Effects of Genetic and Procedural Variation on Measurement of Alcohol Sensitivity in Mouse Inbred Strains

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Crabbe, J.C., Metten, P., Ponomarev, I., Prescott, C., and Wahlsten, D. (2006) Effects of genetic and procedural variation on measurement of alcohol sensitivity in mouse inbred strains. *Behavior Genetics*, 36:536-552.

Abstract:

Mice from eight inbred strains were studied for their acute sensitivity to ethanol as indexed by the degree of hypothermia (HT), indexed as the reduction from pre-injection baseline of their body temperature. Two weeks later, mice were tested for their loss of righting reflex (LRR) after a higher dose of ethanol. The LRR was tested using the "classical" method of watching for recovery in animals placed on their backs in a V-shaped trough and recording duration of LRR. In a separate test, naive animals of the same strains were tested for HT repeatedly to assess the development of rapid (RTOL) and chronic tolerance (CTOL). We have recently developed a new method for testing LRR that leads to a substantial increase in the sensitivity of the test. Strains also have been found to differ in the new LRR test, as well as in the development of acute functional tolerance (AFT) to this response. In addition, our laboratory has periodically published strain difference data on the older versions of the HT and LRR responses. The earlier tests used some of the exact substrains tested currently, while for some strains, different substrains (usually, Nih versus Jax) were tested. We examined correlations of strain means to see whether patterns of strain differences were stable across time and across different test variants assessing the same behavioral construct. HT strain sensitivity scores were generally highly correlated across a 10-23 years period and test variants. The CTOL to HT was well-correlated across studies, and was also genetically similar to RTOL. The AFT, however, was related to neither RTOL nor CTOL, although this may be because different phenotypic end points were compared. The LRR data, which included a variant of the classical test, were not as stable. Measures of LRR onset were reasonably well correlated, as were those taken at recovery (e.g., duration). However, the two types of measures of LRR sensitivity to ethanol appear to be tapping traits that differ genetically. Also, the pattern of genetic correlation between HT and LRR initially reported in 1983 was not seen in current and contemporaneous studies. In certain instances, substrain seems to matter little, while in others, substrains differed a great deal. These data are generally encouraging about the stability of genetic differences.

KEY WORDS: Acute tolerance; chronic tolerance; ethanol; hypothermia; inbred mouse strains; loss of righting reflex; pharmacogenetics.

Article:

INTRODUCTION

Inbred mouse strains have long been used for several purposes in behavioral genetics research. Within a given strain, all same-sex members are genetic clones, as well as obligate homozygotes at each gene. When a panel of several inbred strains is studied for a trait under carefully controlled environmental conditions, the extent to which average among-strain differences exceed within-strain variability can be used to estimate trait effect size (Hegmann and Possidente, 1981). The allelic stability of inbred strains has allowed the cumulation of data on specific strains across many laboratories over many years. This powerful advantage of inbred strain data has led to the recent development of a large informatics project encompassing data from up to 40 standard inbred strains. The Mouse Phenome Project has entered more than 500 phenotypes in a growing database comprised of diverse behavioral and biological variables (Grubb et al., 2004). As biomedical scientists have begun to

manipulate specific genes through gene targeting strategies, inbred strains (particularly C57BL/6J) have been used by many new laboratories as a stable genetic background on which to study effects of gene deletion or over expression.

One consequence of the increased use of laboratory mice in neuroscience has been the ability to test the assumption that allelic genetic stability is directly paralleled by stability in behavioral traits. For some behaviors, we have known this to be true for more than 40 years. A classic example is the finding that C57BL/6 inbred mice prefer weak alcohol solutions to tap water, while DBA/2 mice are near teetotalers, and most strains are low to moderate drinkers (McClearn and Rodgers, 1959). This finding has been verified in several large-scale strain surveys of alcohol preference drinking (Belknap et al., 1993; Fuller, 1964; Rodgers, 1972), and the specific comparison of C57BL/6 versus DBA/2 has proven to be robust in dozens of smaller studies. Many different laboratories have reported this strain difference, and it is true across many procedural variants of alcohol preference tests (e.g., it does not depend on the specific concentration of alcohol in water versus water alone, and is also seen if multiple concentrations of alcohol are offered simultaneously versus water).

Not all behavioral differences among inbred strains are this stable across varied conditions. In an experiment designed to test the hypothesis explicitly, we screened eight genotypes (including several inbred strains and a null mutant) for responses to several traits (Crabbe et al., 1999). We were interested in ascertaining whether the specific laboratory in which a test was performed was a crucial factor affecting outcomes. We therefore tried to eliminate as many differences among the three participating laboratories as possible. We found that for most behaviors, the pattern of strain differences was indeed very stable across laboratories. This included yet another demonstration that C57BL/6J mice drink alcohol that DBA/2J mice shun. For some behaviors, however, the pattern of strain differences was not so stable across laboratories. Notably, the differential pattern of anxiety-like behavior among mouse strains depended on the laboratory performing the tests. Some of these differences may be attributable to the individual experimenter (Wahlsten et al., 2003b), some to the inherent greater variability of particular tasks (and/or their lesser reliability), and some to a lesser degree of genetic influence for some tasks. Whatever the causes, these results suggest that the stability of inbred strain differences for a particular behavior should not be assumed *a priori*.

We have used inbred strains to explore systematically the genetic determinants of the broadly-defined domain of motor coordination. Across several tasks thought to assay sensitivity to ethanol intoxication, multiple inbred strains of mice were found to differ both with and without an ethanol challenge (Crabbe et al., 2003a; 2003b; Metten et al., 2004; Ponomarev and Crabbe, 2004; Rustay et al., 2003; Wahlsten et al., 2003a). These data have revealed striking complexity in the patterns of strain differences. Not surprisingly, different behavioral assays appear to reflect the influence of different constellations of genes, for strain-specific sensitivity to ethanol is by no means uniform across tasks (Crabbe et al., 2005). Interestingly, even within a single assay, the pattern of strain differences can depend on exactly how that task is performed, or on ethanol dose.

One of the most widely used indices of behavioral sensitivity to ethanol in rodents is the loss of the righting reflex (LRR) seen after acute administration of a high dose. In the classic version of this task, a mouse given an anesthetic dose of ethanol is placed on its back in a V-shaped trough, and remains unable to turn onto its stomach for some time thereafter, until a sufficient amount of ethanol is eliminated from the bloodstream. Mice were successfully selectively bred for long (Long Sleep, or LS) or short (Short Sleep, or SS) duration of LRR to produce two selected lines that differ markedly (McClearn and Kakihana, 1981), proving the genetic contribution to this response. Many subsequent studies have explored the neurobiology of this trait (for reviews see Allan and Harris, 1991; Draski and Deitrich, 1996; Phillips and Crabbe, 1991). Recent studies have used LS and SS mice, and specific strains derived from them, to identify the genomic location of several genes influencing this response (Bennett et al., 2002; Markel et al., 1997; Owens et al., 2002). These Quantitative Trait Loci (QTLs) are being actively pursued to identify the specific QTGenes (Mackay, 2001) for each QTL.

Proving a QTG for a QTL underlying LRR to ethanol could be important for finding pathways that could be targeted to develop novel therapeutic medications for humans. In addition, there is evidence from human studies

that sensitivity to the intoxicating effects of ethanol is a marker of genetic susceptibility to develop alcohol dependence (Schuckit, 1994; Schuckit et al., 2004). Because the mouse and human genomes are very similar (approximately 80%), genes identified in mice can be pursued in humans quite readily. It would seem important to know, therefore, whether the LRR is a robust phenotype. If LRR sensitivity to ethanol is as reproducible as ethanol preference drinking across inbred strains, interest in both the neurobiological correlates and the specific QTG discovered would be significantly enhanced.

