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Sensitivity to Ethanol-Induced Motor Incoordination in FAST and SLOW Selectively Bred Mice

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S. L. BOEHM, II, J. C. CRABBE AND T. J. PHILLIPS. *Sensitivity to ethanol-induced motor incoordination in FAST and SLOW selectively bred mice.* PHARMACOL BIOCHEM BEHAV **66**(2) 241–247, 2000.—Earlier studies using the grid test have indicated a negative genetic correlation between sensitivity to ethanol-induced locomotor stimulation and ethanol-induced motor incoordination in FAST and SLOW mice, lines selectively bred for differential sensitivity to ethanol's stimulant effects. Because different tests of motor coordination may not measure the same behavioral competencies or physiological substrates, the present experiments tested adult ethanol- or saline-exposed FAST and SLOW mice of two replicates (FAST-1, FAST-2, SLOW-1, and SLOW-2) using three additional tests of coordination: a stationary dowel, fixed-speed rotarod, and accelerating rotarod. After ethanol treatment, FAST-1 mice fell from the stationary dowel at shorter latencies than SLOW-1 mice, suggesting that they had relatively greater sensitivity to ethanol. However, brain ethanol concentrations (BrECs) were similar at time of fall, and no differences were found between replicate-2 lines. SLOW-1 mice fell from the fixed-speed rotarod at lower BrECs than FAST-1 mice, suggesting possibly greater sensitivity of the SLOW-1 line. Again, no replicate-2 line differences were found. No significant differences were detected for the accelerating rotarod. These results provide little support for a negative genetic relationship between sensitivity to the stimulant and ataxic effects of ethanol using these measures of motor coordination. © 2000 Elsevier Science Inc.

Selected mouse line Ethanol Motor incoordination Locomotor stimulation Correlated trait Stationary dowel Rotarod Genetics

CERTAIN ethanol-related responses are known to be heritable in mice (3). Inbred strain differences (3,11) and the successful replicated selective breeding of mice for high (FAST-1 and FAST-2) and low (SLOW-1 and SLOW-2) sensitivity to ethanol-induced locomotor stimulation (8,18) show that sensitivity to the low-dose locomotor stimulant effects of ethanol is heritable. Another heritable trait is ethanol-induced impairment of motor coordination, or ataxia (2,3,6,11,16–18). Prior research reviewed below suggests that sensitivity to ethanol's locomotor stimulant effects and its motor incoordinating effects may share some common genetic determination.

Correlated responses to selection refer to traits other than the selected trait for which differences between a pair of selected lines are found. Successive generations of selective breeding of the FAST and SLOW lines have altered gene frequencies such that those genes influencing susceptibility to ethanol's locomotor stimulant effects became fixed in a homozygous state in the FAST lines, whereas those influencing resistance became fixed in the SLOW lines (7,18). If FAST and SLOW mice are shown to differ on a new trait, it may be concluded, given certain assumptions (7), that sensitivity to ethanol-induced locomotor activation and the new trait are determined by at least some common genes. This conclusion is strengthened if differences are found between both sets of replicate lines (i.e., FAST-1 vs. SLOW-1 and FAST-2 vs. SLOW-2), or if there is no significant effect of replicate in the presence of a line difference.

A recent study showed that when compared to FAST-1 and FAST-2 mice, SLOW-1 and SLOW-2 mice were more sensitive to ethanol-induced motor incoordination measured

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in the grid test, specifically at the dose of ethanol used for selection (2.0 g/kg) and when coordination errors were calculated, controlling for differences in locomotor activity (17). This result suggests that some of the genes increasing sensitivity to ethanol's incoordinating effects also decrease sensitivity to ethanol's locomotor stimulant effects (or vice versa). Support for a negative genetic correlation between sensitivity to the stimulant and incoordinating effects of ethanol also comes from studies utilizing other genetic animal models. In a comparison of lines selected for differential sensitivity to ethanol-induced sedation, ethanol-treated Short-Sleep mice exhibited greater locomotor stimulation and decreased grid test incoordination compared to ethanol-treated Long-Sleep mice (10). However, these lines do not exist in replicate. In a study comparing a panel of eight inbred mouse strains, strains more sensitive to ethanol-induced locomotor stimulation were less sensitive to ethanol-induced loss of balance on a stationary dowel (2). Moreover, a negative genetic correlation between sensitivity to ethanol-induced locomotor activation and grid test ataxia has been detected among the BXD/Ty recombinant inbred strains (15).

