

Comparison of voluntary ethanol intake by two pairs of rat lines used as genetic models of anxiety

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Abstract

The relationship between anxiety-related behaviors and voluntary ethanol intake was examined in two pairs of rat lines by the oral ethanol self-administration procedure. Floripa high (H) and low (L) rats selectively bred for contrasting anxiety responses in the open-field test, and two inbred strains, spontaneously hypertensive rats (SHR) and Lewis rats which are known to differ significantly when submitted to several behavioral tests of anxiety/emotionality, were used (9-10 animals/line/sex). No differences in the choice of ethanol solutions (2%, days 1-4, and 4%, days 5-8, respectively) in a 2-bottle paradigm were detected between Floripa H and L rats (1.94 ± 0.37 vs 1.61 ± 0.37 g/kg for ethanol intake on day 8 by the Floripa H and L rat lines, respectively). Contrary to expectations, the less anxious SHR rats consumed significantly more ethanol than Lewis rats (respective intake of 2.30 ± 0.45 and 0.72 ± 0.33 g/kg on day 8) which are known to be both addiction-prone and highly anxious. Regardless of strain, female rats consumed more ethanol than males (approximately 46%). The results showed no relationship between high anxiety and voluntary intake of ethanol for Floripa H and L rats. A negative association between these two variables, however, was found for SHR and Lewis rat strains. Data from the literature regarding the association between anxiety and alcohol intake in animal models are not conclusive, but the present results indicate that factors other than increased inborn anxiety probably lead to the individual differences in ethanol drinking behavior.

Key words

- Anxiety
- Ethanol intake
- Spontaneously hypertensive rats
- Lewis
- Selected lines
- Inbred strains

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Introduction

A variety of evidence has associated increased ethanol consumption and anxiety-related behavior. For example, the tension-reduction hypothesis predicts that individuals who are innately anxious or stressed while in a non-drugged state are more sensitive to

the anxiolytic effects of ethanol, and therefore show a higher predisposition to ethanol drinking (1). Whereas clinical studies demonstrate this correlation, there is some evidence that chronic alcohol abuse precedes anxiety and anxiety disorders, and not vice versa (2). The literature about the relationship between anxiety and ethanol intake in

animal models is quite limited. Spanagel et al. (3) reported that anxiety levels were positively correlated with alcohol consumption in Wistar rats selected from a heterogeneous group for individual differences in anxiety-related behavior on the elevated plus maze, suggesting a link between emotionality and alcohol consumption. This was confirmed in our laboratory using the conditioned place preference paradigm (4), since a significant place preference for the ethanol-paired compartment was shown by rats previously selected for anxiety. Also, in this context Möller et al. (5) showed that bilateral lesions of the central amygdala, an important region for anxiety-related behavior (6), reduced both experimental anxiety and voluntary ethanol intake. In addition, inverse strategies have also been applied, with rats selectively bred for high ethanol consumption being evaluated for their anxiety behavior. Steward et al. (7) found that genetically selected alcohol-preferring rats showed a greater innate degree of anxiety than alcohol-non-preferring rats. Later, Colombo et al. (8) found similar results using Sardinian alcohol-preferring and non-preferring rats.

Despite these positive findings, other studies did not find such a correlation. A study using alcohol-accepting and alcohol-avoiding rats showed that these lines did not differ in their anxiety profile (9). Viglinskaya et al. (10) compared several different strains of alcohol drinking and non-drinking rats in tests of anxiety and immobility and concluded that there was no correlation between alcohol consumption and measures of anxiety. An extensive study using 13 behavioral measures in alcohol-preferring and non-preferring rat lines provided mixed support for a link between alcohol drinking and emotionality or anxiety (11). Recently, a study by Fernández-Teruel et al. (12) indicated that apparently there is no direct relationship between trait anxiety, which is higher in Roman low-avoidance rats compared to Roman high-avoidance rats, and alcohol con-

sumption. In addition, Henniger et al. (13), using rats bred and selected for differences in anxiety-related behavior on the elevated plus maze, also showed no relationship between anxiety and ethanol consumption.

The aim of the present study was to determine if rats that differ genetically in anxiety-related behaviors also differ in the acquisition of ethanol intake. In the first part of the study, Floripa H and L rat lines, two directionally selected for 4 generations for high and low scores of central locomotion in the open field, respectively (14), were tested in the paradigm of ethanol self administration. In a second part of the study, spontaneously hypertensive rats (SHR) and Lewis inbred strains that are genetically contrasting regarding anxiety responses (15-17) were also tested in the same ethanol consumption paradigm.

