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MK-801 Potentiates Ethanol's Effects on Locomotor Activity in Mice

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SHEN, E. H. AND T. J. PHILLIPS. *MK-801 potentiates ethanol's effects on locomotor activity in mice.* PHARMACOL BIOCHEM BEHAV **59**(1) 135–143, 1998.—FAST vs. SLOW selected mouse lines and C57BL/6J (B6) vs. DBA/2J (D2) inbred strains differ in their sensitivities to ethanol's locomotor stimulant effects, and provide two unique sets of genetic animal models to study neurophysiological substrates of this behavior. To determine whether NMDA receptor function mediates sensitivity to ethanol's stimulant effects, we assessed the effects of the noncompetitive NMDA antagonist, MK-801, on locomotor activity of naive and ethanol-treated FAST, SLOW, B6, and D2 mice. MK-801 (0.01–0.5 mg/kg, IP) had biphasic effects in all genotypes, with stimulation at moderate doses and decreased activation at the highest dose. FAST mice were more activated by MK-801 than SLOW mice, suggesting that selection differentially altered NMDA receptor function between the lines. B6 and D2 mice did not differ in locomotor responses following MK-801 administration. Stimulant doses of MK-801 decreased or blocked ethanol-stimulated locomotor activity in FAST and D2 mice, and potentiated the locomotor depressant actions of ethanol in SLOW and B6 mice. Potentiation of ethanol's activating properties was observed in one treatment group in D2 mice. These data suggest that NMDA receptors modulate ethanol's stimulant properties, by a more significant involvement in expression of ethanol's locomotor depressant properties. © 1998 Elsevier Science Inc.

Ethanol Locomotor activity FAST and SLOW mice C57BL/6J and DBA/2J mice Selected mouse lines Inbred mouse strains NMDA receptors MK-801

THE precise mechanisms underlying alcohol addiction are currently unknown; however, evidence supports the existence of heritable factors that increase an individual's risk of developing this multifactorial disease. The evidence for genetic factors in alcoholism has led to a search for associated trait markers that may aid detection of at-risk individuals and, if involved in the actions of alcohol (ethanol; EtOH), may further aid understanding of the mechanisms of EtOH addiction. One possible trait marker is psychomotor stimulation by EtOH, which has been reported to parallel subjective euphoric effects (1,19,20) and rising blood alcohol concentrations (37). In rodents, EtOH administration results in a biphasic dose–response profile, with an ascending limb of activation at low EtOH doses and a descending limb at higher doses (17,42). Locomotor activation has been postulated to be mediated by the same neural circuits that mediate euphoria or reward (51), or to at least provide an index of processes underlying these phenomena (18). Because the euphoric or rewarding properties of EtOH may influence subsequent intake, an understanding of the mechanisms by which EtOH produces its locomotor activating effects may aid in understanding processes that lead to addiction.

Sensitivity to EtOH's locomotor stimulant effects is mediated, at least in part, by heritable factors. This is supported by the success of a bidirectional selective breeding program that has produced mouse lines that differ in locomotor response to EtOH (8,38,46). FAST mice were bred for high locomotor activation in response to a low dose of EtOH, whereas SLOW mice were bred for decreased activation and locomotor depression. In a recent in-depth characterization, FAST and SLOW mice were found to differ in several indices of locomotor behavior following EtOH administration, including speed of locomotion, time spent in motion, distance traveled per movement bout, and distance traveled through the center of an open field (46). Phenotypic differences between FAST and SLOW mice arose during the course of selective breeding, as trait-relevant loci became homozygously fixed within each line. Two replicate sets of FAST and SLOW mice were pro-

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duced in parallel from independent breeding populations of a genetically heterogenous stock (HS/Ibg). The replicates were bred largely to aid interpretation of data, because the strength of any conclusion depends, in part, on whether support for a given hypothesis is obtained in only one or both replicates (7).

The contribution of genetic factors to sensitivity to EtOH's stimulant effects is also supported by the existence of inbred strains that vary in locomotor response to EtOH (39). One example is the well-characterized difference between C57BL/6J (B6) and DBA/2J (D2) inbred strains in which D2 mice are highly stimulated and B6 mice are much less stimulated by EtOH (6,16,17). Unlike the systematically produced differences between selected lines, genetic differences between inbred strains arose arbitrarily at the time the strains were generated.

The FAST and SLOW selected lines, and B6 and D2 inbred strains provide unique sets of genetic animal models with which to study mechanisms underlying locomotor responses to EtOH. Previous data demonstrated the involvement of dopamine systems in modulating EtOH's locomotor stimulant effects in FAST mice (45). Other neurotransmitter systems have been implicated in mediating EtOH's stimulant effects [reviewed in (41)], but have not yet been investigated in these genetic animal models. However, the glutamatergic system, which provides excitatory input throughout the central nervous system, has recently become of interest with regard to the actions of EtOH. Biochemical, electrophysiological, and behavioral data suggest that EtOH may exert some of its effects via various glutamate receptor subtypes, particularly the ionotropic NMDA receptor. For example, EtOH has been demonstrated to inhibit NMDA receptor-mediated effects in neuronal cells and tissue (15,24,35,36). Interestingly, a biphasic dose–effect profile has been described in which low EtOH concentrations potentiated, while higher EtOH concentrations blocked NMDA-activated currents (34). Biphasic effects have also been found in biochemical studies using membrane vesicles derived from mouse hippocampus, in which glutamate-stimulated Ca^{2+} influx, demonstrated to be mediated by NMDA receptors (10), was significantly enhanced by low concentrations of EtOH [cited in (12)] and inhibited by higher concentrations of EtOH (11,12). Glutamate-stimulated Ca^{2+} flux has been used as a measure of NMDA receptor function in a brain membrane vesicle homogenate (10). This microsac preparation contains both pre- and postsynaptic components, as well as some unidentified vesicles, but no unbroken cells. It has been reported that microsacs derived from hippocampus and cerebral cortex of SLOW mice were more sensitive to the inhibitory effects of EtOH on glutamate-stimulated Ca^{2+} flux than microsacs derived from FAST mice (12), suggesting that selection has altered some aspects of NMDA receptor function.

