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Coadministration of lithium and celecoxib attenuates the behavioral alterations and inflammatory processes induced by amphetamine in an animal model of mania

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ABSTRACT

The present study evaluated the effects of the coadministration of lithium (Li) and Cel on inflammatory parameters in an animal model of mania induced by dextroamphetamine (D-amph). It was used Wistar rats 60 days old (250–350 g). The animals (n = 10 per group) received D-amph (2 mg/kg) or saline solution of NaCl 0.9% (Sal) intraperitoneally once a day for 14 days. From day eight until 14, the animals from the D-amph and Sal groups received Li (24 mg/kg), Cel (20 mg/kg), Li + Cel or water *via* gavage. Behavioral analyses were performed using the open-field test. The levels of IL-1β, IL-4, IL-10, and TNF-α were evaluated. The administration of D-amph induced hyperactivity in the rats, as well increased the IL-4, IL-10, and TNF-α levels in the serum, frontal cortex, and striatum of rats compared to those of the controls, and treatment with Li plus Cel reversed these alterations. In general, the administration of Li or Cel *per se* did not have effects on the behavioral and biochemical parameters. However, the treatment with Cel *per se* decreased only the IL-10 levels in the serum of animals. Besides, the treatment with Li or Cel decreased the IL-4 levels in the serum and reversed the effects of D-amph on this parameter in the frontal cortex. The treatment with Li reversed the effects of D-amph on the TNF-α levels in all tissues evaluated, and the administration of Cel reversed this alteration only in the striatum. It can be observed that treatment with Li plus Cel was more effective against damages caused by D-amph when compared to the administration of both treatments *per se*, suggesting that the coadministration can be more effective to treat BD rather than Li or Cel itself. The treatment with Li plus Cel was effective against the inflammation induced by D-amph.

1. Introduction

Bipolar disorder (BD) is a chronic psychiatric disorder that involves mood alterations, affecting 1%–3% of the global population (Keck et al., 2001). BD is characterized by an alternation between depressive and manic episodes, with the latter being the clinical mark of BD (American Psychiatric Association, 2013). Lithium (Li) is a drug that is approved by the FDA as a mood stabilizer, which is the "gold standard" of treatment for BD (Geddes and Miklowitz, 2013). Li is useful in the treatment of acute manic episodes and has some antidepressant properties (Manji and Zarate, 2002). Furthermore, other drugs also are used in BD treatment by having antimanic effects, such as anticonvulsants and atypical antipsychotics (Geddes and Miklowitz, 2013).

A selective inhibitor of cyclooxygenase 2 (COX-2), celecoxib (Cel), has been studied as adjuvant therapy for some mood disorders, such as BD (Nery et al., 2008; Arabzadeh et al., 2015; Mousavi et al., 2017). Cel

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is a nonsteroidal anti-inflammatory agent that can reduce the levels of inflammatory markers, such as prostaglandins and cytokines (Shi and Klotz, 2008). It is well described in the literature that alterations in the markers of inflammation are present in patients with psychiatric diseases (Mitchell and Goldstein, 2014). Some authors have discussed several mechanisms that could be involved in the pathophysiology of BD, such as alterations in the monoamine system and inflammatory mechanisms (Haase and Brown, 2014; Bauer et al., 2014; Baumeister et al., 2014).

The microglial cells are responsible for the activation of immune responses in the central nervous system. These cells release cytokines, which are inflammatory mediators, responsible for the modulation of inflammatory signaling (Mallucci et al., 2015; Kang et al., 2015). Previous studies showed alterations in the levels of inflammatory mediators, such as interleukin (IL) 1β, IL-6, IL-18, IL-23, soluble tumor necrosis factor receptor 1 (sTNF-R1), and tumor necrosis factor α (TNF-α), in BD patients (Barbosa et al., 2014; Munkholm et al., 2015; Monfrim et al., 2014; Pae et al., 2004).

The administration of psychostimulants in rats, such as amphetamines (amphs), is a suitable animal model of mania. Amphs induce some behavioral and physiological alterations similar to a manic episode of BD, such as hyperactivity and changes in dopamine and cytokine levels (Pinsonneault et al., 2011; Valvassori et al., 2017; Valvassori et al., 2018). Therefore, the present study aimed to evaluate the effects of the coadministration of Li and Cel on inflammatory parameters in an animal model of mania induced by amph.

2. Materials and methods

2.1. Animals

In the present study, adult male Wistar rats, approximately 60 days old (body weight 250–350 g), from the colony of *Universidade do Extremo Sul Catarinense* (UNESC) were used. The rats were housed with five animals per cage and had *ad libitum* access to water and standard commercial chow. A light/dark cycle (12:12 h; lights on at 7:00 h) and constant temperature (22 ± 1 °C) was maintained in the colony room. All experimental procedures were carried out following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC). The local ethics committee approved this study (*Comissão de Etica no Uso de Animais da Universidade do Extremo Sul Catarinense*, protocol number 056/2015-2). It is important to emphasize that all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

The dosage of drugs used here was based on previous studies (Frey et al., 2006a, 2006b; Kaizaki et al., 2013). Dextroamphetamine (D-amph) was purchased from Sigma-Aldrich (Sigma ®), whereas Li was purchased from Eurofarma (São Paulo city, São Paulo, Brazil) and Cel was purchased from Getz Pharma (Karachi, Pakistan). D-amph was dissolved in saline solution of NaCl 0.9% (Sal). Li and Cel were dissolved in water.

2.3. Experimental design

The animals received D-amph (2 mg/kg, 1 ml/kg) or Sal (NaCl 0.9%) intraperitoneally once a day for 14 days. From day 8–14, the animals from the D-amph and Sal groups received Li (24 mg/kg, 1 ml/kg), Cel (20 mg/kg, 1 ml/kg), Li + Cel or water *via* gavage. Li was administered twice a day (12:12 h), whereas Cel was only administered once a day. At day 15, the rats received an i.p. injection of D-amph or Sal and, 2 h later, were submitted to behavioral analysis. The experimental groups (n = 10 animals per group) in the present study were as follows: 1) Sal + water; 2) Sal + Li; 3) Sal + Cel; 4) Sal + Li + Cel; 5) D-amph + water; 6) D-amph + Li; 7) D-amph + Cel; and 8) D-amph + Li + Cel.

2.4. Behavioral analysis

Behavioral status was evaluated with the open-field test. In this procedure, the hyperactivity induced in the animal model is a parameter that is associated with the symptoms of BD mania (Nestler and Hyman, 2010). The open-field test was carried out in a 40 × 60 cm box, whose 50 cm high walls were made of white fiberboard, except the front wall, which was made of glass. The floor of the box had nine equal squares separated by black lines. The animals were gently placed on the left posterior square and were allowed to freely explore the area for 5 min. During the procedure, the number of crossings and rearings provides relevant information on the rat locomotor (crossings) and exploratory (rearings) activities (Broadhurst, 1960).

2.5. Sample preparation

The rats were sacrificed by decapitation immediately after the open-field test. The frontal cortex and striatum were dissected from the brains of the rats. Peripheral blood samples were immediately collected after decapitation and centrifuged at 10,000 rpm for 15 min to obtain serum. Until the analyses were performed, all samples were stored at −80 °C.