Material and Methods

Animals

Male and female rats (12-13 weeks old) of the inbred SHR and Lewis strains and of the outbred Floripa H and L lines were used. SHR and Lewis rats were obtained from colonies bred in our laboratory under a system of brother-sister mating. Breeding and selection of the Floripa H and L rats were described previously (14). Briefly, genetically heterogeneous animals (obtained through the intercross of three rat strains, Wistar, Hooded and Lewis) were submitted to the open-field, elevated plus-maze and black/white box tests. Animals with high and low scores for central locomotion in the open field were then mated to establish the new H and L lines, respectively. At each generation, the offspring from the two breeding lines was tested in the open field and the best high- and low-score animals were selected for breeding within the H and L lines, respectively. All animals weighing approximately 250-300 g were housed individually

in hanging iron cages at constant temperature ($22 \pm 1^\circ\text{C}$) on a 12-h day/night cycle (lights off at 7:00 pm) with food and water available *ad libitum*. All procedures used in the present study were in strict accordance with the guidelines of the UFSC University Animal Care Committee (CEUA-UFSC).

Ethanol self-administration procedure

A standardized testing procedure for ethanol preference was used as described by Spanagel et al. (3), in which two bottles of either ethanol solution or water were made available continuously as a free choice to the animals. The 24-h consumption of ethanol or water was measured daily at 11:00 am. Body weight was recorded every third day. The position of the bottle was changed daily. The concentration of the ethanol solution was increased after 4 days as follows: day 1-4: 2%; day 5-8: 4% (v/v).

Statistical analysis

Data were analyzed statistically by three-way analysis of variance (ANOVA) for repeated measures, with the level of significance set at $P < 0.05$. When a main factor was found to be significant, *post hoc* comparisons were performed using the Newman-Keuls test.

Results

At the beginning of the experiment, male and female Floripa H rats weighed 314.5 ± 14.0 and 218.2 ± 5.9 g while male and female Floripa L rats weighed 313.6 ± 10.8 and 220 ± 7.3 g, respectively; male and female SHR weighed 313.5 ± 4.5 and 190.4 ± 3.4 g and male and female Lewis rats weighed 315.3 ± 11.92 and 203.6 ± 4.2 g, respectively. At the end of the experiment after 8 days, male and female Floripa H rats weighed 327.3 ± 13.5 and 220.4 ± 4.9 g while male and female Floripa L rats weighed

325.6 ± 9.8 and 229.3 ± 6.5 g, respectively. Male and female SHR weighed 326.6 ± 5.1 and 197.8 ± 3.5 g, while male and female Lewis rats weighed 325.2 ± 12.8 and 209.0 ± 4.6 g, respectively. ANOVA showed no significant differences in this measure.

Figure 1A shows ethanol intake reported as $\text{g kg}^{-1} \text{ day}^{-1}$ for male and female Floripa H

