. Perseverative errors are commonly seen in successive reversal paradigms in which the reinforcer relations are flipped multiple times akin to an ABAB study design (Clarke et al., 2008; Prado et al., 2017; Thomas et al., 2008). In the DGT task, they manifest as the inability to withhold responding on non-reinforced sessions of the reversal groups. In fact, the discrimination reversals for the NIC− and VAR− groups appear to be driven more by an increase in saline responding rather than by a decrease in responding in the previously reinforced nicotine sessions (compare Fig. 3 panels A and B to C and D). We posit that at least two potential factors may account for this pattern of responding. First, the behavioral cost of nose poking to access sucrose in the dipper receptacle is low. Thus, responding in the NIC− and VAR− sessions remained relatively high and seemingly insensitive to the lack of sucrose early in the session. In contrast to the early session responding, recall that total dipper entries in the 20-min session revealed that rats were sensitive overall to the absence of sucrose on drug sessions in the NIC− and VAR− group (see Fig. 4). A second possibility, and not necessarily mutually exclusive

from the first, is that the reward enhancing effects of nicotine and varenicline may produce some perseverative responding in the face of non-reinforcement (Barrett et al., 2018). Barrett et al. (2018) found that varenicline and nicotine enhanced the reinforcing value of a visual stimulus using a behavioral economic approach as well as a progressive ratio schedule of reinforcement. In the present study, the dipper receptacle is associated with sucrose on 50% of the sessions (i.e., saline in the reversal phase). Thus, the stimuli that compose the receptacle have conditioned reinforcing value through this association. Varenicline and nicotine may enhance the reinforcing value of these stimuli early in the session. This enhancement may translate in an increase in goal-tracking behavior until some time has passed without sucrose delivery (i.e., extinction).

Not surprisingly, for the nicotine extinction phase, rats in the NIC− and VAR− groups had lower levels of goal-tracking than the NIC+ and VAR+ groups (i.e., mimicking responding in the reversal phase). The level of goal-tracking behavior seen through session 12 was consistent with other studies testing extinction in the DGT task with similar ligands (i.e., behavior diminishes to lower levels but still persists; Reichel et al., 2010).

In sum, the present study has extended our understanding of the nicotine and varenicline stimulus in a number of notable ways. For example, the varenicline stimulus can fully substitute for the nicotine stimulus when new learning is not required (VAR+). We also discovered that reversal learning with nicotine in the DGT task is possible but it takes extensive training and discrimination reflects more of an increase in saline-evoked responding than a decrease in nicotine-evoked responding early in the session. Further, if the drug-sucrose relation was reversed then substitution for nicotine by the varenicline stimulus appears partial. A conclusion supported by the opposite drug tests in phase 2 training. As noted earlier, it will be of interest to assess the importance of training and substitution doses in the future. We will also be interested in whether training nicotine from the outset to not signal access to sucrose (NIC−) will alter the substitution patterns seen by varenicline when training is either continued (drug sessions not reinforced) or is reversed. Also, we wonder whether full substitution patterns when shifting from NIC+ to VAR+ predicts that the varenicline stimulus will work as well as the nicotine stimulus if it served as the initial training drug in the DGT task.

Disclosure statement

There are no potential conflicts of interest reported by the authors.

Acknowledgments

We would like to thank Jessica Emory, Chris Larsen, and David Kwan for their help with data collection, and Dr. Jennifer E. Murray for her continual guidance and support in so many facets.

Financial support

This study was supported by the National Institute of Health: R01-DA034389.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.04.001.

References

Amodeo, L.R., McMurray, M.S., Roitman, J.D., 2016. Orbitofrontal cortex reflects changes in response–outcome contingencies during probabilistic reversal learning. Neuroscience 1–11. https://doi.org/10.1016/j.neuroscience.2016.03.034.

Barrett, S.T., Geary, T.N., Steiner, A.N., Bevins, R.A., 2018. A behavioral economic analysis of the value-enhancing effects of nicotine and varenicline and the role of nicotinic acetylcholine receptors in male and female rats. Behav. Pharmacol. 29 (6), 493–502. https://doi.org/10.1097/FBP.0000000000000404.