Despite the evidence supporting the view that ethanol-induced locomotor stimulation is negatively genetically correlated with ethanol-induced motor incoordination, at least two studies did not support this relationship. One study compared seven inbred mouse strains along with the Long- and Short-Sleep mice, and found that ethanol-induced grid test ataxia and ethanol-induced increases in locomotor activation were genetically uncorrelated (9). Another study probed a panel of 15 inbred mouse strains, and failed to find evidence of a genetic correlation between ethanol-induced locomotor stimulation and ethanol-induced ataxia assessed on an accelerating rotarod (3). It is not immediately clear why these studies do not completely agree with the body of work supporting this negative genetic relationship. However, we speculate that one probable reason is that different tests of motor coordination do not measure the same physiological substrates.

"Motor coordination" appears to describe a behavior comprising several components, such as gait, muscle strength, balance, ambulation, and coordination. Evidence from our laboratory suggests that different tests of motor coordination may measure different subsets of these components (1,6,16). Thus, it is not improbable that a significant genetic correlation could be identified between locomotor stimulation and one measure of ethanol-induced motor incoordination, but not another. The present experiments further assessed the presence, or absence, of a negative genetic correlation between sensitivity to ethanol-induced locomotor stimulation and ethanol-induced ataxia in FAST and SLOW mice using three different measures of motor coordination: a stationary dowel, a fixed-speed rotarod, and an accelerating rotarod.

METHOD

Subjects

Subjects were naïve 55–85-day-old male and female FAST and SLOW mice (selection generation 37), selectively bred in replicate (FAST-1, FAST-2, SLOW-1, and SLOW-2) for increased or decreased sensitivity to ethanol-induced locomotor activation, respectively. Mice were bred and housed at the Portland Veterans Affairs Medical Center. Details of the selection process have been published elsewhere (8,18). Ethanol-induced locomotor stimulation was assessed by determining the number of photocell beam interruptions in a circular automated activity monitor (Lehigh Valley) after ethanol (2.0 g/kg; 20% v/v) injection on 1 day and saline injection 24 h later (the order of these treatments was reversed for the first six generations of selective breeding). Saline scores were subtracted from ethanol scores to create change scores; these change scores were the phenotypic scores used for selection. Mice exhibiting extremely high activity scores were mated in a rotational within-family breeding scheme to produce FAST offspring for the next generation. Those with extremely low scores were similarly mated to produce SLOW offspring. One male and one female from each of nine families per replicate and line became breeders and brother–sister mating was prohibited.

Each replicate line was independently bred from an original HS/Ibg heterogenous population (8), an eight-way cross of inbred strains. Thus, each pair of replicate lines represents an independent selective breeding project. Because some protocols were quite time consuming, and mice of all lines were not always available, it was not possible to test all four lines contemporaneously. The critical question was whether the lines differed within replicate, so we chose to include the independent comparison of either the FAST-1 and SLOW-1, or the FAST-2 and SLOW-2 selected lines in each experiment. Mice used in these experiments were weaned at 21 $\pm$ 1 days of age, and isosexual groups were housed three to five per cage in clear polycarbonate crages (28 $\times$ 18 $\times$ 13 cm) with corn cob bedding and free access to food and water except during testing. Mice were maintained on a 12 L:12 D cycle (lights on at 0600 h) and an ambient room temperature of 22 $\pm$ 1°C.

General Methods

On test days, mice were moved (in their home cages) to the experimental room, weighed, and allowed at least 30 min to habituate to the test environment. All tests were begun by the second hour of the light phase. Mice were injected intraperitoneally with 2.5 g/kg ethanol (20% v/v in 0.9% saline) or saline before all test trials, but not practice trials. This dose was chosen because previous experiments have shown it to be effective in testing for ethanol sensitivity using the fixed-speed and accelerating rotarods. Injection volumes ranged from 0.3–0.5 ml per mouse. For each task, two measures of ethanol sensitivity were utilized; latency to fall, and BrEC at the time of fall. A shorter latency to fall or lower BrEC at the time of fall indicated greater ethanol sensitivity.

Experiments were performed in duplicate to generate an adequate number of subjects for detection of statistically significant differences. This decision was made a priori due to limitations in the number of subjects that could be tested contemporaneously. Both genders were included in these experiments because sex differences have been identified for some ethanol effects. All experimental procedures were approved by the Veterans Affairs Medical Center Institutional Animal Care and Use Committee, and followed National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Stationary Dowel

The stationary dowel was developed to assess tolerance to ethanol-induced incoordination (13). The apparatus comprised a 1-cm diameter wooden dowel suspended 60 cm above sawdust bedding. Equal 24-cm segments of the dowel were separated by 8.25-cm diameter cardboard disks permitting four subjects to be tested simultaneously. Before inclusion in the experiments, mice were required to pass a 2-min

criterion test in which they were placed on the stationary dowel without prior injection. Only one male FAST-1 mouse was unable to meet this criterion. Mice passing the 2-min criterion tests were injected with ethanol or saline (n = 10–16 per line/replicate/sex/treatment) and immediately tested on the dowel. Latency to fall (seconds) was recorded. Because saline-treated mice can maintain balance for an indefinite period of time, these animals were removed from the stationary dowel after 3 min. No saline-treated mouse ever fell before the conclusion of the 3-min test session.