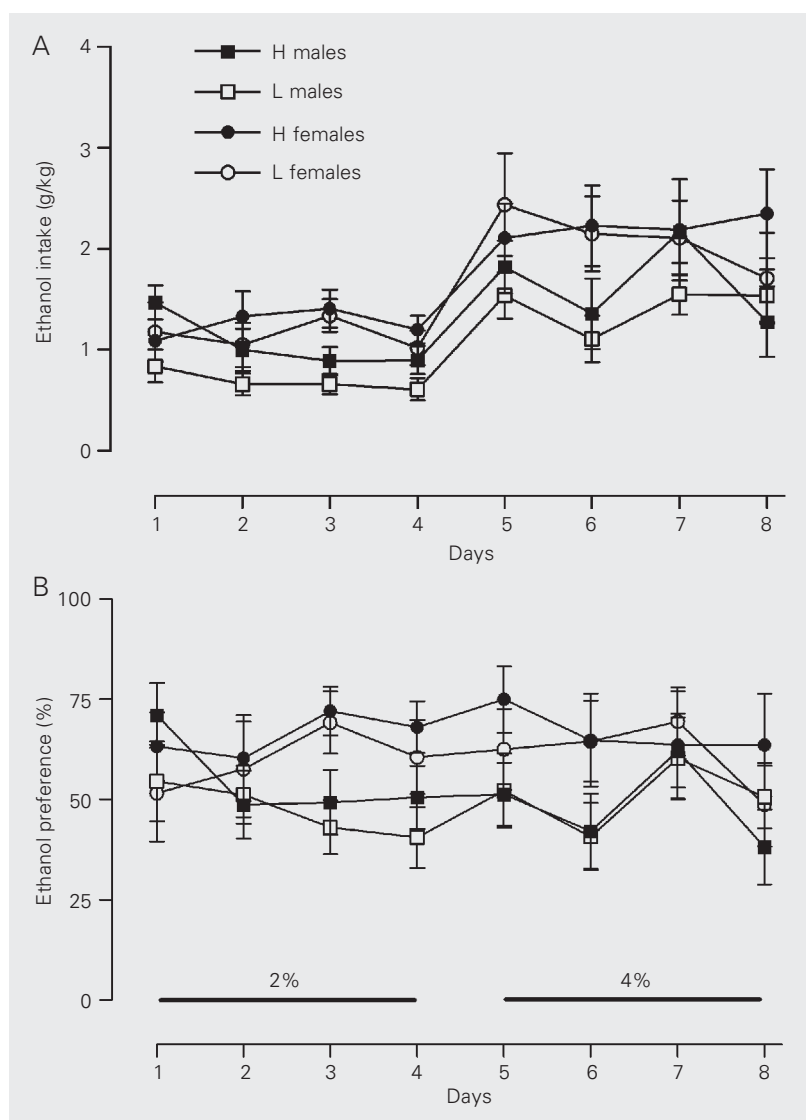


Figure 1. Comparison of ethanol intake (A) and ethanol preference (B) between Floripa high (H) and low (L) lines. The concentration of the ethanol drinking solution was increased from 2 to 4%. Data are reported as g absolute ethanol ingested daily per kg body weight in *panel A* and as percent of ethanol compared to total fluid intake in *panel B*. Each point indicates the mean \pm SEM for 9-10 animals per group. No strain effect was detected but females showed a greater ethanol intake and preference than males ($P < 0.05$, ANOVA).

and L rats exposed to two ethanol concentrations. Analysis of oral ethanol self-administration over a period of 8 days revealed a significant effect of gender ($F(1,37) = 6.48$, $P < 0.01$), with females drinking substantially more alcohol than males, and day (F

(7,259) = 15.60, $P < 0.00001$) because ethanol concentration increased after the 4th day. No main effect of strain was found ($F(1,37) = 1.231$, $P = 0.27$). Also, no significant interactions were found between gender and day ($F(7,259) = 1.61$, $P = 0.130$) or strain and day ($F(7,259) = 0.263$, $P = 0.96$).

ANOVA for ethanol preference (Figure 1B) also revealed a significant effect of gender ($F(1,37) = 4.87$, $P = 0.0335$), with females showing more preference than males. No main effect was found for strain or day ($F(1,37) = 0.49$, $P = 0.48$, and $F(7,259) = 1.32$, $P = 0.23$, respectively). No interaction was found between strain and day ($F(7,259) = 0.46$, $P = 0.86$).

Figure 2A shows the ethanol intake by male and female SHR and Lewis rats. ANOVA of these data showed a significant effect of gender ($F(1,36) = 11.91$, $P < 0.001$), with female SHR and Lewis rats consuming more ethanol than males, and of day factor ($F(7,252) = 9.27$, $P < 0.00001$), possibly related to the change in ethanol concentration. A main strain effect was significant for ethanol consumption ($F(1,36) = 76.62$, $P < 0.00001$), with SHR consuming more ethanol than Lewis rats. ANOVA also revealed a significant day vs strain interaction ($F(7,252) = 2.09$, $P = 0.04$).

ANOVA for ethanol preference (Figure 2B) did not show a significant effect of gender ($F(1,36) = 0.41$, $P = 0.52$). Nevertheless, ANOVA revealed a significant difference between strains in ethanol preference ($F(1,36) = 31.65$, $P < 0.00001$), with SHR displaying a greater preference for ethanol than Lewis rats, and also an effect of day ($F(7,252) = 3.43$, $P < 0.001$). No significant effects were found for the strain vs day interaction ($F(7,252) = 1.59$, $P = 0.13$).

