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The allostatic impact of chronic ethanol on gene expression: A genetic analysis of chronic intermittent ethanol treatment in the BXD cohort

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ABSTRACT

The transition from acute to chronic ethanol exposure leads to lasting behavioral and physiological changes such as increased consumption, dependence, and withdrawal. Changes in brain gene expression are hypothesized to underlie these adaptive responses to ethanol. Previous studies on acute ethanol identified genetic variation in brain gene expression networks and behavioral responses to ethanol across the BXD panel of recombinant inbred mice. In this work, we have performed the first joint genetic and genomic analysis of transcriptome shifts in response to chronic intermittent ethanol (CIE) by vapor chamber exposure in a BXD cohort. CIE treatment is known to produce significant and sustained changes in ethanol consumption with repeated cycles of ethanol vapor. Using Affymetrix microarray analysis of prefrontal cortex (PFC) and nucleus accumbens (NAC) RNA, we compared CIE expression responses to those seen following acute ethanol treatment, and to voluntary ethanol consumption. Gene expression changes in PFC and NAC after CIE overlapped significantly across brain regions and with previously published expression following acute ethanol. Genes highly modulated by CIE were enriched for specific biological processes including synaptic transmission, neuron ensheathment, intracellular signaling, and neuronal projection development. Expression quantitative trait locus (eQTL) analyses identified genomic loci associated with ethanol-induced transcriptional changes with largely distinct loci identified between brain regions. Correlating CIE-regulated genes to ethanol consumption data identified specific genes highly associated with variation in the increase in drinking seen with repeated cycles of CIE. In particular, multiple myelin-related genes were identified. Furthermore, genetic variance in or near dynamin3 (*Dnm3*) on Chr1 at ~164 Mb may have a major regulatory role in CIE-responsive gene expression. *Dnm3* expression correlates significantly with ethanol consumption, is contained in a highly ranked functional group of CIE-regulated genes in the NAC, and has a *cis*-eQTL within a genomic region linked with multiple CIE-responsive genes.

1. Introduction

Alcohol use disorder (AUD) is the complex result of a multitude of central nervous system (CNS) adaptations following long-term, repeated episodes of heavy ethanol consumption and withdrawal. Although AUD is a uniquely human trait possibly requiring decades to develop, key facets of ethanol-induced behaviors and cognate molecular adaptations following acute ethanol exposure can be studied across a spectrum of animal models including monkeys, mice, rats, flies, and worms. (Becker & Hale, 1993; Bettinger, Leung,

Abbreviations: AUD, alcohol use disorder; BXD, B6 and D2 recombinant inbred strains; CIE, chronic intermittent ethanol; PFC, prefrontal cortex; NAC, nucleus accumbens; eQTL, expression quantitative trait locus; CNS, central nervous system; RI, recombinant inbred; GO, gene ontology; IPA, Ingenuity pathway analysis; LRS, likelihood ratio statistic; LOD, log odds ratio.

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Bolling, Goldsmith, & Davies, 2012; Bhandari, Kendler, Bettinger, Davies, & Grotewiel, 2009). Moreover, human genetic studies have shown that acute behavioral responses to ethanol have predictive ability in terms of long-term risks for developing AUD (Schuckit, 1994). Gene targeting studies in animal models also frequently show correlations between alterations in acute behavioral responses and ethanol consumption behaviors (Crabbe, 2012).

The molecular and cellular mechanisms underlying the transition from acute ethanol exposure to abusive behaviors in AUD are unknown. Changes in stress reactivity, gene expression, and neuronal signaling all accompany acute ethanol exposure and have been postulated to lead to chronic adaptations–essentially an allostatic imprint on the CNS (Costin, Wolen, Fitting, Shelton, & Miles, 2013; McBride et al., 2005). Proving causality between molecular changes and long-lasting behaviors has not yet been achieved (Heilig & Egli, 2006; Higley, Koob, & Mason, 2012).

As an approach to identifying causal relationships between molecular effects and chronic ethanol consumption, we have exploited a mouse genetic model of chronic ethanol exposure and progressive consumption, together with a genomic analysis of regional changes in gene expression in the brain. Our goal is to identify possible transcripts and shared processes underlying the transition in the brain from acute ethanol exposure to chronic intermittent ethanol exposure and withdrawal.

