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High Drinking in the Dark Mice: A genetic model of drinking to intoxication

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ABSTRACT

Drinking to intoxication is a critical component of risky drinking behaviors in humans, such as binge drinking. Previous rodent models of alcohol consumption largely failed to demonstrate that animals were patterning drinking in such a way as to experience intoxication. Therefore, few rodent models of binge-like drinking and no specifically genetic models were available to study possible predisposing genes. The High Drinking in the Dark (HDID) selective breeding project was started to help fill this void, with HDID mice selected for reaching high blood alcohol levels in a limited access procedure. HDID mice now represent a genetic model of drinking to intoxication and can be used to help answer questions regarding predisposition toward this trait as well as potential correlated responses. They should also prove useful for the eventual development of better therapeutic strategies.

Introduction

Binge drinking and drinking to intoxication are frequent elements of human alcohol use disorders (AUDs). Furthermore, frequent binge drinking can have deleterious effects and can be a risk factor for future development of problem drinking or AUDs (Courtney & Polich, 2009; Viner & Taylor, 2007). Binge drinking as defined by the National Institute on Alcohol Abuse and Alcoholism is a pattern of drinking that results in blood alcohol concentrations (BACs) at or above the legal limit (0.80 mg/mL) (DHHS-NIH, 2004). This pattern is generally considered to be more than 4 standard drinks in 2 h for women, and more than 5 drinks in 2 h for men. Consequently, there are both a quantity component and a temporal component characterizing this binge type of intake.

In animal research of AUDs, we have long harnessed the power of behavioral genetics to develop rodent models of various aspects of alcoholism. Specifically, mice and rats have been tested for their willingness to voluntarily drink an alcohol solution when it is accessible in the home cage. The most frequent procedure has been a choice between an alcohol solution and water alone, presented continuously across days. These 2-bottle choice tests allow for the determination of a possible preference for alcohol over water, and numerous lines of mice and rats have been selectively bred for a high or low alcohol preference in this paradigm (for review see Crabbe, Phillips, & Belknap, 2010). Inbred mouse strains show a variety of intakes in these tests (Wahlsten, Bachmanov, Finn, & Crabbe, 2006; Yoneyama, Crabbe, Ford, Murillo, & Finn, 2008), and the natural inclination of some inbred strains to drink large amounts of alcohol (e.g., C57BL/6J [B6]) or to mostly abstain (e.g., DBA/2J [D2]), has been employed extensively for studying biological and genetic factors that influence drinking (Belknap & Atkins, 2001; Blizard, 2007; Rosenwasser & Fixaris, 2013; Shelton & Grant, 2002).

One issue of 2-bottle choice procedures is that drinking in these tests does not necessarily result in pharmacologically relevant BACs (Dole & Gentry, 1984; but see Matson & Grahame, 2011). Consequently, rodents engaged in this type of drinking may never actually experience intoxicating blood alcohol levels, which is certainly not true of humans who drink heavily or have an AUD. The drinking in the dark (DID) test is a limited access drinking procedure that results in high BACs in B6 mice (Rhodes, Best, Belknap, Finn, & Crabbe, 2005, and see the article by Thiele in this issue). This test capitalizes on the natural consummatory patterns of rodents by testing mice during the period of the dark cycle when ingestive behaviors are greatest. Briefly, 3 h into the dark phase of the light–dark cycle, the water bottle is removed and is replaced by access to a 20% alcohol solution. Alcohol bottles are left in place for 2 h on the initial day(s) and 4 h on the final day. After the 4 h of drinking on the final day, BACs are assessed. In B6 mice, these blood levels have been shown

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to be at or above the level associated with behavioral intoxication (Rhodes et al., 2005, 2007). As with continuous access preference drinking, intake in the DID test varies widely across inbred mouse strains, and all strains from the C57/C58 lineage resemble B6 in their high intake and BACs (Crabbe, Metten, et al., 2012; Rhodes et al., 2007). Mean strain intake in the DID test correlates strongly, but not completely, with 2-bottle choice preference drinking when examined across a range of inbred strains (Crabbe, Metten, et al., 2012; Rhodes et al., 2007). Thus, there appears to be significant overlap between the genetic influences on these 2 traits, but there are also likely to be other genetic factors at play in DID that are unrelated to continuous access drinking paradigms.

HDID selection

Since intoxicating BACs were the feature of previous high drinking rodent models that had been lacking, we sought to selectively breed mice that drink to high BACs. Consequently, we began selection for high BACs at the end of a 2-day DID test. Mice drank for 2 h on the first day and 4 h on the second day. The starting population was a genetically heterogeneous stock of mice resulting from an 8-way inbred strain cross (for detailed description of the founding HS/Npt population, see Crabbe et al., 2009). High BAC mice were bred with each other, and over successive generations we have developed a selected line of mice that readily drinks to high BACs in the DID test. There is no corresponding line bred for low BAC, as we use the HS mice as a comparator control. A second replicate line of mice was also developed, using the same breeding procedure (Crabbe et al., 2010). Thus, we now have HDID-1 and HDID-2 mice, which allow us better to probe potentially correlated responses to selection. Realized heritability of this trait is relatively low ($h^2 = 0.08–0.09$), but selection has successfully increased BACs across generations (Crabbe et al., 2009). A recent report of this selective breeding effort (Crabbe et al., 2013) shows that BACs have increased 4.7-fold across 27 selected generations in HDID-1 mice (average BAC = 1.4 mg/mL; 80% of mice reach BACs > 1.0 mg/mL) and 4-fold in HDID-2 mice after 19 selected generations (average BAC = 1.1 mg/mL; 50% of mice reach BACs > 1.0 mg/mL). Rate of elimination of an acute injection of ethanol has been found not to differ between either of the HDID lines and HS mice (Crabbe et al., 2009, 2013). This indicates that the higher BACs of the HDID animals are not due to differences in alcohol metabolism. Further substantiating this idea is the reasonably high correlation between intake and BAC in the DID test in these mice. In addition to reaching higher BACs than the HS mice, HDID mice also drink more alcohol than HS mice in g/kg of body weight.

