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## Extinction with varenicline and nornicotine, but not ABT-418, weakens conditioned responding evoked by the interoceptive stimulus effects of nicotine

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### Abstract

The interoceptive stimulus effects of nicotine acquire control over behavior. This observation, among others, suggests that the stimulus effects of nicotine are important in the development and tenacity of tobacco dependence. Despite this importance, there has been little research examining whether non-reinforced presentations (extinction) of a ligand that share stimulus effects of nicotine will weaken responding controlled by nicotine. Rats were trained to discriminate nicotine (0.4 mg/kg) from saline using a discriminated goal-tracking task in which nicotine signaled intermittent access to sucrose; sucrose was withheld on saline sessions. Experiment 1 examined substitution for nicotine by ABT-418, nornicotine, epibatidine, varenicline, or cytosine in 4-min extinction tests. Experiments 2 to 5 [low dose nicotine (0.05 mg/kg), ABT-418, nornicotine, or varenicline, respectively] examined whether substitution for nicotine would persist if extinction tests were increased to 20 min and repeated daily for 6 days. Finally, generalization of this extinction back to the nicotine training stimulus was assessed. Full substitution in brief 4-min extinction tests was seen for ABT-418, nornicotine, epibatidine, varenicline, and cytosine. Low-dose nicotine, ABT-418, nornicotine, and varenicline, evoked only a partial ‘nicotine-like’ response in the first 20-min extinction test. With repeated extinction, only low-dose nicotine, nornicotine, and varenicline continued to substitute. Extinction with nornicotine and varenicline transferred back to nicotine as indicated by a partial conditioned response to the training stimulus. Interpretations regarding ‘nicotine-like’ effects of a ligand depend on the nature of the test. Understanding the processes mediating transfer of extinction learning with potential pharmacotherapies may reveal new treatment targets.

### Keywords

Chantix®; extinction learning; Pavlovian conditioning; smoking cessation; tobacco

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## INTRODUCTION

Illness and disease associated with tobacco consumption is a tenacious world-wide health problem (Esson and Leeder, 2004). Although long-term smoking cessation rates are improved through behavioral and/or pharmacological approaches, the majority of individuals relapse within a year (Hall et al., 2004; Rose, 2009; Schröter et al., 2006). Clearly, better treatment strategies are needed. Given that chronic tobacco use is attributed, at least in part, to nicotine, improved strategies will come from better understanding of behavioral and neural processes underlying nicotine's addictive effects (Bardo and Schnur, 2009).

Of particular interest in the present report are the interoceptive stimulus effects of nicotine acquiring control over behavior. In human participants, their choice behavior in the lab comes under the control of nicotine in a dose-dependent manner (Perkins et al., 1994). Several different tasks with rodents have been used to study the nicotine stimulus in more detail (Bevins, 2009; Smith and Stoleran, 2009). For instance, in a two-lever drug discrimination task, rats receive intermixed nicotine and saline sessions. On nicotine sessions, responses on one lever (left) are reinforced with food; responses on the other (right) are not reinforced. On saline sessions, the reinforcement contingencies for responding are switched. Behavior comes under control of the nicotine stimulus with over 80% of presses on the drug-appropriate lever (e.g., Stoleran, 1989).

In a discriminated goal-tracking (DGT) task, rats also receive intermixed nicotine and saline sessions. On nicotine sessions, sucrose is available intermittently in a recessed dipper receptacle regardless of behavior; sucrose is not available on saline sessions. Nicotine comes to evoke an increase in head entries into the receptacle relative to saline [goal-tracking; (Farwell and Ayres, 1979)]. Nicotine in this task can be considered a conditioned stimulus (CS) that has acquired control over a goal-tracking conditioned response [CR; (Bevins and Palmatier, 2004; Bevins, 2009)]. The neuropharmacological mechanisms by which nicotine controls behavior differ somewhat between the DGT and two-lever discrimination task (Murray and Bevins, 2007a; Murray et al., 2009).

Using the DGT task, the present research investigated *transfer of extinction learning* involving nicotine. Extinction refers to a decrease in the CR when an excitatory CS is no longer reinforced (Pavlov, 1927) and is the basis of intervention approaches such as guided imagery and cue-exposure therapy (Conklin, 2006; Lee et al., 2007). Converging evidence indicates that extinction reflects new learning that interferes or competes with initial learning (Bouton, 2002; Quirk and Mueller, 2008). Previous research with the DGT task has shown that repeated presentation of nicotine alone decreases the goal-tracking CR (Besheer et al., 2004; Murray and Bevins, 2007b, 2009; Wilkinson et al., 2006). To our knowledge, no one has ever examined the use of substitution compounds during extinction of behavioral control by the nicotine stimulus. Accordingly, the present studies examined whether the nicotine-evoked CR could be weakened by presentation of a ligand during extinction that shares stimulus effects with nicotine. This approach is important from a translational perspective because drugs used as smoking cessation aids are typically administered during periods of nicotine abstinence.

To test whether extinction learning transfers across pharmacological compounds, an important prerequisite was to identify ligands that share stimulus effects with the nicotine CS. To this end, we selected ligands that have some agonist action at nicotinic acetylcholine receptors and also partially and/or fully substitute for the stimulus effects of nicotine in the two-lever discrimination task (Damaj et al., 1994; Damaj et al., 1995; Goldberg et al., 1989; LeSage et al., 2009) and assessed them for the first time in the DGT task (see Table 1). ABT-418, nornicotine, and varenicline were tested in the transfer of extinction experiments given that they evoked a full nicotine-like CR in substitution tests, as well as have somewhat distinct

neuropharmacological actions. We chose to examine varenicline over cytisine given the overlap in receptor action between the two ligands, and that varenicline is marketed as the smoking cessation drug Chantix<sup>®</sup>. Epibatidine was not tested for transfer of extinction learning given the report of toxicity with repeated treatment [Wiley et al., 1996 (see p. 227)]. As a control study, transfer of extinction was also assessed with a dose of nicotine (0.05 mg/kg) that partially substituted for the training dose of nicotine.

## METHODS

### Subjects

Male Sprague-Dawley rats from Harlan (Indianapolis, IN, USA) were housed individually in clear 48.3 × 26.7 × 20.3 cm (l × w × h) polycarbonate cages lined with wood shavings in a temperature- and humidity-controlled colony. Water was continuously available in the home cage. Access to chow (Harlan Teklad Rodent Diet) was restricted such that rats were maintained at 85% of free-feeding body weights. Approximately every 4 weeks the 85% target weight was increased by 2 g. All sessions were conducted during the light portion of a 12 h 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).

### Apparatus

Sixteen 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 these studies. 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 26% sucrose solution (w/v) 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. A second infrared emitter/detector unit mounted 14.5 cm from the sidewall containing the receptacle and 4 cm above the rod floor provided a measure of chamber activity. 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) controlled sucrose deliveries and recorded dipper entries and chamber activity.

