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Genotypic and sex differences in anxiety-like behavior and alcohol-induced anxiolysis in High Drinking in the Dark selected mice

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ABSTRACT

Alcohol use disorders and anxiety disorders are highly comorbid in humans. In rodent lines selected for alcohol drinking, differences in anxiety-like behavior are also seen. The High Drinking in the Dark (HDID) lines of mice are selectively bred for drinking to intoxication during limited access to alcohol, and these mice represent a genetic model of risk for binge-like drinking. The present studies investigated whether these selected lines differ from control (HS) mice in basal anxiety behavior or in anxiolytic response to alcohol. We also assessed the genetic correlation between alcohol drinking in the dark (DID) and basal anxiety-like behavior using existing inbred strain data. Mice of both sexes and HDID replicates (HDID-1 and HDID-2) were tested on an elevated zero maze immediately following a DID test. In general, HDID mice showed more time spent in the open arms after drinking alcohol than HS mice, and open-arm time was significantly correlated with blood alcohol concentration. HDID-1 male mice also showed less anxiety-like behavior at baseline (water-drinking controls). In a separate experiment, HDID-1 and HS mice were tested for anxiolytic dose-response to acute alcohol injections. Both genotypes showed increasing time spent in the open arms with increasing alcohol doses, and HDID-1 and female mice had greater open-arm time across all doses. HDID-1 control males showed lower anxiety-like behavior than the HS control males. Inbred strain data analysis also showed no significant genetic relationship between alcohol DID and anxiety. These findings suggest that HDID selection has not produced systematic changes in anxiety-like behavior or sensitivity to alcohol-induced anxiolysis, though there is a tendency in the male mice of the first replicate toward reduced basal anxiety-like behavior. Therefore, anxiety state and sensitivity to alcohol's anxiolytic effects do not appear to contribute significantly to the high drinking behavior of the HDID mice.

Introduction

Alcohol use disorders (AUDs) and anxiety disorders have been shown to have a high degree of comorbidity (for review, see Kushner, Abrams, & Borchardt, 2000; Smith & Randall, 2012). Two broad hypotheses that are not mutually exclusive might account for this. Alcohol has significant anxiolytic effects (e.g., Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008), and there is evidence that higher basal anxiety may promote greater alcohol intake which can lead to abuse (Bolton, Cox, Clara, & Sareen, 2006). Another possibility is that anxiety disorders and AUDs may share some of the same underlying genetic risk factors. Family and twin studies have suggested possible common transmission of both anxiety disorders and AUDs, which may represent shared genetic risk (e.g., Merikangas, Risch, & Weissman, 1994; Tambs, Harris, & Magnus, 1997).

There is also evidence from the animal literature to suggest a relationship between anxiety and alcohol consumption. Rats classified as anxious by performance on an elevated plus maze (EPM) voluntarily drink more alcohol in a subsequent test than those classified as non-anxious (Spanagel et al., 1995). Similarly, some rodent lines selected for high vs. low alcohol preference also show innate differences in basal anxiety and sensitivity to alcohol-induced anxiolysis (Colombo et al., 1995; Stewart, Gatto, Lumeng,

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Li, & Murphy, 1993). These anxiety-related behaviors appear to be correlated responses to selection for high alcohol intake in these lines. However, this relationship is not seen for all rodent lines selected for high vs. low drinking, with some lines showing an opposite relationship or no relationship with drinking (Can, Grahame, & Gould, 2012; Sandbak, Murison, Sarviharju, & Hyytiä, 1998). Another way of examining this relationship is by testing animals selected for anxiety-like behavior for alcohol drinking. Rats selectively bred for high (HAB) or low (LAB) anxiety-related behaviors on an EPM show differences in alcohol preference in a 2-bottle choice test (Henniger, Spanagel, Wigger, Landgraf, & Hölter, 2002). LAB rats have a greater alcohol preference than the HAB rats, but the anxiolytic effect of an injection of alcohol is greater in the HAB rats. Consequently, the possible genetic relationship between alcohol intake and anxiety appears to be complex for both alcohol- and anxiety-related selection phenotypes.

With the exception of the study by Can and colleagues using mice, most of the previous work involves rat lines, and all previous studies have used animals either selected for, or tested on, 2-bottle choice alcohol preference drinking. In the present experiments, we sought to extend these findings to another model animal species and a test of binge-like drinking by determining the relationship between anxiety-like behavior and alcohol drinking in mice selectively bred for drinking to intoxication. The HDID lines of mice were selected for high blood alcohol (ethanol) concentration (BEC) after drinking in the dark (DID), and routinely drink to intoxicating blood levels in a limited-access test (Crabbe et al., 2009, 2014). These mice have been extensively behaviorally phenotyped to determine correlated responses to selection and possible factors promoting their high drinking (for review, see Barkley-Levenson & Crabbe, 2014). Here, we tested whether drinking during the DID test is sufficient to produce alcohol-induced anxiolysis, and whether differences in anxiolytic response to alcohol or basal anxiety may underlie the high drinking phenotype of HDID mice. We also used existing inbred mouse strain data sets to assess the genetic relationship between anxiety-like behavior and alcohol DID.

