INTRODUCTION

Sensitization, defined as an increase in the effect of a drug following repeated exposure, has been suggested to play an important role in drug addiction (e.g. Robinson and Berridge, 1993). Indeed, sensitization occurs to many drugs of abuse including ethanol: sensitization to the locomotor stimulant effects of low doses of ethanol has been documented in several mouse genotypes, such as the inbred strain DBA/2J (Broadbent et al., 1995; Cunningham, 1995).

Whereas a large number of studies have examined the neural mechanisms underlying sensitization to opiates and psychostimulants, little is known about the processes mediating sensitization to ethanol. A recent study suggested that NMDA receptors play an important role in the locomotor stimulant effects of ethanol in DBA/2J mice (Shen and Phillips, 1998) in that the NMDA antagonist dizocilpine completely blocked the stimulant effects of ethanol at doses of 0.2 mg/kg and above. Although a large body of evidence also indicates that dizocilpine prevents the development of sensitization to psychostimulant and opiate drugs (see Wolf, 1998), the role of NMDA receptors in sensitization to ethanol has not been examined. The present study therefore assessed the ability of dizocilpine to prevent development of sensitization to ethanol in DBA/2J mice. While DBA/2J mice are known to develop robust sensitization to ethanol, they have also been characterized as ‘alcohol-avoiders’ based on drinking studies, raising questions regarding the relevance of examining sensitization in these mice. However, recent evidence indicates that DBA/2J mice are sensitive to the positive motivational effects of ethanol in that they show robust preference for locations paired with ethanol (e.g. Cunningham et al., 1992) and i.v. self-administration (Grahame and Cunningham, 1997). Thus, DBA/2J mice are a suitable strain in which to examine the mechanisms mediating sensitization and its potential impact on alcohol-seeking behaviour.

MATERIALS AND METHODS

Subjects

Ninety-six male DBA/2J mice (Jackson Laboratory, Bar Harbor, Maine, USA), obtained at 6 weeks of age, were housed in groups of four with food (standard lab chow, PMI Nutrition International, Brentwood, MO, USA) and water freely available. Animals were maintained on a 12 h light/12 h dark
cycle with lights on at 07:00 and a room temperature at 21 ± 1°C.

**Apparatus**

Locomotor activity was measured in activity cages at approximately the same time of day for each subject. Activity cages were placed inside ventilated, sound-, and light-attenuating chambers. Activity was monitored by six infrared photobeams placed at 5-cm intervals, 2 cm above a grid floor. An Apple II computer recorded activity counts. Paper towels placed under the grid floor were replaced after each trial and the floor and cage wiped with a damp sponge.

**Drugs**

(+)-Dizocilpine hydrogen maleate (RBI, Natick, MA, USA) was dissolved in 0.9% (w/v) NaCl (saline) and administered i.p. in a volume of 10 ml/kg. Ethanol (2 g/kg, 20% v/v in 0.9% w/v saline) was administered i.p. in a volume of 12.5 ml/kg.

**Procedure**

The experiment consisted of three phases: a habituation session, four acquisition trials, and two test trials.

**Habituation.** Mice were habituated to the activity cages in a 5-min session before which they received two injections of 0.9% saline, one (10 ml/kg) 30 min before and one (12.5 ml/kg) immediately before the session.

**Acquisition trials.** Forty-eight hours after the habituation session, animals received their first acquisition trial. Animals received either a saline or an ethanol (2 g/kg) injection immediately before the 5-min trial. In addition, 30 min before the trial, mice were given saline or one of three doses of dizocilpine (0.2, 0.25, or 0.3 mg/kg). Doses of dizocilpine were selected that prevent the stimulant effects of ethanol (Shen and Phillips, 1998). Thus, eight experimental groups were produced: saline/saline; saline/ethanol; dizocilpine (0.2 mg/kg)/saline; dizocilpine (0.2 mg/kg)/ethanol; dizocilpine (0.25 mg/kg)/saline; dizocilpine (0.25 mg/kg)/ethanol; dizocilpine (0.3 mg/kg)/saline; and dizocilpine (0.3 mg/kg)/ethanol. Three additional 5-min trials, identical to the first acquisition trial, were conducted at 48-h intervals.

