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Sensitivity and tolerance to ethanol-induced incoordination and hypothermia in HAFT and LAFT mice

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Abstract

Acute functional tolerance (AFT) manifests as rapid adaptation during a single ethanol exposure, leading to a decrease in the behavioral response to ethanol. In order to investigate the genetic and environmental components of the development of AFT, mice were selectively bred in replicate from HS/Ibg mice. High (HAFT) and low (LAFT) acute functional tolerance selected lines were bred to differ in the rate of development and magnitude of AFT to ethanol's intoxicating effects using a static dowel-balancing task. In the present set of experiments, HAFT and LAFT mice were tested for development of AFT on a fixed-speed rotarod using a protocol similar to that for which they were selected. HAFT mice developed greater AFT to ethanol than did LAFT mice. In a separate experiment, other mice from these lines were tested for initial sensitivity and the development of chronic tolerance to ethanol-induced hypothermia, and ethanol-induced incoordination in the grid test. Previous research has detected possible common genetic control of these phenotypes. No differences between lines were found in initial sensitivity to ethanol or in the development or magnitude of chronic tolerance in either test. These experiments show that genetic factors influencing the development of acute tolerance to ethanol-induced intoxication are at least partially distinct from those influencing initial sensitivity and the development of chronic tolerance to ethanol-induced hypothermia and incoordination. Furthermore, these experiments show that AFT measured by the stationary dowel generalizes to AFT measured by the fixed-speed rotarod.

Keywords: Acute functional tolerance; Ethanol; Incoordination; Hypothermia; Selected lines

1. Introduction

Acute functional tolerance (AFT) refers to a reduction in neural sensitivity to ethanol and is thought to develop within minutes of a single ethanol exposure (Palmer et al., 1985). As described by Mellanby (1919), this type of tolerance is characterized by more severe intoxication at the same blood ethanol concentration (BEC) on the ascending limb of the time–blood ethanol curve than on the descending limb. However, ethanol is absorbed very rapidly after peripheral injection, and it is difficult to measure many behavioral endpoints of intoxication accurately during the ascending limb of the time–blood ethanol curve. Therefore, a modified procedure to measure AFT has been introduced. This method assesses AFT by administering a second dose of ethanol before the ethanol from a first dose has been completely eliminated from the body. BEC is measured at the behavioral endpoint defining intoxication threshold during the initial descending BEC curve, and then when the behavior is again seen during the second descending arm (Gallaher et al., 1982; LeBlanc et al., 1975).

The notion that AFT comprises both genetic and environmental influences is supported by the successful selective breeding of two replicates of the high (HAFT) and low (LAFT) acute functional tolerance selected lines (Erwin and Deitrich, 1996). For this selection, animals were required to

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balance on a static wooden dowel after an initial ethanol injection. After absorption into the brain, ethanol caused the mice to lose the ability to stay on the dowel. BEC at the time of the initial fall from the dowel indexes initial sensitivity to ethanol's intoxicating effects; a low BEC indicates high neural sensitivity, while a high BEC indicates low sensitivity. Animals were then tested periodically until they recovered the ability to balance and blood samples were taken upon recovery of this ability. HAFT and LAFT lines do not differ in initial sensitivity to an acute injection of ethanol. After the blood sample was drawn, the animal was given a second injection of ethanol and blood was taken again when the animal recovered for the second time. AFT was defined as the difference between BEC at the time of regaining balance on the dowel following the second versus the first injection of ethanol. Both lines develop AFT, but the magnitude of AFT differs by two- and four-fold for females and males, respectively (Erwin and Deitrich, 1996; Erwin et al., 2000).

There are a number of forms of tolerance (see Lê et al., 1992), defined operationally by rate of tolerance development. There is, however, some variety in the names given to various forms of tolerance. As noted earlier, AFT develops within minutes of an ethanol exposure. Chronic tolerance develops (generally over days or weeks) following repeated, discrete drug administrations, continuous ethanol vapor inhalation or intermittent self-administration of ethanol. Rapid tolerance, a form of chronic tolerance, was initially defined as a decreased response to a second dose given after all the ethanol from the first injection had been eliminated from the body (Crabbe et al., 1979). As described above, tolerance assessed in an experimental paradigm of administering multiple, sequential doses following each recovery of a behavioral endpoint, without allowing complete elimination of ethanol to occur, has been termed either acute tolerance (Erwin and Deitrich, 1996) or rapid tolerance (Gallaher et al., 1982, 1996). We refer to the paradigm used in the selection of the HAFT and LAFT mice as AFT.

