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Childhood trauma and post-trauma environment affect fear memory and alcohol use differently in male and female mice

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Title: CHILDHOOD TRAUMA AND POST-TRAUMA ENVIRONMENT AFFECT FEAR MEMORY AND ALCOHOL USE DIFFERENTLY IN MALE AND FEMALE MICE

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ABSTRACT

Background: Childhood trauma is associated with the development of adult mental health and substance use disorders, with females generally being more at risk. Alcohol is commonly used for coping with trauma, and alcohol use disorder (AUD) affects ~14.4 million adult Americans annually. Research investigating sex differences in the environmental modification of anxiety and alcohol use following childhood trauma will extend our understanding of the etiology of AUD. Here, we sought to model the interacting effects of a single-episode late childhood trauma with post-trauma environment on adult alcohol use using male and female mice.

Methods: C57Bl6/J mice (d22) exposed to predator odor (TMT) or water were reared in standard environments (SE) or environmental enrichment (EE). Mice were assessed for adolescent anxiety and conditioned fear, and for adult alcohol use in a limited access, response non-contingent, alcohol exposure paradigm.

Results: A single exposure to predator odor was an effective stressor, inducing long-term sex-dependent changes in conditioned fear and alcohol behaviors that interacted with post-trauma environment. Adolescent EE females showed more conditioned freezing to the trauma-associated context. Adult EE mice consumed less total alcohol than SE mice. However, alcohol use across time differed for males and females. Exposure to a childhood stressor increased alcohol use significantly in females, but not males. EE males, but not EE females, drank less than SE counterparts.

Conclusions: Findings from this model recapitulate greater vulnerability to childhood trauma in females and support sex differences in post-trauma development of conditioned fear and alcohol use that are modified by environment.

Keywords: predator odor, sex effects, environmental enrichment, comorbidity, animal models

1. Introduction

Childhood trauma is associated with the development of adult mental health and substance use disorders (SUD) (Gielen et al., 2012; Kendler et al., 2000; Khoury et al., 2010; Lawson et al., 2013; Mergler et al., 2018). In SUD patients, the prevalence of trauma exposure is high, estimated at ~97% (Gielen et al., 2012; Lawson et al., 2013), and experience of childhood trauma ranged from 60-90% (Lawson et al. 2013). A history of early life trauma is known to influence the course and outcome of SUD, for example, by increasing the severity of mental health symptoms (anxiety, depression, suicide attempts), substance use, and relapse (Hyman et al. 2008; Kendler et al 2000; Lawson et al. 2013; Mergler et al. 2018). Moreover, maladaptive processing of a traumatic memory can further facilitate the development of impairing lifetime behaviors, including anxiety, exaggerated startle response, and the inability to extinguish conditioned fear, in addition to substance abuse (Jacobsen et al. 2001; Johnson et al. 2010; Kilpatrick et al. 2003).

Post-traumatic stress disorder (PTSD) is characterized by recurrent and intrusive recollections and dreams of a traumatic event. It is one of the most common comorbidities of SUD, with ~37-57% of SUD patients also having a lifetime PTSD diagnosis (Gielen et al. 2012). An investigation into the differential effects of childhood trauma, and childhood trauma with PTSD, on the course and severity of SUD found both groups to have similar associations with anxiety, depression, suicidal thoughts and attempts compared to no-trauma controls, although highest levels were consistently in the childhood trauma with PTSD population (Mergler et al., 2018). However, the nature and

consequences of childhood trauma may differ by gender of the victim, with females generally being more at risk for developing psychiatric and substance use disorders (Hyman et al., 2008).

Females are twice as likely than males to develop PTSD. PTSD has a 10.4% female vs 5% male lifetime prevalence rate (Kessler et al. 1995), which triples in victims of childhood trauma (Rodriguez et al. 1997; Khoury et al. 2010; Mergler et al. 2018). One of the most commonly used substances for coping with trauma is alcohol (Jacobsen et al. 2001). Alcohol use disorder (AUD) is a chronic relapsing disorder that affects ~14.4 million adults in the US annually (NSDUH, 2018). A pattern of binge drinking, which is excessive drinking in a short amount of time, can be a predictor of AUD development (Hingson et al., 2006). Comorbid PTSD and excessive alcohol use can exacerbate negative effects on lifetime success (e.g. unemployment, Mergler et al., 2018) and treatment (Najavits et al. 2007) outcomes. The shared vulnerability to PTSD and SUD following trauma suggest their neuropathology may overlap.

