DR. MARCUS M. WEERA (Orcid ID : 0000-0002-2451-0350)

Article type : Original Research Article

EFFECTS OF NICOTINE ON ALCOHOL DRINKING IN FEMALE MICE SELECTIVELY-BRED FOR HIGH OR LOW ALCOHOL PREFERENCE

Marcus M. Weera, Ph.D.[1,*], Molly A. Fields, B.S.[1], Danielle N. Tapp, B.S.[2], Nicholas J. Grahame, Ph.D.[2], and Julia A. Chester, Ph.D.[1]

[1] Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907

[2] Department of Psychology, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202

* Corresponding author (present address):

Marcus M. Weera, Ph.D.

Department of Physiology

Louisiana State University Health Sciences Center

1901 Perdido Street

New Orleans, LA 70112-1393

Phone: 504-568-6171

Fax: 504-568-6158

Email: mweera@lsuhsc.edu

Acknowledgements: This study was supported by the Purdue Research Foundation and by the National Institute on Alcohol Abuse and Alcoholism (AA013522 to Richard Bell and AA07611 to David Crabb). The authors report no conflicts of interest.

ABSTRACT

Background: Studies show that repeated nicotine use associates with high alcohol consumption in humans, and that nicotine exposure sometimes increases alcohol consumption in animal models. However, the relative roles of genetic predisposition to high alcohol consumption, the alcohol drinking patterns, and the timing of nicotine exposure both with respect to alcohol drinking and developmental stage remain unclear. The studies here manipulated all these variables, using mice selectively bred for differences in free-choice alcohol consumption to elucidate the role of genetics and nicotine exposure in alcohol consumption behaviors.

Methods: In Experiments 1 and 2, we assessed the effects of repeated nicotine (0, 0.5 or 1.5 mg/kg) injections immediately before binge-like (drinking-in-the-dark; Experiment 1) or during free-choice alcohol access (Experiment 2) on these alcohol drinking behaviors (immediately after injections and during re-exposure to alcohol access 14 days later) in adult high- (HAP2) and low-alcohol preferring (LAP2) female mice (co-exposure model). In Experiments 3 and 4, we assessed the effects of repeated nicotine (0, 0.5 or 1.5 mg/kg) injections 14 days prior to binge-like and free-choice alcohol access on these alcohol drinking behaviors in adolescent HAP2 and LAP2 female mice (Experiment 3) or adult HAP2 female mice (Experiment 4).

Results: In Experiment 1, we found that repeated nicotine (0.5 and 1.5 mg/kg) and alcohol co-exposure significantly increased binge-like drinking behavior in HAP2 but not LAP2 mice during the re-exposure phase after a 14-day abstinence period. In Experiment 2, 1.5 mg/kg nicotine injections significantly reduced free-choice alcohol intake and preference in the 3rd hour post-injection in HAP2 but not LAP2 mice. No significant effects of nicotine

treatment on binge-like or free-choice alcohol drinking were observed in Experiments 3 and 4.

Conclusions: These results show that the temporal parameters of nicotine and alcohol exposure, pattern of alcohol access, and genetic predisposition for alcohol preference influence nicotine's effects on alcohol consumption. These findings in selectively bred mice suggest that humans with a genetic history of alcohol-use disorders may be more vulnerable to develop nicotine and alcohol co-use disorders.

Keywords: Alcohol; Binge-Like Drinking; Genetics; Repeated Nicotine; Selectively-Bred Mice

INTRODUCTION

Alcohol use disorders (AUDs) and tobacco use are highly co-morbid, with an estimated 80-90% of alcoholics being tobacco users (Miller and Gold, 1998). Conversely, tobacco users are more likely to consume alcohol, with an estimated 90% of tobacco users being regular alcohol drinkers (Keenan et al., 1990). Chronic tobacco and alcohol co-use leads to multiplicative negative consequences such as greater risk of developing cancers (Talamini et al., 1999) and greater relapse rates to alcohol drinking and tobacco use compared to the use of either drug alone (McKee and Weinberger, 2013).

The factors that contribute to the high co-morbidity between AUDs and tobacco use are unclear. Epidemiological studies show that tobacco users find alcohol more reinforcing, consume more alcohol, and are more likely to engage in binge drinking than non-users (McKee et al., 2004). Studies have shown that the initiation of tobacco use may precede the initiation of alcohol use by months to years and that an earlier age of tobacco use initiation is associated with heavier future drinking (Chen et al., 2002; Grant, 1998). This suggests that tobacco use may act as a gateway to more severe alcohol drinking. However, some studies

show that the initiations of tobacco and alcohol use often overlap in many individuals and that individuals who co-use tobacco and alcohol tend to consume more alcohol (Grant, 1998). Taken together, these epidemiological findings suggest that the effects of tobacco use alone and the effects of tobacco and alcohol co-use on future alcohol use severity are difficult to dissociate. Furthermore, epidemiological studies are correlational and do not show if tobacco use and/or tobacco and alcohol co-use has a causative effect on future alcohol use severity.

Animal models have been useful in improving our understanding of the effects of nicotine on alcohol use. Studies in rodents have shown that repeated nicotine injections given just prior to alcohol access augment alcohol free-choice (FC) drinking or operant self-administration (OSA) behaviors (Lê et al., 2003; Sajja and Rahman, 2012). These findings suggest that repeated nicotine and alcohol co-exposure may increase alcohol reinforcement and drinking, consistent with the epidemiological findings reviewed above.

Other studies in rodents have investigated the effects of repeated nicotine pre-exposure in alcohol-naïve animals on subsequent (days to weeks) FC drinking or OSA. These studies were designed to mimic the order of exposure in many co-users of tobacco and alcohol, in which initiation of tobacco use precedes alcohol use and tobacco and alcohol co-use. The findings in this line of research have been varied, with reports of repeated nicotine pre-exposure having augmenting effects (Locker et al., 2016; Potthoff et al., 1983) or no effect (Kemppainen et al., 2009; Smith et al., 1999) on subsequent alcohol drinking or operant self-administration.

In humans, the effects of tobacco use on alcohol intake may depend on the pattern of alcohol use behavior (Weitzman and Chen, 2005). Although a variety of alcohol use patterns are increased across-the-board in smokers compared to non-smokers, binge drinking, defined as a session of heavy drinking that produces blood alcohol concentrations (BACs) ≥ 0.08 g/dL in 2h, is a more frequent form of drinking behavior than other patterns of drinking in smokers (McKee and Weinberger, 2013). In rodents, the effects of chronic nicotine on alcohol intake also seem to depend on the pattern of alcohol access. In mice, several days

of nicotine pre-exposure has been shown to increase binge-like drinking (Locker et al., 2016), using a limited-access paradigm termed “drinking-in-the-dark” (DID), which produces BACs ≥ 0.08 g/dL in 2h in C57BL/6J inbred mice (Rhodes et al., 2007, 2005). However, the effects of repeated nicotine on FC drinking have not been explored in mice, perhaps because intake during FC drinking tends to result in relatively modest BACs below the 0.08 g/dL level, even in the high-drinking C57BL/6J mouse (Matson and Grahame, 2013). In rats, repeated nicotine injections have been shown to increase (Blomqvist et al., 1996) or have no effect (Kemppainen et al., 2009) on FC drinking. However, the effects of chronic nicotine on binge-like drinking in rats are unknown, primarily due to a lack of reliable voluntary binge-like drinking procedures in rats (Hosová and Spear, 2017). To our knowledge, the effects of chronic nicotine on both FC and binge-like drinking have not been compared in the same species.

