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Selectively Bred Lines of Mice Show Response and Drug Specificity for Genetic Regulation of Acute Functional Tolerance to Ethanol and Pentobarbital[1]

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ABSTRACT

Genetic regulation of acute tolerance to ethanol may be associated with ethanol consumption and other ethanol-related behaviors in rodents. We have used lines of mice, selectively bred for high and low acute functional tolerance (HAFT and LAFT, respectively) to ethanol-induced loss of balance to test this hypothesis. Replicate HAFT and LAFT lines differ in AFT to ethanol-induced loss of balance by 4.4- and 5-fold, respectively. Frequency distributions and mean AFT scores for those lines, F_1, and backcrosses show a dominance for the HAFT phenotype. Time courses for acquisition and decay showed that AFT to ethanol-induced loss of balance developed rapidly, could be maintained up to 6 h with repeated doses, and decayed 6 h after peak tolerance and discontinuance of ethanol administration. The lines did not differ in initial sensitivity as measured by brain ethanol concentration at loss of balance, indicating that initial sensitivity and AFT to loss of balance were not coselected traits. Surprisingly, HAFT versus LAFT lines did not differ in development of AFT to loss of righting response, or hypothermia, indicating different mechanisms or neuronal systems mediate genetic influences on these measures. Voluntary ethanol consumption was low in both of the replicate lines, but HAFT lines consumed greater amounts of ethanol than LAFT lines. The HAFT and LAFT lines developed AFT to pentobarbital-induced loss of balance, however, there were no line differences in rates or extent of the AFT development. These results show that genetic regulation of AFT development is drug- as well as response-specific.

Exposure to ethanol during a single session of intoxication results in decreased responsiveness to effects of ethanol on the central nervous system. This rapid adaptation, referred to as acute tolerance, is observed in animals and humans and is influenced by environmental factors and by genotype in rodents (LeBlanc et al., 1974; Crabbe et al., 1982; Sdao-Jarvie and Vogel-Sprott, 1991). Studies in rats show that practicing a moving belt task during intoxication accelerates development of tolerance (LeBlanc et al., 1975; Bitran and Kalant, 1991). However, experiments in mice indicate that acute functional tolerance (AFT) to ethanol-induced loss of balance is not altered by practice of the task (Gallaher et al., 1982; Erwin and Deitrich, 1996). By controlling for environmental and pharmacokinetic influences, a number of studies have demonstrated AFT, attributable to a diminution in central nervous system sensitivity (Gallaher et al., 1982, 1996; Erwin and Deitrich, 1996). Likewise, AFT to pentobarbital-induced ataxia (motor incoordination) and loss of righting response has been shown in mice, rats, and humans (Chan and Siemens, 1979; Ellinwood et al., 1983; Campanelli et al., 1988).

It has been suggested that acquisition of tolerance to intoxicating effects of ethanol may promote increased alcohol consumption (Tabakoff and Hoffman, 1988; Kurtz et al., 1996). In studies with genetically heterogeneous mice (HS/Ibg) a correlation was observed between AFT and voluntary ethanol consumption (Erwin et al., 1980). These findings were extended by Waller et al. (1983) who reported that selectively bred ethanol-preferring rats showed greater acquisition of AFT than nonpreferring (NP) animals. Le and Kiianmaa (1988) also reported similar correlations between AFT and ethanol preference in selectively bred alcohol-drinking and alcohol-avoiding rats.

Early studies of Grieve and Littleton (1979) showed differences among inbred mouse strains in acquisition of AFT to ethanol and Gallaher et al. (1996) demonstrated differences in AFT among C57BL × DBA/2 recombinant inbred strains.

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ABBREVIATIONS: AFT, acute functional tolerance; NP, nonpreferring; HAFT, high acute functional tolerance; LAFT, low acute functional tolerance; RI, recombinant inbred; BEC, blood ethanol concentration; BrECLB, brain ethanol concentration at loss of balance; CAFT, control acute functional tolerance; VEC, voluntary ethanol consumption.

Recently, we reported selectively breeding for high AFT (HAFT) and low AFT (LAFT) lines of mice, further demonstrating a marked genetic influence on acquisition of AFT to ethanol (Erwin and Deitrich, 1996). Genetic selection was performed with a foundation population of genetically heterogeneous (HS/Ibg) mice derived from an eight-way cross of inbred strains (McClearn et al., 1970). After seven and four generations of selection, replicate $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$ lines differed in AFT scores 4.3- and 2.3-fold, respectively. The lines did not differ in rates of ethanol clearance or in initial sensitivity to ethanol-induced loss of balance on a dowel. The latter observation is in contrast to that of Crabbe et al. (1996) who reported a significant correlation between initial sensitivity and AFT to ethanol in C57BL/6 × DBA/2 recombinant inbred (RI) strains of mice. However, in experiments with LS × SS RI strains, we did not find a significant correlation between initial sensitivity and AFT to ethanol (V.M.G. and V.G.E., submitted).

In the present study, the HAFT and LAFT lines of mice have been used to determine the extent that there are shared genetic influences between AFT to loss of dowel balance and other ethanol-related behaviors, including voluntary ethanol consumption. We have examined the relationship between initial sensitivity and AFT to ethanol and pentobarbital, and determined time courses for maintenance and decay of AFT to ethanol.

Materials and Methods

Animals. Mice selectively bred for high ($HAFT_1$ and $HAFT_2$) and low ($LAFT_1$ and $LAFT_2$) AFT to ethanol-induced loss of balance on a stationary dowel rod were used in these studies. The replicate selections were developed as previously described (Erwin and Deitrich, 1996) and maintained at the Institute for Behavioral Genetics, Boulder, CO. Each selection was initiated with male (200) and female (200) genetically heterogeneous mice (HS/Ibg), 60 to 70 days of age; this foundation population of HS mice was developed from an eight-way cross of inbred strains, A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII and has been maintained by random mating of 40 families, avoiding common grandparents for >60 generations (McClearn et al., 1970). The replicate selections ($HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$) were in generations 15 and 12, respectively, except as noted in the table and figure legends. In each set of experiments, represented by the tables and figures, separate groups of animals were used.

