

Published in final edited form as:

*Pharmacol Biochem Behav*. 2016 September ; 148: 28–37. doi:10.1016/j.pbb.2016.05.010.

# Developing a model of limited-access nicotine consumption in C57Bl/6J mice

**C.R. Kasten**[*,a], **A.M. Frazee**[a], and **S.L. Boehm II**[a,b]

[a]Department of Psychology, Indianapolis University Purdue University – Indianapolis, 402 N Blackford St LD 124 Indianapolis, IN 46202 United States

[b]Indiana Alcohol Research Center, 545 Barnhill Drive EH 317 Indianapolis, IN United States

## Abstract

Although United States smoking rates have been on the decline over the past few decades, cigarette smoking still poses a critical health and economic threat. Very few treatment options for smoking exist, and many of them do not lead to long-term abstinence. Preclinical models are necessary for understanding the effects of nicotine and developing treatments. Current self-administration models of nicotine intake may require surgical procedures and often result in low levels of intake. Further, they do not lend themselves to investigating treatments. The current study sought to develop a limited-access model of nicotine intake using the Drinking-in-the-Dark paradigm, which results in high levels of binge-like ethanol consumption that can be pharmacologically manipulated. The present study found that mice will consume nicotine under a range of parameters. Intakes under the preferred condition of 0.14 mg/ml nicotine in 0.2% saccharin reached over 6 mg/kg in two hours and were reduced by an injection of R(+)-baclofen. Mecamylamine did not significantly affect nicotine consumption. As nicotine and ethanol are often co-abused, nicotine intake was also tested in the presence of ethanol. When presented in the same bottle, mice altered nicotine intake under various concentrations to maintain consistent levels of ethanol intake. When nicotine and ethanol were presented in separate bottles, mice greatly reduced their nicotine intake while maintaining ethanol intake. In conclusion, these studies characterize a novel model of limited-access nicotine intake that can be pharmacologically manipulated.

### Keywords

nicotine; self-administration; limited-access; baclofen; mecamylamine; C57Bl/6J

[*]Corresponding author: Chelsea Kasten 402 N Blackford St LD 124 Indianapolis, IN 46202 ckasten@iupui.edu; slboem@iupui.edu.



Author Contributions

CK, AF, and SLB were responsible for the study concept, design, data analysis, and contributed to writing and reviewing the manuscript. CK and AF contributed to acquisition of data.

## 1 Introduction[1]

Although cigarette smoking is on the decline, the Centers for Disease Control (CDC) estimates that as of 2013, 17.8% of the US population was classified as current smokers. Further, cigarette smoking accounts for 1 out of 5 deaths, making it the leading cause of preventable disease. The economic cost of first- and secondhand smoke is vast. Almost $170 billion is spent yearly on medical care, whereas another $156 billion is lost in productivity due to smoking related illness (CDC). Approximately 68.8% of current smokers want to quit, yet quitting often takes multiple attempts and less than 10% of those who try to quit smoking successfully quit for at least 6 months (Messer et al., 2008).

Preclinical animal models, specifically those involving self-administration, can serve as a valuable tool to research the mechanisms of nicotine addiction as well as potential pharmacological treatments. Intravenous self-administration of nicotine mimics the rapid onset of nicotine inhalation in smokers (Lynch et al., 2010) and allows for quantification of how reinforcing the drug is. However, intravenous methods of self-administration require specific equipment, surgical catheter implantation, surgery recovery time, monitoring of catheter patency and pH of administered solutions, and often extensive operant training and food restriction to achieve escalation of responses and significant nicotine administration (Caille et al., 2012; Lynch et al., 2010).

Although non-operant oral self-administration studies do not offer the ability to quantify how reinforcing drugs of abuse are, they do allow for observation of self-administration and pharmacological manipulation of oral consumption. Few oral nicotine consumption studies have been conducted in mice. Of these, all but one has utilized 24 hr, 2-bottle choice paradigms. Adriani et al., (2002) observed intakes around 0.8–1.2 mg/kg nicotine using a limited 2hr 2-bottle choice paradigm in male and female CD-1 mice. Although intakes were low, they produced pharmacologically relevant levels of cotinine, the main metabolite of nicotine. However, mice were also water deprived during the 22 hours of the day when nicotine was not present. C57Bl/6J (B6) mice have routinely been shown to consume more nicotine than other strains in 24 hr free- choice access paradigms. Relatively high levels of intake are consistent across nicotine concentration (Glatt et al., 2009; Klein et al., 2004; Locklear et al., 2012) and are not changed when a sweetener is introduced to the nicotine and non-nicotine solution or related to intake of sweetened solutions (Meliska et al., 1995; Robinson et al., 1996). These results may suggest that B6 mice are insensitive to the bitter taste of quinine relative to other inbred strains, particularly at higher concentrations. However, using a brief-access paradigm that reliably quantifies aversion to quinine, Glatt et al. (2009) demonstrated that B6 mice display similar oral aversion as other strains to increasing concentrations of nicotine.

The purpose of the current studies was to develop a limited-access model of rapid nicotine intake in B6 mice that was more suitable for testing potential pharmacotherapies than previous oral nicotine administration paradigms. We chose to model nicotine intake using the Drinking-in-the-Dark (DID) method developed for 20% ethanol. In the DID paradigm,

[1]C57Bl/6J (B6), Centers for Disease Control (CDC), Drinking-in-the-Dark (DID), nicotinic acetylcholine receptors (nAChRs)

B6 mice consume high binge-like levels of ethanol at rates that are much more rapid than during 24 hr free-choice access paradigms (Matson & Grahame, 2011; Rhodes et al., 2005). We sought to characterize whether B6 mice would consume nicotine in a similar limited access, binge-like fashion, and under what specifications their intake was optimized by substituting ethanol for multiple concentrations of nicotine alone or nicotine with saccharin. In humans, smoking one cigarette over 5 minutes leads to absorption of approximately 0.3–2 mgs nicotine and smokers maintain cotinine levels of approximately 250–300 ng/ml (Hukkanen et al., 2005). It is important to note that, unlike ethanol, there is no clinical definition of what constitutes a nicotine "binge." Therefore, we refer to our model as a limited access, restricted period of rapid consumption without indicating clinical relevance.

Further, we sought to pharmacologically manipulate binge-like nicotine intake, as traditional ethanol DID offers a model that lends itself to prompt testing of pharmacological targets for alcohol and substance use disorders, unlike 24 hr drinking models. To do so, we used the nAChR antagonist mecamylamine and the $GABA_B$ agonist R(+)-baclofen. Mecamylamine is often used to precipitate nicotine withdrawal whereas baclofen attenuates the negative effects of nicotine withdrawal (Varani et al., 2014). Both of these drugs reduce ethanol intake in the DID paradigm and baclofen has been shown to reduce smoking behavior in a clinical trial (Franklin et al., 2009; Hendrickson et al., 2009; Kasten et al., 2015; Leggio et al., 2015). Finally, we sought to characterize whether B6 mice would co-consume nicotine and alcohol, as is often the case in human addiction (National Institute on Alcohol Abuse and Alcoholism, 2007), and whether co-consumption could be pharmacologically manipulated.

## 2 Method

### 2.1 Animals

All animals were male B6 mice maintained on a 12:12 light cycle in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). In all studies mice were at least 60 days old at the beginning of testing. Animals had access to food and water at all times, apart from during nicotine and/or ethanol presentation when water was not available. All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (The National Academic Press, 2003).

### 2.2 Drugs

(−)Nicotine hemisulfate salt dissolved in $H_2O$ (~40% w/v freebase) was purchased from Sigma Aldrich (St. Louis, MO). Nicotine was dissolved in quantities of 87.5, 175, and 262.5 μl per 500 ml of tap water to respective salt-form doses of 0.07, 0.14, and 0.21 mg/ml. Respective freebase nicotine doses for these concentrations were 0.028, 0.056, and 0.084 mg/ml. Saccharin was obtained from Sigma Aldrich (St. Louis, MO) and was dissolved in a 0.025% or 0.2% w/v solution using tap water. Ethanol (195 proof) was obtained from Pharmco, Inc. (Brookefield, CT), and solutions (10% v/v and 20% v/v) were made using tap water. R(+)-baclofen hydrochloride and mecamylamine hydrochloride were obtained from Sigma Aldrich (St. Louis, MO) and dissolved in 0.9% saline to concentrations of 0, 3, 6.5,

and 10 mg for R(+)-baclofen studies and 0, 0.75, 1.5, and 3mg for mecamylamine studies. Baclofen and mecamylamine solutions were delivered via intraperitoneal injections in a volume of 0.1ml per 10g of body weight. Experimental procedures including animal and drug usage are outlined in Table 1. All drug injections took place on Day 5 immediately prior to fluid access.