In behavioral studies, noncompetitive NMDA receptor antagonists generally increase locomotor activity in rats (13) and mice (14,33). In addition, results of studies using various inbred strains suggest that genetic factors contribute to differences in locomotor responses to NMDA receptor antagonists (30,52). With regard to EtOH, the noncompetitive NMDA receptor antagonist, MK-801, has been demonstrated to block (31,44), enhance (29), and have no effect on EtOH-stimulated activity (9). These contradictory results may be due in part to differences in drug doses tested. This possibility is supported by data reported by Kuribara (29), who showed enhancement or reduction of locomotor stimulation, depending on the doses of EtOH and NMDA antagonist that were coadministered.

Based on the evidence described above, we hypothesized that NMDA receptors are important modulators of EtOH's locomotor stimulant effects. One way to test this hypothesis in our genetic animal models is to assess whether genotypes more sensitive to the activating effects of EtOH are differentially sensitive to an NMDA receptor ligand compared to genotypes insensitive to EtOH's activating effects. Thus, in one set of studies, we assessed the effects of the noncompetitive NMDA receptor antagonist MK-801 on the locomotor activity of drug-naive animals, comparing FAST and SLOW mice in one experiment, and B6 and D2 mice in another. We predicted that the functional differences in NMDA receptor function observed in biochemical studies using FAST and SLOW mice would be paralleled by differences in locomotor response to MK-801. In addition, we predicted that if NMDA receptors are important determinants of sensitivity to EtOH's stimulant effects, B6 and D2 mice would also differ in their locomotor responses to MK-801. In a second set of studies, we tested the ability of MK-801 to affect locomotor activity of EtOH-treated animals, using several doses of MK-801 and a range of EtOH doses. It was predicted that MK-801 would enhance locomotor stimulation at low doses of EtOH in genotypes sensitive to EtOH's stimulant effects (FAST and D2 mice). Responses of genotypes relatively insensitive to EtOH's stimulant effects were more difficult to predict. Administration of MK-801 could conceivably confer sensitivity to EtOH's stimulant effects in B6 and SLOW mice because it has locomotor stimulant properties of its own. On the other hand, because MK-801 also decreased EtOH's activating effects in some cases, there was a possibility that MK-801 could increase sensitivity to the locomotor depressant effects of EtOH.

METHOD

Animals

FAST and SLOW mice were bred at the Portland VA Medical Center (Portland, OR). Selective breeding of these lines has been thoroughly described (8,38,46). Briefly, replicate sets of FAST and SLOW mice were contemporaneously derived from comparable, but genetically independent, populations of HS/Ibg mice (Boulder, CO), resulting in FAST-1 and SLOW-1, FAST-2 and SLOW-2 lines. Selection was based upon the difference in locomotor activity assessed on two consecutive test days: after saline injection on one day and after EtOH injection [usually 2.0 g/kg; see (38,46)] on the other day. FAST mice were bred for high ACT scores (EtOH activity–saline activity), reflecting large stimulant responses to EtOH. SLOW mice were bred for low (including negative) ACT scores, reflecting resistance to EtOH's stimulant effects, and perhaps greater sensitivity to EtOH's locomotor depressant effects. Currently, each replicate line is maintained as an independent breeding population, but no selection pressure is placed on the lines (relaxed selection), and individuals are bred without regard to ACT scores.

Male FAST and SLOW mice used in these experiments were from second through sixth litters of generation $S_{36}G_{37}$–$S_{36}G_{40}$ breeders, and were housed with littermates, dam, and sire until 21 $\pm$ 1 days of age. They were subsequently housed three to four per cage with animals of the same sex, line, and replicate until testing at 48–90 days of age. Male B6 and D2 mice were purchased from Jackson Laboratory (Bar Harbor, ME), and were acclimated in our facilities for 1–2 weeks prior to testing. The inbred strains were 52–73 days of age at time of testing. All animals were housed in cages of clear polycarbonate (28 $\times$ 18 $\times$ 13 cm) containing corn cob bedding that was changed twice weekly. Bedding and cages were never changed on a test day. Animals were kept on a 12 L:12 D cycle, with

lights on between 0600 and 1800 h, and ambient temperature maintained at 21 ± 2°C. Food and water were available ad lib, except during activity testing.

Locomotor Activity Testing

Locomotor activity was assessed between 0900 and 1600 h in Omnitech activity monitors (Model CCDIGI; Columbus, OH), which detect movement by interruption of photocell beams (eight on each side, 2 cm above the floor). During testing, mice were placed in a clear acrylic plastic box (40 × 40 cm), which was set inside a 40 × 40 cm activity monitor. The box and monitor were housed in a sound-attenuating chamber made of opaque black acrylic plastic. A small fan mounted on the rear right wall of the chamber provided ventilation and masking noise. Fluorescent lights mounted high on the back wall were turned off so that locomotor activity was assessed in darkness. Eight or 16 locomotor activity chambers were used during an experiment, and genotypes and treatment groups were counterbalanced so that each treatment group of each genotype was tested at least once in each monitor. Data were automatically recorded by an IBM-compatible computer in 5-min samples.