2.6. Assessment of the IL-1β, IL-4, IL-10, and TNF-α levels

The cytokine levels were evaluated in the frontal cortex and striatum, which were homogenized in extraction solution containing aprotinin (100 mg of tissue per 1 mL). The cytokine concentrations were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) following the instructions supplied by the manufacturer (DuoSet kits, R&D Systems; Minneapolis). The results are expressed as pg/100 mg of tissue. Total protein was measured with an adapted version of the method described by Lowry et al. (1951), using bovine serum albumin as a standard.

2.7. 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 data are expressed as the mean ± standard error of mean (S.E.) and were analyzed by three-way ANOVA followed by Tukey's test, if necessary. The Statistica 7® software was used to perform all analyses. Differences between groups were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Behavioral analyses

The effects of treatment with Li and Cel on the behavioral parameters in rats in which an animal model of mania induced by D-amph are shown in Fig. 1. Compared to those of the controls, the administration of D-amph increased the number of crossings (Fig. 1A) and rearings (Fig. 1B), and the coadministration of Li plus Cel reversed all behavioral alterations that were induced by D-amph. The administration of Cel or Li in the D-amph groups did not alter the behavioral changes induced by D-amph. The administration of Li, Cel, or Li plus Cel did not change the behavior of the saline-pretreated animals compared to those of the controls. It is important to note that the dose of Li used in the present study was half of the therapeutic dose.

Data from three-way ANOVA revealed significant effects of D-amph administration [crossing: $F(1,80) = 18.84$, $p < 0.001$; rearing: $F(1,80) = 14.38$, $p < 0.001$], treatment [crossing: $F(1,80) = 5.50$,

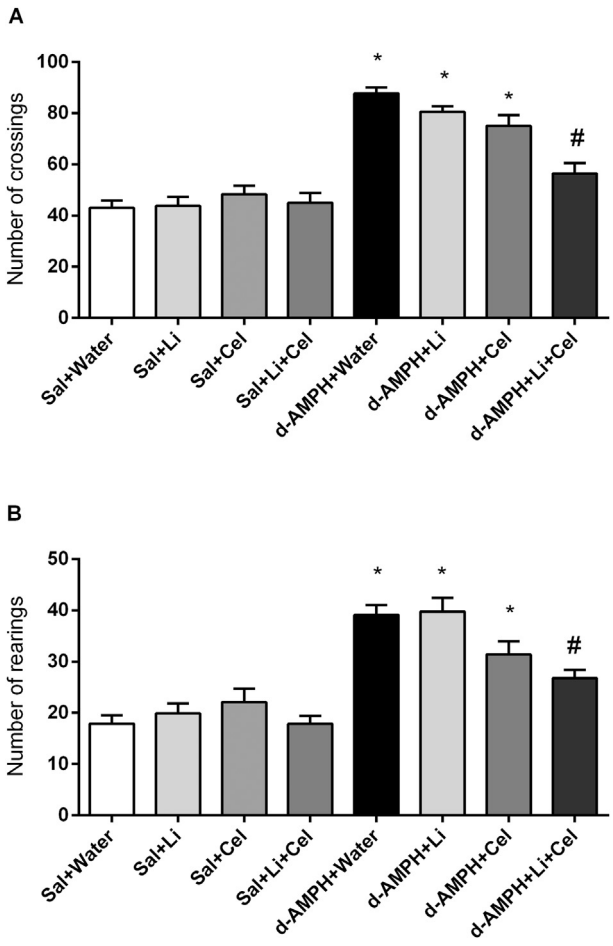

Fig. 1. Effects of Li and Cel administration on the number of crossings (A) and rearings (B) in an animal model of BD induced by D-amph (n = 10 per group). Data were analyzed by three-way ANOVA followed by the Tukey test when F was significant. Values are expressed as the mean ± S.E. *p < 0.05 compared to the Sal group. #p < 0.05 compared to the D-amph group.

p = 0.02; rearing: F(1,80) = 0.08, p = 0.77], D-amph administration × treatment interaction [crossing: F(1,80) = 2.44, p = 0.12; rearing: F(1,80) = 3.63, p = 0.06] and D-amph administration × Cel plus Li interaction [crossing: F(1,80) = 0.55, p = 0.46; rearing: F(1,80) = 0.02, p = 0.88].

3.2. Levels of IL-1β

As shown in Fig. 2, the administration of D-amph and all treatments did not have significant effects on the levels of IL-1β in the serum (Fig. 2A), frontal cortex (Fig. 2B) and striatum (Fig. 2C) of rats compared to those of the controls.

Data from three-way ANOVA revealed no significant effects of D-amph administration [serum: F(1,32) = 7.48, p < 0.05; frontal cortex: F(1,88) = 0.15, p = 0.70; striatum: F(1,38) = 0.14, p = 0.70], treatment [serum: F(1,32) = 0.34, p = 0.56; frontal cortex: F(1,38) = 0.004, p = 0.94; striatum: F(1,38) = 0.07, p = 0.79], D-amph administration × treatment interaction [serum: F(1,32) = 0.13, p = 0.72; frontal cortex: F(1,38) = 0.24, p = 0.62; striatum: F(1,38) = 1.29, p = 0.26] and D-amph administration × Cel plus Li interaction [serum: F(1,32) = 3.22, p = 0.08; frontal cortex: F(1,38) = 0.00, p = 1.00; striatum: F(1,38) = 2.37, p = 0.13].