Fixed-Speed Rotarod

The rotarod was originally developed to measure neurotoxicity (12,14). The apparatus comprised an 8-cm diameter horizontal rotating cylinder suspended 46 cm above sawdust bedding. The cylinder was divided into six sections by white acrylic disks extending 9.6 cm beyond the surface, again allowing for the simultaneous testing of mice. The surface was covered with 320-grit wet–dry sandpaper to reduce slipping. The cylinder was rotated by a motor that could maintain a fixed speed (5 rpm) or a constant rate of acceleration (1 rpm acceleration/3 s). In our experience, untrained mice of some genotypes are unable to walk on the fixed-speed rotarod, but can perform on the accelerating rotarod. We have also found that mice given practice trials on the accelerating rotarod reach a 2-min criterion on the fixed-speed rotarod more easily. Therefore, on the test day, mice were first given three noninjected practice trials on the accelerating rotarod (separated by 30-s rest intervals; see next section). Immediately thereafter, they were given a 2-min criterion test on the fixed-speed rotarod. All mice could maintain balance on the fixed-sped rotarod for 2 min, and were injected with ethanol or saline (n = 12–17 per line/replicate/sex/treatment) and immediately tested on the fixed-speed rotarod. Latency to fall was recorded in seconds. Saline-exposed mice were removed from the apparatus after 3 min and never fell before the 3-min test session had elapsed.

Accelerating Rotarod

The apparatus was identical to the one used for the practice trials prior to fixed-speed rotarod testing. On the test day, mice were given six noninjected practice trials on the accelerating rotarod (separated by 30-s rest intervals). For each practice trial, mice were left on the accelerating rotarod until they fell. Over practice trials, mice gradually became better performers on the apparatus, maintaining balance on the accelerating rotarod for a longer period of time on each subsequent trial. Pilot testing showed that six training trials were sufficient for subjects to achieve a level of performance necessary to avoid a floor effect once ethanol was administered (i.e., to detect a subsequent effect of ethanol). Following practice, mice (n = 8–16 per line/replicate/sex/treatment) were injected with ethanol or saline and immediately placed on the accelerating rotarod; the rotarod and a timer were started 30 s later. Latency to fall was recorded in seconds.

BrECs

Immediately following all tests, mice were euthanized, and whole brains were rapidly removed and frozen on dry ice for analysis of ethanol concentration by gas chromatography (4). Briefly, whole brains were homogenized in 150 μl $ZnSO_4$ (5%), 150 μl $Ba(OH)_2$ (0.3 N), and 300–600 μl dH_2O (1.5× brain weight). Homogenates were centrifuged at 12,000 rpm for 10 min. Supernatant was removed by micropipette, injected onto a Porapak Q 80/100 column (Alltech Assoc. Inc.), and resulting values were compared to a standard ethanol concentration curve (Hewlett Packard, Model 5890a).

Data Analysis

Data for a given apparatus were analyzed by analysis of variance (ANOVA) with line, replicate, and sex as the independent variables in most cases. When appropriate, simple main effects analyses were carried out. The level of statistical significance for all analyses was set at $p < 0.05$. Saline data were not included in the analysis of stationary dowel and fixed-speed rotarod results because saline-treated mice were removed from these apparatuses after 3 min, and their latencies did not vary.

RESULTS

Stationary Dowel

Figure 1 (upper panel) shows latency to fall from the stationary dowel apparatus. The overall three-way analysis (line × replicate × sex) indicated a significant interaction of line and

