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Ontogeny of Ethanol-Induced Locomotor Activity and Hypothermia Differences in Selectively Bred FAST and SLOW Mice

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WOOD, R. D., E. SHEN, J. A. CHESTER AND T. J. PHILLIPS. *Ontogeny of ethanol-induced locomotor activity and hypothermia differences in selectively bred FAST and SLOW mice.* PHARMACOL BIOCHEM BEHAV **62**(2) 339–347, 1999.—The replicate lines of selectively bred FAST and SLOW mice differ in locomotor response to 2 g/kg ethanol (EtOH). FAST mice show enhanced locomotion; SLOW mice exhibit no change or locomotor depression. Little is known about the responses of FAST and SLOW mice to EtOH during development. We assessed the locomotor responses of FAST and SLOW mice at postnatal days (P) 10, 15, 30, and 60. A genetically correlated response, EtOH-induced hypothermia, was also investigated. Although all animals demonstrated their respective selection phenotypes in adulthood, developing FAST mice exhibited ethanol stimulation by P15 (replicate 1) or P30 (replicate 2). At these ages, responses of FAST mice differed from those of SLOW. The stimulant response in FAST mice was adult-like at P30. EtOH-induced hypothermia was seen in SLOW mice by P15. These data suggest that sensitivity to the locomotor stimulant effects of EtOH changes during postnatal development, and may mirror developmental profiles for certain neurotransmitter systems. © 1999 Elsevier Science Inc.

Ethanol Locomotor activity FAST and SLOW mice Development Hypothermia Selected mouse lines Ontogeny Pharmacogenetics

ALCOHOLISM remains a persistent and prevalent disorder, with the incidence of occurrence in the United States estimated at 3.9 to 9% (8). Research on the genetics of alcoholism has progressed from the hope that a single gene determines alcoholism to the realistic expectation that alcoholism is composed of many characteristics that are most likely each mediated by several genes. One way to understand the genetic basis of the multiple effects of alcohol that may influence alcohol addiction has been through the use of selectively bred lines of mice that differ in their responses to ethanol (EtOH). These animals are selectively bred on the basis of differential responsiveness to one effect of ethanol, and any further observed differences between the lines putatively reflect the inherited influence of genes common to the selection phenotype (9). Many of these selected lines have been bred in replicate to strengthen conclusions about the genetic contribution to the phenotype of interest, given the unlikelihood that similar trail-irrelevant alleles would be fixed in both replicates of the line.

The FAST and SLOW mice, selectively bred for 36 generations (38) for differing sensitivity to the locomotor stimulant effects of acute EtOH, and now maintained by random breeding, were developed as an animal model of EtOH-induced euphoria or behavioral disinhibition in humans (1). FAST mice are highly stimulated by ethanol, whereas SLOW mice are unaffected or depressed by alcohol (38). Studies into the neurochemical bases of this differential response have suggested dopaminergic involvement by the finding that haloperidol decreased EtOH-stimulated locomotor activity in FAST mice (36). That this effect was mediated via the dopamine D_2 receptor subtype was suggested by evidence that the more specific D_2 receptor antagonist raclopride also blocked EtOH-stimulated activity in FAST mice (36). The involvement of D_1 receptors seems to be less clear, in that EtOH-stimulated ac-

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tivity was dose dependently decreased in only one replicate of the FAST mice (36). Because of evidence supporting GABA and glutamate modulation of dopaminergic activity in the nucleus accumbens and ventral tegmental area, as well as evidence that these systems themselves affect locomotor activity, it is suspected that GABA and glutamate transmitter systems may also be mediators of the differential responses in FAST and SLOW mice (13,39).

Whereas much is known about the adult response to alcohol in FAST and SLOW selectively bred mice, little is known about how these responses change over development. A developmental approach may be one way to access information on neurochemical mechanisms underlying behavior. Correlations between the developmental emergence of the genetically selected response difference and known changes in neurobiology during development may help elucidate these mechanisms. An example of this rationale can be found in a study by French and colleagues, in which the development of ethanol responsiveness was investigated in mice selectively bred for reduced (Short-Sleep or SS) or enhanced (Long-Sleep or LS) susceptibility to the sedative effects of EtOH (21). The authors found a significant correlation between EtOH-induced decreases in in vivo tyrosine hydroxylase activity in cerebellum, hypothalamus, and brain stem at postnatal days 8 to 12 (P8 to P12), with the appearance of longer sleep times in LS vs. SS mice. Similarly, a significant correlation was noted between decreases in dorsal raphe tryptophan hydroxylase and the emergence of EtOH-induced hypothermia at P16. These findings led to the conclusion that differences in monoamine responsiveness to EtOH may contribute to the selected phenotype in LS and SS mice. Indeed, adult data bear out this hypothesis: a series of studies has shown that the relatively greater sensitivity of LS mice to EtOH has been associated with greater decreases in in vivo tyrosine hydroxylase activity (18–20).

In addition to differing in their locomotor responsiveness to low dose EtOH, FAST and SLOW mice have been found to exhibit differential sensitivity to other EtOH effects. Such genetically correlated responses are of value to the pharmacogenetic researcher because they offer evidence for common mechanisms of action of EtOH (11). Shen and colleagues have previously observed SLOW mice to be more sensitive to the ataxic, sedative, and hypothermic properties of EtOH, and FAST mice to exhibit more severe EtOH withdrawal-induced convulsions (37). These data suggest that FAST mice may be more sensitive to the CNS arousing properties of EtOH, while SLOW mice may be more sensitive to the EtOH's sedative/hypnotic properties.

