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Investigation of a possible sensitization development to a challenge dose of ethanol after 2 weeks following the single injection in mice

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Abstract

In the present study, a possible sensitization development to a single injection of ethanol in mice was investigated. Subjects were adult male Swiss–Webster mice. Ethanol (0.5–4 g/kg) or saline (control) was intraperitoneally injected to mice. Horizontal, vertical and ambulatory locomotor activities were recorded for 30 min immediately following the ethanol or saline injections. After 2 weeks, each group of mice was randomly assigned to two groups. A single challenge dose of ethanol (1 g/kg) was administered to the first group, and saline was injected to the second group. Then, the locomotor activities were recorded for 30 min. In the first experiment, ethanol significantly increased the horizontal and ambulatory activities of the mice at the doses of 0.5 and 1 g/kg, but not at 2 g/kg, while they were decreased at the dose of 4 g/kg. Ethanol (0.5 g/kg) also significantly increased the vertical activity. After 2 weeks, the challenge injection of ethanol (1 g/kg) produced some significant increases in the horizontal and ambulatory activities of the group pretreated with ethanol (2 g/kg). It did not cause any significant change on the locomotor activities of the other three groups treated with lower (stimulant) or higher (depressant) doses of ethanol. In addition, there was no significant difference between locomotor activities of the groups challenged with saline. However, a two-way ANOVA of the data on the challenge injections did not indicate any sensitization development to the effects of ethanol on locomotor activities of the mice. Our results suggest that a locomotor sensitization did not develop to a single injection of ethanol after 2 weeks following the first injection in mice.

Keywords: Ethanol; Locomotor activity; Locomotor sensitization; Mice

1. Introduction

Low doses of ethanol produce locomotor stimulant effects in rodents (Frye and Breese, 1981). Ethanol-induced locomotor stimulation has been proposed to positively correlate with the rewarding effects of ethanol (Wise and Bozarth, 1987; Koob, 1992; Phillips and Shen, 1996). Repeated administration of addictive drugs such as ethanol tends to result in progressive augmentation of the initial locomotor stimulant response to these drugs, and this phenomenon is termed behavioral sensitization (Stewart and Badiani, 1993; Lessov and Phillips, 1998). Sensitization can be viewed as an adaptive change, permitting facilitation within a system and making response induction easier on future experiences by stimuli having access to it (Stewart and Badiani, 1993). The role of sensitization in dependence has been elaborated to explain the changes in motivation for drug seeking, which reflects compulsive use, and the thought that there is a shift in an incentive salience state (defined as a hypersensitive neural state that produces the experience of "drug wanting") (Koob and Le Moal, 1997; Robinson and Berridge, 2001). Some recent studies indicate that sensitization may be important in humans, as well as in laboratory animals, in the development of dependence to psychostimulants such as amphetamine (Strakowski and Sax, 1998; Strakowski et al., 1996; Robinson and Berridge, 2001).

The development of sensitization is dependent on the treatment regimen; repeated, intermittent treatments with relatively high doses of addictive drugs in a different place are more effective to produce sensitization than continuous treatment with low doses of drugs at the same places where

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mice housed (Robinson and Berridge, 2000). Many studies on sensitization development to locomotor stimulant effects of ethanol by repetitive injections in mice have been reported (Itzhak and Martin, 2000; Lessov and Phillips, 1998; Phillips et al., 1995; Souza-Formigoni et al., 1999). Interestingly, even a single dose of amphetamine (Robinson et al., 1982; Vanderschuren et al., 1999) or cocaine (Jackson and Nutt, 1993) has been found enough to induce locomotor sensitization. Vanderschuren et al. (1999) observed a sensitization development to the locomotor stimulant effect of amphetamine after a long, drug-free period, for example, 3 weeks. However, studies that investigate locomotor sensitization induced by a single injection of ethanol after a long, drug-free period were limited. The present study was designed to investigate a possible locomotor sensitization development to a single treatment of ethanol without repetitive injections in mice after a 2-week, ethanol-free period. Ethanol's effects and possible sensitization development were also evaluated on the three different components of locomotor activity, namely horizontal, vertical and ambulatory activities.

