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Sex differences in the synergistic effect of prior binge drinking and traumatic stress on subsequent ethanol intake and neurochemical responses in adult C57BL/6J mice

Deborah A. Finn [a, b, *], Melinda L. Helms [a], Michelle A. Nipper [a], Allison Cohen [b], Jeremiah P. Jensen [a], Leslie L. Devaud [c]

[a] Department of Behavioral Neuroscience, Oregon Health & Sciences University, Portland, OR, United States
[b] VA Portland Health Care System, Portland, OR, United States
[c] School of Pharmacy, Pacific University, Hillsboro, OR, United States

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ABSTRACT

Alcohol-use disorders (AUDs) are characterized by repeated episodes of binge drinking. Based on reports that exposure to predator odor stress (PS) consistently increases ethanol intake, the present studies examined whether prior binge drinking differentially altered responsivity to PS and subsequent ethanol intake in male and female mice, when compared to mice without prior binge exposure. Initial studies in naïve male and female C57BL/6J mice confirmed that 30-min exposure to dirty rat bedding significantly increased plasma corticosterone (CORT) levels and anxiety-related behavior, justifying the use of dirty rat bedding as PS in the subsequent drinking studies. Next, separate groups of male and female C57BL/6J mice received seven binge ethanol sessions (binge) or drank water (controls), followed by a 1-month period of abstinence. Then, 2-bottle choice ethanol intake (10% or 10E vs. water, 23 h/day) was measured in lickometer chambers for 4 weeks. After baseline intake stabilized, exposure to intermittent PS (2×/week × 2 weeks) significantly enhanced ethanol intake after the 2nd PS in male, but not female, binge mice vs. baseline and vs. the increase in controls. However, in a subgroup of females (with low baselines), PS produced a similar increase in 10E intake in control and binge mice vs. baseline. Analysis of lick behavior determined that the enhanced 10E intake in binge male mice and in the female low baseline subgroup was associated with a significant increase in 10E bout frequency and 10E licks throughout the circadian dark phase. Thus, PS significantly increased 10E intake and had a synergistic interaction with prior binge drinking in males, whereas PS produced a similar significant increase in 10E intake in the low baseline subgroup of binge and control females. Plasma CORT levels were increased significantly in both binge and control animals after PS. CORT levels at 24-h withdrawal from daily 10E intake were highest in the groups with elevated 10E licks (i.e., binge males and control females). At 24-h withdrawal, protein levels of $GABA_A$ receptor α1 subunit, corticotropin releasing factor receptor 1, and glucocorticoid receptor in prefrontal cortex (PFC) and hippocampus (HC) were differentially altered in the male and female mice vs. levels in separate groups of age-matched naïve mice, with more changes in HC than in PFC and in females than in males. Importantly, the sexually divergent changes in protein levels in PFC and HC add to evidence for sex differences in the neurochemical systems influenced by stress and binge drinking, and argue for sex-specific pharmacological strategies to treat AUD.

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Introduction

Alcohol-use disorders (AUDs) include behavior of repeated episodes of binge drinking, which is a pattern of drinking that brings blood alcohol (ethanol) concentration (BEC) ≥ 80 mg% (i.e., 80 mg/dL or 0.80 mg/mL). Notably, a history of binge drinking can be a strong predictor of subsequent ethanol dependence (Hingson,

* Corresponding author. VA Portland Health Care System, 3710 SW U.S. Veterans Hospital Road (R&D-49), Portland, OR 97239, United States.
E-mail address: finnd@ohsu.edu (D.A. Finn).

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Heeren, & Winter, 2006). Excessive ethanol use is the 4th leading preventable cause of death in the US, but globally, it accounts for 5.9% of all deaths (~3.3 million in 2012) and is the leading risk factor for premature death and disability among people between the ages of 15 and 49 (NIAAA, 2017).

The experience of stress may represent a risk factor that shifts ethanol consumption from recreational to excessive (see Becker, Lopez, & Doremus-Fitzwater, 2011; Chester, Barrenha, DeMaria, & Finegan, 2006; Gilpin & Weiner, 2017; Lynch, Kushner, Rawleigh, Fiszdon, & Carroll, 1999; Sillaber & Henniger, 2004, and references therein). Human studies document a link between ethanol consumption and stress, with higher levels of consumption in those individuals that experience higher levels of stress (reviewed in Keyes, Hatzenbuehler, Grant, & Hasin, 2012). In rodent animal models, exposure to stress during early development produces a consistent increase in ethanol consumption in adulthood (see Campbell, Szumlinski, & Kippin, 2009 and references therein). Results are more variable regarding the effects of various stressors in adolescent and adult animals on ethanol self-administration when tested during adulthood (e.g., Becker et al., 2011; Brunell & Spear, 2005; Chester et al., 2006; Chester, Barrenha, Hughes, & Keuneke, 2008; Chester, Blose, Zweifel, & Froehlich, 2004; Doremus, Brunell, Rajendran, & Spear, 2005; Lynch et al., 1999; Sillaber & Henniger, 2004; Tambour, Brown, & Crabbe, 2008). However, recent work found that exposure to predator odor stress (PS) increases subsequent ethanol intake in male and female mice and male rats (Cozzoli, Tanchuck-Nipper, Kaufman, Horowitz, & Finn, 2014; Edwards et al., 2013; Manjoch et al., 2016; Roltsch et al., 2014). Exposure to various forms of PS also increases thermal nociception, startle reactivity, and anxiety-related behaviors in ethanol-naïve rats and mice (Belzung, El Hage, Moindrot, & Griebel, 2001; Cohen, Geva, Matar, Zohar, & Kaplan, 2008; Cohen & Zohar, 2004; Gilpin & Weiner, 2017; Hebb et al., 2003; Roltsch et al., 2014; Whitaker & Gilpin, 2015), suggesting that a high arousal and anxiety state may contribute to the PS-induced increase in subsequent ethanol intake. One hallmark of PS is that behavioral and neuroendocrine responses to repeated PS exposure often lead to sensitization rather than habituation, similar to what occurs with post-traumatic stress disorder (PTSD; Staples, 2010). As a result, exposure to PS is considered a traumatic stress, and it is used as a model of PTSD (reviewed in Cohen & Zohar, 2004; Dielenberg & McGregor, 2001; Gilpin & Weiner, 2017; Matar, Zohar, & Cohen, 2013).