The goals of the present study were to characterize the developmental profiles of sensitivity and insensitivity to EtOH's stimulant effects, to determine the approximate age at which the line difference emerges in the replicate lines of FAST and SLOW mice, and to measure the magnitude of the line difference during development. Mice of postnatal ages 10, 15, 30, and 60 days were chosen for study because these ages encompass a broad range of developmental time frames, including the postnatal (P10), preweanling (P15), adolescent (P30), and adult (P60) stages, and because these ages correspond to dynamic changes occurring in neurotransmitter systems believed to mediate EtOH-stimulated locomotor activity (see Discussion). The developmental profile of the correlated response, EtOH-induced hypothermia, was also investigated, as it could be easily studied coincident with study of the selection phenotype. A similar developmental profile of a correlated response in these mouse lines might provide additional evidence of common genetic mediation. The results of these studies will guide predictions and further research on the biological substrates mediating the responses, based on what is known in the literature about the development of different neurotransmitter systems.

METHOD

General Methods

Both genetically independent replicates of the FAST (FAST 1 and FAST 2) and SLOW (SLOW 1 and SLOW 2) mice of both sexes were used in these experiments. All animal handling and experimental use followed the National Institutes of Health guidelines, and procedures were approved by the VA Institutional Animals Care and Use Committee. These mice were bred at the Portland Veterans Affairs (VA) Medical Center (Portland, OR); the breeding and selection procedures for mice used in Experiments 1 and 3 have been described in detail elsewhere (12, 29). Briefly, the replicate FAST and SLOW mice were derived from HS/Ibg genetically heterogeneous stock, and were simultaneously bred for high or low to no locomotor activation following 2.0 g/kg EtOH. Selection was based on magnitude of a locomotor difference score created by subtracting a saline baseline from locomotion after EtOH treatment. Tests after saline and EtOH were conducted 24 h apart and each lasted for 4 min beginning 2 min after injection. A relaxed breeding scheme (no selection pressure) was adopted after 36 generations of selective breeding as the limits of selection appeared to have been reached.

For all three experiments, breeding pairs were checked at the same time once daily to determine the presence of newborn offspring. The day of detection was postnatal day 0 (P0). Offspring remained with their dam and sire until P21, at which time they were weaned into same-sex groups of two to five per cage. The selection and breeding procedures for Experiment 2 were identical to those in Experiments 1 and 3, with three exceptions: 1) the breeding pairs were specifically dedicated to this experiment (i.e., all breeding pairs were used only for Experiment 2, unlike Experiments 1 and 3, in which the offspring of some breeding pairs were used in other unrelated experiments; 2) all litters were culled to 8–10 pups at P1; 3) litters were checked for births on the weekend, unlike Experiments 1 and 2, where litters were checked for births only during the weekdays and those born on weekends were excluded from study.

For all experiments, the colony room temperature was maintained at 21 ± 2°C, and fluorescent ceiling lights were on from 0600–1800 h. The animals were housed in clear polycarbonate cages (28 × 18 × 13 cm) on corncob bedding, which was changed twice weekly. All animals had free access to rodent block food and water.

Of each litter born, one male and one female offspring was chosen at random for one of the ages and doses (treatments); the remaining siblings were assigned to other age and/or treatment groups. Thus, litter effects were mitigated by avoiding assignment of offspring of the same litter to the same treatment group.

Experiment 1. Ontogeny of EtOH-Stimulated Locomotor Activity in FAST and SLOW Mice

Experiment 1 was an initial characterization of the ontogeny of locomotor responsiveness to EtOH in both replicates of FAST and SLOW mice at three ages: P15, 30, and 60.

Locomotor activity testing. All mice were naive at the time of testing, and each mouse was included in only one age and

treatment group (n = 10–17 per group). Fifteen-day-old mice were separated from the dam's cage on the day of testing. All animals were removed from the colony room into the testing room to habituate for at least 1 h prior to testing. The subjects were subsequently weighed and placed into separate holding cages with corncob bedding. After an intraperitoneal (IP) injection of either 2 g/kg ethanol [200 proof ethyl alcohol (Quantum Chemical Corp., Tuscola, IL) diluted to a 20% v/v concentration with 0.9% saline] or saline, mice were immediately placed into an activity chamber. Activity was assessed in Omnitech (Columbus, OH) automated activity monitors. Each activity monitor comprised a clear acrylic box (40 × 40 cm) transected by photocell beams. There were eight beam interruption sites, 5 cm apart along each of the four walls, 2 cm above the floor. For young animals, a platform was placed underneath the acrylic boxes so that the photocell beams were 1.5 cm from the chamber floor. This ensured that the smaller 15-day-old mice would consistently interrupt the photocell beams as they moved around the chamber. To prevent animals from escaping, a clear plastic lid (44 × 44 cm) with 0.64-cm diameter holes was placed atop each box. The activity monitors were set inside black acrylic plastic sound-attenuating chambers with a fluorescent white light mounted on the back wall. A ventilation fan mounted on the rear right wall also provided masking noise. Locomotor activity was assessed via photocell beam interruptions under bright lighting conditions for 10 min between 1300 and 1600 h.

Blood ethanol concentrations (BECs). Upon conclusion of testing, a 20-μl retroorbital sinus blood sample was taken from each EtOH-treated mouse for determination of blood EtOH concentrations (BECs). Following sampling, mice were humanely euthanized by CO_2 inhalation. Blood was immediately placed into microcentrifuge tubes containing ice-cold $ZnSO_4$. Samples were further processed according to a previously described method (28). BECs were determined by gas chromatography (Hewlett-Packard 5890) with flame ionization detection.

Data analysis. Total horizontal distance traveled over the 10-min test session was analyzed initially via five-way ANOVA grouped by line, replicate, drug condition, age, and sex. BECs were analyzed initially via a separate four-way ANOVA grouped by line, replicate, sex, and age. In the absence of significant effects of sex, the data were collapsed over this variable and four- or three-way ANOVAs were performed. Additional data reduction by ANOVAs, simple main effects analyses of significant two-way interactions, and post hoc Tukey mean comparisons were conducted when appropriate.

