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Rewarding and aversive effects of ethanol in High Drinking in the Dark selectively bred mice

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Abstract

Both rewarding and aversive effects contribute to alcohol consumption. Animals genetically predisposed to be high drinkers show reduced sensitivity to the aversive effects of alcohol, and in some instances, increased sensitivity to alcohol's rewarding effects. The present studies tested the High Drinking in the Dark (HDID) selected lines, a genetic model of drinking to intoxication, to determine whether intake in these mice was genetically related to sensitivity to alcohol aversion or reward. Male HDID mice from the first and second replicate lines (HDID-1 and HDID-2, respectively) and mice from the heterogeneous progenitor control population (HS/Npt, or HS) were conditioned for a taste aversion to a salt solution using 2 doses of alcohol, and lithium chloride (LiCl) and saline controls. In separate experiments, male and female HDID-1, HDID-2, and HS mice were conditioned for place preference using alcohol. HDID mice were found to have an attenuated sensitivity to alcohol at a moderate (2 g/kg) dose compared to HS mice, but did not differ on conditioned taste aversion to a high (4 g/kg) dose or LiCl or saline injections. HDID and HS mice showed comparable development of alcohol-induced conditioned place preference. These results indicate that high blood alcohol levels after drinking in the HDID mice is genetically related to attenuated aversion to alcohol, while sensitivity to alcohol reward is not altered in these mice. Thus, HDID mice may find a moderate dose of alcohol to be less aversive than control mice and consequently may drink more because of this reduced aversive sensitivity.

Keywords

alcohol; behavioral genetics; binge drinking; conditioned place preference; conditioned taste aversion; selected lines

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Authors' contributions

CLC, JCC, and AMB-L were responsible for the study concept and design. AMB-L and PJS were responsible for data acquisition. All authors assisted with data analysis and interpretation of findings. AMB-L drafted the manuscript. CLC, PJS, and JCC provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version for publication.

Introduction

Alcohol (ethanol) has some seemingly paradoxical effects, as ethanol treatment can produce responses indicating both reward and aversion. In humans, ethanol's rewarding effects are frequently associated with euphoria, stimulation, and drug liking. In contrast, aversive effects are often associated with dysphoria and sedation (Martin et al. 1993; Morean et al. 2012). These rewarding (positive) and aversive (negative) stimulus effects of ethanol can be present at the same time (for review, see Verendeev and Riley, 2012). Thus, perception of the motivational value of ethanol is presumably determined by an individual's relative sensitivity to the rewarding versus aversive effects. In this manner, sensitivity to both ethanol reward and aversion can modulate ethanol intake.

In animal studies, these motivational effects of ethanol can be readily assessed using procedures based in Pavlovian conditioning such as conditioned place preference (CPP) and conditioned taste aversion (CTA). Previous research has shown that the degree of ethanol-CTA is negatively genetically correlated with home cage preference for ethanol in a panel of inbred strains (Broadbent et al. 2002). Similarly, short-term selected mouse lines bred for strength of ethanol CTA were found to differ in their ethanol consumption, with low CTA mice consuming more ethanol than high CTA mice (Phillips et al. 2005). Mouse lines selected for high and low alcohol preference also show this inverse relationship between drinking and strength of CTA (Chester et al. 2003). In all these studies, the correlated traits are tested in separate groups of naive animals, an advantage of inbred strains and selectively bred lines.

The genetic relationship between ethanol drinking and ethanol CPP has also been examined, but the results are more variable than those seen for CTA (Green and Grahame 2008). Short-term selected mouse lines for high and low ethanol drinking show divergent place conditioning, with the high drinkers having a greater expression of ethanol CPP (Phillips et al. 2005). However, studies of other mouse and rat lines selected for ethanol preference have failed to show this line difference for CPP (Gauvin et al. 2000), and some have shown no difference or even the opposite relationship depending on the conditioning dose used (Grahame et al. 2001). More consistent results have been seen with relating single gene effects to both drinking and CPP. For example, studies of mu opioid receptor (Hall et al. 2001), dopamine D2 receptor (Cunningham et al. 2000), and cannabinoid receptor 1 knockout mice (Naassila et al. 2004; Houchi et al. 2005) have shown that knockout mice of all genotypes have reduced intake and weaker CPP as compared to wild-type controls. Overall, there appears to be a modest, but inconsistent, positive relationship between home cage ethanol consumption and ethanol CPP.