AFT and Initial Sensitivity. Individual mice were tested for AFT with a two-dose procedure (Gallaher et al., 1982, 1996; Erwin and Deitrich, 1996). Animals were trained to remain for >1 min on a 1.5-cm-diameter wooden dowel rod anchored 50 cm above a wood shavings-covered floor of a Plexiglas container (30 × 30 × 60 cm). Virtually all animals achieve this criterion with two or three trials at 5-min intervals. Within 5 to 10 min after meeting criterion, mice were given a 1.75-g/kg dose of ethanol i.p. (15% v/v in saline) and placed on the dowel rod until loss of balance, ~1 to 2 min. Animals are tested for recovery of balance on the rod every 5 min and at regain of balance (remaining on the dowel rod for 1 min) a 25-μl blood sample is obtained from the retroorbital sinus to give a blood ethanol concentration (BEC) at time 1 (t_1). The animal is immediately injected with a second dose of ethanol (2.0 g/kg) and at the time of regaining balance, t_2, a second blood sample is obtained for BEC assay. BEC values, expressed as milligrams of ethanol per deciliter of blood (milligrams/100 ml) are determined spectrophotometrically by a reliable enzyme assay (Lundquist, 1959). The difference between BEC values at t_2 and t_1 ($BEC_2 - BEC_1$) is taken as the measure of AFT (Erwin et al., 1980; Gallaher et al., 1982). Previous studies with this procedure have shown determinations of AFT in individual animals to be reliable and replicable by repeated measures on the same subjects at 1-day and 1-week intervals. Moreover, comparing AFT scores in groups of inbred strains, C57BL/6J or DBA/2J, the measure was reliable, $r = 0.87$, $P < .001$ (Erwin et al., 1980).

In separate experiments, initial sensitivity to ethanol or pentobarbital was determined by measuring brain ethanol or pentobarbital concentrations at the time of loss of balance on the stationary dowel. After drug administration at doses given in the table or figure legends, immediately on loss of balance, animals were decapitated and brains removed. For ethanol assays, brains were rapidly weighed and homogenized in 20 volumes of ice-cold 2% perchloric acid; the homogenates were centrifuged at 5000*g* for 15 min and aliquots of the resulting supernatants were taken for ethanol assays. For pentobarbital assays, brain were rapidly weighed and homogenized in 20 volumes of ice-cold saline; the homogenates were centrifuged at 10,000*g* for 20 min and aliquots of the supernatants were taken for pentobarbital assays. Pentobarbital assays were performed by fluorescence polarization radioimmunoassays using an Adx System provided by Abbott Laboratories Diagnostic Division (Abbott Park, IL).

Locomotor Activity. Procedures for determining the effects of ethanol on locomotor activity were similar to those previously reported (Erwin et al., 1990). Animals were injected i.p. with saline (day 1) and ethanol (15% v/v, day 2) at doses ranging from 1.0 to 3.0 g/kg and immediately placed in Omnitech activity monitors (Omnitech Electronics, Inc., Columbus, OH) to measure spontaneous locomotor activity as horizontal distance (centimeters) traveled after the injections. The activity monitors are enclosed in ventilated boxes, and activity is monitored under reduced lighting at 5-min intervals for 15 min by means of computer. Distance traveled between 5 and 15 min was used for all analyses in that previous studies have shown that BECs peak within the first 5 min after ethanol administration i.p. Ambient temperature was maintained at 22–23°C.

Hypnotic and Hypothermic Sensitivity to Ethanol. Hypnotic sensitivity to ethanol was measured by determining the duration of loss of righting response (sleep time) and the BEC, in milligrams of ethanol per deciliter of blood, at regaining righting response after ethanol administration as described previously (Heston et al., 1974). Operationally, the criterion for righting is the animal being able to change from the supine to prone position three times in 30 s. Hypothermia was measured as the difference in rectal temperature immediately before and at 15- to 30-min intervals beginning 60 min after 4.2-g/kg ethanol dose.

Measurement of Ethanol Preference (Voluntary Ethanol Consumption). Ethanol and water consumption were measured in a standard two-bottle choice paradigm (McClearn and Rodgers, 1961). Mice at 60 to 80 days of age are separated from littermates and individually housed in Plexiglas cages equipped with tops that accommodate two 15-ml plastic drinking cylinders. The cylinders are filled with tap water or 10% v/v 95% USP ethanol in tap water and fitted with stainless steel ball-stop sipper tubes. Filled cylinders are weighed and placed on the cage top ~5 cm apart with sipper tubes extending ~3 cm into the cage. Every 2 days, cylinders are removed and weighed to determine weight (volume) of fluid consumed. Then cylinders are refilled, weighed, and placed back on the cage tops. Positions of the water and 10% ethanol solution are rotated at each 2-day block to allow correction for position effects. Animals are weighed on day 1 and 10 of the experiment; the mean body weight is used to calculate ethanol consumption in grams of ethanol consumed per kilogram body weight per 24 h.

Data Analysis. Statistical analyses were performed with SPSS for Windows, version 7.5; numbers of subjects, F statistics, and P values are shown as needed.

Results

Data in Fig. 1 show frequency distributions for AFT to ethanol in the replicate selected lines. The $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$ lines were in generations 15 and 12 of

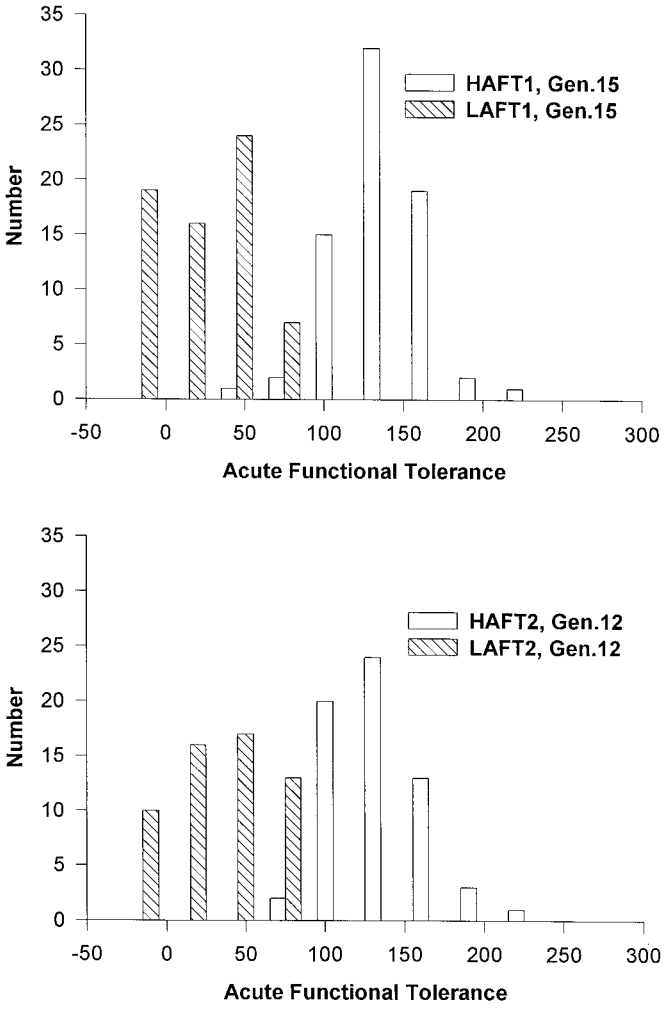

Fig. 1. Frequency distributions for acute functional tolerance in replicate selected lines. The $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$ lines were in generations 15 and 12 of selective breeding for AFT as described in *Materials and Methods*. Distributions are for combined male and female data because there were no sex differences in AFT between the respective HAFT and LAFT lines. Mean AFT values ($BEC_2 - BEC_1$, in milligrams per deciliter) with S.E. were 137 ± 6/23 ± 4 and 132 ± 6/32 ± 7 for $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$, respectively.

