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Ethanol impairs memory by reducing the synaptic connection of the hippocampal spatial neurons

Samira Geravand, Manizheh Karami*

Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran

Abstract

Background and Objective: Ethanol has undesirable effects on memory and synaptic communication. However, its impact on the learned spatial memory is unclear. We investigated the damaging effects of ethanol on place neurons of rat's hippocampal CA1.

Materials and Methods: Sixty four male Wistar rats (250 g) were administered high (1-8 g/kg) or low (0.05-0.1 g/kg) doses of ethanol intraperitoneally (i.p.) and tested (10 min) for the novelty-seeking behavior using the place conditioning box. Sniffing, rearing, grooming, and compartment entering was compared between the first and the last stage, during which the animals had accessibility to the whole device. During the acquisition phase, the rats daily received ethanol (i.p.) and confined in one side of the device for 40 min. The control group solely received saline (1 ml/kg, i.p.). The achievements were analyzed by ANOVA under $\alpha = 0.05$.

Results: The ethanol-acquired animals with the high doses did not recall the information of the familiarization day and displayed a high tendency toward the non-confined side of the box. The rats also showed a reduction in place neuron synaptic strands.

Conclusion: Ethanol disrupts spatial memory and also diminishes CA1 place neuron's fibers.

Keywords: Ethanol, Acquisition, Novelty-seeking behavior, CA1, Place neuron

1. Introduction

Alcohol consumption is increasing in societies. Regardless of the cause of alcohol abuse, its complications are not well-known. From preliminary research (1) to now, the hippocampus is considered central brain's area for the acquisition and use of spatial cognitive maps. O'Keefe and Nadel (2) previously and Okada and Okaichi (3) recently have demonstrated that damage to the hippocampus usually results in impairments on some types of spatial learning and memory tasks. They have also introduced a spatial cell "place neuron" that activates when the animal encounters a new location in its environment. It has also been indicated that the routes from one place to another are memorable (4).

Today evidence suggests that the hippocampus is selectively involved in spatial learning and memory (5, 6). Because ethanol passes through the blood-brain barrier (BBB) and accumulates in the brain, it is likely to have a destructive effect on the consolidation of memory and the formation/use of spatial cognitive plans. A review in the literature shows that the harmful effects of alcohol on mentality have already been reported in humans and animals (7, 8). But, it is unclear that after taking ethanol, the map of the diagnosis of spatial in the brain that has recently

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Corresponding Author: Manizheh Karami **E-mail:** karami@shahed.ac.ir

developed is changing. Besides, its impact on pyramidal neurons of the hippocampal CA1 is not fully known. To answer the above questions, we evaluated the information recall and novelty-seeking behavior in alcohol-injected rats. Also, we examined the place pyramidal neurons of the hippocampal CA1 to signify the destructive effect of alcohol on the synaptic potency.

2. Materials and Methods

2.1. Animals

The study was conducted under the guide for experimental and clinical studies (9). The male Wistar rats weighing 250 g at the start of experiments were provided from Pasteur's Institute of Iran, Tehran. The rats were housed two per cage in a controlled colony room (temperature 21°C ± 3°C). They were kept under a 12:12 h light-dark cycle and fed with water and food (Pars animal food Co., Tehran) *ad libitum*. Each animal was tested only once for the novelty-seeking behavior. The local ethical committee affirmed all experiments.

2.2. Drugs

Pure ethanol (Merck Co., Germany) at different high (1, 2, 4, 8 g/kg) and low (0.05-0.1 g/kg) doses were intraperitoneally (i.p.) administered to the experimental animals (n=6 rats/per dose). The control group (n=6) exclusively received normal saline (1 ml/kg, i.p.).

2.3. Behavioral procedure (Novelty seeking)

The experiments were conducted based on three (familiarization, conditioning, and testing)-stage place conditioning procedure. The 2nd, the conditioning, phase is known as confining in the seeking paradigm. A place preference apparatus in dimensions 30*60*30 cm was designed using an unbiased plan; the two parts of the wooden tool were equally divided by a guillotine door lifted up (12 cm) from the floor throughout the familiarization and test, but remained closed during the conditioning (confining). In the conditioning phase, the animals were initially i.p. injected the ethanol, and confined in one side of the equipment. The stay time and behavioral sign (sniffing, rearing, grooming, or compartment entering) were assessed by the EthoVision system (Auto iris Video Camera LVC-DV323ec, LG Electronics, South Korea) located 120 cm above the tool. A double-blind viewer rechecked all the records; the observer neither knew the treatments nor could speculate the results.