### 2.3 Experiment 1: Limited-Access Nicotine Intake

Nicotine intake was characterized in 18 male B6 mice taken from our breeding colony maintained at IUPUI. These mice were first generation offspring of breeders purchased from Jackson Laboratories. For all studies, fluid was presented daily in 10 ml graduated tubes for two hours, three hours into the dark cycle. Fluid level was read to the nearest 0.05 ml at the beginning and end of the two hour access period. Empty “leak” cages were maintained for each fluid to determine fluid leakage over the 2 hr access periods. Intake was calculated as fluid displacement for each individual mouse minus the fluid leak.

In Experiment 1, animals received 0.21 mg/ml nicotine in water alone, in water containing 0.025% saccharin, or in water containing 0.2% saccharin for five days of access. Following two weeks of water washout, the same animals were given five days of access to 0.14 mg/ml nicotine in 0, 0.025, or 0.2% saccharin. Groups were counterbalanced based on saccharin concentration during 0.21 mg/ml nicotine access. The 0.21 and 0.14 mg/ml doses fall within the high range of nicotine concentrations used in 24 hr free-choice access studies. Further, nicotine-naïve B6 mice should have slight and no aversion to the 0.21 and 0.14 mg/ml concentrations, respectively (Glatt et al., 2008), allowing us to better understand how nicotine concentrations and saccharin edulcoration alters oral nicotine consumption.

During access to 0.14 mg/ml nicotine, mice were placed in an Opto M3 13″×9″ Mouse Cages locomotor activity system (Columbus Instruments, Columbus, OH). The animal’s cage is placed directly into the monitor and locomotor scores are determined based on sensor beam breaks. Each cage monitor was started immediately following introduction of the reinforcer. Locomotor activity scores were collected in 5 minute bins during the two hours of reinforcer access and two hours post-reinforcer access.

### 2.4 Experiment 2: Pharmacological Manipulation of Limited-Access Nicotine Intake

A total of 64 male B6 mice were purchased from Jackson Laboratories. For pharmacological manipulation of nicotine intake, 0.14 mg/ml nicotine in 0.2% saccharin was used because it generated high levels of nicotine intake with low variability. Animals received 5 days of nicotine access. Thirty-two animals were administered 0, 3, 6.5, or 10 mg/kg of R(+)-baclofen. The remaining 32 animals were administered 0, 0.75, 1.5, or 3 mg/kg of mecamylamine. Doses were based on previous ethanol DID findings (Hendrickson et al., 2009; Kasten et al., 2015). On day 5, drug was administered immediately prior to bottles on and a fluid intake was monitored at bottles-on, hour 1, and hour 2 to observe time-specific effects on intake. Dose groups were counterbalanced based on Day 4 nicotine intake and variance in intake across the 4 day acquisition phase.

### 2.5 Experiment 3: Nicotine + Ethanol Limited-Access Intake

Because smoking and alcohol use disorders are highly comorbid, we also investigated co-consumption of nicotine and ethanol intake. Thirty-eight male mice were used to examine co-consumption of nicotine and ethanol. These mice had previously been used in a pilot study that was completed at least 3 weeks prior to the current study and had a 5 or 10 day history of 0.14 mg/ml nicotine in 0.2% saccharin or 0.2% saccharin alone. Three concentrations of nicotine (0.07, 0.14, or 0.21 mg/ml) were presented in two concentrations of saccharin (0.025% or 0.2%). These six concentrations were all combined in an ethanol solution so that the nicotine, ethanol, and saccharin were administered in one bottle. Not all the groups were tested simultaneously. To observe whether mice would consume a nicotine + ethanol combination, the 0.14 mg/ml nicotine + 0.2% saccharin group was piloted prior to the other groups, and given 4 days of reinforcer access. On day 1 the nicotine solution was mixed with 20% ethanol according to standard DID. However, the 20% concentration limited the amount of fluid animals consumed, thereby reducing the amount of nicotine consumed. Therefore, on days 2–4, the ethanol solution was switched to 10% to increase fluid intake. Although the solution was changed, the dependent variables of g/kg of ethanol and mg/kg of nicotine consumed were calculated in the same manner as all other data points. Therefore, the data is included without any adjustments in the statistics and accompanying figure. The 0.07 mg/ml + 0.025%, 0.07 mg/ml + 0.2%, 0.14 mg/ml + 0.025%, 0.21 mg/ml + 0.21 mg/ml + 0.025%, and 0.21 mg/ml + 0.2% groups were all run concurrently. Animals were given access to the nicotine + saccharin solutions in one bottle containing 10% ethanol. Fluid levels were recorded at the beginning and end of the 2 hour access period, and amount of ethanol and nicotine intake was quantified.

### 2.6 Experiment 4: Pharmacological Manipulation of Nicotine + Ethanol Limited-Access Intake

A total of 54 male B6 mice purchased from Jackson Laboratories were used to quantify the effects of baclofen and mecamylamine on concurrent nicotine and ethanol access. These mice were not naïve. As part of a previous unrelated study, they had been exposed to 48 hours of a 2-bottle free choice paradigm with access to two water bottles (n = 36), or one water bottle and one bottle containing up to 0.35 mM quinine (n = 18). The prior quinine exposure took place three weeks prior to the current study and was accounted for in drug and dose assignments.

All mice received fluid access to two bottles for 5 days. One bottle contained 0.14 mg/ml nicotine in 0.2% saccharin, and the other contained 20% ethanol in 0.2% saccharin. Two bottles were used in place of combined solution because intake of the combined solution appeared to be driven by ethanol intake, and we wanted to discern the independent effects of drug on each solution. Bottle position was randomized and changed daily. Immediately prior to bottles-on on day 5, animals received an injection of saline, R(+)-baclofen (3, 6.5, or 10 mg/kg), or mecamylamine (0.75, 1.5, or 3 mg/kg). To conserve animal usage, the same control group was used for each drug. Fluid readings were taken at bottles on, hour 1, and hour 2 to observe time-course specific effects on intake. Dosage groups were counterbalanced based on Day 4 ratio of ethanol to nicotine intake, variance in ratio of

ethanol to nicotine intake over the acquisition phase, day 4 ethanol intake, and day 4 nicotine intake.

### 2.7 Statistics

Statistics were run using SPSS version 23 or GraphPad Prism 5. Statistical significance was set at $p = 0.05$. Significance levels were adjusted for all post-hoc tests. All data is reported as mean ± standard error of the mean (SEM). Nicotine intake and nicotine + ethanol intake was analyzed using a repeated measures Saccharin Concentration*Day or Nicotine Concentration*Saccharin Concentration*Day ANOVA. Cumulative locomotor activity scores during 2 hrs of 0.14 mg/ml nicotine access and for the 2 hrs post-access were analyzed using a Saccharin Concentration*Day ANOVA to assess locomotor activity scores in the same manner as nicotine drinking. Secondary ANOVAs and Tukey's post-hoc tests were used as follow-up tests. Pearsons correlations were also run for Days 1–5 to assess the relationship between nicotine drinking and locomotor activity across all groups.

For all pharmacological tests, nicotine and ethanol acquisition was analyzed using a repeated measures ANOVA for day 1–4 intake. Day 5 intake was analyzed using a repeated measures Dose*Hour ANOVA. One-Way ANOVAs with dose at the between-subjects factor were also used to analyze total Day 5 drinking, as well as hour 1 and hour 2 consumption to assess the *a priori* hypotheses that drug effects would vary over time. Repeated measures Dose*Hour and one-way hourly ANOVAS were also used to assess drug dose effects on ethanol intake ratio, calculated as [ml/kg of ethanol consumed/(ml/kg of ethanol + ml/kg of nicotine consumed)], in the two-bottle tasks. Dunnett's tests were used as post-hoc tests for dose main effects and hourly one-way ANOVAs, with the saline group set as the control.

## 3 Results

### 3.1 Limited-Access Nicotine Intake

A repeated measures Day*Saccharin concentration ANOVA was run to analyze intake of 0.21 mg/ml nicotine. Greenhouse-Geisser statistics are reported due to significant differences in variability between groups (Mauchly's test for sphericity; $p < .001$). There was no significant main effect of day on intake, nor was there a significant interaction ($p$'s > .05). There was a significant main effect of saccharin concentration, with the 0.2% concentration increasing nicotine intake compared to the 0% and 0.025% concentrations ($p$'s < .05) (Fig. 1A).

To analyze intake of 0.14 mg/ml nicotine in differing saccharin concentrations a repeated measures Day*Concentration ANOVA was used. There was a significant main effect of Day with a linear and quadratic pattern ($p$'s < .05). There was also significant main effect of Saccharin Concentration, $F(2,18) = 36.39$, $p < .001$. Mice consumed significantly more nicotine in the 0.2% saccharin solution than in the 0.025% or 0% solutions ($p$'s < .001). There was also a significant Day*Concentration interaction, $F(8,72) = 2.98$, $p < .01$. One-way ANOVAs for each day of intake indicated that nicotine intake was significantly different between saccharin concentrations on all days, with the 0.2% saccharin group consuming more than the 0.025% and 0% saccharin groups on Days 1–5 (Fig. 1B).

