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MK-801- and ethanol-induced activity in inbred long-sleep and short-sleep mice: dopamine and serotonin systems

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Abstract

Low doses of (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801; dizocilpine) or ethanol induce less locomotor activation in inbred long-sleep (ILS) than short-sleep (ISS) mice. These differences may involve altered dopamine and/or 5-hydroxytryptamine (serotonin; 5-HT) neurotransmission. To address this possibility, the dopaminergic and serotonergic mechanisms underlying the locomotor-stimulant effects of MK-801 and ethanol in ILS and ISS mice were studied. Dopamine D1, D2 and 5-HT_{2A} receptor antagonists reduced MK-801-stimulated activity in ILS mice without having any effect in ISS mice. The 5-HT reuptake inhibitor fluoxetine potentiated MK-801-stimulated activity selectively in ILS mice. Strain differences in 5-HT transporters do not explain this selective effect of fluoxetine in ILS mice since [^{3}H]citalopram binding and [^{3}H]5-HT uptake studies found no differences in the affinity, number or function of 5-HT transporters between ILS and ISS mice. Ethanol-induced activity in ISS mice was depressed by dopamine D2 and 5-HT_{2C} receptor antagonists and enhanced by a 5-HT_{1A} receptor antagonist. These results suggest that in ILS mice the locomotor-stimulant effects of MK-801 require increased dopamine and/or 5-HT neurotransmission. Conversely, in ISS mice, the effects of MK-801 appear to be monoamine-independent. Thus, even though both MK-801 and ethanol inhibit *N*-methyl-D-aspartate receptors, their stimulant effects appear to involve different neuronal systems.

Keywords: Ethanol; MK-801; Dopamine; 5-HT (serotonin) 5-Hydroxytryptamine; NMDA receptor; Locomotor activity

1. Introduction

NMDA receptors belong to the ionotropic glutamate receptor family and are important for CNS function and synaptic plasticity. These receptors, formed from a combination of NR1 and NR2 subunits, are ion channels permeable to Na^+ and Ca^{2+} and are blocked by Mg^{2+} in a voltage-dependent manner. (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801; dizocilpine), a noncompetitive, open channel blocker inhibits NMDA receptors by binding to a site inside the channel (see review by Yamakura and Shimoji, 1999). Ethanol is a noncompetitive NMDA receptor antagonist and preferentially inhibits NMDA receptors containing the NR2A and/or NR2B subunits (Chu et al., 1995).

In rodents, low doses of either ethanol or MK-801 stimulate locomotor activity whereas higher doses cause locomotor depression (Diana and Sagratella, 1994; Shen and Phillips, 1998). Despite a number of studies showing that noncompetitive NMDA receptor antagonists stimulate locomotor activity in a monoamine-independent manner (Carlsson and Carlsson, 1989; Mele et al., 1998), the ability of ethanol and MK-801 to increase dopamine and 5-hydroxytryptamine (5-HT; serotonin) release in the brain (Imperato and Di Chiara, 1986; Yan et al., 1996, 1997) favors the notion that increased monoamine neurotransmission underlies the locomotor-stimulant effects of these drugs. This is supported by behavioral studies in rodents showing that blockade of the 5-HT transporter and subsequent increase in synaptic 5-HT levels further enhance locomotor activity

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induced by ethanol and MK-801 (Maj et al., 1996; Risinger, 1997). Conversely, decreasing dopamine and 5-HT transmission by pretreating rats and mice with dopamine D1, D2 or 5-HT_{2A} receptor antagonists attenuates ethanol- and MK-801-induced locomotor activity (Blomqvist et al., 1994; Carlsson et al., 1999; Narayanan et al., 1996; Shen et al., 1995). Thus, these data suggest that dopamine and 5-HT receptor systems regulate the activating effects of ethanol and MK-801 and support the hypothesis that increased dopamine and 5-HT neurotransmission may be involved in the locomotor-stimulant effects of these drugs.

McClearn and Kakihana (1981) selected long-sleep (LS) and short-sleep (SS) mice for differential sensitivity to the sedative effects of ethanol. Likewise, inbred LS (ILS) and inbred SS (ISS) mice, developed by 20 generations of sibling mating of LS or SS mice, also show differential sensitivity to higher doses of ethanol as measured by their loss of righting reflex (Markel et al., 1995). For example, the duration of the loss of righting reflex induced by 4.1 g/kg ethanol in ILS mice was 182.6 min whereas that for the ISS mice was only 6.6 min (Markel et al., 1995). Contrary to the sedative effects of ethanol, we found that the stimulant effects induced by lower doses of ethanol were more prominent in ISS compared to ILS mice (Hanania and Zahniser, 2002; Hanania et al., 2000). Since low initial sensitivity to alcohol in humans is associated with an increased risk for alcoholism (Schuckit, 1994), ILS and ISS mice serve as models for understanding the mechanisms underlying initial sensitivity to lower doses of ethanol. Similar to ethanol, noncompetitive NMDA receptor antagonists such as MK-801, ketamine and phencyclidine also increase locomotor activity to a larger extent in ISS, than ILS mice (Hanania and Zahniser, 2002), thereby supporting a role for NMDA receptors in the stimulant effects of ethanol. Since direct inhibition of monoamine transporters is not involved in the locomotor-stimulant effects of either ethanol or MK-801 in these mouse strains (Hanania and Zahniser, 2002), inhibition of NMDA receptors and subsequent increase in dopamine and/or 5-HT release could underlie the differential locomotor-stimulant effects of MK-801 and ethanol in ILS and ISS mice.

The present study examines such a possibility, i.e. that inhibition of NMDA receptors and subsequent increase in dopamine and/or 5-HT neurotransmission may underlie and explain the differential behavioral effects of MK-801 and ethanol in ILS and ISS mice. To better understand some of the neuroreceptor systems involved and to study the possible mechanisms underlying the differential behavioral effects of MK-801 in ILS and ISS mice, we examined the effects of dopamine and 5-HT receptor antagonists on MK-801-stimulated locomotor in the two mouse strains. Furthermore, since ethanol also inhibits NMDA receptors but increases locomotor activity selectively in ISS mice, we determined whether the locomotor-stimulant effects induced by MK-801 and ethanol in ISS mice are regulated by similar dopamine and/or 5-HT mechanisms.

