Journal of Psychiatric Research 113 (2019) 181–189

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Tamoxifen has an anti-manic effect but not protect the brain against oxidative stress in an animal model of mania induced by ouabain

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ARTICLE INFO

Keywords:
Bipolar disorder
Tamoxifen
Mania
Oxidative stress
Ouabain
Lithium

ABSTRACT

Studies have suggested the involvement of oxidative stress in the physiopathology of bipolar disorder. Preclinical data have shown that PKC inhibitors may act as mood-stabilizing agents and protect the brain in animal models of mania. The present study aimed to evaluate the effects of Lithium (Li) or tamoxifen (TMX) on behavioral changes and oxidative stress parameters in an animal model of mania induced by ouabain (OUA). Wistar rats received a single intracerebroventricular (ICV) injection of OUA or artificial cerebrospinal fluid (ACSF). From the day following ICV injection, the rats were treated for seven days with intraperitoneal injections of saline, Li or TMX twice a day. On the 7th day after OUA injection, locomotor activity was measured using the open-field test, and the oxidative stress parameters were evaluated in the hippocampus and frontal cortex of rats. The results showed that OUA induced hyperactivity in rats, which is considered a manic-like behavior. Also, OUA increased lipid peroxidation and oxidative damage to proteins, as well as causing alterations to antioxidant enzymes in the frontal cortex and hippocampus of rats. The Li or TMX treatment reversed the manic-like behavior induced by OUA. Besides, Li, but not TMX, reversed the oxidative damage caused by OUA. These results suggest that the manic-like effects induced by OUA and the antimanic effects of TMX seem not to be related to the oxidative stress.

1. Introduction

Bipolar disorder (BD) is a severe psychiatric disorder associated with social and functional impairment. Although BD is characterized by the presence of manic, depressive and/or mixed episodes, the clinical hallmark of the diagnosis of BD is the presence of manic episodes (Ketter, 2010; Subramaniam et al., 2013). Previous studies have suggested that oxidative stress is involved in the pathophysiology of BD (Andreazza et al., 2013; Brown et al., 2014; Scola and Andreazza, 2014). Siwek et al (2016) have suggested that oxidative stress parameters increase in the acute phases of BD (mania/hypomania and depression). It is interesting that lithium (Li), considered a gold standard in the treatment of BD, decreases oxidative stress in bipolar patients (Banerjee et al., 2012; de Sousa et al., 2014) and brain from animals submitted to the models of mania (da-Rosa et al., 2012; Valvassori et al., 2015; Jornada et al., 2011).

In addition to protecting against oxidative stress, Li has several molecular targets. Protein kinase C (PKC) is a molecular target of Li that has interested researchers (Cechinel-Recco et al., 2012; Steckert et al., 2012; Armani et al., 2014). PKC plays an essential role in regulating neuronal excitability, neurotransmitter release, and long-term alterations in gene expression and plasticity (Calabrese and Halpain, 2005;

Abbreviations: 3-nitro, 3-nitrotyrosine; 4-HNE, 4-hydroxynonenal; 8-ISO, 8-isoprostane; ACSF, artificial cerebrospinal fluid; BD, bipolar disorder; CAT, catalase; ICV, intracerebroventricular; IP, intraperitoneal; Li, lithium; LPH, lipid peroxidation; OUA, ouabain; PKC, protein kinase C; SOD, superoxide dismutase; TMX, tamoxifen

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https://doi.org/10.1016/j.jpsychires.2019.03.020
Received 30 October 2018; Received in revised form 15 March 2019; Accepted 21 March 2019
0022-3956/ © 2019 Published by Elsevier Ltd.

Zarate and Manji, 2009). Clinical studies have demonstrated that tamoxifen (TMX), a PKC inhibitor, decreases manic symptoms in bipolar patients (Amrollahi et al., 2011; Yildiz et al., 2016; Talaei et al., 2016). Yildiz et al. (2016) found that TMX could treat mania while improving the levels of markers associated with brain energy metabolism. In the same study, the authors suggest the involvement of excessive PKC activation and impaired brain energy metabolism in bipolar mania. It is important to note that alterations in energy metabolism, as well as mitochondrial damage, are closely linked to cellular oxidative stress (Scaini et al., 2016; Cikankova et al., 2017).

In the same away, preclinical studies also have suggested the antimanic effects of TMX. Valvassori et al. (2014) have demonstrated that TMX reversed manic-like behaviors, while regulates alterations in energetic metabolism induced by amphetamine in rats. Besides, a previous study found that Li and TMX modulate behavioral changes, neurotrophic and apoptosis pathway induced by amphetamine in an animal model of mania (Cechinel-Recco et al., 2012). Steckert et al. (2012) also demonstrated that TMX reversed manic-like behaviors and protected the brain against oxidative stress induced by amphetamine in rats. Several data on the efficacy of TMX in reducing manic symptoms of BD suggest that this drug may have potential as a new treatment of BD mania (Armani et al., 2014). However, the antimanic effects of TMX have not been fully elucidating, and more studies are necessary for understanding the mechanisms of action of this drug.

Alterations in brain Na^+K^+-ATPase and cardiac steroids also have been demonstrated in BD, suggesting the hypothesis of their involvement in this disorder (el-Mallakh and Wyatt, 1995; Mynett-Johnson et al., 1998; Traub and Lichtstein, 2000). It is interesting that intracerebroventricular (ICV) administration of ouabain (OUA) in rats induces behaviors-like manic, such as hyperactivity, risk-taking behavior and stereotypic behavior (Lopes-Borges et al., 2015; Valvassori et al., 2017). Classical mood stabilizers and antipsychotics prevented and reversed the manic-like behaviors induced by OUA, mimicking the clinical treatment of mania (El-Mallakh et al., 2006; Jornada et al., 2011). Previous studies from our laboratory research have demonstrated that the OUA induces oxidative stress in brain of rats submitted to the animal model of mania, while Li reversed and prevented this condition (Jornada et al., 2011; Valvassori et al., 2015). Besides, a recent study has demonstrated that increased PKC activity is positively correlated with the manic-like behavior induced by OUA in rats. In the same study, the authors showed that Li and TMX modulate the manic-like behavior by reducing the PKC activity in the animal model of mania induced by OUA (Valvassori et al., 2017).