Experiment 2. Ontogeny of EtOH-Induced Hypothermia and EtOH-Stimulated Locomotor Activity in FAST and SLOW Mice

Experiment 2 sought to replicate the findings from Experiment 1 under changed conditions altered in response to concerns that arose about body size and thermoregulatory differences among mice of different ages. A different locomotor activity testing apparatus was used to address the possibility that the constant activity monitor size used across all ages in Experiment 1 would represent a confound for younger animals. In addition, test duration was increased by 5 min (total test time = 15 min) in case young animals had a different time course of EtOH response than adults, an additional dependent variable—EtOH-induced hypothermia—was included in this study to expand the scope of this work, and an additional age group—P10—was added.

Locomotor activity and hypothermia testing. All mice were naive at time of testing. FAST and SLOW mice were removed from their home cages and carried individually into the testing room in polycarbonate cages (28 × 18 × 13 cm) with corncob bedding and a lid. To avoid potential loss of body temperature in P10 pups, mice in this experiment did not habituate in the testing room for 1 h as in Experiment 1. Immediately after transport to the testing room, animals were weighed and rectal temperatures taken (Sensortek Thermalert model TH-8, Clifton, NJ; P10 rectal probe: IT14; P15 rectal probe: IT18, Physitemp Instruments Inc., Clifton, NJ), after which they received an IP injection of either 2 g/kg EtOH (Pharmco Products, Inc., Brookfield, CT) (20% v/v) or saline. The subjects were then immediately placed into an open field for 15 min. In contrast to Experiments 1 and 3, the open fields used in this experiment were clear Plexiglas cubes with the floors marked into grids. Because mice of these varying ages differed in body size, the floor sizes of the cubes as well as the sizes of their respective grids varied according to the age of the animal: (P10—floor 20 cm^2, grid 1.25 × 2.0 cm; P15—floor 31.2 cm^2, grid 3.8 × 3.8 cm; P30—floor 42 cm^2, grid 4.5 × 5.0 cm; P60—floor 42 cm^2, grid 4.5 × 5.0 cm). Given that P10 mouse pups are unable to adequately thermoregulate when alone (43), the open field for this age group was kept warm via a 60-watt light bulb positioned 30 cm above the floor. This manipulation resulted in a stable ambient temperature of 27°C (±1.2°C). A separate control study was conducted to determine the impact of raising ambient temperature by 5°C on locomotor activity after saline and EtOH treatment in adult FAST and SLOW mice (see Experiment 3).

Activity in the open fields was recorded via videocamera for later counting of line crossings by a trained observer blind with respect to injection substance. At the conclusion of the 15-min test, a rectal temperature was again taken.

Brain ethanol concentrations (BrECs). Whereas retroorbital sinus samples provide a good estimate of BrEC, they are difficult to obtain from 10-day-old mice. Therefore, in Experiment 2, immediately upon removal from the test apparatus, EtOH-treated animals were decapitated following cervical dislocation, and brains were removed for determination of whole brain ethanol concentrations (BrECs). Following rapid freezing on dry ice, the brains were processed as previously described (10). Assays were conducted via gas chromatography (Hewlett-Packard 5890) with flame ionization detection. Saline-treated animals were euthanized via CO_2 inhalation.

Data analysis. The statistical procedures employed were identical to those in Experiment 1. Total line crossings over the 15-min session and ethanol-induced hypothermia were initially analyzed via five-way ANOVA, as described.

Experiment 3. Effects of Increased Ambient Temperature on EtOH-Stimulated Locomotor Activity and Hypothermia in Adult Mice

Experiment 3 was conducted to evaluate possible effects of increased ambient temperatures on the locomotor behavior of adult mice, the condition under which P10 pups were tested in Experiment 2. The impact of raising ambient temperature by 5°C on baseline and EtOH-stimulated locomotor activity and change in body temperature was determined in adult FAST and SLOW mice. The subjects were adult mice of both lines, replicates, and sexes (n = 5–6 per group). This smaller group size was used because sex differences were not expected.

Locomotor activity and hypothermia testing. The procedures used to assess saline and EtOH-stimulated locomotor activity

were identical to those used in Experiment 1, with the following exceptions: only P60 mice were used, rectal temperature was taken prior to and following the activity session, and all sessions lasted for 15 min (to more closely approximate the conditions of Experiment 2). Because the automated activity monitors in this experiment were situated inside sound-attenuating chambers, it was not possible to mount overhead heating lamps. Thus, four out of eight of the activity monitors were equipped with heating pads (Sunbeam, 50 watts), which rested atop the clear plastic lids. The heating pads were set at a medium setting, which resulted in a stable ambient temperature of 27°C (±1.3°C), a temperature comparable to that attained with the heating lamps. To monitor ambient temperatures in the chambers, a temperature probe (Taylor, 9920) was affixed with tape to the left wall of each activity monitor, positioned 16 cm from the floor of the chamber.

Data analysis. ANOVAs and post hoc strategies similar to those in Experiments 1 and 2 were used.

RESULTS

Experiment 1. Ontogeny of EtOH-Stimulated Locomotor Activity in FAST and SLOW Mice

Locomotor activity. Figure 1 graphically depicts the locomotor activity data. Initial analysis of horizontal distance traveled over the 10-min period found no main effects of sex or interactions with sex: therefore, the data were collapsed over this variable. Line differences were evident—FAST and SLOW mice differed from one another in their response to EtOH but not saline. A replicate difference emerged that was dependent on line, age, and drug treatment; FAST 2 mice demonstrated significant EtOH-induced locomotor stimulation at 15 days of age, whereas FAST 1 mice did not exhibit the selected phenotype until 30 days of age. No such replicate differences emerged in SLOW mice; all SLOW mice demonstrated slight but significant locomotor depression in response to EtOH at 60 days of age, but not at earlier ages. The following statistical results supported these characterizations.