2.3.1. Familiarizing

All animals received one habituation session on day 1. They were located in the middle line of the two-side apparatus to move into the entire apparatus for 10 min freely. In this phase, the removable wall of the apparatus was lifted up (12 cm), and the stay time of the rat on each side of the box was calculated.

2.3.2. Confining

After the familiarization, the experimental animals were confined in the box; they were initially i.p. injected the ethanol, and then placed (40 min) in only one side (confining side) of the box. This process was conducted for two times at 6-h intervals during the light phase of a 12-h light/dark cycle (between 09.00 a.m. and 3.00 p.m.). The control group was as confided as the experimental ones, but they solely received saline (1 ml/kg, i.p.).

2.3.3. Testing

The test phase was carried out a day after the confining period. Each animal was tested only once. During the trial, the removable wall was raised 12 cm above the floor, which accords with the familiarization step. To provide more harmonies with the adaptation phase, each animal, freely accessed to both compartments of the apparatus for 10 min. The time spent in confining compartment during this phase was subtracted from that spent in the same part in the familiarization step, and expressed as mean ± S.E.M. Also, the numbers of behavioral signs were counted both in the first and last phases, and deducted, and expressed as mean ± S.E.M. This protocol was as run as in the control and experimental rats.

2.4. Histological investigation

For histological investigations, all animal's brain samples were fixed in 10% formalin solution and processed with a tissue processor through paraffin embedding. Serial sections (3-4 µ) were prepared by the aid of a rotary microtome. The thin brain slices were then stained with Cresyl violet. The slides were eventually evaluated by the light microscope (Olympus, U.S.A) at 4X-100X.

2.5. Statistical analysis

All data were analyzed by the analysis of variance (ANOVA) followed by the LSD *post hoc* test. $P< 0.05$ was considered significant.

3. Results

3.1. Memorizing response to low/high doses of ethanol

The present results indicate that the high concentrations (1-8 g, i.p.) rather than the low doses (0.05-0.1 g/kg, i.p.) of the ethanol were reasonably effective in injuring the animals' information recall (Fig. 1). The comparison made between the experimental and control animals suggest that animals who received high doses of ethanol had more memory loss than the low doses-treated rats. The high doses-treated animals did not remember the information of the familiarization period. They preferentially stopped on the novel (*nov*) side of the device, which did not bound in throughout the confining phase ($p<0.05$).

