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Effects of acute alcohol administration on object recognition learning in C57BL/6J mice

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Abstract

The goal of the present study was to investigate effects of alcohol intoxication on the object recognition learning task. Male C57BL/6J mice habituated to saline injections and exploratory arena received different doses of ethanol (0, 1.6 or 2.4 g/kg) before or after a 10-min training session. During training, animals were exposed to a small object (a marble or a die). On the next day, during a 10-min testing session, animals were exposed to two objects: the familiar object from the previous day and a novel object. Analysis of behavior during testing showed that mice injected with 0 and 1.6 g/kg of ethanol before training spent more time exploring a novel than a familiar object during testing. In contrast, mice injected with 2.4 g/kg ethanol spent equal amounts of time exploring the novel and the familiar object. Mice injected with this dose of ethanol after training did not show a decreased ratio of object exploration during testing. Analysis of behavior during training showed that mice injected with this dose of ethanol spent less time exploring the object, although their locomotor activity was not decreased. Our results show that in C57BL/6J mice, ethanol intoxication interferes with exploratory activity during object exploration, but not with consolidation of memory.

Keywords: Learning; Memory; Amnesia; Ethanol; Exploration; Consolidation

1. Introduction

Acute ethanol intoxication is known to produce memory impairment (for recent reviews, see Browning et al., 1992; Ryabinin, 1998; White et al., 2000). Animal studies suggest that ethanol's effect on memory formation could be due to its effect in the hippocampal formation. Indeed, studies directly comparing learning paradigms have shown preferential attenuation of hippocampus-dependent versus hippocampus-independent tasks following ethanol administration (Matthews et al., 1995; Melia et al., 1996). This idea is contradicted, however, by studies showing that hippocampal lesions do not interfere with ethanol's effect on behavior (Devenport and Hale, 1989). Another contradictory view is that ethanol modulates performance of the animal during the training task rather than impairs the processes of memory acquisition and consolidation (Cunningham and Brown, 1983). The difficulty to understand the mechanisms of ethanol's effect on learning stems partly from the complexity of ethanol's effects on different behavioral components of learning. For example, many learning tasks involve strong external reinforcement (e.g., foot shock, food deprivation or swim stress), which themselves could be perturbed by ethanol intoxication. It would be advantageous to test ethanol's effect in tasks requiring minimal external reinforcement.

One of the few learning tasks requiring minimal external reinforcement is object recognition. This task is based on a natural tendency of animals to preferentially explore novel versus familiar objects (Ennaceur and Delacour, 1988; Myhrer, 1988; Phillips et al., 1988). Hippocampal involvement in this task has been supported by lesion studies (Myhrer, 1988; Phillips et al., 1988; Mumby et al., 1996) (however, see examples of hippocampus-independent object recognition tasks; Aggleton et al., 1986; Mumby et al., 1992). Effects of acute ethanol intoxication on object recognition memory have not been addressed previously.

This study investigates the effects of ethanol on object recognition memory in C57BL/6J mice and shows that high doses of this drug interfere with exploratory activity necessary for this task, but not with consolidation of memory.

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2. Methods

2.1. Subjects

All animal procedures were approved by the Institutional Animal Use committee. Seven- to eight-week-old male C57BL/6J mice were purchased from the Jackson Laboratories (Bar Harbor, ME, USA) and housed three to four per cage under normal light cycle (12:12, lights on at 6:00 AM). Water and food were provided ad libitum. After 1 week of acclimatization to the home cage, mice were habituated to intraperitoneal (ip) injections consecutively by three handling sessions (one per day), three to four sham injections (one per day), three injections of half volume of saline (one per day), and three to four injections of full volume of saline (14 ml/kg, one per day). Our previous studies showed that such procedure leads to a complete habituation of the c-Fos response to acute injection stress in C57BL/6J mice (Ryabinin et al., 1999). Animals were weighed on the last day of habituation to sham injections. Animals were habituated to the training arena by being placed into the arena for 15 min during the last three saline injection habituation sessions. The training arena was a bedding-free Plexiglas cage with dimensions of $44 \times 24 \times 20$ cm placed inside a cardboard box of similar size with a 3.0-cm grid drawn on the floor. The arena was located in a testing room dimly lit by outside light (approximately 20–30 lx inside the arena).