For the MK-801 dose–response experiments, animals were weighed and placed in holding cages for no more than 10 min prior to testing. Animals were then injected with saline or one of several doses of MK-801 (0.01–0.5 mg/kg) and immediately placed in activity test chambers for 60 min. For experiments in which both MK-801 and EtOH were administered, mice were weighed and placed in holding cages, and injected with saline or MK-801 (0.05–0.2 mg/kg). Mice were returned to their holding cages for a 20-min interinjection interval, followed by administration of saline or EtOH (0.5–2.0 g/kg for FAST and SLOW mice; 0.5–1.5 g/kg for B6 and D2 mice) and immediate activity testing for 20 min. All injections were administered IP. Timing of injections and MK-801 doses used in this experiment were based on data collected in the MK-801 dose–response study. EtOH doses were chosen, based on data previously collected under the same testing conditions, to produce no stimulation, moderate, or maximal stimulation in these genotypes (Shen and Phillips, unpublished data). To assess blood ethanol concentrations (BEC), retro-orbital sinus blood samples were obtained from mice that received EtOH injections. Mice were humanely euthanized immediately following blood sampling. All procedures followed guidelines for the care and use of laboratory animals set forth by the National Institutes of Health, and were approved by the Portland VA Institutional Animal Care and Use Committee.

Drug Sources and Preparation

(+)-MK-801 hydrogen maleate was purchased from Research Biochemicals International (Natick, MA), and dissolved in 0.9% saline. Ethanol (200 proof) was obtained from Pharmco Products Incorporated (Brookfield, CT), and diluted to 20% v/v with saline. MK-801 was injected in a volume of 10 ml/kg; EtOH injection volumes were adjusted as appropriate for dose and body weight.

Determination of Blood Ethanol Concentrations

Blood samples (20 μl) obtained from EtOH-treated animals were processed as previously described (40). Briefly, samples were immediately placed on ice in microcentrifuge tubes containing 50 μl ice-cold $ZnSO_4$. Samples were briefly vortexed after addition of 50 μl $Ba(OH)_2$ and 300 μl deionized water, and centrifuged at high speed for 5 min (Beckman Microfuge 12). Supernatant was transferred to glass vials, capped, and assessed for BEC by gas chromatography (Hewlett-Packard 5890) with flame ionization detection.

Data Analyses

Of the many variables recorded by the Omnitech program, horizontal distance traveled (cm) was chosen as the measure of locomotor activity, because it is an easily interpretable metric character and because it is largely uncontaminated by stereotypic behavior. Total distance traveled (TDIS) was calculated for each animal by summing all 5-min data samples collected during the activity test. Because MK-801 had stimulant effects, for experiments in which both MK-801 and EtOH were administered, activity levels of groups receiving MK-801 and EtOH were corrected by subtracting the mean total distance traveled of the appropriate MK-801 dose group from each individual animal's score (CORRDIS). Group differences in TDIS and CORRDIS were assessed by analysis of variance (ANOVA) using the CRUNCH4 statistical package. Significant main effects were analyzed by Tukey mean comparisons, when appropriate. Significant two-way interactions were characterized by simple effects analysis, followed by Tukey mean comparisons. Significance levels were $p < 0.05$ for all statistical tests.

RESULTS

Age and Body Weight

Analysis of body weight and age for each experiment revealed slight but significant differences between genotypes in some cases. When they occurred, significant differences ranged from 1.1 to 2.0 g in body weight, and 7 to 8 days in age. Age and body weight were well matched across treatment groups in all cases, and we could find no evidence for systematic effects of these small differences on locomotor responses in these studies.

MK-801 Dose Response

FAST and SLOW mice. There was a significant main effect of replicate on total distance traveled during the 60-min test in which replicate one mice were generally more active than replicate two mice. There were no interactions involving replicate; thus, data are presented collapsed on this variable in Fig. 1. MK-801 produced a biphasic dose–response profile of locomotor activity, with stimulant effects at moderate doses followed by decreased activation at the highest dose. This pattern of response was evident for both FAST and SLOW mice, but the magnitude of locomotor stimulation exhibited by SLOW mice was much lower than that of FAST mice. Further analysis of the interaction between line and MK-801 dose, $F(6, 252) = 6.6$, $p < 0.001$, showed that FAST mice were significantly stimulated by 0.1, 0.2, and 0.5 mg/kg MK-801 compared to saline controls, while the locomotor activity of SLOW mice was significantly increased by 0.2 mg/kg MK-801 only. There were significant differences between FAST and SLOW mice administered 0.2 and 0.5 mg/kg, with FAST mice showing higher activity levels at both doses even though 0.5 mg/kg was on the descending limb of activation.