selective breeding for AFT as described in *Materials and Methods*. Distributions are for combined male and female data because there were no sex differences in AFT for the respective HAFT and LAFT lines. The data show little overlap in the distributions of the HAFT and LAFT lines. Distributions for the HAFT lines are more normal than for the LAFT lines, suggesting a floor effect for the LAFT lines. Some of the LAFT animals showed a slight negative AFT score that might suggest some increase in sensitivity to ethanol during the intoxication period. As indicated from these distributions, the mean AFT values for the respective HAFT and LAFT lines were similar: $HAFT_1$ and $HAFT_2$ values were 137 ± 6 and 132 ± 6 mg/dl blood, respectively, and $LAFT_1$ and $LAFT_2$ values were 24 ± 4 and 30 ± 7 mg/dl, respectively. As might be expected, the data in Table 1, obtained from generations 11 and 8 for the $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$, respectively, indicate less response in $LAFT_2$ compared with the $LAFT_1$ line.

TABLE 1

Comparisons of initial sensitivity and AFT in HAFT, LAFT, and CAFT lines

Separate groups of males and females (n = 8 to 10 each group) of each line were administered 1.75 g/kg ethanol and at loss of balance, brains were immediately removed and BrECLB (milligrams of ethanol per 100 g brain tissue) determined. In separate groups of mice each line received 1.75 g/kg ethanol and BEC_1 (milligrams of ethanol per deciliter of blood) was determined at regaining balance. Immediately after taking blood sample 1, each animal received 2.0 g/kg ethanol and the BEC_2 (data not shown) at regaining balance was determined. AFT = $BEC_2 - BEC_1$ and actual AFT = BEC_2 − BrECLB.

Line	Sex	BEC_1	AFT	BrECLB	Actual AFT
$HAFT_1$	M	205 ± 5	132 ± 5	156 ± 3**	181
$HAFT_1$	F	213 ± 4	128 ± 6	177 ± 6	164
$LAFT_1$	M	172 ± 6*	19 ± 5*	154 ± 5**	37
$LAFT_1$	F	185 ± 5*	26 ± 7*	189 ± 10	30
$HAFT_2$	M	208 ± 4	124 ± 5	157 ± 10	175
$HAFT_2$	F	201 ± 7	127 ± 11	172 ± 11	156
$LAFT_2$	M	191 ± 5	59 ± 5*	183 ± 10	67
$LAFT_2$	F	195 ± 4	53 ± 6*	175 ± 6	73
CAFT	M	210 ± 6	90 ± 7	155 ± 12*	145
CAFT	F	213 ± 5	105 ± 6	180 ± 11	138

No significant sex differences were observed in AFT values for the replicate HAFT and LAFT or the CAFT lines. Between-subjects ANOVA showed highly significant HAFT versus LAFT differences in AFT: $F_{1,38}$ = 109 and 71 for $HAFT_1$ versus $LAFT_1$ and $HAFT_2$ versus $LAFT_2$, respectively (*). There were no significant between line effects for initial sensitivity defined as BrECLB, however, there was a significant, $P < .05$, effect of sex for BrECLB, $F_{1,38}$ = 8.8 and 10.2 for $HAFT_1$ and $LAFT_1$, respectively (**).

Comparisons of Initial Sensitivity and AFT to Ethanol in Selected Lines and Crosses. As shown in Table 1, initial sensitivity to ethanol-induced loss of balance was determined by measuring BEC at loss of balance on a stationary dowel (BrECLB). BrECLB values were similar to BEC_1 values for the $LAFT_1$ females and for $LAFT_2$ males and females, a result consistent with the observation that LAFT animals develop little AFT during t_1 (~30 min). However, BrECLB values for HAFT lines were significantly lower than the corresponding BEC_1 values, indicating that HAFT animals acquire significant AFT during t_1 (~10 min). Thus, we have used BrECLB rather than BEC_1 to define initial sensitivity to ethanol-induced loss of balance and by comparing this value with BEC_2 we obtained a measure of the actual or true AFT score. Although there were no sex differences between lines for AFT, there were significant sex differences for BrECLB for $HAFT_1$, $LAFT_1$, and the control, unselected line (CAFT). This sex difference may be fortuitous because no sex differences were observed in $HAFT_2/LAFT_2$ lines.

Comparisons of the true AFT values for CAFT animals with those for the HAFT and LAFT lines indicate that the selections differences in AFT are somewhat asymmetric, as previously described by Erwin and Deitrich (1996). If alleles for AFT are dominant, one would expect both heterozygous and homozygous genotypes to produce a similar phenotype; thus, an asymmetric selection with greater response in the LAFT direction might be caused by selection of homozygous recessive alleles that mediate low AFT. We have tested this possibility by comparing AFT scores in $HAFT_1$, $LAFT_1$, $HAFT_1 \times LAFT_1$ F_1 generation, and $F_1 \times LAFT_1$ backcross. As shown in Fig. 2, mean AFT scores for F_1 and $F_1 \times LAFT_1$ backcrosses were more like the values for CAFT (Table 1) and $HAFT_1$ than like $LAFT_1$. These results indicate overall average dominance for alleles mediating high AFT. Consistent with results in Table 1, BEC_1 values were similar for all lines and crosses tested.

