Imipramine can be effective on depressive-like behaviors, but not on neurotrophic factors levels in an animal model of bipolar disorder induced by ouabain

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Abstract

Introduction: Despite the risk of mania switch to long-term administration of antidepressants in bipolar disorder (BD) patients, an acute administration of these drugs can help in depressive episodes. Furthermore, alterations in neurotrophic factor levels seem to be part of the BD's physiopathology. Therefore, the aim of the present study is to evaluate the effect of acute treatment of imipramine on behavior and neurotrophins levels in rats submitted to the animal model of BD induced by ouabain.

Methods: Wistar rats received a single intracerebroventricular (ICV) injection of artificial cerebrospinal fluid or ouabain. The rats were treated for 14 days with saline, lithium, or valproate. On the 13^{th} and 14^{th} days of treatment, the animals received an additional injection of saline or imipramine. Behavior tests were evaluated seven and 14 days after ICV injection. Adrenal gland weight and levels of ACTH were evaluated. Levels of BDNF, NGF, NT-3, and GDNF were measured in the frontal cortex and hippocampus.

Results: The administration of ouabain induced mania- and depressive-like behavior in the animals seven and 14 days after ICV, respectively. The treatment with lithium and valproate reversed the mania-like behavior. All treatments were able to reverse most of the depressive-like behaviors induced by ouabain. Moreover, ouabain increased HPA-axis parameters in serum and decreased the neurotrophin levels in the frontal cortex and hippocampus. All treatments, except imipramine, reversed these alterations.

Conclusion: It can be suggested that acute administration of IMI alone can be effective on depressive-like symptoms but not on neurotrophic factors alterations present in BD.

1 Introduction

Bipolar disorder (BD) is a complex and multifactorial mental illness that affects 1–4% of the world population and presents a high risk of suicide compared to the general population [1, 2]. BD is characterized by a cyclic and chronic presentation of two opposites mood episodes, mania and depression. Mania episodes are characterized by symptoms such as irritability, agitation, and flight of ideas. Oppositely, depression episodes are defined by the presence of depressive humor, anhedonia, and recurring thoughts of death [3].

BD pathophysiology is not fully known; however, previous studies showed that alteration in neurotrophin levels is a factor in this complicated disorder [4]. Neurotrophins play a role in neuroplasticity, memory, and especially in the growth and survival of neurons and glial cells [5]. BD patients show lower serum levels of many neurotrophic factors, especially brain-derived neurotrophic factor (BDNF) [6], nerve growth factor (NGF) [7], and glial cell-line derived neurotrophic factor (GDNF) [8]. It is important to point out that the levels of neurotrophin-3 (NT3) are also reduced in the serum of patients with BD, but only during mania episodes [9].

In this same context, chronic stress was also associated with lower levels of BDNF [10]. Indeed, the hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis is another factor in the pathophysiology of

BD [11]. The HPA axis responds mainly to stress, releasing glucocorticoid hormones by the adrenal gland, including cortisol in humans. The release of this hormone is controlled by the levels of adrenocorticotropic hormone (ACTH), which is controlled by the levels of corticotropin-releasing hormone (CRH) [12]. BD patients showed increased levels of ACTH and cortisol, but not CRH in the serum [13].

The standard gold treatment for BD is lithium (Li), a mood stabilizer, which improves the symptoms of both mood episodes, being more effective in mania [14]. The mechanism behind the therapeutic effect of Li is not fully known. Nevertheless, recent studies demonstrate a direct inhibitory effect of the enzyme glycogen synthase kinase-3 beta, influencing many molecular pathways, such as neurotrophins, oxidative stress, apoptosis, and the HPA axis [15, 16]. It is important to point out that only one out of three patients with BD will achieve remission with Li; therefore, other alternative treatments are necessary [17]. Valproate (VPA), an anticonvulsive, and imipramine (IMI), an antidepressive, are alternative drugs that present effects in reducing the disorder's symptoms in the BD. [18–20]. However, IMI treatment for BD remains controversial since a long-term administration can result in a manic switch, and IMI use is better appropriate for acute bipolar depressive episodes [20, 21].

Animal models are an important tool for studying many disorders and neurological illnesses, especially regarding the pathophysiology and new treatments [22]. The animal model of BD induced by ouabain (OUA), a Na+/K+-ATPase inhibitor, previously described by our research group, can mimic mania-like and depressive-like behavior in the same animal, and Li can reverse these behavior alterations [23]. This animal model is based on the Na+/K+-ATPase theory for BD. Evidence shows that patients with BD present a reduced activity of the enzyme Na+/K+-ATPase [24,25], and this alteration could be responsible for the mood changes characteristic of the disorder [26,27]. Lastly, this animal model can also mimic other pathophysiology pathways of BD, such as alterations in the levels of neurotrophins and oxidative stress [23, 28].

As described before, a previous preclinical study from our research group demonstrated that chronic administration of IMI induced mania-like switching in OUA-induced depressive-like animals [29]. Despite the risk of mania switch to long-term administration of antidepressants in BD patients [30], an acute administration of these drugs can help in depressive episodes. Furthermore, alterations in neurotrophic factor levels, such as BDNF, NGF, NT-3, and GDNF seem to be part of the BD’s physiopathology [4, 31]. Therefore, the present study aims to evaluate the effect of acute treatment of IMI in depressive-like behavior and neurotrophic factors levels in rats submitted to the animal model of BD induced by OUA.

2 Material And Methods

2.1 Animals

The experimental procedures followed the terms of the Brazilian Society for Neuroscience and Behavior (SBNeC), as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals [32]. The local ethics committee *Comissão de Ética no Uso de Animais da Universidade do Extremo Sul*

Catarinense (protocol number #66/2010) approved this study. It was used in male Wistar rats 60 days old (bodyweight 250-350g). The animals were from the colony of *Universidade do Extremo Sul Catarinense*. They were maintained in five animals per cage, under temperature (22 ± 1ºC), relative humidity (45–55%), and day/light cycle (12:12 h, light on at 06:00 h). The rats had free access to food (standard diet for laboratory animals - NUVILAB CR-1®, Brazil) and water.

2.2 Surgical Procedure

The animals were anesthetized via intramuscular with ketamine (80 mg/kg) and xylazine (10 mg/kg). After, were placed in the stereotaxic apparatus, where was removed the skin of the skull of the rats and were placed a 27-gauge 9 mm guide cannula (at 0.9 mm posterior to bregma, 1.5 mm right from the midline and 1.0 mm above the lateral brain ventricle, 3.3 mm ventral to the superior surface of the skull, through a 2-mm hole made at the cranial bone), fixed with dental acrylic cement [33]. In the postsurgical period, the animals recovered within 72h and were treated with tramadol 10mg/Kg at 12/12h by subcutaneous to reduce any pain from the surgery.

2.3 Experimental design

On the fourth day after surgery, the animals received a single injection of 5 µl of artificial cerebrospinal fluid (aCSF) or ouabain 10^{-3}M (dissolved in aCSF) via intracerebroventricular (ICV) [27,34]. In the guide, the cannula was placed a 30-gauge infusion cannula, connected by a polyethylene tube to a microsyringe. Aiming the lateral brain ventricle, the tip of the cannula infusion protruded 1.0 mm beyond the cannula guide. Immediately after ICV injection, the animals were treated via intraperitoneal (IP) (volume of 1ml/kg) with Sal (saline solution, 0.9%), Li (47.5 mg/kg) or VPA (200mg/kg) for 13 days. On the 13th and 14th experimental days, the animals received an extra IP injection of IMI (10mg/kg) or Sal, according to Scheme 1. To improve the explanation of the treatment of each experimental group, a table was created. (Table 1).