2. Materials and methods

2.1. Animals and laboratory

The experiments performed in this study were carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health (USA) and the Declaration of Helsinki. Adult male Swiss–Webster mice (28–35 g) were used in our study. They were placed in a quiet and temperature- and humidity-controlled room (22 ± 2 °C and 60 ± 5%, respectively) in which a 12/12-h light–dark cycle was maintained (light on from 07:00 to 19:00 h). Mice were housed eight per cage in Plexiglas cages. Food and water were available ad libitum.

All experiments were performed at the same time and during the light on period (09:30–11:00 h).

2.2. Drug and apparatus

Ethanol was purchased from Merck Chemical (USA). It was diluted to 20% (v/v) in saline and injected to mice at appropriate volumes. Ethanol solutions were prepared freshly in the morning. Locomotor activities were measured with an open-field activity monitoring system (MAY 9908 model, Activity Monitoring System, Commat, TR). This system had eight Plexiglas cages (42 × 42 × 30 cm) equipped with infrared photocells. Fifteen photocell emitter and detector pairs were located 2 cm above the floor at intervals of 2.5 cm on both counter sides of each activity cage, and another 15 photocell pairs were located 8 cm above the floor. Interruptions of photocell beams were detected by a computer system, and place of animal was calculated by the software at 0.1-s sensitivity. If a calculated place was changed completely, then it presented the ambulatory activity. Other behaviors that caused some interruptions of beams, but not a change in place, presented the horizontal activity. Vertical activity, like rearing, was detected by the photocells located 8 cm above the floor.

2.3. Procedure

Ethanol (0.5, 1, 2 and 4 g/kg) or saline was injected to the naive (never been exposed to any injection or activity cages before) mice intraperitoneally in the test room ($n = 16$ for each group). Locomotor activities (horizontal, vertical and ambulatory) were recorded for 30 min immediately following the ethanol injections. Then, the mice were put in their home cages and were placed in a room that is different from the test room. After 2 weeks, each group of mice randomly assigned into two groups ($n = 8$ for each). The 1st group of mice was injected with saline, while the 2nd group was injected with a single challenge dose of ethanol (1 g/kg). Locomotor activities were also recorded for 30 min immediately following the challenge injections.

2.4. Statistics

Data were expressed as means ± S.E.M. of horizontal, vertical and ambulatory locomotor activities. The dose-dependent effects of ethanol on the locomotor activities on the first day, and differences between the responses of the same mice to the challenge dose (1 g/kg) of ethanol or saline after 2 weeks, were evaluated by a one-way ANOVA followed by Dunnett's test for post hoc analysis. A two-way ANOVA was also used to compare the effects of ethanol and saline challenges on horizontal, vertical and ambulatory activities. The level of statistical significance was set at $P < .05$.

3. Results

The effects of various single doses of ethanol on the horizontal, vertical and ambulatory activities of the mice are shown in Fig. 1A–C. Ethanol (0.5–4 g/kg) caused some significant changes on the horizontal, vertical and ambulatory activities as compared to mice treated with saline [$F(4,75) = 29.443$, $F(4,75) = 8.817$ and $F(4,75) = 34.457$, respectively, $P < .0001$; one-way ANOVA]. Post hoc analysis of the data by Dunnett's test indicated that ethanol (0.5 and 1 g/kg) produced some significant increases in the horizontal ($P < .0001$ and $P = .008$) and ambulatory ($P < .0001$ and $P < .0001$) activities (Fig. 1A and C). However, in the vertical activity, while ethanol (0.5 g/kg) produced a significant increase ($P < .0001$), it did not cause any significant change at dose of 1 g/kg ($P = .317$) (Fig. 1B). Ethanol (2 g/kg) did not cause any significant change on the horizontal, vertical and ambulatory activities ($P = .277$, $P = .996$, $P = .101$, respectively) (Fig. 1A–C). The higher dose of ethanol (4 g/kg)