Besheer, J., Palmatier, M.I., Metschke, D.M., Bevins, R.A., 2004. Nicotine as a signal for the presence or absence of sucrose reward: a Pavlovian drug appetitive conditioning preparation in rats. Psychopharmacology 172 (1), 108–117. https://doi.org/10.1007/s00213-003-1621-9.

Bevins, R.A., Besheer, J., 2014. Interoception and learning: import to understanding and treating diseases and psychopathologies. ACS Chem. Neurosci. https://doi.org/10.1021/cn5001028.

Bevins, R.A., Besheer, J., Pickett, K.S., 2001. Nicotine-conditioned locomotor activity in rats: dopaminergic and GABAergic influences on conditioned expression. Pharmacol. Biochem. Behav. 68 (1), 135–145. https://doi.org/10.1016/S0091-3057(00)00451-2.

Bevins, R.A., Penrod, R.D., Reichel, C.M., 2007. Nicotine does not produce state-dependent effects on learning in a Pavlovian appetitive goal tracking task with rats. Behav. Brain Res. 177 (1), 134–141. https://doi.org/10.1016/j.bbr.2006.10.026.

Bevins, R.A., Barrett, S.T., Polewan, R.J., Pittenger, S.T., Swalve, N., Charntikov, S., 2012. Disentangling the nature of the nicotine stimulus. Behav. Process. 90 (1), 28–33. https://doi.org/10.1016/j.beproc.2011.10.020.

Boakes, R., 1977. In: Davis, H., Hurwitz, H.M.B. (Eds.), Performance on learning to associate a stimulus with positive reinforcement. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 67–101.

Bouton, M.E., Brooks, D.C., 1993. Time and context effects on performance in a Pavlovian discrimination reversal. J. Exp. Psychol. Anim. Behav. Process. 19 (2), 165–179. https://doi.org/10.1037/0097-7403.19.2.165.

Cahill, K., Stead, L.F., Lancaster, T., 2010. Nicotine receptor partial agonists for smoking cessation. In: Cahill, K. (Ed.), Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd., Chichester, UK. https://doi.org/10.1002/14651858.CD006103.pub4.

Cahill, K., Stevens, S., Perera, R., Lancaster, T., 2013. Pharmacological interventions for smoking cessation: an overview and network meta-analysis. Cochrane Database Syst. Rev. 5 (5). https://doi.org/10.1002/14651858.CD009329.pub2.www.cochranelibrary.com. N.PA-N.PA.

CDC, 2014. Smoking and Tobacco Use: Fast Facts. Retrieved from. http://www.cdc.gov/tobacco/data_statistics/fact_sheets/fast_facts/index.htm.

Charntikov, S., Tracy, M.E., Zhao, C., Li, M., Bevins, R.A., 2012. Conditioned response evoked by nicotine conditioned stimulus preferentially induces c-Fos expression in medial regions of caudate-putamen. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology 37 (4), 876–884. https://doi.org/10.1038/npp.2011.263.

Charntikov, S., Falco, A.M., Fink, K., Dwoskin, L.P., Bevins, R.A., 2017. The effect of sazetidine-A and other nicotinic ligands on nicotine controlled goal-tracking in female and male rats. Neuropharmacology 113, 354–366. https://doi.org/10.1016/j.neuropharm.2016.10.014.

Chudasama, Y., Robbins, T.W., 2003. Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J. Neurosci. 23 (25), 8771–8780. https://doi.org/10.1523/JNEUROSCI.23-25-08771.2003.

Clarke, H.F., Robbins, T.W., Roberts, A.C., 2008. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. J. Neurosci. 28 (43) Retrieved from. http://www.jneurosci.org/content/28/43/10972.short.

Coe, J.W., Brooks, P.R., Vetelino, M.G., Wirtz, M.C., Arnold, E.P., Huang, J., ... O'Neill, B.T., 2005. Varenicline: an α4β2 nicotinic receptor partial agonist for smoking cessation. J. Med. Chem. 48 (10), 3474–3477. https://doi.org/10.1021/jm050069n.

De Biasi, M., Dani, J.A., 2011. Reward, addiction, withdrawal to nicotine. Annu. Rev. Neurosci. 34, 105–130. https://doi.org/10.1146/annurev-neuro-061010-113734.