Of the studies examining the genetic relationship between ethanol drinking and the motivational effects of ethanol, most have utilized a continuous access two-bottle choice test of ethanol versus water to measure ethanol consumption. Because ethanol intake is distributed across the 24 hr day, this assay may not result in intoxication even in high drinking animals (Dole and Gentry 1984, but see Matson and Grahame 2011). Limited access binge-like drinking, which results in intoxicating blood ethanol concentrations (BECs), has been much less well studied in relation to ethanol reward and aversion. One

study of inbred strains (Rhodes et al. 2007) showed a negative genetic correlation between drinking on the final day of a limited access Drinking in the Dark (DID) test and published strain data on ethanol conditioned taste aversion (Broadbent et al. 2002), consistent with the findings from two-bottle choice experiments. Very few studies have examined both CPP and ethanol DID, but one study did find that histamine H3 receptor knockout mice have both reduced DID intake and diminished CPP as compared to wild-type mice, suggesting a positive genetic relationship (Nuutinen et al. 2011).

In the present studies, we sought to extend these findings by assessing ethanol-motivated behaviors in animals that have been specifically bred for drinking to intoxication. The High Drinking in the Dark (HDID) lines have been selected for reaching high BECs during the DID test and can be thought of as a genetic model for binge-like drinking (Crabbe et al. 2009). Mice of these genotypes regularly drink to intoxicating BECs in the DID test (i.e., above 0.8 mg/ml) and the high intake and BECs have been shown not to be due to underlying differences in ethanol metabolism (Crabbe et al. 2009 and unpublished data). Here, we tested naïve mice of both replicate lines (HDID-1 and HDID-2) and the control heterogeneous progenitor line (HS/Npt, or HS) for ethanol CTA and ethanol CPP to determine whether changes in sensitivity to ethanol aversion or reward have arisen as correlated responses to selection. We predicted that reduced aversive sensitivity and/or increased ethanol reward sensitivity might be present in the HDID mice and might therefore be related to their high BEC and high drinking phenotype.

Materials and Methods

Animals and Husbandry

Mice in the CPP study were bred and housed at Oregon Health & Science University (Portland, OR) and mice in the CTA studies were bred and housed in the Veterinary Medical Unit at the Veterans Affairs Medical Center (Portland, OR). All mice were between 50 and 78 days of age and were experimentally naïve at the start of testing. 50 day old mice were used in the CTA experiments because the habituation and fluid-restriction phases were relatively long and all mice were fully mature by the start of conditioning phase. HDID-1 mice from the 21st selection generation were used for the CTA experiment. The HDID-2 CTA experiment was run in 2 separate passes, with the first pass using mice from selection generation 14 and the second pass using mice from selection generation 17. The CPP experiment used offspring from breeder pairs taken from the 19th and 12th selection generations for HDID-1 and HDID-2 mice, respectively. HS/Npt mice were used as the control comparator line for the HDID mice in all experiments. HS/Npt mice are the starting population from which the HDID lines were selected and are the product of a systematic 8-way cross of inbred strains described in detail elsewhere (Crabbe et al. 2009). These mice can therefore be considered an outbred heterogeneous stock, and are bred with no selective pressure and thus maintain random assortment of genes. Mice were kept on a 12/12 hour light/dark cycle with lights on at 07:00 hour for the CPP study, 08:00 hour for the HDID-1 CTA study, and 06:00 hour for the HDID-2 CTA studies. All procedures were approved by the local Institutional Animal Care and Use Committees and were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

CTA procedure

The taste conditioning procedure was adapted from that used by Broadbent and colleagues (2002). Testing consisted of 3 phases: habituation, fluid restriction, and conditioning. At the start of the habituation phase (Days 1-7), mice were singly housed and provided with water via a 25-ml graduated cylinder fitted with a stainless steel drinking spout or a polycarbonate bottle with a stainless steel drinking spout fixed to the screw-top lid depending on experiment. Water consumption was monitored to ensure that mice learned how to drink from the sipper tube. During the fluid restriction phase (Days 8-14), water bottles were removed and access to water was provided during a 2-hour period at the same time each day. Mice were weighed daily and water consumption was recorded for the fluid access period. On the first day of the conditioning phase (Days 15-27), mice were weighed and then provided with a drinking tube containing a 0.2 M sodium chloride (NaCl) solution for 1 hour. After 1 hour, fluid consumption was recorded and tubes were removed. Approximately 5 hours after removal of the tube, mice received 30 minutes of access to water to prevent dehydration. On the second day of conditioning, mice received 2 hours of water access as during the fluid restriction phase. This alternating pattern of NaCl conditioning trials and water access was continued such that NaCl was presented on Days 15, 17, 19, 21, 23, 25, and 27 and water was provided on the intervening days. NaCl was presented without a paired injection on Day 15 to allow familiarization of the animals with the novel drinking solution. On the remaining conditioning trials, removal of the NaCl solution after the hour of drinking was immediately followed by intraperitoneal (IP) injection with saline, 2 g/kg ethanol, 4 g/kg ethanol, or 3 mEq/kg lithium chloride (LiCl) as determined by drug group assignment. Saline injections were used as a control for changes in NaCl consumption over time or due to handling. LiCl injections were designed to serve as a positive control to determine whether the genotypes differ in their ability to condition a taste aversion, as this LiCl dose has been shown to produce CTA in mice (Risinger and Cunningham 2000). CTA was assessed by comparing NaCl solution consumption on the first injection-paired trial (Day 17) to consumption on the last conditioning trial (Day 27). A decrease in NaCl consumption across conditioning trials was considered to indicate the development of a taste aversion. This procedure is preferable to conditioning a taste aversion to ethanol itself because it eliminates the potential influence of pre-absorptive effects such as taste and olfaction.