2. Materials and methods

2.1. Animals

Adult (80–90 days old) male ILS and ISS mice were obtained from the Institute for Behavioral Genetics, Boulder, CO. The mice were housed in groups of four to five with food and water available ad libitum. The animals were exposed to a 12-h light–dark cycle. All experiments were conducted between 7:00 am and 5:00 pm. All animal use procedures were in strict accordance with the European Community guidelines for the use of experimental animals and were approved by the Institutional Animal Care and Use Committee, University of Colorado Health Sciences Center.

2.2. Locomotor activity

Drug-naïve ILS and ISS mice were transferred to the behavioral testing room and were allowed to habituate for 60 min with the lights turned off in clear acrylic open-field activity chambers (16 × 16 × 15 in.) with 8 × 8 photobeams (San Diego Instruments, San Diego, CA). Following habituation, mice were injected with saline, vehicle or dopamine/5-HT drugs and their locomotor activity was measured in the dark for 30 min, followed by a second injection of either MK-801 (0.3 mg/kg) or ethanol (2 g/kg), and activity was further monitored for 60 min. Doses for dopamine/5-HT drugs used in this study were chosen based on other studies from the literature. Horizontal locomotor activity, measured as the distance traveled (cm), was calculated from the number of consecutive photobeam breaks. Each mouse was tested only once. MK-801, raclopride, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrocholoride (SCH 23390), fluoxetine, *N*-(2-(4-(2-methoxyphenyl)-1-piperzinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635) and ketanserin (dissolved in saline) were administered i.p. at 1 ml/100 g body weight. (*R*)-(+)-alpha-(2,3-dimethoxyphenyl)-1-{2-(4-phenylrthyl)]-4-piperidine-methanol (M 100907) was dissolved in saline containing 2% dimethyl sulfoxide (DMSO). 6-Chloro-5-methyl-1-[2-(2-(2methypyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242048) was dissolved in 45% 2-hydroxypropyl β-cyclodextrin containing 2% DMSO. Ethanol (15%, v/v in saline) was administered i.p. at 1.5 ml/100 g body weight.

The data were analyzed so as to determine the effects of the dopamine/5-HT drugs on (1) spontaneous "baseline locomotor activity" and (2) "MK-801- or ethanol-stimulated activity". The effects of the dopamine/5-HT drugs on baseline activity were measured by averaging the distance traveled during the 30-min period following either saline (or vehicle) or drug injection and comparing these data for each mouse strain. To measure the effects of the dopamine/5-HT drugs on MK-801- or ethanol-stimulated activity, the distance that the mice traveled during the 60-min or 15-min period following MK-801 or ethanol injection, respectively,

was averaged and compared between the drug-pretreated and appropriate control groups.

2.3. [^{3}H]citalopram binding

Mice were sacrificed by cervical dislocation. Their cerebral cortex was dissected, homogenized in 50 mM Tris buffer (pH 7.2) and centrifuged at 20,000 × *g* and 4 °C for 20 min. The pellets, resuspended in 50 mM Tris–HCl buffer containing 120 mM NaCl and 5 mM KCl (pH 7.2), were added to assay tubes and incubated for 60 min at room temperature. Direct saturation curves were generated using nine concentrations of [^{3}H]citalopram (0.1–10 nM). Competition curves were generated in the presence of 5-HT (10^{-3}–10^{-8} M) and [^{3}H]citalopram (~ 3.0 nM). Nonspecific binding was measured in the presence of 10 μM fluoxetine. The reactions were terminated by rapid vacuum filtration over GF/B filters (Brandel, Gaithersburg, MD) and three washes with ice-cold 50 mM Tris–HCl buffer. The retained radioactivity was measured by liquid scintillation spectrometry. Proteins were determined by the method of Bradford (1976) using bovine serum albumin as the standard. B_{max}, K_d and IC_{50} values were determined from nonlinear curve fitting (GraphPad Software, San Diego CA). K_i values for 5-HT were calculated from the IC_{50} values using the Cheng and Prusoff correction and the mean K_d value determined for [^{3}H]citalopram.

2.4. [^{3}H]5-HT uptake

Cerebral cortices and hippocampi were dissected, homogenized in ice-cold 0.32 M sucrose buffer containing 3.2 mM sodium phosphate monobasic and 12.7 mM sodium phosphate dibasic (pH 7.4) and centrifuged at 800 × *g* and 4 °C for 12 min. The supernatant was recentrifuged at 22,000 × *g* and 4 °C for 15 min. The resulting P_2 pellet was resuspended in phosphate buffer containing 126 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.4 mM $MgSO_4$, 3.2 mM sodium phosphate monobasic, 12.7 mM sodium phosphate dibasic, 11.1 mM glucose and 1.1 mM ascorbic acid (pH 7.4). Assay tubes containing homogenate, assay buffer and 1 μM pargylene (to inhibit monoamine oxidase) were incubated at 37 °C for 10 min. Nonspecific uptake was determined in the presence of 10 μM fluoxetine. Assays were initiated by adding [^{3}H]5-HT (final concentration 5 nM) to assay tubes, and uptake reactions were terminated after 3 min by vacuum filtration over GF/B filters presoaked with 0.05% polyethyleneimine to reduce filter

