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## Systemic administration of racemic baclofen reduces both acquisition and maintenance of alcohol consumption in male and female mice

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### Abstract

Baclofen is a GABA<sub>B</sub> receptor agonist with proposed use as a treatment for alcohol use disorder (AUD). In preclinical studies, racemic baclofen decreases alcohol consumption in both mice and rats; however, there is a significant disparity in the efficacy of the drug across species. We previously demonstrated that baclofen is enantioselective, with the racemic enantiomer successfully reducing binge-like alcohol consumption during Drinking-in-the-Dark (DID) in C57BL/6J (B6) mice, as well as 24-h consumption during two-bottle choice (2BC) preference drinking in replicate 1 High Alcohol Preferring (HAP) mice. Here we extend these findings by investigating the effects of racemic baclofen on the acquisition and maintenance of alcohol consumption, locomotor activity, and saccharin drinking in two different mouse genotypes and drinking paradigms. Adult male and female B6 mice were allowed free access to 20% (v/v) alcohol for 2 h daily in a 14-day DID procedure. Adult male and female replicate 2 HAP (HAP2) mice were allowed 24-h access to 10% (v/v) alcohol versus tap water in a 2BC procedure for 14 days. Systemic injections of baclofen (0.0 or 3.0 mg/kg) were given 3 h into the dark cycle on days 1–5 in alcohol acquisition experiments and days 6–10 in alcohol maintenance experiments. We found that racemic baclofen significantly reduces acquisition of DID and 2BC alcohol drinking in male and female B6 and HAP2 mice, whereas it only significantly reduces the maintenance of DID alcohol intake in B6 mice. Racemic baclofen did not alter home cage locomotor activity but did alter saccharin intake, suggesting it may have nonspecific effects. The current data add to literature suggesting that smaller doses of racemic baclofen may be an effective treatment of AUD. Future work should focus on the longitudinal efficacy of racemic baclofen in high-drinking mouse genotypes to further investigate whether it is effective for those with a genetic predisposition to AUD.

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Declaration of competing interest

The authors declare no conflict of interest.

## Keywords

Alcohol; Binge; Mice; Racemic baclofen; Two-bottle choice

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## Introduction

Despite there being three different Food and Drug Administration-approved medications for the treatment of alcohol use disorder (AUD; disulfiram, naltrexone, and acamprosate), the age-standardized death rates due to alcohol in the US have significantly increased over the past three decades (US Burden of Disease Collaborators et al., 2018). In fact, excessive alcohol drinking is a leading cause of preventable death in the United States (US Burden of Disease Collaborators et al., 2018). Further, AUD is the most common substance use disorder across the globe (Degenhardt et al., 2018). Improvement of current treatment strategies for AUD is warranted due to the alarmingly high and increasing rates of alcohol-related deaths. Baclofen (Keberle, Faigle, & Wilhelm, 1969, pp. 471–548) is a selective gamma-aminobutyric acid-B ( $GABA_B$ ) receptor agonist recently investigated as a treatment for AUD (De Beaupaire et al., 2019; Pierce, Sutterland, Beraha, Morley, & van den Brink, 2018). Because of alcohol's actions on the GABA system (for review, please see Koob, 2004 and Förstera, Castro, Moraga-Cid, & Aguayo, 2016), and the previous reports demonstrating evidence for baclofen in reducing alcohol drinking and related behaviors (Echeverry-Alzate et al., 2021; Kasten & Boehm, 2014; Rabat, Henkous, Corio, Noguez, & Beracochea, 2019; for review see Colombo & Gessa, 2018), baclofen may be a promising treatment option for AUD. Additionally, human studies have found greater efficacy with baclofen for reducing alcohol consumption in populations that exhibit greater AUD severity compared to lower AUD severity (Leggio, Garbutt, & Addolorato, 2010). However, much remains unknown about how baclofen affects drinking behaviors in models of excessive alcohol consumption.

$GABA_B$  receptors are heterodimeric G-protein coupled receptors that bind the neurotransmitter GABA (Evenseth, Gabrielsen, & Sylte, 2020). Baclofen is a  $GABA_B$  receptor agonist existing in two enantiomers [R(+)- and S(-)- baclofen], each with relatively high binding affinity for  $GABA_B$  receptors and a relatively short half-life in humans and a half-life of 1.5–3.4 h in rodents ( $K_D = 47$  nM; ~5 h; Drew, Johnston, & Weatherby, 1984; Kent, Park, & Lindsley, 2020; Lal et al., 2009) and in *in vivo* studies (between 1.5 and 3.4 h; Lal et al., 2009). Both enantiomers are eliminated from blood plasma at similar rates, and both cross the blood brain barrier (BBB). However, racemic baclofen is transported to the site of action at four times the rate of S(-)- baclofen, meaning it more readily crosses the BBB (van Bree, Heijligers-Feijen, de Boer, Danhof, & Breimer, 1991). Previous research has shown discrepancies in drug efficacy when using non-enantiomer specific baclofen. For example, whereas systemically administered baclofen has been shown to *increase* alcohol drinking and operant responding in mice and rats (Czachowski, Legg, & Stansfield, 2006; Moore et al., 2007; Smith, Boyle, & Amit, 1999; Smith, Robidoux, & Amit, 1992), systemic or site-specific ventral tegmental area (VTA) administration has been shown to *decrease* (Anstrom, Cromwell, Markowski, & Woodward, 2003; Janak & Gill, 2003; Liang et al., 2006; Maccioni, Lorrai, Leite-Morris, & Colombo, 2018; Moore

& Boehm, 2009) alcohol drinking and operant responding in mice and rats. Furthermore, our group and others have shown bidirectional effects of baclofen when the enantiomers are selectively tested in rodents. Specifically, systemic and brain site-specific racemic baclofen reduced alcohol drinking or self-administration in mice (Kasten, Blasingame, & Boehm, 2015; Kasten & Boehm, 2014) and rats (Echeverry-Alzate et al., 2021; González-Marín, Lebourgeois, Jeanblanc, Diouf, & Naassila, 2018; Lorrain, Maccioni, Gessa, & Colombo, 2016; Quintanilla, Perez, & Tampier, 2008), whereas S(-)- baclofen increased alcohol drinking in mice (Kasten & Boehm, 2014; Kasten et al., 2015) and rats (Echeverry-Alzate et al., 2021; but see Lorrain et al., 2016). Thus, it appears that it may be the racemic enantiomer that is most effective at reducing alcohol-motivated behaviors in rodents, and perhaps the most efficacious of the enantiomers in the treatment of AUD.

Nevertheless, much remains unknown about how racemic baclofen affects alcohol drinking in preclinical models. While the current body of research is incredibly informative, demonstrating a reduction in drinking in multiple doses, it was done in males only, without adequate power to detect sex effects, and without assessment of baclofen efficacy in high-alcohol drinking models. Further, since commonly used doses of baclofen that reduce alcohol drinking also reduce locomotor activity (Cryan et al., 2004), we sought to establish a subthreshold dose of baclofen aiming to reduce alcohol drinking without altering locomotor activity. To further investigate and expand upon this literature, the current experiments sought to assess the effect of a subthreshold dose of systemically administered racemic baclofen on the development and maintenance of alcohol drinking in two mouse models of high alcohol consumption. Specifically, we assessed baclofen effects on binge-like alcohol drinking using a limited-access model of alcohol drinking, Drinking-in-the-Dark (DID; Thiele, Crabbe, & Boehm, 2014), in both male and female C57BL/6J (B6) mice, as well as a free-access two-bottle choice (2BC) model of alcohol drinking in male and female selectively bred High Alcohol Preferring (HAP) 2 mice.

