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Effects of lithium and valproate on behavioral parameters and neurotrophic factor levels in an animal model of mania induced by paradoxical sleep deprivation

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ABSTRACT

The present study aimed to evaluate the effects of treatment with lithium (Li) and valproate (VPA) on behaviors and brain BDNF, NGF, NT-3, NT-4 and GDNF levels in mice submitted to paradoxical sleep deprivation (PSD), which induces an animal model of mania. Male C57BL/6J mice received an intraperitoneal (i.p.) injection of saline solution (NaCl 0.09%, 1 ml/kg), Li (47.3 mg/kg, 1 ml/kg) or VPA (200 mg/kg, 1 ml/kg) once a day for seven days. Animals were randomly distributed into six groups (n = 10 per group): (1) Control + Sal; (2) Control + Li; (3) Control + VPA; (4) PSD + Sal; (5) PSD + Li; or (6) PSD + VPA. Animals were submitted to 36 h of PSD, and then, they were submitted to the open field test. The frontal cortex and hippocampus were dissected from the brain. The manic-like behaviors in the mice were analyzed. Treatment with Li and VPA reversed the behavioral alterations induced by PSD. PSD decreased BDNF, NGF, and GDNF levels in the frontal cortex and hippocampus of mice. The administration of Li and VPA protected the brain against the damage induced by PSD. However, PSD and the administration of Li and VPA did not affect the levels of NT-3 and NT-4 in either brain structure evaluated. In conclusion, the PSD protocol induced manic-like behavior in rats and induced alterations in neurotrophic factor levels. It seems that neurotrophic factors and sleep are essential targets to treat BD.

1. Introduction

Bipolar disorder (BD) is a chronic mood disorder with alternating mania/hypomania and depressive episodes. According to the DSM V, the hallmark of the diagnosis of BD is the presence of a manic episode (American Psychiatric Association, 2013). The literature describes that lithium (Li) is the gold standard for treating BD (Cheniaux, 2011). However, Li can cause side effects in many patients, impairing adherence to treatment. Some side effects of this drug are excessive urination, hypothyroidism, weight gain, hyperparathyroidism, hypercalcemia, tremor, nausea, anorexia, diarrhea, epigastric discomfort, and sexual problems (Coryell, 2009; Curran and Ravindran, 2014). Therefore, some anticonvulsants, such as sodium valproate (VPA), have been used in the treatment of BD (Fountoulakis et al., 2007). Previous studies have demonstrated that treatment with Li and VPA can be effective in acute manic episodes and can provide some protection against recurrent mood alterations (Manji and Zarate, 2002; Davis et al., 2005). Interestingly, Li and VPA are structurally dissimilar but have many molecular targets in common, such as glycogen synthase kinase-3 (GSK-3), histone deacetylase, protein kinase C, and neurotrophic factors (Zarate et al., 2006).

Some evidence suggests the involvement of neurotrophic factors in the pathophysiology of psychiatric disorders, including BD (Scola and Andreazza, 2015; Wiener et al., 2017). In the central nervous system,

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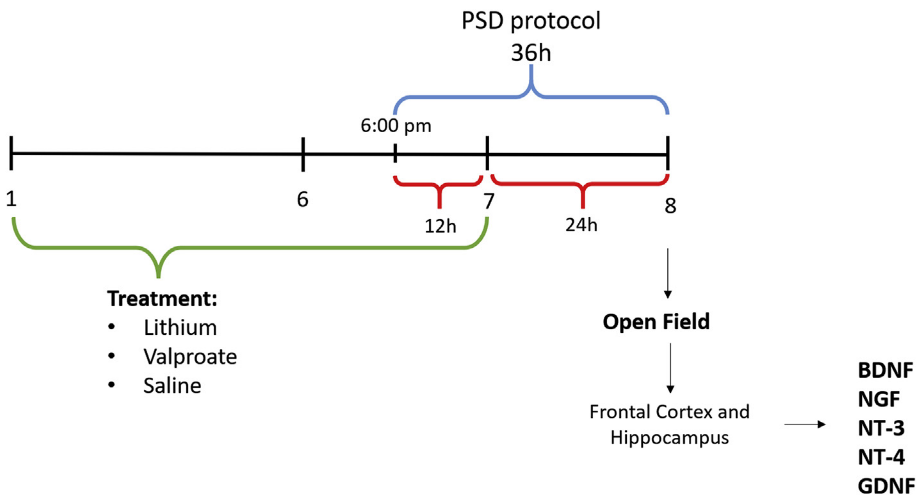

Scheme 1. Experimental design. Effects of PSD on the: behavioral parameters; BDNF, NGF, NT-3, NT-4 and GDNF levels in the Frontal Cortex and Hippocampus of animals. BDNF: brain-derived neurotrophic factor; GDNF; glial cell line-derived neurotrophic factor; NT-3: neurotrophin-3; NT-4: neurotrophin-4; NGF: nerve growth factor; PSD: paradoxical sleep deprivation.

neurotrophic factors regulate neurotransmitter release and neuronal excitability, which are critical mediators of neuronal plasticity (Wiener et al., 2015; Scola and Andreazza, 2015). The neurotrophic factors more studied in mood disorders are brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF) (Walz et al., 2008; Wiener et al., 2017; Safari et al., 2017; Valvassori et al., 2017b). Previous studies reported that these neurotrophins are decreased during manic episodes in BD patients compared with levels in healthy controls or euthymic patients (Cunha et al., 2006; Takebayashi et al., 2006; Barbosa et al., 2011a). Furthermore, alterations in neurotrophic factor levels are observed even during pharmacological treatment in BD patients (Zhang et al., 2010).

In manic and depressive episodes, insomnia, or a decrease in the need for sleep, and hypersomnia have been observed in BD patients (American Psychiatric Association, 2013; Kaplan and Harvey, 2013). Sleep deprivation (SD) can lead healthy subjects to present manic-like symptoms, in addition to increasing manic episodes in BD patients (Kaplan and Harvey, 2013). Furthermore, sleep disturbances have been linked to a reduction in neurotrophin levels (Schmitt et al., 2016). Paradoxical sleep deprivation (PSD) in rodents is considered a suitable animal model of mania because animals submitted to this protocol present some manic-like symptoms. Some manic-like behaviors induced by PSD are hyperactivity, aggressive behavior, and hypersexuality (Gessa et al., 1995; Benedetti et al., 2008; Valvassori et al., 2017c).

Moreover, it has been described that treatment with Li can reverse manic-like behaviors induced by PSD (Kaplan and Harvey, 2013; Bunney and Bunney, 2013; Valvassori et al., 2017c). Although the literature demonstrates that PSD induces alterations in behavior and BDNF levels, information on the effects of Li and VPA in this context is scarce. In addition, there are few studies on PSD and changes in neurotrophic factors such as NGF, NT-3, NT-4, and GDNF. Therefore, the present study aimed to evaluate the effects of treatment with Li and VPA on behavior and BDNF, NGF, NT-3, NT-4 and GDNF levels in the brains of mice submitted to the animal model of mania induced by PSD.

