Published in final edited form as:

Psychopharmacology (Berl). 2015 July ; 232(14): 2443–2454. doi:10.1007/s00213-015-3878-1.

The role of varenicline on alcohol-primed self-administration and seeking behavior in rats

Patrick A. Randall¹, Anel A. Jaramillo^{1,2}, Suzanne Frisbee¹, and Joyce Besheer^{1,2,3}

¹Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599-7178, USA

²Curriculum in Neurobiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599-7178, USA

³Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599-7178, USA

Abstract

Rationale—Varenicline, a smoking-cessation agent, may be useful in treating alcohol use disorders. An important consideration when studying factors that influence drinking/relapse is influence of the pharmacological effects of alcohol on these behaviors. Pre-exposure to alcohol (priming) can increase craving, drinking and seeking behaviors.

Objectives—The primary goal of this work was to determine the effects of varenicline on alcohol-primed self-administration and seeking behavior in male Long Evans rats.

Methods—First, we assessed whether varenicline (0, 0.3, 1, 3 mg/kg, IP) has alcohol-like discriminative stimulus effects and whether varenicline alters sensitivity to alcohol in rats trained to discriminate a moderate alcohol dose (1 g/kg, IG) vs. water. Second, animals trained to self-administer alcohol underwent assessments to test the effects of: (i) varenicline (0, 0.3, 1, 3 mg/kg, IP) on self-administration, (ii) alcohol priming (0, 0.3, 1 g/kg, IG) on self-administration and seeking behavior, (iii) varenicline (1 mg/kg) in combination with alcohol priming (1 g/kg) on these behaviors.

Results—Varenicline did not substitute for alcohol, but disrupted the expression of sensitivity to alcohol. Varenicline decreased self-administration, but only at a motor impairing dose (3 mg/kg). Alcohol priming decreased self-administration and seeking behavior. Varenicline (1 mg/kg) blocked this effect under self-administration conditions, but not seeking conditions, which effectively resulted in increased alcohol intake.

Conclusions—These findings suggest the importance of further behavioral and mechanistic studies to evaluate the use of varenicline in treating alcohol use disorders and its potential impact on drinking patterns in smokers using varenicline as a smoking cessation aid.

Keywords

alcoholism; Chantix; ethanol; nicotine; priming; reinstatement; drinking

Introduction

Alcohol use disorders are a continuing world-wide public health issue. For this reason there is great interest in better understanding behaviors that lead to excessive drinking and developing effective treatments for reducing ongoing drinking and preventing relapse. To date, the US Food and Drug Administration (FDA)-approved treatments for alcohol dependence are naltrexone (opioid antagonist), disulfiram (aldehyde dehydrogenase inhibitor), and acamprosate (actions on GABA and glutamate). These compounds have different mechanisms of action and each have demonstrated efficacy in a limited subset of the patient population (Liang and Olsen 2014). However, there is continued emphasis on developing novel therapeutics and investigating potential novel pharmacological targets for the treatment of alcohol use disorders (AUDs).

One target that has been receiving increased attention is the nicotinic acetylcholine system. This is of particular interest given that clinical studies show high comorbidity between smoking and heavy drinking (~80% of heavy drinkers also smoke) and that heavy drinking may influence nicotine dependence (Chatterjee and Bartlett 2010; Dani and Harris 2005; Falk et al. 2006; Grant 1998; McKee et al. 2007). For this reason, there has been growing interest in the pharmacological compound varenicline for the treatment of AUDs. Varenicline is currently FDA-approved for use as a smoking cessation agent (Cahill et al. 2012), and is a partial nicotinic acetylcholine receptors (nAChRs) agonist at $\alpha 4\beta 2$, $\alpha 3\beta 2$ and $\alpha 6$ and a full agonist at $\alpha 3\beta 4$ and $\alpha 7$ nAChRs (Coe et al. 2005; Rollema et al. 2009). In clinical studies, varenicline has been shown to have efficacy in decreasing alcohol craving and consumption, particularly in smokers (Fucito et al. 2011; Mitchell et al. 2012). Additionally, evidence from human, nonhuman primates and rodent studies have shown that varenicline may also be useful in decreasing alcohol consumption and alcohol-seeking behaviors (Childs et al. 2012; Feduccia et al. 2014; Hendrickson et al. 2010; Kaminski and Weerts 2014; Litten et al. 2013; McKee et al. 2009; Mitchell et al. 2012; Steensland et al. 2007; Wouda et al. 2011). Importantly, varenicline appears to selectively attenuate alcohol-related behaviors as compared to naltrexone (Steensland et al. 2007).

An important consideration when studying factors that influence drinking and relapse is the influence of the pharmacological effects of alcohol on these behaviors, specifically the role of alcohol priming (e.g., alcohol pre-exposure leading to additional alcohol consumption or increased craving). Numerous clinical studies have demonstrated that alcohol priming not only increases craving in alcoholics (Hodgson et al. 1979; Ludwig and Wikler 1974), but also increases alcohol seeking behavior (Bigelow et al. 1977). Importantly, these effects are not limited to alcohol-dependent individuals. For example, non-dependent social drinkers report increased desire for alcohol following an alcohol priming dose (Kirk and de Wit 2000). In addition, non-alcoholics tend to choose alcohol over other types of reinforcement and show increased alcohol consumption following an alcohol priming dose (Chutuaape and

de Wit 1994; de Wit and Chutuape 1993; Hobbs et al. 2005). In preclinical animal models, an experimenter-administered priming dose of alcohol effectively reinstates alcohol-seeking behavior (Gass and Olive 2007; Le et al. 1999; Le et al. 1998; Vosler et al. 2001).