Repeatability of LRR tested with the classic method has been demonstrated (Markel et al., 1995). Markel et al. tested > 1000 F2 mice from the intercross of inbred LS X inbred SS F1 hybrid mice for LRR duration after ethanol. One week later, all mice were retested on this trait. The nonparametric phenotypic correlation of transformed LRR durations (censored to remove animals who received incomplete injections) was 0.47.

Another commonly used behavioral—physiological response to ethanol is the reduction in body temperature seen under normal ambient temperatures, which constitute a mild cold stress for rats and mice. Thermoregulation is an attractive target because of its clear dose-effect characteristics, and because the underlying physiology and pharmacology are relatively well understood (Kalant and Lê, 1984). The acute hypothermic response (HT) to ethanol in mice shows significant genetic influence in studies with inbred strains (Crabbe et al., 1982), and lines of mice have been selectively bred for this response (Crabbe et al., 1987). The QTLs for ethanol-induced HT have also been reported (Crabbe et al., 1996).

With prolonged exposure to ethanol, tolerance to many of its behavioral effects rapidly develops (Kalant et al., 1971), including HT and LRR, and tolerance, too, is genetically influenced (Crabbe et al., 1982; Crabbe, 1994; Erwin et al., 1992; Grieve and Littleton, 1979; Keir and Deitrich, 1990). Tolerance is often described as an attenuation of response with repetition. While "chronic tolerance" (CTOL) is generally used to describe the reduced sensitivity seen after a few, discrete injections or a period of several days of drinking (or inhaling) alcohol, reduced HT in mice can be seen after a second, daily administration of ethanol intraperitoneally (Crabbe et al., 1979), and this rapid tolerance (RTOL) also differs among strains (Crabbe et al., 1982). A new method for assessing LRR allows the detection of an even more rapid form of tolerance, acute functional tolerance (AFT), which occurs during the course of a single drug administration (Mellanby, 1919). Inbred strains differ markedly in the magnitude of AFT to the LRR response (Ponomarev and Crabbe, 2004). Because AFT (at least, to LRR) begins to accrue immediately and appears nearly complete by 10-20 minutes after injection (Ponomarev and Crabbe, 2004), AFT cannot be detected for ethanol-induced hypothermia due to the relatively long onset (several minutes) between administration of ethanol and the neurobiological changes underlying changes in thermoregulatory set point.

If genetic animal models are to be useful in the search for genes affecting drug sensitivity, the data linking specific genotypes to specific outcomes must be reproducible. In this report, we synthesize the results of several of the previously published analyses cited above and add new inbred strain data for four responses: acute sensitivity to ethanol-induced LRR, acute ethanol-induced hypothermia, and RTOL and CTOL to body temperature reductions by ethanol. We have previously assessed each of these responses, but our earlier experiments differ from those reported here for one or more reasons. In the past (Crabbe et al., 1982, 1994b; Crabbe, 1983; Ponomarev and Crabbe, 2004), we have used different substrains of standard mouse inbred strains and/or different methods. Over the years, we have used different apparatus, and both the specific animal feed and bedding has changed. Of course, personnel are largely or completely nonoverlapping from 1982. For the LRR, we report new data on the classic LRR method, which we compare with the new method for assaying this trait (Ponomarev and Crabbe, 2002, 2004). The tasks, strains and substrains employed and the specific variables selected are described below, and the reader can find full details in the published papers covering the older, previously reported data, or by contacting the authors.

METHODS

Animals, Husbandry, and General Procedures

All experiments were conducted in Portland. Mice of the following inbred strains were obtained at 5-6 weeks of age from The Jackson Laboratory (Bar Harbor, ME, USA): 129S1/SvImJ (129), A/J (A), BALB/cByJ (BALB), C3H/HeJ (C3H), C57BL/6J (B6), DBA/2J (DBA/2 or D2), BTBR T^{+} *tf*/J (BTBR), and FVB/NJ (FVB). Mice were housed in plastic cages lined with Bed-o-Cob® bedding, usually three mice per cage, with others of their strain and sex. Food (Purina 5001) and water were provided ad libitum. Ambient temperature was 21 ± 1ºC, and colony lights were on for 12 hours (0600-1800). Bedding was changed weekly, but always after testing. All housing and test procedures were approved by the Institutional Animal Care and Use Committee at the Portland VA Medical Center and were in accordance with USPHS Guidelines.

Hypothermia Sensitivity and Tolerance

Experiment 1

A total of 96 naive mice from the eight strains (n=12/strain, equally divided between males and females) were tested at 61-63 days of age. The current methods for testing ethanol-induced hypothermia have been described in detail elsewhere (Crabbe et al., 1989). One hour before testing, mice were moved from the colony room to a testing room. Each mouse was weighed and placed in an individual ventilated 8 × 9 × 9 cm polycarbonate chamber for 1 hour. A baseline rectal temperature in degrees Fahrenheit was taken using a Sensortek Thermalert TH8 digital thermometer (Sensortek Inc., Clifton, NJ, USA), the mouse was injected intraperitoneally with 3 g/kg ethanol (Pharmco Products, Brookfield, CT, USA diluted 20% v/v in saline), and returned to its chamber. Temperatures were taken again at 30, 60, and 120 minutes after injection. At the end of the experiment, mice were returned to their original home cages in the colony. Hypothermia data from this experiment are identified subsequently as from "2004."

Experiment 2

A naive group of 95 male and female mice (n = 6/ sex/strain except FVB/NJ females with n = 5) of the same strains was tested for rapid and chronic tolerance to ethanol's hypothermic effects using a protocol identical to that for Experiment 1, except that the mice were repeatedly tested for five successive days. A blood sample (20 µL) was collected from the lateral tail vein of each mouse on days 1 and 5, immediately after the 120 minutes temperature assessment and assayed for blood ethanol concentration (BEC) by gas chromatography (Rustay and Crabbe, 2004). Hypothermia data from this experiment are identified subsequently as from "2005."

Loss of Righting Reflex

Experiment 3

Mice from Experiment 1 were allowed two weeks' respite, then were tested for sensitivity to the sedative effects of ethanol, measured as the LRR using our variant of the classical method (Keir and Deitrich, 1990; McClearn and Kakihana, 1981). Mice were moved to the testing room and left for 1 hour. Each mouse was then weighed and injected with ethanol (4 g/kg, 20% v/v, ip) and held until it appeared to have become intoxicated. At this point, the mouse was placed on its back in a V-shaped trough. A mouse was declared to have lost its righting reflex if it remained on its back for at least 30 seconds within 3 minutes after injection. The 3 minutes criterion for LRR was employed because we have found that nearly all mice that require longer than 3 minutes do not ever LRR and show substantially lower brain ethanol concentrations than those that do LRR (Ponomarev & Crabbe, 2002). We attribute the failure to LRR in 15% or more of mice after ip injections to the well-known failure of many ip injections fully to reach the peritoneal cavity even with experienced personnel performing the injections (Ponomarev and Crabbe, 2002). In our experiment, 21/96 of mice failed to LRR within 3 minutes (a rate higher than we usually see), and their data were excluded from the experiment. These mice were blood sampled and showed low BECs at 180 seconds. The remaining mice were then observed until they righted themselves, at which point recovery time was noted and the mouse was immediately placed again on its back. Mice righting themselves a second time within 30 seconds were deemed to have regained righting reflex. A blood sample was collected as above upon recovery of righting reflex. The LRR data from this experiment are identified subsequently as from "2005."