2. Materials and methods

2.1. Animals

The animals used in the present study were male C57BL/6J mice, ± 60 days old, with a body weight between 20 and 25 g; mice were provided by the colony of *Universidade do Extremo Sul Catarinense* and were grouped five mice per cage. The mice were maintained under a 12-h light/dark cycle with water and food *ad libitum*. Experimental procedures were performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) guidelines. The local ethics committee approved this study (*Comissão de Ética no Uso de Animais da Universidade do Extremo Sul Catarinense*) under protocol 007/2016-1.

2.2. Treatments

Over a period of seven days, mice received an intraperitoneal (i.p.) saline solution (Sal, NaCl 0.09%, 10 ml/kg), Li (47.5 mg/kg, 10 ml/kg) or VPA (200 mg/kg, 10 ml/kg) injection once per day. All doses and treatments used in the present study were based on previous results from our research group (Jornada et al., 2010; Valvassori et al., 2015).

Note: The animals treated with Li had plasma levels of Li between 0.6 and 1.2 mEq/L, similar to the therapeutic dose for BD patients (Valvassori et al., 2015).

2.3. Paradoxical sleep deprivation (PSD) protocol

Five days after the start of treatment, the PSD protocol was started at 6:00 p.m. For this protocol, the cages (38 × 31 × 17 cm), containing 12 platforms each (3.5 cm diameter), were filled with a volume of water 1 cm deep, forcing the animals to stay on the platforms. However, the mice could freely move from one platform to another. When the mice enter the paradoxical phase of sleep, muscular atony occurs, and the animals were awoken by falling into the water. The animals were grouped 5 animals per cage, with food and water available *ad libitum*. This protocol adopted a period of 36 h of PSD (Scheme 1) because this amount of time undergoing PSD can increase the locomotor activity of animals, which is considered a manic-like behavior (Tufik et al., 2009; Armani et al., 2012). During the PSD protocol, the mice were removed from the platform for treatment and were replaced immediately after the injection. The mice in the control group were maintained in their original cages, in which the floors were lined with sawdust.

2.4. Experimental groups

The animals were randomly distributed into the six following groups ($n = 10$ per group): (1) Control + Sal; (2) Control + Li; (3) Control + VPA; (4) PSD + Sal; (5) PSD + Li; and (6) PSD + VPA.

2.5. Open field test

To evaluate hyperactivity in mice, the open field test was used immediately after the PSD protocol (Scheme 1). The open field apparatus is a box that consists of a 40 × 40 cm plywood arena surrounded by 40 cm high wooden walls containing a frontal glass cover. The bottom of the apparatus arena was divided into sixteen equal portions (10 × 10 cm each) with black lines. The number of crossings (horizontal exploration) in each portion, the number of rearings (vertical exploration), and the number of visits to the center of the open field (risk-taking behavior) were evaluated. The center of the open field was delimited by a central quadrant of 10 × 10 cm in the center of the arena. The animals were put into the left rear quadrant and then left to explore the arena for 5 min.

2.6. Brain samples

The animals were decapitated immediately after completing the open field test (Scheme 1). Mouse brains were dissected in the frontal cortex and hippocampus, rapidly frozen, and stored at −80 °C until assayed. The samples of the frontal cortex and hippocampus of the mice were homogenized in KCl KH_2PO_4 (12 mM KCl, 0.038 mM KH_2PO_4, pH = 7.4).

2.7. Protein determination

All biochemical measures were normalized to the protein content with bovine albumin as a standard (Lowry et al., 1951).

2.8. Measurement of BDNF, NGF, NT-3, NT-4, and GDNF levels

For the analysis of neurotrophic factors, brain tissues were homogenized in phosphate-buffered solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycolbis (2-aminoethyl ether)-N,N,N'N'-tetraacetic acid (EGTA). The homogenates were centrifuged at 10,000 g for 20 min, and the supernatants were collected to quantify the neurotrophic factor levels. BDNF, NGF, NT-3, NT-4, and GDNF levels in the frontal cortex and hippocampus were evaluated by sandwich enzyme-linked immunosorbent assay using commercial kits according to the manufacturer's instructions [NGF and BDNF levels were assessed with a kit from Chemicon (USA), and GDNF, NT-3, and NT-4 levels were assessed with a kit from Biosensis (USA)].

2.9. Statistical analysis

The software used in the statistical analyses were STATISTICA 8 (Statsoft ©) and Prism 6 (GraphPad©). All data are presented as the mean ± S.E.M. Differences among experimental groups were determined by two-way ANOVA followed by Tukey's post hoc test. $P \leq 0.05$ was considered statistically significant. Correlations were analyzed using the Pearson correlation test. The Pearson correlation coefficient was used to analyze the strength of the relationship between continuous variables.

3. Results

3.1. Behavioral measures

Fig. 1 shows that PSD increased crossings, rearings, and visits to the center of the open field. Pretreatment with Li decreased these manic-like behaviors induced by PSD, i.e., prevented the mice from experiencing behavioral alterations induced by PSD. The administration of VPA decreased the PSD-induced increase in crossings, rearings, and visits to the center of the open field. However, VPA only completely prevented the PSD-induced increase in crossings and rearings and partially blocked risk-taking behavior. It is important to note that treatment with Li increased the number of visits to the center of the open field in the mice.

Two-way ANOVA revealed significant effects of PSD protocol [Crossings: $F(1.42) = 30.06$, $p < 0.001$; Rearings: $F(1.42) = 8.65$, $p < 0.01$; Center visits: $F(1.42) = 50.89$, $p < 0.001$] and treatment [Crossings: $F(2.42) = 25.82$, $p < 0.001$; Rearings: $F(2.42) = 13.12$, $p < 0.001$; Center visits: $F(2.42) = 13.16$, $p < 0.001$] and a significant PSD × treatment interaction [Crossings: $F(2.42) = 13.16$, $p < 0.001$; Rearings: $F(2.42) = 22.23$, $p < 0.001$; Center visits: $F(2.42) = 44.48$, $p < 0.001$].

3.2. BDNF levels

Fig. 2 shows the effects of pretreatment with Li and VPA on the levels of BDNF in the frontal cortex and hippocampus of mice submitted to PSD. PSD decreased the levels of BDNF in the frontal cortex and hippocampus of mice. Treatment with Li prevented alterations induced by PSD, increasing BDNF levels in both evaluated brain structures in PSD-submitted animals compared to those of the PSD + Sal group. Furthermore, pretreatment with VPA increased BDNF levels in PSD-submitted animals in both brain structures compared to those in the PSD + Sal group. It is important to note that in the PSD + VPA group, the increased BDNF levels in the hippocampus were significantly higher than those in the Control + Sal group.

