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# Information Recall Deficit in the Novel Task after Treatment of Rats with Ethanol

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## ABSTRACT

**Background and Objective**: Identifying the details of the injurious effects of alcohol seems to be decisive in nowadays with growing abuse of the substance. This study mainly aimed to assess the adverse effects of ethanol on the recalling of information in the Wistar rats.

**Materials and Methods**: Male Wistar rats (Pasteur Institute of Iran) were evaluated using the novelty seeking behavior based on the place conditioning task. The animals in the conditioning phase were confined in only one side of the equipment. They (n=8 per group) were intraperitoneally injected ethanol at high (1-8 g/kg) or low (0.1-0.8 g/kg) concentrations prior to the testing. The control group solely received saline solution (1 ml/kg, i.p.) in the testing day. The liver samples of the experimental animals were examined to provide the setback systemic injury evidence for the ethanol.

**Results**: Based on the findings, the ethanol treated rats did not remember the previous data and showed more interest to the novel side ($p<0.05$). The liver samples of the experimental animals illustrated no unpleasant feature at the low concentrations of ethanol with the exception of the pyknotic nuclei for the higher concentrations three days after drug injection.

**Conclusion**: Recalling of the facts need permanent comparisons between the receiving reminiscences from environ with the information which already exist in the memory boxes. This study may show the prompt troubling influence of ethanol on the memory retrieval along with the long-lasting systemic hazard of the biggest gland.

**Key Words:**
Ethanol
Novelty seeking behavior
Information recall
Rat

## 1. Introduction

We know that some regions of brain are critical for the consolidation of memory and use of spatial cognitive plans. For example, the hippocampus is introduced as a key area for the acquisition and use of spatial cognitive maps (1). The use of some toxins or materials has deleterious effects on memory formation or recall of information. Alcohol is one of them; the deleterious effects of alcohol on memory in human and animals have been well studied (2).

Alcohol was identified for the first time by an Iranian scientist Zakaria Razi many years ago. But, today there is alcohol abuse in the world. In fact, the alcohol abuse is growing in many communities (1).

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Lot of researches up to now has been designed to investigate the hazardous effect of alcohol on the memory processes; *e.g.* the authors have explored the hold up of memory retrieval by evaluation of the inhibitory avoidance response induced by pre-training ethanol in mice (3). They have suggested that the hippocampal N-methyl-d-aspartate (NMDA) receptors play a role in the recall process.

Since we know that ethanol crosses the blood-brain barrier and accumulates in the brain we aimed to show the prompt effect of ethanol on the recall of information in Wistar rats. We investigated the seeking behavior of ethanol-treated animals using a novel behavioral design. The mentioned technique is newly adapted by our laboratory to show the effects of harmful substances on the brain by assessment of emotional-behavioral components. The notable point is that the material likely troubles the memory retrieval which is dependent on spatial and working memory processes. Also we wanted to demonstrate the pathological effect of the material on the biggest gland of the ethanol-treated animals jointly with the behavioral measurements to verify the long-lasting systemic hazard of the substance.

## 2. Materials and Methods

### 2.1. Animals

The animals used were male Wistar rat weighing 250-350 g. They were housed two per cage in a controlled colony room (temperature 21°C ± 3°C). They were maintained under a 12:12 h light-dark cycle with water and food (bought from Pars animal food Co., Tehran) provided *ad libitum*. The animals were tested only once. All the experimental procedure was in accordance with animal care guidelines affirmed by the local ethical committee at Shahed University.

### 2.2. Drugs

Ethanol (Merck Co., Germany) was intraperitoneally (i.p.) injected pre-testing at different high (1-8 g/kg) and low (0.1-0.8 g/kg) concentrations and parallel to the procedure, the control group exclusively received saline prior to the test.

### 2.3. Behavioral procedure (in novelty seeking manner)

The experimental animals were tested using the novelty seeking behavior based on a three-stage place conditioning paradigm. The investigative behavior consists of components like sniffing, rearing, grooming and moving from one side of the assessment device to the other side, but, the main feature of the behavior is the residing time in the compartments of the apparatus.

The phases of the conditioning paradigm as the following are the familiarization, the conditioning, and the testing. For each drug dose, the animals were confined in only one side of the equipment in the conditioning phase, lasting three days.