Time Courses for Maintenance and Decay of AFT in HAFT and LAFT Lines. Understanding the time courses

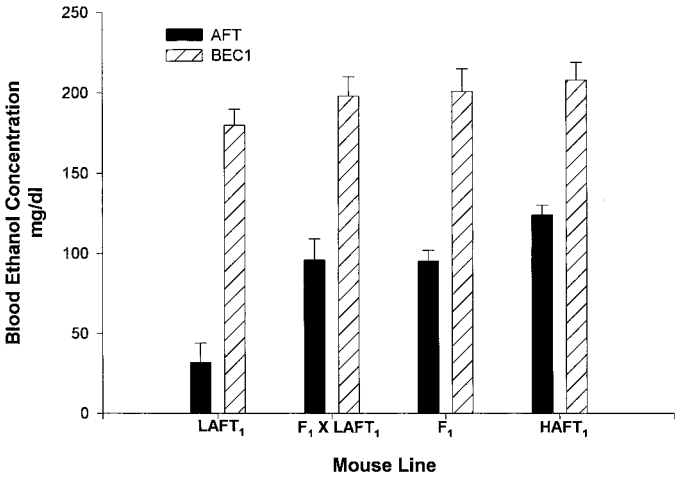

Fig. 2. Comparison of initial sensitivity and acute functional tolerance in selected lines and crosses. AFT and BEC_1 values for regain of balance on the dowel were obtained as described in *Materials and Methods* and Table 1 with 8 to 10 mice per line or cross. ANOVA shows a highly significant line effect for AFT ($F_{3,37} = 10.86$, $P < .01$). Student-Newman-Keuls post hoc tests show AFT values for HAFT and LAFT differ, $P < .05$, from F_1, or $F_1 \times LAFT_1$ backcross.

for maintenance and decay of AFT are essential in identifying neuroadaptive processes that mediate AFT to ethanol. As shown in Fig. 3, the times to regain balance after four consecutive doses of ethanol were plotted as a function of the corresponding BEC values obtained at the time of regain of balance. The resulting plots show the time course for acquisition and maintenance of AFT in $HAFT_1$ and $LAFT_1$ mice. The $LAFT_1$ mice maintained AFT at a level of ~30 mg/dl, whereas the $HAFT_1$ mice rapidly acquired and maintained AFT of ~130 mg/dl for up to 400 min of exposure to ethanol.

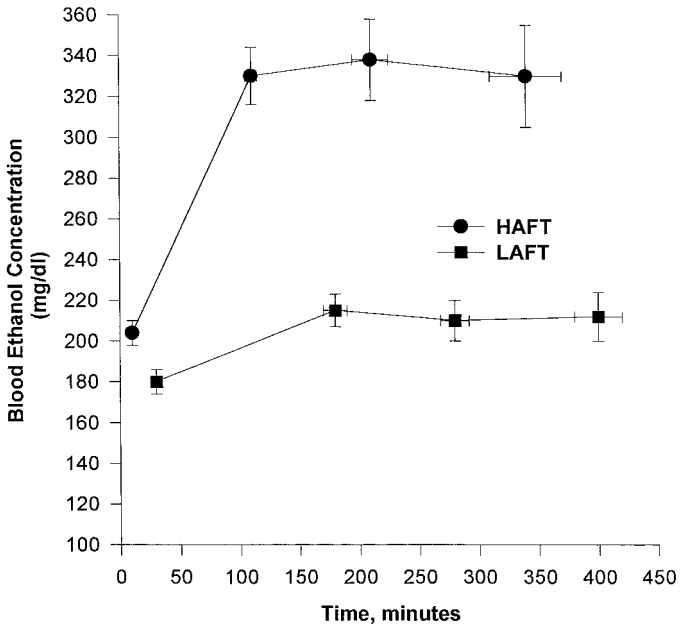

Fig. 3. Time course for maintenance of acute functional tolerance in HAFT and LAFT lines. $HAFT_1$and $LAFT_1$ mice ($n = 10$ per group) were injected with an initial dose of 1.75 g/kg ethanol; at the time of regain of balance on the stationary dowel, blood samples were obtained for BEC determinations and the mice received 2.0 g/kg ethanol. Subsequently, at regain of balance, blood samples were taken and the mice received 1.0 g/kg ethanol; the 1-g/kg dose was repeated once.

These results show different maximum amounts of AFT for the HAFT and LAFT lines.

For determination of decay of tolerance, it was important to know whether maximum AFT developed after a single 3.75-g/kg ethanol dose. After this dose, in separate groups of mice ($n = 6–9$ mice per group), BECs at loss of balance (BrECLB) were compared with the BEC at the time, ~2 h, of regain of balance. Development of AFT in $HAFT_1$ animals was virtually identical (129 ± 14 mg/dl at 2 h; Fig. 4) to that obtained with the two-dose procedure, indicating that it is not necessary to use a two-dose paradigm to produce AFT to ethanol-induced loss of balance. In early studies, we reported that decay of AFT to ethanol was rapid with C3H mice returning to naïve sensitivity to loss of dowel balance at 6 to 24 h after regaining balance at peak tolerance (Erwin, 1986). In the present study with $HAFT_1$ animals, we have confirmed (Fig. 4) those earlier results. At 6 and 24 h after receiving 3.75 g ethanol/kg, mice were given a challenge dose of 1.75 g/kg to determine brain sensitivity (BrECLB) for loss of balance. The data show that at 6 and 24 h the ethanol-pretreated animals had 35 and 10 mg/dl residual tolerance, respectively.

In another experiment, Table 2, it was shown that the ED_{50} values for ethanol-induced loss of balance were similar at 6 h after saline and 3.75 g/kg ethanol pretreatment. In this experiment, neither saline- nor ethanol-treated $HAFT_1$ or $LAFT_1$ differed from their respective naïve control animals. These experiments show there is a rapid decay of AFT with little residual at 6 h and none at 24 h.

Acquisition of Acute Tolerance to Ethanol-Induced Loss of Righting Response and Hypothermia in Replicate HAFT and LAFT Lines. To determine whether genes that regulate acquisition of AFT to loss of dowel balance generalizes to other central nervous system inhibitory effects of ethanol, acquisition of acute tolerance to ethanol-induced loss of righting response was determined on the HAFT and

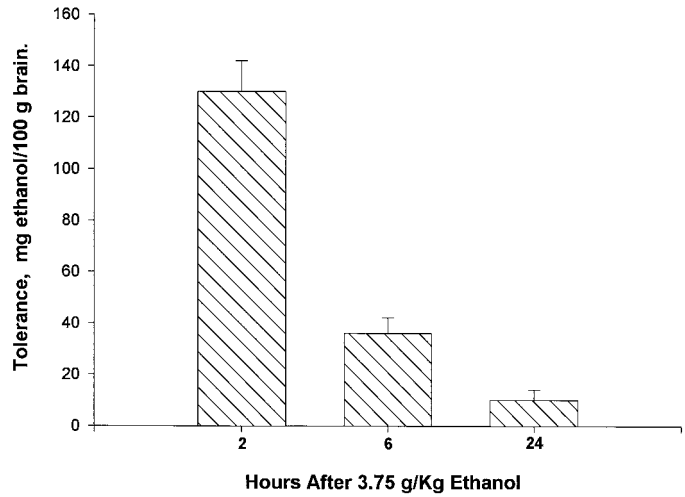

Fig. 4. Initial and residual tolerance as a function of time after a single 3.75-g/kg dose of ethanol in $HAFT_1$ mice. AFT was measured in separate groups ($n = 6–9$ males) of $HAFT_1$ mice as described in Table 1. Peak tolerance was obtained at 2 h (mean time of regain of balance) after 3.75 g/kg ethanol. Values represent milligrams per deciliter difference between BrECLB after a 1.75-g/kg ethanol dose (initial sensitivity) and the BrEC at regain of balance. At 6 and 24 h after 3.75 g/kg ethanol when BEC values were not detectable, mice received a challenge dose of ethanol (1.75 g/kg) and BrECLB was compared with BrECLB in naïve animals to measure residual tolerance.