The effects of varenicline on alcohol-primed self-administration and alcohol seeking behavior have not been previously evaluated, which is important given the impact of priming cues on subsequent drug self-administration and seeking behavior. Therefore, the primary goal of this work was to determine the effects of varenicline on alcohol-primed behavior. Specifically, using operant self-administration methods, the effects of varenicline on maintenance of alcohol self-administration and alcohol-seeking behavior (under “probe-extinction” conditions) following alcohol priming injections were assessed. Interestingly, it has been suggested that drug-related priming effects may be related to the discriminative stimulus/interoceptive effects of the drug (Colpaert 1987; Colpaert et al. 1979; DiChiara and Reinhart 1995; Gerber and Stretch 1975; Jackson et al. 2003; Stolerman 1992; Stretch and Gerber 1973). For example, in self-administration models, drugs with similar discriminative stimulus effects as the self-administered drug may also prime drug seeking behavior (Anker and Carroll 2010; DiChiara and Reinhart 1995; Fattore et al. 2003; Gerber and Stretch 1975). Therefore, we first sought to determine whether varenicline has alcohol-like discriminative stimulus effects and whether varenicline pretreatment alters sensitivity to alcohol using standard drug discrimination methods. Rats were trained to discriminate a moderate alcohol dose (1 g/kg) from water. This alcohol training dose was used as it is comparable to alcohol intake achieved in the self-administration experiments. This assessment allows us to determine whether varenicline-induced changes in alcohol priming-related behavior may be related to altered sensitivity to the alcohol priming cue. Within the context of the existing literature showing varenicline-induced decreases in alcohol drinking and self-administration (as discussed above), we hypothesized that varenicline would prevent alcohol priming-induced increases in self-administration and seeking.

Materials and Methods

Animals

34 Long-Evans rats singly housed in ventilated cages were used for these studies. For the discrimination studies in Experiment 1, rats (n=12) were maintained at 325–340 g. For all the self-administration experiments, rats weighed 350–400 grams at the beginning of testing and had food and water available ad libitum in the home cage. The following is the breakdown of the rats used in the self-administration experiments (Experiments 2 (n=12), 3 (n=10), 4 (n=9), 5 (n=12) and 6 (n=9)). In an effort to reduce the number of rats required for this work, rats in Experiment 2 were also used in Experiment 5 and rats in Experiment 3 were also used in Experiments 4 and 6 (one rat was not included in these latter experiments due to inconsistent baseline performance). The design of studies and animal use is shown in Figure 1. The colony room was maintained on a 12 hour light/dark schedule (lights on at 07:00) with all experiments being conducted during the light portion of the schedule. Animals were under continuous care and monitoring by veterinary staff from the Division of Laboratory Animal Medicine (DLAM) at UNC-Chapel Hill. All procedures were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and institutional

guidelines. All protocols were approved by the UNC Institutional Animal Care and Use Committee (IACUC). UNC-Chapel Hill is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Behavioral Training Procedures

Alcohol discrimination training – See Figure 1 for timeline—Rats were trained using the same behavioral chambers and procedures as previous studies from this laboratory (Besheer et al. 2012; Besheer et al. 2014; Besheer et al. 2009). Briefly, immediately following intragastric gavage (IG) administration of alcohol (1 g/kg) or water rats were placed in the chambers. Following a 10 minute delay, both response levers were extended into the chamber (beginning of the 15 minute session). On alcohol sessions (i.e., following alcohol administration (1 g/kg, IG)), completion of a fixed ratio 10 (FR10) schedule of reinforcement (e.g., every 10th response) on the alcohol-appropriate lever resulted in presentation of sucrose. Similarly, on water sessions (i.e., following IG water administration), completion of an FR10 on the water-appropriate lever resulted in sucrose delivery. During both alcohol and water sessions, responding on the inappropriate lever was recorded but had no programmed consequence. Testing began once the following criteria were met: the percentage of alcohol- and water-appropriate responses emitted prior to the first reinforcer and during the entire session was >80% for 8/10 consecutive days.

Testing—Test sessions were similar to training sessions except that they were 2 minutes in duration (following 10 min delay) and completion of an FR10 on either lever resulted in sucrose reinforcement, allowing for assessment of the effects of treatment on overall response rate. Test sessions were interspersed with standard training sessions.

Alcohol self-administration training – See Figure 1 for timeline—Rats were trained using the same behavioral chambers and procedures as previous studies from this laboratory (Besheer et al. 2013; Besheer et al. 2010; Cannady et al. 2013), with the addition of infrared photobeams (that divided the chamber into 4 parallel zones) to measure locomotor activity during sessions (number of beam breaks). Self-administration sessions (30 min) took place 5 days per week (M-F) with both response levers remaining on a fixed ratio 2 (FR2) schedule of reinforcement such that every second response on either lever resulted in delivery of a corresponding reinforcement (i.e., alcohol or water). A sucrose fading procedure was used in which alcohol was gradually added to the 10% (w/v) sucrose solution. The exact order of exposure was as follows: 10% sucrose (w/v)/2% (v/v) alcohol (10S/2A), 10S/5A, 10S/10A, 5S/10A, 5S/15A, 2S/15A. There were two sessions at each concentration. Following sucrose fading, sweetened alcohol (2S/15A) and water continued as reinforcers for the remainder of the study.

Experimental Procedures (see Figure 1)

Experiment 1: Effects of varenicline on the discriminative stimulus effects of alcohol—To assess whether varenicline has alcohol-like discriminative stimulus effects, rats received varenicline (0, 0.3, 1, 3 mg/kg, IP; n=6) 30 min prior to the start of the discrimination test session. To determine whether varenicline alters the discriminative stimulus effects of the alcohol training dose (1 g/kg), rats received varenicline (0, 0.3, 1, 3

mg/kg, IP; n=6) 20 min prior to alcohol (1 g/kg, IG; i.e., 30 min before the start of the discrimination test session). Within each assessment, doses were assigned in a repeated measures design with each rat receiving all treatments in a randomized order, with at least two baseline sessions between testing days.

Experiment 2: Effects of varenicline on maintenance of alcohol self-

administration—To assess the effects of varenicline pretreatment on the maintenance of alcohol self-administration, on test days, rats received varenicline (0, 0.3, 1, 3 mg/kg, IP) 30 minutes prior to a self-administration session. Doses were assigned in a repeated measures design with each rat receiving all treatments in a randomized order, with at least two baseline sessions between testing days. At the conclusion of varenicline testing, the rats were tested to examine locomotor behavior independent of the self-administration procedure as described in Besheer et al. (2013). Rats received varenicline (0 or 3 mg/kg, IP) 30 min before placement in the locomotor activity chamber. Each rat experienced two 30-minute locomotor sessions in which the varenicline dose order was randomized. These locomotor assessments were interspersed with self-administration sessions with at least 3 days between tests. On the locomotor test days, self-administration sessions were withheld.

