Sex differences in binge alcohol drinking and the behavioral consequences of protracted withdrawal in C57BL/6J mice

Jean K. Rivera-Irizarry[1]*, Lia J. Zallar[2]*, Olivia B. Levine[1], Mary Jane Skelly[3a], Jared E. Boyce[3b], Thaddeus Barney[3], Ruth Kopyto[2], & Kristen E. Pleil[1,2,3^]

[1]Neuroscience Graduate Program, Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medicine, Cornell University
[2]Pharmacology Graduate Program, Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medicine, Cornell University
[3]Department of Pharmacology, Weill Cornell Medicine, Cornell University
[a]Current affiliation: Psychology Department, Iona College
[b]Current affiliation: Medical Scientist Training Program, University of Wisconsin School of Medicine and Public Health
^correspondence can be directed to krp2013@med.cornell.edu
*denotes equal contribution

Highlights

- Female mice consistently consumed more alcohol than males, with a more robust sex difference in consuming high (20%) versus lower (10%) concentration alcohol.
- Female alcohol intake was biased towards the first 2-hrs of each drinking session. While females consumed more water than males at the 24-hr timepoint, there was no difference in the first 2-hrs of drinking sessions suggesting elevated female alcohol intake is not driven by increased liquid consumption.
- Alcohol preference was elevated for 10% versus 20% alcohol but there was no difference between the sexes.
- Protracted alcohol abstinence decreased avoidance behavior and increased impulsivity in females only, with no effect in males.
- There was no effect of protracted alcohol abstinence on anhedonia in either sex.

Summary

The overconsumption of alcohol is a widespread public health issue linked to numerous diseases and mental health issues including anxiety among others. Binge drinking, defined as having 4-5 drinks in a 2-hour period, is more common in men than women but that demographic gap is shrinking. Mice are commonly used as an animal model of alcohol consumption and binge drinking to study behaviors associated with/resulting from alcohol drinking. We found that female mice consume more alcohol compared to their male counterparts, and furthermore, that following long-term alcohol consumption and abstinence, females but not males displayed reduced anxiety and increased compulsive behavior. Although high levels of anxiety that remained sustained for long periods of time are considered to negatively affect mental/physical health, some amount of anxiety is healthy to reduce behaviors that may compromise well-being including potentially binge drinking. We propose that this behavioral outcome may denote increased risk-taking behavior and that the reduction of anxiety in females may lead to negative outcomes.

Abstract

Background

Binge alcohol drinking is a risk factor linked to numerous disease states including alcohol use disorder (AUD). While men binge drink more alcohol than women, this demographic gap is quickly shrinking, and preclinical studies demonstrate that females consistently consume more alcohol than males. Further, women are at increased risk for the co-expression of AUD with neuropsychiatric diseases such as anxiety and mood disorders. However, little is understood about chronic voluntary alcohol drinking and its long-term effects on behavior in females. Here, we sought to characterize sex differences in chronic binge drinking and the effects of protracted alcohol withdrawal on anxiety- and affective-related behaviors in males and females.

Methods

We assessed binge alcohol drinking patterns in male and female C57BL/6J mice using a modified Drinking in the Dark (DID) paradigm in which mice received home cage access to one bottle of 10% or 20% alcohol (EtOH) or water for 2 hrs per day on Days 1-3 and to two bottles (EtOH/H2O + H2O) for 24 hrs on Day 4 for eight weekly cycles. Mice were then tested for the effects of protracted abstinence on avoidance, affective, and compulsive behaviors.

Results

Female mice consistently consumed more alcohol than males, with a more robust sex difference for 20% than 10% EtOH. Higher alcohol intake in females was biased toward the early access period (2-4 hrs) but was not dependent on higher overall water/fluid intake. Alcohol preference was higher in 10% vs. 20% EtOH but there was no difference between sexes. Protracted alcohol abstinence decreased avoidance behavior (elevated plus maze, open field, novelty suppressed feeding) and increased compulsive behavior (marble burying) in females but not males, and there was no effect of alcohol history on stress coping and negative affective behaviors (sucrose preference, forced swim test, tail suspension) in either sex.

Conclusion

Female mice engaged in higher volume binge drinking than their male counterparts; further, protracted abstinence from chronic binge drinking resulted in a female-specific behavioral disinhibition that may be related to greater alcohol intake. These results have implications for sex-dependent alcohol drinking patterns and long-term negative neuropsychiatric/physiological health outcomes in humans.

Introduction

Excessive alcohol use is a critical public health issue with wide ranging social and health-related negative consequences. Alcohol use disorder (AUD), which affects nearly 30 million people in the United States alone (1), is highly co-expressed with other neuropsychiatric diseases including anxiety and mood disorders (2, 3). The negative outcomes associated with alcohol are primarily driven by the pattern of consumption and cumulative intake volume (3). In particular, binge alcohol drinking, defined as the consumption of alcohol resulting in a blood ethanol concentration (BEC) of >0.08 mg/dl, is one of the most significant predictors of later development of AUD and other co-morbid neuropsychiatric diseases (2, 4), and persistent cycles of binge drinking to intoxication followed by withdrawal persists among individuals with AUD (3, 5). Binge drinking and AUD are more common among men than women, although this gap in prevalence has been shrinking in recent years as women's alcohol drinking is increasing at a faster rate than men's (3, 6). Indeed, current epidemiological data show that adolescent girls are already as likely to express AUD as adolescent boys (7); and, females in other mammalian species including rodents drink as much or more than their male counterparts when intake is adjusted to bodyweight (8-12). Nonetheless, the complexities of binge drinking behavior remain poorly understood in females, including in rodent models such as the commonly used C57Bl/6J mouse strain that have been employed robustly in males. In particular, it is unclear whether females consume more than males due to differences in alcohol preference or motivated voluntary consumption, as the studies performed to date report a mix of results showing elevated female preference at least some time points of alcohol exposure (10, 11) or no sex differences (9, 13-15), depending on the alcohol exposure paradigm and length.

Understanding alcohol drinking behavior is particularly important because of its contributions to the expression of other maladaptive behaviors associated with neuropsychiatric diseases, such as anxiety, anhedonia, and compulsive behaviors. Studies have found that chronic alcohol exposure has divergent effects on behavior depending on rodent species/strain, alcohol administration paradigm, and state of alcohol dependence or duration of withdrawal (recently reviewed in detail by Bloch and colleagues (16)). The effects of protracted abstinence (<1 week) from chronic (4 weeks+) alcohol exposure on emotion-related behaviors in male C57BL/6J mice varies widely, with some showing increased avoidance, anhedonia, and compulsive behaviors (17-22) and others reporting no effects in some or all measures (10, 20, 23). However, these studies vary widely in the alcohol exposure paradigms, durations of alcohol exposure, and behavioral testing time points employed. In particular, relatively little is known about the behavioral effects of chronic voluntary binge alcohol drinking beyond a week into abstinence. Furthermore, women are at an increased risk of co-expression of AUD with anxiety and affective disorders (24-26), but there is a dearth of females used in the literature exploring the effects of protracted alcohol exposure on behavior, using many common protocols including vapor inhalation models and voluntary binge drinking models such as intermittent access (IA) and Drinking in the Dark (DID; (16). As such, the field has not been able to assess the role of sex in the relationship between alcohol and emotional states.

Here, we assessed sex differences in binge alcohol consumption, pattern, and preference, as well as the effects of binge alcohol drinking on stable behavioral phenotypes during protracted abstinence. We found that females consumed more alcohol than their male counterparts, an effect that was not dependent on total volume intake. Additionally, we found that compared to water-drinking controls, alcohol-exposed mice displayed a consistent reduction in avoidance behavior without alterations in affective behaviors. Moreover, this aberrant risk-taking behavior was biased toward expression in females. As females consumed higher levels of alcohol across the eight weeks of exposure, these results may reflect increased susceptibility to aberrant plasticity in females or a dose-dependent effect of alcohol.

Methods

Animals

Male and female C57BL/6J mice were purchased from Jackson Laboratories (stock #00064) at eight weeks of age. Mice were housed in same-sex cages of five on a reverse 12:12 hr light cycle colony room with lights off at 7:30am daily, given *ad libitum* access to water and standard chow, and allowed to acclimate for at least one week. Mice were singly housed one week prior to the beginning of binge alcohol (20% or 10% EtOH) or water (H2O) drinking. Following eight weekly cycles, 20% EtOH and H2O groups underwent a battery of behavioral tests over six weeks of abstinence. All behavioral testing occurred during the dark phase of the light-dark cycle, beginning approximately three hrs into the start of the dark phase. For all tests occurring outside of the home cage, mice were habituated to the testing room for one hr before behavioral testing, and assays were conducted under 250 lux lighting conditions and analyzed using Ethovision XT (Noldus). All experimental procedures were approved by the Institutional Animal Care and Use Committees at Weill Cornell Medicine.

Binge alcohol drinking

Here, we used a modified version of the standard Drinking in the Dark (27) DID) procedure, allowing us to assess alcohol preference within the DID paradigm across eight weekly cycles (**Fig. 1a**). Home cage water bottles were replaced with 50 ml bottles containing alcohol (20% or 10%) or water (H2O) as a control three hrs into the dark phase of the light cycle for four consecutive days each weekly DID cycle. On Mon-Wed, mice received two hours of access, as in the standard DID paradigm (8). On Thurs, mice received a modified access to two bottles (one EtOH and one water for EtOH drinking groups, and two water bottles for H2O DID) for 24 hrs, and bottle weights were taken 2 hr, 4 hr, and 24 hr into access. EtOH/water bottles were then replaced with home cage water bottles on Fri morning until the following DID cycle, which began 72 hr later the following Mon. In the latter half of the paradigm, a subset of 10% EtOH mice underwent unsuccessful fear conditioning (due to technical issues that prevented the floor grid from providing shocks) before cycles 5 and 8; because these mice did not differ from their counterparts in any measures recorded, all 10% EtOH DID mice are included within as a comparison to 20% EtOH and H2O DID mice for alcohol drinking. Following eight cycles, the 20% EtOH and H2O DID cohort mice underwent a battery of behavioral tests (**Fig. 5a**).

Sucrose preference test

Seventy-two hr after the last alcohol/water access of eight-cycle DID (20% EtOH and H2O), mice were assessed for their preference for 1% sucrose over three consecutive days, followed by 2% sucrose for the subsequent three days, using a 24 hr two-bottle choice assay as previously described (28). The home cage water bottle was replaced with two 50 ml bottles, one containing 1% sucrose and one containing water, and bottles were weighed and replaced in switched positions every 24 hr for three days. The following three days, the same procedure was repeated with bottles containing water and 2% sucrose. Sucrose and water intake were recorded, and consumption and preference were analyzed for days 2-3 for each sucrose concentration. Data were excluded if a bubble in the bottle spout prevented access to the sucrose on that day.

Open field test

Approximately two weeks following the last alcohol exposure, mice were tested for avoidance behavior and locomotion in the open field test (OF) as previously described (28). The test was conducted in a plexiglass arena (50 × 50 × 34.5 cm) with a gray floor and each mouse was placed in one corner of the arena and allowed to freely explore for 30 min. Total time spent in the center of the arena (all four paws in the 25 cm × 25 cm area in the center of the arena) and periphery were quantified to calculate the percent center time. The total distance traveled in the maze (cm) was used to measure locomotion while the percent time in the center of the maze was used to assess avoidance-like behavior.

Elevated plus maze
Approximately 2.5 weeks following the last alcohol exposure, the elevated plus maze (EPM) test was conducted as previously described (28) in a plexiglass maze with two open and two closed arms (35 cm l × 5.5 cm w, with 15 cm h walls for closed arms) extending from a central platform (5.5 cm ×5.5 cm) elevated 50 cm above the floor. Mice were placed in the center of the elevated plus maze (EPM) facing an open arm and allowed to freely explore the maze for five min. The percent time spent in the open arms was used to assess avoidance behavior and ambulatory locomotion was also analyzed. Due to a technical issue, EPM data for some mice could not be analyzed and were therefore excluded.

Light-dark box
Approximately three weeks following the last alcohol exposure, the light/dark box test was conducted as previously described (28) in a rectangular box divided into two equal compartments (20 cm l × 40 cm w × 34.5 cm h): one dark with a closed lid and the other with an open top and illuminated by two 60 W bulbs placed 30 cm above the box. The two compartments were separated by a divider with a 6 cm x 6 cm cut-out passageway at floor level. At the beginning of a trial, each mouse was placed in a corner of the light compartment and allowed to freely explore the apparatus for 10 min. The number of light side entries and total time spent in the light compartment as compared to the dark compartment were used to assess avoidance behavior.

Marble burying task
Approximately 3.5 weeks following the last alcohol exposure, mice were placed in a clean, standard rat cage (26 cm l x 48 cm w x 20 cm h) with 5 cm of fresh, unscented bedding containing 20 evenly-spaced marbles. Following 30 min, the number of marbles buried, with a threshold of at least ⅔ of the marble being covered by bedding, was assessed by the experimenter and recorded.