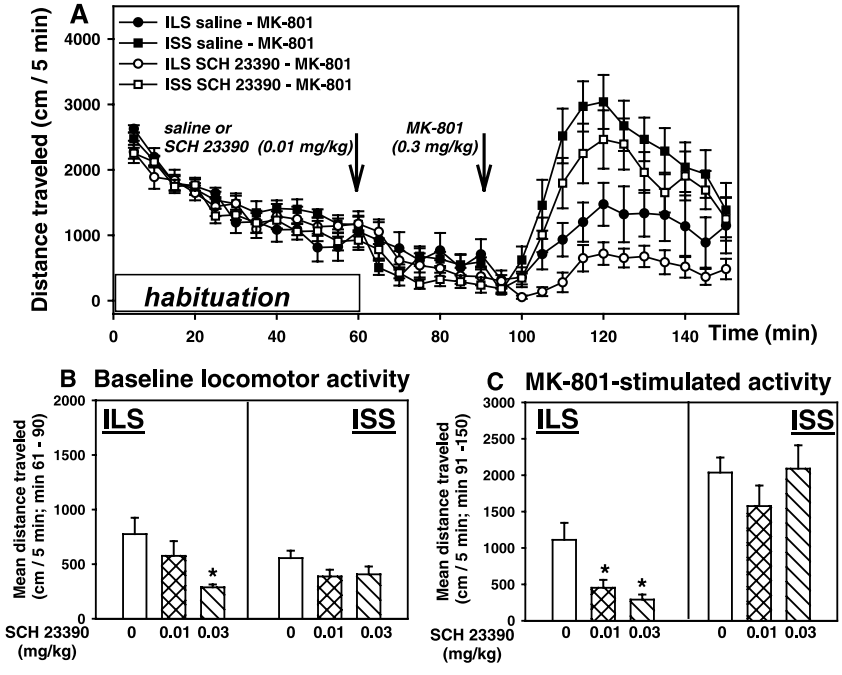

Fig. 1. (A) Time–response curve showing the effects of pretreatment with the dopamine D1 receptor antagonist SCH 23390 (0.01 mg/kg) on MK-801 (0.3 mg/kg)-stimulated locomotor activity in ILS and ISS mice. Mice were placed in the activity chambers and allowed to habituate with the lights off for 60 min prior to injection. Mice were injected i.p. (arrow) with saline or SCH 23390 (n = 9–12 mice per group), and activity was measured in the dark for 30 min. Following the first injection, mice were injected i.p. (arrow) with MK-801, and activity was measured in the dark for an additional 60 min. (B) Mean values ± S.E.M. for the effects of SCH 23390 on baseline locomotor activity in ILS and ISS mice during the 30 min (min 61–90) after saline or SCH 23390 injection. ANOVA analysis followed by Tukey's post hoc analysis showed a significant effect of SCH 23390 on saline-stimulated activity in ILS mice only at the 0.03 mg/kg dose ($F(2,28) = 7.513$, $P < 0.05$). No significant effect of SCH 23390 was found on baseline locomotor activity in ISS mice ($F(2,30) = 1.144$, $P > 0.05$). (C) Mean values ± S.E.M. for the effects of pretreatment with SCH 23390 on MK-801-induced locomotor activity in ILS and ISS mice during the 60 min (min 91–150) after MK-801 injection. ANOVA analysis followed by Tukey's post hoc analysis showed a significant effect of SCH 23390 on MK-801-stimulated activity in ILS ($F(2,28) = 4.226$, $P < 0.05$), but not ISS ($F(2,30) = 1.982$, $P > 0.05$), mice.

binding and three washes with ice-cold 0.32 M sucrose solution. Radioactivity and proteins were measured as above.

2.5. Statistical analysis

Locomotor activity data were analyzed using either analysis of variance (ANOVA) followed by Tukey's post hoc tests or Student's *t*-tests. [^{3}H]citalopram binding and [^{3}H]5-HT uptake data were analyzed using Student's *t*-tests. In all tests, $P < 0.05$ was considered to be statistically significant.

2.6. Materials

(+)-MK-801 hydrogen maleate, raclopride, SCH 23390, ketanserin, 2-hydroxypropyl β-cyclodextrin, pargyline and DMSO were purchased from Sigma/RBI (St. Louis, MO, USA). Ethanol was purchased from AAPER Alcohol and Chemical (Shelbyville, KY, USA). [^{3}H]citalopram and 5-[1,2-^{3}H(N)]-hydroxytryptamine creatinine sulfate were purchased from NEN Life Sciences Products (Boston, MA, USA). WAY 100635, M 100907 and SB 242084 were synthesized at Solvay Pharmaceuticals Research Laboratories (Weesp, The Netherlands). Fluoxetine was a gift from Eli Lilly (Indianapolis, IN, USA).

3. Results

3.1. Effect of dopamine receptor antagonists on MK-801-stimulated locomotor activity

To test whether dopamine receptor activation is involved in the locomotor-stimulant effects of MK-801 in ILS and ISS mice, we tested the effects of pretreatment with antagonists selective for either D1 or D2 receptors. Two doses of the dopamine D1 receptor antagonist SCH 23390 (0.01 and 0.03 mg/kg) were tested. The time course for the effects of pretreatment with either saline or SCH 23390 (0.01 mg/kg) on basal and MK-801 (0.3 mg/kg)-stimulated locomotor activity is shown for both mouse strains in Fig. 1A. The lower dose of SCH 23390 tested did not alter baseline locomotor activity in ILS mice; however, at the higher dose, a significant decrease was observed (Fig. 1B). Neither dose of SCH 23390 affected baseline activity of ISS mice (Fig. 1B). Pretreatment with both doses of SCH 23390 significantly attenuated MK-801-stimulated locomotor activity in ILS mice (Fig. 1C). In contrast, MK-801-induced activity in ISS mice pretreated with SCH 23390 was similar to that of the saline-pretreated control group (Fig. 1C).

We next tested the effects of pretreatment with the dopamine D2 receptor antagonist raclopride (0.1 and 0.3 mg/kg) on the locomotor-stimulant effects of MK-801 in