Experiment 3: Effects of alcohol priming on maintenance of alcohol self-

administration—To characterize the effects of alcohol priming on ongoing self-administration, rats received alcohol (0.3, 1.0 g/kg, IG) or water (IG) 10 minutes prior to a self-administration session. Doses were administered in a repeated measures design with each rat receiving each treatment in a randomized order, with at least two baseline sessions between testing days.

Experiment 4: Effects of varenicline on alcohol self-administration following priming

—To determine the effects of varenicline on alcohol-primed self-administration, rats received varenicline (0 or 1 mg/kg, IP) 20 minutes prior to the alcohol (0 or 1 g/kg, IG) injection and 10 minutes later underwent a self-administration session. Each rat received each treatment in a randomized order, with at least two baseline sessions between testing days.

Experiment 5: Effects of alcohol priming on alcohol-seeking behavior

—In order to assess the impact of alcohol priming on the resilience of alcohol seeking behavior under different conditions, rats were tested under “probe-extinction” conditions. For these tests, all cues were activated following completion of each FR2 (i.e., cue light illuminated, pump sound activated), however no reinforcers were delivered (i.e., no alcohol or water). On test days, rats received the alcohol priming dose (0, 0.3, 1 g/kg, IG) 10 minutes prior to the session. Rats received each treatment in a randomized order, with at least two baseline sessions between tests.

Experiment 6: Effects of varenicline on alcohol-seeking behavior following priming

—To determine the effects of varenicline on alcohol-primed seeking behavior, rats received varenicline (0 or 1 mg/kg, IP) 20 minutes prior to the alcohol (0 or 1 g/kg, IG) injection. 10 min later each rat underwent a “probe-extinction” session as described in

Experiment 5. Rats received each treatment in a randomized order, with at least two baseline sessions between testing days.

Drugs

Alcohol (95% w/v, Pharmaco-AAPER, Shelbyville, KY) and sucrose were diluted with distilled water for self-administration sessions. For the alcohol discrimination studies and for all priming injections in the self-administration studies, alcohol was diluted to 20% (v/v) with distilled water which also served as the water control. Varenicline tartrate (Abcam Pharmaceuticals, Cambridge, UK) was dissolved in 0.9% saline solution which also served as the vehicle control. Doses for Experiments 1 and 2 were selected based on previous studies (Ginsburg and Lamb 2013; LeSage et al. 2009; Panlilio et al. 2014). Doses for Experiments 4 and 6 were determined based on the outcome of Experiments 1 and 2. For priming and alcohol discrimination experiments, alcohol was administered via intragastric gavage (IG) with volume varied by rat weight to achieve desired dose. Varenicline was administered via intraperitoneal (IP) injection at a volume of 1 ml/kg.

Statistical Analyses

For the discrimination assessments (Experiment 1), alcohol-appropriate responses (prior to delivery of the first reinforcer) and response rates were analyzed with one-way repeated measures analysis of variance (RM ANOVA). For the self-administration experiments, alcohol intake (g/kg) was approximated based on body weight and number of reinforcements delivered. For all single treatment experiments (i.e., varenicline alone or alcohol prime alone), total alcohol responses, water responses, alcohol intake (g/kg) and total locomotion were analyzed with one-way repeated measures ANOVA. For experiments in which two treatment types were used (i.e., alcohol prime and varenicline), two-way repeated measures factorial ANOVA (with prime condition and varenicline dose as factors) was used. To assess changes in response patterns over time, multi-factor ANOVA was used with prime condition, varenicline condition and time point as factors. Post-hoc analysis (Tukey) was used to determine differences between specific treatment conditions. Statistical significance was determined at $p = 0.05$.

Results

Experiment 1: Effects of varenicline on the discriminative stimulus effects of alcohol

Varenicline administered alone had no alcohol-like discriminative stimulus effects, as alcohol-appropriate responses were below the substitution threshold (80%) and did not significantly differ from saline (Figure 2A). A significant effect on response rate was observed ($F[3,15]=3.60$, $p=0.04$; Figure 2B), however, posthoc analyses did not detect any significant differences. In contrast, varenicline pretreatment altered sensitivity to the discriminative stimulus effects of the alcohol training dose (1 g/kg). Repeated measures (RM) ANOVA showed a significant varenicline-induced decrease in alcohol-appropriate responses ($F[3,15] = 9.682$, $p < 0.01$, Figure 2C), with decreased alcohol-appropriate responses following 1.0 and 3.0 mg/kg varenicline doses as compared to saline ($p < 0.05$). Additionally, RM ANOVA showed a significant varenicline-induced reduction in response rate ($F[3,15] = 7.677$, $p < 0.01$, Figure 2D), at the highest varenicline dose (3.0 mg/kg) as

compared to saline ($p < 0.05$), indicating the likely disruption of motor behavior. Together, these results show that varenicline does not substitute for the discriminative stimulus effects of alcohol, and when administered prior to alcohol disrupts sensitivity to/alters the discriminative stimulus effects of a moderate alcohol dose (1 g/kg).

Experiment 2: Effects of varenicline on maintenance of alcohol self-administration

RM ANOVA showed a significant effect of varenicline pretreatment on total alcohol lever responses ($F[3,33] = 32.788$, $p < 0.001$, Figure 3A), alcohol intake (g/kg; $F[3,33] = 22.929$, $p < 0.001$, Table 2) and total locomotion ($F[3,33] = 11.775$, $p < 0.001$, Table 3). Post hoc analyses for each measure showed a significant reduction at the highest varenicline dose (3 mg/kg) relative to vehicle ($p < 0.05$). Analysis of alcohol responding across the session showed a main effect of time ($F[5,55] = 33.409$, $p < 0.01$, Figure 3B) and a varenicline dose by time interaction ($F[15,165] = 7.951$, $p < 0.01$). Post-hoc analysis (Tukey) showed that rats treated with 3.0 mg/kg varenicline responded significantly less on the alcohol lever in the first 10 minutes of the session compared to saline ($p < 0.05$). Varenicline did not produce any effects on water lever responses (Table 1). In the independent locomotion assessment, varenicline (3 mg/kg) significantly decreased locomotion (i.e., total distance traveled) compared to saline (mean $\pm$ S.E.M. beam breaks – saline: 2624.65 ± 152.44 ; varenicline: 1462.90 ± 179.66 ; $t[11] = 5.821$, $p < 0.001$).