A Day*Concentration ANOVA was used to analyze locomotor activity during the two hours of 0.14 mg/ml nicotine access. One animal was removed because it was repeatedly found to be hanging on the cage top outside of the range of the beams, making its activity count inaccurate. There was no significant interaction of Day*Saccharin Concentration. However, there was a main effect of Day with a quadratic and cubic trend ($p$'s < .05). There was also a significant main effect of saccharin concentration, $F(2,17) = 6.23$, $p < .01$. Tukey's post-hoc tests revealed that the 0.2% saccharin group was significantly more active than the 0.025% saccharin group ($p < .01$) and trended towards being more active than the nicotine alone group ($p = .057$) (Fig. 1C). Correlations assessing the direct relationship between daily nicotine intake and locomotor score revealed no significant relationship on Days 1 or 2 ($p$'s > .05). Nicotine intake was significantly correlated with locomotor activity on Days 3–5, $r(20) = 0.566$, 0.628, and 0.484, respectively ($p$'s < .05).

Locomotor activity for the 2 hrs post-reinforcement (0.14 mg/ml nicotine) access was also analyzed using a Day*Saccharin Concentration ANOVA. There was a main effect of day with a significant linear trend ($p$'s < .01). The main effect of saccharin concentration also reached significance, $F(2,17) = 3.73$, $p = .046$. Across days, the 0.2% saccharin group was significantly more active than the 0.25% saccharin group for the 2 hrs post-reinforcement access ($p = .042$). There was also a significant Day*Concentration interaction, $F(8,68) = 2.22$, $p < .05$. Follow-up one-way ANOVAs for each day revealed significant concentration effects on Days 2, 4, and 5 ($p$'s < .05). The 0.2% saccharin group was significantly more active than the 0.025% saccharin group on Days 2 and 5 ($p$'s < .05) and trended towards being significantly more active on Day 4 ($p = .051$). The 0.2% saccharin group was also significantly more active than the nicotine alone group on Day 5 ($p < .05$) (Fig. 1D). Correlations between daily nicotine intake and post-reinforcement activity revealed a significant relationship only on Day 5 of access, $r(20) = 0.582$, $p < .01$.

### 3.2 Pharmacological Manipulation of Limited-Access Nicotine Intake: Baclofen

A repeated measures ANOVA on day 1–4 intake revealed no effect of day on nicotine acquisition; $F(3,93) = 1.48$, $p > .05$ (Fig. 2A). A repeated measures Hour*Dose ANOVA on day 5 revealed no significant effect of hour on intake ($p > .05$). There was a trend towards an Hour*Dose interaction, but it did not reach significance ($p = .075$). However, on day 5 there was a significant effect of Dose on total 2 hour intake; $F(3,28) = 9.9$, $p < .001$. Dunnett's post-hoc tests revealed that all doses of baclofen significantly reduced total nicotine intake compared to the saline group ($p$'s < .01) (Fig. 2B). ANOVAs on hourly day 5 intake revealed a significant effect of dose at each hour ($p$'s < .01). At hour 1, all doses of baclofen significantly reduced nicotine intake ($p$'s < .01). However, at hour 2, although the 6.5 and 10 mg/kg baclofen doses significantly reduced nicotine intake ($p$'s < .001), the 3 mg/kg dose only trended towards reducing intake ($p = .059$) (Fig. 2C).

### 3.3 Pharmacological Manipulation of Limited-Access Nicotine Intake: Mecamylamine

A repeated measures ANOVA on day 1–4 intake revealed a significant effect of day on nicotine acquisition; $F(3,93) = 2.91$, $p < .05$ (Fig. 2D). Follow-up paired-samples t-tests adjusted for multiple comparisons revealed that intake on Day 4 was significantly higher than Day 2 intake ($p < .05$), and trended towards being higher than Day 1 intake ($p = .06$). A

repeated measures Hour*Dose ANOVA revealed no significant effect of dose on total day 5 intake; $F(3,28) = 1.36$, $p > .05$ (Fig. 2E). There was a significant effect of hour; $F(1,28) = 23.93$, $p < .001$), with intake being significantly higher in hour 1. There was also an interaction between Hour*Dose; $F(3,28) = 3.30$, $p < .05$). One-way ANOVAs at each hour revealed no significant effect of Dose at either hour ($p$'s > .05) (Fig. 2F).

### 3.4 Nicotine + Ethanol Limited-Access Intake: Same Bottle

A repeated measures Nicotine Concentration*Saccharin Concentration*Day ANOVA revealed a main effect of day on ethanol intake; $F(3,96) = 2.75$, $p < .05$. There was a significant linear trend in the data, with ethanol intake increasing over days ($p < .05$). There was also a main effect of saccharin dose; $F(1,32) = 12.19$, $p = .001$. Animals consumed more ethanol when given access to the solution with 0.2% saccharin compared to animals given access to solution with 0.025% saccharin ($p < .05$). There was no main effect of nicotine concentration on ethanol intake, or any significant interactions between the variables ($p$'s > .05) (Fig. 3A, 3C).

A repeated measures Nicotine Concentration*Saccharin Concentration*Day ANOVA revealed a main effect of day on nicotine consumption, which was linear in pattern; $F(3,96) = 15.52$, $p < .001$. As with ethanol intake, there was a significant effect of saccharin concentration; $F(1,32) = 4.63$, $p < .05$. Animals that received access to the 0.2% saccharin concentration consumed more nicotine than those that received access to the 0.025% concentration ($p < .05$). There was also a main effect of nicotine; $F(2,32) = 77.15$, $p < .001$. Animals receiving the 0.14 mg/ml concentration drank significantly more nicotine than those receiving the 0.07 mg/ml concentration, and the animals receiving 0.21 mg/ml drank significantly more than both other groups ($p$'s < .001). There was a significant interaction of Nicotine Concentration*Day; $F(6,96) = 4.850$, $p < .001$. One-way ANOVAs for each day revealed a significant effect of Nicotine Concentration each day, which followed the same pattern as the nicotine main effect ($p$'s < .05). Although the omnibus Nicotine Concentration*Saccharin Concentration*Day ANOVA trended towards significance ($p = .057$), there were no other significant interactions (Fig. 3B, 3D).

### 3.5 Pharmacological Manipulation of Nicotine + Ethanol Limited-Access Intake: Separate Bottles, Baclofen

A repeated measures ANOVA revealed a significant effect of day with a linear and cubic pattern on ethanol acquisition; $F(3,90) = 8.04$, $p < .001$ (Fig. 4A). An Hour*Dose repeated measures ANOVA on day 5 revealed no interaction of Hour*Dose. However, there was an effect of hour, with animals consuming more ethanol in the first hour; $F(1,27) = 19.287$, $p < .001$. There was also a significant main effect of dose; $F(3,27) = 5.03$, $p < .01$. Dunnett's post-hoc test revealed that the 6.5 and 10 mg/kg dose of R(+)-baclofen significantly reduced total ethanol intake compared to the control group ($p$'s < .05) (Fig. 4B). One-way ANOVAs at each hour revealed only a significant effect of dose at hour one, which followed the same pattern as effect of dose on total intake ($p$'s < .05) (Fig. 4C).

A repeated measures ANOVA revealed no effect of day on nicotine acquisition ($p > .05$) (Fig. 4D). An Hour*Dose repeated measures ANOVA on day 5 revealed no significant main

effects or interactions of the variables on nicotine intake ($p$'s > .05). One-way ANOVAs also revealed no significant effect of R(+)-baclofen at either hour on nicotine intake ($p$'s > .05) (Figs. 4E, F).

Finally, an Hour*Dose repeated measures ANOVA revealed no significant main effects or interactions on ethanol intake ratio following drug administration ($p$'s > .05). There were also no significant effects of dose at either hour ($p$'s > .05). Total mean ethanol intake ratio ± SEM was 0.86 ± 0.08 for 0 mg/kg, 0.84 ± 0.04 for 3 mg/kg, 0.74 ± 0.11 for 6.5 mg/kg, and 0.91 ± 0.04 for 10 mg/kg.

### 3.6 Pharmacological Manipulation of Nicotine + Ethanol Limited-Access: Separate Bottles, Mecamylamine

A repeated measures ANOVA revealed a significant effect of day with a linear trend on acquisition of ethanol intake; $F(3,90) = 13.44$, $p < .05$ (Fig. 5A). An Hour*Dose repeated measures ANOVA on day 5 revealed only a main effect of hour on ethanol intake, with more ethanol being consumed in the first hour; $F(1,27) = 16.86$, $p < .001$. One-way ANOVAS to assess effect of dose on hourly intake revealed no significant effect of dose at either hour on ethanol intake ($p$'s > .05) (Figs. 5B,C).