3.3. Levels of IL-4

The levels of IL-4 are shown in Fig. 3. The administration of D-amph

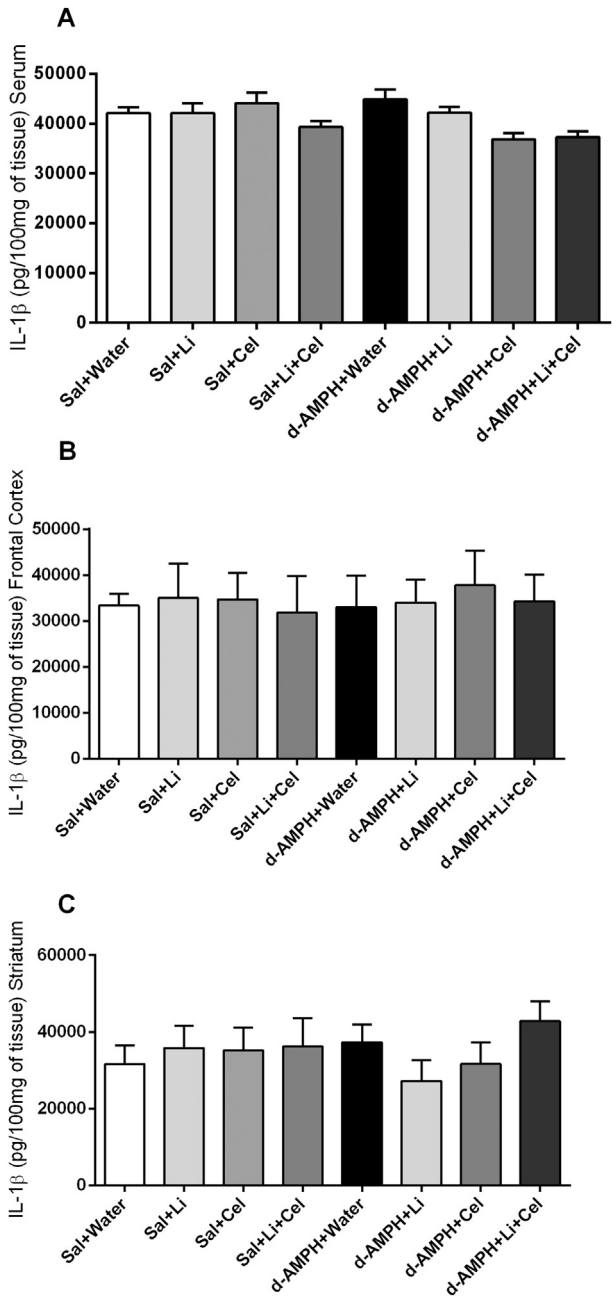

Fig. 2. Effects of the administration of Li and Cel on the levels of IL-1β in the serum (A), frontal cortex (B) and striatum (C) in an animal model of BD induced by D-amph (n = 10 per group). Data were analyzed by three-way ANOVA followed by the Tukey test when F was significant. Values are expressed as the mean ± S.E.

increased the IL-4 levels in the serum (Fig. 3A), frontal cortex (Fig. 3B) and striatum (Fig. 3C) when compared to those of the control group. The treatment with Li or Cel did not alter the D-amph-induced IL-4 increase. However, the administration of Li plus Cel reversed the increases in the IL-4 levels induced by D-amph in both brain structures and the serum of rats.

Data from three-way ANOVA revealed significant effects of D-amph administration [serum: F(1,38) = 10.62, p < 0.05; frontal cortex: F(1,38) = 7.53, p < 0.05; striatum: F(1,38) = 10.38, p < 0.05], treatment [serum: F(1,38) = 8.57, p < 0.05; frontal cortex: F(1,38) = 7.53, p < 0.05; striatum: F(1,38) = 3.55, p = 0.07], D-amph administration × treatment interaction [serum: F(1,38) = 1.52, p = 0.22; frontal cortex: F(1,38) = 1.21, p = 0.28; striatum: F(1,38) = 0.94, p = 0.33]

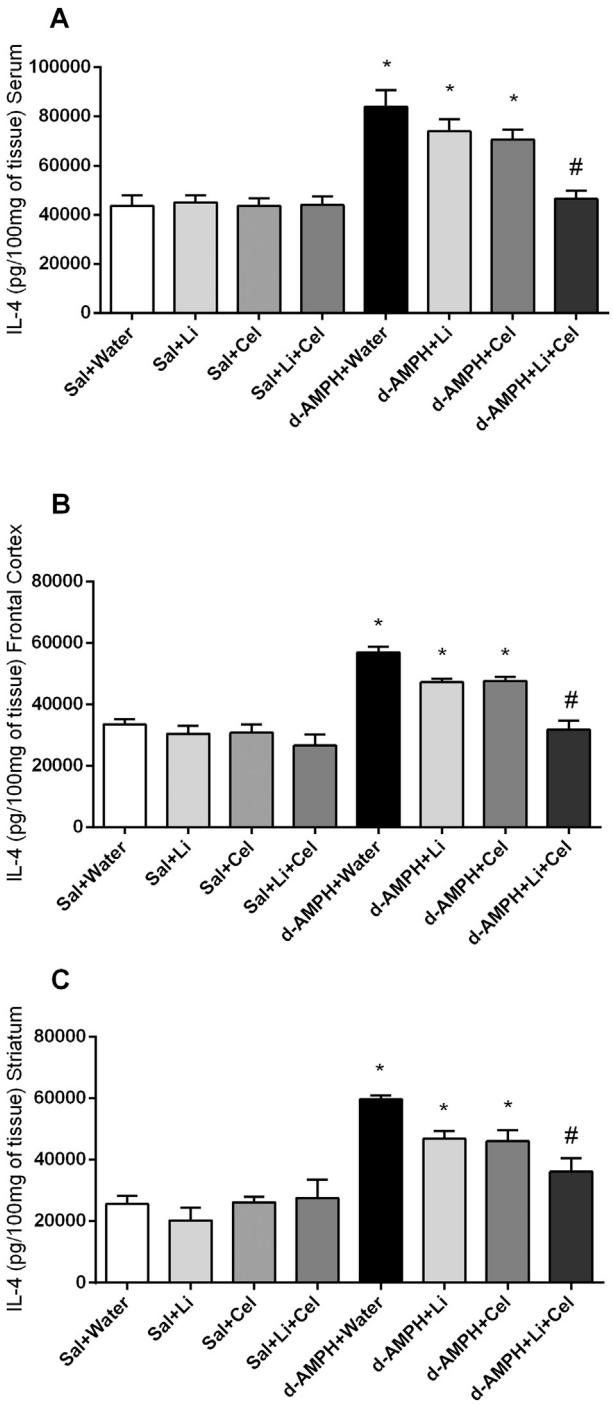

Fig. 3. Effects of the administration of Li and Cel on the levels of IL-4 in the serum (A), frontal cortex (B) and striatum (C) in an animal model of BD induced by D-amph (n = 10 per group). Data were analyzed by three-way ANOVA followed by the Tukey test when F was significant. Values are expressed as the mean ± S.E. *p < 0.05 compared to the Sal group. #p < 0.05 compared to the D-amph group.

