

Experimental anxiety and the reinforcing effects of ethanol in rats

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Abstract

In order to examine the relationship between anxiety and reinforcing effects of alcohol, drug-naive male Wistar rats weighing 250-300 g were classified as "anxious" and "non-anxious" in the elevated plus-maze test. A conditioned place preference test was then used to investigate the reinforcing effects of ethanol (EtOH) on these animals. On 2 alternate days, groups of "anxious", "non-anxious" and "normal" rats received intraperitoneal (*ip*) injections of EtOH (0.5, 1.0 or 1.5 g/kg) immediately before a 15-min confinement to the white compartment. On the 2 intervening days the same rats received *ip* injections of saline before confinement to the opposite compartment. On day 5, a 15-min free-choice test was carried out with no injections. Rats classified as "anxious" showed a significant, though not dose-dependent preference for all doses of ethanol compared to saline-treated animals. These data demonstrate that rats regarded as "anxious" are more sensitive to the reinforcing effects of EtOH than "non-anxious" and "normal" Wistar rats and emphasize the relevance of the basal levels of anxiety of rats when trying to detect the reinforcing effects of EtOH.

Key words

- Anxiety
- Plus-maze
- Place preference
- Ethanol
- Wistar rats

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Presented at the 6th World Congress
of Biological Psychiatry, Nice,
France, June 22-27, 1997.

Research supported in part by CNPq.
S.L. Blatt was the recipient of a
fellowship from CNPq.

Received July 30, 1998
Accepted January 11, 1999

Introduction

The reinforcing properties of drugs of abuse are seen as the major factor leading to drug-seeking behavior and to a subsequent addictive state. However, probably due to a more complex pharmacodynamic action of ethanol (EtOH) than of other addictive drugs, the demonstration of reinforcing effects of EtOH in animal models has been difficult to achieve (1,2). Thus, in the conditioned place preference (CPP) paradigm, which has been used widely to study the rewarding effects of various drugs of abuse (3-5), studies with ethanol have generated conflicting results (1,2,6). In this context, it is important to

mention that an additional and prevalent notion holds that acute anti-anxiety actions of EtOH are important for the reinforcing properties of this drug. Therefore, the tension-reduction hypothesis, though controversial, proposes that there is a high comorbidity for several anxiety disorders and alcohol abuse in clinical and epidemiological studies (7-9).

One of the most widely used animal models of anxiety is the elevated plus-maze which has been pharmacologically and ethologically validated (10,11). Using this procedure, recent studies have shown remarkable strain-dependent differences in the anxiety response of inbred rats (12) as well as normal Wistar rats (13). Moreover, basic studies on labora-

tory animals attempting to validate the tension-reduction hypothesis have produced conflicting results. For example, in heterogeneous populations of Wistar rats, high baseline levels of experimental anxiety have been reported to predict high voluntary EtOH self-administration (14), whereas more contradictory results have been reported with rat lines bred for EtOH preference (15,16). Since little is known about the possible interaction between experimental anxiety and the effects of alcohol, in the present study we investigated the relationship between different levels of anxiety and the reinforcing effects of EtOH in rats. For this purpose, drug-naïve Wistar rats were tested in the elevated plus-maze for their initial level of anxiety. Rats were then classified as anxious and non-anxious and subsequently tested in a conditioned place preference paradigm.

Material and Methods

Animals

Male Wistar rats weighing 250-300 g from our colony were used. The animals were housed in groups of 6 in plastic cages and maintained in a room with controlled temperature ($23 \pm 1^\circ\text{C}$) and a 12-h light cycle (lights on 7:00 h) with food and tap water available *ad libitum*.

Drugs

Ethanol (Merck) was diluted in NaCl to 10.0% (w/v) concentration. The control solution consisted of an equivalent volume of saline. All drugs were administered intraperitoneally.

Apparatus

Elevated plus-maze test. The wooden plus-maze consisted of two opposite 50-cm long x 10-cm wide open arms and two 50 x

10 x 40 cm (length, width and height) enclosed arms. The arms extended from a central 10 x 10-cm platform. The maze was elevated to a height of 50 cm above the floor. The enclosed arms were painted black. To determine the exploratory activity of rats, white tapes (about 2 mm wide) were used to divide the floor of each open arm into three equal sectors.

