Alcohol 58 (2017) 73–82

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Variable effects of chronic intermittent ethanol exposure on ethanol drinking in a genetically diverse mouse cohort

Marcelo F. Lopez [a, *], Michael F. Miles [d, e, f], Robert W. Williams [g], Howard C. Becker [a, b, c]

[a] Charleston Alcohol Research Center, Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC 29425, USA
[b] Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425, USA
[c] RHJ Department of Veterans Affairs Medical Center, Charleston, SC 29425, USA
[d] Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA
[e] Department of Neurology, Virginia Commonwealth University, Richmond, VA 23298, USA
[f] Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA 23298, USA
[g] Department of Genetics, Genomics and Informatics, University of Tennessee Health Science Center, Memphis, TN, USA

ARTICLE INFO

Article history:
Received 29 March 2016
Received in revised form
5 September 2016
Accepted 21 September 2016

Keywords:
Ethanol dependence
Voluntary ethanol intake
Recombinant inbred BXD mice

ABSTRACT

The BXD family of mice were generated by crossing and inbreeding ethanol-preferring C57BL/6J and ethanol-avoiding DBA/2J strains that differ greatly in genome sequence and other behaviors. This study evaluated variations in the level of voluntary ethanol intake in a cohort of 42 BXD strains and both progenitor strains using a model of alcohol dependence and relapse drinking. A total of 119 BXDs (85 males, 34 females) (n ~ 4 per genotype; 1/genotype/sex/group) were evaluated along with males from both progenitor strains (n = 14–15/genotype). Mice were evaluated for intake using limited access (2 h/day) 2-bottle (15% v/v ethanol vs. water) model for 6 weeks (baseline intake). Each animal received 4 weekly cycles of chronic intermittent ethanol (CIE) vapor exposure (CIE group) or air control exposure (CTL group) (16 h/day × 4 days) interleaved by 5-day drinking test cycles. Blood ethanol concentrations (BEC) ranged from 150 to 300 mg/dl across genotypes. Baseline intake varied greatly among cases—from ~0.8 to ~2.9 g/kg. As expected, CIE exposure induced a significant increase in ethanol drinking in C57BL/6J relative to baseline as well as air controls that remained relatively stable over the four test cycles. In contrast, DBA/2J cases did not show a significant increase in consumption. Heritability of variation in baseline consumption, calculated from C57BL/6J and DBA/2J strains is about 54% but this increases following treatment to 60–80%. As expected from the marked difference between progenitors, ethanol intake and level of escalation varied greatly among BXDs after exposure (~−1.3 to 2.6 g/kg). Interestingly, the magnitude and direction of changes in ethanol intake did not relate to BEC values of the preceding CIE exposure cycle. Overall, these data indicate significant variation in consumption and even escalation, much of it under genetic control, following repeated CIE treatment.

1. Introduction

There is considerable evidence regarding genetic influence on alcohol use disorders (Cloninger, 1987; Dick & Foroud, 2002; Kendler, Aggen, Prescott, Crabbe, & Neale, 2012; Merikangas et al., 1998). Understanding the basis of individual differences in response to alcohol effects, tolerance or sensitivity, and risk for development of dependence are critical to better understand alcoholism and to implement preventive measures and develop appropriate treatment. For example, sensitivity to adverse (e.g., sedative) effects of alcohol intoxication can influence the amount of alcohol consumed and the risk of developing future alcohol abuse and dependence. Subjects with a positive family history for alcoholism have been found to show more subjective stimulation and less sedation that can also predict later alcohol problems (King, Houle, de Wit, Holdstock, & Schuster, 2002; Quinn & Fromme, 2011; Schuckit & Smith, 2000).

Animal models have been extremely valuable in advancing the study of the role of genetic factors in alcoholism. Some studies have used lines of rats or mice that, starting with outbred subjects, were

* Corresponding author. Charleston Alcohol Research Center, Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, 67 President St., MSC 861, Charleston, SC 29425, USA.
E-mail address: lopezm@musc.edu (M.F. Lopez).

http://dx.doi.org/10.1016/j.alcohol.2016.09.003
0741-8329/

created through selected breeding based on their alcohol (ethanol) preference (P, NP rats), level of intake (HAD, LAD rats; HAP, LAP mice), acute response to ethanol (FAST, SLOW mice), drinking to intoxication (HDID mice), ethanol withdrawal sensitivity (WSP, WSR mice), to name a few (Crabbe, Harris, & Koob, 2011; Crabbe, Phillips, & Belknap, 2010). Interestingly, in some cases the selection for a particular trait, for example high ethanol preference, also results in a change in other parameters that favor ethanol intake such as tolerance to alcohol's sedative effects (Crabbe et al., 2010). This suggests that genetic influences for various alcohol-related phenotypes may be related, thereby providing clues about common mechanisms underlying increased risk for alcohol abuse (Crabbe et al., 2010, 2012; Cunningham et al., 1991; Metten et al., 1998; Risinger, Malott, Prather, Niehus, & Cunningham, 1994). An alternative approach is the direct manipulation of a gene or groups of genes of interest to evaluate its impact on alcohol intake (Crabbe, Phillips, Harris, Arends, & Koob, 2006; Crabbe et al., 2010).