## Materials and methods

### Animals

Naïve adult male and female B6 mice (postnatal day [PND] 65 at drinking start) were acquired from The Jackson Laboratory (Bar Harbor, Maine, United States). Naïve adult male and female HAP2 mice (PND 68–98 at drinking start) were bred on-site at the IUPUI School of Science (for more information on the genetic selection and characterization of HAP2 mice, please see Oberlin, Best, Matson, Henderson, & Grahame, 2011). Animals were individually housed in a vivarium with a 12-h/12-h reverse light–dark cycle for at least one week prior to the start of experiments. Mice were housed in wired top standard shoebox mouse cages (18.4 cm wide, 29.2 cm long, 12.7 cm tall) and were given food (Lab Diet 5001, Rodent Diet) and tap water *ad libitum* apart from water bottle removal during the DID sessions. 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).

## Drugs

190 proof alcohol was purchased from Pharmco, Inc. (Brookfield, Connecticut) and was added to tap water to create a 20% v/v alcohol solution used in the DID experiments. 200 proof alcohol was purchased from Pharmco, Inc. and was added to tap water to create a 10% v/v alcohol solution for use in 2BC experiments. 200 proof alcohol was used for the 2BC experiments because HAP mice are selectively bred using this alcohol concentration. Saccharin (99%) was purchased from Sigma Aldrich (St. Louis, Missouri, United States) and was added to tap water to create a 0.2% (w/v) saccharin concentration. Racemic baclofen hydrochloride was purchased from Sigma Aldrich and dissolved in 0.9% saline purchased from Teknova (Newark, Delaware, United States) to make drug doses of 0.0 or 3.0 mg/kg (injected at volumes of 10 mL/kg). Our previous work found that an acute dose of 3 mg/kg racemic baclofen did not affect DID or 2BC alcohol or saccharin intake. Therefore, we chose this “subthreshold” dose to pilot the effects of repeated administration. Preliminary data in male B6 mice showed that 3.0 mg/kg decreased alcohol consumption during drinking acquisition and maintenance. Animals were divided into four groups in each experiment: males that received saline or baclofen and females that received saline or baclofen.

## Drinking-in-the-dark (DID)

DID is a limited-access model of binge-like alcohol consumption (Thiele et al., 2014). B6 mice received one 10-mL ball-bearing sipper tube containing 20% v/v alcohol in tap water into their home cages in place of the regular water bottle. This occurred 3 h into the dark cycle for 2 h on days 1–14. On days 15 and 16, mice received access to 0.2% saccharin using identical procedures as we have previously done (Bauer, Garcy, & Boehm, 2020; Bauer, McVey, Germano, Zhang, & Boehm, 2022) to assess nonspecific effects of baclofen on reward consumption. Consumption was measured by reading the sipper tubes to the nearest 0.025 mL and adjusting for leak based on volumes from sipper tubes on empty cages on the same drinking rack. Body weights were taken one day prior to the first baclofen administration to determine injection volume and weekly thereafter to determine g/kg alcohol consumption. Injections were given immediately before fluid access, 3 h into the dark cycle.

## Two-bottle choice (2BC)

2BC is a free-access model of alcohol consumption whereby animals receive unlimited access to both alcohol (10% v/v) and tap water in their home cages 24 h a day. HAP2 mice were allowed free access for a total of 14 days. On days 15 and 16, mice underwent 2BC of saccharin (0.2%; Bauer et al., 2020, 2022). Weights were taken the day before injection day 1 and weekly thereafter. Injections were given 2 h and 45 min into the dark cycle to stay consistent with the timing of injections relative to the dark cycle for the DID experiments, and evidence suggests that intake in HAP2 mice is highest at 3 h into the dark cycle and remains stable for 6 h (Matson & Grahame, 2013). Consumption was measured by reading the sipper tubes to the nearest 0.025 mL. 2BC preference scores were calculated by dividing alcohol consumed by total alcohol and water consumed.

### Acquisition model

30 B6 mice (15 male and 15 female) underwent DID of a 20% alcohol solution for 14 days, and 36 HAP2 mice (18 male and 18 female) mice underwent 2BC of a 10% alcohol solution for 14 days. On days 1–5, all mice were given daily systemic injections of racemic baclofen (0 or 3 mg/kg) 3 h into the dark cycle. On days 15 and 16, mice were injected with the same dose of baclofen and given access to 0.2% saccharin instead of alcohol. Drinking was monitored at 2 h for DID and at 2 and 24 h post-injection for 2BC on all injection days. On days 6–14, drinking was monitored at 2 h for DID and 24 h for 2BC.

### Maintenance model

32 B6 mice (16 male and 16 female) underwent DID with a 20% (v/v) alcohol solution for 14 days and 32 HAP2 mice (16 male and 16 female) mice underwent 2BC with a 10% (v/v) alcohol solution for 14 days. On days 1–5, drinking and locomotor activity (see below) were monitored at 2 h for DID and 24 h for 2BC. On days 6–10, mice were given a systemic injection of racemic baclofen (0 or 3 mg/kg) 3 h into the dark cycle and immediately presented with alcohol. During these days, drinking was monitored at 2 h post-injection for DID and at 2 and 24 h post-injection for 2BC. On days 11–14, drinking was monitored at 2 h for DID and 24 h for 2BC (no injections) to monitor the duration of the maintenance effect. Finally, on days 15 and 16, mice were injected with the same dose of baclofen or saline vehicle and given DID (B6) or 2BC (HAP2) access to 0.2% saccharin instead of alcohol.

### Locomotor monitoring

Home cage locomotor activity was monitored during all drinking sessions in B6 and HAP2 mice to capture any locomotor effects of baclofen that might interfere with alcohol drinking. Data were collected using the Opto M–3 system (Columbus Instruments), which tabulates locomotor activity by counting beam breaks over a set time interval using infrared beams that surround the perimeter of the home cage. The specific parameters have been described in depth elsewhere (Bauer et al., 2020; Linsenhardt & Boehm, 2012). Ambulatory data were collected every 5 min. Equipment malfunction resulted in missing data from day 14 of the HAP2 maintenance experiment and most data from the B6 experiments. Thus, B6 locomotor data were not analyzed.

### Statistical analyses

Alcohol drinking, saccharin drinking, and locomotor activity data were analyzed separately by experiment (acquisition and maintenance), fluid type (alcohol and saccharin), and genotype (B6 and HAP2). Three-way ANOVAs were conducted with sex and baclofen dose as between-group factors and day as the within-group factor. B6 mice were assessed for consumption. HAP2 mice were assessed for drinking and locomotor activity at 2 h and 24 h on baclofen injection days and 24 h on non-injection days. RM three-way ANOVAs of dose, day, and sex were run for all analyses. If sex did not interact with any other factor, we collapsed on sex as a factor and ran RM two-way ANOVAs. In addition to the aforementioned analyses, we also analyzed the data on injection days only; however, the overall interpretation did not change (results not included). Greenhouse-Geisser corrections

were applied and follow-up *post hoc* tests were performed as necessary. Data were analyzed using GraphPad Prism 8. Differences were considered significant at  $p < 0.05$  and corrected for *post hoc* analyses.

## Results

### B6 acquisition

The effect of baclofen in B6 mice on acquisition of alcohol drinking during DID is shown in Fig. 1A and B. RM three-way ANOVA of sex, dose, and day on consumption revealed significant main effects of dose [ $F(1, 27) = 16.15, p < 0.01$ ], day [ $F(13, 351) = 29.97, p < 0.01$ ], and sex [ $F(1, 27) = 4.31, p < 0.05$ ], as well as significant interactions of day  $\times$  dose day [ $F(13, 351) = 10.52, p < 0.01$ ] and day  $\times$  sex  $\times$  dose [ $F(13, 351) = 2.14, p < 0.05$ ]. Due to the three-way interaction of sex  $\times$  day  $\times$  dose, we analyzed the data for male and female mice separately. In male mice, we ran an RM two-way ANOVA of day and dose, revealing a main effect of dose [ $F(1, 13) = 20.87, p < 0.01$ ], day [ $F(4.63, 60.14) = 16.61, p < 0.01$ ], and an interaction of dose  $\times$  day [ $F(13, 169) = 3.59, p < 0.01$ ]. Follow-up *post hoc* Bonferroni-corrected *t* tests found that baclofen significantly reduced drinking compared with saline on day 5 [ $t(11.23) = 5.99, p < 0.01$ ; Fig. 1A]. In female mice, an RM two-way ANOVA of day and dose revealed a main effect of day [ $F(5.36, 74.98) = 14.92, p < 0.01$ ] and a significant interaction of day and dose [ $F(13, 182) = 9.39, p < 0.01$ ]. Bonferroni-corrected *t* tests revealed that baclofen significantly reduced drinking compared with saline on days 1, 3, 4, and 5 ( $p$ 's  $< 0.05$ ); Fig. 1B.