A four-way ANOVA grouped on line, replicate, age, and treatment revealed a complex significant four-way interaction, $F(2, 507) = 3.02$, $p < 0.05$. To determine the age at which the stimulant or suppressant response to EtOH emerged, and when the lines diverged, separate three-way ANOVAs grouped on treatment, line, and age were assessed within each replicate. Both of these analyses revealed significant three-way interactions [replicate 1: $F(2, 251) = 3.02$, $p < 0.05$; replicate 2: $F(2, 256) = 17.9$, $p < 0.001$]. Significant two-way interactions of treatment with line and treatment with age were seen in each of the three-way ANOVAs [replicate 1: treatment × line, $F(1, 251) = 109.8$, $p < 0.001$, treatment × age, $F(2, 251) = 12.6$, $p < 0.001$; replicate 2: treatment × line, $F(1, 256) = 230.7$, $p < 0.001$, treatment × age, $F(2, 256) = 10.3$, $p < 0.001$]. Further simple main effects analyses of these significant interactions showed that the stimulant response to EtOH was evident in FAST 2 mice by P15 but not until P30 in FAST 1 mice. Also, these were the ages at which the FAST lines diverged from the SLOW lines of the same replicate. SLOW mice developed the suppressant response to EtOH at P60, regardless of replicate.

BECs. BEC data are presented in Table 1. Initial analyses of these data revealed no sex effects; thus, the data were collapsed over this variable. A significant replicate by age interaction emerged, $F(2, 245) = 4.9$, $p < 0.05$, with replicate 2 animals showing significantly higher BECs at P15 than replicate 1. However, for both replicates and lines, P15 BECs were higher than their respective P30 counterparts. In addition, P15 replicate 2 mice differed from P60 replicate 2 mice.

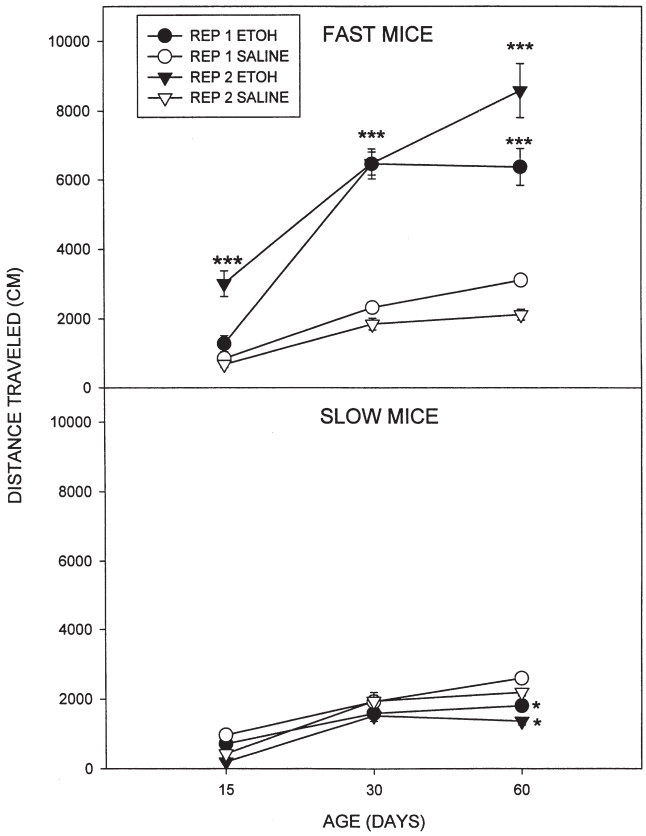

FIG. 1. Experiment 1: developmental changes in locomotor responses to saline and EtOH injections in FAST and SLOW mice at P15, 30, and 60. Distance traveled (mean ± SEM) was used as the measure of baseline (saline) or EtOH-stimulated locomotor activity in FAST and SLOW mice of both replicates at three ages. The data are collapsed over sex. FAST 2 mice developed the locomotor stimulant response by P15, whereas FAST 1 mice did not until P30. The magnitude of the stimulant response appeared adult-like by P30. Activity of SLOW mice of both replicates was significantly suppressed by EtOH at P60. Symbols indicate significant differences between EtOH and saline groups of the same replicate; $*p < 0.05$, $***p < 0.001$.

Experiment 2. Ontogeny of EtOH-Induced Hypothermia and EtOH-Stimulated Locomotor Activity in FAST and SLOW Mice

Activity data. Figure 2 graphically depicts the locomotor activity data. Data for P10 mice are presented in inset figures with expanded scales to better depict their low activity levels. The results of Experiment 2 essentially replicated Experiment 1. As in Experiment 1, no sex effects were observed; therefore, the data were collapsed over this variable. FAST and SLOW mice differed significantly in their responses to EtOH without differing in locomotion after saline treatment. Again, FAST mice differed in their age of response to EtOH's locomotor stimulant effects based upon replicate, with FAST 2 mice exhibiting the selected phenotype at P15 and FAST 1 mice at P30. SLOW mice demonstrated significant locomotor suppression to EtOH by P15. No significant differences emerged between the 10- and 15-min epochs.