Ethanol Pharmacokinetics

Experiment 4

Six to eight naive male mice from the eight inbred strains were tested at 107.0±0.9 days old. Each mouse was injected with ethanol (3 g/kg, ip, 20% v/v) and a blood sample was taken as described at 30, 60, and 120 minutes. Mice from Experiment 1 were not used so that their body temperature data would not be influenced by the additional stress from repeated blood sampling. While in previous studies we have found sex differences in BEC, we have not seen significant strain X sex interactions. Samples were analyzed as described.

Statistical Analyses and Trait Variables

Present Data

Hypothermia was indexed as the change from baseline body temperature at 30, 60, and 120 minutes after injection with 3 g/kg. Thus, positive scores indicate HT and larger numbers indicate greater initial sensitivity to ethanol. Daily HT scores were evaluated and the degree of tolerance was defined as (day 1 HT30)—(day 2 HT30) for RTOL, or (day 1 HT30)—(day 5 HT30) for CTOL. Positive scores for the tolerance measures thus indicate a lesser hypothermic response on subsequent days than on day 1.

Sensitivity to LRR was defined as the duration of LRR (LRR DUR) in minutes (time of recovery minus time at onset) and BEC at the time of recovery from LRR (LRR BEC). Longer duration and lower BEC at recovery indicate greater sensitivity to ethanol.

Historical Data

Hypothermia and Loss of Righting Reflex

We have published strain surveys for initial sensitivity and tolerance to ethanol's hypothermic effects previously (Crabbe et al., 1982, 1994b; Crabbe, 1983). Those strain mean data for the 3 g/kg ethanol dose were included in the present correlational analyses. Data from these studies is referred to as from "1982," "1983," or "1994." Previously published studies used 9-13 weeks old mice, given ethanol ip (15 or 20% v/v in saline), except for the 1994 experiment (Crabbe et al., 1994b). Housing and testing environment procedures were as described above (For further details see each cited reference). Occasionally, additional variables are discussed (e.g., 60 minutes data), for which further information can be found in the published papers. We also used a variant of the classic method for assessing LRR. These mice were given 4 g/kg ethanol at 4-6 weeks of age and LRR DUR was recorded on mice resting on their backs in their home cage (Crabbe, 1983). In a later study, mice were tested to establish the ED_{50} to LRR in the classic trough (LRR ED_{50} 1994) using the up-and-down method (Dixon and Mood, 1948). The LRR DUR was not recorded in this study (Crabbe et al., 1994b).

We have also recently reported LRR sensitivity for these strains using the new, more sensitive method (Ponomarev and Crabbe, 2004). Mice were restrained in a cylinder immediately after 3 g/kg ethanol injection and turned 90° each 5 seconds until they failed to right, then retested at 3-10 minutes intervals until they regained righting reflex. Blood samples (20 µL) were collected from the peri-orbital sinus at initial loss and at recovery. Sensitivity to LRR indexed as a dose threshold (LRR NEW BEC, i.e., BEC at time of LRR) and in the traditional way as the duration of the effect (LRR NEW DUR in minutes) were included in the analysis. With this new method, animals lose righting reflex sooner (i.e., at lower BEC), and after lower doses of ethanol, than when tested by the classic method.

Acute functional tolerance

Using the new method, mice regain righting reflex at a higher BEC than their BEC at LRR. The difference between these values represents the magnitude of within-session, AFT (Ponomarev and Crabbe, 2004).

Pharmacokinetic Measures

Blood ethanol concentrations measured in Experiments 2 and 4 were included in correlational analyses to evaluate the possible influence of pharmacokinetics on trait sensitivity. Variables included in this analysis to index strain differences in effective ethanol dose after 3 g/kg injection were BEC 120 minutes from Experiment

2 (BEC120 2005), and BEC 30 minutes after injection from Experiment 4 (BEC 30 Exp 4). Several other BEC values appear in the published papers.

Experiments 1-4

Strain and sex differences, and effect sizes, were analyzed with ANOVA.

Genetic Correlations

The extent to which one can attribute the influence of a common set of genes on two traits can be estimated from the correlation of inbred strain means if the strains have been maintained and tested in equivalent environments (Hegmann and Possidente, 1981). With only eight strains, a correlation of $r = 0.71$ is required to achieve the usual level of statistical significance ($p < 0.05$). However, we were interested in the overall pattern of relationships among the tasks we explored, so we discuss some non-significant correlations as well as those that were significant. As has always been our practice in such analyses (Crabbe, 1983; Crabbe et al., 1994b, 2005; Metten and Crabbe, 2005), we also examined scatterplots for all correlations. With the low power of the correlational analyses where N= 8, single outlier strains can have a marked effect on correlation values, producing either false positive or false negative results, and we did not want to overlook potentially interesting relationships. The range of a genetic correlation is technically –1.0 to + 1.0, but the magnitude of each correlation is actually bounded by the true reliabilities of each correlated task, and of the correlation itself. Our overall goal was to see whether the same inbred strains would differ in a given response to ethanol roughly the same pattern across many years and across a reasonable range of environmental conditions (e.g., different technicians, different methods of assessing the same trait). The magnitude of any given pairwise correlation was not our major concern, nor was its statistical significance: therefore, we report probability values without multiple comparison protection (with such protection, only two correlations reported in this manuscript were statistically significant, both from the 1982 data set). Representative variables were chosen from the HT sensitivity and tolerance data for correlational analyses. We are happy to provide to any interested investigator any unpublished data we used for this report.

We also performed factor analyses to clarify the structure underlying the correlational data (Preacher and MacCallum, 2002). The strain means were subjected to an exploratory factor analysis using PROC FACTOR in SAS (Version 8, SAS Institute, 1994). The program excluded five of twenty strains from the analysis due to sparse data.

RESULTS

Experiment 1. Hypothermic Sensitivity

There are many ways to index temperature sensitivity in mice (Crabbe et al., 1994a). Initial analyses of variance considered strain and sex for three potential indices—two measures of change from baseline temperature (at 30 and 60 minutes), and the area under the 2 hours response time curve. Results were very similar for all three measures, and we included HT30 in the correlational analyses. Data from one animal (a C3H/HeJ female) were excluded due to apparent injection failure.

There were significant effects of sex on several variables, including body weight, baseline temperature, and temperature at 120 minutes (all $F > 13.1$, $p < 0.001$); none of these main effects interacted with strain. Female mice weighed about 4-5 g less than males, and their baseline and 120 minute temperatures were slightly higher than males (~0.25°C). There were no significant sex differences or interactions with strain for any other variables, including calculated HT scores at any time (all $F < 3.0$, $p > 0.05$). Using univariate ANOVAs, strains differed significantly in their sensitivity to the temperature-lowering effects of ethanol for all three measures ($F(7,87) > 13.0$, $p < 0.0001$). The most sensitive strains were 129 and BALB, with average change from baseline temperature at 30 minutes = 3.35 ± 0.23 and 3.24 ± 0.28°C, respectively. The least sensitive strains were D2 and C3H, with average HT30 scores of 1.03 ± 0.19 and 1.59±0.20ºC, respectively. The proportion of individual differences that could be attributed to genetic influences (strain effect size, omega squared, from the ANOVA) was >47% (Sokal and Rohlf, 1981). Neither body weight nor baseline temperature was significantly genetically correlated with hypothermia scores ($-0.17 < r < 0.62$, $p > 0.36$).