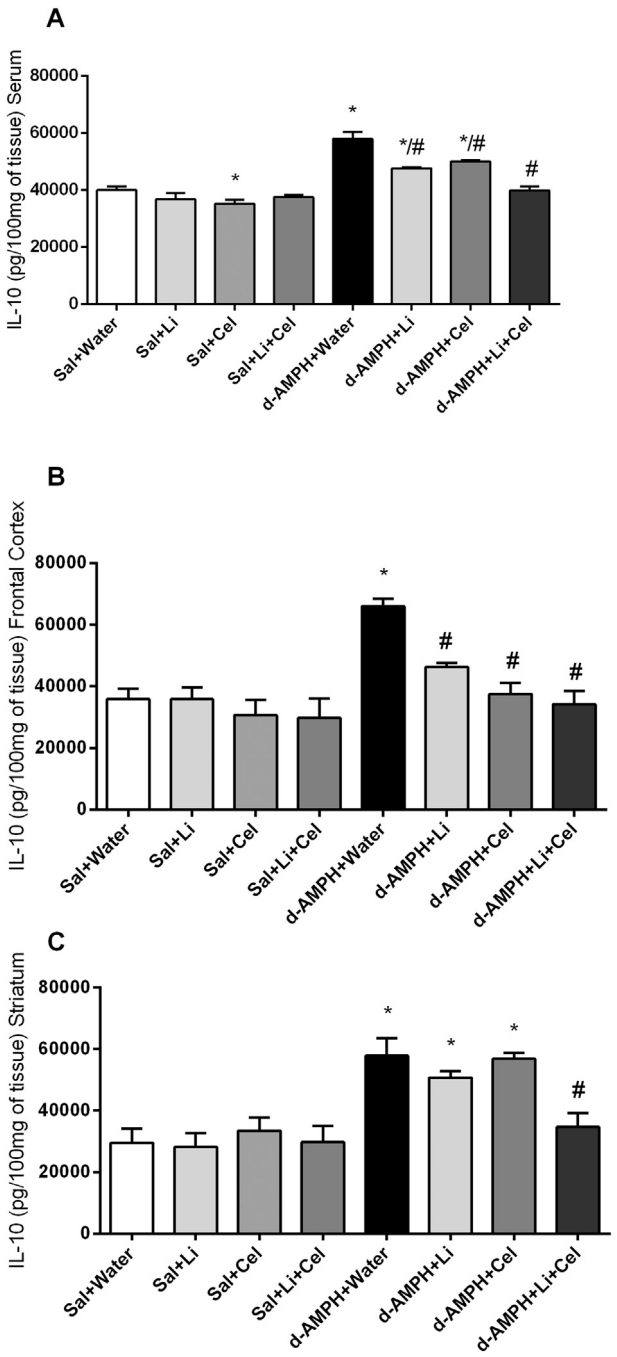

Fig. 4. Effects of the administration of Li and Cel on the levels of IL-10 in the serum (A), frontal cortex (B) and striatum (C) in an animal model of BD induced by D-amph (n = 10 per group). Data were analyzed by three-way ANOVA followed by the Tukey test when F was significant. Values are expressed as the mean ± S.E. *p < 0.05 compared to the Sal group. #p < 0.05 compared to the D-amph group.

and D-amph administration × Cel plus Li interaction [serum: F(1,38) = 1.19, p = 0.28; frontal cortex: F(1,38) = 0.57, p = 0.45; striatum: F(1,38) = 0.14, p = 0.71].

3.4. Levels of IL-10

The IL-10 levels are shown in Fig. 4. The administration of D-amph induced increases in the IL-10 levels in the serum (Fig. 4A), frontal cortex (Fig. 4B) and striatum (Fig. 4C) compared to those of the controls. The administration of Li or Cel alone partially reversed the IL-10 increase induced by D-amph in the serum of rats. The Li plus Cel treatment reversed the increase in IL-10 in the serum of rats. The administration of Cel *per se* decreased the IL-10 levels in the serum compared to the controls. In the frontal cortex, treatment with Li or Cel or Li plus Cel reversed the increases in the IL-10 levels induced by D-amph. In the striatum, the administration of Li or Cel alone did not alter the D-amph-induced IL-10 increase. However, the administration of Li plus Cel reversed the D-AMPH-induced IL-10 increase.

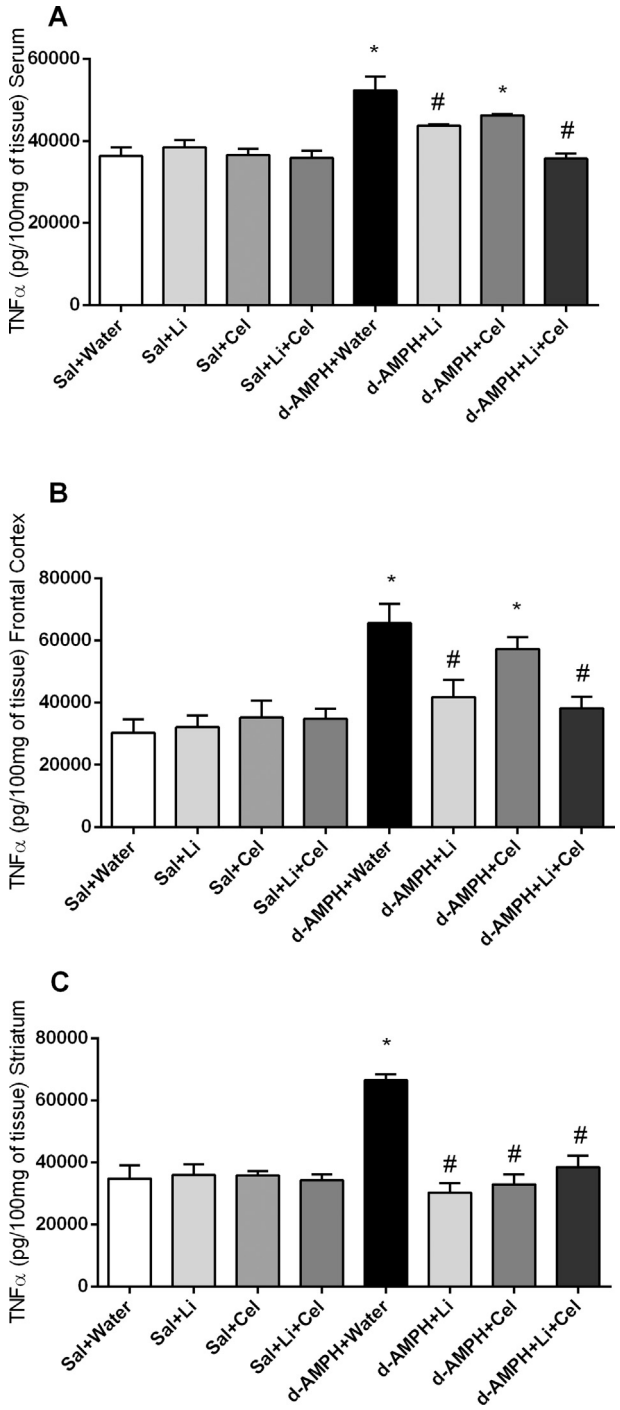

Fig. 5. Effects of the administration of Li and Cel on the levels of TNF-α in the serum (A), frontal cortex (B) and striatum (C) in animals submitted to an animal model induced by D-amph (n = 10 per group). Data were analyzed by three-way ANOVA followed by the Tukey test when F was significant. Values are expressed as the mean ± S.E. *p < 0.05 compared to the Sal group. #p < 0.05 compared to the D-amph group.

Data from three-way ANOVA revealed significant effects of D-amph administration [serum: F(1,32) = 7.29, p < 0.01; frontal cortex: F (1,38) = 6.94, p < 0.05; striatum: F(1,38) = 3.51, p = 0.07], treatment [serum: F(1,32) = 22.20, p < 0.001; frontal cortex: F (1,38) = 3.91, p = 0.055; striatum: F(1,38) = 4.05, p = 0.051], D-amph administration × treatment interaction [serum: F(1,32) = 1.91, p = 0.18; frontal cortex: F(1,38) = 1.90, p = 0.18; striatum: F (1,38) = 2.06, p = 0.16] and D-amph administration × Cel plus Li interaction [serum: F(1,32) = 1.65, p = 0.21; frontal cortex: F (1,38) = 2.37, p = 0.13; striatum: F(1,38) = 1.12, p = 0.30].

3.5. Levels of TNFα

Fig. 5 shows the TNFα levels. The administration of D-amph induced an increase in TNFα levels in the serum (5A), frontal cortex (5B), and striatum (5C) compared to the controls. Treatment with Li reversed the D-amph-induced increase in TNF-α levels in the serum, frontal cortex, and striatum of rats. The treatment with Cel alone did not change the D-amph-induced TNFα levels increased in the serum and frontal cortex but reversed this alteration in the striatum. The coadministration of Li and Cel reversed the increase in TNFα levels induced by D-amph in the serum, frontal cortex, and striatum of rats.