B6 and D2 mice. Similar to its effects in FAST and SLOW mice, MK-801 altered the locomotor activity of B6 and D2 mice in a dose-dependent biphasic manner (see Fig. 2). However, there were no genotype-dependent differences in magnitude of stimulation produced by MK-801. A significant main

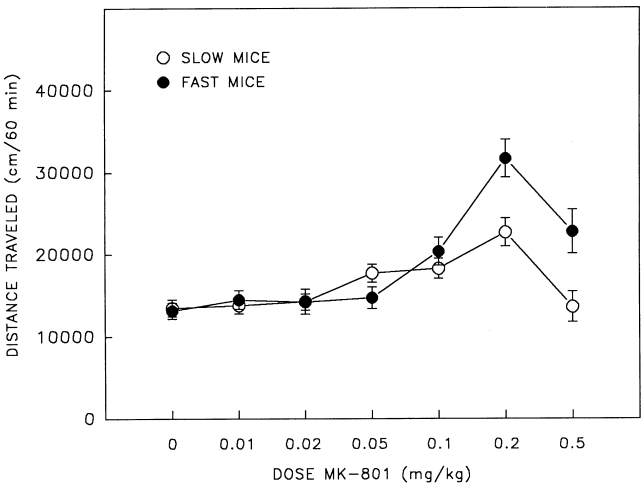

FIG. 1. Effects of increasing doses of the noncompetitive NMDA receptor antagonist, MK-801, on locomotor activity of SLOW and FAST mice. Data are presented collapsed on replicate. Mice were injected with saline or a single dose of MK-801 and immediately placed in activity monitors. Cumulative distance traveled during the 60-min test is presented. Vertical lines are SEM; $n = 10$ mice per line, replicate, and dose.

effect of MK-801 dose, $F(6, 123) = 57.3, p < 0.001$, was due to large and significant increases in the activity of B6 and D2 mice following administration of 0.1, 0.2, and 0.5 mg/kg MK-801, compared to saline controls. Peak activation occurred at 0.2 mg/kg MK-801, with 0.5 mg/kg falling on the descending limb of a biphasic dose–response curve.

MK-801 and EtOH Coadministration

Analysis of time-course data in the previous experiments suggested that the locomotor effects of MK-801 occurred within 15–20 min of injection (data not shown). Thus, MK-801 was injected 20 min prior to the second injection and activity test. Furthermore, doses of MK-801 were chosen for this experiment based on their ability to produce low, moderate, or high levels of activation.

FAST and SLOW mice. BECs obtained from EtOH-treated animals were in the expected range for most animals; however, two SLOW-1, two FAST-1, and one FAST-2 mouse had BECs that were at least 2.5 standard deviations from the mean, indicating either a misplaced injection or an incorrect dose. These animals were subsequently excluded from all data analyses.

Figure 3 shows the locomotor responses of FAST and SLOW mice to MK-801 alone and in combination with EtOH. A four-way ANOVA (line, replicate, MK-801 dose, and EtOH-dose) on total distance traveled (TDIS) revealed a significant main effect of replicate and significant interactions involving replicate, suggesting replicate-dependent differences in response to MK-801 and EtOH administration. However,

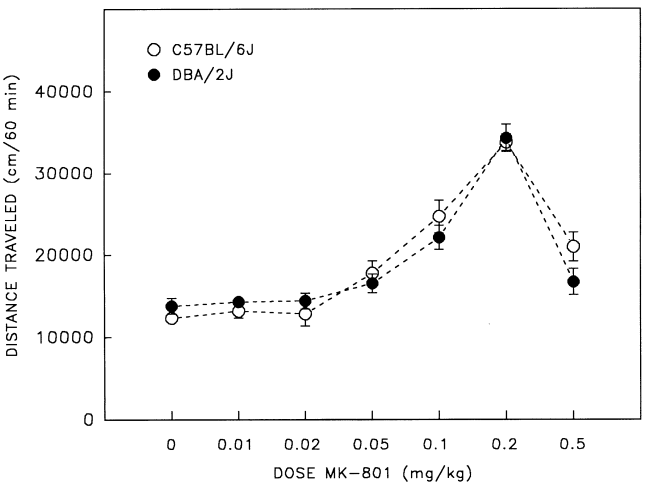

FIG. 2. Effects of increasing doses of MK-801 on locomotor activity of B6 and D2 mice. Mice were injected with saline or one dose of MK-801 and placed in activity monitors for a test duration of 60 min. Cumulative distance traveled is presented. Vertical lines are SEM; $n = 10$ mice per strain and dose.