### B6 maintenance

The effect of baclofen on maintenance of alcohol drinking during DID in B6 mice is shown in Fig. 1C and D. An RM three-way ANOVA of sex, dose, and day across days 1–14 revealed a significant main effect of dose [ $F(1, 29) = 14.49, p < 0.01$ ], day [ $F(13, 377) = 10.54, p < 0.01$ ] and sex [ $F(1, 29) = 11.42, p < 0.01$ ], suggesting that all factors influenced alcohol consumption across days. There was also a significant interaction of dose  $\times$  day [ $F(13, 377) = 4.509, p < 0.01$ ] and dose  $\times$  sex [ $F(1, 29) = 6.64, p < 0.05$ ]. Due to the interaction of sex  $\times$  dose, we separated our analyses by sex. In male mice, an RM two-way ANOVA of day and dose revealed a significant main effect of day [ $F(3.51, 52.62) = 6.4, p < 0.01$ ] and a significant interaction of day  $\times$  dose [ $F(13, 195) = 2.46, p < 0.01$ ]. However, follow-up *post hoc* Bonferroni-corrected *t* tests did not reveal a significant effect of baclofen on alcohol drinking on any day ( $p$ 's  $> 0.05$ ); Fig. 1C. In female mice, a similar RM two-way ANOVA of day and dose revealed a significant main effect of day [ $F(2.96, 41.37) = 5.12, p < 0.01$ ], dose [ $F(1, 14) = 21.18, p < 0.01$ ], and an interaction of day  $\times$  dose [ $F(13, 182) = 2.312, p < 0.01$ ]. Subsequent *post hoc* Bonferroni-corrected *t* tests indicated that baclofen significantly reduced intake on days 7, 8, and 10 ( $p$ 's  $< 0.05$ ); Fig. 1D.

### HAP2 acquisition

The effect of baclofen in HAP2 mice on acquisition of alcohol drinking during 2BC is shown in Fig. 2A and B. An overall RM three-way ANOVA did not reveal any significant interactions involving sex, so data were collapsed on this factor. To mirror the intake time frame of the B6 experiments, and due to the short half-life of racemic baclofen, we assessed

differences in drinking at 2 h and 24 h. An RM two-way ANOVA of dose and day on 2-h alcohol consumption across injection days revealed a significant main effect of dose [ $F(1, 33) = 25.15, p < 0.01$ ]; Fig. 2A. An RM two-way ANOVA of dose and day on 24-h alcohol consumption across days revealed a significant main effect of day [ $F(4.88, 161.0) = 15.40, p < 0.01$ ] and dose [ $F(1, 33) = 19.59, p < 0.05$ ], but no significant interactions; Fig. 2B. The effect of baclofen in HAP2 mice on acquisition of alcohol preference drinking during 2-h and 24-h 2BC is shown in Table 1. Five preference scores were not calculated because two saline and three baclofen mice did not consume either water or alcohol on one of the injection days. Again, the overall RM three-way ANOVA did not reveal any significant interactions involving sex. A mixed-effects model of day and dose showed no significant effects at 2 h. However, at 24 h, an RM two-way ANOVA of day and dose found a significant main effect of day [ $F(2.94, 96.84) = 5.25, p < 0.01$ ] and a main effect of dose [ $F(1, 33) = 5.66, p < 0.05$ ], but no significant interactions. Preference outside of injection days was monitored at 24 h only. RM two-way ANOVA of day and dose revealed a significant main effect of day [ $F(2.34, 77.11) = 9.8, p < 0.01$ ]; Table 1. Thus, baclofen reduced preference at 24 h on injection days only.

The effect of baclofen on locomotor activity during the acquisition of 2BC drinking in HAP2 mice is shown in Fig. 2C and D. Sex did not interact with any other factor in the overall RM three-way ANOVA. An RM two-way ANOVA of dose and day on 2-h locomotor activity revealed a significant main effect of day [ $F(4, 132) = 6.10, p < 0.01$ ] but not dose; Fig. 2C. An RM two-way ANOVA of dose and day on 24-h locomotor activity during the acquisition of alcohol consumption revealed a significant main effect of day [ $F(5.35, 176.4) = 4.73, p < 0.01$ ] but not dose; Fig. 2D. Thus, baclofen treatment during acquisition did not affect locomotion.

### HAP2 maintenance

The effect of baclofen in HAP2 mice on maintenance of alcohol drinking during 2BC is shown in Fig. 3A. To mirror the intake time frame of the B6 experiments, and due to the short half-life of racemic baclofen, we assessed differences in drinking at 2 and 24 h. Sex did not interact with any other factor in the overall RM three-way ANOVA. An RM two-way ANOVA of dose and day on 2-h alcohol consumption for all injection days (days 6–10) revealed a significant main effect of dose [ $F(1, 29) = 13.01, p < 0.01$ ] and day [ $F(3, 86.7) = 3.05, p < 0.05$ ]; Fig. 3A. Next, RM three-way ANOVA of 24-h drinking did not result in any significant interactions involving sex, so data were again collapsed on this factor for subsequent analysis. An RM two-way ANOVA of dose and day on 24-h alcohol consumption revealed a significant main effect of day [ $F(5.16, 149.8) = 5.16, p < 0.01$ ] and a significant interaction of day  $\times$  dose [ $F(13, 377) = 2.01, p < 0.05$ ]. However, *post hoc* Bonferroni-corrected *t* tests failed to detect significant effects of baclofen on any day ( $p$ 's  $> 0.05$ ); Fig. 3A. The effect of baclofen on maintenance of alcohol preference drinking during 2- and 24-h 2BC in HAP2 mice is shown in Table 2. Sex did not interact with any other factor in the overall RM three-way ANOVA. An RM two-way ANOVA of dose and day across days 6–10 revealed a main effect of day [ $F(3.39, 98.29) = 3.12, p < 0.05$ ] and dose [ $F(1, 29) = 18.02, p < 0.01$ ] on 2-h preference. For 24-h preference, a subsequent RM two-way ANOVA of dose and day across days 1–14 revealed a significant main effect of day

[ $F(4.08, 118.4) = 3.35, p < 0.05$ ] and a day  $\times$  dose interaction [ $F(13, 377) = 2.55, p < 0.01$ ]. Subsequent *post hoc* Bonferroni-corrected *t* tests did not detect effects of baclofen on any day ( $p$ 's  $> 0.05$ ).

