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Bivalent Effects of MK-801 on Ethanol-Induced Sensitization Do Not Parallel Its Effects on Ethanol-Induced Tolerance

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The relationship between the effects of the *N*-methyl-D-aspartate (NMDA) antagonist MK-801 on acute responses to ethanol and its ability to block ethanol sensitization and tolerance was examined in DBA/2J mice. Cross-sensitization between these drugs was also studied. Repeated administration of 0.1 mg/kg MK-801 with ethanol potentiated, whereas 0.25 mg/kg attenuated, sensitization to ethanol's locomotor stimulant effects; rearing was similarly affected. There was evidence for cross-sensitization between ethanol and 0.25 mg/kg MK-801. MK-801 potentiated ethanol's ataxic effects in the grid test, but had no effect on tolerance to this effect. MK-801's effects on ethanol sensitization appeared to be related to its own behavioral effects, rather than NMDA receptor blockade per se. Further, these studies demonstrate dissociation between ethanol sensitization and tolerance.

Many abused drugs acutely stimulate locomotor activity in rodents (Amalric & Koob, 1993; Phillips, Burkhart-Kasch, Gwiazdon, & Crabbe, 1992; Wise & Bozarth, 1987) and induce sensitization (an increase in this stimulant response) upon repeated administration (Phillips, Dickinson, & Burkhart-Kasch, 1994; Pierce & Kalivas, 1997). Drug-induced sensitization has also been demonstrated in nonrodent species, including humans (Levens & Akins, 2001; Newlin & Thomson, 1990; Powell & Holtzman, 2001). The behavioral changes associated with sensitization may play a role in the intensification of craving that accompanies drug addiction (Robinson & Berridge, 1993).

Tolerance (decreased drug responsiveness upon repeated administration) may also play a role in addiction by imparting reduced sensitivity to behaviorally impairing drug effects. For example, decreases in initial ataxic, thermic, hypnotic, and other depressant effects have been shown after the repeated administration of several abused drugs (MacKenzie-Taylor & Rech, 1991; Rustay et al., 2001; Sannerud et al., 1993; Windh, Little, & Kuhn, 1995). Sensitization and tolerance to these drugs' behavioral effects are likely to be indications of the neuroadaptive changes associated with repeated drug experience. Further, behavioral sensitization may be manifest as a consequence of tolerance development to the effects of ethanol that inhibit locomotion. Whether directly associated or not, these two behavioral alterations may share common neural mechanisms.

Understanding the neurochemistry underlying these behavioral adaptations can lead to the discovery of drugs to prevent them, which may result in the development of pharmacotherapies for the treatment of human addicts. The *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors has been suggested as a necessary substrate for the development of both ethanol sensitization and tolerance (Broadbent & Weitemier, 1999; Camarini, Frussa-Filho, Monteiro, & Calil, 2000; Vanderschuren & Kalivas, 2000; Wu, Mihic, Liu, Le, & Kalant, 1993). This has led researchers to draw parallels between sensitization and other forms of NMDA-dependent plasticity, such as long-term potentiation (Morris, Anderson, Lynch, & Baudry, 1986) and drug-induced alterations in dopamine receptor sensitivity (Li, White, & Wolf, 2000). However, the doses of glutamatergic drugs that are sufficient to block ethanol sensitization and tolerance also have profound effects on the acute responses to ethanol. For example, in DBA/2J (D2) mice, MK-801, a noncompetitive NMDA receptor antagonist, dose dependently potentiated ethanol's locomotor depressant and ataxic effects (Kuribara, 1994; Shen & Phillips, 1998). This complicates the interpretation of MK-801's effects on sensitization, as it may be important for an animal to experience the acute stimulant effect of ethanol in order for sensitization to develop. The blockade of ethanol sensitization without an effect on the acute response has not been demonstrated with any pharmacological compound. It has been argued for psychostimulant and opiate sensitization that interoceptive cues associated with MK-801 itself may modify sensitization because the drugs are no longer recognized as the same drugs after being given in close association with MK-801 (Carlezon, Kosten, & Nestler, 2000; Sripada, Gaytan, Swann, & Dafny, 2001). Likewise, tolerance may be affected by MK-801 through its exacerbation of ethanol's sedative–ataxic effects, rather than by blockade of a pharmacological mechanism associated with tolerance.

Cross-sensitization, enhanced responsiveness to one drug after sensitization to another, is thought to reflect similarity in neuroadaptive responses to repeated exposure to each drug (Itzhak & Martin, 1999; Phillips, Roberts, & Lessov, 1997). If MK-801 attenuates ethanol sensitization by preventing an NMDA receptor-associated neuroadaptation, then cross-sensitization would not be expected between these two drugs. Because ethanol and MK-801 have the ability to inhibit NMDA-induced increases in intracellular calcium concentrations in vitro (Dildy-Mayfield & Leslie, 1991),

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actions that may underlie their locomotor stimulant properties, it is also possible that common changes in NMDA receptor function could lead to cross-sensitization between these two drugs.

One goal of the present experiments was to test the hypothesis that the effect of MK-801 on ethanol sensitization is associated with its impact on the acute locomotor response to ethanol. A second goal was to test the hypothesis that MK-801's effects on ethanol tolerance parallel its effects on ethanol sensitization. Finally, cross-sensitization between MK-801 and ethanol was studied to explore the notion that these drugs have similar neurochemical consequences when repeatedly administered. The word *sensitization* will hereafter specifically refer to locomotor sensitization, as opposed to sensitization to other drug effects, including stereotypic, euphoric, and reinforcing drug effects. These different forms of sensitization are dissociable and are likely to have different neural mechanisms (Leith & Kuczenski, 1982).

