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Affect-related related behaviors in mice selectively bred for high and low voluntary alcohol consumption

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Abstract

There is considerable evidence for the existence of comorbidity between alcohol-use disorders and depression in humans. One strategy to elucidate hereditary factors affecting the comorbidity of these disorders is to use genetic animal models, such as mouse lines selectively bred for voluntary ethanol consumption. We hypothesized that mice from lines that were bred for high-alcohol preference would manifest increased depression-like phenotypes compared to low-alcohol preferring mice. Mice that were bi-directionally selected and bred on the basis of their High-(HAP) or Low-Alcohol Preference (LAP) were tested in the open-field (OFT), dark-light box (DLB), forced swim (FST), and learned helplessness tests (LH). The study was conducted in two independently derived replicates. In the OFT, both HAP2 and HAP3 mice showed higher levels of general locomotion compared to LAP mice. However, only HAP2 mice spent more time in the center compared to LAP2 mice. In the DLB, there was a slightly higher anxiety-like phenotype in HAP mice. In both FST and LH, we observed higher depression-like behaviors in HAP mice compared to LAP mice, but this was limited to the Replicate 2 mice. Overall, we identified affect-related behavioral changes in mouse lines bred for high-alcohol preference. Notably, the Replicate 3 lines that showed fewer depression-like behaviors also manifest smaller differences in alcohol intake. These data suggest that there may be overlap between genes that predispose to excessive alcohol intake and those underlying affective-related behaviors in the mouse.

Keywords

ethanol; mood disorder; depression; anxiety; alcoholism; genetics

It is well recognized that alcohol use disorders and major depression are both highly heritable (Mayfield et al. 2008; Sullivan et al. 2000). Various epidemiological studies also indicate considerable comorbidity between alcohol abuse and depression (Grant et al. 1995; Kessler et al. 1994). Additionally, there is epidemiological evidence for the co-transmission of alcoholism and depression in families (Merikangas et al. 1994). A number of the same

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genes have been associated with both the development of alcoholism and depression. For example, in humans this includes polymorphisms in the serotonin transporter promoter (5-*HTTLPR*) (Karg et al. 2011; McHugh et al. 2010), the muscarinic acetylcholine receptor M2 (*CHRM2*) (Luo et al. 2005; Wang et al. 2004), acetaldehyde dehydrogenase 2 (*ALDH2*) (Lee et al. 2010), and the circadian locomotor output cycles kaput (*CLOCK*) (Sjöholm et al. 2010). In the mouse, transgenic manipulation of many genes, including corticotropin-releasing factor (Olive et al. 2003; Van Gaalen et al. 2002), corticotropin-releasing factor receptor type-1 (Bale et al. 2002; Kishimoto et al. 2000; Sillaber et al. 2002; Smith et al. 1998), the serotonin transporter (Boyce-Rustay et al. 2006; Kelai et al. 2003; Lira et al. 2003; Muller et al. 2011), neuropeptide Y type-2 receptor (Thiele et al. 2004; Tschenett et al. 2003), and delta and mu-opioid receptors (Becker et al. 2002; Filliol et al. 2000; Roberts et al. 2000; Roberts et al. 2001) have been implicated in modifying both alcohol preference and affect-related behaviors (for a review of alcohol preference related genes in the mouse see (Crabbe et al. 2006)). These individual genetic findings support a shared genetic susceptibility between alcohol use disorders and depression. However, the extent to which the same genetic factors lead to combined predisposition for both disorders is not completely understood. Alcohol abuse can lead to disruptions in social and professional relationships that may play a role in vulnerability to depression. Stress, anxiety and depression can also lead individuals to abuse alcohol as a form of self-medication (Bolton et al. 2009; Schuckit and Smith 2006; Swendsen and Merikangas 2000; Swendsen et al. 2000). Apart from such relationships between these conditions, the same environmental factors can also possibly elevate the risk for both conditions at the same time, leading to apparent comorbidity (Prescott et al. 2000). Because of such complex interactions, it is helpful to use representative animal models to experimentally analyze genetic and other biological relationships between these conditions.

In this study, we tested male and female mice that were the progeny of two selectively bred low alcohol preferring (LAP) and high alcohol preferring (HAP) mouse lines (Grahame et al. 1999; Oberlin et al. 2011) in a battery of affect-related behavioral tests. These tests were conducted in two independently selected line pairs. On the basis of the known comorbidity between alcohol use disorders and depression in humans, we hypothesized that HAP mice would show more depressive-like behaviors when compared to their LAP counterparts.

METHODS

Animals

13 male and 16 female Low-Alcohol Preferring Replication 2 (LAP2), 16 male and 16 female High Alcohol Preferring Replication 2 (HAP2), 16 male and 15 female Low-Alcohol Preferring Replication 3 (LAP3), 16 male and 16 female High-Alcohol Preferring Replication 3 (HAP3) mice were transported to the University of Maryland School of Medicine from the Indiana University-Purdue University School of Medicine. Mice were housed in our vivarium for 5 weeks and were between 13–16 weeks of age at the beginning of testing. Procedures were conducted during the light cycle. All subjects went through the same behavioral test battery procedure and each subject was tested in all tests. Subjects were alcohol and experiment-naïve prior to testing. All experimental procedures were approved

by the University of Maryland, Baltimore ACUC, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Behavioral Testing

The behavioral test battery was conducted in an order in which less stressful tests preceded more stressful tests. Tests proceeded in the following order: open-field test, dark-light box test, forced swim test, and the learned helpless test.

Open-Field Test (OFT)

Distance moved in the OFT is useful for assessing general activity levels, and time in center of the arena is considered an inverse measure of anxiety-like behavior (Gould et al. 2009). Locomotor activity of animals was assessed for 30 minutes in 50×50×38cm arenas under 30–35 lux illumination. The test was recorded, and the total distance moved and the time spent in the center area which corresponds to 25% of the total area were calculated with TopScan software from CleverSys Inc (Reston, VA).