Sex differences in patterns of drug use are also commonly reported in both the human and animal literature. In humans, females are more vulnerable to key phases of addiction including escalation of use, drug bingeing, and relapse, potentially due to interactions with hormonal phases (reviewed in (Anker and Carroll, 2011; Fattore et al., 2008). Supporting evidence for this has also been found in animal models of addiction. Across drug classes (e.g. nicotine, alcohol, cocaine), female lab animals (rats, mice, monkeys) self-administer more drug than males (see Table 1 in Fattore et al. 2008). In spite of known differences in patterns of use, most studies focus on male subjects (Fattore et al., 2008; Giacometti and Barker, 2020). Investigations of sex-dependent responses to late childhood/preadolescent trauma will be an important contribution to the animal literature. There

exists, however, a large body of literature on prenatal and early postnatal stress in rodents. Such studies demonstrate alterations to offspring stress response resulting from environmental programming via epigenetic mechanisms (e.g. DNA methylation, histone modifications), which then increase genetic vulnerabilities to neuropsychiatric disorders such as PTSD (Meaney et al., 2007; Rodgers and Bale, 2015; Seckl and Meaney, 2006) and responsivity and intake of drugs (e.g. alcohol, reviewed by Campbell et al. 2009).

The use of animal models that recapitulate the complex etiology of human disease has greatly advanced our understanding and ability to treat them. Exposure to a predator odor has been adopted as an animal model of PTSD as it uses a psychological stressor that is life-threatening but does not involve physical pain, which is consistent with the etiology of PTSD in humans (reviewed in Clinchy et al., 2010). Employment of an ethologically relevant predator odor (e.g. TMT) as an environmental stressor has also been shown to successfully induce persistent changes in anxiety and alcohol use behaviors in C57Bl6/J mice, and changes in neural activation patterns in limbic system regions involved in fear, memory, and anxiety, such as the amygdala and hippocampus) (Cozzoli et al., 2014; Galliot et al., 2012; Hacquemand et al., 2010; Hwa et al., 2019; Sotnikov et al., 2014). Fear learning in animals is usually assessed using classical (Pavlovian) conditioning paradigms that pair an acute stressor (the unconditioned stimulus) with a specific environment or cue (the conditioned stimulus). In rodents, the standard threat response is freezing behavior, and this conditioned response upon re-exposure to the threat-associated context is used to indicate fear memory (discussed in Izquierdo et al., 2016). The C57Bl6/J inbred mouse strain will voluntarily drink unsweetened alcohol (Yoneyama et al., 2008). This genetic predisposition to alcohol use makes C57 mice an ideal model of non-contingent self-administration of alcohol effects. However,

it should be noted that findings generated using an alcohol-preferring inbred strain of mice may be limited in their interpretation to genetically predisposed alcohol-preferring subjects.

The original Drinking in the Dark (DID) animal paradigm was designed to model excessive binge-like drinking (reviewed by Thiele and Navarro, 2014), and the intermittent DID (iDID) paradigm used in this study is a prolonged and intermittent version of this (Rodriguez-Ortega et al. 2018). Binge drinking is defined by the National Institute on Alcohol Abuse and Alcoholism (2018) as resulting in blood ethanol concentrations (BECs) of 0.08% (80 mg/dl). Modeling repeated episodes of binge drinking without dependence is important as it has specific negative behavioral (e.g. injury/accident, increased risk taking) and neurobiological (e.g. alterations in GABA, glutamate, dopamine, corticotropin releasing factor) consequences (see Table 1 in Sprow and Thiele, 2012). For example, increased expression of CRF and GABA receptors in the amygdala and medial prefrontal cortex induced by early life stress (maternal separation) have been implicated in greater binge drinking and impulsive behaviors (Gondré-Lewis et al., 2016). Binge drinking in adolescence is a strong predictor of elevated alcohol use in adulthood, with female mice showing greater vulnerability than male mice (Strong et al. 2010). As it may signal a transition phase to AUD, models of binge drinking without dependence offer an opportunity to develop interventions specific to this key phase.

