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Research article

Environmental enrichment blocks reinstatement of ethanol-induced conditioned place preference in mice

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HIGHLIGHTS

- The effect of training dose on the extinction of ethanol-induced CPP was investigated in mice.
- The effect of training dose on the relapse of ethanol-induced CPP was investigated in mice.
- Environmental enrichment blocked the reinstatement of ethanol-induced CPP.
- Environmental enrichment did not affect the extinction rate of ethanol-induced CPP.

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ABSTRACT

This study aimed to explore the effect of environmental enrichment (EE) on the reinstatement of ethanol-induced conditioned place preference (CPP) in C57Bl/6J mice. To investigate the effect of training dose on the extinction and relapse of ethanol-induced CPP, doses of ethanol were applied and we found 0.8 g/kg and 1.6 g/kg training doses lead to significant CPP. In the reinstatement procedure, previously extinguished 1.6 g/kg ethanol CPP could be markedly reinstated by a priming injection of 0.8 g/kg. In contrast, priming with 0.4 g/kg of ethanol failed to reinstate the CPP induced by 0.8 g/kg. To investigate whether concomitant EE exposure could prevent the reinstatement of ethanol-induced CPP, one half of the mice were housed in standard environment (SE) and the other half in EE during the extinction and reinstatement session in the second experiment. Our study showed that reinstatement of ethanol-induced CPP was blocked by EE and the extinction rate was the same between SE and EE mice. These findings suggest that EE can block reinstatement of ethanol-induced CPP in mice, and aiding in the identification of new therapeutic strategies for alcohol addiction.

1. Introduction

Ethanol addiction is a chronic relapsing brain disease characterized by compulsive drug-seeking and drug-taking behaviors despite adverse consequences [28]. One of the major challenges in the treatment of alcoholism and other addictions is the prevention of relapse. After detoxification, up to 85% of the ethanol-dependent patients return to excessive drinking [4]. To date, the anti-craving drugs acamprosate and naltrexone as well as the aversion therapeutic drug disulfiram are available for ethanol dependence treatment, but these pharmacotherapy are still very constrained because of their limited efficacy, side effects and low patient compliance [7]. Considering the current therapeutic limitations, it is necessary to develop pharmacologic and non-pharmacologic strategies for alcoholism.

Environmental conditions can dramatically influence the behavioral and neurochemical effects of drugs of abuse. Epidemiological and preclinical studies suggest that negative environmental conditions such as stress increases the vulnerability to addiction [23], while positive conditions such as environmental enrichment (EE) can reduce the activating and reinforcing effects of psychostimulants and may prevent the development of drug addiction [30]. Studies in rodents have clearly demonstrated the capacity for EE on addiction-related behaviors. Exposure to EE decreases the development of conditioned place preference (CPP) or self administration

Abbreviations: EE, environmental enrichment; SE, standard environment; IE, impoverished condition; CPP, conditioned place preference; SA, self administration.

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(SA) to cocaine [14,29], heroin [12], morphine [35], ethanol [9,11], sucrose [5], amphetamine [32], but not to methamphetamine [34]. In addition to these preventive effects, EE during abstinence dramatically eliminate the already established addiction-related behaviors. Grimm et al. first found EE attenuated cue-induced reinstatement of sucrose seeking in rats [15], then solinas et al. found that 10 days of EE after the development of CPP prevented cocaine-induced reinstatement [29]. Later, Other studies demonstrated that EE has curative effects and prevent relapse in SA procedure to cocaine [8], nicotine [1], methamphetamine [17,22] and sucrose [16]. These results highlight the potential effect of EE on the prevention of relapse to drug addiction. Although in most experiments EE reduced the effects of drugs of abuse and decreased the vulnerability to develop addiction, there have been no reports suggesting an influence of EE modulation on the reinstatement of ethanol-induced CPP.

CPP paradigm is widely used to evaluate the rewarding and psychological dependence-generating properties of drugs [19]. It is based on classical Pavlovian conditioning, in which animals develop an association between the rewarding action of a drug and specific environmental cues. This simple and rapid procedure has been widely used to model acquisition, expression, extinction, and reinstatement of ethanol-seeking behavior in rodents [20]. In this study, we explored the influence of EE on the reinstatement of ethanol-induced CPP in adult male C57Bl/6J mice. We first investigated the effect of training dose on the extinction and relapse of ethanol-induced CPP. In the second experiment, after the test session, we housed half mice in SE and the other half in EE during the extinction and reinstatement session to investigate whether concomitant EE exposure prevents the reinstatement of ethanol-induced CPP.

