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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.

Keywords

Binge; Drinking in the dark; Ethanol consumption; Genetics; Selective breeding

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.

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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 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 of the drinking session. To address this question we looked at the partitioning of intake during the first and second halves of the 4-h test across all selected generations. Both HDID-1 and HDID-2 mice do appear to drink more alcohol (approximately 60% of their total intake) during the second half of the session than the first, but this difference in pattern was apparent even in the first selected generation and has not changed across generations of selection (Crabbe et al., 2009, in press). Thus, it appears to be a feature of the starting population rather than a correlated response to selection. We have further examined the pattern of drinking in these mice by using continuous recording apparatus (lickometers and BioDAQ episodic intake monitors [Research Diets, New Brunswick, NJ]) during the DID test. From this continuously recorded data, the specifics of the drinking microstructure can be analyzed, including the size, duration, and frequency of drinking bouts, as well as the temporal distribution of these bouts

throughout the session. When testing mice in the BioDAQ system, which constantly records the weight of the drinking bottles, we define bouts as at least a 0.02-g change in drinking bottle weight with no more than 1 min between registered weight changes. Using this system, we have found that male HDID-1 mice show a greater average alcohol bout size than HS mice, while not differing in alcohol bout duration. In most tests, they also show a greater number of bouts, but this characteristic is not always seen (Barkley-Levenson & Crabbe, 2012). The larger bout size in these mice represents an enhanced drinking efficiency. Separate experiments using lickometer chambers have shown that the increased bout size of the HDID-1 mice, with no change in bout duration, appears to be due to a larger alcohol volume consumed per lick by these mice (unpublished data). This type of "gulping" phenotype has been previously associated with excessive alcohol intake in other animal models (e.g. Grant et al., 2008; Samson, 2000). Preliminary studies suggest that the HDID-2 mice do not share this larger bout size phenotype, and instead reach high BACs and high alcohol intake by having more frequent drinking bouts.

We also recently analyzed the hourly ingestion of alcohol during the 2-day DID test in both replicate lines by assaying BACs each hour in separate groups of mice. HDID-1 mice reached their highest BAC (1.4 mg/mL) after 3 h of drinking, while HDID-2 mice did not reach their peak until 4 h (BAC = 1.7 mg/mL). Interestingly, separate groups of mice tested after the initial 2-h drinking session on Day 1 reached BACs = 1.2 mg/mL in both replicate lines (Crabbe et al., 2013).

Another facet of alcohol consumption that has been examined in the HDID lines is escalation of drinking with intermittent access. Offering 24-h periods of intermittent access to alcohol has been shown to produce an increase in intake over that seen in continuous-access paradigms (Hwa et al., 2011; Melendez, 2011; Wise, 1973). Comparison of every-other-day, 24-h access versus continuous access drinking in HDID-1, HDID-2, and HS mice failed to show escalation of intake in any genotype with intermittency (Crabbe, Harkness, et al., 2012). When HDID-1 mice were given continuous access to 10% ethanol versus water for 6 weeks, and then switched to a schedule where ethanol was only offered 1 day a week, their ethanol intake did escalate during the next 6 weeks. However, there was no difference in the magnitude of the escalation effect as compared to HS controls (Rosenwasser et al., 2013). This supports the idea that the effects of intermittency are not constant across all animal models, and that some genotypes may be more resistant to these effects than others.

We have also used the chronic intermittent alcohol exposure procedure developed by Becker and Lopez (2004) to assess whether the genotypes differ in escalation of drinking following repeated cycles of alcohol vapor exposure and withdrawal. Here, ethanol versus water choice is first offered in daily 2-h sessions near the time of "lights off" for several weeks. Ethanol vapor is then applied chronically (for 4 days) and intermittently (16 h/day) to induce physical dependence, and preference drinking is reassessed during withdrawal. We found that HDID-1 mice drank more than HS in all these limited access sessions. Both HDID-1 and HS mice increased their alcohol consumption following the 3rd chronic intermittent vapor exposure, but that the magnitude of escalation did not differ between the lines (Crabbe, Metten, et al., 2012). Thus, escalation of drinking due to both intermittency of

access and following chronic intermittent exposure to alcohol vapor appear to be traits that are genetically distinct from BACs following DID.