Previous research has detected possible common genetic determinants (genetic correlations) of numerous ethanol-related phenotypes. Initial sensitivity, as well as acquisition of acute and chronic tolerance, are influenced by some common genetic factors (Crabbe et al., 1982; Khanna et al., 1985, 1990). Moreover, it appears that chronic tolerance to ethanol-induced hypothermia and incoordination share some common genetic control (Lê and Kiianmaa, 1990).

Selected lines are powerful tools for examining potential genetic correlations. Genetic selection for a particular trait results in complementary lines that differ principally in the genes responsible for that trait. Finding differences between the two lines on other traits suggests that the same (or a subset of) genes responsible for the selected trait are influencing these other traits as well, i.e., the traits are under common genetic control. When replicate selected lines are available, as with the HAFT and LAFT mice, finding a correlated response in both replicate comparisons increases the likelihood of a true genetic correlation as opposed to a chance finding.

In the present set of experiments, HAFT and LAFT mice from both replicates were tested for sensitivity to ethanol and development of AFT on a fixed-speed rotarod. Our goal was to see whether the selection phenotype (tolerance to intoxication on a static dowel) would generalize to another common test of motor incoordination. Furthermore, sensitivity and chronic tolerance to the incoordinating and hypothermic effects of ethanol were examined in HAFT and LAFT mice. Finding a line difference in chronic tolerance development would suggest that the genes responsible for AFT development are also contributing to differential chronic tolerance. There is evidence that the development of tolerance to ethanol can lead to increases in ethanol consumption (Tabakoff and Hoffman, 1988). Elucidation of the mechanisms of tolerance development could therefore lead to a better understanding of and treatment possibilities for alcoholism.

2. General materials and methods

2.1. Animals and husbandry

HAFT and LAFT mice aged 80–135 days, from selection generations 7–12 (S_7–S_{12}) were used in all experiments. These mice were developed from a genetically heterogeneous stock of HS/Ibg mice by Dr. V.G. Erwin at the University of Colorado Health Sciences Center (Denver, CO). HAFT-1, HAFT-2, LAFT-1 and LAFT-2 mice from second and third litters were received from Dr. Erwin and were maintained at the Veterinary Medical Unit, Department of Veterans Affairs Medical Center (Portland, OR). Mice were not bred at the VA Medical Center. Same-sex groups of HAFT or LAFT mice (Replicate 1 or 2) were housed on corncob bedding, one to five per polypropylene cage. The colony room was kept on a 12-h light:dark cycle with lights on at 6 a.m. Room temperature ranged from 20°C to 21°C. Mice had free access to food and water except during experimental procedures. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with NIH guidelines.

2.2. Drugs

Ethanol solutions, 15% and 20% (v/v), were made by mixing ethanol (Pharmco, 200 proof) and 0.9% saline. Solutions were made fresh daily. All injections were given intraperitoneally according to body weight, and control mice used for comparison were given equivalent volumes of 0.9% saline.

2.3. Blood (BEC) and brain (BrEC) ethanol concentration determination

Ethanol concentration was determined from blood (Experiment 1) and whole brain (Experiments 2 and 3)

by gas chromatography (Model 5890a; Hewlett Packard; see Boehm II et al., 2000a). For blood samples, mice were gently restrained and 20 μl of blood was taken from the periorbital sinus. Fifty microliters of $ZnSO_4$, 50 μl of $Ba(OH)_2$, and 30 μl of dH_2O were added to the samples, which were then centrifuged at 12,000 rpm for 5 min. Supernatant was removed, analyzed and compared to a standard ethanol concentration curve. For brain samples, mice were euthanized and the whole brain was removed and immediately frozen. Briefly, whole brains were homogenized in 150 μl of $ZnSO_4$ (5%), 150 μl of $Ba(OH)_2$ (0.3 N) and 300–600 μl of dH_2O (1.5 × brain weight). Homogenates were centrifuged at 12,000 rpm for 10 min. Supernatant was removed, analyzed and compared to a standard ethanol concentration curve.

2.4. Statistical analysis

Animals arrived for testing one replicate at a time (i.e., HAFT-1 and LAFT-1, or HAFT-2 and LAFT-2). As a result, it was not possible to test both replicates concurrently in any of the experiments. Therefore, each replicate line was analyzed separately using an analysis of variance (ANOVA). When interactions were present, simple main effect analyses were performed by separate one- or two-way ANOVAs between variables. Differences were considered significant at $P < .05$. Systat (Chicago, IL) version 9.0 was used for all analyses.

