Seizure Sensitivity and GABAergic Modulation of Ethanol Sensitivity in Selectively Bred FAST and SLOW Mouse Lines

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ABSTRACT
FAST and SLOW selected mouse lines were bred for differences in locomotor response to low-dose ethanol. FAST mice exhibit an extreme stimulant response and SLOW mice exhibit locomotor depression at the same ethanol dose. We tested the hypothesis that γ-aminobutyric acid (GABA) systems modulate ethanol’s stimulant effects by examining convulsant responses to GABA_\text{A} receptor ligands, and by assessing the effects of GABA_\text{A} and GABA_\text{B} ligands on locomotor activity in the presence and absence of EtOH. FAST mice were more sensitive to the convulsant effects of GABA_\text{A} drugs, and to one of two non-GABAergic drugs also tested. FAST and SLOW mice differed in locomotor responses to benzodiazepines, but not to other GABA_\text{B} receptor ligands. Ethanol’s stimulant effects were not selectively altered by bicuculline or picrotoxin. The selected lines differed in sensitivity to the locomotor depressant effects of the GABA_\text{B} agonist, baclofen. Ethanol-stimulated activity of FAST mice was inhibited by baclofen, and this effect was reversed by administration of the GABA_\text{B} antagonist, CGP-35348. These GABA_\text{B} receptor-mediated effects were replicated in DBA/2J inbred mice that exhibit extreme sensitivity to ethanol’s stimulant effects. In summary, we found moderate to strong evidence that some sites on the GABA_\text{A} receptor complex were altered as a consequence of selection of FAST and SLOW mice, but found little support for GABA_\text{B} mediation of EtOH-stimulated activity. In contrast, we found moderate evidence for differential alteration of GABA_\text{A} receptor function; however, GABA_\text{B} receptor involvement in ethanol-stimulated activity was strongly supported by results in the selected lines and an inbred strain.

Determination of the neural mechanisms underlying the acute effects of alcohol (ethanol) and identification of the genetic factors mediating these effects are primary goals of current alcohol research. In part, this interest derives from recent evidence suggesting that sensitivity to alcohol’s acute effects in humans may be predictive of later propensity to develop alcoholism (Pollock et al., 1986; Schuckit, 1988, 1994). Locomotor stimulation is one of many acute alcohol effects that are studied to gain insight into alcohol’s mechanisms of action. In addition, locomotor activation in mice is a putative model of alcohol-induced euphoria in humans and may be particularly relevant for understanding alcohol’s addictive properties (Lukas and Mendelson, 1988; Wise and Bozarth, 1987).

Selectively bred mouse and rat lines have been useful preparations for studying alcohol genetics and alcohol pharmacology. The FAST and SLOW mouse lines were created by bidirectional selective breeding for extreme sensitivity and insensitivity to the locomotor stimulant effects of a moderate dose of ethanol (EtOH; Crabbe et al., 1987; Phillips et al., 1991; Shen et al., 1995b). The activity selection project, responses of the FAST and SLOW lines across 36 generations of selection, and results of studies designed to identify correlated responses to selection have been recently documented (Shen et al., 1995b; Shen et al., 1996). A strength of this selection project is that replicated lines were produced. When differences found in one set of lines (e.g., FAST-1 vs. SLOW-1) are also found in the other set (e.g., FAST-2 vs. SLOW-2), strong evidence of a genetically correlated response to selection is obtained (Crabbe et al., 1990). Moderate evidence is provided by a difference found in only one set of replicated lines.

Previous research exploring mechanisms of EtOH stimulant sensitivity in FAST and SLOW mice examined glutamate (Daniell and Phillips, 1994; Shen and Phillips, 1998) and dopamine systems (Shen et al., 1995a). The non-competitive NMDA antagonist, MK-801, stimulated the locomotor activity of FAST mice to a greater degree than that of SLOW mice and, in general, potentiated the expression of EtOH’s locomotor depressant properties (Shen and Phillips, 1998).

ABBREVIATIONS: BEC, blood ethanol concentration; B6, C57BL/6J; D2, DBA/2J; GABA, γ-aminobutyric acid; EtOH, ethanol; LS, Long-Sleep; SS, Short-Sleep

Accepted for publication June 5, 1998 This paper is available online at http://www.jpet.org

0022-3565/98/2872-0606$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 287, No. 2

0022-3565/98/2872-0606$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 287, No. 2

0022-3565/98/2872-0606$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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Ethanol decreased l-glutamate-stimulated increases in intracellular free calcium concentrations in brain microsac preparations from SLOW mice, but not in microsacs from FAST mice (Daniell and Phillips, 1994). Whereas dopaminergic agonists and antagonists had similar effects on the locomotion of FAST and SLOW lines in the absence of EtOH treatment, the stimulant response of FAST mice to EtOH was decreased by dopamine receptor antagonists (Shen et al., 1995a) and enhanced by dopamine agonists (Phillips and Shen, 1996).

