Alcohol 47 (2013) 381–389

Contents lists available at SciVerse ScienceDirect

Alcohol

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

Ethanol drinking in Withdrawal Seizure-Prone and -Resistant selected mouse lines

John C. Crabbe [a,b,*], Stephanie E. Spence [a,b], Lawrence C. Huang [a,b], Andy J. Cameron [a,b], Jason P. Schlumbohm [a,b], Amanda M. Barkley-Levenson [a,b], Pamela Metten [a,b]

[a] Portland Alcohol Research Center, Veterans Affairs Medical Center, Oregon Health & Science University, Portland, OR 97239, USA
[b] Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239, USA

ARTICLE INFO

Article history:
Received 8 April 2013
Received in revised form
7 May 2013
Accepted 8 May 2013

Keywords:
Ethanol withdrawal
Selective breeding
Ethanol preference
Drinking in the dark
Mouse
Genetics

ABSTRACT

Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mouse lines were bidirectionally selectively bred, respectively, to have severe or mild ethanol withdrawal handling-induced convulsions (HICs) after cessation of 3 days of ethanol vapor inhalation. Murine genotypes with severe withdrawal have been found to show low ethanol consumption, and high consumers show low withdrawal. An early drinking study with WSP and WSR mice showed modest evidence consistent with this genetic correlation, but there were several limitations to that experiment. We therefore conducted a thorough assessment of two bottle ethanol preference drinking in both replicate pairs of WSP/WSR selected lines in mice of both sexes. Greater preference drinking of WSR-2 than WSP-2 female mice confirmed the earlier report. However, in the parallel set of selected lines, the WSP-1 mice drank more than the WSR-1s. Naive mice tested for preference for sucrose, saccharin and quinine did not differ markedly for any tastant. Finally, in a test of binge-like drinking, Drinking in the Dark (DID), WSP mice drank more than WSR mice and attained significantly higher (but still modest) blood ethanol concentrations. Tests of acute withdrawal after DID showed a mild, but significant elevation in handling-induced convulsions in the WSP line. These results provide further evidence that 2-bottle ethanol preference and DID are genetically distinguishable traits.

Published by Elsevier Inc.

Introduction

Mice show substantial genetic differences in ethanol withdrawal severity as indicated by handling-induced convulsions (HICs) after ethanol administration ceases. For example, inbred strains differ markedly in HIC severity after exposure to ethanol vapor for 72 h (Metten & Crabbe, 2005) or after periods of intermittent ethanol vapor exposure (Metten, Sorensen, Cameron, Yu, & Crabbe, 2010). The strain differences cannot be explained by differences in alcohol metabolism, because each strain was exposed to ethanol vapor concentrations designed to result in equivalent blood ethanol concentrations (BECs). Strains also differ in HIC severity following an acute, anesthetic dose of ethanol (Metten & Crabbe, 1994), and recombinant inbred strains derived from the intercross of the high-withdrawal DBA/2J and low-withdrawal C57BL/6J inbred strains show a range of acute (Buck, Metten, Belknap, & Crabbe, 1997) and chronic (Crabbe, 1998) withdrawal HIC scores.

Murine genotypes also show pronounced differences in willingness to ingest ethanol solutions offered under a variety of conditions. C57BL/6J (B6) mice are well-established high drinkers (Wahlsten, Bachmanov, Finn, & Crabbe, 2006) but show modest withdrawal HICs, while DBA/2J (D2) mice are abstainers, but show severe withdrawal HICs (Metten & Crabbe, 2005; Wahlsten et al., 2006). A review of several studies with populations of mice derived from B6 and D2 intercrosses reported a consistent, substantial negative genetic correlation between g/kg intake of ethanol in a two bottle preference test for 10% ethanol vs. water and severity of acute or chronic withdrawal HICs (Metten et al., 1998). Pooled across 6 experiments using B6/D2-derived populations, the mean genetic correlation was $r = -0.39$ [two-tailed $p = 3 \times 10^{-5}$; (Metten et al., 1998)]. However, the negative correlation was less striking in populations derived from multiple genotypes, including other inbred strains. This finding suggested an important role of alleles from the B6 and D2 lineages, but left open the possibility that some other genotypes might also show the inverse relationship between drinking and withdrawal.

In 1985, we initiated a long-term, replicated selective breeding project to create mouse lines bred for severe (Withdrawal Seizure-Prone; WSP) or mild (Withdrawal Seizure-Resistant; WSR) withdrawal, based on HIC scores following 3 days of ethanol vapor

* Corresponding author. Portland VA Medical Center, Research (R&D 12), 3710 SW US Veterans Hospital Road, Portland, OR 97239, USA. Tel.: +1 503 273 5298; fax: +1 503 273 5851.
E-mail address: crabbe@ohsu.edu (J.C. Crabbe).

0741-8329/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.alcohol.2013.05.002

inhalation (Crabbe, Kosobud, Young, Tam, & McSwigan, 1985). These lines were selected from HS/Ibg, a genetically segregating heterogeneous stock derived from systematic intercrosses of 8 inbred strains, including B6 and D2 (McClearn, Wilson, & Meredith, 1970). Female mice from the 17th and 19th selected generation of WSP-1, WSP-2, WSR-1, and WSR-2 and both unselected control lines (WSC-1 and WSC-2) were tested in two different ethanol preference drinking paradigms where water was always offered as an alternative. We found that WSR mice generally drank more ethanol than WSP, consistent with the negative genetic correlation in B6/D2 populations, although the pattern of drinking depended upon the paradigm employed and varied somewhat over time (Kosobud, Bodor, & Crabbe, 1988). However, there were several features of these early drinking studies that were not optimal: the relatively short duration of the test; no examination of male mice; the use of mice for one experiment that had previous experience with an ethanol solution; and the use of an unusual drinking protocol in the other experiment. Furthermore, WSP/WSR mice were subsequently directionally selected through generation 26 (S_{26}), and many more generations have since ensued under relaxed selection, allowing for the possible effects of genetic drift to accumulate, which could have changed the pattern of correlated responses to selection in these lines (Falconer & Mackay, 1996). Therefore, in the current experiments, we systematically examined two bottle ethanol preference drinking across a range of concentrations using what has become our standard preference drinking protocol (Phillips, Crabbe, Metten, & Belknap, 1994). We tested additional, naive mice for their taste sensitivity and preference for sucrose, saccharin and quinine solutions. Finally, we tested naive mice using a relatively new form of binge-like ethanol intake called drinking in the dark (DID; Rhodes, Best, Belknap, Finn, & Crabbe, 2005).