Genetic factors contribute to the use and co-use of tobacco and alcohol in humans (Madden and Heath, 2002). Studies in animal models also show that common genetic factors contribute to alcohol- and nicotine-motivated behaviors. For example, selectively-bred alcohol preferring (P) rats are more sensitive to nicotine reinforcement than non-preferring (NP) and Wistar rats (Hauser et al., 2013; Lê et al., 2006). In addition, inbred strains of mice with a genetic predisposition for higher alcohol drinking also consume more nicotine (Robinson et al., 1996). However, the role of genetic predisposition in chronic nicotine’s effects on alcohol drinking requires further study to identify risk factors and targeted treatments for addiction.

The current study had 4 experiments designed to address 3 main goals. The first goal was to explore the role of genetic propensity toward alcohol preference in nicotine-alcohol interactions. We used mice selectively-bred (from the HS/IBG progenitor stock of heterogeneous mice) for high (HAP2) or low (LAP2) preference for alcohol in a continuous free-choice paradigm (Grahame et al., 1999; Oberlin et al., 2010). We hypothesized that HAP2 mice would be more sensitive to the effects of chronic nicotine on alcohol drinking

than LAP2 mice based on previous studies indicating genetic overlap between alcohol and nicotine-motivated behaviors (Hauser et al., 2013; Lê et al., 2006; Robinson et al., 1996).

The second goal was to compare temporal parameters of repeated nicotine injections, either together with (co-exposure model), or prior to alcohol access (pre-exposure model), on binge-like and FC drinking behavior in mice. The binge-like drinking procedure used in the present study is a variant of the DID procedure mentioned previously (Rhodes et al., 2007). For FC drinking, the procedure used in the present study has been shown to produce pharmacologically relevant (> 0.1 g/L) levels of alcohol drinking across the dark phase of the light-dark cycle in HAP2 mice (Matson and Grahame, 2013). Based on the studies reviewed above, we hypothesized that repeated nicotine injections concurrent with alcohol access (co-exposure) would produce a more robust augmentation of subsequent alcohol drinking than repeated nicotine injections prior to alcohol access (pre-exposure). We also hypothesized that both co- and pre-exposure to repeated nicotine would have a greater effect on binge-like than free-choice drinking.

The third goal was to compare age of nicotine pre-exposure (adolescent vs. adult) on subsequent binge-like and FC drinking in mice. Adolescence is a unique period during which the brain is highly malleable by drugs (Spear, 2016), however, studies in adolescent rodents have indicated conflicting results. For example, in a study in which adolescent mice were given 22h per day access to nicotine drinking solution or water and 2h per day access to water (first 6d) and alcohol (subsequent 4d) for 10d, mice that were given nicotine solution showed greater alcohol intake (DID procedure) than mice in the water group (Locker et al., 2016). In a rat study in which adolescent animals were given repeated nicotine or saline injections received FC access to alcohol in adulthood, no differences were observed between the nicotine and saline groups (Kemppainen et al., 2009). Based on these studies and other findings that show that adolescents are more sensitive than adults to drugs' long-term effects (Spear, 2016), we hypothesized that nicotine pre-exposure during adolescence would produce a greater augmentation of alcohol drinking than pre-exposure during adulthood and that this effect would be more evident in the DID procedure.

We focused our studies on female mice. A body of accumulating evidence suggests that females are more sensitive to nicotine's effects than males, possibly due to hormonal differences between the sexes (Lynch et al., 2002). In humans, women tend to become nicotine-dependent faster and typically have lower success rates in smoking cessation than men (Pogun et al., 2017). Female C57BL/6J mice consume more nicotine, and show greater escalation of consumption over time and greater withdrawal symptoms, than male mice (Locklear et al., 2012). Female rats show greater motivation to self-administer nicotine than male rats (Donny et al., 2000). Additionally, the heritability of smoking has been shown to be higher in women than in men (Pogun et al., 2017) and alcohol intake is higher in female HAP2 and LAP2 mice than in males (Chester and Weera, 2017; Oberlin et al., 2010); thus, the use of females might improve the detection of nicotine-alcohol interactive effects in HAP2 and LAP2 mice.

MATERIALS AND METHODS

Subjects

Experimentally-naïve female HAP and LAP mice from replicate line 2 (HAP2 and LAP2) were used in Experiments 1-3, and female HAP2 mice were used in Experiment 4. For Experiments 1, 2, and 3, mice were generated and tested at Purdue University using breeders obtained from the Indianapolis Alcohol Research Center (IARC). For Experiment 4, mice were generated and tested at the IARC. All mice were weaned between postnatal days (PND) 21-23 and housed with same sex littermates in clear polycarbonate cages (11.5 x 7.5 x 5 in) in groups of 2-4 until the start of drinking procedures. During all drinking procedures, mice were singly-housed in a reversed 12:12 light-dark cycle and were given 14d to habituate to the housing environment before the start of drinking procedures. Mice had access to food and water *ad libitum*, except during DID (see below).

Ages reported are ages at the start of experimental procedures (at the start of drinking in Experiments 1 and 2; at the start of injections in Experiments 3 and 4). In Experiment 1, HAP2 and LAP2 mice from generations 47 and 52 were between PND 106-

149. In Experiment 2, HAP2 and LAP2 mice from generations 47 and 50 were between PND 123-167. In Experiment 3, HAP2 and LAP2 mice from generation 47 were between PND 35-38. In Experiment 4, HAP2 mice from generation 50 were between PND 87 and 93. All procedures were approved by the Purdue University Animal Care and Use Committee.

Drugs

(-)-Nicotine base (Acros Organics, Geel, Belgium) was diluted in 0.9% saline. All nicotine doses are reported as free base. Alcohol was diluted from 95% alcohol (Koptec, King of Prussia, PA) to 10% (FC drinking) and 20% (DID) alcohol solutions in tap water. Nicotine doses were chosen based on prior studies that showed that nicotine injections altered alcohol drinking behaviors (e.g., Sajja and Rahman, 2012). All injections were given intraperitoneally at 10 mL/kg. Alcohol and water drinking bottles were always read on the cage to prevent spillage.

Experiment 1: The effects of repeated nicotine injections on immediate binge-like alcohol drinking (DID) and on DID after a 14d abstinence period (re-exposure).

Fig. 1A shows the timeline for the experimental procedures. Mice received 14d of habituation to single-housing and reversed light-dark cycle, 4d of DID acquisition, 3d of habituation (saline) injections, 10d of nicotine (0.5 or 1.5 mg/kg) or saline injections immediately before DID, a 14d abstinence period, and 2d of re-exposure to DID. Mice were assigned to experimental groups [0.0 (saline), 0.5, or 1.5 mg/kg nicotine] in a counterbalanced fashion based on their 2h alcohol intake on the 3rd day of habituation injections (Kasten et al., 2015). Nicotine injection procedures were modeled after the procedures of Sajja and Rahman (2012), who showed that repeated nicotine injections increased limited-access two-bottle choice drinking in C57BL/6J mice.

For the DID procedure, standard water bottles were replaced with one bottle of 20% alcohol 3h into the dark cycle for 2h. Alcohol drinking bottles were made from 10-mL serological pipettes fitted with stainless steel sipper tubes. Meniscus readings were accurate to 0.1 mL. During habituation and nicotine/saline treatment, injections were given

immediately before alcohol presentation. Alcohol intakes were recorded at 1 and 2h post-injection. Mice were weighed immediately before injections and received fresh alcohol daily during the DID procedure.

After the last day of nicotine injections, mice from the second replication of this experiment were retained for 14d with access to water only (in regular water bottles) and then were re-exposed to 2d of DID to assess potential effects of the prior chronic nicotine and alcohol co-exposure on binge-like alcohol drinking after a 14d abstinence period. The rationale for this approach was to assess potential long-term effects of nicotine and alcohol co-exposure on binge-like drinking. Mice were given 14d of abstinence to prevent potentially confounding effects of acute alcohol and/or nicotine withdrawal.

