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Preprint

Sex, but not early life stress, effects on two-bottle choice alcohol drinking behaviors in mice

Thomas W. Perry, Elizabeth A. Sneddon, Amanda N. Reichert, Kristen M. Schuh, Natalie A. Shand,

Jennifer J. Quinn, & Anna K. Radke

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Abstract

In humans, early life stress (ELS) is associated with an increased risk for developing both alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD). We have previously used an infant footshock model to explore this shared predisposition. Infant footshock produces stress-enhanced fear learning (SEFL) in rats and mice and increases aversion-resistant alcohol drinking in rats. The goal of the current study was to extend this model of comorbid PTSD and AUD to male and female C57BL/6J mice. Acute ELS was induced using 15 foot-shocks on postnatal day 17. In adulthood, after PND 90, ethanol drinking behavior was tested in one of three two-bottle choice drinking paradigms: continuous access, limited access drinking in the dark, or intermittent access. In continuous access, mice were given 24 h access to 5% or 10% ethanol and water. Each ethanol concentration was provided for five consecutive drinking sessions. In limited access drinking in the dark, mice were given 2 h of access to 15% ethanol and water across 15 sessions. Ethanol was provided 3 h into the dark cycle to maximize task engagement when mice are most active. In intermittent access, mice were presented with 20% ethanol and water Monday, Wednesday, and Friday, for four consecutive weeks. In a fifth week of intermittent access drinking, increasing concentrations of quinine (10 mg/L, 100 mg/L, and 200 mg/L) were added to the ethanol to test aversion-resistant drinking. Our results indicate that infant footshock does not influence adult ethanol consumption in mice. Infant footshock did not affect ethanol-only consumption or preference in any of the three drinking paradigms. Further, and in contrast to our previous results in rats, infant footshock did not appear to influence consumption of quinine-adulterated ethanol. The biological sex of the mice did affect ethanol-only consumption in all three drinking paradigms, with females consuming more ethanol than males. Preference for ethanol vs. water was higher in females only under continuous access conditions. Our results suggest that infant footshock alone may not be sufficient to increase drinking levels in mice. We hypothesize that infant footshock may require a secondary, adolescent stress exposure to influence ethanol drinking behavior. Further research is needed to create a valid model of PTSD-AUD comorbidity in male and female mice.

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Introduction

Alcohol use disorder (AUD) is a complex psychiatric disorder influenced by both genetic and environmental factors (Mayfield et al., 2008; Tawa et al., 2016; Young-Wolff et al., 2011). Stress is one environmental variable that has been strongly associated with alcohol abuse and the development of AUD (Guinle & Sinha, 2020; Sinha, 2001). Among individuals with PTSD, AUD has been reported to be up to three times more prevalent than it is in the general public (Kessler et al., 1995; Gilpin & Weiner, 2017; Smith & Cottler, 2018). This suggests that PTSD and AUD may be products of common alterations brought about by stress. Early life stress (ELS) in humans is associated with an increased risk of alcohol dependence, early onset alcohol use, and PTSD-AUD comorbidity (Lee et al., 2018; Enoch, 2011; Pechtel & Pizzagalli, 2011). These associations indicate that in early stages of development the brain may be particularly susceptible to stress-induced changes (Lupien, 2009).

Consistent with clinical literature, in preclinical models, chronic ELS in the form of isolation or maternal separation has generally been found to increase levels of alcohol consumption later in adulthood (Becker et al., 2011; McCool & Chappell, 2007; Cruz et al., 2008). Additionally, a number of studies have found that ELS increases aversion-resistant drinking when alcohol is paired with an aversive stimulus such as quinine (Bertanga et al., 2021; Radke et al., 2020; Shaw et al., 2020). This drinking despite a negative consequence has been used to model compulsive-like drinking, a central characteristic of AUD (Hopf et al., 2010; Hopf & Lesscher, 2014; Radke et al., 2017; Sneddon et al., 2019). Sex has been reported to play a role in the relationship between ELS and alcohol drinking behaviors; however, the exact influence of sex remains unclear (Bertanga et al., 2021; Radke et al., 2020; Shaw et al., 2020).

In a recent study using Long-Evans rats, we found that acute ELS in the form of repeated footshock on postnatal day (PND) 17 increases aversion-resistant drinking in an intermittent access alcohol drinking paradigm (Radke et al., 2020). This form of acute infantile trauma has previously been established as a method to sensitize fear learning, a phenomenon known as stress-enhanced fear learning (SEFL) and has been used as a preclinical model for PTSD and PTSD susceptibility (e.g., Quinn et al., 2014; Poulos et al., 2014). The common influence of infant footshock on disordered drinking behavior

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and fear learning, but not other forms of hippocampal-dependent learning or cognitive flexibility (Sneddon et al., 2021), suggests that an infant SEFL paradigm may be a viable stress model for investigating AUD-PTSD comorbidity, as well as the shared neural mechanisms underlying both disorders.