Two-way ANOVA revealed significant effects of the PSD protocol [BDNF = Frontal Cortex: $F(1.42) = 7.52$, $p < 0.01$; Hippocampus: $F(1.42) = 6.56$, $p < 0.05$] and treatment [BDNF = Frontal Cortex: $F(2.42) = 11.57$, $p < 0.001$; Hippocampus: $F(2.42) = 40.51$, $p < 0.001$] and a significant PSD × treatment interaction [BDNF = Frontal Cortex: $F(2.42) = 13.73$, $p < 0.001$; Hippocampus: $F(2.42) = 13.12$, $p < 0.001$].

3.3. NGF levels

Fig. 3 shows the effects of PSD and pretreatment with Li and VPA on NGF levels in the frontal cortex and hippocampus of mice. The mice submitted to the PSD protocol showed a significant decrease in NGF levels in the frontal cortex and hippocampus. Li and VPA increased the NGF levels in both brain structures in animals submitted to PSD compared to those in the PSD + Sal group.

Two-way ANOVA revealed significant effects of the PSD protocol [NGF = Frontal Cortex: $F(1.42) = 18.84$, $p < 0.001$; Hippocampus: $F(1.42) = 18.68$, $p < 0.001$] and treatment [NGF = Frontal Cortex: $F(2.42) = 24.66$, $p < 0.001$; Hippocampus: $F(2.42) = 12.11$, $p < 0.001$] and a significant PSD × treatment interaction [NGF = Frontal Cortex: $F(2.42) = 10.25$, $p < 0.001$; Hippocampus: $F(2.42) = 16.93$, $p < 0.001$].

3.4. NT-3 levels

Fig. 4 shows the results of the effects of pretreatment with Li and VPA on the NT-3 levels in the frontal cortex and hippocampus of mice submitted to PSD. We did not find significant differences in NT-3 levels in any of the experimental groups or brain structures evaluated.

Two-way ANOVA revealed effects of the PSD protocol [NT-3 = Frontal Cortex: $F(1.42) = 0.007$, $p = 0.93$; Hippocampus: $F(1.42) = 2.08$, $p = 0.16$] and treatment [NT-3 = Frontal Cortex: $F(2.42) = 0.01$, $p = 0.99$; Hippocampus: $F(2.42) = 4.27$, $p = 0.02$] and PSD × treatment interaction [NT-3 = Frontal Cortex: $F(2.42) = 0.38$, $p = 0.68$; Hippocampus: $F(2.42) = 0.64$, $p = 0.53$].

3.5. NT-4 levels

Fig. 5 shows the results of the effects of pretreatment with Li and VPA on the NT-4 levels in the frontal cortex and hippocampus of mice submitted to PSD. We did not find significant differences in NT-4 levels in any of the experimental groups or brain structures evaluated.

Two-way ANOVA revealed effects of the PSD protocol [NT-4 = Frontal Cortex: $F(1.42) = 2.97$, $p = 0.09$; Hippocampus: $F(1.42) = 0.09$, $p = 0.77$] and treatment [NT-4 = Frontal Cortex: $F(2.42) = 1.39$, $p = 0.26$; Hippocampus: $F(2.42) = 0.21$, $p = 0.81$] and

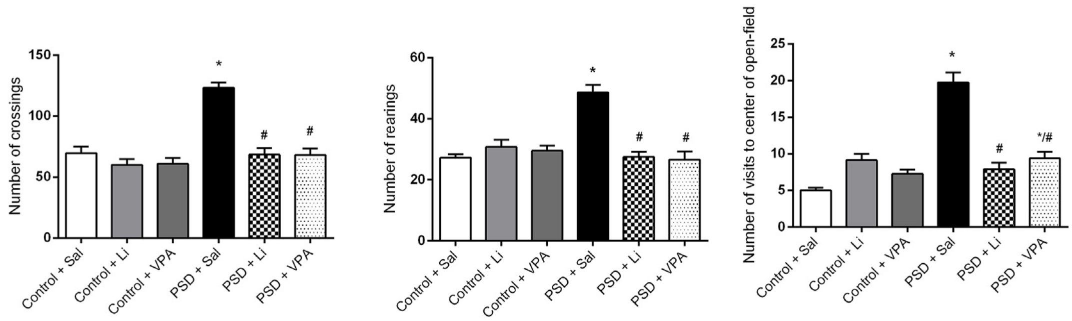

Fig. 1. Effects of Li and VPA administration on the number of crossings, rearings, and visits to the center of the open field in animals submitted to sleep deprivation (n = 10 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

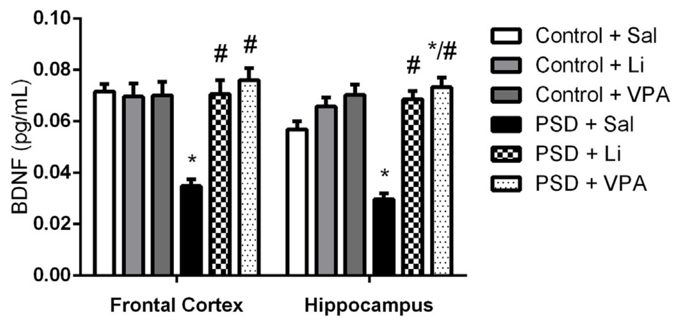

Fig. 2. Effects of Li and VPA administration on BDNF levels in the frontal cortex and hippocampus of animals subjected to sleep deprivation (n = 6 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

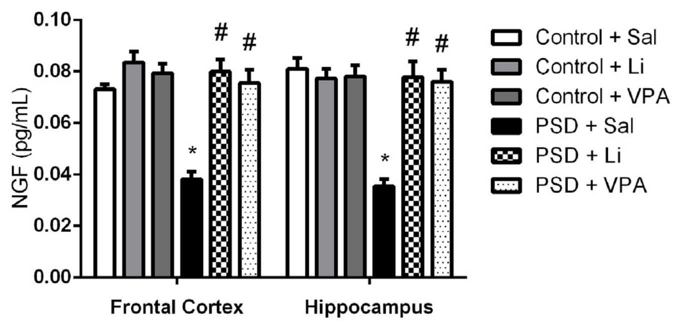

Fig. 3. Effects of Li and VPA administration on NGF levels in the frontal cortex and hippocampus of animals subjected to sleep deprivation (n = 6 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

PSD × treatment interaction [NT-4 = Frontal Cortex: F(2.42) = 0.51, p = 0.60; Hippocampus: F(2.42) = 1.19, p = 0.31].