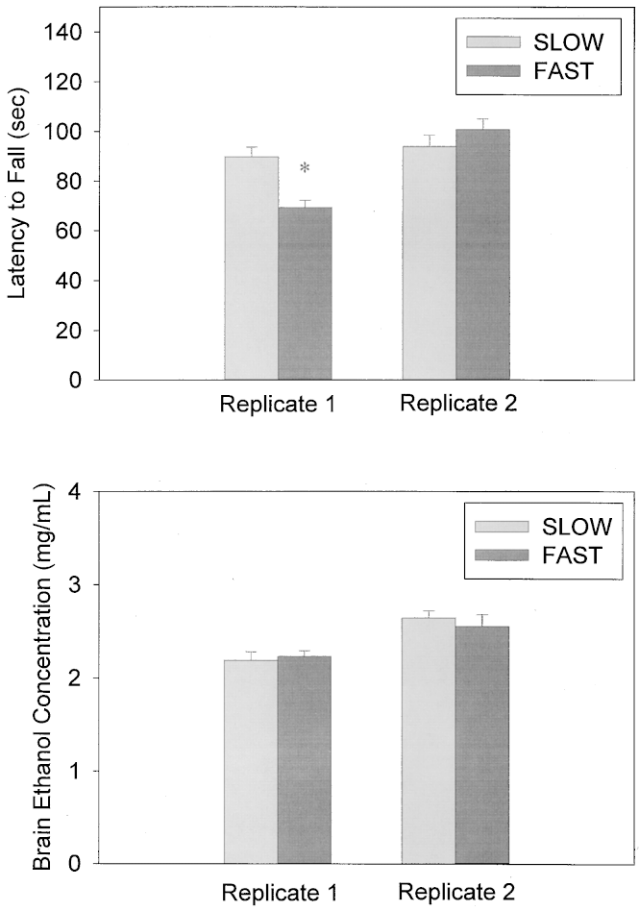

FIG. 1. Latencies to fall (upper panel) and BrECs at the time of fall (lower panel) from the stationary dowel for ethanol-treated FAST and SLOW replicate-1 and -2 mice. Values represent means ± SEM for 24–30 mice per replicate line collapsed on sex. Differences were considered significant at $*p < 0.05$ from simple main-effects analysis. For comparative purposes, saline-injected mice were tested but removed from the stationary dowel after 180 s (data not shown).

replicate, $F(1, 105) = 11.5$, $p < 0.01$, and no other significant effects. Simple main effects analysis revealed that FAST-1 mice fell from the stationary dowel at shorter latencies than SLOW-1 mice ($p < 0.001$), but this difference was not present between the replicate-2 lines.

BrECs are shown in the lower panel of Fig. 1. Three-way ANOVA detected a main effect of sex, $F(1, 108) = 7.1$, $p < 0.01$, and a main effect of replicate, $F(1, 108) = 21.2$, $p < 0.001$. Female mice fell from the stationary dowel at higher BrECs than male mice (2.51 ± 0.06 vs. 2.29 ± 0.07, respectively), and replicate-1 mice fell at lower BrECs than replicate-2 mice. There were no other significant effects or interactions.

Fixed-Speed Rotarod

Latency to fall from the fixed-speed rotarod test is shown in Fig. 2 (upper panel). Three-way ANOVA detected a main effect of replicate, $F(1, 105) = 9.7$, $p < 0.01$, but no other significant effects or interactions. Replicate-2 mice had longer latencies to fall than did replicate-1 mice.

BrECs at the time of fall are shown in the lower panel of Fig. 2. Three-way ANOVA detected only a significant interaction of line × replicate, $F(1, 102) = 4.6$, $p < 0.05$. Simple main effects analysis showed that SLOW-1 mice fell at lower BrECs than did FAST-1 mice ($p < 0.05$), but that FAST-2 and SLOW-2 mice did not differ.

Accelerating Rotarod Practice Trials

Figure 3 (upper and lower panels) shows latency to fall from the accelerating rotarod over six undrugged training trials. The overall five-way repeated-measures ANOVA (line × replicate × sex × treatment × trial) detected several significant two-way and three-way interactions involving all factors except treatment group. Because the sex factor only interacted with replicate and trial and not line, data were combined across sex and treatment groups and separate two-way repeated-measures ANOVAs were performed for each set of replicate lines with line and trial as grouping factors. Analysis of replicate-1 mice (Fig. 3, upper panel) revealed significant main effects of line, $F(1, 114) = 7.3$, $p < 0.01$, and trial, $F(5,$