Experiment 2: The effects of repeated nicotine injections on immediate FC drinking and on FC drinking after a 14d abstinence period (re-exposure).

Figure 2A shows the timeline for the experimental procedures. Mice received 14d of habituation to single-housing and reversed light-dark cycle, 14d of acquisition of FC drinking, 3d of habituation (saline) injections, 9d of nicotine (0.5 or 1.5 mg/kg) or saline injections at 3h into the dark cycle, a 14d abstinence period, and 6d of re-exposure to FC drinking. During the acquisition and re-exposure phases, mice were given one bottle of water and one bottle of 10% alcohol in 25-mL graduated plastic cylinders fitted with stainless steel sipper tubes. Meniscus readings were accurate to 0.5 mL. Alcohol and water were replaced and bottle positions were swapped every 2d to prevent development of side preference. After 14d of acquisition of FC drinking, 25-mL bottles were replaced by 10-mL bottles (described above) for the habituation and nicotine/saline injections phases of the experiment. Mice still had continuous access to alcohol and water, but intakes were recorded only at 1, 2, and 3h after habituation and nicotine/saline injections. Mice were assigned to experimental groups [0.0 (saline), 0.5, or 1.5 mg/kg nicotine] in a counterbalanced fashion based on their 3h alcohol intake on the 3rd day of habituation injections. Mice then received 9 consecutive days of nicotine or saline injections at 3h into the dark cycle. During habituation and nicotine/saline

injections, drinking fluids were replaced daily and mice were weighed immediately before the start of the dark cycle. After the last day of nicotine injections, mice from the second replication of this experiment were retained for 14d with access to water only (in regular water bottles) and then re-exposed to 6d of FC drinking to assess potential effects of the prior chronic nicotine and alcohol co-exposure on FC drinking after a 14d abstinence period. Mice received 9 instead of 10d of injections as in Experiment 1 due to an injection error on the 10th day of the first replication of this experiment. Drinking data on this day was discarded and no mice from this replication were retained for the re-exposure. Then, mice in the second replication of this experiment received 9 injections and were retained for the re-exposure drinking test.

Experiment 3: The effects of repeated nicotine injections during adolescence on FC drinking and DID in adulthood (HAP2 and LAP2 mice).

Figure 3A shows the timeline for the experimental procedures. Group-housed adolescent mice received nicotine (0.5 or 1.5 mg/kg) or saline injections every other day beginning at PND 35-38 for 20d [10 injections total; based on the method of Kemppainen et al. (2009)]. Drug treatment groups were counterbalanced across mouse lines and cages. On the day after the final injection, mice were singly-housed in reversed 12:12 light-dark cycle and were habituated for 14d. On PND 69-72, regular water bottles were replaced by two 25-mL bottles of 10% alcohol and water for 14d (FC drinking). Every 2d, drinking fluids were replaced with fresh alcohol or water, drinking bottle positions were swapped, and mice were weighed. Alcohol and water intakes were recorded daily for the first 6d and every 2d from days 8-14 of drinking.

At the end of FC drinking, mice remained singly-housed with regular water bottles for 14d, followed by 5d of exposure to the DID procedure. Mice were given 5d of DID instead of 2 (Experiment 1) to assess if adolescent nicotine exposure produced any latent effects on DID. On each day, water bottles were replaced by one bottle of 20% alcohol 3h into the dark cycle for 2h. Alcohol intakes were recorded at the end of the 2h drinking session.

Experiment 4: The effects of repeated nicotine injections in adulthood on FC drinking and DID (HAP2 mice only).

Figure 4A shows the timeline of experimental procedures. This experiment was conducted in Dr. Nicholas Grahame's laboratory at IUPUI in a similar fashion to Experiment 3 except only HAP2 adult mice were used because (1) untreated adult LAP mice usually do not drink enough alcohol in FC drinking to encounter its pharmacological effects (Oberlin et al., 2010). Also, nicotine treatment did not alter alcohol intake in LAP2 mice in Experiments 1-3 (see Results). In addition, adult mice were singly housed during the injection period that started 20d prior to the start of FC alcohol drinking on PND 87-93 (recall that in Experiment 3 mice needed to mature into adulthood before single housing and alcohol drinking exposure).

During FC drinking, mice had access to 10% alcohol and water concurrently from either 25- or 50-mL bottles. Bottles were read daily for the first 9d, and every third day thereafter. During the 2h of DID, mice had access to 20% alcohol from 10-mL serological pipettes fitted with stainless steel sipper tubes. These drinking tubes were clamped to the stainless-steel wire cage lid.

Statistical Analyses

Alcohol intake was expressed as grams of alcohol per kilogram of body weight (g/kg). For FC drinking experiments, alcohol preference ratio [volume of alcohol intake (mL)/total fluid intake (mL)] was calculated. All data were analyzed using analysis of variance (ANOVA) in the Statistical Package for Social Sciences software (IBM Corporation, Armonk, NY). Omnibus ANOVA (between-subject factors: line and treatment group; within-subject factors: day and hour, where appropriate) with significant interactions were followed-up by lower order ANOVAs and Tukey's *post-hoc* where appropriate. The significance level was set at $p < 0.05$. All data are shown as mean ± SEM.

RESULTS

Experiment 1: The effects of chronic nicotine injections on immediate binge-like alcohol drinking (DID) and on DID after a 2-week abstinence period.

DID during Acquisition and Habituation Injections:

Given that DID is not well-characterized in HAP and LAP mice, here we report DID in these mice during acquisition and habituation injections. Mean (± SEM) DID intake collapsed across the 4 acquisition days in HAP2 mice was 2.47 ± 0.13 (hour 1) and 1.76 ± 0.17 (hour 2) and, in LAP2 mice, was 0.76 ± 0.09 (hour 1) and 0.82 ± 0.07 (hour 2). Analysis of DID during acquisition [line (2) x day (4) x hour (2) ANOVA] yielded main effects of line [$F(1,89) = 107.1$; $p < 0.001$; HAP2 > LAP2] and hour [$F(1,89) = 8.7$; $p < 0.005$] and a line x hour interaction [$F(1,89) = 12.3$; $p = 0.001$]. The interaction was due to significantly decreased alcohol intake in the second hour in HAP2 mice [Hour effect: $F(1,44) = 12.9$; $p = 0.001$] but not LAP2 mice.

Analysis of DID during habituation injections [line (2) x day (3) x hour (2) ANOVA] yielded a main effect of line [$F(1,89) = 109.8$; $p < 0.001$; HAP2 > LAP2] and a line x hour interaction [$F(1,89) = 5.9$; $p < 0.05$]. Mean (± SEM) DID intake collapsed across the 3 habituation days in HAP2 mice was 1.61 ± 0.10 (hour 1) and 1.39 ± 0.10 (hour 2) and, in LAP2 mice, was 0.51 ± 0.06 (hour 1) and 0.64 ± 0.05 (hour 2).

DID Immediately After Nicotine or Saline Injections:

Across 10d of nicotine/saline treatment, HAP2 mice in the 1.5 mg/kg nicotine group showed greater alcohol intake than HAP2 mice in the 0.5 mg/kg nicotine group in both hours 1 and 2 across (Fig. 1B, D). An ANOVA of line (2) x treatment (3) x day (10) x hour (2) yielded significant main effects of line [$F(1,85) = 69.7$; $p < 0.001$], treatment [$F(2,85) = 3.5$; $p < 0.05$], day [$F(9,765) = 4.0$; $p < 0.001$], and hour [$F(1,85) = 9.8$; $p < 0.005$]. There were also significant day x line [$F(9,765) = 4.6$; $p < 0.001$], day x hour [$F(9,765) = 3.3$; $p = 0.001$], line x treatment [$F(2,85) = 6.1$; $p < 0.005$] and line x treatment x day x hour [$F(18,765) = 1.7$, $p <$

0.05] interactions. This four-way interaction was further explored with line x treatment x day ANOVAs within each hour.