2.2. Injections

On the training day, mice received intraperitoneal injections of 0, 13 or 20% ethanol in saline (v/v, 14 ml/kg) resulting in 0, 1.6 or 2.4 g/kg of ethanol. In the pretraining injection experiment, animals were injected 2 min before being placed into the training arena. In the posttraining injection experiment, animals received the injections immediately after being removed from the training arena. Experimental groups contained following number of animals. Pretraining injection: 0 g/kg — 16 mice, 1.6 kg — 12 mice, 2.4 g/kg — 12 mice; posttraining injection: 0 g/kg — 11 mice, 1.6 g/kg — 12 mice, 2.4 g/kg — 12 mice. Animals were treated identically during the preceding habituation sessions except that ethanol was substituted for saline in the experimental groups. Similarly, during testing, animals were injected with saline in the same order as on the day of training.

2.3. Training

Training was performed by placing the animals individually one by one in the middle of the training arena with one object located on one side of the arena 8 cm away from the far wall. The object was either a white $1 \times 1 \times 1$ cm die or a blue flattened marble of similar size. These objects were selected on the basis of previous observations, which demonstrated a lack of preferential exploration for one object over the other. The objects were not attached to the apparatus. However, none of the mice in our experiments moved the objects from its original position. Objects were washed with water after each individual session during both training and testing. Training time lasted for 10 min.

2.4. Testing

Testing was performed by placing the animal in the middle of the same arena with two objects. Objects were placed on opposite sides 8 cm from the short walls. The choice of object as new or familiar and the position (side of the arena) of the object was switched from one animal to another. Testing lasted for 10 min.

2.5. Behavioral analysis

Animals' behavior was filmed on a video camera mounted on a tripod 1 m over the training arena during both training and testing (the camera was also present in the same location during habituation). Video tapes were analyzed by an experimenter blind to each animal's experimental condition, although the decreased coordination of animals injected 2.4 g/kg of ethanol allowed some measure of distinction from other groups.

The following parameters were measured. Object exploration time: time the animal spent with its nose within 0.5 cm from the object, this measure was observed continuously for five consecutive 2-min intervals. Locomotor activity: number of line crossings counted using the 3-cm grid. Rearing: time the animal spent standing in an upright position without leaning against a wall. Leaning: time the animal spent in an upright position leaning against a wall. Grooming: time the animal spent grooming.

The ratio of object exploration times was calculated as object exploration time with a novel object during testing divided by object exploration time with a familiar object during testing. Such ratio is considered to be the main measure of retention in this task (Ennaceur and Delacour, 1988).

Data were analyzed using multifactorial ANOVA using dose (0, 1.6 or 2.4 g/kg) and time of injection (after or before training) as between-subjects factors and interval of training (1–5) as a repeated measure. Where appropriate post hoc analysis was performed using Fisher's PLSD test with P values of less than .05 considered statistically significant.

3. Results

3.1. Object exploration

Analysis of object exploration showed that saline-injected mice spent only approximately 5% of the time in the arena exploring the objects (Fig. 1). During the training