To extend the utility of this preclinical model, the current study explored how acute ELS in the form of infant footshock influences consumption of alcohol alone and when paired with an aversive outcome in C57BL/6J mice. We used a standard infant SEFL protocol (15 footshocks on postnatal day (PND) 17) that we have previously found increases fear learning and resistance to extinction in mice (Sneddon et al., 2021). In adulthood, mice were exposed to alcohol in one of three two-bottle choice drinking tasks: limited access drinking in the dark, continuous access, or intermittent access. To model aversion-resistant drinking, alcohol was adulterated with increasing concentrations of quinine, an aversive bittering agent, in the drinking in the dark and intermittent access paradigms (Radke, 2017). Additionally, considering published findings (Hennessy et al., 2009) and data from our own labs (Reichert et al., unpublished data) showing that exposure to conspecifics following trauma, otherwise known as social buffering, can modulate the effect of trauma, we investigated whether social buffering could alter the effects of infantile trauma on intermittent access drinking. Finally, due to robust evidence of enhanced vulnerability to both stress-related disorders and addictive behaviors in females, we examined sex as a potential moderator of the effects of infant footshock on alcohol drinking in all experiments.

Methods

Subjects

161 C57BL/6J mice (88 males 73 females) generated from breeding pairs from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were provided food and water *ad libitum*, unless otherwise specified in the experimental methods. Mice were on a 12:12 light/dark cycle. Other than PND 17 footshock exposure, all behavioral tests were conducted during adulthood (PND 60+). During infancy, all mice were briefly anesthetized with isoflurane and given an ear snip for identification purposes. Prior to

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home cage drinking, mice were group housed (2-4 mice/cage). One week before the first drinking session mice were individually housed in standard shoe box Udel polysulfone rectangular mouse cages (18.4 cm x 29.2 cm x 12.7 cm) outfitted with 2-bottle cage tops. All animals were cared for in accordance with the guidelines set by the National Institute for Health. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Miami University.

Early life stress exposure

Mice were placed in a novel MED-Associates conditioning chamber (Context A) on PND 17 (after Quinn et al., 2014). Context A was brightly lit, contained a uniform grid floor, was scented with vanilla, and was cleaned with odorless 5% sodium hydroxide. Mice received either 15 footshocks (1 mA, 1 sec) (ELS group) or no footshocks (Non-ELS group) during a 60-min session beginning at 180 sec following placement in the chamber. Shocks were delivered according to a variable inter-shock interval of 120-240 sec. Progressive scan video cameras containing visible light filters (VID-CAMMONO-2A; Med Associates, Inc.) monitored mice throughout the session.

For the continuous access and intermittent access experiments, mice were returned to a cage with their littermates in a brightly lit room separate from the conditioning chambers following shock exposure. Mice were run in squads of 4 and the entire litter was returned to the home cage 3 h after the final squad's session.

For the intermittent access experiment, we examined the effects of social buffering on stress enhanced fear learning (Reichert et al., unpublished data) and alcohol drinking in adulthood. Immediately following the ELS exposure session, mice were placed into their assigned social buffering condition for 90 minutes. During this time, pups in the isolation condition were housed individually while pups in the littermates condition were housed as a group. All buffering took place in polysulfone holding cages (19 x 29.2 x 12.7 cm) that are similar to the housing cages but without bedding. Holding cages were in a room with overhead lighting and were placed on top of Sunbeam-brand heated mats, half on the pad and half off.

Fear Conditioning and Memory Test

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Before the commencement of drinking, on PND 60, mice that took part in intermittent access drinking were fear conditioned in a novel context. This context consisted of four identical conditioning chambers (32.4 x 25.4 x 21.6 cm; Med-Associates, Inc., Georgia, VT) housed inside sound-attenuating cabinets. The chambers were distinct from the PND 17 footshock and isolation contexts in several ways: the room was dark except lit by red lamps, the room smelled of vinegar, there were black Plexiglass triangular inserts within the chamber, and the floor had staggered stainless steel rods (18 rods, two rows, .05 cm vertically apart). Mice were placed into the fear conditioning chamber and received one footshock (0.25 mA, 1s) 180 seconds following placement within the chamber. Thirty seconds following footshock, mice were returned to the homecage. On PND 90 mice were administered a fear extinction session in this context. Freezing behavior was measured to test fear memory (data not shown).

Two-bottle choice ethanol drinking

For all experiments, mice were given access to reverse osmosis (RO) drinking water and ethanol in RO water (v/v) in their home cage. Mice were weighed at the beginning of every session and solutions were made fresh for each drinking session. Bottles were alternated each session to equate side biases. Two "dummy" cages were outfitted with bottles to account for spillage and evaporation.