### Drugs

(-)-Nicotine hydrogen tartrate, ABT-418 hydrochloride, (±)-nornicotine, and (-)-cytisine were purchased from Sigma (St. Louis, MO, USA). (±)-Epibatidine was purchased from Tocris Cookson, Inc. (Ellisville, MO, USA). Varenicline dihydrochloride used in Experiment 1 was generously provided by Pfizer (Groton, CT, USA); varenicline for Experiment 5 was a gift from NIDA (RTI International, Research Triangle Park, NC, USA). Table 1 indicates vehicles and subtype specificity of these compounds. All drugs were mixed at 1 ml/kg and injected subcutaneously (SC). Nicotine doses are reported in the base form; doses for the remaining compounds are reported by salt form.

### Experiment 1: Substitution with nAChR agonists

**Discrimination Training:** Rats (n=16; 342 ± 4 g before food restriction) were handled for at least 3 min per day for 3 consecutive days and then given a SC injection of 0.4 mg/kg nicotine in the home cage once per day for 3 consecutive days to attenuate the initial locomotor suppressant effects of nicotine (cf. Bevins et al., 2001). Training of the discrimination began the following day. Nicotine or saline was administered SC 5 min before the start of the 20-min session. During nicotine sessions, sucrose was available for 4 sec on 36 separate occasions.

Timing of sucrose deliveries was varied across sessions by creating 4 different programs that had different inter-sucrose intervals. The average time for the first sucrose delivery across programs was 137 sec, with a range of 124–152 sec. Subsequent deliveries of sucrose ranged from 4–80 s with a mean of 25 s. No sucrose was available on saline sessions, but there were 4-sec ‘empty’ intervals within the programs to maintain consistency between nicotine and saline sessions (see Data Analyses). Session types and programs were randomly assigned for each rat with the restriction that no more than 2 nicotine or 2 saline sessions occurred consecutively. Training continued for 10 nicotine and 10 saline sessions.

**Testing:** The first 4 consecutive days of a 5-day testing cycle were 2 nicotine and 2 saline training sessions randomly intermixed. If the criterion for discrimination was met across these training sessions (see Dependent Measures and Criterion), then day 5 was a 4-min extinction test (no sucrose) of the assigned solution. Table 2 shows the progression of testing. After establishing a nicotine generalization curve, substitution testing of each ligand, along with the training dose of nicotine and vehicle for a benchmark, started. Each rat had a randomly assigned order within each ligand, and all solutions within a phase were tested before starting the next ligand. If the discrimination criterion was not met in that cycle, the rat remained in the home cage on day 5.

**Dependent Measures and Criterion:** The primary dependent measure during training was the number of dipper entries per second before the first sucrose delivery for nicotine sessions or a comparable interval from saline sessions. On test sessions, the dipper entry rate was taken from the first 2 min so as to approximate the average interval to the first sucrose delivery in nicotine training sessions. To qualify to test, dipper entry rates on each nicotine session had to be at least 0.01 entries per second higher than on each saline session within the testing cycle (cf. Murray and Bevins, 2007a). Rate of activity was also analyzed. For comparison purposes, this measure of chamber activity was derived from the same interval of time as the dipper entry measure.

**Data Analyses:** For discrimination training, dipper entries and chamber activity from the last 3 days were analyzed with two-way (Drug [nicotine or saline] × Session) repeated measures analyses of variance (ANOVAs). For generalization and substitution testing phases, dipper entries and chamber activity were analyzed with separate one-way repeated measures ANOVAs for each compound. Paired t-tests were used for planned comparisons and were limited to contrasting each test value with vehicle. Test doses that differed significantly from vehicle were then compared to the nicotine training dose. Full substitution was declared when doses of the test compound significantly differed from vehicle, but not from the nicotine training dose. Partial substitution was declared when a dose differed statistically from vehicle and the nicotine training dose. The median effective dose (ED<sub>50</sub>) was calculated using linear regression on doses from the ascending limb of the dose-effect function.

## **Experiment 2: Transfer of extinction with 0.05 mg/kg nicotine**

**Discrimination Training:** Rats (n=24; 274 ± 2 g before food restriction) were handled and trained as described in Experiment 1. Training continued for 14 nicotine and 14 saline sessions.

**Extinction and Transfer Test:** Following the last day of training, rats (n=8/group) were assigned to receive 1 of 3 solutions during the extinction phase (saline, or 0.05 or 0.4 mg/kg nicotine). Group assignment was made with the restriction that responding during discrimination training did not vary significantly between groups. During extinction, rats received their assigned solution SC 5 min before the start of a 20-min session; no sucrose was available. Extinction lasted for 6 daily sessions. Twenty-four hours after the last extinction

session was the transfer test. On the transfer test, all rats received 0.4 mg/kg nicotine (i.e., the initial training dose) 5 min before the 20-min session. Sucrose was withheld on this test day.

**Dependent Measures and Data Analyses:** Dependent measures for training were the same as described in Experiment 1. For the extinction and transfer test we used total number of dipper entries during the 20-min session. Data were analyzed with ANOVAs, post-hoc comparisons were conducted with Fisher's Protected Least Significance Difference (LSD) tests.

**Experiment 3: Transfer of extinction with ABT-418**—Unless noted, this experiment proceeded like Experiment 2. Following the nicotine/saline discrimination training, rats ( $n=40$ ;  $288 \pm 2$  g before food restriction) were assigned to 1 of 4 extinction groups (saline, 0.4 mg/kg nicotine, 0.3 mg/kg ABT-418, or 0.6 mg/kg ABT-418) with the restriction that dipper entries did not differ significantly between groups in discrimination training ( $n=10$ /group). In the extinction phase, the assigned solution was administered 5 min before the start of the session.

**Experiment 4: Transfer of extinction with nornicotine**—Unless noted, this experiment proceeded like Experiment 2. Following discrimination training, rats ( $n=39$ ;  $297 \pm 1$  g before food restriction) were assigned to 1 of 4 extinction groups (saline, 0.4 mg/kg nicotine, 1 mg/kg nornicotine, or 3 mg/kg nornicotine) with the restriction that responding did not differ significantly between groups ( $n=9$  in 0.4 mg/kg nicotine group;  $n=10$  in the remaining groups). In the extinction phase, the assigned solution was administered 15 min before the start of the session.

**Experiment 5: Transfer of extinction with varenicline**—This experiment proceeded like Experiment 2 except where noted. Following nicotine/saline discrimination training, rats ( $n=50$ ;  $302 \pm 3$  g before food restriction) were assigned to 1 of 5 extinction groups (saline, 0.4 mg/kg nicotine, 0.1 mg/kg varenicline, 1 mg/kg varenicline, or 3 mg/kg varenicline) with the restriction that responding did not differ significantly between groups ( $n=10$ /group). In the extinction phase, the assigned solution was administered 30 min before the start of the session.

## RESULTS

### Experiment 1: Substitution with nAChR agonists

**Discrimination Training**—Rats acquired the discrimination between nicotine and saline as evidenced by higher dipper entry rates on the last 3 nicotine sessions (mean  $\pm$  1 SEM;  $0.19 \pm 0.02$ ) in comparison to the last 3 saline sessions ( $0.07 \pm 0.01$ ). There was a main effect of Drug (nicotine or saline),  $F(1,15)=45.79$ ,  $p<0.001$ , but not of Session,  $F(2,30)=2.41$ ,  $p=.107$ , or Drug by Session interaction,  $F<1$ . Rats were more active on the last 3 nicotine sessions ( $0.47 \pm 0.03$ ) in comparison to the last 3 saline sessions ( $0.31 \pm 0.01$ ). There was a main effect of Drug,  $F(1,15)=21.50$ ,  $p<.001$ . Neither the main effect of Session or Drug  $\times$  Session interaction was significant,  $F_s<1$ , indicating that nicotine increased chamber activity.