Method

Subjects

Male DBA/2J (D2) mice, an inbred strain chosen for its tendency to develop robust ethanol sensitization (Cunningham & Noble, 1992; Phillips et al., 1994), were purchased from The Jackson Laboratory (Bar Harbor, ME), and housed in the Portland Veterans Affairs animal facility at least 11 days prior to study initiation. At the time of testing, mice were between 57 and 77 days of age and weighed 17–31 g. Behavioral testing occurred between 8 a.m. and 4 p.m.; the lights were on from 6 a.m. to 6 p.m. in the colony room in which the mice were housed. Room temperature was maintained between 20 and 22 °C. Mice were housed isosexually in groups of 2 to 4 in clear, air-filtered polycarbonate (28 cm long × 18 cm wide × 13 cm high) cages with corncob bedding. Water bottles and food (rodent lab blocks), suspended from wire lids, were available ad libitum, except during activity testing.

Behavioral Test Apparatus

Activity monitors. Clear acrylic plastic boxes (40 cm long × 40 cm wide × 30 cm high), covered by plastic lids (44 × 44 cm, with 0.64-cm holes for ventilation), were placed in Accuscan (Columbus, OH) activity monitors. Consecutive interruptions of eight pairs of intersecting photocell beams, placed 2 cm above the floor, measured distance traveled (in centimeters) in 5-min intervals during 20-min sessions. Interruptions of a second set of photocell beams located 6 cm above the floor measured rearing. The monitors were set inside individual black acrylic chambers (Flair Plastics, Portland, OR), each containing foam insulation for exclusion of external noise, a fluorescent light mounted on the rear wall, and a ventilation fan that also helped to mask extraneous laboratory sounds.

Grid test. The grid test apparatus was similar to the activity monitors, with two major exceptions: (a) the acrylic boxes were smaller (15 cm long × 15 cm wide × 20 cm high), and (b) the floors consisted of 1.25-cm wire mesh grids suspended 1 cm above stainless steel plates. Simultaneous contact with the wire mesh and metal plate (caused by paw slippage through the mesh) resulted in the closing of an electrical circuit and counted as an error. Because of the smaller size of the acrylic boxes, only four pairs of intersecting photobeams measured activity counts in the chamber. Each grid test apparatus was placed into a black acrylic box identical to those housing the activity monitors.

Drugs

All drugs were injected intraperitoneally with 0.4-mm, 27 gauge hypodermic needles (Sherwood Medical, St. Louis, MO). Ethanol (Pharmco Products, Brookfield, CT; 20% vol/vol) was diluted from 100% in 0.9% (wt/vol) saline and injected at a dose of 2 g/kg. MK-801 (Sigma, St. Louis, MO) was dissolved in 0.9% saline and injected in a 10-ml/kg volume. Justifications of the MK-801 and ethanol doses used in each experiment are given below.

Behavioral Procedures

On each test day in all experiments, mice were moved from the colony room to the testing room and left undisturbed for 45–60 min to permit acclimation to the testing environment. At the end of each experiment, the mice were killed by carbon dioxide asphyxiation. All procedures complied with the Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Experiment 1. To examine whether experiencing the acute stimulant response to ethanol would alter the ability of MK-801 to attenuate ethanol sensitization, it was necessary to identify a pharmacologically active dose of MK-801 without significant effects on ethanol's acute stimulant response. The 2-g/kg ethanol dose was chosen for this experiment because of its robust locomotor stimulant and sensitizing effects in D2 mice (Cunningham & Noble, 1992; Phillips et al., 1994; Phillips, Huson, Gwiazdon, Burkhart-Kasch, & Shen, 1995). Each day, mice were weighed before receiving two injections, spaced 20 min apart. After the first injection, mice were placed in clear polycarbonate (28 cm long × 18 cm wide × 13 cm high) holding cages with corncob bedding for the 20-min inter-injection interval. After the second injection, mice were placed into the activity monitors, and behavior was recorded for 20 min in 5-min intervals. For the first 3 days, all injections were saline. These days were included to achieve maximum habituation to the test procedures. Data from Day 3 provided a measure of habituated baseline activity. On Day 4, separate groups received one of the following pretreatment doses of MK-801: 0, 0.05, 0.10, 0.15, and 0.20 mg/kg, 20 min prior to injection with 2 g/kg ethanol. The doses of MK-801 were chosen from previous experimental results, which showed that doses up to and including 0.25 mg/kg produced locomotor stimulation in D2 mice, and that doses above and including 0.20 mg/kg affected the acute response to ethanol in a nonadditive manner (Shen & Phillips, 1998). The 20-min pretreatment period was chosen because the peak locomotor response to MK-801 in D2 mice occurred 20 min after intraperitoneal injection (Shen & Phillips, 1998). Each dose category included 6–12 mice.

Experiments 2 and 3. The purpose of Experiment 2 was to determine the effect on ethanol sensitization of 0.10 mg/kg MK-801, the dose identified in Experiment 1 as having no significant effects on the stimulant response to ethanol. The unexpected results of Experiment 2 prompted Experiment 3, in which we ascertained whether we could replicate the results of previous experiments that used 0.25 mg/kg MK-801, a dose that has profound effects on ethanol's stimulant response and that has been reported to block ethanol sensitization (Broadbent & Weitemier, 1999; Camarini et al., 2000).

In each experiment, there were four treatment groups; a description of these groups is presented in Table 1. During the treatment phase (Days 4–15), Group SS received only saline injections; it was included to examine any changes in baseline activity occurring over time. Groups SE and MS received saline plus repeated 2 g/kg ethanol or MK-801 injections, respectively, to determine the presence and magnitude of sensitization to these drugs. Finally, Group ME received daily injections of both MK-801 and ethanol; this group was used to assess the effects of MK-801 on the development of ethanol sensitization.