3.6. GDNF levels

Fig. 6 shows the results of pretreatment with Li and VPA on the GDNF levels in the frontal cortex and hippocampus of mice submitted to PSD. PSD induced a decrease in GDNF levels in the frontal cortex and hippocampus of animals compared to those in the Control + Sal group. Moreover, treatment with Li and VPA prevented this alteration induced by PSD, increasing GDNF levels in both brain structures evaluated in PSD-submitted mice compared to those in the PSD + Sal group. However, the administration of VPA increased GDNF levels in the hippocampus of mice compared to those in the control group.

Two-way ANOVA revealed significant effects of the PSD protocol

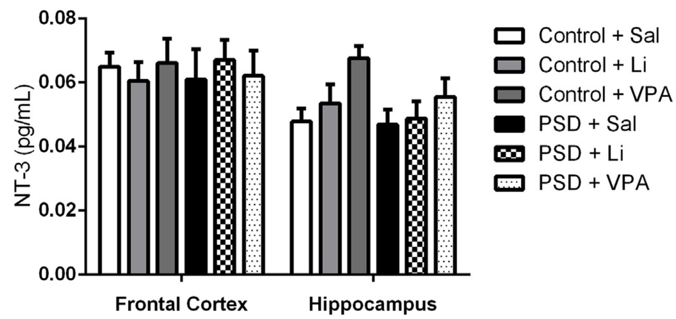

Fig. 4. Effects of the administration of Li and VPA on the levels of NT-3 in the frontal cortex and hippocampus of animals submitted to sleep deprivation (n = 6 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

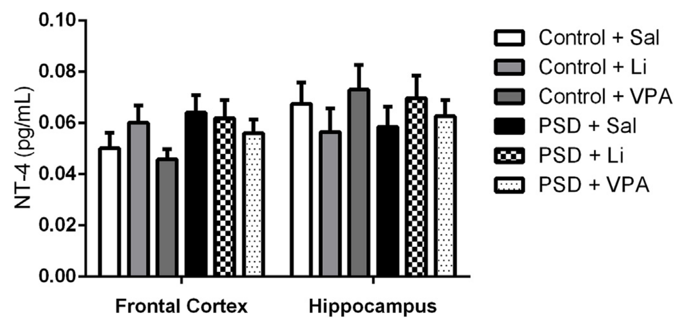

Fig. 5. Effects of Li and VPA administration on NT-4 levels in the frontal cortex and hippocampus of animals subjected to sleep deprivation (n = 6 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

[GDNF = Frontal Cortex: F(1.42) = 3.62, p = 0.06; Hippocampus: F (1.42) = 57.60, p < 0.001] and treatment [GDNF = Frontal Cortex: F (2.42) = 15.15, p < 0.001; Hippocampus: F(2.42) = 36.40, p < 0.001] and a significant PSD × treatment interaction [GDNF = Frontal Cortex: F(2.42) = 8.45, p < 0.001; Hippocampus: F (2.42) = 8.34, p = 0.01].

3.7. Correlation between locomotor activity and neurotrophic factor levels

The correlation between the number of crossings and BDNF levels is demonstrated in Fig. 7. BDNF levels in the frontal cortex (Fig. 7A) and hippocampus (Fig. 7B) of mice were negatively correlated with the number of crossings, Pearson correlation for BDNF: [frontal cortex (n = 36; r2 = 0.4450; p < 0.0001), hippocampus (n = 36;

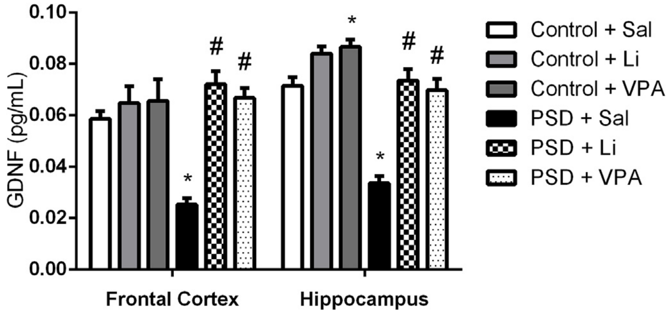

Fig. 6. Effects of Li and VPA administration on the levels of GDNF in the frontal cortex and hippocampus of animals submitted to sleep deprivation (n = 6 per group). Data were analyzed by two-way ANOVA, followed by Tukey's test when F was significant. Values are expressed as the mean ± S.E.M. *p < 0.05 compared to the control group. #p < 0.05 compared to the SD group.

$r2 = 0.4195$; $p < 0.0001$)].

Fig. 8 demonstrates the correlation between the number of crossings and NGF levels. NGF levels in the frontal cortex (Fig. 8A) and hippocampus (Fig. 8B) of mice were negatively correlated with the number of crossings, Pearson correlation for NGF: [frontal cortex (n = 36; $r2 = 0.5258$; $p < 0.0001$), hippocampus (n = 36; $r2 = 0.5562$; $p < 0.0001$)].

Fig. 9 shows the correlation between NT-3 levels in the frontal cortex (Fig. 9A) and hippocampus (Fig. 9B) of mice. It was found that NT-3 levels in the frontal cortex of animals did not correlate with the number of crossings; however, in the hippocampus, NT-3 levels were negatively correlated with the number of crossings, Pearson correlation for NT-3: [frontal cortex (n = 36; $r2 = 0.01778$; $p = 0.3715$), hippocampus (n = 36; $r2 = 0.1009$; $p = 0.0278$)].

The NT-4 levels were not correlated with the number of crossings in any structure evaluated, as seen in Fig. 10A (frontal cortex) and **10B** (hippocampus), Pearson correlation for NT-4: [frontal cortex (n = 36; $r2 = 0.03884$; $p = 0.1794$), hippocampus (n = 36; $r2 = 0.04743$; $p = 0.1463$)].

The correlation between the number of crossings and GDNF levels is demonstrated in Fig. 11. GDNF levels in the frontal cortex (Fig. 11A) and hippocampus (Fig. 11B) of mice were negatively correlated with the number of crossings, Pearson correlation for GDNF: [frontal cortex (n = 36; $r2 = 0.2085$; $p = 0.0011$), hippocampus (n = 36; $r2 = 0.6032$; $p < 0.0001$)].

4. Discussion

In the present study, PSD induced characteristics that are considered manic-like behaviors, hyperactivity and risk-taking behavior, in mice. Furthermore, PSD decreased BDNF, NGF, and GDNF levels in the frontal cortex and hippocampus of mice. The administration of Li and VPA protected animals against behavioral alterations and biochemical damage in the brain induced by PSD. However, PSD and the administration of Li or VPA did not affect the levels of NT-3 and NT-4 in either brain structure evaluated. Indeed, it has been described in the literature that PSD induces hyperactivity and risk-taking behavior in rats (Radhakrishnan et al., 2015; Valvassori et al., 2017c; Arent et al., 2015; Streck et al., 2015). Previous studies also showed that mood stabilizers prevented behavioral alterations induced by PSD (Valvassori et al., 2017c; Arent et al., 2015; Streck et al., 2015).