Dark-Light Box Test (DLB)

The DLB is validated as a test of anxiety-like behavior (Hascoet and Bourin 2009). Our DLB apparatus measures 35×35×35cm with an inner dark 11×35×35cm compartment with a gate between the dark (15 lux) and light (650 lux) compartments. Mice were initially placed in the dark compartment and the gate was opened. Subjects were then free to move between compartments during the ten minute test session. Sessions were recorded and an observer measured the time spent in the light compartment by subjects and counted the number of transitions from the dark compartment to the light compartment. The data from two female Replicate 2 HAP mice in this test were lost due to camera errors. Time in the light compartment is considered an inverse measure of anxiety-like behavior (Hascoet and Bourin 2009).

Forced Swim Test (FST)

Subjects were placed in Plexiglas cylinders (30cm height × 20cm diameter) filled with 15cm of water at a temperature of 24±1°C for six minutes. Sessions were recorded and an observer who was blind to the identity of the groups scored the last four minutes of each session. Mobility was defined as any purposeful movements other than those necessary to maintain balance or the head above the water. Antidepressants increase activity in the FST, and lower levels of activity in this test are considered to be a reflection of depressive-like state (Can et al. under review; Hascoët and Bourin 2009).

Learned Helplessness Test (LH)

The LH test involves inducing behavioral despair in a shuttle box with inescapable electric shocks and then re-testing the mice in the same apparatus during one or more escapable shock sessions (Anisman and Merali 2009; Dao et al. 2010). The number of escape failures during the escapable shock session is considered a measure of a depressive-like state in the subjects. This test consisted of two subsequent stages. Subjects within each replicate/line/sex combination were randomly assigned into two groups. The control group was habituated to

the test chambers, but not shocked, during the first phase (Anisman and Merali 2009; Dao et al. 2010).

Stage 1 – Induction of helplessness—Subjects in the inescapable-shock group were exposed to 120 inescapable 15 second shocks of 0.3 mA in one compartment of a two-compartment Mouse Shuttle Cage (Coulbourn Instruments; Whitehall, PA). The gate between the compartments was closed during this stage. Interval between shocks was 15 seconds. Control subjects were placed in the same shuttle compartment with the gate closed for one hour, but no shock was administered.

Stage 2 – Testing—The testing procedure was performed one day after the induction phase and consisted of five initial trials that were followed by 25 experimental trials. The variable intertrial interval averaged 20 seconds. In the very first five trials (pre1–pre5), the gate opened simultaneously with the commencement of shock (0.3 mA), and stayed open for the duration of the shock. In the following trials, 1–25, the gate opened three seconds after the initiation of the shock. In both types of trials, if the mouse passed through the gate to the other compartment, the shock was terminated. If a subject did not pass through the gate during the shock (escape failure), the shock was terminated after 15 seconds and the gate was lowered. Data are reported for trials 1–25 as described previously (Dao et al. 2010).

Statistical Analyses

Statistical analyses of the behavioral data were performed using GraphPad PrismV5 or SPSS17. We utilized the analysis framework suggested by Crabbe et al. (1990) to interpret correlated responses in selectively bred animals. For each behavior, we initially analyzed all factors including replicate, line, sex and group (the latter only applicable for LH) with a three or four-way ANOVA. Planned comparisons between lines in each replicate were made using Bonferroni corrected t-tests. In the statistical analysis of the OFT, DLB, and FST two pairwise comparisons were made, and we consequently adjusted the statistical significance level by a factor of two. In the statistical analyses of the LH, we made two separate sets of four comparisons and therefore adjusted the statistical significance level by a factor of four. $P < 0.05$ was considered statistically significant. See Supplemental Information Table S1, for the detailed results of statistical analyses.

RESULTS

OFT

For total distance moved, a three-way ANOVA indicated that the main effects of line ($p < 0.0001$) and replicate ($p < 0.0001$) but not sex were statistically significant. There was a significant interaction between line and replicate ($p < 0.05$). None of the other interactions were statistically significant. Comparisons between lines in each replicate revealed that HAP mice traveled a greater distance than LAP mice in both replicates, (for the Replicate 2, $p < 0.0001$ and for the Replicate 3, $p < 0.05_{\text{Bonferroni-corrected}}$) (Figure 1a, b). To assess the effect of line within each replicate, we calculated omega-squared values. The omega-squared value for line was $\omega^2 = 0.406$ for Replicate 2, and $\omega^2 = 0.077$ for Replicate 3.

We also analyzed time spent in the center area of the arena. The main effects of sex ($p<0.01$), and replicate ($p<0.0001$) were statistically significant; however, the main effect of line was not. The three-way interaction between sex, replicate and line as well as the two way-interactions between sex and replicate and sex and line were not significant. However, there was a significant replicate by line interaction ($p<0.01$). Pairwise comparisons demonstrated that in the Replicate 2, HAP mice spent more time in the center area of the open-field than LAP mice ($p<0.0001$ Bonferroni-corrected). In the Replicate 3, HAP mice did not differ from their LAP counterparts in terms of time spent in the center (Figure 1c, d).

DLB

A three-way ANOVA of the time spent in the light compartment indicated that the main effect of sex was not significant, but the main effects both line ($p<0.05$) and replicate ($p<0.001$) were statistically significant. None of the possible interactions between sex, line, and replicate were significant. There were no significant pairwise differences between LAP and HAP mice either in the Replicate 2 or Replicate 3 line pairs. Thus, as the main effect of line indicated, we observed that while LAP mice spent more time in the light compartment compared to HAP mice when data from both replicates are combined, these differences did not reach statistical significance in the planned pairwise comparisons between lines in each replicate (Figure 2a, b).