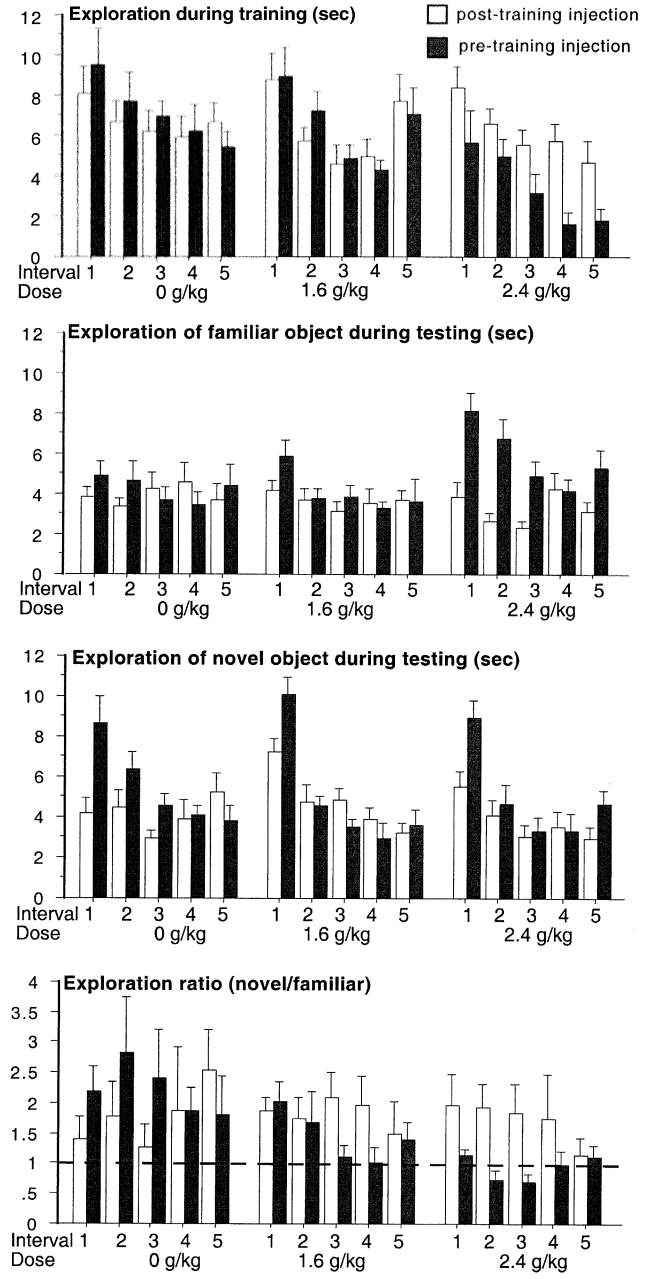

Fig. 1. Object exploration during training and testing as a function of ethanol dose (0–2.4 g/kg), session interval (1–5) and injection time (after training — open bars; before training — closed bars). Note the lower exploration during training and lower ratio of novel versus familiar object in mice injected 2.4 g/kg before training. Dashed line indicates chance exploration ratio.

session, exploration gradually decreased with time, as reflected by the significant effect of interval on exploration [$F(4,334)=9.4$, $P<.0001$]. The time of saline injection (before or after training) did not affect the amount of time mice spent exploring the object. However, mice injected 2.4 g/kg of ethanol before the session spent significantly less time exploring the object than other groups. This was reflected by the significant effect of dose on exploration times [$F(2,334)=10.3$, $P<.0001$] and the significant interaction between dose and injection time [$F(2,334)=6.6$, $P<.01$]. Post hoc analysis confirmed that animals preinjected with 2.4 g/kg ethanol explored the object significantly less than other groups.

During testing, the majority of mice spent more time exploring the novel object. However, mice injected 2.4 g/kg before training spent similar amount of time exploring novel and familiar objects. This was reflected by the significant effects of dose on the exploration ratio [$F(2,334)=4.3$, $P<.05$] and the significant interaction between dose and time of injection [$F(2,334)=3.8$, $P<.05$]. A post hoc analysis confirmed that the exploration ratio was significantly lower in this group than in other groups. Analysis of exploration of the familiar object (but not of the novel object) showed significant interaction between dose and injection time [$F(2,334)=8.2$, $P<.001$]. Post hoc analysis showed that this interaction occurred because of significantly higher exploration times with the familiar object in animals injected with the higher ethanol dose prior to training than in all other groups.

In addition, there was a significant effect of interval on exploration of the novel [$F(4,33)=26.3$, $P<.0001$] and of the familiar [$F(4,33)=3.8$, $P<.01$] object caused by higher exploration times during the first minutes of testing. A significant interaction between injection time and interval for exploration of familiar [$F(4,334)=3.3$, $P<.05$] and novel [$F(4,334)=6.2$, $P<.0001$] objects was due to higher exploration of objects during the first intervals in the arena in mice injected any solution before training versus animals injected posttraining. This was confirmed by post hoc analysis showing significant difference in exploration of both familiar and novel objects between pre- and postinjected animals during Intervals 1 and 2. However, no effects of injection time on exploration ratio or interactions between injection time and interval for this measure were found ($P>.05$). Similarly, effects of other factors or interactions between them were not statistically significant.

3.2. Other behaviors

Animals injected before training showed higher locomotor activity scores than animals injected posttraining during both the training [$F(1,53)=18.2$, $P<.0001$] and the testing [$F(1,53)=12.1$, $P<.01$] sessions. There was also a significant effect of dose [$F(2,53)=10.8$, $P<.001$] and significant interaction between dose and injection time [$F(2,53)=6.8$, $P<.01$] on locomotor activity during training. Post hoc analysis indicated that these effects were due to significant differences in locomotor activity between animals preinjected with 1.6 g/kg of ethanol and other groups (Fig. 2).