The effect of baclofen on locomotor activity during the maintenance of 2BC drinking in HAP2 mice is shown in Fig. 3C and D. Sex did not interact with any other factor in the overall RM three-way ANOVA. Day 14 locomotor activity was not collected due to equipment malfunction. The RM two-way ANOVA of dose and day on 24-h locomotor activity during the maintenance of alcohol consumption (days 1–13) revealed a significant main effect of day [ $F(5.36, 155.5) = 14.37, p < 0.01$ ] and dose [ $F(1, 29) = 4.436, p < 0.05$ ]; Fig. 3D. To determine whether baclofen's apparent locomotor suppressing effect occurred as a result of drug administration or was a consequence of baseline differences, we also assessed the effect of baclofen on 24-h locomotor activity on injection days only (days 6–10). RM two-way ANOVA of dose and day for days 6–10 revealed a main effect of day [ $F(3.206, 92.97) = 3.934, p < 0.01$ ] and dose [ $F(1, 29) = 4.331, p = 0.0046$ ]. Together, these results confirm the presence of baseline locomotor differences and a modest locomotor suppressant effect of baclofen. Further, to probe locomotor activity during the 2-h window, a two-way RM ANOVA of dose and day (days 6–10) revealed a main effect of dose [ $F(1, 29) = 10.87, p < 0.01$ ] and a significant interaction of day and dose [ $F(4, 116) = 2.7, p = 0.03$ ]. *Post hoc* Bonferroni-corrected *t* tests indicated a significant difference between drug doses on day 6 [ $t(26.95) = 4.25, p < 0.01$ ] and day 8 [ $t(24.80) = 3.32, p < 0.05$ ]; Fig. 3C.

### Saccharin drinking

Following each of the drinking procedures, mice received two days of saccharin drinking (days 15 and 16) to assess whether baclofen also altered saccharin drinking; Fig. 4. Three-way ANOVAs were initially run on sex, dose, and day finding no significant interaction or main effect of sex. Thus, we collapsed on sex as a factor. In B6 mice, RM two-way ANOVA of dose and day during acquisition revealed a main effect of day [ $F(1, 29) = 9.38, p < 0.01$ ]; Fig. 4A. RM two-way ANOVA of dose and day during maintenance revealed a main effect of dose [ $F(1, 31) = 32.58, p < 0.01$ ]; Fig. 4B. In the HAP2 acquisition experiment, RM two-way ANOVAs of dose and day revealed a significant effect of dose at 2 h [ $F(1, 33) = 7.78, p < 0.01$ ] and 24 h [ $F(1, 33) = 5.66, p < 0.05$ ]; Fig. 4C and D. In the HAP2 maintenance experiment, RM two-way ANOVAs of dose and day revealed a significant main effect of time at 24 h [ $F(1, 29) = 30.21, p < 0.01$ ], but not at 2 h ( $p$ 's  $> 0.05$ ); Fig. 4F and E. These data are important because they demonstrate that the effect of repeated baclofen administration on alcohol drinking is not specific to alcohol.

### Discussion

Several studies have assessed the efficacy of various baclofen doses on alcohol consumption in mouse models (Crabbe et al., 2017; Kasten et al., 2015; Villas Boas et al., 2012; for review see Colombo & Gessa, 2018). However, the goal of the present study was to expand upon the current literature and establish a subthreshold dose of systemic racemic baclofen as a potential treatment for AUD. Specifically, we sought to assess whether systemic administration of racemic baclofen would decrease alcohol consumption in B6 and HAP2

mice, two models of high alcohol intake, during an early acquisition period of alcohol intake, and whether racemic baclofen could disrupt stable drinking when administered during a maintenance period of alcohol intake. Additionally, we sought to investigate whether racemic baclofen would alter daily home-cage locomotor activity during the drinking sessions of the HAP2 experiments and saccharin consumption in both genotypes.

Ultimately, the present study demonstrates that systemic injections of 3.0-mg/kg racemic baclofen transiently reduced alcohol intake in B6 and HAP2 mice. Specifically, in B6 mice, administration of the drug reduced alcohol intake during acquisition and maintenance of alcohol drinking; in HAP2 mice, alcohol intake decreased both 2- and 24-h readings when treatment occurred during the acquisition of alcohol consumption, but only during the 2-h reading in the maintenance phase of alcohol consumption. Further, racemic baclofen modestly decreased HAP2 locomotor activity at the 24-h reading of the maintenance phase. Lastly, 3.0-mg/kg racemic baclofen altered saccharin intake in both genotypes; however, this was inconsistent across experiments. Taken together, our findings suggest that while racemic baclofen decreases alcohol intake, there is no evidence for long-term efficacy after cessation of drug administration and that other factors such as genetics or high levels of sustained alcohol intake may influence its efficacy.

### **B6 acquisition and maintenance**

Racemic baclofen has been shown to decrease alcohol drinking at varying doses (5–10 mg/kg) in C57BL/6J mice following systemic administration and intracranial infusions into the nucleus accumbens shell (Kasten & Boehm, 2014; Kasten, Frazee, & Boehm, 2016). Here we chose to use a 3-mg/kg dose of racemic baclofen because unpublished pilot data from our lab demonstrated that an acute dose of 3 mg/kg did not affect DID or 2BC alcohol or saccharin intake. However, repeated administration of this dose reduced alcohol consumption during acquisition and maintenance in male C57BL/6J mice. Factors not assessed at this dose include whether repeated baclofen altered saccharin intake or differences between male and female B6 mice. Given that there are saccharin and locomotor effects following higher doses of systemic administration of racemic baclofen, we sought to investigate those factors in the present study.

Similar to our pilot work, we found a significant decrease in alcohol intake in both males and females after systemic administration of 3-mg/kg racemic baclofen during our acquisition (days 1–5; Fig. 1A and B) and maintenance (days 6–10; Fig. 1C and D) experiments. The decrease in alcohol intake seen here is consistent with previous reports using mouse models (Crabbe et al., 2017; Kasten et al., 2015; Villas Boas et al., 2012). However, in previous studies using B6 mice, a 3-mg/kg dose did not significantly decrease alcohol intake or lever pressing for alcohol (Besheer, Lepoutre, & Hodge, 2004; Kasten et al., 2015). Thus, the present study is the first to show that a 3-mg/kg dose of racemic baclofen significantly reduces alcohol intake in C57BL/6J and HAP2 mice. Other rodent models have shown that a 3-mg/kg dose is effective. For example, Lorrai et al. (2016) found that 3-mg/kg racemic baclofen decreased alcohol intake and lever pressing for alcohol in alcohol-preferring rats, supporting the present findings.

Further, the decrease in alcohol intake seen here did not have a lasting effect. During our assessment of acquisition, B6 mice returned to normal drinking levels on day 7 (Fig. 1A and B), the second day without a drug injection. This pattern of alcohol consumption was stable across days 6–14, suggesting that repeated systemic administration of racemic baclofen during the first 5 days of drinking disrupts the acquisition of alcohol intake but does not affect the maintenance of alcohol intake. Our acquisition data are in line with previous studies using alcohol-preferring rats (Colombo et al., 2002) and higher doses in mice (Kasten et al., 2015). Similarly, maintenance of alcohol intake was unaffected when given an alcohol pre-exposure period. Following five days of drug treatment, the decrease in alcohol intake returned to baseline drinking (days 1–5; Fig. 1C and D). In contrast, there is evidence that baclofen disrupts maintenance intake in P rats (Colombo et al., 2002).

### HAP2 acquisition and maintenance

In male and female HAP2 mice, systemic injections of racemic baclofen decreased repeated alcohol intake during acquisition at the 2- and 24-h time points (Fig. 2A and B). Alcohol preference was also significantly reduced at 2 h but not at 24 h of access, in contrast to the findings of Kasten et al. (2015); Tables 1 and 2. Further, like the findings in B6 mice, acquisition of alcohol intake was disrupted in both male and female HAP2 mice; however, maintenance was not. Intake in the 3.0-mg/kg dose group returned to levels similar to those in the saline condition on the first day without racemic baclofen treatment. A surprising finding, however, was the lack of drug effect during the maintenance experiment at 24-h readings (Fig. 3B), suggesting that pre-exposure to alcohol eliminates the drug effect found in the acquisition experiment. Additionally, while this was the case at 24-h readings, there was a significant decrease in alcohol consumption 2 h after drug treatment (Fig. 3A). Additionally, the 2-h effect appears to show a developing tolerance to a 3.0-mg/kg injection of racemic baclofen across the five days (Fig. 3A). A tolerance to intrathecal baclofen therapy has been reported in clinical studies (Heetla, Staal, Kliphuis, & van Larr, 2009); however, no studies have shown a tolerance effect using systemic racemic baclofen. Ultimately, this conclusion is speculative and would require further investigation. Thus, we can conclude that racemic baclofen is not as efficacious in decreasing repeated alcohol consumption following early acquisition of 2BC but can reduce drinking in the first 2-h window of 2BC in HAP2 mice.