A three-way ANOVA of the number of transitions from the dark compartment to the light compartment revealed no significant main effect of sex or of line. However, the main effect of replicate was statistically significant ($p<0.01$). None of the possible interactions between sex, line, and replicate were significant. The planned pairwise comparisons between lines in each replicate did not reveal any significant differences (Figure 2c, d).

FST

A three-way ANOVA indicated main effects of replicate ($p<0.0001$) and line ($p<0.0001$), but not sex on immobility time. The three-way interaction between replicate, line, and sex and the two-way interaction between replicate and sex were not statistically significant. However, the two-way interactions between replicate and line ($p<0.01$) and line and sex ($p<0.05$) were both statistically significant. Pairwise comparisons revealed that the Replicate 2 HAP mice spent more time immobile than Replicate 2 LAP mice ($p<0.0001$ Bonferroni-corrected). However, the difference between HAP and LAP mice in the Replicate 3 did not reach statistical significance (Figures 3 a, b).

LH

Overall, we did not observe any significant differences between lines in the control groups of both replicates in number of failures to escape. Replicate 2 HAP mice showed more failures to escape from shocks in the inescapable-shock group compared to identically treated LAP mice, indicating a depression-like phenotype. However, as was the case in the FST, we did not find any such difference between the Replicate 3 LAP and HAP lines (Figure 4a, b).

Specifically, a four-way ANOVA revealed that the main effect of group ($p < 0.0001$) and line ($p < 0.05$) were significant. The main effects of sex or replicate were not significant. Among the interaction effects, replicate by line ($p < 0.0001$), replicate by group ($p < 0.05$), and replicate by line by group ($p < 0.05$) were statistically significant but not the interaction of replicate by line by sex. None of the other possible interactions were significant.

Since sex had neither a main nor an interaction effect; we followed the previous analysis with two separate two-way ANOVAs, in which group and line were factors, for each replicate. In the Replicate 2, the two-way ANOVA revealed that the main effect of group ($p < 0.0001$) and the main effect of line ($p < 0.0001$) along with the group by line interaction ($p < 0.01$) were statistically significant. In order to assess the effects of treatment group and line in the Replicate 2, we first conducted planned pairwise comparisons between control and inescapable-shock groups in each line. t-tests showed that both LAP and HAP mice in the inescapable-shock group failed more than their control group counterparts (for LAP mice $p < 0.01$ Bonferroni-corrected; for HAP mice $p < 0.001$ Bonferroni-corrected). Pairwise comparisons between lines within each treatment group indicated that there was no statistically significant difference between LAP and HAP mice in the control group. On the other hand, in the inescapable-shock group, HAP mice failed to escape in a significantly greater number of trials compared to LAP mice ($p < 0.001$ Bonferroni-corrected).

In the Replicate 3, while the main effect of group ($p < 0.0001$) was statistically significant; the main effect of line was not. The group by line interaction was also not statistically significant. The planned pairwise comparisons showed that LAP mice exposed to inescapable-shock failed to escape in more trials than control LAP mice ($p < 0.01$ Bonferroni-corrected); however there was no significant difference between control and HAP mice exposed to inescapable-shock. Pairwise comparisons between lines within each treatment group indicated that there was no statistically significant difference between LAP and HAP mice in either the control group or in the group exposed to inescapable-shock.

DISCUSSION

In this study, we examined two pairs of alcohol naïve mouse lines that were selectively bred for their low and high voluntary alcohol consumption in a battery of behavioral tests to assess general activity, depression-like, and anxiety-related behaviors. Our results provide support for the hypothesis that alleles resulting in high alcohol intake also increase the likelihood of mice showing depression-, but not anxiety-like behaviors.

The LAP and HAP mice lines were produced by bi-directional selection for alcohol preference from an outbred HS/Ibg mice colony (Grahame et al. 1999). The initial progenitor population of HS/Ibg mice was bred by crossing eight inbred strains and therefore carry a considerable amount genetic heterogeneity (McClern et al. 1970). Our study was conducted with two replicate line pairs that were independently derived from the same heterogeneous HS/Ibg mouse colony using an identical selection strategy (Oberlin et al. 2011). Differences between any single pair of lines can result from genetic drift. We interpreted correlated responses following the model suggested by Crabbe et al. (1990), estimating the degree of genetic correlation between alcohol drinking and traits tested here

among these selectively bred mouse lines. Briefly, this model posits that the discovery of the same correlated response in both independent replicates constitutes *strong* evidence for the existence of such relationship. If a correlated response is found only in one replicate, but not in the other, and, as long as the main effect of selection is significant in the overall statistical analysis, the evidence for the correlated response is considered is *moderate*. Thus, when the results from individual replicates do not agree, the conclusions are informed by the overall analysis. If there is no main effect of selection when correlated responses from both replicates are taken into account jointly, but the selection lines are significantly different in only one of the replicates for the correlated response, the evidence for the correlated response is interpreted as *weak* (Crabbe 2007; Crabbe et al. 1990). Though not specifically defined in this model, another possible case is that a correlated response is significant in neither replicate when analyzed separately, but the main effect of selection is significant when results from both replicates are combined. Following the general logic of this model, we considered this type of evidence as weak. We summarized our results using this interpretation approach in Table I.

