



# Anxiolytic effects of swimming exercise and ethanol in two behavioral models: beneficial effects and increased sensitivity in mice

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## ABSTRACT

Several behavioral mechanisms have been suggested to explain the effects of ethanol or physical exercise on anxiety. The purpose of the current study was to assess the effects of chronic and acute administration of ethanol on swimming exercise in mice, sequentially submitted to the elevated plus-maze and open-field tests. In the first experiment, sedentary or physical exercise groups received chronic treatment with ethanol (0.1, 0.2, 0.4, 2 or 4 g ethanol/kg/day by oral gavage) for 14 days before the tests. In the second experiment, groups received a single dose of ethanol (ip: 0.6, 0.8, 1.0 or 1.2 g/kg), ten minutes before the start of behavioral tests. The present study found an anxiolytic-like effect after chronic ethanol treatment or swimming exercise, evidence of beneficial effects. Moreover, we conclude that exercise can increase behavioral sensitivity to ethanol in acute treatment. The experiments described here show that the effects of ethanol on the behavior displayed in the elevated plus-maze and open-field are not only dose-dependent but also modified by swimming exercise. These results may provide valuable insights into possible molecular mechanisms governing these adaptations.

**Keywords:** Behavior. Elevated plus-maze. Ethanol. Open-field. Swimming exercise.

## INTRODUCTION

Many of the changes that happen in response to physical exercise and to ethanol have been reported in tests commonly used to assess anxiety-like or defensive behavioral response in the elevated plus-maze and open-field tests (Fukushiro et al., 2010; Hopkins & Buccì, 2010; Pohorecky, 2010). At present, the neurobiological

mechanisms underlying the decreased anxiety influenced by physical exercise are still unclear.

One of the most widely used animal models of anxiety is the elevated plus-maze (EPM), which has been pharmacologically and ethologically validated (Lister, 1987; Montgomery, 1955; Pellow & File, 1986). The behavioral variables that are typically recorded when rodents are in the elevated plus-maze are the time spent and number of entries made into the open and enclosed arms. This behavior reflects a conflict between the rodents' preference for protected areas (closed arms) and their innate motivation to explore novel environments (Lister, 1987). Thus, anxious animals will spend most of the time in the enclosed arms, while less anxious animals will explore open areas more frequently and for longer times (Pellow & File, 1986). The differences in anxiotypic behavior expressed by these animals are not limited to their performance on the EPM. The novel environment is an established measure of general anxiotypic behavior, and levels of locomotion, rearing and grooming in this paradigm can be used as indices of an anxiety-like state in rats (Courvoisier et al., 1996). Locomotion and rearing are exploratory activities, and high levels of such behavior suggest a low-anxiety state in rodents (Courvoisier et al., 1996; Cruz et al., 2010).

Studies have shown that chronic physical activity can alter the anxiety level in a variety of contexts. Most of the evidence supporting the reduction of anxiety by exercise is found in human studies and only a few reports that have examined the relationship between chronic physical exercise and anxiety levels in animals (Binder et al., 2004; Dishman et al., 1996; Tharp & Carson, 1975; Weber & Lee, 1968). Furthermore, animal studies regarding the neurophysiological mechanisms of the anxiolytic actions of exercise have shown conflicting results. In studies allowing animals voluntary access to a running wheel, there are reports of anxiolytic effects (Binder et al., 2004; Dishman et al., 1996, 1997), no effect (Pietropaolo et al., 2006) or anxiogenic effects (Burghardt et al., 2004) following exercise.

An anxiety-like effect has been observed in laboratory animals after chronic ethanol treatment (File, 1994; Valdez et al., 2002) and is also one of the withdrawal signs in alcoholics (Koob et al., 1998). These symptoms

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were observed as an increase in time spent in the enclosed arms of the EPM and a decrease in locomotion, measured in the open field (Zhang et al., 2007). File (1994) indicated that noise stress exposure during induction of ethanol dependence could abolish anxiety-like consequences of withdrawal in the EPM and social interaction tests. According to numerous data in the literature chronic ethanol administration induces an anxiogenic effect in the EPM (Cole et al., 2000; File et al., 1993).

Despite the enormous negative health and socioeconomic impact of alcohol use and abuse on the world population, light-to-moderate alcohol consumption has several human health beneficial effects. These include reduced risk of coronary heart disease, type 2 diabetes, and some types of cancer (Athyros et al., 2007; Hendriks & van Tol, 2005; Pedersen et al., 2008; Slama et al., 2002). It is well established that moderate use of alcohol improves mood, enhances feelings of happiness and freedom from care and decreases stress, tension, and depression (Baum-Baicker, 1985; Poikolainen et al., 1996). Significant increases in cognitive performance and improved short term memory also occur (Baum-Baicker, 1985; Launer et al., 1996).

