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Chronic Intermittent Ethanol Exposure Modulation of Glutamatergic Neurotransmission in Rat Lateral/Basolateral Amygdala is Duration-, Input-, and Sex-Dependent

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Abstract—The basolateral amygdala (BLA) controls numerous behaviors, like anxiety and reward seeking, via the activity of glutamatergic principal neurons. These BLA neurons receive excitatory inputs primarily via two major anatomical pathways – the external capsule (EC), which contains afferents from lateral cortical structures, and the stria terminalis (ST), containing synapses from more midline brain structures. Chronic intermittent ethanol (CIE) exposure/withdrawal produces distinct alterations in these pathways. Specifically, 10 days of CIE (via vapor inhalation) increases presynaptic function at ST synapses and postsynaptic function at EC synapses. Given that 10-day CIE/withdrawal also increases anxiety-like behavior, we sought to examine the development of these alterations at these inputs using an exposure time-course in both male and female rats. Specifically, using 3, 7, and 10 days CIE exposure, we found that all three durations increase anxiety-like behavior in the elevated plus maze. At BLA synapses, increased presynaptic function at ST inputs required shorter exposure durations relative to post-synaptic alterations at EC inputs in both sexes. But, synaptic alterations in females required longer ethanol exposures compared to males. These data suggest that presynaptic alteration at ST-BLA afferents is an early neuroadaptation during repeated ethanol exposures. And, the similar patterns of presynaptic-then-postsynaptic facilitation across the sexes suggest the former may be required for the latter. These cooperative interactions may contribute to the increased anxiety-like behavior that is observed following CIE-induced withdrawal and may provide novel therapeutic targets to reverse withdrawal-induced anxiety.

Key words: basolateral amygdala, anxiety, paired-pulse ratio, strontium substitution, sex differences, ethanol dependence.

INTRODUCTION

Anxiety disorders and alcohol use disorders (AUDs) frequently co-occur and the clinical significance of this relationship has been reported in epidemiological studies for decades (Ross, 1995; Merikangas et al., 1998; Kushner et al., 2000; Burns and Teesson, 2002; Grant et al., 2015). Interestingly, the relationship between anxiety disorders and AUDs is more strongly associated with alcohol dependence than with alcohol abuse (Kushner et al., 2000; Hasin et al., 2007). This is likely because alcohol-dependent individuals who suddenly stop or drastically reduce their drinking experience a wide range of physical (e.g., heightened respiration, blood pressure, seizures, delirium tremors) and psychological (e.g., anxiety, dysphoria, agitation) symptoms (Finn and Crabbe, 1997; Becker, 2000). The anxiety that emerges from alcohol withdrawal is so severe that people often relapse and self-medicate with alcohol to seek relief from their symptoms (Schellekens et al., 2015; Driessen et al., 2001). Therefore, withdrawal-induced anxiety symptoms associated with terminating long-term alcohol exposure are strong contributing factors for relapse in alcohol-dependent individuals. Similar to humans, animals show increased anxiety-like behavior during withdrawal, which may likewise contribute to the enhanced alcohol consumption observed during this time (Valdez et al., 2002).

One commonly used and well-validated animal model of producing alcohol (ethanol) dependence is via vapor inhalation (Goldstein and Pal, 1971). This model consistently produces a dependence-like phenotype and yields behaviors (e.g., increased anxiety, enhanced ethanol consumption) that are frequently cited as markers of ethanol withdrawal in the rodent literature (Finn and Crabbe, 1997; Kliethermes et al., 2004; O'Dell et al., 2004). In addition to overt behavioral signs that emerge following chronic ethanol vapor exposure, neurophysiological adaptations also occur in the lateral/basolateral amygdala

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(BLA), a brain region that is an integral component of the fear/anxiety circuit (Phillips and LeDoux, 1992; Janak and Tye, 2015; Davis et al., 1994).