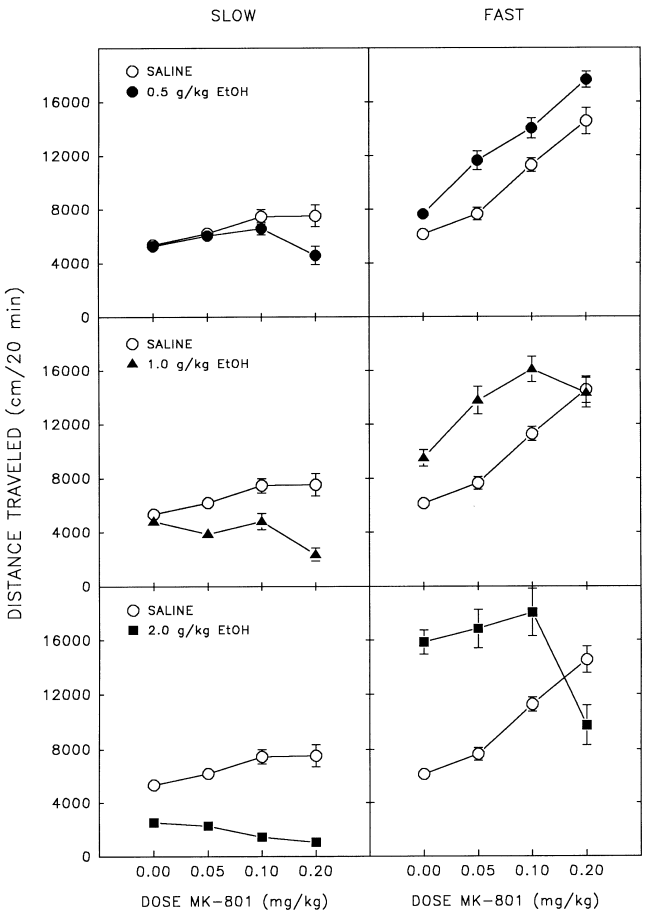

FIG. 3. Effects of MK-801 administration on locomotor activity of EtOH-treated SLOW (left panels) and FAST mice (right panels). Mice were injected with saline or MK-801, followed 20 min later by an injection of saline or EtOH. Cumulative distance traveled during the 20-min activity test is presented. Open symbols in each panel represent animals that received saline or MK-801, and saline (no EtOH); these groups are repeated in each panel for comparison with EtOH-treated animals. SEM larger than symbol size are shown; $n =$ 9–11 per line, replicate, and treatment group.

when the responses of the replicated lines (SLOW-1 vs. SLOW-2; FAST-1 vs. FAST-2) were compared, these differences were largely in magnitude but not direction of effects. Because the stimulant effects of MK-801 made interpretation of data more difficult in some cases, analysis of a difference score that corrected for activation by MK-801 (CORRDIS) was also performed. Results of four-way ANOVA on CORRDIS were nearly identical to those seen after analysis of TDIS. Because replicate differences were only in magnitude of effects for both variables, data are presented collapsed on replicate in Fig. 3 (TDIS) and Table 1 (CORRDIS). As seen in Fig. 3, the effects of MK-801 alone on FAST and SLOW mice were consistent with those observed in the dose–response study, with FAST mice being more sensitive to the locomotor stimulant effects of MK-801. Because characterization of the responses of each set of lines was desired, data were subsequently analyzed by two-way ANOVA grouped on MK-801 and EtOH treatment for FAST and SLOW mice separately.

For SLOW mice, characterization of a significant MK-801 × EtOH interaction, $F(9, 302) = 4.5$, $p < 0.001$, for TDIS by simple effects and Tukey post hoc mean comparisons showed that 2.0 g/kg EtOH significantly decreased activity relative to saline-treated mice. No other EtOH dose affected the locomotor activity of SLOW mice. In contrast, MK-801 (0.1 and 0.2 mg/kg) significantly increased the activity of SLOW mice when given alone. Further analysis of these data was conducted on CORRDIS to account for the stimulant effects of MK-801 alone. Analysis of CORRDIS also yielded a significant interaction between MK-801 and EtOH, $F(9, 302) = 3.9$, $p < 0.001$. As shown in Table 1, pretreatment with 0.2 mg/kg MK-801 significantly decreased CORRDIS of mice administered 0.5 g/kg EtOH, compared to mice administered this dose of EtOH alone. For mice given 1.0 and 2.0 g/kg EtOH, pretreatment with the two highest doses of MK-801 also significantly decreased CORRDIS. These data suggest that MK-801 administration potentiated the locomotor depressant effects of EtOH in SLOW mice.

TABLE 1

MEAN (± SEM) CORRDIS IN FAST AND SLOW MICE*

Treatment Group	SLOW Mice	FAST Mice
0.5 g/kg EtOH		
MK-801 dose (mg/kg)		
0	−82.5 ± 310.9	1539.2 ± 415.6
0.05	−171.3 ± 500.9	3982.5 ± 744.9
0.1	−890.2 ± 468.2	2791.0 ± 780.8
0.2	−3056.2 ± 670.1‡	3093.6 ± 823.0
1.0 g/kg EtOH		
MK-801 dose (mg/kg)		
0	−545.1 ± 459.6	3355.7 ± 609.8
0.05	−2330.6 ± 453.7	6133.1 ± 1051.5
0.1	−2656.2 ± 621.2‡	4805.9 ± 891.6
0.2	−5174.4 ± 584.4‡	−233.9 ± 1358.5†
2.0 g/kg EtOH		
MK-801 dose (mg/kg)		
0	−2826.4 ± 451.1	9674.8 ± 942.8
0.05	−3892.4 ± 461.9	9225.2 ± 1483.2
0.1	−6021.8 ± 343.2‡	6742.1 ± 1672.0
0.2	−6470.5 ± 493.9‡	−4807.3 ± 1619.4‡

*CORRDIS is total distance traveled corrected for MK-801 activity levels (see text).

†$p < 0.05$ compared to group that received 0 mg/kg MK-801 within the same EtOH group.

‡$p < 0.01$ compared to group that received 0 mg/kg MK-801 within the same EtOH group.

Significant differences were determined by Tukey mean comparisons after simple effects analyses of two-way interactions (MK-801 × EtOH) in two-factor ANOVA for each line.

For FAST mice, characterization of a significant MK-801 × EtOH interaction, $F(9, 301) = 12.3$, $p < 0.001$, for TDIS revealed that MK-801 (0.1 and 0.2 mg/kg) and EtOH (2.0 g/kg) each significantly increased locomotor activity when given alone (see Fig. 3). As in the case of SLOW mice, further analysis of these data was conducted on CORRDIS. Despite MK-801's stimulant effects, pretreatment with the highest dose of MK-801 did not enhance locomotor activation by EtOH (1.0 and 2.0 g/kg), but significantly decreased the locomotor activity of EtOH-treated mice, compared to animals that received EtOH alone (see Table 1). As seen in Fig. 3, activity levels of mice administered MK-801 and EtOH neared, but did not go below baseline activity (animals that received saline only). Thus, MK-801 reversed or blocked EtOH's stimulant effects in FAST mice in a robust manner, suggesting modulation of locomotor stimulation by NMDA receptors.