**Nicotine Generalization**—Conditioned responding evoked by nicotine was sensitive to test dose,  $F(5,75)=14.84$ ,  $p<.001$  (see Figure 1A). Relative to saline, dipper entries were higher at all nicotine doses,  $t_s \geq 3.21$ ,  $p_s \leq .001$ , indicating nicotine control of the conditioned response. When compared to the training dose of nicotine, dipper entries were lower at 0.025 mg/kg nicotine,  $t=3.94$ ,  $p=.001$ . The  $ED_{50}$  for nicotine was 0.031 mg/kg. Chamber activity was also sensitive to nicotine dose,  $F(5,75)=7.32$ ,  $p<.001$  (see Table 3). Activity counts were higher on 0.1, 0.2, and 0.4 mg/kg nicotine than saline,  $t_s \geq 2.39$ ,  $p_s \leq .03$ .

**ABT-418 Substitution**—ABT-418 substituted for the nicotine CS,  $F(4,60)=19.33$ ,  $p<.001$  (Figure 1B). Specifically, dipper entries were higher than saline at 0.3, 0.6, and 1 mg/kg



ABT-418,  $t_{s \geq 4.93}$ ,  $p_{s \leq .001}$ . Conditioned responding to 0.3 and 1 mg/kg ABT-418 was lower than 0.4 mg/kg nicotine,  $t_{s \geq 2.92}$ ,  $p_{s \leq .011}$ , indicating partial substitution at these doses. Full substitution was seen at 0.6 mg/kg ABT-418 as the CR at this dose did not differ from nicotine,  $t(15)=1.41$ ,  $p=.18$ . The  $ED_{50}$  for ABT 418 was 0.51 mg/kg. Activity was increased at the higher ABT-418 doses,  $F(4,60)=7.64$ ,  $p<.001$  (see Table 3). Specifically, counts were higher on 0.3, 0.6, and 1 mg/kg ABT-418 than saline,  $t_{s \geq 2.49}$ ,  $p_{s \leq .025}$ . Activity at 0.6 and 1 mg/kg ABT-418 were similar to the nicotine training dose,  $t_{s \leq 1.50}$ ,  $p_{s \geq .15}$ .

**Nornicotine Substitution**—Nornicotine substituted for the nicotine CS,  $F(4,52)=5.61$ ,  $p<.001$  (Figure 1C). Dipper entries were above saline at all nornicotine doses,  $t_{s \geq 2.48}$ ,  $p_{s \leq .028}$ . The CR evoked by 3 and 6 mg/kg nornicotine did not differ from the nicotine training dose,  $t_{s \leq 1}$ , denoting full substitution. Responding was significantly lower at 0.3 and 1 mg/kg nornicotine than nicotine indicating partial substitution,  $t_{s \geq 2.29}$ ,  $p_{s \leq .04}$ . The  $ED_{50}$  for nornicotine was 3.21 mg/kg. Activity was unaffected by nornicotine,  $F(4,52)=1.6$ ,  $p=.19$  (Table 3).

**Epibatidine Substitution**—Epibatidine also substituted for the nicotine CS,  $F(6,78)=12.38$ ,  $p<.001$ , (see Figure 1D). Conditioned responding evoked by 1 and 2  $\mu$ g/kg epibatidine was significantly above saline,  $t_{s \geq 2.78}$ ,  $p_{s \leq .016}$ . Responding evoked by 1  $\mu$ g/kg epibatidine was significantly below 0.4 mg/kg nicotine,  $t(13)=3.17$ ,  $p=.007$ . Although the CR evoked by 2  $\mu$ g/kg epibatidine appeared lower than the training dose of nicotine, there was sufficient variability at this dose that it did not differ from nicotine,  $t(13)=1.96$ ,  $p=.07$ . The  $ED_{50}$  for epibatidine was 0.97  $\mu$ g/kg. The overall ANOVA indicated an effect of epibatidine on activity,  $F(6,78)=3.56$ ,  $p=.004$  (Table 3). However, planned comparisons against the saline baseline did not reveal any significant differences,  $t_{s \leq 2.10}$ ,  $p_{s \geq .056}$ .

**Varenicline Substitution**—Varenicline fully substituted for the nicotine CS,  $F(4,52)=5.35$ ,  $p<.001$  (Figure 1E). Relative to saline, responding was higher at all varenicline doses tested,  $t_{s \geq 3.27}$ ,  $p_{s \leq .006}$ . At 0.3, 1, and 3 mg/kg, conditioned responding did not differ statistically from nicotine,  $t_{s \leq 1.57}$ ,  $p_{s \geq .14}$ , indicating full substitution. Responding evoked by 0.1 mg/kg varenicline was lower than nicotine,  $t(13)=2.18$ ,  $p=.049$ . The  $ED_{50}$  for varenicline was 1.49 mg/kg. Varenicline increased chamber activity,  $F(4,52)=2.85$ ,  $p=.033$  (Table 3), relative to saline at 1 mg/kg,  $t(13)=3.57$ ,  $p=.003$ . However, activity at this dose did not differ from the nicotine ( $t<1$ ).

**Cytisine Substitution**—Cytisine substituted for the nicotine CS,  $F(4,44)=8.93$ ,  $p<.001$  (Figure 1F), with responding at 0.3, 1, and 3 mg/kg above saline,  $t_{s \geq 2.4}$ ,  $p_{s \leq .034}$ . Responding evoked by 0.3 and 3 mg/kg cytisine was lower than nicotine,  $t_{s \geq 3.0}$ ,  $p_{s \leq .012}$ ; 1 mg/kg cytisine evoked a CR comparable to nicotine,  $t(11)=1.8$ ,  $p=.97$ . The  $ED_{50}$  for cytisine was 1.88 mg/kg. Chamber activity was not significantly altered by cytisine,  $t(11)=2.45$ ,  $p=.06$  (Table 3).

## Experiment 2: Transfer of extinction with 0.05 mg/kg nicotine

**Discrimination Training**—By the end of training, dipper entry rates had stabilized and were higher on the last 3 nicotine sessions ( $0.20 \pm 0.007$ ) in comparison to the last 3 saline sessions ( $0.09 \pm 0.007$ ). There was a main effect of Drug (nicotine or saline),  $F(1,23)=91.89$ ,  $p<.001$ . The main effect of Session and the Drug by Session interaction were not significant,  $F_{s \leq 2.25}$ ,  $p_{s \geq .12}$ . Rats were more active on nicotine ( $0.46 \pm 0.02$ ) than on saline sessions ( $0.21 \pm 0.01$ ) in these later training sessions. There was a main effect of Drug,  $F(1,23)=91.85$ ,  $p<.001$ , but no main effect of Session or Drug by Session interaction,  $F_{s(2,46)} \leq 1.67$ ,  $p_{s \geq .199}$ .