In the OFT, a strong effect of selection on general locomotion was observed. The main effect of line was significant and HAP mice moved a greater distance than their LAP counterparts in both replicates. However, the effect size of line was greater in the Replicate 2 compared to the Replicate 3, as indicated by an omega-squared analysis. Similar differences in locomotor activity between alcohol-preferring and non-preferring mice have been observed previously. For example, the C57BL/6J X FVB/NJ hybrid strain drinks more alcohol in a free choice setting than either parental strains, which are already noted for their high alcohol preference (Blednov et al. 2010; Blednov et al. 2005). This hybrid strain showed dramatically higher levels of locomotion in both novel environment and homecage-like environments when compared to a low-alcohol preferring strain under alcohol-naïve conditions (Ozburn et al. 2010). Similarly, the alcohol-naïve C57BL/6J mice were found to show greater locomotor activity than non-alcohol preferring DBA/2J mice in novel settings (Orsini et al. 2004). Among selectively bred rat lines, alcohol-preferring P rats manifest greater locomotion compared to their non-preferring NP counterparts; though there exist no such difference between alcohol-preferring AA and non-preferring ANA rats (Badishtov et al. 1995).

These multiple lines of evidence support a correlated response between alcohol preference and increased general locomotor activity in a novel arena. However, it is not clear how this higher level of locomotion is related to alcohol-preference. One possibility is that HAP mice manifest a greater propensity toward novelty, which in turn translates into higher activity in the novel arena. This notion has support in both the human and the animal literature. In humans, it has been found that novelty-seeking and higher exploratory activities in a novel setting during childhood are reliable predictors of later alcohol abuse (Cloninger et al. 1988). Saccharin preference in rodents and sweet preference in humans as a form of novelty seeking have been repeatedly associated with alcohol preference. For instance, alcoholic human adults were found to prefer higher concentration sucrose solutions compared to normal controls (Kampov-Polevoy et al. 1998). HAP1 mice that were selected from the same progenitor colony as the ones used in this study prefer saccharin solution over regular water more than LAP1 mice (Grahame et al. 1999), and there is a strong positive correlation between saccharin and alcohol preference in both HAP and LAP mice lines, and among

various inbred mouse strains (Belknap et al. 1993; Oberlin et al. 2011). Similar differences in saccharin preference were also found between alcohol-preferring AA and P rats their non-preferring ANA and NP counterparts (Sinclair et al. 1992).

There were two anxiety-related measures recorded in this study; the amount of time spent in the center area of the open-field and the time spent in the light compartment of the dark-light box. In the OFT, the Replicate 2 HAP mice spent considerably more time in the center field than LAP mice, suggesting a lower anxiety-like phenotype. However, no such difference was observed in the Replicate 3 between LAP and HAP mice. Overall analysis of the OFT center time revealed that the main effect of selection was not significant in terms of the time spent in center in this test. On the basis of these results, we considered the evidence for HAP mice having a lower anxiety-like phenotype as weak, since it was supported only in one replicate (Table I). The second measure was the time spent in the light compartment of the DLB. The DLB is a validated test of anxiolytic-like activity in mice, based on the inherent aversion of mice toward highly illuminated spaces (Bourin and Hascoët 2003). In the DLB, the more time spent in the light compartment is considered an indication of less anxiety-like phenotype. Even though, the mean time spent in the light compartment was statistically higher for LAP mice than HAP mice when both replicates were combined, we have found no statistically significant differences between the lines when they were compared pairwise in each replicate. The overall analysis, however, did indicate a significant main line effect, whereby LAP mice demonstrated less anxiety-like behavior. Since only the main effect of selection was significant we consider the evidence for the lower anxiety-like phenotype of LAP mice as weak (Table I). The second measure that we analyzed in the DLB, the number of transitions from dark to the light compartment, did not show any significant effect of line, further weakening the evidence for an anxiety-like phenotype difference.

The findings from these two tests were, apart from resulting in weak evidence, contradictory: the open-field test indicated a lower anxiety-like phenotype for HAP mice and the DLB test showed the same thing for LAP mice only in one of the outcome measures. The tension-reduction hypothesis predicts that is that excessive alcohol drinking might be a form of self-medication to relieve anxiety in individuals with predisposition toward anxiety (Sher et al. 2005). However, previous attempts to demonstrate a relationship between anxiety-related behaviors and alcohol preference in animal models have resulted in inconsistent results. For example, selectively bred Sardinian alcohol-preferring rats had been found to enter the open arms of the elevated plus maze less than their non-alcohol-preferring counterparts (Colombo et al. 1995). In another study, alcohol-preferring AA rats showed more entries to open arms of the elevated plus maze compared to non-alcohol-preferring ANA rats, demonstrating a lower anxiety-like phenotype (Moller et al. 1997). A more recent study examining all of the rat lines selected for differences in alcohol consumption found no consistent relationship between genetic differences in drinking and anxiety as measured by the elevated plus maze task or by the multivariate concentric square field assay (Roman et al. 2011). Thus, the current findings agree with previous studies in showing inconsistent relationships between anxiety and alcohol consumption.

We assessed behavior of LAP and HAP mice in two separate depression related tests. In the FST, the Replicate 2 HAP mice manifested greater immobility times compared to LAP mice,

demonstrating a depression-like phenotype. This difference did not reach statistical significance in the Replicate 3. However, analysis of LAP and HAP lines across replicates also confirmed the association of alcohol preference and longer immobility times in the FST. This constituted a moderate level of evidence for a correlated response between depression-like behaviors and alcohol preference in the Crabbe et al. model (Table I) (Crabbe et al. 1990). In the second depression related behavioral assessment, the LH test, we did not observe any significant line differences in the control groups in regard to number of trials with failure to escape when presented with escapable shocks. This suggests that differences in escape performances between LAP and HAP mice previously exposed to inescapable shock would not be due to learning deficits. However, in the inescapable-shock group, that is the subjects that were given a series of inescapable shocks prior to the test trial, HAP mice failed to escape in a greater number of trials, often reaching to the maximum possible of 25 trials, compared to LAP mice in the Replicate 2. In the Replicate 3, however, we did not observe a significant difference between the lines. As with the FST results, we considered this as a moderate level of evidence for the existence a correlated response between depression-like behaviors and alcohol preference in the LH test (Table I). Thus, we found clear differences between LAP and HAP mice in the Replicate 2, indicating a depression-like phenotype in the alcohol-preferring mice. While this was not the case in the Replicate 3 when assessed alone, the analyses across replicates supports the hypothesis that a depression-like phenotype is associated with higher alcohol preference.