Experiment 3: Effects of alcohol priming on maintenance of alcohol self-administration

RM ANOVA demonstrated that alcohol priming significantly decreased total alcohol lever responses ($F[2,18] = 3.703$, $p < 0.05$, Figure 4A) and alcohol intake (g/kg) ($F[2,18] = 6.407$, $p < 0.01$, Table 2), with a significant reduction in both measures following the highest alcohol priming dose (1 g/kg) compared to water pretreatment ($p < 0.05$). Analysis of alcohol responses across the session showed a significant main effect of time ($F[5,40] = 4.454$, $p < 0.01$, Figure 4B), but no alcohol dose by time interaction. Total water lever responses (Table 1) and total locomotion (Table 3) were not affected by alcohol priming. Interestingly, alcohol intake (g/kg) following the water prime was lower than baseline (Table 2), indicating that self-administration behavior was likely affected by the oral fluid administration. Indeed, previous studies have demonstrated that alcohol intake following oral administration of 2 ml of a subthreshold dose of alcohol decreases subsequent alcohol intake compared to a zero volume control (Czachowski et al. 2006).

Experiment 4: Effects of varenicline on maintenance of alcohol self-administration following priming

A two-way RM ANOVA showed a significant main effect of alcohol prime on alcohol responses ($F[1,8] = 17.898$, $p < 0.01$; Figure 5A), and alcohol intake ($F[1,8] = 13.620$, $p < 0.01$; Table 2), with a significant reduction in both measures following alcohol (1 g/kg) priming injection relative to water injection ($p < 0.05$). A significant main effect of varenicline treatment on alcohol responses ($F[1,8] = 8.713$, $p < 0.05$) and alcohol intake ($F[1,8] = 12.816$, $p < 0.01$; Table 2) was also observed, with significantly greater alcohol responding following varenicline (1 mg/kg) pretreatment relative to saline ($p < 0.05$; Figure 5A) and an alcohol priming-induced reduction in alcohol lever responses following vehicle pretreatment ($p < 0.05$; Figure 5A). Interestingly, the alcohol-priming effect was not observed

following varenicline pretreatment, suggesting that varenicline blocked or counteracted the effects of priming. Similar to the data pattern observed in Experiment 3, alcohol intake (g/kg) following the water prime was lower than baseline (Table 2). There were no effects on total water lever responses (Table 1) or total locomotion (Table 3).

Examination of the pattern of alcohol responses across the session by RM factorial ANOVA showed a significant decrease in alcohol responding across the session ($F[5,40] = 17.270$, $p < 0.01$, Figure 5B). There was no priming by varenicline treatment interaction on alcohol lever responses. Additionally, alcohol lever responses across time showed a greater decrease following alcohol priming (1 g/kg) compared to water injection ($F[1,32] = 6.042$, $p < 0.05$). Conversely, alcohol lever responses were higher across the session following varenicline treatment (1 mg/kg) compared to saline treatment ($F[1,32] = 11.392$, $p < 0.01$). There was also a time period by varenicline interaction ($F[5,40] = 3.355$, $p < 0.05$), with significantly greater responding in minutes 5–10 following varenicline treatment (1 mg/kg) vs. saline ($p < 0.05$).

Experiment 5: Effects of alcohol priming on alcohol-seeking behavior

Baseline alcohol intake (session prior to testing) was 0.80 ± 0.1 g/kg. Alcohol priming significantly reduced alcohol-seeking behavior as indicated by a RM ANOVA ($F[2,22] = 9.785$, $p < 0.001$, Figure 6A), with less alcohol lever responses compared to water ($p < 0.05$). Analysis of alcohol responses across the session showed a significant main effect of time ($F[5,55] = 21.966$, $p < 0.01$, Figure 6B) and an alcohol dose by time interaction ($F[10,110] = 3.257$, $p < 0.01$). Post-hoc analysis showed that rats primed with alcohol (0.3 and 1.0 g/kg) responded less in the first 5 minutes compared to water pretreatment. Priming had no effects on total water lever responses (Table 1) or total locomotion (Table 3).

Experiment 6: Effects of varenicline on alcohol-seeking behavior following priming

Baseline alcohol intake (session prior to testing) was 0.76 ± 0.1 g/kg. Two-way RM ANOVA showed a main effect of alcohol priming on total alcohol lever responses ($F[1,8] = 20.537$, $p < 0.05$, Figure 7A), with a significant reduction observed following alcohol (1 g/kg) compared to water ($p < 0.05$). Furthermore, there was a significant main effect of varenicline treatment on total alcohol lever responses ($F[1,8] = 5.999$, $p < 0.05$), with a significant increase following varenicline (1 mg/kg) compared to saline treatment ($p < 0.05$). There was no priming by varenicline treatment interaction on alcohol lever responses. Post-hoc analysis confirmed an alcohol priming-induced reduction in alcohol lever responses following both vehicle and varenicline pretreatment ($p < 0.05$). Analysis of response patterns across the probe-extinction session showed a reduction in alcohol lever responses over time ($F[5,40] = 8.356$, $p < 0.01$, Figure 7B). Furthermore, following alcohol priming, there was a greater reduction in lever pressing compared to water priming ($F[1,32] = 12.617$, $p < 0.05$). Conversely, lever pressing remained higher over time following varenicline treatment compared to saline ($F[1,32] = 5.109$, $p < 0.05$). There were no interactions between treatments or between treatments and time period. Total water lever responses (Table 1) and total locomotion (Table 3) was not affected by any treatment condition.