Therefore, the present study investigated the effects of TMX or Li on manic-like behavior and oxidative stress parameters [lipid peroxidation (LPH), 4-hydroxynonenal (HNE), 8-isoprostane (8-ISO), carbonyl, 3-nitrotyrosine (3-Nitro), superoxide dismutase (SOD) and catalase (CAT)] in an animal model of mania induced by OUA.

2. Material and methods

2.1. Animals

In the present study were used adult male Wistar rats, approximately 60 days old, from the breeding colony, maintained at the *Universidade do Extremo Sul Catarinense*. The animals were housed five per cage under controlled conditions of temperature (22 ± 1 °C), relative humidity (45–55%) and day/light cycle (12:12 h, light on at 06:00 h). Rat chow (standard diet for laboratory animals - NUVILAB CR-1®, Brazil) and tap water were available *ad libitum*. 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 Ética no Uso de Animais da Universidade do Extremo Sul Catarinense, protocol number: 073/2017–1. It is important to emphasize that all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Surgical procedure

Animals were anesthetized via intramuscular with ketamine (80 mg/kg) and xylazine (10 mg/kg). In a stereotaxic apparatus, the skin of the rat skull was removed and a 27-gauge 9 mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from the midline and 1.0 mm above the lateral brain ventricle. Through a 2-mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull and fixed with dental acrylic cement. Animals recovered from surgery within three days.

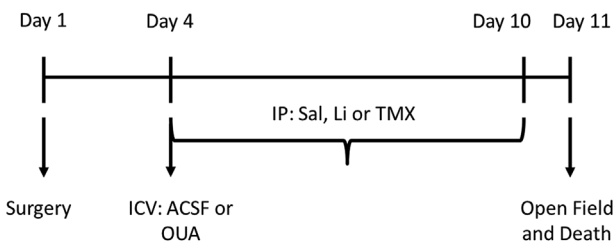
2.3. Experimental design

In this experimental model, we reproduced the treatment of acute manic episode according to previously proposed (Jornada et al., 2011). Animals (n = 48) received a single ICV injection of 5 µl of 10^{-3} M ouabain dissolved in artificial cerebrospinal fluid (ACSF) or 5 µl of ACSF alone on the fourth day following surgery (El-Mallakh et al., 1995; Jornada et al., 2011). A 30-gauge cannula was placed inside the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the cannula infusion protruded 1.0 mm beyond the cannula guide aiming the right lateral brain ventricle. From the day following the injection of ouabain or aCSF, the rats were treated for 7 days with intraperitoneal (IP) injections of saline, lithium or tamoxifen in 6 experimental groups of 8 animals per group: 1) ACSF ICV + saline IP (ACSF + Sal), 2) ACSF ICV + lithium IP (ACSF + Li), 3) ACSF ICV + tamoxifen IP (ACSF + TMX), 4) ouabain ICV + saline IP (OUA + Sal), 5) ouabain ICV + lithium IP (OUA + Li), 6) ouabain ICV + tamoxifen IP (OUA + TMX). Animals in the Li group received injections of lithium (47.5 mg/kg – 1 mL/kg), and in the TMX group received TMX (1 mg/kg – 1 mL/kg) twice a day (Valvassori et al., 2014). The animals were killed 24 h after the last injection of Li, TMX, or Sal (0.9% NaCl— 1 mL/kg) (see Scheme 1).

Note: In previous studies from our and others research groups (Einat et al., 2007; Jornada et al., 2011; Steckert et al., 2012; Valvassori et al., 2014), it can be observed that the doses of Li and TMX used in the present study have a significant effect against the manic-like behaviors and biochemical alterations induced in animal models of mania. Furthermore, the dose of Li and TMX used in the present study reversed the ouabain-induced increase in the phosphorylation and activity of PKC (Valvassori et al., 2017).

2.4. Behavior patterns of rat in the open field test

The task was performed in an open field, a 60 × 60 cm box, whose 50 cm-height-walls was made of fiberboard, except the frontal wall, which was made of glass, and the floor of the box has nine equal square



Scheme 1. Experimental design. In the day 1, the animals were subjected to stereotaxic surgery. In the day 4, the animals received a single ICV administration of ACSF or OUA, and started the treatment with Sal, Li or TMX intraperitoneally during 7 days. 24 h after the last injection, the animals were subjected to open field test and death. ICV = intracerebroventricular; ACSF = artificial cerebrospinal fluid; OUA = ouabain; Sal = Saline; Li = lithium; TMX = tamoxifen.

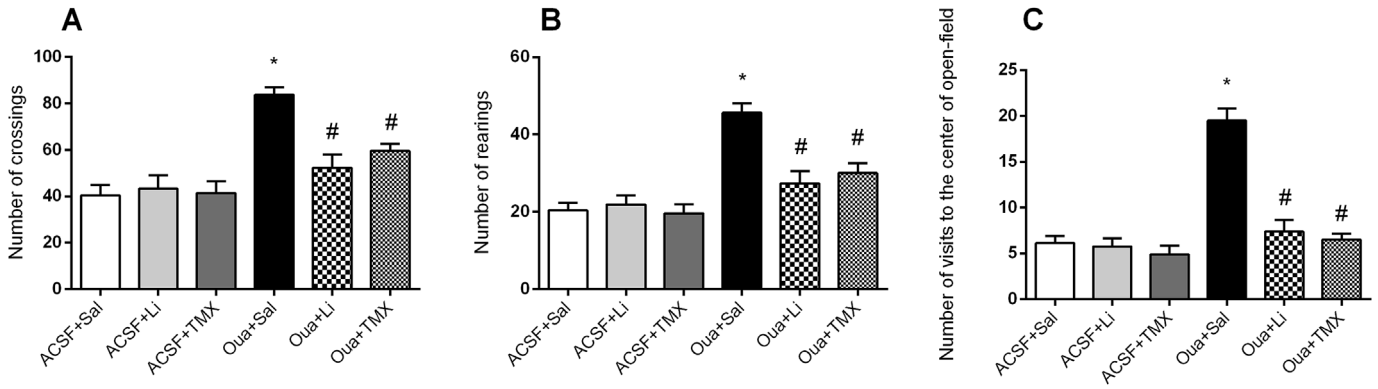


Fig. 1. Effects of administration of Li and TMX on the number of crossings (A), rearings (B) and center visits (C) in animals submitted to ouabain-induced animal model (n = 10 per group). Data were analyzed by Two-ways analysis of variances followed by the Tukey test when F was significant. Values are expressed as mean ± S.E. *p ≤ 0.05 compared to ACSF group. #p ≤ 0.05 compared to OUA group.