A repeated measures ANOVA revealed no effect of day on nicotine acquisition ($p > .05$) (Fig. 5D). An Hour*Dose repeated measures ANOVA on day 5 revealed no significant main effects or interaction of mecamylamine dose*hour on nicotine intake ($p$'s > .05). One-way ANOVAs also revealed no significant effect of mecamylamine at either hour on nicotine intake ($p$'s > .05) (Figs. 5E,F).

Finally, an Hour*Dose repeated measures ANOVA revealed no significant main effects or interactions on ethanol intake ratio following drug administration ($p$'s > .05). There were also no significant effects of dose at either hour ($p$'s > .05). Total mean ethanol intake ratio ± SEM was 0.86 ± 0.08 for 0 mg/kg, 0.82 ± 0.07 for 0.75 mg/kg, 0.65 ± 0.10 for 1.5 mg/kg, and 0.63 ± 0.14 for 3 mg/kg.

## 4 Discussion

The current studies were completed to identify what concentrations of nicotine B6 mice will consume in a limited-access paradigm, whether nicotine and ethanol would be co-consumed, and whether R(+)-baclofen and mecamylamine are able to reduce nicotine alone and nicotine + ethanol consumption in a manner similar to that of ethanol consumption. The current studies demonstrated that male B6 mice will binge-consume nicotine under multiple conditions, with intakes reaching averages at and above 6 mg/ml of nicotine salt concentrations (approximately 2.4 mg/ml freebase) when nicotine is added to a 0.2% saccharin solution (Fig. 1). This intake is significantly reduced by R(+)-baclofen, but not significantly altered by mecamylamine (Fig. 2). The mice also co-consumed nicotine and ethanol, but greatly reduced their nicotine intake when ethanol was concurrently presented in a separate bottle (Figs. 3–5). During concurrent nicotine and ethanol access in separate bottles, R(+)-baclofen significantly reduced ethanol intake, but no longer significantly altered nicotine intake (Fig. 4). Mecamylamine again did not alter nicotine intake, but also

failed to reduce concurrent ethanol intake (Fig. 5), although it has been previously shown to reduce ethanol intake at these doses using a similar procedure (Hendrickson et al., 2009).

Although cotinine levels were not assessed, the mice in the current study drank nicotine at levels far surpassing that which have previously been shown to produce pharmacologically relevant cotinine levels. Adriani et al. (2002) demonstrated that an intake of 1 mg/kg over 1 hr resulted in cotinine levels seen following a 0.2–0.3 mg/kg i.p. nicotine injection, and that intakes of 3 mg/kg over 1 hr resulted in locomotor effects. In the current study, we saw significantly increased locomotor activity during and after access to 0.14 mg/ml nicotine in 0.2% saccharin. Under these conditions, mice were consuming over 8 mg/kg of nicotine by Day 3 of access (Fig. 1). Although locomotor activity did not increase over days during access, it did become significantly correlated with nicotine intake by Day 3 across all groups, indicating a change in response to nicotine intake at an individual level. Activity post-reinforcement did change in a linear pattern across days, also possibly indicating an adaptation to repeated nicotine intake (Fig. 1C, D).

It is unclear if the current levels and duration of drinking would result in upregulation of nAChRs. Previous studies have observed upregulation of nAChRs following injection of 0.45–4 mg/kg nicotine twice daily, micropump infusion of 9.6 mg/kg nicotine over 24 hours, and 24 hr oral consumption of nicotine (Wonnacott, 1990; Sparks & Pauly, 1999; Nashmi et al., 2007; Ribeiro-Carvalho et al., 2008). These studies used 10 to 30 days of nicotine exposure, which is much longer than the exposure employed in the current studies. However, with extended access it is likely that upregulation would be seen given the high levels of intake in the current studies. Similar intakes have also been shown to result in somatic withdrawal symptoms during 28 days of access, but not 14, indicating that the duration of access in the current studies may not produce somatic withdrawal (Grabus et al., 2004 Locklear et al., 2012). Questions of cotinine levels, somatic withdrawal, and nAChR upregulation can be answered with time-course studies. Cotinine has a half-life of approximately 38 minutes in B6 mice (Siu & Tyndale, 2007), therefore samples should be taken to assess cotinine levels at half-hour time points in different subgroups of mice. Although the metabolic capacity of the mouse is likely to differ from that of humans, daily smokers maintain cotinine levels of around 250–300 ng/ml, which can be reached in mice (Grabus et al., 2004; Hukkanen et al., 2005).

One concern in the present study is the need to edulcorate nicotine solutions with saccharin so that they become palatable. Although mice drink low, but potentially relevant (Adriani et al., 2002; Klein et al., 2004) levels of nicotine without saccharin present, saccharin significantly increases the intake of 0.14 mg/ml nicotine. This is contrary to Robinson et al. (1996), who found that addition of a sweetener did not significantly increase nicotine intake across various concentrations in B6 mice. However, the previous study used a 2-bottle choice paradigm and saccharin was added to both the nicotine and non-nicotine solution without comparing how addition of saccharin only to the nicotine solution would affect intake. Saccharin on its own can be pharmacologically manipulated, which confounds the interpretation of drug effects on nicotine intake. Testing each drug on saccharin intake can help to interpret drug effects on reinforcers that need to be supplemented with saccharin. In the current study the 3 mg/kg dose of R(+)-baclofen significantly reduced nicotine

consumption, although we have previously shown that this dose is not sufficient to reduce consumption of 0.2% saccharin alone in the DID paradigm. These results were also unlikely to be due to locomotor suppression, as the high dose of 10 mg/kg R(+)-baclofen does not reduce home cage locomotor activity during access to 0.2% saccharin or 20% ethanol, even though it reduces concurrent reinforcer intake (Kasten et al., 2015). The 6.5 mg/kg dose also significantly reduced nicotine intake (Fig. 2). Unpublished data from our lab has shown that 6.5 mg/kg of R(+)-baclofen significantly reduces 20% ethanol, but not 0.2% saccharin intake in the DID paradigm, suggesting a reinforcer-specific effect at this dose. Unexpectedly, addition of saccharin to the 20% ethanol solution did not cause an increase in ethanol intake over consumption levels that have been previously demonstrated in our lab (Fritz et al., 2014; Kasten et al., 2015; Moore et al., 2007).

Using a lower concentration of nicotine may alleviate the need for adding saccharin to the fluid. Aversion begins to occur at a concentration of around .05 mg/ml nicotine in naïve mice (Glatt et al., 2009). However, at this low concentration mice may consume significantly less nicotine, as it would require approximately 3 times the fluid intake as the 0.14 mg/ml concentration. Using concentrations of nicotine higher than 0.21 mg/ml without the addition of saccharin may bifurcate the population into groups that are sensitive and insensitive to the aversive taste of nicotine and lead to great variance in intakes. For example, female rats that consume more quinine also consume more nicotine, potentially indicating decreased taste sensitivity (Nesil et al., 2015). Therefore, edulcorating nicotine with saccharin may be of less concern than using nicotine alone.

Concurrent access to nicotine and ethanol in the same or separate bottles led to different nicotine intakes. When nicotine and ethanol were given in the same bottle, mice consumed the same amount of ethanol regardless of nicotine concentration, leading to significantly different nicotine intakes ranging from around 2 mg/kg to 6 mg/kg (Fig. 3). When nicotine and ethanol were simultaneously presented in different bottles mice consumed the same amount of ethanol as when nicotine was given in the same bottle, but drastically reduce their nicotine intake (Figs. 4 & 5). From the same bottle, mice consuming 20% ethanol in 0.14 mg/ml nicotine + 0.2% saccharin averaged an ethanol intake of 2.97 g/kg (Fig. 2C). From separate bottles mice consumed 3.67 mg/kg and 3.62 mg/kg of ethanol across the acquisition days for the baclofen and mecamylamine studies, respectively. When presented in the same bottle, the ethanol + 0.14 mg/ml nicotine + 0.2% saccharin mice consumed an average 4.55 mg/kg of nicotine. When given in separate bottles in the baclofen and mecamylamine studies, mice consumed an average of 1.21 mg/kg and 1.19 mg/kg nicotine across acquisition days, respectively. Similar patterns in operant lever responses of female alcohol preferring P rats for nicotine and ethanol solutions were observed by Hauser et al. (2012). Under various conditions the rats responded for nicotine + saccharin or nicotine + ethanol + saccharin. However, responses for ethanol alone were significantly higher when the animals were given a choice of 15% ethanol alone, 15% ethanol + 0.07 mg/ml nicotine, or 15% ethanol + 0.14 mg/ml nicotine. These results strongly indicate a preference for ethanol alone over access to a separate nicotine solution. B6 mice may simply prefer 20% ethanol in 0.2% saccharin over the nicotine + saccharin solution. Making ethanol less salient by increasing the concentration or removing the saccharin may begin to equalize nicotine and ethanol intake.