TABLE 2

ED_{50} values for ethanol-induced loss of balance as a function of ethanol treatment

Separate groups of mice were injected i.p. with doses of ethanol from 0.75 to 1.75 g/kg in 0.1- to 0.2-g/kg increments at 360 min after saline or 3.75 g/kg ethanol (a time when BEC values are <36 mg/dl). Four mice from each group were injected at the lowest dose and then doses were increased in separate mice until approaching the ED_{50} value for loss of balance (inability to remain on the dowel for 1 min) 3 min after the ethanol dose. At approximately the ED_{25} to ED_{75} doses, an $n = 8$ to 10 mice per line was used to estimate the ED_{50} values.

Line	Treatment	ED_{50}
		g/kg
$HAFT_1$	Naive	1.25
$LAFT_1$	Naive	0.80[a]
$HAFT_1$	360 min (saline)	1.30
$HAFT_1$	360 min (ethanol)	1.35
$LAFT_1$	360 min (saline)	0.80[a]
$LAFT_1$	360 min (ethanol)	0.90[a]

[a] Naive, saline, or ethanol-treated HAFT mice were less sensitive than LAFT; an ED_{50} dose in HAFT was equivalent to an ED_{100} dose in LAFT and an ED_{50} dose in LAFT was an ED_{20} dose in HAFT. Neither saline or ethanol-treated HAFT or LAFT differed from their respective naive mice.

LAFT lines. As noted in Fig. 5, separate groups of $HAFT_1$/$LAFT_1$ and $HAFT_2$/$LAFT_2$ mice received an initial dose of 3 or 4 g/kg ethanol followed by a 2.0-g/kg dose at regaining of righting response. The duration of loss of righting response (sleep time) was determined after each initial and subsequent ethanol injection. BEC values (milligrams of ethanol per deciliter of blood) were determined at each regain of righting response. Mice from the replicate HAFT and LAFT lines developed acute tolerance to the hypnotic effects of ethanol. From the first regain (~20 to 30 min) to the last regain (~300 min), there were significant, $P < .001$, increases in BEC values at regain of righting response (64–72 and 41–48 mg/dl) for $HAFT_1$/$LAFT_1$ and $HAFT_2$/$LAFT_2$ lines, respectively. There was a significant, $F_{3,38} = 11.0$, $P < .001$, line effect for BEC_1, indicating the initial hypnotic sensitivity was greater in $HAFT_1$/$LAFT_1$ than in $HAFT_2$/$LAFT_2$. However, the slopes were similar for the replicate lines, indicating that the rates of acquisition of acute tolerance were similar.

Development of rapid tolerance (24 h after ethanol) to ethanol-induced hypothermia has been demonstrated in inbred mouse strains (Crabbe et al., 1979). Thus, it was of interest to determine whether HAFT and LAFT lines developed AFT to ethanol-induced hypothermia and if these lines differed in extent of hypothermia or in rates of acquisition of AFT to hypothermia. Results in Fig. 6 show a similar 3.5°C loss in rectal temperature in $HAFT_1$ and $LAFT_1$ mice at 60 min after a 4.2-g/kg dose of ethanol; the rates of recovery from 60 to 180 min were similar for both lines. Similar results were obtained with $HAFT_2$ and $LAFT_2$ lines (data not shown). A second experiment compared development of AFT with hypothermia in HAFT and LAFT lines by determining the BEC_1 and BEC_2 at the times rectal temperatures returned to 36°C after two consecutive ethanol doses (3 then 1.5 g/kg). The data in Table 3 show development of AFT to ethanol-induced hypothermia ($BEC_2 - BEC_1$), but interestingly, the selected lines did not differ in acquisition of AFT to this ethanol response. It is evident that selection of HAFT and LAFT lines did not segregate alleles that influence AFT to ethanol-induced hypothermia.

Dose-Response Function for Ethanol-Induced Changes in Locomotor Activity in $HAFT_1$ and $LAFT_1$ Mice. The dose-response data in Fig. 7 show the classical biphasic effect of ethanol on locomotor activity with significant increases at 1.5 and 2.0 g/kg and a decrease at 3.0 g/kg. ANOVA showed a significant, $P < .001$, dose effect for both $HAFT_1$ and $LAFT_1$ ($F_{5,42} = 13.6$ and $F_{5,43} = 18$, respectively). There was no significant line by dose interaction, but ethanol activation was significantly greater, $P < .05$, in $LAFT_1$ than in $HAFT_1$ ($F_{1,14} = 4.2$ and $F_{1,12} = 6$, at doses of 1.5 and 2.0 g/kg, respectively). A dose of 3.0 g/kg produced a significant decrease in locomotor activity in both lines with no significant line difference, indicating similar sensitivities to locomotor inhibitory effects of ethanol. At 2.0 g/kg there was no significant difference in ethanol-induced locomotor activation in the replicate $HAFT_2$/$LAFT_2$ lines (data not shown). Thus, it is likely that differences in $HAFT_1$ and $LAFT_1$ are fortuitous and unrelated to selection of AFT to ethanol-induced loss of dowel balance.