While the exact reason for this lower efficacy in the 24-h maintenance period is unclear, one factor to consider is baclofen's short half-life, which is between 1.5 and 3.4 h in *in vivo* studies using rodents or humans, respectively (Lal et al., 2009). Changes in drinking were only modestly seen at the 2-h readings during the maintenance experiment. In contrast, a significant decrease was seen at the 2- and 24-h time points in the acquisition experiment. Thus, one could speculate that the drug's short half-life would result in less efficacy at 24-h intakes. However, the clear effect at 24 h during the acquisition experiment suggests that the drug half-life may not be the only explanation for this inconsistency. Another possible reason for this could be differences in the expression of patterns of GABA<sub>B</sub> receptors in high-drinking mouse models (Villas Boas et al., 2012). Villas Boas et al. (2012) showed that compulsive mice, or "mice that had a loss of control over ethanol intake", had increased transcription levels of *Gabbr1* and *Gabbr2*, which express GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits,

respectively (Ribeiro et al., 2012). Further, additional work from this lab found that baclofen only reduced alcohol consumption in light-drinking male Swiss mice. While both genotypes in the current study consume levels of alcohol that yield pharmacologically relevant blood ethanol concentrations (BECs) (Ardinger, Grahame, Lapish, & Linsenhardt, 2020; Rhodes, Best, Belknap, Finn, & Crabbe, 2005), HAP2 mice model a genetic predisposition to consume excessive amounts of ethanol (Matson & Grahame, 2013). Differences in the expression of these genes could explain why baclofen was more efficacious in the maintenance of alcohol consumption when the acquisition of alcohol was not disrupted. However, given the scope of our study, we cannot make definitive conclusions. Moving forward, it would be of interest to assess the balance of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits in select brain regions of B6 versus HAP2 mice.

### Locomotor activity

Evidence suggesting that locomotor activity is altered after administration of racemic baclofen has been inconsistent. Effects are dependent on species (Colombo & Gessa, 2018), drug dose (Broadbent & Harless, 1999; Kasten et al., 2015), and whether subjects are alcohol-naïve or not (Cryan et al., 2004; Kasten et al., 2015). Many of the previous reports have found that racemic baclofen either decreases or does not affect locomotor activity at all (Silverman et al., 2015). In HAP2 mice, we found that repeated systemic administration of 3.0-mg/kg racemic baclofen altered 24-h locomotor activity during days 6–10 of the 2BC maintenance experiment, but this could have been due to initial baseline locomotor differences between groups on days 1–5. Additionally, we did not find this same effect during the acquisition of alcohol experiments in HAP2 mice (Fig. 2B and C) This is in line with previous studies, which found no effect on locomotor activity in HAP2 mice following a 10-mg/kg systemic injection of racemic baclofen (Kasten et al., 2015).

### Saccharin drinking

Saccharin intake was assessed for 2 day at the end of each experiment. Immediately following the acquisition of alcohol intake model in B6 mice, two days of saccharin intake was not significantly different between treatment groups (Fig. 4A). However, this finding was inconsistent across experiments, as baclofen decreased saccharin intake following the maintenance of alcohol intake model (Fig. 4B). Inconsistencies in the effect of racemic baclofen on saccharin intake are present in the literature (Echeverry-Alzate et al., 2021; Kasten & Boehm, 2014; Kasten et al., 2015; Quintanilla et al., 2008), but to our knowledge there are no studies showing that the dose used here would alter saccharin intake in mice when administered acutely. Further, Crabbe et al. (2017) showed that a 5-mg/kg dose decreased water intake in B6 mice. This, and other studies showing a reduction in intake of alternative solutions, suggests that racemic baclofen is not specific to alcohol and may alter fluid consumption in general, perhaps in a dose-dependent manner.

Further, during our experiment assessing the acquisition of alcohol intake in HAP2 mice, saccharin intake was significantly reduced in HAP2 male mice at the 24-h reading (Fig. 4C). In contrast, we did not see a significant difference between treatment groups in saccharin intake during the maintenance of alcohol intake experiment (Fig. 4D). Kasten et al. (2015) found that a 10-mg/kg dose of racemic baclofen decreased total saccharin intake in HAP1

male and female mice. To our knowledge, this is the only study that has assessed the effect of racemic baclofen on HAP2 mice at the 3-mg/kg dose. Thus, we can conclude that a 3-mg/kg dose may be enough to sufficiently alter saccharin consumption, specifically in males, suggesting that racemic baclofen is not specific to alcohol. One limitation is that saccharin intake was assessed immediately following alcohol drinking and in the same mice. While we did not include a water-drinking wash-out period, Kasten et al. (2015) assessed the effect of racemic baclofen with a 1- or 2-week water-drinking wash-out period and found that racemic baclofen still altered saccharin intake at the 10-mg/kg dose. In line with this, Echeverry-Alzate et al. (2021) also found that racemic baclofen decreased sucrose responding at a 1.5-mg/kg dose following a 1-day wash-out period. Thus, evidence beyond our model suggests racemic baclofen is not selective to ethanol. Future studies should include a wash-out drinking period to ensure that consumption of alternative solutions following racemic baclofen treatment is solely due to drug treatment and no other variables. Here we only assessed saccharin intake for 2 days to explore the potential effects racemic baclofen could have on alternative solutions. This was added given the decrease in alcohol intake seen in the current study; however, conducting studies assessing the acquisition and maintenance of saccharin drinking in a separate group of animals would allow for further investigation of the magnitude of this effect over time.

### Sex effects

Historically, the effect of racemic baclofen on sex differences has been understudied in both clinical and preclinical models. Thus, most of the findings have been focused on males or have not been powered enough to analyze the data by sex, even though the number of women diagnosed with AUD is rapidly increasing (Grant et al., 2017). In preclinical models, female mice tend to drink more alcohol than male mice in various paradigms (Crabbe et al., 2009; Grahame, Li, & Lumeng, 1999; Middaugh, Kelley, Bandy, & McGroarty, 1999), and there is some evidence that racemic baclofen may affect males and females differently (Jeanblanc et al., 2020). Thus, we felt it was important to assess sex differences in the present study. We found that racemic baclofen decreased alcohol consumption similarly in both males and females. Additionally, we found that females consumed more alcohol (B6) and had a higher preference for alcohol (HAP2) than males in some instances; however, racemic baclofen was not more effective in males compared to the females, which was found by Jeanblanc et al. (2020) in Wistar rats. While this study did not find sex differences between the efficacy of racemic baclofen, future studies should continue to incorporate both sexes to fully understand the drug's profile.

### Limitations

While our data add to the current body of literature, there are some limitations. First, it should be noted that we did not collect blood from these animals at any point. Thus, in the present study we cannot assess the effect of racemic baclofen on corresponding BECs. B6 mice typically reach BECs up to 100 mg/dL during DID (Rhodes et al., 2005), and HAP2s can reach BECs greater than 100 mg/dL (Matson & Grahame, 2013). Kasten et al. (2015) found that racemic baclofen decreased BECs at 10 mg/kg, which corresponded to their intake. While one could speculate that BECs would also be decreased in the current study, we cannot draw definitive conclusions without BEC values.