Environmental enrichment (EE) is a rodent paradigm that uses environmental complexity and novelty via systematic rotation of objects (e.g. toys, tunnels) to promote more naturalistic behaviors and development. EE has profound effects on the brain which include increased synaptic plasticity, dendritic complexity, and neurogenesis, as well as more resistance to the development

of disease, anxiety, abnormal repetitive behaviors, and substance abuse (Hannan 2014; Bechard et al. 2016; Nithianantharajah and Hannan 2006; Laviola et al. 2008). EE has been found to induce anxiolytic effects following a stressor (TMT: Sotnikov et al. 2014), reduce alcohol intake (Rodriguez-Ortega et al. 2018), and reduce alcohol intake following an acute stressor (restraint: Marianno et al., 2017). EE can also alter activation of addiction and stress related brain regions, such as the amygdala and hippocampus (Bakos et al., 2009; Pang et al., 2019; Sotnikov et al., 2014). The effects of environment on brain mechanisms and behaviors involved in addiction following late childhood trauma are not well known. Research investigating novel behavioral models of experience-induced changes in alcohol use will extend our understanding of the etiology of AUD.

Here, we sought to model the interacting effects of a single-episode childhood trauma with post-trauma environment on alcohol use in male and female mice. Young C57Bl6/J mice (d22) were exposed to predator odor and then reared in EE or standard lab environments. Males and females were assessed as adolescents for anxiety and conditioned fear memory, and as adults for alcohol use in a limited access, response non-contingent alcohol exposure paradigm. We use TMT-conditioned fear memory as a representative model of traumatic memory, and thus refer to mice exposed to the TMT as trauma-exposed mice. We hypothesized that trauma-exposed mice would drink more than non-exposed mice and that enriched post-trauma environments would reduce alcohol use compared to standard housed mice. Results support sex differences in post-trauma development of fear memory and alcohol use that are modified by environment.

2. Material and Methods

2.1 Animals

Male and female C57BL6/J mice were originally obtained from The Jackson Laboratory and housed in monogamous breeding pairs. A total of 54 offspring (male n=27, female n=27) from 9 litters were weaned at 22 days of age, exposed to TMT (n=28) or water (n=26) (see TMT exposure in *Behavioral Tests*), and housed in either standard environments (SE) (n=29) or EE (n=25) conditions, separated by sex. Final group numbers for females: EE TMT- (n=5), EE TMT+ (n=6), SE TMT- (n=8), and SE TMT+ (n=8); and, for males: EE TMT- (n=6), EE TMT+ (n=8), SE TMT- (n=7), and SE TMT+ (n=6). Litters were split so that half went into EE and the other half went into standard environments (SE), whereas all siblings from a litter were exposed to the same TMT- treatment. A timeline of our general methods is depicted in Figure 1. All procedures were approved by the State College of New York at Geneseo Institutional Animal Care and Use Committee and followed the Guidelines of the Care and Use of Laboratory Animals.

2.2 Environmental Enrichment (EE)

Pet kennels (23L x 15W x 13H) were modified to have two levels using galvanized mesh. Habitrail tunnels, various toys (Legos, plastic rings), manipulatable objects (toilet paper rolls, Kleenex), and foraging (bird seed) materials were rotated weekly to maintain novelty as well as complexity. Kennels also always had a running wheel, rodent chow, water, and bedding on the bottom floor.

2.3 Behavioral Tests

2.3.1 Acute Fear and Conditioned Fear Memory

Immediately after weaning (d22), individual mice were placed in an inescapable glass terrarium (D- 15 cm x H- 17.7 cm, Siyaglass) for a 10-minute session that we videotaped and later observed for acute fear response as indicated by freezing behavior (absence of all movement except respiration). A piece of tissue (1x1 cm of Kimwipe) soaked in 3µL of water or a predator odor: TMT (synthetic fox pheromone: 2,4,5-trimethylthiazoline (TMT), Purity>97%, BioSRQ, Sarasota, FL, USA) was taped to the ceiling of the enclosure. We exposed 54 subjects at 22 days of age to either TMT (TMT+) or water (TMT-); however, two video files were incomplete upon playback and thus were not used in the d22 analyses. The total time spent freezing was calculated and used an index of acute fear response (d22 freezing).

At 35 days of age, TMT-exposed mice (n = 14 TMT+ males, n = 14 TMT+ females) were placed back into the trauma-associated environment for assessment of context-induced fear memory. During this 10-min session, no TMT was present. The total time spent freezing was calculated and used an index of conditioned fear memory (d35 freezing).