The purpose of the present study was to examine the effects of ethanol, combined with swimming exercise, on behavior in mice, such as anxiety and defensive responses in the elevated plus-maze and open field.

## MATERIAL AND METHODS

### 1. Animals

Male Swiss mice, 45 days old (25-30g) were used in all experiments. Animals were housed (ten/cage) in air-conditioned rooms (23±1°C) with a 12/12 h light-dark cycle, with free access to food and water. All experimental procedures followed a protocol approved by the local institutional Animal Care and Use Committee.

### 2. Chronic ethanol treatment

In the first experiment, mice were randomly assigned to one of two experimental groups: control or swimming. Both groups were subdivided into 6 treatment groups of 10 mice, which received daily treatment with ethanol (at 0, 0.1, 0.2, 0.4, 2 or 4 g ethanol/kg/day) by gavage (po) for 14 days before the behavioral tests. Fresh stock ethanol solutions for the treatments were prepared each day by diluting ethanol (P.A.) to 14% ethanol in 0.9% NaCl (saline). The various doses were prepared by diluting this stock in saline. Control (no ethanol) animals received an equivalent volume of saline. Mice were exposed to ethanol or saline immediately after the exercise period. Twenty-four hours after the last treatment, mice were subjected to tests of anxiety-like behavior. This delay was based on previous results with rats, where the maximum effect was recorded 24 hours after the last dose of ethanol (Kliethermes, 2005; Pandey et al., 1999). The 14-day treatment with ethanol was performed during the last two weeks of exercise training (see below).

### 3. Acute ethanol treatment

In the second experiment, animals were again randomly allocated to two experimental groups, control or swimming, each of which was divided into 5 treatment groups that received a single dose of ethanol, ip (0, 0.6, 0.8, 1.0 or 1.2 g/kg), immediately after the final swimming session. The doses were prepared from 14% ethanol. Behavioral tests started ten minutes later.

### 4. Adaptation to water

All animals were allowed to adapt to water before the experiments. The adaptation consisted in keeping the animals in shallow water at 32±1°C, on 5 days of one week, in 10-min sessions, between 8.00 a.m. and 5.30 p.m. The purpose of the adaptation was to reduce the stress without, however, promoting physical training.

### 5. Exercise training

Swimming groups were trained to swim 30 min/day, 5 days a week, over 8 weeks, in a progressively increasing moderate free-style swimming program without weight loading. This program was validated previously (Dawson & Horvath, 1970; Liu et al., 2010). Daily swimming was performed in a large water tank (100 cm × 40 cm × 90 cm) at 32±1°C, filled to a depth of 60 cm. During the first 7 days, the mice swam continuously for 10 min. At the end of the 7th day, the exercise session was increased to 20 min. From day 14, the animals performed 30 min continuous exercise daily, until the end of the training period. Twenty-four hours after the last exercise session, both fully-trained and sedentary animals were subjected individually to behavioral tests. All experiments were performed between 7:30 and 11:30 a.m. and were carried out in a sound-attenuated and temperature-controlled (23±1°C) room, illuminated with one 40-W fluorescent light placed 1.3 m above of the elevated plus-maze or open field.

### 6. Behavioral tests

#### 6.1. Elevated plus-maze (EPM)

The first behavioral test procedure was performed on an elevated plus-maze, introduced by Pellow & File (1986) to measure anxiety in rats and subsequently adapted for mice by Lister (1987). The apparatus consisted of a wooden maze shaped like a plus sign, with two opposite open arms (21.5 x 7.5 cm) and two opposite enclosed arms (30 x 21.5 x 7.5 cm), all extending from a square central platform (7.5 x 7.5 cm). The floor of the maze was painted with impermeable epoxy resin, to avoid urine impregnation. The maze was raised 35 cm above the floor. A rim of Plexiglas (0.3 cm) circumscribed the open arms, to prevent accidental falls. Each animal was tested for 5 min, starting on the platform, facing an enclosed arm. The number of entries and the time spent in both open and enclosed arms were measured. The maze was carefully wiped with a damp cloth after each animal.