HDID-1 and HDID-2 mice were tested in different experiments (see below) at different times due to animal availability and procedural necessity. Separate control groups of HS mice were consequently tested with each experiment, and the HDID-1 and -2 mice are only compared to the control group that was tested concurrent with their respective line.

Experiment 1: CTA in HDID-1 and HS

80 male mice were used (n=10/genotype/drug group) for this experiment. The taste conditioning procedure was performed as described above. Drinking sessions took place between 1230-1430h on water days and between 1230-1330h on conditioning days.

Experiment 2: CTA in HDID-2 and HS

The first pass of this experiment used 79 male mice and the second pass used 77 male mice (n=9–10/genotype/drug group for each pass). Drinking sessions took place between 1030–1230h on water days and between 1030–1130h on conditioning days.

Conditioned Place Preference apparatus

The apparatus used is described in detail elsewhere (Cunningham et al. 2006). Briefly, twelve conditioning boxes (30 ×15 ×15 cm) made of acrylic and aluminum were each enclosed in a light- and sound-attenuating chamber (Coulbourn Instruments Model E10–20). General activity and the time spent on each side of the box was recorded by six sets of infrared photo emitters and detectors mounted 2.2 cm above the floor at 5-cm intervals along the wall. The experiment was conducted with no light in the chamber. The conditioning stimuli (CS) consisted of interchangeable grid and hole floor halves. The grid floor was made out of 2.3-mm stainless steel rods mounted 6.4 mm apart in acrylic rails. The hole floor was made out of perforated stainless steel sheets (16 gauge) consisting of 6.4-mm round holes with 9.5 mm staggered centers. These cues were chosen based on previous studies showing that naïve DBA/2J mice spend equal amounts of time on each cue (Cunningham et al. 2003).

Experiment 3: Ethanol CPP

138 male and female HDID-1, HDID-2, and HS mice were used. Although sex differences were not anticipated for either CPP or CTA, female mice were readily available at the time of CPP testing and were therefore included in this experiment in order to attain as complete a dataset as possible. All genotypes and sexes were tested at the same time. This experiment consisted of three phases: pretest (one session), conditioning (eight ethanol sessions and eight saline sessions), and place preference testing (three sessions). Before each session mice were placed into a sex-specific weigh bucket, weighed, and received an IP injection immediately before being placed in the apparatus. Each squad consisted of a single sex, and the order of squads was counterbalanced.

The pretest was conducted to confirm that there were no inherent biases to either floor cue. All mice were weighed, injected with saline IP, and placed in the center of the apparatus with both floors (half grid/half hole) for a single 30 min session. The position of each floor was counterbalanced and number of beam breaks and time spent on each floor were recorded.

An unbiased one-compartment place conditioning procedure was used (Cunningham et al. 2003, 2006). During conditioning sessions, mice were weighed and given an IP injection before being placed on a single floor cue for 5 min. Within the six groups, mice were assigned to either GRID+ or GRID– conditioning subgroups (n=9–12/genotype/sex/conditioning subgroup). The GRID+ subgroup received ethanol paired with the grid floor (CS+) and saline paired with the hole floor (CS–). The GRID– subgroup received ethanol paired with the hole floor (CS+) and saline paired with the grid floor (CS–). CS+ and CS– trials alternated over 16 days (counterbalanced order), with place preference tests performed after the fourth, sixth, and eighth pairs of conditioning sessions. Place preference tests were

performed identically to the pretest and number of beam breaks and time spent on each floor were recorded. This procedure of multiple preference tests was used in order to allow us to capture possible genotypic differences in both learning rate and asymptote during the acquisition of CPP.