2.3.2 Light-Dark Box (LDB)

Mice were tested for adolescent anxiety at 29 days of age in a Light-Dark Box test (San Diego Instruments, San Diego, CA). This test capitalizes on the rodent's natural aversion to open, brightly lit environments in favor of dark, enclosed environments. A clear acrylic open field enclosure (40.6 cm W x 40.6 cm D x 38.1 cm H) outfitted with photo beam arrays held a Dark Box insert (20.3 cm W x 20.3 cm D x 19 cm H) with a door (6.5 cm x 6.5 cm). The mouse's position is automatically quantified using a photobeam activity system (PAS). After habituating to the room for 30 minutes, mice were individually placed into the light side of the Light-Dark Box for a 10-minute session of

free exploration. The total time spent in the light side of the arena was used as an index of anxiety, with more time in the light indicative of lower anxiety.

2.3.3 Intermittent Drinking in the Dark (iDID)

Drinking sessions took place four times per week for a total of 18 sessions. Starting at 56 days of age, mice were habituated to the testing room for 30 min and then spent the next 2 hours inside individual cages that differed from the home cage in sight (striped walls) and smell (vanilla scent), and flooring (foam mats). In this cage, mice had access to 10% unsweetened EtOH (Fisher Scientific) diluted with water (Millipore) via graduated pipettes (10mL, Fisher Scientific) modified into sipper tubes. Post-session volume was subtracted from pre-session volume, then calculated as g/kg/2h. Total alcohol intake was calculated by summing daily (n=18) EtOH g/kg/2h. However, for repeated measures analyses, we averaged 2 days of EtOH g/kg/2h into a single bin (e.g. Bin 1 = days 1 and 2), and thus these analyses were run with 9 bins rather than 18 days.

2.4 Statistical analyses

Analyses were conducted using SPSS (v26) and figures were made using GraphPad Prism (8.0). Significance for all analyses was considered at $p = 0.050$ or less. One day behavioral tests (i.e. TMT-exposure, LDB, Conditioned Fear) and total alcohol intake (g/kg) were analyzed using an ANOVA with Treatment (TMT+, TMT-), Housing (EE, SE), Sex (M, F), and all interactions as factors in the model. Significant interactions were further explored using independent t-tests with Welch's correction for between group comparisons. Homogeneity of variance was assessed using Levine's test. When transformations of data were also unsuccessful in meeting this assumption, each independent factor was assessed separately using the non-parametric Mann-Whitney U test.

Daily self-administration data were analyzed using a Repeated Measures ANOVA, with Time as the within-subjects factor and Treatment, Housing, Sex, as between-subjects factors, and all interactions as factors in the model. Mauchly's test of sphericity ensured these data met assumptions of homogeneity. Separate Pearson's correlation analyses were conducted to relate freezing (d22) with 1. LDB, 2. freezing (d35), and 3. total alcohol intake (g/kg), in order to assess the relationship between acute fear response to early trauma and subsequent development of anxiety and alcohol use. A Pearson's analysis using freezing (d35) data from TMT-exposed mice and their total alcohol intake (g/kg) assessed the relationship between persistent fear memory of early trauma and adult alcohol use.

3. Results

3.1 Acute and conditioned fear response

Treatment with TMT at d22 was an effective acute stressor, as TMT-exposed (TMT+) mice froze significantly more than TMT-naïve (TMT-) mice (TMT Treatment: Mann-Whitney U: TMT+ mice: n=27, mean = 198.2 (12.0); TMT- mice: n=25, mean (SE) = 30.6 (12.4), $p<0.0001$; see Fig. 2A). There were no differences in d22 freezing due to Housing or Sex (Mann-Whitney U tests; p's > 0.05). TMT+ males and females were re-exposed to the context (no TMT was present) at 35 days of age and showed differences in conditioned freezing, revealed by main effects of Sex (F>M: $F(1,24) = 9.8$, $p = 0.005$), and Housing (EE>SE: $F(1, 24) = 5.0$, $p = 0.034$) and a Housing by Sex interaction (ANOVA: $F(1, 24) = 14.03$, $p = 0.001$, see Fig. 2B). Follow up tests indicate that EE females displayed heightened freezing behavior during re-exposure compared to all other groups (EE females vs.: SE females: $t(9)$, $t=4.1$, $p = 0.002$; EE males: $t(6) = 5.4$, $p = 0.001$; SE males: $t(9) = 2.7$, $p = 0.02$), which did not differ from each other (all p's > 0.05). Neither acute fear (d22

freezing) nor conditioned fear (d35 freezing) correlated with anxiety or alcohol use (Pearson's correlations: all p's > 0.05; data not shown).

