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ANXIETY RESPONSE AND RESTRAINT-INDUCED STRESS DIFFERENTIALLY AFFECT ETHANOL INTAKE IN FEMALE ADOLESCENT RATS

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Abstract—Anxiety disorders are more likely to occur in women than in men, usually emerge during adolescence and exhibit high comorbidity with alcohol use disorders (AUD). Adolescents with high levels of anxiety or heightened reactivity to stress may be at-risk for developing AUD. An approach to analyze if high levels of inborn anxiety predict greater ethanol drinking is to assess the latter variable in subjects classified as high- or low-anxiety responders. The present study assessed ethanol drinking in adolescent, female Wistar, rats classified as high-, low- or average-anxiety responders and exposed or not to restraint stress (RS, Exp. 1). Classification was made through a multivariate index derived from testing anxiety responses in an elevated plus maze and a light–dark box tests. RS was applied after animals had been initiated to ethanol drinking. Intake of sweetened ethanol was unaffected by level of anxiety response. Adolescents with high levels of inborn anxiety exhibited significantly higher intake of unsweetened ethanol than counterparts with standard levels of anxiety, yet this effect was inhibited by RS exposure. Experiment 2 assessed FOS immunoreactivity after RS. Stress induced a significant increase in FOS immunoreactivity at the paraventricular nucleus, yet this effect was unaffected by level of anxiety response. Female adolescents with high levels of basal anxiety may be at-risk for exhibiting increased predisposition for ethanol intake and preference. The study also indicates that stress may exert differential effects on adolescent ethanol intake as a function of the level of anxiety response.

Key words: adolescents, ethanol intake, stress, anxiety, Fos immunoreactivity.

INTRODUCTION

Anxiety disorders, which are more likely to occur in women than in men (Pisu et al., 2016), usually emerge during adolescence (Cunningham et al., 2002) and exhibit high comorbidity with alcohol use and alcohol use disorders (AUD) (Hobbs et al., 2011). A recent study indicated that social anxiety disorder significantly predicted AUD in both African American and European American adolescents (Sartor et al., 2016). This is consistent with the postulate that individuals with high levels of anxiety may be more sensitive to the negative reinforcing effects of alcohol (hereinafter referred to as ethanol) and thus may be at-risk for developing AUDs (Kushner et al., 1994).

Ethanol intake has been measured in animals selectively bred to exhibit high- or low-inborn anxiety (Henniger et al., 2002) and vice versa, anxiety responses have been analyzed in animals selectively bred for high- and low-ethanol intake. Rats selected for high anxiety-response drank more ethanol than rats selected for their predisposition to explore dangerous environments (Izidio and Ramos, 2007) and the ethanol-preferring P rats exhibit significantly greater sensitivity to exteroceptive nociceptive stimulation and spend less time in the open arms of an elevated plus maze than their ethanol-nonpreferring (NP) counterparts (Stewart et al., 1993). An anxious phenotype has also been described in the genetically selected Marchigian Sardinian alcohol-preferring rats (Ciccocioppo et al., 2006). These animals exhibited reduced propensity to explore the open arms of the elevated plus maze and the central zone of the open field (Roman et al., 2012). Similarly, the Warsaw alcohol high-preferring (WHP) rats exhibited enhanced acoustic startle response than their low-preferring WLP counterparts (Acewicz et al., 2012). Other studies, however, have failed to replicate these results (Da Silva et al., 2004) or yielded a negative association between anxiety and ethanol intake (Henniger et al., 2002). For instance, the WHP rats exhibited less anxiety (i.e., more

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Abbreviations: AA, average-anxiety responder; ANOVAs, analyses of variance; Arc, arcuate nucleus; AUD, alcohol use disorders; BLA, basolateral amygdala nucleus; CEA, central amygdala nucleus; EPM, elevated plus maze; Fos-ir, FOS immunoreactivity; HA, high-anxiety responder; LA, low-anxiety responder; LDB, light–dark box; mPFC, medial prefrontal cortex; PD, postnatal day; PVC, polyvinyl chloride; PVN, paraventricular nucleus; RS, restraint stress; WHP, Warsaw alcohol high-preferring.

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time spent in the central section of an open field) than the WLP rats (Acewicz et al., 2014).

Another approach to analyze if high levels of inborn anxiety predict greater ethanol drinking is to submit subjects to a validated animal model of anxiety [e.g., elevated plus maze or light–dark box, EPM and LDB, respectively] (Kumar et al., 2013). The animals are classified as high- or low-anxiety responders as a function of performance on the test and then ethanol drinking is assessed. This approach has yielded evidence for an association between inborn anxiety and ethanol intake at adulthood (Spanagel et al., 1995; Bahi, 2013). For instance, Primeaux et al. (2006) found greater preference for 4% ethanol and 6% ethanol in rats classified as anxious as a function of performance on the elevated plus maze, compared with non-anxious rats.