There were EtOH dose-dependent increases in BECs for both FAST and SLOW mice, and SLOW mice had consistently higher BECs than FAST mice. This line difference in BEC was significant, $F(1, 450) = 27.2$, $p < 0.01$, and there was a significant line × EtOH interaction, $F(2, 450) = 4.5$, $p < 0.05$. Further analysis of this interaction revealed significantly higher BECs in SLOW mice compared to FAST mice at each EtOH dose tested, and significant EtOH dose-dependent increases in BEC within each line. However, the BEC differences were extremely small. BECs (±SE) for SLOW mice were 0.40 ± 0.01, 1.06 ± 0.02, and 2.39 ± 0.02 mg/ml; BECs for FAST mice were 0.38 ± 0.02, 0.97 ± 0.02, and 2.26 ± 0.02 mg/ml, for 0.5, 1.0, and 2.0 g/kg EtOH, respectively, collapsed on MK-801 treatment. MK-801 administration did not significantly alter BECs, suggesting that alterations in locomotor responses to EtOH were not due to effects of MK-801 on EtOH pharmacokinetics.

B6 and D2 mice. Three-way ANOVA grouped on strain, MK-801, and EtOH treatment revealed a significant three-way interaction for TDIS during the 20-min test, $F(9, 288) = 2.06$, $p < 0.05$, and examination of the data suggested that the strains differed in locomotor responses to EtOH alone and to the combination of MK-801 and EtOH, but not to MK-801 alone (see Fig. 4). Consistent with results of the dose–response study, MK-801 (0.1 and 0.2 mg/kg) significantly increased the locomotor activity of D2 mice with magnitudes equal to those seen in B6 mice.

Statistical analyses of TDIS for B6 mice revealed a significant MK-801 × EtOH interaction, $F(9, 144) = 5.0$, $p < 0.01$. B6 mice were not significantly affected by any dose of EtOH, but were significantly activated by 0.1 and 0.2 mg/kg MK-801. Analysis of CORRDIS also revealed a significant MK-801 × EtOH interaction, $F(9, 144) = 5.0$, $p < 0.001$. As seen in Table 2, higher doses of MK-801 significantly reduced locomotor activity of mice given 1.0 or 1.5 g/kg EtOH. These data suggest potentiation of EtOH's locomotor depressant properties by MK-801 in this mouse strain.

For D2 mice, analysis of a significant MK-801 × EtOH interaction, $F(9, 144) = 10.3$, $p < 0.01$, revealed that, in addition to stimulant effects of MK-801 alone, EtOH alone also dose dependently increased TDIS of D2 mice with significant effects at 1.5 g/kg (see Fig. 4). As seen in Table 2, CORRDIS

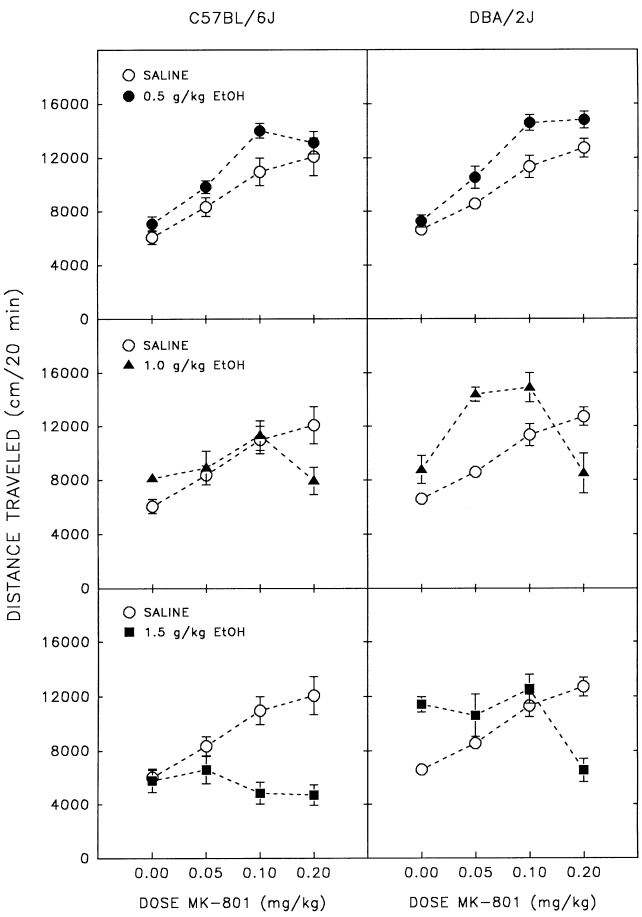

FIG. 4. Effects of MK-801 administration on locomotor activity of EtOH-treated B6 (left panels) and D2 mice (right panels). Mice were injected and tested as described in text and Fig. 3 legend. SEM larger than symbol size are shown; $n = 10$ mice per strain and treatment group.

analyses for 1.0 g/kg EtOH-treated mice showed a significant increase in activity by pretreatment with 0.05 mg/kg MK-801, and a significant decrease in response to 0.2 mg/kg MK-801. The stimulant effects of 1.5 g/kg EtOH were not enhanced by MK-801, but were reduced by 0.2 mg/kg, despite the robust locomotor stimulant effects of this MK-801 dose when given on its own. In summary, potentiation of locomotor stimulation by coadministration of MK-801 and EtOH occurred in one case, but antagonism of EtOH's activating properties appears to be the more prevalent effect.