3.2 Light-Dark Box

Adolescent anxiety, as assessed by the amount of time spent in the light side of the light-dark box, did not differ due to TMT Treatment, Housing, or Sex (Mann-Whitney U tests, all p's > 0.05; data not shown).

3.3 Alcohol use

Mice reared in EE consumed less total alcohol (g/kg) across sessions compared to SE mice ($F(1, 46) = 4.05$, $p = 0.050$; see Fig. 3A). No further significance was found for the effects of TMT Treatment, Sex, or any interactions, on total alcohol intake. When considered with the factor of time, there was an escalation of alcohol use for all groups (Time: $F(8, 368) = 11.0$, $p < 0.0001$). Mice exposed to TMT drank more than mice not exposed (TMT Treatment: $F(1, 46) = 8.8$, $p = 0.005$), and EE mice drank less alcohol than SE mice (Housing: $F(1, 46) = 3.8$, $p = 0.050$). A Time by Treatment interaction ($F(8, 368) = 2.0$, $p = 0.036$) and a Time by Housing by Sex interaction ($F(8, 368) = 2.4$, $p = 0.013$) revealed further differences. To further explore this 3-way interaction, we ran subsequent analyses separated by sex. In male mice, alcohol intake increased over Time ($F(8, 184) = 5.0$, $p <0.0001$) and was greater in males reared in SE vs EE Housing ($F(1, 23) = 6.4$, $p = 0.018$; see Fig. 3B). TMT treatment did not significantly affect the pattern of alcohol use in male mice. In female mice, alcohol intake increased over Time ($F(8, 184) = 6.7$, $p < 0.0001$) and was greater in TMT+ females vs TMT- females (TMT Treatment: $F(1, 23) = 9.6$, $p = 0.005$; see Fig. 3C). Unlike the males, housing did not affect the pattern of female alcohol use.

4. Discussion

The current findings indicate lasting, sex-dependent, effects of a single-episode childhood trauma on fear memory and alcohol use. Exposing young mice (d22) to a predator odor successfully induced an initial fear response, as indicated by heightened freezing behavior compared to control mice. Post-trauma adolescent environment modified conditioned fear memory in a sex-dependent way, such that EE females demonstrated the most freezing upon re-exposure to the trauma-associated context.

TMT-induced changes in anxiety were selective, as the Light-Dark Box test, a test of anxiety unrelated to the traumatic experience, revealed no group differences. Interestingly, previous rodent studies that also employed predator stress exposure during late childhood (3-4 weeks old) reported decreased anxiety and fear-related behaviors in adulthood, thus demonstrating protective effects (Chen et al., 2014; Hacquemand et al., 2010). However, short-term predator effects assessed in an elevated plus-maze 2 days after the exposure to predator stress did not alter anxiety-like behaviors (Chen et al., 2014). Thus, the age of trauma-exposure and the time of behavioral assessment are factors that may have contributed to the lack of group differences in the light-dark box test. Future studies using the current animal model may wish to explore long-term predator odor effects on anxiety by including additional anxiety tests later in development.

The current model recapitulates key human sex differences in vulnerability to childhood trauma (Blanco et al., 2018; Hyman et al., 2008). Adolescent EE female mice showed more conditioned fear memory compared to the other groups. This finding, to the best of our knowledge, is novel

and is now in need of replication. We speculate that stress and environment may alter the functioning of limbic structures important in emotional memory (e.g. hippocampus, amygdala) in a sex-dependent way. A previous study found exposure to predator odor enhanced performance in a hippocampal-dependent test of spatial memory through co-activation of the amygdala (Galliot et al., 2010). Prior research has found female C57 mice are more responsive than males to the ability of predator odor stress to increase corticosterone and allopregnanolone, which index HPA and endogenous neurosteroids, respectively (Cozzoli et al., 2014). Sex-dependent changes in markers of brain plasticity are also induced by EE. Compared to male rats, female rats reared in EE show a more pronounced increase in hippocampal BDNF, a neurotrophic factor important in learning and memory (Bakos et al., 2009). EE differentially influences neuronal morphology in males and females, with more dendritic branching in the hippocampus of EE females (Juraska et al., 1989, 1985).