Drugs

Ethanol (OHSU Research Stores, Portland, OR: 20% v/v) for the CPP study was prepared using a 95% stock solution diluted with 0.9% saline and was administered IP at a dose of 2g/kg. This dose and administration of ethanol has been successful in achieving CPP in DBA/2J and C57BL/6J mice (Cunningham et al. 2006). Saline was administered IP at a volume of 12.5 ml/kg. Ethanol (Decon Laboratories Inc., King of Prussia, PA: 20% v/v) for the CTA studies was prepared using 100% ethanol diluted with saline and administered at a 2 or 4 g/kg dose. LiCl (0.15 M; Sigma-Aldrich, St. Louis, MO) was diluted in sterile distilled water and administered IP at a dose of 3 mEq/kg. Saline injections were given at a volume of 10 ml/kg. NaCl drinking solution (0.2M; Sigma-Aldrich, St. Louis, MO) was prepared by dissolving NaCl in tap water.

Statistical Analyses

Statistical analyses were carried out using multifactorial analyses of variance (ANOVAs) in Systat (version 13; Systat Software, Inc., Chicago, IL) or StatView (version 5.0, SAS Institute, Cary, NC). Lower order ANOVAs were used to follow up significant interactions. Significance was set at $\alpha=0.05$ for all tests unless otherwise specified and a Bonferroni correction for multiple comparisons was used for post-hoc analyses. For CTA experiments, daily intake was converted to ml per kg of body weight. CTA change scores were computed by subtracting intake at baseline (i.e., conditioning trial 1) from intake during the final conditioning trial. CTA daily intake data were analyzed by repeated measures ANOVA, with between subjects factors of genotype and drug group and the within subjects factor of conditioning trial. Baseline intake of the NaCl solution on conditioning trial 1 (before any injections were given) was analyzed by two-way ANOVA with the factors of genotype and drug group to determine whether there were any initial group differences in consumption. Change scores were analyzed by two-way ANOVA with genotype and drug group as factors. CPP test data for time spent on the grid floor were analyzed by three-way ANOVA for the pretest and each preference test with genotype, sex, and conditioning subgroup as factors. A significant difference between conditioning subgroups was used to indicate the development of a place preference. A repeated measures ANOVA with between subjects factors of sex, genotype, and conditioning subgroup was also used to analyze potential changes across tests 1–3. Locomotor activity data were analyzed by two-way ANOVA for the pretest and each preference test with genotype and sex as factors.

Results

Experiment 1: CTA in HDID-1 and HS

Six animals (1 HDID-1 animal each from the saline, LiCl, and 2 g/kg ethanol groups, and 1 HS animal each from the saline, LiCl, and 4 g/kg groups) were excluded from analysis because they received unintentional water access during the night prior to conditioning trial

4. Figure 1 shows intake of the NaCl solution across conditioning trials in the HDID-1 (1a) and HS (1b) mice. Statistical analyses showed a main effect of genotype ($F_{1,66}=4.9$, $p=0.030$), drug group ($F_{3,66}=37.2$, $p<0.001$), and conditioning trial ($F_{5,330}=119.7$, $p<0.001$). Significant two-way interactions were found for trial $\times$ drug group ($F_{15,330}=14.3$, $p<0.001$) and genotype $\times$ drug group ($F_{3,66}=4.9$, $p=0.004$). A significant three-way interaction of conditioning trial $\times$ genotype $\times$ drug group ($F_{15,330}=1.8$, $p=0.040$) was also found. Follow-up ANOVAs examining each drug group separately showed main effects of genotype ($F_{1,17}=21.8$, $p<0.001$) and trial ($F_{5,85}=34.6$, $p<0.001$), and a significant trial $\times$ genotype interaction ($F_{5,85}=4.2$, $p=0.002$) in the 2 g/kg ethanol treated animals. HDID-1 mice had significantly greater NaCl solution consumption than HS on conditioning trials 3–6 ($F_{1,17-18}=16.4$, $p=0.001$), indicating that magnitude of CTA at this dose was smaller for HDID-1 than the HS animals. For the 4 g/kg ethanol group there was a main effect of trial ($F_{5,85}=129.8$, $p<0.001$), but only a trend towards a main effect of genotype ($F_{1,17}=3.7$, $p=0.072$) and no significant trial $\times$ genotype interaction. These results indicate the development of comparable CTA in both HDID-1 and HS mice at this dose. There was a main effect of conditioning trial ($F_{5,80}=22.3$, $p<0.001$) in the LiCl group, but no significant trial $\times$ genotype interaction, indicating that LiCl CTA was similar between genotypes. Among vehicle-treated animals there was no main effect of genotype or significant trial $\times$ genotype interaction. There was a significant effect of trial ($F_{5,80}=3.2$, $p=0.011$), but post-hoc analyses showed this to be due solely to lower NaCl consumption on Trial 3 than Trial 1 ($p=0.027$). These results indicate that the handling and injection procedure had no systematic effect on NaCl intake over time.