Ethanol is thought to produce at least some of its effects via interactions with the GABA_A receptor (reviewed by Deitrich et al., 1989) although the precise site or sites of interaction are not known. The GABA_A receptor is a macromolecular complex that contains several specific binding sites for GABA, picrotoxin, benzodiazepines, anesthetic steroids and barbiturates. Studies during the course of selection of the FAST and SLOW lines determined them to be differentially sensitive to the locomotor stimulant effects of several alcohols and two barbiturates (pentobarbital and phenobarbital), with FAST mice exhibiting larger stimulant responses in each case (Phillips et al., 1992). These results suggested to us that GABA_A receptor modulated systems might play an important role in determining the differences in EtOH sensitivity between these sets of selected lines.

In the current work, two approaches were used to examine differences in sensitivity to GABA systems between the FAST and SLOW lines. Chemically induced convulsions were utilized as a simple means of identifying sensitivity differences, and the effects of GABAergic drugs on locomotion in the absence and presence of EtOH were assessed. In the latter studies, drugs interacting with GABA_B receptors were also investigated to follow up interesting reports of GABA_B receptor involvement in EtOH’s stimulant effects (Allan and Harris, 1989; Cott et al., 1976; Humeniuk et al., 1993).

The convulsant effects of seven drugs were tested. Since the focus of this particular work was primarily on GABAergic systems, the drugs were chosen for actions at three sites on the GABA_A receptor subtype. To address the specificity of GABA mediation of line differences in convulsant sensitivity, the effects of two drugs thought to act through non-GABAergic mechanisms were also evaluated. Previous work demonstrated a positive relationship between convulsant sensitivity and the locomotor stimulant effects of EtOH in selectively bred Long-Sleep (LS) and Short-Sleep (SS) mice (Phillips et al., 1989a). Thus, we hypothesized that FAST mice would be more sensitive to GABAergic convulsants than SLOW mice, but predicted that there would be no systematic line differences in sensitivity to non-GABAergic convulsants.

Effects of GABAergic drugs on locomotion and in altering the effects of EtOH on locomotor activity were studied as more directly relevant to the selection phenotype. Based on our previous results with barbiturates and alcohols, we predicted that FAST mice would be more sensitive to any locomotor stimulant effects and SLOW mice would be more sensitive to the locomotor depressant effects of the GABA_A receptor drugs. GABA_A receptor involvement was tested by determining the effects of the agonist, baclofen, on locomotor activity of FAST and SLOW mice. Based on previous reports in the literature, we predicted that FAST and SLOW mice would be differentially sensitive to baclofen’s locomotor effects and that baclofen administration would decrease EtOH-stimulated locomotion in FAST mice. The involvement of a particular neurotransmitter system in mediating locomotor activation by EtOH is more strongly supported when results are similar among 2 or more sets of independently derived genetic models. Thus, when our expectations were met in FAST and SLOW mice, we further tested our hypothesis by assessing baclofen’s effects in a pair of inbred strains with extreme sensitivity and insensitivity to the stimulant effects of EtOH (Dudek et al., 1991; Phillips and Crabbe, 1991).

Methods

Animals. Selective breeding of FAST and SLOW mice has been described in detail (Shen et al., 1995b). Briefly, two replicate sets of FAST and SLOW mice were concurrently bred for high and low (or negative) magnitudes of locomotor activation, respectively, in response to 2.0 g/kg EtOH. After 36 generations of selective breeding, the limits of selection appeared to have been reached and mice were placed on a relaxed breeding scheme (no selection pressure) on which they are currently maintained. Inbreeding is minimized by avoiding mating of animals with common grandparents. FAST and SLOW mice from generations S_66G_39-S_66G_49 and generations S_22-S_26G_37 were used in studies of sensitivity to chemical convulsants and studies of locomotor activity, respectively. Male mice were used in all convulsant studies. Males and females were used in locomotor activity studies, depending on availability, but only one sex was used in any one experiment. There was one exception; males and females were tested for locomotor responses to picrotoxin in the absence of EtOH due to low availability of either sex. We have not observed systematic sex differences in the selection response (Shen et al., 1995b), nor have we observed changes in selection response due to relaxed breeding (Shen et al., 1996). FAST and SLOW mice were produced and raised in the breeding facilities at the Portland VA Medical Center. They were reared by dam and sire until 21±2 days of age, when they were separated into isosexual groups of 2 to 4 mice per cage. C57BL/6J and DBA/2J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and allowed to acclimate in our facilities for 1 to 2 weeks before activity testing. Animals were housed in cages made of clear polycarbonate (28 x 18 x 13 cm) with corn cob bedding that was changed twice weekly. Cages were never changed on a test day. Animals were 7–19 weeks old at time of testing, and were offered unlimited water and rodent block food at all times except during testing. Ambient temperature in breeding, colony and testing rooms was a constant 21±2°C, and the light cycle in all rooms was 12-hr light/12-hr dark, with full illumination beginning at 6 a.m.