3. Experiment 1: Rotarod AFT

3.1. Methods

Male HAFT and LAFT Replicate 1 (S_7) and Replicate 2 (S_8) mice ($n = 12–16$ per replicate per line) were tested for AFT on a fixed-speed rotarod. Briefly, the rotarod comprises a 2.5-in. rotating (5 rpm) cylinder covered with 320 grit sandpaper to reduce slipping. The test protocol followed that of Erwin and Deitrich (1996) as closely as possible, except for the difference in apparatus. On all test days, mice were moved into the testing room and allowed to acclimate for 30 min. Mice were given practice trials (three per day, with 30-s rests between trials) until each was able to stay on the rotarod for 2 min. This criterion was reached by all animals in 3–4 days of practice. On the test day, mice were given practice trials until the 2-min criterion was reached (one to two trials for all mice). Mice were then administered 1.75 g/kg ethanol (15% v/v in 0.9% saline) and immediately placed on the rotarod. Latency to fall was recorded. Mice were then re-tested on the apparatus every 5 min until recovery. First recovery was defined as the ability of the mouse to remain on the rod for two consecutive 30-s trials. Upon first recovery, a periorbital blood sample (20 μl) was taken, and a second dose of 2.0 g/kg ethanol was given. Sixty minutes after the second injection, re-tests every 5 min commenced to identify second recovery. Second recovery consisted of regaining balance on the rotarod (two 30-s trials) at which time a second periorbital blood sample was drawn.

3.2. Results

Fig. 1 shows the results of experiment 1. BECs are shown for both first and second recoveries. In addition, the magnitude of AFT was determined by calculation of an AFT score ($BEC_2 - BEC_1$). Because the replicates were tested at different times, each replicate was analyzed separately. Sensitivity was assessed with a one-way ANOVA of Line for BEC at Recovery 1 (BEC_1) for each replicate. These analyses revealed no significant effects of Line on this measure of sensitivity in either replicate.

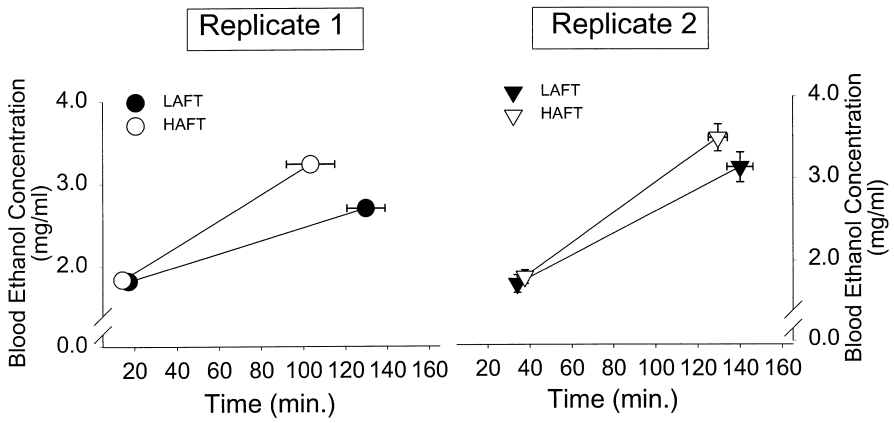

Fig. 1. Sensitivity and AFT on the fixed-speed rotarod in Replicates 1 and 2 mice. BEC is shown (*y*-axis) versus time after injection (*x*-axis). The earliest point on each line represents the time and BEC at the initial recovery of function. Similarly, the second point represents the time and BEC for recovery after the second injection (see text). Lines did not differ at first recovery for either replicate, but HAFT-1 mice had a higher BEC than did LAFT-1 mice at the second recovery ($P < .001$). Replicate 2 mice did not differ significantly at either point. Values represent means ± S.E.M. for 12 mice per line (Replicate 1), and 13–16 mice per line (Replicate 2). Absent S.E.M. bars are smaller than symbol size.