Experiment 2. Hypothermic Sensitivity and Tolerance

Data from nine animals were excluded from analyses either because their body weight or baseline temperatures changed significantly over days or they died during the experiment. These mice were distributed across 6 of 8 strains. Some data from four additional animals were removed for other reasons. One BALB female had an incomplete injection on day 5 (day 5 and CTOL data removed). One each of C3H female, D2 male, B6 male animals on day 3 had extremely high HT scores (> 6.2°C) for unexplained reasons, and their data from day 3 on were removed. Final sample sizes included n = 4–6/sex/strain/day). Strain mean body weights generally decreased during the experiment by 0–4%. The A/J strain showed mean body weight reduction of just less than 10%, and the significant strain difference observed was due to this strain ($F(7,74) = 3.9$, $p = 0.001$).

Strain mean baseline temperatures ranged between 37.3 and 39.0°C each day. A repeated measures ANOVA on baseline temperatures showed that all main effects ($F(1–7,66) > 12.1$, $p<0.0001$) and interactions of strain, sex, and day were significant ($F(4–28,264) > 1.6$, $p < 0.03$) except the between-subjects interaction of strain *X* sex ($F(7,66) =1.3$, $p = 0.27$), so we employed our usual measure of change from baseline, yielding a positively valenced hypothermia score for each animal at each time point. Figure 1 shows average temperatures over time each day in each strain. Figure 2 presents the HT30 scores for each strain over days. As with baseline temperatures, hypothermia scores at 30 minutes showed significant main effects of Strain ($F(7,66) = 14.4$, $p < 0.0001$) and Day ($F(4,264) =18.3$, $p < 0.0001$), and interactions of strain by sex, day by strain, and day by sex, ($F(4–28,66–264) > 2.8$, $p < 0.05$) but neither the main effect of sex nor the three-way interaction was significant ($p > 0.12$). To interpret this pattern of interactions, we next analyzed each day separately. Main effects of strain were significant each day $F(7,66–70) > 4.8$, $p < 0.01$). A main effect of sex was observed only on Day 3 ($F(1,67) = 8.3$, $p < 0.01$), while an interaction of strain by sex was seen only on days 1, 4 and 5 ($F(7,66–70) > 2.5$, $p < 0.05$). The most sensitive strain on Day 1 was BALB, with average HT30 of 3.64±0.26ºC, but the B6 strain was slightly more sensitive (3.49±0.48ºC) than 129S1 (3.12±0.28ºC). Similar to Experiment 1, the least sensitive strains were D2 and C3H, with average HT30 scores of 1.14 ± 0.22 and 1.32±0.23ºC, respectively. The proportion of individual differences on day 1 that could be attributed to genetic influences was > 42%.

Next, we calculated tolerance scores for days 2 (RTOL) and 5 (CTOL). Figure 3 presents RTOL and CTOL to ethanol's hypothermic effects by strain for the 30 minutes time point. The RTOL differed among strains ($F(7,70) = 4.96$, $p \leq 0.0001$), with BALB and 129S1 having the greatest RTOL scores (2.01 ± 0.29 and 1.30 ± 0.36°C, respectively) and C3H and D2 having the lowest RTOL scores (-0.25 ± 0.42 and -0.18±0.24ºC, respectively). CTOL scores showed a similar pattern. Strains differed in CTOL magnitude ($F(7,66)=8.07$, $p<0.0001$), with BALB and 129S1 having the greatest CTOL scores (2.17 ± 0.36 and 1.67 ± 0.33°C, respectively) and D2 and C3H having the lowest CTOL scores (-0.45 ± 0.27 and -0.16 ± 0.25°C, respectively).

Because the day 1 portion of Experiment 2 was identical in method to that for Experiment 1, we correlated strain means for several variables as indices of genetic reliability. Body weights, baseline temperatures, and HT scores were significantly correlated 0.89–0.97 across the two experiments. For example, HT30 and HT60 scores in Experiments 1 and 2 were correlated r = 0.90 and 0.95, respectively ($p < 0.01$).

Experiment 3. Loss of Righting Reflex

For the LRR experiment using the classic method, failure to lose righting reflex within 180 seconds has often been taken to indicate an incomplete ip injection (Crabbe et al., 1994b). For the new method, which assesses the moment of LRR with greater sensitivity, this has been shown to be an objective criterion for removal of subjects due to incomplete ip injections, based on subsequent pharmacokinetic data in such animals (Ponomarev and Crabbe, 2002). Loss of subjects in Experiment 3 was distributed nonsystematically across strains ($X^2 = 8.72$, $p > 0.05$). After removal of these animals, data were examined for outliers. Data for three other animals were removed because their durations of LRR or BECs were more than three standard deviations above the mean of all subjects. Significant effects of strain, sex, and their interaction were detected on body weight ($F(1–7,56) > 2.9$, $p \leq 0.01$), with females of each strain weighing less than males. Onset of LRR occurred at 20–176 seconds

among remaining subjects (mean ± - SEM: 96.3 ± 3.9 seconds, see Table I). A significant main effect of strain was detected ($F(7,56)=2.35$, $p < 0.05$), but no effects of sex were found ($F(1–7,56) < 2.13$, $p > 0.15$).

Significant main effects of strain and sex were found on LRR DUR ($F(1–7,56) > 8.2$,$p <0.001$), with males generally having longer durations than females (86.4 ± 3.76 versus 75.9 ± 3.30 minutes, respectively). A significant interaction was present ($F(7,56) = 2.5$, $p < 0.05$), which seemed to be due to a shorter duration for BALB males relative to females (65.7 ± 6.56 versus 77.4 ± 13.68, respectively; see Table I). Table I also shows the significant strain differences in the LRR DUR, collapsed on sex. The most sensitive strains were D2 and B6, with average durations of 95.9 ± 5.95 and 92.1 ± 4.62 minutes, respectively. The least sensitive strains were C3H and BALB, with average durations of 49.9 ± 5.21 and 70.9 ± 6.88 minutes, respectively. The strain effect size was 35%. There were no significant effects of strain, sex, or their interaction on the BEC at recovery of righting reflex ($F(1\text{-}7,56) < 2.0$, $p \geq 0.10$).