### 2.4. Familiarizing

All animals received one habituation session on day 1 (before confining). They were located in the middle-line of the two-side apparatus to freely move into the entire apparatus for 10 min. In this phase, the removable wall of the device was raised 12 cm above the floor. The time spent by rats in each side of the device was recorded by an Ethovision system (Auto iris Video Camera LVC-DV323ec, LG Electronics, South Korea) located 120 cm above the apparatus. A double-blind observer who had no knowledge of the treatments nor could speculate the results then calculated the traced behavior.

### 2.5. Confining

This phase as has been mentioned in above section was started a day after the familiarization. It consists of twice pairings with one side of the box. The animals were simply confined for 40 min in the selected part twice a day with a 6-h interval. This phase was carried out during the light phase of a 12-h light/dark cycle (e.g., at 09.00 a.m. and at 15.00 p.m.).

### 2.6.Testing

Test phase was carried out on day 5, 24 h after the last confining session. Each animal was tested only once. For testing, the removable wall was raised 12 cm above the floor in accordance with the familiarization step. To provide harmony with the adaptation phase, each animal was allowed free access to both compartments of the apparatus for 10 min. The time spent (s) in the compartments in the day of testing were subtracted between those obtained for the familiarization and the testing phases and the result was expressed as mean ± S.E.M. The behavioral signs were also counted; change in number of the sign/10 min performed in the apparatus to those achieved for the familiarization was expressed as mean ± S.E.M. It should be notified that this protocol was as performed for the control (saline treated) group as for the experimental groups except that the control rats simply received saline (1 ml/kg, i.p.) prior to the testing.

### 2.7. Surgery procedure

A midline incision with a length of about 2 cm in the upper abdomen area was performed for treatment groups under anesthetization. The liver samples were then dissected out and collected in 10% formalin solution for further histological analysis.

### 2.8. Histological analysis

For histological investigations, the samples of liver were processed with tissue processor through paraffin embedding. The serial sections (3-5 µm) were prepared with a rotary microtome. The slides were then stained using Hematoxylin and Eosin (H&E) and cleared with xylene. After mounting, the slides were evaluated both with light photomicroscope (Olympus) at the desired magnification and by Image J (UTHSCSA, version 2.03, USA), the free image processing and analysis program for Microsoft Windows to provide quantification for 100 $\mu m^2$ units.

### 2.9. Statistical analysis

All data were analyzed by the analysis of variance (ANOVA) followed by the Tukey's *Post hoc* test. $P< 0.05$ was considered as significant.

## 3. Results

### 3.1. Effect of high and low concentrations of ethanol on the animals' information recall in the novelty seeking paradigm

The present results indicate the troubling effect of the drug ethanol on the animal's act. The ethanol at the high concentrations used in the study (1-8 g/kg) was effective in damaging the memory retrieval. A comparison made between the test animals versus the controls show that the ethanol treated rats did not remember the information of the introduction day and were more concentrated in the novel side; the side that during conditioning had no constraining in ($p<0.05$). So, the behavioral data show a major difference in the score of placing in the novel part between the ethanol received rats to the control group (Fig. 1).

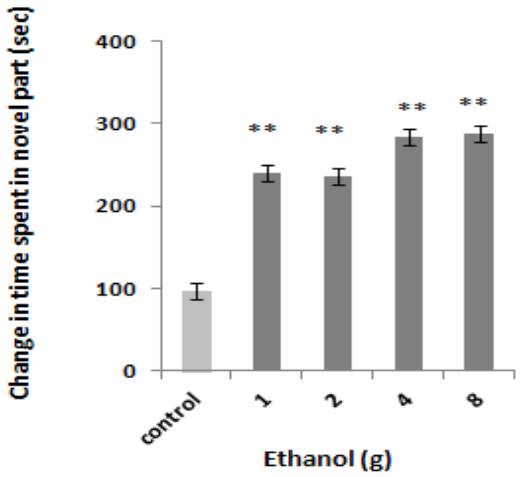


**Figure 1:** The figure shows the response to the high concentrations of ethanol (1-8 g/kg) or saline (control) in Wistar rats. At first, the animals were habituated in the area of studying (place conditioning apparatus). Three days later, after the conditioning phase, they were tested. The experimental animals throughout the conditioning phase were confined just in one side of the box. They were eventually given the ethanol (1-8 g/kg) prior to the testing. All animals' performances in the apparatus were recorded both in the first and in the last days and eventually the data were compared

between the drug receiving groups and the controls. Data are expressed as the score of change in time spent in seeking for the novel place and expressed as mean ± S.E.M. A difference between the drug-administered groups versus the vehicle was observed. *Post hoc* analysis by Tukey showed the differences (***P* < 0.01) versus the control.