Table 1
Experimental groups. ICV = intracerebroventricular; aCSF = artificial cerebrospinal fluid; OUA = ouabain; IP = intraperitoneal; Sal = saline; Li = lithium; VPA = valproate; IMI = imipramine.

Group	Model (ICV)	Treatment (IP)		
	Day 1	Day 1–12	Day 13	Day 14
aCSF + Sal	aCSF	Sal	Sal + Sal	Sal
aCSF + Li	aCSF	Li	Li + Sal	Sal
aCSF + VPA	aCSF	VPA	VPA + Sal	Sal
aCSF + IMI	aCSF	Sal	Sal + IMI	IMI
aCSF + Li + IMI	aCSF	Li	Li + IMI	IMI
aCSF + VPA + IMI	aCSF	VPA	VPA + IMI	IMI
OUA + Sal	OUA	Sal	Sal + Sal	Sal
OUA + Li	OUA	Li	Li + Sal	Sal
OUA + VPA	OUA	VPA	VPA + Sal	Sal
OUA + IMI	OUA	Sal	Sal + IMI	IMI
OUA + Li + IMI	OUA	Li	Li + IMI	IMI
OUA + VPA + IMI	OUA	VPA	VPA + IMI	IMI

2.4 Open field test (OFT)

The OFT was performed in an apparatus with a floor of 60 × 60 cm, divided into nine equal squares (20 x 20) separated by black lines. The walls were made of fiberboard, with 50 cm in height (except the frontal wall, which was made of glass). In the protocol, the animals were placed, gently, in the apparatus to explore the area for 5 min. The following parameters were evaluated: the number of crossings (the total number that rat crossed the squares during the entire test period of the test), and the number of rearings (the total number that the rats showed erect postures during the whole test period) [35]. The animals were submitted to open field on the 7th and 14th days of the experiment. The administration of drugs always happened after the OFT. The same animals submitted to the OFT were also evaluated in the forced swimming test (FST) **(see** scheme 1**)**.

2.5 Forced Swimming Test (FST)

The protocol of FST occurs in two individual exposures to a cylindrical transparent tank (80 cm tall and 30 cm in diameter) with water under a temperature of 22–23°C. In the apparatus, the level of water cannot allow the rats to touch the bottom of the tank or escape. The researchers changed the water after exposure of each rat to the tank. The first exposure was the training section, in which the researcher put the rat in the water for 15 min. After 24h of the training section, the animals were placed in the tank again

for the test section for 5 min. In the test, we evaluated the time of immobility (i.e., no additional activity was observed other than that required to keep the rat's head above the water), and the time of swimming (i.e., movement, usually horizontal, throughout the tank) [36].

Note

The training session was carried out 13 days after, and the test session 14 days after the ICV administration in rats. Animals that were submitted to pharmacological treatment received their daily doses after the training session. The animals in the IMI groups received a dose of the antidepressant at 23h30min and 1h before the behavioral test **(see** scheme 1**)**.

2.6 Sweet Food Consumption (SFC)

The SFC test is commonly used to measure anhedonic-like behavior in experimental animals [37]. The apparatus is the same as the OFT. The animals were submitted to five training sessions and two test sessions, one session per day, 3 minutes each session. In all sessions, ten Froot Loops® (wheat cereal, corn starch, and sugar) were placed in the center of the open field. The rats were fasted for 22 hours before the training sessions to stimulate the intake of the new food. It is important to note that immediately after the last training session, the animals were provided with free food. The training sessions were carried out to adapt the rats to the cereal, and during the two test sessions, the amount of cereal eaten by the animals was counted. In the present study, the consumption of fractions of the Froot Loops (e.g., 1/3 or 1/4) was considered.

Note

The training sessions started on the 7th day after ICV administration, and the two test sessions were performed on the 13th and 14th day after ICV administration in rats. The animals that underwent pharmacological treatment received their daily doses after the training sessions. The animals in the IMI groups received a dose of the antidepressant 1h30min before the behavioral tests **(see** scheme 2**)**. The animals evaluated in the SFC were not submitted to any other behavioral test or biochemistry analysis.

2.7 Biological samples.

Immediately after FST, the animals received ketamine (80 mg/kg) and xylazine (10 mg/kg) via intramuscular, and the blood then was drawn by cardiac puncture. The blood was collected to evaluate the ACTH levels in the serum. The brain was dissected in the frontal cortex and hippocampus to the evaluation of BDNF, NGF, NT-3, and GDNF levels. Moreover, it was evaluated the weight of the adrenal gland.

2.8 Protein determination

In the present study, the biochemical measures were normalized according to the method of Lowry and colleagues [38] with some modifications [39].

2.9 Parameters of the HPA axis

The levels of adrenocorticotropic hormone (ACTH) were determined in the serum of the rats through radioimmunoassay-based kits developed by MP Biomedicals, LLC (Santa Ana, California, USA). The weight of the adrenal gland was measured with a precision scale.

2.10 Levels of BDNF, NGF, NT-3, and GDNF

The frontal cortex and hippocampus were homogenized in a solution of phosphate-buffered (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol bis (2-aminoethyl ether)-N, N, N'N'-tetraacetic acid (EGTA). After centrifugation at 10,000 g for 20 min, the supernatants were collected. The levels of BDNF, NGF, NT-3, and GDNF were analyzed through sandwich enzyme-linked immunosorbent assay using commercial kits according to the manufacturer's instructions [BDNF and NGF levels: kit from Chemicon (USA); NT-3 and GDNF levels: kit from Biosensis (USA)].

2.11 Statistical analysis

The variables were analyzed according to their distribution through Shapiro-Wilk's test for normality. The Levene test assessed the homogeneity of variances among groups. All results are presented as the means and standard deviation of the mean. Differences among the experimental groups were determined by a three-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results are considered significant when $p \leq 0.05$.

3 Results

Seven days after ICV injection, OUA increased the number of crossing and rearings of rats in the open field test, characterizing a maniac-like behavior. Furthermore, Li and VPA treatment were able to reverse the alterations caused by OUA (Fig. 1). This behavioral alteration was not found 14 days after OUA administration, having no significant differences between aCSF and OUA groups (Fig. 2).

Data from two-way ANOVA seven days after ICV administration: crossing [OUA: F (1, 114) = 194,8 P < 0,0001; treatment: F (2, 114) = 95,80 P < 0,0001; OUA x treatment: F (2, 114) = 106,1 P < 0,0001], and rearings [OUA: F (1, 114) = 106,5 P < 0,0001;treatment: F (2, 114) = 100,2 P < 0,0001; OUA x treatment: F (2, 114) = 96,88 P < 0,0001]. Data from three-way ANOVA fourteen days after ICV administration: crossing [treatment: F (2, 108) = 1,527 P = 0,2218; OUA: F (1, 108) = 5,128 P = 0,0255; IMI: F (1, 108) = 2,677 P = 0,1047; treatment x OUA F (2, 108) = 0,09475 P = 0,9097; treatment x IMI: F (2, 108) = 0,2438 P = 0,7841; OUA x treatment: F (1, 108) = 5,490 P = 0,0210; treatment x OUA x IMI: F (2, 108) = 1,556 P = 0,2157], and rearing [treatment: F (2, 108) = 1,986 P = 0,1422; OUA: F (1, 108) = 6,702 P = 0,0110; IMI:F (1, 108) = 0,5997 P = 0,4404; treatment x OUA: F (2, 108) = 0,1513 P = 0,8598; treatment x IMI: F (2, 108) = 0,5146 P = 0,5992; OUA x treatment: F (1, 108) = 5,398 P = 0,0220; treatment x OUA x IMI: F (2, 108) = 1,288 P = 0,2800].