Novelty suppressed feeding
Approximately four weeks following the last alcohol exposure, mice underwent the novelty suppressed feeding (NSF) test. Mice were exposed to a fruit loop in their home cages for two consecutive days, then fasted overnight for 18-20 hrs prior to testing. During the assay, the mouse was placed into a novel environment (plexiglass arena; 50 × 50 × 34.5 cm) containing fresh bedding and a fruit loop on a piece of weighing paper in the center of the arena. The latency to retrieve and bite the fruit loop was recorded by an experimenter. As soon as the mouse began to bite the fruit loop, or 5 min had elapsed, the mouse was removed and placed back into its home cage containing pre-weighed standard chow pellets, and the amount of chow consumed over the next 10 min was recorded.

Tail suspension test
Approximately 4.5 weeks following the last alcohol exposure, mice underwent the tail suspension test (TST). Each mouse was hung by its tail for six min, and an experimenter blind to condition hand scored the last four min for active coping (hindlimb kicking, body shaking, attempts by the mouse to reach its tail or the wall) or passive coping (immobility and minor hindlimb movements) using BORIS (Behavioral Observation Research Interactive Software).

Forced swim test
Approximately 5 weeks following last alcohol exposure, for the forced swim test (FST), each mouse was placed in a glass cylinder (1.5 L beaker, 15 cm h, 11 cm d) containing room temperature water for six min. The last four min were scored for swimming, climbing, and immobile behaviors by an experimenter bling to condition using BORIS. Swimming was scored as forceful movements of the hindlimbs. Climbing was scored as a vertical (or tilted) position against the cylinder wall surface with the head pointed upwards and vigorous

kicking. Minor movements in the forelimbs and light kicking of one hindlimb to stay afloat were scored as immobility.

Paw withdrawal

Approximately 5.5 weeks following the last alcohol exposure, mice were tested for pain sensitivity using a hot plate paw withdrawal assay. Mice received three trials separated by 10 min, in which they were placed within a cylinder on a hot plate of a temperature of 52 °C or 58 °C, and the latency to lift or lick its hind paw or jump away from the hotplate was recorded and the mouse was removed from the hot plate and returned to its home cage. If the mouse did not withdraw its paw within 30 sec, it was removed from the hot plate to prevent tissue damage. Paw withdrawal latency averaged across the three trials was calculated.

Statistical analysis

Statistical analyses were performed in GraphPad Prism 9. Data for all dependent measures were examined for normal distributions within group and equality of variance across groups using Q-Q plots. Analysis of variance (ANOVA) was used to evaluate the effects of and interactions between sex, EtOH concentration, and DID cycle on EtOH/water consumption and preference and sex and EtOH on behavioral measures in protracted withdrawal. For all ANOVAs, significant effects were further probed with post hoc paired or unpaired t-tests with Holm–Sidak corrections for multiple comparisons, and multiplicity-adjusted P values are reported. Statistical comparisons were always performed with an alpha level of 0.05 and using two-tailed analyses. Data are presented as mean ± SEM, and raw data points are included in all graphs where reasonable.

Results

Distinct patterns of alcohol and water consumption depending on sex and alcohol concentration

Here we used a modified DID binge drinking paradigm to assess the effects of sex on water and alcohol consumption and preference across multiple concentrations of alcohol (**Fig. 1a,b**). We first measured raw cumulative intake of EtOH solution or H2O in the 20% EtOH DID, 10% EtOH DID, and H2O DID mice (**Fig. 1c-e**). A 2xRM-ANOVA on raw cumulative intake of 20% EtOH showed main effects of sex (F (1, 18) = 5.97, **P* = 0.025) and cycle (F (7, 126) = 1141.0, P < 0.0001) and an interaction between the two (F (7, 126) = 4.45, ***P = 0.0002). Post hoc t-tests with H-S corrections showed that raw consumption was higher in F than M on cycles 5-8 (cycle 5: t144 = 2.60, *P = 0.050; cycle 6: t144 = 3.00, *P = 0.019; cycle 7: t144 = 3.50, **P = 0.005; cycle 8: t144 = 3.41, **P = 0.006; all other cycles: Ps > 0.30; **Fig. 1c**). In contrast, cumulative raw intake of 10% EtOH was not different in M and F, as there was an effect of DID cycle (F (7, 126) = 642.90, P < 0.0001) but no effect of sex or interaction (Ps > 0.35) in a 2xRM-ANOVA (**Fig. 1d**). And, cumulative raw intake of water was not different between M and F in H2O DID (2xRM-ANOVA: main effect of DID cycle (F (7, 126) = 303.0, P < 0.0001) but no effect of sex or interaction (Ps > 0.50; **Fig. 1e**). These results show that raw intake was greater in F than M only for high concentration (20%) EtOH. When EtOH or water consumption was normalized to bodyweight, females cumulatively consumed more 20% EtOH, 10% EtOH, and H2O than M (**Fig. 1f-h**). This emerged by cycle 3 for 20% EtOH DID, cycle 4 for 10% EtOH DID, and cycle 5 for H2O DID. A 2xRM-ANOVA for 20% EtOH DID showed main effects of sex (F (1, 18) = 29.37, ****P < 0.0001) and cycle (F (7, 126) = 862.3, P < 0.0001) and an interaction between the two (F (7, 126) = 28.42, ****P < 0.0001). Post hoc t-tests with H-S corrections showed that normalized consumption was higher in F than M on cycles 3-8 (*Ps < 0.05, as indicated; cycles 1 and 2: Ps > 0.15; **Fig. 1f**). A 2xRM-ANOVA for 10% EtOH DID showed main effects of sex (F (1, 18) = 14.42, ****P < 0.0001) and cycle (F (7, 126) = 776.7, P < 0.0001) and an interaction between the two (F (7, 126) = 16.05, ****P < 0.0001). Post hoc t-tests with H-S corrections showed that normalized consumption was higher in F than M by cycles 4-8 (*Ps < 0.05, as indicated; cycles 1-3: Ps > 0.10; **Fig. 1g**). A 2xRM-ANOVA for H2O DID show main effects of sex (F (1, 18) = 9.52, **P = 0.006) and cycle (F (7, 126) = 360.7, P < 0.0001) and an interaction between the two (F (7, 126) = 9.85, ****P < 0.0001). Post hoc t-tests with

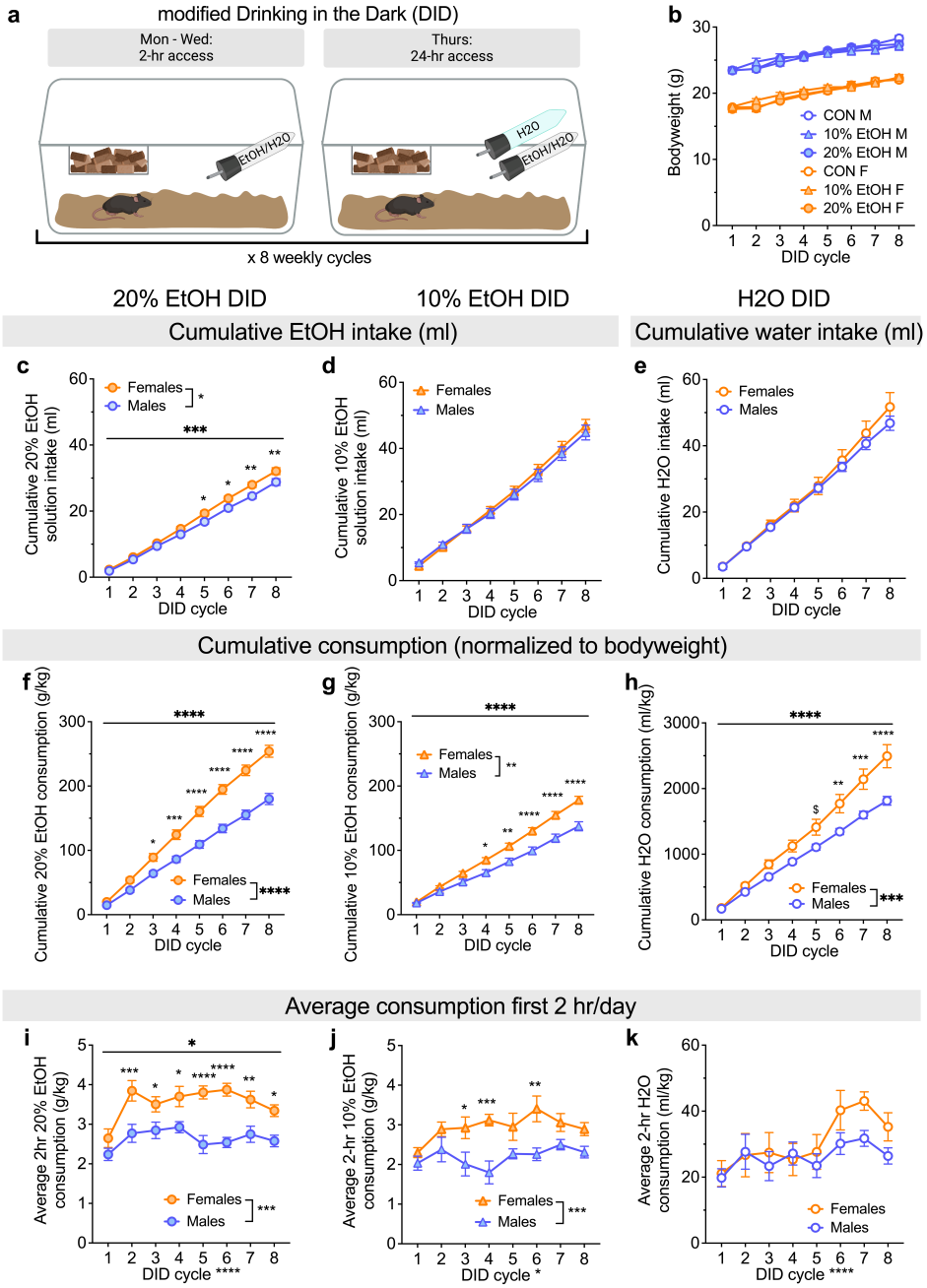

Figure 1: Alcohol and water consumption are sex- and alcohol concentration-dependent. A) Schematic depicting the modified Drinking in the Dark (DID) binge drinking paradigm in C57BL/6J mice. **B)** Bodyweight gain was similar across DID cycles in H2O, 10% EtOH, and 20% EtOH DID mice of both sexes. **C)** Cumulative raw intake of 20% EtOH solution (ml) was higher in females compared to males on DID cycles 5-8. **D)** There was no sex difference in cumulative raw 10% EtOH intake (ml) across DID cycles. **E)** There was no sex difference in cumulative raw H2O intake (ml) across DID cycles. **F)** Cumulative EtOH consumption normalized to bodyweight (g/kg) was higher in females compared to males on DID cycles 3-8. **G)** Females consumed more 10% EtOH (g/kg) compared to males on DID cycles 4-8. **H)** Females consumed more H2O (ml/kg) compared to males during DID cycles 6-8, with a trend on cycle 5. **I)** Females displayed higher average 20% EtOH consumption (g/kg) during the first two hrs/day of access on DID cycles 2-8. **J)** Females displayed higher average 10% EtOH consumption (g/kg) on the first two hrs/day of access on DID cycles 3, 4, and 6. **K)** There was no difference between males and females on average H2O consumption during the first two hrs/day of water access. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 in 2xRM-ANOVA main effects and interactions of sex and DID cycle, as well as post hoc t-tests with H-S corrections between M and F. $P < 0.10 for post hoc t-tests with H-S corrections between M and F.

H-S corrections showed that normalized H2O consumption was higher in F than M by cycles 6-8 (*Ps < 0.05, as indicated) and trended toward higher on cycle 5 ($P = 0.064; **Fig. 1h**), suggesting that females drank more (cumulative) water than males across the entire mDID paradigm, but increased intake was more robust for increasing EtOH concentrations. Examination of the average EtOH/water consumption for the first 2 hrs/day on each DID cycle (average of days 1-3 and the first 2 hrs on day 4) showed that females consumed more EtOH than males but not more water than males during this early access period (**Fig. 1i-k**). A 2xRM-ANOVA for 20% EtOH DID showed main effects of sex (F (1, 18) = 29.37, ****P* = 0.0001) and cycle (F (7, 126) = 6.60, ****P < 0.0001) and an interaction between the two (F (7, 126) = 212, **P* = 0.046). Post hoc t-tests with H-S corrections showed that average 2 hr consumption was higher in F than M on cycles 2-8 (Ps < 0.05, as indicated; cycle 1: P = 141; **Fig. 1i**). For 10% EtOH DID, a 2xRM-ANOVA showed main effects of sex (F (1, 18) = 17.73, ****P* = 0.0005) and cycle (F (7, 126) = 2.13, *P = 0.045) but no interaction between the two (P = 0.167). Post hoc t-tests with H-S corrections showed that average 2 hr consumption was higher in F than M on cycles 3, 4, and 6 (Ps < 0.05, as indicated) but not other cycles (Ps > 0.15; **Fig. 1j**). For H2O DID, a 2xRM-ANOVA showed a main effect of cycle (F (7, 126) = 4.56, *****P* < 0.0001) but no effect of sex or interaction (Ps > 0.20; **Fig. 1k**). These results confirm that females' increased consumption of EtOH compared to males was more robust for 20% than 10% EtOH. Further, they suggest while females display higher cumulative fluid intake across the mDID paradigm for all EtOH/H2O conditions (**Fig. 1f-h**), their higher consumption of EtOH during the first two hr of each drinking day (**Fig. 1i,j**) was not attributable to higher fluid intake during that time (**Fig. 1k**).