After the mice were weighed, either MK-801 or saline was injected, followed 20 min later by a second injection of either ethanol or saline. On days when an activity test was scheduled, mice were placed individually into holding cages after the first injection and into the activity monitors after the second injection. On nontest days, injections were administered in the colony room, and mice were returned to their home cages following

Table 1
Schedule of Injections for the Experimental Groups in Experiments 2 and 3

	Habituation			Treatment phase											Challenge phase		
Group	**1**	**2**	**3**	**4**	5	6	**7**	8	9	**10**	11	12	**13**	14	15	**16**	**17**	**18**
SS	**SS**	**SS**	**SS**	**SS**	SS	SS	**SS**	SS	SS	**SS**	SS	SS	**SS**	SS	SS	**SE**	**SS**	**MS**
SE	**SS**	**SS**	**SS**	**SE**	SE	SE	**SE**	SE	SE	**SE**	SE	SE	**SE**	SE	SE	**SE**	**SS**	**MS**
MS	**SS**	**SS**	**SS**	**MS**	MS	MS	**MS**	MS	MS	**MS**	MS	MS	**MS**	MS	MS	**SE**	**SS**	**MS**
ME	**SS**	**SS**	**SS**	**ME**	ME	ME	**ME**	ME	ME	**ME**	ME	ME	**ME**	ME	ME	**SE**	**SS**	**MS**

Note. Numbers refer to the day of the experiment. SS = two saline injections spaced 20 min apart; SE = a saline injection followed by a 2 g/kg ethanol injection; MS = an MK-801 injection (0.10 or 0.25 mg/kg) followed by a saline injection; ME = an injection of MK-801 followed by an ethanol injection. On test days, indicated by boldface, mice were placed into the activity monitors immediately after the second injection and distance traveled was recorded in 5-min time intervals for 20 min. During the treatment phase, mice received differential treatments.

each injection. As in Experiment 1, during the first 3 days, the groups received only saline injections. From Days 4 through 15, groups received the drug treatments described in Table 1 (under "Treatment phase"). Locomotor activity data collected for 20 min on Days 1–4, 7, 10, and 13 (indicated by bold lettering in Table 1), provided measures of acute drug responses (Day 4) and of the patterns of behavioral changes accompanying the repeated drug treatments.

An ethanol challenge test was conducted on Day 16. Locomotor behavior was measured after all groups received saline followed by ethanol, to determine the effects of the repeated drug treatments on the response to ethanol alone. On Day 17, all groups received two saline injections to determine if there were effects of repeated drug treatments on baseline behavior. Finally, on Day 18, all groups received injections of MK-801 followed by saline injections to identify possible cross-sensitization between ethanol and MK-801. Each group in these experiments comprised 15–16 mice.

In Experiments 2 and 3, we also examined the effects of MK-801 and ethanol on rearing. Although rearing behavior has often been described as a measure of exploration, it is also a behavior inhibited by drug-induced ataxic effects (i.e., moderate to severe ataxia competes with the ability to rear). In fact, Crabbe, Gallaher, Phillips, and Belknap (1994) found that sensitivity to ethanol-induced decreases in rearing were genetically correlated with sensitivity to ethanol-induced loss of righting reflex. We speculated that MK-801's effects on ethanol sensitization would be related to its effects on tolerance to ethanol-induced inhibition of rearing.

Experiment 4. Rearing data from Experiments 2 and 3 suggested that MK-801 potentiates the ataxic effects of ethanol, and that this potentiation could provide an explanation for some of the observed effects on ethanol sensitization. However, rearing data do not directly index ataxia. Therefore, this experiment was performed by the methods described for Experiments 2 and 3 (Table 1), except that mice were tested in the grid test to obtain a direct measure of ataxia. Further, this apparatus simultaneously quantifies locomotor behavior. The effects of the 0.10- and 0.25-mg/kg doses of MK-801 were tested simultaneously in this study. The two MK-801 dose groups were designated 0.1M-S and 0.25M-S if a saline injection followed the MK-801 injection, and 0.1M-E and 0.25M-E if ethanol followed MK-801. The MK-801 challenge dose on Day 18 was 0.25 mg/kg, followed by saline for all groups. Each treatment group in this experiment contained 16 mice.

Data Analysis

The dependent variables for Experiments 1–3 were distance traveled (in centimeters) and rearing counts. When data from the different 5-min segments of the locomotor activity response curves were analyzed, we confirmed that the stimulant response to 2 g/kg ethanol occurred primarily during the first 10 min after injection (Shen, Harland, Crabbe, & Phillips, 1995). Therefore, we present analysis and results for only this portion of the 20-min test session. For the grid test, data from the entire 20-min test were analyzed, because we found the peak ataxic response to 2 g/kg ethanol to occur between 5 and 15 min after injection. The dependent variables for the grid test were number of errors and activity counts. An error was defined as simultaneous contact with the grid floor and the underlying metal plate, as described above. An activity count was defined as an interruption of one of the lower sets of photobeams. In addition, we analyzed the ratio of total errors to total activity counts. This measure of ataxia corrects for differences in activity, because more active mice had a greater chance of committing misstep errors through the grid (e.g., Shen, Dorow, Huson, & Phillips, 1996). This ratio is hereafter referred to as the *ataxia ratio*.

Data from the first 2 habituation days were not included in any analyses; however, the baseline data from Day 3, when the activity levels among groups were well matched, were included in the analyses of Experiment 1 data, and in the analyses of the pretreatment phase data (Days 4–15) in Experiments 2–4. Data from Experiment 1 were analyzed with repeated-measures analysis of variance (ANOVA), with dose as the between-groups variable and day as the repeated measure. For Experiments 2–4, data for the pretreatment phases were analyzed with repeated-measures ANOVA, with pretreatment (MK-801, saline) and treatment (ethanol, saline) as the between-groups variables and day as the repeated measure. Data from each challenge day were analyzed separately, with only pretreatment and treatment as between-groups variables. For the grid test experiment, the pretreatment variables consisted of three levels (0.10, 0.25, saline). Tukey's honestly significant difference tests and planned contrasts were used to determine specific differences between groups. Significance levels were set at $\alpha = .05$. All statistical analyses were conducted with STATISTICA for Windows (StatSoft, Tulsa, OK).