OUA administration induced depressive-like behavior 14 days after ICV administration, as demonstrated by the increase in time of immobility and decrease in time of swimming in the forced swimming test. Li

and VPA treatment partially reversed these behavioral changes, while IMI treatment completely reversed all these alterations. The combined treatment of Li/VPA and IMI reduced the time of immobility and increased the time of swimming beyond the basal levels. Interestingly, Li and VPA *per se* and combined with IMI also altered these parameters in aCSF group (Figs. 3A and 3B). Still, in Fig. 3, it is also possible to observe that OUA decreased the sweet food consumption 14 days after ICV administration, another characteristic of depressive-like behavior. All treatments reversed this parameter (Fig. 3C).

Data from three-way ANOVA: immobility time [treatment: F (2, 108) = 109,9 P < 0,0001; OUA: F (1, 108) = 503,6 P < 0,0001; IMI: F (1, 108) = 662,9 P < 0,0001; treatment x OUA: F (2, 108) = 38,96 P < 0,0001; treatment x IMI: F (2, 108) = 19,43 P < 0,0001; OUA x IMI: F (1, 108) = 299,6 P < 0,0001; treatment x OUA x IMI: F (2, 108) = 1,188 P = 0,3087] swimming time [treatment: F (2, 108) = 109,9 P < 0,0001; OUA: F (1, 108) = 503,6 P < 0,0001; IMI: F (1, 108) = 662,9 P < 0,0001; treatment x OUA: F (2, 108) = 38,96 P < 0,0001; treatment x IMI: F (2, 108) = 19,43 P < 0,0001; OUA x IMI: F (1, 108) = 299,6 P < 0,0001; treatment x OUA x IMI: F (2, 108) = 1,188 P = 0,3087] sweet food consumption [treatment:F (2, 108) = 18,93 P < 0,0001; OUA: F (1, 108) = 14,16 P = 0,0003; IMI: F (1, 108) = 8,584 P = 0,0041; treatment x OUA: F (2, 108) = 21,17 P < 0,0001; treatment x IMI: F (2, 108) = 19,97 P < 0,0001; OUA x IMI:F (1, 108) = 28,94 P < 0,0001; treatment x OUA x IMI: F (2, 108) = 22,29 P < 0,0001].

ACTH levels and adrenal gland weight increased 14 days following OUA administration (Fig. 4). All treatments reversed these alterations, except IMI treatment, which did not reverse the increase in adrenal gland weight.

Data from three-way ANOVA: ACTH levels [treatment: F (2, 108) = 50,20 P < 0,0001; OUA: F (1, 108) = 19,88 P < 0,0001; IMI: F (1, 108) = 47,97 P < 0,0001; treatment x OUA: F (2, 108) = 31,53 P < 0,0001; treatment x IMI: F (2, 108) = 46,69 P < 0,0001; OUA x IMI: F (1, 108) = 26,27 P < 0,0001; treatment x OUA x IMI: F (2, 108) = 34,67 P < 0,0001] and adrenal gland weight [treatment: F (2, 108) = 240,6 P < 0,0001; OUA: F (1, 108) = 163,4 P < 0,0001; IMI: F (1, 108) = 6,956 P = 0,0096; treatment x OUA: F (2, 108) = 178,1 P < 0,0001; treatment x IMI: F (2, 108) = 4,323 P = 0,0156; OUA x IMI: F (1, 108) = 0,6171 P = 0,4339; treatment x OUA x IMI: F (2, 108) = 0,1537 P = 0,8577].

BDNF and NGF levels decreased in the frontal cortex and hippocampus 14 days following OUA administration. Both BDNF and NGF levels alterations were reversed by almost all treatments, except IMI treatment, which did not reverse these effects induced by OUA (Figs. 5–6**)**. Besides, the combined treatment of Li-IMI and/or VPA-IMI also increased the levels of these neurotrophins in the frontal cortex and/or hippocampus of aCSF group.

Data from three-way ANOVA: BNDF levels frontal cortex [treatment: F (2, 48) = 34,53 P < 0,0001; OUA: F (1, 48) = 38,08 P < 0,0001; IMI: F (1, 48) = 32,52 P < 0,0001; treatment x OUA: F (2, 48) = 9,469 P = 0,0003; treatment x IMI: F (2, 48) = 3,883 P = 0,0273; OUA x IMI: F (1, 48) = 6,982 P = 0,0111; treatment x OUA x IMI: F (2, 48) = 4,148 P = 0,0218], BDNF levels hippocampus [treatment: F (2, 48) = 58,36 P < 0,0001; OUA: F (1, 48) = 36,35 P < 0,0001; IMI: F (1, 48) = 7,635 P = 0,0081; treatment x OUA: F (2, 48) = 14,77 P < 0,0001; treatment x IMI: F (2, 48) = 8,691 P = 0,0006; OUA x IMI: F (1, 48) = 7,127 P = 0,0103; treatment x OUA x IMI:

F (2, 48) = 3,524 P = 0,0373], NGF levels frontal cortex [treatment: F (2, 48) = 25,37 P < 0,0001; OUA: F (1, 48) = 65,92 P < 0,0001; IMI: F (1, 48) = 10,23 P = 0,0024; treatment x OUA: F (2, 48) = 7,563 P = 0,0014; treatment x IMI: F (2, 48) = 3,714 P = 0,0316; OUA x IMI: F (1, 48) = 0,07025 P = 0,7921; treatment x OUA x IMI: F (2, 48) = 5,350 P = 0,0080] and NGF hippocampus [treatment: F (2, 48) = 23,08 P < 0,0001; OUA: F (1, 48) = 20,02 P < 0,0001; IMI: F (1, 48) = 8,524 P = 0,0053; treatment x OUA: F (2, 48) = 11,23 P < 0,0001; treatment x IMI: F (2, 48) = 9,752 P = 0,0003; OUA x IMI: F (1, 48) = 5,349 P = 0,0251; treatment x OUA x IMI: F (2, 48) = 6,608 P = 0,0029].

Regarding NT-3, the only alteration observed in the frontal cortex was in the OUA + IMI group, which decreased the levels of this neurotrophin when compared to the control group. In the hippocampus, NT3 levels decreased 14 days following OUA administration, and the treatments with Li, VPA, the association with Li + IMI or Li + VPA reversed this alteration. However, a single injection of IMI did not alter the OUA-induced NT-3 decreased. The combined treatment of Li-IMI also increased this neurotrophin beyond the basal levels in the aCSF group (Fig. 7).