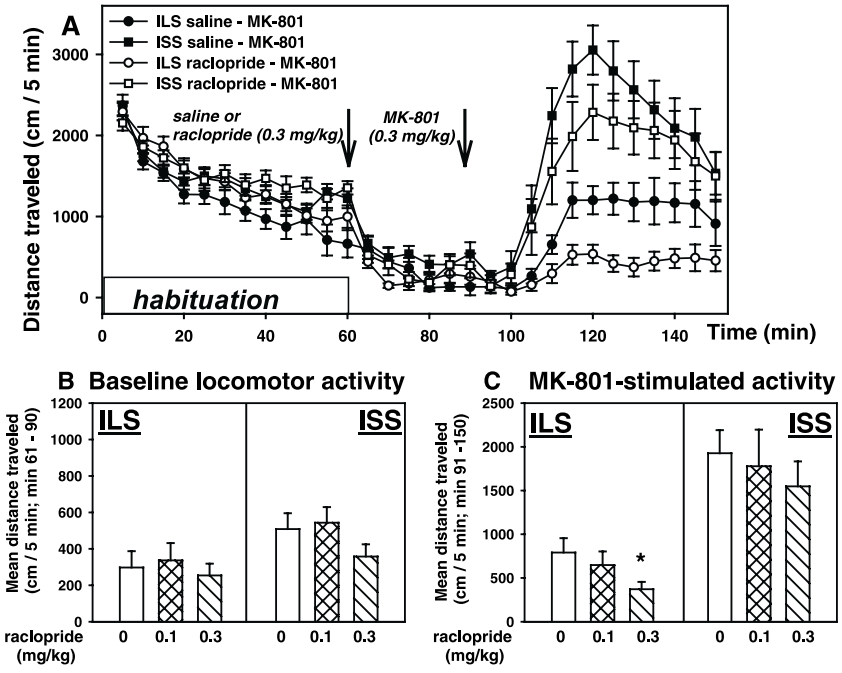

Fig. 2. (A) Time–response curve showing the effects of pretreatment with the dopamine D2 receptor antagonist raclopride (0.3 mg/kg) on MK-801 (0.3 mg/kg)-stimulated locomotor activity in ILS and ISS mice ($n = 10–12$ mice per group). See Fig. 1 for methodological details. (B) Mean values ± S.E.M. for the effects of raclopride on baseline locomotor activity in ILS and ISS mice during the 30 min after saline or raclopride injection. ANOVA analysis found no significant effect of raclopride on baseline activity in either ILS ($F(2,28) = 0.259$, $P > 0.05$) or ISS ($F(2,30) = 1.583$, $P > 0.05$) mice. (C) Mean values ± S.E.M. for the effects of pretreatment with raclopride on MK-801-induced locomotor activity in ILS and ISS mice during the 60 min after MK-801 injection. ANOVA analysis followed by Tukey's post hoc analysis found a significant effect of raclopride on MK-801-stimulated activity in ILS mice at the 0.3 mg/kg dose ($F(2,28) = 3.738$, $P < 0.05$). No significant effect of raclopride was found on MK-801-induced activity in ISS mice ($F(2,30) = 0.371$, $P > 0.05$).

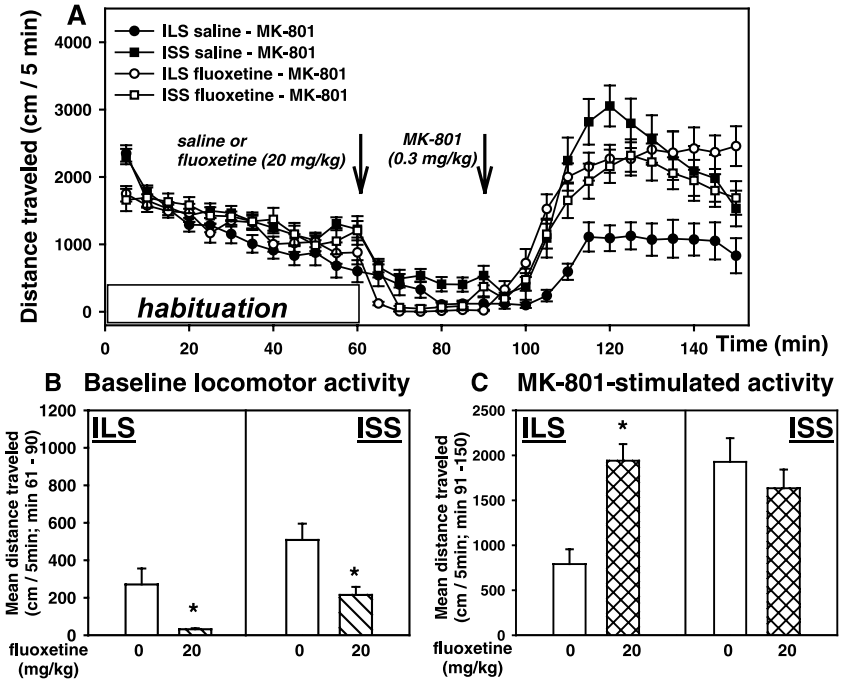

Fig. 3. (A) Time–response curve showing the effects of pretreatment with the 5-HT uptake inhibitor fluoxetine (20 mg/kg) on MK-801 (0.3 mg/kg)-stimulated locomotor activity in ILS and ISS mice ($n = 10–11$ mice per group). See Fig. 1 for methodological details. (B) Mean values ± S.E.M. for the effects of fluoxetine on saline-induced locomotor activity in ILS and ISS mice during the 30 min after saline or fluoxetine injection. Fluoxetine significantly inhibited baseline locomotor activity in both mouse strains ($P < 0.05$). (C) Mean values ± S.E.M. for the effects of pretreatment with fluoxetine on MK-801-induced locomotor activity in ILS and ISS mice during the 60 min after MK-801 injection. Fluoxetine significantly potentiated MK-801-induced activity in ILS mice ($P < 0.05$) without having any effect on ISS mice ($P > 0.05$).

ILS and ISS mice. The time course for the effects of the higher dose of raclopride on MK-801-stimulated locomotor activity is shown in Fig. 2A. Raclopride alone had no effect on baseline activity in either mouse strain (Fig. 2B). Compared to the saline-pretreated group, pretreatment with 0.3 mg/kg raclopride significantly reduced MK-801-stimulated locomotor activity in ILS mice (Fig. 2C). Similar to SCH 23390, neither dose of raclopride altered MK-801-induced locomotor activity in ISS mice (Fig. 2C).

3.2. Effect of a 5-HT uptake inhibitor on MK-801-stimulated locomotor activity

The ability of MK-801 to increase 5-HT release (Yan et al., 1997) is thought to be involved in its locomotor-stimulant effects. Here we tested whether increasing synaptic 5-HT levels with 20 mg/kg fluoxetine, a 5-HT uptake blocker, would further potentiate MK-801-stimulated activity in ILS and ISS mice. The time course for the effects of fluoxetine is shown in Fig. 3A. By itself, fluoxetine markedly depressed baseline locomotor activity in both mouse strains (Fig. 3B). Despite the reduced baseline activity, fluoxetine enhanced MK-801-stimulated locomotor activity in ILS mice compared to the saline-pretreated group (Fig. 3C). MK-801-stimulated locomotor activity in ISS mice pretreated with fluoxetine was similar to that of the saline-pretreated group (Fig. 3C). Lower doses of fluoxetine had no effect on either basal or MK-801-stimulated locomotor activity in either mouse strain (data not shown).

3.3. 5-HT transporters in ILS and ISS mice

To test whether strain differences in the number or affinity of 5-HT transporters mediate the differential behav-

Table 1
[^{3}H]citalopram binding in ILS and ISS mouse cortical membranes

Genotype	N	B_{max} (fmol/mg protein)	K_d (nM)	K_i, 5-HT (μM)
ILS	3–4	268 ± 37	2.0 ± 0.7	0.47 ± 0.01
ISS	3–4	316 ± 67	2.3 ± 0.7	0.52 ± 0.02

[^{3}H]citalopram saturation binding curves using cortical membranes prepared from drug-naïve ILS and ISS mice found no significant strain differences in the B_{max} or K_d values. Similarly, 5-HT competition curves constructed with [^{3}H]citalopram in cortical membranes showed no strain differences in K_i values.