Data from three-way ANOVA revealed significant effects of D-amph administration [serum: F(1,32) = 5.50, p < 0.05; frontal cortex: F (1,38) = 2.12, p = 0.15; striatum: F(1,38) = 8.26, p < 0.01], treatment [serum: F(1,32) = 16.34, p < 0.001; frontal cortex: F (1,38) = 11.16, p < 0.01; striatum: F(1,38) = 12.51, p < 0.01], D-amph administration × treatment interaction [serum: F(1,32) = 0.86, p = 0.36; frontal cortex: F(1,38) = 0.04, p = 0.84; striatum: F (1,38) = 20.64, p < 0.001] and D-amph administration × Cel plus Li interaction [serum: F(1,32) = 0.03, p = 0.86; frontal cortex: F (1,38) = 0.28, p = 0.60; striatum: F(1,38) = 27.11, p < 0.001].

4. Discussion

According to the present results and previous studies, D-amph administration leads to behavioral hyperactivity (Frey et al., 2006a, 2006b; Valvassori et al., 2010; Valvassori et al., 2015). The results of the present study showed that the administration of D-amph induced hyperactivity in rats, which can be considered a manic-like behavior, reinforcing the validity of this animal model. The treatment with a therapeutic dose of Li (47.5 mg/kg) reverses the effects of the administration of D-amph on the manic-like behaviors in rats (Varela et al., 2013; Valvassori et al., 2015). Based on that, the authors did not evaluate the therapeutic dose of Li. Furthermore, treatment with Li at half of the therapeutic dose, 24 mg/kg, did not alter the manic-like behavior induced by D-amph. The authors opted to use a half dose of Li to observe the effects of this dose *per se* and its association with Cel. In addition, treatment with Cel alone did not affect the manic-like behavior induced by D-amph. The authors opt by 20 mg/kg of Cel based in previous results, which showed that this dose has significant effects on the behavioral and inflammation parameters in rats (Kaizaki et al., 2013). However, the association between Cel and Li at 24 mg/kg reversed the manic-like behavior.

The possible role of anti-inflammatory agents used as an adjuvant in BD treatment has been studied previously. Borre et al. (2012) demonstrated that treatment for two weeks with Cel reversed hyperactivity in rats. A randomized clinical trial with placebo and Cel showed that BD patients who received this drug obtained a significant improvement in depressive symptoms after the first week of treatment (Nery et al., 2008). Furthermore, some studies have demonstrated that COX-2 inhibitors, such as Cel, have effects on the inflammatory markers and neurotransmitter systems involved in mood disorders (Casolini et al., 2002; Ross et al., 2002; Mirjany et al., 2002; Raison et al., 2006). Ross et al. (2002) showed that COX-2 inhibitors reversed the manic-like behavior induced by amph. It could be suggested that, based on the present study, Cel can act synergistically with Li to reverse the manic-like behavior induced by D-amph.

Thomas and Kuhn (2005) demonstrated that the use of amphs induces an increase in COX-2 expression, an essential inflammatory mediator in the striatum. Studies have shown that there is a rise in the TNFα and IL-6 levels associated with manic and depressive episodes (O'Brien et al., 2006; Hung et al., 2007; Ortiz-Dominguez et al., 2007). Some authors have found alterations in cytokine levels in the serum of

BD patients (Rapaport et al., 1999; Bauer et al., 2005; Leboyer et al., 2012). The present study also found an increase in cytokine levels in the serum of animals, mimicking the observations from clinical studies. Some studies have suggested that a decrease in synthesis or increases in dopamine levels can modulate microglial activation in the striatum of animals, suggesting that there is a relationship between D-amph administration and alterations in cytokine levels (LaVoie and Hastings, 1999; Thomas et al., 2008). Pro-inflammatory cytokines may activate the microglia, and thus leading to neuronal apoptosis, causing impairs in the mechanisms that modulate the emotional regulation and cognitive functions, which could result in cognitive dysfunctions and mood alterations (Rosenblat et al., 2014; Rosenblat, 2019).

It has been suggested that bipolar patients present dopaminergic signaling alterations and an increase in dopamine levels during manic episodes (Joyce et al., 1995; Vogel et al., 2004). Concomitantly with the manic-like behavior induced by D-amph, there was an increase in the levels of anti- and pro-inflammatory cytokines (IL-4, IL-10, and TNFα) in the serum, frontal cortex, and striatum of rats. Previous studies also have demonstrated that there is an increase in the levels of both anti- and pro-inflammatory cytokines (IL-4, IL-6, IL-10, and TNFα) in the serum, frontal cortex, and striatum of rats in an animal model of BD induced by D-amph (Valvassori et al., 2015; Valvassori et al., 2018). Furthermore, studies also demonstrated the increases in pro-inflammatory cytokines in structures such as frontal-cortex, hippocampus and striatum in animals that showed manic-like behavior, such as hyperlocomotion, hyperactivity and decreased anxious-like behavior in pharmacological, genetical and environmental animal model of mania (Valvassori et al., 2017; Valvassori et al., 2018; Han et al., 2018). Interestingly, although differences between methodologies induce the model, the manic-like behavior seems to be accompanied by alterations in the cytokine levels. Considering that these cytokines alterations could impair structures of the limbic system, involved in decision-making, social interaction, and other types of behavior, it may be the cause of mood alterations.

IL-1β and TNFα are pro-inflammatory interleukins released during the early stages of the inflammatory process (Oshiro and Morris, 1997; Braddock and Quinn, 2004). In this study, the BD animal model exhibited increases in the TNFα levels. However, IL-1β levels were not significantly different in any tissue evaluated. In contrast, IL-4 and IL-10 are anti-inflammatory interleukins (Curfs et al., 1997; Sommer and White, 2010). The administration of D-amph in the present study also increased the levels of these interleukins, mimicking the inflammatory aspects of BD patients. Previous studies have shown that D-amph administration increases the TNF-α, IL-4, IL-6, and IL-10 levels without changing the IL-1β levels (Valvassori et al., 2015; Valvassori et al., 2017). It has been shown that D-amph induces damage in the dopaminergic neurons, which can lead to microglial activation and interleukin release (Frey et al., 2006b; Yamamoto et al., 2010; Feier et al., 2012). Thomas and Kuhn (2005) showed that amph increases the striatal expression of COX-2, which is an inflammatory mediator. Therefore, in the present study, D-amph may have been involved in the inflammatory process due to damage to the dopaminergic system and COX-2 activation.

It is important taking into account that both anti- and pro-inflammatory cytokines were increased after chronic administration of D-amph. The maintenance of immune homeostasis is attributed to the balance of pro-inflammatory cytokines and anti-inflammatory cytokines. The increase of pro-inflammatory cytokines usually results in an increase of anti-inflammatory cytokines; however, the increase of anti-inflammatory cytokines usually cannot fully compensate for the effect of pro-inflammatory cytokines (Nold et al., 2010). Therefore, a possible explanation for the increased D-amph-induced of both pro- and anti-inflammatory cytokines could be an alteration of the homeostasis of the immune system induced by D-amph.