Results

Experiment 1

The robust stimulant response to ethanol on Day 4, relative to the baseline activity measured on Day 3, was dose dependently inhibited by increasing doses of MK-801 (see Figure 1), $F(5, 55) = 5.6$, $p < .01$, for the Dose × Day interaction. Tukey's post hoc tests indicated that mice treated with 0.15 or 0.20 mg/kg MK-801 in combination with ethanol exhibited no stimulant response to ethanol ($ps < .01$); the stimulant responses of mice treated with 0.05, 0.075, and 0.1 mg/kg were not significantly affected. Because the 0.1-mg/kg dose was the highest dose that did not significantly alter the ethanol response, this dose was used in Experiment 2. We speculated that this dose would attenuate ethanol sensitization, but to a lesser degree than higher doses, if

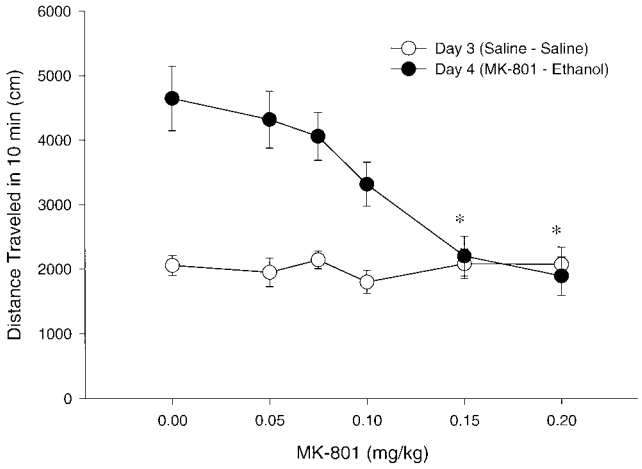

Figure 1. MK-801 dose-dependently inhibited ethanol-stimulated activity. Day 3 data are distance traveled for 10 min immediately after the second of two saline injections, spaced 20 min apart. On Day 4, activity was measured after an injection of 2 g/kg ethanol, preceded 20 min by the indicated doses of MK-801. Asterisks indicate significant ($\alpha = .05$) differences in activity compared with the 0 mg/kg MK-801-treated mice of the ethanol treatment groups, as determined by Tukey's post hoc tests. Data are means (± *SEM*).

ethanol sensitization is associated with neuroadaptive changes that are blocked by MK-801 binding to NMDA receptors. However, if MK-801's effects on ethanol-induced sensitization are due to the combined behavioral effects of MK-801 and ethanol (i.e., the mice do not experience ethanol's initial stimulant effects when MK-801 is administered at higher doses) then ethanol sensitization should be left intact when this lower MK-801 dose is used, unlike the result seen with higher doses.

Experiment 2

Locomotor activity. MK-801 and ethanol had significant effects on locomotor activity during the treatment phase: Days 4–15 (see Figure 2A), $F(4, 236) = 2.7$, $p < .04$, for the Pretreatment × Day interaction, $F(4, 236) = 34.4$, $p < .01$, for the Treatment × Day interaction. Planned contrasts were used to test two a priori hypotheses: (a) on Day 4, Groups SE, MS, and ME would show increased locomotion relative to Group SS, demonstrating the acute effects of these treatments; and (b) the locomotor responses would be altered on Day 13 compared with Day 4, demonstrating sensitization or tolerance. Mice in Groups SE, MS, and ME traveled greater distances than did Group SS mice on Day 4 ($ps < .01$), demonstrating significant stimulant responses to ethanol, MK-801, and their combination. The responses of Groups SE, MS, and ME were not significantly different from each other. Contrasts of the groups' responses on Day 13 versus Day 4 indicated that the responses of Groups SE and ME were sensitized after 10 days of repeated treatment ($ps < .01$). This was not the case for the other groups; the responses of groups MS and SS were not significantly altered on Day 13 compared with Day 4. Thus, MK-801 administered alone did not induce sensitization.

All mice were challenged with 2 g/kg ethanol on Day 16. The response to ethanol was dependent on the combination of MK-801 and ethanol treatments during the treatment phase, $F(1, 58) = 6.9$, $p < .02$, for the Pretreatment × Treatment interaction. Groups SE and ME exhibited larger ethanol stimulant responses than Group SS ($ps < .01$), supporting the development of ethanol-induced sensitization. Unexpectedly, the sensitized response of Group ME was larger than that of Group SE ($p < .01$), which suggests that ethanol sensitization was potentiated by repeated pretreatment with 0.1 mg/kg MK-801 during the treatment phase. Similarities in locomotor behavior on Day 17 after saline treatment indicated that repeated treatments with MK-801, ethanol, or their combination did not significantly alter basal activity. Finally, the groups also responded similarly to MK-801, indicating that there was no significant sensitization or cross-sensitization in mice that had