Wisor et al. (2008) demonstrated that sleep deprivation (SD) suppresses the expression of the CLOCK gene in the brain by increasing TNFα levels. Interestingly, Li protects against TNFα increases induced by PSD in the brains of rats (Valvassori et al., 2017b). In addition, Li can normalize anxiety in mice with the CLOCKΔ19 mutation (a gene involved in the circadian rhythm) (Kristensen et al., 2018; Coque et al., 2011). Herein, the administration of Li increased the number of visits to the center of the open field, suggesting a decrease in anxiety in the animals. A possible explanation for the therapeutic effects of Li could be its effects on TNFα and, consequently, in protecting the brain against PSD-induced CLOCK gene alterations.

Other data showed that PSD animals had decreased BDNF in the frontal cortex and hippocampus. Li and VPA protected against this neurotrophic alteration induced by PSD. Neurotrophic functions are associated with various physiological functions, including sleep (Faraguna et al., 2008). In turn, sleep disturbances, such as insomnia and SD, can be related to alterations in levels of neurotrophic factors (Schmitt et al., 2016). Indeed, previous studies demonstrated that subjects suffering from insomnia exhibited decreased BDNF levels in serum compared with those of sleep healthy controls. Additionally, serum BDNF levels were correlated with the severity of insomnia (Giese et al., 2013, 2014).

PSD has been considered an environmental stressor that can lead to the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Schmitt et al., 2016; Han et al., 2012; Valvassori et al., 2017b). The literature shows that the deregulation of the HPA axis can lead to sleep alterations and decreases in the levels of neurotrophic factors. Therefore, sleep can be a crucial mediator of the relationship between stress and neurotrophic factor levels, and sleep alterations can lead to decreases in neurotrophic factor levels, such as BDNF levels (Schmitt et al., 2016). A previous study from our research group found that

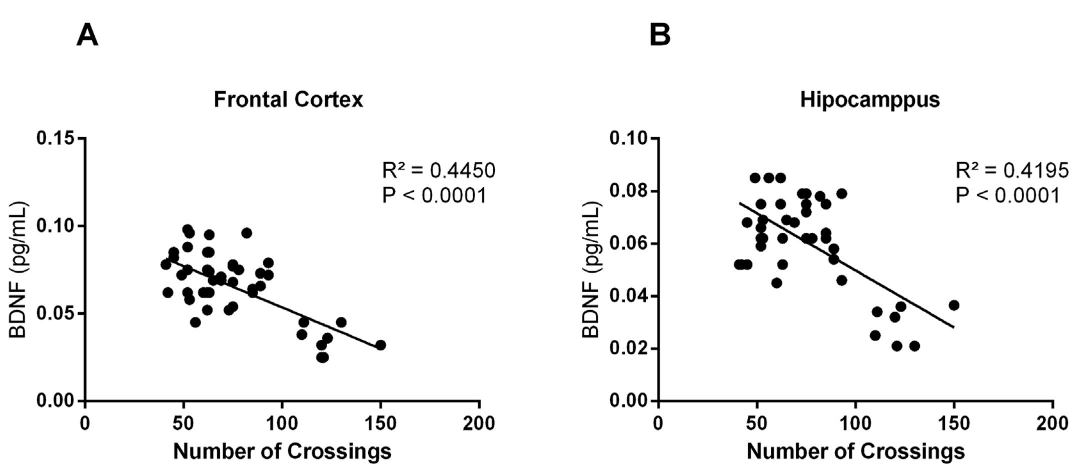

Fig. 7. Correlations between the number of crossings and levels of BDNF in the frontal cortex (a) and hippocampus (b) of rats submitted to sleep deprivation. The results were assessed using the Pearson correlation test.

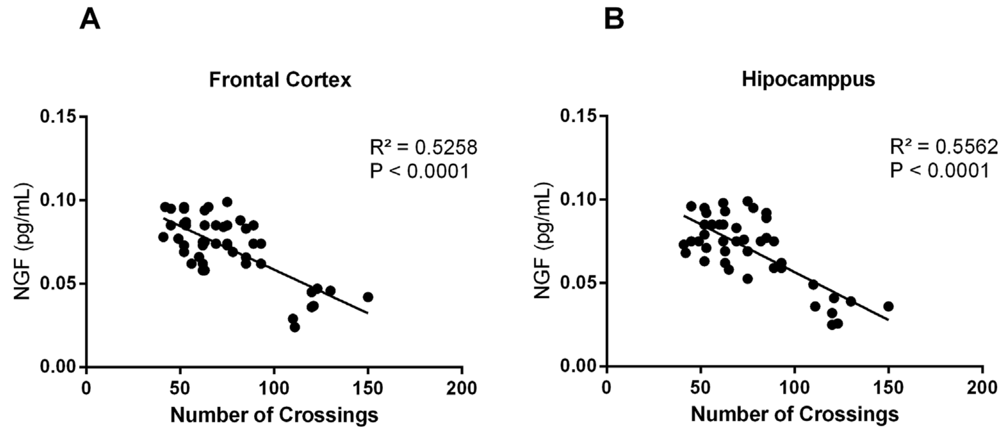

Fig. 8. Correlations between the number of crossings and levels of NGF in the frontal cortex (a) and hippocampus (b) of rats submitted to sleep deprivation. The results were assessed using the Pearson correlation test.

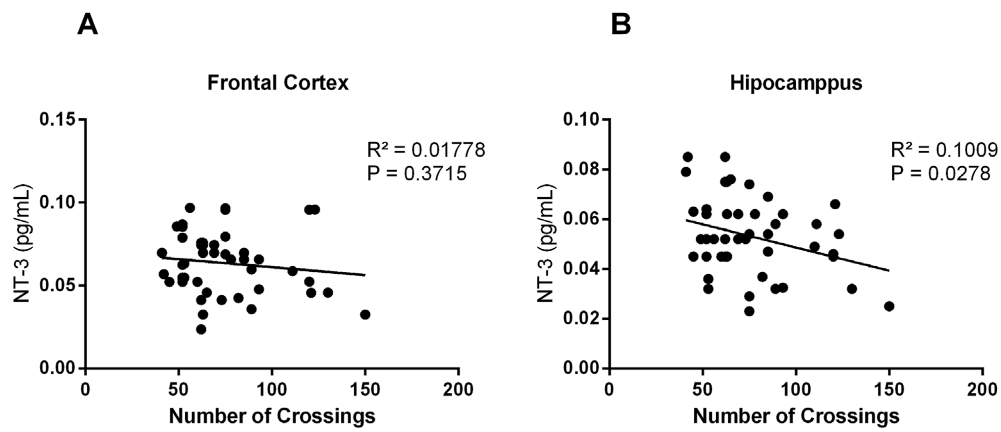

Fig. 9. Correlations between the number of crossings and levels of NT-3 in the frontal cortex (a) and hippocampus (b) of rats submitted to sleep deprivation. The results were assessed using the Pearson correlation test.