Experimental procedures

We have previously published a detailed description of our animal husbandry and colony procedures, as well as of the ethanol preference and tastant drinking protocols we employed for Experiments 1 and 2 (Crabbe, Spence, Brown, & Metten, 2011). The reader is referred to that paper for details, and a summary of the methods is presented below.

Animals and husbandry

Mice from the Withdrawal Seizure-Prone (WSP-1 and -2) and -Resistant (WSR-1 and -2) selected lines were bred in our colonies in the Portland VA Veterinary Medical Unit. All mice were naive at the beginning of each experiment and were from the 26th selected generation and filial generations ranged from 98 to 127 (e.g., $S_{26}G_{98}$). These two pairs of replicate lines have been maintained without selection pressure using a rotational, within-family mating scheme with 9–27 breeding pairs/generation of animals since selection ceased at S_{26}. The lines differed at least 10-fold in chronic ethanol withdrawal severity after 11 selected generations (Crabbe et al., 1985), and periodic comparisons have shown no decline in the magnitude of withdrawal differences between WSP and WSR lines [(Phillips, Feller, & Crabbe, 1989) and unpublished data]. All mice were between 50 and 98 days old at the start of testing.

Mice were maintained in standard plastic cages on Bed-o-cob bedding (Andersons, Maumee, OH, USA) with stainless steel wire bar tops with a recess for chow. Rodent chow 5001 (PMI Nutrition International, Brentwood, MO, USA) and tap water were available *ad libitum* and colonies and testing rooms were maintained on a 12 h:12 h light:dark schedule at a temperature of 21 ± 1 °C. Two weeks before the start of an experiment, mice were transferred to a procedure room with the same environmental conditions and were individually housed. Animals in Experiment 3 were acclimated during this time to a reversed light:dark schedule of 21:30 lights on: 09:30 lights out. All procedures were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee and were performed according to NIH Guidelines for the Care and Use of Laboratory Animals.

Experiment 1: two bottle ethanol consumption and preference

Sixty-nine mice were tested ($n = 7$–10 per selected line, replication, and sex), using our standard method (Phillips et al., 1994). The water bottle was replaced with two 25 ml graduated cylinders with stainless steel drinking spouts, both containing tap water, for two days. During the next 16 days, one cylinder contained tap water and the other an ethanol solution. The preference test commenced with 3% ethanol (v/v) in tap water on the left side vs. water on the right side. Twenty-four hours later, intake was recorded, and the bottles were left in place until 48 h. Ethanol (Decon Laboratories, Inc., King of Prussia, PA) and water cylinder positions were then switched. Mice were exposed to ethanol vs. water for 16 days, 4 days each at 3%, 6%, 10%, and 20%, with daily readings and positions switched each 48 h. Body weights were taken the day the experiment started, and at every concentration switch. Two spillage control cages with fluids (but without mice) were used, one at each end of the rack.

Each day's data were first corrected by subtracting the average loss of each fluid from the two control cages. We computed consumption (g ethanol/kg body weight) and preference ratio (volume from the ethanol tube/total fluid volume consumed from ethanol + water). We also report water (or total fluid) consumption and body weight. Data from the occasional leaking tubes were treated as missing, as described in detail elsewhere (Crabbe et al., 2011).

Experiment 2: tastant preference

Sixty-four naive mice were tested ($n = 6$–10 per selected line, replication, and sex) using the same procedures described for Experiment 1. Mice were serially offered three tastants (dissolved in tap water) vs. tap water for 24 days. Each tastant was offered for 8 days, first at a low and then at a higher concentration. Tastants and concentrations, in the order of presentation, were: quinine hemisulfate salt monohydrate (Sigma–Aldrich, St. Louis, MO), at 0.1 mM (0.004%) and then 0.8 mM (0.032%); saccharin sodium salt hydrate (Sigma–Aldrich, St. Louis, MO) at 3.2 mM (0.066%) and then 10 mM (0.21%); and sucrose (Fisher Scientific, Pittsburgh, PA) at 49.7 mM (1.7%) and then 124 mM (4.25%). Six days of water only drinking were given between each tastant. All procedures were performed as in Experiment 1, and data were treated as described for Experiment 1. Only preference ratios were analyzed (see Results section).

Experiment 3: drinking in the dark (DID)

Eighty-five naive mice were tested ($n = 10$–13 per selected line, replication, and sex). We used the 4 day DID test originally described in Rhodes et al. (2005). Details of the apparatus and procedure are available in http://www.scripps.edu/cnad/inia/modelmousedrinkingindark.pdf. Mice were individually housed and placed on a reversed light–dark cycle for two weeks. All subsequent procedures were performed under red light. Mice were weighed and scored for baseline handling-induced convulsions (see below) 4 h before the start of the drinking test. Starting 3 h after lights off, each water bottle was replaced with a single 10 ml stoppered Falcon disposable clear polystyrene serological pipet (Fisher Scientific) filled with a 20% (v/v) ethanol solution fit with