The hour 1 ANOVA yielded significant main effects of line [$F(1,85) = 44.9$; $p < 0.001$; HAP2 > LAP2] and day [$F(9,765) = 2.8$, $p < 0.005$], and significant day x line [$F(9,765) = 2.0$; $p < 0.05$] and line x treatment [$F(2,85) = 4.9$; $p = 0.01$] interactions. The line x treatment interaction was due to a significant main effect of treatment in HAP2 mice only [$F(2,42) = 3.8$, $p < 0.05$]. Tukey's *post-hoc* showed that HAP2 mice that received 1.5 mg/kg nicotine injections showed greater hour 1 alcohol intake (average across 10d of treatment) than HAP2 mice that received 0.5 mg/kg nicotine injections ($p < 0.05$; Fig. 1B). An analysis of line within each treatment showed that HAP2 mice consumed more alcohol (average across 10d of treatment) than LAP2 mice in all treatment groups (ps < 0.05; Fig. 1B, C).

The hour 2 ANOVA (line x treatment x day) yielded significant main effects of line [$F(1,85) = 67.1$; $p < 0.001$], treatment [$F(2,85) = 3.1$; $p < 0.05$], and day [$F(9,765) = 4.6$; $p < 0.001$], and significant day x line [$F(9,765) = 4.5$; $p < 0.001$] and line x treatment [$F(2,85) = 4.6$; $p < 0.05$] interactions. The day x line interaction was due to a significant effect of day in HAP2 mice only [$F(9,396) = 5.2$; $p < 0.001$]. The line x treatment interaction was due to a significant main effect of treatment in HAP2 mice only ($p < 0.05$). Tukey's *post-hoc* showed that HAP2 mice that received 1.5 mg/kg nicotine injections consumed more alcohol (average across 10d of treatment) than HAP2 mice that received 0.5 mg/kg nicotine injections ($p < 0.05$; Fig. 1D). An analysis of line within each treatment showed that HAP2 mice showed greater alcohol intake than LAP2 mice in all treatment groups (ps < 0.005; Fig. 1D, E).

Given that it was previously found that a single acute injection of nicotine (0.5 mg/kg) decreased alcohol intake in C57BL/6J mice in a DID procedure (Hendrickson et al., 2009), we also performed a within-subjects analysis comparing intake at both hours on the last habituation day to intake at both hours after the first nicotine/saline treatments (day 1) as another way to assess initial sensitivity to nicotine's effects on DID. We found that a 0.5 mg/kg nicotine injection on the first day significantly reduced hour 1 alcohol intake in HAP2 mice (Fig. 1B). ANOVA on hour 1 data [line (2) x treatment (3) x day (2)] yielded significant

main effects of line [$F(1,85) = 41.5$, $p < 0.001$] and day [$F(1,85) = 17.2$, $p < 0.001$] and a line x treatment x day interaction [$F(2,85) = 3.1$, $p < 0.05$]. Follow up ANOVAs indicated a treatment x day interaction in HAP2 only [$F(2,42) = 3.7$, $p < 0.05$]. One way ANOVA of treatment within each day in HAP2 mice indicated a treatment effect on the first day of treatment [$F(2,42) = 4.5$, $p < 0.05$] and Tukey's *post-hoc* analyses showed that alcohol intake in the 0.5 mg/kg nicotine group was significantly lower than the saline group ($p < 0.05$; Fig 1B).

ANOVA on hour 2 data [line (2) x treatment (3) x day (2)] yielded a significant main effect of line only [$F(1,85) = 79.2$, $p < 0.001$; HAP2 > LAP2; Fig. 1D,E].

DID After 2-week Abstinence Period (Re-exposure):

During re-exposure to DID following a 2-week abstinence period, HAP2 mice in the nicotine treatment groups generally showed greater hour 1 alcohol intake than HAP2 mice in the saline group. On day 1 of re-exposure, HAP2 mice in the 1.5 mg/kg nicotine group showed greater hour 1 alcohol intake than HAP2 mice in the saline and 0.5 mg/kg nicotine groups. On day 2 of re-exposure, HAP2 mice in the 0.5 mg/kg nicotine group showed greater hour 1 alcohol intake than HAP2 mice in the saline group (Fig. 1B). Because we observed hour-dependent effects above, we analyzed alcohol intake in hours 1 and 2 separately. An ANOVA of line (2) x treatment (3) x day (2) on hour 1 alcohol intake during DID re-exposure yielded significant main effects of line [$F(1,38) = 116.7$; $p < 0.001$], treatment [$F(2,38) = 5.3$; $p < 0.01$], and day [$F(1,38) = 26.2$; $p < 0.001$], and a significant day x line x treatment interaction [$F(2,38) = 5.0$; $p < 0.05$]. Follow up ANOVAs indicated a significant treatment x day interaction [$F(2,18) = 6.6$; $p < 0.01$] in HAP2 mice only. Follow-up analyses of treatment within each day yielded significant treatment effects on both days 1 ($p < 0.05$) and 2 ($p < 0.01$). On day 1 of re-exposure, Tukey's *post-hoc* analyses showed that HAP2 mice that previously received 1.5 mg/kg nicotine injections consumed more alcohol than HAP2 mice that received saline ($p = 0.06$) or 0.5 mg/kg nicotine ($p = 0.05$). On day 2 of re-exposure, Tukey's *post-hoc* showed that HAP2 mice that received 0.5 mg/kg nicotine

injections consumed significantly more alcohol than HAP2 mice that received saline (p < 0.01). The difference in alcohol intake between the saline and 1.5 mg/kg nicotine groups was not significant (p = 0.09).

For hour 2 data, an ANOVA of line x treatment x day yielded significant main effects of line [F(1,38) = 21.6; p < 0.001; HAP2 > LAP2] and treatment [F(2,38) = 4.1; p < 0.05]. Tukey's *post-hoc* for treatment showed that mice in the 1.5 mg/kg nicotine group showed lower alcohol intake than mice in the saline group in hour 2 of re-exposure (p < 0.05; collapsed across line and day; Fig. 1D, E).

Experiment 2: The effects of repeated nicotine injections on immediate FC drinking and on FC drinking after a 2-week abstinence period (re-exposure).

FC Drinking Immediately After Nicotine or Saline Injections:

Across 9d of nicotine/saline treatment, HAP2 mice that received 1.5 mg/kg nicotine injections showed lower hour 3 alcohol intake and preference compared to HAP2 mice in the saline and 0.5 mg/kg nicotine groups (Fig. 2D, F). ANOVAs of line (2) x treatment (3) x hour (3) x day (9) on alcohol intake and preference yielded significant main effects of line [F(1,85) = 206.2 and 461.3, on intake and preference, respectively; ps < 0.001; HAP2>LAP2] and a significant line x treatment x hour interaction [F(4,170) = 6.0 and 4.6 for intake and preference, respectively; ps < 0.001 and 0.005]. Since there was no four-way interaction with day, the alcohol intake and preference data were analyzed collapsed across days. Follow-up analyses of line x treatment within each hour (1, 2, and 3) post-injection yielded significant main effects of line (HAP2 > LAP2) at all 3 time points [F(1,85) = 89.7, 206.0, 143.1 on intake, and 237.4, 248.5, 345.9 on preference; ps < 0.001; Fig. 2B-G]. At hour 3 only, there was a significant main effect of treatment [F(2,85) = 3.4; p < 0.05] for alcohol intake and significant line x treatment interactions for alcohol intake and preference [F(2,85) = 5.7 and 7.7; ps = 0.005 and < 0.001, respectively]. Follow-up one-way ANOVA of treatment on hour 3 alcohol intake and preference within each line yielded significant effects of treatment only in HAP2 mice [F(2,40) = 4.0 and 5.4, ps < 0.05 and 0.01, respectively]. Tukey's *post-hoc*

analysis showed that HAP2 mice that received 1.5 mg/kg nicotine showed lower alcohol intake and preference than HAP2 mice that received 0.0 and 0.5 mg/kg nicotine in the 3rd hour post-injection ($p < 0.05$; Fig. 2D, G). Analyses of line within each treatment showed that HAP2 mice showed significantly greater alcohol intake than LAP2 mice at all treatments (ps < 0.05; Fig. 2B-G).