Table 2
[^{3}H]5-HT uptake into cortical and hippocampal synaptosomes

Genotype	N	Specific [^{3}H]5-HT uptake (fmol/mg protein)	
		Cortex	Hippocampus
ILS	4	397 ± 47	744 ± 140
ISS	4	387 ± 11	742 ± 104

Specific [^{3}H]5-HT (5 nM) uptake was measured using cortical and hippocampal synaptosomes prepared from ILS and ISS mice. No significant strain differences were found in [^{3}H]5-HT uptake in either brain region.

Table 3
The effects of 5-HT receptor antagonists on baseline and MK-801-induced locomotor activity in ILS and ISS mice

Drugs	*N*	Baseline locomotor activity		MK-801-induced activity	
		ILS	ISS	ILS	ISS
5-HT$_2$ receptor antagonist					
Saline	11	271 ± 85	508 ± 87	1055 ± 227	2394 ± 324
Ketanserin (1.0 mg/kg)	9–15	245 ± 128	470 ± 276	218 ± 62*	1848 ± 343
5-HT$_{2A}$ receptor antagonist					
Vehicle	12	743 ± 102	625 ± 77	1321 ± 153	2702 ± 246
M 100907 (0.05 mg/kg)	10	488 ± 139	608 ± 98	651 ± 157*	2241 ± 277
(1.0 mg/kg)	10	532 ± 123	547 ± 133	525 ± 109*	1811 ± 332
5-HT$_{2C}$ receptor antagonist					
Vehicle	8	116 ± 41	598 ± 106	436 ± 206	2186 ± 269
SB 242084 (3.0 mg/kg)	8	110 ± 19	558 ± 106	420 ± 119	2694 ± 313
5-HT$_{1A}$ receptor antagonist					
Saline	10–12	426 ± 199	870 ± 108	650 ± 175	3051 ± 204
WAY 100635 (2.0 mg/kg)	10	346 ± 111	799 ± 115	599 ± 177	2325 ± 265
(5.0 mg/kg)	8	210 ± 77	594 ± 71	547 ± 151	1938 ± 318*

None of the 5-HT receptor antagonists tested had any significant effect on baseline activity in ILS and ISS mice ($P>0.05$). Pretreatment with either ketanserin (10 mg/kg) or M 100907 (0.05–1.0 mg/kg) significantly decreased MK-801-induced activity in ILS mice (*$P<0.05$), whereas WAY 100907 (5 mg/kg) significantly decreased MK-801-induced activity in ISS mice (*$P<0.05$). No significant effect for SB 242084 (3.0 mg/kg) on MK-801-induced activity was found in either mouse strain ($P>0.05$).

ioral effects found with fluoxetine, we constructed [^{3}H]citalopram saturation binding curves using cortical membranes prepared from drug-naïve ILS and ISS mice. The binding curves were indicative of a single binding site. No significant strain differences were found in the B_{max} or K_d values (Table 1). To test whether there was a strain difference in the affinity of 5-HT transporters for 5-HT, 5-HT competition curves were constructed with [^{3}H]citalopram in cortical membranes. No strain differences in K_i values were observed (Table 1).

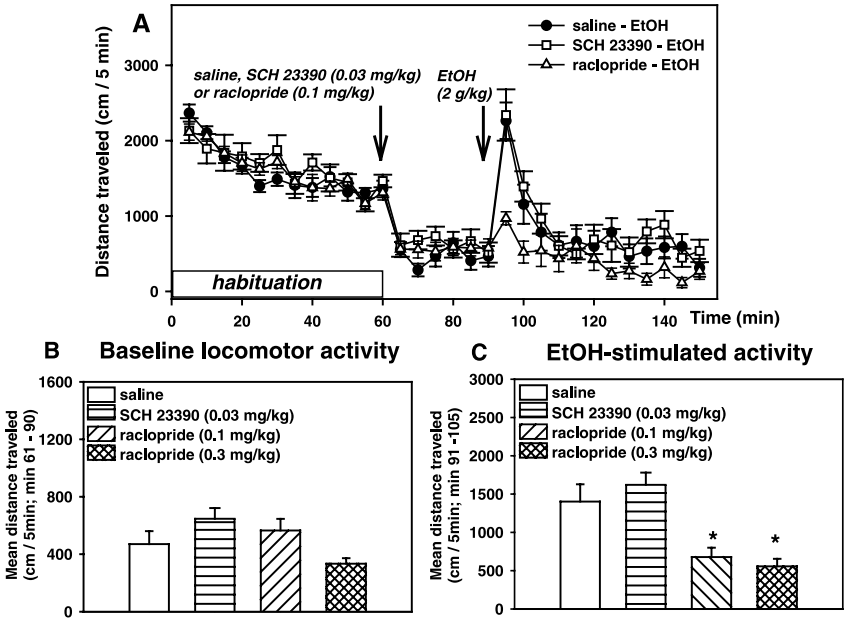

Fig. 4. (A) Time–response curve showing the effects of pretreatment with the dopamine D1 receptor antagonist SCH 23390 (0.03 mg/kg) or the D2 receptor antagonist raclopride (0.1 mg/kg) on ethanol (2 g/kg)-stimulated locomotor activity in ISS mice ($n=8–12$ mice per group). See Fig. 1 for methodological details. (B) Mean values ± S.E.M. for the effects of SCH 23390 and raclopride on baseline locomotor activity in ISS mice during the 30 min after saline or drug injection. ANOVA analysis showed no significant effects for either SCH 23390 or raclopride on baseline locomotor activity in ISS mice ($F(3,38)=2.831$, $P>0.05$). (C) Mean values ± S.E.M. for the effects of pretreatment with SCH 23390 or raclopride on ethanol-induced locomotor activity in ISS mice during the 15 min (min 91–105) after ethanol injection. ANOVA analysis followed by Tukey's post hoc test showed that raclopride, but not SCH 23390, significantly depressed ethanol-induced locomotor activity in ISS mice ($F(3,38)=8.975$, $P<0.05$).

To test whether functional differences in 5-HT transporters are involved in the differential behavioral effects found with fluoxetine, we measured specific [^{3}H]5-HT uptake into cortical and hippocampal synaptosomes prepared from ILS and ISS mice. Again, no significant strain differences were found in [^{3}H]5-HT uptake in either brain region (Table 2).