We next dissected Day 4 two bottle choice (2-BC) drinking behavior, examining both consumption and preference across the 2 hr, 4 hr, and 24 hr time points (**Figs. 2 and 3**). During the first 2 hrs of Day 4 of 20% EtOH DID, females drank more EtOH but not water than males (**Fig. 2a**). A 2xRM-ANOVA for EtOH intake showed main effects of sex (F (1, 18) = 16.29, ****P* = 0.0008) and cycle (F (7, 126) = 5.72, ****P < 0.0001) but no interaction between the two (P = 0.268). Post hoc t-tests with H-S corrections showed that Day 4 2-hr consumption was higher in F than M on cycles 2, 5, and 7 (*Ps < 0.05, as indicated) and trended toward an increase on cycles 4, 6, and 8 ($Ps < 0.10) but did not differ on cycles 1 and 3 (Ps > 0.30; **Fig. 2a**). In contrast, water consumption did not differ between females and males, as there was a main effect of cycle (F (7, 126) = 4.25, ***P = 0.0003) but no effect of sex or interaction (Ps > 0.25). Four-hr consumption showed a similar pattern (**Fig. 2b**), with a 2xRM-ANOVA for 4-hr 20% EtOH showing main effects of sex (F (1, 18) = 24.75, *****P* < 0.0001) and cycle (F (7, 126) = 4.13, ***P = 0.0004) but no interaction between the two (P = 0.714). Post hoc t-tests with H-S corrections showed that 4-hr consumption was higher in F than M on cycles 2, 3, 4, 5, and 8 (*Ps < 0.05, as indicated) and trended toward an increase on the remaining cycles ($Ps < 0.10). In contrast, water consumption did not differ between females and males, as there was a main effect of cycle (F (7, 126) = 4.03, ***P = 0.0005) but no effect of sex or interaction (Ps > 0.05). For 24-hr 20% EtOH consumption (**Fig. 2c**), a 2xRM-mixed effects model showed main effects of sex (F (1, 18) = 28.12, *****P* < 0.0001) and cycle (F (7, 116) = 2.40, *P = 0.025) but no interaction between the two (P = 0.495). Post hoc t-tests with H-S corrections showed that 24-hr consumption was higher in F than M on cycles 2-5 (*Ps < 0.05, as indicated) and trended toward an increase on cycle 7 ($P = 0.087). In contrast, water consumption did not differ between females and males, as there was a main effect of cycle (F (7, 116) = 5.06, ***P < 0.0001) but no effect of sex or interaction (Ps > 0.05). During the first 2 hrs of Day 4 of 10% EtOH DID, females drank more EtOH but not water than males (**Fig. 2d**). A 2xRM-ANOVA for EtOH intake showed main effects of sex (F (1, 18) = 23.48, ****P* = 0.0001) and cycle (F (7, 126) = 2.26, *P = 0.033) but no interaction between the two (P = 0.790). Post hoc t-tests with

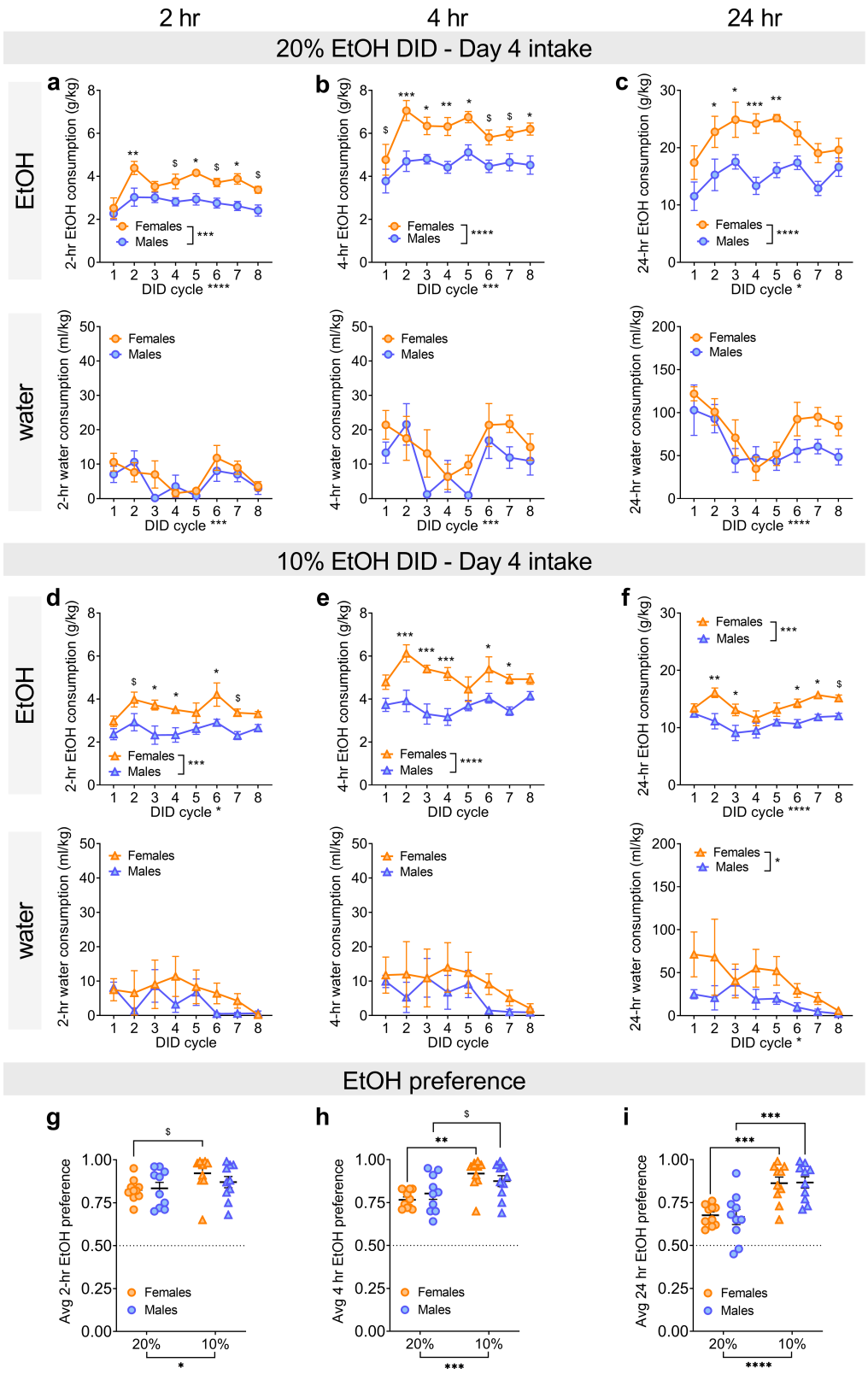

Figure 2: Females consume more alcohol than males across EtOH concentrations at 2-, 4-, and 24-hour timepoints on Day 4 access but have similar alcohol preference. A-C) EtOH (top row) and water (bottom row) consumption at 2-hr, 4-hr, and 24-hr time points on Day 4, 2-bottle choice (2-BC) access in 20% EtOH DID across cycles. **A)** During the first 2 hrs, females rapidly escalate EtOH intake and consumed more 20% EtOH (g/kg) than males during DID cycles 2, 5, and 7, with a trend towards increased intake on cycles 4 and 6. There was no difference in water consumption between the sexes. **B)** The sex difference in 20% EtOH consumption was even more robust for the first 4 hrs of Day 4 access, with females consuming more than males on DID cycles 2-5, and 8 and a trend towards increased

intake cycles 1, 6, and 7. There was no difference in water consumption. **C)** Females consumed more 20% EtOH (g/kg) than males during the 24-hr period of Day 4 access on DID cycles 2-5. There was no difference in water consumption. **D-F)** EtOH (top row) and water (bottom row) consumption at 2-hr, 4-hr, and 24-hr time points on Day 4, 2-bottle choice (2-BC) access in 10% EtOH DID across cycles. **D)** During the first 2 hrs, females consumed more 10% EtOH (g/kg) than males on DID cycles 3, 4, and 6, with a trend towards increased intake in cycles 2 and 7. There was no difference in water consumption. **E)** During the first 4 hrs, females consumed more 10% EtOH (g/kg) than males during DID cycles 2, 3, 4, 6, and 7. There was no difference in water consumption. **F)** During the 24-hr access period, females consumed more 10% EtOH than males during DID cycles 2, 3, 6, and 7, with a trend toward increased intake in cycle 8. Water consumption was overall higher in females compared to males. **G-I)** Average EtOH preference in 20% and 10% DID at 2-hr (**G**), 4-hr (**H**), and 24-hr (**I**) time points, showing that males and females similarly displayed higher EtOH preference for 10% compared to 20% EtOH. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 in 2xRM-ANOVA main effects and interactions of sex and DID cycle (**A-F**) and 2xANOVA main effects of EtOH concentration (**G-I**) and post hoc t-tests with H-S corrections. $P < 0.10 for post hoc t-tests with H-S corrections between M and F (**A-F**) and EtOH concentration within sex (**G-I**).

H-S corrections showed that Day 4 2-hr consumption was higher in F than M on cycles 3, 4, and 6 (*Ps < 0.05, as indicated) and trended toward an increase on cycles 2 and 7 ($Ps < 0.10) but did not differ on cycles 1, 5, and 8 (Ps > 0.20). In contrast, water consumption did not differ between females and males, as there was a trend toward an effect of cycle (F (7, 126) = 2.00, P = 0.061) but no effect of sex or interaction (Ps > 0.35). Four-hr 10% EtOH consumption showed a similar pattern (**Fig. 2e**), with a 2xRM-ANOVA for 4-hr 10% EtOH showing a main effect of sex (F (1, 18) = 28.67, ****P < 0.0001) and a trend toward the effect of cycle (F (7, 126) = 2.00, P = 0.060) but no interaction between the two (P = 0.132). Post hoc t-tests with H-S corrections showed that 4-hr 10% EtOH consumption was higher in F than M on cycles 2, 3, 4, 6, and 7 (*Ps < 0.05, as indicated) but not the other cycles (Ps > 0.10). In contrast, water consumption did not differ between females and males, as there were no effects or interaction (Ps > 0.10). For 24-hr 10% EtOH consumption (**Fig. 2f**), a 2xRM-ANOVA showed main effects of sex (F (1, 18) = 16.32, ***P = 0.0008) and cycle (F (7, 126) = 4.94, ****P < 0.0001) but no interaction between the two (P = 0.220). Post hoc t-tests with H-S corrections showed that 24-hr consumption was higher in F than M on cycles 2, 3, 6, and 7 (*Ps < 0.05, as indicated) and trended toward an increase on cycle 8 ($P = 0.058) but was not different on cycles 1, 4, and 5 (Ps > 0.20). Water consumption also differed between females and males in 10% EtOH DID, as there were main effects of sex (F (1, 18) = 4.44, *P = 0.049) and cycle (F (7, 126) = 2.12, *P = 0.046) but no interaction (P = 0.705). However, post hoc t-tests with H-S corrections revealed no differences between males and females in any specific cycle (Ps > 0.30).

We next assessed whether sex or EtOH concentration affected EtOH preference at the 2, 4, and 24 hr time points on Day 4 2-BC (**Fig. 2g-i**). While EtOH preference was significantly above chance for both sexes at both 10% and 20% concentrations (one-sample t-tests compared to the null hypothesis of 0.5 preference score: all Ps < 0.01, not indicated), there were differences between concentrations that depended on sex. We found that 2-hr EtOH preference (averaged across all cycles) was higher for 10% than 20% EtOH (**Fig. 2g**). A 2xANOVA showed a main effect of EtOH concentration (F (1, 36) = 4.72, P = 0.037) but no effect of sex or interaction (Ps > 0.30). Post-hoc t-tests with H-S corrections showed a trend for higher 10% than 20% EtOH preference in females (t36 = 2.24, $P = 0.062) but not males (P = 0.411). This phenotype was stronger at 4 hrs (**Fig. 2h**), as a 2xANOVA showed a main effect of EtOH concentration (F (1, 36) = 15.77, ***P = 0.0003) but no effect of sex or interaction (Ps > 0.15). Post-hoc t-tests with H-S corrections showed a higher 10% than 20% EtOH preference in females (t36 = 3.82, **P = 0.001) and a trend in males (t36 = 1.80, $P = 0.081). At 24 hrs, both sexes robustly displayed a stronger 10% than 20% EtOH preference (**Fig. 2i**). A 2xANOVA showed a main effect of EtOH concentration (F (1, 36) = 32.33, ****P < 0.0001) but no effect of sex or interaction (Ps > 0.80). Post-hoc t-tests with H-S corrections showed higher 10% than 20% EtOH preference in both sexes (F: t36 = 3.88, ***P = 0.0004; M: t36 = 4.17, ***P = 0.0004).