We explored relationships between freezing behavior and anxiety and alcohol use. These correlational analyses showed freezing during the TMT exposure on day 22 did not predict later anxiety in the light-dark box or adult alcohol use. Freezing on day 22 also did not correlate with freezing on day 35 during re-exposure to the context. Thus, the initial stress response was not a useful indicator of which individuals would go on to develop greater anxiety or alcohol use. Conditioned freezing on day 35 was also not a useful predictor of total adult alcohol use.

Compared to standard housed mice, mice reared in EE drank less alcohol overall. Males and females did not differ in the total amount of alcohol consumed. Although, in general, female C57 mice have been found to drink more than males (e.g. Strong et al. 2010), when ethanol preference

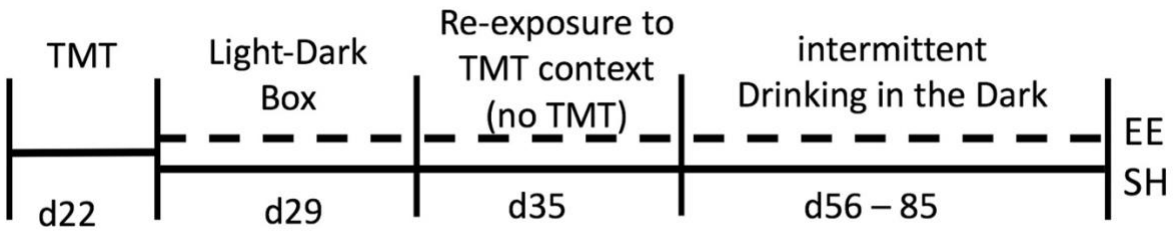
was assessed after predator odor stress, no sex differences were found beyond the initial day of exposure (see Table 3 in Cozzoli et al. 2014). When alcohol intake was assessed across time, both male and female C57 mice exposed to predator odor stress consumed more alcohol across time, agreeing with Cozzoli et al. (2014). However, employing the environment as a modifier revealed novel sex differences in post-trauma alcohol consumption. In females, TMT-exposed subjects drank more as adults than non-exposed mice and this behavior was resistant to beneficial changes associated with rearing environment. In males, irrespective of TMT-exposure, alcohol use was lower in EE mice. An earlier study that used a similar intermittent access to alcohol paradigm but tested only male mice also found alcohol use to be readily influenced by the environment, such that EE was associated with both preventative and therapeutic benefits (Rodriguez-Ortega et al. 2018). These data thus add to the growing literature suggesting heightened female vulnerability following exposure to stress and suggest that males may benefit more from post-trauma environmental interventions than females.

The lack of direct assessment of neurobiological hypotheses is a limitation of the current study. Future studies will investigate the effects of TMT and rearing environment on the function of the amygdala and hippocampus in binge-drinking males and females. We did not track estrous cycle and thus cannot relate it to our findings of fear memory or drinking behaviors. Although previous research in rodents has found no fluctuation of alcohol consumption across continuous and intermittent drinking paradigms due to estrous cycle (Priddy et al., 2017; Satta et al., 2018) these studies did not assess stress as a factor. Thus, future studies using the current model may wish to include estrous cycle monitoring as a way to assess the influence of sex hormones on behavioral outcomes. Finally, we did not directly assess BECs following drinking sessions to ensure