Convulsant behavior. Timed tail vein infusions were used to assess convulsant sensitivity. All testing was performed between 9 a.m. and 12 noon. Mice were moved to a testing room 45 to 60 min before testing. Just before testing, each mouse was weighed, placed in a clear acrylic plastic restraining tube, and its tail was immersed in a warm water bath (40–45°C) for 30 to 45 sec to dilate the tail veins. A butterfly needle (27 g, 3/8 in.; Abbott Laboratories) was inserted into the lateral tail vein at approximately the midlength point of the tail. Proper insertion was verified by the appearance of blood in the infusion tubing. Infusion began immediately and lasted for 1.5 min as calculated by the infusion pump (model 355). Infusion rates (0.25–0.5 ml/min) and drug concentrations (0.01–5 mg/ml) were chosen from prior work (Kosobud and Crabbe, 1990) to keep the infusion time under three min and the infusion volume under 1.0 ml for each drug. In those cases when the endpoint convulsion was not terminal, mice were immediately euthanized by cervical dislocation.
Convulsant drugs. The drugs used were picrotoxin (Sigma), t-butyl-bicycle-2,2,2-phosphorothionate (TBPS; Research Biochemicals) and pentylentetrazol (PTZ; Sigma), all thought to act at the picrotoxin GABA_A site, bicuculline (Research Biochemicals), which binds to the GABA_A site; methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM; Research Biochemicals), which is an inverse agonist at the benzodiazepine receptor site, kainic acid (Sigma), an excitatory amino acid agonist, and strychnine sulfate (Sigma), thought to produce convulsions via a glycine receptor associated chloride channel. All drugs were prepared on the day of the experiment. PTZ, picrotoxin, DMCM and strychnine sulfate were dissolved in saline. Bicuculline was dissolved in a few μl of concentrated hydrochloric acid (37%), diluted with saline, and adjusted to pH 7.26–7.41 with 5 M NaOH. Kainic acid was dissolved in distilled water, and the pH adjusted with 5 M NaOH. TBPS was suspended in 2% DMSO in distilled water without pH adjustment, and was vortexed just before each infusion.

Locomotor activity testing. Locomotor activity was assessed using Omnitech Digiscan Activity Monitors (Columbus, OH), interfaced with an IBM-compatible computer. Animals were placed in a clear acrylic plastic test box (40 cm x 40 cm) set inside an activity monitor that had 8 photocell beams and detectors evenly spaced along each of its 4 sides, approximately 2 cm above the floor. The test box and activity monitor were housed inside a sound-attenuating chamber made of opaque black acrylic plastic. The chamber was equipped with a small ventilation fan mounted on the rear right wall that also provided masking noise. Locomotor activity was assessed with white fluorescent lights on during studies with GABA_A drugs, and with lights off during GABA_A experiments, solely because this was consistent with other ongoing work that these studies originally accompanied. Since we are not attempting to compare results across drugs, we do not believe that this environmental difference impacts interpretation of the current results. Ethanol’s stimulant effects can be equally well measured in the light (Shen et al., 1995a) or the dark (Dudek et al., 1991). Sound attenuation was made possible with foam lining on the walls, ceiling, and door of the testing chamber. Data were automatically recorded in 5-min time samples.

Animals were injected i.p. with the appropriate drug or drug combinations and immediately placed into the center of the testing apparatus to begin the activity test. Each animal was naive when tested, and was used only once. In some cases, two drug injections were required and time intervals between injections varied with each drug depending on a previously determined time course of drug effects on locomotion. Specific details of each experiment are described more fully in the Results section and in the appropriate figure legends.

Drugs used in locomotor activity studies. Diazepam was a generous gift from Ed Gallaher. Muscimol, an agonist for the GABA_A receptor, and picrotoxin were purchased from Sigma. Bicuculline, an antagonist at the GABA_A site, and the GABA_B receptor agonist, baclofen, were obtained from Research Biochemicals. Midazolam, a short-acting benzodiazepine, was obtained from Hoffman-LaRoche. CGP-35348 was a generous gift from Ciba-Geigy. Midazolam, a short-acting benzodiazepine, was obtained from Pharmco Products and was diluted to 20% (v/v) in saline. All drug solutions were prepared on the day of the experiment.

Determination of blood ethanol concentrations. In experiments involving EtOH treatment, a 20 μl blood sample was obtained from the retro-orbital sinus of each EtOH-treated mouse immediately following the conclusion of the activity test. Each blood sample was immediately placed in a microcentrifuge tube containing 50 μl ice-cold ZnSO₄ and further processed as previously described (Shen and Phillips, 1988). Analysis of BEC was performed using gas chromatography with flame ionization detection (Hewlett-Packard 5890).

Data analyses. For the convolution studies, latency to each convolution was converted to dose (mg/kg) for each animal. Distance traveled (cm) was the dependent measure for the locomotor activity studies. Analysis of variance (ANOVA), performed using the CRUNCH4 statistical package, was used to detect significant differences between genotypes, replicates, and drug treatments as appropriate for each study. Data for both replicates are presented in the figures when the line × replicate interaction was statistically significant; otherwise data are shown collapsed on replicate. Significant two-way interactions were further characterized by simple effects analysis followed by Tukey post-hoc mean comparisons when appropriate. A P < .05 was considered to indicate a significant difference in all statistical tests.