## 6.2. Open field

The open-field apparatus consisted of a circular wooden box (61 cm in diameter and 24 cm high) with a square grid marked on the floor and the top open. Animals were placed in the center of the open field and allowed to explore for 5 min. The following parameters were recorded: time spent entering squares (ambulation); time for which the animal did not move at all (freezing); time rearing (rising on the hind paws) and time the animal performed self-cleaning (grooming). The total ambulation and freezing times were determined as a measure of activity. Exploratory behavior in the open field has also been used as a measure of defensive behavior, where increased line crossings and rearing responses are suggestive of a decrease in defensive behavior (Royce, 1977).

## 7. Statistical analysis

All data are expressed as mean  $\pm$  S.E.M. of 10 animals per group. The data were analyzed with a repeated-measures analysis of variance (ANOVA), with group and dose as the independent variables, and performance in each session (anxiety-state indices) as the dependent variables. Post hoc analyses with Newman-Keuls paired *t* tests were used to test for differences within groups across the doses of ethanol. A probability level of 0.05 was used to test for statistical significance.

## RESULTS

### 1. Effects of chronic ethanol and/or swimming exercise measured in EPM test in mice

Analysis of variance revealed significant main effects of group (control vs. swimming exercise,  $F_{1,18} = 16.869$ ;  $p < 0.001$ ) and ethanol dose ( $F_{4,56} = 14.615$ ;  $p < 0.001$ ) on the time spent by the mice in the open arms of the plus-maze. In contrast, the group  $\times$  ethanol interaction did not have a significant effect ( $F_{4,56} = 1.139$ ;  $p > 0.05$ ; Fig. 1A). Indeed, post-hoc analysis showed that ethanol increased the proportion of open arm-time in the control group, at the doses 1.0 and 1.2 g/kg, and so did exercise in the swimming group. However, the time spent in open arms was not significantly changed by combined swimming and ethanol treatment ( $p > 0.05$ ). Considering the number of entries into the open arms, there were significant main effects of the group ( $F_{1,18} = 23.153$ ;  $p < 0.001$ ) and ethanol dose ( $F_{4,56} = 94.617$ ;  $p < 0.001$ ), but again there was no significant group  $\times$  ethanol interaction ( $F_{4,56} = 0.8922$ ;  $p > 0.05$ ; Fig. 1B). The ethanol increased the percent open-arm entries at the doses 1.0 and 1.2 g/kg, in the control group, as did swimming exercise, but there was no increase after combined treatment ( $p > 0.05$ ). Analysis of variance revealed significant differences between groups ( $F_{1,18} = 15.037$ ;  $p < 0.001$ ) and ethanol doses ( $F_{4,56} = 41.865$ ;  $p < 0.001$ ), in the time spent by the mice in the enclosed arms of the EPM; once more, the group interaction was not significant ( $F_{4,56} = 1.352$ ;  $p > 0.05$ ; Fig. 1C). The ethanol, at doses of 0.2 and 0.4 g/kg, or swimming exercise alone, induced a decrease in the % time spent in the enclosed arms, while

this time did not change significantly when swimming was combined with the ethanol treatment ( $p > 0.05$ ). Considering the number of entries into the enclosed arms, significant differences were found between different groups ( $F_{1,18} = 16.055$ ;  $p < 0.001$ ) and ethanol doses ( $F_{4,56} = 20.252$ ;  $p < 0.001$ ), but the group  $\times$  ethanol interaction was not significant ( $F_{4,56} = 0.3309$ ;  $p > 0.05$ ; Fig. 1D). Ethanol, in doses of 0.2 and 0.4 g/kg, or swimming exercise, induced a fall in the % number of entries into enclosed arms, but there was no fall after combined treatment ( $p > 0.05$ ).