(20 × 20) separated by black lines. The animals were gently placed to explore the arena for 5 min. The following behavioral parameters were assessed in the open field test: Crossings: the total number of square crossings during the entire test period. Rearings: the total number of erect postures during the whole test period (Broadhurst, 1960; Ericson et al., 1991). Visits to center: Total number of visits to the center of open-field. The center square of 20 cm × 20 cm was defined as the "center" area of the field (Einat et al., 2007).

2.5. Evaluation of oxidative stress parameters in the rat brains

The samples, frontal cortex, and hippocampus of rats were homogenized in KCl KH_2PO_4 (12 mM KCl, 0.038 mM KH_2PO_4, pH = 7.4).

2.5.1. Lipid peroxidation

2.5.1.1. Lipid hydroperoxide (LPH). LPH activity was measured using the assay kit by Cayman Chemical (Item nº: 705002). The LPH was isolated from homogenized samples of frontal cortex and hippocampus of rats, using a solution of methanol in chloroform (0 °C, 1500 g, 5 min). Afterward, the samples were incubated at 21 °C with 0.1 units per unit chromogen mixture of chloroform extract. Samples were applied to the plates to read the absorbance of 500 nm. The absorbance was compared with a standard curve to determine the amount of hydroperoxide lipid peroxidation of the samples.

2.5.1.2. 4-Hydroxynonenal (4-HNE). 4-HNE was measured using the assay kit by Cell Biolabs (Inc., San Diego, CA, USA; STA-338). Protein adducts of 4-HNE formed by modification of lysine, histidine or cysteine were quantified using enzyme immunoassay according to Kimura et al. (2005).

2.5.1.3. 8-Isoprostane (8-ISO). 8-ISO was measured using the assay kit by Cayman Chemical (Item nº. 516351). 8-isoprostane levels were quantified using ACETM Competitive EIAs with 8-iso-acetylcholinesterase conjugate as a tracer and 8-iso-specific rabbit antiserum. As 8-iso and the tracer compete for limited anti-serum binding, the color intensity caused by tracer binding was inversely proportional to the amount of 8-iso. Its absorbance defined 8-isoprostane levels at 450 nM.

2.5.2. Oxidation and nitration of the proteins

2.5.2.1. Protein carbonyl. The carbonyl groups were quantified based on their reaction with dinitrophenylhydrazine (DNPH) to assess the level of oxidative damage to proteins, as previously described by Levine et al. (1994). With the addition of 20% trichloroacetic acid, the proteins were precipitated and then dissolved in DNPH; their absorbance was read at 370 nM.

2.5.2.2. 3-Nitrotyrosine (3-nitro). 3-nitro quantitation was performed using the assay kit by Cell Biolabs (STA-305). After incubating the sample for a short period, the anti-nitrotyrosine antibody was added, followed by a secondary HRP-conjugated antibody. Comparison to a standard curve determined the quantity of 3-nitro in the samples. Its absorbance defined 3-nitrotyrosine levels at 450 nM.

2.5.3. Activity of antioxidant enzymes

2.5.3.1. Superoxide dismutase (SOD) activity. The method utilized to evaluated SOD activity employs xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a formazan dye that is assayed spectrophotometrically at 492 nm at 37 °C. The inhibition in the production of the chromogen is proportional to the activity of SOD present in the sample; one unit of SOD causes 50% inhibition of the rate of the reduction of INT under the conditions of the assay (Bannister and Calabrese, 1987).

2.5.3.2. Catalase (CAT) activity. CAT is an enzyme able to degrade peroxides, including hydrogen peroxide (H_2O_2), and its activity assessment is based upon establishing the rate of H_2O_2 degradation spectrophotometrically at 240 nm at 25 °C. CAT activity was calculated regarding micromoles of H_2O_2 consumed per minute per milligram of protein, using a molar extinction coefficient of 43.6 M^{-1} cm^{-1} (Aebi, 1984).

2.6. Statistical analysis

Results of the present study are showed as the means ± standard error. 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. Data were analyzed by Two-ways analysis of variances followed by the Tukey test when F was significant. A value of p ≤ 0.05 was considered significant.

3. Results

In Fig. 1, OUA increased the number of (A) crossings, rearings (B) and, center visits (C) in rats. The treatment with Li and TMX reversed these behavioral alterations induced by OUA. The administration of Li or TMX in ACSF-treated animals did not change behavioral measures, indicating that the effects of these drugs in OUA-treated rats were not associated with sedation.

Two-way ANOVA revealed significant effects of ouabain administration [Crossings: $F(1.42) = 29.91$, $p < 0.001$; Rearings: $F(1.42) = 34.40$, $p < 0.001$; Center visits: $F(1.42) = 37.29$, $p < 0.001$] and treatment [Crossings: $F(2.42) = 6.35$, $p = 0.003$; Rearings: F

