Expression of TrkB receptors in the nucleus accumbens and prefrontal cortex of mice exposed to ethanol and voluntary physical activity

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ABSTRACT

Alcohol use disorder (AUD) is known to be influenced by environmental factors. Voluntary physical activity (VPA) has been proven to be rewarding and to play a role in preventing drug relapse. In a previous study, we found that VPA mitigated ethanol-rewarding effects by preventing ethanol-induced conditioned place preference (CPP). There is evidence demonstrating alterations in the neurotrophic signaling of brain-derived neurotrophic factor (BDNF) in mice subjected to both CPP conditioning and VPA. Considering the putative participation of the BDNF signaling in ethanol-CPP and the high-affinity BDNF receptors TrkB 145 kDa and 90 kDa, we sought to address whether the expression of these receptors is changed in the nucleus accumbens (NAc) and prefrontal cortex (PFC) of ethanol-treated mice exposed to VPA. Mice were assigned into four groups. They were housed in home cages with locked (“Sedentary”) or unlocked running wheels (VPA), and treated with saline or 1.8 g/kg ethanol during the conditioning phase. The groups are referred as Saline-Sedentary, Saline-VPA, Ethanol-Sedentary and Ethanol-VPA. TrkB receptors do not play a relevant role in ethanol-induced CPP or in VPA-induced protection against ethanol CPP. VPA and ethanol exposure decreased TrkB 145/90 ratio in the PFC. No differences were found among groups in the NAc. Considering that TrkB 90 kDa can counterbalance the excessive neurotrophic signaling mediated by BDNF-TrkB 145 kDa in the brain, the lower TrkB 145/90 ratio might represent a putative protection to stressful events involving BDNF-induced glutamatergic hyperexcitability and excitotoxicity.

KEYWORDS: ethanol, voluntary physical activity, running wheels, TrkB 145 kDa receptor, TrkB 90 kDa receptor, conditioned place preference, neuroprotection.

INTRODUCTION

Alcoholism is a major health issue worldwide and one of the most frequent substance use disorders. Non-pharmacological approaches such as voluntary physical activity (VPA) should be recommended as part of the range of therapeutic approaches for the treatment and prevention of alcohol use disorder (AUD). Although positive correlation between alcohol consumption and physical exercise has been demonstrated [1, 2], evidence, both in human and animal models, show that physical activity can be a protective factor against AUD [3, 4]. For instance, we found that VPA prevented the development of ethanol-induced CPP in mice [5]. Neurochemical alterations involving neurotrophic factor signaling have been identified in mice...
subjected to VPA [6], as well as in mice subjected to CPP to ethanol [7]. A few studies have examined changes in BDNF-TrkB neurotrophic signaling related to ethanol-rewarding effects [7, 8]. Heterozygous BDNF knockout mice consumed more ethanol and expressed higher ethanol-induced CPP, while BDNF overexpression decreased ethanol intake [7]. Exposure to running wheels increased hippocampal BDNF mRNA in mice [6]. BDNF is released in the synaptic cleft and binds with high affinity to the transmembrane receptors TrkB, which can be divided into two different receptor subtypes: TrkB 145 kDa (or full length) and TrkB 90 kDa (or truncated). TrkB 145 kDa receptor activation by its agonist BDNF elicits several alterations, including neuronal depolarization, increase in synapses number per neuron, dendritic and axonal growth and apoptosis inhibition. On the other hand, BDNF binding to TrkB 90 kDa induces antagonistic effects when compared to TrkB 145 kDa [9, 10].

Based on these studies, we sought to verify whether the previously demonstrated prevention of ethanol-CPP by VPA [5] would be related to TrkB signaling in brain structures related to drug and natural reward.

**MATERIALS AND METHODS**

**Subjects**

Male Swiss Webster outbred mice, 75 days old, were maintained in polycarbonate cages (42 cm length × 28 cm width × 21.5 cm height), grouped in 4 mice per cage, with food and water ad libitum throughout the entire experiment, under controlled temperature (21 ± 1 C) and light/dark cycle of 12:12 h (lights on at 7:00 AM). The behavioral experiments were conducted during daytime (between 8:00 AM and 12:00 AM). All the experimental procedures were conducted according to the guidelines of the Brazilian National Council for Control of Animal Experimentation (CONCEA), in accordance with the Brazilian National Law number 11,794 of October 8, 2008, Decree 6899 of July 15, 2009, after the approval from the Ethics Committee of Animal Use (Protocol 172/2013) of the Institute of Biomedical Sciences of the University of Sao Paulo.

**Assessment of VPA in running wheels**

For the VPA groups, four unlocked running wheels were introduced in each home cage (42 cm length, 28 cm width, 21.5 cm height; 4 mice/cage). The wheels were provided during the conditioning phase of the CPP protocol. Running distance was not recorded, as the animals were grouped. Likewise, the “Sedentary” groups were maintained in cages with four locked running wheels per cage. Mice were randomly assigned to one of the two activity conditions and treated with saline or ethanol during the conditioning phase: Saline-Sedentary (wheels locked, treated with saline); Saline-VPA (wheels unlocked, treated with Saline); Ethanol-Sedentary (wheels locked, treated with ethanol); Ethanol-VPA (wheels unlocked, treated with ethanol).