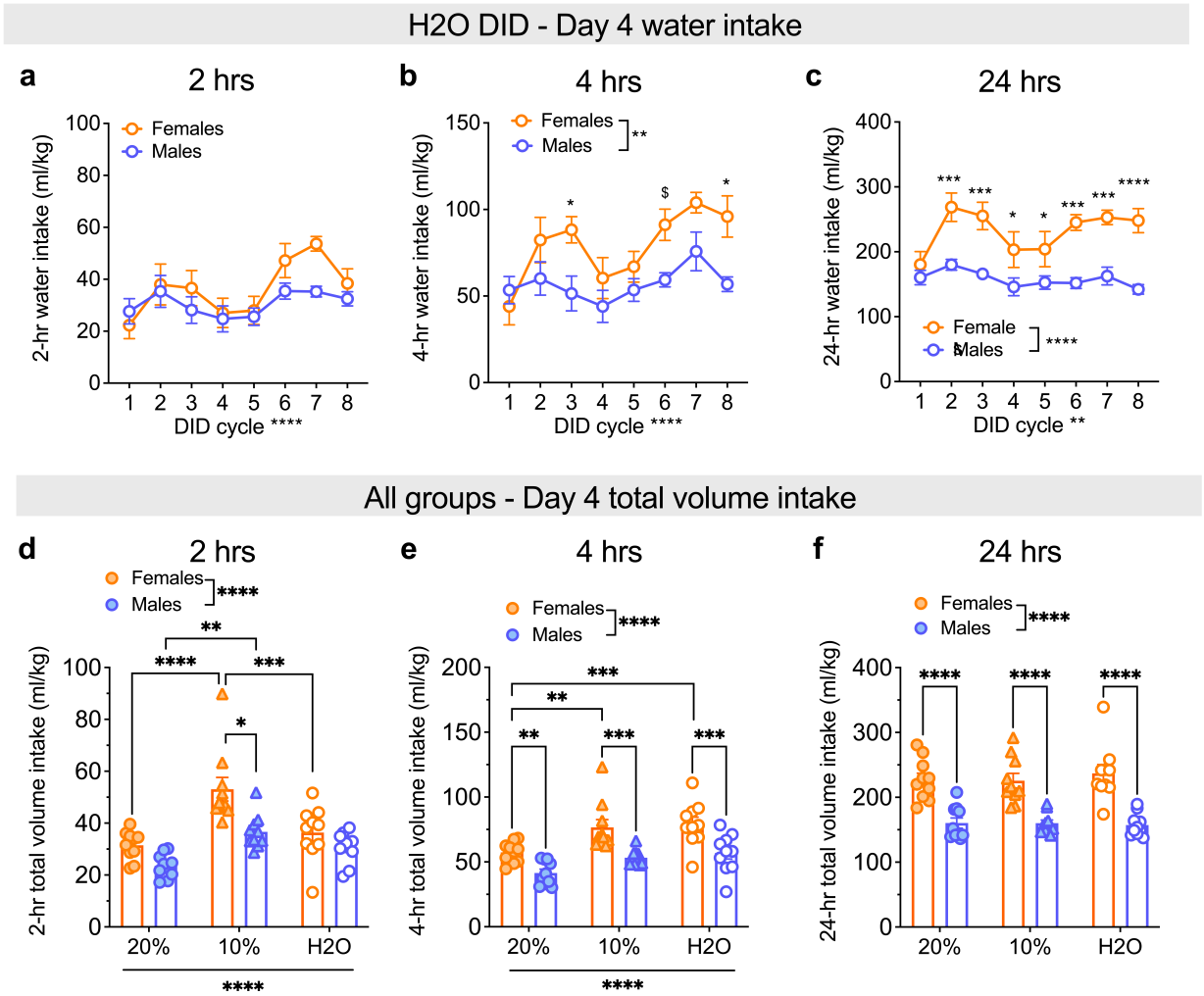

Figure 3: Females' higher fluid intake than males emerges across the day but does not account for their greater alcohol intake at early time points. A-C) Water consumption at 2-hr, 4-hr, and 24-hr time points on Day 4, 2-bottle choice (2-BC) access in H2O DID across cycles. **A)** There were no sex differences in H2O consumption (ml/kg) during the first 2 hrs of Day 4 access. **B)** Females consumed more H2O (ml/kg) than males during the first 4 hrs of Day 4 on cycles 3 and 8, with a trend towards increased consumption during cycle 6. **C)** Females consumed more H2O (ml/kg) than males during the 24-hr period on cycles 2-8. **D-F)** Average total volume intake for Day 4 EtOH and water in 20% EtOH, 10% EtOH, and H2O DID. **D)** During the first 2 hrs, total volume intake was highest for 10% EtOH, and females consumed more fluid than males in 10% EtOH DID. **E)** Within 4 hrs, females consume greater volume than males for all conditions, and intake was higher for H2O, particularly in females. **F)** At the 24-hr time point, females consumed more fluid than males in all DID conditions; there were no differences in fluid intake between DID conditions in either sex. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 in 2xRM-ANOVA main effects and interactions of sex and DID cycle (**A-C**) and 2xANOVA main effects of sex and EtOH concentration (**D-F**), as well as post hoc t-tests with H-S corrections as indicated. $P < 0.10 for post hoc t-tests with H-S corrections between M and F.

We also examined water consumption at these 2, 4, and 24 hr time points in mice that underwent H2O DID for comparison (**Fig. 3a-c**). For 2-hr consumption (**Fig. 3a**), a 2xANOVA revealed a main effect of cycle (F (7, 126) = 4.83, ****P < 0.0001) but no effect of sex or interaction (Ps > 0.10). For 4-hr H2O consumption (**Fig. 3b**), a 2xANOVA showed a main effect of sex (F (1, 18) = 9.74, ***P* = 0.006) and cycle (F (7, 126) = 5.44, ****P < 0.0001) and a trend for interaction between the two (F (7, 126) = 1.82, P = 0.089). Post hoc t-tests with H-S corrections showed that Day 4 4-hr consumption was higher in F than M on cycles 3 and 8 (*Ps < 0.05, as indicated) and trended toward higher on cycle 6 ($P = 0.093) but did not differ on cycles 1, 2, 4, 5, or 7 (Ps > 0.15). For 24r-hr H2O DID water consumption (**Fig. 3c**), a 2xRM-mixed effects model showed main effects of sex (F (1, 18) = 28.73, ****P* < 0.0001) and cycle (F (7, 116) = 3.31, **P = 0.003) but no interaction between the

two (P = 0.242). Post hoc t-tests with H-S corrections showed that 24-hr consumption was higher in F than M on all cycles but the first (cycle 1: P = 0.259; all other cycles: *Ps < 0.05, as indicated). These results show that water intake in females is higher than in males, but this emerges across the 24-hr period after the first two hr of access. Given these results, we further assessed total volume intake across 2, 4, and 24 hr time points of Day 4 averaged across cycles) for all mice (**Fig. 3d-f**). For the 2-hr time point (**Fig. 3d**), a 2xANOVA showed main effects of sex (F (1, 54) = 20.50, ****P < 0.0001) and EtOH concentration (F (2, 54) = 20.96, ****P < 0.0001) but no interaction (P = 0.127). Post hoc t-tests with H-S corrections showed that total intake for the first two hrs on Day 4 was higher in F than M for 10% EtOH (t54 = 4.27, ***P = 0.0002) and trended toward higher for 20% EtOH (t54 = 2.06, $^{\$}$P = 0.087) but was not different for H2O DID mice (P = 0.137). Further, total volume intake in 10% EtOH DID mice was higher than in 20% EtOH and H2O DID for females (***Ps ≤ 0.0001, as indicated) and than 20% DID mice for males (**P = 0.004; all other Ps > 0.10). For the 4-hr time point (**Fig. 3e**), a 2xANOVA showed main effects of sex (F (1, 54) = 36.31, ****P < 0.0001) and EtOH concentration (F (2, 54) = 11.30, ****P < 0.0001) but no interaction (P = 0.638). Post hoc t-tests with H-S corrections showed that total intake for the first two hrs on Day 4 was higher in F than M for all three groups (**Ps < 0.01, as indicated). Further, total volume intake in 20% EtOH DID mice was lower than in 10% EtOH and H2O DID for females (**Ps < 0.01, as indicated) and than H2O DID mice for males (*P = 0.035; all other Ps > 0.10). For the 24-hr time point (**Fig. 3f**), a 2xANOVA showed a main effect of sex (F (1, 54) = 85.01, ****P < 0.0001) but not EtOH concentration or interaction (Ps > 0.70). Post hoc t-tests with H-S corrections showed that total intake for the first two hrs on Day 4 was higher in F than M for all three groups (****Ps < 0.0001). Thus, total volume intake normalized across the last 20 hrs of access for all groups, leading to similar overall volume intake across conditions that were higher in females than males.

Overall, results show that effect of sex and EtOH concentration on EtOH consumption were not due to differences in overall fluid intake (which emerged across the day but were not present during the early hours of access). Therefore, we examined the pattern of EtOH and H2O intake in the EtOH DID mice (**Fig. 4**). We found that mice of both sexes drank more alcohol in the first two hours than the second two hours, but this was a more prominent effect in 10% compared to 20% EtOH. In 20% EtOH DID females (**Fig. 4a**), a 2xRM-ANOVA showed main effects of the first vs. second two hrs ("time"; F (1, 9) = 27.25, ***P = 0.0005) and DID cycle (F (7, 63) = 2.97, **P = 0.009), as well as an interaction (F (7, 63) = 2.41, *P = 0.030). Post hoc t-tests with H-S corrections showed that consumption was higher on the first two compared to the second two hrs on cycles 2, 4, 5, 6, and 7 (**Ps < 0.01, as indicated) but not cycles 1, 3, or 8 (Ps > 0.15). We observed a similar, but more robust, pattern for 10% EtOH DID females (**Fig. 4b**). A 2xRM-ANOVA showed a main effect of time (F (1, 9) = 263.1, ****P < 0.0001) and a trend for DID cycle (F (7, 63) = 2.14, P = 0.052) but no interaction (P = 0.121). Post hoc t-tests with H-S corrections showed that consumption was higher in the first two compared to the second two hrs on all cycles (**Ps < 0.01, as indicated). In contrast, water intake for 20% and 10% EtOH females did not differ between the first vs. second 2 hrs of access on Day 4 or DID cycle, as 2xRM-ANOVAs showed no effects or interactions (all Ps > 0.10; **Fig. 4c,d**). In addition, there were no differences in the first vs. second two hrs of water consumption on H2O DID in females (**Fig. 4e**), as a 2xRM-ANOVAs showed an effect of cycle (F (7, 63) = 5.33, ****P < 0.0001) but not an effect of time or interaction (Ps > 0.05). These results suggest that females specifically frontload their intake for EtOH, particularly at lower concentrations. In concert with our results showing that total volume intake is highest in 10% EtOH DID females in the first two hrs and then equilibrates by 24 hrs (**Fig. 3d-f**), this frontloading may be performed to achieve similar EtOH intake levels to 20% EtOH consumption. Males also displayed frontloading behavior for EtOH, especially 10%, however, they also displayed a mild frontloading of H2O. In 20% EtOH DID males (**Fig. 4f**), a 2xRM-ANOVA showed main effects of time (F (1, 9) = 27.68, ***P = 0.0005) but not DID cycle or interaction (Ps > 0.15) on EtOH intake. Post hoc t-tests with H-S corrections showed that consumption was higher on the first two compared to the second two hrs on cycles 2, 3, 4, and 6 (*Ps < 0.05, as indicated) but not cycles 1, 5, 7, or 8 (Ps > 0.15). As in females, we observed a more robust pattern for 10% EtOH DID than 20% DID males (**Fig. 4g**). A 2xRM-