Eaton, J.B., Peng, J.-H., Schroeder, K.M., George, A.A., Fryer, J.D., Krishnan, C., ... Lukas, R.J., 2003. Characterization of human 4 2-nicotinic acetylcholine receptors stably and heterologously expressed in native nicotinic receptor-null SH-EP1 human epithelial cells. Mol. Pharmacol. 64 (6), 1283–1294. https://doi.org/10.1124/mol.64.6.1283.

Faessel, H.M., Obach, R.S., Rollema, H., Ravva, P., Williams, K.E., Burstein, A.H., 2010. A review of the clinical pharmacokinetics and pharmacodynamics of varenicline for smoking cessation. Clin. Pharmacokinet. 49 (12), 799–816. https://doi.org/10.2165/11537850-000000000-00000.

Farwell, B.J., Ayres, J.J.B., 1979. Stimulus-reinforcer and response-reinforcer relations in the control of conditioned appetitive headpoking ("goal tracking") in rats. Learn. Motiv. 10 (3), 295–312. https://doi.org/10.1016/0023-9690(79)90035-3.

Garrison, G.D., Dugan, S.E., 2009. Varenicline: a first-line treatment option for smoking cessation. Clin. Ther. 31 (3), 463–491. https://doi.org/10.1016/j.clinthera.2009.03.021.

Hasselmo, M.E., Giocomo, L.M., 2006. Cholinergic modulation of cortical function. J. Mol. Neurosci. 30 (1–2), 133–136. https://doi.org/10.1385/JMN:30:1:133.

Jutkiewicz, E.M., Brooks, E.A., Kynaston, A.D., Rice, K.C., Woods, J.H., 2011. Patterns of nicotinic receptor antagonism: nicotine discrimination studies. J. Pharmacol. Exp. Ther. 339 (1), 194–202. https://doi.org/10.1124/jpet.111.182170.

Kenney, J.W., Gould, T.J., 2008. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. Mol. Neurobiol. 38 (1), 101–121. https://doi.org/10.1007/s12035-008-8037-9.

Lange-Asschenfeldt, C., Schable, S., Suvorava, T., Fahimi, E.G., Bisha, M., Stermann, T., ... Kojda, G., 2016. Effects of Varenicline on alpha4-Containing Nicotinic Acetylcholine Receptor Expression and Cognitive Performance in Mice. https://doi.org/10.1016/j.neuropharm.2016.03.025.

LeSage, M.G., Shelley, D., Ross, J.T., Carroll, F.I., Corrigall, W.A., 2009. Effects of the

nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. Pharmacol. Biochem. Behav. 91 (3), 461–467. https://doi.org/10.1016/j.pbb.2008.08.024.

Levin, M.E., Weaver, M.T., Palmatier, M.I., Caggiula, A.R., Sved, A.F., Donny, E.C., 2012. Varenicline dose dependently enhances responding for nonpharmacological reinforcers and attenuates the reinforcement-enhancing effects of nicotine. Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and Tobacco 14 (3), 299–305. https://doi.org/10.1093/ntr/ntr213.

McDannald, M.A., Jones, J.L., Takahashi, Y.K., Schoenbaum, G., 2015. Learning theory: a driving force in understanding orbital function. Neurobiol. Learn. Mem. 108, 22–27. https://doi.org/10.1016/j.nlm.2013.06.003.Learning.

Murray, J.E., Walker, A.W., Li, C., Wells, N.R., Penrod, R.D., Bevins, R. a, 2011. Nicotine trained as a negative feature passes the retardation-of-acquisition and summation tests of a conditioned inhibitor. Learning & Memory (Cold Spring Harbor, N.Y.) 18 (7), 452–458. https://doi.org/10.1101/lm.2177411.

Palmatier, M.I., Wilkinson, J.L., Metschke, D.M., Bevins, R.A., 2004. Stimulus properties of nicotine, amphetamine, and chlordiazepoxide as positive features in a pavlovian appetitive discrimination task in rats. Neuropsychopharmacology 731–741. https://doi.org/10.1038/sj.npp.1300629.