Data from three-way ANOVA: NT3 levels frontal cortex [treatment: F (2, 48) = 20,05 P < 0,0001; OUA: F (1, 48) = 0,5237 P = 0,4728; IMI: F (1, 48) = 3,560 P = 0,0652; treatment x OUA: F (2, 48) = 11,95 P < 0,0001; treatment x IMI: F (2, 48) = 2,470 P = 0,0953; OUA x IMI: F (1, 48) = 0,2482 P = 0,6206; treatment x OUA x IMI:F (2, 48) = 1,137 P = 0,3294] and NT3 levels hippocampus [treatment: F (2, 48) = 32,69 P < 0,0001; OUA: F (1, 48) = 14,02 P = 0,0005; IMI: F (1, 48) = 37,45 P < 0,0001; treatment x OUA: F (2, 48) = 13,97 P < 0,0001; treatment x IMI: F (2, 48) = 4,848 P = 0,0121; OUA x IMI: F (1, 48) = 8,040 P = 0,0067; treatment x OUA x IMI: F (2, 48) = 1,226 P = 0,3026].

GDNF levels decreased 14 days following OUA administration in the frontal cortex, and all treatments reversed this alteration. The combined treatment of Li-IMI also increased this neurotrophin beyond the basal levels in the aCSF group. In the hippocampus, there were no statistical differences between groups in GDNF levels. The combined treatment of Li-IMI and VPA-IMI increased this neurotrophin beyond the basal levels in the aCSF group (Fig. 8).

Data from three-way ANOVA: GDNF levels frontal cortex: [tretment: F (2, 48) = 39,33 P < 0,0001; OUA: F (1, 48) = 35,51 P < 0,0001; IMI: F (1, 48) = 19,78 P < 0,0001; treatment x OUA: F (2, 48) = 16,23 P < 0,0001; treatment x IMI: F (2, 48) = 3,981 P = 0,0251; OUA x IMI: F (1, 48) = 0,2959 P = 0,5890; treatment x OUA x IMI: F (2, 48) = 11,31 P < 0,0001] and GDNF levels hippocampus: [treatment: F (2, 48) = 17,10 P < 0,0001; OUA: F (1, 48) = 26,88 P < 0,0001; IMI: F (1, 48) = 9,309 P = 0,0037; treatment x OUA: F (2, 48) = 0,7656 P = 0,4707; treatment x IMI: F (2, 48) = 1,590 P = 0,2146; OUA x IMI: F (1, 48) = 14,54 P = 0,0004; treatment x OUA x IMI: F (2, 48) = 2,085 P = 0,1355].

4 Discussion

Previously described by El-Mallakh [26], the Na+/K+-ATPase hypothesis for BD suggests that a decrease in the activity of this enzyme can be responsible for the maniac and depressive symptoms of this disorder. The decrease in the Na+/K+-ATPase activity can deregulate the membrane excitability, altering

the homeostasis and cellular concentration of important ions for the membrane depolarization, such as Na+, K+, and Ca++ [26,27]. Based on this theory, a previous study published by our research group presents the validation of the animal model of BD induced by OUA, a Na+/K+-ATPase inhibitor. In that study, OUA induced a maniac-like behavior and depressive-like behavior seven and 14 days after ICV injection, respectively. The present study is consistent with our previous data, reinforcing the validity of the animal model of BD induced by OUA [23].

A study in patients showed that the activity of Na+/K+-ATPase in erythrocytes seems to be increased in patients with BD [40]. However, a meta-analysis pointed out that the activity of Na+/K+-ATPase in erythrocytes seems to be decreased, and the level of activity could be related to the humor episode [24]. In the brain, the expression and/or levels of Na+, K + ATPase α2, and α3 isoforms seem to be higher in the prefrontal and parietal cortices of patients compared with controls [41,42]. These studies contributed to the idea that the dysregulation in the Na+/K+-ATPase enzyme can have a role in BD's pathophysiology. However, the action mechanisms of mood stabilizers, anticonvulsants, and antidepressants drugs on Na + K + ATPase alteration in BD needs to be elucidated.

Our results show that the treatment with Li and VPA reversed the mania-like behavior and the decrease in sweet food consumption induced by OUA. Furthermore, the alterations caused by OUA on the forced swimming test were partially reversed by Li and VPA treatment. The effects of Li and VPA on the mania-like behavior are well described in the literature, and it's consistent with the present findings [16,23,34,43,44]. On the other hand, the effect of mood stabilizers in the depressive episode of BD is still controversial. Some studies support the use of Li for bipolar depression [14,45] while a recent meta-analysis considered Li ineffective [20]. The mechanisms of action of Li and VPA are complex, but it's known that antioxidant and anti-inflammatory proprieties, and regulation in pathways such as GSK-3β signaling, could be some of the targets of these medications [16,43,46,47]. Therefore, more studies are necessary to evaluate the real antidepressant effect of these drugs in BD.

The use of antidepressants in the depressive episode of BD is complex and remains uncertain. A recent meta-analysis found that IMI is better than placebo for the treatment of acute depression in BD type I [20], suggesting that tricyclic antidepressants can be effective for acute bipolar depression. However, the use of antidepressants in BD treatment must be carefully considered, due to the risk of mania-switch [30]. A recent study by our research group showed that the long-term administration of IMI could induce a mania switch in depressive-like rats submitted to the animal model of BD induced by OUA [29]. Considering the risk of mania switch, it was evaluated the effect of the acute administration of IMI, and the treatment of Li/VPA plus IMI in the alterations caused by OUA in the forced swimming and sweet food consumption test. The present results show that IMI alone or with Li or VPA reversed the depressive-like behavior induced by OUA administration, which is congruent with previous studies [48–51].

In bipolar patients, the deregulation of the HPA axis is reported, indicating an important role of this pathway in BD pathophysiology [13,52]. Similarly, in this study with rodents, ACTH levels and adrenal gland weight was found to have increased 14 days following the OUA administration, indicating a

hyperfunction of the HPA axis. The treatment of Li and VPA alone and combined with IMI reversed both alterations, whereas IMI alone only reversed the ACTH levels. The animal model of BD induced by OUA and other animal models of mania and depression well established in the literature, such as paradoxical sleep deprivation and chronic unpredicted mild stress, also demonstrate alterations in ACTH levels and adrenal gland weight. Besides, Li can act to reverse these alterations [23,53,54]. Congruent with our results, evidence has shown that IMI could reverse alterations in ACTH levels in animals that present depressive-like behavior [48]. Low evidence was found about the effect of VPA in both parameters evaluated, and the influence of IMI on adrenal gland weight in animal models of mania or depression. However, we suppose that the short-period treatment with IMI may have contributed to the non-reverse of the adrenal gland weight by IMI.

It is described in the literature hypotheses on the interactions between neurotrophins and HPA-axis dysregulation, leading to depressive symptoms [55]. Indeed, herein the levels of BDNF, NGF, GDNF, and NT3 were found deceased in the frontal cortex and/or hippocampus after OUA administration, which accompanied the depressive-like behaviors. Consistent with our results, previous studies have found a reduction in neurotrophin levels in brain structures of animals submitted to models of mania, depression, and BD [28,56–58]. Alterations in neurotrophin levels also are present in BD patients. BD patients were found to have lower serum levels of BDNF, NGF, and GDNF [6–8]. Interestingly, the decrease in BDNF levels seems to be correlated with cognitive impairments in BD, whereas NT3 and NT4 were found humor-dependent altered [9,59]. Since alterations in the levels of neurotrophins are an important part of BD neurobiology, it is also important that drugs used for the treatment of this disorder act on this pathophysiological mechanism.