Discussion

The current experiments demonstrate several important findings. First, in the dose range tested, varenicline does not have alcohol-like effects similar to a moderate dose of alcohol (1 g/kg). However, varenicline is capable of disrupting the discriminative stimulus effects of alcohol (1 g/kg) at a dose that did not alter response rate (1 mg/kg; Figures 2C and D). Next, varenicline pretreatment decreased alcohol self-administration (Figure 3), but only at a high dose (3 mg/kg) that also reduced locomotion during the self-administration session (Table 3), in an independent assay, and as reflected by reduced response rate in the discrimination study (Figure 2D). Therefore, self-administration was not selectively reduced by varenicline pretreatment. Consistent with this finding, is other work in which a dose of 1.8 mg/kg varenicline or greater non-selectively decreased alcohol self-administration behavior (Ginsburg and Lamb 2013). The lack of a selective effect of varenicline on alcohol self-administration is in contrast to other operant alcohol self-administration studies that show a varenicline-induced decrease in self-administration (Steensland et al. 2007; Wouda et al. 2011). Several factors may contribute to these differential effects, including alcohol self-administration history (daily g/kg, duration of training), response requirements, and strain. Indeed, recently, varenicline has been shown to have differential effects on alcohol and food related-behaviors depending on the operant testing conditions (Ginsburg and Lamb 2014). Additionally, varenicline has also been shown to reduce voluntary alcohol drinking under home cage drinking conditions (Feduccia et al. 2014; Steensland et al. 2007); however, given the lack of a specific work requirement (i.e., lever press, nose poke) under those conditions, direct comparison to the present work is difficult. Lastly, we found that varenicline prevented an alcohol-primed decrease in self-administration (Figure 5), resulting in potentiated alcohol self-administration relative to vehicle pretreatment. In contrast, under “probe-extinction” conditions, the alcohol-primed decrease in alcohol-seeking behavior was unaffected by varenicline (Figure 7).

An important aspect of the current studies was to assess the role of alcohol priming under self-administration and alcohol seeking (“probe-extinction”) conditions. Alcohol priming (1 g/kg) reliably decreased alcohol self-administration (Experiment 3 and 4). These findings are in line with several previous studies that suggest that this effect is evidence for rats titrating intake based on the priming dose (Czachowski et al. 2003; Czachowski et al. 2006; Samson and Czachowski 2003). However, it is important to note, that even though self-administration is reduced following priming (1 mg/kg), the amount of total alcohol exposure following the self-administration session (which includes the priming dose), is greater than the water-primed rats (~1.3 g/kg vs. 0.6 g/kg, respectively). Interestingly, priming was more effective at decreasing behavior under alcohol seeking conditions (Experiment 5), as both alcohol doses tested (0.3 and 1 g/kg) similarly decreased seeking behavior (Figure 6). This suggests that alcohol seeking behavior is more sensitive than self-administration behavior to alcohol priming. Consistent with this finding, is work by Jackson et al. (2003) in which alcohol priming (0.5 g/kg) significantly decreased alcohol-seeking behavior during probe-extinction tests similar to the strategy incorporated in the present work. However, together, these findings are in contrast to those from reinstatement studies in which alcohol-seeking behavior is potentiated following alcohol priming at doses similar to those employed in the

present work (Le et al. 1999; Le et al. 1998; Samson and Chappell 2002). These different data patterns suggest that following extended periods of explicitly trained extinction and/or the consequent absence from alcohol (as employed in reinstatement studies), the functional impact of an alcohol priming cue to modulate behavior is increased compared to probe extinction trials as used in this work and by Jackson et al. (2003).

Interestingly, the consequences of varenicline pretreatment on alcohol priming varied under the different testing conditions. That is, under self-administration conditions (e.g., alcohol is available) varenicline blocked the effects of alcohol priming on self-administration, which resulted in potentiated self-administration relative to the vehicle control (i.e., self-administration at levels similar to the water-primed controls; Figure 5). In contrast, under seeking conditions (i.e., “probe-extinction”) varenicline pretreatment did not alter alcohol-primed seeking behavior (Figure 7). A plausible explanation for the potentiation of self-administration behavior is that varenicline decreased sensitivity to the interoceptive effects of the alcohol priming dose, such that the priming cue no longer effectively regulated behavior. Indeed, this potential explanation is supported by the findings of Experiment 1. This disruption in sensitivity to alcohol may be related to varenicline having interoceptive effects of its own that compete with alcohol. Indeed, varenicline has been shown to have methamphetamine-like discriminative stimulus effects (Desai and Bergman 2014). Furthermore, Ginsburg and Lamb (2013) demonstrate that varenicline (1 mg/kg) increases alcohol self-administration, potentially through stimulant-like effects. However, this explanation cannot account for the lack of effect of varenicline pretreatment under seeking conditions.

In order to better understand the differences between the effects of varenicline on self-administration and seeking conditions, response patterns across the session were examined. Under self-administration conditions (Experiment 4), a significant varenicline-induced increase in alcohol responses occurred early in the session (Figure 5B), consistent with previous work (Ginsburg and Lamb 2013). In contrast, under seeking conditions (Experiment 6) no varenicline-induced increase in responding was observed at any point during the session, although a trend was observed early in the session (Figure 7B). These differences in response patterns may, in part, be explained by different testing conditions. The combination of varenicline, alcohol priming, and the availability of alcohol in Experiment 4 may have led to the appropriate conditions under which motivation for alcohol-reinforced lever pressing was enhanced. In contrast, alcohol was not available in Experiment 6 (i.e., extinction session), potentially contributing to the decrease in responding observed. These findings demonstrate the importance of testing conditions in evaluating the effects of priming and the role of varenicline.

In the present work, the effects of a single varenicline dose to modulate a single alcohol priming dose were assessed. The varenicline dose was selected based on the results of the initial self-administration experiment (Experiment 2), in that it was necessary to select a dose that did not negatively impact motor behavior. The alcohol priming dose was selected based on the finding that pretreatment with a moderate alcohol dose (1 g/kg) effectively reduced subsequent self-administration and alcohol-seeking behavior (Experiments 3 and 5, respectively). However, it will be informative for future work to examine a dose range of

both compounds. Additionally, the current experiments were undertaken in a non-dependence model of alcohol intake. Varenicline has been shown to be effective at reducing alcohol consumption in individuals that have been heavy drinkers for many years (for review see (Erwin and Slaton 2014)); therefore, it will be interesting to examine the effects of varenicline on operant self-administration under conditions of dependence. Moreover, the behavioral profile of varenicline in the present study (i.e., lack of selective effect of varenicline on operant alcohol self-administration, disruption of sensitivity to the discriminative stimulus effects of alcohol, and reduced ability of alcohol priming to appropriately regulate self-administration behavior), suggests that varenicline may actually present a risk factor for non-dependent drinkers (e.g., social drinkers) using varenicline as a smoking cessation aid. Interestingly, one of the label indications for varenicline is the recommendation that individuals prescribed varenicline to quit smoking reduce their alcohol consumption while taking the drug. As such, further mechanistic and behavioral studies are needed to characterize the therapeutic value of varenicline in the treatment of alcohol use disorders, and also the potential impact on drinking patterns in social drinkers, especially those using varenicline as a smoking cessation aid.