**Extinction**—For conditioned responding across the six days of extinction (Figure 2, left panel) there was a main effect of Group,  $F(2,21)=27.44$ ,  $p=.001$ , a main effect of Session,  $F$

(1,21)=46.50,  $p=.001$ , and a Group by Session interaction,  $F(10,105)=5.46$ ,  $p<.001$ . Specifically, dipper entries on 0.4 mg/kg nicotine were higher than 0.05 mg/kg nicotine and saline on all sessions ( $LSD_{mmd}=20.62$ ). More so, 0.05 mg/kg nicotine engendered higher levels of responding relative to saline on all but the last extinction session suggesting partial substitution throughout most of extinction. Rats extinguished with 0.4 mg/kg nicotine were more active ( $274 \pm 17.8$ ) than those given saline ( $171 \pm 11.6$ ) or 0.05 mg/kg nicotine ( $185 \pm 19.1$ ) during extinction. There was a main effect of Group,  $F(2,21)=11.58$ ,  $p=.001$ ,  $LSD_{mmd}=43.56$ , and a main effect of Session,  $F(5,105)=2.83$ ,  $p=.019$ , but not a Group by Session interaction,  $F<1$ .

**Transfer Test**—The right panel of Figure 2 shows the results from the transfer test when all rats were tested with the 0.4 mg/kg training dose of nicotine. The overall ANOVA was significant,  $F(2,21)=9.14$ ,  $p<.001$ . Conditioned responding for the saline and the 0.05 mg/kg nicotine group was higher than rats extinguished with 0.4 mg/kg nicotine ( $LSD_{mmd}=37.45$ ). Although extinction with 0.05 mg/kg nicotine appeared to reduce the CR on the transfer test, this difference was not significant. Activity did not differ on the transfer test,  $F<1$ .

### Experiment 3: Transfer of extinction with ABT-418

**Discrimination Training**—Discrimination performance stabilized by the end of training with average dipper entry rates higher on the last 3 nicotine sessions ( $0.25 \pm 0.015$ ) compared to the last 3 saline sessions ( $0.06 \pm 0.004$ ). There was a main effect of Drug,  $F(1,39)=157.19$ ,  $p<.001$ , and Session,  $F(2,78)=7.24$ ,  $p=.001$ , but not a Drug by Session interaction,  $F(2,78)=1.53$ ,  $p=.223$ . Rats were more active on nicotine ( $0.49 \pm 0.02$ ) than on saline ( $0.27 \pm 0.01$ ) in these later sessions. There was a main effect of Drug,  $F(1,39)=105.56$ ,  $p<.001$ , but there was no main effect of Session or Drug by Session interaction,  $F_s \leq 1.96$ ,  $p_s \geq .15$ .

**Extinction**—Conditioned responding across extinction is shown in the left panel of Figure 3. There were significant main effects of Group,  $F(3,36)=33.04$ ,  $p=.001$ , and Session,  $F(5,180)=26.97$ ,  $p=.001$ , and a Group by Session interaction,  $F(15,180)=7.04$ ,  $p<.001$ . Dipper entries on 0.4 mg/kg nicotine were higher than saline, 0.3, and 0.6 mg/kg ABT-418 on all sessions ( $LSD_{mmd}=19.32$ ). Relative to saline and 0.3 mg/kg ABT-418, the 0.6 mg/kg dose of ABT-418 evoked some conditioned responding only on the first extinction session. Activity was also higher during this phase for rats extinguished with 0.4 mg/kg nicotine ( $341 \pm 19.7$ ) relative to rats that received saline ( $176 \pm 9.7$ ) or 0.3 ( $207 \pm 17.6$ ) or 0.6 ( $191 \pm 7.1$ ) mg/kg ABT-418 as indicated by a main effect of Group,  $F(3,36)=27.39$ ,  $p=.001$ ,  $LSD_{mmd}=41.85$ . The main effect of Session was also significant,  $F(5,180)=2.71$ ,  $p=.02$ , but the Group by Session interaction,  $F(15,180)=1.16$ ,  $p=.31$ , was not significant.

**Transfer Test**—Extinction with ABT-418 did not transfer back to the nicotine CS (Figure 3, right panel). The one-way ANOVA was significant,  $F(3,36)=4.7$ ,  $p=.007$ , with post-hoc contrasts showing that conditioned responding in the 0.4 mg/kg nicotine group was lower than all other groups ( $LSD_{mmd}=40.54$ ). Activity between groups did not differ on the transfer test,  $F<1$ .

### Experiment 4: Transfer of extinction with nornicotine

**Discrimination Training**—Discrimination performance stabilized by the end of training with dipper entry rates higher on the last 3 nicotine sessions ( $0.23 \pm 0.016$ ) compared to the last 3 saline sessions ( $0.07 \pm 0.006$ ). There was a main effect of Drug,  $F(1,39)=164.88$ ,  $p<.001$ , but there was no main effect of Session or a Drug by Session interaction,  $F_s < 1$ . Activity was higher on nicotine ( $0.57 \pm 0.02$ ) than saline ( $0.35 \pm 0.02$ ) in these later sessions as evidenced by a main effect of Drug,  $F(1,38)=70.34$ ,  $p<.001$ . The main effect of Session,  $F(2,76)=2.10$ ,  $p=.13$ , and Drug by Session interaction,  $F(2,76)=2.41$ ,  $p=.10$ , were not significant.



**Extinction**—Figure 4 (left panel) shows that conditioned responding across extinction varied by group. The main effects of Group,  $F(3,35)=10.25$ ,  $p=.001$ , Session,  $F(5,175)=18.50$ ,  $p=.001$ , and the Group by Session interaction,  $F(15,175)=2.28$ ,  $p=.006$ , were significant. Conditioned responding was higher in the group receiving 0.4 mg/kg nicotine than all other groups across the six extinction sessions ( $LSD_{mmd}=19.03$ ). More so, 1 and 3 mg/kg nornicotine evoked higher levels of responding relative to saline on all extinction sessions. Activity was also higher for rats treated with 0.4 mg/kg nicotine ( $341 \pm 16.9$ ) than those treated with saline ( $237 \pm 18.7$ ) or 1 ( $232 \pm 22.6$ ) or 3 ( $227 \pm 15.7$ ) mg/kg nornicotine as indicated by a main effect of Group,  $F(3,35)=8.76$ ,  $p<.001$ ,  $LSD_{mmd}=53.29$ .

**Transfer Test**—Extinction with nornicotine transferred back to the nicotine CS (Figure 4, right panel). The ANOVA was significant,  $F(3,35)=4.13$ ,  $p=.013$ , with conditioned responding for the 0.4 mg/kg nicotine and 3 mg/kg nornicotine groups lower than saline ( $LSD_{mmd}=24.64$ ). Nicotine was not different from the 1 or 3 mg/kg nornicotine groups. Activity between groups did not differ on the transfer test,  $F<1$ .

### Experiment 5: Transfer of extinction with varenicline

**Discrimination Training**—Dipper entry rates stabilized by end of training with mean responding in the last 3 nicotine sessions ( $0.22 \pm 0.01$ ) higher than the last 3 saline sessions ( $0.05 \pm 0.003$ ). There was a main effect of Drug,  $F(1,49)=315.98$ ,  $p<.001$ , but no main effect of Session or Drug by Session interaction,  $F_s \leq 2.48$ ,  $p_s \geq .08$ . Activity on nicotine ( $0.47 \pm 0.02$ ) was higher than on saline ( $0.29 \pm 0.01$ ) in these later sessions. There was a main effect of Drug,  $F(1,53)=123.4$ ,  $p<.001$ , but no main effect of Session or Drug by Session interaction,  $F_s < 1$ .