Experiment 1: Continuous access ethanol drinking

Forty mice (20 male and 20 female) were given access to 5% or 10% ethanol ~6 h into the light cycle (i.e., 11:30 AM) (**Figure 1A**). Each concentration of ethanol was provided for 5 consecutive, 24-h continuous drinking sessions and bottle weights were recorded every 24 h.

Experiment 2: Limited access drinking in the dark

Fifty-five mice (28 male and 27 female) were presented with 15% ethanol following a limited access, two-bottle choice protocol for 2 h a day, five day/week (Monday-Friday) (**Figure 2A**). Bottles were placed on the home cage 3 h after lights out and bottle weights were recorded before and after each session.

Experiment 3: Intermittent access ethanol drinking

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Sixty-six mice (40 male and 26 female) were presented with 20% ethanol following a two-bottle choice, intermittent access protocol for 5 weeks (**Figure 3A**). Before commencement of ethanol drinking, mice were fear conditioned in a novel context on PND 60 and tested on PND 90 (data not shown). This context consisted of four identical conditioning chambers (32.4 x 25.4 x 21.6 cm; Med-Associates, Inc., Georgia, VT) housed inside sound-attenuating cabinets. The chambers were distinct from the PND 17 footshock and isolation contexts in several ways: the room was dark except lit by red lamps, the room smelled of vinegar, there were black Plexiglass triangular inserts within the chamber, and the floor had staggered stainless steel rods (18 rods, two rows, .05 cm vertically apart). Mice were placed into the fear conditioning chamber and received one footshock (0.25 mA, 1sec) 180 sec following placement within the chamber. Thirty seconds following footshock, mice were returned to the home cage. For IA, ethanol drinking sessions took place on Monday, Wednesday, and Friday. On intervening sessions (Tuesday, Thursday, Saturday, and Sunday), mice had access to two bottles of drinking water only. Bottles weights were recorded at the beginning of each ethanol-drinking session as well as after 30 min and 24 h. After 4 weeks (12 sessions) of drinking, quinine hemisulfate was added to the ethanol bottle in increasing concentrations of 10 mg/L, 100 mg/L, and 200 mg/L (equivalent to 25, 250, and 500 µM).

Data Analysis

Bottle weights were converted to grams (g) of ethanol or milliliters (mL) of water for each mouse. Consumption was calculated as = *(Initial Bottle Weight – Final Bottle Weight) – Average Weight Loss of Dummy Bottles*. Preference was calculated as = *(Volume of Ethanol [Volume of Ethanol + Volume of Water]) x 100*. Consumption and preference were averaged across mice for each group and analyzed using Three-Way Repeated Measures (RM) ANOVA with infant footshock, sex, and concentration as factors. All results were first analyzed with session as a factor, but as no significant effects were found, data were averaged across session for final analysis and visualization. In the intermittent access experiment, social buffering condition (i.e., isolated vs. littermates) was included as a factor, but data were ultimately collapsed across this factor as there were no significant effects. The Greenhouse-Geisser correction was applied when the assumption of sphericity was violated ($\varepsilon < 0.75$). Follow-up Holm-

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Sidak's tests were used as appropriate to assess differences between groups. Aversion-resistant drinking was defined *a priori* as a significant reduction from baseline (= 0 g/L) consumption and was assessed in all groups with a Dunnett's test to correct for multiple comparisons.

Results

Experiment 1: Continuous access ethanol drinking

Sex, but not stress, impacted ethanol consumption in the continuous access drinking test, with females consuming more than males (**Figure 1B**). A three-way ANOVA revealed no effect of infant footshock on average ethanol consumption across the two concentrations of ethanol presented ($F_{(1, 36)}$ = 0.085, p = 0.773). There was, however, a significant main effect of sex on ethanol consumption ($F_{(1, 36)}$ = 21.300, p < 0.001) and a main effect of ethanol concentration on consumption ($F_{(1, 36)}$ = 10.550, p = 0.003). An interaction between ethanol concentration, sex, and infant footshock neared significance ($F_{(1, 36)}$ = 3.943, p = 0.055). There were no other significant interactions between the factors (all p > 0.409). Holm Sidak's multiple comparison tests comparing consumption of the 5% and 10% concentrations for each group, demonstrated a greater consumption of 5% vs. 10% ethanol in 15-shock females only (p = 0.008).

Similar to consumption, preference for ethanol vs. water was higher in females than males but was not affected by stress (**Figure 1C**). A three-way ANOVA revealed no significant effect of infant footshock ($F_{(1, 36)}$ = 0.009, p = 0.925) or concentration ($F_{(1, 36)}$ = 3.125, p = 0.086) on ethanol preference. There was, however, a significant main effect of sex on ethanol preference ($F_{(1, 36)}$ = 11.220, p = 0.002). There was also a significant interaction between ethanol concentration, sex, and infant footshock ($F_{(1, 36)}$ = 5.182, p = 0.029). There were no other significant interactions between the factors (all p > 0.487). Holm Sidak's multiple comparison tests comparing preference for ethanol across concentrations in each group found that 15-shock females had significantly higher preference for 5% vs. 10% ethanol (p = 0.047).