There were EtOH dose-dependent increases in BEC, and D2 mice had significantly higher BEC than B6 mice [significant effect of strain: $F(1, 216) = 44.0$, $p < 0.01$]. Analysis of a significant strain × EtOH interaction, $F(2, 216) = 8.8$, $p < 0.01$, revealed significant dose-dependent increases in BEC within each strain, and significant strain differences in BEC (D2 > B6) at 1.0 and 1.5 g/kg EtOH. However, differences in mean BECs between strains were slight. For 0.5, 1.0, and 1.5 g/kg EtOH, respectively, mean BEC values (±SE) were 0.34 ± 0.01, 0.94 ± 0.01, and 1.55 ± 0.02 mg/ml for B6 mice; and were 0.36 ± 0.02, 1.03 ± 0.01, and 1.70 ± 0.02 mg/ml for D2 mice, collapsed on MK-801 treatment. There were no effects of MK-801 administration on BEC, suggesting that alterations in locomotor activity by MK-801 were not due to changes in EtOH metabolism.

TABLE 2

MEAN (± SEM) CORRDIS IN C57BL/6J AND DBA/2J MICE*

Treatment Group	C57BL/6J Mice	DBA/2J Mice
0.5 g/kg EtOH		
MK-801 dose (mg/kg)		
0	991.8 ± 517.6	631.4 ± 462.3
0.05	1501.4 ± 473.2	1970.9 ± 827.9
0.1	3039.6 ± 554.0	3256.9 ± 584.1
0.2	1044.2 ± 833.6	2091.9 ± 619.8
1.0 g/kg EtOH		
MK-801 dose (mg/kg)		
0	2056.9 ± 372.5	2146.3 ± 1051.6
0.05	545.7 ± 1247.2	5820.4 ± 542.7†
0.1	328.1 ± 1100.1	3573.4 ± 1101.8
0.2	−4144.1 ± 1013.9‡	−4213.1 ± 1467.9‡
1.5 g/kg EtOH		
MK-801 dose (mg/kg)		
0	−247.6 ± 857.7	4815.8 ± 559.3
0.05	−1752.3 ± 1011.5	2050.5 ± 1559.3
0.1	−6111.8 ± 830.8‡	1213.3 ± 1049.3†
0.2	−7357.5 ± 781.8‡	−6152.2 ± 857.7‡

*See text and Table 1 for definition of CORRDIS.

†$p < 0.05$ compared to group that received 0 mg/kg MK-801 within the same EtOH group.

‡$p < 0.01$ compared to group that received 0 mg/kg MK-801 within the same EtOH group.

Significant differences were determined by Tukey mean comparisons after simple effects analyses of two-way interactions (MK-801 × EtOH) in two-factor ANOVA for each strain.

DISCUSSION

In these experiments, we used two sets of genetic animal models, FAST and SLOW selected lines and B6 and D2 inbred strains, to assess the role of the NMDA-type glutamate receptor in mediating differences in sensitivity to EtOH's locomotor stimulant effects, and in modulating locomotor responses to EtOH. MK-801 administration produced a biphasic dose effect profile in all genotypes, with locomotor activation at lower doses and decreased activation at the highest dose tested. These results are consistent with other published literature (14,23,33), some of which suggest that decreased activation at higher doses may be due to increased incidence of stereotypic behaviors (33,49). Analysis of stereotypy measures recorded by the automated monitors during the dose–response studies supported this suggestion (data not shown), although the extent to which these measures accurately reflect visually rated stereotypic behavior in mice is unclear.

FAST and SLOW mice of both replicates differed in their sensitivities to the locomotor stimulant effects of MK-801, providing strong evidence that NMDA receptor function was altered as a consequence of selection and is thus involved in differential sensitivities of FAST and SLOW mice to EtOH's stimulant properties. However, B6 and D2 mice did not differ in locomotor response to MK-801. Differences in derivation of selected lines vs. inbred strains may explain the discrepancy in results between these genetic models. Theoretically, all trait-relevant loci are differentially fixed between bidirectionally selected lines. In contrast, even robust phenotypic differ-

ences between two inbred strains are largely due to arbitrary differences in genotype, and inbred strains are less likely to be different at all trait-relevant loci. D2 and B6 mice could differ at gene loci mediating sensitivity to EtOH-induced activation other than loci influencing NMDA receptor systems, and still express differences in EtOH-stimulated activity. In summary, the data reported here suggest that innate differences in NMDA receptor function may contribute, but are not necessary, for differential sensitivity to the activating effects of EtOH.