Drinking on trial 1 was analyzed individually to determine whether there were any baseline group differences in consumption of the NaCl solution. There was a main effect of genotype ($F_{1,66}=6.1$, $p=0.016$), with HDID-1 mice (Fig. 1a) drinking more NaCl at baseline than HS mice (Fig. 1b). Because of this initial line difference in drinking, we decided to also analyze CTA change score as a way to minimize any potential impact of baseline variation. CTA change scores (intake on the final trial minus intake on the first trial) for each group are shown in Figure S1. A significant interaction of genotype $\times$ drug group was found ($F_{3,66}=4.5$, $p=0.006$), as well as a main effect of drug group ($F_{3,66}=36.3$, $p<0.001$). Follow-up ANOVAs showed that analysis of change scores yielded the same pattern of results as the repeated measures ANOVA, with HDID-1 mice trending towards a smaller magnitude of CTA for 2 g/kg ethanol than the HS animals, though this difference failed to reach the Bonferroni-corrected significance threshold of $\alpha=0.0125$ ($F_{1,17}=6.7$, $p=0.020$). HDID-1 and HS mice did not differ significantly on change score for any of the other conditioning groups, reinforcing the idea that HDID-1 mice are specifically resistant to a taste aversion conditioned with moderate dose ethanol.

Experiment 2: CTA in HDID-2 and HS

Three mice in Experiment 2 died during the course of the study and their data have been excluded from analysis. Figure 2 shows intake of the NaCl solution across conditioning trials in the HDID-2 (2a) and HS (2b) mice. Statistical analyses showed a pattern of results similar to that seen with the first replicate line in Experiment 1. There were main effects of genotype ($F_{1,141}=36.8$, $p<0.001$), drug group ($F_{3,141}=26.8$, $p<0.001$), and conditioning trial

($F_{5,705}=54.1$, $p<0.001$), as well as significant two-way interactions of genotype $\times$ trial ($F_{5,705}=3.5$, $p=0.004$) and drug group $\times$ trial ($F_{15,705}=7.8$, $p<0.001$). Although the three-way interaction of genotype $\times$ drug group $\times$ trial was not statistically significant, we chose to use the same data analysis strategy as for Experiment 1 due to our *a priori* interest in interpreting results across the replicate lines. Follow up ANOVAs of each drug group individually showed a main effect of genotype ($F_{1,36}=6.2$, $p=0.018$) and trial ($F_{5,180}=11.3$, $p<0.001$), and a significant genotype $\times$ trial interaction ($F_{5,180}=2.8$, $p=0.019$) in the 2 g/kg ethanol group. HDID-2 and HS mice did not differ in intake on trials 1-4, but HDID-2 mice had significantly greater intake than HS mice on trials 5 and 6 ($F_{1,37}=7.9$, $p=0.008$), indicating a reduced magnitude of CTA at this dose. For the 4 g/kg ethanol-treated animals, there were main effects of genotype ($F_{1,34}=16.0$, $p<0.001$) and trial ($F_{5,170}=93.4$, $p<0.001$), but no significant interaction of genotype $\times$ trial, indicating the development of a similar CTA in both lines. The LiCl group showed main effects of genotype ($F_{1,35}=17.6$, $p<0.001$), trial ($F_{5,175}=11.3$, $p<0.001$), and a significant genotype $\times$ trial interaction ($F_{5,175}=3.2$, $p=0.009$). Follow-up ANOVAs showed that HDID-2 mice consumed significantly more NaCl solution than HS mice on trials 3, 4, and 6 ($F_{1,36}=11.8$, $p=0.002$; Bonferroni-corrected $\alpha=0.008$), indicating transiently weaker conditioning to LiCl in the HDID-2 mice. The vehicle group showed a main effect of genotype ($F_{1,36}=4.2$, $p=0.048$), but no significant effect of trial or significant genotype $\times$ trial interaction.

Analysis of baseline NaCl drinking on trial 1 showed no main effect of drug group, a trend towards a main effect of line ($p=0.076$), and no significant genotype $\times$ drug group interaction, indicating that groups were relatively well matched for baseline intake. CTA change from baseline scores for each group are shown in Figure S2. Analysis of CTA change scores showed main effects of genotype ($F_{1,144}=7.7$, $p=0.006$) and drug group ($F_{3,144}=18.5$, $p<0.001$), and a significant genotype $\times$ drug group interaction ($F_{3,144}=2.9$, $p=0.035$). Follow-up ANOVAs of each drug group individually showed that HDID-2 mice conditioned with 2 g/kg ethanol had a significantly smaller change score than HS mice ($F_{1,37}=7.8$, $p=0.008$). Mice in the 4 g/kg ethanol, LiCl, and saline groups did not differ significantly between the genotypes for the CTA change score, although there was a trend towards a greater change score for the HS mice in the vehicle-treated group ($p=0.076$).