Experiment 2: Limited access drinking in the dark

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When assessing limited access consumption, there were no effects of stress, but females consumed more than males (**Figure 2B**). A two-way ANOVA revealed no significant effect of infant footshock on ethanol consumption ($F_{(1, 51)} = 0.245$, $p = 0.623$). Analysis, however, indicate a significant main effect of sex ($F_{(1, 51)} = 22.100$, $p < 0.001$). The interaction between the factors was not significant ($F_{(1, 51)} = 0.328$, $p = 0.569$). For ethanol preference, there were no main effects of sex ($F_{(1, 51)} = 0.165$, $p = 0.687$) or stress ($F_{(1, 51)} = 1.637$, $p = 0.207$) and the interaction was not significant ($F_{(1, 51)} = 0.012$, $p = 0.912$) (**Figure 2C**).

Experiment 3: Intermittent access ethanol drinking

There were no effects of social buffering observed on any measure, therefore data from mice housed alone vs. with littermates following the ELS session on PND 17 were collapsed for visualization and analysis. Ethanol consumption averaged across the first twelve sessions of intermittent access drinking was higher in females but not affected by stress (**Figure 3B**). A two-way ANOVA revealed no significant effect of infant footshock on average consumption of ethanol ($F_{(1, 62)} = 0.019$, $p = 0.890$). There was, however, a significant main effect of sex on average ethanol consumption ($F_{(1, 62)} = 8.553$, $p = 0.005$). No significant interaction effect was indicated ($F_{(1, 62)} = 2.450$, $p = 0.123$). Ethanol preference was also similar across groups, regardless of sex or stress exposure (**Figure 3C**). A two-way ANOVA revealed no significant effect of infant footshock ($F_{(1, 62)} = 0.014$, $p = 0.908$), or sex ($F_{(1, 62)} = 0.118$, $p = 0.732$) on average preference for ethanol vs. water. No significant interaction effect was indicated, ($F_{(1, 62)} = 2.795$, $p = 0.100$)

For aversion-resistant drinking on sessions 12-15, females continued to consume more than males, and quinine reduced consumption equally in all groups (**Figure 3D**). A three-way ANOVA revealed no significant effect of infant footshock on consumption of quinine adulterated ethanol across sessions 12-15. ($F_{(1, 62)} = 1.633$, $p = 0.206$). There were, however, significant main effects of sex ($F_{(1, 62)} = 13.480$, $p < 0.001$) and quinine concentration ($F_{(2.680, 166.2)} = 59.130$, $P < 0.001$) on consumption. No significant interaction effects were indicated (all $p > 0.718$).

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To assess aversion resistant consumption, two-way ANOVAs followed by Dunnett's multiple comparisons tests were performed for each sex. In males, there was a significant main effect of quinine concentration ($F_{(2.728, 103.7)} = 53.110$, $p < 0.0001$) and a significant main effect of stress ($F_{(1, 38)} = 4.603$, $p = 0.038$), but no interaction between the two factors. Compared to baseline, consumption was decreased in no-shock and 15-shock males at the 100 and 200 g/L concentrations (all $p < 0.0001$), but not at a quinine concentration of 10 g/L ($P = 0.704$ and 0.967). In females, there was a significant main effect of quinine concentration ($F_{(2.506, 60.14)} = 16.250$, $p < 0.0001$). Neither the main effect of stress or interaction reached the threshold for significance. Aversion resistance was observed at the 10 mg/L concentration in no-shock ($p = 0.851$) and 15-shock ($p = 0.647$) females while consumption was reduced at the 100 and 200 g/L concentrations in both groups (all $p < 0.05$).

Discussion

The central finding of this study is that acute exposure to footshock on PND 17, using a protocol that enhances adult fear learning (Sneddon et al., 2021), does not affect ethanol drinking behavior in C57BL/6J mice. Infant footshock had no significant effect on ethanol-only consumption or preference in two-bottle choice limited access, intermittent access, or continuous access drinking paradigms. In addition, infant footshock did not affect the consumption of quinine-adulterated ethanol in the intermittent access paradigm. There was, however, a clear effect of sex on drinking behaviors, such that females consumed more ethanol than males when accounting for bodyweight. However, preference for ethanol vs. water was only higher in females under continuous access conditions.

In preclinical studies, stress has been found to have wide-ranging effects on ethanol drinking behavior (e.g., Becker et al., 2011; Radke et al., 2014), although at least one prior review of the literature estimated that acute stress increased drinking in fewer than 50% of studies (Becker et al., 2011). The limited and/or variable effects of acute stress exposure on drinking are in contrast with chronic stress exposure, which increase drinking behaviors much more consistently, especially when administered in early stages of development (Becker et al., 2011; Radke et al., 2014). The current set of experiments

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examined the effects of acute, infant footshock on adult drinking behaviors in mice, based on prior demonstrations that this protocol can potentiate alcohol drinking behaviors in rats (Meyer et al., 2013; Radke et al., 2020). Thus, taken together, these studies highlight the variability inherent in studies of acute stress effects on alcohol drinking behaviors.