Acknowledgments

This work was supported, in part, by funds from the National Institutes of Health AA019682 (JB) and the Bowles Center for Alcohol Studies.

References

- Anker JJ, Carroll ME. Reinstatement of cocaine seeking induced by drugs, cues, and stress in adolescent and adult rats. *Psychopharmacology (Berl)*. 2010; 208:211–22. [PubMed: 19953228]
- Besheer J, Fisher KR, Cannady R, Grondin JJ, Hodge CW. Intra-amygdala inhibition of ERK(1/2) potentiates the discriminative stimulus effects of alcohol. *Behav Brain Res*. 2012; 228:398–405. [PubMed: 22209853]
- Besheer J, Fisher KR, Jaramillo AA, Frisbee S, Cannady R. Stress hormone exposure reduces mGluR5 expression in the nucleus accumbens: functional implications for interoceptive sensitivity to alcohol. *Neuropsychopharmacology*. 2014; 39:2376–86. [PubMed: 24713611]
- Besheer J, Fisher KR, Lindsay TG, Cannady R. Transient increase in alcohol self-administration following a period of chronic exposure to corticosterone. *Neuropharmacology*. 2013; 72:139–47. [PubMed: 23643750]
- Besheer J, Grondin JJ, Cannady R, Sharko AC, Faccidomo S, Hodge CW. Metabotropic glutamate receptor 5 activity in the nucleus accumbens is required for the maintenance of ethanol self-administration in a rat genetic model of high alcohol intake. *Biol Psychiatry*. 2010; 67:812–22. [PubMed: 19897175]
- Besheer J, Grondin JJ, Salling MC, Spanos M, Stevenson RA, Hodge CW. Interoceptive effects of alcohol require mGlu5 receptor activity in the nucleus accumbens. *J Neurosci*. 2009; 29:9582–91. [PubMed: 19641121]
- Bigelow GE, Griffiths RR, Liebson IA. Pharmacological influences upon human ethanol self-administration. *Adv Exp Med Biol*. 1977; 85B:523–38. [PubMed: 596286]
- Cahill K, Stead LF, Lancaster T. Nicotine receptor partial agonists for smoking cessation. *Cochrane Database Syst Rev*. 2012; 4:CD006103. [PubMed: 22513936]
- Cannady R, Fisher KR, Durant B, Besheer J, Hodge CW. Enhanced AMPA receptor activity increases operant alcohol self-administration and cue-induced reinstatement. *Addict Biol*. 2013; 18:54–65. [PubMed: 23126443]

- Chatterjee S, Bartlett SE. Neuronal nicotinic acetylcholine receptors as pharmacotherapeutic targets for the treatment of alcohol use disorders. *CNS Neurol Disord Drug Targets*. 2010; 9:60–76. [PubMed: 20201817]
- Childs E, Roche DJ, King AC, de Wit H. Varenicline potentiates alcohol-induced negative subjective responses and offsets impaired eye movements. *Alcohol Clin Exp Res*. 2012; 36:906–14. [PubMed: 22339626]
- Chutuaape MA, de Wit H. Relationship between subjective effects and drug preferences: ethanol and diazepam. *Drug Alcohol Depend*. 1994; 34:243–51. [PubMed: 8033763]
- Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollemma H, Lu Y, Mansbach RS, Chambers LK, Rovetti CC, Schulz DW, Tingley FD 3rd, O'Neill BT. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem*. 2005; 48:3474–7. [PubMed: 15887955]
- Colpaert FC. Drug discrimination in behavioral toxicology. *Zentralbl Bakteriol Mikrobiol Hyg B*. 1987; 185:48–51. [PubMed: 3124387]
- Colpaert FC, Niemegeers CJ, Janssen PA. Discriminative stimulus properties of cocaine: neuropharmacological characteristics as derived from stimulus generalization experiments. *Pharmacol Biochem Behav*. 1979; 10:535–46. [PubMed: 37526]
- Czachowski CL, Legg BH, Samson HH. Assessment of sucrose and ethanol reinforcement: the across-session breakpoint procedure. *Physiol Behav*. 2003; 78:51–9. [PubMed: 12536010]
- Czachowski CL, Prutzman S, DeLory MJ. Volume and dose effects of experimenter-administered ethanol preloads on ethanol seeking and self-administration. *Alcohol*. 2006; 40:35–40. [PubMed: 17157718]
- Dani JA, Harris RA. Nicotine addiction and comorbidity with alcohol abuse and mental illness. *Nat Neurosci*. 2005; 8:1465–70. [PubMed: 16251989]
- de Wit H, Chutuaape MA. Increased ethanol choice in social drinkers following ethanol preload. *Behav Pharmacol*. 1993; 4:29–36. [PubMed: 11224168]
- Desai RI, Bergman J. Methamphetamine-like discriminative-stimulus effects of nicotinic agonists. *J Pharmacol Exp Ther*. 2014; 348:478–88. [PubMed: 24389640]
- DiChiara TJ, Reinhart PH. Distinct effects of Ca²⁺ and voltage on the activation and deactivation of cloned Ca(2+)-activated K⁺ channels. *J Physiol*. 1995; 489 (Pt 2):403–18. [PubMed: 8847636]
- Erwin BL, Slaton RM. Varenicline in the treatment of alcohol use disorders. *Ann Pharmacother*. 2014; 48:1445–55. [PubMed: 25095786]
- Falk DE, Yi HY, Hiller-Sturmhofel S. An epidemiologic analysis of co-occurring alcohol and tobacco use and disorders: findings from the National Epidemiologic Survey on Alcohol and Related Conditions. *Alcohol Res Health*. 2006; 29:162–71. [PubMed: 17373404]
- Fattore L, Spano MS, Cossu G, Deiana S, Fratta W. Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. *Eur J Neurosci*. 2003; 17:1723–6. [PubMed: 12752390]
- Feduccia AA, Simms JA, Mill D, Yi HY, Bartlett SE. Varenicline decreases ethanol intake and increases dopamine release via neuronal nicotinic acetylcholine receptors in the nucleus accumbens. *Br J Pharmacol*. 2014; 171:3420–31. [PubMed: 24628360]
- Fucito LM, Toll BA, Wu R, Romano DM, Tek E, O'Malley SS. A preliminary investigation of varenicline for heavy drinking smokers. *Psychopharmacology (Berl)*. 2011; 215:655–63. [PubMed: 21221531]
- Gass JT, Olive MF. Reinstatement of ethanol-seeking behavior following intravenous self-administration in Wistar rats. *Alcohol Clin Exp Res*. 2007; 31:1441–5. [PubMed: 17666107]
- Gerber GJ, Stretch R. Drug-induced reinstatement of extinguished self-administration behavior in monkeys. *Pharmacol Biochem Behav*. 1975; 3:1055–61. [PubMed: 817297]
- Ginsburg BC, Lamb RJ. Effects of varenicline on ethanol- and food-maintained responding in a concurrent access procedure. *Alcohol Clin Exp Res*. 2013; 37:1228–33. [PubMed: 23413834]
- Ginsburg BC, Lamb RJ. Relative potency of varenicline or fluvoxamine to reduce responding for ethanol versus food depends on the presence or absence of concurrently earned food. *Alcohol Clin Exp Res*. 2014; 38:860–70. [PubMed: 25396255]