TABLE 1

BLOOD (EXPERIMENT 1) OR BRAIN (EXPERIMENT 2) EtOH CONCENTRATIONS (mean mg/ml + SE)

	Blood EtOH (mg/ml)—Experiment 1		
Experiment 1	P15	P30	P60
FAST 1	2.21† (0.08)	2.06 (0.18)	2.22 (0.06)
FAST 2	2.43*†‡ (0.07)	1.99 (0.02)	2.12 (0.08)
SLOW 1	2.41† (0.13)	2.08 (0.25)	2.19 (0.11)
SLOW 2	2.67*†‡ (0.09)	2.06 (0.04)	2.12 (0.07)

	Brain EtOH (mg/ml)—Experiment 2			
Experiment 2	P10	P15†	P30	P60
FAST 1	1.74 (0.09)	2.21 (0.05)	1.86 (0.06)	1.81 (0.15)
FAST 2	1.97 (0.20)	2.52 (0.25)	1.72 (0.14)	1.92 (0.09)
SLOW 1	2.08 (0.12)	2.20 (0.12)	1.93 (0.09)	1.95 (0.12)
SLOW 2	2.00 (0.09)	1.81 (0.11)	1.51 (0.11)	1.97 (0.12)

*Significantly different from Replicate 1 mice of the same line, $p < 0.05$.

†Significantly different from P30 mice of the same replicate and line, $p < 0.05$.

‡Significantly different from P60 mice of the same replicate and line, $p < 0.05$.

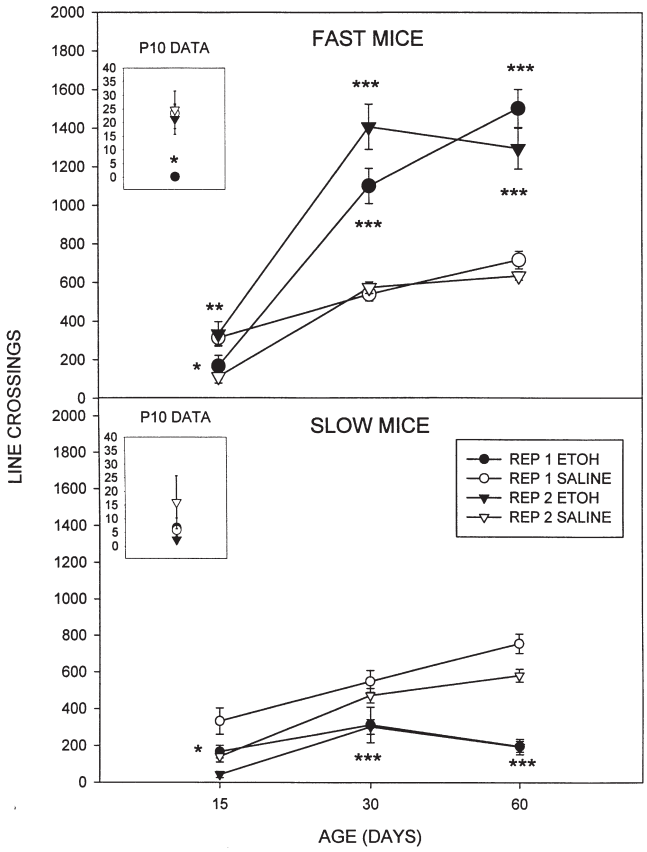

FIG. 2. Experiment 2: developmental changes in locomotor responses to saline and EtOH in FAST and SLOW mice at P10, 15, 30, and 60. Line crossings (mean ± SEM) were used as the measure of baseline (saline) or EtOH-stimulated locomotor activity in FAST and SLOW mice of both replicates at four ages (Inset = P10). The data are collapsed over sex. As in Experiment 1, FAST 2 mice developed the locomotor stimulant response by P15, whereas FAST 1 mice did not until P30. The magnitude of the stimulant response appeared adult-like by P30. Activity of SLOW mice of both replicates was suppressed by EtOH at P15. Symbols indicate significant differences between EtOH and saline groups of the same replicate; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

As in Experiment 1, a complex four-way interaction of line, replicate, age, and treatment was seen, $F(3, 358) = 2.9, p < 0.05$. Similar to Experiment 1, to determine the age at which the stimulant or suppressant response to EtOH emerged, and when the lines diverged, separate three-way ANOVAs grouped on treatment, line, and age were assessed within replicate. Each of these analyses revealed significant three-way interactions [replicate 1: $F(3, 182) = 31.6, p < 0.001$; replicate 2: $F(3, 176) = 26.3, p < 0.001$]. Significant two-way interactions of treatment with line and treatment with age were seen in both of the three-way ANOVAs [replicate 1: treatment × line, $F(1, 182) = 86.2, p < 0.001$, treatment × age, $F(3, 182) = 31.6, p < 0.001$; replicate 2: treatment × line, $F(1, 176) = 141.6, p < 0.001$, treatment × age, $F(3, 176) = 8.6, p < 0.001$]. Further simple main effects analyses of these interactions revealed that FAST 2 mice were stimulated by EtOH when compared with saline by P15. FAST 1 mice, however, were not stimulated by EtOH until P30. These were also the ages at which the FAST lines diverged from their SLOW counterparts. Interestingly, P10 and 15 FAST 1 mice demonstrated a locomotor depressant response to EtOH. With regard to SLOW mice, the locomotor suppressant effect of 2.0 g/kg EtOH was evident at P15 in both replicates.

Hypothermia data. Figure 3 graphically depicts the hypothermia data. Ethanol-induced hypothermia was defined as a significant decrease from baseline following EtOH injection. An age-related difference in baseline body temperature was seen, with P10 and 15 pups having lower basal body temperature compared to P30 and 60 pups. Initial analysis of the hypothermia data revealed no significant effects of sex; thus, further analyses were collapsed over this variable. The greater hypothermic response to EtOH in SLOW mice, which has previously been documented (37), did not emerge until 15 days of age, and intensified with increasing age. These descriptions were supported by the following analyses.