In a prior study using Long-Evans rats, we demonstrated that PND 17 footshock increased aversion-resistant drinking in male and female subjects but did not alter consumption of unadulterated alcohol (Radke et al., 2020). Our current results in mice thus replicate at least some of those prior findings in rats, as consumption and preference were not altered in any of the three drinking paradigms tested. However, considering that some stressors, including infant footshock and chronic predatory stress, have a selective effect on aversion-resistant drinking behavior (Radke et al., 2020; Shaw et al., 2020), we expected stress to increase aversion-resistance in the intermittent access experiment. Instead, our results demonstrated that, regardless of footshock, mice continued consumption of ethanol at quinine concentrations of 10 mg /L and reduced consumption of ethanol at quinine concentrations of 100 and 200 mg /L.

Although not in line with our original predictions, there are two notable explanations for why infant footshock may have failed to increase aversion-resistant drinking in the current study. First, the effect of infant footshock may be model-species dependent. There are several notable differences between Long-Evans rats and C57BL/6J mice. Long-Evans rats are an outbred line, and thus in a given set of subjects there is increased genetic diversity compared to the inbred C57BL/6J line. The effect of stress on ethanol consumption has been found to vary between mouse strains (Radke et al., 2014). Furthermore, PTSD-AUD comorbidity is not ubiquitous in individuals who have experienced trauma (Gilpin & Weiner, 2017; Young-Wolff et al., 2011). Thus, the effect of early life trauma is likely dependent on genetic predispositions (Radke et al., 2020; Young-Wolff et al., 2011).

Another notable difference between C57BL/6J mice and Long-Evans rats is that C57BL/6J mice are bred to be high sucrose preferring mice and are known to consume more ethanol than other strains of mice. In contrast, Long-Evans mice have no predisposition to maladaptive ethanol drinking. Thus, the

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lack of effect of infant footshock on ethanol consumption in C57BL/6J mice may be a result of a ceiling effect, whereby aversion-resistant drinking has already reached a peak. A second consideration is that our previous study in rats included an intermittent access ethanol drinking period during adolescence (PND 35-55) that was absent from the current set of experiments in mice (Radke et al., 2020). Consumption of ethanol could be described as a stressor in and of itself. Ethanol exposure has been found to increase activity in the hypothalamic-pituitary-adrenal (HPA) axis, and withdrawal from ethanol has been associated with increased levels of corticotropin releasing factor (Radke et al., 2014). Thus, ethanol exposure in adolescence may be necessary to reveal the effects of infant footshock on aversion-resistant drinking, possibly by acting as a secondary stressor that potentiates the effects of infant footshock.

A major goal of this line of work is to establish a viable model of co-morbid PTSD and AUD in male and female mice. Infant footshock is appealing for its relatively selective effects on PTSD-relevant behaviors, including fear learning and anxiety behaviors (Quinn et al., 2014; Poulos et al., 2014) as well as resistance to fear extinction (Sneddon et al., 2021). Another promising model to study these behaviors was recently reported by Shaw and colleagues (2020). They reported that chronic predatory stress during adolescence increases fear related behavior and aversion-resistant consumption of ethanol in C57BL/6J mice. While chronic predatory stress is different from infant footshock, the similar effects of these stressors on fear related behavior indicates that some fear related stressors are sufficient to affect drinking behavior in C57BL/6J mice.

Consistent with a large body of previous literature our results show a clear effect of sex on ethanol consumption, with females consuming more ethanol than males (Finn, 2020; Radke et al., 2021a; Radke et al., 2021b). Further, males and females displayed similar preference for ethanol vs. water in the limited access and intermittent access experiments. This finding is consistent with previous studies of sex differences in alcohol drinking behaviors in our lab that have found divergences in the effects of biological sex on ethanol consumption vs. preference (Sneddon et al., 2019; Sneddon et al., 2022), and may give insight into the origin of sex differences in ethanol drinking. Sex differences in alcohol preference have been found to differ based on ethanol concentration (Radke et al., 2021; Sneddon et al.,

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2022), but it is unclear if the lack of an effect on preference seen here resulted from the different concentrations of ethanol used in the three experiments or from the intermittent access vs. continuous access conditions. It is also interesting to note that while sex has consistent effects on alcohol drinking behaviors here and in our prior studies, it has not been observed to interact with the effects of infant footshock on behavior in rats or mice (Radke et al., 2020; Sneddon et al., 2021).