**Test trials.** The first test trial, conducted 48 h after the final acquisition trial, was designed to examine the development of between-group sensitization. All animals received an injection of saline 30 min before the test, and an injection of ethanol (2 g/kg) immediately before the test. Activity was then measured for 30 min. Dizocilpine was not administered before the test trial.

In a second test, the effects of chronic ethanol treatment and of dizocilpine on blood-ethanol levels were examined. Blood samples were taken from four groups of mice: the saline/saline, saline/ethanol, dizocilpine (0.3 mg/kg)/saline, and dizocilpine (0.3 mg/kg)/ethanol groups. The saline/saline and saline/ethanol groups were pretreated with saline, whereas the dizocilpine groups were pretreated with 0.3 mg/kg of dizocilpine. In addition, all groups received an injection of 2 g/kg ethanol 30 min after the first injection. Blood-ethanol samples were taken from each group 10 and 60 min after administration of ethanol.

**Blood ethanol measurement**

Blood-ethanol levels were measured using a gas chromatographic procedure (Phillips et al., 1989). Twenty μl of tail blood were expelled into a cold microcentrifuge tube containing 50 μl of 5% (w/v) zinc sulphate. Fifty μl of 0.3 N barium hydroxide and 300 μl of deionized water were added and the samples centrifuged for 5 min. The supernatant was removed and frozen until ethanol levels were measured.

**Data analysis**

Mean (± SEM) activity scores/min from the acquisition trials and first test trial are presented below. All data were statistically assessed by analysis of variance (ANOVA) followed by Tukey HSD tests. Probability levels of 0.05 or less were considered to be significant.

**RESULTS**

Due to an incorrect injection, one animal was excluded from the experiment.

**Acquisition trials**

Activity levels of the eight groups on each of the four acquisition trials are shown in Fig. 1. Analysis of activity levels on the first trial revealed that dizocilpine alone (left panel) increased activity at all doses. Similarly, administration of ethanol stimulated activity (squares, right panel). All doses
DIZOCILPINE PREVENTS SENSITIZATION T O ETHANOL

Fig. 1. Activity levels on acquisition trials.
Mice received two injections before trials. The four groups represented in the left panel received saline i.p. immediately before each trial. The four groups represented in the right panel received ethanol (2 g/kg) i.p. immediately before each trial. In addition, each group was pretreated i.p. 30 min before each trial with either saline or one of three doses of dizocilpine (0.2, 0.25, or 0.3 mg/kg). n = 10–13/group. Values are means ± SEM of activity counts per minute. Trial pretreatment: — saline; ···○·· dizocilpine (0.20 mg/kg); ···○·· dizocilpine (0.25 mg/kg); ···△·· dizocilpine (0.30 mg/kg).

of dizocilpine, however, prevented ethanol’s stimulant effects (right panel). Analysis of the data from the first trial in a two-way ANOVA (Dizocilpine × Ethanol) indicated a main effect of Ethanol [F(1,187) = 137.4, P < 0.001], main effect of Dizocilpine [F(3,187) = 5.9, P < 0.001], and an interaction [F(3,187) = 71.6, P < 0.001]. Follow-up analysis of the saline groups in a one-way ANOVA revealed a main effect of Dizocilpine [F(3,44) = 35.9, P < 0.001]. Tukey tests confirmed that all doses of dizocilpine stimulated activity compared to animals receiving saline alone (Ps < 0.001). Similar analysis of the ethanol groups revealed a main effect of Dizocilpine [F(3,43) = 49.0, P < 0.001]. Tukey tests demonstrated that all doses of dizocilpine were effective in preventing ethanol-stimulated activity (Ps < 0.001).