3.4. Effect of 5-HT receptor antagonists on MK-801-stimulated locomotor activity

The effects of pretreatment with antagonists selective for several of the 5-HT receptor subtypes on baseline and MK-801-stimulated locomotor activity in ILS and ISS mice are summarized in Table 3. The nonselective 5-HT$_{2A/2C}$ receptor antagonist ketanserin (1 mg/kg) had no effect on baseline activity in either mouse strain. However, compared with their saline-pretreated counterparts, ketanserin-pretreated ILS mice exhibited significantly reduced MK-801-stimulated locomotor activity. No effect of ketanserin was seen on MK-801-stimulated locomotor activity in ISS mice. To examine whether the inhibitory effects of ketanserin were mediated through inhibition of 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors, we first tested the selective 5-HT$_{2A}$ receptor antagonist M 100907. Similar to ketanserin, M 100907 (0.05–1 mg/kg) alone did not alter baseline locomotor activity of ILS or ISS mice. Both doses of M 100907 significantly reduced MK-801-stimulated locomotor activity in ILS mice. Neither dose affected MK-801-stimulated locomotor activity in ISS mice, although there was a nonsignificant trend towards decreased activity at the higher dose tested. To test whether 5-HT$_{2C}$ receptors are also involved in the inhibitory effects of ketanserin, we used the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (3 mg/kg). As shown in Table 3, SB 242084 did not alter baseline activity in either mouse strain. Similarly, SB 242084-pretreated ILS and ISS mice showed MK-801-induced locomotor activity similar to their respective control group.

WAY 100635, a selective 5-HT$_{1A}$ receptor antagonist, was also tested. By itself, WAY 100635 (2–5 mg/kg) showed a nonsignificant trend to depress baseline locomotor activity in both mouse strains. Pretreatment with WAY 100635 failed to alter MK-801-induced locomotor activity in ILS mice. However, both doses of WAY 100635 reduced MK-801-stimulated locomotor activity in ISS mice. This reduction was statistically significant at the higher dose tested (Table 3).

3.5. Effect of dopamine receptor antagonists on ethanol-stimulated locomotor activity in ISS mice

The above-mentioned data show that the locomotor activity induced by the noncompetitive NMDA receptor antagonist MK-801 in ISS mice is insensitive to dopaminergic modulation. Since ethanol only increases locomotor activity in ISS but not ILS mice (Hanania and Zahniser, 2002), we tested whether ethanol-stimulated activity in ISS mice is modulated by dopamine receptor antagonists. The time course for the effects of pretreatment with either SCH 23390 or raclopride on locomotor activity induced by 2 g/kg ethanol is shown in Fig. 4A. As in our previous experiments (Figs. 1B and 2B), neither SCH 23390 (0.01 mg/kg) nor raclopride (0.1–0.3 mg/kg) altered baseline locomotor activity in ISS mice (Fig. 4B). Similarly, SCH 23390 had no effect on ethanol-induced activity (Fig. 4C). However, both doses of raclopride significantly reduced locomotor activity induced by ethanol in ISS mice (Fig. 4C).

3.6. Effect of 5-HT receptor antagonists on ethanol-stimulated locomotor activity in ISS mice

We next tested whether ethanol-induced locomotor activity in ISS mice is modulated by 5-HT receptor antagonists (Fig. 5). The 5-HT$_{2A}$ receptor antagonist M100907, the 5-HT$_{2C}$ receptor antagonist SB 242084 and the 5-HT$_{1A}$

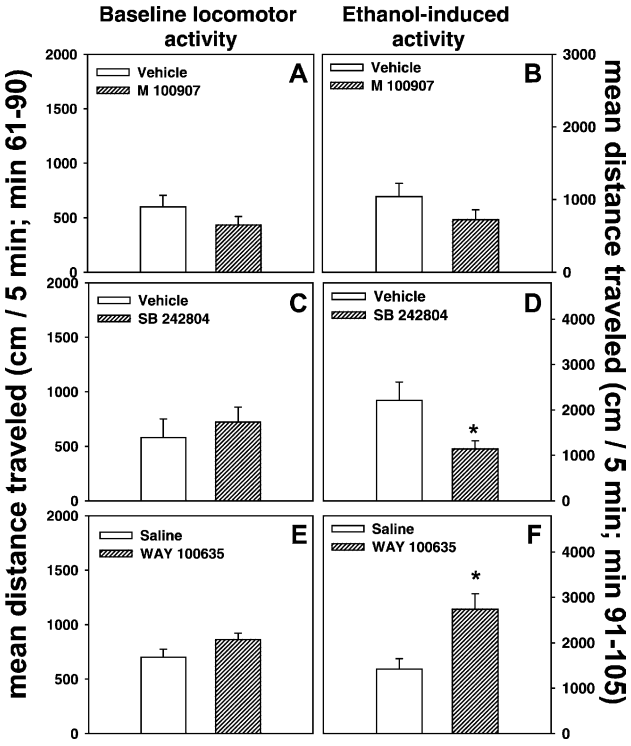

Fig. 5. Summary of the effects of pretreatment with 5-HT receptor antagonists on ethanol-stimulated locomotor activity in ISS mice (n = 8–10 mice per group). (A, C, E) Mean values ± S.E.M. for the effects of M 100907, SB 242084 and WAY 100635 on baseline locomotor activity in ISS mice during the 30 min after saline, vehicle or drug injection. No significant effect on any of these drugs was found compared to saline or vehicle-induced locomotor activity (P>0.05). (B, D, F) Mean values ± S.E.M. for the effects of pretreatment with M 100907, SB 242084 and WAY 100635 on ethanol-induced locomotor activity in ISS mice during the 15 min after ethanol injection. Compared to the saline- or vehicle-treated group, SB 242084- and WAY 100635-treated ISS mice showed significantly depressed and enhanced locomotor activity, respectively (P<0.05). M 100907-pretreated mice showed similar activity to that of the vehicle-treated group.

receptor antagonist WAY 100635 had no effect on baseline locomotor activity (Fig. 5A,C,E). Similar to MK-801, ethanol-induced locomotor activity in ISS mice was insensitive to M 100907 (Fig. 5B). However, compared to the control group, ethanol-treated ISS mice exhibited decreased and increased activity following pretreatment with SB 242084 and WAY 100635, respectively (Fig. 5D,F).