FC Drinking After 2-week Abstinence Period (Re-exposure):

Nicotine treatment did not significantly affect alcohol intake or preference during re-exposure to FC drinking following a 2-week abstinence period (Fig. 2H, I). ANOVAs of line (2) x treatment (3) x day (3) on alcohol intake and preference during re-exposure to FC drinking yielded significant main effects of line [$F(1,40) = 636.0$ and 662.4, on intake and preference, respectively; ps < 0.001; HAP2 > LAP2] and a significant main effect of treatment [$F(2,40) = 4.8$; $p < 0.05$] on alcohol intake. Tukey's *post-hoc* analysis of treatment on alcohol intake collapsed by line did not yield any significant effects. However, the main effect of treatment appears to be due to increased alcohol intake in mice that received chronic nicotine and alcohol co-exposure. There were no significant effects of day on alcohol intake or preference (Fig. 2H, I).

Experiment 3: The effects of chronic nicotine injections in adolescent HAP2 and LAP2 mice on subsequent (adult) FC drinking and DID.

Nicotine treatment in HAP2 and LAP2 mice during adolescence did not affect alcohol intake in adulthood, as measured by FC drinking and DID (Fig. 3B-D). An ANOVA of line (2) x treatment (3) x day (10) on FC alcohol intake and preference yielded significant main effects of line [$F(1,66) = 1314.6$ and 621.8, on intake and preference, respectively; ps < 0.001; HAP2 > LAP2; Fig. 3B, C]. An ANOVA of line (2) x treatment (3) x day (5) on alcohol intake during the 5 days of DID also yielded a significant main effect of line [$F(1,65) = 166.5$; $p < 0.001$; HAP2 > LAP2; Fig. 3D].

Experiment 4: The effects of chronic nicotine injections in adult HAP2 mice on subsequent FC drinking and DID.

Nicotine treatment in adult HAP2 and LAP2 mice did not affect subsequent alcohol intake, as measured by FC drinking and DID (Fig. 4B-D). An ANOVA of treatment (3) x day (9) on FC alcohol intake and preference did not show any main effects or interaction (Fig. 4B, C). An ANOVA of treatment (3) x day (4) on DID alcohol intake showed a main effect of day only [$F(3, 48) = 3.4$, $p < 0.05$], driven by generally declining alcohol intake over days (Fig. 4D).

DISCUSSION

Overall, we report that the effects of repeated nicotine injections on alcohol intake are dependent on temporal parameters, alcohol drinking paradigm, and genetic predisposition for alcohol preference. One main goal of this study was to compare the effects of repeated nicotine co-exposure on alcohol consumption (DID in Experiment 1 and FC in Experiment 2) with the effects of repeated nicotine pre-exposure in the absence of concurrent alcohol access (Experiments 3 and 4), using both binge-like and FC drinking procedures. We found that repeated nicotine and binge-like alcohol co-exposure increased binge-like alcohol intake in HAP2 mice during re-exposure to DID after a 2-week abstinence period (Experiment 1). However, repeated nicotine and FC alcohol co-exposure did not affect FC drinking after a 14d abstinence period (Experiment 2). Repeated nicotine pre-exposure alone during adolescence (Experiment 3) or adulthood (Experiment 4) did not produce any effects on subsequent FC or binge-like drinking.

During the nicotine/saline treatment phase of Experiment 1, acute injection of nicotine (0.5 mg/kg) on day 1 decreased DID alcohol intake during hour 1 in HAP2 mice only. This finding is similar to those of Hendrickson and colleagues (2009), who found that a single injection of nicotine (0.5 mg/kg) decreased hour 1 DID alcohol intake in C57BL/6J mice. A possible explanation offered by the authors was that an acute nicotine pretreatment

may have desensitized nicotinic acetylcholine receptors (nAChRs), which are important mediators of alcohol's reinforcing effects (Hendrickson et al., 2013). Overall, averaged across 10d of nicotine/saline treatments, HAP2 mice that received 1.5 mg/kg nicotine had higher alcohol intake than mice that received 0.5 mg/kg nicotine injections in both hours 1 and 2 post-injection (Fig. 1B, D). The reason for this outcome was due to the fact that alcohol intake in the 0.5 mg/kg group remained relatively low whereas intake in the 1.5 mg/kg group tended to increase across the 10d of treatment (analyses indicated that neither nicotine group significantly differed from saline). One possible explanation for this finding is that the different doses of nicotine may have caused different adaptations of nAChRs, such as upregulation/downregulation of different nAChR subtypes and/or alterations of subunit composition (Govind et al., 2012, 2009). Previous studies have shown that different nicotine treatment doses may result in different nAChR-mediated motivation-related outcomes such as dopamine neuron firing (Besson et al., 2007; Tolu et al., 2013).

After 2 weeks of forced abstinence, in the first hour only on day 1 of re-exposure to DID, HAP2 mice with a history of 1.5 mg/kg nicotine treatment showed significantly elevated alcohol intake (compared to the saline group and 0.5 mg/kg group, which did not differ from saline). In the first hour only on day 2 of re-exposure to DID, all groups showed increased intake but intake in the nicotine-treated groups were higher than the saline-treated group, with a significant difference between the 0.5 mg/kg nicotine vs. saline group. Interestingly, nicotine-treated groups in both lines (main effect of treatment) showed reduced alcohol intake in hour 2 of DID (collapsed across both re-exposure days). This intake behavior may be similar to the 'front-loading' phenomenon observed following weeks of daily DID access in C57BL/6J mice. These animals shift overall intake to the beginning of the 2h daily access period, which may represent increased motivation and/or compulsion to consume alcohol (Linsenbardt and Boehm, 2014). This interpretation is consistent with a recent finding in rats that chronic nicotine in alcohol dependent rats increased motivation and compulsion to seek alcohol in an OSA paradigm (Leão et al., 2015), and supports studies in humans that show that binge drinking is highly associated with tobacco use (McKee and Weinberger, 2013).

However, a subsequent study by (Linsenbardt and Boehm, 2015) reported that the "front-loading" effect was not specific to alcohol because it was also observed in HAP1 mice that had repeated DID exposures to water only. Additional studies are necessary to characterize the specificity of the nicotine pre-exposure effect on DID alcohol drinking in HAP2 and HAP3 mice using other reinforcers like water and saccharin.

Nicotine injections produced some acute effects on FC drinking in Experiment 2. HAP2 mice that received 1.5 mg/kg nicotine showed lower alcohol intake and preference than controls in the 3rd hour post-injection. This is a novel finding because previous studies of immediate nicotine effects on alcohol drinking in mice have not measured alcohol intake beyond 2h post-injection (Sajja and Rahman, 2012). The decreased alcohol intake in HAP2 mice is consistent with Sharpe and Samson (2002), in which repeated nicotine injections immediately prior to alcohol OSA decreased both alcohol seeking and consumption in rats. The time-dependent effects of nicotine in this experiment are also reminiscent of studies in rats which found that an acute nicotine injection immediately prior to alcohol OSA decreased responding, but an acute nicotine injection 3-4h prior to alcohol OSA increased responding (Doyon et al., 2013a; Hauser et al., 2012).