Lastly, Li and VPA treatment, and the combined treatment of Li with IMI and VPA with IMI (but not IMI alone) increased the neurotrophin levels in the frontal cortex and hippocampus, which is congruent with the previous study from Dal-Pont and colleagues [60]. Li is known for its neuroprotective and neurotrophic effects [15]. By contrast, Varela and colleagues [61] described that VPA was not able to reverse the effects of OUA on BDNF levels in the hippocampus of rats. This difference in results perhaps could have occurred because of methodological differences between the studies, given that the previous study used seven days of treatment, while the present research has a protocol of 13 days of treatment with VPA. The fact that IMI did not reverse the effects of OUA in the neurotrophic levels may be due to the short period of administration since this antidepressive can increase the levels of mature BDNF in the hippocampus of Wistar rats in the chronic treatment of 14 days [62]. These results indicate that the acute antidepressive-like effect of this drug did not act on the neurotrophic system.

In conclusion, the acute treatment with IMI ameliorates depressive-like behavior and ACTH levels, but not neurotrophic parameters, in rats submitted to the animal model of BD induced by OUA. In contrast, the treatment with Li and VPA alone, or combined with IMI, can act in both behavioral and neurotrophic alterations caused by OUA. The fact that IMI could not reverse the decrease in neurotrophic parameters caused by OUA may be due to the time of treatment. Furthermore, our results support the validity of face,

construct, and predictive of the animal model of BD induced by OUA, reinforcing its relevance for the study of this disorder and the screening of new substances for depressive and manic episodes.

Declarations

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Author Contribution

Samira S. Valvassori and João Quevedo contributed to design and development; methodological design; supervision (responsible for organizing and executing the project); analysis/interpretation and critical review. Jefté Peper-Nascimento, Wilson R. Resende, and Gustavo C. Dal-Pont participated in data collection and processing; biochemical analyzes of the samples as well as performed the statistical analyzes. Taise Possamai-Della and Jorge M. Aguiar-Geraldo performed statistical analyzes and contributed to the analysis/interpretation, literature survey, and writing.

Data Availability

All data generated or analyzed during this study are included in the manuscript.

Code Availability

Not applicable.

Declarations

Ethics Approval

The experimental procedures followed the terms of the Brazilian Society for Neuroscience and Behavior (SBNeC), as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The local ethics committee *Comissão de Ética no Uso de Animais da Universidade do Extremo Sul Catarinense* (protocol number #66/2010) approved this study.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Conflicts of interest

JQ received clinical research support from LivaNova; has speaker bureau membership with Myriad Neuroscience, Janssen Pharmaceuticals, and Abbvie; is consultant for Eurofarma; is stockholder at Instituto de Neurociencias Dr. Joao Quevedo; and receives copyrights from Artmed Editora, Artmed Panamericana, and Elsevier/Academic Press. All other authors have no conflicts of interest.

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Schemes

Schemes 1 and 2 are available in the Supplementary Files section

Figures

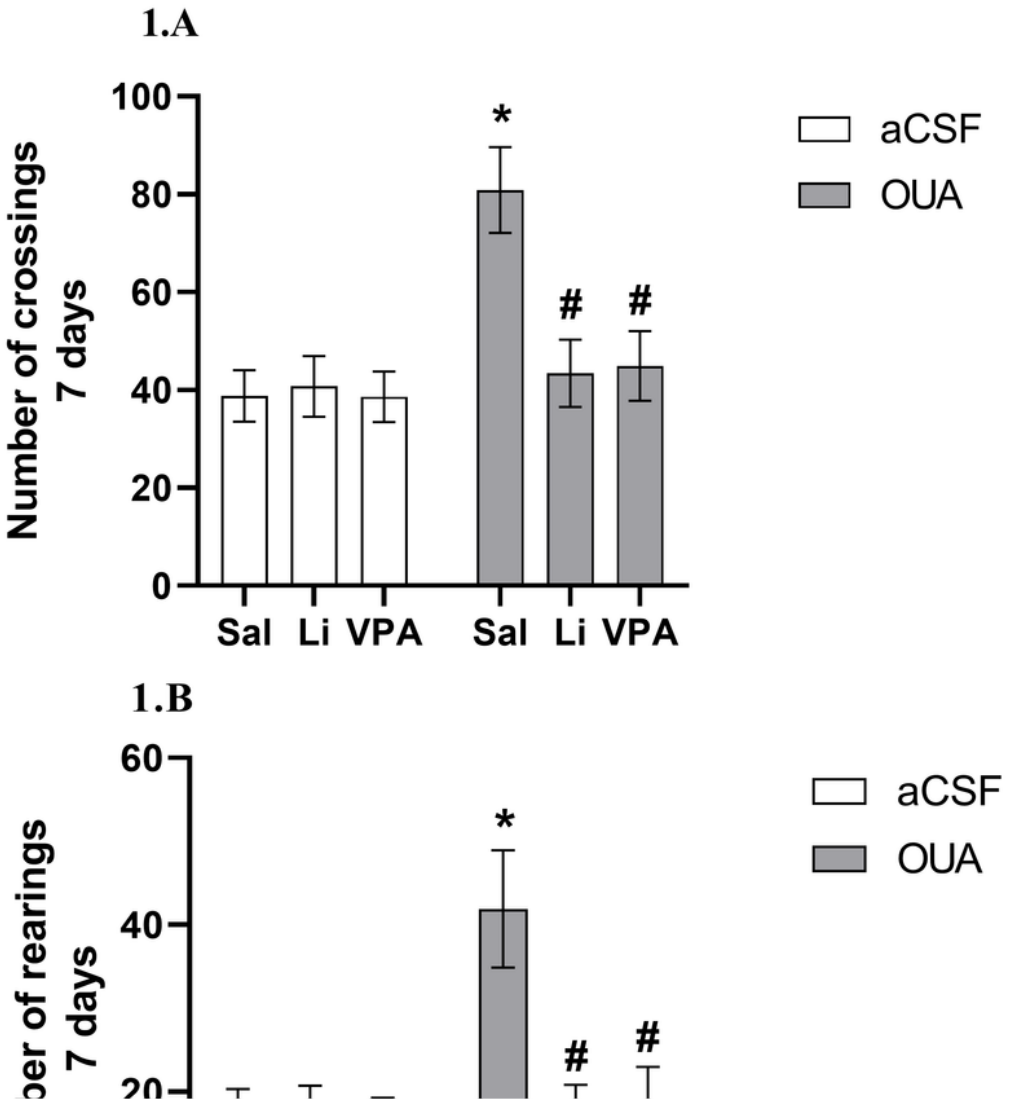

Figure 1

Effects of Li and VPA treatment on open field test seven days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to two-way ANOVA followed by Tukey's post-hoc test