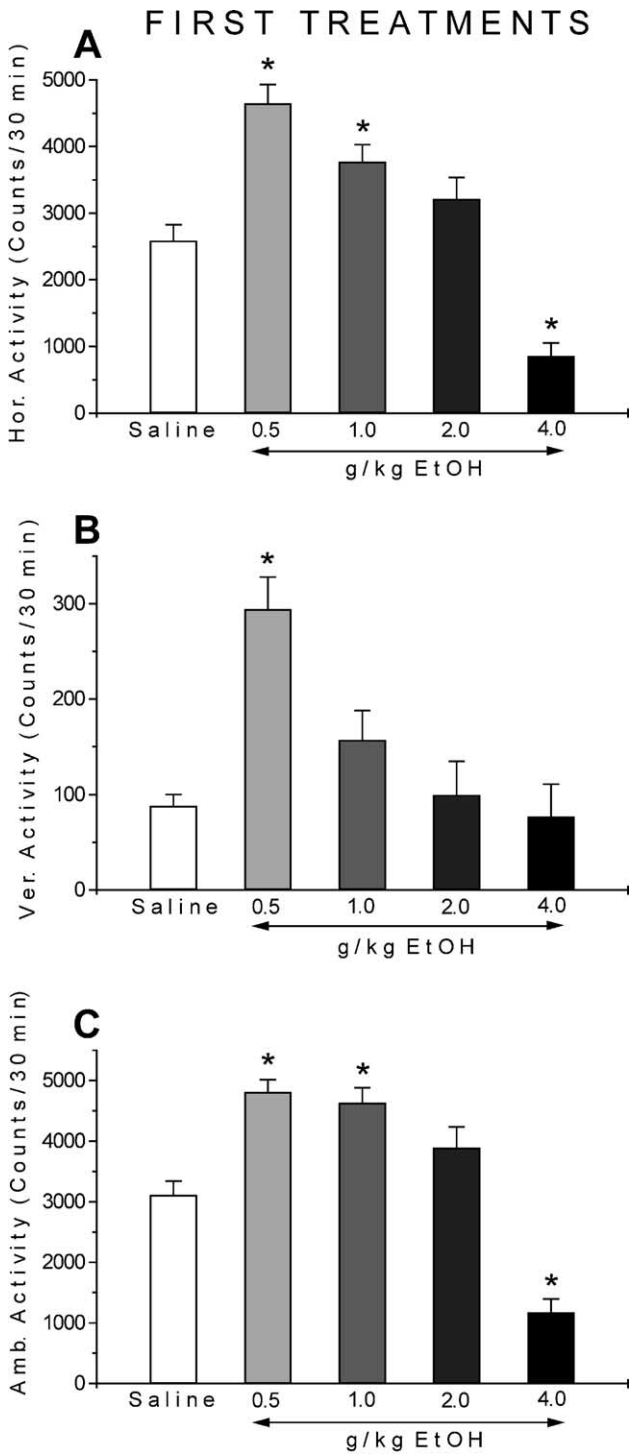

Fig. 1. Acute effects of ethanol on locomotor activity in mice. Means ± S.E.M. of horizontal (A), vertical (B) and ambulatory (C) activity counts recorded for 30 min immediately following the saline or ethanol injections ($n = 16$ for each group). * $P < .05$, significantly different from saline (Dunnett's test).

decreased significantly horizontal ($P < .0001$) and ambulatory ($P < .0001$) activities of the mice (Fig. 1A and C). This dose of ethanol did not cause any significant change on the vertical activity ($P = .997$) (Fig. 1B).