- Grant BF. Age at smoking onset and its association with alcohol consumption and DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse*. 1998; 10:59–73. [PubMed: 9720007]
- Hendrickson LM, Zhao-Shea R, Pang X, Gardner PD, Tapper AR. Activation of $\alpha 4^*$ nAChRs is necessary and sufficient for varenicline-induced reduction of alcohol consumption. *J Neurosci*. 2010; 30:10169–76. [PubMed: 20668200]
- Hobbs M, Remington B, Glautier S. Dissociation of wanting and liking for alcohol in humans: a test of the incentive-sensitisation theory. *Psychopharmacology (Berl)*. 2005; 178:493–9. [PubMed: 15517194]
- Hodgson R, Rankin H, Stockwell T. Alcohol dependence and the priming effect. *Behav Res Ther*. 1979; 17:379–87. [PubMed: 486041]
- Jackson A, Duka T, Stephens DN. Effects of alcohol and lorazepam during extinction of alcohol self-administration in rats. *J Psychopharmacol*. 2003; 17:293–9. [PubMed: 14513921]
- Kaminski BJ, Weerts EM. The effects of varenicline on alcohol seeking and self-administration in baboons. *Alcohol Clin Exp Res*. 2014; 38:376–83. [PubMed: 24033702]
- Kirk JM, de Wit H. Individual differences in the priming effect of ethanol in social drinkers. *J Stud Alcohol*. 2000; 61:64–71. [PubMed: 10627098]
- Le AD, Poulos CX, Harding S, Watchus J, Juzysch W, Shaham Y. Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injections of alcohol and exposure to stress. *Neuropsychopharmacology*. 1999; 21:435–44. [PubMed: 10457541]
- Le AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y. Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. *Psychopharmacology (Berl)*. 1998; 135:169–74. [PubMed: 9497022]
- LeSage MG, Shelley D, Ross JT, Carroll FI, Corrigan WA. Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacol Biochem Behav*. 2009; 91:461–7. [PubMed: 18817808]
- Liang J, Olsen RW. Alcohol use disorders and current pharmacological therapies: the role of GABAA receptors. *Acta Pharmacol Sin*. 2014; 35:981–93. [PubMed: 25066321]
- Litten RZ, Ryan ML, Fertig JB, Falk DE, Johnson B, Dunn KE, Green AI, Pettinati HM, Ciraulo DA, Sarid-Segal O, Kampman K, Brunette MF, Strain EC, Tiouririne NA, Ransom J, Scott C, Stout R. A double-blind, placebo-controlled trial assessing the efficacy of varenicline tartrate for alcohol dependence. *J Addict Med*. 2013; 7:277–86. [PubMed: 23728065]
- Ludwig AM, Wikler A. “Craving” and relapse to drink. *Q J Stud Alcohol*. 1974; 35:108–30.
- McKee SA, Falba T, O’Malley SS, Sindelar J, O’Connor PG. Smoking status as a clinical indicator for alcohol misuse in US adults. *Arch Intern Med*. 2007; 167:716–21. [PubMed: 17420431]
- McKee SA, Harrison EL, O’Malley SS, Krishnan-Sarin S, Shi J, Tetrault JM, Picciotto MR, Petrakis IL, Estevez N, Balchunas E. Varenicline reduces alcohol self-administration in heavy-drinking smokers. *Biol Psychiatry*. 2009; 66:185–90. [PubMed: 19249750]
- Mitchell JM, Teague CH, Kayser AS, Bartlett SE, Fields HL. Varenicline decreases alcohol consumption in heavy-drinking smokers. *Psychopharmacology (Berl)*. 2012; 223:299–306. [PubMed: 22547331]
- Panlilio LV, Hogarth L, Shoaib M. Concurrent access to nicotine and sucrose in rats. *Psychopharmacology (Berl)*. 2014
- Rollema H, Hajos M, Seymour PA, Kozak R, Majchrzak MJ, Guanowsky V, Horner WE, Chapin DS, Hoffmann WE, Johnson DE, McLean S, Freeman J, Williams KE. Preclinical pharmacology of the $\alpha 4\beta 2$ nAChR partial agonist varenicline related to effects on reward, mood and cognition. *Biochem Pharmacol*. 2009; 78:813–24. [PubMed: 19501054]
- Samson HH, Chappell A. Reinstatement of ethanol seeking responding after ethanol self-administration. *Alcohol*. 2002; 26:95–101. [PubMed: 12007584]
- Samson HH, Czachowski CL. Behavioral measures of alcohol self-administration and intake control: rodent models. *Int Rev Neurobiol*. 2003; 54:107–43. [PubMed: 12785286]

- Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE. Varenicline, an $\alpha 4 \beta 2$ nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci U S A*. 2007; 104:12518–23. [PubMed: 17626178]
- Stolerman I. Drugs of abuse: behavioural principles, methods and terms. *Trends Pharmacol Sci*. 1992; 13:170–6. [PubMed: 1604709]
- Stretch R, Gerber GJ. Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. *Can J Psychol*. 1973; 27:168–77. [PubMed: 4197125]
- Vosler PS, Bombace JC, Kosten TA. A discriminative two-lever test of dizocilpine's ability to reinstate ethanol-seeking behavior. *Life Sci*. 2001; 69:591–8. [PubMed: 11510953]
- Wouda JA, Riga D, De Vries W, Stegeman M, van Mourik Y, Schetters D, Schoffelmeer AN, Pattij T, De Vries TJ. Varenicline attenuates cue-induced relapse to alcohol, but not nicotine seeking, while reducing inhibitory response control. *Psychopharmacology (Berl)*. 2011; 216:267–77. [PubMed: 21331520]

Experiment 1:

Experiments 2 and 5:

Experiments 3, 4, and 6:

Figure 1.

The experimental design and timeline for the experiments showing training history for the self-administration (SA) experiments and within group testing. The three separate timelines represent different group of rats.

Figure 2.

Discriminative stimulus effect of alcohol in combination with varenicline and varenicline alone. Mean ($\pm$ SEM) alcohol appropriate-responses (A) and response rate (B) following varenicline injection in alcohol discrimination-trained rats. Varenicline did not have alcohol-like discriminative stimulus effects. Mean ($\pm$ SEM) alcohol-appropriate responses (C) and response rate (D) following varenicline pretreatment prior to the alcohol training dose (1 g/kg). Horizontal dashed line (>80%) represents full expression of the discriminative stimulus effects of alcohol. *- $p < 0.05$, different from saline treatment.

Figure 3. Effects of varenicline on alcohol self-administration. Mean ($\pm$ SEM) alcohol responses (A) and alcohol responses across the session (B) following varenicline injection. * - $p < 0.05$ different from saline treatment.

Figure 4.

Effects of alcohol priming on alcohol self-administration. Mean ($\pm$ SEM) alcohol responses (A) and alcohol responses across the session (B) following alcohol priming doses. * - $p < 0.05$ different from water treatment.

Figure 5.

Effects of alcohol priming combined with varenicline treatment on alcohol self-administration. Mean ($\pm$ SEM) alcohol responses (A) and alcohol responses across the session (B) following varenicline injection and alcohol priming in rats trained to self-administer alcohol. * - $p < 0.05$, different from water (A) or saline (B).

Figure 6.

Effects of alcohol priming on alcohol seeking behavior. Mean ($\pm$ SEM) alcohol responses (A) and alcohol responses across the session (B) following alcohol priming and under probe-extinction conditions. * - $p < 0.05$, different from water pretreatment.

Figure 7.

Effects of alcohol priming combined with varenicline on alcohol seeking behavior. Mean ($\pm$ SEM) alcohol responses (A) and alcohol responses across the session (B) following alcohol priming and varenicline pretreatment, under probe-extinction conditions. * - $p < 0.05$, different from water pretreatment.

Table 1

Total (mean±S.E.M.) water responses for the alcohol self-administration experiments.

Experiment 2	Varenicline dose (mg/kg, IP)			
	0	0.3	1	3
	2.4±0.6	2.0±0.8	1.9±0.8	2.2±0.7
Experiment 3	Alcohol prime dose (g/kg, IG)			
	0	0.3	1	
	2.2±0.7	1.9±0.6	2.6±0.6	
Experiment 4	Varenicline dose (mg/kg)/Alcohol prime dose (g/kg, IG)			
	0/0	0/1	1/0	1/1
	2.1±0.7	2.4±1.0	1.5±0.6	1.9±0.5
Experiment 5	Alcohol prime dose (g/kg, IG)			
	0	0.3	1	
	2.3±0.8	2.6±0.6	1.9±0.7	
Experiment 6	Varenicline dose (mg/kg)/Alcohol prime dose (g/kg, IG)			
	0/0	0/1	1/0	1/1
	2.3±0.8	1.6±0.6	2.2±1.1	2.1±1.6

Table 2

Alcohol intake (g/kg; mean±SEM) for self-administration experiments.

Experiment 2	Baseline intake	Varenicline dose (mg/kg, IP)			
		0	0.3	1	3
Experiment 3	0.87±0.07	0.93±0.14	1.07±0.11	0.76±0.09	0.30±0.10*
		Alcohol prime dose (g/kg, IG)			
		0	0.3	1	
Experiment 4	0.80±0.1	0.56±0.05	0.43±0.08	0.28±0.04*	
		Varenicline dose (mg/kg)/Alcohol prime dose (g/kg, IG)			
		0/0	0/1	1/0	1/1
	0.84±0.03	0.57±0.05	0.35±0.06*	0.77±0.08	0.63±0.05

Baseline intake (g/kg) from session prior to beginning of testing.

* denotes significant difference from 0 (p<0.05)

Table 3

Locomotion (total beam breaks; mean±S.E.M.) for the self-administration experiments.

Experiment 2	Varenicline dose (mg/kg, IP)			
	0	0.3	1	3
	774±36.5	862.7±60.6	724.4±48.4	492.3±88.1 *
Experiment 3	Alcohol prime dose (g/kg, IG)			
	0	0.3	1	
	685.2±83.6	729.4±72.6	755.5±68.7	
Experiment 4	Varenicline dose (mg/kg)/Alcohol prime dose (g/kg, IG)			
	0/0	0/1	1/0	1/1
	748.7±77.9	764.8±54.8	829.2±65.1	760.6±45.1
Experiment 5	Alcohol prime dose (g/kg, IG)			
	0	0.3	1	
	751.6±68.6	788.2±49.5	766.4±64.3	
Experiment 6	Varenicline dose (mg/kg)/Alcohol prime dose (g/kg, IG)			
	0/0	0/1	1/0	1/1
	848.4±39.9	954.2±96.2	860.7±52.3	916.5±68.2

* denotes significant difference from 0 (p<0.05)