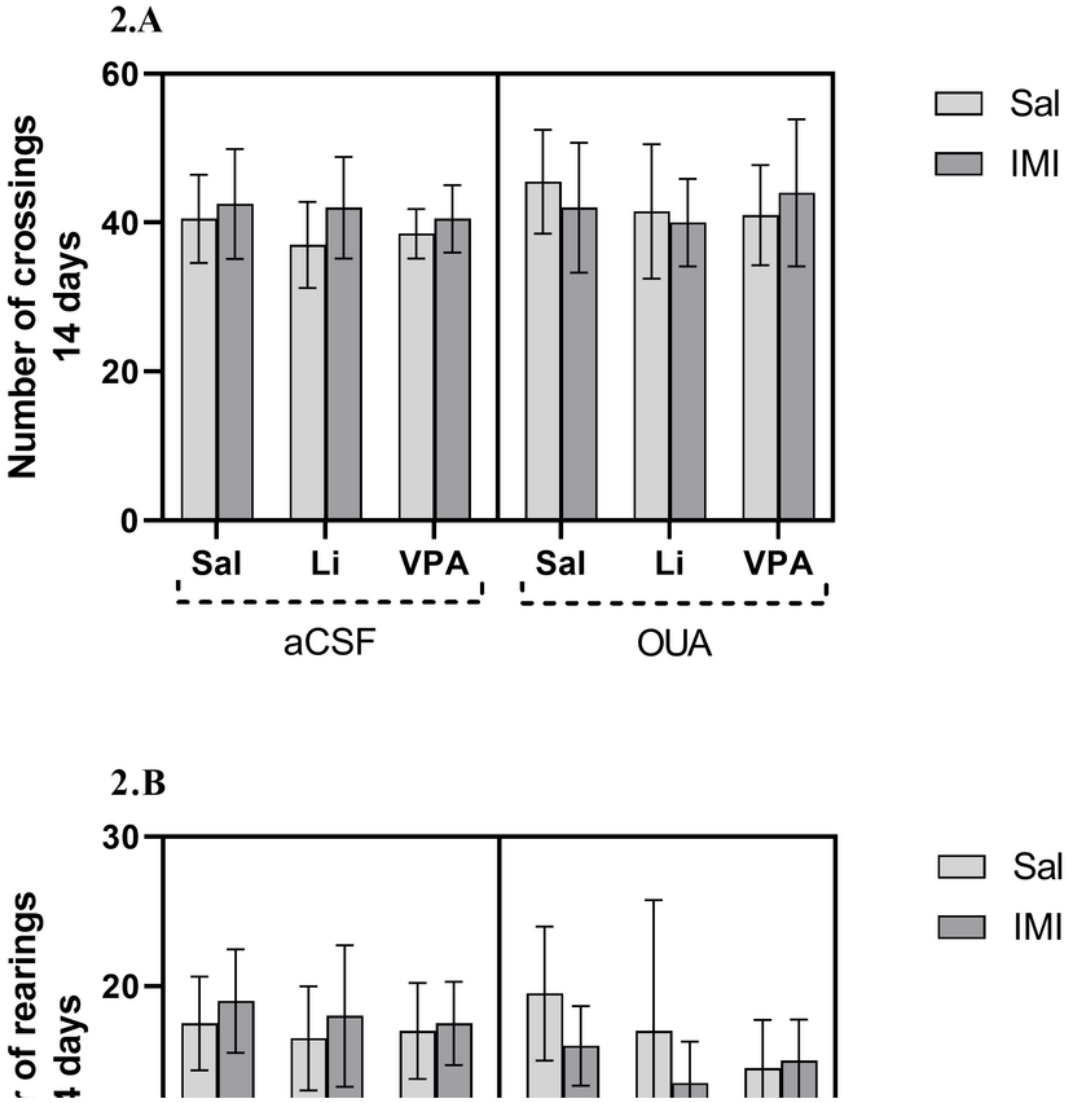

Figure 2

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on open field test fourteen days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

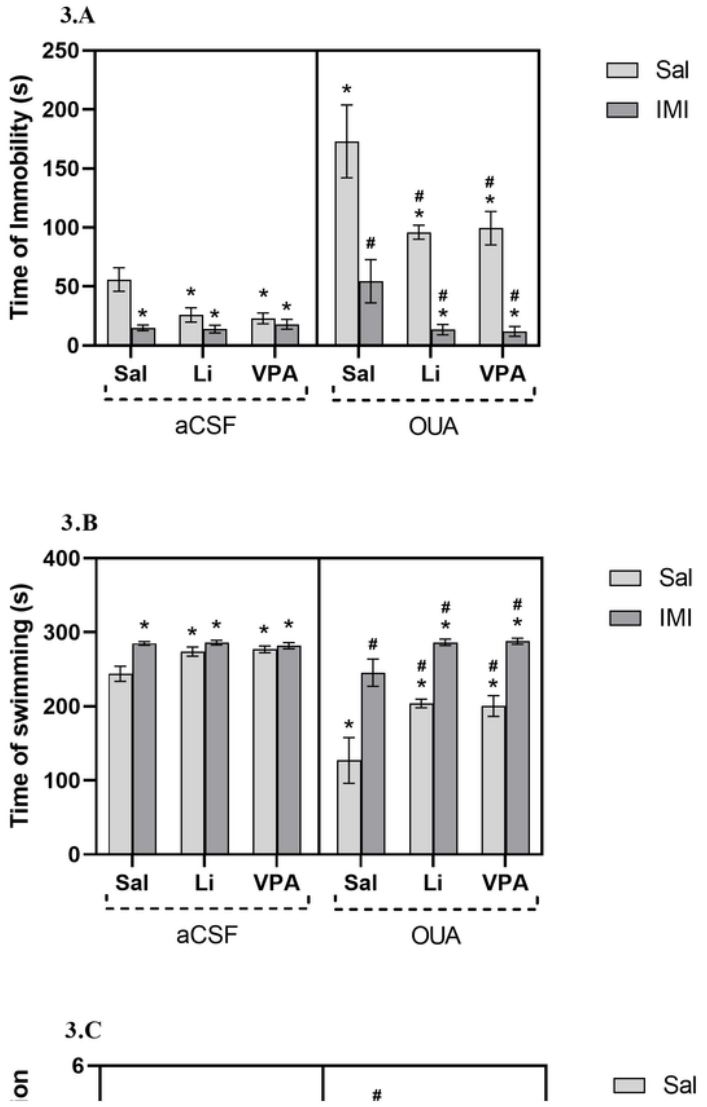

Figure 3

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on forced swimming test (3A-3B) and sweet food consumption (3C) test fourteen days after OUA ICV injection in rats. Data are represented