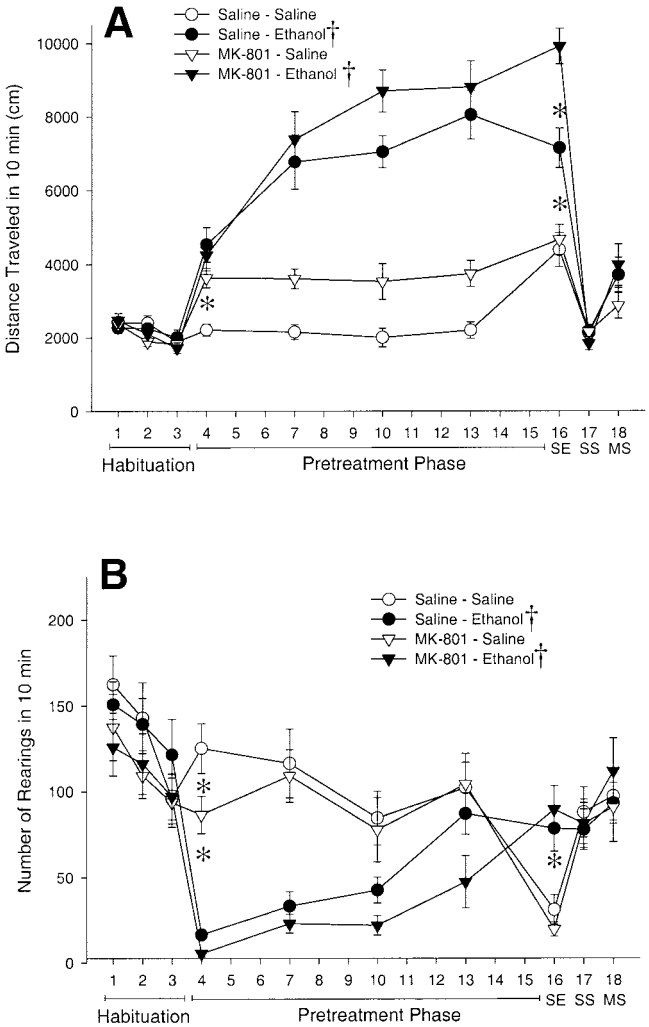

Figure 2. Effects of 0.1 mg/kg MK-801 on ethanol sensitization (A) and rearing (B). Group means (± *SEM*) for the first 10 min of the 20 min test are presented. On Days 1–3, mice received saline injections. On Days 4–15, mice received the treatments indicated in the legend. On Days 16, 17, and 18, mice received saline then ethanol (SE), saline then saline (SS), and MK-801 then saline (MS), respectively. Asterisks separate significant ($\alpha = .05$) differences between group means; daggers indicate significant increases in activity or rearing (Day 13 compared with Day 4) for the indicated groups.

been repeatedly treated with 0.1 mg/kg MK-801 or ethanol, respectively.

Rearing. The effect of the drug treatments on rearing is shown in Figure 2B. During the treatment phase, ethanol significantly attenuated rearing, $F(4, 236) = 14.5$, $p < .01$, for the Treatment × Day interaction. On Day 4, rearing was significantly lower in Groups MS, SE, and ME compared with Group SS ($ps < .01$), indicating that MK-801 also reduced rearing. However, the effect of ethanol on rearing was more profound than that of MK-801 ($p < .01$). Contrasts of the ethanol-treated groups' responses on Day 13 versus Day 4 indicated that the rearing behavior of Groups SE and ME began to recover after 10 days of repeated treatment ($ps < .02$), in parallel with the development of sensitization. The responses of Groups MS and SS were not significantly changed.

When challenged with ethanol on Day 16, groups receiving ethanol during the treatment phase displayed more rearing, regardless of MK-801 treatment condition, $F(1, 58) = 30.2$, $p < .0001$, for the treatment main effect, suggesting that tolerance had developed to ethanol's initial depressant effect on rearing. However, there was no main effect of pretreatment or Pretreatment × Treatment interaction, indicating that 0.1 mg/kg MK-801 had only minor effects on rearing behavior. The groups did not differ in response to saline or MK-801 on Days 17 and 18, indicating that the drug treatments did not alter baseline rearing or the rearing response to MK-801.

Experiment 3

The potentiation of ethanol-induced sensitization by 0.1 mg/kg MK-801 was unexpected, as previous studies found that higher doses of MK-801 blocked ethanol sensitization (Broadbent & Weitemier, 1999; Camarini et al. 2000). To verify that the result obtained with 0.1 mg/kg MK-801 was not idiosyncratic to our experimental design, we sought to replicate these previous results using 0.25 mg/kg MK-801, which was shown in previous studies to block ethanol sensitization.

Locomotor activity. Locomotor response patterns during the treatment phase (Figure 3A) were dependent on the MK-801 and ethanol treatment conditions during these days, $F(4, 236) = 13.2$, $p < .01$, for the Pretreatment × Treatment × Day interaction. The stimulant response of Group MS was greater than that of all other groups on Day 4 ($ps < .01$). Similarly, the stimulant effect of ethanol was supported by greater locomotion in Group SE compared with Group SS on Day 4 ($p < .01$). The inhibition of the ethanol stimulant response by MK-801 was indicated by a significantly lower response in Group ME compared with Group SE ($p < .01$). Contrasts of groups' responses on Day 13 versus Day 4 showed that Group ME had a significantly larger response on Day 13 versus Day 4 ($p < .01$), supporting the development of sensitization. Group SE showed a statistical trend toward sensitization ($p = .067$). In Groups SS and MS, the locomotor responses on Days 4 and 13 were not significantly different.

Response to the ethanol challenge on Day 16 was dependent on the prior MK-801 and ethanol treatment conditions during the treatment phase, $F(1, 59) = 13.4$, $p < .01$, for the Pretreatment × Treatment interaction. Sensitization of Group SE was indicated by their larger response compared with that of Group SS ($p < .01$). Group SE also exhibited more activity than Groups MS and ME ($ps < .03$). The lower response of Group ME compared with