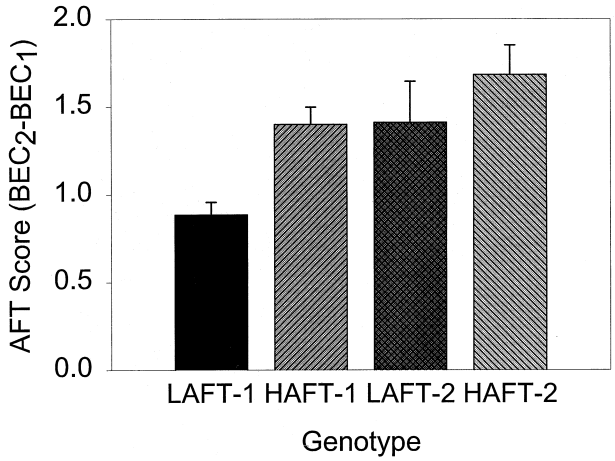

Fig. 2. AFT score for each of the replicate lines tested on the fixed-speed rotarod. The AFT score was calculated by subtracting the BEC at Recovery 1 from the BEC at Recovery 2 (see Fig. 1). The higher the AFT score, the greater the degree of AFT. HAFT-1 mice had a significantly higher AFT score than LAFT-1 mice. However, HAFT-2 and LAFT-2 did not differ. Values represent mean ± S.E.M. for 12 mice per line (Replicate 1), and 13–16 mice per line (Replicate 2).

These results were similar to those obtained during selection on the static dowel task. A common way to report AFT is the difference between BEC at Recoveries 2 and 1. An analysis of our calculated AFT score ($BEC_2 - BEC_1$) revealed greater AFT development in HAFT mice over LAFT mice in Replicate 1 [$F(1,22) = 17.56$, $P < .001$]. In Replicate 2, HAFT and LAFT mice developed AFT to the same degree [$F(1,27) = 0.93$, $P > .30$] (see Fig. 2).

Although the replicate lines were tested separately, a two-way ANOVA (Line × Replicate) for AFT score was done to determine if there was an overall main effect of Line. This would provide better evidence for a true genetic correlation (Crabbe et al., 1990). This analysis revealed a main effect of Line [$F(1,49) = 5.70$, $P < .03$] and Replicate [$F(1,49) = 6.07$, $P < .02$]; however, there was no Line × Replicate interaction [$F(1,49) = 0.54$, $P > .46$]. Because the genetic difference was of sufficient magnitude in the first replicate pair of lines to create a significant main effect when the second replicate pair was included in the ANOVA, these results provide strong evidence for the notion that the mechanisms necessary for the development of AFT on the static dowel task may also be mediating AFT development on the fixed-speed rotarod (see Crabbe et al., 1990).

4. Experiment 2: Sensitivity and chronic tolerance to ethanol-induced ataxia

4.1. Methods

Male and female HAFT and LAFT mice ($n = 7–12$ per group, replicate and line) were tested in the grid test apparatus (Belknap, 1975). There were insufficient numbers of male and female mice to allow comparisons between sexes. Therefore, males and females were combined for analyses. Replicate 1 mice were from S_{12}, while Replicate 2 mice were from S_7. The apparatus comprised a hardware cloth grid (1.5 cm) suspended 1 cm above a metal plate floor. A $15 \times 15 \times 20$ cm clear Plexiglas box was placed on top of the hardware cloth within which the mouse can ambulate. Missteps occurred when a mouse's foot slipped through the grid and touched the metal floor, completing an electrical circuit. An Apple IIe computer recorded missteps. The entire apparatus was contained within a Digiscan activity monitor, which measured horizontal activity through photocell beam breaks. Photocell beams were located two per side. Ataxia was measured as the ratio of missteps to activity counts times 100 for each test day.

Mice were divided into two groups (EtOH or SAL). A test schedule for each of the groups of mice is depicted in Table 1. Mice in both groups were tested on Days 1 and 2 after saline injections to habituate them to the apparatus and handling. Mice in the SAL group were given saline on all test days except on the final test day (Day 11) on which they received ethanol (2.5 g/kg, 20% v/v). EtOH group mice were given saline on Days 1 and 2, and ethanol (2.5 g/kg) on all other test days. Mice were only tested on Days 1, 2, 3, 5, 7, 9 and 11. On Days 4, 6, 8 and 10, mice were neither tested nor injected and remained in their home cages in the colony room. On all test days, mice were moved into the procedure room and allowed to sit undisturbed for at least 30 min. Mice were then weighed and placed into individual holding cages. Injections were given and each mouse was placed into the apparatus for the 15-min test. After testing, mice were placed back in their home cages and returned to the colony room. When testing was completed on day 11, animals were sacrificed and their brains were removed and frozen on dry ice for determination of BrECs.