A four-way ANOVA on baseline body temperature grouped by line, replicate, age, and treatment showed a significant main effect of age without interactions, $F(3, 243) = 71.5, p < 0.001$. Post hoc analyses determined that P10 and 15 mice had lower initial body temperature relative to P30 and 60 mice (mean ± SEM = P10: 32.9 ± 0.22; P15: 33.7 ± 0.27; P30: 36.2 ± 0.25; P60: 37.1 ± 0.07). With regard to EtOH-induced hypothermia, a four-way ANOVA grouped on line, replicate, age, and treatment revealed a significant line by age by treatment interaction, $F(3, 284) = 3.2, p < 0.05$. The two-way interactions of treatment with line, $F(1, 383) = 15.7, p < 0.001$, and age, $F(3, 383) = 7.2, p < 0.001$ were also significant, and to determine the ontogeny of the hypothermic response to EtOH, simple main effect analyses of treatment at each level of line and age were performed. SLOW mice were significantly hypothermic to EtOH by P15 compared with their saline-treated controls. Ethanol-treated FAST mice appeared to demonstrate slight hypothermia at P30 and 60 when compared with their saline-treated counterparts; however, the change from baseline was negligible. To determine the age at which the lines di-

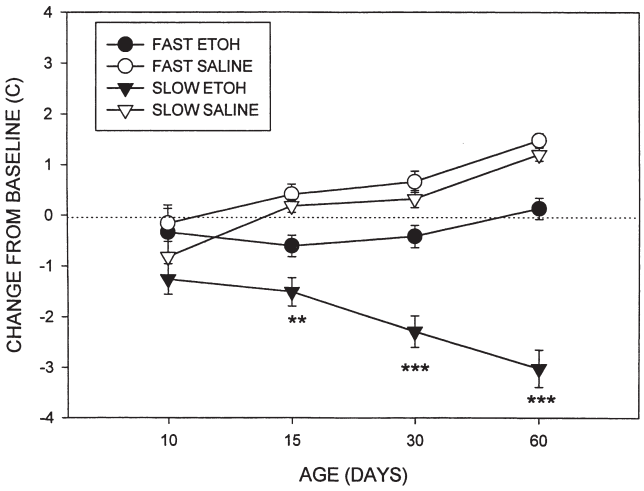

FIG. 3. Experiment 2: developmental changes in hypothermic responses to EtOH in FAST and SLOW mice. Both replicates were tested over four ages with hypothermia measured by temperature change from baseline (mean ± SEM). The data are collapsed over sex. SLOW mice were hypothermic in response to EtOH by P15, the magnitude of the response increased with age. Although it appears as though FAST mice were also hypothermic, note that saline-treated mice were hyperthermic and that EtOH-treated FAST mice actually experienced little change from baseline. Hypothermia denoted by symbols: Significantly different from saline group of the same line; $^{**}p < 0.01$; $^{***}p < 0.001$.

verged, the significant line by age interaction was further examined by simple main effect analyses. Not surprisingly, no line differences were evident in response to saline. However, the lines diverged in their hypothermic response to EtOH at P15, with SLOW mice demonstrating significantly greater hypothermia than FAST mice.

BrECs. As in Experiment 1, no sex effects emerged from the BrEC data; thus, data were collapsed over this measure. Subsequent analysis found that, similar to Experiment 1, BrECs were elevated in P15 animals relative to P30 mice. However, while the effect in Experiment 1 was limited to P15 FAST and SLOW animals of replicate 2, animals of both FAST and SLOW replicates demonstrated heightened BrECs at P15 in Experiment 2 (see Table 1). This characterization was supported by a three-way ANOVA grouped on line, replicate, and age that revealed a main effect of age, $F(3, 187) = 5.6$, $p < 0.005$, and no interaction effects. Tukey's post hoc analyses of this main effect revealed that P15 pups exhibited higher BrECs than P30 mice. There was a trend for P15 pups to exhibit higher BrECs than P10 and P60 mice as well ($ps < 0.09$).

Experiment 3. Effects of Increased Ambient Temperature on EtOH-Stimulated Locomotor Activity in Adult Mice

There were no significant effects of heating the activity chambers on body temperature or locomotor activity in EtOH or saline-treated adult FAST and SLOW mice. Separate five-way ANOVAs grouped on line, replicate, sex, treatment, and chamber temperature status were carried out on the dependent variables, change in body temperature and locomotor activity.

As has been seen in some earlier generations of these lines (38), a main effect of sex was found for distance traveled, $F(1, 160) = 7.0$, $p < 0.01$ (females more stimulated than males); however, no significant interactions of sex with any other factor including line were present. A two-way interaction of treatment by line, $F(1, 160) = 403.4$, $p < 0.001$, was determined to be due to a heightened response of FAST mice to EtOH relative to their saline-treated counterparts and to SLOW mice of both treatment conditions, while EtOH-treated SLOW mice demonstrated suppressed activity relative to their saline-treated counterparts; this is the selection phenotype (see Fig. 4). Analysis of the change in body temperature from baseline revealed that, while a significant interaction of treatment by line was seen, $F(1, 141) = 203.9$, $p < 0.001$ (EtOH-treated SLOW mice demonstrated hypothermia while FAST did not), no significant effects of raising ambient chamber temperature were seen on this measure (data not shown).