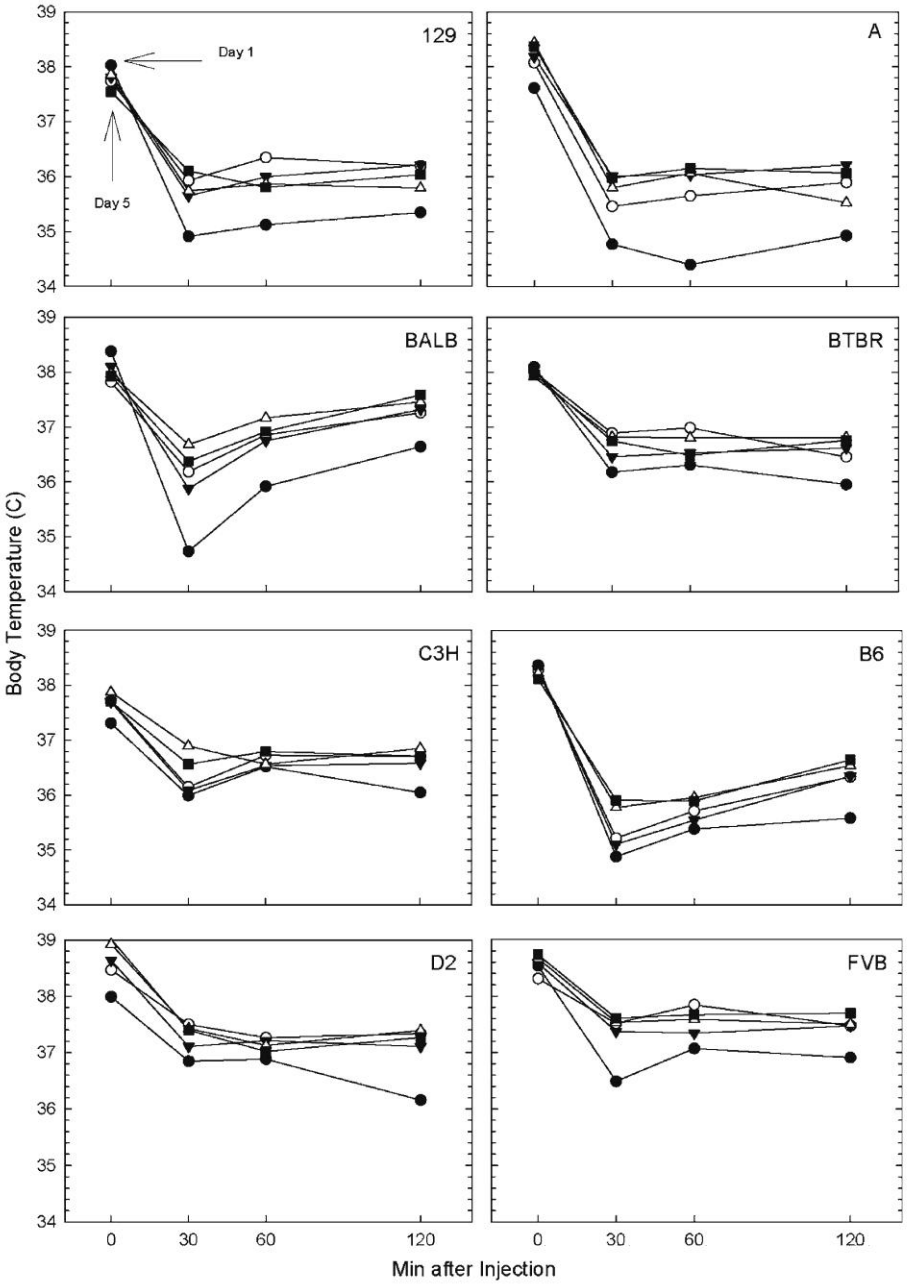

Fig. 1. Ethanol hypothermia sensitivity in Experiment 2. Mean strain body temperature (°C) at baseline (0 minute) and 30, 60, and 120 minutes after 3 g/kg ethanol is shown. Average SEM = 0.21, and is not depicted for clarity. Solid circles, day 1; open circles, day 2; solid triangles, day 3; open triangles, day 4; solid squares, day 5.

Experiment 4. Ethanol Metabolism

Strains differed significantly in BEC ($F(7,47) = 3.8$, $p<0.01$), and BEC declined systematically over time ($F(2,94) = 35.4$, $p < 0.0001$). There was also a significant strain *X* time interaction ($F(14,94) = 2.7$, $p<0.01$).As we were interested in relating behavioral scores 30 minutes after injection to BEC, we next performed a one-way ANOVA on these data. Again, strains differed significantly ($F(7,52) = 5.7$, $p<0.0001$).The range of strain mean values for BEC at 30 minutes was from 1.7 (BTBR) to 2.8 mg/ml (129).

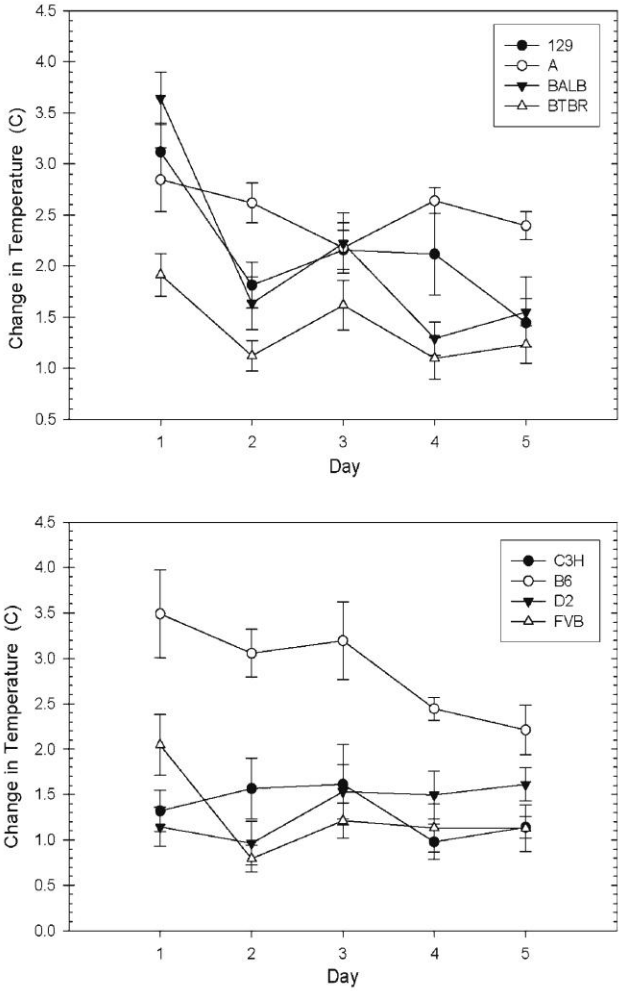

Fig. 2. Change in hypothermic response in Experiment 2. Strain mean ± SE change from baseline temperature 30 minutes after injection (HT30) is shown over days. Reductions in change scores over days indicate tolerance development.

Genetic Correlations

The correlations among strain mean HT, HT tolerance, LRR using both the classic and new methods, and acute functional tolerance in the new LRR test are given in Table II. Measures of BEC are also included. Correlations significant at $p < 0.05$ are shown in bold type, and those at $p < 0.01$ in bold italics.

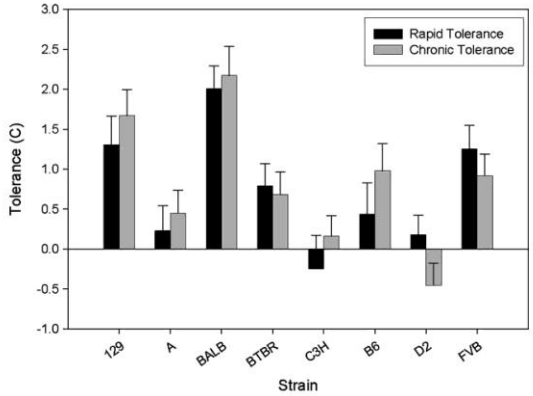

Fig. 3. Rapid (RTOL) and chronic (CTOL) tolerance to ethanol hypothermia in Experiment 2. Mean ± SE RTOL (dark bars) and CTOL (grey bars) are shown. RTOL, HT30 (day 2)–HT30 (day 1). CTOL, HT30 (day 5)–HT30 (day 1). Larger positive scores indicate greater tolerance development.

Hypothermic sensitivity and tolerance

Sensitivity to ethanol-induced HT was highly genetically correlated across experiments, with correlations among the four variables ranging from 0.50 to 0.90 (Fig. 4 and Table II). The exception was the low correlation (r = 0. 25, 14 strains) between HTs at 30 minutes in 1994 and 1982. This was not an aberration, as correlations among these two data sets for other doses and times on day 1 were also low (data not shown). However, both the 1982 and 1994 data correlated well with both 2004 and 2005 data.