In addition to effects on baseline activity, MK-801 altered locomotor responses to EtOH in all genotypes. In FAST and D2 mice, combinations of the higher doses of MK-801 and EtOH resulted in antagonism or blockade of EtOH-stimulated activity to near baseline levels. Enhancement of the stimulant properties of EtOH was observed in one treatment group in D2 mice. These data suggest modulation of EtOH's stimulant effects by NMDA receptors. In SLOW and B6 mice, MK-801 appeared to enhance the locomotor depressant effects of EtOH, although an alternative interpretation of these data is that EtOH antagonized the locomotor stimulant effects of MK-801. Because MK-801 potentiated EtOH's locomotor depressant effects in SLOW and B6 mice, it is possible that the antagonism of EtOH-stimulated activity in FAST and D2 mice is also due to potentiation of locomotor depression. The findings reported here are consistent with those of Liljequist (31) and of Robledo et al. (44), in which EtOH-stimulated activity was reduced or blocked by NMDA antagonist administration. Kuribara reported both robust potentiation and blockade of EtOH's stimulant effects after MK-801 administration, depending on the combination of doses administered (29). Our results generally did not replicate the low-dose enhancement of locomotor activity reported by Kuribara, who used a different genotype, route of EtOH administration, and duration of activity test than used here.

The exact mechanisms by which EtOH and NMDA receptors interact to produce their behavioral effects are unknown. As discussed previously, evidence suggests that EtOH has NMDA antagonist effects, much like MK-801, resulting in inhibition of glutamate-stimulated Ca^{2+} flux. In addition, the site of EtOH interaction with the NMDA receptor may be separate from that of the MK-801 binding site (48), and may be a novel hydrophobic site on the receptor (5). Glutamate-stimulated Ca^{2+} flux in microsacs derived from SLOW mice was more sensitive to inhibition by EtOH than that of microsacs derived from FAST mice (12). Thus, it may be that insensitivity to EtOH's stimulant effects, or perhaps greater sensitivity to EtOH's depressant or sedative effects, is due in part to enhanced NMDA receptor sensitivity to EtOH. The blockade or antagonism of EtOH's stimulant effects by MK-801 in FAST and D2 mice might, therefore, be due to increased numbers of bound NMDA receptors, or a greater functional inhibition, by administration of both ligands. Although the locomotor activity of FAST mice was not decreased below baseline levels (indicating locomotor depression), higher MK-801 doses may have produced such an effect. Consistent with the notion that NMDA receptors may contribute to sedation by EtOH, it has been demonstrated that sedative-hypnotic effects of high EtOH doses can be potentiated by high doses of MK-801 (21,50).

In addition to noncompetitive antagonists such as MK-801, competitive antagonists at the NMDA receptor have been reported to have effects on locomotor activity in rats and mice. The most consistent effect of competitive antagonist administration appears to be decreased activity (14,23,28), although increases in activity (3,47), as well as no effect (3,4) have been reported. The locomotor stimulant effects of cocaine (43), amphetamine (3), and EtOH (31) were reduced by administration of competitive NMDA receptor antagonists. For cocaine and amphetamine, these effects are likely to occur via glutamatergic modulation of dopaminergic projections to nucleus accumbens (43). Given the results of competitive antagonist administration on EtOH-, cocaine-, and amphetamine-stimulated activity in other studies, and the results of the studies described here, it is expected that competitive antagonist administration would also reduce or inhibit EtOH's locomotor stimulant effects in FAST and D2 mice. However, it is possible that this would not be the case, because noncompetitive and competitive antagonists appear to most consistently produce opposite effects on spontaneous locomotor activity. These differences in behavioral output in response to these types of NMDA antagonists have been postulated to be due to differences in binding sites, thereby producing different effects on receptor function (4,28). Failure to observe alterations in EtOH-stimulated activity by competitive antagonists would not necessarily refute the role of NMDA receptors in this behavior, but could be explained by differences in the interaction of EtOH with the alternate binding sites.

Finally, because MK-801 has locomotor stimulant effects of its own, the question arises as to whether other drugs with locomotor stimulant properties, such as cocaine, amphetamine, or morphine, would produce the same effects as MK-801. Because the effects of these psychomotor stimulant drugs are modulated, at least to some extent, by both dopaminergic and glutamatergic systems (27), it is possible that one would observe similar effects to results reported here. However, the mechanism by which this would occur could possibly be different. For example, as discussed above, the combined effects of MK-801 and EtOH may be due to the ability of both drugs to affect the NMDA receptor directly. On the other hand, cocaine administration could alter EtOH's stimulant properties either by indirect effects on glutamatergic systems or by alteration of dopaminergic function via its actions on the dopamine transporter.

The mesoaccumbens-pallidal circuit comprising dopaminergic connections between ventral tegmental area (VTA) and nucleus accumbens (N Acc), and GABAergic projections from N Acc to ventral pallidum/substantia innominata (VP/SI) has been implicated in the regulation of spontaneous, novelty- and psychostimulant-induced locomotor activity (2,25). Although a neural circuit for EtOH's stimulant effects has not been determined, the involvement of dopaminergic function in EtOH-stimulated activity in several mouse genotypes (22,26,32), including FAST mice (45), suggests the possibility that the meso-accumbens pallidal circuit also mediates EtOH-stimulated activity. The data presented here are consistent with this possibility, because glutamatergic inputs from several limbic areas are thought to be important modulators of this circuit and of locomotor behavior via actions within the N Acc (25). The commonality of at least a subset of neural mechanisms or neuroanatomical sites mediating psychomotor stimulant effects of drugs of abuse has been proposed in several forms (27,51) but has not been confirmed, especially with regard to EtOH's stimulant effects. A systematic investigation of neural circuits involved in EtOH-stimulated activity is currently under consideration. Whether these investigations point to neural circuits in common with or unique from those mediating psychomotor stimulant-induced activation, we will continue to gain additional information regarding EtOH's mechanisms of action that may be relevant to alcohol addiction.

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