Table 1
Procedure schedule for the grid test

	Test day										
Treatment group	1	2	3	4	5	6	7	8	9	10	11
EtOH											
Injection	S	S	E		E		E		E		E
Test in monitors	+	+	+	–	+	–	+	–	+	–	+
SAL											
Injection	S	S	S		S		S		S		E
Test in monitors	+	+	+	–	+	–	+	–	+	–	+

Procedure for animals tested in the grid test apparatus. All animals received saline (S) on Days 1 and 2. Animals chronically treated with ethanol (EtOH group) were given 2.5 g/kg ethanol (E) for the remaining 5 days of testing. Saline-treated animals (SAL group) received saline on all test days except on Day 11 when they were given 2.5 g/kg ethanol.

4.2. Results

Results are shown in Fig. 3. Sensitivity to ethanol was assessed by analyzing ataxia ratios on Day 3 in the EtOH group using a one-way ANOVA with Line as the between-groups factor; only significant effects are reported. There were no differences in sensitivity in either set of replicate lines. Between-group analyses of Day 11 scores allowed comparisons of mice receiving their fifth ethanol injection and mice receiving their first, when both groups have had equal exposure to the apparatus. In Replicate 1 mice, two-way ANOVA (Line × Treatment) of the ataxia ratios (misteps/activity count × 100) on Day 11 revealed a main effect of Treatment [$F(1,34) = 8.91$, $P < .01$] as well as a Line × Treatment interaction [$F(1,34) = 8.86$, $P < .01$]. Fig. 3 shows that LAFT-1 mice that had received repeated ethanol administrations had a lower ataxia ratio than LAFT-1 mice receiving their first ethanol injection, consistent with the development of tolerance. Conversely, HAFT-1 mice given repeated ethanol did not differ from HAFT-1 mice receiving their first ethanol exposure. Analysis of Day 11 ataxia ratio in Replicate 2 revealed a different pattern. There was a main effect of Line [$F(1,35) = 23.87$, $P < .001$] and a Line × Treatment interaction [$F(1,35) = 7.59$, $P < .01$]. HAFT-2 mice in the EtOH group developed tolerance to ethanol's ataxic effects, while LAFT-2 mice given prior ethanol treatments showed a significantly greater response than those receiving their first injection. LAFT-2 mice had greater overall impairment than HAFT-2 mice.

Tolerance development was also determined by examining the ataxia ratios from Days 3 to 11 in only the groups repeatedly treated with ethanol using a two-way ANOVA with Line as the between-groups factor, and Day as the within-group factor. In Replicate 1 mice, the only significant factor was that of Day [$F(4,68) = 3.74$, $P < .05$], with both HAFT-1 and LAFT-1 mice showing a decrease in ataxia ratios over the course of the experiment (i.e., tolerance to ethanol's ataxic effects). In Replicate 2, however, the same ANOVA revealed a main effect of Line [$F(1,16) = 16.64$, $P < .002$] and Day [$F(4,64) = 2.70$, $P < .04$], and a significant Line × Day interaction [$F(4,64) = 4.65$, $P < .01$]. HAFT-2 mice given chronic ethanol exhibited less ataxia over the days of the experiment, while LAFT-2 mice seemed to become more sensitive, showing greater ataxia over days.

Table 2
BrECs (mg/ml) for each of the replicate lines

Treatment group	HAFT-1	LAFT-1	HAFT-2	LAFT-2
EtOH	2.75 ± 0.07	2.64 ± 0.11	2.34 ± 0.06	2.37 ± 0.04
SAL	2.79 ± 0.18	2.69 ± 0.14	2.27 ± 0.12	2.44 ± 0.09

BrECs 15 min after an injection of 2.5 g/kg ethanol. There were no differences between animals given their fifth injection (EtOH group) and those receiving their first dose (SAL group) in either replicate. Values are means ± S.E.M. for 7–12 mice per treatment group per line per replicate.

Locomotor activity data from this experiment were analyzed with a two-way ANOVA in ethanol-treated animals only. This revealed main effects of Line [$F(1,17) = 13.70$, $P < .01$] and Day [$F(4,68) = 3.88$, $P < .01$] in Replicate 1 mice (data not shown). Overall, HAFT-1 mice were more active than LAFT-1 mice and activity increased over days. There was also a Day × Line interaction [$F(4,68) = 3.78$, $P < .01$]. Analysis of changes across days within each line showed that LAFT-1 activity did not change over days, while HAFT-1 mice showed increasing activity over the course of ethanol treatment. Similar analysis in Replicate 2 mice chronically treated with ethanol revealed no significant main effects or interactions, suggesting that both HAFT-2 and LAFT-2 lines had similar activity responses to ethanol treatment.