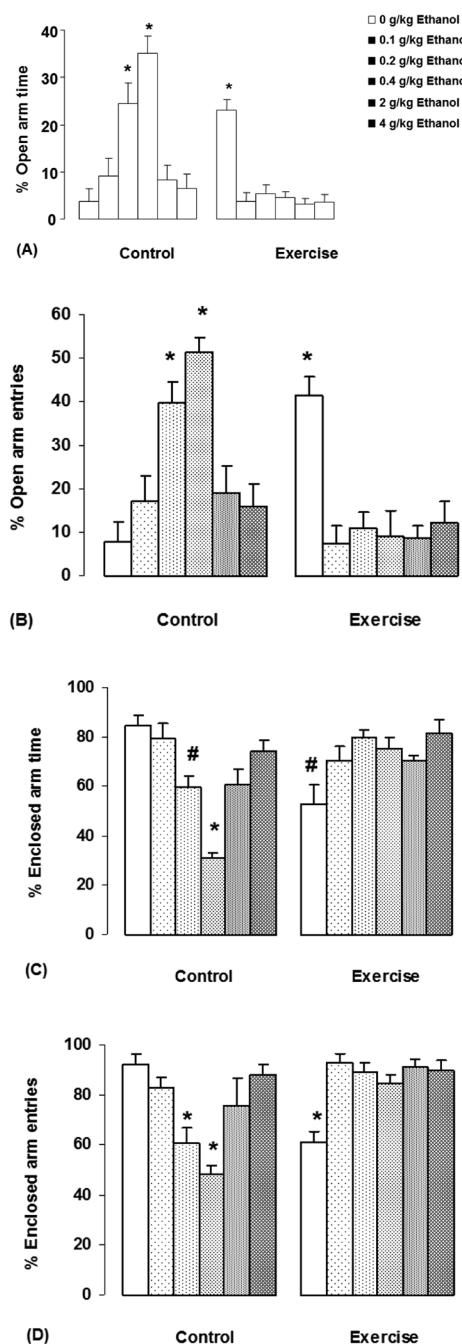


Fig. 1. Behavioral responses in the EPM of control and swimming mice 14 days after ethanol treatment: (A) % time in open arms; (B) % number of entries in open arms; (C) % time in enclosed arms; (D) % number of entries in enclosed arms. Bars represent mean  $\pm$  SEM ( $n=10$ /group). Statistical tests: ANOVA followed by Newman-Keuls ( $\#p < 0.05$ ;  $*p < 0.001$ ).

## 2. Effects of chronic ethanol and/or swimming exercise measured in open field in mice

Analysis of variance revealed significant main effects of group (control vs. swimming,  $F_{1,18} = 7.488$ ;  $p < 0.01$ ) and ethanol dose ( $F_{4,56} = 7.173$ ;  $p < 0.01$ ) on time spent in ambulation in the open field. In contrast, there was no significant group $\times$ ethanol interaction ( $F_{4,56} = 0.1766$ ;  $p > 0.05$ ) (Fig. 2A). Ethanol, at doses of 0.2 and 0.4 g/kg, or swimming exercise significantly increased the % ambulation. Considering the freezing time, there were significant differences between groups ( $F_{1,18} = 15.882$ ;  $p < 0.001$ ) and ethanol dose ( $F_{4,56} = 27.358$ ;  $p < 0.001$ ), while their interaction caused no significant change ( $F_{4,56} = 0.3996$ ;  $p > 0.05$ ; Fig. 2B). Ethanol at doses of 0.2 and 0.4 g/kg or swimming exercise significantly decreased the % freezing time. Analysis of variance revealed a significant effect of group $\times$ ethanol interaction on the time spent by the mice in rearing ( $F_{4,56} = 3.946$ ;  $p < 0.01$ ), but the effects of group ( $F_{1,18} = 1.238$ ;  $p > 0.05$ ) or ethanol dose ( $F_{4,56} = 0.9870$ ;  $p > 0.05$ ; Fig. 2C) alone were not significant. The ethanol increased the % rearing, at the doses of 0.2 and 0.4 g/kg, in the swimming exercise group. There were no significant differences in time spent in grooming in the open field, between groups ( $F_{1,18} = 0.9270$ ;  $p > 0.05$ ) or ethanol doses ( $F_{4,56} = 1.265$ ;  $p > 0.05$ ), and no interaction effect ( $F_{4,56} = 0.8477$ ;  $p > 0.05$ ) (Fig. 2D).