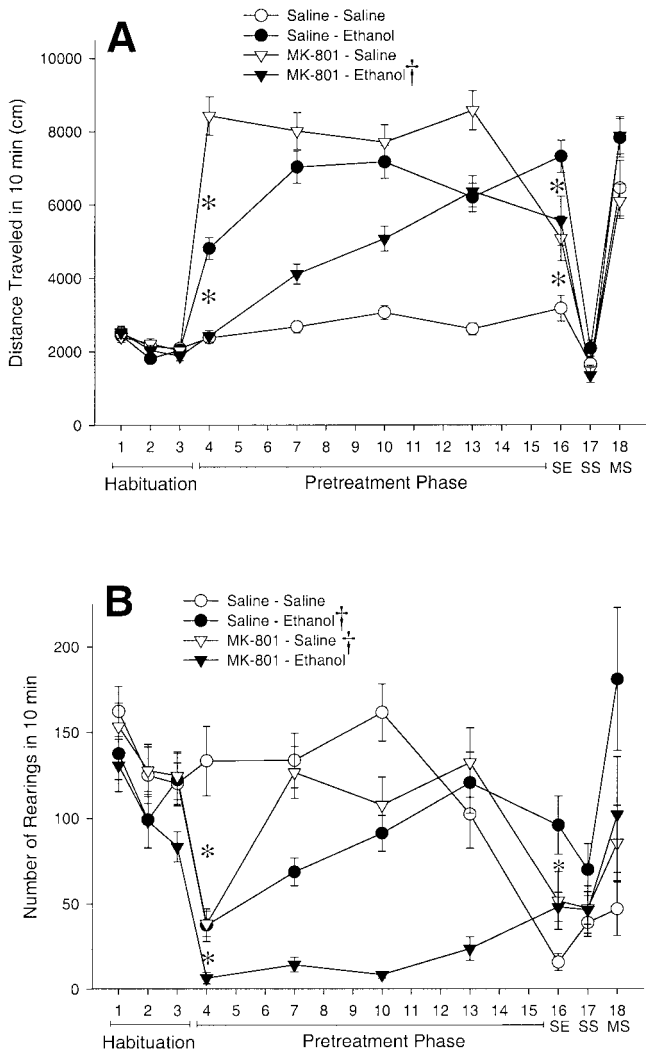

Figure 3. Effects of 0.25 mg/kg MK-801 on ethanol sensitization (A) and rearing (B). Group means (± *SEM*) for the first 10 min of the 20 min test are presented. On Days 1–3, mice received saline injections. On Days 4–15, mice received the treatments indicated in the legend. On Days 16, 17, and 18, mice received saline then ethanol (SE), saline then saline (SS), and MK-801 then saline (MS), respectively. Asterisks separate significant ($\alpha = .05$) differences between group means; daggers indicate significant increases in activity or rearing (Day 13 compared with Day 4) for the indicated groups.

Group SE supports an attenuation of sensitization by 0.25 mg/kg MK-801, as has been found in previous studies. This result was characterized as an attenuation rather than blockade of ethanol sensitization, because Group ME exhibited a larger stimulant response to the ethanol challenge than did Group SS ($p < .01$). However, Group SS did not show the usual ethanol-induced activation (compared with the response of Group SS on Day 16 in Experiment 2, and Group SE on Day 4 in Experiments 2 and 3). Without this abnormally low stimulant response, the blockade of sensitization by the high dose of MK-801 would be apparent; the reasons for this blunted response remain unclear. This blunted response of Group SS also affects interpretation of the enhanced

response to ethanol of Group MS, compared with that of Group SS ($ps < .02$). The larger stimulant response of Group MS suggests that repeated 0.25 mg/kg MK-801 treatment resulted in cross-sensitization to the subsequent injection of ethanol. However, the group difference is likely partially due to an abnormally low stimulant response in Group SS.

On saline challenge Day 17, prior MK-801 treatment reduced baseline activity, regardless of the ethanol treatment condition, $F(1, 59) = 5.2$, $p < .03$, for the Pretreatment main effect. On MK-801 challenge Day 18, mice that had received prior ethanol treatment were more active than ethanol-naive mice, regardless of prior MK-801 pretreatment condition, $F(1, 59) = 7.5$, $ps < .01$, for the main effect of treatment. This suggests that repeated ethanol treatment resulted in cross sensitization to 0.25 mg/kg MK-801.

Rearing. Patterns of rearing responses during the treatment phase (Figure 3B) were dependent on the MK-801 and ethanol treatment conditions, $F(4, 236) = 2.5$, $p < .05$, for the Pretreatment × Treatment × Day interaction. Planned contrasts revealed that the responses of Groups SE, MS, and ME were lower than those of Group SS on Day 4 ($ps < .01$), indicating that all drug treatments decreased rearing. The effect of this dose of MK-801 was similar to that of ethanol, unlike the 0.1-mg/kg dose. Groups SE and MS reared more compared with Group ME ($ps < .01$), indicating that MK-801 potentiated ethanol's effects on rearing. By Day 13, Groups MS and SE showed significantly more rearing compared with Day 4 ($ps < .01$), suggesting that these mice were able to recover from the initial drug effects. However, the extreme depression in rearing behavior induced by the combination of MK-801 and ethanol did not abate with additional drug treatment.

Rearing behavior after ethanol challenge on Day 16 was influenced by prior MK-801 and ethanol treatment conditions, $F(1, 59) = 10.3$, $p < .01$, for the Pretreatment × Treatment interaction. All groups exhibited more rearing behavior than Group SS ($ps < .05$). Mice in Group SE exhibited more rearing than all other groups ($ps < .02$), suggesting that tolerance to ethanol's effects on rearing developed in this group. Groups MS and ME tended to exhibit more rearing than Group SS, which would indicate the development of tolerance in these groups, although these differences did not reach statistical significance ($ps < .06$ and .09, respectively). The groups did not differ significantly in response to saline on Day 17, indicating that the drug treatments did not alter baseline rearing. On MK-801 challenge Day 18, mice that had received prior ethanol treatment reared more than ethanol-naive mice, regardless of prior MK-801 pretreatment condition, $F(1, 59) = 6.3$, $p < .02$ for the treatment main effect.

Experiment 4

The effect of MK-801 and ethanol on rearing behavior suggests that MK-801's ability to alter sensitization paralleled its potentiating effects on motor incoordination. However, because rearing is not typically used as a measure of coordination, we more directly assessed this relationship by using the grid-test apparatus. Figure 4A shows that mice from all groups had very low ataxia ratios after saline treatment on Days 1–3. The ataxic responses during the treatment phase were dependent on the MK-801 and ethanol treatment conditions, $F(8, 240) = 2.5$, $p < .02$, for the Pretreatment × Treatment × Day interaction. On Day 4, the ataxic response of Groups 0.1M-S and 0.25M-S did not differ significantly from that of Group SS, indicating that MK-801 alone did not have significant ataxic effects. However, mice from ethanol-treated Groups SE, 0.1M-E, and 0.25M-E had significantly higher ataxia ratios than any of the non-ethanol-treated groups ($ps < .03$). Further, Groups 0.1M-E and 0.25M-E had larger ataxia ratios than Group SE, suggesting that MK-801 enhanced the acute ataxic effects of ethanol ($ps < .04$). Groups 0.1M-E and 0.25M-E had significantly lower ratios on Day 13 than on Day 4, supporting the development of within-groups tolerance ($ps < .03$). Group SE did not develop significant tolerance by this measure, although the