The chronic intermittent ethanol vapor model (CIE) has been widely used in rodent studies (Lopez & Becker, 2005, 2012; O'Dell, Roberts, Smith, & Koob, 2004; Roberts, Heyser, Cole, Griffin, & Koob, 2000) as a tool to approximate the repeated cycles of heavy consumption and withdrawal that are seen in humans during development of AUD. CIE exposed rats or mice will show alterations in the amount and pattern of ethanol consumption, generally increasing their ethanol consumption following CIE vapor (Becker, 2013; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Lopez & Becker, 2005; O'Dell et al., 2004). Genomic studies have correlated patterns of gene expression following CIE with coincident changes in ethanol consumption (Osterndorff-Kahanek, Ponomarev, Blednov, & Harris, 2013). For example, recent CIE studies on brain derived neurotrophic factor (BDNF) signaling events and miRNA regulatory mechanisms hint at one elegant potential mechanism for neuro-adaptation to chronic ethanol (Darcq et al., 2015; Logrip, Janak, & Ron, 2009; Smith et al., 2016). The CIE model provides a powerful tool for discovery and hypothesis testing of processes and mechanisms underlying progressive ethanol consumption. However, to identify more causal links between CIE behavioral adaptations and specific molecular mechanisms, we combined the CIE model with both a genetic and genomic analysis. Such a “genetical genomics” approach has been widely used with other genetic studies on ethanol behaviors (Hitzemann et al., 2014; Tabakoff et al., 2009), but only rarely with a matched analysis of ethanol-evoked changes in gene expression (Putman et al., 2016; Wolen et al., 2012).

Here, we report an initial analysis of CIE influences on ethanol consumption and gene expression changes in two brain regions of mice from the BXD recombinant inbred panel. Our results identify significant similarities and differences between acute and CIE genomic responses to ethanol. We characterized the functional groups associated with CIE genomic responses and highlight possible genetic intervals crucial for both CIE-evoked changes in gene expression and ethanol consumption. Our findings highlight the promise of this integrated behavioral, genetic and genomic analysis of CIE and suggest future work that may identify novel targets for therapeutic development in AUD.

2. Materials and methods

2.1. Animals

Male and female C57BL/6J and DBA/2J mice for CIE experiments were purchased from Jackson Laboratory at 10 weeks old (Bar Harbor, ME). After 1 week acclimation to the animal facility, mice were singly housed for 72 h prior to the drinking experiments. Male and female BXD RI strains for CIE (n = 43 strains) at 12–16 weeks old were supplied by the University of Tennessee Health Sciences Center (Memphis, TN). BXD mice were single housed immediately, and began drinking experiments after 72 h acclimation to single housing. Please refer to Supplemental Table 1 and Lopez, Miles, Williams, and Becker (2016) in this Special Issue for additional details regarding strain number and attrition during the CIE protocol. All mice were housed individual in an AALAC-accredited facility under 12 h light/dark cycles with free access to food and water. All animal housing and care was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Council, 2011).

2.2. Chronic intermittent ethanol (CIE)

Chronic intermittent ethanol procedures were performed at Medical University of South Carolina with approval by the Institutional Animal Care and Use Committee, according to well-established procedures shown to cause increased ethanol consumption (Lopez & Becker, 2005). Since the CIE model is a complex and time consuming behavioral analysis, as an initial genetic analysis of CIE-evoked behaviors and gene expression alterations, we utilized a design where single animals per strain/treatment were used for most strains to maximize the number of strains for genetic and genomic analysis. After 6 weeks of limited access (2 h/day) baseline drinking with 2-bottle choice 15% v/v ethanol and water, mice (n = 119) representing 43 BXD RI strains and progenitors were divided into two groups: CIE and control. CIE mice received ethanol vapor in Plexiglass inhalation chambers (60 × 36 × 60 cm) for 16 h/day for 4 days. Control mice were also placed in the inhalation chambers for 16 h/day for 4 days, but did not receive ethanol vapor. After 4 days in the inhalation chamber, mice underwent 72 h of complete ethanol abstinence, followed by 5 days limited access drinking (2-bottle choice 15% v/v ethanol and water, 2 h/day) (Lopez & Becker, 2005). This cycle was repeated such that BXD mice underwent 4 sessions of inter-cycle ethanol consumption and 5 sessions of inhalation chamber exposure. Ethanol levels in the inhalation chambers were set to produce blood ethanol concentrations of 200–300 mg/dl. Prior to each vapor chamber session, mice were injected intraperitoneally with 1 mmol/kg pyrazole, an alcohol dehydrogenase inhibitor used to stabilize blood ethanol concentration. Blood was collected from mice after the third day in the inhalation chamber during each inhalation chamber cycle. Mice were sacrificed 72 h after the 5th inhalation chamber session. A schematic of the CIE protocol employed here is given in Fig. 1. A table of the BXD strains used in this analysis is included in Supplemental Table 1.

2.3. Tissue harvesting and RNA isolation

Surviving mice (n = 72) were sacrificed 72 h after CIE procedures by decapitation. Brains were immediately removed, and specific regions dissected using a brain punch micro-dissection and snap frozen in liquid nitrogen. Tissue samples were shipped on dry ice to Virginia Commonwealth University, and stored at −80 °C until RNA isolation. Total RNA was isolated from prefrontal cortex (PFC) and nucleus accumbens (NAC), as described (Kerns et al.,