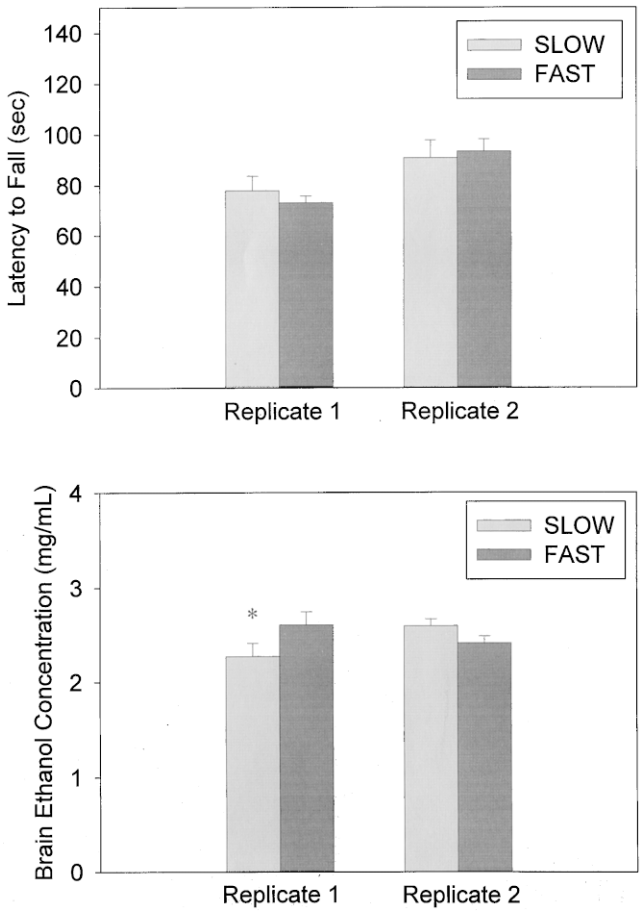

FIG. 2. Latencies to fall (upper panel) and BrECs at the time of fall (lower panel) from the fixed-speed rotarod for ethanol-treated FAST and SLOW replicate-1 and -2 mice. Values represent means ± SEM for 26–32 mice per replicate line collapsed on sex. Differences were considered significant at $*p < 0.05$ from simple main-effects analysis. For comparative purposes, saline-injected mice were tested but removed from the fixed-speed rotarod after 180 s (data not shown).

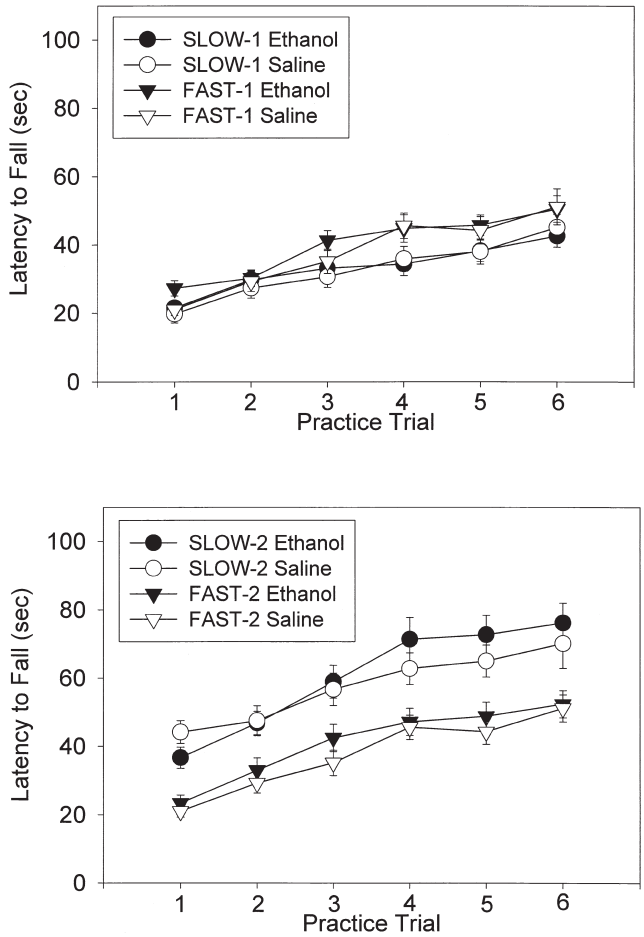

FIG. 3. Latency to fall on accelerating rotarod practice trials 1–6 for replicate-1 (upper panel) and -2 (lower panel) FAST and SLOW mice. Values represent means ± SEM for 18–31 mice per replicate line and treatment (ethanol or saline) group collapsed on sex. Ethanol and saline group designations refer to treatments postpractice trial 6. Differences were considered significant at $*p < 0.05$ from simple main-effects analysis.