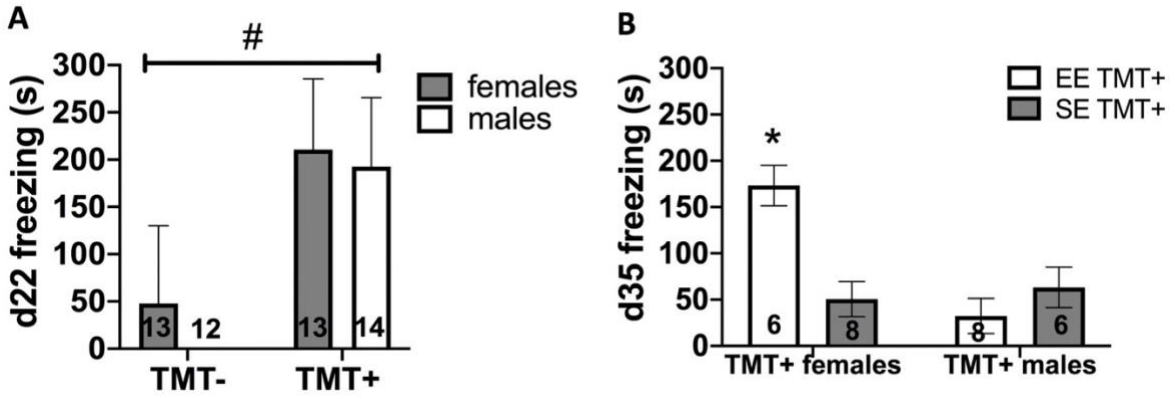
concentrations of pharmacological relevance. However, a previous study that measured corresponding BECs in C57 mice after 30 minutes of access to a 5% EtOH solution found intoxicating levels were reached (~2.0 mg/mL, Strong et al. 2010), suggesting that the similar levels of 10% EtOH solution consumed in the current study were likely also intoxicating.

In summary, the findings in this study show females to be more sensitive to the effects of adverse late childhood experiences on fear memory and adult alcohol use than males. Moreover, post-trauma environment interacted with these sex effects. Female mice reared in EE after TMT-exposure showed heightened conditioned fear as adolescents compared to all other groups and, unlike in males, the pattern of female alcohol use showed no benefits from EE. These data argue for the investigation of sex differences in response to trauma in fear and alcohol behaviors, and the use of the environment as a tool to reveal important interactive effects, with implications for successful treatment of comorbid stress and alcohol use disorders.

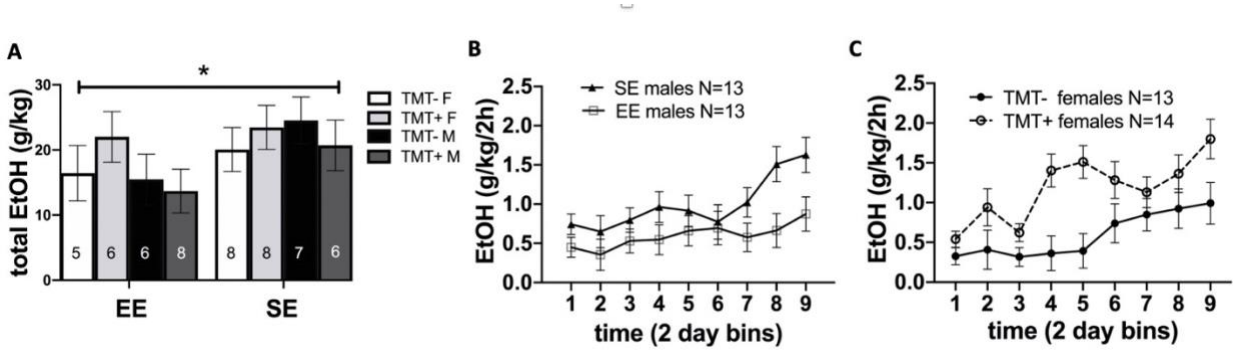
FIGURES



[1] A timeline of the general methods.



[2] Freezing behavior in response to a predator odor (TMT). **A** At 22 days of age, mice exposed to predator odor (TMT+) froze more than control mice (TMT-). **B** Two weeks later (d35), re-exposure to the trauma-associated context elicited conditioned freezing in TMT-exposed mice that was greatest in females reared with EE. * indicates $p < 0.0001$ for TMT+ vs TMT-; # indicates $p < 0.05$ for EE females vs all other groups



[3] Shows the amount of alcohol consumed adjusted for body weight (g/kg). **A** Overall, mice reared in EE consumed less alcohol than SE mice (Housing, $p<0.05$). **B** EE males drank less than SE males over time (Time*Housing, $p<0.05$), but TMT-exposure did not influence male drinking. **C** In females, TMT-exposure was associated with increased adult alcohol intake across time (Time*TMT, $p<0.05$), and post-trauma housing was not significant.

* indicates $p < 0.05$ for EE vs SE

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