Alcohol sensitivity, tolerance, and withdrawal

Correlated responses to HDID selection beyond drinking behaviors have also been studied. In humans, sensitivity to the various acute effects of alcohol has been proposed as a marker for risk of subsequent abuse and seems to be related to current drinking status (e.g. Holdstock, King, & de Wit, 2000; Newlin & Thomson, 1990; Schuckit et al, 2011). We tested the HDID and HS mice for performance on multiple behavioral tasks following acute injection with alcohol. Alcohol similarly impaired performance on the balance beam and accelerating rotarod in all genotypes, while HDID-1 mice showed greater locomotor stimulation and a higher number of footslips on the parallel rod floor test after alcohol injection than either HDID-2 or HS mice (Crabbe, Kruse, et al., 2012). These results are consistent with previous findings of a genetic relationship between alcohol-induced locomotor stimulation and drinking (Risinger, Malott, Prather, Niehus, & Cunningham, 1994). The divergent findings between the replicate lines suggest that although these behaviors are not necessary for the HDID selection phenotype *per se*, they may play a role in the drinking behaviors of the HDID-1 mice. However, while some examined traits showed an increased sensitivity to ethanol in the HDID mice relative to HS, others showed an attenuated sensitivity. Both replicate lines, for example, had a reduced hypothermic response to acute alcohol injection as compared to HS controls (Crabbe, Kruse, et al., 2012). Alcohol-induced loss of righting reflex (LORR) was also examined in these genotypes, and differing effects were observed in both replicates: HDID-1 mice had longer LORR durations than HS, and HDID-2 mice had shorter durations (Crabbe, Kruse, et al., 2012). Taken together, these findings suggest that in the HDID-1 mice, at least the propensity to drink to high BACs is largely associated with increased sensitivity to alcohol's stimulant effects and decreased sensitivity to its sedative effects. However, this genetic relationship between alcohol sensitivity measures and HDID drinking to intoxication is complex and may vary depending on the assay used.

Sensitivity to the aversive and rewarding effects of alcohol has been measured in the HDID mice to determine how perception of the motivational effects of alcohol may have been altered by selection. We have found that both HDID lines and HS mice develop an alcohol-conditioned place preference with a 2-g/kg conditioning dose, but the magnitude of this preference, thus, the perceived rewarding value, does not differ between the lines (Barkley-Levenson, Cunningham, Smitasin, & Crabbe, 2013). In contrast, the magnitude of an alcohol-conditioned taste aversion differed between the lines, with HDID-1 and HDID-2 mice having attenuated aversion to a 2-g/kg dose of alcohol compared to HS mice. At a higher conditioning dose (4 g/kg), however, both HDID replicates and HS mice developed a robust aversion. These findings, and the finding that all genotypes showed significant taste aversion conditioned by lithium chloride, indicate that all genotypes are capable of the associative learning necessary to condition a taste aversion, but that the HDID mice are less sensitive to the aversive effects of a moderate dose of alcohol. This apparent inverse genetic correlation between alcohol consumption and sensitivity to an alcohol-conditioned taste aversion is consistent with data from other selected lines and inbred strains (for review, see

Green & Grahame, 2008) and suggests a possible mechanism by which HDID mice are able to drink to pharmacologically relevant BACs (i.e., they may be relatively unresponsive to the interoceptive feedback from intoxicating BACs).

Tolerance to alcohol (a reduction in response to the same, repeated dose) and the presence of withdrawal symptoms in the absence of alcohol are both part of the diagnostic criteria for AUDs in humans. We have examined the development of tolerance to repeated alcohol injections in the HDID lines, as well as withdrawal responses following both acute and chronic alcohol exposure, and following the DID test. Across 3 daily injections, male mice of both replicates showed a trend toward greater tolerance to the hypothermic effects of alcohol than HS mice, whereas female mice tended to show less tolerance (Crabbe, Colville, et al., 2012). Further studies will be needed to determine the basis of this sex difference, as well as to test the development of tolerance to other effects of alcohol.

Withdrawal has been assessed in HDID and HS mice using handling-induced convulsions (HICs). HICs emerged during the hours following either acute injection with 4-g/kg alcohol or 72-h continuous chronic vapor exposure. Severity of withdrawal, however, did not differ among the genotypes (Crabbe, Colville, et al., 2012). Both replicates of HDID demonstrated higher HIC scores at baseline than HS mice, which indicate a potentially correlated response to selection of greater central nervous system excitability at the very low level represented by basal HICs. These findings differ from those obtained from other selected lines and inbred strains, wherein continuous access drinking has been shown to be negatively genetically correlated with withdrawal severity (e.g. Lopez, Grahame, & Becker, 2011; Metten et al., 1998). This relationship does not necessarily extend to DID, however, as mouse lines selected for alcohol withdrawal severity actually show a modest positive genetic correlation between withdrawal and DID (Crabbe et al., 2013). The divergence in correlated responses to preference drinking selection and HDID selection again underscores the existence of unique genetic contributions captured by each.