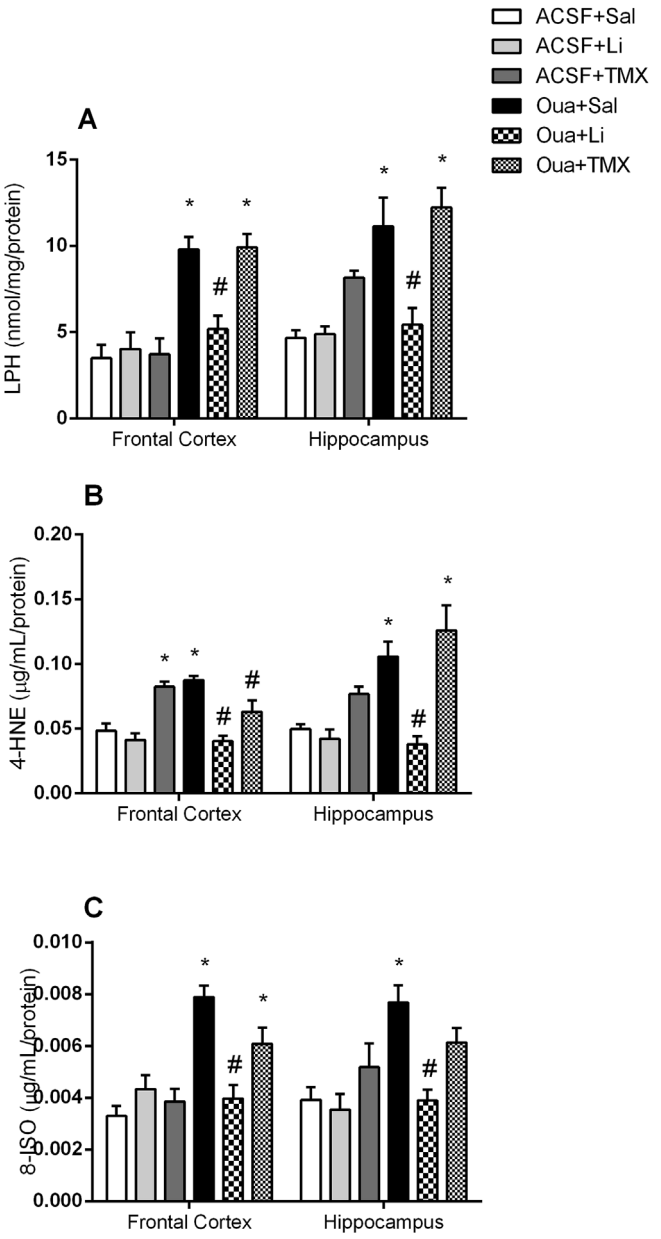


Fig. 2. Effects of administration of Li and TMX on the levels of LPH (A), 4-HNE (B) and 8-ISO (C) in frontal cortex and hippocampus of rats submitted to ouabain-induced animal model (n = 6 per group). Data were analyzed by Two-ways analysis of variances followed by the Tukey test when F was significant. Values are expressed as mean ± S.E. *p ≤ 0.05 compared to ACSF group. #p ≤ 0.05 compared to OUA group.

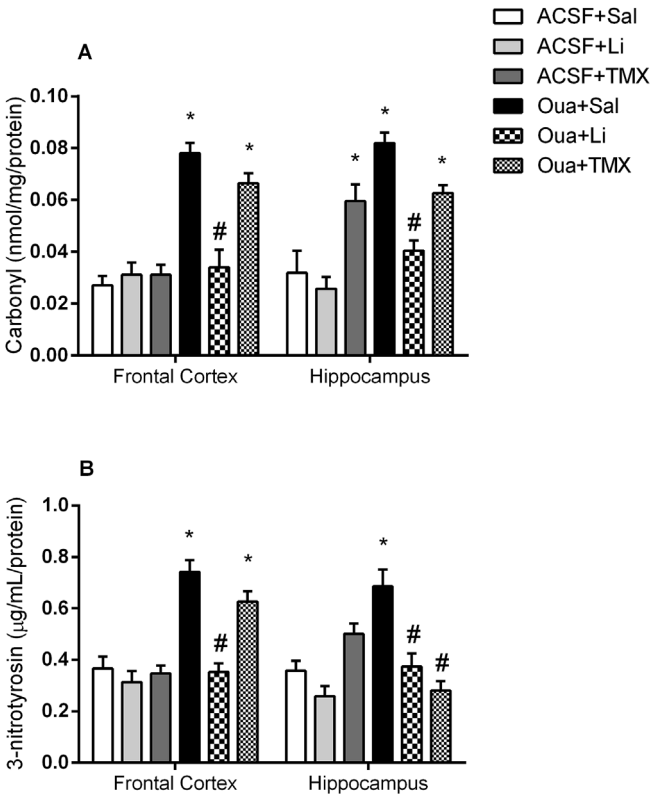


Fig. 3. Effects of administration of Li and TMX on the levels of Protein Carbonyl (A) and 3-nitro (B) frontal cortex and hippocampus of rats submitted to ouabain-induced animal model (n = 6 per group). Data were analyzed by Two-ways analysis of variances followed by the Tukey test when F was significant. Values are expressed as mean ± S.E. *p ≤ 0.05 compared to ACSF group. #p ≤ 0.05 compared to OUA group.

(2.42) = 8.43, p < 0.001; Center visits: F(2.42) = 31.82, p < 0.001] and a significant ouabain administration × treatment interaction [Crossings: F(2.42) = 5.18, p = 0.009; Rearings: F(2.42) = 5.69, p = 0.006; Center visits: F(2.42) = 18.20, p < 0.001].

It can be observed in Fig. 2 that OUA administration increased (A) LPH, (B) 4-HNE and (C) 8-ISO levels in the frontal cortex and hippocampus of rats. The treatment with Li reversed all these alterations. The treatment with TMX reversed the 4-HNE increases induced by OUA in the frontal cortex of the rats. It is important to note that TMX *per se* increased the levels of 4-HNE in the frontal cortex of rats.

Data from the two-way ANOVA revealed significant effects of ICV ouabain administration [LPH = frontal cortex: F(1.30) = 45.80, $p < 0.001$; hippocampus: F(1.30) = 21.94, $p < 0.001$], [HNE = frontal cortex: F(1.30) = 1.83, $p = 0.185$; hippocampus: F (1.28) = 18.75, $p < 0.001$]) and [8-ISO = frontal cortex: F (1.30) = 14.38, $p < 0.001$; hippocampus: F(1.28) = 11.82, $p = 0.001$]), treatment [LPH = frontal cortex: F(2.30) = 4.51, $p = 0.019$; hippocampus: F(2.30) = 13.61, $p < 0.001$], [HNE = frontal cortex: F(2.30) = 18.76, $p < 0.001$; hippocampus: F (2.28) = 22.66, $p < 0.001$] and [8-ISO = frontal cortex: F (2.30) = 1.80, $p = 0.128$; hippocampus: F(2.28) = 7.80, $p = 0.002$] and a significant ouabain administration × treatment interaction [LPH = frontal cortex: F(2.30) = 6.38, $p = 0.004$; hippocampus: F (2.30) = 4.78, $p = 0.015$], [HNE = frontal cortex: F(2.30) = 14.07, $p < 0.001$; hippocampus: F(2.28) = 6.22, $p = 0.005$] and [8-ISO = frontal cortex: F(2.30) = 6.81, $p = 0.003$; hippocampus: F (2.28) = 5.93, $p = 0.007$]).

As shown in Fig. 3, OUA administration increased (A) carbonyl and (B) 3-nitro levels in the frontal cortex and hippocampus of rats. The treatment with Li reversed all alterations in both brain structures evaluated. The treatment with TMX only reversed the increases of 3-nitro induced by OUA in the hippocampus. Furthermore, TMX *per se* increased the levels of carbonyl in the hippocampus of rats.