Results

Convulsant Sensitivity

Data for all seizure signs produced by each convulsant, except DMCM, are presented in figure 1; however, some convulsants produced different subsets of all possible seizure signs. There were significant line differences in response to all of the convulsants with GABA_A receptor complex sites of action, with the exception of DMCM. In some instances the line difference emerged in only one of the replicate pairs of lines, and was present for some, but not for other, seizure types. In these cases, ANOVA grouped on line and replicate revealed significant line × replicate interactions. Sensitivity differences between selected lines and replicates, as well as F-values for significant main effects of line and line × replicate interactions, are summarized in table 1. FAST mice were significantly more sensitive to the convulsant effects of picrotoxin, bicuculline, TBPS, and PTZ. The dose of strychnine required to produce some seizure signs was also lower in FAST than in SLOW mice, indicating greater sensitivity of the FAST line. This line difference was apparent in only the second replicate pair of lines. In contrast to all other drugs, SLOW mice of one replicate were significantly more sensitive to THE seizures induced by kainic acid. Finally, DMCM consistently produced only face and forelimb seizures, and there was no difference in sensitivity between FAST and SLOW mice. The mean DMCM doses required to produce face and forelimb seizures were 1.21 ± 0.05 mg/kg and 1.32 ± 0.05 mg/kg (mean ± S.E.) in FAST and SLOW mice, respectively.

Locomotor Activity

Effects of GABA_A drugs. Of all the GABA_A drugs tested, only the benzodiazepine receptor agonists, diazepam and midazolam, produced significant line differences in distance traveled. Cumulative distance traveled during 15 min of activity testing is presented for midazolam and diazepam in figures 2 and 3, respectively. In addition, examination of time-course data indicated that locomotor stimulant effects could be seen during the first 5 min after injection. Since this is the time period upon which selective breeding of FAST and SLOW mice was based, 5-min data are shown as well (see figs. 2 and 3 insets). Midazolam significantly increased the locomotor activity of FAST mice at 5 min (P < .05 for 2.5 mg/kg; P < .01 for 5–10 mg/kg) and during the first 15 min (5 mg/kg, P < .05) of the test, but had no significant effect on SLOW mice at either time point. In contrast, although line differences were consistently observed, the pattern of response to diazepam was dependent on the time frame analyzed. At 5 min post-injection, FAST mice were significantly activated (P < .05 for 4 and 16 mg/kg; P < .01 for 8 mg/kg).
and SLOW mice were significantly depressed by diazepam (8 mg/kg, P < .05). Significant locomotor depression was still observed for SLOW mice when cumulative 15-min data were analyzed (P < .01 for all doses); however, the activity of FAST mice was not significantly altered by any diazepam dose because the stimulation observed early in the activity test was masked by subsequent mild locomotor depression. Doses of diazepam tested in these studies differed between FAST and SLOW mice because preliminary studies suggested robust line differences in responsiveness to diazepam, leading to an extension of the doses used to test FAST mice. To conserve animals, two lower diazepam doses were excluded from the dose range tested in FAST mice.

Locomotor activity of FAST and SLOW mice differed significantly at both time points for common doses tested (P < .001), but did not differ in baseline activity.

Bicuculline, picrotoxin and muscimol each decreased the locomotor activity of FAST and SLOW mice. However, the lines did not differ in sensitivity to the locomotor depressant effects of these GABA<sub>A</sub> ligands. Data are presented in figures 4 through 6 for comparison with benzodiazepine results. In the cases of picrotoxin and muscimol, significant main effects of line were detected (table 2) due to generally higher activity levels of FAST mice. Results for 5-min and 15-min total distance were similar in each case and only 15-min data are presented. Statistical analyses revealed significant main effects of dose for each drug (bicuculline, F[4,140] = 43.8; picrotoxin, F[4,195] = 36.6; muscimol, F[4,179] = 16.6, P < .001 for each drug). Bicuculline doses of 2.25 and 3 mg/kg, 1 and 2 mg/kg picrotoxin, and 2 mg/kg muscimol all significantly decreased locomotor activity of FAST and SLOW mice (P < .01 for all drug doses, compared to appropriate saline control). Some young mice (6 weeks old) were inadvertently included in the picrotoxin study, but after careful analysis of the data, we determined that age was not a significant factor in this experiment.

To test the hypothesis that GABA<sub>A</sub> systems mediate EtOH's locomotor stimulant properties, the effects of picrotoxin and bicuculline on EtOH-treated FAST and SLOW mice were assessed. Bicuculline and picrotoxin significantly decreased activity of both FAST and SLOW mice at higher doses tested, but selective alteration of EtOH-stimulated activity was not observed in FAST mice at any dose of either antagonist (data not shown). SLOW mice exhibited only locomotor depression to EtOH, and no significant effects of picrotoxin or bicuculline were seen, likely due to confounding floor effects. The results that neither GABA<sub>A</sub> ligand selectively altered EtOH-stimulated activity in FAST mice sug-
gests that GABA_A receptors are not important mediators of EtOH-stimulant effects in these mice.

**Effects of GABA_B drugs in FAST and SLOW mice.** The GABA_B agonist baclofen had locomotor depressant effects on EtOH-naïve FAST and SLOW mice (fig. 7). For replicate 1 animals, significant locomotor depression was observed after administration of 2.5 and 5 mg/kg baclofen in SLOW-1 mice, and after only 5 mg/kg in FAST-1 mice (P < .01 for each dose, compared to appropriate saline control). In replicate 2, the locomotor activity of both FAST and SLOW mice was reduced to the same extent by 2.5 and 5 mg/kg baclofen (P < .01). Thus, a line sensitivity difference in response to baclofen was observed in replicate 1 mice only.