Experiment 3: Ethanol CPP

Time spent on the grid floor during the pretest and each preference test is shown in Figure 3. Analysis of time spent on the grid floor during the pretest (Fig. 3a) showed no main effect of conditioning subgroup, sex, or genotype, and no significant interactions, indicating that animals of each group had no initial bias towards either of the tactile cues (All $F < 1.7$). Analysis of tests 1–3 (Fig. 3) showed main effects of conditioning subgroup ($F_{1,126}>27.4$, $p<0.0001$), but no significant effects of genotype or sex and no significant interactions. A repeated measures ANOVA of grid time across all CPP tests (tests 1–3) showed a significant 4-way interaction of test $\times$ conditioning subgroup $\times$ genotype $\times$ sex ($F_{4,252}=2.4$, $p=0.047$). A follow-up ANOVA showed a significant 3-way interaction of test $\times$ conditioning subgroup $\times$ genotype for males only ($F_{4,132}=2.48$, $p=0.047$). ANOVAs were run for each test for males only and showed a significant conditioning subgroup $\times$ genotype interaction only on test 3 ($F_{2,66}=3.43$, $p=0.038$), which was due to significant place conditioning in both HDID lines

but not in the HS males. Place conditioning on tests 1 and 2 was comparable across genotypes. Analysis of locomotor activity during each test showed only a main effect of genotype ($F_{2,132} = 7.5$, $p < 0.001$), with HDID-1 mice having lower activity than HDID-2 and HS mice on the pretest and all preference tests (Fig. 4).

Discussion

In the CTA experiments, both HDID replicates had weaker ethanol CTA to a 2 g/kg conditioning dose than HS mice. In contrast, all genotypes conditioned a strong aversion to the 4 g/kg dose, indicating that HDID mice do not simply have deficits in the associative learning necessary to develop a CTA. Consequently, the attenuated CTA to a moderate dose of ethanol is presumably due to a difference in sensitivity to the aversive effects of that dose. This is further supported by the fact that HDID-1s, and to some extent HDID-2s, developed a CTA similar to HS mice when treated with LiCl. The specificity of the genotypic difference to the 2 g/kg ethanol dose is consistent with previous mouse studies showing that the inverse genetic relationship between ethanol preference drinking and ethanol conditioned taste aversion is more robust with a 2 g/kg dose than a 4 g/kg dose (e.g. Chester et al. 2003).

There is some evidence that the HDID-2 mice also had less LiCl CTA than the HS mice, an effect which was not seen with the HDID-1s. This could suggest that the second replicate is less sensitive to mildly aversive drugs in general; testing additional compounds would be necessary to resolve this. This potentially attenuated aversive sensitivity does not seem to reflect a deficit in learning, as the HDID-2 mice readily conditioned an aversion to the 4 g/kg ethanol dose and show no deficits in ethanol CPP. These mice also show no significant difference from HS mice in baseline consumption of the saline solution in the CTA test, suggesting that the present results cannot be explained by reduced exploration or increased novelty aversion in this line. That the HDID-2 mice show this more generalized attenuation of aversion than the HDID-1 mice is not entirely surprising: these two lines were independently selectively bred and thus it can be expected that different combinations of genes were affected in the two iterations of the selection experiment. The broadly-defined similarity of relative insensitivity to moderate-dose ethanol CTA in the HDID-1 and HDID-2 lines argues strongly for the role of some of the same genes that lead to high binge-like drinking. However, the HDID-1 and HDID-2 lines differ for many other genes as well, due to unavoidable chance effects. Because the genetic contributions to individual differences in ethanol CTA and high drinking in the dark are not completely the same, the pattern of ethanol CTA results is not expected to be identical in the two HDID lines.