as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

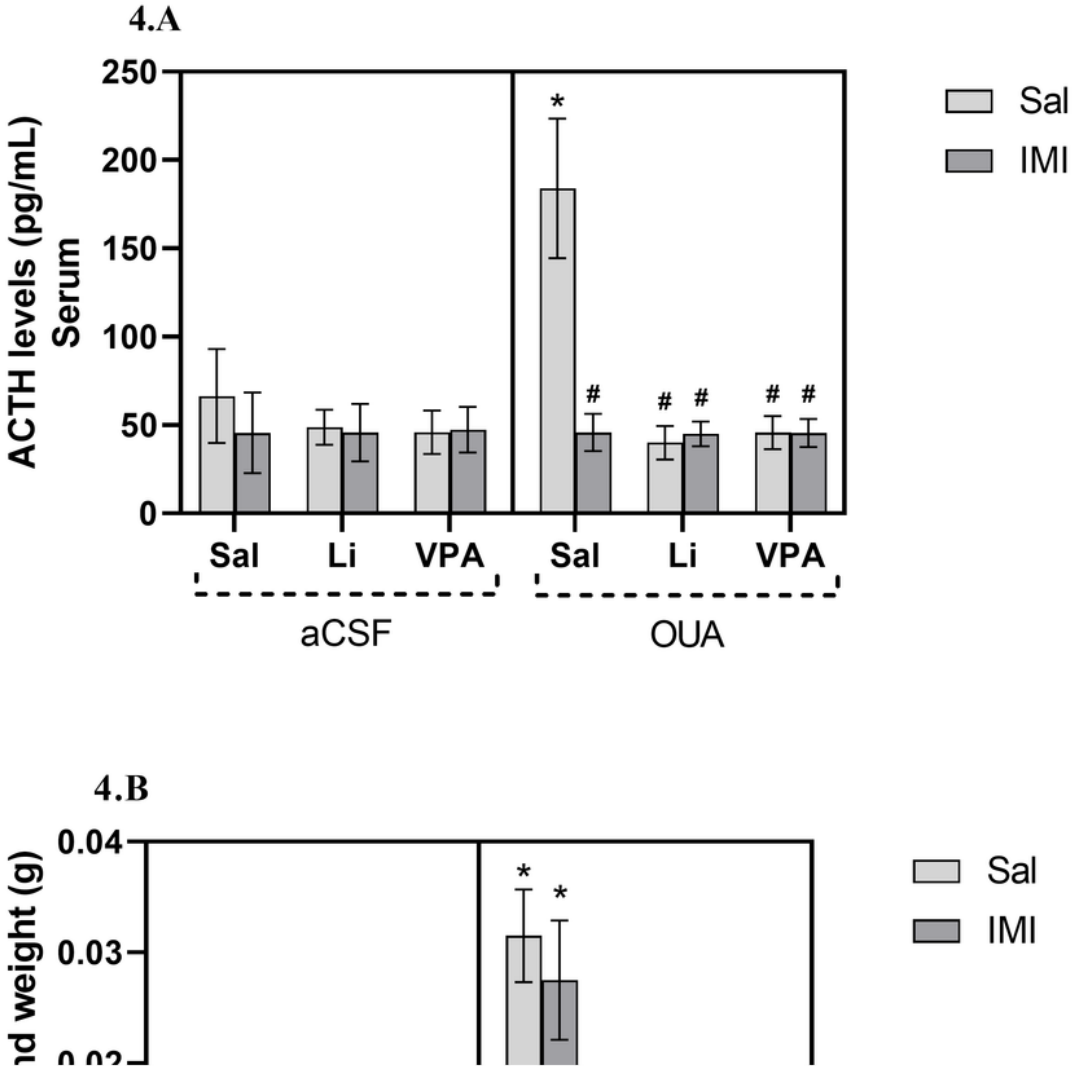

Figure 4

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on ACTH levels (4A) and adrenal gland weight (4B) fourteen days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

Figure 5

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on BDNF levels in frontal cortex (5A) and hippocampus (5B) fourteen days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

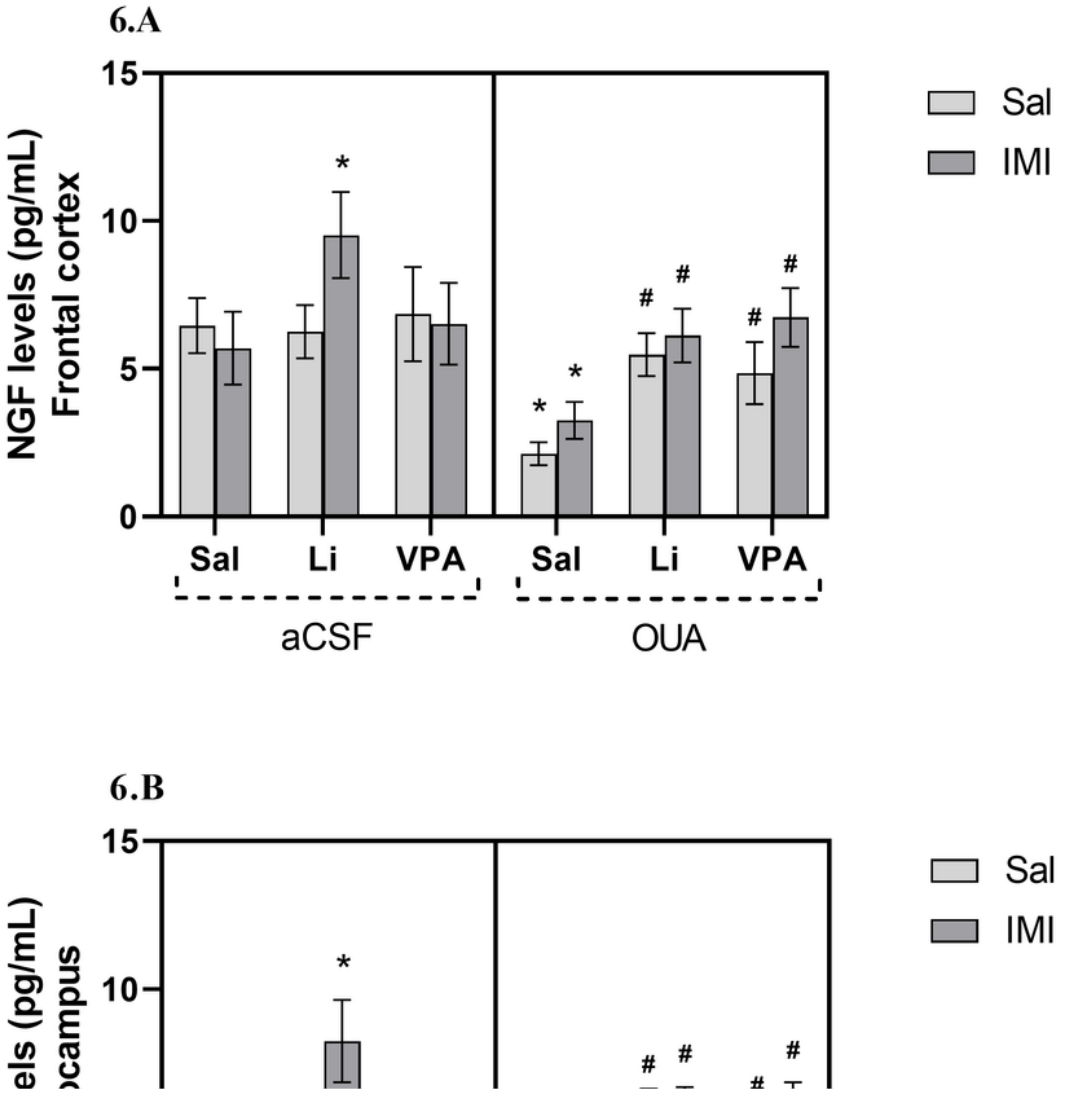

Figure 6

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on NGF levels in frontal cortex (6A) and hippocampus (6B) fourteen days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