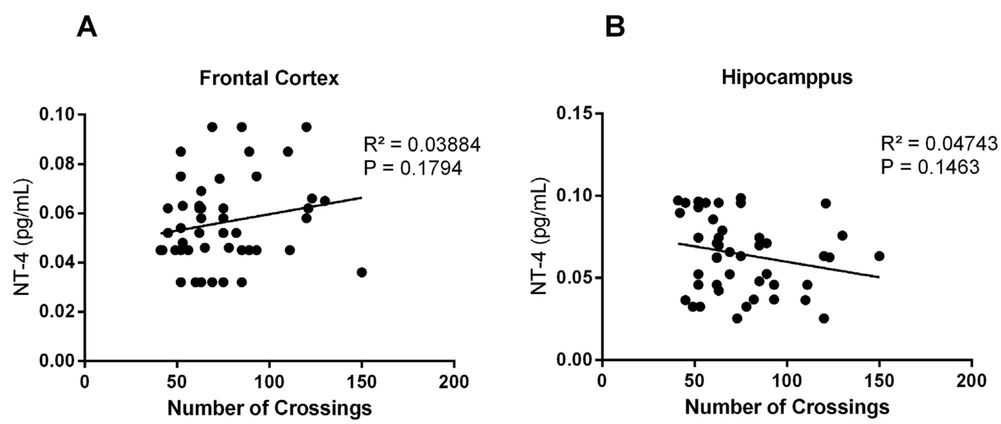

Fig. 10. Correlations between the number of crossings and levels of NT-4 in the frontal cortex (a) and hippocampus (b) of rats submitted to sleep deprivation. The results were assessed using the Pearson correlation test.

animals submitted to PSD showed an increase in corticosterone and adrenocorticotrophic hormone levels (ACTH) (Valvassori et al., 2017b). Chaves et al. (2019) demonstrated that the administration of corticosterone in mice decreased the levels of BDNF in the hippocampus. Therefore, it can be suggested that the increased corticosterone in PSD animals could be associated with the decrease in the level of BDNF observed in the present study.

In a previous study, Li protected against alterations in serum corticosterone and ACTH induced by PSD in mice (Valvassori et al., 2017b). Therefore, a possible hypothesis is that by modulating the HPA axis, Li increased the BDNF levels in the present study. In addition, several studies have demonstrated the effects of Li and VPA on BDNF in the brains of animals submitted to animal models of mania (Frey et al., 2006; Jornada et al., 2010). Frey et al. (2006) showed that Li and VPA reversed the decrease in BDNF levels induced by amphetamine in the brains of rats. In turn, Jornada and its coauthors (2010) showed that in addition to its antimanic effects, Li and VPA also protected the brains of rats against the decrease in BDNF levels induced by ouabain. Together with our results, these studies suggest that manic-like behaviors are accompanied by decreased BDNF levels, and the therapeutic effects of

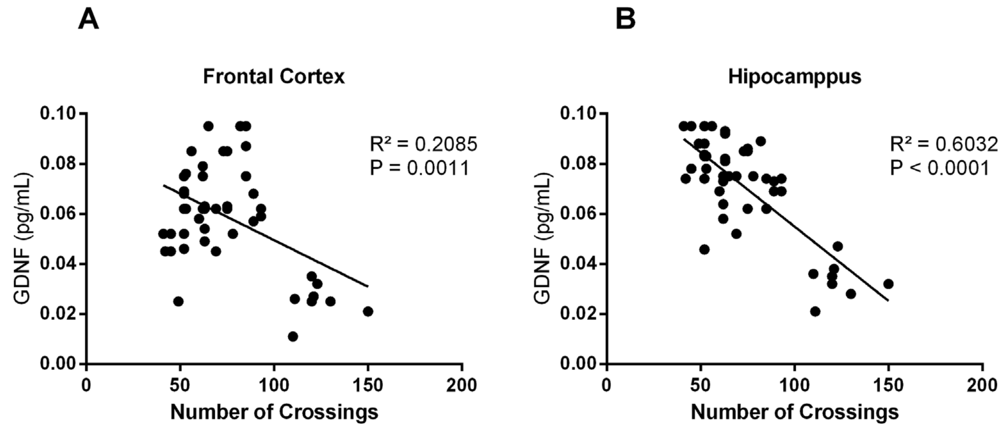

Fig. 11. Correlations between the number of crossings and levels of GDNF in the frontal cortex (a) and hippocampus (b) of rats submitted to sleep deprivation. The results were assessed using the Pearson correlation test.

mood-stabilizers include modulating the levels of this neurotrophin.

Herein, PSD also decreased NGF levels in the frontal cortex and hippocampus of mice. Li or VPA prevented NGF alterations induced by PSD. In accordance with our results, another preclinical study demonstrated that rodents submitted to PSD showed decreases in NGF levels (Sei et al., 2000). NGF is an essential regulator of neuronal development and survival (Levi-Montalcini and Angeletti, 1968). A previous clinical study demonstrated that BD individuals experiencing mania had lower NGF levels than euthymic patients or controls (Barbosa et al., 2011b). A preclinical study also showed reductions in NGF levels in rats with manic-like behaviors induced by ouabain (OUA) (Varela et al., 2015). Varela et al. (2015) demonstrated that the administration of Li and VPA reversed alterations in NGF levels in the frontal cortex of rats that showed manic-like behavior induced by OUA. Therefore, the modulation of NGF seems to be an important target for mood stabilizers, protecting the brains of BD patients.

The effects of GDNF are associated with dopaminergic neurons (Lin et al., 1993; Ogłodek et al., 2016). Decreases in GDNF levels were reported in the blood of BD patients during a manic episode (Takebayashi et al., 2006). In the present study, decreases in GDNF levels were accompanied by manic-like behaviors. Li and VPA prevented the manic-like behavior induced by PSD and protected the brains of mice against GDNF decreases. A previous study demonstrated that Li or VPA treatment reversed and prevented manic-like behavior and increased GDNF levels in the brains of rats submitted to ouabain administration (Varela et al., 2015). These findings suggest a relationship between manic-like behavior, alterations in GDNF levels, and the therapeutic effects of Li and VPA.

In conclusion, alterations in sleep through the PSD protocol induced manic-like behavior in rats and induced changes in neurotrophic factor levels. The administration of Li and VPA protected against behavioral and neurotrophic alterations induced by PSD. Therefore, it seems that neurotrophic factors and sleep are essential targets to treat BD. However, more studies are necessary to better understand these mechanisms.