Features of the HDID mice

One goal of working with the HDID lines is to determine other alcohol-related domains that may share common genetic determinants with BAC in the DID test. To this end, we are behaviorally phenotyping these lines for performance on a variety of other alcohol-related tasks. Since the HDID lines are a relatively new animal model, there is still much to be studied with regard to potentially correlated responses to selection, and even more in the biological and behavioral differences that may underlie the enhanced drinking they exhibit. We have examined more possibly correlated traits in the first replicate line than in the second, but results from both replicates are reported here wherever possible.

Drinking phenotypes

The drinking phenotypes of the HDID mice are perhaps the best studied of their behaviors. The initial variable of interest was, of course, the targeted selection phenotype: high BACs after DID. This is seen in HDID-1 and HDID-2 mice of both sexes, and corresponds with high g/kg alcohol intake as well. In HDID-1 mice, intoxication can also be demonstrated behaviorally, with mice showing impaired performance on a balance beam task when tested immediately after DID (Crabbe et al., 2009). HDID alcohol preference drinking in 2-bottle choice, continuous access procedures have also been tested. HDID-1 and HS mice showed modest differences in alcohol intake in this procedure when tested serially for alcohol concentrations ranging from 3% to 25%, with HDID-1 mice drinking more alcohol than HS mice at the 9% concentration only. For higher concentrations (30–40%), HS mice actually showed greater g/kg intake than HDID-1 mice (Crabbe, Spence, Brown, & Metten, 2011). HDID-1 mice in a separate study tended toward greater g/kg intake of, and preference for, 10% alcohol than HS mice, though this difference failed to reach levels of statistical significance (Rosenwasser, Fixaris, Crabbe, Brooks, & Ascheid, 2013). In limited access 2-bottle choice procedures, HDID-1 mice had a slightly greater preference for 15% and 20% alcohol solutions than HS mice (Barkley-Levenson & Crabbe, 2012; Crabbe et al., 2011). However, greater g/kg intake of 15% ethanol in the HDID-1 than HS mice was not seen until after 60 days of testing when giving daily 2-h access sessions early during the circadian dark (Crabbe et al., 2011). These findings suggest that selection may have resulted in modest changes in continuous access drinking at moderate alcohol concentrations, and are consistent with the idea of some shared genetic component of DID and 2-bottle choice drinking. However, there are undoubtedly differences in underlying mechanisms between these behaviors as well. One key difference between continuous access preference drinking and DID is that only the latter is likely to result in intoxicating BACs. This difference in intoxication experience may be a factor in why selection for preference drinking and for high BACs in the DID test are not perfectly symmetrical.

In addition to alcohol consumption, we have examined consumption of other fluids as well. HDID-1 mice have been shown to drink less water than HS mice (Barkley-Levenson & Crabbe, 2012; Crabbe et al., 2011), which suggests that enhanced alcohol drinking in this line is somewhat fluid-specific and does not appear to represent increased drinking in general. Alcohol-naïve HDID-1 and HS mice have also been tested for drinking of multiple tastant solutions in 2-bottle choice procedures with continuous access to water. HDID-1 and HS mice both avoid bitter quinine solutions and prefer sweet saccharin and sucrose solutions (Crabbe et al., 2011). Thus, changes in taste sensitivity or seeking of caloric content are unlikely to explain the enhanced drinking to intoxication in HDID-1 mice. The absence of a genotypic difference in sweet preference (i.e., sucrose or saccharin solutions) appears to be unique to these lines. Data from other rodent lines selected for alcohol preference, as well as findings from human alcoholics and family-history positive individuals, have suggested a genetic correlation between sweet taste sensitivity and alcohol intake (e.g. Grahame, Li, & Lumeng et al., 1999; Kampov-Polevoy, Garbutt, & Khalitov, 2003). The majority of the rodent data come from lines selected for preference for 10% alcohol solutions. The 20% alcohol solution used in our selection procedure presumably differs from 10% alcohol in its taste and perceived sweetness, and the difference in alcohol concentrations used for selection may have been a factor in why HDID and HS mice do not show this genetic relationship between drinking and sweet preference.

Because selection has been for high BAC rather than overall intake, we are interested in the pattern of consumption over the 4-h DID test session in HDID and HS mice. We thought it conceivable that selection for high BACs at the conclusion of the test would result in mice that allocate the majority of their intake to later parts