**Extinction**—As shown in the left panel of Figure 5, conditioned responding varied by group across extinction sessions. There was a main effect of Group,  $F(4,45)=32.47$ ,  $p<.001$ , Session,  $F(5,225)=62.17$ ,  $p<.001$ , and a Group by Session interaction,  $F(20,225)=4.97$ ,  $p<0.001$ . Dipper entries were higher for the 0.4 mg/kg nicotine group than the other groups on all six extinction sessions ( $LSD_{mmd}=19.03$ ). Further, 0.1 and 1 mg/kg varenicline evoked higher responding than saline across the six extinction sessions, whereas 3 mg/kg varenicline evoked more dipper entries than saline only on the first day of extinction. For activity, there was a main effect of Group,  $F(4,45)=12.76$ ,  $p<.001$ , a main effect of Session,  $F(5,225)=6.01$ ,  $p<.001$ , and a Group by Session interaction,  $F(20,225)=2.87$ ,  $p<.001$ . Given the effects of varenicline on activity we show the mean activity counts during extinction and testing in Table 4. Activity in the 0.4 mg/kg nicotine group was higher than the other groups throughout extinction except for the 1 mg/kg varenicline group which did not differ from nicotine on sessions 2 and 5 ( $LSD_{mmd}=32.73$ ). Further, activity on 1 mg/kg varenicline was significantly elevated relative to 0.4 mg/kg nicotine on the first extinction day. Notably, repeated testing with 3 mg/kg varenicline decreased activity relative to saline on sessions 3, 4, and 6 suggesting that motor impairment may be interfering with dipper entries (i.e., the CR) at this dose.

**Transfer Test**—Extinction with varenicline transferred back to the nicotine CS (Figure 5, right panel). The one-way ANOVA was significant,  $F(4,45)=10.40$ ,  $p<.001$ . Subsequent contrasts indicated that responding on the 0.4 mg/kg nicotine group was lower than saline, 0.1, and 1 mg/kg varenicline, but not the 3 mg/kg varenicline group ( $LSD_{mmd}=28.14$ ). Dipper entries for the saline group were higher than the 0.1 and 3 mg/kg varenicline groups (i.e., transfer of extinction). In comparison to saline, activity was decreased on the transfer test for the group that had 3 mg/kg varenicline in the extinction phase,  $F(4,45)=3.79$ ,  $p=.01$ ,  $LSD_{mmd}=48.15$ . Thus, the decreased dipper entries on the transfer test for this group likely reflects a motor impairing effect of repeated daily treatment with the high dose varenicline rather than transfer of extinction.

## DISCUSSION

In the first experiment, we selected nAChR agonists that differ in their affinity to receptor subtypes (see Table 1), but all at least partially substitute for nicotine using a two-lever discrimination task [see Smith and Stolerman (2009) and Wooters et al., (in press) for reviews]. We essentially replicated these findings in Experiment 1, with perhaps some minor and potentially interesting differences (see later). All ligands tested evoked a goal-tracking CR statistically comparable to the 0.4 mg/kg training dose of nicotine. Full substitution in brief 4-min extinction tests was seen for ABT-418 at 0.6 and 1 mg/kg, nornicotine at 3 and 6 mg/kg, epibatidine at 2 µg/kg, varenicline at 1 and 3 mg/kg, and cytosine at 1 mg/kg. This substitution reflects overlap in stimulus effects with nicotine and not merely dissimilarity with saline (i.e., a drug versus no-drug discrimination). If the latter possibility was the case, then any substance that has perceptible stimulus effects should evoke a robust conditioned response. Past research is inconsistent with this prediction and suggests that discrimination performance is specific to the nicotine stimulus. For instance, doses of atomoxetine or amphetamine that has demonstrable behavioral effects evoke little to no conditioned responding in nicotine CS-trained rats (Besheer et al., 2004; Reichel et al., 2007).

Similar to Experiment 1, full substitution for the nicotine stimulus in rats has been reported for ABT-418 (Damaj et al., 1995), nornicotine (Goldberg et al., 1989), and epibatidine (Damaj et al., 1994) using the two-lever discrimination procedure and brief extinction tests (2 to 5 min) to assess generalization. In contrast, published studies have only reported partial substitution by varenicline and cytosine for the discriminative stimulus effects of nicotine. For example, 0.3, 1, 3, and 5 mg/kg varenicline produced 38–63% of nicotine-appropriate responding for rats trained to discriminate 0.4 mg/kg nicotine from saline on a two-lever task (LaSage et al., 2009; Smith et al., 2007). In these reports, cytosine at 1 and 3 mg/kg produced partial substitution (21–56% nicotine-appropriate responding), with evidence of response impairment at the higher dose [see Chandler and Stolerman (1997) and Stolerman et al., (1984) for additional examples of partial substitution with cytosine]. This difference in substitution may indicate a differential role for the  $\alpha 4\beta 2^*$  nAChRs in the stimulus effects of nicotine controlling behavior in the two different tasks. Similar dissociations involving the glutamate and the cannabinoid systems also exist (Murray and Bevins, 2007a; Murray et al., 2009), warranting further research on these neuropharmacological differences.

The finding of complete generalization from substitution tests is taken to indicate that the ligand is ‘nicotine like’ (see Smith and Stolerman, 2009; Wooters et al., in press). The present research was designed to test the extent to which these ligands were ‘nicotine like’ through extended extinction and whether the extinction would transfer back to the nicotine CS. To our knowledge this research is the first to assess extinction of stimulus control of responding by a drug state using ligands that appear to share stimulus properties with the training drug. The findings challenge the conclusions from more traditional generalization tests.

Conclusions regarding the ‘nicotine like’ effects of each ligand were changed by the outcome of the first 20-min extinction session. For instance, ABT-418 at a dose that fully substituted for the nicotine stimulus in the brief generalization test of Experiment 1 (i.e., 0.6 mg/kg) only showed partial substitution in a 20-min extinction session; the lower dose did not generalize to nicotine when using total responding across the 20 min. We replicated the results of Experiment 1 with ABT-418, nornicotine, and varenicline. For instance, in Experiment 3 ABT-418 at 0.3 mg/kg evoked a partial CR in the first 2 min of the test (mean of  $0.093 \pm 0.021$  dipper entries per sec versus saline at  $0.046 \pm 0.006$ ). The CR evoked by 0.6 mg/kg ABT-418 ( $0.202 \pm 0.026$ ) was comparable to nicotine ( $0.237 \pm 0.037$ ) at the start of the extinction session. A somewhat different pattern was found with nornicotine (Experiment 4) and varenicline (Experiment 5) on the first day of extinction. The test doses of these ligands evoked a partial

CR when assessing substitution in a 20-min session, regardless of whether the dose partially or fully substituted for nicotine in the brief generalization test. In short, when there is some experience with non-reinforcement ABT-418, nornicotine, and varenicline, at best, share only partial stimulus effects with nicotine.