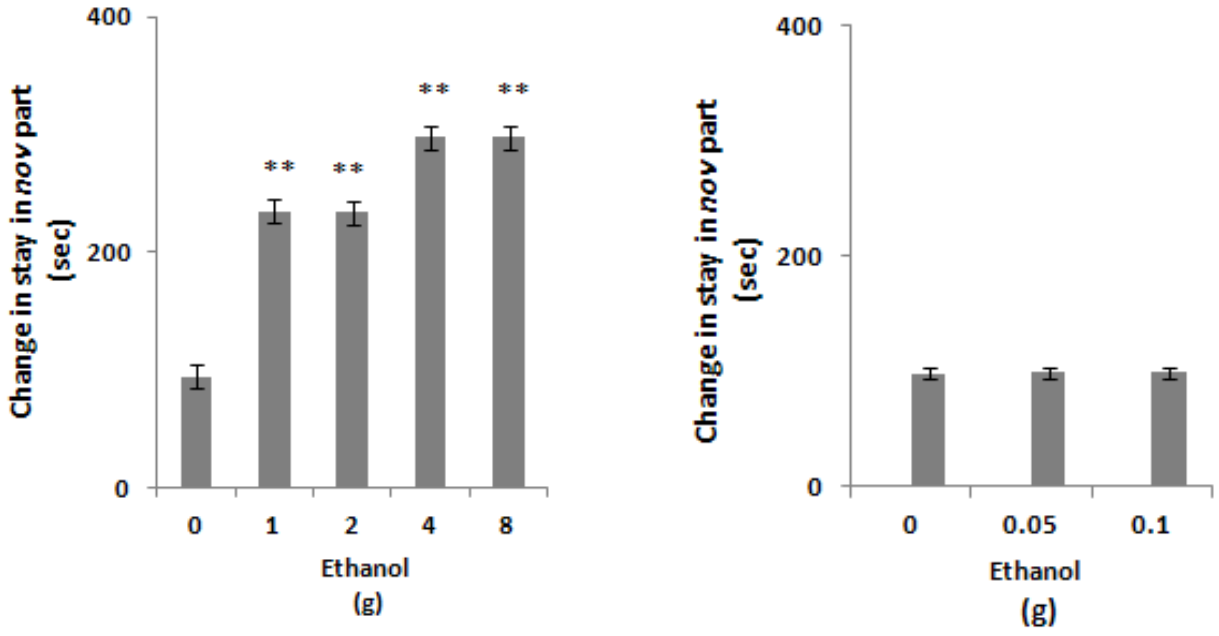

Figure 1. The figure shows the response to ethanol low/high doses or saline (control) in Wistar rats. At first, the animals were habituated in the area of studying (place conditioning apparatus). Three days later, they were tested (in the day of the test). Throughout the conditioning (confining) phase, the rats were given ethanol high (1-8 g/kg, i.p.) or low (0.05- 0.1 g/kg, i.p.) and confined just in one side of the box. The performances of the rats were recorded both in adaptation and the test days. Data are expressed as the score of change in the time spent during testing, and seeking the novel part of the device, and expressed as mean ± S.E.M. A difference between the ethanol-administered groups *vs*. the vehicle is indicated by the ANOVA at the high level of the substance. *Post hoc* analysis by LSD showed the differences (**$p < 0.01$) to the control (legend 0).

3.2. Novelty behavior due to low/high doses of ethanol

Based on the achievements, all animals that received the ethanol regardless of the treatments exhibited behavioral signs such as sniffing and rearing in the *nov* side of the device ($p<0.05$) (Fig. 2).