The amygdala receives sensory information through multiple projections with major pathways arriving via the external capsule ('lateral' inputs in a coronal slice) and the stria terminalis ('medial' inputs) (Rainnie et al., 1991; Davis et al., 1994; Bauer et al., 2002). This information is first processed in the BLA, and is then relayed to downstream brain regions, ultimately resulting in a physiological/psychological response (e.g., anxiety) (Davis et al., 1994; Janak and Tye, 2015). The BLA is comprised primarily of pyramidal-shaped glutamatergic projection neurons and non-pyramidal-shaped GABAergic interneurons, and manipulating activity of these neurons dramatically alters anxiety-like behavior in rodents (Sanders and Shekhar, 1995a, 1995b; Sajdyk and Shekhar, 1997a, 1997b). There is also evidence demonstrating that the BLA may be involved with the anxiogenic effects of ethanol observed during withdrawal (Läck et al., 2007, 2008). Our laboratory has also shown that alcohol dependence/withdrawal modulates glutamatergic synaptic transmission onto BLA projection neurons in an input-dependent manner (Christian et al., 2012, 2013). Specifically, 24 h after 10 days of chronic intermittent ethanol (CIE) vapor exposure, glutamatergic afferents arriving along the external capsule/lateral pathway express postsynaptic alterations characterized by increased AMPA receptor function that correlate with increased receptor phosphorylation and trafficking. These effects contrast with glutamatergic afferents arriving via the stria terminalis/medial pathway which express presynaptic adaptations represented by increased glutamate release probability, increased synaptic glutamate concentrations, a larger pool of readily releasable vesicles, and decreased failure rates at these terminals.

Our laboratory and others demonstrated that withdrawal from alcohol produces increases in anxiety-like behavior, and this may be associated with adaptations in glutamatergic synaptic transmission occurring in the BLA during dependence. However, the time-course of these behavioral and neurophysiological alterations is unknown. This is significant because in the fear conditioning literature, a temporal relationship exists between pre- and post-synaptic plasticity. More specifically, presynaptic activation of the stria terminalis inputs facilitates postsynaptic long-term potentiation at external capsule synapses, which may be important for fear learning (Cho et al., 2012; Fonseca, 2013). It is possible that ethanol dependence and withdrawal may also differentially modulate presynaptic facilitation at stria inputs and postsynaptic plasticity expressed at the external capsule in time-specific ways.

Sex differences to a variety of ethanol-related behaviors have been reported in both preclinical and clinical studies (Devaud and Chadda, 2001; Nolen-Hoeksema, 2004; Devaud et al., 2006; Morales et al., 2015; Jury et al., 2017). For example, our laboratory has recently shown that while dependence produced by 10 days of CIE exposure increased ethanol consumption in males (as has been reported numerous times by others (Woolley et al., 1997; Carnicella et al., 2008; Simms et al., 2008; Meyer et al., 2013; Kimbrough et al., 2017), female ethanol drinking remains unaffected (Butler et al., 2014; Rosenwasser et al., 2014; Morales et al., 2015). Despite behavioral evidence demonstrating differences between males and females in alcohol use and sensitivity, we and few others have examined neurophysiological changes that may emerge following ethanol dependence in females. Therefore, the current series of experiments examined the time course of ethanol adaptations that occur presynaptically from stria terminalis afferents and postsynaptically via external capsule afferents onto BLA principal neurons after various durations of CIE vapor exposure and 24-h withdrawal in male and female Sprague–Dawley rats. These data will provide further characterization of synaptic adaptations that occur on BLA principal neurons after various CIE exposures that likely contributes to anxiety-like behavior during withdrawal, which may ultimately lead to relapse.

EXPERIMENTAL PROCEDURES

Animals

Five-week-old male and female Sprague–Dawley rats were obtained from Envigo (Indianapolis, IN) and were given unlimited access to standard rat chow and water throughout the experimental procedure. Upon arrival, rats were pair-housed and maintained on a reverse 12:12-h light–dark cycle (lights on at 9 PM). All animal care procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Wake Forest Animal Care and Use Committee.

Chronic intermittent ethanol (CIE) vapor exposure

Pair-housed rats were exposed to chronic intermittent ethanol (CIE) vapor for 3, 7, or 10 days, using standard procedures from our laboratory (Läck et al., 2007; Christian et al., 2012; Morales et al., 2015). Briefly, home cages were placed in larger, custom-built Plexiglas chambers (Triad Plastics, Winston-Salem, NC), and at the beginning of the light cycle (9pm EST), ethanol vapor was pumped into the chambers and maintained at 15–20 mg/L throughout the exposure for 12-h day. Air-exposed control animals were similarly housed, except they received room-air only while in the chambers. Animals were weighed daily; and, tail blood samples were collected once during the CIE exposure to monitor blood ethanol concentrations (BECs) and adjust ethanol vapor levels as necessary (Table 1). Blood ethanol concentrations were determined using a standard, commercially available alcohol dehydrogenase/NADH enzymatic assay (Diagnostic Chemicals Limited, Oxford CT). At arrival, body weights (in grams ± SEM) for males and females were 99.15 ± 1.15 and 86.25 ± 0.57, respectively. After 3, 7, and 10 days of CIE exposure, males weighed 183.91 ± 2.59, 173.21 ± 1.18, and 161.89 ± 2.89, while air-exposed males weighed 219.05 ± 5.01. After 3, 7, and 10 days of CIE exposure, females weighed 138.85

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