4. Discussion

There were several striking findings in this study. First, dopamine and 5-HT receptor antagonists differentially regulate MK-801-stimulated locomotor activity in ILS and ISS mice. Specifically, our data suggest that MK-801 increases activity in a monoamine-dependent manner in ILS mice, but in a monoamine-independent manner in ISS mice. Second, in ISS, mice locomotor activity induced by ethanol and MK-801, both noncompetitive NMDA receptor antagonists, is mediated by different neuronal mechanisms.

4.1. Regulation of MK-801-stimulated activity by dopamine receptor antagonists

The locomotor-stimulant effects of MK-801 have been linked to increased dopamine release and metabolism in the brain (Liljequist et al., 1991; Svensson et al., 1991). This idea is supported by data showing that MK-801-stimulated activity is inhibited by dopamine D1 and/or D2 receptor antagonists (Lapin and Rogawski, 1995; Narayanan et al., 1996). The mesolimbic loop may be a target for the actions of MK-801, such that blockade of NMDA receptors on γ-aminobutyric acid (GABA)-containing interneurons in the ventral tegmental area disinhibits mesoaccumbens dopamine neurons, thereby causing increased dopamine release in the nucleus accumbens and increased locomotor activity. In support of this hypothesis, MK-801 increases neuronal firing in the ventral tegmental area, as well as dopamine release and metabolism in the nucleus accumbens (French and Ceci, 1990; Marcus et al., 2001). Furthermore, locomotor activity induced by intra-ventral tegmental area administration of MK-801 is inhibited by the $GABA_B$ receptor agonist baclofen (Narayanan et al., 1996). In the present study, MK-801-stimulated locomotor activity in ILS mice was blocked by SCH 23390 and raclopride, suggesting that the effects of MK-801 in these mice are dependent on increased dopamine neurotransmission mediated by co-activation of D1 and D2 receptors.

By contrast, locomotor activity in MK-801-treated ISS mice was insensitive to D1 and D2 receptor antagonists. Previously, we found that 0.3 mg/kg is the lowest dose of MK-801 that induces locomotor stimulation in both mouse strains (Hanania and Zahniser, 2002). At this threshold dose, ISS mice exhibit greater locomotor stimulation than ILS mice. Thus, this dose of MK-801 may cause higher dopamine release in ISS than in ILS mice so that higher concentrations of SCH 23390 or raclopride would be required to produce an effect in ISS mice. However, higher doses of both antagonists depressed baseline activity of ISS mice (data not shown), making it impossible to assess whether their inhibitory effects on MK-801-stimulated locomotor activity were specific or nonspecific. Alternatively, the stimulatory effects of MK-801 in ISS mice could be dopamine-independent. MK-801 can increase locomotor activity in monoamine-depleted mice (Carlsson and Carlsson, 1989). Furthermore, our previous results demonstrated that cocaine and amphetamine, drugs that increase levels of dopamine, as well as 5-HT and norepinephrine, do not stimulate activity in ISS mice (Hanania and Zahniser, 2002).

4.2. Regulation of MK-801-stimulated activity by fluoxetine

The 5-HT uptake inhibitor fluoxetine inhibited baseline locomotor activity in ILS and ISS mice but potentiated MK-801-stimulated activity selectively in ILS mice, suggesting that 5-HT transporters may differ between the two mouse strains. However, [^{3}H]citalopram binding and [^{3}H]5-HT uptake studies showed no strain differences in cortical or hippocampal 5-HT transporters. Although we did not rule out the possibility that 5-HT transporters differ in other brain areas of ILS and ISS mice, it seems unlikely that the strain differences in the effects of fluoxetine on MK-801-stimulated locomotor activity are due to differences in 5-HT transporter density or activity. A more likely explanation is that synaptic levels of 5-HT, which would be increased by fluoxetine, differentially modulate MK-801-induced activity in the two mouse strains (see below).

4.3. Regulation of MK-801-stimulated activity by 5-HT receptor antagonists

The involvement of 5-HT in the behavioral activating effects of MK-801 is not surprising since MK-801 can enhance 5-HT release in the brain (Yan et al., 1997). Furthermore, 5-HT transporter inhibitors like fluoxetine and citalopram potentiate MK-801-stimulated locomotor activity in rats (Maj et al., 1996). Thus, increased 5-HT neurotransmission and activation of 5-HT receptors could be involved in the behavioral effects of MK-801. In support of this, fluoxetine-induced potentiation of MK-801-stimulated activity is inhibited by 5-HT_2 and 5-HT_3 receptor antagonists (Maj et al., 1996). Also, MK-801-stimulated activity is attenuated by the 5-HT_{2A} receptor antagonist M 100907 (Carlsson et al., 1999; Ninan and Kulkarni, 1998) and potentiated by the 5-HT_{2C} receptor antagonist SB 242084 (Hutson et al., 2000). Similar to these findings, we found that both ketanserin and M 100907 depressed MK-801-stimulated activity in ILS mice, supporting a stimulatory role for 5-HT_{2A} receptors in the locomotor activating effects of MK-801 in this strain.

Two mechanisms could explain the potentiating effects of 5-HT_{2A} receptors on MK-801-stimulated locomotor activity.

First, direct glutamate–5-HT interactions could explain the ability of 5-HT_{2A} receptors to modulate the effects of MK-801. For example, Carlsson (1995) found that the 5-HT_2 receptor agonist-mediated potentiation of MK-801-stimulated activity in monoamine-depleted mice was inhibited by M 100907. Thus, by inhibiting NMDA receptors and producing a hypoglutamate state, MK-801 could increase 5-HT_{2A} receptor-mediated activity (Carlsson et al., 1999). Cortical 5-HT_{2A} receptors could also be involved in the behavioral effects of MK-801. Activation of these receptors causes depolarization of cortical neurons (Araneda and Andrade, 1991), thereby relieving the Mg^{2+} block and rendering the NMDA receptors open and available for binding of MK-801. This notion is further supported by studies showing that activation of 5-HT_{2A} receptors increased NMDA receptor-mediated activity of cortical neurons in an M 100907-sensitive manner (Neuman and Zebrowska, 1992). Alternatively, interactions between 5-HT and dopamine could be involved in the locomotor-stimulant effects of MK-801. For example, fluoxetine-mediated potentiation of MK-801-stimulated activity in rats is blocked by dopamine D1 and D2 receptor antagonists (Maj et al., 1996). In vivo microdialysis found that 5-HT increases dopamine release in rat striatum (Benloucif and Galloway, 1991); and MK-801-, as well as K^+-, induced dopamine release in the nucleus accumbens and medial prefrontal cortex is inhibited by M 100907 (Schmidt and Fadayel, 1996; Pehek et al., 2001). Thus, fluoxetine-mediated enhancement of MK-801-induced locomotor activity in ILS mice could be also mediated through enhanced dopamine neurotransmission.