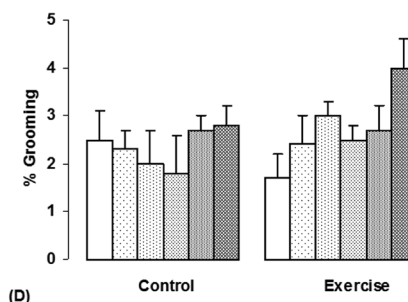
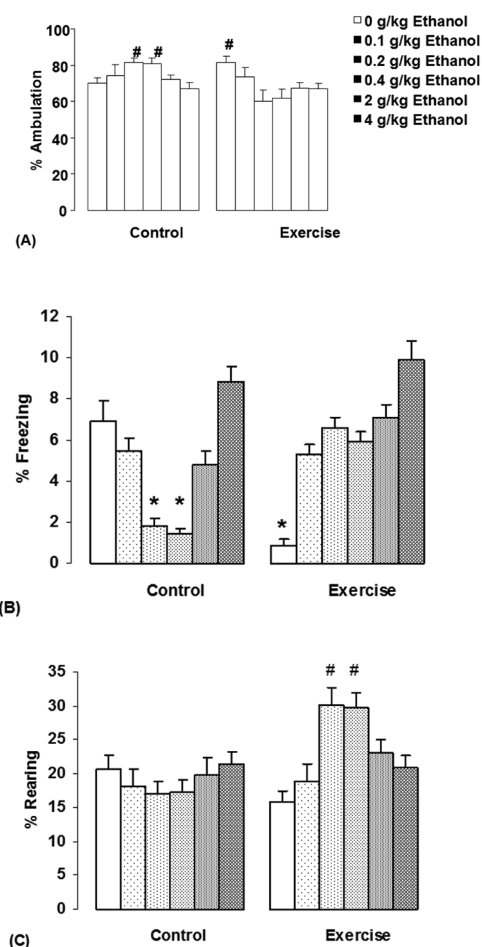
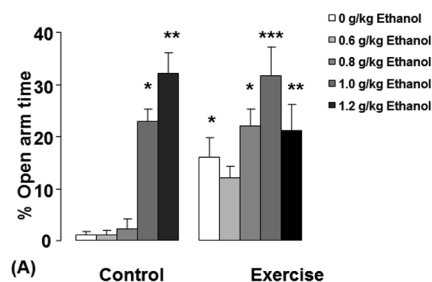


Fig. 2. Behavioral responses in the open field of control and swimming exercise groups of mice, 14 days after treatment with ethanol: (A) % ambulation; (B) % freezing; (C) % rearing; (D) % grooming. Bars represent the mean $\pm$ SEM (n=10/group). Statistical comparisons: ANOVA followed by Newman-Keuls (# $p < 0.05$  and \* $p < 0.001$ ).

## 3. Effects of acute ethanol and/or swimming exercise measured in EPM test in mice

Analysis of variance revealed significant main effects of group (control vs. exercise,  $F_{1,18} = 13.446$ ;  $p < 0.001$ ), ethanol dose ( $F_{4,56} = 38.915$ ;  $p < 0.001$ ) and group $\times$ ethanol interaction ( $F_{4,56} = 26.947$ ;  $p < 0.001$ ; Fig. 3A) on the time spent by the mice in the open arms of the plus-maze. Indeed, post-hoc analysis showed that ethanol increased the % open arm time at the two highest doses (1.0 and 1.2 g/kg) in the control group. Additionally, ethanol, at doses of 0.8, 1.0 and 1.2 g/kg in the swimming group, and swimming exercise alone, increased the time spent in the open arms. Regarding the number of entries into the open arms, analysis of variance revealed significant differences between ethanol doses ( $F_{4,56} = 31.728$ ;  $p < 0.001$ ), as well as a group $\times$ ethanol interaction ( $F_{4,56} = 37.458$ ;  $p < 0.001$ ), however, the group alone did not have a significant effect ( $F_{1,18} = 1.528$ ;  $p > 0.05$ ; Fig. 3B). Ethanol raised the % entries only at the doses of 1.0 and 1.2 g/kg in the control group and 0.8, 1.0 and 1.2 in the swimming group. There was no significant difference between groups ( $F_{1,18} = 0.1982$ ;  $p > 0.05$ ) or ethanol doses ( $F_{4,56} = 0.201$ ;  $p > 0.05$ ), or any group interaction ( $F_{4,56} = 0.3252$ ;  $p > 0.05$ ), for the time spent by the mice in enclosed arms (Fig. 3C). Regarding the number of entries into the enclosed arms, significant effects were found for ethanol dose ( $F_{4,56} = 22.982$ ;  $p < 0.001$ ) and group $\times$ ethanol interaction ( $F_{4,56} = 11.876$ ;  $p < 0.001$ ). However, the groups did not differ significantly ( $F_{1,18} = 1.7200$ ;  $p > 0.05$ ; Fig. 3D). The ethanol reduced the % entries into enclosed arms at the doses of 1.0 and 1.2 g/kg in the control group and at 0.8, 1.0 and 1.2 g/kg in the swimming exercise group.