Data from the two-way ANOVA revealed significant effects of ICV OUA administration [Carbonyl = frontal cortex: F(1.29) = 61,83, p < 0,001; Hippocampus: F(1.29) = 17,47, p < 0,001] and [3-Nitro = frontal cortex: F(1.30) = 29,82, p < 0,001; Hippocampus F (1.29) = 1,09, p = 0,3] and treatment [Carbonyl = frontal cortex: F (2.29) = 10,52, p < 0,001; Hippocampus: F(2.29) = 10,97, p < 0,001] and [3-Nitro = frontal cortex F(2.30) = 11,74, p < 0,001; Hippocampus F (2.29) = 3,44, p = 0,04] and a significant OUA administration × treatment interaction [Carbonyl = frontal cortex: F (2.29) = 13,95, p < 0,001; Hippocampus: F(2.29) = 4,87, p = 0,01] and [3-Nitro = frontal cortex: F(2.30) = 8,36, p = 0,001;

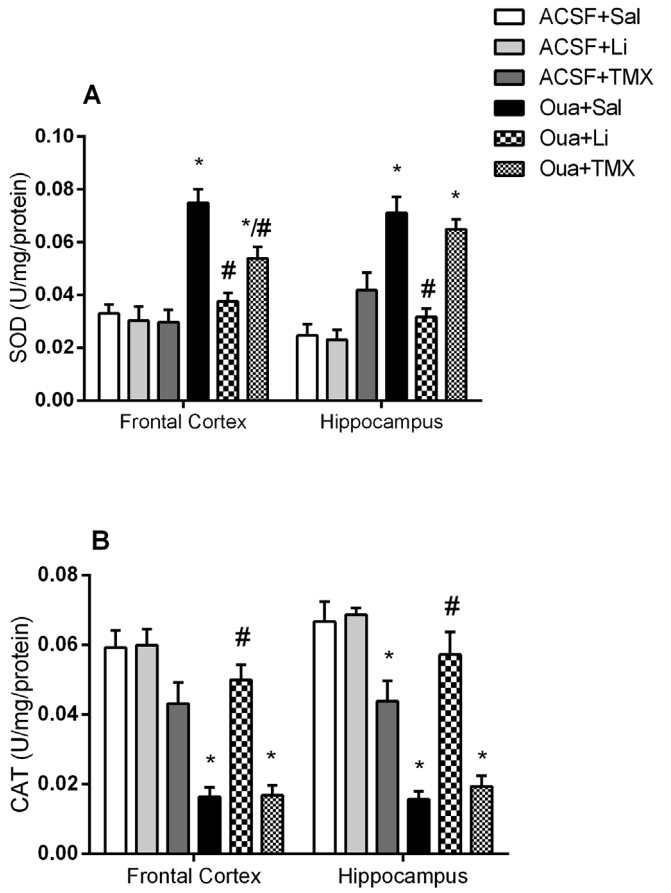


Fig. 4. Effects of administration of Li and TMX on the activity of SOD (A) CAT (B) in frontal cortex and hippocampus of rats submitted to ouabain-induced animal model (n = 6 per group). Data were analyzed by Two-ways analysis of variances followed by the Tukey test when F was significant. Values are expressed as mean ± S.E. *p ≤ 0.05 compared to ACSF group. #p ≤ 0.05 compared to OUA group.

Hippocampus F (2.29) = 10,49, p < 0,001].

As shown in Fig. 4, OUA administration increased SOD activity (A) and decreased CAT activity (B) in the frontal cortex and hippocampus of rats. The treatment with Li reversed these enzymes alterations in both brain structures evaluated. The treatment with TMX partially reversed the alterations in the SOD activity induced by OUA in the frontal cortex of animals. The treatment with TMX *per se* decreased the CAT activity in the hippocampus of rats.

Data from the two-way ANOVA revealed significant effects of ICV OUA administration [Superoxide Dismutase = frontal cortex: F (1.29) = 35,90, p < 0,001; Hippocampus: F(1.29) = 21,43, p < 0,001] and [Catalase = frontal cortex: F(1.30) = 54,18, p < 0,001; Hippocampus F(1.29) = 58,40, p < 0,001] and treatment [Superoxide Dismutase = frontal cortex: F(2.29) = 9,73, p < 0,001; Hippocampus: F(2.29) = 8,27, p = 0,001] and [Catalase = frontal cortex: F(2.30) = 16,91, p < 0,001; Hippocampus F (2.29) = 22,23, p < 0,001] and a significant OUA administration × treatment interaction [Superoxide Dismutase = frontal cortex: F(2.29) = 6,98, p = 0,003; Hippocampus: F(2.29) = 7,16, p = 0,002] and [Catalase = frontal cortex: F(1.29) = 7,04, p = 0,003; Hippocampus F (2.29) = 9,31, p < 0,001].

In Fig. 5 can be observed the correlation between locomotor activity, and LPH, 4-HNE, and 8-ISO levels. It was observed a positive correlation between locomotor activity and LPH levels in frontal cortex (**A**) and hippocampus (**B**). The 4-HNE did not show correlation with locomotor activity in frontal cortex (**C**) and hippocampus (**D**). However, the locomotor activity was positively correlated with 8-ISO levels in the frontal cortex (**E**) and hippocampus (**F**).

Data from pearson correlation for LPH: [hippocampus (n = 36; $r^2 = 0.1431$; $p < 0.05$), frontal cortex (n = 36; $r^2 = 0.2634$; $p < 0.05$)], 4-HNE: [hippocampus (n = 36; $r^2 = 0.1030$; p = 0.0642), frontal cortex (n = 36; $r^2 = 0.09897$; $p < 0.0617$)] and 8-isoprostane: [hippocampus (n = 36; $r^2 = 0.2415$; $p < 0.05$), frontal cortex (n = 36; $r^2 = 0.3283$; $p < 0.005$)].

The results of correlation between locomotor activity and levels of Carbonyl and 3-nitro were shown in Fig. 6. The carbonyl levels showed a positive correlation with the locomotor activity in the frontal cortex (**A**) and hippocampus (**B**) of rats. Furthermore, 3-nitro presented a positive correlation with the locomotor activity of animals in the frontal cortex (C). In the hippocampus (**D**) did not show correlation.