The procedure of the test was as described previously (13). Briefly, each rat was placed in the center of the maze and time spent in the open and closed arms was recorded over a 5-min test period by an observer sitting at a distance of approximately 1 m from the apparatus. In addition, the following parameters were recorded: the latency period to the first entry into one of the open arms of the maze and the number of sectors crossed in the open arms. To consider an animal as anxious, the two parameters measured had to correlate. Thus, rats that crossed fewer sectors than 10 and spent a total time in open arms of less than 60 s were considered to be anxious. The non-anxious group consisted of rats that crossed more than 20 sectors and spent more than 120 s in the open arms. The cut-off times applied were established using the mean value of time spent in the open arms ± 3 SEM. One week after selection, anxious, non-anxious and normal rats received control solution or EtOH (0.5, 1.0 or 1.5 g/kg) 15 min prior to testing in the conditioned place preference test. A total of 263 rats were used in the plus-maze test for the selection procedure which provided 65 anxious, 68 non-anxious and 130 normal rats.

Conditioned place preference

Place conditioning and testing was conducted using a 30 x 30 x 22 cm two-chambered acrylic cage. One white end was illuminated with a 40-W white light bulb and had a floor grid and the other end was black and had a smooth white floor.

A biased place-conditioning procedure was used. The conditioning period of this experiment lasted 4 consecutive days. On days 1 and 3, animals received control solution, were immediately placed in the black end chamber and confined there for 15 min. On days 2 and 4, rats were injected with EtOH (0.5, 1.0 or 1.5 g/kg) and immediately confined to the white chamber for the same 15-min period. The apparatus was cleaned after each conditioning session with a cloth dampened with 10% ethanol.

During the next day (the fifth day) uninjected rats were given free access to the two sections of the apparatus and the number of seconds each subject spent in each chamber was recorded for 15 min. The location of the animal was defined by the position of the four paws in the compartment.

For this procedure, three main groups of rats were formed and divided into 4 experimental groups, each consisting of at least 10 rats.

Measurement of blood ethanol

A separate group of rats submitted to the same injection schedule as in the CPP test was used to determine blood EtOH concentration. Fifteen minutes after the last *ip* injection of EtOH (0.5, 1.0 or 1.5 g/kg) or saline, animals were anesthetized with ether and blood was collected by cardiac puncture. Plasma aliquots were stored at 2-4°C for later determination of EtOH levels. The determination of alcohol in the blood was based on the titration reaction technique of Nicloux (17).

Statistical analysis

The data are reported as means \pm SEM and were analyzed statistically by analysis of variance (ANOVA) followed by the Newman-Keuls *post hoc* test. Differences were considered to be significant when $P \leq 0.05$.

Results

Table 1 summarizes the results of the selection procedure of drug-naive rats in the plus-maze test for their basal level of anxiety. The subsequent division into anxious, non-anxious and normal rats resulted in statistically well-differentiated groups [$F_{2,89} = 31.069$, $P < 0.0001$]. Thus, rats classified as non-anxious exhibited shorter latencies to entry into the open arms, crossed more sectors, and spent more time in the open arms of the maze compared to normal rats. All of these measurements suggest reduced aversiveness in the non-anxious subgroup. The opposite was true for rats assigned to the anxious subgroup.

Figure 1 shows the results of place preference conditioning for anxious, non-anxious and normal rats in the elevated plus-maze. As can be seen, a significant preference for the treatment-paired compartment was observed in anxious rats injected with EtOH (0.5, 1.0 or 1.5 g/kg, *ip*) [$F_{6,64} = 3.52$, $P < 0.045$]. In non-anxious rats, EtOH, 0.5 g/kg, caused an increase of time spent on the drug-paired side; however, this response did not reach statistical significance (Figure 1). These results indicate the reinforcing properties of EtOH only in anxious rats as measured in the place preference paradigm.