Circadian effects

Because the HDID selection phenotype relies so heavily on behaviors performed during a particular circadian window, circadian patterns in these mice have been assessed to determine any potential alterations arising from selection. HDID-1 and HS mice showed largely similar circadian waveforms under a standard light:dark cycle of 12-h light/12-h dark and when free-running in constant darkness, though HDID-1 mice had a shorter free-running cycle than HS mice in constant light (McCulley, Ascheid, Crabbe, & Rosenwasser, 2013). HDID-1 mice had less wheel running activity during the early dark phase and increased activity during the late dark phase than HS mice under a standard light cycle. The fact that HDID-1 mice are less active than HS mice during the circadian time point when DID is tested could potentially be related to their drinking phenotype. It is possible that greater baseline activity could represent a competing behavior for drinking in the DID test, though this is purely speculative. In general, HDID selection does not seem to have produced major changes in circadian patterns.

Gene expression changes

Most biological and genetic features still need to be examined in these mice, but there has been one study published on the gene expression networks in the HDID lines and HS mice (Iancu et al., 2013). Analysis of differential gene expression for 9393 transcripts of ventral striatum tissue from alcohol-naïve mice showed numerous transcripts that differed between the selected lines and the controls, with only a subset of these transcripts showing differential expression in the same direction in both replicates. Possible quantitative trait loci (QTLs) were also determined for both replicates. This analysis identified multiple chromosomal locations associated with the HDID phenotype. A comparison of the putative QTLs for the DID phenotype with QTLs previously identified for preference drinking revealed little overlap. It is possible that differences in founding populations contribute to the dissimilarity of QTL results. HS/Npt mice have not been used as the starting population for any other alcohol-selected lines, and consequently QTL results may differ in part because the alleles available during HDID selection are different from those found in the other inbred strain crosses used for mapping. This possibility is supported by the fact that potential QTLs identified here also failed to overlap with possible QTLs for DID identified previously using a B6 × FVB/NJ F2 population (Phillips et al., 2010). However, mice in that study were tested for DID after more than 3 weeks of daily, 2-bottle choice drinking whereas Iancu and colleagues used naïve HDID and HS mice for their QTL mapping experiment. Consequently, failure to reproduce the same QTLs for DID could be influenced by the population difference, the procedural difference, or both. It should be noted that the putative QTLs identified in the B6 × FVB/NJ mapping study for DID and preference drinking also showed little overlap, which reinforces the likelihood of genetic contributions to binge-like drinking that are distinct from those promoting continuous-access preference drinking.

Analysis of the networks represented by the correlated expression patterns for different genes found selection-based changes in connectivity for multiple networks. Interestingly, the changes in network structure between the HDID and HS mice were more pronounced in the first replicate than in the second, corresponding to the greater number of generations subjected to selective breeding (22 generations for HDID-1,15 for HDID-2, and 0 for HS). The analysis also identified many individual genes whose expression was particularly responsive to selection. Some of these genes had previously been implicated in studies of alcohol intake and related behaviors (e.g. genes encoding the GABA receptor subunit $\gamma 1$, the Y2 receptor for neuropeptide Y, and neurotensin), and these genes and other related genes within the network may be useful starting points for studying the specific biological features that engender drinking to intoxication in the HDID lines.

HDID mice as a model of binge-like drinking

When developing rodent models of alcohol abuse, it is not possible to recapitulate the entirety of symptoms seen in human alcoholics, nor is it necessarily desirable to attempt to do so. Excessive amounts of drinking at any single point in time, for example, is not actually required to be present for clinical diagnosis of an AUD, but animal models of this particular trait are clearly of great potential translational interest. Animal models of discrete aspects of

human AUDs allow us to study and manipulate specific traits and behaviors in the absence of many potential confounding factors. Some have proposed criteria for an animal model of excessive alcohol consumption: 1) drinking should result in BACs of 100 mg% or greater, 2) excessive alcohol consumption should occur under free-choice drinking procedures (i.e., in the presence of water or another palatable solution), 3) alcohol should be consumed for its post-ingestive pharmacological effects rather than for taste or caloric content, 4) behavioral intoxication should be evident after excessive consumption, 5) tolerance should develop with chronic excessive intake, and 6) dependence should develop as seen by withdrawal symptoms following cessation of chronic excessive intake (Bell, Rodd, Lumeng, Murphy, & McBride, 2006). Although we do not endorse the necessity for any animal model to achieve all these criteria, we describe the advantages and disadvantages of the HDID mice in relation to them and other existing animal models.