Data from pearson correlation for Carbonyl: [hippocampus (n = 36; $r^2 = 0.1987$; $p < 0.05$), frontal cortex (n = 36; $r^2 = 0.5107$; $p < 0.0001$)], 3-nitrotirosin: [hippocampus (n = 36; $r^2 = 0.09235$; p = 0.0716), frontal cortex (n = 36; $r^2 = 0.2631$; $p < 0.005$)].

Fig. 7 demonstrates the results of correlation between locomotor activity and of antioxidant enzymes activity. It can be observed a positive correlation between SOD activity and the number of crossings in the frontal cortex **(A)** and hippocampus **(B)** of animals. However, the CAT showed a negative correlation with the locomotor activity of animals in frontal cortex **(C)** and hippocampus **(D)**.

Data from pearson correlation for SOD: [hippocampus (n = 36; $r^2 = 0.2670$; $p < 0.05$), frontal cortex (n = 36; $r^2 = 0.4298$; $p < 0.0001$)] and CAT: [hippocampus (n = 36; $r^2 = 0.3627$; $p < 0.001$), frontal cortex (n = 36; $r^2 = 0.3092$; p = 0.0004)].

4. Discussion

In the present study was demonstrated that OUA, a Na^+K^+ATPase inhibitor, induces manic-like behavior, which can be observed through the increase of crossings, rearings and center visits of open-field. Moreover, the administration of Li or TMX reversed the hyperactivity induced by OUA, indicating an anti-manic effect of TMX and Li. The induction of hyperactivity in rats by OUA has been proposed as a good model of mania (El-Mallakh and Wyatt, 1995; Jornada et al., 2011). Indeed, clinical studies have reported Na^+K^+ATPase alterations in BD patients (El-Mallakh and Wyatt, 1995; Huff et al., 2010; Banerjee et al., 2012). In corroboration with our results, other authors also demonstrated that TMX, an inhibitor of PKC, has antimanic effects on the behavioral parameters in animal models of mania induced by OUA, sleep deprivation and amphetamine (Armani et al., 2012; Valvassori et al., 2014, 2017). Previous studies have demonstrated that activation of PKC enhances the release of dopamine (Robinson, 1991; Cowel, 2000). It is known that dopamine decreases Na^+K^+ATPase activity through PKC-dependent phosphorylation (Barati et al., 2017). Furthermore, the increased PKC activity induce hyperlocomotion in rats and seems to be correlated with Na^+K^+ATPase alterations (Valvassori et al., 2017). It has been explored the effects of selective estrogen receptor modulators (SERMs), which present estrogenic properties in the central nervous system (Khan, 2018; Hughes-Davies et al., 2009; Hayes, 2009). Moreover, it is observed higher levels of G-protein-coupled estrogen receptor-1 (GPER-1) in euthymic BD patients, when compared to the control group (Orhan et al., 2018). Previous studies demonstrated that the use of SERMs, e.g. TMX, could treat cognitive and pathophysiological alterations in patients with psychiatric disorders, such as BD (Yildiz et al., 2008; Talaei et al., 2016). The action anticancer of TMX occurs by 4-hydroxytamoxifen, its activity metabolite, and desmethyl analog endoxifen, both generate through CYP 2D6 and CYP3A4/3A5 isozymes action (Shagufta and Ahmad, 2018). However, studies showed that independently of their effects on the estrogen receptors, the TMX and your metabolites (4-hydroxytamoxifen and endoxifen) impair the function of the dopamine transporter (DAT) (Mikelman et al., 2017). Furthermore, the TMX is a PKC inhibitor and modulate a lot of

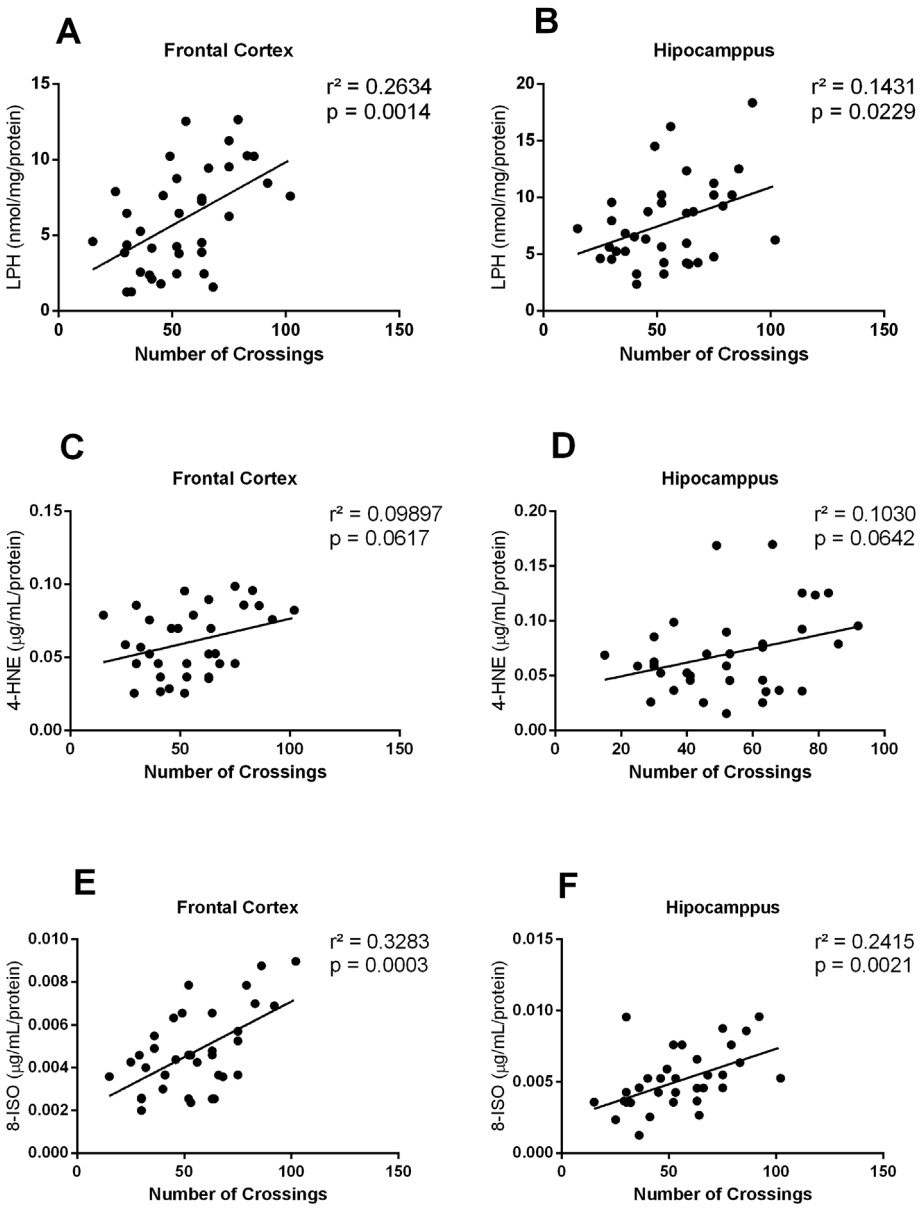


Fig. 5. Correlations between locomotor activity (number of crossings) and LPH levels (A and B) 4-HNE levels (C and D) and 8-ISO levels (E and F) in frontal cortex and hippocampus of animals submitted to OUA-induced animal model. Results were assessed using the Pearson correlation test.