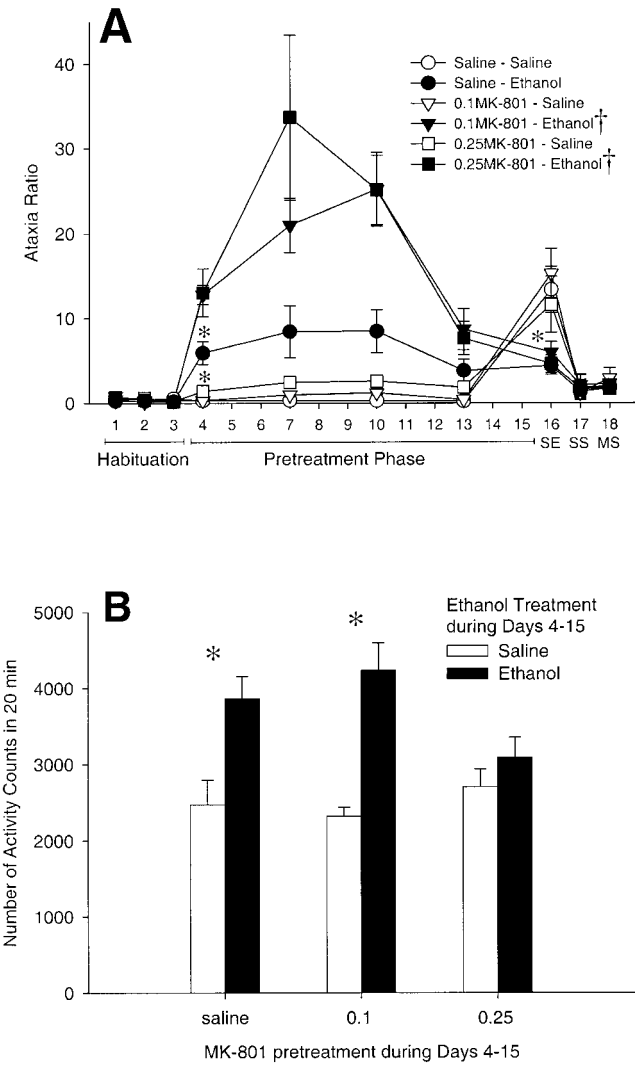

Figure 4. Effects of 0.1 and 0.25 mg/kg MK-801 on the development of ethanol tolerance in the grid-test apparatus. A: Mean (± *SEM*) ataxia ratio scores (see text for a description of this measure) over 20 min. B: Mean (± *SEM*) activity levels in response to 2 g/kg ethanol on Day 16. On Days 1–3, mice received saline injections. On Days 4–15, mice received the treatments indicated in the legend, but activity measurements were only taken on Days 4, 7, 10, and 13. On Days 16, 17, and 18, mice received saline then ethanol (SE), saline then saline (SS), and MK-801 then saline (MS), respectively. Asterisks separate significant ($\alpha = .05$) differences between group means; daggers indicate significant increases in ataxia (Day 13 compared with Day 4) for the indicated groups.

between-groups measure of tolerance on Day 16 indicated that tolerance had developed in this group (see below).

Analysis of the ethanol challenge data on Day 16 revealed that repeated ethanol treatment resulted in tolerance to ethanol's ataxic effects, $F(1, 74) = 21.7$, $p < .0001$, for the main effect of treatment. However, tolerance was not influenced by prior MK-801 treatment. Analysis of activity data, shown in Figure 4B, indicated that ethanol-induced activity was dependent on the MK-801 and ethanol treatment conditions, $F(1, 74) = 4.0$, $p < .03$, for the Pretreatment × Treatment interaction. Planned contrasts indicated that only Groups SE and 0.1M-E had significantly higher responses than Groups SS and 0.1-S, respectively (ps $< .01$), suggesting that the sensitizing effect of repeated ethanol was blocked by coadministration of the 0.25-mg/kg dose of MK-801, replicating the findings of Experiment 3. In addition, Group 0.1M-E had slightly more activity counts than Group SE, similar to the findings of Experiment 2, but this difference was not statistically significant.

Discussion

These studies demonstrated that a low dose of MK-801, which did not have significant effects on the acute stimulant response to ethanol, not only failed to block, but potentiated ethanol sensitization in D2 mice. In contrast, a higher dose of MK-801, which completely inhibited ethanol's acute effect, significantly attenuated ethanol sensitization. These results demonstrate that ethanol sensitization can occur in the presence of NMDA receptor blockade. We postulate that the opposite effects of the two MK-801 doses on the development of ethanol sensitization are associated with their own behavioral or interoceptive effects when coadministered with ethanol. The potentiation of sensitization with 0.1 mg/kg MK-801 could be explained by a slight enhancement of the stimulant effects of ethanol, whereas the blockade with 0.25 mg/kg may be associated with the profound potentiation of the ataxic–sedative effects. The mechanism of this potentiation may be these drugs' common stimulant properties, which may be due to their effects on common neural systems. This would be consistent with previous studies (Kuribara, 1994; Shen & Phillips, 1998; Vanover, 1999; Wilson, Bosy, & Ruth, 1990) showing that the stimulant effects of MK-801 and ethanol were potentiated when low doses of these drugs were coadministered, but the ataxic, locomotor depressant, and sedative-hypnotic effects were potentiated during coadministration of higher doses. Although an enhancement of ethanol's acute stimulant effects by 0.1 mg/kg MK-801 was not evident on Day 4, by the last treatment test day, mice that received coadministration of this dose and ethanol began to show augmented responses compared with mice that received only ethanol. The augmented responses may have contributed to the potentiation of the locomotor sensitization to ethanol observed on Day 16.