In the present study, treatment with a COX-2 inhibitor alone, Cel, reversed the TNF-α increase induced by D-amph in the serum, frontal cortex, and striatum. Also, Cel alone decreased the D-amph-induced IL-10 increase in the frontal cortex and the serum of rats. A previous study demonstrated that the administration of 5 mg/kg Cel for five days did not reverse the increases in TNF-α in the brain that were induced by amph (El-Sayed El-Sisi et al., 2016). It is important to emphasize that Cel alone did not reverse the increases in the levels of IL-4 that are induced by D-amph in all tissues that were assessed (Cui et al., 2006). It is suggested that the therapeutic effects of Cel are dependent on the cerebral area and on the cytokines that are evaluated. It is important to emphasize that the different areas of brains have a specific population of neurons, synthesize different levels of neurotransmitters and has different signaling pathway, depending on its function (Valenzuela et al., 2011). Therefore, the differences in the effects of Li or Cel among brain area on cytokines levels could be explained by the heterogeneity among different brain structures.

In this study, Li (24 mg/kg) reversed the D-amph-induced increases in the TNF-α levels in all tissues that were assessed. Furthermore, Li reversed the IL-10 levels in the frontal cortex and partially reduced the levels in the serum. Previous studies have shown that treatment with Li reduces the production of TNFα and increases the release of IL-4 and IL-10 (Yuskaitis and Jope, 2009; Himmerich et al., 2013; Rapaport and Manji, 2001; Al-Azemi et al., 2010; Liu et al., 2011; Green and Nolan, 2012). A previous study demonstrated that Li (47.5 mg/kg) reversed the increases in the IL-4, IL-6, IL-10 and TNF-α levels in the brain and serum of rats (Valvassori et al., 2015). In addition, Li inhibit and decrease COX-2 expression, TNF-α, and IL-1β synthesis (Himmerich et al., 2013; Wang et al., 2013; Zheng et al., 2017). Furthermore, Li also increases the synthesis of IL-2 and IL-10 (Himmerich et al., 2013). Therefore, it is evident that the anti-inflammatory role of Li is involved in its therapeutic effect as a mood stabilizer. In the present study, the combined treatment with Li and Cel was more effective on reversing the alterations in the cytokine levels induced by D-amph, reversing the increases in TNF-α, IL-4, and IL-10 in all tissues that were evaluated. As previously described, Li and Cel have modulatory effects on interleukins (Wang et al., 2013; El-Sayed El-Sisi et al., 2016). Therefore, it may be suggested that by acting together, the two drugs potentiated their anti-inflammatory effects.

It is important to emphasize that, in the present study, Li and Cel *per se* inhibited amph-increased TNFα, which is a pro-inflammatory cytokine. In the other hand, Li or Cel *per se* did not attenuate amph-induced hyperactivity. From these two observations, it can be suggested that the inhibition of pro-inflammatory cytokines is not directly related to the anti-manic effects of Li or Cel. However, it is important to note that the activations of the immune system can activate the signaling cell death pathway, impairing the cerebral function and accelerate the progression of the disorder (Lotrich et al., 2014, Green, 2006; Bauer et al., 2014). Besides the maintenance of symptoms, the treatment of BD should include cerebral protection.

It is described some triggers of inflammation in BD, such as epigenetic factors, microglial activation, environmental stressors, and others (Fries et al., 2019). However, studies suggested that the temporal relationship between BD and inflammation is bidirectional (Perugi et al., 2014). BD may predate inflammatory comorbidity, or the inflammatory comorbidity may start before the start of BD symptoms, or both may start at the same time (Rosenblat and McIntyre, 2016). The inflammatory process and high levels of cytokines can impair the health of BD patients with progressive worsening of cognitive dysfunction and functional impairment (Lotrich et al., 2014, Green, 2006; Bauer et al., 2014). Therefore, it is suggested that the treatment of neuroinflammation aims to reduce the neuroprogression and provide a better quality of life to BD patients.

5. Conclusion

The search for more effective treatments for BD is ongoing. Treatment with Li plus Cel was effective in reducing the inflammation

induced by D-amph in rats. Therefore, this data may suggest that the inhibition of COX-2 may be a therapeutic strategy for BD treatment. However, more studies are necessary to elucidate these mechanisms better.

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References

Al-Azemi, M., Omu, F.E., Kehinde, E.O., Anim, J.T., Oriowo, M.A., Omu, A.E., 2010. Lithium protects against toxic effects of cadmium in the rat testes. J. Assisted Reprod. Genet. 27, 469–476.

Diagnostic and Statistical Manual of Mental Disorders (DSM5). In: American Psychiatric Association, Psychiatric Association, American (Eds.), Bipolar Disorder and Relationship, 5th ed. American Psychiatric Press, Washington, DC, pp. 123–155.

Arabzadeh, S., Ameli, N., Zeinoddini, A., Rezaei, F., Farokhnia, M., Mohammadinejad, P., Ghaleiha, A., Akhondzadeh, S., 2015. Celecoxib adjunctive therapy for acute bipolar mania: a randomized, double-blind, placebo-controlled trial. Bipolar Disord. 17, 606–614.

Barbosa, I.G., Bauer, M.E., Machado-Vieira, R., Teixeira, A.L., 2014. Cytokines in bipolar disorder: paving the way for neuroprogression. Neural Plast 2014, 360481.

Bauer, M.S., Altshuler, L., Evans, D.R., Beresford, T., Williford, W.O., Hauger, R., 2005. Prevalence and distinct correlates of anxiety, substance, and combined comorbidity in a multi-site public sector sample with bipolar disorder. J. Affect. Disord. 85, 1–15.

Bauer, I.E., Pascoe, M.C., Wollenhaupt-Aguiar, B., Kapczinski, F., Soares, J.C., 2014. Inflammatory mediators of cognitive impairment in bipolar disorder. J. Psychiatr. Res. 56, 18–27.

Baumeister, D., Russell, A., Pariante, C.M., Mondelli, V., 2014. Inflammatory biomarker profiles of mental disorders and their relation to clinical, social and lifestyle factors. Soc Psiquiatria Psychiatr Epidemiol 49 (6), 841–849.

Borre, Y., Lemstra, S., Westphal, K.G., Morgan, M.E., Olivier, B., Oosting, R.S., 2012. Celecoxib delays cognitive decline in an animal model of neurodegeneration. Behav. Brain Res. 234 (2), 285–291.

Braddock, M., Quinn, A., 2004. Targeting IL-1 in inflammatory disease: new opportunities for therapeutic intervention. Nat. Rev. Drug Discov. (4), 330–339.

Broadhurst, P.L., 1960. Experiments in psychogenetics. In: Eisenk, H.J. (Ed.), Experiments in Personality. Am J Psychiatry. vol. 1. pp. 31–71.

Casolini, P., Catalani, A., Zuena, A.R., Angelucci, L., Cui, X., Yang, S.C., Sharma, S., Heuze-Vourc'h, N., Dubinett, S.M., 2002. Inhibition of COX-2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat. J. Neurosci. Res. 68 (3), 337–343.IL-4 regulates COX-2 and PGE2 production in human non-small cell lung cancer. Biochem. Biophys. Res. Commun. 343 (4), 995–1001.

Cui, C., Sakata-Haga, H., Ohta, K., Nishida, M., Yashiki, M., Sawada, K., Fukui, Y., 2006. Histological brain alterations following prenatal methamphetamine exposure in rats. Congenit. Anom. (Kyoto). 46 (4), 180–187.