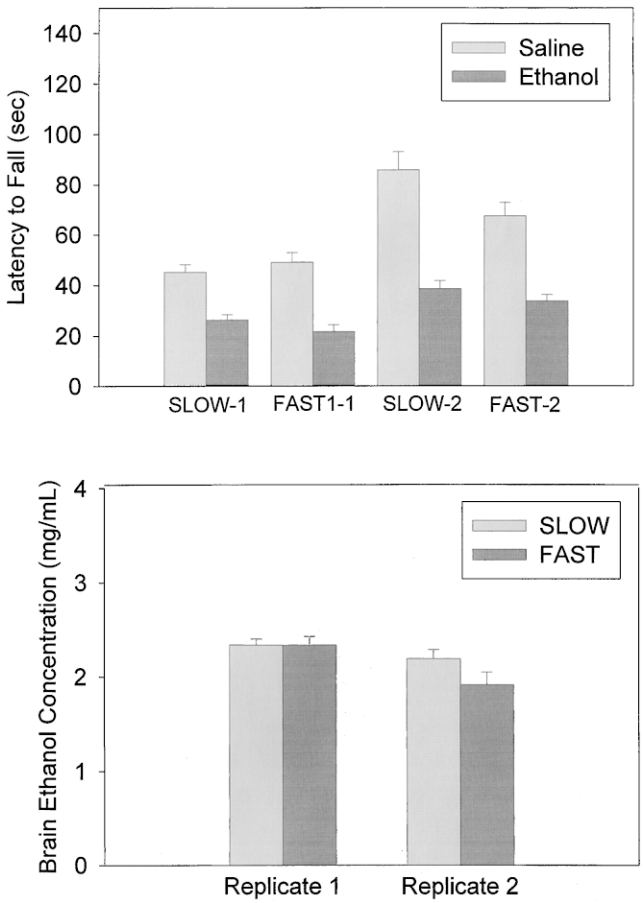

FIG. 4. Latency to fall (upper panel) and BrECs at the time of fall (lower panel) from the accelerating rotarod (trial 7) for ethanol-treated replicate-1 and -2 FAST and SLOW mice. Mice were injected with ethanol or saline prior to trial 7. Values represent means ± SEM for 18–31 mice per replicate line and treatment (ethanol or saline) group collapsed on sex. Differences were considered significant at $*p < 0.05$ from simple main-effects analysis.

570) = 66.5, $p < 0.001$, but no interactions of these factors. The performance of FAST-1 mice was greater than that of SLOW-1 mice, and all replicate-1 mice showed improved performance with practice. Analysis of replicate-2 mice (Fig. 3, lower panel) also detected main effects of line, $F(1, 79) = 38.2$, $p < 0.001$, and trial, $F(5, 395) = 73.9$, $p < 0.001$, but no significant interaction of line and trial. In this case, the performance of SLOW-2 mice was greater than that of FAST-2 mice, and both lines again showed improved performance over trials. The reversal in order of performance for both sets of replicate lines strongly suggests that the difference in accelerating rotarod ability is not a genetically correlated response to selection.

Accelerating Rotarod Trial 7

When ethanol or saline was administered before trial 7, four-way ANOVA revealed main effects of line, $F(1, 184) = 5.4$, $p < 0.03$, replicate, $F(1, 184) = 38.0$, $p < 0.01$, and treatment, $F(1, 184) = 163.2$, $p < 0.01$, but no effect of sex. In addition, analysis detected a marginally significant interaction of line × replicate, $F(1, 184) = 3.9$, $p = 0.05$, but no other significant two-way or three-way interactions of these factors (Fig. 4, upper panel), FAST mice fell at shorter latencies than did SLOW mice, but the absence of a line × treatment interaction indicated that FAST mice did not show an enhanced sensitivity to the ataxic effects of ethanol. Replicate-1 mice fell from the accelerating rotarod at shorter latencies than did replicate-2 mice, and ethanol-treated mice fell at shorter latencies than did saline-treated mice.

BrECs at the time of fall are shown in the lower panel of Fig. 4. Three-way between subjects ANOVA detected only a significant main effect of replicate, $F(1, 95) = 7.5$, $p < 0.01$. At the time of fall, replicate-1 mice had higher BrECs than did replicate-2 mice.

DISCUSSION

Results of the present experiments are summarized in Table 1. Neither pair of replicate FAST and SLOW mice differed in ethanol sensitivity when tested on the accelerating rotarod. However, when tested on the stationary dowel, FAST-1 mice fell at shorter latencies than did SLOW-1 mice, suggesting that FAST-1 mice are more sensitive to ethanol's incoordinating effects. FAST-2 and SLOW-2 mice did not differ in latency to fall from the stationary dowel, nor did either set of replicate lines differ in BrEC at the time of fall. When tested on the fixed-speed rotarod, FAST and SLOW mice did not differ in latency to fall. However, SLOW-1 mice fell at lower BrECs than did FAST-1 mice, suggesting greater neurosensitivity to ethanol in SLOW-1 mice; FAST-2 and SLOW-2 mice did not differ in BrEC at the time of fall from the fixed-speed rotarod.