Analysis of BrEC in each replicate after the Day 11 ethanol injections revealed no differences between the lines in either replicate (Table 2). Thus, the behavioral differences in response to ethanol between genotypes and groups represent brain sensitivity to ethanol, not differential ethanol pharmacokinetics.

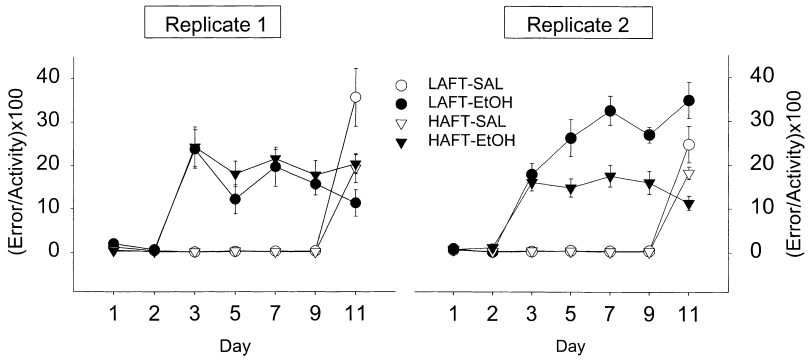

Fig. 3. Ataxia ratios in the grid test for both HAFT and LAFT lines of Replicates 1 and 2. The ataxia ratio is calculated by dividing the number of foot slips through the grid by the total number of photocell beam breaks and multiplying by 100 (see text). All mice received saline on Days 1 and 2. Ethanol-treated mice were given 2.5 g/kg of 20% ethanol. On days when mice were not tested, mice were undisturbed in the colony room. On Day 11, all mice received ethanol injections. See Table 1 for the complete injection and test schedule. Values represent means ± S.E.M. for 7–12 mice per treatment group, line and replicate. For statistical analyses and results, see text.

5. Experiment 3: Sensitivity and chronic tolerance to ethanol-induced hypothermia

5.1. Methods

Male and female HAFT and LAFT mice of Replicate 1 ($n = 10–11$ per sex per line; S_7) were injected with 3.0 g/kg ethanol (20% v/v) for three consecutive days. A separate, nearly identical study was performed using female Replicate 2 mice (S_7), inadvertently given 3.5 g/kg ethanol ($n = 10$ per line). Only females were used due to the unavailability of males at the time of testing. That Replicate 1 and 2 mice received different doses of ethanol is not of concern because replicates were analyzed separately. On Days 1 and 3, mice were moved into the testing room, weighed and placed into individual ventilated Plexiglas cages. Mice were allowed to sit undisturbed for at least 30 min prior to the start of testing. Baseline temperatures were taken just prior to the injection of ethanol. Following injections, mice were returned to the hypothermia chambers and temperatures were again taken at 30 and 60 min after injection. For all temperature measurements, a 0.5-mm probe was inserted into the rectum of each mouse to measure core body temperature. Each probe was connected to a Sensortek (Clifton, NJ) Thermalert TH-8 temperature monitor. Readings were taken 5 s after insertion of the probe to allow the temperature reading to stabilize on the monitor. On Day 2, mice were moved into the testing room, habituated for at least 30 min and injected with 3.0 (Replicate 1) or 3.5 (Replicate 2) g/kg ethanol. They were then placed back in their home cages and returned to the colony room. Temperature measurements were not taken on Day 2. Following the final test session on Day 3, mice were sacrificed and their brains were removed for analysis of BrEC by gas chromatography.

5.2. Results

A three-way ANOVA (Line × Sex × Day) of Replicate 1 data was performed to investigate differences in baseline temperature. This analysis revealed significant main effects of Sex [$F(1,38) = 18.36$, $P < .001$] and Day [$F(1,38) = 22.62$, $P < .003$]. Male and female LAFT-1 mice had Day 1 baseline temperatures (mean ± S.E.M.) of 37.3 ± 0.24°C and 37.7 ± 0.13°C, respectively. Similarly, male HAFT-1 mice had lower baseline temperatures than female HAFT-1 mice, 36.9 ± 0.23°C and 37.5 ± 0.14°C, respectively. Both sexes and both lines showed a higher baseline temperature on Day 3 than Day 1. Further, a significant Day × Line interaction was found [$F(1,38) = 4.43$, $P < .05$], with HAFT showing a greater increase in baseline temperature on Day 3 than LAFT mice. Because of these baseline differences, Fig. 4 is expressed as the temperature change from baseline to show an unambiguous index of hypothermic sensitivity and tolerance (Crabbe et al., 1982).