Such low levels of nicotine intake are very unlikely to be pharmacologically relevant and make it difficult to assess any effects of drugs, such as baclofen, which reduce nicotine intake on their own. Along with ethanol preference, capacity to consume more fluid may have interfered with the animals' ability to increase their nicotine intake. During consumption of 0.14 mg/ml nicotine alone, 10% ethanol + 0.14 mg/ml nicotine in the same bottle, and 20% ethanol and 0.14 mg/ml nicotine in different bottles animals consumed approximately 35 ml/kg of total fluid over the acquisition period. Increasing the nicotine concentration to 0.21 mg/ml in the separate bottle paradigm may allow for increased nicotine consumption with decreased fluid intake. Mice in the mecamylamine groups appeared to increase their nicotine fluid intake without decreasing their ethanol fluid intake. However, there was no significant difference in ethanol intake ratio (section 3.6). It should also be noted that on the test day the control group reduced its nicotine intake by more than 1 mg/ml whereas the group that received 1.5 mg/kg mecamylamine kept steady nicotine intake from days 4–5, indicating an injection effect on the test day. There was a similar effect on ethanol intake from days 4–5 (see Fig. 5).

Another important variable is the time course of nicotine intake versus the time course of ethanol intake. The data herein suggest relatively consistent levels of nicotine intakes within the first and second hours of consumption, whereas ethanol intake tends to be higher in the first hour (Figs. 4 & 5). Using volumetric drinking devices, Linsenbardt and Boehm (2014) characterized a very distinct pattern of DID drinking in B6 mice that changes from steady intake across the first session to a front-loading pattern with a late increase in drinking on day 14 of access. Assessing discrete patterns of nicotine intake alone versus nicotine + ethanol intake using volumetric drinking devices may further reveal important time-course characteristics of nicotine intake across repeated access sessions and how concurrent ethanol access changes patterns of nicotine and ethanol intake.

As demonstrated herein, the limited-access model offers ability to pharmacologically manipulate nicotine intake without the concerns of the 24 hr paradigms, such as interference of or need for water access, intake being spread across the day, or determining injection time-point. Current therapeutics, such as varenicline, can be tested to further verify the model for testing of potential pharmaceutical treatments for nicotine intake. Although it visually appears that mecamylamine increases nicotine-alone intake in the first hour of access, there were no significant effects of mecamylamine at any time point in any study (Figs. 2 & 5). This includes our inability to replicate a significant reduction in ethanol intake by the 3 mg/kg mecamylamine dose demonstrated to be effective by Hendrickson et al. (2009). However, because mice were concurrently consuming nicotine this may be due to an interaction between nicotine and ethanol. Notably, our results reveal that R(+)-baclofen reduces nicotine intake at all doses when nicotine is given alone (Fig. 2). Although it could be argued that there is a floor effect preventing baclofen from reducing nicotine intake when ethanol was concurrently presented in a different bottle, it is important to note that nicotine intake only visually trends towards decreasing in the 10 mg/kg dose (Fig. 4). Once again, this may suggest interactive effects between nicotine and ethanol. Also of note is that the 6.5 mg/kg dose of R(+)-baclofen, which has not previously been tested in ethanol DID, significantly reduced ethanol intake.

As previously discussed, rates of smoking are significantly higher in alcoholics, which is possibly due to similar underlying mechanisms. A history of alcohol use disorders significantly increases number of cigarettes smoked and aspects of cigarette smoking, such as increased time smoking per cigarette and number of puffs (Keenan et al., 1990) . It has been proposed that treating comorbid smoking is essential to recovery from alcohol use disorders (Prochaska, 2010). A recent review by Roche et al. (2016) outlines many different behavioral factors that contribute to smoking in heavy drinkers. These include the tendency for heavy-drinking smokers to show more stimulation than sedation to the effects of ethanol, use of one substance leading to craving for the other substance, increased motivation or loss of resistance to use both substances while using one substance, and using one substance to enhance the positive reinforcing effects or reduce the negative reinforcing effects of the other substance. Co-abuse may lead to less sedation, enhanced calming and euphoric effects, and reduced cognitive impairing effects of ethanol leading to development of cross-tolerance and comorbid alcohol and tobacco use disorders (Roche et al., 2016). Translational pre-clinical to human ethanol + nicotine co-consumption conclusions are difficult to draw from the current research due to limitations such as use of one in-bred strain, a consistent 5 day exposure period, and differences seen when ethanol and nicotine were combined in one bottle versus given concurrently in two separate bottles. However, the factors described by Roche et al. (2016) as being important for heavy-drinking smokers can be modeled using parameters of the current work. Stimulation response to ethanol can be accounted for as a potential moderator of concurrent nicotine intake across time using the two-bottle concurrent access model. The changes in topography of ethanol and nicotine drinking may also be observed over time in the two-bottle current access model. Following varying lengths of two-bottle concurrent exposure, nicotine alone, or ethanol alone, multiple behavioral models may be used to examine nicotine and ethanol cross-tolerance and whether it changes as function of duration of exposure. Operant-responding and extinction paradigms can be used to assess motivation and craving for nicotine + ethanol using the nicotine doses described within.

Nicotine and ethanol also share similar molecular and neurological pathways. Preclinical models have demonstrated that nicotine and ethanol, like other drugs of abuse, activate the dopaminergic pathway from the ventral tegmental area to the nucleus accumbens and other regions as well as increase dopamine release in the nucleus accumbens. In the case of nicotine and ethanol these effects are mediated in part by nAChRs, which are widely expressed in the ventral tegmental area on dopaminergic and GABAergic neurons (Hendrickson et al., 2013). Pharmaceuticals used to treat smoking or alcoholism have later been found to be effective in treating both disorders in heavy smoking and drinking clinical populations, such as varenicline and baclofen (Fucito et al., 2011; Franklin et al., 2009; Leggio et al., 2015; McKee et al., 2009; Mitchell et al., 2012). Agonism of GABAergic receptors in this region may generally work to inhibit intake of drugs of abuse, whereas agonism of nAChRs by varenicline may work in a similar way to mediate intake via GABAergic and dopaminergic agonism (Hendrickson et al., 2013). In the case of antagonism with mecamylamine, which is used to induce nicotine withdrawal, animals may continue to consume nicotine in an attempt to overcome the aversive effects of nAChR antagonism. Conversely, baclofen blocks mecamylamine-induced withdrawal symptoms

(Varani et al., 2011; 2014). Differences in mechanism of action and effects on withdrawal symptomology may explain the opposing effects of mecamylamine and R(+)-baclofen on oral nicotine consumption.

In conclusion, these studies demonstrate that male B6 mice will binge-consume nicotine under multiple parameters, R(+)-baclofen is able to reduce binge nicotine consumption, and that mice will co-consume ethanol and nicotine in a binge paradigm, although nicotine intake is greatly reduced when reinforcers are given in separate bottles. The current binge-like model presents a simple and quick pre-clinical paradigm to assess potential treatments for smoking cessation, as well as smoking and ethanol disorders. Avenues for future research include reducing nicotine and saccharin concentrations, analyzing the time course of nicotine intake during concurrent ethanol access, and assessing the parameters of the models in females, which have previously been shown to have higher levels of 24-hour free-choice intake (Glatt et al., 2009; Klein et al., 2004; Locklear et al., 2012; Meliska et al., 1995). Most importantly, a time-course of cotinine levels should be assessed during and after nicotine exposure. Although this model shows high levels of intake, it is important to keep in mind that physiological changes, such as rates of metabolism or alterations in receptor expression, have not yet been quantified. Further, it is unclear whether this model leads to behavioral changes, such as nicotine withdrawal symptoms or changes in anxiety and cognition that are commonly associated with cigarette smoking.

## Acknowledgments

The authors would like to thank Drs. Eric and Engleman and Brandon Fritz for their advice. This work was supported by NIH grants AA007611 (SLB) and AA007462 (CRK).

Funding Support: This work was supported by NIH grants AA007611 (SLB) and AA007462 (CRK).