One explanation for the absence of differences in LH in Replicate 3 lines is that the depression-like phenotype of the Replicate 2 HAP lines could be due to genetic drift, that is, random fixing of alleles related to these depression-like behaviors but unrelated to the higher alcohol-preference. Another explanation is related to the difference in selection generation and the overall levels of alcohol-preference between the Replicate 2 and 3. The Replicate 2 mice that were used in this study were taken from the 35th selection generation; the Replicate 3 mice, however, were taken from the 14th generation. Though HAP mice from both replicates were consuming more alcohol than their LAP counterparts, parents of the Replicate 2 HAP mice were consuming about twice as much alcohol as the Replicate 3 HAP mice parents (Oberlin et al., 2011). Currently, the Replicate 3 mice are still responding to selection pressure, while the Replicate 2 HAP mice have reached an asymptotic level of alcohol preference (Oberlin et al. 2011). This raises the possibility that the lack of correlated responses in depression and anxiety tests between lines may have arisen from the incomplete selection for alcohol-preference in the Replicate 3. However, it should be noted that the possibility of genetic drift influencing these results cannot be excluded.

Elucidation of the genetic factors and neurobiological mechanisms underlying alcohol use disorders and depression interaction is vital, with very important consequences for human health. In this study, we investigated this relationship using a genetic mouse model, fully implementing the correlated response model (Crabbe et al. 1990). While, we have found a strong effect of selection for alcohol preference on general locomotion, the selection pressure for alcohol preference had weak and conflicting effects on anxiety-related behaviors. We found moderate evidence for a genetic link between alcohol-preference and affect-related behavioral changes in the mouse. It should be noted that the depression-like phenotype manifested by the HAP mice in the FST and LH do not necessarily imply a

constitutively depressed mouse, but alternatively could be interpreted as a manifestation of depression-like behaviors only when exposed to a situation that renders behavioral despair. Indeed, it has been previously reported that HAP mice from both the Replicate 2 and 3 manifest a greater preference for sweet solutions than do their LAP counterparts as well as mice from their Hs/lbg progenitor line (Oberlin et al. 2011). This could be interpreted as increased hedonic behavior, though it would be important to assess the sucrose preference response to chronic unpredictable stress prior to making any firm conclusion regarding the meaning of this finding. It may be that HAP and LAP mice, rather than manifesting a full repertoire of depression-related traits, may differ on some endophenotypic measures of depression but not others (Gottesman and Gould 2003; Gould and Gottesman 2006). Another point that should be kept in mind is that the bi-directional selection procedure for alcohol preference cannot distinguish between rewarding and negative motivational effects of alcohol consumption by itself (Cunningham et al. 2000). For instance, while a high-alcohol preferring line may prefer alcohol for its positive reinforcing effects, the lack of preference in the low-preferring line may be due not to lack of the reinforcing effects of alcohol in this line but rather to avoidance of the taste or negative after effects of alcohol consumption (Camarini et al. 2010). These effects can only be distinguished with additional procedures (e.g. conditioned taste aversion, conditioned place preference) (Chester et al. 2003; Green and Grahame 2008). For example, testing the Replicate 1 LAP and HAP mice in the conditioned place preference procedure revealed that low alcohol preference in the LAP mice might be due to avoidance of the taste of alcohol (Grahame et al. 2001).

The comorbidity between alcohol abuse and depression has been shown in humans (Hasin et al. 2005). However, demonstration of strong evidence for clear causal relationships, between these two conditions remains elusive. While epidemiological studies indicate that it is more likely than not that alcohol abuse precedes depression (Boden and Fergusson 2011) in the absence of empirical studies, it is difficult to dissect the relative contributions of causal relationships between these conditions and their shared genetics. Our findings in this study may help clarifying these issues. Further research may confirm these findings with new lines of alcohol-preferring animals and more importantly extend it by identifying genetic factors and underlying neurobiological mechanisms related to this relationship.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Anisman, H.; Merali, Z. Learned Helplessness in Mice. In: Gould, TD., editor. *Mood and Anxiety Related Phenotypes in Mice*. New York, NY: Humana Press; 2009.
- Badishtov BA, Overstreet DH, Kashevskaya OP, Viglinskaya IV, Kampov-Polevoy AB, Seredenin SB, Halikas JA. To drink or not to drink: Open field behavior in alcohol-preferring and-nonpreferring rat strains. *Physiol Behav*. 1995; 57(3):585–589. [PubMed: 7753899]