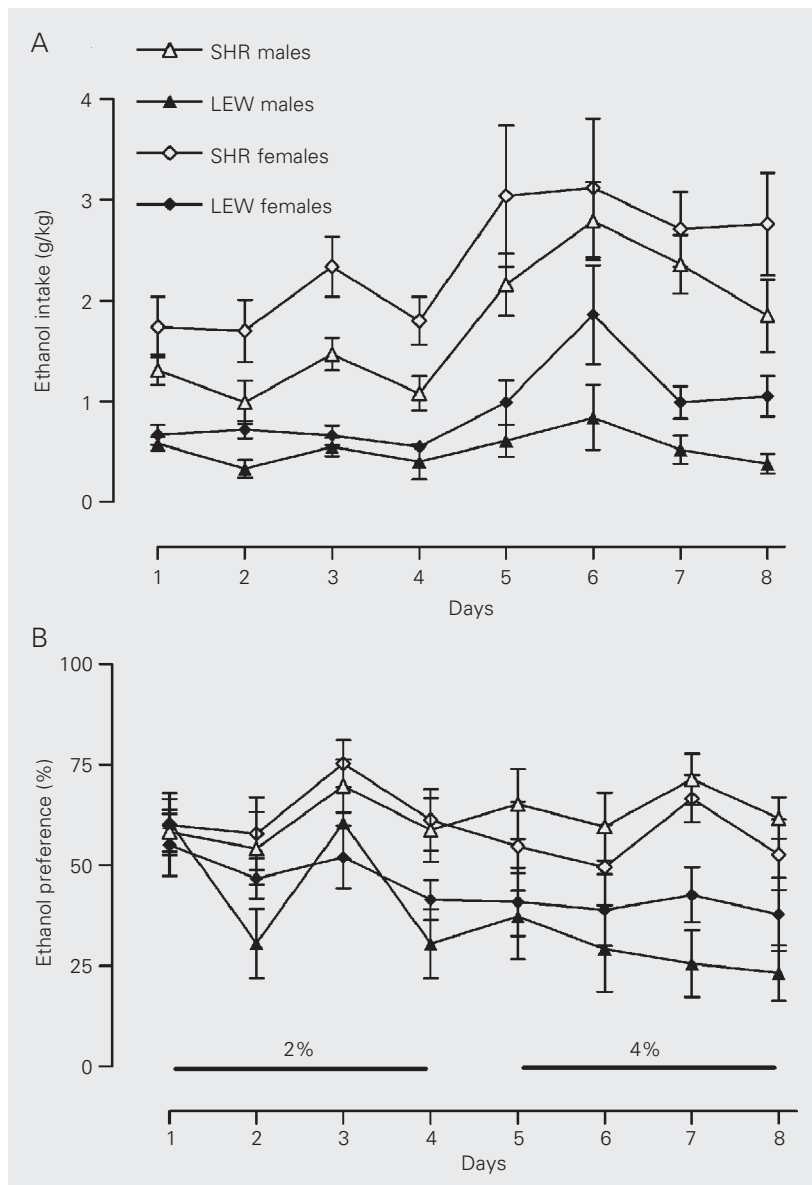


Figure 2. Comparison of ethanol intake (A) and ethanol preference (B) between spontaneously hypertensive rats (SHR) and Lewis (LEW) rats. The concentration of the ethanol drinking solution was increased from 2 to 4%. Data are reported as g absolute ethanol ingested daily per kg body weight in *panel A* and as percent of ethanol compared to total fluid intake in *panel B*. Each point indicates the mean \pm SEM of 9-10 animals per group. SHR showed a greater ethanol intake and preference than LEW rats and female rats consumed more ethanol than male rats ($P < 0.05$, ANOVA).

Discussion

In the present study, we compared two pairs of rat strains - previously known to show contrasting anxiety-related responses

in a series of paradigms (14-17) - in an oral ethanol self-administration procedure. The expected relationship between the initiation of ethanol intake and the known levels of innate anxiety was not found. No differences in ethanol intake behavior were found between the Floripa H and L rat lines genetically selected for low and high emotionality, respectively. Moreover, contrary to what was expected, SHR consumed more ethanol than Lewis rats, a strain known to present more anxiety-like behaviors than their SHR counterparts of both sexes (16,17). In addition, confirming other studies, female rats displayed a higher ethanol drinking pattern than male rats (18-21) regardless of strain. Although at variance with the study of Spanagel et al. (3), which reported that "anxious" rats consumed significantly more ethanol than "non-anxious" rats selected on the elevated plus-maze test, the present results confirm and extend recent findings suggesting that increased levels of inborn anxiety and high alcohol consumption are not necessarily related (12,13).

It is important to emphasize that in the present study the association between anxiety levels and ethanol preference was examined in two distinct pairs of rat lines genetically displaying contrasting anxiety responses, namely the Floripa H and L rats and the Lewis and SHR rats. The Floripa H and L lines were selected over 4 generations for high and low scores of central locomotion in the open field. As a result, throughout generations these lines developed differences not only regarding the selected behavior but also regarding other anxiety-related measures tested on the elevated plus maze and the black/white box (14). In all cases, Floripa H rats displayed higher levels of approach towards the aversive environments of each test. Although in a less marked manner, rats of the 4th generation of selection also showed moderate differences in peripheral locomotion in the open field, a measure thought to reflect general locomotor activity (14). More

recent experiments, however, have indicated that such differences tend to disappear in further generations (Ramos A, unpublished data).