The potentiation of the ataxic–sedative effects with high doses of MK-801 may prevent mice from experiencing the interoceptive cues associated with ethanol administration, which may be important for sensitization to develop. In concordance with this, Sripada et al. (2001) have suggested that the ability of NMDA receptor antagonists to block sensitization to psychostimulants may be due to their ability to alter these cues. An association between highly discriminable drugs and their ability to produce state-dependent effects has been suggested (Overton, 1984), and MK-801's discriminative stimulus and state-dependent effects have been demonstrated (Grant, Colombo, Grant, & Rogawski, 1996; Jackson, Koek, & Colpaert, 1992; Tricklebank, Singh, Oles, Preston, & Iversen, 1989). A state-dependency explanation has been offered for MK-801's effects on bromocriptine, morphine, and amphetamine sensitization (Carlezon et al., 2000; Carlezon, Mendrek, & Wise, 1995; Ranaldi, Munn, Neklesa, & Wise, 2000); this explanation states that, in order for sensitization to be expressed, animals must be tested in the same pharmacological state as during drug administration. Stephens, Elliman, and Dunworth (2000) have demonstrated that such a state-dependent sensitization can be observed by coadministering a drug that does not act at NMDA receptors (chlordiazepoxide) with amphetamine. For these reasons, the importance of testing locomotor activity during the repeated treatment phases of sensitization has been emphasized (Tzschentke & Schmidt, 1998, 2000). By collecting data during these treatment days, it is possible to determine whether sensitization to the combination of MK-801 and another drug is occurring, which would suggest, if sensitization was not apparent on the drug challenge day, that the blockade of sensitization had become state-dependent. Data from the treatment days in the present studies (Figures 2 and 3) indicate that sensitization did occur to the combination of both doses of MK-801 with ethanol; the responses of the coadministration groups were significantly higher on the last treatment test day compared with the first treatment test day. For the low MK-801 dose-plus-ethanol group, the expression of behavioral sensitization to ethanol was not state dependent; this group still displayed a sensitized response when challenged with ethanol alone. Mice in the high MK-801 dose-plus-ethanol group appeared to develop sensitization to the combination of the two drugs (Figure 3), although this effect was somewhat delayed. However, on Day 16, when MK-801 was removed and the mice were administered ethanol alone, a sensitized response was not seen. These observations support a state-dependent explanation of MK-801's effects on sensitization. Alternatively, the progressive increase in locomotion could have been due to the development of tolerance to the ataxic effects of this drug combination.

The differential effects of the two doses of MK-801 on rearing in the present experiments support the idea that enhancement of ethanol's intoxicating effects with higher MK-801 doses provides a behavioral explanation for its ability to block sensitization. The high dose of MK-801 significantly enhanced ethanol's effect on rearing, whereas the low dose did not. In addition, although mice that received the low dose of MK-801 and ethanol showed a significant recovery of rearing behavior by Day 13, mice that received coadministration of 0.25 mg/kg MK-801 with ethanol did not show significant changes in this response during the treatment phase. The effects of MK-801 on sensitization are thus paralleled by its ability to potentiate ethanol's effect on rearing, and by the ability of the mice to become tolerant to the effects of the combination of MK-801 and ethanol on rearing behavior. In addition, although measures of activity and rearing may seem mutually exclusive, that is, an animal cannot engage in forward locomotion and rear at the same time, these experiments suggest that this negative correlation is not absolute. First, although ethanol increased locomotion and decreased rearing acutely (a negative correlation between forward locomotion and rearing), both rearing and distance traveled were increased with repeated administrations (a positive correlation). Second, repeated MK-801 resulted in no

changes in forward locomotion, but there was an increase in rearing over days. Conversely, in Experiment 3, repeated administration of the combination of ethanol and MK-801 resulted in no changes in rearing, but there was a progressive increase in forward locomotion over days.

We used the grid test as a more direct measure of ataxia, both to measure the ataxic effects of each drug and of the drug combinations and to examine whether tolerance could have influenced the results obtained in our activity studies. MK-801 potentiated ethanol's ataxic effect in the grid test, although not dose dependently. In addition, there were no differences in the pattern of tolerance development between the groups treated with ethanol and either dose of MK-801, nor was there any effect of MK-801 on the development of ethanol tolerance when measured during the ethanol challenge. Thus, the effects of MK-801 on ethanol tolerance in the grid test did not parallel its effects on ethanol sensitization or tolerance to ethanol's effects on rearing.

The lack of an effect of MK-801 on ethanol tolerance in the grid test suggests that tolerance and sensitization are dissociable, as 0.25 mg/kg MK-801 blocked ethanol-induced sensitization to grid test activity levels, which replicated the findings of Experiment 3. Other researchers have reported a blockade of tolerance to the hypothermic, hypnotic, and incoordinating effects of ethanol by MK-801 (Szabo, Tabakoff, & Hoffman, 1994; Wu et al., 1993). It is likely that tolerance as measured by the grid test is qualitatively different than that measured by techniques such as the rotarod and loss of righting reflex. Szabo et al. (1994) have suggested that learning-dependent, in contrast to learning-independent, tolerance is sensitive to blockade by MK-801. It is possible that the form of tolerance measured by the grid test is learning independent, and thus insensitive to MK-801. However, the current studies were not designed to distinguish between these types of tolerance.

Mice treated repeatedly with ethanol or 0.25 mg/kg MK-801 displayed cross-sensitization to the alternative drug. However, whereas mice sensitized to ethanol, they did not sensitize to MK-801 alone, which is consistent with previous studies in D2 mice (Broadbent & Weitemier, 1999). In addition, the enhanced response to ethanol in MK-801-treated mice relative to that of saline-treated mice may be a result of an unusually low response to ethanol in the saline-treated mice in this experiment. During the MK-801 challenge day, the higher response to MK-801 in ethanol-treated groups suggests that cross-sensitization may be at least asymmetrical. Although behavioral sensitization did not develop to MK-801 alone and the conclusion about symmetrical cross-sensitization is tenuous, these results suggest that the neurochemical systems altered during repeated ethanol treatment overlap with those underlying MK-801's stimulant response. Given that repeated ethanol injections result in cross-sensitization to MK-801, it seems unlikely that coadministration of MK-801 with ethanol blocks ethanol sensitization through blockade of the NMDA receptor.

In conclusion, these experiments show that MK-801, depending on the dose, can produce either a potentiation or an attenuation of ethanol sensitization, in the same experimental paradigm. The potentiation of sensitization with the low dose of MK-801 is in contrast with an NMDA-dependent mechanism for ethanol sensitization. Although the ability of MK-801 to potentiate ethanol-induced reductions in rearing paralleled its ability to block sensitization, MK-801 had no effect on tolerance to ethanol's ataxic effects when directly measured by the grid test. This represents a mechanistic dissociation between ethanol tolerance and sensitization.

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