Curfs, J.H., Meis, J.F., Hoogkamp-Korstanje, J.A., 1997. A primer on cytokines: sources, receptors, effects, and inducers. Clin. Microbiol. Rev. (4), 742–780.

El-Sayed El-Sisi, A., Sokkar, S.S., El-Sayed El-Sayad, M., Sayed Ramadan, E., Osman, E.Y., 2016. Celecoxib and omega-3 fatty acids alone and in combination with risperidone affect the behavior and brain biochemistry in amphetamine-induced model of schizophrenia. Biomed. Pharmacother. 82, 425–431.

Feier, G., Valvassori, S.S., Lopes-Borges, J., Varela, R.B., Bavaresco, D., Scaini, G., Morais, M.O., Andersen, M.L., Streck, E.L., Quevedo, J., 2012. Behavioral changes and brain energy metabolism dysfunction in rats treated with methamphetamine or dextroamphetamine. Neurosci. Lett. 530, 75–79.

Frey, B.N., Valvassori, S.S., Réus, G.Z., Martins, M.R., Petronilho, F.C., Bardini, K., Dal-Pizzol, F., Kapczinski, F., Quevedo, J., 2006a. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J. Psychiatry Neurosci. 31 (5), 326–332 Sep.

Frey, B.N., Martins, M.R., Petronilho, F.C., Dal-Pizzol, F., Quevedo, J., Kapczinski, F., 2006b. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bipolar Disord. 8, 275–280.

Fries, G.R., Walss-Bass, C., Bauer, M.E., Teixeira, A.L., 2019. Revisiting inflammation in bipolar disorder. Pharmacol. Biochem. Behav. 177, 12–19 Feb.

Geddes, J.R., Miklowitz, D.J., 2013. Treatment of bipolar disorder. Lancet 381, 1672e82.

Green, M.F., 2006. Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. J Clin Psychiatry 67, 3–8 Suppl 9.

Green, H.F., Nolan, Y.M., 2012. GSK-3 mediates the release of IL-1β, TNF-α and IL-10 from cortical glia. Neurochem. Int. 61 (5), 666–671.

Haase, J., Brown, E., 2014. Integrating the monoamine, neurotrophin and cytokine hypotheses of depression—a central role for the serotonin transporter. Pharmacol. Ther. 147, 1–11.

Han, L., Wang, L., Tang, S., Yuan, L., Wu, S., Du, X., Xiang, Y., Qu, X., Liu, H., Luo, H., Qin, X., Liu, C., 2018. ITGB4 deficiency in bronchial epithelial cells directs airway inflammation and bipolar disorder-related behavior. J. Neuroinflammation 15 (1), 246 Aug 31.

Himmerich, H., Bartsch, S., Hamer, H., Mergl, R., Schönherr, J., Petersein, C., Munzer, A., Kirkby, K.C., Bauer, K., Sack, U., 2013. Impact of mood stabilizers and antiepileptic drugs on cytokine production in-vitro. J. Psychiatr. Res. 47, 1751–1759.

Hung, Y.J., Hsieh, C.H., Chen, Y.J., Pei, D., Kuo, S.W., Shen, D.C., Scheu, W.H., Chen, Y.C., 2007. Insulin sensitivity, proinflammatory markers and adiponectin in young males with different subtypes of depressive disorder. Clin. Endocrinol. 67, 784–789.

Joyce, P.R., Fergusson, D.M., Woollard, G., Abbott, R.M., Horwood, L.J., Upton, J., 1995. Urinary catecholamines and plasma hormones predict mood state in rapid cycling bipolar affective disorder. J. Affect. Disord. 33 (4), 233–243 Apr 4.

Kaizaki, A., Tien, L.T., Pang, Y., Cai, Z., Tanaka, S., Numazawa, S., Bhatt, A.J., Fan, L.W., 2013. Celecoxib reduces brain dopaminergic neuronaldysfunction, and improves sensorimotor behavioral performance in neonatal rats exposed to systemic lipopolysaccharide. J. Neuroinflammation Apr 5 (10), 45.

Kang, J.W., Kim, S.J., Cho, H.I., Lee, S.M., 2015. DAMPs activating innate immune responses in sepsis. Ageing Res. Rev. 24 (Pt A), 54–65.

Keck, P.E., McElroy, S.L., Arnold, L.M., 2001. Bipolar disorder. Med Clin North Am 85 (3), 645–661.

LaVoie, M.J., Hastings, T.G., 1999. Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine. J. Neurosci. 19 (4), 1484–1491.

Leboyer, M., Soreca, I., Scott, J., Frye, M., Henry, C., Tamouza, R., Kupfer, D.J., 2012. Can bipolar disorder be viewed as a multi-system inflammatory disease? J. Affect. Disord. 141 (1), 1–10.

Liu, K.J., Lee, Y.L., Yang, Y.Y., Shih, N.Y., Ho, C.C., Wu, Y.C., Huang, T.S., Huang, M.C., Liu, H.C., Shen, W.W., Leu, S.J., 2011. Modulation of the development of human monocyte-derived dendritic cells by lithium chloride. J. Cell. Physiol. 226, 424 – 433.

Lotrich, F.E., Butters, M.A., Aizenstein, H., Marron, M.M., Reynolds 3rd, C.F., Gildengers, A.G., 2014. The relationship between interleukin-1 receptor antagonist and cognitive function in older adults with bipolar disorder. Int J Geriatr Psychiatry 29, 635–644.

Lowry, O.H., Rosebrough, N.J., Farr, A., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.

Mallucci, G., Peruzzotti-Jametti, L., Bernstock, J.D., Pluchino, S., 2015. The role of immune cells, glia and neurons in withe and gray matter pathology in multiple sclerosis. Prog. Neurobiol. 127-128C, 1–22.

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.

Mirjany, M., Ho, L., Pasinetti, G.M., 2002. Role of cyclooxygenase-2 in neuronal cell cycle activity and glutamate-mediated excitotoxicity. J. Pharmacol. Exp. Ther. 301 (2), 494–500.

Mitchell, R.H., Goldstein, BI., 2014. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. J. Am. Acad. Child Adolesc. Psychiatry 53 (3), 274–296.

Monfrim, X., Gazal, M., De Leon, P.B., Quevedo, L., Souza, L.D., Jansen, K., Oses, J.P., Pinheiro, R.T., Silva, R.A., Lara, D.R., Ghisleni, G., Spessato, B., Kaster, M.P., 2014. Immune dysfunction in bipolar disorder and suicide risk: is there an association between peripheral corticotropin-releasing hormone and interleukin-1β? Bipolar Disord. 16 (7), 741–747.

Mousavi, S.Y., Khezri, R., Karkhaneh-Yousefi, M.A., Mohammadinejad, P., Gholamian, F., Mohammadi, M.R., Zeinoddini, A., Akhondzadeh, S., 2017. A randomized, double-blind placebo-controlled trial on effectiveness and safety of celecoxib adjunctive therapy in adolescents with acute bipolar mania. J Child Adolesc Psychopharmacol 27 (6), 494–500 Aug.

Munkholm, K., Weikop, P., Kessing, L.V., Vinberg, M., 2015. Elevated levels of IL-6 and IL-18 in manic and hypomanic states in rapid cycling bipolar disorder patients. Brain Behav. Immun. 43, 205–213.