The RTOL in 2005 was correlated with the earliest estimate of CTOL reported in 1982 (day 3: r = 0.76). It was also correlated with CTOL measures on both day 3 and day 5 from 2005, as well as CTOL at 5 days in the 1982 data ($r \geq 0.67$). Overall, the pattern of correlations among RTOL and CTOL measures indicated strong similarity of the genetic contributions to these traits across experiments and replications. The pattern of correlations among these variables for HT60 scores (and RTOL and CTOL scores based on this time point) were even higher than the correlations reported here ($0.72 \leq r \leq 0.95$ for HT and $0.56 \leq r \leq 0.93$ for tolerance), strengthening our belief that these variables are genetically related.

Loss of righting reflex and acute functional tolerance

The measure of duration of LRR obtained with the classical method in 1983 correlated well with the data from 2004 (r = 0.63, six strains) and from the new LRR method (r = 0.81, $p < 0.01$, 11 strains). It was somewhat puzzling that the 2004 data and new LRR method data were only weakly related (r = 0.42, eight strains). The other available data for estimating LRR sensitivity were the estimate of ED_{50} from 1994 using the classical method and the BEC at LRR (LRR NEW BEC), which we believe to be the best estimate from the new method (Ponomarev and Crabbe, 2004). These two indices were essentially uncorrelated with each other (r = 0.08), or with the classic (or new) measures of duration of LRR. The BEC at which animals initially show LRR and the LRR DUR would appear to be different traits, in terms of their genetic influences. The AFT using the new method was significantly correlated with BEC at onset (data not shown: insensitive strains showing less AFT, r=0.68, p <— 0.001), and was negatively correlated with the duration of LRR in all three data sets.

Table I. Loss of Righting Reflex in Experiment 3

Strain	Sex	*N*	Onset to LRR (seconds)	LRR Duration (minutes)	BEC (mg/ml)
129S1/SvImJ	Both	7	87.6 ± 12.4	90.1 ± 10.6	3.67 ± 0.11
	F	3	103.3 ± 28.4	69.5 ± 14.1	3.65 ± 0.19
	M	4	75.8 ± 5.3	105.5 ± 10.3	3.69 ± 0.15
A/J	Both	12	74.2 ± 6.2	79.6 ± 4.3	3.40 ± 0.11
	F	6	63.0 ± 8.5	75.6 ± 6.5	3.34 ± 0.20
	M	6	85.3 ± 6.6	83.7 ± 5.7	3.46 ± 0.11
BALB/cByJ	Both	9	86.0 ± 10.1	70.9 ± 6.9	3.20 ± 0.12
	F	4	76.0 ± 15.2	77.4 ± 13.7	3.09 ± 0.18
	M	5	94.0 ± 14.0	65.7 ± 6.6	3.28 ± 0.17
BTBR T^+ *tf*/J	Both	6	127.7 ± 3.2	87.2 ± 7.2	3.30 ± 0.08
	F	4	127.8 ± 4.7	77.3 ± 1.2	3.32 ± 0.12
	M	2	127.5 ± 4.5	107.0 ± 13.8	3.27 ± 0.14
C3H/HeJ	Both	9	102.2 ± 8.8	49.9 ± 5.2	3.52 ± 0.15
	F	3	85.7 ± 6.1	43.2 ± 10.8	3.01 ± 0.18
	M	6	110.5 ± 11.8	53.3 ± 5.9	3.78 ± 0.11
C57BL/6J	Both	9	90.9 ± 14.3	92.1 ± 4.6	3.48 ± 0.07
	F	4	79.0 ± 19.9	87.9 ± 3.9	3.58 ± 0.12
	M	5	100.4 ± 21.3	95.4 ± 7.8	3.39 ± 0.08
DBA/2J	Both	11	109.1 ± 10.8	95.9 ± 5.9	3.25 ± 0.07
	F	5	92.2 ± 18.9	78.9 ± 4.9	3.29 ± 0.11
	M	6	123.2 ± 9.9	110.0 ± 5.0	3.21 ± 0.10
FVB/NJ	Both	9	105.9 ± 10.1	89.1 ± 3.3	3.40 ± 0.10
	F	3	107.3 ± 5.2	90.2 ± 4.4	3.36 ± 0.22
	M	6	105.2 ± 15.4	88.6 ± 4.8	3.43 ± 0.12

Relationships among traits

HT sensitivity (day 1) was generally correlated with both RTOL and CTOL. These correlations were generally > 0.6–0.7 and tended to be larger within a data set, but were evident even across data sets. The exception was the data from 1994, where the single measure of initial HT sensitivity never correlated with a tolerance measure

($r \leq 23$). As noted above, correlations based on data 60 minutes after injection were even higher (data not shown). HT sensitivity was unrelated to AFT. Neither measures of LRR duration, ED_{50}, nor BEC at onset were strongly correlated with measures of HT sensitivity, RTOL or CTOL. Finally, AFT was unrelated to either RTOL or CTOL.

Table II. Strain Mean Correlations

	HT30 1982	*HT30 1994*	*HT30 2004*	*HT30 2005*	*RTOL 2005*	*CTOL D3 1982*	*CTOL D3 2005*	*CTOL D5 1982*	*CTOL D5 2005 DECR*	*LRR ED_{50} 1994*	*LRR NEW BEC*	*LRR DUR 1983*	*LRR DUR 2004*	*LRR NEW DUR*	*AFT (LRR NEW)*	*BEC 60 1982*	*BEC 120 2005*
HT30 1994	.25																
HT30 2004	.69	.62															
HT30 2005	.60	.50	***.90***														
RTOL 2005	−.02	−.12	.61	.60													
CTOL D3 1982	.77	.12	***.91***	.74	.76												
CTOL D3 2005	.28	.23	***.84***	.68	***.89***	***.93***											
CTOL D5 1982	***.66***	−.09	***.89***	.66	.67	.81	***.92***										
CTOL D5 2005	.23	.11	***.82***	***.83***	***.87***	.78	***.86***	.71									
LRR ED 1994	**− *.69***	−.26	−.43	−.17	−.06	***−.70***	−.35	***−.65***	.02								
LRR NEW BEC	−.08	.33	.20	−.01	−.55	−.26	−.17	−.34	−.22	.08							
LRR DUR 1983	.06	.03	.32	.39	.47	.03	.25	−.31	.46	.15	−.02						
LRR DUR '04	.31	.24	. 05	.14	.21	.19	.05	.01	−.03	−.46	−.50	.63					
LRR NEWDUR '04	.26	−.23	.26	.24	.33	.01	.18	−.14	.40	.19	.32	***.81***	.42				
AFT (LRR)	.00	.30	.09	−.10	−.48	−.16	−.09	−.04	−.33	−.17	***.68***	−.56	−.49	−.28			
BEC 60 1982	.38	.37	.32	.00	−.47	.17	.03	−.01	−.26	−.13	.35	.06	−.19	−.03	***.69***		
BEC 120 2005	.74	.63	.59	.62	.18	.65	.26	.53	.37	−.62	.12	.77	.51	.67	−.15	.27	
BEC 30 Exp 4	−.32	−.50	.08	.27	.25	−.04	−.00	−.22	.34	.50	−.14	.77	.11	.39	−.30	−.92	.28

Correlations significant at $p \leq 0.05$ are in **bold** text, those at $p \leq 0.01$ in ***bold italic*** text, and those at $p \leq 0.001$ are ***underlined***. Boxed correlations are significant with Bonferroni correction for multiple comparisons. Differences in statistical significant for correlations of similar magnitude reflect differences in *n*, which varies from 5–20 strains. RTOL refers to the difference between days 1 and 2. For CTOL measures, D3 indicates day 3 vs day 1, while D5 indicates day 5 vs day 1. See text for methods, other definitions of variables, and principles regarding interpretation of correlations.