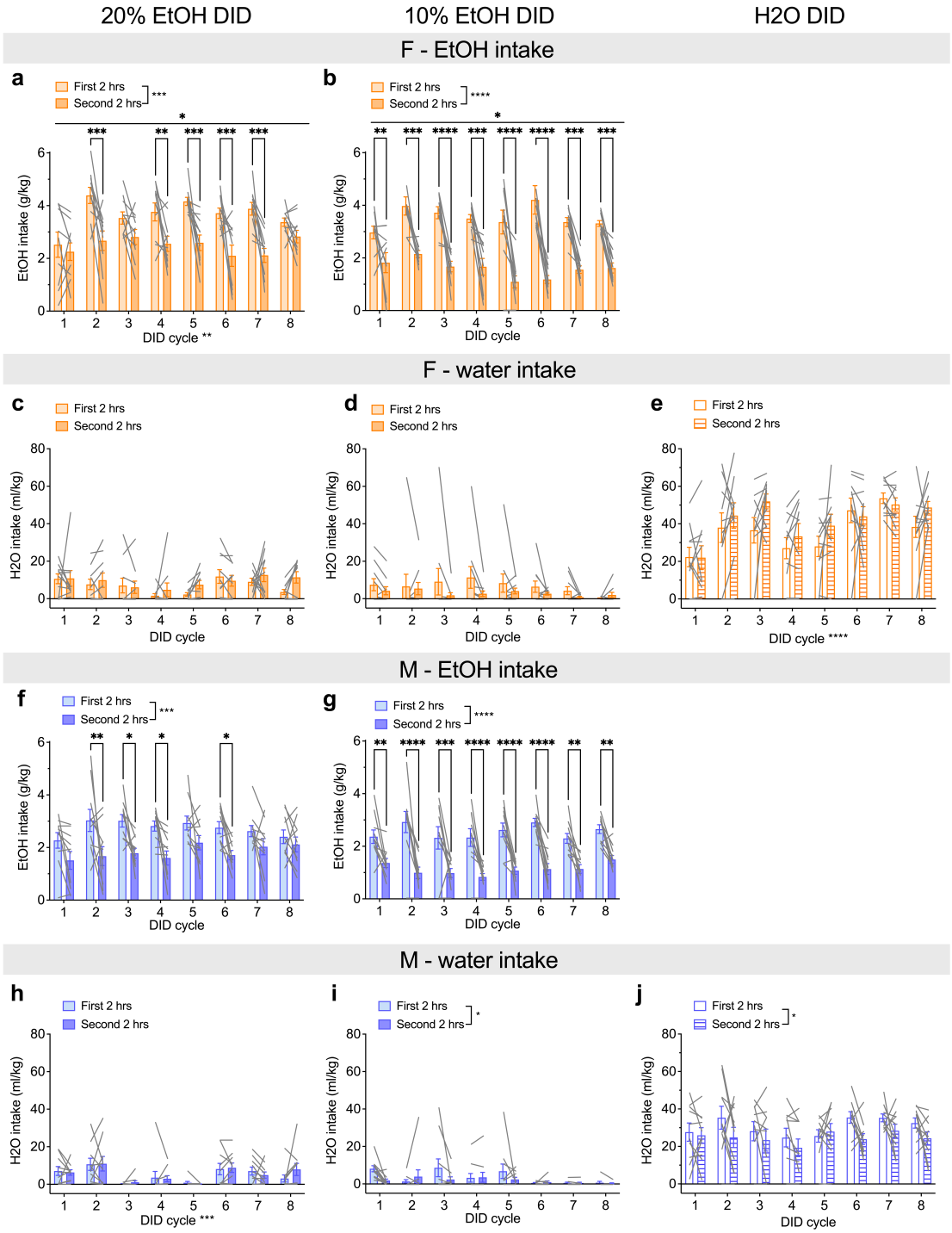

Figure 4: Sex and alcohol concentration differences in front-loading behavior during DID. A-E) EtOH and water intake in females during the first vs. second 2 hrs of Day 4 for 20% EtOH, 10% EtOH, and H2O DID. **A)** Females consumed more 20% EtOH (g/kg) during the first vs. second 2 hrs of Day 4 DID on cycles 2 and 4-7. **B)** Females consumed more 10% EtOH (g/kg) during the first vs. second 2 hrs of Day 4 DID on all cycles. **C-E)** There was no difference in female H2O consumption (ml/kg) during the first vs. second 2 hrs in 20% EtOH DID (**C**), 10% EtOH DID (**D**), or H2O DID (**E**). **F-J)** EtOH and water intake in males during the first vs. second 2 hrs of Day 4 for 20% EtOH, 10% EtOH, and H2O DID. **F)** Males consumed more 20% EtOH (g/kg) during the first vs. second 2 hrs of Day 4 DID on cycles 2-4 and 6. **G)** Males consumed more 10% EtOH (g/kg) during the first vs. second 2 hrs of Day 4 DID on all cycles. **H-J)** There was no difference in male H2O consumption (ml/kg) during the first vs. second 2 hrs in 20% EtOH DID (**H**), but intake was higher on the first vs. second two hrs for 10% EtOH DID (**I**) and H2O DID (**J**). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 in 2xRM-ANOVA main effects and interactions of time point and DID cycle, as well as post hoc t-tests with H-S corrections between the first and second 2 hrs.

ANOVA for EtOH consumption in 10% EtOH DID males showed a main effect of time (F (1, 9) = 79.30, ****P < 0.0001) but no effect of DID cycle or interaction (Ps > 0.20). Post hoc t-tests with H-S corrections showed that consumption was higher in the first two compared to the second two hrs on all cycles (**Ps < 0.01, as indicated). In contrast to females that only frontloaded EtOH, males' water intake was modestly higher in the first two hrs compared to the second two hrs in 10% EtOH DID and H2O DID (but not 20% EtOH DID). A 2xRM-ANOVA for water intake on 20% DID in males showed a main effect of cycle (F (7, 63) = 4.36, ***P = 0.0005) but no effect of time or interaction (Ps > 0.65; **Fig. 4h**). However, for 10% EtOH DID (**Fig. 4i**), there was a main of time (F (1, 9) = 5.13, *P = 0.050) but not cycle or interaction (Ps > 0.05). Post hoc t-tests with H-S corrections showed that consumption trended toward being higher on the first two compared to the second two hrs on cycles 1 and 3 (Ps < 0.10) but no other cycles (Ps > 0.35). Similarly, for H2O DID (**Fig. 4j**), there was a main effect of time (F (1, 9) = 6.41, *P = 0.032) but not cycle or interaction (Ps > 0.30). Post hoc t-tests with H-S corrections showed that consumption trended toward being higher on cycle 6 but no other cycles (cycle 6: P = 0.065; all other Ps > 0.10).

No effect of chronic alcohol drinking on reward sensitivity

Following 20% EtOH or H2O DID, mice underwent a battery of behavioral assays to assess the effects of chronic binge alcohol drinking, beginning with sucrose preference starting 72 hrs after the last EtOH/water exposure (**Fig. 5**). Mice underwent 3 days of 1% sucrose preference, followed by 3 days of 2% sucrose preference, and we observed that females consumed more sucrose but not water than males at both doses, but EtOH drinking did not affect this behavior. A 3xRM-ANOVA on sucrose and water consumption for the 1% sucrose preference test revealed main effects of substance (F (1, 29) = 609.8, *P* < 0.0001) and sex (F (1, 29) = 48.38, ****P < 0.0001), as well as an interaction between the two (F (1, 29) = 20.03, ****P < 0.0001); however, there was no effect of or interactions involving EtOH as a variable (*P*s > 0.20; **Fig. 5b**). To follow up on the substance x sex interaction, we performed 2xANOVAs within each substance. For sucrose, we found an effect of sex (F (1, 29) = 41.50, *P* < 0.0001) but no effect of EtOH or interaction between the two (*P*s > 0.15). Post hoc t-tests with H-S corrections showed that CON and EtOH F consumed more sucrose than their male counterparts (t29 = 5.80, ****P < 0.0001; t29 = 3.43, **P = 0.002, respectively). We found similar results for 2% sucrose (**Fig. 5c**). A 3xRM-ANOVA showed main effects of substance (F (1, 35) = 931.1, *P* < 0.0001) and sex (F (1, 35) = 65.54, ****P < 0.0001), as well as an interaction between the two (F (1, 35) = 50.56, ****P < 0.0001); however, there was no effect of or interactions involving EtOH as a variable (*P*s > 0.40). To follow up on the substance x sex interaction, 2xANOVAs within each substance were performed. For sucrose, we found an effect of sex (F (1, 35) = 60.76, *P* < 0.0001) but no effect of EtOH or interaction between the two (*P*s > 0.55). Post hoc t-tests with H-S corrections showed that CON and EtOH F consumed more sucrose than their male counterparts (t35 = 5.86, ****P < 0.0001; t35 = 5.18, ****P < 0.0001, respectively). For water, we found no effects or interaction (*P*s > 0.10). Analysis of preference for 1% sucrose over water showed no effects of sex or EtOH or an interaction between these variables (Ps > 0.25; **Fig. 5d**). Analysis of preference for 2% sucrose over water showed no effects of sex or EtOH or an interaction between these variables (Ps > 0.10; **Fig. 5e**). Altogether, these results suggest that while females had higher consumption of sucrose compared to males, both sexes showed a high preference for sucrose at both concentrations, and these measures were not affected by a history of EtOH drinking.

Female-specific behavioral disinhibition following a history of binge alcohol drinking

We next assessed avoidance behavior across assays performed 2-4 weeks post-EtOH/H2O DID. We found that across behavioral tests, females displayed a reduction in avoidance behavior (**Fig. 6**). In the open field test (OF), we found that 20% EtOH females, but not males, spent more time in the center of the OF than their H2O DID control (CON) counterparts (**Fig. 6a, b**). A 2xANOVA on the % time spent in the center showed a main

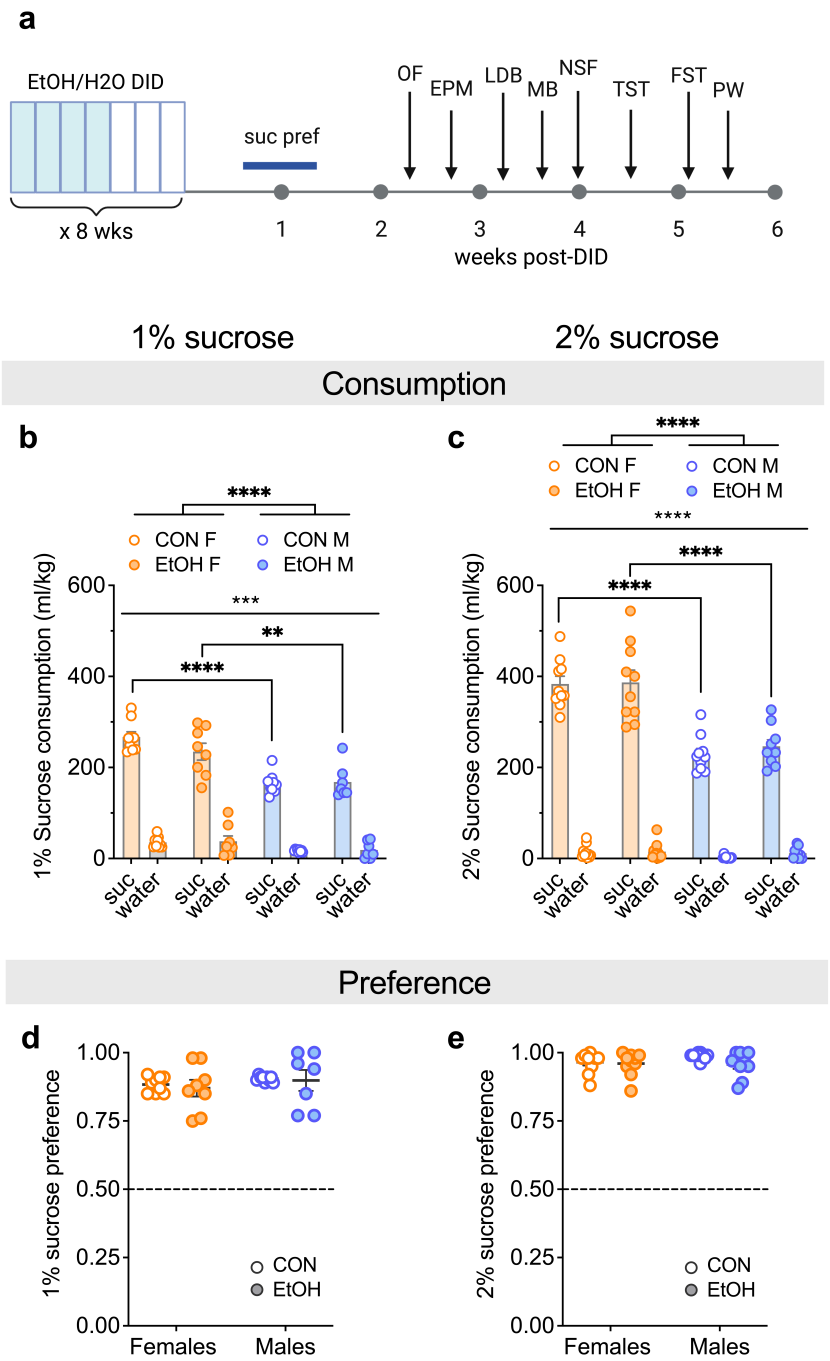

Figure 5: Females display higher sucrose consumption than males regardless of alcohol history. A) Experimental timeline. **B-C)** Sucrose and water consumption during the sucrose preference test, beginning 72 hr after the last EtOH/H2O drinking session, using 1% sucrose (**B**) and 2% sucrose (**C**), showing that females consumed more sucrose, but not water, than males, with no effect of EtOH drinking history. **D-E)** Sucrose preference was similar between H2O DID CON mice and 20% EtOH DID groups and between males and females for 1% (**D**) and 2% (**E**) sucrose. **P < 0.01, ***P < 0.001, ****P < 0.0001 in 3xRM-ANOVA main effects and interactions of substance, sex, and EtOH, as well as post hoc t-tests with H-S corrections as indicated.