- Bale TL, Picetti R, Contarino A, Koob GF, Vale WW, Lee KF. Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. *Journal of Neuroscience*. 2002; 22(1):193–199. [PubMed: 11756502]
- Becker A, Grecksch G, Kraus J, Loh HH, Schroeder H, Holtt V. Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. *Naunyn-Schmiedeberg Arch Pharmacol*. 2002; 365(4):296–302. [PubMed: 11919654]
- Belknap J, Crabbe J, Young E. Voluntary consumption of ethanol in 15 inbred mouse strains. *Psychopharmacology*. 1993; 112(4):503–510. [PubMed: 7871064]
- Blednov Y, Ozburn A, Walker D, Ahmed S, Belknap J, Harris R. Hybrid Mice as Genetic Models of High Alcohol Consumption. *Behav Genet*. 2010; 40(1):93–110. [PubMed: 19798565]
- Blednov YA, Metten P, Finn DA, Rhodes JS, Bergeson SE, Harris RA, Crabbe JC. Hybrid C57BL/6J × FVB/NJ mice drink more alcohol than do C57BL/6J mice. *Alcoholism*. 2005; 29(11):1949–1958. [PubMed: 16340451]
- Boden JM, Fergusson DM. Alcohol and depression. *Addiction*. 2011; 106(5):906–914. [PubMed: 21382111]
- Bolton JM, Robinson J, Sareen J. Self-medication of mood disorders with alcohol and drugs in the National Epidemiologic Survey on Alcohol and Related Conditions. *Journal of Affective Disorders*. 2009; 115(3):367–375. [PubMed: 19004504]
- Bourin M, Hascoët M. The mouse light/dark box test. *European Journal of Pharmacology*. 2003; 463(1–3):55–65. [PubMed: 12600702]
- Boyce-Rustay JM, Wiedholz LM, Millstein RA, Carroll J, Murphy DL, Daws LC, Holmes A. Ethanol-related behaviors in serotonin transporter knockout mice. *Alcoholism*. 2006; 30(12):1957–1965. [PubMed: 17117959]
- Camarini R, Pautassi RM, Mendez M, Quadros IM, Souza-Formigoni ML, Boerngen-Lacerda R. Behavioral and Neurochemical Studies in Distinct Animal Models of Ethanol's Motivational Effects Current Drug Abuse Reviews. 2010; 3(4):205–221.
- Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The Mouse Forced-Swim Test. *Journal of Visualized Experiments*. (under review).
- Chester JA, Lumeng L, Li T-K, Grahame NJ. High- and Low-Alcohol-Preferring Mice Show Differences in Conditioned Taste Aversion to Alcohol. *Alcoholism: Clinical and Experimental Research*. 2003; 27(1):12–18.
- Cloninger CR, Sigvardsson S, Bohman M. Childhood Personality Predicts Alcohol Abuse in Young Adults. *Alcoholism: Clinical and Experimental Research*. 1988; 12(4):494–505.
- Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, Gessa GL. Sardinian alcohol-preferring rats: A genetic animal model of anxiety. *Physiol Behav*. 1995; 57(6):1181–1185. [PubMed: 7652041]
- Crabbe J. Alcohol Psychopharmacogenetics. In: Jones, BC.; Mormede, P., editors. *Neurobehavioral Genetics*. Boca Raton, FL: CRC Press; 2007. p. 457–468.
- Crabbe JC, Phillips TJ, Kosobud A, Belknap JK. Estimation of Genetic Correlation: Interpretation of Experiments Using Selectively Bred and Inbred Animals. *Alcoholism: Clinical and Experimental Research*. 1990; 14(2):141–151.
- Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF. REVIEW: Alcohol-related genes: contributions from studies with genetically engineered mice. *Addiction Biology*. 2006; 11(3–4): 195–269. [PubMed: 16961758]
- Cunningham CL, Fidler TL, Hill KG. Animal Models of Alcohol's Motivational Effects. *Alcohol Research & Health*. 2000; 24(2):85. [PubMed: 11199282]
- Dao DT, Mahon PB, Cai X, Kovacsics CE, Blackwell RA, Arad M, Shi J, Zandi PP, O'Donnell P, Knowles JA, Weissman MM, Coryell W, Scheftner WA, Lawson WB, Levinson DF, Thompson SM, Potash JB, Gould TD. Mood Disorder Susceptibility Gene CACNA1C Modifies Mood-Related Behaviors in Mice and Interacts with Sex to Influence Behavior in Mice and Diagnosis in Humans. *Biological Psychiatry*. 2010; 68(9):801–810. [PubMed: 20723887]
- Filliol D, Ghazizadeh S, Chluba J, Martin M, Matthes HWD, Simonin F, Befort K, Gaveriaux-Ruff C, Dierich A, LeMeur M, Valverde O, Maldonado R, Kieffer BL. Mice deficient for delta- and