PTSD is now classified as a trauma- or stressor-related disorder, and several studies document an association between PTSD and the development of an AUD (reviewed in Gilpin & Weiner, 2017). While PTSD symptoms may precede the onset of AUD (Gilpin & Weiner, 2017), the prevalence of AUD among those with PTSD is estimated at 28% for women and 52% for men (Norman et al., 2012). In general, comorbid PTSD/AUD is associated with greater psychological distress, diminished social functioning, poorer treatment response, more frequent hospitalizations, more physical health problems, and increased AUD-related problems than with either disorder alone (Norman et al., 2012). Thus, PTSD symptoms appear to promote excessive ethanol drinking, whereas ethanol abuse worsens PTSD symptoms. This comorbidity negatively influences recovery prognosis and effective pharmacotherapeutic strategies.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, and abnormalities in the HPA axis are observed in both AUD and PTSD, suggesting that the overlap in HPA involvement may constitute a neurobiological mechanism underlying the comorbidity of these disorders (Gilpin & Weiner, 2017; Norman et al., 2012). For example, some individuals with PTSD have an increased number and sensitivity of glucocorticoid receptors (GRs), enhanced negative feedback of the HPA axis, and elevated corticotropin releasing factor (CRF) levels in cerebrospinal fluid (Pittman et al., 2012; Zoladz & Diamond, 2013). Using the PS model of PTSD, exposure to PS significantly increased plasma corticosterone (CORT) and adrenocorticotropic hormone (ACTH) levels in male and female rodents (Cozzoli et al., 2014; Whitaker & Gilpin, 2015), with a greater increase in female vs. male mice (Cozzoli et al., 2014) and a greater increase in "Avoider" rats with a high anxiety phenotype (Whitaker & Gilpin, 2015). CRF peptide levels also were elevated significantly following PS in the central nucleus of the amygdala and the ventromedial prefrontal cortex (vmPFC) of these rats (Itoga et al., 2016; Schreiber, Lu, Baynes, Richardson, & Gilpin, 2017). Thus, PS produces similar changes in HPA axis responsivity as seen in PTSD, supporting the suggestion that PS may be used to model PTSD in rodent studies.

There are sex differences in HPA axis responsivity to stress and in CRF receptor 1 (CRF-R1) signaling, which may influence susceptibility to PTSD and AUD. Epidemiological studies indicate that lifetime prevalence of PTSD is twice as high in females as in males (11% vs. 5.4%; 9.7% vs. 3.6%; Bangasser & Valentino, 2014; Kilpatrick et al., 2013; Valentino, Bangasser, & Van Bockstaele, 2013). Using animal models, sex differences in the coupling of CRF-R1 with second messenger cascades render females more responsive to acute stress and less able to adapt to chronic stress as a result of compromised CRF-R1 internalization (Valentino, Bangasser, et al., 2013; Valentino, Van Bockstaele, & Bangasser, 2013). In female rats, the decreased ability of CRF-R1 to associate with β-arrestin 2 results in less internalization of CRF-R1 and biased signaling through stimulatory G-protein (Gs) and protein kinase A pathways following stress in locus coeruleus (LC) neurons. In male rats, stress promotes a greater association of CRF-R1 with β-arrestin 2, facilitating internalization of CRF-R1 in LC neurons and a bias toward signaling through β-arrestin 2 (i.e., Gs independent). These sex differences in CRF-R1 signaling, which are unrelated to adult hormone status of males or females, have a functional influence on LC neurons, with the magnitude of LC activation by stress being greater in female vs. male rats (Valentino, Bangasser, et al., 2013; Valentino, Van Bockstaele et al., 2013). Thus, mechanisms underlying the PS-associated increase in ethanol intake likely differ in males and females.

Despite the comorbidity of AUD and PTSD in humans, few studies have examined the possibility that binge ethanol exposure could accentuate the physiological and behavioral effects of stress. We contend that prior binge ethanol drinking is a risk factor that interacts with stress to augment subsequent ethanol-drinking behavior. To test this assertion, initial studies in naïve male and female C57BL/6J mice confirmed that 30-min exposure to dirty rat bedding significantly increased plasma CORT levels and anxiety-related behavior, which provided a rationale for the use of dirty rat bedding as PS in the current studies. Then, the subsequent drinking studies examined whether prior binge drinking differentially altered responsivity to intermittent PS and subsequent ethanol intake in adult male and female C57BL/6J mice, when compared to mice without previous binge ethanol exposure. Accompanying changes in levels of select proteins in the PFC and hippocampus (HC) also were examined and compared with levels in a separate group of age-matched naïve mice to explore neurochemical responses following prior binge drinking and intermittent PS exposure. The proteins examined were related to HPA axis and stress (CRF-R1 and GR, see above) and a γ-aminobutyric acid$_A$ receptor (GABA$_A$R) subunit that is the most prominent subtype in the adult brain and is important in ethanol dependence (GABA$_A$R α1 subunit; reviewed in Kumar et al., 2009), and that exhibits sex differences following chronic ethanol administration (Devaud, Fritschy, & Morrow, 1998). We predicted that prior binge ethanol drinking would differentially alter