Advantages of the model

One of the greatest advantages of the HDID mice as a model of binge-like or excessive alcohol intake is that they will readily drink to intoxicating BACs without the need for any major procedural manipulations (e.g. fluid restriction, sucrose-fading, etc.). In the present selection generations (S27 and S19 for HDID-1 and HDID-2, respectively), over 80% of the HDID-1 animals and over 50% of the HDID-2 animals had BACs at or above 100 mg% (Crabbe et al., 2013). Thus, most of these mice when tested in the DID procedure experienced pharmacologically relevant blood alcohol levels. This is certainly a strength when compared to most other drinking procedures, where it is uncertain that the animals are actually becoming intoxicated. The observed heightened blood levels also correlate with g/kg alcohol intake and are not readily explained by changes in alcohol metabolism. Therefore, HDID mice satisfy the first proposed requirement of a model of excessive drinking. HDID-1 mice have also been shown to meet the 3rd and 4th criteria. Because the HDID-1 mice do not differ from HS mice in preference for or avoidance of sweet, caloric sweet, and bitter solutions, it appears that their high alcohol intake is not driven by differences in taste sensitivity (Crabbe et al., 2011). Alcohol consumption during DID by the HDID-1 mice is also sufficient to produce behavioral intoxication as demonstrated by increased ataxia (Crabbe et al., 2009).

The nature of tolerance development in HDID mice is more equivocal. The 5th criterion of Bell et al. clearly refers to tolerance that results from chronic drinking. This criterion makes sense when applied to animals that have continuous access to alcohol, but its relevance for animals tested repeatedly in short access sessions is less clear. We discussed earlier studies of ethanol tolerance in HDID-1 and HS mice induced by injections, but such studies have not been applied to animals that have been drinking. We have performed a single study in HDID-1 mice offered 20% ethanol in the DID test for 4 h on 3 successive days. Each day, mice were tested immediately after drinking for performance on a balance beam. Mice drank 5.3, 4.7, and 4.8 g/kg ethanol on the 3 successive days. Foot slip errors declined steadily across days of testing (1.9, 1.2, and 0.7, respectively). The significant decline in intoxication, particularly between Days 2 and 3, shows that tolerance develops from drinking in HDID-1 mice (Crabbe, in press). This experiment has not been conducted in HDID-2 mice. On the other hand, the minimal escalation in intake seen in HDID mice

during either standard or 2-bottle choice DID tests given daily across multiple weeks (e.g. Crabbe et al., 2011; Crabbe, Metten, et al., 2012; Metten, Brown, & Crabbe, 2011) suggests that tolerance is not developing in these animals, though this has yet to be explicitly tested.

Both HDID replicate lines show increased handling-induced convulsions during acute withdrawal after a single DID test (Crabbe et al., 2013). Thus, the 6th criterion of Bell et al. is met in both replicate HDID lines. Whether withdrawal symptoms are exacerbated following more chronic DID experience in these mice has not yet been examined.

In addition to meeting a number of the proposed criteria for a rodent model of excessive drinking, the HDID mice have several other advantages as a model. As mentioned previously, DID and continuous access preference drinking have some distinct genetic contributions. HDID mice, consequently, provide a means to study genes relevant to excessive drinking that may not be captured by other genetic models that were developed using 2-bottle choice procedures. Furthermore, these lines are currently the only existing models of genetic risk for binge-like drinking. An additional strength of the HDID lines is the existence of 2 independently selected replicates, as this allows for better assessment of potentially correlated responses to selection. In addition, the selection appears to have produced some differences in the patterning of intake in the 2 replicates, so the genetic pathways revealed may allow us access to a greater proportion of the human individual differences in AUD phenotypes. Finally, the selection phenotype is based on a simple 2-day procedure that allows for high-throughput testing. This limited access feature of the DID test has led to its adaptation to pharmacological assessments of potential therapies and studies designed to explore the neurobiological underpinnings (for review, see Sprow & Thiele, 2012; Thiele, this volume).