Notably, nornicotine (1 and 3 mg/kg) and varenicline (0.1 and 1 mg/kg) continued to partially substitute for the nicotine stimulus on the subsequent 5 extinction sessions. ABT-418, in contrast, did not evoke a CR after the first day of extinction. Thus, with repeated extinction, the neuropharmacological processes controlling substitution by ABT-418 differ from varenicline or nornicotine either qualitatively or quantitatively. Although the present study was not designed to dissociate these two possibilities, it is clear that having some experience with non-reinforcement had a larger impact on responding to ABT-418 than either varenicline or nornicotine. Thus, conclusions regarding how 'nicotine like' these ligands are changed once assessed across repeated extinction sessions. Nornicotine and varenicline appear to share some longer-term stimulus effects with nicotine, but ABT-418 does not.

Since the weakening of conditioned responding produced by the non-reinforced presentations of a previously excitatory CS reflects the acquisition of new learning that competes or interferes in some way with previous learning (Bevins et al., 1999; Bouton, 1991, 2002; Davis and Myers, 2002; Delamater, 2004; Pavlov, 1927; Quirk and Mueller, 2008; Rauhut et al., 2001; Rescorla, 1997, 2001), transfer tests assessed whether extinction learning that occurred with the substitution ligand would generalize back to the nicotine training stimulus. Extinction learning involving nornicotine and varenicline transferred to the nicotine stimulus as evidenced by a reduced CR relative to controls that received saline during the extinction phase. This transfer was not complete as conditioned responding was still above nicotine controls in the groups that received extinction with nornicotine or varenicline—excluding the 3 mg/kg varenicline group that showed a non-specific motor effect. There was no effect of receiving ABT-418 in extinction on subsequent responding to nicotine on the transfer test. Taken together, these findings suggest that continued expression of a CR may be required for extinction learning and its transfer. This conclusion regarding the importance of response expression to extinction is consistent with most of the Pavlovian conditioning research using exteroceptive stimuli (e.g., Rescorla, 1993, 2001; Robleto et al., 2004).

Extinction with 0.05 mg/kg nicotine produced a partial goal-tracking CR in the first 5 of the 6 extinction sessions. Further, there was a tendency for decreased conditioned responding in this group when tested with 0.4 mg/kg nicotine in the extinction transfer test (see Figure 2). The 0.05 mg/kg dose was selected because it consistently evokes a partial goal-tracking CR in brief generalization tests using 0.4 mg/kg nicotine as the training dose (see Experiment 1; Murray and Bevins, 2007a, 2007b; Struthers et al., 2009). Further, the ED<sub>50</sub> for this training dose of nicotine was 0.031 mg/kg in the present study, but in past work the ED<sub>50</sub> has typically been higher. For instance, the ED<sub>50</sub> was 0.050 mg/kg in Murray and Bevins (2007a), 0.079 mg/kg in Murray and Bevins (2007b), and 0.059 and 0.05 mg/kg in Struthers et al., (2009). Thus, a dose of nicotine near the ED<sub>50</sub> appears to be at the cusp of effective for supporting extinction learning for the 0.4 mg/kg nicotine stimulus. Future work will need to parametrically vary the extinction dose of nicotine to better define the threshold value for the current experimental parameters.

Of import, conclusions regarding the shared stimulus properties of ligands clearly depend on the test—brief extinction differs from extended extinction, which differs from repeated extinction and generalization tests of extinction learning. Whether similar observations would be made in other drug discrimination procedures beside the DGT task will need to await further research. Such discrimination procedures of interest include the widely used two-lever discrimination task described earlier (e.g., Smith and Stolerman, 2009), as well as the

discriminated taste aversion procedure (e.g., Skinner et al., 1998) and an occasion setting version of the DGT task used in the present study (e.g., Palmatier et al., 2005).

In that occasion setting variant of the DGT procedure, a brief light CS is added to the drug and no-drug training sessions. On nicotine sessions the light CS immediately precedes sucrose access; the same light is presented on saline sessions, but sucrose is not delivered in those sessions. The goal-tracking CR is isolated to the light CS only on nicotine session (Palmatier et al., 2005). In this variant of the procedure, nicotine serves as a Pavlovian feature or occasion setter [see Bevins (2009) for a review]. The ability of nicotine trained in this manner to facilitate conditioned responding evoked by the discrete exteroceptive CS is not altered by repeated non-reinforced presentations (i.e., extinction) of nicotine alone (Palmatier and Bevins, 2007). This outcome clearly differs from the present study where nicotine is considered more of a contextual CS that enters into direct association with the sucrose and is clearly susceptible to extinction. Given this observation, there is no reason to expect that repeated non-reinforced presentation of a ligand that shares stimulus effects with nicotine will have any impact on light-evoked CR in the presence of the nicotine occasion setter. Perhaps the nicotine discriminative stimulus in the two-lever discrimination task will be more like the nicotine occasion setter than the CS. Whichever is the case, we should note that it should be possible to degrade the occasion setting function of nicotine by presenting the light CS without sucrose in the presence of a 'nicotine-like' ligand. This possibility has not been tested.

In a recent paper, Jed Rose (2009) suggested that a less-considered mechanism by which pharmacotherapies such as varenicline or nicotine replacement may work is by decreasing the reinforcing or rewarding value of cigarettes. Although a complete description of his theory is beyond the scope of this discussion, it relies on the idea that these pharmacotherapies given before quitting smoking reduce the reinforcing effects nicotine thus creating a disjoint between smoking, cigarettes, and reinforcement. Albeit speculative, we would add that this disjoint created by the pharmacotherapy may also result in partial extinction of conditioned rewarding value that the nicotine stimulus has acquired over years by being paired with such appetitive stimuli as food, drink, social interactions, work breaks, relief from stress, etc. (cf. Alessi et al., 2002). Notably, this Pavlovian extinction would continue upon smoking cessation. Perhaps drug development targeting this aspect of the addiction process will discover new targets and increase the efficacy of treatment.