In the present study, no effect of the selective 5-HT_{1A} receptor antagonist WAY 100635 was seen in ILS mice. The highest dose of WAY 100635 (5 mg/kg) decreased MK-801-stimulated activity in ISS mice. However, since baseline activity was also affected, it was impossible to assess whether the inhibitory effect of WAY 100635 on MK-801-induced activity was a specific pharmacological effect.

4.4. Regulation of ethanol-stimulated activity dopamine receptor antagonists

Similar to MK-801, low doses of ethanol also stimulate locomotor activity in ISS but not ILS mice (Hanania and Zahniser, 2002). However, unlike MK-801, we found that ethanol-stimulated locomotor activity in ISS mice was modulated by dopamine and 5-HT receptor antagonists. Like MK-801, ethanol-mediated increases in dopamine and 5-HT release in the brain (Di Chiara and Imperato, 1985; Yan et al., 1996; Yim and Gonzales, 2000) are also accompanied by enhanced locomotor activation in rodents (Imperato and Di Chiara, 1986). Furthermore, dopamine receptor antagonists attenuate ethanol-stimulated activity in mice (Risinger et al., 1992; Shen et al., 1995). Brodie et al. (1999) reported that ethanol directly increases firing of ventral tegmental area dopamine neurons. Furthermore, DBA/2J mice, which show greater ethanol-induced locomotor activity compared to C57 mice (Kiianmaa et al., 1983), are also more sensitive to ethanol-mediated excitation of ventral tegmental area dopamine neurons (Brodie and Appel, 2000). Therefore, it is likely that in ISS mice ethanol increases dopamine neurotransmission through activation of ventral tegmental area neurons, thereby increasing locomotor activity. This could explain the ability of the D2 receptor antagonist raclopride to attenuate this behavior.

4.5. Regulation of ethanol-stimulated activity by 5-HT receptor antagonists

In the present study, the 5-HT_{1A} receptor antagonist WAY 100635 potentiated ethanol-stimulated locomotor activity in ISS mice. This effect was not surprising because 5-HT_{1A} receptor agonists depress ethanol-induced activity in mice (Blomqvist et al., 1994). Furthermore, by reducing the inhibitory tone of 5-HT_{1A} somatodendritic receptors (Brodie et al., 1995; Fornal et al., 1996; Gobert et al., 2000), WAY 100635 could cause a net increase in the firing rate of 5-HT afferents to the ventral tegmental area. This would result in disinhibition of mesoaccumbal-cortical dopamine pathways, thereby potentiating the locomotor-stimulant effects of ethanol in ISS mice.

Contrary to the enhancing effects of 5-HT_{1A} receptor antagonist, the 5-HT_{2C} receptor antagonist SB 242084 inhibited ethanol-induced activity in ISS mice. The role for this receptor in ethanol-mediated behavior has not been well studied. Nonetheless, Szeliga and Grant (1998) found that agonists and antagonists at the 5-$HT_{2A/2C}$ receptors failed to produce discriminative stimulus effects similar to ethanol. On the other hand, Pandey et al. (1996) found a higher number of 5-HT_{2C} receptors and a greater phosphoinositide hydrolysis mediated by 5-HT_{2C} receptors in alcohol-preferring P rats compared to alcohol-nonpreferring NP rats, thus linking this receptor to alcohol drinking behavior. Electrophysiological studies found that 5-HT potentiates ethanol-mediated excitation of ventral tegmental area dopamine neurons via a 5-HT_2 receptor mechanism (Brodie et al., 1995). Thus, inhibiting 5-HT_{2C} receptors might reduce the stimulatory effects of ethanol. Alternatively, 5-HT_{2C} receptors have a tonic inhibitory tone on mesocortical and mesolimbic dopamine neurotransmission (Di Giovanni et al., 2000; Gobert et al., 2000), and SB 242084 can increase the firing rate of ventral tegmental area neurons and dopamine release in the nucleus accumbens (Di Matteo et al., 1999). Therefore, if ethanol- and SB 242084-mediated dopamine release is additive, the behavioral effect of the combined drugs could mimic the effect of high dose ethanol, i.e. locomotor depression. Further studies would be needed to test these hypotheses.

4.6. Locomotor activity induced by MK-801 vs. Ethanol in ISS mice

The differential modulation of MK-801- and ethanol-induced activity in ISS mice by dopamine and 5-HT receptor

antagonists suggests that different neuronal mechanisms underlie the behavioral activating effects of MK-801 and ethanol. Whereas the actions of MK-801 may be mediated primarily through inhibition of NMDA receptors, the actions of ethanol likely involve, but are not solely dependent upon, inhibition of NMDA receptors. In FAST and SLOW mice, Shen and Phillips (1998) found a positive correlation supporting a role for NMDA receptors in the locomotor-stimulant actions of ethanol. On the other hand, we found that the contribution of NMDA receptor inhibition to the locomotor-stimulant effects of ethanol in LSXSS recombinant inbred mice was ≤ 10% (Zahniser et al., 1999).

In addition to inhibiting NMDA receptors, ethanol also potentiates $GABA_A$ receptor function. For example, Allan and Harris (1986) reported differential enhancement by ethanol of $GABA_A$ receptor-mediated chloride flux in brain membrane vesicles from LS and SS mice. Ethanol also potentiates $GABA_B$ receptor activity. For example, in the presence of ethanol, $GABA_B$ receptor-induced decreases in the firing rate of ventral tegmental area GABA neurons are further potentiated (Steffensen et al., 2000). This group also found that ethanol inhibits NMDA receptor responses in the nucleus accumbens via activation of $GABA_B$ receptors. The exact mechanisms by which dopamine and 5-HT receptor antagonists modulate activity induced by ethanol, but not MK-801, in ISS mice is unclear. However, the fact that multiple neuroreceptor systems are involved in the actions of ethanol could explain the differences in the behavioral effects of ethanol and MK-801. In any case, our data support the idea that in ISS mice ethanol increases locomotor activity by a monoamine-dependent mechanism whereas MK-801-induced locomotor stimulation is monoamine-independent.

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