Perkins, K.A., Kunkle, N., Michael, V.C., Karelitz, J.L., Donny, E.C., 2016. Assessing discrimination of nicotine in humans via cigarette smoking. Nicotine Tob. Res. 18 (9), 1830–1836. https://doi.org/10.1093/ntr/ntw082.

Pittenger, S.T., Zeplin, L.C., Dwoskin, L.P., Bevins, R.A., 2015. The effect of switching pharmacological intervention during extinction on nicotine-evoked conditioned responding in rats. Psychopharmacology 232 (23), 4347–4358. https://doi.org/10.1007/s00213-015-4067-y.

Polewan, R.J., Savala, S.A., Bevins, R.A., 2013. Interoceptive conditioning with the nicotine stimulus: extinction learning as a method for assessing stimulus similarity across doses. Behav. Pharmacol. 24 (1), 45–54. https://doi.org/10.1097/FBP.0b013e32835d5278.

Potts, L.A., Garwood, C.L., 2007. Varenicline: the newest agent for smoking cessation. Am. J. Health Syst. Pharm. https://doi.org/10.2146/ajhp060428.

Prado, V.F., Janickova, H., Al-Onaizi, M.A., Prado, M.A.M.M., 2017. Cholinergic circuits in cognitive flexibility. Neuroscience 345, 130–141. https://doi.org/10.1016/j.neuroscience.2016.09.013.

R Core Team, 2016. R: A language and environment for statistical computing (3.3.2.). R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org.

Reichel, C.M., Murray, J.E., Barr, J.D., Bevins, R.A., 2010. Extinction with varenicline and nornicotine, but not ABT-418, weakens conditioned responding evoked by the interoceptive stimulus effects of nicotine. Neuropharmacology 58 (8), 1237–1245. https://doi.org/10.1016/j.neuropharm.2010.03.005.

Roh, S., Hoeppner, S.S., Schoenfeld, D., Fullerton, C.A., Stoeckel, L.E., Evins, A.E., 2014. Acute effects of mecamylamine and varenicline on cognitive performance in non-smokers with and without schizophrenia. Psychopharmacology 231 (4), 765–775. https://doi.org/10.1080/15374410802148095. (The).

Smith, J.W., Mogg, A., Tafi, E., Peacey, E., Pullar, I.A., Szekeres, P., Tricklebank, M., 2007. Ligands selective for α4β2 but not α3β4 or α7 nicotinic receptors generalise to the nicotine discriminative stimulus in the rat. Psychopharmacology 190 (2), 157–170. https://doi.org/10.1007/s00213-006-0596-8.

Thomas, E., Snyder, P.J., Pietrzak, R.H., Jackson, C.E., Bednar, M., Maruff, P., 2008. Specific impairments in visuospatial working and short-term memory following low-dose scopolamine challenge in healthy older adults. Neuropsychologia 46 (10), 2476–2484. https://doi.org/10.1016/j.neuropsychologia.2008.04.010.

Tighe, T.J., Graves, D.M., Riley, C.A., 1968. Successive reversals of a classically conditioned heart-rate discrimination. J. Exp. Anal. Behav. 11 (2), 199–206.

Troisi, J.R., 2013. Acquisition, extinction, recovery, and reversal of different response sequences under conditional control by nicotine in rats. J. Gen. Psychol. 140 (3), 187–203. https://doi.org/10.1080/00221309.2013.785929.

Volkow, N.D., Wise, R.A., 2005. How Can Drug Addiction Help us Understand Obesity? vol. 8(5)https://doi.org/10.1038/nn1452.

Ward, R.D., Kynaston, A.D., Bailey, E.M., Odum, A.L., 2008. Discriminative control of variability: effects of successive stimulus reversals. Behav. Process. 78 (1), 17–24. https://doi.org/10.1016/j.beproc.2007.11.007.

Wooters, T.E., Bevins, R.A., Bardo, M.T., 2009. Neuropharmacology of the interoceptive stimulus properties of nicotine. Current Drug Abuse Reviews 2 (3), 243–255. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/20443771.

World Health Organization, 2010. Addiction to nicotine. Gender, Women, and The Tobacco Epidemic 137–150.