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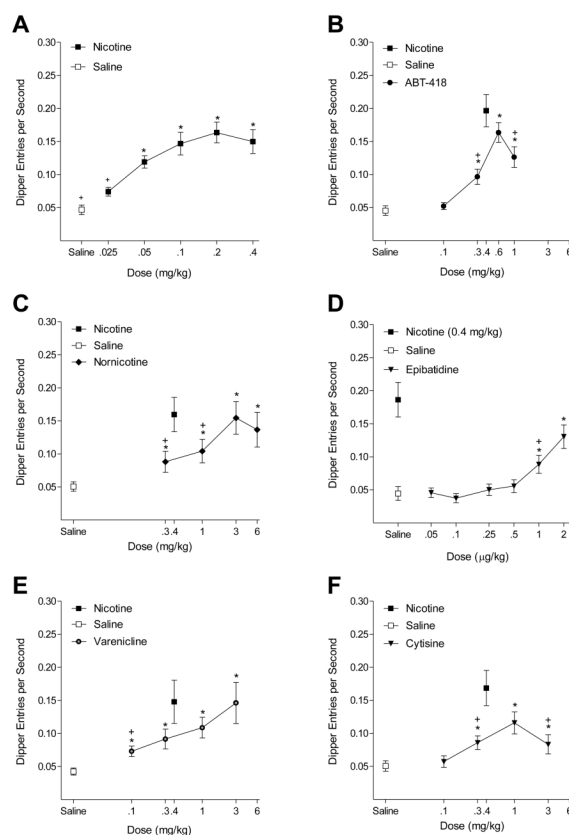
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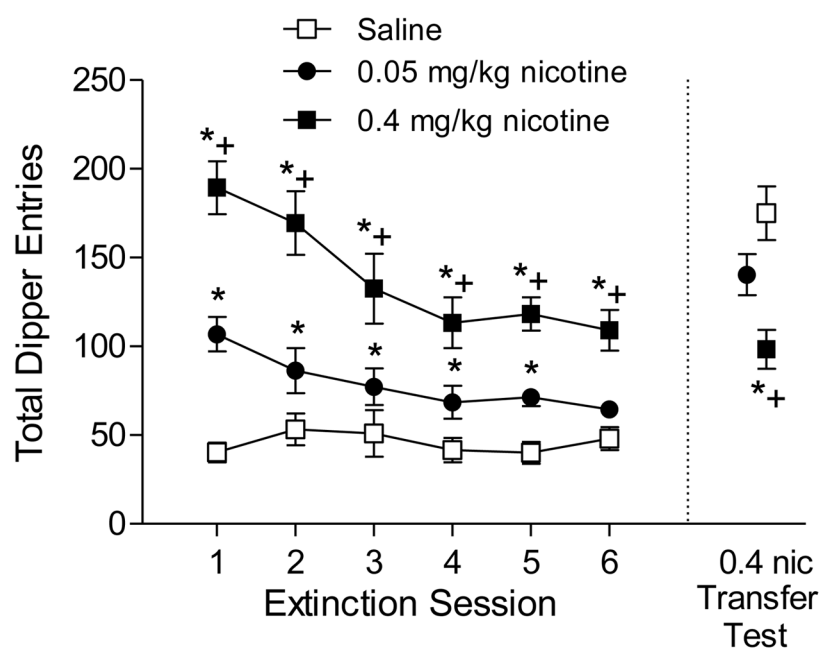


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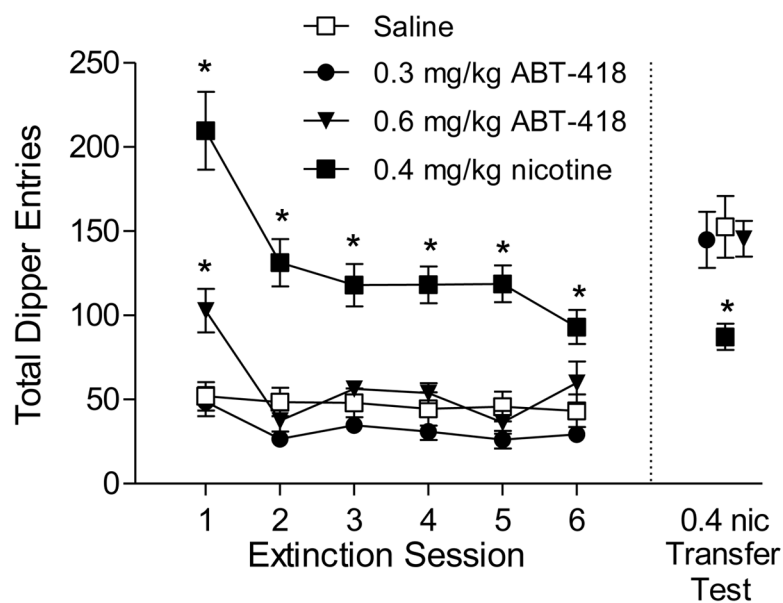
**Figure 1.**

Mean dipper entries ( $\pm 1$  SEM) during nicotine generalization testing (A) and substitution testing with ABT-418 (B), nornicotine (C), epibatidine (D), varenicline (E), and cytosine (F).

\* Significant difference from saline. + Significant difference from 0.4 mg/kg nicotine.

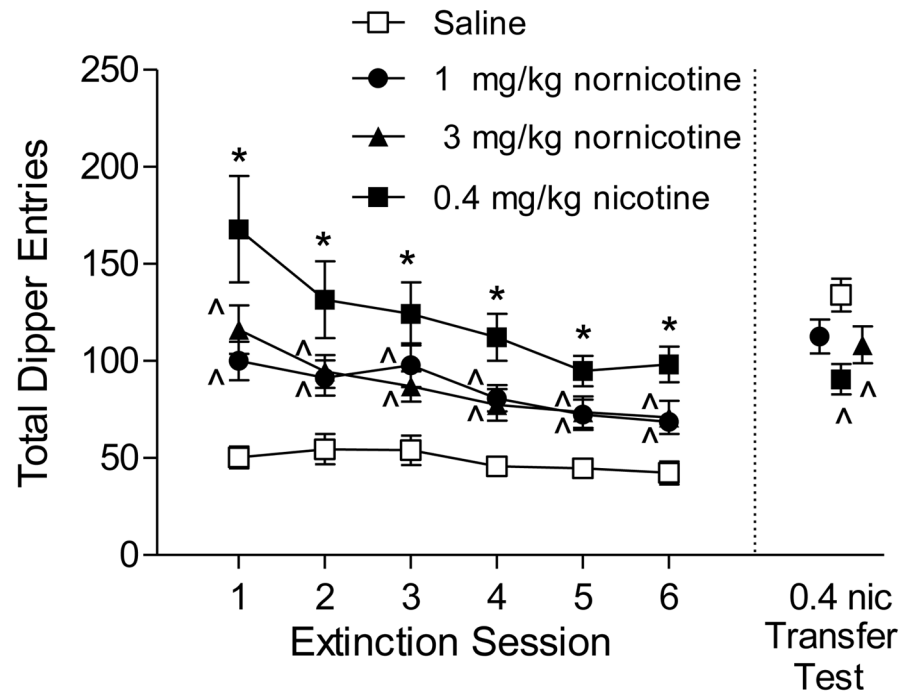


**Figure 2.** Mean  $\pm$  SEM dipper entries during extinction training with nicotine and the transfer of associative substitution test. \* Significant difference from saline. + Significant difference from 0.05 mg/kg nicotine.



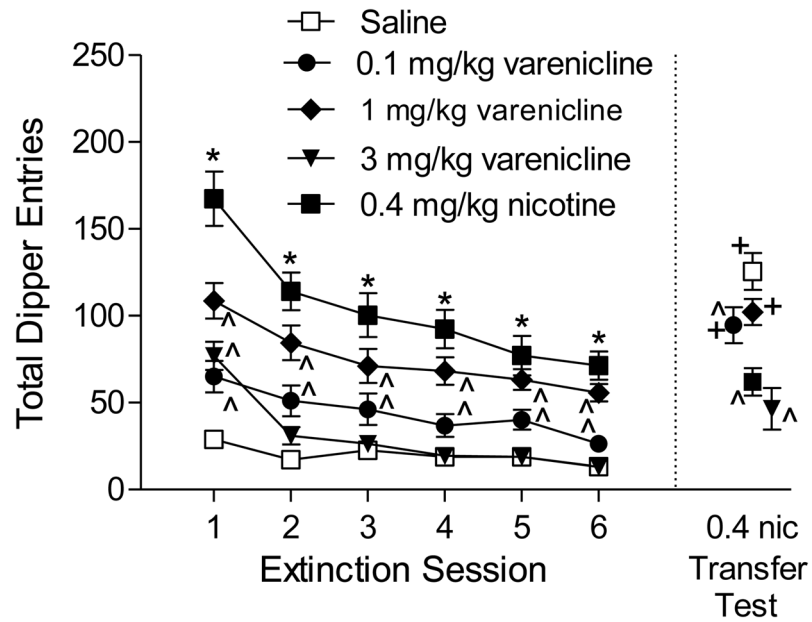
**Figure 3.**

Mean  $\pm$  SEM dipper entries during extinction training with ABT-418 and the transfer of associative substitution test. \* Significant difference from all other compounds.