Alcohol exhibited a suppressive effect on rearing during training [$F(2,53)=9.3$, $P<.001$]. Animals receiving injections posttraining showed significantly less rearing during training [$F(1,53)=7.3$, $P<.01$] and significantly more

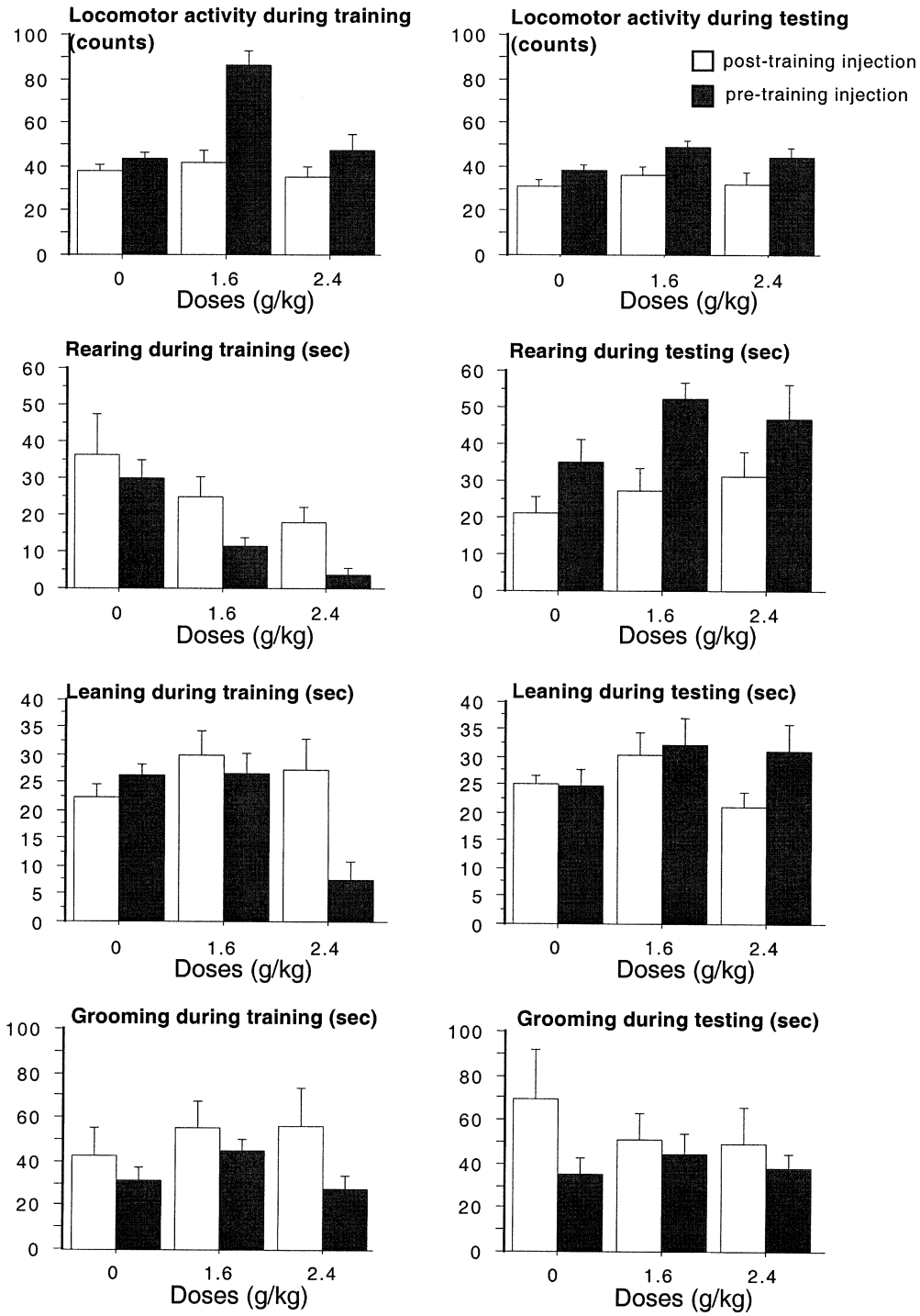

Fig. 2. Horizontal locomotor activity, vertical activity (rearing and leaning) and grooming as a function of ethanol dose (0–2.4 g/kg) and injection time (after training — open bars; before training — closed bars). Note the higher levels of locomotor activity in animals injected 1.6 g/kg prior to learning and lack of locomotor-suppressive effects in animals injected 2.4 g/kg of ethanol.

rearing during testing [$F(1,53)=9.1$, $P<.01$]. There was a significant effect of dose [$F(2,53)=4.3$, $P<.05$], injection time [$F(1,53)=4.3$, $P<.05$], and interaction between dose and injection time [$F(2,53)=4.8$, $P<.05$] on the leaning measure during training. Post hoc analysis indicated that these effects on leaning were due to significantly lower scores in animals preinjected with 2.4 g/kg than in all other groups.