The use of inbred strains of mice has also helped evaluate different genetic components of ethanol effects and intake. While C57BL/6J has been characterized as an ethanol preferring strain, the DBA/2J strain has been characterized as ethanol avoiding based on their voluntary ethanol intake levels (Belknap, Crabbe, & Young, 1993; Crabbe, Young, & Kosobud, 1983). These mouse strains also differ in other ethanol-related effects. For example, DBA/2J mice are more sensitive than C57BL/6J mice to behavioral and physiological manifestations of ethanol withdrawal (Metten et al., 1998). The BXD recombinant inbred strains were generated by inbreeding F2 generation subjects obtained by crossing ethanol-preferring C57BL/6J (B) and ethanol avoiding DBA/2J (D), The BXD progeny strain inherits stretches of DNA from either the B or D parent, and each strain has its own unique and fixed pattern of B and D genotypes across the entire genome. The BXDs were originally developed in the 1970s with around 30 RI lines and more recently expanded to ~150 RI strains (Peirce, Lu, Gu, Silver, & Williams, 2004; Taylor, 1978; Wang et al., 2016), and are now used to map and define gene variants that control a wide range of traits (Houtkooper et al., 2013; Wang et al., 2016). BXD strains have been evaluated along their progenitor strains for voluntary ethanol intake (Gill, Liu, & Deitrich, 1996; Phillips, Crabbe, Metten, & Belknap, 1994; Rodriguez, Plomin, Blizard, Jones, & McClearn, 1994, 1995), ethanol-induced conditioned place preference (Risinger & Cunningham, 1998), and acute response to ethanol and withdrawal from chronic ethanol exposure (Buck, Rademacher, Metten, & Crabbe, 2002; Metten et al., 1998; Philip et al., 2010; Phillips et al., 1994; Putman et al., 2016). Thus, this resource has been very valuable for examining genetic contributions to various pharmacological effects of ethanol and motivation effects of ethanol (Crabbe et al., 1983; Philip et al., 2010; Phillips et al., 1994).

Studies conducted with rodents have demonstrated that dependence results in escalation of voluntary ethanol drinking (Becker, 2013). In most studies, dependence was produced via chronic intermittent ethanol (CIE) and ethanol consumption was measured in the home cage. Using this approach, a mouse model of ethanol dependence and relapse drinking that involves repeated cycles of CIE exposure and demonstrates escalation of voluntary ethanol drinking has been developed using male C57BL/6J mice (Becker & Lopez, 2004; Griffin, Lopez, & Becker, 2009; Lopez & Becker, 2005). This escalation of drinking produced more than a 2-fold increase in blood ethanol levels and brain ethanol concentrations (Becker & Lopez, 2004; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009). Further, increased consumption appears specific to ethanol because CIE exposure did not alter intake of sucrose or saccharin (Becker & Lopez, 2004; Lopez, Griffin, Melendez, & Becker, 2012). CIE exposed mice also show tolerance to ethanol's aversive effects (Lopez et al., 2012) and are less sensitive to devaluation of ethanol's reinforcing effect (Lopez, Becker, & Chandler, 2014), which can help maintaining higher levels of voluntary ethanol intake.

Few studies have examined genetic factors that may influence this CIE-induced escalation of voluntary ethanol intake. Indeed, most of the above mentioned studies were conducted using the C57BL/6J strain. The study presented here is a first attempt to explore the influence of genetic background on ethanol consumption both prior to and during the course of CIE exposure. Specifically, a panel of BXD strains were used to evaluate baseline ethanol intake and changes in intake after repeated cycles of CIE (or air control) exposure under a limited access free-choice drinking paradigm. The study also included male C57BL/6J mice that served as a positive control, since this model of ethanol dependence and relapse drinking was developed using these mice. This initial foray into the genetics of CIE escalation was designed in collaboration with researchers of the NIAAA-funded INIA-Stress consortium to generate not only critical phenotype data (ethanol intake, blood ethanol levels during CIE exposure), but also to provide tissue to generate endocrine, neurochemical and genetic/genomic parameters for these BXD strains.

We have intentionally limited resampling within single strains to a bare minimum (usually two cases per strain) in the interest of screening larger numbers of diverse genotypes. This is the polar extreme of studying only one or two strains (C57BL/6J and DBA/2J) in great depth. Our approach provides a good view of the range of variation across a genetically diverse family but of course, does not provide precise estimate of strain averages. In contrast, the analysis of a single strain provides much more accurate data for one genotype, but results will often not generalize well. In this study, we generally do not make claims about phenotypes of individual BXD stains, but we can compute heritability of the consequences of CIE using the well replicated data from the progenitor strains and we can also estimate general effects of sex on CIE-associated traits.

2. Methods

2.1. Subjects

A total of 119 mice (85 males and 34 females) were used in this study. Mice representing 42 BXD genotypes, D2B6F1 hybrids (DBA/2J females crossed with C57BL/6J males) as well as both progenitor strains (C57BL/6 and DBA/2) were included in the design of the study. All of these mice were obtained from University of Tennessee (UT) and were 12–16 weeks old upon arrival. The mice were kept in quarantine for 3 weeks before use. Adult (10 weeks old upon arrival) C57BL/6J and DBA/2J stock obtained from The Jackson Laboratory (Bar Harbor, ME) served as positive and negative progenitor control (N = 8/group; see Table 1). The general study design typically involved the use of 4–8 more cases per genotype, and 1–2 cases per experimental cell as defined by genotype, sex, and group (CIE, CTL). This design does not allow us to accurately define means per strain and treatment (except for the progenitors). We used data from both progenitor strains and both sexes to estimate heritability under precisely the same conditions used to treat all BXD cases. It is reasonable to assume the heritabilities computed from contrast of the progenitors will also apply to their inbred progeny. Mice were individually housed with free access to food (Harland Teklad, Madison, WI) and water throughout all phases of the experiments. Body weights were recorded weekly during ethanol drinking weeks or daily during chronic intermittent ethanol (CIE) or air exposure (detailed below). Mice were housed in a temperature and humidity-controlled animal facility under a reversed 12-hr light/dark cycle (lights on at 0200 h). Mice were not food or water deprived at any time during the study. All procedures were