**Ethanol-induced CPP**

Ethanol (95% v/v, Merck, Rio de Janeiro, Brazil) was dissolved in saline (0.9% sodium chloride w/v) to make a solution of 20% v/v ethanol and was administered intraperitoneally (i.p.) at the dose of 1.8 g/kg. Control mice were i.p. injected with equivalent volumes of 0.9% saline. The CPP apparatus consisted of a rectangular box (44 × 14 × 14 cm) divided into three chambers: two equal-sized (17.5 × 13 × 14 cm) end-compartments. The conditioning protocol [5] consisted of: (1) Habituation (H1) – each animal received a saline injection (i.p.) and had free access to all the compartments of the apparatus during 15 min. (2) Conditioning phase (8 conditioning sessions: C1–C8) – The conditioning sessions started 24 h after habituation. Each mouse was exposed to four conditioning pairings with ethanol (1.8 g/kg, i.p.) alternated daily with four pairings with saline (i.p.) for 5 min per conditioning trial. Half of the mice received ethanol in one compartment (rod floor), and the other half in a mesh floor compartment. Half of these animals received ethanol on the first conditioning day, while the other half was injected with saline. For saline groups, mice were injected with saline in all conditioning sessions. (3) Test trial – conducted twenty-four hours after the last conditioning session. On the test day, drug-free mice were placed into the central compartment, with free access to all compartments for 15 min. Two hours
after the test, animals were euthanized by cervical dislocation, and the brains were immediately removed and placed on dry ice. The brains were stored at -80°C.

**Protein expression and Western blot**

Dissection of prefrontal cortex (PFC) and nucleus accumbens (NAc), protein extraction and Western blot were performed as previously described [5]. The polyclonal primary antibodies anti-TrkB (1:200,000, Millipore) were used. The polyclonal primary antibody α-tubulin (1:40,000, Sigma-Aldrich) was used for normalization of the target proteins. The reagent enhanced chemiluminescence ECL (GE Healthcare Amersham) was used for immunoreactive protein band detection. Protein bands were exposed on ECL Hyperfilm (GE Healthcare Bio-Sciences, Little Shalfont, Buckinghamshire, UK). Optical density was quantified by the software ImageJ 1.48 V (National Institute of Health, Bethesda, MD, USA). For the Western blot data analysis, the optical density of the bands for the control group (Saline-Sedentary) was averaged for each gel, and the optical densities of the bands from the other groups were calculated as a percentage of the control value obtained from the same gel.

**RESULTS**

A two-way Analysis of Variance (2-way ANOVA) was performed to determine whether or not there were statistically significant differences in TrkB 145 kDa and TrkB 90 kDa levels among the groups in the NAc. There were no main effects or interactions (all p values > 0.05) (Figure 1A and B). Levene’s test detected heterogeneity of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Levels of TrkB 145 kDa (A), TrkB 90 kDa (B) and TrkB 145/TrkB 90 ratio (C) in the NAc of Sedentary and VPA mice conditioned to saline or ethanol. Values are expressed in mean ± SEM. N = 8-10/group.
DISCUSSION

The main finding of the present study is a lower TrkB 145kDa/TrkB 90 kDa ratio in mice exposed to ethanol or VPA. To understand the possible meaning of this result, it is important to specify the role of each TrkB receptor subtypes. The TrkB 145-kDa receptor – also called full length receptor – is a transmembrane catalytic receptor that homodimerizes after binding to its agonist BDNF, catalyzing a self-phosphorylation reaction. The BDNF-TrKB 145-kDa binding leads to the activation of intracellular biochemical events that culminate in synaptic plasticity, including: dendritic and axonal growth, higher number of synapses per neuron, long-term potentiation, neuronal proliferation,

variances among the groups and Kruskal-Wallis test was used to evaluate differences in the NAc TrkB 145/90 ratio. There were no main effects or interactions (all p values > 0.05) (Figure 1C). Kruskal-Wallis test was used to evaluate differences in TrkB 145 kDa and TrkB 90 kDa levels in the PFC because Levene’s test detected heterogeneity of variances among the groups. No main effects or interactions were found (all p values > 0.05) (Figure 2A and B). A two-way ANOVA was used to compare TrkB 145/90 ratio among groups in the PFC (Levene’s tests values were > 0.05). A main effect of treatment (p = 0.0107) and VPA (p = 0.0465) was found, but no interaction (p = 0.5065).

Figure 2. Levels of TrkB 145 kDa (A), TrkB 90 kDa (B) and TrkB 145/TrkB 90 ratio (C) in the PFC of Sedentary and VPA mice conditioned to saline or ethanol. *p < 0.05, comparison between VPA and Sedentary groups (2-way ANOVA); #p < 0.05, comparison between Ethanol and Saline groups (2-way ANOVA). Values are expressed in mean ± SEM. N = 8-10/group.
Running wheels and ethanol alter TrkB's expression ratio

involving excessive BDNF-induced depolarization, such as hypoglycemia, ischemia, traumatic brain injuries and epilepsy [11, 12]. In line with this hypothesis, exposure to running wheels or to low ethanol doses elicits neuroprotective effect. For instance, running wheels' exposure attenuated seizure susceptibility in rats [13, 14], and protected the brain of gerbils against neuronal damage induced by ischemia [15]. In addition, previous data demonstrated that ethanol administration attenuates cerebral ischemia in mice [16]. In humans, moderate consumption of ethanol is associated with reductions in cerebrovascular morbidity and mortality [17].

CONCLUSION

In conclusion, the present study demonstrated that ethanol and VPA can change the balance of TrkB 145-kDa and 90 kDa in the PFC. The lower TrkB 145/90 ratio might represent a putative protection to stressful events involving BDNF-induced glutamatergic hyperexcitability and excitotoxicity. Future studies involving expression of BDNF and the activated form of TrkB (p-TrkB) might yield a more complete picture regarding the role of BDNF signaling in both ethanol and VPA-induced reward.

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CONFLICT OF INTEREST STATEMENT

Marcos Brandão Contó declares that he has no conflict of interest. Vânia D’Almeida declares that she has no conflict of interest. Rosana Camarini declares that she has no conflict of interest.

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