The strongest evidence for a genetic correlation is obtained when both sets of replicate lines differ for the new trait (7). Strong evidence of a genetic correlation was not obtained for the present measures of ethanol-induced motor incoordination. Moderate evidence of a genetic correlation is indicated by a significant main effect of line and interaction of line × replicate, followed by the discovery that a difference exists between only one pair of replicates (7). Moderate evidence of a genetic correlation was also not obtained. Weak evidence is provided by a significant line × replicate interaction and a difference between only one replicate pair of lines in the absence of a main effect of line (7). Our data provide, at best, only weak evidence for a genetic correlation between sensitivity to ethanol-induced locomotor stimulation and ethanol-induced ataxia. In both the stationary dowel and fixed-speed rotarod tests, only replicate-1 FAST and SLOW mice differed in ethanol sensitivity, behaviorally in one case (stationary dowel) and

TABLE 1

RELATIVE ETHANOL SENSITIVITY OF FAST AND SLOW MICE

	Relative Ethanol Sensitivity*	
Test	Indicated by Latency	Indicated by BrEC
Stationary dowel	FAST-1 > SLOW-1	FAST-1 = SLOW-1
	FAST-2 = SLOW-2	FAST-2 = SLOW-2
Fixed-speed rotarod	FAST-1 = SLOW-1	FAST-1 < SLOW-1
	FAST-2 = SLOW-2	FAST-2 = SLOW-2
Accelerating rotarod	FAST-1 = SLOW-1	FAST-1 = SLOW-1
	FAST-2 = SLOW-2	FAST-2 = SLOW-2

*Greater than sign indicates a significantly higher ethanol sensitivity.

according to BrEC at induction of ataxia in the other (fixed-speed rotarod). Thus, these results offer only weak support for a positive genetic correlation in one case (i.e., the stationary dowel test), and a negative genetic correlation in the other (i.e., the fixed-speed rotarod test), between sensitivity to ethanol-induced locomotor activation and ethanol-induced motor incoordination. The present results are different from those obtained for sensitivity to ethanol-induced (2.0 g/kg) grid test ataxia in which both pairs of replicate lines differed in sensitivity using a ratio of errors to activity (17).

One possible explanation for the above results concerns the characteristics of the different measures of ethanol sensitivity. The grid test apparatus indexes ethanol-induced motor incoordination by counting foot slips through a 1 cm^2 wire-mesh floor. Whereas the present tests terminated within 4.5 min after an ethanol injection, the grid test apparatus continued measuring ethanol-induced motor incoordination for another 5.5 min. Thus, the duration of the grid test study was longer than that of the present experiments, perhaps allowing line differences in ethanol-induced incoordination to develop over the 10-min interval. We are currently performing experiments in which ethanol-induced grid test ataxia is measured on a minute-to-minute basis to better equate it with the present tests. In one study, we found that 5-HT_{1B} null mutant mice were less sensitive to ethanol-induced grid test ataxia than wild types when a cumulative 10-min test was used. When these data were examined at specific time points, the strains were found to differ during minutes 3, 4, and 5 following injection (1). A time-course analysis such as this might reveal the time interval after exposure when ethanol is most effective at inducing grid test ataxia in FAST and SLOW mice.

Although dose and strain dependent, mouse BrECs rise rapidly and peak at roughly 5 min postinjection. Because the dowel and rotarod tests were terminated within 4.5 min after injection, ethanol sensitivity was likely assessed during the rising phase of the blood ethanol absorption curve. If there are no difference in ethanol absorption between the FAST and SLOW selected lines, a shorter latency to fall should be paralleled by a lower BrEC at the time of fall. Both a shorter latency to fall and a lower BrEC would clearly support differences in neurosensitivity to ethanol. However, whereas FAST-1 mice exhibited shorter latencies to fall from the stationary dowel than did SLOW-1 mice, BrEC at the time of fall did not differ between the lines. Furthermore, SLOW-1 mice had lower BrEC at the time of fall from the fixed-speed rotarod than did FAST-1 mice, but they did not fall at shorter latencies. These significant results could reflect statistical false-positives or they could suggest a subtle difference in rate of ethanol absorption between FAST-1 and SLOW-1 mice. However, these data do not indicate a consistent difference in the rate of ethanol absorption. We believe our data argue against any difference. If this is true, then the apparent greater sensitivity of FAST-1 (vs. SLOW-1) mice on the fixed-speed rotarod would represent a false-positive result.

Several studies have measured blood ethanol clearance rates at fixed time points after injection, and suggest that differences may exist between FAST and SLOW mice. Retro-orbital sinus bloods were taken immediately following tests for ethanol's motor incoordinating (grid test), hypothermic, and loss of righting effects (17), and ethanol's locomotor stimulant effects (18,19). Except for in the grid test, SLOW-1 and -2 mice had significantly higher blood ethanol concentrations compared to FAST-1 and -2 mice in each behavioral assay. In addition, an ethanol clearance rate study was performed in which a single dose of 2.0 g/kg ethanol was administered and retro-orbital sinus blood samples were collected at 15, 30, 60, 120, and 180 min postinjection (18). Results showed that SLOW mice (both replicates) had higher blood ethanol concentrations than FAST mice at each time point. Together, these data may suggest that selection of the FAST and SLOW lines differentially altered some aspect of ethanol clearance. However, in each of the above studies, the magnitude of the difference in blood ethanol concentration was very small compared to that of the behavioral response. Thus, it is unlikely that differences in ethanol clearance can fully account for any of ethanol's differential effects in FAST and SLOW mice.