To see whether the patterns we discerned by examining the bivariate correlation matrix were also seen in a global statistical analysis, we also examined these mean data with a factor analysis. A four-factor solution accounted for 89.7% of the variation in the measures, with eigenvalues ranging from 5.6 to 1.9. An oblique rotation resulted in fairly distinct factors, with intercorrelations ranging from —0.24 to 0.18. The loadings from this solution are displayed in Table III.

Role of Ethanol Pharmacokinetics

In our earlier analyses, we assessed the potential role of strain differences in ethanol pharmacokinetics by examining strain mean correlations of behavioral sensitivity variables with BEC. Our earlier analyses did not find patterns of significant positive correlation, which would have suggested that sensitive strains were those that achieved higher BEC. As we reported in 1982, 1983, and 1994, BEC values were not systematically related to behavioral data within those experiments. In the current experiments, we were surprised to see that the BEC values at 120 minutes from Experiment 2 tended to be correlated with data for HT sensitivity at 30 minutes from all four data sets ($.59 \leq r \leq 0.74$). However, correlations from Experiment 4 (BEC 30 minutes) were not significantly correlated with any variable (see Table II).

DISCUSSION

There are several different ways to think about the robustness of genetic differences. For some measures, individuals of known genotype can be assessed on multiple occasions and test—retest reliability or repeatability computed. The genetic contribution to such an assessment of reliability depends upon knowledge of trait heritability. For many traits, a second test can never be exactly the same as the first. Because repeated administration of drugs is known to lead to increases (sensitization; Phillips, 1997) or decreases (tolerance; Kalant et al., 1971) in response magnitude, estimating the reliability and degree of common genetic influence on drug responses is more difficult. The use of different populations of the same inbred strains is a useful way to estimate genetic relatedness among traits (Hegmann and Possidente, 1981), and the magnitude of the strain effect from an ANOVA is a useful estimate of genetic effect size from each trait (Sokal and Rohlf, 1981).

We found that a simple measure of acute ethanol sensitivity, HT, displayed substantial genetic influence across multiple panels of 8-20 inbred strains. In the two most recent studies with the same eight strains (Experiments 1 and 2), the genetic effect sizes for HT were remarkably similar (47% and 42%, respectively) as were the strain means ($r = 0.90$). We did not estimate genetic effect sizes in our 1982 or 1994 studies, but perusal of the reported ANOVA F values suggests that they were at least this large. What is more, HT sensitivity was fairly well genetically correlated across studies conducted by a single laboratory over a period of more than 20 years. The general procedures for trying to standardize the HT testing protocol (i.e., those taken to minimize any strain-specific environmental influences introduced by husbandry, handling or testing) have been quite similar since 1982. However, the substrain for many strains changed after the 1982 and 1983 studies. We used to obtain free NIH-substrain mice through an Interagency Agreement between the (then) Veterans Administration and the National Institutes of Health. More recent studies have used purchased JAX sub-strain mice. And, what is perhaps the most likely important source of differences, personnel performing the tests were different for every study. Even though Experiments 1 and 2 were performed by the same experimenters, they had different roles. Given all these potential sources of nongenetic influences, we find it quite reassuring that the strain correlations were as large as they were. The HT study that agreed least well with the others was the 1994 strain survey (Crabbe et al., 1994b). We believe the most likely reasons for this are procedural differences between this and the other studies. A complete discussion of procedural differences among studies is available upon request.

The HT scores at 30, 60, and 120 minutes were highly genetically correlated in both Experiments 1 and 2 (Fig. 4). Furthermore, the correlations were higher between 30 and 60, and 60 and 120, than the correlations between 30 and 120 (data not shown). We have seen this pattern in several other large data sets with different genotypes as well (Crabbe, 1994; Crabbe et al., 1994a, 1996). All genotypes generally show their greatest HT response by 45 minutes after injection of 3 g/kg ethanol, and by the later time points, the magnitude of genetic effects is progressively diminished as animals reestablish thermal homeostasis. Nonetheless, strain sensitivities were highly related across years, substrains and differences in procedure.

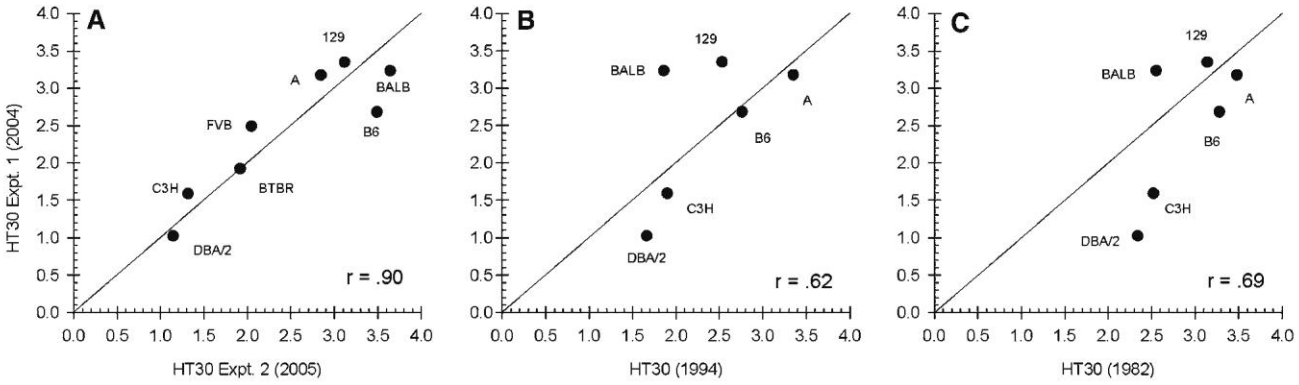

Fig. 4. Correlation of acute hypothermic response across experiments. Strain mean values for HT30 from Experiment 1 are plotted versus (from left to right) those from the same or similar strains from (A) Experiment 2, (B) 1994, and (C) 1982. Each panel shows the one-to-one line. Note that there were additional strains in common between 1994 and 1982 other than the six represented here. While the correlation between 1982 and 1994 data for six strains appears high here, adding the additional strains reduces the correlation to 0.25 (Table II).

Table III. Results from Exploratory Factor Analysis in SAS with Promax rotation

Measure	Factor 1	Factor 2	Factor 3	Factor 4		
HT30 1982	−0.03	0.10	**0.95**	0.09		
HT30 1994	−0.20	0.08	**0.90**	0.13		
HT30 2004	−0.02	**0.83**	**0.48**	−0.11		
HT30 2005	**0.37**	**0.78**	**0.49**	−0.05		
RTOL 2005	0.22	**0.93**	**−0.32**	0.05		
CTOL D3 1982	−0.30	**0.90**	0.09	0.15		
CTOL D3 2005	−0.17	**1.00**	−0.09	−0.01		
CTOL D5 1982	**−0.49**	**0.86**	0.07	0.02		
CTOL D5 2005	**0.32**	**0.95**	0.07	**−0.32**		
LRR ED 1994	**0.69**	−0.04	−0.20	**−0.68**		
LRR NEW BEC	**−0.56**	−0.29	**0.53**	**−0.43**		
LRR DUR 1983	**0.94**	−0.05	0.16	**0.33**		
LRR DUR 2004	**0.35**	−0.22	0.17	**0.90**		
LRR NEW DUR	**1.02**	−0.18	**0.32**	0.02		
AFT (LRR)	**−0.92**	−0.11	0.18	−0.12		
BEC 60 1982	**−0.69**	−0.11	**0.58**	−0.04		
BEC 120 2005	0.28	0.11	**0.73**	**0.54**		
BEC 30 Exp 4	**0.92**	−0.06	−0.22	−0.15		
Loadings are shown in bold if >0.32 (i.e. 10% of variance in measurement).						
Boxes indicate factors characterized primarily by one type of measure.						
Factor Intercorrelations						
Factor	**1**	**2**	**3**	**4**		
1		0.03	−0.24	0.10		
2			0.14	0.18		
3				0.15		
					Total	Percentage of variance explained
Eigenvalues	5.30	5.58	3.35	1.92	16.15	0.8972