For each mouse, an average of the temperature change from baseline for measurements taken at 30 and 60 min was calculated for each day, and analyses were performed on these values. For Replicate 1 mice, a three-way (Line × Sex × Day) ANOVA of the average change from baseline scores showed no effects of Sex or Line, but a significant main effect of Day [$F(1,38) = 16.18$, $P < .001$]. Fig. 4 shows that both lines showed less hypothermic response on Day 3 than Day 1, demonstrating the development of chronic tolerance in both lines. The lines did not differ in their response on Day 1, suggesting equal sensitivity to the hypothermic effects of ethanol in both lines. BECs did not differ between any of the groups in this experiment.

Fig. 4 also shows the response of female Replicate 2 mice to 3.5 g/kg ethanol ip. A two-way (Line × Day) ANOVA of the average change from baseline temperature revealed only a main effect of Day [$F(1,18) = 69.56$, $P < .001$], with both lines again showing an attenuated hypothermic response on Day 3. For Replicate 2, as with Replicate 1 mice, BECs at the end of the experiment did not

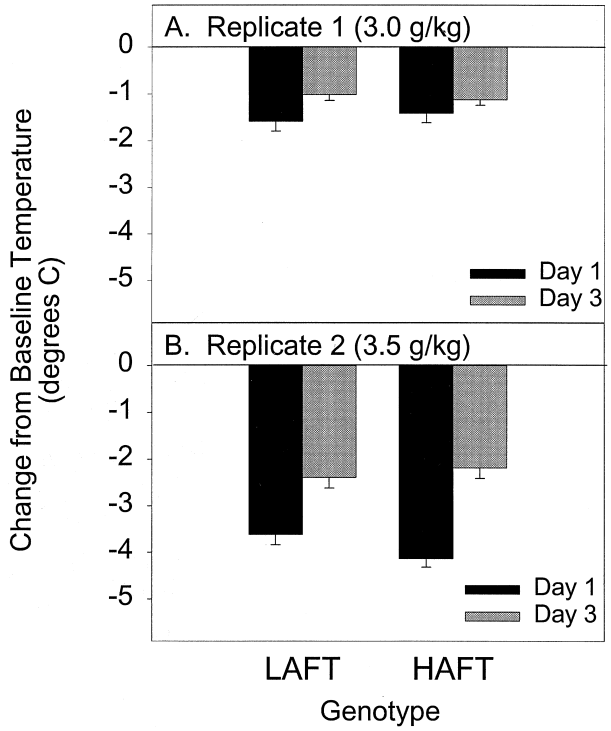

Fig. 4. (A) Change from baseline temperature in HAFT-1 and LAFT-1 mice given 3.0 g/kg ethanol (20% v/v). Each bar is the average of measurements taken at 30 and 60 min postinjection. Tolerance is inferred from a reduction in the hypothermic response on Day 3 (light bars) from that seen on Day 1 (dark bars). Day 3 values differed significantly from Day 1. HAFT-1 and LAFT-1 mice did not differ in the degree of chronic tolerance development to ethanol's hypothermic effects. For data analyses and results, see text. Values are means ± S.E.M. for 21 mice per line. (B) Change from baseline temperature in female HAFT-2 and LAFT-2 mice given 3.5 g/kg ethanol (20% v/v). Each bar is the average of measurements taken at 30 and 60 min post-injection. Day 3 (dark bars) values are significantly different from Day 1 (light bars). However, the HAFT and LAFT lines did not differ in the degree of chronic tolerance development. For data analysis and results, see text. Values represent means ± S.E.M. for 10 mice per line.

differ between the two lines. Further, the two lines did not differ in baseline body temperature.

6. Discussion

The results of the experiments presented here provide evidence that different types of tolerance may be mediated through separable mechanisms. Although HAFT and LAFT mice develop AFT differently on the static dowel test and the fixed-speed rotarod, they did not differ in the degree of chronic tolerance development. This was true for both the ataxic and hypothermic effects of ethanol, suggesting that chronic tolerance to ataxia and hypothermia were not co-selected traits with AFT to ethanol's ataxic effects.