2. Materials and methods

2.1. Animals

All experiments were performed on male C57Bl/6J mice, 8 weeks old at the beginning, obtained from Vital River Laboratory Animal Center in China. The animals were housed 4 per cage in a 12-h light/dark cycle (light on at 7:00 a.m.) with food and water available *ad libitum*. The room temperature was maintained at 22 ± 1 °C and relative humidity at 45–50%. The animals were allowed to acclimate to environmental conditions for at least 2 weeks before test. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local committee of animal use and protection. Efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

Ethanol (0.4, 0.8, 1.6 g/kg) were dissolved in saline (0.9% NaCl) and injected intraperitoneally at a volume of 10 ml/kg. All saline injections were equal in volume to ethanol injections.

2.3. Housing environmental conditions

After arrival, all mice were housed in standard environment (SE), that is, mice were housed in common cages (29 × 18 × 16 cm) covered with soft woodchip bedding. After the expression of CPP, half of the mice were kept in SE, whereas the other half were switched to EE, which consisted of larger cages (55 × 40 × 20 cm) containing a constantly running wheel for voluntary exercise, and four–five non-chewable plastic objects that were changed every four days with new objects of different shape and color. SE mice were housed in group of 4 and EE mice were housed in group of 12. To avoid stress, littermates were not changed during the course of the study.

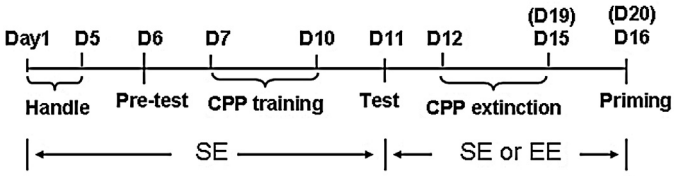

Fig. 1. Experimental design and time schedule for the conditioned place preference experiments.

2.4. Conditioned place preference

CPP was conducted in four identical rectangular PVC boxes, each containing three chambers separated by guillotine doors. The two large end chambers (20 cm long × 20 cm wide × 20 cm high) were separated with a small center choice chamber (20 cm long × 7 cm wide × 20 cm high). The two end chambers were differed in wall color (black *vs* white) and floor texture (grid *vs* bar). The center choice chamber was separated from two large chambers by gray walls that had doorways cut in them. The location of animals in the box was monitored with a video camera-based computerized imaging system (Yishu Company, Shanghai, China).

The place conditioning procedure was similar to that used previously to successfully produce CPP with ethanol [2,3,31] and processed in the following phases (Fig. 1):

Habituation During this phase, mice were handled and injected intraperitoneally with saline (10 ml/kg body weight) for 5 days. The time schedule was the same as the conditioning phase.

Pretest On day 6, mice were placed in the center chamber with the sliding doors raised and allowed to explore the entire compartment freely for 30 min. Time (seconds) spent in each chamber were recorded automatically.

Conditioning Beginning on day 7, the mice were allocated to stay for a period of 25 min in the two large end chambers twice daily for 4 consecutive days, with the mice received ethanol (0.8 g/kg or 1.6 g/kg, i.p.) on their initially non-preferred side and saline on their initially preferred side [31]. Ethanol and saline conditioning sessions were separated by at lease 5 h, consistent with the time course of ethanol effects [13].

Test Testing was conducted on day 11 as in the pretest procedure.

Extinction Starting on day 12, the mice were exposed daily to the apparatus, with free assess to all chambers without any injections, for 30 min a day to extinguish CPP. Once the mean significantly less than the CPP test for three consecutive days, indicating complete extinction was established. That is, all mice underwent the same number of extinction sessions independently of their individual scores.

Reinstatement The effects of a priming injection with ethanol (half of the dose used during conditioning) were evaluated 24 h after extinction. Immediately after injection, the mice were placed in the central zone of the CPP apparatus with free ambulation for 30 min.

2.5. Data presentation and analysis

In the CPP experiments, the preference scores were expressed as the time (seconds) spent in ethanol-paired chamber. All values were expressed as means ± SEM and the statistical analyses were conducted using Graph Pad Prism 4.0 software. Results were analyzed with Student's *t*-test, one-way or two-way analysis of variance (ANOVA). A significance level of 0.05 was used for all tests.