intracellular cascades (Yildiz et al., 2016; Talaei et al., 2016). It is well described in the literature that activation of PKC enhances the release of dopamine, and the reverse is true; therefore, inhibition of PKC decreases the release of dopamine (Robinson, 1991; Cowel, 2000). The ICV OUA administration increases the dopamine release in the brain of rats (Sui et al., 2013). Li and TMX reversed the PKC pathway changes induced by OUA (Valvassori et al., 2017). Together, these that suggested that inhibiting PKC, Li and TMX could be indirectly decreasing the levels of dopamine extracellular, and, consequently, decreasing dopamine oxidation and oxidative damage to biomolecules.

In this study, the OUA administration induced lipid and protein damage in frontal cortex and hippocampus of rats, observed through increases of LPH, HNE, 8-ISO, carbonyl protein, and 3-nitro levels. These results are in agreement with previous studies that demonstrated that OUA induces oxidative damage to biomolecules in the brain of rats (Brüning et al., 2012; Brocardo et al., 2010; Jornada et al., 2011; Valvassori et al., 2015a, 2016; Valvassori, 2015b). It is important to note that oxidative lipid damage has been associated with decreases in the activity of the Na^+K^+-ATPase in bipolar patients (Banerjee et al., 2012). Therefore, the animal model of mania induced by OUA seems an excellent model to mimics such condition observed in bipolar patients and to test new potential mood stabilizers with antioxidant properties. The previous study demonstrated that a decrease in the $Na^+ K^+$ ATPase induces an increase in mitochondrial superoxide and the increasing levels of lipid peroxidation. The intracellular reactive oxygen species increase may produce neuronal damage by initiating damage to cellular macromolecules, such as lipids, proteins, and DNA (Valvassori et al., 2015a). Besides, previous studies (Jornada et al., 2011) as well in this study, it was demonstrated that OUA increases SOD and decreases CAT activities. It was observed that alterations in Na^+K^+ATPase activity, which lead to oxidative stress, also change the others antioxidant enzymes activity, such as glutathione peroxidase, and glutathione reductase (Brüning et al., 2012; Brocardo et al., 2010; Valvassori et al., 2016). These antioxidant enzymes alterations could be impairing even more de damage induced by OUA.

On the other hand, the treatment with Li reversed the oxidative and nitrosative damage to lipid and protein induced by OUA in frontal cortex and hippocampus of rats. These results are in agreement with a

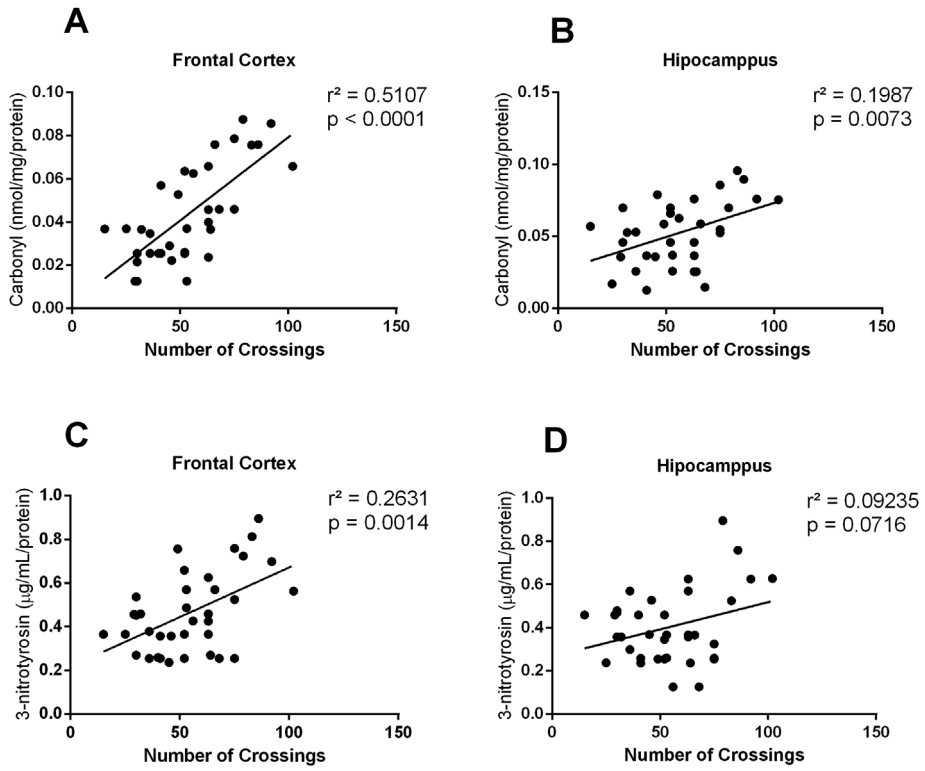


Fig. 6. Correlations between locomotor activity (number of crossings) and levels of Carbonyl (A and B) and 3-nitro (C and D) in frontal cortex and hippocampus of animals submitted to OUA-induced animal model. Results were assessed using the Pearson correlation test.