Materials and methods

Animals and husbandry

Male and female mice of the HDID-1, HDID-2, and HS lines were bred and housed in the Veterinary Medical Unit at the Veterans Affairs Medical Center (Portland, OR, USA). All mice were between 51 and 80 days of age and were experimentally naïve at the start of testing. Mice received *ad libitum* access to food (Purina 5001 chow, LabDiet, St. Louis, MO) and water unless otherwise specified. HDID-1 mice from the 22nd and 27th selection generations were used in Experiment 1 and mice from the 23rd and 28th selection generation were used in Experiment 2. HDID-2 mice from the 19th selection generation were used in Experiment 1. HS/Npt (HS) mice are the starting population from which the HDID lines were selected and are the product of a systematic 8-way inbred strain cross (see Crabbe et al., 2009 for details). These mice are not subjected to selective pressure and represent a genetically heterogenous population used as a comparator control for the HDID lines. For both Experiments 1 and 2, mice were tested in multiple passes (replicate experiments), with some or all of the sexes and genotypes included in each pass. For Experiment 1, all mice were kept on a 12-h/12-h reverse light/dark cycle with lights off at 9:30 AM. For Experiment 2, one pass of mice was kept on a 12-h/12-h forward light/dark cycle with lights on at 6:00 AM, and a second pass of mice was kept on a reverse light/dark cycle with lights off at 10:30 AM. Both groups were tested at approximately the same time during their circadian light phase, as our laboratory and most others routinely test anxiety-like behavior during the light cycle. All procedures were approved by the local Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Experiment 1: anxiety-like behavior after DID

Seventy-nine male and female mice of the HDID-1, HDID-2, and HS lines were used in this study (n = 6–9/line/sex/group). Mice were tested in 4 passes, with mice of all sexes and genotypes used in each pass except that only female HDID-1 mice were tested in the first pass. At the beginning of the experiment, mice were singly housed and habituated to reverse light/dark for 2 weeks. During this time, mice were given water from polycarbonate bottles with stainless steel sipper tubes attached. After the acclimation period, mice were given a modified version of our standard 2-day DID test. The 2-day DID was chosen because this is the test used in our selection procedure and we were interested in whether alcohol-induced anxiolysis is experienced by these mice under conditions comparable to HDID selection. The DID test is described in detail elsewhere (Crabbe et al., 2009). Briefly, 2–3.5 h after lights off, water bottles were removed and replaced with 10-mL graduated cylinders fitted with stainless steel ball-bearing sipper tubes containing either 20% alcohol or water depending on group assignment. Start times were staggered by 10-min intervals for every 2 mice to allow for testing on the elevated zero maze (EZM) immediately after drinking on the second day. At the start of the drinking session, fluid levels were recorded and tubes were left in place for 2 h. After 2 h, fluid levels were recorded again and tubes were removed and water bottles were returned to the cages. The next day, the procedure was repeated identically except that tubes were left in place for 4 h. At the end of the 4 h, mice were tested on the EZM in squads of 2 (one mouse per maze) for 5 min. Immediately following the EZM test, a 20 μL blood sample was taken from the retro-orbital sinus of each alcohol-group mouse to determine BEC.

Experiment 2: dose–response to alcohol-induced anxiolysis

One hundred thirty-three male and female HDID-1 and HS mice (n = 5–10/line/sex/dose) were used in this experiment. Male mice were tested in two passes, and female mice were tested in a single pass. Prior to the start of testing, mice were pseudorandomly assigned to a dose group (saline, 0.5, 1, or 1.5 g/kg alcohol). Behavioral testing in Experiment 2 started at approximately 3 h after lights on for both passes. On the day of testing, mice were moved into the procedure room, weighed, and allowed to habituate for 1 h. Mice were then injected intraperitoneally (i.p.) in squads of 2 with saline or the appropriate dose of alcohol as determined by group assignment. Mice were placed in individual holding cages for 10 min and then given a 5-min test on the EZM.

Experiment 3: genetic correlation of alcohol DID and basal anxiety-like behavior in inbred strains

Our laboratory has previously published 4-day DID consumption data for 23 inbred mouse strains (Crabbe et al., 2012). Intake data for 2-day DID are not presently available for all of these strains, so the 4-day DID data were used for the correlational analysis. In order to correlate DID intake with anxiety-like behavior, inbred strain data were mined from extant data sets in the lab and those available on the Mouse Phenome Database (MPD, The Jackson Laboratory, http://phenome.jax.org/). Criteria for selection of anxiety data were: at least 10 strains in common with the DID data set, inclusion of both males and females, and a measure of anxiety-like behavior