The findings furthermore signify that the ethanol at the low concentrations (0.1-0.8 g/kg) was quite harmful for the animal's remembrance. Assessments completed between the experimental animals versus the controls demonstrate that the ethanol treated rats did not remember the information of the introduction day. Based on the between group comparison test (Tukey's *post hoc*), the group which treated by the highest concentration of the drug (0.8 g) was more interested in the novel side ($p<0.05$) (Fig. 2).

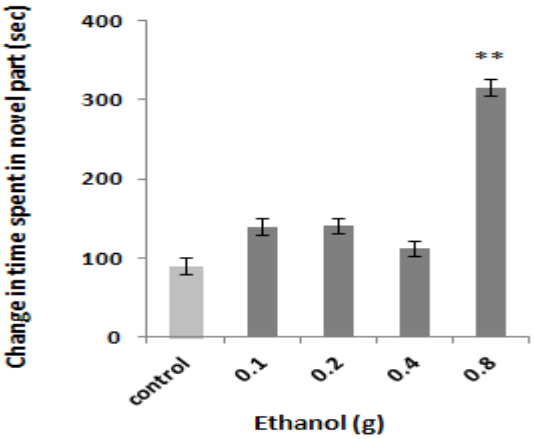


**Figure 2:** This figure shows the response to ethanol low concentrations (0.1-0.8 g/kg) or saline (control) in Wistar rats. At first, the animals were habituated in the place conditioning apparatus. They were then tested three days later. Throughout the conditioning phase they were trained behaviorally; they were confined just in one side of the box. They eventually were given ethanol (0.1-0.8 g/kg) prior to the testing. All animals' performances in the apparatus were recorded both in the adaptation and the test days and finally the data were compared between the drug receiving groups and the controls. Data are expressed as the score of change in time spent in seeking for the novel place and expressed as mean ± S.E.M. A difference between the drug-administered groups versus the vehicle was observed. *Post hoc* analysis by Tukey showed the differences (***P* < 0.01) versus the control.

### 3.2. Effect of ethanol on the animals' liver tissue promptly or three days after drug usage

The rats' liver samples of all ethanol treated groups (0.1-0.8 g/kg or 1-8 g/kg) taken from the experimental animals in the testing day or three days later exhibited no unpleasant feature at the low concentrations of ethanol with the exception of the pyknotic nuclei for the higher concentrations three days after drug injection (Fig. 3).