The effects of challenge injections of ethanol (1 g/kg) and saline on the locomotor activities of the mice pretreated with saline or ethanol are shown in Figs. 2A–C and 3A–C, respectively. Ethanol or saline pretreatment to the mice altered the horizontal and ambulatory locomotor responses

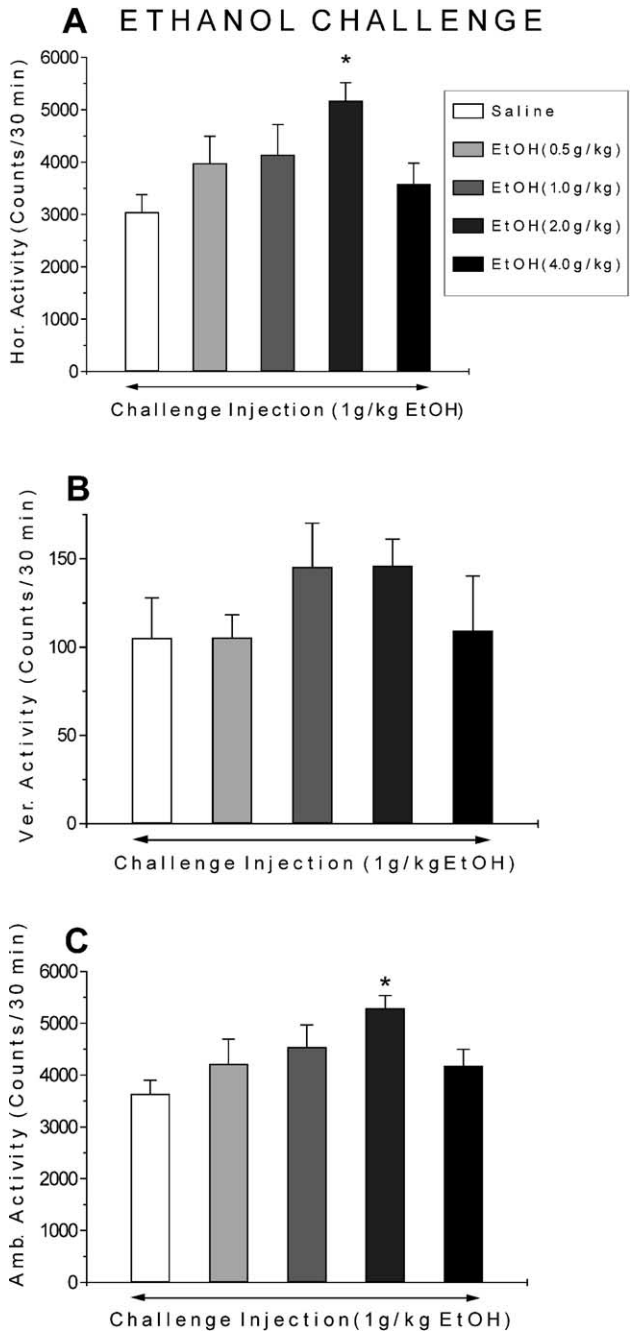

Fig. 2. The effects of ethanol challenge (1 g/kg) on locomotor activity of the mice, which administered saline or ethanol (0.5–4 g/kg) 2 weeks ago. Means ± S.E.M. of horizontal (A), vertical (B) and ambulatory (C) activity counts recorded for 30 min immediately following the saline or ethanol injections ($n = 8$ for each group). * $P < .05$, significantly different from saline (Dunnett's t test).