The role of GABA_B receptors in mediating EtOH-stimulated activity was assessed by administration of the GABA_B agonist, baclofen, to saline or EtOH-treated FAST mice. SLOW mice were not included in this study because they do not show a stimulant response to EtOH. Analysis of a significant baclofen × EtOH interaction (F[4,180] = 3.4, P < .05) revealed that baclofen dose-dependently decreased the stimulant effects of EtOH in FAST mice without affecting saline activity (fig. 8). Specifically, 1.25 mg/kg (P < .05), 2.5 and 5.0 mg/kg (P < .01 for both doses) significantly decreased activity of EtOH-treated mice, providing evidence that GABA_B receptors are important for the expression of EtOH-stimulated activity.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Seizure sign</th>
<th>Line differences</th>
<th>Replicate differences</th>
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<tr>
<td></td>
<td>Seizure</td>
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<td>Replicate</td>
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<tr>
<td></td>
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<td></td>
<td>FF</td>
<td>[1, 40] = 0.2</td>
<td>Rep 1 only</td>
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<td>FF</td>
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<td>REP</td>
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<td>Picrotoxin</td>
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<td>[1, 43] = 38.0</td>
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* MYO, myoclonus; FF, face and forelimb clonus; RB, running/bouncing clonus; THE, tonic hindlimb extension.

The role of GABA_B receptors in mediating EtOH-stimulated activity was assessed by administration of the GABA_B agonist, baclofen, to saline or EtOH-treated FAST mice. SLOW mice were not included in this study because they do not show a stimulant response to EtOH. Analysis of a significant baclofen × EtOH interaction (F[4,180] = 3.4, P < .05) revealed that baclofen dose-dependently decreased the stimulant effects of EtOH in FAST mice without affecting saline activity (fig. 8). Specifically, 1.25 mg/kg (P < .05), 2.5 and 5.0 mg/kg (P < .01 for both doses) significantly decreased activity of EtOH-treated mice, providing evidence that GABA_B receptors are important for the expression of EtOH-stimulated activity.
Fig. 4. Lack of line sensitivity differences in locomotor response to the GABA\(_A\) antagonist, bicuculline. S\(_{22}\)-S\(_{29}\) FAST and SLOW male mice were injected with saline or bicuculline, and after a 15-min wait, were injected with saline and immediately tested for locomotor activity for 15 min. Fluorescent lights were on in the test chambers. This protocol was used to mimic testing conditions in a separate experiment designed to assess the effects of bicuculline on EtOH-treated animals. Doses of 2.5 and 3 mg/kg bicuculline significantly decreased locomotor activity of both selected lines (P < .01). Vertical bars are S.E.; n = 16 per line and treatment group.

In order to confirm that the effects of baclofen were due to specific actions on the GABA\(_B\) receptor, we tested the hypothesis that concurrent administration of the GABA\(_B\) antagonist, CGP-35348, with baclofen would decrease or block the inhibitory effect of baclofen on EtOH-stimulated activity. Results of these studies are presented in fig. 9. All mice, with the exception of saline controls, were administered EtOH 15 min after administration of the GABA\(_B\) drugs. Comparisons of saline control mice with mice given EtOH only or mice given baclofen only confirmed that significant EtOH stimulation occurred (t\(_{[38]} = 10.1\), P < .01) and that baclofen completely blocked the stimulant effect of EtOH (t\(_{[38]} = 1.01\)). Administration of CGP-35348 dose-dependently reversed baclofen’s inhibitory effects on EtOH-stimulated activity, but did not affect expression of EtOH-stimulated activity on its own (significant CGP-35348 \times baclofen interaction, F[2,108] = 4.5, P < .05). The effect of CGP-35348 on baclofen-treated mice was significant at both 50 (P < .05) and 100 mg/kg (P < .01). These results suggest that inhibition of EtOH’s stimulant effects by baclofen was due to specific actions at the GABA\(_B\) receptor, and provide further support for this receptor’s role in mediating the locomotor stimulant actions of EtOH.

In a separate study, we confirmed that CGP-35348 did not have any effects on the locomotor activity of baclofen-treated FAST mice in the absence of EtOH, by assessing the effects of saline, 50 or 100 mg/kg CGP-35348 administered with 4 mg/kg baclofen. The test protocol was the same as that used in the study described above, but mice were administered saline rather than EtOH in the second injection. A saline control group was also included. No significant differences among groups were detected (data not shown), indicating that the reversal of baclofen’s inhibitory effects on EtOH activation could not have been due to independent effects of CGP-35348 on locomotion.

There were no changes in BECs due to administration of baclofen alone or in combination with CGP-35348 (data not shown). Thus, administration of these GABA\(_B\) ligands did not alter the metabolism of EtOH and pharmacokinetic factors may be excluded as an explanation for the observed changes in locomotor activity in these mice.