Analysis of our data revealed that female mice had higher blood ethanol concentrations than male mice in the stationary dowel test, but not the fixed-speed or accelerating rotarod tests. Whereas the stationary dowel data might reflect a statistical false positive, it may suggest a potential sex difference in ethanol absorption. Ethanol absorption has not been directly studied in male and female mice of these selected lines. However, FAST and SLOW mice of both sexes were tested in the above time-course experiment (18), and female mice had significantly higher blood ethanol concentrations at each time point. We presently do not know how to explain these putative sex differences. However, because sex did not interact with replicate or line in the present study, we do not believe this difference is important in interpreting genetic differences in ethanol's effects on motor incoordination using the stationary dowel.

Three different tests of ethanol-induced motor incoordination were employed in the present series of studies. Whereas each of these measures motor coordination in mice, ataxia is complex consisting of a constellation of components including gait, balance, grip strength, and ambulation. A growing body of evidence from this laboratory suggests that one test of motor coordination may not measure the same subset of coordinated motor abilities as another. A survey of 25 BXD/Ty recombinant inbred strains where different animals were tested for either fixed-speed rotarod or grid test sensitivity to 2.0 g/kg ethanol found essentially zero genetic correlation between strain sensitivities (6). Thus, strains more impaired on the grid test were not necessarily more impaired on the fixed-speed rotarod. Furthermore, experiments examining ethanol-induced ataxia in mouse lines selected for high (HOT) and low (COLD) sensitivity to ethanol-induced hypothermia suggest that not all tests of ataxia are measuring the same physiological substrates (16). In another study, 5-HT_{1B} null mutant mice were found to be less sensitive than their wild-type counterparts when measured by the grid test and the balance beam, but did not differ when tested on the stationary dowel, fixed-speed rotarod, accelerating rotarod, screen test, loss of righting reflex test, and the grip strength test (1,5). Thus, increased or decreased sensitivity to ethanol-induced ataxia measured on one test may not generalize to another.

Each test of motor coordination we employed likely requires a unique combination of specific abilities. The stationary dowel appears to measure balance and grip strength. In contrast, the fixed-speed and accelerating rotarods appear to measure balance, gait, and locomotor ability. Theoretically, as the mouse becomes intoxicated, it loses its ability to make postural adjustments as the rod rotates. The accelerating rotarod may be a more complex task as balance maintenance becomes increasingly more difficult for the mouse. The grid test represents a measure of motor coordination requiring precise ambulation, as the mouse must place its foot in an exact location (or avoid placing its foot where there is no support) to avoid foot slippage through the grid. Perhaps it is this coordinated locomotion that was affected by selection.

Overall, the experiments reported here did not provide strong or even moderate evidence of a genetic correlation between ethanol-induced locomotor stimulation and ethanol-induced locomotor ataxia when measured on the stationary dowel, fixed-speed rotarod, or accelerating rotarod. However, this result does not eliminate the possibility that some components of ethanol-induced motor coordination are negatively genetically correlated with sensitivity to ethanol-induced locomotor stimulation as has been found among FAST and SLOW mice (17), Long-Sleep and Short-Sleep mice (10), and BXD recombinant inbred mice (15) using the grid test. Several other indices of motor coordination have not been utilized in comparisons of the FAST and SLOW lines that could provide a more complete evaluation of this potential association. For example, effects of ethanol on grip strength, balance beam coordination, ability to perform the screen test, or rope climb have not been assessed. It is possible that differential ethanol effects on grip strength might play a role in performance on some of these balance tasks.

Finally, it has been shown that SLOW mice are more sensitive to the sedative and hypothermic effects of ethanol seen at higher doses (17). During selective breeding, SLOW mice were selected on the basis of small (and sometimes negative) difference scores, which would indicate ethanol-induced locomotor depression. Thus, increased ethanol-induced ataxia in SLOW mice may be related to reduced ambulation due to a greater sensitivity to ethanol-induced sedation. Moreover, it is possible that FAST and SLOW mice fall from the stationary dowel, fixed-speed rotarod, and accelerating rotarod for different reasons, although at about the same time (i.e., ethanol increases ambulation in FAST mice, whereas it decreases ambulation in SLOW mice). Clearly, much work remains to fully define the genetic relationship between sensitivity to ethanol-induced locomotor stimulation and ethanol-induced motor incoordination.

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