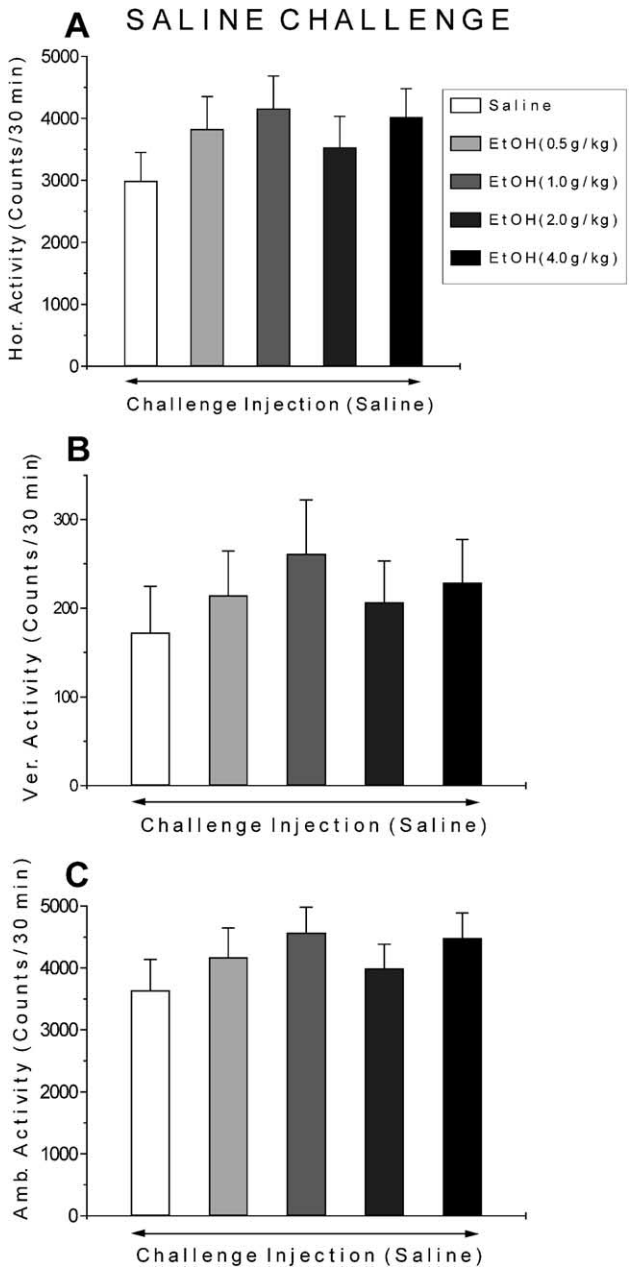

Fig. 3. The effects of saline challenge on locomotor activity of the mice, which administered saline or ethanol (0.5–4 g/kg) 2 weeks ago. Means ± S.E.M. of horizontal (A), vertical (B) and ambulatory (C) activity counts recorded for 30 min immediately following the saline or ethanol injections ($n = 8$ for each group).

to the challenge injection of ethanol [$F(4,35) = 3.287$, P=.022 and $F(4,35) = 3.017$, P=.031, respectively; one-way ANOVA] (Fig. 2A and C); but the pretreatment did not alter these responses to the challenge injection of saline [$F(4,35) = 0.881$ and $F(4,35) = 0.751$, respectively, $P > .05$; one-way ANOVA] (Fig. 3A and C). Vertical activity of the mice was not affected by ethanol (1 g/kg) or saline challenge [$F(4,35) = 1.035$ and $F(4,35) = 0.390$, respectively, P>.05; one-way ANOVA]. Post hoc analysis of the ethanol challenge data by Dunnett's test indicated a significant difference in the horizontal (P=.005) and ambulatory (P=.007) activities of the mice treated with 2 g/kg single dose of ethanol 2 weeks ago. Challenge injection (1 g/kg ethanol) to the other three groups (pretreated with 0.5, 1 and 4 g/kg ethanol) did not cause any significant change on the horizontal, vertical and ambulatory activities as compared with saline-pretreated mice.

For challenge injections, the two-way ANOVA performed on the vertical activity revealed a significant effect for treatment [$F(1,70) = 14.172$, P=.0003] but not for pretreatment dose [$F(4,70) = 0.710$, P=.587] and their interaction [$F(4,70) = 0.254$, P=.900]. The two-way ANOVA performed on the horizontal and ambulatory activities did not indicate any significant effect for treatment [$F(1,70) = 0.888$, P=.349 and $F(1,70) = 0.654$, P=.421, respectively], pretreatment dose [$F(4,70) = 2.405$, P=.057 and $F(4,70) = 2.013$, P=.102, respectively] and their interaction [$F(4,70) = 1.452$, P=.226 and $F(4,70) = 1.252$, P=.297, respectively].