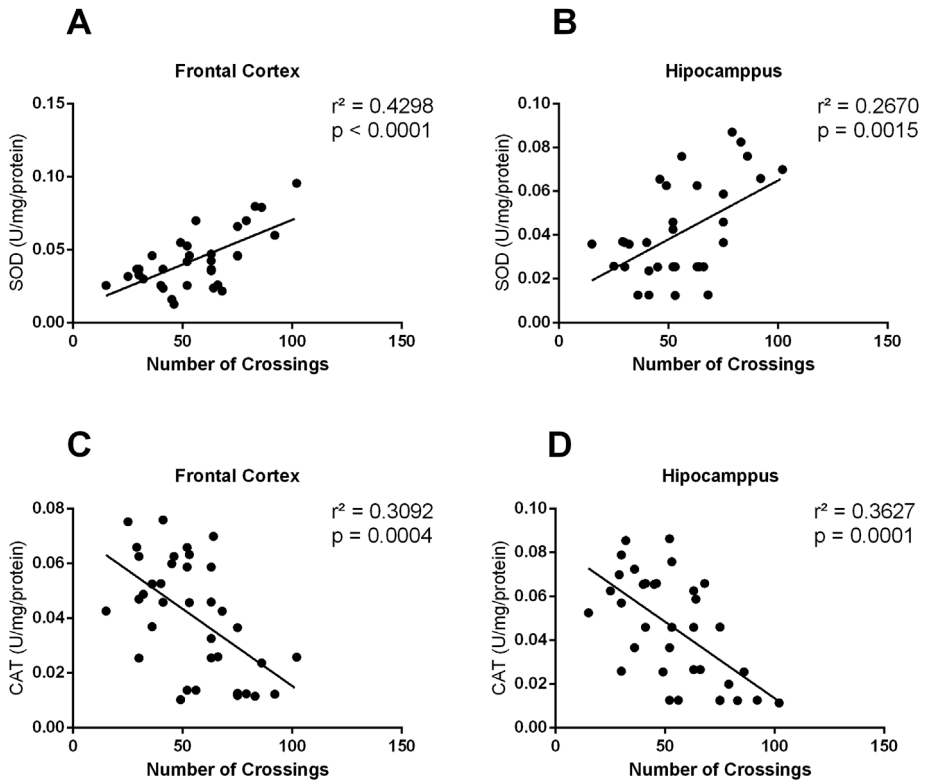


Fig. 7. Correlations between locomotor activity (number of crossings) and activity of antioxidant enzymes in frontal cortex and hippocampus of rats submitted to ouabain-induced animal model: SOD (A and B) and CAT (C and D). Results were assessed using the Pearson correlation test.

previous study (Jornada et al., 2011), which demonstrates that Li reverses the ouabain-induced increase in superoxide production and lipid damage from submitochondrial particles, and lipid and oxidative protein damage from the total cell of the hippocampus and frontal cortex. It is well described in the literature that lithium has antioxidant properties, modulating antioxidant enzymes and prevent oxidative

damage (Jornada et al., 2011). However, the exact mechanism by which Li exert its antioxidant effects is not well elucidating. As described in the introduction, one of the mechanisms of action of Li that has been studied by the researchers of the area is the inhibition of PKC (Cechinel-Recco et al., 2012; Steckert et al., 2012; Armani et al., 2014). Therefore, the hypothesis of the present study was: The inhibition of PKC could be some effect on the oxidative damage induced by OUA in the brain. Therefore, TMX, a PKC inhibitor, was tested in the animal model of mania induced by OUA, which is known produces oxidative damage to biomolecules from the brain.

However, the administration of TMX only reversed the OUA-induced increased in 4-HNE in frontal cortex and 3-nitro in the hippocampus. In the 8-ISO analysis, the group OUA + TMX was not different from OUA + Sal neither control group, suggesting a partial reversion. Regarding antioxidant enzymes, the treatment with TMX partially reversed the increased of SOD induced by OUA. It is crucial to emphasize that, in the present study, TMX did not has effects on most of the oxidative stress parameters evaluated. The previous study from our research laboratory showed that TMX reversed the manic-like behavior triggered by amphetamine and protected the brain against the oxidative damage induced in the animals. However, as in the present study, these protective effects were dependent on cerebral area and technique evaluated (Steckert et al., 2012). Together with our results, the data from Steckert et al. (2011) suggest that the anti-manic effect of TMX is not related to antioxidant properties.

It is important to note that the administration of TMX alone increased 4-HNE levels, a parameter of lipid oxidative damage, in the frontal cortex of rats. A previous study (Steckert et al., 2011) demonstrated that TMX *per se* induces increased of superoxide in submitochondrial particles from frontal cortex, amygdala, and hippocampus of rats. Besides, in this same study, the authors found that TMX *per se* induces oxidative damage to lipid in the frontal cortex and striatum of rats. Lee et al. (2012) also found that TMX produced oxidative stress, through increases in ROS production, overcoming the antioxidant capacity, leading to lipid damage depending on the dose. In the same way, Tomkova et al. (2018) showed that administration of PKC inhibitors increases mitochondrial ROS levels. These findings suggest that decreases in PKC activity can lead to oxidative stress.

The ICV administration of OUA has been shown a useful animal model of mania, allowing the evaluation of several physiological parameters involved in BD, such as PKC pathways. The search for more adequate treatments is still essential for a better quality of life for BD patients. The TMX has effects on the OUA-induced manic-like behaviors in rats, but it did not seem to be effective against oxidative stress. However, more studies are necessary to understand better the mechanisms of this substance on the pathophysiology and behaviors involved in BD.

Positive points of study: 1) The evaluation of PKC signaling and TMX effects can be helpful to the development of new drugs the treatment of BD. 2) The present study evaluates the administration of TMX in an animal model of mania, showing some effects of this drug on the behavioral manifestations similar to observed in BD mania. 3) Moreover, we showed the correlation between manic-like behavior and oxidative stress, both found in the BD patients, which reinforces the validity of the model. 4) These results can contribute to understanding the mechanisms of TMX and the involvement of PKC on behavioral manifestation, as well as pathophysiological parameters observed in BD mania.

Limitations of study: 1) This paper evaluated only two structures of the limbic system and did not assess specific regions of these structures, can be a bias of the study. 2) In the present study did not evaluate female rats, making the results more limited. 3) Although animal models are a powerful tool to study psychiatric disorders, the animals of the laboratory are very similar to each other, which makes the study end up distancing itself a little from the clinical reality. This final point could be an essential factor to false positive or negative results.

Acknowledgements

This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES - Finance Code 001), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina* (FAPESC), *Instituto Cérebro e Mente* and *Universidade do Extremo Sul Catarinense* (UNESC). JQ and SSV are CNPq Research Fellows. GCD is holder of a FAPESC studentship and FFG is holder of a CNPq Studentship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.03.020.

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