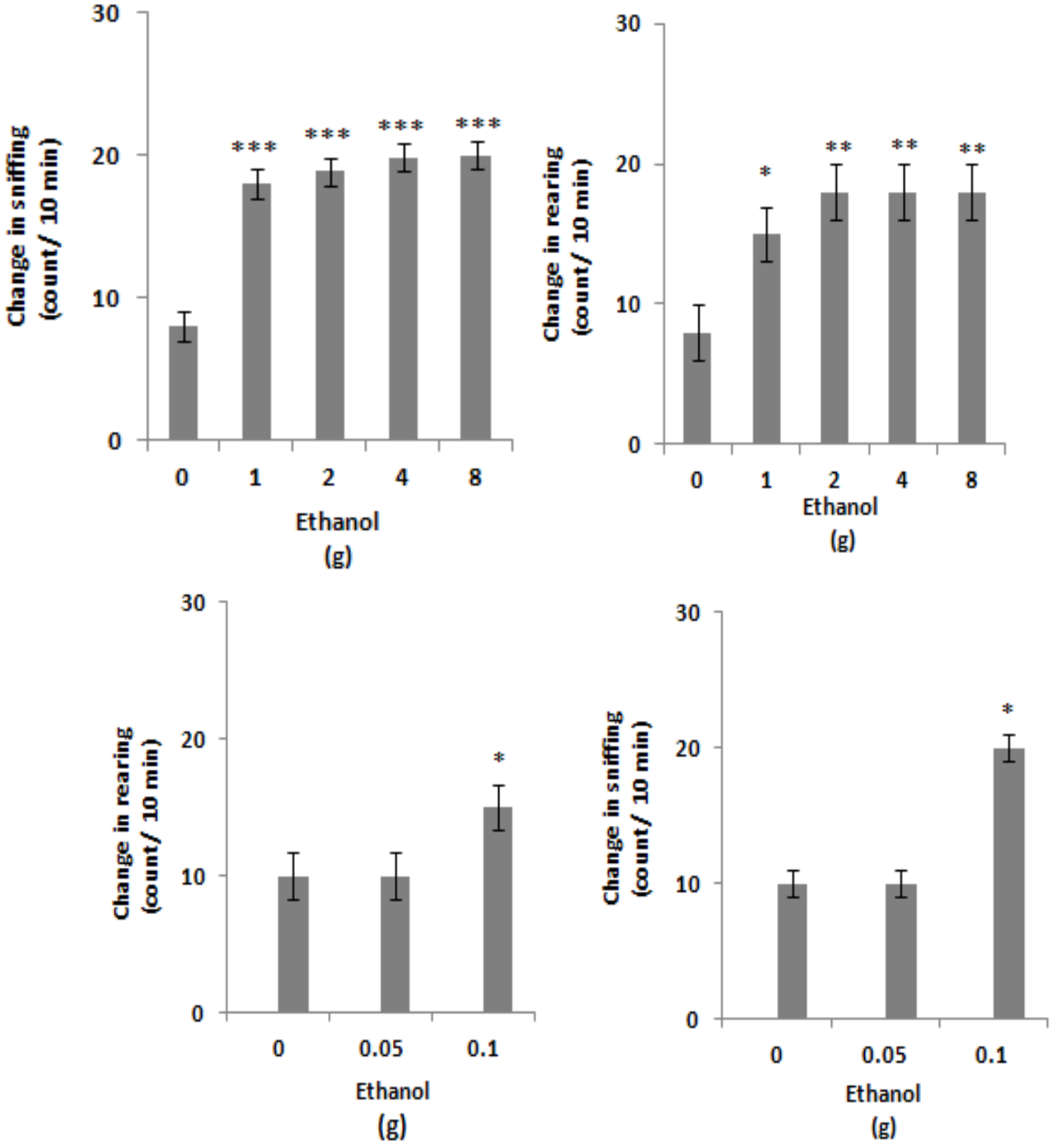

Figure 2. The figure shows the seeking response to the low/high concentrations of ethanol (respectively 0.05-0.1/1-8 g/kg) or saline (control) in Wistar rats. At first, the animals were habituated in the area of studying (place conditioning apparatus). Three days later, after the conditioning (confining) phase, they were tested. The experimental animals throughout the conditioning phase were administered the ethanol, and confined in one side of the box. They were finally tested in the last day. All performances of the animals in the apparatus were recorded both in the first and the last days, and in the end, the data were compared between the ethanol-received groups and the control. Data are expressed as the change in novel seeking behaviors between the 1st and the last days, and expressed as mean of count/10 min ± S.E.M. The difference between the experimental groups *vs.* the vehicle is indicated by the ANOVA. The *Post hoc* analysis by LSD showed the differences (*p< 0.05, **p < 0.01, ***p < 0.001) to the control (legend 0).

3.3. Effect of low/high doses of ethanol on CA1 place neurons

According to current findings, rats that received ethanol at high doses showed destructive effects on hippocampal CA1 spatial neurons. This feature was absent in animals treated with low-dose ethanol. Also, these neurons had a decrease in synaptic strands, as evidenced with Cresyl violet staining (Fig. 3).