Given the short half-life of nicotine in mice, it is important to note that the attenuating effect of nicotine on alcohol consumption 3h after injection observed in Experiment 2 may not be a direct effect caused by nicotine itself but rather by nicotine's primary metabolite, cotinine, which has a much longer half-life than nicotine (up to 1h in mice) and is available in high concentrations in the brain (Siu and Tyndale, 2007). Cotinine has been shown to modulate nAChRs (Terry et al., 2015), promote striatal dopamine release (Dwoskin et al., 1999), and decrease impulsive- and compulsive-like behavior (Terry et al., 2012). Given that alcohol consumption is modulated by nAChRs and the striatal dopamine system (Hendrickson et al., 2013), and that high alcohol consumption is associated with high impulsivity (Oberlin and Grahame, 2009), these effects of cotinine may reduce alcohol consumption.

Assuming that the mice in this study metabolized alcohol at a rate recorded previously (0.85-0.90 g/kg/hr; Chester and Barrenha, 2007), DID alcohol intake may have produced pharmacologically-relevant BACs in LAP2 mice. Thus, the DID procedure may be a particularly useful model for exploring mechanisms regulating alcohol drinking in people without a genetic family history of AUDs. Although the DID procedure appears to have increased alcohol intake in LAP2 mice, HAP2 mice still drank double the amount of LAP2 mice. Similar findings in HAP1 and LAP1 mice tested in a DID procedure were previously reported (Crabbe et al., 2011), showing that, not surprisingly, genetic mechanisms that underlie FC alcohol drinking also influence binge-like drinking.

Several other mechanisms may underlie nicotine's effects on alcohol drinking in HAP2 mice. Repeated nicotine exposure has been shown to produce cross-tolerance to alcohol and increased self-administration of alcohol, which can be mediated by metabolic enzymes and/or by alterations in synaptic plasticity in the brain (Abburi et al., 2016; Florek et al., 2015). As mentioned above, a study recently showed that concurrent nicotine and alcohol exposure increases motivation for alcohol self-administration and compulsive-like drinking in rats. These behavioral findings were accompanied with nAChR-dependent activation of neuronal ensembles in stress-related brain areas such as central amygdala, dorsomedial prefrontal cortex, and bed nucleus of the stria terminalis (Leão et al., 2015). The stress system, including the glucocorticoid system, plays an important role in nicotine's augmenting effects on alcohol self-administration (Doyon et al., 2013) and in the development of compulsive-like alcohol self-administration (Vendruscolo et al., 2012).

HAP and LAP mouse lines differ in behavioral and hormonal response to stressors (Breit and Chester, 2016; Chester et al., 2014). Notably, we have shown a strong inverse genetic correlation between propensity for high alcohol preference and corticosterone levels after footshock and conditioned fear-related stimuli (LAP1/2 > HAP1/2 lines; Chester et al., 2014). Nicotine, like alcohol and other drugs of abuse, is a chemical stressor that stimulates the release of corticosterone (Armario, 2010) and it is possible that line differences in stress

system responsivity to nicotine partly contribute to the observed line differences in sensitivity to nicotine's effect on alcohol drinking behavior.

Exposure to nicotine has been shown to produce cross-sensitization to alcohol's rewarding effects (Biala and Budzynska, 2008), perhaps by sensitizing midbrain dopamine neurons to alcohol's stimulating effects (Ding et al., 2012). The delayed nature of nicotine's effects on alcohol drinking suggests that alterations in gene expression may be involved. Nicotine exposure can alter epigenetic mechanisms that regulate a variety of genes, including the addiction-related FosB gene (Nestler, 2001). One study showed that nicotine exposure increased the ability of cocaine to increase FosB expression through inhibition of histone deacetylase (Levine et al., 2011). Given that increased FosB expression increases the rewarding effects of drugs (Nestler, 2001), alterations in epigenetic regulation of the FosB gene by nicotine and alcohol exposure may contribute to the increased alcohol drinking seen in Experiment 1. For further discussion on the molecular sites of nicotine-alcohol interactions, please refer to the following excellent reviews: Hendrickson et al., 2009; Tarren and Bartlett, 2017.

Finally, another goal of the present study was to assess the effects of repeated nicotine pre-exposure during adolescence vs. adulthood on alcohol drinking. We did not find any effects of repeated nicotine pre-exposure (injections) during adolescence (Experiment 3) or adulthood (Experiment 4) on either FC or binge-like drinking. This supports previous findings in rats (Kemppainen et al., 2009), but is inconsistent with the findings of Locker et al. (2016) in mice. Nicotine was injected by experimenters in the present study as well as in rats (Kemppainen et al., 2009), who reported similar findings. On the other hand, mice received nicotine in their drinking water in Locker et al. (2016). These differences in the mode of nicotine delivery may have produced different biological consequences that, in turn, produced different influences on alcohol drinking behaviors. For example, the chronic nature of nicotine access (22h/d) in the study by Locker et al. (2016) may have resulted in a different pattern and/or level of nAChR upregulation than repeated nicotine injections. A study previously showed that chronic nicotine delivery via osmotic minipumps results in

greater upregulation of nAChRs than repeated nicotine injections (Ulrich et al., 1997). Secondly, there was a gap of at least 14d between the end of repeated nicotine exposure and the start of alcohol drinking procedures in the present study and in Kemppainen et al. (2009), which may have permitted the reversal of neuroadaptations produced by chronic nicotine that may have altered subsequent alcohol drinking. In comparison, there was an overlap of 4d between chronic nicotine pre-exposure in the drinking water and introduction of alcohol-containing bottles for 2h in Locker et al. (2016). Further studies are needed to address these speculations.

It is important to note that the experiments in the present study were conducted in female mice, which often show greater responses to nicotine than males (see Introduction); however, sex effects may be dependent on genotype (Bernardi et al., 2016). Given the sex differences in nicotine sensitivity (see review by Pogun et al., 2017) and in nicotine-alcohol interactions (see review by Adams, 2017), future studies should include male subjects for comparison. In addition, we plan to test the effects of adolescent alcohol exposure on subsequent nicotine responses in HAP/LAP lines to shed additional light on age-related and genetic vulnerabilities associated with co-abuse of alcohol and nicotine. Finally, future work should build upon the current findings by examining nicotine-alcohol interactions in the replicate 3 HAP/LAP selected lines to further support conclusions that nicotine's effects on alcohol-drinking behaviors are regulated by genes selected for high or low alcohol preference. In summary, our results indicate that co-exposure to repeated nicotine injections and alcohol, but not repeated pre-exposure to nicotine alone (in adolescents or adults), increases binge-like alcohol intake in HAP2 but not LAP2 mice after a period of abstinence. These findings in selected mouse lines indicate that nicotine's effects on motivation to binge drink alcohol depend on genetic predisposition toward alcohol preference. Chronic binge drinking and tobacco use in humans with a genetic history of AUDs may lead to an escalation of subsequent binge drinking and more severe alcohol-related problems.

REFERENCES

Abburi C, Wolfman SL, Metz RAE, Kamber R, McGehee DS, McDaid J (2016) Tolerance to ethanol or nicotine results in increased ethanol self-administration and long-term depression in the dorsolateral striatum. eNeuro 3.

Adams S (2017) Psychopharmacology of tobacco and alcohol comorbidity: a review of current evidence. Curr Addict Rep 4:25–34.

Armario A (2010) Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome. Trends Pharmacol Sci 31:318–325.

Bernardi RE, Zohsel K, Hirth N, Treutlein J, Heilig M, Laucht M, Spanagel R, Sommer WH (2016) A gene-by-sex interaction for nicotine reward: evidence from humanized mice and epidemiology. Transl Psychiatry 6:e861.

Besson M, Granon S, Mameli-Engvall M, Cloëz-Tayarani I, Maubourguet N, Cormier A, Cazala P, David V, Changeux J-P, Faure P (2007) Long-term effects of chronic nicotine exposure on brain nicotinic receptors. Proc Natl Acad Sci U S A 104:8155–8160.