It was interesting, however, that these mice did differ in the development of AFT on the fixed-speed rotarod. The fixed-speed rotarod and the static dowel (the apparatus on which the lines were selected) require different responses by the animal to be successful. In performing the static dowel task, it is our impression that it benefits the animal to remain motionless; the more active an animal is, the more likely it seems to be that it will fall off. Conversely, the fixed-speed rotarod requires the animal to stay in motion; a heavily sedated animal will immediately fall off the apparatus. If certain attributes of activity were concurrently selected during selection of the HAFT and LAFT mice on the static dowel task, one would predict that HAFT mice might show lower levels of activity than LAFT mice. However, in a test of this hypothesis, Erwin et al. (2000) demonstrated that these two lines of mice do not differ in their locomotor stimulant response to ethanol.

Activity of the two lines was also measured in the current study when mice were tested in the grid test apparatus. This test gives a direct measure of both foot missteps and overall locomotor activity. HAFT-2 and LAFT-2 mice showed no differences in their locomotor stimulant response to their initial ethanol injection when measured over the 15-min test. Further, the activity counts of both lines remained constant over the course of the experiment. From these data, it is apparent that the resulting ataxia ratios (see Fig. 3), which are calculated from the total errors and the overall activity, are not being driven by changes in the locomotor response in either HAFT-2 or LAFT-2 animals. That is, the ataxia ratios were the direct result of increases (LAFT-2) or decreases (HAFT-2) in the total number of errors over the days of the experiment (data not shown).

Replicate 1 mice showed a slightly different activity response in the grid test. LAFT-1 mice showed stable activity counts over the entire experiment, with mice given repeated ethanol injections showing slightly higher counts than those given saline. HAFT-1 mice, however, had a higher baseline activity level on Day 2, and when given repeated injections of ethanol, showed sensitization to its locomotor-activating effects. The ethanol-treated HAFT-1 mice showed a parallel increase in foot missteps leading to the stable ataxia ratios seen on Days 3–11 (see Fig. 3). With the exception of HAFT-1 mice, the activity of the replicate lines did not change over days. This suggests that the genes responsible for the development of AFT are not having differential effects on locomotor activity in the HAFT and LAFT mice.

The fact that the two replicates did not show identical responses in some tests (i.e., locomotor response across days, AFT development) does not necessarily mean that there is no genetic correlation between AFT on the static dowel and the other behaviors tested. It is possible that the differences between replicates are due to a chance fixation of alleles favoring a particular response in one replicate and not the other. However, it is also possible that the trait is only weakly correlated, making it difficult to detect in both replicates. Overall, detecting a significant main effect between groups, as was the case with AFT on the fixed-speed rotarod, provides the best indication of a true genetic correlation between traits even when the effect is greater in one replicate than the other (see Crabbe et al., 1990).

It has been shown that tasks assessing incoordination may be measuring different aspects of the overall trait (Boehm II et al., 2000a,b; Crabbe et al., 1996; Schafer and Crabbe, 1996). Incoordination is a very complex phenotype that comprises aspects of muscle strength, gait, balance, proprioception, and locomotor activity. Each measure of drug-induced "ataxia" may be measuring one or more of these components. For example, the rotarod may be sensitive to gait, balance and overall activity, while being little affected by muscle strength. Alternatively, the grid test apparatus may be influenced more by the gait and activity level of the animal while not being affected much by balance. There is evidence that the fixed-speed rotarod and the grid test may be measuring a different subset of coordinated motor abilities. Crabbe et al. (1996) found that in 25 BXD/Ty recombinant inbred strains of mice tested on both of these apparatus, there was virtually no correlation found between mean strain ataxia measured on one apparatus and the other. That is, performance on one of the tasks did not predict performance on the other. Also, Boehm II et al. (2000a) showed that serotonin 1_B receptor null mutant mice differed from their wild-type controls in the grid test, but not on the fixed-speed rotarod.

Due to the different composition of each of the measures of motor incoordination, it would be useful to measure the development of chronic tolerance to ethanol on an apparatus that is known to support differential AFT development. This would allow a more straightforward interpretation of whether or not the two types of tolerance share a common mechanism. For example, testing HAFT and LAFT mice for chronic tolerance development on the static dowel apparatus (or fixed-speed rotarod) would minimize the problem of different apparatus measuring different components of motor incoordination. At present, chronic tolerance studies using HAFT and LAFT mice have only been performed

with measures that have not demonstrated differential development of AFT (e.g., hypothermia, grid test), due to the difficulty of testing AFT with these particular measures. Future studies using these tactics may better serve to elucidate the genetic similarities and differences between chronic tolerance and AFT.

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