Next, we only used a single dose in these experiments. As stated in our methods, a “subthreshold” dose of 3.0-mg/kg racemic baclofen was used because previous work in our lab showed that acute administration did not affect DID or 2BC alcohol or saccharin intake. These experiments were conducted to further probe unpublished findings in the lab. Thus, we did not include other doses of racemic baclofen. Kasten et al. (2015) included 0-, 1-, 3-, and 10-mg/kg doses and found that significant changes were only found at the 10-mg/kg dose. As such, future studies need to include various doses of racemic baclofen to gain a more well-rounded understanding of the drug effects on intake and locomotion.

Another limitation is the length of our acquisition and maintenance models. While we see clear effects of racemic baclofen across experiments, we only assessed early acquisition (first five days). Here we focused on early acquisition to replicate the work of Kasten et al. (2015) and other unpublished data from our lab. Further, our drinking was stable and did not show an escalation during these five days. However, to capture the maintenance of alcohol consumption more accurately, future studies should extend the acquisition period of alcohol consumption to ensure more stable drinking and potential dependence before administering racemic baclofen.

## Conclusion

In conclusion, these studies add to the literature demonstrating that a 3-mg/kg dose of racemic baclofen effectively decreases 2-h alcohol intake in both male and female B6 and HAP2 mice. However, we did not find that drug treatment after acquisition of drinking behavior consistently disrupted maintenance of alcohol drinking in HAP2 mice. Additionally, we found inconsistencies in baclofen suppression of saccharin intake and locomotor activity, suggesting that this dose of baclofen may result in more variable results than higher doses previously reported in mouse studies. Ultimately, these data highlight that racemic baclofen effectively suppressed alcohol drinking in male and female mice. Even so, further studies are needed to assess the inconsistencies found here and understand the specific mechanism by which baclofen could be altering drinking and locomotor activity in B6 and HAP2 mice.

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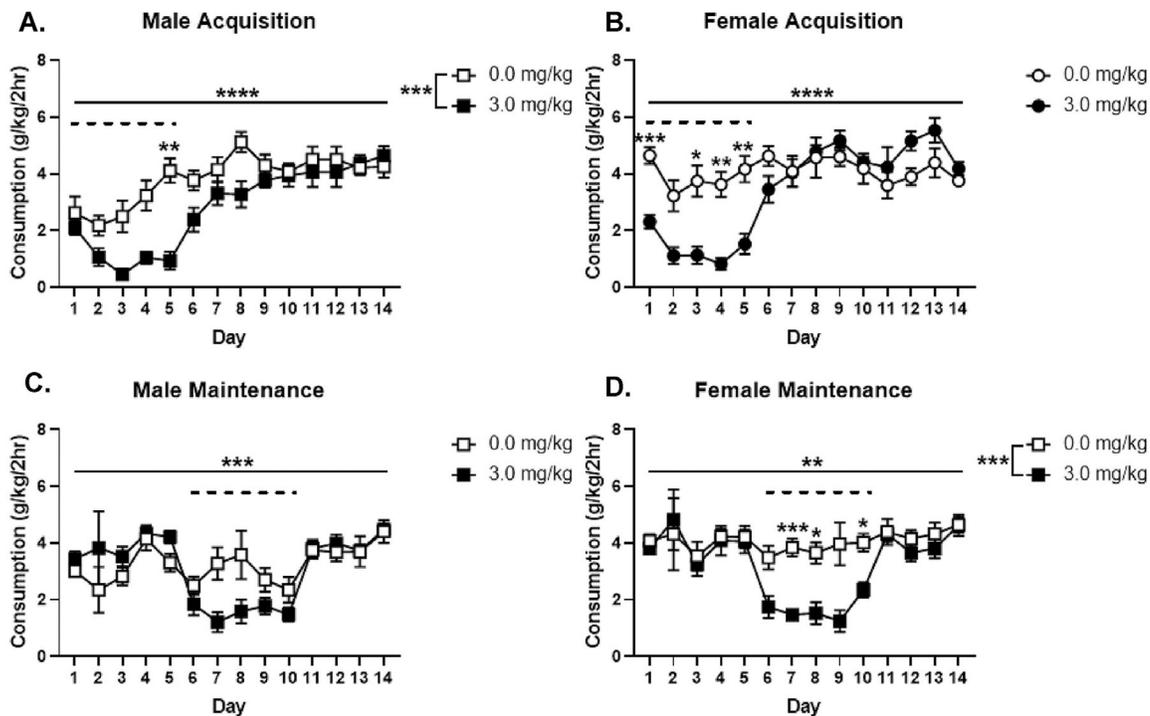
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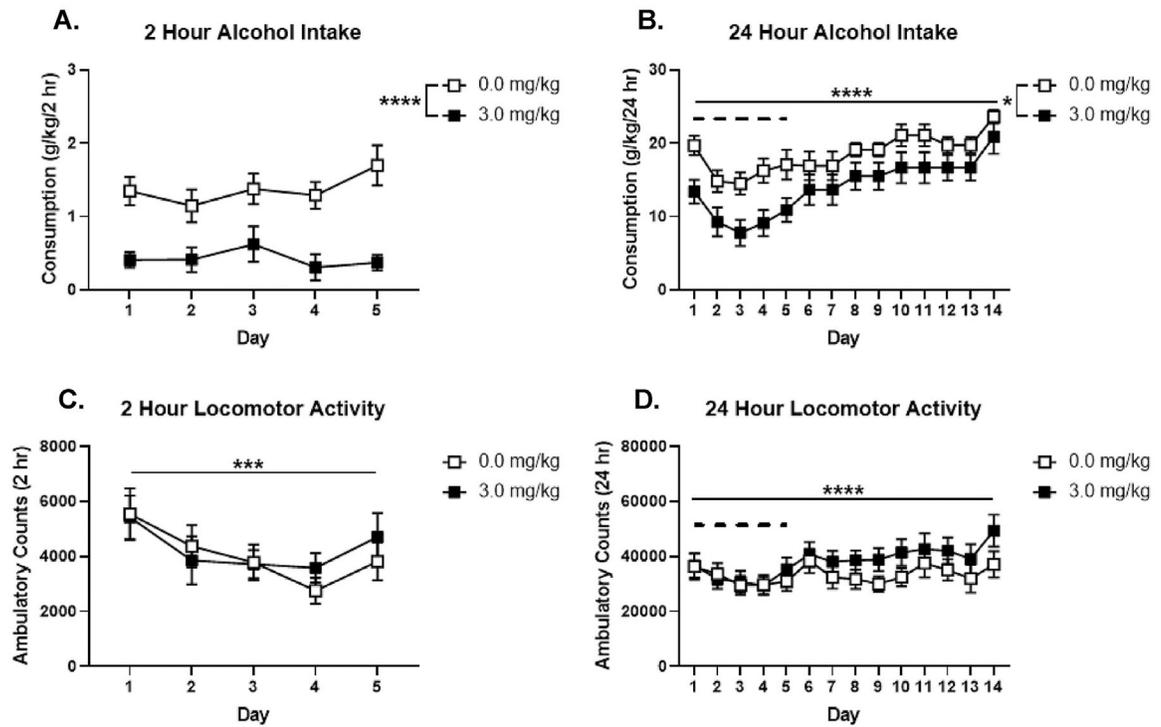
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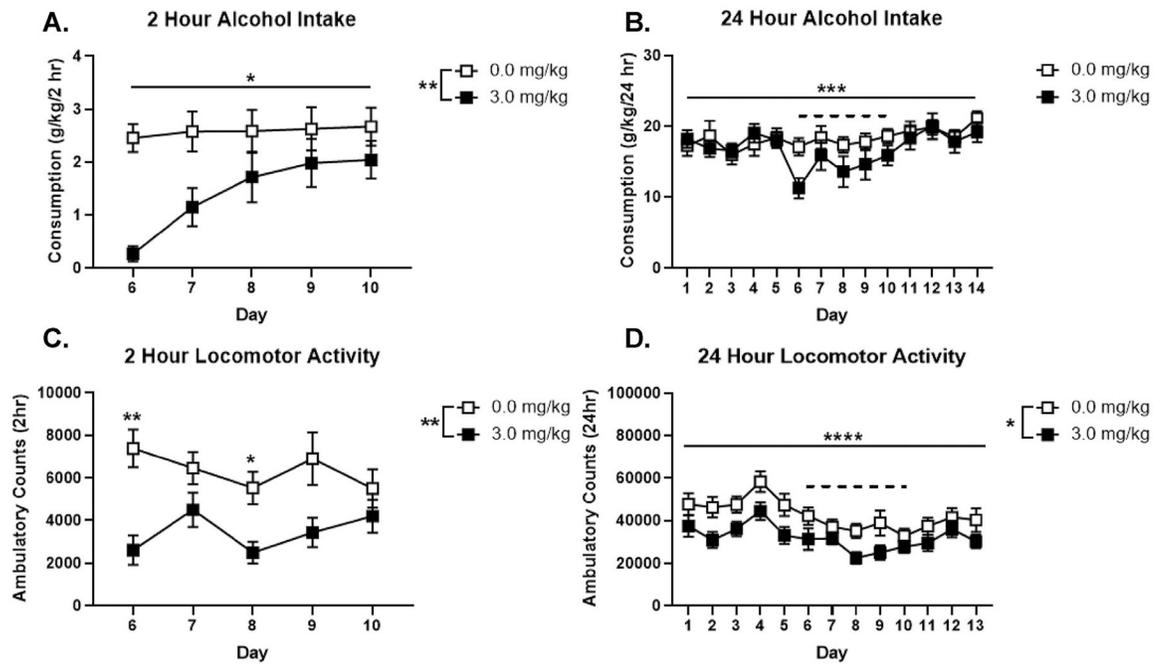
**Fig. 1. Effect of baclofen on alcohol consumption During DID in B6 mice.**