When the animals were exposed for the first time to the oral ethanol self-administration procedure, there was no significant difference in ethanol intake between Floripa H and L rat lines. As mentioned before, these findings are in agreement with the study by Henniger et al. (13), which also used rat lines selectively bred for differences in anxiety responses, although there are some methodological differences between the two studies: in our study the rats had a choice between water and low concentrations of ethanol solutions (2 and 4%, respectively) in a 2-bottle paradigm, while in the study of Henniger et al. (13), rats had a choice between water and higher ethanol concentrations (5, 10 and 20%, respectively) in a 4-bottle paradigm from the beginning to the end. Although we did not measure blood ethanol levels, it should be pointed out that rats usually prefer alcohol solutions of lower rather than higher concentrations (i.e., >6% (v/v) ethanol solutions), probably due to taste aversion. Furthermore, in the study by Henniger et al. (13), the high anxiety behavior and low anxiety behavior lines were selected in the elevated plus-maze test. Thus, it is important to note that the elevated plus-maze and the open-field tests are likely to assess different forms of anxiety (22,23). In a recent review, Prut and Belzung (24) concluded that the open-field test may be an animal model of non-pathological anxiety, sensitive to anxiolytic-like effects of classical benzodiazepine and 5-HT₁ receptor agonists but not to the effects of compounds displaying anxiolytic-like effects regarding the clinical entity termed "anxiety disorders".

The relationship between high anxiety and high ethanol intake was not confirmed in the present study using the inbred strains SHR and Lewis. However, a negative association was found, contrary to the aforemen-

tioned tension-reduction hypothesis, with the less anxious SHR consuming more alcohol than the more anxious Lewis rats. Recent studies have shown that these strains constitute a useful genetic model for the study of anxiety, with Lewis rats consistently displaying more anxiety-like behaviors in the open field, the elevated plus maze and the black/white box, three tests of emotionality/anxiety, than their SHR counterparts of both sexes (16,17). Interestingly, the two strains do not differ in any measure of general locomotion in the open field and the elevated plus maze (17). Besides, Lewis rats are routinely used in drug research and are considered to be genetically predisposed to discriminate positive reinforcement of different classes of drugs such as cocaine (25), morphine (26) and ethanol (27). Therefore, it was surprising to find here that the SHR strain showed higher voluntary intake of ethanol than the Lewis strain. It is known that SHR are more sensitive to the hypnotic effects of ethanol and consume more ethanol than the normotensive Wistar-Kyoto rats (28). This is consistent with the data reported by Wood et al. (29), which show that genetically hypertensive mice drink more ethanol than normotensive mice. However, recent findings by Cailhol and Mormède (20) using a continuous free access procedure have shown that, after progressive initiation to ethanol, SHR drink less alcohol than Wistar Kyoto hyperactive rats. The strain difference in ethanol drinking behavior found in the present study may be due to taste aversion, since other studies have shown that strains with higher preference for sweet and/or bitter solutions also tend to display higher consumption of ethanol (30,31). Further experiments comparing saccharin and quinine pref-

erence in SHR and Lewis rats are being carried out in our laboratory in order to clarify this issue.

Certainly, conflicting results among studies on ethanol self-administration might be partly explained by methodological, species and/or strain differences. The interaction between different rat lines and behavioral measures was elegantly analyzed by Overstreet et al. (11). These investigators confirmed that there are behavioral differences between several alcohol-preferring and non-preferring rat lines/strains, with the former animals exhibiting less anxiogenic behavior than the latter, in direct contrast with earlier suggestions comparing just one pair of rat lines. Indeed, alcohol abuse and anxiety constitute a complex phenotype that is shaped by environmental and genetic factors and by an intricate interaction of these factors.

The important finding in the present study, however, is that our data, considered as a whole, fail to support the hypothesis that high ethanol intake is closely related to an inborn increased anxiety-related behavior of the animals, independent of genetic background. These data support and extend the interpretation put forward by Henniger et al. (13), that these factors might not necessarily be co-selected when animals are selectively bred either for differences in emotionality or for differences in ethanol preference. In this context, it is important to mention that findings from the National Comorbidity Survey (32) did not demonstrate a significant correlation between anxiety disorders and alcohol use disorders. Thus, the present results provide evidence for the lack of a high/general correlation between anxiety and voluntary ethanol intake in laboratory animals.

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