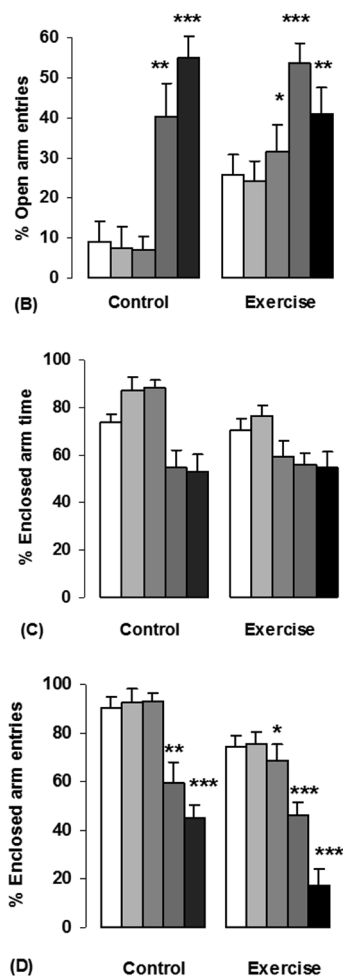


Fig. 3. Behavioral responses on the elevated plus-maze of control and swimming mouse groups after acute ethanol treatment: (A) % open arm time; (B) % open arm entries, (C) % enclosed arm time; (D) % enclosed arm entries. Bars represent the mean±SEM (n=10/group). Statistical tests: ANOVA followed by Newman-Keuls (\*p<0.05; \*\*p<0.01, \*\*\*p<0.001).

#### 4. Effect of acute ethanol and/or swimming exercise measured in open-field test in mice

Analysis of variance revealed significant main effects of group (control vs. swimming exercise,  $F_{1,18} = 12.050$ ;  $p < 0.001$ ), ethanol dose ( $F_{4,56} = 12.386$ ;  $p < 0.001$ ) and group×ethanol interaction ( $F_{4,56} = 23.475$ ;  $p < 0.001$ ), on the time spent by the mice in ambulation in the open field (Fig. 4A). Ethanol raised this % time only at the doses of 1.0 and 1.2 g/kg in the control group and 0.8, 1.0 and 1.2 g/kg in the swimming exercise group. Regarding the freezing time, there were significant effects of group ( $F_{1,18} = 6.325$ ;  $p < 0.001$ ), ethanol dose ( $F_{4,56} = 9.831$ ;  $p < 0.001$ ) and group×ethanol interaction ( $F_{4,56} = 12.191$ ;  $p > 0.05$ ; Fig. 4B). Percent freezing time was significantly reduced by ethanol, at doses of 1.0 and 1.2 g/kg in the control group and at 0.8, 1.0 and 1.2 g/kg in the exercise group, or by swimming alone. There were no significant effects of group ( $F_{1,18} = 0.8159$ ;  $p > 0.05$ ), ethanol dose ( $F_{4,56} = 1.4650$ ;  $p > 0.05$ ) or group interaction ( $F_{4,56} = 0.6799$ ;  $p > 0.05$ ) in the time spent in rearing in the open field (Fig. 4C). Moreover, there was

no significant effect of group ( $F_{1,18} = 1.447$ ;  $p > 0.05$ ), ethanol dose ( $F_{4,56} = 0.3018$ ;  $p > 0.05$ ) or group×ethanol interaction ( $F_{4,56} = 1.481$ ;  $p > 0.05$ ) in the time spent in grooming (Fig. 4D).