Development of within-group sensitization was assessed by examining changes in the activity levels of each group across the four acquisition trials. As expected, mice treated with ethanol alone (squares, right panel, Fig. 1) increased their activity levels across trials, suggesting development of within-group sensitization. The activity of dizocilpine/ethanol groups tended to remain constant or increase slightly across trials (right panel). Analysis of the data from the ethanol groups in a two-way ANOVA (Dizocilpine × Trial) revealed a main effect of Dizocilpine [F(3,43) = 53.6, P < 0.001], a main effect of Trial [F(3,129) = 14.5, P < 0.001] and an interaction [F(9,129) = 2.3, P < 0.025]. Follow-up analyses of each group in separate one-way ANOVAs indicated a main effect of Trial in the saline/ethanol group [F(3,33) = 25.7, P < 0.001], confirming development of within-group sensitization. The dizocilpine (0.3 mg/kg)/ethanol group was the only other group that demonstrated significant increases in activity levels across trials [F(3,33) = 3.7, P < 0.05].

Activity levels of the saline groups tended to remain constant or decrease across trials (left panel, Fig. 1). Analysis of the data in a two-way ANOVA revealed a main effect of Dizocilpine [F(3,44) = 92.0, P < 0.001] and an interaction [F(9,132) = 2.4, P < 0.025]. One-way ANOVAs conducted on each group indicated a main effect of Trial only in the saline/saline group [F(3,33) = 7.8, P < 0.01], suggesting that habituation occurred across trials.

Test trials

Activity levels in each 10-min period of the 30-min sensitization test are shown in Fig. 2. Development of between-group sensitization was assessed by challenging all groups with 2 g/kg of ethanol and comparing activity levels of saline and ethanol groups pretreated with the same dose of dizocilpine on acquisition trials. Between-group sensitization was evident in the saline/ethanol group in that activity levels of this group were elevated compared to the saline/saline group (top left panel, Fig. 2). In contrast, animals treated with ethanol in the presence of dizocilpine on acquisition trials showed similar activity levels to their ethanol-naive control groups, suggesting that sensitization did not develop. A three-way ANOVA (Dizocilpine × Ethanol × Time) demonstrated a main effect of Ethanol [F(1,187) = 10.7, P < 0.01], a significant Dizocilpine × Ethanol interaction [F(3,87) = 4.5, P < 0.01], and a main effect of Time [F(2,174) = 826.8, P < 0.001], but no Dizocilpine × Ethanol × Time interaction. Follow-up one-way ANOVAs comparing mean 30-min test activity
levels of saline and ethanol groups treated with the same dose of dizocilpine revealed significant differences only between the saline/saline and saline/ethanol groups \([F(1,22) = 22.0, P < 0.001]\), indicating that between-group sensitization developed only in the saline/ethanol group.

To assess the possibilities that: (1) sensitization may be due to greater blood-ethanol levels in animals repeatedly exposed to ethanol; (2) the lack of sensitization observed in dizocilpine/ethanol groups may be due to changes in blood-ethanol levels by dizocilpine, blood-ethanol levels were measured in four groups of animals during a second test. Administration of ethanol alone to mice in the saline/saline and saline/ethanol groups resulted in similar blood-ethanol levels 10 min after ethanol injection [mean (± SEM) of 1.53 (± 0.08) and 1.60 (± 0.09) mg/ml respectively], suggesting that the sensitization observed in the saline/ethanol group is not due to alterations in blood-ethanol levels. Administration of 0.3 mg/kg of dizocilpine and ethanol to the dizocilpine (0.3 mg/kg)/saline and dizocilpine (0.3 mg/kg)/ethanol groups resulted in blood-ethanol levels of 1.98 (± 0.07) and 1.74 (± 0.13) mg/ml respectively 10 min after ethanol injection. Analysis of the data from the four groups in a two-way ANOVA (Dizocilpine × Ethanol) revealed only a main effect of Dizocilpine \([F(1,44) = 9.8, P < 0.01]\), indicating that blood-ethanol levels were higher in groups pretreated with dizocilpine.