HAs may be more sensitive to aversive and stressful stimulation (Muigg et al., 2008), and adolescents have been found to be more sensitive to stress, and to stress–ethanol interactions, than adults. Five days of restraint stress (RS) significantly enhanced ethanol intake and reduced ethanol-induced sleep time in adolescent but not in adults (Fernandez et al., 2016). A single, 90-min session of RS increases anxiety, as shown by reduced social investigation, in both adults and adolescent rats. This effect of stress is reversed by ethanol in adolescent, but not in adult, rats (Varlinskaya and Spear, 2012). Another relevant interaction between adolescence, stress and ethanol is that male (Siegmund et al., 2005) or female (Fullgrabe et al., 2007) rats that started to drink ethanol during adolescence, but not during adulthood, were sensitive to foot-shock induced facilitation of ethanol drinking. This could result from an ethanol-induced alteration in the brain circuits involved in the stress response. Rats given vapor ethanol exposure during postnatal days (PD) 28–42, for instance, exhibited reduced expression of corticotropin releasing factor mRNA in the paraventricular nucleus (PVN), after a challenge with ethanol in early adulthood (Allen et al., 2011). These results highlight the importance of assessing stress-induced drinking early in development. Stress during adolescence may facilitate onset and escalation of drinking, likely to a greater extent than stress during adulthood, and sub-populations characterized by high levels of inborn anxiety may be particularly vulnerable to ethanol drinking and stress-induced drinking.

The present study assessed ethanol drinking throughout the duration of adolescence, in female rats classified as high-, low- or AAs and exposed or not to RS (Exp. 1). Classification was made through a multivariate index of anxiety, and RS was applied after animals had been initiated to ethanol drinking. It has been indicated that animals need several intake sessions to learn about ethanol's anti-anxiety effects (Samson et al., 1998). Experiment 2 assessed neural activation (FOS immunoreactivity, Fos-ir) after RS in the three anxiety groups, in the basolateral and central amygdala (BLA and CEA, respectively), and in the PVN and arcuate nucleus (Arc).

These brain areas are involved in the stress response and regulate baseline levels of anxiety response. Briski and Gillen (2001) and later Keshavarzy et al. (2015) observed significant Fos-ir in PVN and Arc after a 2-h or a 1-h RS session, respectively [also see Kwon et al. (2006)]. Rats classified as HAs, but not those classified as LAs, exhibited decreased expression of central corticotropin-releasing factor in the PVN, after chronic RS (5 weeks, 3 h/day; Wisowska-Stanek et al., 2016). The role of the amygdala in mediating anxiety responses and in the acquisition of conditioned fear has been studied at length (Maren and Quirk, 2004). Rats bilaterally lesioned in CEA exhibited a blunted response to experimentally induced anxiety and a significant reduction in ethanol intake (Moller et al., 1997). The CEA also endures plastic changes (for instance, after chronic drug treatment) that result in greater anxiety and sensitivity to stress (Koob, 2009). The BLA, in turn, projects to several other areas, including CEA, ventral hippocampus and medial prefrontal cortex (mPFC). A recent study found an increase in anxiety-like behavior after the optogenetic activation of the BLA-mPFC pathway, whereas the inhibition of this circuit was associated with decreased anxiety-like behavior (Felix-Ortiz et al., 2016). Alterations in anxiety were also observed after manipulating the projections between the BLA and the CEA (Tye et al., 2011) or between the BLA and the ventral hippocampus (Felix-Ortiz et al., 2013). Moreover, several studies have reported a reduction of experimental anxiety after microinjection of benzodiazepines into the BLA or CEA, underscoring a casual role of these structures in the expression of anxiety-like behaviors (Menard and Treit, 1999; Engin and Trait, 2008).

Our hypotheses were that HAs would exhibit greater ethanol intake and that this would be exacerbated by RS. We expected these behavioral differences to translate into greater neural response to RS; i.e., greater RS-induced Fos-ir, with likely regional differences, in high-anxiety than in low-anxiety or average-anxiety females. We focused on females due to the greater prevalence of anxiety disorders in women and because, among college students who drink, women are more likely than men to develop AUD (Perkins, 2002). Female rats also consume more ethanol than males (Lancaster et al., 1996; Doremus et al., 2005).

EXPERIMENTAL PROCEDURES

Experimental designs

A 3 (level of baseline anxiety response: high-anxiety, average-anxiety and low-anxiety; HA, AA and LA, respectively) × 2 (stress exposure: stressed or non-stressed; S and NS, respectively) factorial design was employed in both Experiments 1 and 2. Number of animals in each group was as follows: HA-S = 12, HA-NS = 14; AA-S = 9, AA-NS = 18; LA-S = 9, LA-NS = 17 (Experiment 1), and HA-S = 5, HA-NS = 5; AA-S = 5, AA-NS = 5; LA-S = 5, LA-NS = 5 (Experiment 2). The uneven number of subjects in the groups of Exp. 1 was because group assignment as a function of anxiety response occurred after completion of experimental procedures. A separate group of rats was left untreated (UT, $n = 5$) in Experiment 2. These

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