Alternatively, the lesser sensitivity of HDID-2 mice to LiCl on 3 of the 6 test trials could merely reflect transient differences. The HDID-2s (14th and 17th selected generations) are not as far along as the HDID-1s (21st generation) in the selection process. Because the difference between HDID-1 and HS mice in the primary selected trait (DID-BEC) was greater than the difference between HDID-2 and HS, some putative correlated responses to selection that appear early (i.e., in HDID-2) might not survive as the intensity of selection increases (HDID-1). We examined the data for the two passes of HDID-2 mice tested and saw a stronger tendency for reduced HDID-2 LiCl CTA in S14 than in S17 (data not

shown), which is consistent with the hypothesis that LiCl sensitivity in HDID-2 was a “false positive” correlate. This is supported by a difference in the selection phenotype between these generations as well (average BECs for S14 and S17 plus/minus the standard deviation: 0.790 ± 0.038 and 1.171 ± 0.043 mg/ml, respectively; HDID-1 mice of S21 had a BEC of 1.22 ± 0.057 mg/ml. Data are unpublished). Regardless, the consistent and significantly reduced sensitivity to the 2 g/kg ethanol dose in both replicates across trials is strong evidence for a correlated response to selection. Thus, while it is possible that the random assortment of genes during the HDID-2 selection has given rise to attenuated aversive sensitivity to other drugs as well, the apparent shared genetic contributions to ethanol aversion and drinking to intoxication are likely present in both lines.

Mice of all genotypes and sexes developed a modest but significant ethanol CPP, indicating that ethanol is perceived as rewarding by these animals. The lack of any difference in magnitude between the lines on tests 2 and 3 suggests that initial ethanol reward sensitivity does not differ between HDID and HS mice. The finding of greater time on the drug-paired floor in the HDID males than the HS males on test 4 suggests possible subtle genotypic differences in ethanol CPP. Specifically, each CPP test essentially acts as an extinction trial since the conditioning cues are being presented in the absence of drug. Thus, the difference in CPP on test 4 could indicate a difference between HDID and HS males in sensitivity to ethanol extinction, with the HDID mice extinguishing more slowly than the HS. Future testing using an actual extinction procedure after CPP acquisition could be used to investigate this possibility further. Another possibility would be to perform a single CPP expression test after the full number of conditioning trials to assess whether a genotypic difference in CPP remains in the absence of repeated drug-free tests.

One limitation of the CPP study is that only a single dose of ethanol was tested. It is therefore possible that at a different dose we would have been able to distinguish genotypic differences in place conditioning, as were seen with the CTA experiments. The place conditioning seen here seemed to reach a performance ceiling by the first test and did not increase with additional conditioning trials, so it might be possible to see differences between the lines at a lower dose. However, the magnitude of the CPP was relatively modest and CPP has been previously noted as often lacking a graded dose-response curve (e.g. Cunningham et al. 1992), so a lower dose might simply eliminate CPP expression entirely. It could prove useful in the future, though, to test these genotypes of mice using a more dose-sensitive paradigm such as the reference dose procedure (Grolewski et al. 2008) to see if we are able to observe any differences between the lines. It may also prove beneficial to test these genotypes for CPP using a different conditioning agent (e.g. a natural reinforcer or a different drug of abuse), to determine whether the present results generalize to other rewarding substances.

When examining CPP data, it is important to consider the fact that locomotor activity can affect the expression of a place preference, with higher levels of activity impairing the demonstration of CPP (Gremel and Cunningham 2007). We did observe a genotypic difference in activity in the CPP tests (Fig. 4), with the HDID-1 mice showing lower activity than the other two genotypes. This difference in activity is consistent with previous findings from these lines (Crabbe et al. 2012a), but is unlikely to have affected the CPP results since

even the most active lines had locomotor activity well below levels shown to interfere with CPP expression. It should also be noted that CPP is not an entirely unambiguous measure of drug reward, and that additional testing (e.g. ethanol-reinforced responding on a progressive ratio) is needed in order to completely rule out possible differences in reward sensitivity. From the present results, however, it would appear that drinking to intoxication in the HDID lines does not reflect an alteration in perceived rewarding effects of ethanol. This is consistent with much of the previous work from rodent selected lines that also failed to show alterations in ethanol CPP as a correlated response to selection for ethanol preference or intake. Single gene effects have been shown to influence both ethanol drinking and CPP, and it is possible that future experiments examining individual gene contributions to the HDID phenotype will yield similar results, but an overall genetic relationship between drinking to intoxication and ethanol CPP does not appear to be present.