**A–B. Acquisition Model.** Male and female B6 mice were treated with either 0.0 or 3.0-mg/kg baclofen ( $n = 7\text{--}8/\text{dose}/\text{sex}$ ) on days 1–5 for acquisition. **A.** Main effect of dose ( $***p < 0.001$ ), day ( $****p < 0.0001$ ), and an interaction of dose and day ( $p < 0.0001$ ). We ran *post hoc* Bonferroni-corrected *t* tests assessing whether intake differed by baclofen dose at each day. We found that on day 5, baclofen significantly reduced alcohol drinking compared to saline ( $**p < 0.01$ ). **B.** Main effect of day ( $****p < 0.0001$ ) and a significant interaction of dose and day ( $p < 0.0001$ ). We ran *post hoc* Bonferroni-corrected *t* tests assessing whether intake differed by baclofen dose at each day. We found that on day 1 ( $***p < 0.001$ ), day 3 ( $*p < 0.05$ ), day 4 ( $**p < 0.01$ ), and day 5 ( $**p < 0.01$ ) baclofen significantly reduced alcohol intake compared with saline. **C–D. Maintenance Model.** Male and female HAP2 mice were treated with either 0.0 or 3.0-mg/kg baclofen ( $n = 7\text{--}8/\text{dose}/\text{sex}$ ) on days 6–10 for maintenance. **C.** Main effect of day ( $***p < 0.001$ ) and a significant interaction of day and dose ( $p < 0.01$ ). **D.** Main effect of day ( $**p < 0.01$ ), dose ( $***p < 0.001$ ), and an interaction of dose and day ( $p < 0.01$ ). We ran *post hoc* Bonferroni-corrected *t* tests assessing whether intake differed by baclofen dose at each day. We found that on day 7 ( $**p < 0.01$ ), day 8 ( $*p < 0.05$ ), and day 10 ( $*p < 0.05$ ), baclofen significantly reduced drinking as compared to saline. Data are displayed as mean  $\pm$  SEM. Treatment days are denoted with dashed lines over injection days.



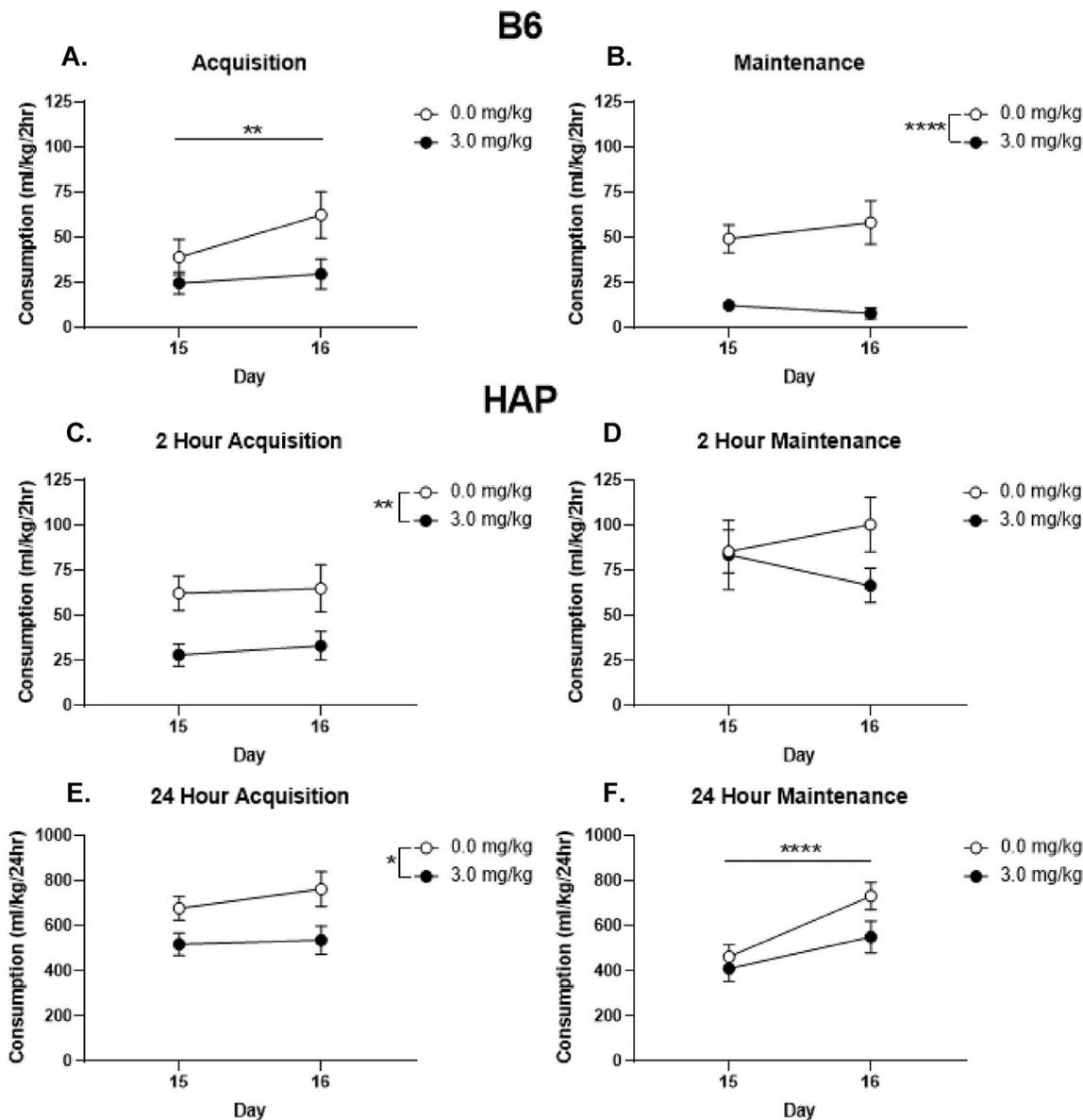
**Fig. 2. Effect of baclofen on alcohol consumption and locomotor activity during 2- and 24-h drinking in the Acquisition Model in HAPs.**

Male and female HAP2 mice were treated with either 0.0 or 3.0-mg/kg baclofen ( $n = 7-8/\text{dose/sex}$ ) on days 1–5. Alcohol consumption and locomotor activity were monitored during the first 2 h following injection on days 1–5 and at 24 h on days 1–14. **A.** Main effect of dose ( $****p < 0.0001$ ). **B.** Main effect of day ( $****p < 0.0001$ ) and dose ( $*p < 0.05$ ). **C.** Main effect of day ( $**p < 0.01$ ). **D.** Main effect of day ( $****p < 0.0001$ ). Data are collapsed on sex and displayed as mean  $\pm$  SEM. Treatment days are denoted with dashed lines over injection days.