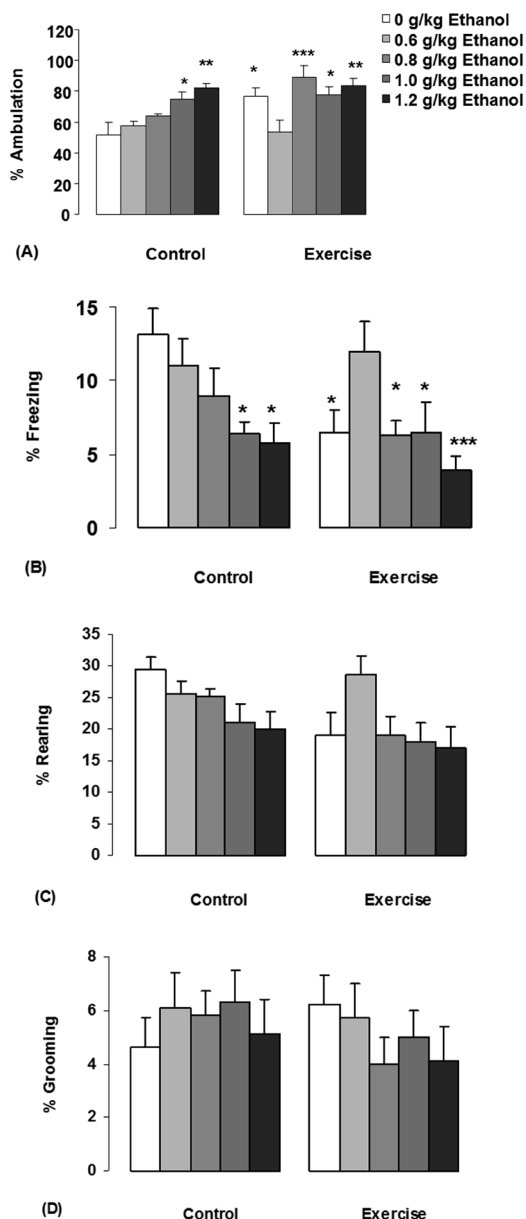


Fig. 4. Behavioral responses in the open field of control and swimming exercise groups of mice after acute ethanol treatment: (A) % ambulation; (B) % freezing; (C) % rearing; (D) % grooming. Bars represent the mean±SEM (n=10/group). Statistical comparisons: ANOVA followed by Newman-Keuls (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001).

#### DISCUSSION

The results of the first experiment suggest that chronic ethanol (0.2 – 0.4 g/kg) or swimming exercise induced an anxiolytic-like effect in mice in the elevated plus-maze (EPM) test. These symptoms were observed as an increase in both % time and % open-arm entries (Fig. 1A and 1B). These results strongly indicate that swimming exercise or treatment with low doses of ethanol results in

an improved coping with aversive situations, leading to a reduced anxiety level. In contrast, the alterations produced by combining swimming with the ethanol treatment showed clear signs of reduction of such anxiolytic effects. These data suggest that the effects of low doses of ethanol may differ from those normally produced in animal models of alcoholism (Cole et al., 2000; File, 1994; Koob et al., 1998; Valdez et al., 2002; Zhang et al., 2007). Only a few studies have been carried out to investigate the neurobehavioral changes produced by the prolonged intake of low doses of ethanol.

Prolonged ethanol treatment (at 0.2 – 0.4 g/kg) or swimming exercise increased ambulation and reduced freezing in the open field, indicating a reduction of fear and an increase in exploratory activity. In addition, swimming exercise increased rearing behavior, but only in mice treated chronically with moderate amounts of ethanol (Fig. 2C). Thus, the increase in spontaneous rearing seen in the present study can be attributed to decreased anxiety-related behavior. In tests of open-field behavior, low levels of locomotion, rearing, freezing and other behavior such as grooming and shivering are conventionally viewed as isomorphic with the hypervigilance, hesitancy, fear, and autonomic responsiveness characteristic of human anxiety (Brühl et al., 2011).

These results indicate that swimming exercise and/or low doses of ethanol reduce anxiety-like behavior in two animal tests of anxiety, without a significant change in total activity levels. However, swimming exercise combined with chronic ethanol exposure did not increase the anxiolytic-like behavior in the EPM in mice, twenty-four hours after the last treatment. Several physiological mechanisms have been suggested to explain the beneficial effects of ethanol and exercise on anxiety (Dishman et al., 1996; Martinsen & Raglin, 2007; Paluska & Schwenk, 2000). To examine the effects of chronic exercise on brain activity, researchers have used experimental designs that utilize low-intensity exercise for prolonged periods of time over an extended training period. Increases in open-field locomotion, consistent with reduced anxiety, have been reported in albino rats following swimming exercise (Tharp & Carson, 1975; Weber & Lee, 1968) and after treadmill running (Dishman et al., 1996; Tharp & Carson, 1975).

The present study addressed several questions, including whether the effects of ethanol treatment changed when it was combined with swimming exercise and whether distinct changes were seen in different animal models of anxiety-like or defensive behavior measured in EPM and open-field tests. The anxiolytic action of ethanol was seen only at low, and not at higher, doses, supporting the suggestion of an inverted U dose response relation (Aguayo et al., 2002). A wide variety of withdrawal syndromes have been defined, and the neurobiology of many chemical and behavioral dependencies has been characterized (Daniel et al., 2004; Koob & Volkow, 2010; Taylor et al., 2007; Ussher et al., 2001; 2004).