Limitations of the model

The main current limitation of the HDID mice is the lack of choice. When water was offered to HDID-1 mice in Generation S9 in addition to ethanol in the 4-day DID test, they reached lower BACs than groups of mice tested in the traditional single-bottle test (Crabbe et al., 2009). Therefore, we do not know whether HDID lines meet the 2nd criterion proposed by Bell and colleagues for a model of excessive drinking. In human AUDs, alcohol is generally consumed even when other liquids are available. The drinking of the HDID mice therefore loses some translational utility because the phenotype as usually assessed is dependent upon alcohol being the only fluid present. However, fewer than half of the mice from S9 were at that time drinking to BACs greater than 100 mg/mL. We plan to repeat this test with water available in the current generation of mice, who now are reaching BACs twice as great as mice from S9.

A second limitation is that drinking occurs during a limited window of availability, though this can also be a strength as noted above. It has been suggested that even repeated, short exposures to the high levels of drinking seen in HDID mice may be insufficient to produce some of the neural changes underlying the addictive process. The fact that behavioral tolerance clearly develops across 3 daily intoxication tests suggests strongly that neural adaptations are elicited by limited access DID testing, but their extent as compared to more chronic levels of alcohol exposure is unknown. One final potential complication is that the

HDID-1 mice show consistently more consumption during the latter half of the drinking session. This has been true throughout the 2-day DID tests used during selection and has not changed across generations, despite 4–5-fold increases in BACs achieved across generations. This drinking pattern is distinct from other high-drinking rodent models, where "front-loading" via high early session intake is often reported (e.g. Bell et al., 2011). Given that the tendency toward later session consumption was seen in the first cohort of mice tested for selection, it may be an innate characteristic of the founding population. This possibility is supported by the fact that unselected HS mice still show increased drinking during the second half of the 4 h session as compared to the first half (Barkley-Levenson & Crabbe, 2012). During the very first exposure to alcohol, we have seen that HDID-1 mice drink significantly more alcohol during the first 30 min of the session than during any other time bin and reach intoxicating BACs after only 2 h (Barkley-Levenson & Crabbe, 2012; Crabbe et al., 2013). With subsequent alcohol experience, drinking shifts toward the latter part of the drinking session and peak BACs are reached after 3 h of drinking on the second day of a 2-day DID test. We believe that selection has altered patterning of drinking throughout the session in some way, and that the patterning is driving the elevation in BACs taken at the end of drinking. Studies of the specific drinking microstructure of the HDID lines are ongoing and will likely resolve this issue.

Future directions

The HDID lines meet many of the proposed criteria for a model of excessive drinking and show promise for helping to determine specific genetic and neurobiological features that promote drinking to intoxication. Many relevant aspects of the model have yet to be tested rigorously in the HDID-2 line. More data would improve the face validity of the model in 3 important areas. First, the blood levels HDID mice will attain after self-administration of alcohol when other choices are available needs to be explored systematically. Second, we should begin to explore the DID drinking in more chronic paradigms. When the DID model was developed in C57BL/6J mice, we tested their intake for 13 successive days and saw no tendency for the amount ingested to increase over time (Rhodes et al., 2005). However, we have not repeatedly tested recent generations of HDID mice for many days. Nor have we taken animals initiated to binge-like drinking and switched them to conditions where 2-bottle choice, or continuous access, is offered. It would be very useful to find conditions that lead to escalations in drinking in these mice. Finally, it would be useful to test whether HDID mice will self-administer intoxicating doses of alcohol in the face of punishment, such as mild electric shock (Crabbe, 2012). The transition to shock-resistant drinking has been achieved thus far only with rat models that involve long exposures and complex operant manipulations (e.g., Seif et al., 2013) but would represent an important advance. Due to the relative newness of these mouse lines, many more experiments will need to be performed to behaviorally phenotype the lines and to examine possible correlated responses to selection. This will ideally provide greater insight into what factors contribute to drive the high intake and high BACs in the HDID mice. It will also help us determine whether different neural mechanisms underlie the similar phenotypes of the 2 replicate lines. We have just initiated a project where we are breeding an additional selected line from a cross of the HDID-1 and HDID-2 animals, based on the successful application of this to lines of mice selected for preference drinking (Oberlin, Best, Matson, Henderson, & Grahame,

2011). Because there is apparent genetic divergence between the replicates, this crossed line will allow us to maximize the contributions of trait-relevant genes that have been captured by selection and may produce animals with an enhanced phenotype. At present, it is unclear why the HDID mice, unlike most other genotypes that are not particularly averse to alcohol, drink to intoxication. The most crucial future studies will likely be those that seek to understand what is driving intake and BACs in these animals. With a better understanding of what motivates the binge-like drinking in mice, the ultimate hope is that we may identify key substrates to target in the treatment of human AUDs.

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