**Fig. 3. Effect of baclofen on alcohol consumption and locomotor activity during 2- and 24-h drinking in the Maintenance Model in HAPs.**

Male and female HAP2 mice were treated with either saline or 3.0-mg/kg baclofen ( $n = 7-8/\text{dose}/\text{sex}$ ) on days 6–10. Alcohol consumption and locomotor activity were monitored during the first 2 h following injection on days 6–10 and at 24 h on days 1–14. **A.** Main effect of dose ( $**p < 0.01$ ) and day ( $*p < 0.05$ ). **B.** Main effect of day ( $***p < 0.05$ ). **C.** Main effect of dose ( $**p < 0.01$ ) and a significant interaction of dose and day ( $p < 0.05$ ). There was a significant difference between treatment groups on days 6 and 8 ( $*p$ 's  $< 0.05$ ). **D.** Main effect of dose ( $*p < 0.05$ ), day ( $****p < 0.0001$ ). Data are displayed as mean  $\pm$  SEM. Treatment days are denoted with dashed lines over injection days.



**Fig. 4. Effect of baclofen on saccharin consumption following DID.**

Male and female B6 or HAP mice were treated with either baclofen or saline following alcohol drinking on days 15 and 16. **A. B6 Acquisition.** RM two-way ANOVA of day and dose revealed a significant main effect of day (\*\* $p < 0.01$ ). **B. B6 Maintenance.** RM two-way ANOVA of day and dose revealed a significant effect of dose (\*\*\*\* $p < 0.0001$ ). **C. 2-h HAP acquisition.** RM two-way ANOVA of day and dose revealed a main effect of dose (\*\* $p < 0.01$ ). **D. 2-h HAP maintenance.** RM two-way ANOVA of day and dose revealed no significant differences ( $p$ 's  $> 0.05$ ). **E. 24-h HAP acquisition.** RM two-way ANOVA of day and dose revealed a main effect of dose (\* $p < 0.05$ ). **F. 24-h HAP maintenance.** RM two-way ANOVA of day and dose revealed a main effect of day (\*\*\* $p < 0.01$ ). Data are collapsed on sex and displayed as mean  $\pm$  SEM.

**Table 1**

Effect of baclofen on acquisition of 24-h continuous access alcohol preference in HAP2 mice

<b>2-Hour</b>		
<b>Dose</b>	<b>0 mg/kg</b>	<b>3 mg/kg</b>
<b>Day 1</b>	36.97 ± 31.51	43.56 ± 37.00
<b>Day 2</b>	46.99 ± 35.62	37.10 ± 38.03
<b>Day 3</b>	45.30 ± 34.55	50.93 ± 38.49
<b>Day 4</b>	40.93 ± 41.88	50.63 ± 43.16
<b>Day 5</b>	52.11 ± 35.12	42.17 ± 40.21
<b>24-Hour</b>		
<b>Dose *</b>	<b>0 mg/kg</b>	<b>3 mg/kg</b>
<b>Day 1</b>	50.43 ± 22.53	57.66 ± 23.54
<b>Day 2</b>	48.27 ± 28.60	40.76 ± 29.05
<b>Day 3</b>	44.07 ± 29.15	43.38 ± 30.67
<b>Day 4</b>	45.81 ± 27.72	50.80 ± 28.41
<b>Day 5 **</b>	57.25 ± 27.09	57.20 ± 27.68
<b>Day 6</b>	64.85 ± 23.25	54.44 ± 30.21
<b>Day 7</b>	64.85 ± 23.25	54.44 ± 30.21
<b>Day 8</b>	76.49 ± 18.25	61.99 ± 25.16
<b>Day 9</b>	76.49 ± 18.25	61.99 ± 25.16
<b>Day 10</b>	79.41 ± 16.53	66.60 ± 28.64
<b>Day 11</b>	79.41 ± 16.53	66.60 ± 28.64
<b>Day 12</b>	83.28 ± 14.50	69.69 ± 23.56
<b>Day 13</b>	83.28 ± 14.50	69.69 ± 23.56
<b>Day 14 ****</b>	76.18 ± 12.32	68.64 ± 20.43

Alcohol and water drinking data were calculated for all days. Male and female mice that were treated with either saline or baclofen received both water and alcohol bottles during the two-bottle choice session, and alcohol preference was calculated. Preference was calculated as follows: [(Alcohol Consumption)/(Water Consumption + Alcohol Consumption) × 100].

Main effect of days 1–5 for 24-h preference (\*\* $p < 0.01$ ) and days 6–14 (\*\*\*\* $p < 0.0001$ ). Main effect of dose for days 1–5 at 24 h (\* $p < 0.05$ ). Data are displayed as mean ± SD.

**Table 2**

Effect of baclofen on maintenance of continuous access alcohol preference in HAP2 mice

<b>2-Hour*</b>		
<b>Dose***</b>	<b>0 mg/kg</b>	<b>3 mg/kg</b>
<b>Day 6</b>	82.22 ± 28.50	15.63 ± 35.21
<b>Day 7</b>	70.89 ± 35.82	37.50 ± 45.34
<b>Day 8</b>	68.67 ± 40.19	46.67 ± 45.46
<b>Day 9</b>	76.00 ± 36.15	55.48 ± 46.10
<b>Day 10</b>	91.11 ± 25.87	64.93 ± 42.59
<b>24-Hour*</b>		
<b>Dose</b>	<b>0 mg/kg</b>	<b>3 mg/kg</b>
<b>Day 1</b>	73.24 ± 21.49	80.79 ± 11.90
<b>Day 2</b>	72.03 ± 27.08	78.90 ± 18.34
<b>Day 3</b>	73.00 ± 25.64	81.64 ± 14.69
<b>Day 4</b>	71.35 ± 26.10	84.95 ± 13.89
<b>Day 5</b>	77.61 ± 18.97	83.47 ± 16.05
<b>Day 6</b>	79.66 ± 19.46	75.04 ± 27.98
<b>Day 7</b>	79.91 ± 20.91	77.16 ± 24.76
<b>Day 8</b>	83.92 ± 19.85	72.16 ± 27.16
<b>Day 9</b>	83.45 ± 19.52	73.81 ± 32.21
<b>Day 10</b>	88.52 ± 12.65	78.92 ± 21.82
<b>Day 11</b>	90.33 ± 17.60	86.83 ± 16.87
<b>Day 12</b>	85.58 ± 19.25	83.61 ± 15.73
<b>Day 13</b>	85.65 ± 19.96	83.22 ± 18.59
<b>Day 14</b>	92.11 ± 9.55	86.64 ± 11.36

Alcohol and water drinking data were calculated for all days. Male and female mice that were treated with either saline or baclofen received both water and alcohol bottles during the two-bottle choice session, and alcohol preference was calculated. Preference was calculated as follows: [(Alcohol Consumption)/(Water Consumption + Alcohol Consumption) × 100].

Main effect of day (\* $p < 0.05$ ) and main effect of dose (\*\* $p < 0.001$ ). Data are displayed as mean ± SEM.