5. Limitations of the study

The authors discuss some points of the manuscript using circadian rhythm disruption, but the circadian rhythm itself was not evaluated in this study. However, sleep is part of the circadian rhythm, once it is the total period of one day; consequently, PSD affects the circadian rhythm. The present study did not use female rats; therefore, it is important to study differences in PSD and neurotrophin alterations among sexes.

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References

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (DSM5). In: American Psychiatric Association (Ed.), Bipolar Disorder and Relationship, fifth ed. American Psychiatric Press, Washington, DC, pp. 123–155.

Arent, C.O., Valvassori, S.S., Steckert, A.V., Resende, W.R., Dal-Pont, G.C., Lopes-Borges, J., Amboni, R.T., Bianchini, G., Quevedo, J., 2015. The effects of n-acetylcysteine and/or deferoxamine on manic-like behavior and brain oxidative damage in mice submitted to the paradoxal sleep deprivation model of mania. Jun. J. Psychiatr. Res. 65, 71–79.

Armani, F., Andersen, M.L., Andreatini, R., Frussa-Filho, R., Tufik, S., Galduróz, J.C., 2012. Successful combined therapy with tamoxifen and lithium in a paradoxical sleep deprivation-induced mania model. Feb. CNS Neurosci. Ther. 18 (2), 119–125.

Barbosa, I.G., Huguet, R.B., Sousa, L.P., Abreu, M.N., Rocha, N.P., Bauer, M.E., Carvalho, L.A., Teixeira, A.L., 2011a. Circulating levels of GDNF in bipolar disorder. Neurosci. Lett. 502, 103–106.

Barbosa, I.G., Huguet, R.B., Neves, F.S., Reis, H.J., Bauer, M.E., Janka, Z., Palotás, A., Teixeira, A.L., 2011b. Impaired nerve growth factor homeostasis in patients with bipolar disorder. World J. Biol. Psychiatry 12 (3), 228–232.

Benedetti, F., Fresi, F., Maccioni, P., Smeraldi, E., 2008. Behavioural sensitization to repeated sleep deprivation in a mice model of mania. Behav. Brain Res. 187, 221–227.

Bunney, B.G., Bunney, W.E., 2013. Mechanisms of rapid antidepressant effects of sleep deprivation therapy: clock genes and circadian rhythms. Biol. Psychiatry 73, 1164–1171.

Chaves, R.C., Mallmann, A.S.V., Oliveira, N.F., Oliveira, I.C.M., Capibaribe, V.C.C., da Silva, D.M.A., Lopes, I.S., Valentim, J.T., de Carvalho, A.M.R., Macêdo, D.S., Vasconcelos, S.M.M., Gutierrez, S.J.C., Barbosa Filho, J.M., de Sousa, F.C.F., 2019. Reversal effect of Riparin IV in depression and anxiety caused by corticosterone chronic administration in mice. May. Pharmacol. Biochem. Behav. 180, 44–51.

Cheniaux, E., 2011. O tratamento farmacológico do transtorno bipolar: uma revisão sistemática e crítica dos aspectos metodológicos dos estudos clínicos modernos. Rev. Bras. Psiquiatr. 33, 72–80.

Coque, L., Mukherjee, S., Cao, J.L., Spencer, S., Marvin, M., Falcon, E., Sidor, M.M., Birnbaum, S.G., Graham, A., Neve, R.L., Gordon, E., Ozburn, A.R., Goldberg, M.S., Han, M.H., Cooper, D.C., McClung, C.A., 2011. Specific role of VTA dopamine neuronal firing rates and morphology in the reversal of anxietyrelated, but not depression-related behavior in the ClockDelta19 mouse model of mania. Neuropsychopharmacology 36, 1478–1488.

Coryell, W., 2009. Maintenance treatment in bipolar disorder: a reassessment of lithium as the first choice. Bipolar Disord. 11, 77–83.

Cunha, A.B., Frey, B.N., Andreazza, A.C., Goi, J.D., Rosa, A.R., Goncalves, C.A., Santin, A., Kapczinski, F., 2006. Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neurosci. Lett. 398, 215–219.

Curran, G., Ravindran, A., 2014. Lithium for bipolar disorder: a review of the recent literature. Expert Rev. Neurother. 14, 1079–1098.

Davis, L.L., Bartolucci, A., Petty, F., 2005. Divalproex in the treatment of bipolar depression: a placebo-controlled study. Apr. J. Affect. Disord. 85 (3), 259–266.