Biala G, Budzynska B (2008) Calcium-dependent mechanisms of the reinstatement of nicotine-conditioned place preference by drug priming in rats. Pharmacol Biochem Behav 89:116–125.

Blomqvist O, Ericson M, Johnson DH, Engel JA, Söderpalm B (1996) Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. Eur J Pharmacol 314:257–267.

Breit KR, Chester JA (2016) Effects of chronic stress on alcohol reward- and anxiety-related behavior in high- and low-alcohol preferring mice. Alcohol Clin Exp Res 40:482–490.

Chen X, Unger JB, Palmer P, Weiner MD, Johnson CA, Wong MM, Austin G (2002) Prior cigarette smoking initiation predicting current alcohol use: evidence for a gateway drug effect among California adolescents from eleven ethnic groups. Addict Behav 27:799–817.

Chester JA, Barrenha GD (2007) Acoustic startle at baseline and during acute alcohol withdrawal in replicate mouse lines selectively bred for high or low alcohol preference. Alcohol Clin Exp Res 31:1633–1644.

Chester JA, Kirchhoff AM, Barrenha GD (2014) Relation between corticosterone and fear-related behavior in mice selectively bred for high or low alcohol preference. Addict Biol 19:663–675.

Chester JA, Weera MM (2017) Genetic correlation between alcohol preference and conditioned fear: Exploring a functional relationship. Alcohol 58:127–137.

Crabbe JC, Spence SE, Brown LL, Metten P (2011) Alcohol preference drinking in a mouse line selectively bred for high drinking in the dark. Alcohol 45:427–440.

Ding Z-M, Katner SN, Rodd ZA, Truitt W, Hauser SR, Deehan GA, Engleman EA, McBride WJ (2012) Repeated exposure of the posterior ventral tegmental area to nicotine increases the sensitivity of local dopamine neurons to the stimulating effects of ethanol. Alcohol 46:217–223.

Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, Mielke MM, Hoffman A, McCallum S (2000) Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. Psychopharmacology (Berl) 151:392–405.

Doyon WM, Dong Y, Ostroumov A, Thomas AM, Zhang TA, Dani JA (2013a) Nicotine decreases ethanol-induced dopamine signaling and increases self-administration via stress hormones. Neuron 79:530–540.

Dwoskin LP, Teng L, Buxton ST, Crooks PA (1999) (S)-(-)-Cotinine, the major brain metabolite of nicotine, stimulates nicotinic receptors to evoke [3H]dopamine release from rat striatal slices in a calcium-dependent manner. J Pharmacol Exp Ther 288:905–911.

Florek E, Kulza M, Piekoszewski W, Gomółka E, Jawień W, Teżyk A, Napierała M (2015) Influence of tobacco smoke exposure on pharmacokinetics of ethyl alcohol in alcohol preferring and non-preferring rats. Pharmacol Rep PR 67:921–927.

Govind AP, Vezina P, Green WN (2009) Nicotine-induced upregulation of nicotinic receptors: Underlying mechanisms and relevance to nicotine addiction. Biochem Pharmacol, 78:756–765.

Govind AP, Walsh H, Green WN (2012) Nicotine-induced upregulation of native neuronal nicotinic receptors Is caused by multiple mechanisms. J Neurosci 32:2227–2238.

Grahame NJ, Li TK, Lumeng L (1999) Selective breeding for high and low alcohol preference in mice. Behav Genet 29:47–57.

Grant BF (1998) 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 10:59–73.

Hauser SR, Bracken AL, Deehan GA, Toalston JE, Ding Z-M, Truitt WA, Bell RL, McBride WJ, Rodd ZA (2013) Selective breeding for high alcohol preference increases the sensitivity of the posterior VTA to the reinforcing effects of nicotine. Addict Biol.

Hauser SR, Getachew B, Oster SM, Dhaher R, Ding Z-M, Bell RL, McBride WJ, Rodd ZA (2012) Nicotine modulates alcohol-seeking and relapse by alcohol-preferring (P) rats in a time-dependent manner. Alcohol Clin Exp Res 36:43–54.

Hendrickson LM, Guildford MJ, Tapper AR (2013) Neuronal nicotinic acetylcholine receptors: common molecular substrates of nicotine and alcohol dependence. Front Psychiatry 4:29.

Hendrickson LM, Zhao-Shea R, Tapper AR (2009) Modulation of ethanol drinking-in-the-dark by mecamylamine and nicotinic acetylcholine receptor agonists in C57BL/6J mice. Psychopharmacology (Berl) 204:563–572.

Hosová D, Spear LP (2017) Voluntary binge consumption of ethanol in a sweetened, chocolate-flavored solution by male and female adolescent Sprague Dawley rats. Alcohol Clin Exp Res 41:541–550.

Kasten CR, Blasingame SN, Boehm II SL (2015) Bidirectional enantioselective effects of the GABAB receptor agonist baclofen in two mouse models of excessive ethanol consumption. Alcohol 49:37–46.

Keenan RM, Hatsukami DK, Pickens RW, Gust SW, Strelow LJ (1990) The relationship between chronic ethanol exposure and cigarette smoking in the laboratory and the natural environment. Psychopharmacology (Berl) 100:77–83.

Kemppainen H, Hyytiä P, Kiianmaa K (2009) Behavioral consequences of repeated nicotine during adolescence in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol Clin Exp Res 33:340–349.

Lê AD, Li Z, Funk D, Shram M, Li TK, Shaham Y (2006) Increased vulnerability to nicotine self-administration and relapse in alcohol-naive offspring of rats selectively bred for high alcohol intake. J Neurosci 26:1872–1879.

Lê AD, Wang A, Harding S, Juzytsch W, Shaham Y (2003) Nicotine increases alcohol self-administration and reinstates alcohol seeking in rats. Psychopharmacology (Berl) 168:216–221.

Leão RM, Cruz FC, Vendruscolo LF, Guglielmo G de, Logrip ML, Planeta CS, Hope BT, Koob GF, George O (2015) Chronic nicotine activates stress/reward-related brain regions and facilitates the transition to compulsive alcohol drinking. J Neurosci 35:6241–6253.

Levine A, Huang Y, Drisaldi B, Griffin EA, Pollak DD, Xu S, Yin D, Schaffran C, Kandel DB, Kandel ER (2011) Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. Sci Transl Med 3:107ra109.

Linsenbardt DN, Boehm SL (2015) Relative fluid novelty differentially alters the time course of limited-access ethanol and water intake in selectively bred high-alcohol-preferring mice. Alcohol Clin Exp Res 39:621–630.

Linsenbardt DN, Boehm SL (2014) Alterations in the rate of binge ethanol consumption: implications for preclinical studies in mice. Addict Biol 19:812–825.

Locker AR, Marks MJ, Kamens HM, Klein LC (2016) Exposure to nicotine increases nicotinic acetylcholine receptor density in the reward pathway and binge ethanol consumption in C57BL/6J adolescent female mice. Brain Res Bull 123:13–22.

Locklear LL, McDonald CG, Smith RF, Fryxell KJ (2012) Adult mice voluntarily progress to nicotine dependence in an oral self-selection assay. Neuropharmacology 63:582–592.

Lynch WJ, Roth ME, Carroll ME (2002) Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology (Berl) 164:121–137.

Madden PAF, Heath AC (2002) Shared genetic vulnerability in alcohol and cigarette use and dependence. Alcohol Clin Exp Res 26:1919–1921.

Matson LM, Grahame NJ (2013) Pharmacologically relevant intake during chronic, free-choice drinking rhythms in selectively bred high alcohol-preferring mice. Addict Biol 18:921–929.

McKee SA, Hinson R, Rounsaville D, Petrelli P (2004) Survey of subjective effects of smoking while drinking among college students. Nicotine Tob Res 6:111–117.