DISCUSSION

The primary aim of this study was to pinpoint an age at which the activity response to ethanol of FAST and SLOW mice diverged. In addition, the emergence of a correlated phenotype, sensitivity to ethanol-induced hypothermia, and the developmental changes in expression of both the activity and hypothermia phenotypes were characterized. Briefly, the results of this series of experiments revealed that, while all lines demonstrated their respective selection phenotypes in adulthood, developmental effects were evident. Developing FAST 2 mice exhibited a locomotor stimulant-response to EtOH by 15 days of age, while FAST 1 mice did not show this response until 30 days of age. The magnitude of the locomotor stimulant response to EtOH in FAST mice of both replicates appeared adult-like by P30. With regard to the correlated response to selection, EtOH-induced hypothermia, the previously observed larger hypothermic response of SLOW mice (37) was observed in adult animals in this study as well, and the age of onset of this response appeared to be P15. In both developmental experiments, P15 was found to be an age at which blood and brain EtOH concentrations were elevated

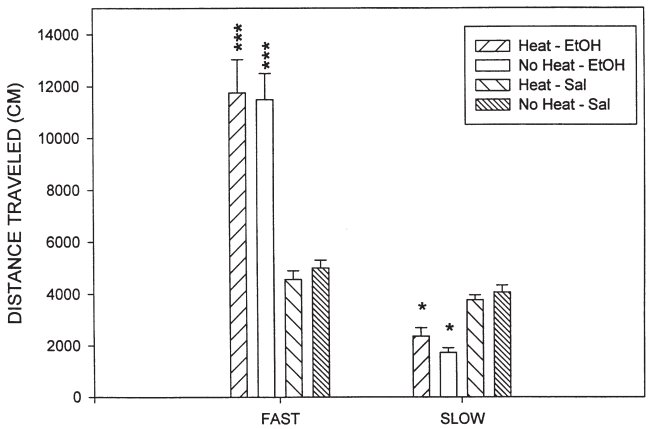

FIG 4. Experiment 3: the effect of raising ambient temperature on distance traveled (CM mean ± SEM) as a measure of baseline (saline) or EtOH-stimulated locomotor activity in adult FAST and SLOW mice of both replicates. FAST and SLOW mice demonstrated their characteristic phenotypes in response to EtOH; raising ambient temperature had no significant effect on locomotor response. Symbols indicate significant differences from saline group of same condition; $^{*}p < 0.05$, $^{***}p < 0.001$.

relative to both younger and older age groups. Adult females in Experiment 3 were significantly more stimulated by EtOHs than males. This is a common finding for stimulant response to EtOH and other drugs, possibly related to hormonal factors (15,17,45). Our laboratory has previously observed sex differences in sensitivity to EtOH-stimulated locomotor activity in FAST and SlOW mice [see (38)]. Despite the use of scaled activity monitors in Experiment 2, consistent line and replicate differences in 15-day-old animals were observed between Experiments 1 and 2, thus speaking to the robustness of our findings. Indeed, our laboratory has previously observed consistent line differences in adult FAST and SLOW mice despite the use of different types of open fields (29). The inclusion of both Experiments 1 and 2 in this study and their similar results, therefore, lend credibility to the present findings.

One interpretation for the replicate differences in the locomotor data is that of a general developmental delay in one replicate relative to the other (i.e., FAST 2 mature earlier than FAST 1). One way of assessing general developmental delay is the presence of body weight differences. Thus, if P15 FAST 1 mice weighed less than P15 FAST 2 mice, the argument of a general developmental delay would be warranted. Although no significant differences were found in body weight between the replicates of FAST mice at this (or any) age, post hoc power analysis of replicate differences in EtOH-treated FAST mice at P15 revealed a power of only 0.35, suggesting a limited power to detect body weight differences in our experiments. Blood EtOH concentrations in P15 FAST 2 mice were higher than their replicate 1 counterparts, suggesting that differences in EtOH absorption or metabolism might underlie the locomotor activity differences observed between the replicates at P15. However, brain EtOH concentrations in Experiment 1 P15 FAST mice did not reflect replicate differences, weakening this argument. As testament to the fact that developmental changes in EtOH-related behaviors may not be attributable to differences in EtOH pharmacokinetics, Smolen and colleagues found no significant line differences in ethanol or acetaldehyde metabolism, despite line differences in the developmental emergence of differential behavioral sensitivity to EtOH in Long-Sleep and Short-Sleep mice (41). Furthermore, FAST and SLOW differences in BECs are rarely found in our laboratory, despite substantial line differences in the locomotor stimulant response to EtOH. Nevertheless, a more thorough follow-up study assessing EtOH pharmacokinetics (i.e., uptake as well as metabolism) in developing FAST and SLOW mice would lend sufficient evidence to address this argument.

It is not known why P10 and 15 FAST 1 mice in Experiment 2 showed evidence of a locomotor sedative effect to EtOH. Although heightened BrECs might explain such a finding, no line, replicate, or interaction effects were observed for this measure. It appears as though young FAST 1 mice may be especially behaviorally sensitive to 2 g/kg EtOH. Thus, one intriguing explanation for the replicate differences seen in the ontogeny of EtOH-stimulated locomotor activity might be that FAST 1 mice were incapable of expressing EtOH stimulation due to their enhanced sensitivity to its sedative effects. A dose–response study in P10 FAST and SLOW mice would be an effective test of this hypothesis.

The line difference in sensitivity to EtOH-induced hypothermia, a response that has been found to be genetically correlated with selection for sensitivity to the locomotor stimulant effects of EtOH (37), was present by P15. It is tempting to speculate that P15 is the true age of emergence of this correlated phenotype. However, one could argue that the absence of a significant line difference in this measure at P10 may be due to factors related to heating mouse pups of this age because this manipulation was employed to prevent P10 mice from isolation-induced hypothermia. We do not currently know the answer to this question, but studies in heated and unheated P8–P11 pups are underway in a set of lines selected for differential sensitivity to the hypothermic effects of ethanol.

An ultimate goal of the current series of studies is to correlate the emergence and changes in magnitude of EtOH-related phenotypes in the FAST and SLOW mice with known periods of growth or change in neurobiological substrates suspected of mediating the adult response. As stated in the introduction, such correlations would strengthen existing hypotheses regarding the mechanism(s) underlying these EtOH-related characteristics. Some speculations are called for based on the behavioral data collected in the present study. However, no neurobiological substrates were directly assessed in our study, and thus, any hypotheses generated need to be tested by more direct means.