## References

Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology. 2002; 27(2):212–224. DOI: 10.1016/s0893-133x(02)00295-6 [PubMed: 12093595]

Alcohol Alert. 2007. [Press release] Retrieved from http://pubs.niaaa.nih.gov/publications/AA71/AA71.htm

Caille S, Clemens K, Stinus L, Cador M. Modeling nicotine addiction in rats. Methods Mol Biol. 2012; 829:243–256. DOI: 10.1007/978-1-61779-458-2_15 [PubMed: 22231818]

Franklin TR, Harper D, Kampman K, Kildea S, Jens W, Lynch K, Childress AR. The GABA B agonist baclofen reduces cigarette consumption in a preliminary double-blind placebo-controlled smoking reduction study. Drug and alcohol dependence. 2009; 103(1–2):30–36. DOI: 10.1016/j.drugalcdep.2009.02.014 [PubMed: 19398283]

Fritz BM, Companion M, Boehm SL. “Wired,” yet intoxicated: modeling binge caffeine and alcohol co-consumption in the mouse. Alcohol Clin Exp Res. 2014; 38(8):2269–2278. DOI: 10.1111/acer.12472 [PubMed: 24961658]

Fucito L, Toll B, Wu R, Romano D, Tek E, O’Malley S. A preliminary investigation of varenicline for heavy drinking smokers. Psychopharmacology. 2011; 215(4):655–663. DOI: 10.1007/s00213-010-2160-9 [PubMed: 21221531]

Glatt AR, Denton K, Boughter JD Jr. Variation in nicotine consumption in inbred mice is not linked to orosensory ability. Chem Senses. 2009; 34(1):27–35. DOI: 10.1093/chemse/bjn049 [PubMed: 18775876]

Hauser SR, Katner SN, Deehan GA Jr, Ding ZM, Toalston JE, Scott BJ, Rodd ZA. Development of an oral operant nicotine/ethanol co-use model in alcohol-preferring (p) rats. Alcohol Clin Exp Res. 2012; 36(11):1963–1972. DOI: 10.1111/j.1530-0277.2012.01800.x [PubMed: 22486609]

Hendrickson L, Zhao-Shea R, Tapper A. Modulation of ethanol drinking-in-the-dark by mecamylamine and nicotinic acetylcholine receptor agonists in C57BL/6J mice. Psychopharmacology. 2009; 204(4):563–572. DOI: 10.1007/s00213-009-1488-5 [PubMed: 19247637]

Hendrickson LM, Guildford MJ, Tapper AR. Neuronal Nicotinic Acetylcholine Receptors: Common Molecular Substrates of Nicotine and Alcohol Dependence. Frontiers in Psychiatry. 2013; 429doi: 10.3389/fpsyt.2013.00029

Hukkanen J, Jacob P 3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005; 57(1):79–115. DOI: 10.1124/pr.57.1.3 [PubMed: 15734728]

Kasten CR, Blasingame SN, Boehm SL 2nd. Bidirectional enantioselective effects of the GABAB receptor agonist baclofen in two mouse models of excessive ethanol consumption. Alcohol. 2015; 49(1):37–46. DOI: 10.1016/j.alcohol.2014.11.005 [PubMed: 25557834]

Keenan RM, Hatsukami DK, Pickens RW, Gust SW, Strelow LJ. The relationship between chronic ethanol exposure and cigarette smoking in the laboratory and the natural environment. Psychopharmacology. 1990; 100(1):77–83. DOI: 10.1007/bf02245794 [PubMed: 2296630]

Klein LC, Stine MM, Vandenbergh DJ, Whetzel CA, Kamens HM. Sex differences in voluntary oral nicotine consumption by adolescent mice: a dose-response experiment. Pharmacol Biochem Behav. 2004; 78(1):13–25. DOI: 10.1016/j.pbb.2004.01.005 [PubMed: 15159130]

Leggio L, Zywiak WH, Edwards SM, Tidey JW, Swift RM, Kenna GA. A preliminary double-blind, placebo-controlled randomized study of baclofen effects in alcoholic smokers. Psychopharmacology (Berl). 2015; 232(1):233–243. DOI: 10.1007/s00213-014-3652-9 [PubMed: 24973894]

Linsenbardt DN, Boehm SL 2nd. Alterations in the rate of binge ethanol consumption: implications for preclinical studies in mice. Addict Biol. 2014; 19(5):812–825. DOI: 10.1111/adb.12052 [PubMed: 23742054]

Locklear LL, McDonald CG, Smith RF, Fryxell KJ. Adult mice voluntarily progress to nicotine dependence in an oral self-selection assay. Neuropharmacology. 2012; 63(4):582–592. DOI: 10.1016/j.neuropharm.2012.04.037 [PubMed: 22583831]

Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL. Animal Models of Substance Abuse and Addiction: Implications for Science, Animal Welfare, and Society. Comparative Medicine. 2010; 60(3):177–188. [PubMed: 20579432]

Matson LM, Grahame NJ. Pharmacologically relevant intake during chronic, free-choice drinking rhythms in selectively bred high alcohol-preferring mice. Addiction Biology. 2011; 18(6):921–929. DOI: 10.1111/j.1369-1600.2011.00412.x [PubMed: 22126215]

McKee SA, Harrison ELR, O'Malley SS, Krishnan-Sarin S, Shi J, Tetrault JM, Balchunas E. Varenicline Reduces Alcohol Self-Administration in Heavy-Drinking Smokers. Biol Psychiatry. 2009; 66(2):185–190. doi:http://dx.doi.org/10.1016/j.biopsych.2009.01.029. [PubMed: 19249750]

Messer K, Trinidad DR, Al-Delaimy WK, Pierce JP. Smoking Cessation Rates in the United States: A Comparison of Young Adult and Older Smokers. American Journal of Public Health. 2008; 98(2): 317–322. DOI: 10.2105/AJPH.2007.112060 [PubMed: 18172143]

Mitchell J, Teague C, Kayser A, Bartlett S, Fields H. Varenicline decreases alcohol consumption in heavy-drinking smokers. Psychopharmacology. 2012; 223(3):299–306. DOI: 10.1007/s00213-012-2717-x [PubMed: 22547331]

Moore EM, Serio KM, Goldfarb KJ, Stepanovska S, Linsenbardt DN, Boehm SL Ii. GABAergic modulation of binge-like ethanol intake in C57BL/6J mice. Pharmacology Biochemistry and Behavior. 2007; 88(1):105–113. doi:http://dx.doi.org/10.1016/j.pbb.2007.07.011.

Nashmi R, Xiao C, Deshpande P, McKinney S, Grady SR, Whiteaker P, Lester HA. Chronic nicotine cell specifically upregulates functional alpha 4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. J Neurosci. 2007; 27(31):8202–8218. DOI: 10.1523/jneurosci.2199-07.2007 [PubMed: 17670967]

Prochaska JJ. Failure to treat tobacco use in mental health and addiction treatment settings: A form of harm reduction? Drug and alcohol dependence. 2010; 110(3):177–182. doi:http://dx.doi.org/10.1016/j.drugalcdep.2010.03.002. [PubMed: 20378281]

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiology & Behavior. 2005; 84(1):53–63. doi:http://dx.doi.org/10.1016/j.physbeh.2004.10.007. [PubMed: 15642607]

Ribeiro-Carvalho A, Lima CS, Filgueiras CC, Manhães AC, Abreu-Villaça Y. Nicotine and ethanol interact during adolescence: Effects on the central cholinergic systems. Brain Research. 2008; 1232:48–60. doi:http://dx.doi.org/10.1016/j.brainres.2008.07.062. [PubMed: 18692029]

Roche DJ, Ray LA, Yardley MM, King AC. Current insights into the mechanisms and development of treatments for heavy drinking cigarette smokers. Curr Addict Rep. 2016; 3(1):125–137. DOI: 10.1007/s40429-016-0081-3 [PubMed: 27162709]

Siu EC, Tyndale RF. Characterization and comparison of nicotine and cotinine metabolism in vitro and in vivo in DBA/2 and C57BL/6 mice. Mol Pharmacol. 2007; 71(3):826–834. DOI: 10.1124/mol.106.032086 [PubMed: 17158199]

Smoking & Tobacco Use. Dec 11. 2015 Retrieved from http://www.cdc.gov/tobacco/data_statistics/fact_sheets/fast_facts/

Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. Psychopharmacology (Berl). 1999; 141(2):145–153. [PubMed: 9952038]

Varani AP, Moutinho LM, Calvo M, Balerio GN. Ability of baclofen to prevent somatic manifestations and neurochemical changes during nicotine withdrawal. Drug Alcohol Depend. 2011; 119(1–2):e5–12. DOI: 10.1016/j.drugalcdep.2011.05.017 [PubMed: 21733642]

Varani AP, Moutinho Machado L, Balerio GN. Baclofen prevented the changes in c-Fos and brain-derived neutrophic factor expressions during mecamylamine-precipitated nicotine withdrawal in mice. Synapse. 2014; 68(11):508–517. DOI: 10.1016/j.neuropharm.2014.11.01310.1002/syn.21763 [PubMed: 25042794]

Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends in Pharmacological Sciences. 1990; 11(6):216–219. doi:http://dx.doi.org/10.1016/0165-6147(90)90242-Z. [PubMed: 2200178]

## Highlights

- C57Bl/6J mice will consume high levels of nicotine in a limited-access model.
- Nicotine intake can be attenuated by R(+)-baclofen, but not mecamylamine.
- Mice will alter nicotine intake to maintain consistent ethanol intake.