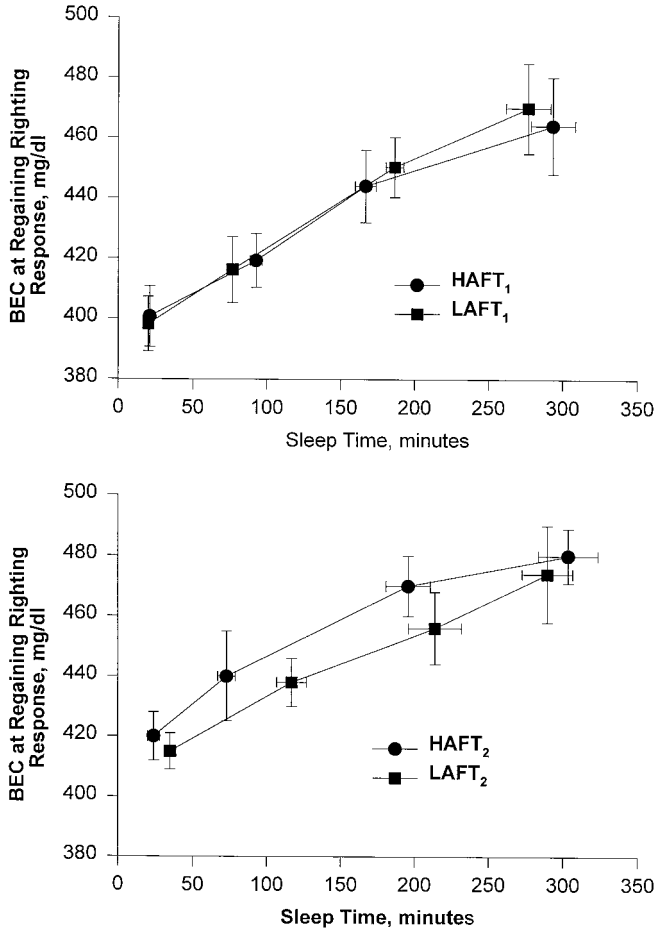

Fig. 5. Acquisition of acute tolerance to ethanol-induced loss of righting response in replicate HAFT and LAFT lines. Separate groups ($n = 10$–12 per group, sexes combined) of $HAFT_1$/$LAFT_1$ and $HAFT_2$/$LAFT_2$ mice were used in this experiment. One group received an initial dose of 3 g/kg and the other received an initial dose of 4 g/kg ethanol. At regain of righting response, each group received a 2.0-g/kg dose. The duration of loss of righting response (sleep time) was determined after each initial and subsequent injection and BEC values (milligrams of ethanol per deciliter of blood) were determined at each regain of righting response.

Voluntary Ethanol Consumption in HAFT and LAFT Mice. As noted in Table 4, voluntary ethanol consumption was determined by the standard two-bottle choice for 8 days and the data show that HAFT and LAFT animals do not consume large quantities of ethanol. Indeed the ethanol con-

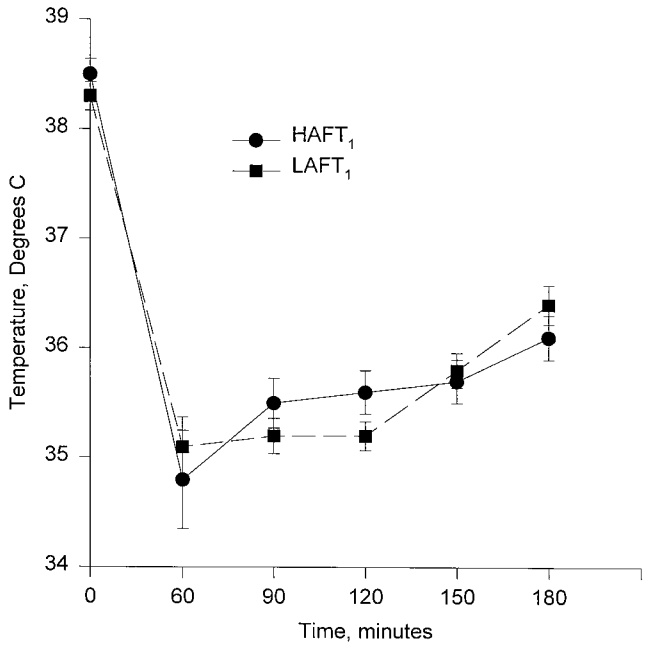

Fig. 6. Time course for ethanol-induced hypothermia in HAFT and LAFT Mice. $HAFT_1$ and $LAFT_1$ mice (n = 6 per group) received 4.2 g/kg ethanol and rectal temperatures were recorded at the times shown.

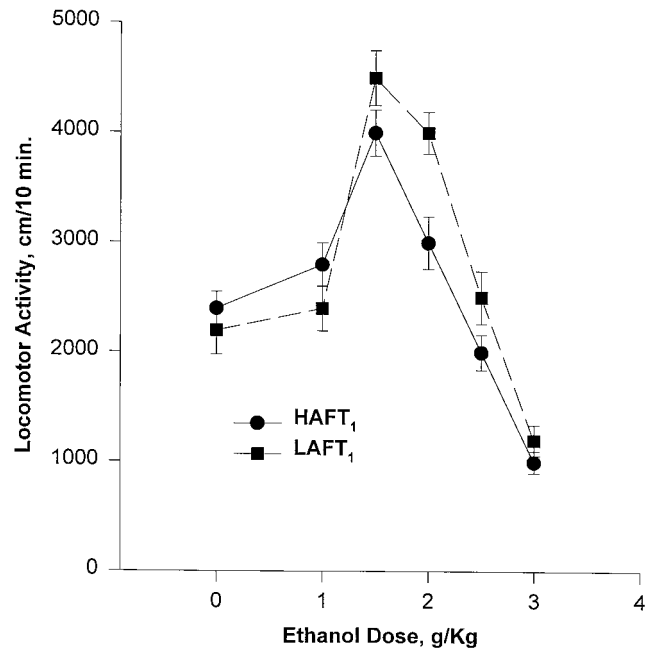

Fig. 7. Dose-response function for ethanol-induced changes in locomotor activity in $HAFT_1$ and $LAFT_1$ mice. On day 1, separate groups of mice (n = 6–10 males) received saline injections and locomotor activity (distance traveled in centimeters) was measured for 15 min as described in *Materials and Methods*. On day 2, the mice were injected with 0 (saline), 1.0, 1.5, 2.0, 2.5, or 3.0 g/kg ethanol and the mean locomotor activity recorded at 5 to 15 min.

TABLE 3

Development of AFT to ethanol-induced hypothermia in HAFT and LAFT mice

Rectal temperatures were measured at 15-min intervals, beginning at 60 min after a 3-g/kg dose of ethanol. The mean temperature at 60 min was 35.4 ± 0.2 and 35.0 ± 0.3°C for $HAFT_1$ and $LAFT_1$, respectively (n = 6 per line). When the rectal temperature returned to 36°C at t_1 a blood sample was obtained for BEC_1 and the mice were injected immediately with a 1.5-g/kg dose of ethanol. A value for BEC_2 was obtained when the rectal temperature returned to 36°C (t_2). AFT to hypothermia was defined as $BEC_2 - BEC_1$. Values for HAFT and LAFT lines were not significantly different.