We suspect that the selected line differences in FAST and SLOW mice involve at least dopamine, GABA, and/or glutamate receptor systems in the mesoaccumbens pathway that is part of the brain reward circuitry (13,35,36). As reviewed by Phillips and Shen (30), it is the dopaminergic receptors in the nucleus accumbens that appear to be critical in mediating basal as well as EtOH-stimulated forward locomotion. More specifically, there is strong evidence for D_2 receptor involvement in EtOH's stimulatory effects in FAST mice, with some additional (albeit weaker) evidence for involvement of D_1 receptors or possibly some interaction of both receptor subtypes. Thus, further analysis of which developing DA systems may be important in determining the developmental emergence of the selected line difference in FAST and SLOW mice would do well to focus on D_1 and D_2 development in the nucleus accumbens.

The first appearance of DA cells in mesencephalic regions has been reported as early as gestational day 13 in rat (34) as well as mouse (6,24), and they appear adult-like in rat by gestational day 19 (31). There is limited information; however, changes in the mesolimbic dopamine systems do occur postnatally. For instance, mouse nigral tyrosine hydroxylase (TH) activity has been shown to increase to a 25% "overshoot" above adult levels at P15. This peak has been shown to decline to adult-like levels by P28, a time at which adult-like TH levels were also seen in the striatum (4). Of particular relevance to our study are reports that D_2 receptor density increases during the first 2–3 weeks of life in the rat nucleus accumbens and striatum (27), and that rat D_2 receptor mRNA reaches adultlike levels in accumbens at P28 (42). Further, measurement of rat D_2 receptor binding protein with [^{3}H]raclopride revealed an adult-like pattern at P30 (33). Although similar mouse data are not available, given the adult-like appearance of locomotor stimulant response at P30 in our study, it is tempting to speculate that D_2 systems may be important mediators in the development of this response. In a study of the behavioral functionality of D_2 autoreceptors, as measured by the ability of DA agonists at doses selective for D_2 autoreceptors to inhibit locomotor activity, Lin and Walters (26) reported that rat D_2 autoreceptors are behaviorally functional at 21, but not 10 days of age. It is noteworthy that administration of the mixed D_1/D_2 agonist, apomorphine, revealed only a nonsignificant trend toward decreasing locomotor activity at P21 in the latter study. However, apomorphine was behaviorally active in 35-day-old and adult rats, suggesting that, unlike D_2 autoreceptors, D_1 receptor or D_1/D_2 receptor interactions may not be mature by P21 in young rats.

There is the suggestion of a role for GABA and glutamate systems in determining sensitivity to EtOH's low-dose stimulatory effects [for review, see (30)]. A role for GABA systems in the developmental emergence of EtOH-induced locomotor stimulation is difficult to characterize. It is known that transient peaks occur in $GABA_B$ receptor binding in rat brain regions during the first 3 weeks of postnatal life, declining to adult levels thereafter (44), and that rat $GABA_A$ receptor subunit mRNAs are low to undetectable during the first postnatal week but rise over the next few days of the second week (5). Rat $GABA_A$ receptors peak in mesencephalic brain regions at around P21 (46). GABA transporter mRNAs show dense distribution during the first week of mouse life but decline in hippocampus during the second and third weeks (16). Mouse GABAergic development has been shown to follow a similarly complex pattern (14). For instance, Type II benzodiazepine receptors increase to asymptote during the first postnatal week and Type I sites increase progressively to adulthood (3). These studies suggest a dynamic process of change occurring in the GABA system that may be important in mediating the effects seen in the present experiments; however, further research will be needed to more fully characterize the potential role of this neurotransmitter in the development of EtOH-induced hyperlocomotion.

With regard to the neonatal development of glutamate systems, one study has shown that glutamate/DA interactions as measured by DA release in rat striatum are barely detectable at P8, but rise to adult-like levels at P45 (2). Whereas no studies have directly assessed the development of glutamate interactions in mouse striatum, several other brain regions have been investigated. A predominant finding is that of a transient peak in glutamate activity that occurs during the first 2–3 weeks of postnatal life. This peak has been reported in mice as well as rats. For instance, Suransarri and Oja (32) have reported heightened [^{3}H]MK-801 binding in P14 mice relative to adults, and Shibata and colleagues have shown maximal mRNA levels for two glutamate transporter subtypes at this age, also in mice (40). Further, the density of glutamate binding sites in three deep nuclei of developing mouse cerebellum is high at P5 and 10, but appears adult-like when assessed at P25 (22). Glazewski and colleagues have reported increased binding values for hippocampal NMDA receptors within the second and third week of postnatal life in mouse (23). Additionally, [^{3}H]MK-801 binding in rat forebrain has been shown to reach adult levels at P14 (7,25). Because the earliest age of onset of heightened locomotor activity in our study was P15, it is exciting to speculate that developmentally regulated changes in glutamate systems play an important role in determining the onset of EtOH-induced locomotor stimulation, while D_2 systems, through their continued maturation, allow for an increase in the magnitude of the response over age. Some ways to test for glutamate involvement in the development of the locomotor stimulant response to EtOH would be to determine whether the age of onset of EtOH-stimulated locomotor activity correlates with MK-801 locomotor stimulation [MK-801 stimulates FAST mice; see (39)], as well as to assess the ability of MK-801 to attenuate the locomotor stimulant response to EtOH at various ages. The involvement of DA systems might be explored by administering agonists and/or antagonist to the D_2 receptor at a variety of ages encompassing P15 and P30 in the FAST mice.

In summary, these data demonstrate the utility of the developmental approach to understanding underlying mechanisms of EtOH-related behaviors. These data strengthen and extend our current hypotheses about the neurobiological underpinnings of alcohol stimulation and reward, and will serve as heuristic tools for future research. The combined study of developmental neuropsychobiology and genetically selected mouse lines appears to be an effective tool for the dissection of behavior.

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