Effects of GABA\(_B\) drugs on inbred strains. Given our findings in FAST mice indicating GABA\(_B\) receptor mediation of EtOH-stimulated activity, we sought to strengthen evidence for this conclusion by testing a pair of inbred strains known to differ drastically in their sensitivity to this EtOH effect. Results from the relatively insensitive B6 strain and sensitive D2 strain confirmed such a role for GABA\(_B\) receptors (fig. 10). Because B6 mice have demonstrated a slight stimulant response to EtOH in some studies (Crabbe et al., 1982), they were tested with the more stimulated D2 strain. However, in the current study B6 mice were not activated by EtOH, and an assessment of baclofen’s effects on EtOH stimulation in this strain was not possible. Interestingly, the combination of 2.5 mg/kg baclofen and EtOH appeared to
potentiate locomotor depression in B6 mice, whereas this baclofen dose had a small enhancing effect on locomotion in non-EtOH treated mice. The highest baclofen dose significantly decreased the locomotor activity of both saline and EtOH-treated B6 mice (P < .01). On the other hand, consistent with results obtained in FAST mice, baclofen blocked the expression of EtOH-stimulated activity in D2 mice at doses that had no effect on baseline activity. Further analysis of a significant baclofen × EtOH interaction (F[4,90] = 10.4, P < .01) in D2 mice revealed that both 0.625 and 1.25 mg/kg baclofen significantly reduced EtOH-stimulated activity (P < .01). Higher doses significantly reduced the activity of EtOH-treated D2 mice (P < .01). The activity of saline-treated D2 mice was also reduced by 5 mg/kg baclofen (P < .01), but 2.5 mg/kg had a slight stimulant effect on saline-treated animals. The strains did not differ significantly in locomotor response to baclofen alone.

As seen in figure 11, the GABA_B specificity of baclofen’s inhibitory effect on EtOH-stimulated activity in D2 mice was supported by the result that CGP-35348 (50 and 100 mg/kg; P < .01) blocked this effect of baclofen, but did not alter locomotor stimulation by EtOH on its own (significant CGP-35348 × baclofen interaction, F[2,54] = 6.2, P < .01). Similar to results in FAST mice, EtOH significantly increased loco-

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### TABLE 2

Summary of selected line and inbred strain sensitivity differences in locomotor response to GABA_A and GABA_B receptor ligands

| Drug       | Activity response | Sensitivity order | ANOVA results | Replicate effects
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GABA_A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>Stimulation</td>
<td>FAST &gt; SLOW</td>
<td></td>
<td>Both reps</td>
</tr>
<tr>
<td>5-min data</td>
<td></td>
<td></td>
<td>[1, 179] = 53.8</td>
<td>[4, 179] = 5.6^*</td>
</tr>
<tr>
<td>15-min data</td>
<td></td>
<td></td>
<td>[1, 179] = 56.2^*</td>
<td>[4, 179] = 3.5^d</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Stimulation</td>
<td>FAST &gt; SLOW</td>
<td></td>
<td>Both reps</td>
</tr>
<tr>
<td>5-min data</td>
<td></td>
<td></td>
<td>[1, 108] = 61.9^*</td>
<td>[2, 108] = 12.4^*</td>
</tr>
<tr>
<td>15-min data</td>
<td></td>
<td></td>
<td>[1, 114] = 53.5^*</td>
<td>[2, 114] = 7.2^c</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>Depression</td>
<td>N.S.</td>
<td></td>
<td>Both reps</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[1, 140] = 2.3</td>
<td>[1, 140] = 1.0</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>Depression</td>
<td>N.S.</td>
<td></td>
<td>Both reps</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[1, 195] = 25.7^*</td>
<td>[4, 195] = 1.3</td>
</tr>
<tr>
<td>Muscimol</td>
<td>Depression</td>
<td>N.S.</td>
<td></td>
<td>Both reps</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[1, 179] = 43.4^*</td>
<td>[4, 179] = 1.8</td>
</tr>
<tr>
<td><strong>GABA_B</strong></td>
<td>Depression</td>
<td>SLOW &gt; FAST</td>
<td></td>
<td>Rep 1 only</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[1, 94] = 82.7^*</td>
<td>[4, 94] = 4.0^d</td>
</tr>
<tr>
<td>Baclofen</td>
<td></td>
<td></td>
<td>[1, 90] = 0.02</td>
<td>[4, 90] = 1.4</td>
</tr>
</tbody>
</table>

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^a Lists replicate(s) in which significant line differences were observed.

^b In this case, a significant three-way interaction of line, replicate and dose (F[4,187] = 2.7, P < .05) was observed, and data were subsequently analyzed separated by replicate. F values reported for this experiment are from the two-way ANOVA grouped on line and dose for replicate 1 animals only.

^c P < .05, ^d P < .01, ^e P < .001.