Line	Time to Recover Rectal Temperature		BEC_1	AFT to Hypothermia
	min			
	t_1	t_2	*mg/dl*	
$HAFT_1$	100 ± 11	202 ± 15	250 ± 16	58 ± 12
$LAFT_1$	87 ± 10	173 ± 16	248 ± 14	68 ± 11

TABLE 4

VEC in HAFT and LAFT mice

VEC was determined by the standard two-bottle choice for 8 days, rotating the water and 10% ethanol in water bottles every 2 days. Values represent the mean grams of ethanol consumed per kilogram body weight per 24 h during days 4 to 8. Each line was represented by n = 6 to 10 males and females.

Line	Voluntary Ethanol Intake
	g/kg/24 h
$HAFT_1$	1.21 ± 0.30
$LAFT_1$	0.54 ± 0.08[a]
$HAFT_2$	1.46 ± 0.51
$LAFT_2$	0.52 ± 0.11[a]

[a] ANOVA showed a significant main effect by line, $F_{3,68} = 3.8$, $P < .02$. There was no significant main effect for sex nor line by sex interaction, thus sexes were combined for the ANOVA by line.

sumption values are similar to those observed with the NP DBA/2 strain. However, the data show a significant main effect by line ($F_{3,\ 68} = 3.8$, $P < .02$) with the HAFT lines drinking more ethanol than the respective LAFT lines. There was no significant main effect for sex or line by sex interaction. In separate experiments with 66 HAFT and LAFT mice of both sexes, the phenotypic correlation (r) between AFT and ethanol consumption was 0.401, $P < .001$, indicating a 16% covariance in these ethanol-related behaviors.

Initial Sensitivity and AFT to Pentobarbital-Induced Loss of Dowel Balance in HAFT and LAFT Mice. Initial sensitivity to pentobarbital was determined by measuring the brain concentration at the time of loss of dowel balance (~1–2 min) after a 40-mg/kg dose i.p. These values (~25 μg/g brain) are shown as the time zero in Fig. 8. Differences between the initial sensitivity values and the brain concentrations at regain of balance after 30-, 40-, 60-, and 80-mg/kg doses, show a significant acquisition of tolerance to pentobarbital over time (~3 to 4 h). Across all lines, there was a significant dose effect ($F_{3,89} = 4.4$, $P < .02$) with $HAFT_1/LAFT_1$ and $HAFT_2/LAFT_2$ lines developing acute tolerance (~8 and 10 μg pentobarbital/g brain for the replicates, respectively). Surprisingly, there were no significant line (HAFT versus LAFT) differences in acquisition of acute tolerance to pentobarbital; however there was a significant line difference in brain sensitivity between $HAFT_1$ and $LAFT_1$ ($F_{1,42} = 22.6$, $P < .0001$); this line difference was not observed in $HAFT_2$ versus $LAFT_2$, suggesting that the difference in sensitivity does not indicate a coselected trait.

Discussion

Selectively bred lines and inbred strains of mice have been used to determine genetic influences on ethanol and pentobarbital response traits. The use of inbred strains or intercrosses of two inbred strains to determine the extent of shared genetic influence on more than one ethanol-related behavior (i.e., pleiotropy) is limited by the chance fixation of different alleles in the strains. However, lines of mice, selectively bred from a genetically heterogeneous population that has been derived from crosses of eight inbred strains, poten-

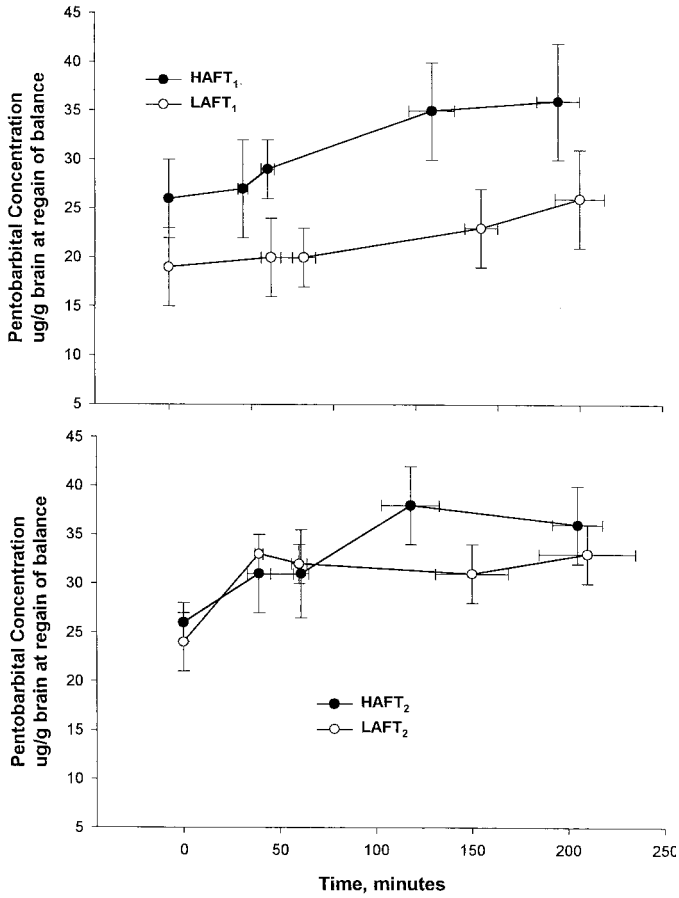

Fig. 8. Comparisons of initial sensitivity and acquisition of AFT to pentobarbital in HAFT and LAFT mice. Separate groups (n = 8–10 per group, sexes combined) of replicate lines of mice received 30-, 40-, 60-, or 80-mg/kg doses of pentobarbital i.p. In one set of experiments with 40 mg/kg, whole brains were removed immediately after loss of balance on the dowel rod to determine brain concentrations of pentobarbital (BrPbLB, micrograms of pentobarbital per gram whole brain) as a measure of initial sensitivity; this value is shown as the time zero. In a similar manner, separate groups of each mouse line received pentobarbital and the mean duration of loss of balance (TRB) in minutes was determined. At regaining balance, whole brains were taken to determine brain pentobarbital concentrations, BrPbRB.

tially will yield a greater number of genes (alleles) that contribute to differences in an ethanol-related behavior influenced by multiple genes (McClearn and DeFries, 1973). Thus, after >10 generations of selective breeding the HAFT and LAFT lines contain multiple genes that regulate high or low capacity to develop AFT to ethanol. The HAFT and LAFT lines should differ in traits or ethanol-related behaviors that are regulated by those genes that influence AFT to ethanol-induced loss of dowel balance. In a finite breeding population there are chance fixations of irrelevant alleles, however, fortuitous fixation of alleles unrelated to AFT in two distinct selections is less probable. Therefore, we have used the conservative criterion that both replicate lines of HAFT and LAFT must differ for a trait to be coselected with shared genetic influence (Crabbe et al., 1990). The current article examines whether there are shared genetic influences (overlap in relevant alleles) between acquisition of AFT to ethanol-induced loss of balance and initial sensitivity to loss of balance and loss of righting response; development of AFT to ethanol-induced loss of righting response; acquisition of AFT to ethanol-induced hypothermia; locomotor activation or inhibition; voluntary ethanol consumption; and development of AFT to pentobarbital-induced loss of balance. These traits were studied because there is evidence to suggest that they may be related.