- gamma-opioid receptors exhibit opposing alterations of emotional responses. *Nature Genetics*. 2000; 25(2):195–200. [PubMed: 10835636]
- Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry*. 2003; 160(4):636–645. [PubMed: 12668349]
- Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. *Genes Brain Behav*. 2006; 5(2):113–119. [PubMed: 16507002]
- Gould, TD.; Dao, DT.; Kovacsics, CE. The open field test. In: Gould, TD., editor. *Mood and Anxiety Related Phenotypes in mice: characterization using behavioral tests*. Totowa, NJ: Humana Press; 2009.
- Grahame NJ, Li TK, Lumeng L. Selective Breeding for High and Low Alcohol Preference in Mice. *Behav Genet*. 1999; 29(1):47–57. [PubMed: 10371758]
- Grahame NJ, Chester JA, Rodd-Henricks K, Li T-K, Lumeng L. Alcohol place preference conditioning in high- and low-alcohol preferring selected lines of mice. *Pharmacology Biochemistry and Behavior*. 2001; 68(4):805–814.
- Grant, FB.; Harford, CT. Comorbidity between DSM-IV alcohol use disorders and major depression : results of a national survey. Shannon, IRLANDE: Elsevier; 1995.
- Green AS, Grahame NJ. Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? *Alcohol*. 2008; 42(1):1–11. [PubMed: 18164576]
- Hascoet, M.; Bourin, M. The Mouse Light-Dark Box Test. In: Gould, TD., editor. *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests*. Humana Press Inc; 2009. p. 197-223.
- Hascoët, M.; Bourin, M. The Forced Swimming Test in Mice: A Suitable Model to Study Antidepressants. In: Gould, TD., editor. *Mood and Anxiety Related Phenotypes in Mice*. Humana Press; 2009. p. 85-118.
- Hasin DS, Goodwin RD, Stinson FS, Grant BF. Epidemiology of Major Depressive Disorder: Results From the National Epidemiologic Survey on Alcoholism and Related Conditions. *Arch Gen Psychiatry*. 2005; 62(10):1097–1106. [PubMed: 16203955]
- Kampov-Polevoy AB, Garbutt JC, Davis CE, Janowsky DS. Preference for Higher Sugar Concentrations and Tridimensional Personality Questionnaire Scores in Alcoholic and Nonalcoholic Men. *Alcoholism: Clinical and Experimental Research*. 1998; 22(3):610–614.
- Karg K, Burmeister M, Shedden K, Sen S. The Serotonin T transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis Revisited: Evidence of Genetic Moderation. *Arch Gen Psychiatry*. 2011; 68(5):444–454. [PubMed: 21199959]
- Kelai S, Aissi F, Lesch KP, Cohen-Salmon C, Hamon M, Lanfumey L. Alcohol intake after serotonin transporter inactivation in mice. *Alcohol and Alcoholism*. 2003; 38(4):386–389. [PubMed: 12814910]
- Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen H-U, Kendler KS. Lifetime and 12-Month Prevalence of DSM-III-R Psychiatric Disorders in the United States: Results From the National Comorbidity Survey. *Arch Gen Psychiatry*. 1994; 51(1):8–19. [PubMed: 8279933]
- Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J. Deletion of *Crrh2* reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nature Genetics*. 2000; 24(4):415–419. [PubMed: 10742109]
- Lee S-Y, Hahn C-Y, Lee J-F, Huang S-Y, Chen S-L, Kuo P-H, Lee IH, Yeh TL, Yang YK, Chen S-H, Ko H-C, Lu R-B. MAOA Interacts With the ALDH2 Gene in Anxiety–Depression Alcohol Dependence. *Alcoholism: Clinical and Experimental Research*. 2010; 34(7):1212–1218.
- Lira A, Zhou MM, Castanon N, Ansorge MS, Gordon JA, Francis JH, Bradley-Moore M, Lira J, Underwood MD, Arango V, Kung HF, Hofer MA, Hen R, Gingrich JA. Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. *Biological Psychiatry*. 2003; 54(10):960–971. [PubMed: 14625138]
- Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case–control structured association study. *Hum Mol Genet*. 2005; 14(16):2421–2434. [PubMed: 16000316]

- Mayfield RD, Harris RA, Schuckit MA. Genetic factors influencing alcohol dependence. *British Journal of Pharmacology*. 2008; 154(2):275–287. [PubMed: 18362899]
- McClearn, GE.; Wilson, JR.; Meredith, W. The use of isogenic and Heterogenic mouse stocks in behavioral research. In: Lindzey, G.; Thiessen, DD., editors. *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*. New York: Appleton-Century-Crofts; 1970. p. 3-22.
- McHugh RK, Hofmann SG, Asnaani A, Sawyer AT, Otto MW. The serotonin transporter gene and risk for alcohol dependence: A meta-analytic review. *Drug and Alcohol Dependence*. 2010; 108(1–2): 1–6. [PubMed: 20060655]
- Merikangas KR, Risch NJ, Weissman MM. Comorbidity and co-transmission of alcoholism, anxiety and depression. *Psychol Med*. 1994; 24(1):69–80. [PubMed: 8208896]
- Moller C, Wiklund L, Thorsell A, Hyytia P, Heilig M. Decreased measures of experimental anxiety in rats bred for high alcohol preference. *Alcoholism*. 1997; 21(4):656–660. [PubMed: 9194920]
- Muller JM, Morelli E, Ansorge M, Gingrich JA. Serotonin transporter deficient mice are vulnerable to escape deficits following inescapable shocks. *Genes Brain Behav*. 2011; 10(2):166–175. [PubMed: 20955517]
- Oberlin B, Best C, Matson L, Henderson A, Grahame N. Derivation and Characterization of Replicate High- and Low-Alcohol Preferring Lines of Mice and a High-Drinking Crossed HAP Line. *Behav Genet*. 2011; 41(2):288–302. [PubMed: 20853157]
- Olive F, Mehmert K, Koenig H, Camarini R, Kim J, Nannini M, Ou C, Hodge C. A role for corticotropin releasing factor (CRF) in ethanol consumption, sensitivity, and reward as revealed by CRF-deficient mice. *Psychopharmacology*. 2003; 165(2):181–187. [PubMed: 12397512]
- Orsini C, Buchini F, Piazza P, Puglisi-Allegra S, Cabib S. Susceptibility to amphetamine-induced place preference is predicted by locomotor response to novelty and amphetamine in the mouse. *Psychopharmacology*. 2004; 172(3):264–270. [PubMed: 14600800]
- Ozburn A, Harris R, Blednov Y. Behavioral Differences Between C57BL/6J × FVB/NJ and C57BL/6J × NZB/B1NJ F1 Hybrid Mice: Relation to Control of Ethanol Intake. *Behav Genet*. 2010; 40(4): 551–563. [PubMed: 20364436]
- Prescott CA, Aggen SH, Kendler KS. Sex-Specific Genetic Influences on the Comorbidity of Alcoholism and Major Depression in a Population-Based Sample of US Twins. *Arch Gen Psychiatry*. 2000; 57(8):803–811. [PubMed: 10920470]
- Roberts AJ, McDonald JS, Heyser CJ, Kieffer BL, Matthes HWD, Koob GF, Gold LH. mu-opioid receptor knockout mice do not self-administer alcohol. *J Pharmacol Exp Ther*. 2000; 293(3):1002–1008. [PubMed: 10869404]
- Roberts AJ, Gold LH, Polis L, McDonald JS, Filliol D, Kieffer BL, Koob GF. Increased ethanol self-administration in delta-opioid receptor knockout mice. *Alcoholism*. 2001; 25(9):1249–1256. [PubMed: 11584142]
- Roman E, Stewart RB, Bertholomey ML, Jensen ML, Colombo G, Hyytiä P, Badia-Elder NE, Grahame NJ, Li T-K, Lumeng L. Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test. *Addiction Biology* no-no. 2011
- Schuckit MA, Smith TL. An evaluation of the level of response to alcohol, externalizing symptoms, and depressive symptoms as predictors of alcoholism. *J Stud Alcohol*. 2006; 67(2):215–227. [PubMed: 16562403]
- Sher KJ, Grekin ER, Williams NA. The development of alcohol use disorders. *Annu Rev Clin Psychol*. 2005:1493–523.
- Sillaber I, Rammes G, Zimmermann S, Mahal B, Zieglgänsberger W, Wurst W, Holsboer F, Spanagel R. Enhanced and Delayed Stress-Induced Alcohol Drinking in Mice Lacking Functional CRH1 Receptors. *Science*. 2002; 296(5569):931–933. [PubMed: 11988580]
- Sinclair JD, Kampov-Polevoy A, Stewart R, Li TK. Taste preferences in rat lines selected for low and high alcohol consumption. *Alcohol*. 1992; 9(2):155–160. [PubMed: 1599627]
- Sjöholm LK, Kovanen L, Saarikoski ST, Schalling M, Lavebratt C, Partonen T. CLOCK is suggested to associate with comorbid alcohol use and depressive disorders. *Journal of Circadian Rhythms*. 2010; 8(1)
- Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Chen RP, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee KF. Corticotropin releasing factor receptor