Nery, F.G., Monkul, E.S., Hatch, J.P., Fonseca, M., Zunta-Soares, G.B., Frey, B.N., Bowden, C.L., Soares, J.C., 2008. Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. Hum Psychopharmacol 23 (2), 87–94.

Nestler, E.J., Hyman, S.E., 2010. Animal models of neuropsychiatric disorders. Nat. Neurosci. 13 (10), 1161–1169 Oct.

Nold, M.F., Nold-Petry, C.A., Zepp, J.A., Palmer, B.E., Bufler, P., Dinarello, C.A., 2010. IL-37 is a fundamental inhibitor of innate immunity. Nat. Immunol. 11, 1014–1022.

O'Brien, S.M., Scully, P., Scott, L.V., Dinan, T.G., 2006. Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. J Affect Dis 90, 263–267.

Ortiz-Dominguez, A., Hernandez, E., Berlanga, C., Gutiérrez-Mora, D., Moreno, J., Heinze, G., Pávon, L., 2007. Immune variations in bipolar disorder: phasic differences. Bipolar Disord. 9, 596–602.

Oshiro, Y., Morris, D.L., 1997. TNF-alpha release from human peripheral blood mononuclear cells to predict the proinflammatory activity of cytokines and growth factors. J. Pharmacol. Toxicol. Methods 37 (1), 55–59.

Pae, C.U., Lee, K.U., Han, H., Serretti, A., Jun, T.Y., 2004. Tumor necrosis factor alpha gene-G308A polymorphism associated with bipolar I disorder in the Korean population. Psychiatry Res. 125 (1), 65–68.

Perugi, G., Quaranta, G., Belletti, S., Casalini, F., Mosti, N., Toni, C., Dell'Osso, L., 2014. General medical conditions in 347 bipolar disorder patients: clinical correlates of metabolic and autoimmune-allergic diseases. J. Affect. Disord. 170C, 95–103.

Pinsonneault, J.K., Han, D.D., Burdick, K.E., Kataki, M., Bertolino, A., Malhotra, A.K., Gu, H.H., Sadee, W., 2011. Dopamine transporter gene variant affecting expression in

human brain is associated with bipolar disorder. Neuropsychopharmacology 36 (8), 1644–1655.
Raison, C.L., Capuron, L., Miller, A.H., Rapaport, M.H., Guylai, L., Whybrow, P., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27 (1), 24–31.Immune parameters in rapid cycling bipolar patients before and after lithium treatment. J. Psychiatr. Res. 33, 335 – 340.
Rapaport, M.H., Manji, H.K., 2001. The effects of lithium on ex vivo cytokine production. Biol. Psychiatry 50, 217–224.
Rapaport, M.H., Guylai, L., Whybrow, P., 1999. Immune parameters in rapid cycling bipolar patients before and after lithium treatment. J. Psychiatr. Res. 33 (4), 335–340.
Rosenblat, J.D., 2019. Targeting the immune system in the treatment of bipolar disorder. Psychopharmacology(Feb 13). https://doi.org/10.1007/s00213-019-5175-x.
Rosenblat, J.D., McIntyre, R.S., 2016. Bipolar disorder and inflammation. Psychiatr Clin North Am. 39 (1), 125–137 Mar.
Rosenblat, J.D., Cha, D.S., Mansur, R.B., McIntyre, R.S., 2014. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 53, 23–34.
Ross, B.M., Brooks, R.J., Lee, M., Kalasinsky, K.S., Vorce, S.P., Seeman, M., Fletcher, P.J., Turenne, S.D., 2002. Cyclooxygenase inhibitor modulation of dopamine-related behaviours. Eur. J. Pharmacol. 450 (2), 141–151.
Shi, S., Klotz, U., 2008. Clinical use and pharmacological properties of selective COX-2 inhibitors. Eur. J. Clin. Pharmacol. 64, 233–252.
Sommer, C., White, F., 2010. Cytokines, chemokines, and pain. In: Beaulieu, P., Lussier, D., Porreca, F. (Eds.), Pharmacology of Pain, 1st Ed. IASP Press, Seattle, pp. 279–302.
Thomas, D.M., Kuhn, DM., 2005. Cyclooxygenase-2 is an obligatory factor in methamphetamine-induced neurotoxicity. J. Pharmacol. Exp. Ther. 313 (3), 870–876.
Thomas, D.M., Francescutti-Verbeem, D.M., Kuhn, D.M., 2008. Methamphetamine-induced neurotoxicity and microglial activation are not mediated by fractalkine receptor signaling. J. Neurochem. 106 (2), 696–705.
Valenzuela, C.F., Puglia, M.P., Zucca, S., 2011. Focus on: neurotransmitter systems. Alcohol Res Health 34 (1), 106–120.
Valvassori, S.S., Rezin, G.T., Ferreira, C.L., Moretti, M., Gonçalves, C.L., Cardoso, M.R., Streck, E.L., Kapczinski, F., Quevedo, J., 2010. Effects of mood stabilizers on mitochondrial respiratory chain activity in brain of rats treated with d-amphetamine. J. Psychiatr. Res. 44 (14), 903–909.
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. Bipolar Disord. 17 (5), 507–517.
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., 2017. 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. Jun 19 (4), 246–258.
Valvassori, S.S., Resende, W.R., Varela, R.B., Arent, C.O., Gava, F.F., Peterle, B.R., Dal-Pont, G.C., Carvalho, A.F., Andersen, M.L., Quevedo, J., 2018. The effects of histone deacetylase inhibition on the levels of cerebral cytokines in an animal model of mania induced by dextroamphetamine. Mol. Neurobiol. 55 (2), 1430–1439 Feb.
Varela, R.B., Valvassori, S.S., Lopes-Borges, J., Fraga, D.B., Resende, W.R., Arent, C.O., Zugno, A.I., Quevedo, J., 2013. Evaluation of acetylcholinesterase in an animal model of mania induced by D-amphetamine. Psychiatry Res. 209 (2), 229–234.
Vogel, M., Pfeifer, S., Schaub, R.T., Grabe, H.J., Barnow, S., Freyberger, H.J., Cascorbi, I., 2004. Decreased levels of dopamine D3 receptor mRNA in schizophrenic and bipolar patients. Neuropsychobiology 50, 305–310.
Wang, H.M., Zhang, T., Li, Q., Huang, J.K., Chen, R.F., Sun, X.J., 2013. Inhibition of glycogen synthase kinase-3β by lithium chloride suppresses 6-hydroxydopamine-induced inflammatory response in primary cultured astrocytes. Neurochem. Int. 63, 345–353.
Yamamoto, B.K., Moszczynska, A., Gudelsky, G.A., 2010. Amphetamine toxicities: classical and emerging mechanisms. Ann. N. Y. Acad. Sci. 1187, 101–121.
Yuskaitis, C.J., Jope, R.S., 2009. Glycogen synthase kinase-3 regulates microglial migration, inflammation, and inflammation-induced neurotoxicity. Cell. Signal. 21, 264–273.
Zheng, J., Liu, Z., Li, W., Tang, J., Zhang, D., Tang, X., 2017. Lithium posttreatment confers neuroprotection through glycogen synthase kinase-3β inhibition in intracerebral hemorrhage rats. J. Neurosurg. 127 (4), 716–724 Oct.