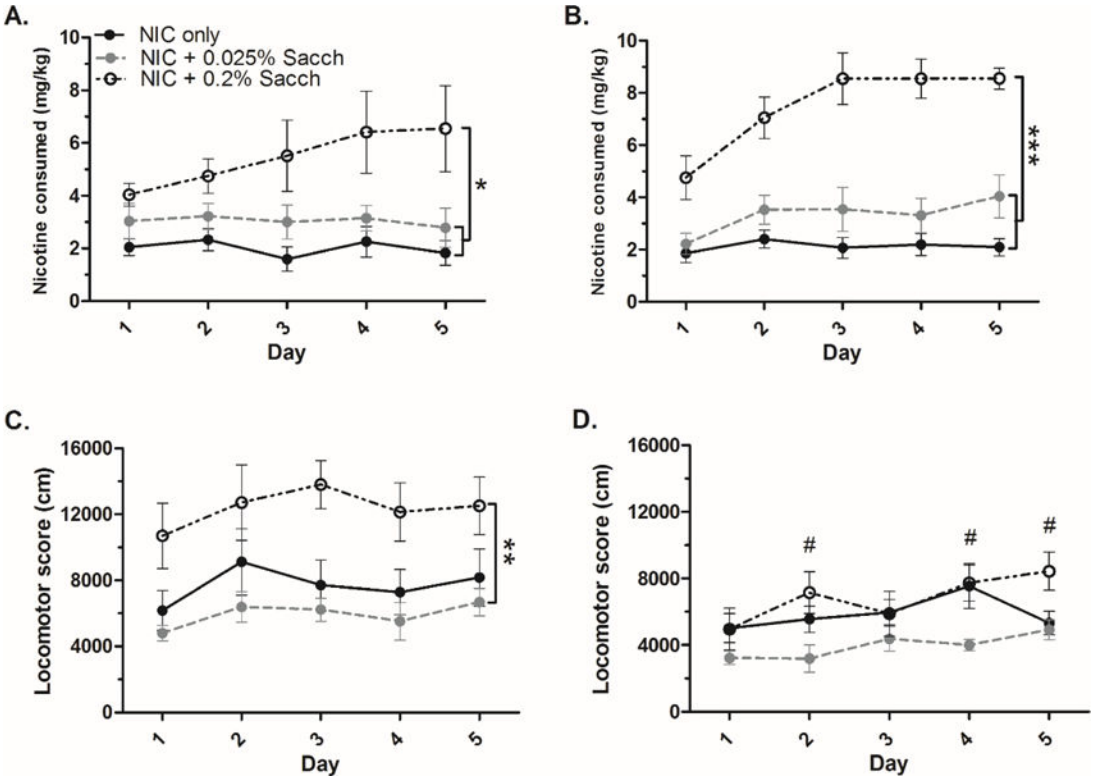


**Figure 1.**

*Figure 1* shows the amount of nicotine consumed at 0.21 mg/ml (**A**) or 0.14 mg/ml (**B**) in no saccharin, 0.025% saccharin, or 0.2% saccharin. The higher saccharin concentration increased intake of both nicotine concentrations. The higher saccharin concentration also altered locomotor activity during the 2 hours of access (**C**) and for the two hours after access (**D**) to 0.14 mg/ml nicotine. Asterisk (*) indicates $p < .05$, double asterisk (**) indicates $p < .01$, triple asterisk (***) indicates $p < .001$, and hashtag (#) indicates significant one-way ANOVA for day.

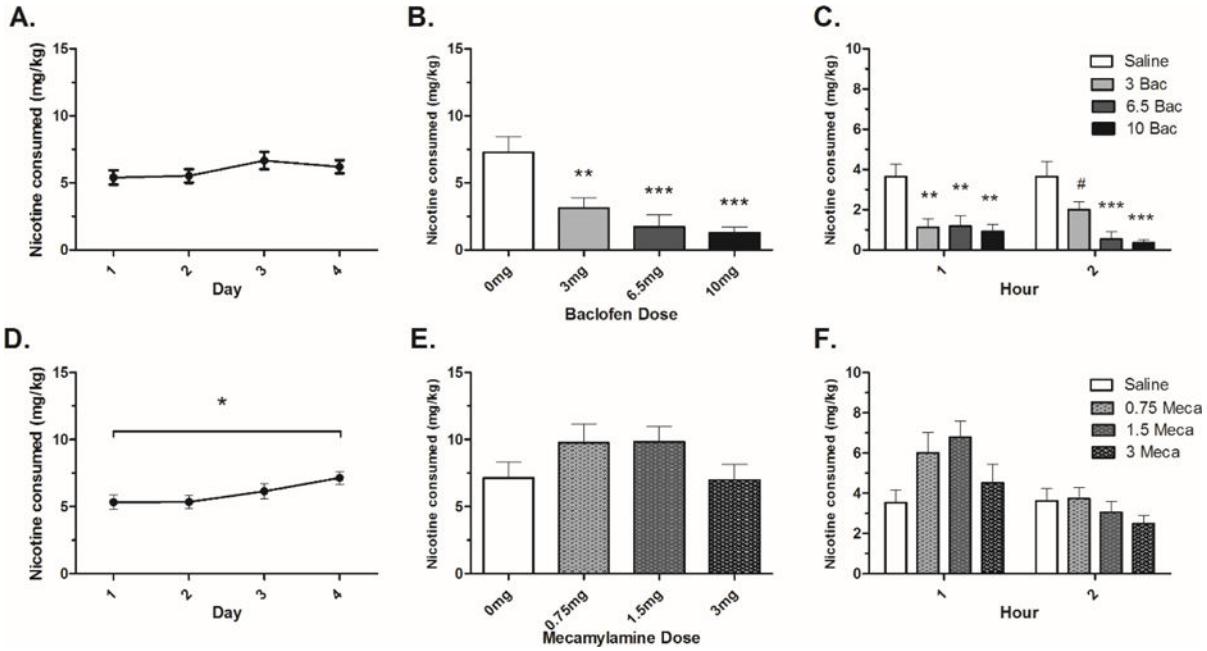


**Figure 2.**

*Figure 2* indicates the effect of baclofen and mecamylamine on 0.14 mg/ml nicotine + 0.2% saccharin intake in one-bottle DID. Panel **A** shows acquisition of intake for the baclofen experiment. All doses of baclofen significantly reduced total nicotine intake (**B**) with hourly effects (**C**). Panel **D** shows acquisition of intake for the mecamylamine experiment. Mecamylamine administration did not significantly affect total (**E**) or hourly (**F**) nicotine intake at any dose. Asterisk (*) indicates $p < .05$, double asterisk (**) indicates $p < .01$, triple asterisk (***) indicates $p < .001$, hashtag (#) indicates $p = .059$, n's = 8 for panels **B** and **C**, n's = 11 for panels **E** and **F**.