In summary, our study found no effect of infant footshock on ethanol drinking behaviors in mice. While this could suggest that infant footshock selectively increases drinking in a rat model, we hypothesize that early life trauma may require a secondary, adolescent stressor in order to affect drinking behavior. Further research should be conducted investigating the specific conditions necessary to increase alcohol drinking behaviors following acute ELS. It should be noted that contradictory results and poor replicability is common in studies investigating the relationship between stress and alcohol exposure. Thus, in order to understand the intricate differences in the relationship between stressors and ethanol consumption, future studies could also include measurements of physical indicators of the stress response such as corticosterone levels. Such continued development and refinement of mouse models of co-morbid PTSD and AUD will be beneficial to uncovering the shared neural mechanisms of these two debilitating disorders.

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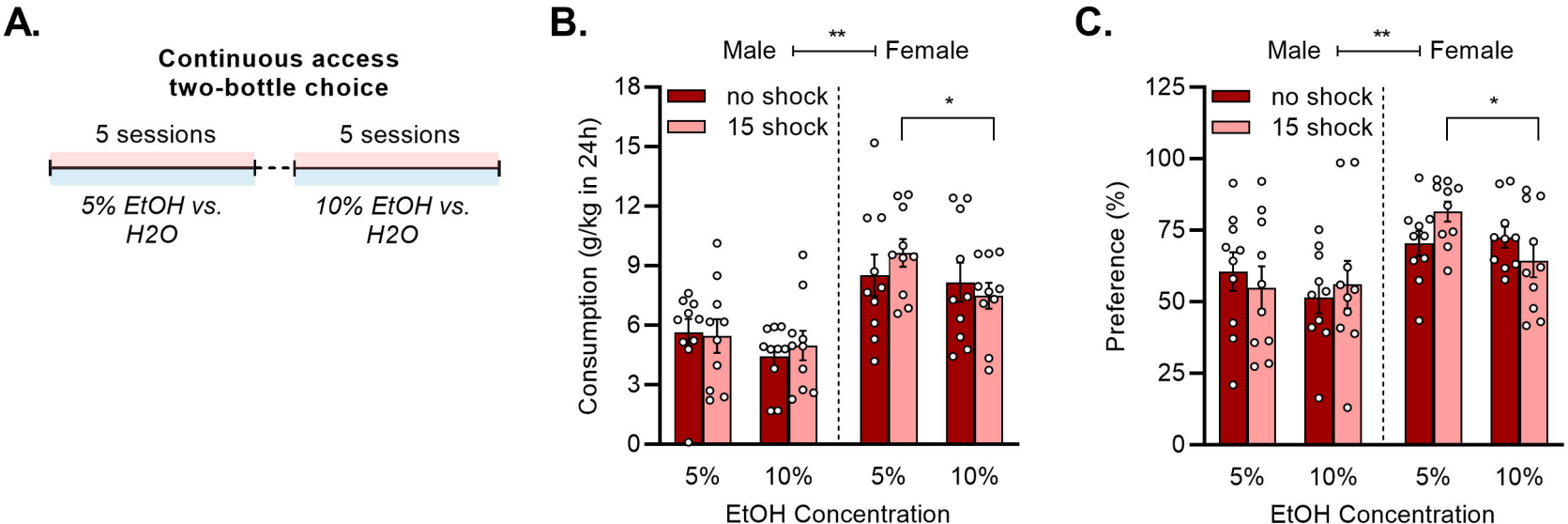
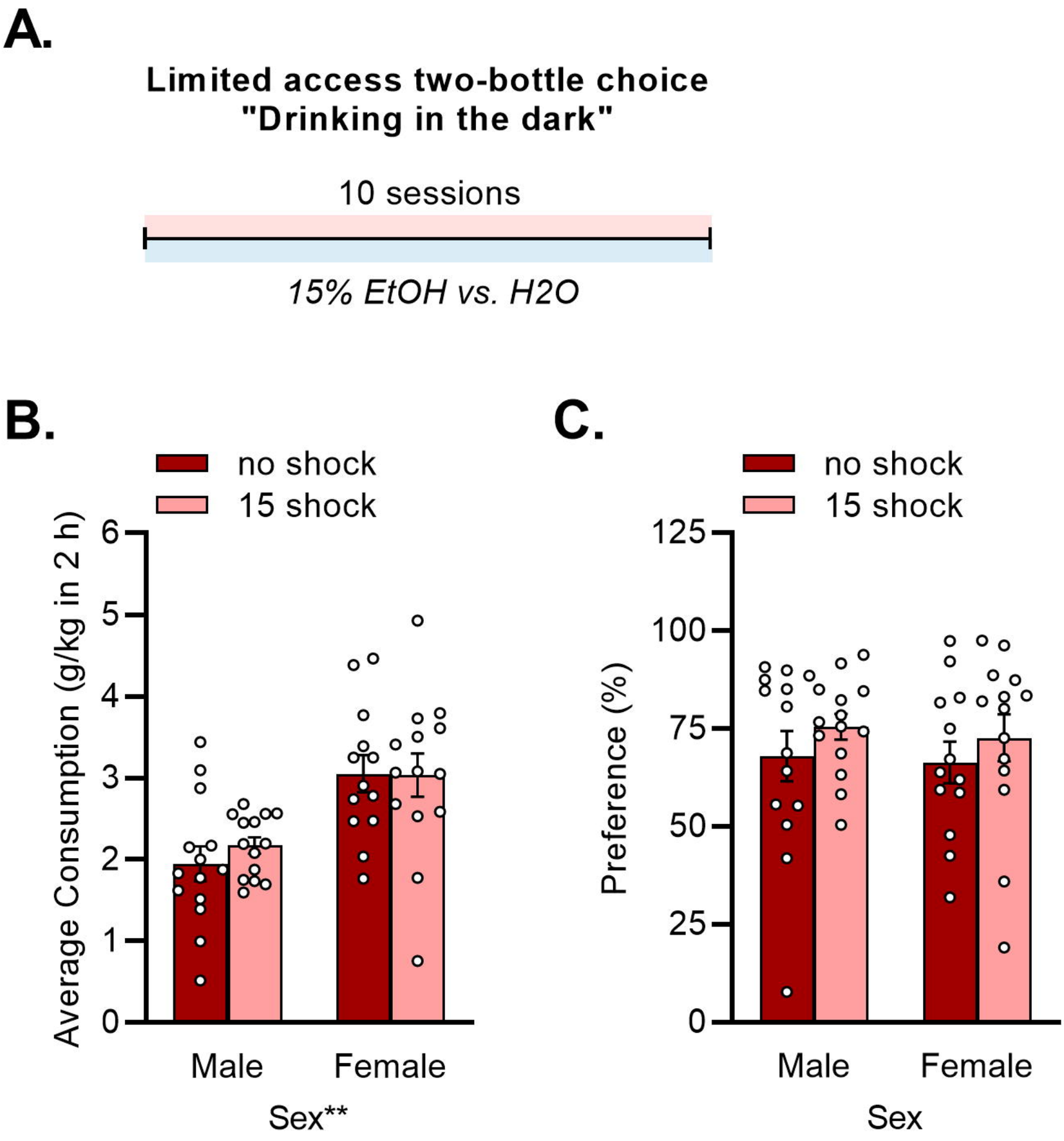
Figure Legends