In the process of selective breeding for lines of mice that differ in acquisition of AFT to ethanol-induced loss of balance, we previously reported (Erwin and Deitrich, 1996) and continue to observe an asymmetry in selection response. As noted in *Results*, this asymmetry might result from dominant effects of the high AFT alleles where only those individuals homozygous for low AFT alleles would be distinguished by a low AFT score. Because heterozygotes and individuals homozygous for high AFT would give a similar AFT score, selection would proceed slower in the high AFT than in the low AFT direction. This possibility was tested by comparing AFT values for $HAFT_1 \times LAFT_1$ F_1 crosses and $F_1 \times LAFT_1$ backcrosses with CAFT and $HAFT_1/LAFT_1$. The results show a clear dominance for AFT in the high direction and are consistent with the hypothesis.

It has been suggested that tolerance is a neuroadaptive process occurring in response to ethanol-induced impairment, and the greater the impairment, the more rapid the acquisition of tolerance (Kalant, 1977). Recent studies of Crabbe et al. (1996) support this hypothesis. They found positive genetic correlations in C57BL × DBA/2 RI strains between initial sensitivity and acute tolerance to a rotating dowel balance test, indicating that the more sensitive strains develop greater acute tolerance. Moreover, tolerance to ethanol-induced grid test ataxia and hypothermia were positively correlated. However, the results of the present study (Table 1) show clearly that $HAFT_2$ and $LAFT_2$ lines do not differ in initial sensitivity as defined by BrECLB, and the $HAFT_1$ and $LAFT_1$ lines differ in the opposite direction predicted by the results of Crabbe et al. (1996). Differences in BEC_1 cannot be taken as initial sensitivity differences because the HAFT and CAFT, but not LAFT, lines have developed significant AFT during the time of loss and first regain of balance. In addition, results with 24 LS × SS RI strains show no significant genetic correlation between initial sensitivity, also defined as BrECLB, and development of AFT to ethanol-induced loss of dowel balance (V.G.E., submitted). Our results are consistent with those of Kurtz et al. (1996), who showed that preferring rats developed within-session tolerance to hypnotic effects of ethanol, whereas NP rats, which exhibited a greater degree of initial sensitivity, did not develop within-session (acute) functional tolerance. Differences in our results compared with others might be that their studies were conducted in very different panels of RI strains and selected lines. Another difference is that they used a rotating dowel rod rather than a stationary dowel rod. The apparent small differences in method of response assessment might not be trivial. In a recent study, we have demonstrated that the replicate HAFT and LAFT lines do not differ in acquisition of AFT on the rotorod test than on the stationary dowel (R.A.D., P. Bludeau, and V.G.E., submitted).

The time courses for development and decay of AFT show that these processes occur rapidly, within minutes to a few hours; there was no "carry over" tolerance to loss of dowel balance at 24 h after acquisition of peak AFT. This finding distinguishes AFT from rapid or chronic tolerance to ethanol that show changes in sensitivity at times greater than 24 h

after ethanol exposure (Crabbe et al., 1979; Khanna et al., 1991). Because it is possible pharmacokinetic differences might alter rates of development or decay of tolerance to ethanol, we determined whether HAFT and LAFT lines differed in peak blood levels or clearance following ethanol administration. In previous studies (Erwin and Deitrich, 1996) the HAFT and LAFT lines possessed identical peak ethanol blood levels and clearance rates.

The present study clearly demonstrates response specificity for genetic regulation of AFT. Consistent with results in Fig. 5, we have reported that HAFT and LAFT lines do not differ in initial sensitivity to ethanol-induced loss of righting response (R.A.D., P. Bludeau, and V.G.E., submitted). Moreover, the results demonstrate that both replicate lines of HAFT and LAFT mice develop acute tolerance to hypnotic sensitivity to ethanol. Surprisingly, the rates and magnitude of AFT development to loss of righting response were similar in the HAFT versus LAFT lines even though these lines differ up to 4-fold in AFT to loss of dowel balance. Additionally, the HAFT and LAFT lines did not differ in sensitivity to ethanol-induced hypothermia or in the rates of recovery from hypothermia. These surprising results suggest differences in mechanisms that mediate adaptation to different ethanol responses. The data in Fig. 8 show that HAFT and LAFT lines did not differ in acquisition of pentobarbital-induced AFT to loss of balance. Earlier studies (Khanna et al., 1991) found ethanol-tolerant (rapid tolerance) rats did not display cross-tolerance with pentobarbital with a tilt-plane motor impairment response. These results indicate that mechanisms influencing neuroadaptation to ethanol differ from those regulating AFT to pentobarbital and further show that acquisition of AFT to ethanol involves adaptation to the specific drug, not simply adaptation to the task, i.e., loss of balance.

Because differences in ethanol actions on motor function might contribute to selected differences in performance of the dowel test, we examined whether ethanol-induced changes in locomotor activity might be a coselected trait with AFT. Ethanol dose-response functions show that HAFT and LAFT lines respond similarly with locomotor activation at low doses and with inhibition at high (3g/kg) doses. These results indicate those genetic processes regulating development of ethanol-induced AFT do not influence locomotor responses to ethanol. Another ethanol-related behavior, voluntary ethanol consumption (VEC), reported to be associated with acute tolerance (Erwin et al., 1980; Waller et al., 1983) was measured as a coselected trait in the HAFT and LAFT lines. Consistent with those previous observations, the present results show that VEC values are significantly greater in HAFT than in LAFT lines, even though none of the lines consumed large quantities of ethanol. In addition, correlational studies showed a significant, $r = 0.4$, $P < .001$, correlation between AFT to ethanol-induced loss of balance and VEC. The results indicate some overlap in genes that influence these ethanol-related behaviors. These observations may have important implications in ultimately revealing processes that contribute to the development of alcoholism (Tabakoff and Hoffman, 1988). The development of AFT to ethanol may contribute to factors that increase ethanol consumption by reducing aversive effects that otherwise might limit its intake.

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