McKee SA, Weinberger AH (2013) How can we use our knowledge of alcohol-tobacco interactions to reduce alcohol use? Annu Rev Clin Psychol 9:649–674.

Miller NS, Gold MS (1998) Comorbid cigarette and alcohol addiction: epidemiology and treatment. J Addict Dis 17:55–66.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128.

Oberlin B, Best C, Matson L, Henderson A, Grahame N (2010) Derivation and characterization of replicate high- and low-alcohol preferring lines of mice and a high-drinking crossed HAP line. Behav Genet 41:288–302.

Oberlin BG, Grahame NJ (2009) High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task. Alcohol Clin Exp Res 33:1294–1303.

Pogun S, Yararbas G, Nesil T, Kanit L (2017) Sex differences in nicotine preference. J Neurosci Res 95:148–162.

Potthoff AD, Ellison G, Nelson L (1983) Ethanol intake increases during continuous administration of amphetamine and nicotine, but not several other drugs. Pharmacol Biochem Behav 18:489–493.

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol Behav 84:53–63.

Rhodes JS, Ford MM, Yu C-H, Brown LL, Finn DA, Garland T, Crabbe JC (2007) Mouse inbred strain differences in ethanol drinking to intoxication. Genes Brain Behav 6:1–18.

Robinson SF, Marks MJ, Collins AC (1996) Inbred mouse strains vary in oral self-selection of nicotine. Psychopharmacology (Berl) 124:332–339.

Sajja RK, Rahman S (2012) Neuronal nicotinic receptor ligands modulate chronic nicotine-induced ethanol consumption in C57BL/6J mice. Pharmacol Biochem Behav 102:36–43.

Sharpe AL, Samson HH (2002) Repeated nicotine injections decrease operant ethanol self-administration. Alcohol 28:1–7.

Siu ECK, Tyndale RF (2007) Characterization and comparison of nicotine and cotinine metabolism in vitro and in vivo in DBA/2 and C57BL/6 mice. Mol Pharmacol 71:826–834.

Smith BR, Horan JT, Gaskin S, Amit Z (1999) Exposure to nicotine enhances acquisition of ethanol drinking by laboratory rats in a limited access paradigm. Psychopharmacology (Berl) 142:408–412.

Spear LP (2016) Consequences of adolescent use of alcohol and other drugs: Studies using rodent models. Neurosci Biobehav Rev 70:228–243.

Talamini G, Bassi C, Falconi M, Sartori N, Salvia R, Rigo L, Castagnini A, Francesco VD, Frulloni L, Bovo P, Vaona B, Angelini G, Vantini I, Cavallini G, Pederzoli P (1999) Alcohol and smoking as risk factors in chronic pancreatitis and pancreatic cancer. Dig Dis Sci 44:1303–1311.

Tarren JR, Bartlett SE (2017) Alcohol and nicotine interactions: pre-clinical models of dependence. Am J Drug Alcohol Abuse 43:146–154.

Terry AV, Buccafusco JJ, Schade RF, Vandenhuerk L, Callahan PM, Beck WD, Hutchings EJ, Chapman JM, Li P, Bartlett MG (2012) The nicotine metabolite, cotinine, attenuates glutamate (NMDA) antagonist-related effects on the performance of the five choice serial reaction time task (5C-SRTT) in rats. Biochem Pharmacol 83:941–951.

Terry AV, Callahan PM, Bertrand D (2015) R-(+) and S-(-) isomers of cotinine augment cholinergic responses in vitro and in vivo. J Pharmacol Exp Ther 352:405–418.

Tolu S, Eddine R, Marti F, David V, Graupner M, Pons S, Baudonnat M, Husson M, Besson M, Reperant C, Zemdegs J, Pagès C, Hay YAH, Lambolez B, Caboche J, Gutkin B, Gardier AM, Changeux J-P, Faure P, Maskos U (2013) Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. Mol Psychiatry 18:382–393.

Ulrich YM, Hargreaves KM, Flores CM (1997) A comparison of multiple injections versus continuous infusion of nicotine for producing up-regulation of neuronal [3H]-epibatidine binding sites. Neuropharmacology 36:1119–1125.

Vendruscolo LF, Barbier E, Schlosburg JE, Misra KK, Whitfield TW, Logrip ML, Rivier C, Repunte-Canonigo V, Zorrilla EP, Sanna PP, Heilig M, Koob GF (2012) Corticosteroid-dependent plasticity mediates compulsive alcohol drinking in rats. J Neurosci 32:7563–7571.

Weitzman ER, Chen Y-Y (2005) The co-occurrence of smoking and drinking among young adults in college: national survey results from the United States. Drug Alcohol Depend 80:377–386.

FIGURE LEGENDS

Figure 1. The effects of repeated nicotine injections on immediate binge-like alcohol drinking (DID) and on DID after a 14d abstinence period (re-exposure). (A) Timeline schematic for Experiment 1. Mice were given alcohol access 2h every day except during the 14d abstinence period. **(B – E)** DID immediately after nicotine or saline injections. [a] HAP2 mice that received 1.5 mg/kg nicotine injections showed greater average (across 10d) alcohol intake than mice that received 0.5 mg/kg nicotine injections (p <0.05). [b] HAP2 mice that received 0.5 mg/kg nicotine showed significantly lower alcohol intake than mice that received saline on day 1 of nicotine/saline injections. [c] HAP2 mice that received 1.5 mg/kg nicotine injections showed greater alcohol intake than mice that received 0.5 mg/kg (p = 0.05) nicotine injections on the first day of DID (hour 1) following a 2-week abstinence period. [d] HAP2 mice that received 0.5 mg/kg nicotine injections showed greater alcohol intake than mice that received saline injections on the second day of DID (hour 1) following a 14d abstinence period (p < 0.01). N's = 13-16/line/treatment group (5-8/line/treatment group for re-exposure phase).

Figure 2. The effects of repeated nicotine injections on immediate FC drinking and on FC drinking after a 14d abstinence period (re-exposure). (A) Timeline schematic for Experiment 2. Mice were given FC alcohol access throughout the experiment except during the 14d abstinence period. **(B – D)** Immediate post-nicotine alcohol intake and **(E – G)** alcohol preference over 9d of FC drinking. HAP2 mice in the 1.5 mg/kg nicotine group showed significantly lower alcohol intake (D) and preference (G) than HAP2 mice in the 0.0 or 0.5 mg/kg nicotine groups in hour 3 post-injection. **(H)** Alcohol intake and **(I)** preference over 6d of FC drinking after a 14d abstinence period (re-exposure phase). There were no significant effects of treatment on re-exposure FC alcohol intake or preference. All data are presented collapsed by day as there were no significant effects of day. *p < 0.05; (B – G) N = 14-17/line/treatment group; (H, I) N = 7-8/line/treatment group.

Figure 3. The effects of repeated nicotine injections during adolescence on FC drinking and DID in adulthood (HAP2 and LAP2 mice). (A) Timeline schematic for Experiment 3. Mice were given alcohol access only during FC drinking and DID. **(B)** FC alcohol intake and **(C)** preference over 14d. **(D)** DID alcohol intake over 5d. All data are presented collapsed by day as there were no significant effects of day. N = 11-12/line/treatment group.

Figure 4. The effects of repeated nicotine injections in adulthood on alcohol FC drinking and DID (HAP2 mice only). (A) Timeline schematic for Experiment 4. Mice were given alcohol access only during FC drinking and DID. **(B)** FC alcohol intake and **(C)** preference over 14d. **(D)** DID alcohol intake over 5d. Chronic nicotine pre-exposure had no effect on FC drinking and DID in adult HAP2 mice. All data are presented collapsed by day as there were no significant effects of day. N = 11-14/treatment group.