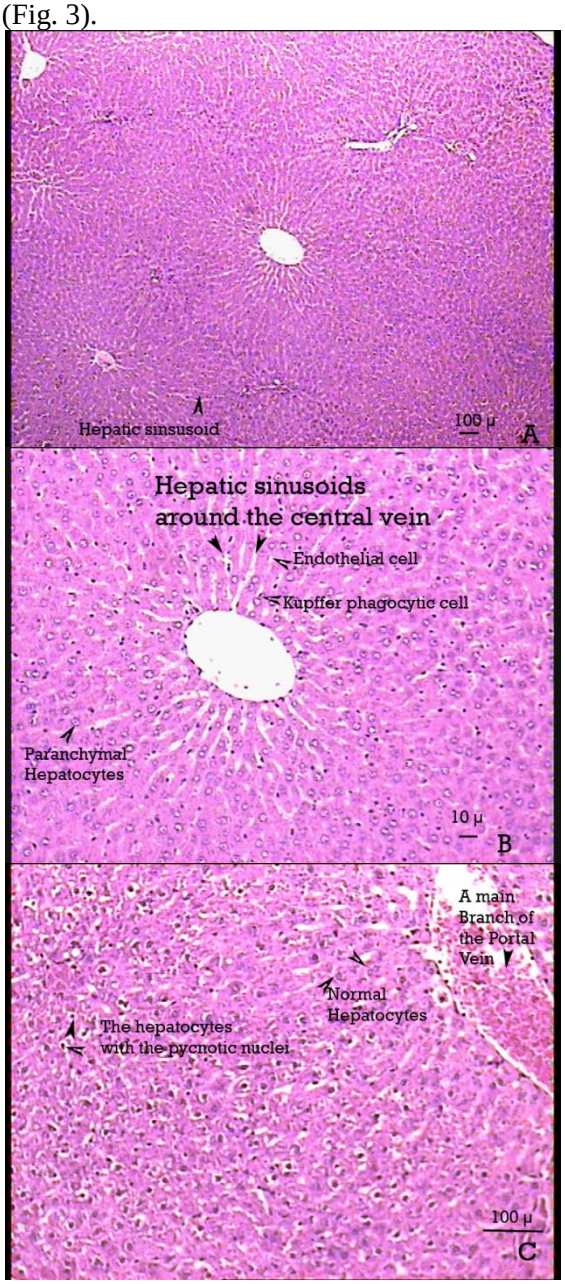


**Figure 3:** Photomicrographs are taken from the liver samples of ethanol treated (A-B) rats. The samples showed no damage (A-C) with the exception of the pyknotic nuclei three days after drug injection at high dose (C).

## 4. Discussion

This study was planned to specify the harmful effect of ethanol on the rat recall of the memorized observation. The deleterious effect of the chemical material was shown by aid of behavioral assessment (Figures 1 and 2). The ethanol received animals, in contrast to the controls, showed a significant preferred novelty behavior. In the complementary study, the lasting (three days after injection) effect of once usage of the substance was shown as the presence of pyknotic nuclei for the hepatocytes (Fig. 3). To discuss the findings, we may state that one of the main memory impairing effects of ethanol is introduced as impairment of hippocampal cholinergic system (3). It is precious to know that the hippocampus, a part of the limbic system located deep in the temporal lobe, has a central role in learning and memory formation (4). This part of the brain receives a large cholinergic input (5). It is more interesting to know that there is a close relationship between the cholinergic and nitric oxide (NO) systems to form memory. This fact adequately suggests a critical role for the hippocampal cholinergic input as well as the NO in the memory process and the information recall (6, 7). It is probable that this area of the brain takes influence by substances of abuse (8).

It is worthy to remember that the alcohol through mesocortical pathway increases dopamine in the nucleus accumbens and induces the rewarding effects (9). It has previously been demonstrated that alcohol consumption adversely affects the spatial memory (4).

Apart from the previously published effect of this substance based on the present findings we may strongly signify its harm effect on recalling of previously learned information. As has been revealed, the ethanol treated rats did not remember the information of the introduction day and showed more interest to the novel side. This study may show clearly the possible troubling influence of acute usage of ethanol on information remind in the rat. The detailed recall needs permanent comparisons between the receiving memories from environ with the information which already exists in memory boxes. This is especially interesting when we refer to the previous articles emphasizing that the deleterious effects of ethanol on the central and the peripheral nervous systems can occur after chronic alcohol abuse (10).

We additionally verified the side effect of the material on the biggest gland (liver) of the ethanol-treated animals together with the behavioral measurements to verify the lasting systemic danger of the substance. The parenchymal hepatocytes had pyknotic nuclei at the higher concentrations of the substance three days later to the drug injection. The liver samples of the experimental animals illustrated no unusual change at the low concentrations of the ethanol. The findings may indicate the acute mal-influence of the matter on the gland beside the prompt injurious power on the memory. Although there is evidence about the harmful effects of ethanol on liver (11, 12), but, most of the previous works have not paid attention on the acute effects of the substance on the liver. However, the highlighted point of this study is showing the lasting destructive effects of acute but not over-consumption of ethanol.

We may conclude that the alcohol treatment for once has deleterious effect on recall process along with the long-lasting systemic hazard at the biggest gland. We may also suggest a study with a prolonged injection pattern of ethanol using the novelty task to provide evidence for indicating damage of brain areas.

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