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![Fig. 7. Effects of baclofen on locomotor responses of male FAST and SLOW mice. Animals from S33 were injected with baclofen 15 min before a second injection of saline, followed by testing in lighted activity chambers. Total distance traveled during the 20-min activity test is shown separately for the replicate lines. Replicate 1 SLOW mice were more sensitive to the locomotor depressant effects of baclofen (significant effects at 2.5 and 5 mg/kg, P < .01) than FAST mice whose locomotor activity was decreased by 5 mg/kg (P < .01). Baclofen (2.5 and 5 mg/kg, P < .01) decreased locomotor activity of replicate 2 FAST and SLOW mice to the same degree. S.E. larger than symbol size are shown; n = 10–11 per line, replicate and dose.](image1)

![Fig. 8. Dose-dependent reduction of EtOH-stimulated activity by the GABA_B agonist, baclofen, in S33–S34 FAST male mice. Significant reductions and complete blockade were observed in animals given 2.5 and 5.0 mg/kg baclofen, respectively (P < .01). Mice were injected with saline or baclofen 15 min before injection of saline or EtOH (2.0 g/kg). A 20-min activity test in darkened activity chambers (fluorescent lights off) immediately followed the second injection. Vertical bars are S.E.; n = 20 per treatment group.](image2)
motor activity ($t_{18} = 2.84, P < .01$), and baclofen completely blocked this activation ($t_{18} = .8$) compared to the saline control group. B6 mice were not included in this experiment because they were not activated by EtOH in the previous study.

D2 mice had significantly higher BECs than B6 mice (mean ± S.E. = 1.64 ± 0.02 and 1.52 ± 0.01 mg/ml for D2 and B6 mice, respectively; main effect of strain, $F(1,88) = 34.5$, $P < .001$). The small magnitude of this difference makes it unlikely to account for the majority of the difference in stimulant sensitivity between the strains. There were no alterations in BEC due to administration of the GABA$_A$ ligands, arguing against pharmacokinetic factors as an explanation for the effects of these drugs on the behavior of EtOH-treated mice.

Discussion

The role of GABAergic systems, specifically GABA$_A$ and GABA$_B$ receptors, in mediating the stimulant actions of EtOH were investigated in FAST and SLOW selected mouse lines, and subsequently in B6 and D2 inbred strains (GABA$_B$ studies only). Consistent with work in earlier generations of these mice (Phillips et al., 1992), differential sensitivities between FAST and SLOW mice were observed in response to some GABA$_A$ ligands tested. However, the GABA$_A$ receptor antagonists, bicuculline and picrotoxin, did not selectively reduce the EtOH stimulant response. On the other hand, FAST and SLOW mice differed in locomotor responses to baclofen, and EtOH's stimulant effects in FAST mice were blocked by administration of this GABA$_B$ agonist. These GABA$_B$ effects were replicated in D2 inbred mice that display high sensitivity to the stimulant actions of EtOH. Taken together, these data suggest a role for GABA systems in mediating or modulating locomotor stimulant actions of EtOH. However, precise mechanisms of action, and interactions of GABA systems with other neurotransmitter systems within the nervous system that are responsible for this behavioral phenomenon are yet to be elucidated.

Studies of convulsant sensitivity in FAST and SLOW mice
generally showed line differences in one replicate, but not the other, which we interpret as moderate evidence for genetically correlated selection responses (Crabbe et al., 1990). This type of result may indicate spurious genetic correlations arising from chance fixation of trait-irrelevant alleles, may indicate that the subset of genes common to both phenotypes is small in number or effect, or that some of the important alleles were lost in one or the other set of replicate lines. Our data consistently demonstrated a greater sensitivity of FAST mice to the convulsant effects of the drugs tested, with the exception of kainic acid and DMCM. These data are in agreement with our previous suggestion that FAST mice are generally more sensitive to central nervous system excitation, as supported by a more severe EtOH withdrawal syndrome in FAST compared to SLOW mice (Shen et al., 1996). However, given the kainic acid and DMCM results we would not conclude that FAST mice are more susceptible to convulsions in general. Our current results are also consistent with previous reports of an association between EtOH stimulant sensitivity and seizure susceptibility in selectively bred LS and SS mice (Bowers et al., 1991; Peris et al., 1989; Phillips et al., 1989a). However, data obtained in the LSXSS recombinant inbred strain panel did not support such an association (Wehner et al., 1991). If, as suggested above, genetic factors common to both phenotypes are small in number or effect, the ability to detect a genetic correlation may be maximized in selected lines, but more difficult to detect in inbred strains, leading to such inconsistencies.

Administration of benzodiazepines generally resulted in locomotor stimulation that differed between FAST and SLOW mice. Locomotor depression was elicited by the other GABAergic agents tested, bicuculline and picrotoxin and muscimol. The reason for these discrepancies is unclear. One possible explanation is that the two phenotypes, though both mediated in part by GABA receptors, are ultimately mediated by different neural pathways. For example, doses of picrotoxin and bicuculline necessary to induce convulsions are much higher than doses that alter locomotor activity. High doses of these drugs may affect neural pathways different from those affected by low doses, perhaps by preferentially acting on GABA<sub>A</sub> receptors with different subunit composition located in a distinct neuroanatomical location.

GABA<sub>B</sub> agonist administration blocked EtOH-stimulation in two mouse genotypes with extreme sensitivity to this acute EtOH effect. Whereas FAST mice were specifically bred for this extreme stimulant response, this characteristic of D2 mice arose by chance during inbreeding. The similar results obtained in these independent genetic models provide more compelling evidence for GABA<sub>B</sub> mediation of EtOH-stimulated activity than either model alone. In addition, the current results are consistent with effects observed in other mouse genotypes (Cott et al., 1976; Humeniuk et al., 1993), and with effects on other acute measures of EtOH action (Allan and Harris, 1989). Restoration of EtOH-stimulated activity by CGP-35348 in D2 and FAST mice demonstrated GABA<sub>B</sub> receptor specificity of this effect. Taken together, these data strongly support a role for GABA<sub>B</sub> systems in mediating or modulating EtOH-stimulated locomotor activity.