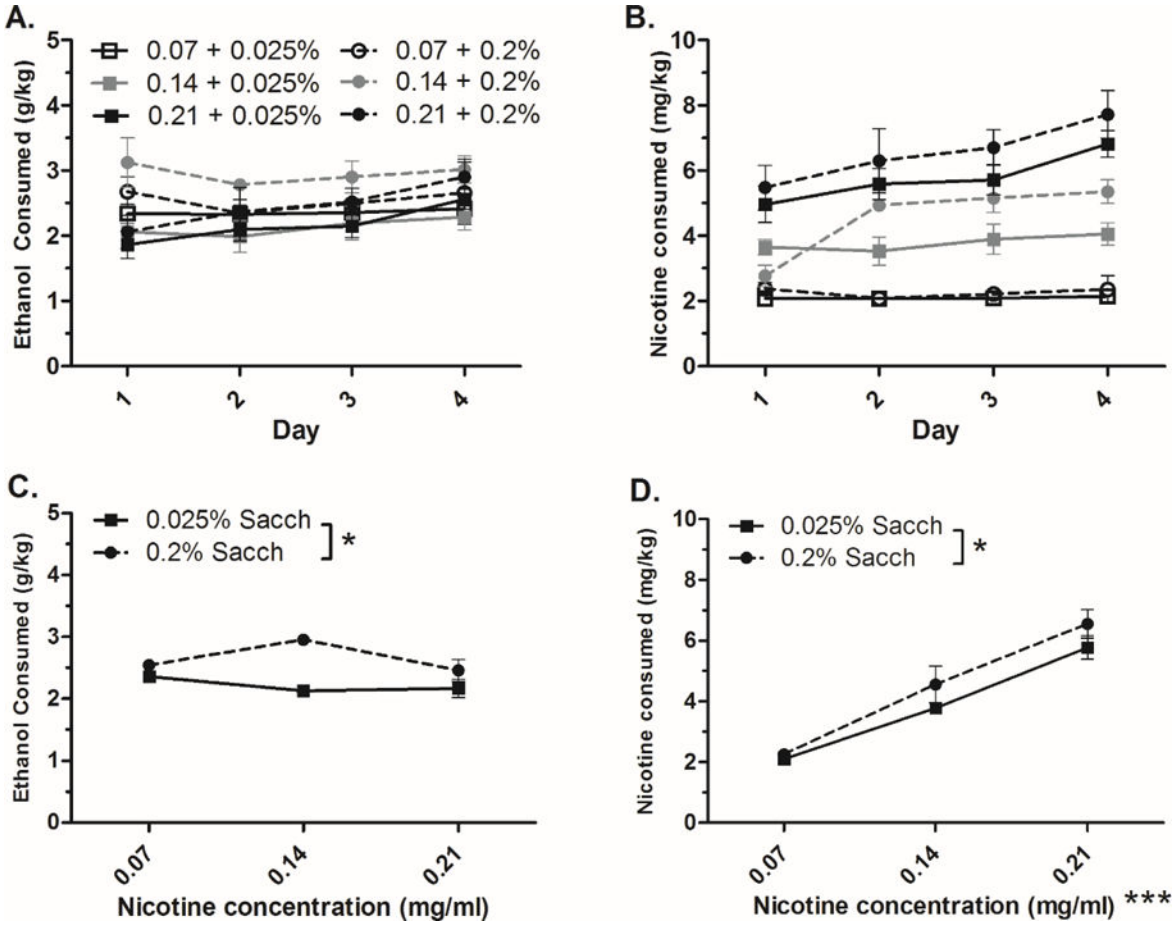


**Figure 3.**

*Figure 3* indicates daily ethanol (**A**) and nicotine (**B**) intake of combined nicotine + ethanol. There was a main effect of saccharin concentration on average ethanol (**C**) and nicotine (**D**) consumption. Nicotine intake was also significantly different across nicotine concentrations. Asterisk (*) indicates $p < .05$, triple asterisk (***) indicates $p < .001$, n's = 6–7.

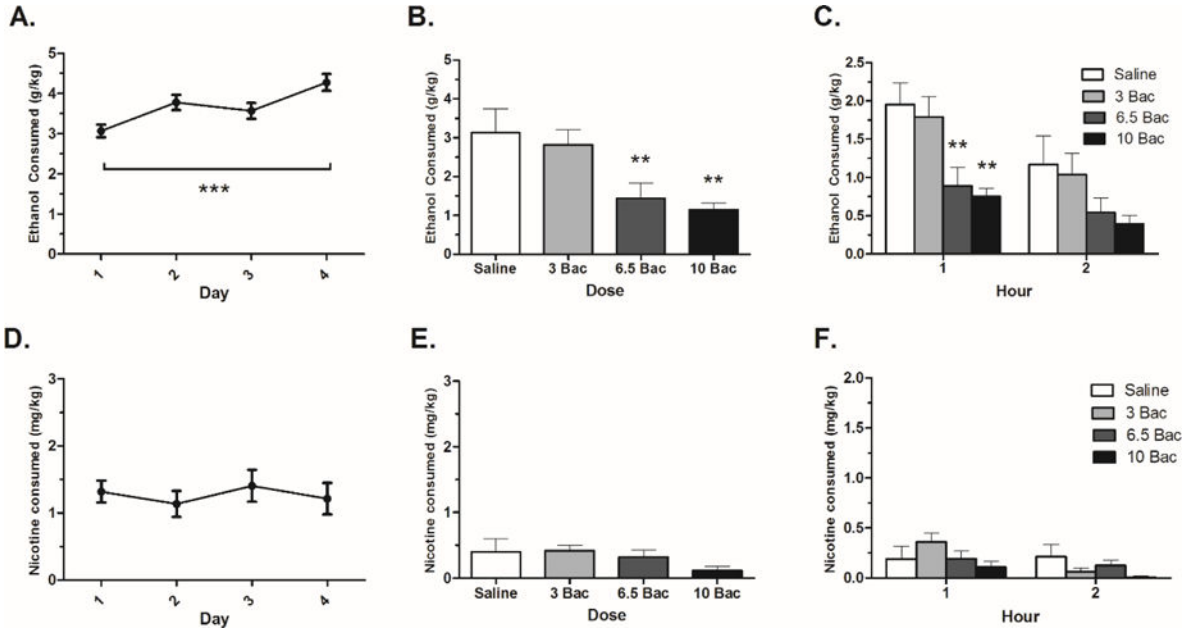


**Figure 4.**

*Figure 4* shows the effects of baclofen on consumption of 20% ethanol and 0.14 mg/ml nicotine in separate bottles during concurrent access. Both bottles contained 0.2% saccharin. Ethanol acquisition is shown in Panel **A.** Baclofen reduced total (**B**) and hourly (**C**) 20% ethanol intake. Nicotine acquisition is shown in Panel **D**. Baclofen did not affect total (**C**) and hourly (**D**) 0.14 mg/ml nicotine intake. Double asterisk (**) indicates $p < .01$, triple asterisk (***) indicates $p < .001$, n's = 7–8.

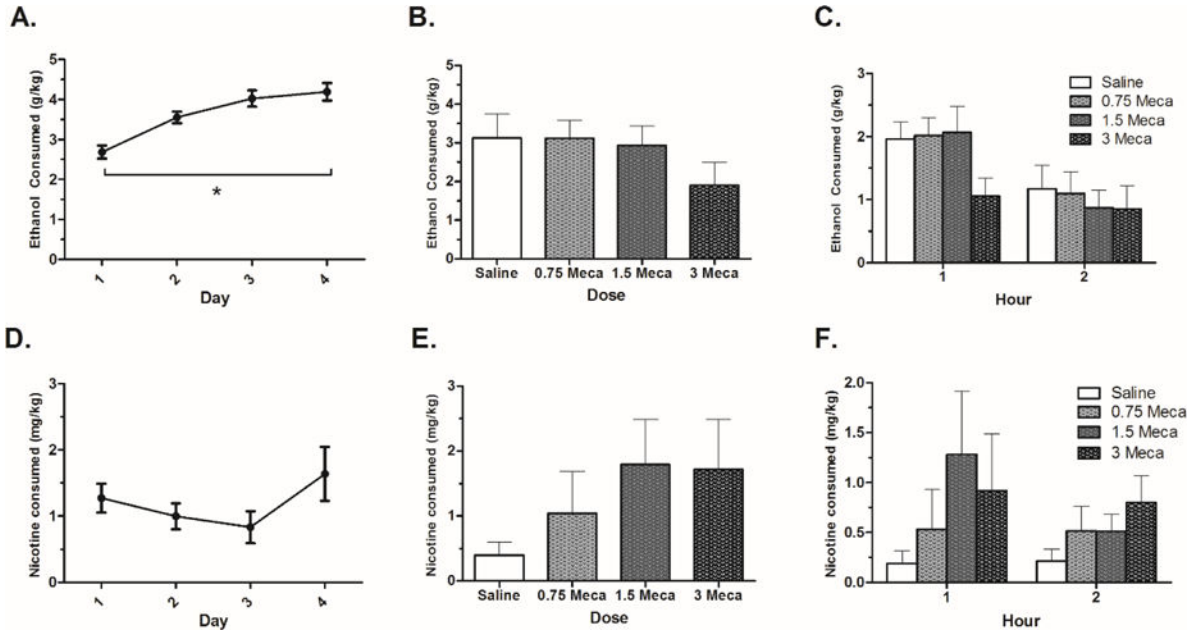


**Figure 5.**

*Figure 5* shows the effect of mecamylamine on consumption of 20% ethanol and 0.14 mg/ml nicotine in separate bottles during concurrent access. Both bottles contained 0.2% saccharin. Ethanol acquisition is shown in Panel **A.** Mecamylamine did not affect total (**B**) or hourly (**C**) 20% ethanol intake. Nicotine acquisition is shown in Panel **D**. Mecamylamine did not affect total (**E**) or hourly (**F**) 0.14 mg/ml nicotine intake, n's = 7–8.

## Table 1

| | Study Length | Fluid Access | Drug administered | Group size |
|---|---|---|---|---|
| **Experiment 1** | 5 Days | 0.21 or 0.14 mg/ml nicotine in 0, 0.025, or 0.2% saccharin | n/a | n's = 6 |
| **Experiment 2** | 5 Days | 0.14 mg/ml nicotine in 0.2% saccharin | R(+)-baclofen (0, 3, 6.5, 10 mg/kg) or mecamylamine (0, 0.75, 1.5, 3 mg/kg) | R(+)-baclofen n's = 8 Mecamylamine n's = 11 |
| **Experiment 3** | 4–5 Days | 0.07, 0.14, or 0.21 mg/ml nicotine in 0.025 or 0.2% saccharin. All bottles contained 10% ethanol. | n/a | n's = 6–7 |
| **Experiment 4** | 5 Days | One bottle of 0.14 mg/ml nicotine in 0.2% saccharin and one bottle of 20% ethanol in 0.2% saccharin | R(+)-baclofen (0, 3, 6.5, 10 mg/kg) or mecamylamine (0, 0.75, 1.5, 3 mg/kg) | All n's = 7–8 |