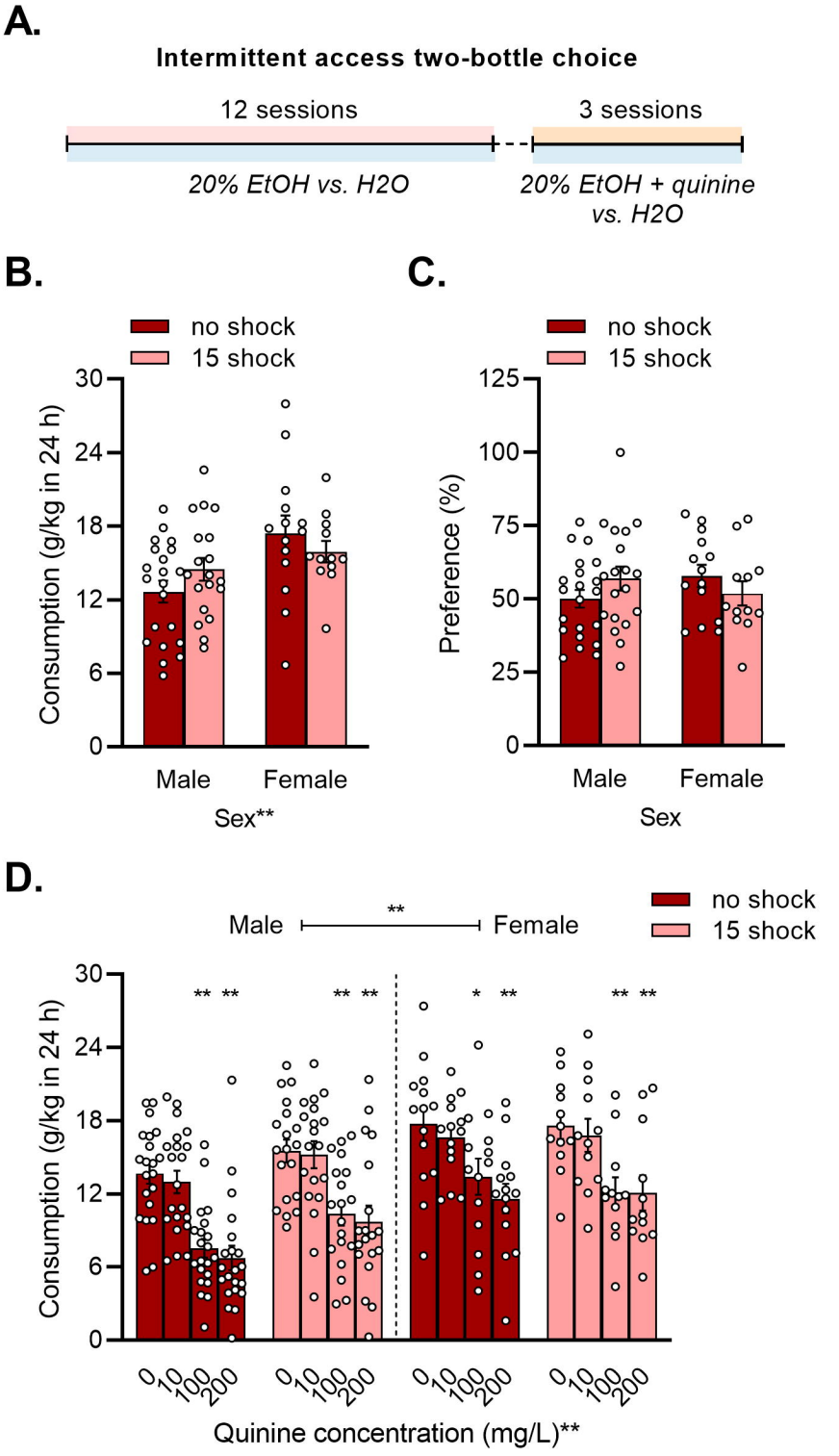
Figure 1. Sex, but not infant footshock, influences ethanol consumption and preference in a continuous access drinking task. (**A**) Mice drank 5% and 10% ethanol for five 24-h sessions each. (**B**) Infant footshock did not impact ethanol consumption (g/kg). Females consumed significantly more ethanol than males, **$p < 0.001$ main effect of sex. Female mice that received footshock consumed more 5% ethanol than 10% ethanol, *$p = 0.008$ Holm-Sidak's test. (**C**) Infant footshock did not impact preference for ethanol vs. water. Ethanol preference was higher in females vs. males, **$p = 0.002$ main effect of sex. Ethanol preference was higher in female mice that received footshock at the 5% vs. 10% ethanol concentration, *$p = 0.047$ Holm-Sidak's test.