Animals injected posttraining showed less grooming than animals injected before training independently of the dose [$F(1,53)=4.7$, $P<.05$]. Effects of other factors or interactions were not significantly different.

4. Discussion

Our results confirm that acute ethanol administration interferes with learning. Moreover, injecting ethanol prior and posttraining allowed us to compare ethanol ability to interfere with memory acquisition versus consolidation. In the present study, administration of 2.4 g/kg of ethanol only prior to training inhibited preferential exploration of novel versus familiar object during testing. This inhibition of preferential exploration of a novel object could not be attributed to a decrease in locomotor activity on the day of testing. In fact, all other parameters measured on the day of testing besides exploration of the objects were not affected by dose of the drug, suggesting that animals of the 2.4 g/kg group behaved normally on the next day after the injection, but did not have a memory for the familiar object. In contrast, animals injected ethanol posttraining showed higher exploration times with the novel object resulting in preferential exploration of novel versus familiar object. This finding strongly argues for ethanol's interference during memory acquisition, rather than during consolidation.

Depending on particularities of procedure, the object recognition task can test either hipoccampus-dependent (Myhrer, 1988; Mumby et al., 1996; Clark et al., 2000; Rampon et al., 2000) or -independent memory (Aggleton et al., 1986; Mumby et al., 1992). The procedure used in the present study is closer to those showing hippocampal dependence. Therefore, this result could be in agreement with previous studies showing suppressive effects of acute ethanol on other hippocampal forms of memory, such as contextual fear conditioning, passive avoidance and spatial working memory in laboratory animals (Bammer and Chesher, 1982; Hernandez and Powell, 1986; Nabeshima et al., 1988; Melchior et al., 1993; Givens, 1995; Matthews et al., 1995; Melia et al., 1996). However, our study suggests that ethanol suppresses acquisition of memory not by interfering with the process of memory encoding, but by interfering with exploratory activity necessary to encode the memory. Thus, the time spent exploring a single object on the day of training was significantly decreased in the 2.4-g/kg group in comparison to other animals. Decreased exploration of this object could lead to decreased encoding of its properties, and, hence, lack of memory.

What are the mechanisms of ethanol's suppression of object exploration? This effect seems not to be mediated by suppression of locomotor activity because locomotor activity counts were not different in animals injected 2.4 versus 0 g/kg of ethanol. Similarly, injection of 1.6 g/kg of ethanol increased locomotor activity during training, which did not result in increased object exploration, a result in agreement with previous findings of locomotor-stimulating effects of ethanol in C57BL/6J mice (Crabbe et al., 1982; Middaugh et al., 1987, 1992; Bachtell and Ryabinin, 2001). Ethanol's effects on vertical activity (leaning and rearing) observed in this study are also in agreement with previous studies (Krsiak, 1976; Smoothy and Berry, 1984). Rearing and leaning can also be used as indicators of exploratory activity, but ethanol effects on these measures could be contaminated by effects on coordination and vestibular function. Since our measure of object exploration is simple proximity of the animal's nose to the object, it is difficult to envision how lack of coordination would interfere with object exploration. Perhaps a more likely possibility is that ethanol attenuated object exploration by decreasing motivation to explore the object. Interestingly, the present study was performed because of the initial concern that ethanol might interfere with motivational aspects involved in other types of learning. Our results suggest, however, that although object recognition requires much less external reinforcement than many other learning tasks, the minimal motivation that is required to explore the objects is perturbed by ethanol intoxication. Therefore, the seeming advantage of object recognition task over other ways to assess ethanol's effects on learning was not confirmed.

Decreased motivation to explore the objects could be mediated by either sedative or aversive (anxiogenic) effects of ethanol. Interestingly, different levels of locomotor activity, rearing and grooming observed in mice injected any dose of ethanol (including 0 g/kg) before or after training suggest that these animals had different levels of anxiety. However, this difference did not seem to interfere with the ratio of exploration of novel versus familiar object during testing. It seems more likely, therefore, that ethanol's effect on object exploration was due to sedative than due to aversive effects of ethanol.

Mechanisms of these effects should be addressed in further independent studies where the aversive effects of ethanol could be minimized, possibly after alcohol self-administration in mice.

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