4. Discussion

The present study suggests some significant increases in the horizontal and ambulatory activities of the mice by a challenge dose of ethanol after 2 weeks following the single injection. However, there are no significant effects for treatment, pretreatment dose and their interaction by a two-way ANOVA. Thus, these locomotor increases could not be interpreted as a sensitization development.

In the present study, in contrast to horizontal and ambulatory activities, ethanol (1 and 4 mg/kg) did not produce any significant change in the vertical activity of the mice during the first injections. Furthermore, vertical activity was also not affected by challenge injection of ethanol. These findings indicate that ethanol may have different effects on the various components of locomotor activity in mice. In addition, horizontal and ambulatory activities may be more sensitive to the locomotor effects of ethanol in mice.

Our results indicating low doses of ethanol significantly stimulated locomotor activity in mice confirm previous findings. Many studies have reported that high doses of ethanol reduced locomotor activity in rodents, whereas low doses between 0.5 and 1.6 g/kg served as stimulant in several strains of mice (Matchett and Erickson, 1977; Koechling et al., 1990, 1991; Ng and George, 1994).

Psychomotor stimulant properties of the central nervous system acting drugs are accepted as addictive (Wise and Bozarth, 1987; Koob, 1992; Phillips and Shen, 1996). Although ethanol is commonly classified as a sedative compound, at relatively small doses, it has stimulant effects on locomotor activity (Kornetsky et al., 1988). However, acute locomotor stimulant properties of ethanol may not be necessary to induce development of sensitization. A repeated injection study by Broadbent et al. (1995) indicated that sensitization development could not be prevented even by

blocking ethanol's acute locomotor stimulant effect by haloperidol in mice. It is known that, in repetitive treatment protocols, relatively high doses are more effective to induce behavioral sensitization (Robinson and Berridge, 2000). In the present study, we used a dose range of ethanol from stimulant to sedative (0.5–4 g/kg). This dose range of ethanol was also used in various sensitization studies performed in mice (Masur and Boerngen, 1980; Risinger and Oakes, 1996; Lessov and Phillips, 1998). Like repetitive injection studies (Masur and Boerngen, 1980; Risinger and Oakes, 1996; Lessov and Phillips, 1998), we did not observe any significant locomotor sensitization by the low and stimulant doses of ethanol. In our study, we observed some increases in horizontal and ambulatory locomotor responses to a higher but ineffective dose (2 g/kg) of ethanol. However, these increases in locomotor activities could not be interpreted as a sensitization development when the data was analyzed by a two-way ANOVA. Inappropriate challenging time (2 weeks) or different methodological practice (i.e. repetitive or intermittent exposure to ethanol) may be responsible for no sensitization developments in the present study.

In sensitization studies, one of the important parameters is the time between the last administration of repeating treatments and challenge injection. It is typical that sensitization is more evident when that period is longer than a week (Manley and Little, 1997; Robinson and Berridge, 2000). Lessov and Phillips (1998) suggested that repetitive ethanol injections induce locomotor sensitization even at the 29th day posttreatment. We administered the challenge injections 2 weeks later from the first injections. A period of 2 weeks for challenge injections was commonly used in many sensitization studies with repetitive injections (Pecins-Thompson and Peris, 1993; Manley and Little, 1997; Lessov and Phillips, 1998; Itzhak and Martin, 1999).

In the present study, ethanol (1 g/kg) caused submaximal locomotor stimulation in the first experiment. We used only this dose of ethanol for challenge experiments. If the more stimulant (0.5 g/kg) or depressant (4 g/kg) doses of ethanol were selected as the challenge doses, the possible sensitization producing effects on the locomotor activity could be masked during the challenge experiments.

In conclusion, our results suggest some increases in horizontal and ambulatory activities, but not in vertical activities, of the mice to a single dose of ethanol after 2 weeks following the first injection. However, these increases cannot be interpreted as a locomotor sensitization development.

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