In contrast, blood-ethanol levels were similar in all groups at the 60-min sample. Mean levels (± SEM) for the saline/saline, dizocilpine (0.3 mg/kg)/saline, saline/ethanol, and dizocilpine (0.3 mg/kg)/ethanol groups were respectively 1.75 (± 0.08), 1.68 (± 0.1), 1.59 (± 0.12), and 1.54 (± 0.08) mg/ml. A two-way ANOVA (Dizocilpine × Ethanol) did not indicate significant differences between these four groups.

**DISCUSSION**

Both within- and between-group sensitization developed in animals treated with ethanol alone, confirming previous reports (Broadbent et al., 1995; Cunningham, 1995). By contrast, animals treated with dizocilpine did not develop sensitization to ethanol. Although the dizocilpine (0.3 mg/kg)/ethanol group showed significant increases in activity across acquisition trials, suggesting within-group sensitization, the increasing activity levels may reflect development of tolerance to dizocilpine’s suppressant effect on ethanol stimulation. Indeed since: (1) this group was treated with the highest dose of dizocilpine, one would predict the least sensitization; (2) sensitization was not observed in this group on the test trial, the simplest explanation of the activity increases seems to lie in the development of tolerance to dizocilpine.

When administered in combination with ethanol, dizocilpine completely prevented the stimulant effects of ethanol, confirming previous reports (Liljequist, 1991; Shen and Phillips, 1998). Thus, an argument could be made that simply blocking
ethanol-stimulated activity prevents development of sensitization. Evidence that (1) the magnitude of the stimulant effect of ethanol is not correlated with development of sensitization across strains of mice (Phillips et al., 1995) and (2) blockade of the acute stimulant response to ethanol by the GABA$_A$ agonist THIP and the D$_2$ antagonist haloperidol (Broadbent et al., 1995; Broadbent and Harless, 1999) does not prevent sensitization, however, suggests dissociation of the neural mechanisms mediating the acute stimulant response and sensitization.

The possibility that dizocilpine prevents development of sensitization to ethanol by decreasing blood-ethanol levels was assessed in the second test. Rather than decreasing ethanol levels, levels were unchanged or slightly increased by dizocilpine administration, suggesting that the lack of sensitization was not due to lower ethanol levels. However, since ethanol has a biphasic dose–response curve, with high doses of ethanol producing less stimulation of activity, the possibility that dizocilpine prevented sensitization by increasing blood-ethanol levels and thus, reducing activity levels on acquisition trials, must be considered. While this possibility cannot be entirely dismissed, it appears unlikely that changes in blood-ethanol levels can account entirely for the observed lack of sensitization, since: (1) increases in blood-ethanol levels in the dizocilpine/ethanol group were relatively small compared to saline/ethanol controls; (2) higher doses of ethanol, which would be expected to produce similar blood-ethanol levels to those found in the dizocilpine/ethanol group, have been reported to produce sensitization (Cunningham et al., 1992; Broadbent and Harless, 1997); (3) as discussed above, changes in the acute stimulant effects of ethanol on acquisition trials do not necessarily prevent the development of sensitization.

In summary, the current study provided initial evidence that NMDA receptors play an important role in the development of sensitization to ethanol. A substantial literature indicates that NMDA receptors are also critically important in the development of sensitization to amphetamine, cocaine, and morphine (for review see Wolf, 1998). Thus, commonalities may exist in the neural processes mediating sensitization to ethanol and other drugs of abuse. Further studies examining the ability of competitive NMDA antagonists, as well as specific AMPA and metabotropic glutamate receptor antagonists to prevent ethanol sensitization, drugs which have recently been shown to prevent sensitization to psychostimulants (Karler et al., 1991; Ohmori et al., 1994; Kim and Vezina, 1998), will provide additional evidence of similarities in the mechanisms mediating sensitization to ethanol and other abused drugs.

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REFERENCES