Alcohol dependence is characterized by the presence of withdrawal symptoms (physical and psychological) after drinking ceases, which results from physical dependence on alcohol. Withdrawal from alcohol in humans is characterized by central nervous system hyperexcitability, seizures, autonomic dysregulation, anxiety, restlessness,

nausea, sleeplessness and depression. Moreover, after withdrawal from exercise, some studies have found signs of depression and anxiety, or other indications of negative affective states in both mice and humans (Aidman & Woolard, 2003; Malisch et al., 2009). In the current study, an anxiolytic-like effect was observed in mice treated with low doses of ethanol. These results agree with previous studies that suggest a beneficial effect of ethanol in low doses. In a large multicenter study by Athyros et al. (2007), it was found that, with moderate alcohol consumption (20 to 45 grams of ethanol per day), the prevalence of type 2 diabetes, coronary heart disease, peripheral arterial disease and overall cardiovascular disease was significantly less than in a non-drinking control group.

The results of the second experiment suggest that either acute ethanol or swimming exercise induced anxiolytic-like effects in mice in both the elevated plus-maze and open-field tests. In the EPM, these effects were observed as an increase in % open-arm time and % open-arm entries (Fig. 3A and 3B). In addition, ethanol or swimming increased ambulation time and reduced the amount of time spent in immobility in the open field (Fig. 4A and 4B). These findings are consistent with other studies showing that low to moderate doses of ethanol stimulate motor activity in rodents (Boerngen-Lacerda & Souza-Formigoni, 2000; Correa et al., 2003). Moreover, it has been proposed that motor stimulation reflects the positive reinforcing, or euphorogenic, properties of ethanol, since both phenomena result from activation of common neuronal pathways (Da Silva et al., 2005; Ghosland et al., 2005; Wise & Bozarth, 1987).

The experiments described here show that the effects of acute ethanol on behavior displayed in the EPM and open-field tests are not only dose-dependent but also depend on the swimming exercise. Corroborating our finding on increased sensitivity to anxiolytic effects, it was reported that repeated exposure (14 days) to forced swimming stress in the inbred mouse strain C57BL/6J increased sensitivity to the sedative/hypnotic and hypothermic effects of ethanol (Boyce-Rustay et al., 2007). Increased sensitivity to the sedative/hypnotic effects of ethanol is one factor associated with reduced ethanol drinking in mice (Naassila et al., 2002; Palmer et al., 2004). Chronic swim stress significantly potentiated sleep time responses to 4 g/kg ethanol measured twenty-four hours after the final stressor in C57BL/6J mice (Boyce-Rustay et al., 2007; Boyce-Rustay et al., 2008). However, work examining the effects of stress on sensitivity to the behavioral effects of acute ethanol challenge has not produced a clear consensus (Brown et al., 2001; Cunningham & Bischof, 1987; Roberts et al., 1995). Supporting the notion that swimming exercise may be one factor contributing to this variability, the main finding of the present study was that repeated exposure to swimming exercise produced alterations in ethanol-related behavior.

In summary, the present study found anxiolytic-like effects after low-dose chronic ethanol treatment or swimming exercise, evidence of the beneficial effects of these treatments. Moreover, we conclude that exercise can increase behavioral sensitivity to ethanol in acute treatment. Such work may provide valuable insights into possible molecular mechanisms governing these adaptations.

## RESUMO

*Efeitos ansiolíticos do exercício de natação e etanol em dois modelos comportamentais: efeitos benéficos e aumento na sensibilidade em camundongos*

Vários mecanismos comportamentais foram propostos para explicar os efeitos do etanol ou do exercício sobre a ansiedade. O objetivo do presente estudo foi avaliar os efeitos da administração crônica e aguda de etanol sobre o exercício de natação em camundongos, seqüencialmente submetidos aos testes do labirinto em cruz elevado e campo aberto. No primeiro experimento, os grupos de sedentários e exercício físico receberam tratamento crônico com etanol (0,1; 0,2; 0,4; 2 e 4 g de etanol/kg/dia através de gavagem oral) durante 14 dias antes dos testes. No segundo experimento, os grupos receberam uma única dose de etanol, i.p. (0,6; 0,8; 1,0 ou 1,2 g de etanol/kg), dez minutos antes do início dos testes comportamentais. O presente estudo encontrou efeitos ansiolíticos após tratamento crônico com etanol ou exercício de natação, provas dos efeitos benéficos. Além disto, concluímos que o exercício pode aumentar a sensibilidade comportamental ao etanol no tratamento agudo. Os experimentos aqui descritos mostram que os efeitos do etanol sobre o comportamento exibido no labirinto em cruz elevado ou campo aberto não são apenas dose-dependente, mas também depende do exercício de natação. Este trabalho pode fornecer "insights" valiosos sobre os possíveis mecanismos moleculares que regem essas adaptações.

*Palavras-chave:* Campo aberto. Comportamento. Etanol. exercício de natação. Labirinto em cruz elevado.

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