Faraguna, U., Vyazovskiy, V.V., Nelson, A.B., Tononi, G., Cirelli, C., 2008. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J. Neurosci. 28, 4088–4095.
Fountoulakis, K.N., Vieta, E., Siamouli, M., Valenti, M., Magiria, S., Oral, T., Fresno, D., Giannakopoulos, P., Kaprinis, G.S., 2007. Treatment of bipolar disorder: a complex treatment for a multi-faceted disorder. Ann. Gen. Psychiatr. 6, 27.
Frey, B.N., Valvassori, S.S., Réus, G.Z., Martins, M.R., Petronilho, F.C., Bardini, K., Dal-Pizzol, F., Kapczinski, F., Quevedo, J., 2006. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J. Psychiatry Neurosci. 31, 326–332.
Giese, M., Unternaehrer, E., Brand, S., Calabrese, P., Holsboer-Trachsler, E., Eckert, A., 2013. The interplay of stress and sleep impacts BDNF level. PLoS One 8, e76050.
Giese, M., Unternahrer, E., Huttig, H., Beck, J., Brand, S., Calabrese, P., Holsboer-Trachsle, E., Eckert, A., 2014. BDNF: an indicator of insomnia? Mol. Psychiatry 19, 151–152.
Gessa, G.L., Pani, L., Fadda, P., Fratta, W., 1995. Sleep deprivation in the rat: an animal model of mania. Eur. Neuropsychopharmacol. 5, 89–93.
Han, K.S., Kim, L., Shim, I., 2012. Stress and sleep disorder. Exp. Neurobiol. 21, 141–150.
Jornada, L.K., Moretti, M., Valvassori, S.S., Ferreira, C.L., Padilha, P.T., Arent, C.O., Fries, G.R., Kapczinski, F., Quevedo, J., 2010. Effects of mood stabilizers on hippocampus and amygdala BDNF levels in an animal model of mania induced by ouabain. Jun. J. Psychiatr. Res. 44 (8), 506–510.
Kaplan, K.A., Harvey, A.G., 2013. Behavioral treatment of insomnia in bipolar disorder. Am. J. Psychiatry 170, 716–720.
Kristensen, M., Nierenberg, A.A., Ostergaard, S.D., 2018. Face and predictive validity of the ClockΔ19 mouse as an animal model for bipolar disorder: a systematic review. Jan. Mol. Psychiatry 23 (1), 70–80.
Levi-Montalcini, R., Angeletti, P.U., 1968. Nerve growth factor. Physiol. Rev. 48 (3), 534–569.
Lin, L.F., Doherty, D.H., Lile, J.D., Bektesh, S., Collins, F., 1993. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. May 21. Science 260 (5111), 1130–1132.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. Nov. J. Biol. Chem. 193 (1), 265–275.
Manji, H.K., Zarate, C.A., 2002. Molecular and cellular mechanisms underlying mood stabilization in bipolar disorder: implications for the development of improved therapeutics. Mol. Psychiatry 7 (Suppl. 1), S1–S7.
Ogłodek, E.A., Just, M.J., Szromek, A.R., Araszkiewicz, A., 2016. Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. Pharmacol. Rep. 68, 945–951.
Radhakrishnan, A., Aswathy, B.S., Kumar, V.M., Gulia, K.K., 2015. Sleep deprivation during late pregnancy produces hyperactivity and increased risk-taking behavior in offspring. Jan. Brain Res. 30 (1596), 88–98.
Safari, R., Tunca, Z., Özerdem, A., Ceylan, D., Yalçın, Y., Sakizli, M., 2017. Glial cell-derived neurotrophic factor gene polymorpisms affect severity and functionality of bipolar disorder. J. Integr. Neurosci. 16 (4), 471–481.
Schmitt, K., Holsboer-Trachsler, E., Eckert, A., 2016. BDNF in sleep, insomnia, and sleep deprivation. Ann. Med. 48 (1–2), 42–51.
Scola, G., Andreazza, A.C., 2015. The role of neurotrophins in bipolar disorder Progress. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 56, 122–128.
Sei, H., Saitoh, D., Yamamoto, K., Morita, K., Morita, Y., 2000. Differential effect of short-term REM sleep deprivation on NGF and BDNF protein levels in the rat brain. Sep. 22. Brain Res. 877 (2), 387–390.
Streck, E.L., Scaini, G., Jeremias, G.C., Rezin, G.T., Gonçalves, C.L., Ferreira, G.K., Réus, G.Z., Resende, W.R., Valvassori, S.S., Kapczinski, F., Andersen, M.L., Quevedo, J., 2015. Effects of mood stabilizers on brain energy metabolism in mice submitted to an animal model of mania induced by paradoxical sleep deprivation. Neurochem. Res. J. 40 (6), 1144–1152.
Takebayashi, M., Hisaoka, K., Nishida, A., Tsuchioka, M., Miyoshi, I., Kozuru, T., Hikasa, S., Okamoto, Y., Shinno, H., Morinobu, S., Yamawaki, S., 2006. Decreased levels of whole blood glial cell line-derived neurotrophic factor (GDNF) in remitted patients with mood disorders. Int. J. Neuropsychopharmacol. 9 (5), 607–612.
Tufik, S., Andersen, M.L., Bittencourt, L.R., Mello, M.T., 2009. Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. Sep. An. Acad. Bras. Cienc. 81 (3), 521–538.
Valvassori, S.S., Resende, W.R., Dal-Pont, G., Sangaletti-Pereira, H., Gava, F.F., Peterle, B.R., Carvalho, A.F., Varela, R.B., Dal-Pizzol, F., Quevedo, J., 2017b. Lithium ameliorates sleep deprivation-induced mania-like behavior, hypothalamic-pituitary-adrenal (HPA) axis alterations, oxidative stress and elevations of cytokine concentrations in the brain and serum of mice. Bipolar Disord. 19 (4), 246–258.
Valvassori, S.S., Dal-Pont, G.C., Resende, W.R., Jornada, L.K., Peterle, B.R., Machado, A.G., Farias, H.R., de Souza, C.T., Carvalho, A.F., Quevedo, J., 2017c. Lithium and valproate act on the GSK-3β signaling pathway to reverse manic-like behavior in an animal model of mania induced by ouabain. May 1. Neuropharmacology 117, 447–459.
Valvassori, S.S., Tonin, P.T., Varela, R.B., Carvalho, A.F., Mariot, E., Amboni, R.T., Bianchini, G., Andersen, M.L., Quevedo, J., 2015. Lithium modulates the production of peripheral and cerebral cytokines in an animal model of mania induced by dextroamphetamine. Aug. Bipolar Disord. 17 (5), 507–517.
Varela, R.B., Valvassori, S.S., Lopes-Borges, J., Mariot, E., Dal-Pont, G.C., Amboni, R.T., Bianchini, G., Quevedo, J., 2015. Sodium butyrate and mood stabilizers block ouabain-induced hyperlocomotion and increase BDNF, NGF and GDNF levels in brain of Wistar rats. Feb. J. Psychiatr. Res. 61, 114–121.
Walz, J.C., Frey, B.N., Andreazza, A.C., Ceresér, K.M., Cacilhas, A.A., Valvassori, S.S., Quevedo, J., Kapczinski, F., 2008 Apr. Effects of lithium and valproate on serum and hippocampal neurotrophin-3 levels in an animal model of mania. J. Psychiatr. Res. 42 (5), 416–421 Epub May 21.
Wiener, C.D., de Mello Ferreira, S., Moreira, F.P., Bittencourt, G., de Oliveira, J.F., Molina, M.L., Jansen, K., de Mattos Souza, L.D., Rizzato Lara, D., Portela, L.V., da Silva, R.A., Oses, J.P., 2015. Serum levels of nerve growth factor (NGF) in patients with major depression disorder and suicide risk. J. Affect. Disord. 184, 245–248.
Wiener, C.D., Molina, M.L., Moreira, F.P., Dos Passos, M.B., Jansen, K., da Silva, R.A., de Mattos Souza, L.D., Oses, J.P., 2017. Brief psychoeducation for bipolar disorder: evaluation of trophic factors serum levels in young adults. Nov. Psychiatry Res. 257, 367–371.
Wisor, J.P., Pasumarthi, R.K., Gerashchenko, D., Thompson, C.L., Pathak, S., Sancar, A., Franken, P., Lein, E.S., Kilduff, T.S., 2008. Sleep deprivation effects on circadian clock gene expression in the cerebral cortex parallel electroencephalographic differences among mouse strains. Jul 9. J. Neurosci. 28 (28), 193–201 7.
Zarate Jr., C.A., Singh, J., Manji, H.K., 2006. Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder. Jun 1. Biol. Psychiatry 59 (11), 1006–1020.
Zhang, X., Zhang, Z., Sha, W., Xie, C., Xi, G., Zhou, H., Zhang, Y., 2010. Effect of treatment on serum glial cell line-derived neurotrophic factor in bipolar patients. J. Affect. Disord. 126, 326–329.