Figure 2. Sex but not infant footshock influences ethanol consumption in a limited access drinking task. (**A**) Mice drank 15% ethanol for ten 2-h sessions. (**B**) There was a significant influence of sex on ethanol consumption (g/kg), *$p < 0.001$ main effect of sex. (**C**) Neither sex nor stress altered preference for ethanol vs. water.

Figure 3. Sex but not infant footshock influences ethanol consumption in an intermittent access drinking task. (**A**) Mice drank 20% ethanol for twelve 24-h sessions. Quinine was added to the ethanol solution on sessions 13-15 in increasing concentrations (10, 100, and 200 mg/L). (**B**) Females consumed more ethanol than males, **$p < 0.001$ main effect of sex. Infant footshock did not impact ethanol consumption. (**C**) Neither sex nor stress altered preference for ethanol vs. water. (**D**) Females consumed more ethanol mixed with quinine than males, **$p < 0.001$ main effect of sex. Quinine decreased ethanol consumption regardless of sex or footshock exposure, **$p < 0.001$ main effect of quinine concentration. Consumption was reduced at the 100 and 200 mg/L concentrations, but not 10 mg/L, in all groups, * $p < 0.05$, **$P < 0.01$ Dunnett's test.


A.
Continuous access
two-bottle choice
5 sessions
5 sessions
5% EtOH vs.
H2O
10% EtOH vs.
H2O
B.
Male
**
Female
no shock
15 shock
*
Consumption (g/kg in 24h)
0
3
6
9
12
15
18
5%
10%
5%
10%
EtOH Concentration
C.
Male
**
Female
no shock
15 shock
*
Preference (%)
0
25
50
75
100
125
5%
10%
5%
10%
EtOH Concentration


A.
Limited access two-bottle choice
"Drinking in the dark"
10 sessions
15% EtOH vs. H2O
B.
no shock
15 shock
Average Consumption (g/kg in 2 h)
0
1
2
3
4
5
6
Male
Female
Sex**
C.
no shock
15 shock
Preference (%)
0
25
50
75
100
125
Male
Female
Sex


A.
Intermittent access two-bottle choice
12 sessions
3 sessions
20% EtOH vs. H2O
20% EtOH + quinine vs. H2O
B.
no shock
15 shock
Consumption (g/kg in 24 h)
30
24
18
12
6
0
Male
Female
Sex**
C.
no shock
15 shock
Preference (%)
125
100
75
50
25
0
Male
Female
Sex
D.
Male
**
Female
no shock
15 shock
Consumption (g/kg in 24 h)
30
24
18
12
6
0
** **
** **
* **
** **
0
10
100
200
Quinine concentration (mg/L)**