In our examination of the potential role of ethanol pharmacokinetics in explaining the strain differences in behavioral sensitivity, we examined not only the two BEC values reported above (from Experiments 2 and 4), but also values taken from the earlier studies (data not shown). Considering all BEC behavior correlations, the only ones that appeared even potentially meaningful were those from Experiment 2 with most HT and TOL variables (see Table II). These correlations were not statistically significant, but represented only 6-8 inbred strain means, which could reflect too little power to detect real relationships. These BEC assessments were taken 120 minutes after injection, a time quite a bit removed from the behavioral measures. By so long after injection, the range of strain means had become rather small (1.9-2.4 mg/ml) and represented only about 13 % of the range through which BEC linearly predicts HT response (about 0.5-4.5 mg/ml). We believe that attempts to correlate BEC with behavior when the range of BEC is rather small underlie our frequent finding of no strong relationship in the earlier studies, and that the correlation seen here in Experiment 2 is an outlier. Thus, we believe that pharmacokinetic differences among strains do not explain a substantial degree of their behavioral sensitivity.

The classic method for assessing LRR reports the duration of the effect, or, sometimes, the BEC at the moment the animal regains its ability to right. Behavioral endpoints taken several minutes after injection of substantial ethanol doses are likely to reflect a combination of the initial sensitivity of the naive brain to the drug, and the development of acute functional tolerance (Erwin and Deitrich, 1996; Keir and Deitrich, 1990). There were five indices of sensitivity to LRR at recovery available for correlational analyses. Duration measured in minutes correlated reasonably well across the three studies (1983, 2004, and 2005) despite the distinct methodological differences described earlier. The BEC at recovery in Experiment 3 showed no significant differences among strains and was weakly and negatively correlated with BEC at recovery using the new method (r = -0.38, data not shown), suggesting that this is not as informative an index of sensitivity using the classic method. Interestingly, duration measured in 1983 correlated well with the BEC at recovery using the new method, which may suggest that placing animals on their backs in the home cage encourages them to respond differently than if placed in the V-shaped trough. As expected, all three duration measures were negatively correlated with AFT, suggesting that a strain that developed a great deal of acute functional tolerance regained function more quickly, even though the AFT data are based on BEC values, not time.

The new method for assessing LRR sensitivity relies on estimating the brain ethanol concentration at the moment an animal loses its ability to right itself. Three indices of onset sensitivity were available for correlational analyses. It should be noted that animals tested for LRR ED_{50} in 1994 were not naive, but had been tested 1 week earlier for activity and HT following 1-3 g/kg ethanol (Crabbe et al., 1994b). The ED_{50} for the strain to lose righting reflex was negatively correlated with the onset in seconds to LRR in Experiment 3 (six strains), and not correlated with the BEC at onset from the new method. The latter two indices correlated reasonably well (r = 0. 59, eight strains). This suggests that both the new and classic methods are tapping similar physiological systems when onset of LRR is the index. In general, onset measures were not correlated with duration measures, suggesting that these two types of indices are measuring different genetic components of the response. The degree of AFT developed was positively correlated with the onset in seconds and onset BEC in the latter two studies (r > 0.61, 8-20 strains). Animals losing function more quickly (and therefore at lower BEC) were more likely to show pronounced AFT and recover at higher BEC.

The genetic correlation between RTOL and CTOL is generally consistent with other available data. For example, we have selectively bred mice to display High (HRT) versus Low (LRT) Rapid Tolerance to ethanol intoxication on the accelerating rotarod. The HRT mice also show greater CTOL, consistent with common genetic influences on RTOL and CTOL (Rustay and Crabbe, 2004). Similarly, RTOL and CTOL to ethanol hypothermia were significantly correlated across 20 inbred strain means (data not shown, from Crabbe et al., 1982). Numerous studies show similarity of pharmacological responses of measures of RTOL and CTOL (Barbosa and Morato, 2000, 2001; Kalant, 1996; Khanna et al., 1991, 1994). However, in HRT and LRT mice, there was no effect of either MK-801 or D-cycloserine on RTOL (N.R. Rustay and J.C. Crabbe, unpublished), although these NMDA effectors usually block or stimulate RTOL and CTOL. Finally, it should be noted that one would not expect to find complete overlap between genetic influences on CTOL (for example) using different behavioral endpoints such as HT and rotarod ataxia.

That AFT appears to be different from RTOL and CTOL is also consistent with most studies, although the literature is sparse. Mice have been selectively bred for high-AFT (HAFT) and low-AFT (LAFT) using two successive recoveries of ability to balance on a dowel after ethanol (Erwin and Deitrich, 1996). However, these animals did not differ in the degree of CTOL to two different effects of ethanol, HT and a test of ambulatory ataxia, the grid test (Rustay et al., 2001). Furthermore, and unlike RTOL and CTOL, AFT does not respond to some of the same pharmacological manipulations that affect RTOL and CTOL (Khanna et al., 1992), although altering the test conditions subsequently suggested that AFT also was inhibited by NMDA antagonists (Khanna et al., 2002).

The factor analysis tended to support the relationships and interpretations just described. The LRR variables loaded predominantly on Factor 1, tolerance measures on Factor 2, and HT sensitivity measures on Factor 3. The rather small factor inter-correlations suggest that while measures of the same underlying construct revealed

stable patterns of strain influences, there was little stable evidence of genetic relationships across measures. One exception (the strong negative loading of AFT LRR on Factor 1) is explained because the AFT LRR variable is computed from the LRR NEW BEC.

In summary, different cohorts of inbred mouse strains were found to produce rather reproducible patterns of strain differences when tested for similar responses to ethanol over more than 20 years. Although different methods were used to assess and index similar underlying traits, the strain differences were robust against the relatively minor range of procedural variations we employed. They were evident even though some of the older data used different substrains in some cases, and they were able to detect genetic "signal" from the "noise" introduced by multiple experimenters. The underlying genetic consistency was strong enough to allow us to determine that a rather large procedural variation on one test, the LRR, appeared to be assessing the same underlying phenotype as the classic method, but that onset and recovery measures from either version were under dissociable genetic influences. And, the cumulative data from such strain surveys was shown to be helpful in distinguishing different temporal forms of ethanol tolerance, which will aid their mechanistic distinction. For the most part, strain differences were in sensitivity of the brain to ethanol rather than of pharmacokinetic origin. However, the degree of genetic similarity revealed depended on the specific response studied, and should not be assumed to be true for all behavioral tasks. Furthermore, these studies were conducted by a single laboratory, and may not accurately predict the generalization of this genetic robustness across laboratories.

These results are relevant for modeling individual differences in drug sensitivity in mice. Genes that predict individual human risk must be found in the natural environmental setting in which they are expressed. The predictive value of any such hypothetical gene may, or may not, extend beyond that specific set of environmental features. If groups of clones of mice cannot be shown to have characteristic differences in drug sensitivity across a reasonable range of laboratory environmental conditions, then there is little hope of finding genetic markers or haplotypes with predictive value across genetically heterogeneous groups of humans. The results of our retrospective analysis suggest that genetic animal models can indeed reproducibly measure drug responses, which makes further studies likely to be valuable as long as care is taken with measurement methods.

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