Although our data support the involvement of GABA<sub>B</sub> receptors in expression of EtOH-stimulated activity, we obtained only moderate evidence for differential alteration of GABA<sub>B</sub> receptor systems during selective breeding of the FAST and SLOW lines, and no evidence for differences between B6 and D2 mice. Two of our previous studies are of relevance here. First, we have demonstrated the involvement of dopaminergic systems in mediating EtOH-stimulated activity, even in the absence of line differences in sensitivity to dopamine antagonists alone (Shen et al., 1995a). In another set of studies, MK-801 altered locomotor responses of all EtOH-treated genotypes, and differentially altered the locomotor activity of EtOH-naive FAST and SLOW but not B6 and D2 mice (Shen and Phillips, 1998). Overall, these data sets have suggested to us that innate differences in receptor function (whether NMDA, DA, or GABA<sub>B</sub>) may contribute, but are not necessary, for differential sensitivity to EtOH's stimulant effects.

Locomotor depressant effects of baclofen in FAST mice in the dose-response study were not replicated in the subsequent experiment that tested baclofen's effects in saline or EtOH-treated mice. One possible explanation for this discrepancy is that the dose-response study was conducted with lights on, while the EtOH study was conducted with lights off. It has previously been demonstrated that changes in lighting conditions affect expression of EtOH-stimulated activity (Crabbe et al., 1988) and it is likely that locomotor activity in response to other drugs is sensitive to such environmental changes.

The baclofen dose required to block EtOH-stimulated activity was much lower in D2 mice compared to FAST mice. This can be attributed to the lower dose of EtOH used, the slightly lower magnitude of stimulation seen in D2 mice, or may perhaps reflect differential sensitivity to baclofen among these genotypes. A lower dose of CGP-35348 was required to completely reverse the inhibition of EtOH-stimulated activity in D2 mice compared to FAST mice, probably due to the lower dose of baclofen required to block locomotor activation. B6 mice were not activated by acute EtOH administration in

...
our studies, and doses that altered the activity of EtOH-treated D2 mice did not affect EtOH-treated B6 mice. However, the highest dose of baclofen tested produced a sharp decrease in locomotor activity in both saline and EtOH-treated groups of both inbred strains, demonstrating a locomotor depressant effect of baclofen at higher doses. There may have been potentiation of locomotor depression in B6 mice by one dose combination of baclofen and EtOH. This would not be surprising given that B6 mice are sometimes demonstrated to have an enhanced sensitivity to the locomotor depressant effects of EtOH (Phillips and Crabbe, 1991).

The possible involvement of GABA<sub>A</sub> and GABA<sub>B</sub> receptors in EtOH-stimulated activity is interesting because these receptors are thought to be crucial components of the neural circuitry that mediates locomotor activation in response to novelty, psychomotor stimulants, and other drugs of abuse (Amalric and Koob, 1993; Hooks and Kalivas, 1995; Kalivas et al., 1990). The mesoaccumbens-pallidal circuit comprises dopaminergic neurons that project from ventral tegmental area (VTA) to nucleus accumbens where they form connections with GABAergic neurons that in turn project to ventral pallidum/substantia innominata (VP/SI). Neuronal activity in the nucleus accumbens is modulated by glutamatergic projections, partly from hippocampus and prefrontal cortex. GABA<sub>B</sub> receptors are thought to be present in VP/SI where they mediate output to the extrapyramidal system. GABA<sub>B</sub> receptors are thought to modulate activity of the dopaminergic projections by actions at their cell bodies in VTA. The results reported here, along with previous reports of dopaminergic (Phillips and Shen, 1996; Shen et al., 1995a) and glutamatergic (Shen and Phillips, 1998) involvement in EtOH-stimulated activity of FAST mice, suggest that the mesoaccumbens-pallidal circuit may mediate EtOH’s stimulant effects. This possibility is appealing in that it provides a common mechanism for the actions of several drugs of abuse, and may be related to the addictive potential of these drugs. In addition to acute locomotor effects, some components of the mesoaccumbens-pallidal circuit may be involved in the phenomenon of locomotor sensitization to psychostimulants (Pierce and Kalivas, 1997). Our immediate future plans include pursuing this possibility for EtOH-induced locomotor sensitization. In addition, we intend to investigate GABA<sub>B</sub> receptor differences between FAST and SLOW mice using radioligand binding and to establish the locus of baclofen’s effects on EtOH-stimulated activity in the central nervous system using central administration procedures.

Acknowledgments
We thank Dr. Ed Gallaher and Ciba-Geigy for their generous gifts of diazepam and CPG-35348, respectively. The expert technical assistance provided by Mary Huson and Carrie McKinnon are greatly appreciated; many thanks to Christina Lessov for emergency statistical assistance.

References

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