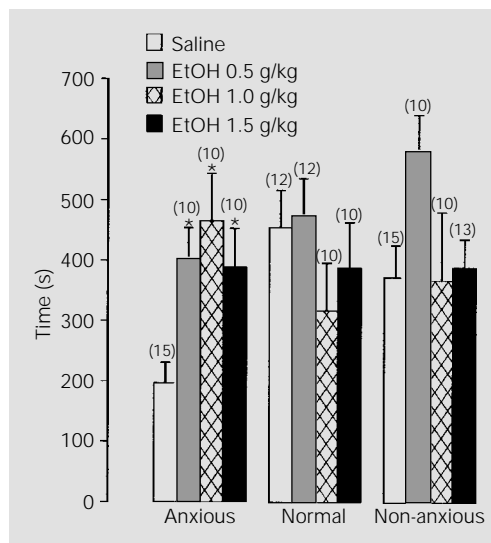
Since no variations were detected in blood EtOH levels among the different groups of rats, the data were pooled for each dose. Blood EtOH concentrations measured in

Table 1 - Selection experiment according to the exploratory activity of naive undrugged Wistar rats in an elevated plus-maze.

Data are reported as the mean \pm SEM. * $P \leq 0.05$ compared to the normal group (one-way ANOVA and Newman-Keuls test).

	Latency of first open-arm entry (s)	Number of crossed sectors in open arms	Total time spent in open arms (s)	Number of rats
Non-anxious	6.6 \pm 0.9	25.3 \pm 0.9*	138.4 \pm 7.6*	48
Normal	9.0 \pm 1.6	15.8 \pm 0.2	93.4 \pm 3.4	44
Anxious	63.8 \pm 12.4*	6.0 \pm 0.5*	42.1 \pm 3.4*	45

Figure 1 - Mean time (s) spent on the test day in the previously drug-paired compartment of the place preference apparatus. Place conditioning sessions were held for 4 days for anxious, non-anxious and normal rats injected *ip* with saline or ethanol (EtOH) (0.5, 1.0 or 1.5 g/kg). Data are reported as the mean \pm SEM. Numbers in parentheses indicate the size of the groups. * $P \leq 0.05$ compared to the control group (Newman-Keuls test).



animals 15 min after *ip* injection of EtOH (0.5, 1.0 and 1.5 g/kg) yielded the following means: 7.9, 15.9 and 19.4 mg/dl, respectively.

Discussion

The wide variation in anxiety levels observed in the plus-maze in an outbred Wistar rat population is consistent with the results of our earlier study (13) and suggest a genetic heterogeneity for the anxiety response.

The most important finding of the present study is that EtOH induces reinforcing effects as measured in the conditioned place preference paradigm only in rats classified as anxious. These results confirm the notion that EtOH may possess weak reinforcing properties in normal rats. For example, most place-conditioning studies show no effects of low EtOH doses and conditioned place aversions at higher doses (18,19). A few studies have shown EtOH-induced CPP in rats with a history of pre-exposure to EtOH (6,20,21) or when the drug is combined with food (22,23) or morphine (20,24). Bozarth (1) reported place-preference without any EtOH pre-exposure after 15 drug-compartment pairings, suggesting that a longer conditioning period might be necessary to asso-

ciate weak positive reinforcing properties of EtOH with a distinct compartment.

As mentioned previously, the relationship between anxiety and EtOH has been a matter of considerable controversy. The present data showing that anxious rats developed place preference after a short period of conditioning, 4 days, favor the hypothesis that the anxiety state may contribute to the vulnerability to EtOH intake. Thus, in a recent study Spanagel et al. (14) showed a significantly higher intake and preference for EtOH in rats selected as anxious in the plus-maze test, which led them to suggest that the degree of anxiety may underlie, at last in part, the initial motivation to drink alcohol. These results agree with the study of Stewart et al. (16) which indicated a higher degree of anxiety in EtOH-preferring than non-preferring rats.

In addition, blood EtOH levels measured within the time range of 15 min for the behavioral test were dose-dependent and seemed to be sufficient to influence the place preference conditioning.

Although not tested in the present investigation, it is important to emphasize that studies in this laboratory using EtOH in a quite similar dosage range (0.8-1.2 g/kg, *ip*) did not find differences in anxiety measurements recorded in the plus-maze test (25). Therefore, it is likely that the results of place preference conditioning were not confounded by the potential anxiolytic effect of EtOH. Also, the locomotor activity of these animals was measured and dissociated from a sedative action of EtOH at the doses used here in our study (data not shown).

In conclusion, the present data demonstrate a significant place preference for the EtOH-paired compartment by rats previously selected to be anxious. In addition, these results emphasize the relevance of basal level of anxiety of rats in terms of sensitivity to the reinforcing effects of EtOH and may contribute to the understanding of alcohol dependence.

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