effect of EtOH (F (1, 36) = 4.95, *P = 0.033) but no effect of sex or interaction (Ps > 0.15). Post hoc t-tests with H-S corrections showed that EtOH mice entered the center more quickly than CON mice in females but not males (F: t36 = 2.59, *P = 0.027; M: t36 = 0.56, P = 0.582). Examination of the % time in the center of the OF across the 30 min assay confirmed this effect (**Fig. 6b**). A 3xRM-ANOVA on the % time spent in the center of the open field revealed main effects of EtOH (F (1, 216) = 13.44, ***P = 0.0003) and time (F (5, 216) = 6.71,

****P < 0.0001), but no effect of sex (P > 0.40). However, there was a sex x EtOH interaction (F (1, 216) = 5.64, *P = 0.018) but no other interaction (Ps > 0.25). We followed up using 2xRM-ANOVAs within sex. In females, there were main effects of EtOH (F (1, 18) = 8.10, *P = 0.011) and time (F (5, 90) = 5.48, ***P = 0.0002), as well as an interaction between the two (F (5, 90) = 3.04, *P = 0.014). Post hoc t-tests with H-S corrections showed that EtOH F spent more time in the center in the 20-25 min time bin (t108 = 4.33, ***P = 0.002) and a trend in the 15-20 min time bin (t108 = 2.59, $P = 0.053). In males, there was a main effect of time (F (5, 90) = 5.60, ***P = 0.0002) but no effect of EtOH or interaction (Ps > 0.60). When the % time spent in the center of the OF was averaged across the 30 min test, a 2xANOVA showed a main effect of EtOH (F (1, 36) = 4.95, *P = 0.033) but no effect of sex or interaction (Ps > 0.15). Post hoc t-tests with H-S corrections showed that EtOH F spent more time in the center than CON F (t36 = 2.59, *P = 0.027) but there was no difference between CON and EtOH M (t36 = 0.56, P = 0.582). A 2xANOVA on the latency to enter the center of the OF showed a main effect of EtOH (F (1, 36) = 16.50, ***P = 0.0003) but no effect of sex or interaction (Ps > 0.20). Post hoc t-tests with H-S corrections showed that EtOH mice entered the center more quickly than CON mice in both sexes (F: t36 = 3.62, **P = 0.002; M: t36 = 2.12, *P = 0.041; **Fig. 6c**). A 2xANOVA on the total distance traveled during the assay showed no effects of EtOH or sex or interaction (Ps > 0.75; **Fig. 6d**), suggesting that differences in avoidance behavior, particularly in females, were not due to locomotor effects.

In the elevated plus maze (EPM), a 2xANOVA on the % time spent in the open arms showed a main effect of EtOH (F (1, 16) = 12.05, **P = 0.003), a trending effect of sex (F (1, 16) = 4.17, P = 0.058), and a trend for interaction (F (1, 16) = 3.21, P = 0.092). Post hoc t-tests with H-S corrections showed that EtOH F spent more time in the open arms of the EPM than CON F (t16 = 3.72, **P = 0.004) but there was no difference between CON and EtOH M (t16 = 1.19, P = 0.252; **Fig. 6e**). A 2xANOVA on the total distance traveled showed no effects of sex or EtOH or interaction between the two (Ps > 0.25; **Fig. 6f**), suggesting no differences in locomotion. In the light-dark box (LDB), a 2xANOVA on the % time spent in the light side showed no effect of sex or EtOH (Ps > 0.40) but there was an interaction between the two (F (1, 36) = 5.65, *P = 0.023). Post hoc t-tests with H-S corrections showed no effects in direct comparisons (Ps > 0.05; **Fig. 6g**). A 2xANOVA on the number of entries to the dark side, as a measure of locomotion, showed a trending effect of sex (F (1, 36) = 3.98, P = 0.054) but no effect of EtOH or interaction between the two (Ps > 0.15; **Fig. 6h**). In the novelty-suppressed feeding assay (NSF), a 2xANOVA on the latency to eat the fruit loop in the novel environment revealed an effect of sex (F (1, 36) = 8.90, **P = 0.005) but not EtOH (P > 0.10), and there was an interaction between the two (F (1, 36) = 19.06, ***P = 0.0001). Post hoc t-tests with H-S corrections showed that CON F had longer latencies than EtOH F (t36 = 4.24, ***P = 0.0003) and CON M (t36 = 5.20, ****P < 0.0001) but there was no difference between CON M and EtOH M (P > 0.30; **Fig. 6i**). A 2xANOVA on the post-test home cage chow consumption showed no effects of sex or EtOH or interaction between the two (Ps > 0.30; **Fig. 6j**), suggesting there were no differences in hunger driving effects of sex and EtOH on NSF. Altogether, results from avoidance behavior assays suggest that a history of binge alcohol drinking in females, but not males, leads to a decrease in behavioral inhibition. We also examined marble burying behavior, finding that EtOH F also display increased compulsive-like behavior (**Fig. 6k**), as a 2xANOVA on the proportion of marbles buried showed effects of sex (F (1, 36) = 13.46, ***P = 0.001) and EtOH (F (1, 36) = 5.81, P = 0.021) and an interaction between the two (F (1, 36) = 9.24, **P = 0.004). Post hoc t-tests with H-S corrections showed that CON F buried fewer marbles than EtOH F (t36 = 3.85, ***P = 0.001) and CON M (t36 = 5.20, ****P < 0.0001) but there was no difference between CON M and EtOH M (P > 0.30).

Effects of sex, but not EtOH drinking, on coping strategy

Finally, we tested mice in the forced swim test (FST), tail suspension (TS), and paw withdrawal assay for pain 4-6 weeks following EtOH/H2O exposure (**Fig. 7**). We found that females displayed more active coping strategies, as a 2xANOVA on the % time immobile in the FST showed an effect of sex (F (1, 36) = 7.79, **P = 0.008) but no effect of EtOH or interaction between the two (Ps > 0.45). Post hoc t-tests with H-S corrections

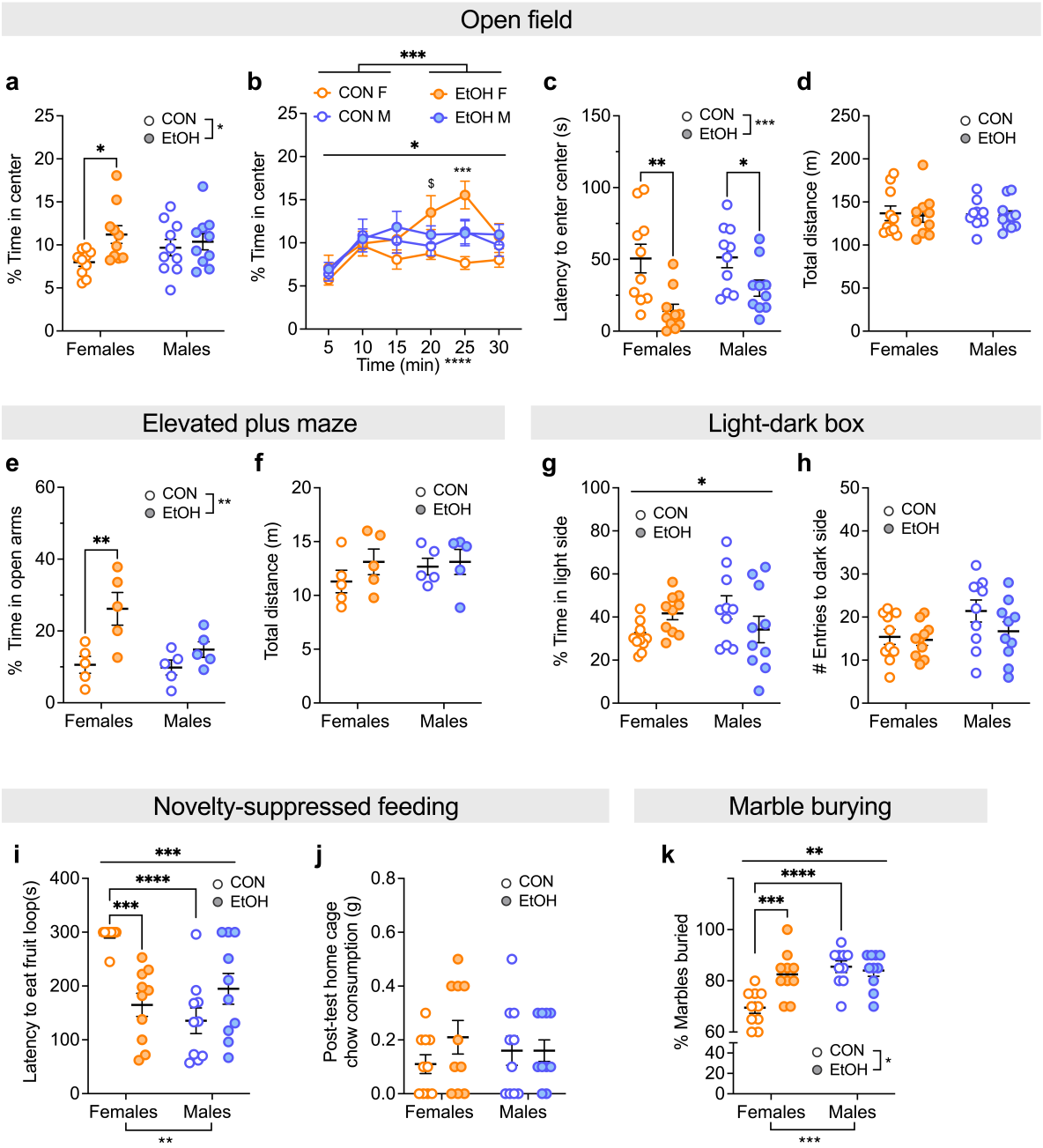

Figure 6: Females, but not males, display reduced avoidance and increased compulsive behavior during protracted abstinence (2-4 weeks) from chronic binge alcohol drinking. A-D) Open field test (OF). **A)** EtOH females, but not males, spent a greater % time in the center of the OF compared to CONs. **B)** This effect emerged across the 30 min assay. **C)** EtOH mice of both sexes had a shorter latency to enter the center of the OF. **D)** There was no effect of sex or alcohol history on locomotion in the OF. **E-F)** Elevated plus maze (EPM). **E)** EtOH females, but not males, spent a greater % time in the open arms of the EPM compared to CONs. **F)** There was no effect of sex or alcohol history on total distance traveled (m) in the EPM. **G-H)** Light-dark box (LDB). **G)** There was an interaction between sex and alcohol history for % time spent in the light side of the LDB but no differences in direct comparisons. **H)** There was no effect of alcohol history or sex on locomotion as measured by the number of dark side entries. **I-J)** Novelty-suppressed feeding (NSF). **I)** EtOH females, but not males, had a reduced latency to eat the fruit loop compared to CONs. In addition, males had shorter latencies than females. **J)** There was no effect of sex or alcohol history on post-test home cage food consumption. **K)** Marble burying (MB). EtOH females, but not males, buried more marbles than CONs. In addition, males buried more marbles than females. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 in 2xANOVA main effects of and interactions between sex and EtOH, 3xRM-ANOVA main effects of and interactions between sex, EtOH, and time, and post hoc t-tests with H-S corrections as indicated. $P < 0.10 for post hoc t-tests with H-S corrections between M and F.

showed trends for greater immobility in M than F in CON and EtOH groups (CON: t36 = 1.93, P = 0.099; EtOH: t36 = 2.02, P = 0.099; **Fig. 7a**). A 2xANOVA on the % time swimming showed an effect of sex (F (1, 36) = 8.48, **P = 0.006) but no effect of EtOH or interaction between the two (Ps > 0.45). Post hoc t-tests with H-S corrections showed trends for greater swimming in F than M in CON and EtOH groups (CON: t36 = 1.95, P = 0.072; EtOH: t36 = 2.17, P = 0.072; **Fig. 7b**). A 2xANOVA on the % time climbing showed no effects of sex or EtOH or interaction between the two (Ps > 0.65; **Fig. 7c**). We also found more active coping in females than males on the TST, as a 2xANOVA on the % time passive showed an effect of sex (F (1, 36) = 9.09, **P = 0.005) but no effect of EtOH or interaction between the two (Ps > 0.15). Post hoc t-tests with H-S corrections showed that CON F displayed less time passive than CON M (t36 = 3.12, **P = 0.007) but there was no difference between EtOH F and EtOH M (P > 0.25; **Fig. 7d**). These results showed that females displayed more active coping while males showed more passive coping, but there were no effects of EtOH on this behavior. Finally, we measured thermal pain sensitivity, finding no effect of EtOH or sex at either low- or high-temperature stimuli (**Fig. 7e**). A 2xANOVA on paw withdrawal latency for 52 °C showed no effects of sex or EtOH or interaction between the two (Ps > 0.65). Similarly, at 58 °C there were no effects or interactions (Ps > 0.65), confirming that EtOH did not affect thermal pain sensitivity.