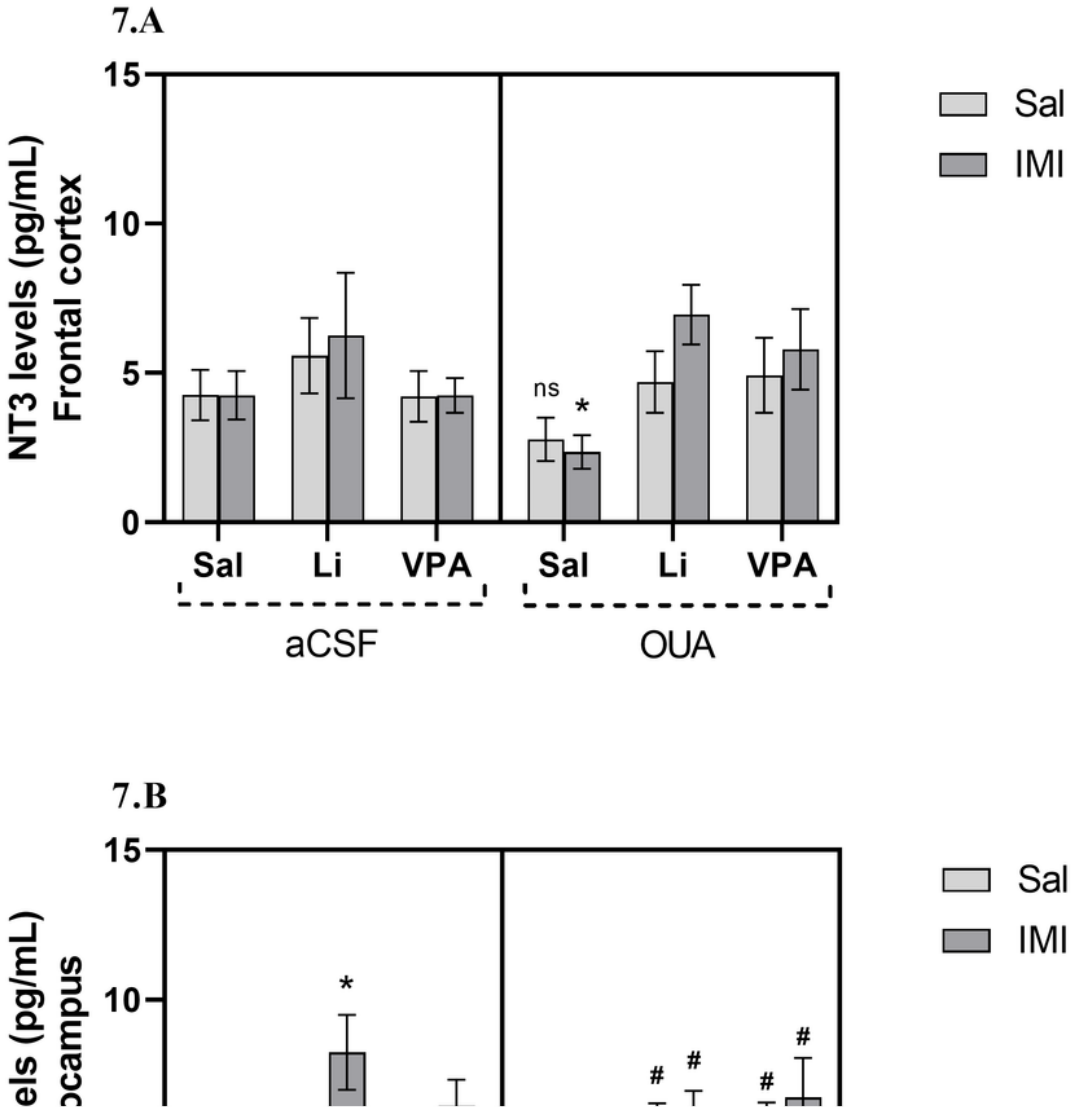

Figure 7

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on NT-3 levels in frontal cortex (7A) and hippocampus (7B) fourteen days after OUA ICV injection in rats. Data are represented as means ±

standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

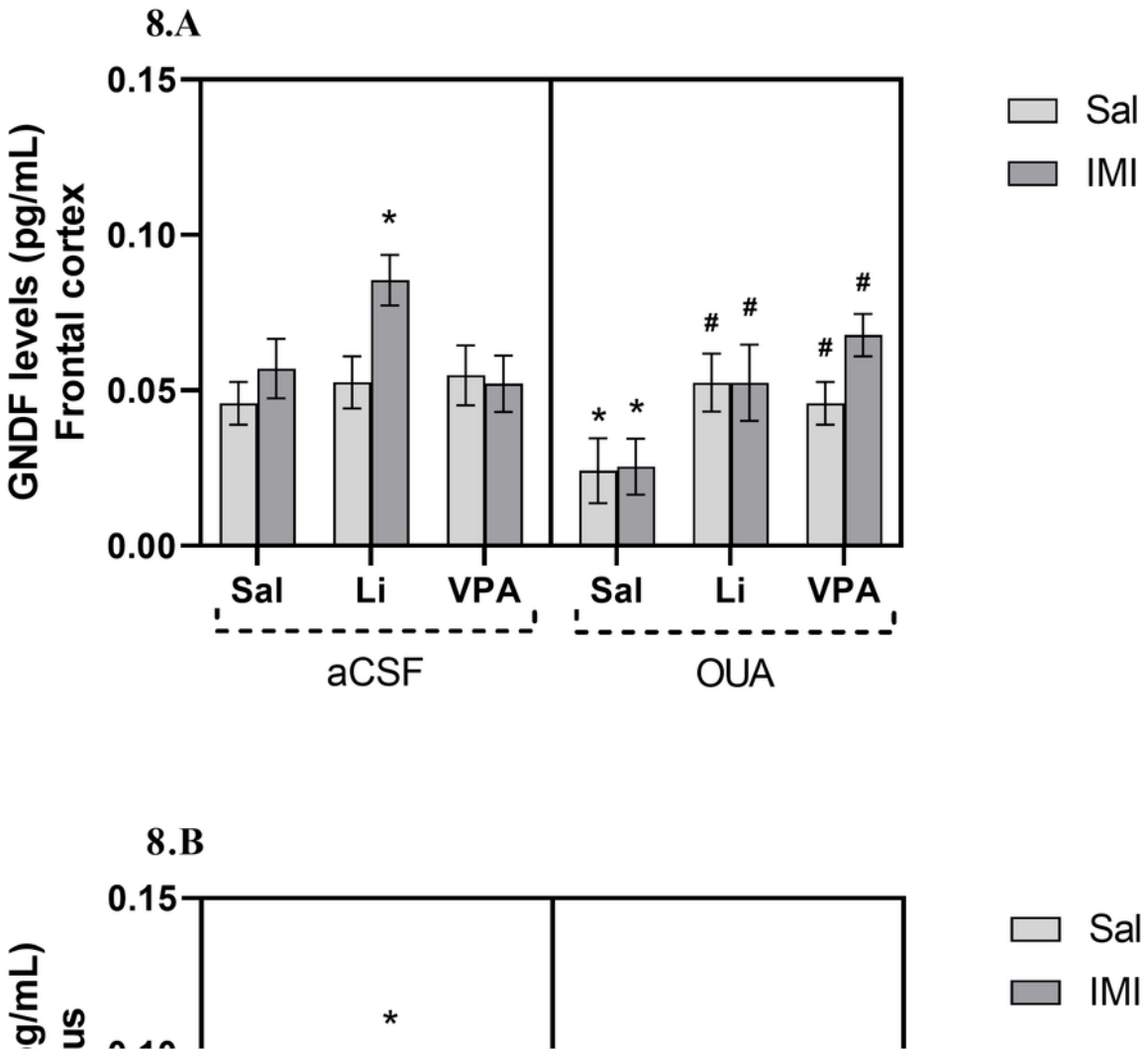

Figure 8

Effects of Li, VPA, IMI and the combined treatment of Li/VPA with IMI on GDNF levels in frontal cortex (8A) and hippocampus (8B) fourteen days after OUA ICV injection in rats. Data are represented as means ± standard deviation; *p < 0.05 compared to de aCSF group; #p < 0.05 compared to the OUA group, according to three-way ANOVA followed by Tukey's post-hoc test

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.pptx
- Scheme2.pptx