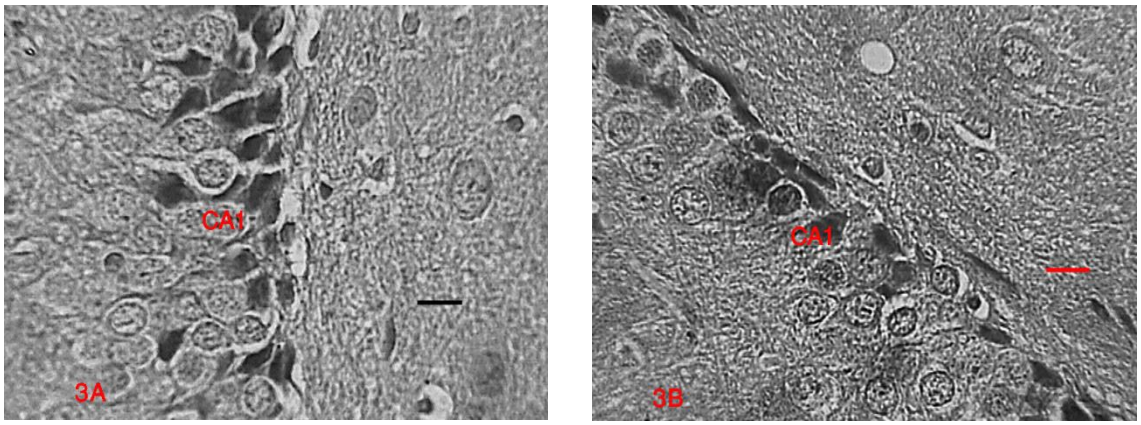

Figure 3. Panels show the brain of ethanol-treated rat (at high doses) at the CA1 level. The small pyramidal place neurons showed the change in the synaptic connection (B compared with control A). The Bars beside the samples show the µm values (100 micrometer).

4. Discussion

We studied the harmful effects of ethanol in memorized observation recalling. The harmful effect of the substance was analyzed with the help of our innovative novelty-seeking design. According to present results, animals who received ethanol at high doses did not remind the memories of the first day with the box during testing, which had the same conditions as the familiarization day. Compared to the control group of rats, these animals showed more seeking behavior for new environments, which is a sign of the lack of consolidation for the newly experienced memories. The spatial neurons of these animals also showed more significant decrease in the synaptic splits.

First, it should be acknowledged that the destructive effects of ethanol have already been reported (8). However, the impact of ethanol on the recall of learning based memories is still not well-known. Also, the space cells which create the place diagnosis circuitry for the brain are not yet fully described. Thus, we studied the degrading effect of ethanol on hippocampal CA1 place neurons. The hippocampus, a part of the limbic system, which is located deep in the temporal lobe, has a central role in learning and memory formation (10). It receives huge cholinergic input (11). Besides, this area of the brain is influenced by the substances of abuse (12). Surprisingly, this part of the brain has interactions between particular neurotransmitter systems. The nitric oxide (NO), the free radical retrograde neurotransmitter, plays a unique role in regulating the presynaptic terminals. It also involves in synaptic plasticity and long-term memory reinforcement (13, 14). But, does the NO interact with dopamine in the presynaptic part, is still not known. As it has previously been shown alcohol through the mesocortical pathway increases dopamine (DA) in the nucleus accumbens and induces the rewarding effects (15). This process involves in the dependence and addiction phenomena (16).

Our choice was the trained-based simple dependency, the conditioned place preference (CPP). Furthermore, we considered the fact that the hippocampal place neurons entity plays important roles in performing the recall and spatial learning memory. Because the detailed recall needs permanent comparisons between the receiving memories from environ with the information already exists in memory boxes, the present data unexpectedly show that alcohol consumption, damaged the CA1 place neurons, and reduced the volume of synaptic connections.

We additionally signify the deleterious effect of ethanol on recalling of previously learned information; because the ethanol-treated rats did not remember the information of the introduction day and showed more interest to the *nov* side of the CPP box. Because the detailed reminding needs permanent comparisons between the receiving memories from environment with the information that already exists in memory boxes (17), this study may show the possible troubling influence of ethanol on information recall in the rat.

To explain, it has been previously demonstrated that alcohol consumption adversely affects spatial memory (10). Along with this, we measured the novelty-seeking behavior of the low/high dose ethanol-treated animals that signifying the novelty seeking for the part being familiar at first, but did not remember at testing. This, of course, was not observed in lower dose (0.05 µg/rat) of low-dose category, indicating a dose-dependent effect. The rats' performance in the retrieval test, thus, is dependent on the baseline effect of the ethanol level. We suggest the study of ethanol impact over the periods between five and more days to find differences in the significance and variance of the results as a function of the periods that the animal can remember the events. Others have noted the local degradation effects of the hippocampus in the study of remote memory (18) or considered the effect of the type of reminder tool and method in memory retrieval (19). We may also suggest a study with a prolonged injection pattern of ethanol in the novelty approach to indicate damage to brain areas. We may conclude that alcohol as a significant source of enormous medical, social, and economic burdens throughout the world (20) has a deleterious effect on reminding process and show its destructive effect on CA1 place neurons.

Conflict of Interest

The authors report no conflicts of interest

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