**Figure 4.**

Mean  $\pm$  SEM dipper entries during extinction training with nornicotine and the transfer of associative substitution test. \* Significant difference from all other compounds. ^ Significant difference from saline.



**Figure 5.**

Mean  $\pm$  SEM dipper entries during extinction training with varenicline and the transfer of associative substitution test. \* Significant difference from all other compounds. ^ Significant difference from saline. + Significant difference from 0.4 mg/kg nicotine.



**Table 1**

Vehicles and receptor specificity for compounds.

Compound	Vehicle	Receptor Subtype Affinity ( $K_i$ /nM)
Nicotine	saline, pH to 7.0 $\pm$ .2 using NaOH	$\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 3\beta 2 > \alpha 7$ (Hahan <i>et al</i> 2003)
ABT-418	saline	$\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 3\beta 2 > \alpha 7$ (Hahan <i>et al</i> 2003)
Nornicotine	saline	$\alpha 6/3\beta 2\beta > \alpha 7 > \alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 4\beta 2\text{ha}5 > \alpha 3\beta 3\beta 3 > \alpha 3\beta 2$ (Papke <i>et al</i> 2007)
Epibatidine	H <sub>2</sub> O	$\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 3\beta 2 > \alpha 7$ (Hahan <i>et al</i> 2003)
Varenicline	saline	$\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 7$ (Smith <i>et al</i> 2007)
Cytisine	saline	$\alpha 4\beta 2 > \alpha 3\beta 4 > \alpha 7$ (Smith <i>et al</i> 2007)

**Table 2**

Order of substitution testing phases for Experiment 1.

Test Type <sup>*</sup>	Injection	Doses (mg/kg) <sup>‡</sup>	IPI <sup>#</sup>
Generalization	Nicotine	0, 0.025, 0.05, 0.1, 0.2, 0.4	5 min
Substitution	ABT-418	0, 0.1, 0.3, 0.6, 1	5 min
	nicotine <sup>‡</sup>	0.4	5 min
Substitution	Nornicotine	0, 0.3, 1, 3, 6	15 min
	nicotine <sup>‡</sup>	0.4	5 min
Substitution	Epibatidine	0, 0.00005, 0.0001, 0.00025, 0.0005, 0.001, 0.002	10 min
	nicotine <sup>‡</sup>	0.4	5 min
Substitution	Varenicline	0, 0.1, 0.3, 1, 3	30 min
	nicotine <sup>‡</sup>	0.4	5 min
Substitution	Cytisine	0, 0.1, 0.3, 1, 3	15 min
	nicotine <sup>‡</sup>	0.4	5 min

\* All tests within each phase are randomly intermixed. One phase is completed before the next begins.

<sup>‡</sup> Epibatidine (μg/kg)

<sup>#</sup> injection-to-placement interval

<sup>‡</sup> Baseline response levels were tested for comparison purposes.

Table 3

Activity data for Experiment 1.

	Nicotine	Saline	Generalization or Substitution				
Nicotine (mg/kg)			0.025	0.05	0.1	0.2	
	0.43 ± 0.3 <sup>^</sup>	0.32 ± 0.3	0.32 ± 0.2	0.37 ± 0.2	0.43 ± 0.3 <sup>^</sup>	0.50 ± 0.3 <sup>^</sup>	
ABT-418 (mg/kg)			0.1	0.3	0.6	1	
	0.46 ± 0.3	0.27 ± 0.3	0.26 ± 0.2	0.37 ± 0.2 <sup>^+</sup>	0.41 ± 0.4 <sup>^</sup>	0.45 ± 0.5 <sup>^</sup>	
Nomicotine (mg/kg)			0.3	1	3	6	
	0.44 ± 0.5	0.29 ± 0.2	0.30 ± 0.3	0.34 ± 0.3	0.36 ± 0.4	0.29 ± 0.2	
Epibatidine (µg/kg)			0.05	0.1	0.25	0.5	1 2
	0.37 ± 0.5	0.29 ± 0.4	0.22 ± 0.2	0.29 ± 0.3	0.26 ± 0.3	0.29 ± 0.3	0.35 ± 0.3 0.36 ± 0.2
Varenicline (mg/kg)			0.1	0.3	1	3	
	0.43 ± 0.3	0.30 ± 0.4	0.32 ± 0.3	0.32 ± 0.4	0.43 ± 0.4 <sup>^</sup>	0.38 ± 0.4	
Cytisine (mg/kg)			0.1	0.3	1	3	
	0.43 ± 0.5	0.33 ± 0.3	0.36 ± 0.4	0.30 ± 0.2	0.36 ± 0.4	0.26 ± 0.2	

<sup>^</sup> Significantly different than saline<sup>+</sup> Significantly different than nicotine

**Table 4**

Activity data from associative extinction and testing in Experiment 5.

Group	Extinction Session					
	1	2	3	4	5	6
Saline	198.4±14.9 <sup>+</sup>	175.5±10.7 <sup>+</sup>	197.6±18.9 <sup>+</sup>	224.2±20.1 <sup>+</sup>	181.4±15.1 <sup>+</sup>	189.9±22.0 <sup>+</sup>
0.4 mg/kg nicotine	274.6±13.6 <sup>^</sup>	274.6±15.5 <sup>^</sup>	271.0±9.2 <sup>^</sup>	272.1±11.0 <sup>^</sup>	262.4±10.7 <sup>^</sup>	256.3±8.7 <sup>^</sup>
0.1 mg/kg varenicline	224.1±20.6 <sup>+</sup>	206.8±18.4 <sup>+</sup>	209.6±22.8 <sup>+</sup>	212.9±20.6 <sup>+</sup>	210.2±20.7 <sup>+</sup>	232.5±13.8 <sup>+</sup>
1 mg/kg varenicline	327.6±16.2 <sup>+</sup>	244.7±16.1 <sup>^</sup>	225.5±19.1 <sup>+</sup>	222.0±17.4 <sup>+</sup>	239.9±14.9 <sup>^</sup>	213.7±14.6 <sup>+</sup>
3 mg/kg varenicline	187.8±12.2 <sup>+</sup>	151.6±9.8 <sup>+</sup>	162.0±13.4 <sup>+</sup>	156.6±14.4 <sup>+</sup>	159.8±12.4 <sup>+</sup>	149.1±14.9 <sup>+</sup>
						215.7±14.5 <sup>^</sup>

<sup>^</sup> Significantly different than saline

<sup>+</sup> Significantly different than nicotine