- 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron*. 1998; 20(6):1093–1102. [PubMed: 9655498]
- Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry*. 2000; 157(10):1552–1562. [PubMed: 11007705]
- Swendsen JD, Merikangas KR. The comorbidity of depression and substance use disorders. *Clinical Psychology Review*. 2000; 20(2):173–189. [PubMed: 10721496]
- Swendsen JD, Tennen H, Carney MA, Affleck G, Willard A, Hromi A. Mood and alcohol consumption: An experience sampling test of the self-medication hypothesis. *J Abnorm Psychol*. 2000; 109(2):198–204. [PubMed: 10895557]
- Thiele TE, Naveilhan P, Ernfors P. Assessment of ethanol consumption and water drinking by NPY Y2 receptor knockout mice. *Peptides*. 2004; 25(6):975–983. [PubMed: 15203244]
- Tschenett A, Singewald N, Carli M, Balducci C, Salchner P, Vezzani A, Herzog H, Sperk G. Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. *European Journal of Neuroscience*. 2003; 18(1):143–148. [PubMed: 12859347]
- Van Gaalen MM, Stenzel-Poore MP, Holsboer F, Steckler T. Effects of transgenic overproduction of CRH on anxiety-like behaviour. *European Journal of Neuroscience*. 2002; 15(12):2007–2015. [PubMed: 12099906]
- Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S, Kwon JM, Wu W, Dick DM, Rice J, Jones K, Nurnberger JI, Tischfield J, Porjesz B, Edenberg HJ, Hesselbrock V, Crowe R, Schuckit M, Begleiter H, Reich T, Goate AM, Bierut LJ. Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum Mol Genet*. 2004; 13(17):1903–1911. [PubMed: 15229186]

OFT

Figure 1. Open field test

(A, B) Total distance moved in meters in the open-field test. (C, D) Time that the subjects spent in the center portion of the open-field arena defined as 25% of the total area in the open-field test. LAP refers to “Low-Alcohol Preferring”, HAP refers to High-Alcohol Preferring”. * $p < 0.05$, *** $p < 0.001$; Bonferroni corrected unpaired t-test. Data are expressed as mean $\pm$ SEM. n: 29–32 animals per group.

Figure 2. Dark-light test

(A, B) Time spent in the light compartment in the dark-light box test. (C, D) Number of transitions from dark compartment to the light compartment. LAP refers to “Low-Alcohol Preferring”, HAP refers to High-Alcohol Preferring”. Data are expressed as mean $\pm$ SEM. n: 29–32 animals per group.

Figure 3. Forced swim test

Immobility time in the forced swim test. (A) Replicate 2 mice. (B) Replicate 3 mice. LAP refers to “Low-Alcohol Preferring”, HAP refers to High-Alcohol Preferring”. **** $p < 0.0001$; Bonferroni corrected unpaired t-test. Data are expressed as mean $\pm$ SEM. n: 29–32 animals per group.

Figure 4. Learned helplessness

The number of trials in which subjects failed to escape from the shock in the learned helplessness test. Inescapable-shock group subjects received 120 shocks in one compartment of the shuttle-box prior to the test trial. Control group subjects were placed in the same experimental chamber but did not receive any shocks before the test. (A) Replicate 2 mice. (B) Replicate 3 mice. LAP refers to “Low-Alcohol Preferring”, HAP refers to High-Alcohol Preferring”. *** $p < 0.001$; Bonferroni corrected unpaired t-test. Data are expressed as mean $\pm$ SEM. n: 12–20 animals per group.

Table 1

Summary of the results of the behavioral tests.

Behavioral Test and Measure	Main Effect of Line	Line X Replicate Interaction	Pairwise Line Differences		Evidence for the Correlated Response
			REP2	REP3	
OFT Distance Moved	+	+	+	+	STRONG
OFT Center Time	-	+	+	-	WEAK
DLB Time in Light	+	-	-	-	WEAK
DLB Transitions	-	-	-	-	NONE
FST Immobility Time	+	+	+	-	MODERATE
LH Escape Failures	+	+	+	-	MODERATE

(+) sign denotes a statistically significant result of the particular analysis. (-) sign denotes lack of a statistically significant result of the particular analysis. Line refers to LAP (Low-Alcohol Preferring) or HAP (High-Alcohol Preferring) lines. REP2 and REP3 refer to the selection Replication 2 and 3 of LAP-HAP mice.
Format adapted from (Crabbe et al. 1990)