The results of the present experiments demonstrate that the genetic relationship between ethanol motivated behaviors and binge-like drinking is similar to that seen previously with two-bottle choice continuous access drinking. Specifically, these findings are consistent with the literature on the negative genetic correlation between preference drinking and ethanol CTA in both mouse and rat selected lines (e.g. Broadbent et al. 2002; Brunetti et al. 2002; Chester et al. 2003; Froehlich et al. 1988; Phillips et al. 2005; see review by Cunningham et al. 2009) and extend this association to another model of ethanol intake. The consistency of findings for the binge-like and continuous access, preference drinking traits is consistent with data suggesting a moderate genetic correlation between these two traits. Although inbred strain correlations between DID and preference drinking are generally high (Rhodes et al. 2007; Crabbe et al. 2012b), HDID mice show only modestly greater preference drinking than HS (Crabbe et al. 2011; Rosenwasser et al. 2012). Our results suggest the existence of similar genetic contributions underlying both sensitivity to ethanol aversion and drinking to intoxication (DID), but not ethanol reward as measured by CPP. Previous work has shown little evidence of genetic correlation between ethanol CTA and CPP (e.g. Phillips et al. 2005), and quantitative trait loci mapping for both behaviors suggests only limited chromosomal overlap (Cunningham 1995; Risinger and Cunningham 1998), so it is not surprising that HDID selection has resulted in line differences in one behavior and not the other.

It is important to note that the present results demonstrate a negative *genetic* correlation between the HDID phenotype and ethanol CTA. In order to determine whether drinking to high BECs and CTA are related within a given individual, it would be necessary to test CTA and ethanol DID in the same animals. It is possible to speculate, however, as to how changes in aversive sensitivity could produce increased intake. In humans, for example, heavy drinkers given ethanol in a laboratory setting report lower sedation and higher drug “liking” than low drinkers, and individual differences in these responses predict subsequent binge drinking frequency in the high drinking individuals (King et al. 2011). In our model, it could be that a reduced sensitivity to the aversive effects of ethanol at moderate doses such as those ingested during the DID test allows HDID mice to drink in a manner that produces higher BECs and greater intoxication than HS mice. We have shown previously that HDID-1 mice drink ethanol in significantly larger “bouts” (i.e., consume a larger g/kg dose

of ethanol in the same amount of time) than HS mice during the DID test (Barkley-Levenson and Crabbe 2012). It might be that this pattern of intake is possible for the HDID-1 mice because they are less sensitive to the negative pharmacological effects of these large drinking bouts. Another possibility is that the reduced aversion of the HDID mice allows them to more readily drink enough ethanol to experience its rewarding effect. Thus, while we report here that experimenter-administered ethanol is equally rewarding to both HDID and HS mice, in a home cage drinking situation where the mice determine the dose administered, HDID mice might be more likely to actually experience ethanol reward which may in turn enhance motivation to continue drinking. The specific genetics underlying sensitivity to ethanol's aversive effects are not yet well understood, but the $\alpha 2$ subunit of GABA type-A receptors has been implicated in mediating ethanol CTA (Blednov et al. 2012). Consequently, these subunits could be an interesting target for future pharmacological and genetic studies in the HDID and HS lines. Overall it remains unknown exactly what drives the high intake and high BECs in the HDID mice, but the current studies provide strong evidence that reduced sensitivity to ethanol's aversive effects may be a significant contributor to the HDID phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

NaCl solution intake in ml/kg body weight across the 6 conditioning trials. Panel a shows intake for the HDID-1 mice and panel b shows intake for the HS mice. Ethanol conditioning groups (2 and 4 g/kg) are shown in filled symbols and control groups (vehicle and LiCl) are shown in open symbols. Means $\pm$ SEM shown. * indicates a statistically significant difference ($p < 0.001$) in NaCl solution intake from corresponding HS group.

Fig. 2.

NaCl solution intake in ml/kg body weight across the 6 conditioning trials. Panel a shows intake for the HDID-2 mice and panel b shows intake for the HS mice. Ethanol conditioning groups (2 and 4 g/kg) are shown in filled symbols and control groups (vehicle and LiCl) are shown in open symbols. Means $\pm$ SEM shown. * indicates a statistically significant difference ($p < 0.01$) in NaCl solution intake from corresponding HS group.

Fig. 3.

Ethanol conditioned place preference expression during the pretest and Tests 1–3 (after 4, 6, or 8 pairings, respectively). Panels a and b show time spent on the grid floor by male (a) and female (b) animals of each genotype. G+ indicates mice that received 2 g/kg ethanol paired with the grid floor (GRID+ conditioning subgroup) and G- indicates mice that received 2 g/kg ethanol paired with the hole floor (GRID- conditioning subgroup). The difference between G+ and G- group scores reflects the magnitude of conditioned place preference. Panels c and d show percent time spent on the drug-paired floor (collapsed on conditioning subgroup) for males (c) and females (d) of each genotype. The dashed line at 50% indicates the point of no preference for either floor. Means $\pm$ SEM shown.

Fig. 4.

Average locomotor activity counts (beam breaks) per minute during the pretest and each place preference test (T1–3) for males (a) and females (b). Means $\pm$ SEM shown.