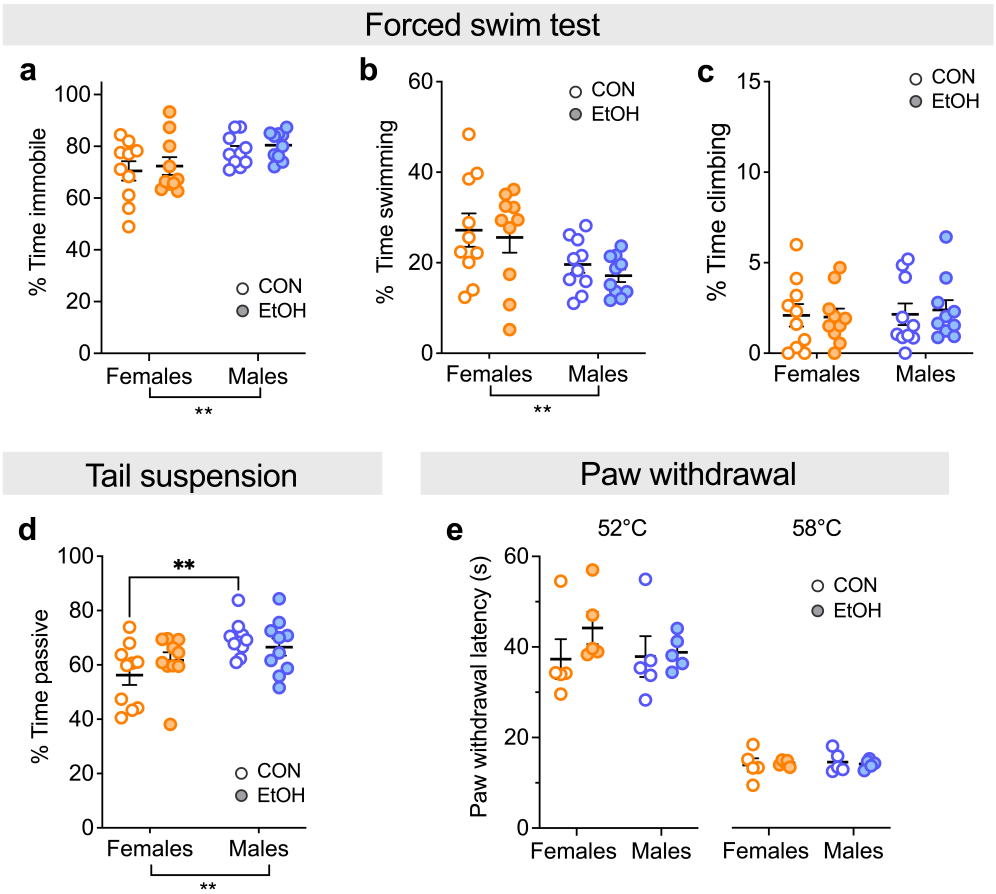

Figure 7: Sex, but not protracted abstinence from chronic binge drinking, impact behavioral measures of coping strategy and negative affect tested 4-6 weeks following EtOH/H2O exposure. A-C) Forced swim test (FST). Females displayed less % time immobile (**A**) and more time swimming (**B**) but not climbing (**C**) than males, regardless of alcohol history. **D)** Tail suspension test (TST). Females displayed less % time passive than males. **E)** Hot plate paw withdrawal. There was no effect of sex or alcohol history on the paw withdrawal latency for either low (52 °C) or high (58 °C) hot plate temperatures. **P < 0.01 in 2XANOVA main effects of and interactions between sex and EtOH and post hoc t-tests with H-S corrections between M and F.

Discussion

Here, we found that female mice consume more alcohol compared to their male counterparts in a modified DID paradigm across eight cycles (**Figs. 1 & 2**), consistent with what we and others have previously shown for DID and other binge alcohol drinking models in mice(8, 9). This difference was more robust for 20% compared to 10% EtOH, even though preference for alcohol over water did not differ between sexes at either alcohol concentration. Further, females' higher alcohol intake was biased toward early access period drinking (2-4 hrs), when water intake was similar between sexes (**Figs. 1-4**). In addition, we found that protracted abstinence (2-4 weeks) from chronic binge drinking led to behavioral disinhibition in female mice only; specifically, we observed a reduction in anxiety-like behavior and an increase in compulsive-like behavior in alcohol-drinking females but not males compared to water control mice in the OF, EPM, NSF, and MB assays (**Fig. 6**). Furthermore, we found basal sex differences in assays measuring reward sensitivity/anhedonia and coping behavior but no effects of alcohol abstinence in the sucrose preference, FST, and TS assays (**Figs. 5 & 7**). Together, these results suggest that anxiety-related behaviors are most vulnerable to pathological behavioral outcomes in protracted alcohol abstinence from chronic binge drinking, particularly in females.

We found that females have increased alcohol consumption compared to their male counterparts on multiple measures but intriguingly, females' elevated alcohol consumption compared to males is biased toward the early hours of access (2-4 hours; **Fig. 2A-F**); a similar front-loading sex difference has been previously reported in a rat operant model (29). When normalized to bodyweight, increased alcohol intake in females compared to males was present during early (2-4 hrs) access by week 2 for the 20% EtOH cohort and week 3 for the 10% EtOH cohort (**Fig. 1F, G**). One potential explanation for increased alcohol intake in females is that they may consume overall more fluid including water, and here we report that females do indeed drink more water/fluid across the day than males (**Fig. 3C**). However, this phenomenon does not occur during early access (first 2-4 hours) in drinking sessions (**Fig. 1K**) which suggests that the increased fluid intake hypothesis does not account for increased alcohol intake in females. Further, total volume intake across the day is static regardless of water, 10% EtOH, or 20% EtOH (**Fig. 3F**). Therefore, the total volume is capped for the 24-hour period and differences in alcohol consumption are a matter of distribution of the consumption. For example, we report more 10% EtOH frontloading than 20% (**Fig. 4A, B, F, G**), suggesting this distribution of intake toward the beginning of the session is related to achieving interoceptive effects of alcohol that potentially match those following 20% EtOH drinking. Finally, females consume more cumulative raw 20% EtOH than males, which we do not see with water (**Fig. 1C, E**). Therefore, elevated female alcohol consumption compared to males is not likely due to increased overall volume intake. Another potential explanation for why female mice consume more alcohol than their male counterparts is that they require more alcohol consumption to achieve BECs associated with the optimal interoceptive effects of alcohol. However, females across mammalian species, from mice to humans, display higher BECs for similar amounts of orally ingested alcohol due to several body composition factors affecting ethanol pharmacokinetics, including lower water and higher fat content that affect volume distribution and absorption and lower gastric ethanol metabolism (30-33). Thus, it is unlikely that the sex differences we observed in binge alcohol drinking were due to pharmacokinetic factors, but it is possible that higher BECs are required in females to achieve similar interoceptive effects. Future studies will be able to directly determine the relationship between alcohol's actions and metabolism in the periphery and the behavioral effects associated with alcohol drinking and abstinence. Indeed, binge drinking behavior is of particular concern because along with neuropsychiatric risk, high-volume alcohol consumption leads to a greater risk for multi-factorial peripheral health problems including cancer, cardiovascular issues, and liver disease in women compared to men (7). Therefore, our results suggest critical sex differences in binge alcohol drinking behavior that may suggest female vulnerability to the effects of high-volume alcohol consumption.

In this study, we found that the behavioral effects of protracted abstinence following chronic binge drinking are specific to females, who demonstrate a behavioral disinhibition phenotype including reduced avoidance behavior and increased compulsive behavior (**Fig. 6**). The results of previous work on the affective behavioral effects of chronic alcohol C57BL/6J mice are highly variable and male dominated (16). Variation in results is likely due to several factors, including the paradigm utilized, length of the experiment, sex of the animals, behavioral assays employed, and time-points into abstinence tested. Indeed, variability in the method of alcohol exposure may lead to significant differences in outcomes of affective behaviors following alcohol in both sexes, which may explain outcome differences in our study compared to other investigations. Although results reported in the literature are highly mixed, males have been shown to display increased avoidance, anhedonia, and compulsive behaviors in early abstinence (>1 week) following chronic alcohol across vapor inhalation (34-39), continuous access (21, 40), intermittent drinking (13), and DID (18, 22, 41, 42). It is important to simultaneously consider that there are contrasting data showing no effects of all these paradigms, with the exception of continuous access, on avoidance behaviors in early abstinence in males (10, 13, 18, 42-45). Behavior during protracted withdrawal in males is somewhat less but still variable to a degree, with increased avoidance following chronic vapor (17) and DID (15, 18), with no effects following continuous (20, 21) and on most measures during intermittent access (10), and fairly consistent increased anhedonia across alcohol vapor (17, 19) and continuous drinking (20, 21, 40), with some exceptions for DID (18, 23). Notably, most effects reported in the literature demonstrate increased avoidance in male C57BL/6J mice during abstinence (16). This is in direct contrast to our findings in that there was no effect on behavior in males and furthermore, females appear to display decreased avoidance. There is a report, however, of mild behavioral disinhibition in males and females following 7 weeks of intermittent alcohol access (10). It is possible that the variability of avoidance-related alterations following chronic alcohol consumption in males is due to differences in testing timepoints.

Our results indicating female-specific behavioral effects of protracted abstinence led us to question whether the amount of cumulative alcohol intake plays a role in the behavioral effects we observed here; that is, whether males would display a similar phenotype with additional alcohol drinking that achieved the cumulative alcohol intake levels of females, or whether there are fundamental sex differences in the neural plasticity underlying the behavioral effects. Some studies report increased avoidance in males following acute abstinence from long term (8+ weeks) voluntary alcohol drinking (41) while others show no effect in some or all measures (42, 45) but to our knowledge, none have investigated these behaviors in protracted abstinence. There may be an inherent sex difference in avoidance and compulsive-like behavioral outcomes in protracted abstinence from chronic alcohol consumption. Indeed, females show increased stress responsivity to various physiological triggers (46-48), and women are more likely to use alcohol to regulate negative emotional states than men (49, 50).

There is a relative dearth of studies on the behavioral effects of chronic drinking in females, but most point to no effect on avoidance in early or protracted abstinence on most measures (10, 34, 42), with some exceptions in continuous (51) and intermittent access (13) models. The literature on the effects of alcohol abstinence on anhedonia is limited and female biased, but investigations of protracted abstinence following chronic continuous access show an anhedonic phenotype in both sexes (20, 21, 40, 51-54), with limited effects on avoidance behavior in females only (20, 51-53). This is notable given our findings that protracted abstinence from intermittent access binge drinking produces reduced avoidance and no negative affect in females. Because these are some of the only studies looking at the long-term consequences of chronic alcohol exposure in females, future studies directly comparing the nature of alcohol access, duration/amount of exposure, and specific behavioral time points will be useful for resolving which factors lead to specific behavioral outcomes in females. In addition, given that females in our study displayed a broad reduction in avoidance behavior typically associated with a maladaptive behavioral disinhibition phenotype, and there is an

established relationship between behavioral disinhibition/risk-taking and alcohol drinking in humans and rodents (55, 56), future studies specifically dissecting risk-taking and impulsive behavior will help refine our understanding of the results in our study. Therefore, there may be sex differences underlying the physiological and behavioral responses to alcohol.

Perspective and Significance

Altogether, our results demonstrate sex differences in binge drinking and subsequent behavior in abstinence, pointing to the need for further investigations into the mechanisms underlying these phenomena. Understanding anxiety-related behavior, anhedonia, and risk-taking during abstinence in both sexes is critical for informing knowledge on the effects of chronic binge drinking.

Declarations

Ethics approval and consent to participate
All animal procedures were performed in accordance with the Weill Cornell Medicine Institutional Animal Care and Use Committee guidelines and approval.

Consent for publication
Not applicable.

Availability of data and material
Source data will be publicly deposited upon publication.

Competing interests
The authors declare no competing interests.

Funding
This research was supported by: NIH grants K99/R00 AA023559 and R01 AA027645, a NARSAD Young Investigator Award, a Stephen and Anna-Maria Kellen Foundation Junior Faculty Award 26608 (K.E.P.); NIH grant F31 AA029293 (L.J.Z.); NIH grant T32 DA039080 (J.K.R.I., L.J.Z., O.B.L.).

Authors' contributions
K.E.P. designed all experiments, J.K.R.I., O.B.L., M.J.S, J.E.B, R.K., and K.E.P. performed and analyzed behavior data. K.E.P. oversaw experiments. K.E.P., L.J.Z., and T.B. wrote the manuscript, and all authors edited and approved the final version of the manuscript.

Acknowledgements
Not applicable.

References

1. SAMHSA CfBHSaQ. National Survey on Drug Use and Health. Table 5.6A—Alcohol use disorder in past year: among people aged 12 or older; by age group and demographic characteristics, numbers in thousands. 2021.
2. Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. Arch Gen Psychiatry. 1997;54(4):313-21.
3. Vsevolozhskaya OA, Anthony JC. Transitioning from First Drug Use to Dependence Onset: Illustration of a Multiparametric Approach for Comparative Epidemiology. Neuropsychopharmacology. 2016;41(3):869-76.
4. Patrick ME, Evans-Polce RJ, Parks MJ, Terry-McElrath YM. Drinking Intensity at Age 29/30 as a Predictor of Alcohol Use Disorder Symptoms at Age 35 in a National Sample. J Stud Alcohol Drugs. 2021;82(3):362-7.
5. Gowin JL, Sloan ME, Morris JK, Schwandt ML, Diazgranados N, Ramchandani VA. Characteristics Associated With High-Intensity Binge Drinking in Alcohol Use Disorder. Front Psychol. 2021;12:750395.
6. Pollard MS, Tucker JS, Green HD. Changes in Adult Alcohol Use and Consequences During the COVID-19 Pandemic in the US. JAMA Netw Open. 2020;3(9):e2022942.
7. White AM. Gender Differences in the Epidemiology of Alcohol Use and Related Harms in the United States. Alcohol Res. 2020;40(2):01.
8. Levine OB, Skelly MJ, Miller JD, Rivera-Irizarry JK, Rowson SA, DiBerto JF, et al. The paraventricular thalamus provides a polysynaptic brake on limbic CRF neurons to sex-dependently blunt binge alcohol drinking and avoidance behavior in mice. Nat Commun. 2021;12(1):5080.
9. Sneddon EA, White RD, Radke AK. Sex Differences in Binge-Like and Aversion-Resistant Alcohol Drinking in C57BL/6J Mice. Alcohol Clin Exp Res. 2019;43(2):243-9.
10. Bloch S, Rinker JA, Marcus MM, Mulholland PJ. Absence of effects of intermittent access to alcohol on negative affective and anxiety-like behaviors in male and female C57BL/6J mice. Alcohol. 2020;88:91-9.
11. Yu W, Hwa LS, Makhijani VH, Besheer J, Kash TL. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. Alcohol. 2019;77:135-45.
12. Sneddon EA, Ramsey OR, Thomas A, Radke AK. Increased Responding for Alcohol and Resistance to Aversion in Female Mice. Alcohol Clin Exp Res. 2020;44(7):1400-9.
13. Quijano Cardé NA, De Biasi M. Behavioral characterization of withdrawal following chronic voluntary ethanol consumption via intermittent two-bottle choice points to different susceptibility categories. Alcohol Clin Exp Res. 2022;46(4):614-27.
14. Centanni SW, Morris BD, Luchsinger JR, Bedse G, Fetterly TL, Patel S, et al. Endocannabinoid control of the insular-bed nucleus of the stria terminalis circuit regulates negative affective behavior associated with alcohol abstinence. Neuropsychopharmacology. 2019;44(3):526-37.
15. Flanigan ME, Hon OJ, D'Ambrosio S, Boyt KM, Hassanein L, Castle M, et al. Subcortical serotonin 5HT. Nat Commun. 2023;14(1):1800.
16. Bloch S, Holleran KM, Kash TL, Vazey EM, Rinker JA, Lebonville CL, et al. Assessing negative affect in mice during abstinence from alcohol drinking: Limitations and future challenges. Alcohol. 2022;100:41-56.
17. Starski P, Hong SI, Peyton L, Oliveros A, Wininger K, Hutchison C, et al. Ethanol induces maladaptive impulse control and decreased seeking behaviors in mice. Addict Biol. 2020;25(3):e12754.
18. Lee KM, Coehlo M, McGregor HA, Waltermire RS, Szumlinski KK. Binge alcohol drinking elicits persistent negative affect in mice. Behav Brain Res. 2015;291:385-98.
19. Kimbrough A, Lurie DJ, Collazo A, Kreifeldt M, Sidhu H, Macedo GC, et al. Brain-wide functional architecture remodeling by alcohol dependence and abstinence. Proc Natl Acad Sci U S A. 2020;117(4):2149-59.
20. Dao NC, Suresh Nair M, Magee SN, Moyer JB, Sendao V, Brockway DF, et al. Forced Abstinence From Alcohol Induces Sex-Specific Depression-Like Behavioral and Neural Adaptations in Somatostatin Neurons in Cortical and Amygdalar Regions. Front Behav Neurosci. 2020;14:86.
21. Stevenson JR, Schroeder JP, Nixon K, Besheer J, Crews FT, Hodge CW. Abstinence following alcohol drinking produces depression-like behavior and reduced hippocampal neurogenesis in mice. Neuropsychopharmacology. 2009;34(5):1209-22.
22. Bloodgood DW, Hardaway JA, Stanhope CM, Pati D, Pina MM, Neira S, et al. Kappa opioid receptor and dynorphin signaling in the central amygdala regulates alcohol intake. Mol Psychiatry. 2021;26(6):2187-99.

23. Olney JJ, Marshall SA, Thiele TE. Assessment of depression-like behavior and anhedonia after repeated cycles of binge-like ethanol drinking in male C57BL/6J mice. Pharmacol Biochem Behav. 2018;168:1-7.
24. Anthenelli RM. Focus on: Comorbid mental health disorders. Alcohol Res Health. 2010;33(1-2):109-17.
25. Bott K, Meyer C, Rumpf HJ, Hapke U, John U. Psychiatric disorders among at-risk consumers of alcohol in the general population. J Stud Alcohol. 2005;66(2):246-53.
26. Goldstein RB, Dawson DA, Chou SP, Grant BF. Sex differences in prevalence and comorbidity of alcohol and drug use disorders: results from wave 2 of the National Epidemiologic Survey on Alcohol and Related Conditions. J Stud Alcohol Drugs. 2012;73(6):938-50.
27. Thiele TE, Navarro M. "Drinking in the dark" (DID) procedures: a model of binge-like ethanol drinking in non-dependent mice. Alcohol. 2014;48(3):235-41.
28. Rivera-Irizarry JK, Skelly MJ, Pleil KE. Social Isolation Stress in Adolescence, but not Adulthood, Produces Hypersocial Behavior in Adult Male and Female C57BL/6J Mice. Front Behav Neurosci. 2020;14:129.
29. Flores-Bonilla A, De Oliveira B, Silva-Gotay A, Lucier KW, Richardson HN. Shortening time for access to alcohol drives up front-loading behavior, bringing consumption in male rats to the level of females. Biol Sex Differ. 2021;12(1):51.
30. Kezer CA, Simonetto DA, Shah VH. Sex Differences in Alcohol Consumption and Alcohol-Associated Liver Disease. Mayo Clin Proc. 2021;96(4):1006-16.
31. Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med. 1990;322(2):95-9.
32. Baraona E, Abittan CS, Dohmen K, Moretti M, Pozzato G, Chayes ZW, et al. Gender differences in pharmacokinetics of alcohol. Alcohol Clin Exp Res. 2001;25(4):502-7.
33. Desroches D, Orevillo C, Verina D. Sex- and strain-related differences in first-pass alcohol metabolism in mice. Alcohol. 1995;12(3):221-6.
34. Jury NJ, DiBerto JF, Kash TL, Holmes A. Sex differences in the behavioral sequelae of chronic ethanol exposure. Alcohol. 2017;58:53-60.
35. Sidhu H, Kreifeldt M, Contet C. Affective Disturbances During Withdrawal from Chronic Intermittent Ethanol Inhalation in C57BL/6J and DBA/2J Male Mice. Alcohol Clin Exp Res. 2018;42(7):1281-90.
36. Warden AS, Wolfe SA, Khom S, Varodayan FP, Patel RR, Steinman MQ, et al. Microglia Control Escalation of Drinking in Alcohol-Dependent Mice: Genomic and Synaptic Drivers. Biol Psychiatry. 2020;88(12):910-21.
37. Maldonado-Devincci AM, Kampov-Polevoi A, McKinley RE, Morrow DH, O'Buckley TK, Morrow AL. Chronic Intermittent Ethanol Exposure Alters Stress Effects on (3α,5α)-3-hydroxy-pregnan-20-one (3α,5α-THP) Immunolabeling of Amygdala Neurons in C57BL/6J Mice. Front Cell Neurosci. 2016;10:40.
38. Pleil KE, Lowery-Gionta EG, Crowley NA, Li C, Marcinkiewcz CA, Rose JH, et al. Effects of chronic ethanol exposure on neuronal function in the prefrontal cortex and extended amygdala. Neuropharmacology. 2015;99:735-49.
39. Rose JH, Karkhanis AN, Chen R, Gioia D, Lopez MF, Becker HC, et al. Supersensitive Kappa Opioid Receptors Promotes Ethanol Withdrawal-Related Behaviors and Reduce Dopamine Signaling in the Nucleus Accumbens. Int J Neuropsychopharmacol. 2016;19(5).
40. Gong MF, Wen RT, Xu Y, Pan JC, Fei N, Zhou YM, et al. Attenuation of ethanol abstinence-induced anxiety- and depressive-like behavior by the phosphodiesterase-4 inhibitor rolipram in rodents. Psychopharmacology (Berl). 2017;234(20):3143-51.
41. Belmer A, Patkar OL, Lanoue V, Bartlett SE. 5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol. Sci Rep. 2018;8(1):2099.
42. Rath M, Guergues J, Pinho JPC, Zhang P, Nguyen TG, MacFadyen KA, et al. Chronic Voluntary Binge Ethanol Consumption Causes Sex-Specific Differences in Microglial Signaling Pathways and Withdrawal-associated Behaviors in Mice. Alcohol Clin Exp Res. 2020;44(9):1791-806.
43. Daut RA, Busch EF, Ihne J, Fisher D, Mishina M, Grant SG, et al. Tolerance to ethanol intoxication after chronic ethanol: role of GluN2A and PSD-95. Addict Biol. 2015;20(2):259-62.
44. Holmes A, Fitzgerald PJ, MacPherson KP, DeBrouse L, Colacicco G, Flynn SM, et al. Chronic alcohol remodels prefrontal neurons and disrupts NMDAR-mediated fear extinction encoding. Nat Neurosci. 2012;15(10):1359-61.

45. Cox BR, Olney JJ, Lowery-Gionta EG, Sprow GM, Rinker JA, Navarro M, et al. Repeated cycles of binge-like ethanol (EtOH)-drinking in male C57BL/6J mice augments subsequent voluntary EtOH intake but not other dependence-like phenotypes. Alcohol Clin Exp Res. 2013;37(10):1688-95.
46. Bangasser DA, Valentino RJ. Sex differences in molecular and cellular substrates of stress. Cell Mol Neurobiol. 2012;32(5):709-23.
47. Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol. 2014;35(3):303-19.
48. Bangasser DA, Wiersielis KR. Sex differences in stress responses: a critical role for corticotropin-releasing factor. Hormones (Athens). 2018;17(1):5-13.
49. Guinle MIB, Sinha R. The Role of Stress, Trauma, and Negative Affect in Alcohol Misuse and Alcohol Use Disorder in Women. Alcohol Res. 2020;40(2):05.
50. Peltier MR, Verplaetse TL, Mineur YS, Petrakis IL, Cosgrove KP, Picciotto MR, et al. Sex differences in stress-related alcohol use. Neurobiol Stress. 2019;10:100149.
51. Vranjkovic O, Winkler G, Winder DG. Ketamine administration during a critical period after forced ethanol abstinence inhibits the development of time-dependent affective disturbances. Neuropsychopharmacology. 2018;43(9):1915-23.
52. Pang TY, Du X, Catchlove WA, Renoir T, Lawrence AJ, Hannan AJ. Positive environmental modification of depressive phenotype and abnormal hypothalamic-pituitary-adrenal axis activity in female C57BL/6J mice during abstinence from chronic ethanol consumption. Front Pharmacol. 2013;4:93.
53. Holleran KM, Wilson HH, Fetterly TL, Bluett RJ, Centanni SW, Gilfarb RA, et al. Ketamine and MAG Lipase Inhibitor-Dependent Reversal of Evolving Depressive-Like Behavior During Forced Abstinence From Alcohol Drinking. Neuropsychopharmacology. 2016;41(8):2062-71.
54. Pang TY, Renoir T, Du X, Lawrence AJ, Hannan AJ. Depression-related behaviours displayed by female C57BL/6J mice during abstinence from chronic ethanol consumption are rescued by wheel-running. Eur J Neurosci. 2013;37(11):1803-10.
55. Balogh KN, Mayes LC, Potenza MN. Risk-taking and decision-making in youth: relationships to addiction vulnerability. J Behav Addict. 2013;2(1).
56. Momeni S, Sharif M, Agren G, Roman E. Individual differences in risk-related behaviors and voluntary alcohol intake in outbred Wistar rats. Behav Pharmacol. 2014;25(3):206-15.