

Depressive-Like Behavior Induced by Long-Term Ouabain Administration Accompanied Alteration in Neuroinflammation Parameters in Rats

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Abstract

Objective: The present study aims to investigate the effects of Lithium (Li) on manic- and depressive-like behaviors and inflammatory parameters in rats submitted to the bipolar disorder (BD) model induced by ouabain (OUA).

Material and methods: Adult male rats received a single intracerebroventricular (ICV) injection of OUA or artificial cerebrospinal fluid (aCSF). On the fourth day after the ICV injection, the rats received intraperitoneal injections of saline (NaCl 0.9%) or Li (47.5 mg/kg), two times a day, for 14 days. On the seventh day after OUA injection, the locomotor activity was assessed (open field test), and on the fourteenth day, locomotion was evaluated again, which was followed by the forced swimming test to evaluate depressive-like behavior. After euthanasia, inflammatory parameters were evaluated in the frontal cortex and hippocampus.

Results: After seven days of OUA administration, the animals showed a hyperactive behavior that was reversed by treatment with Li. After 14 days of ICV injections, rats exhibited a depressive behavior. Regarding the inflammatory parameters, measured after 14 days of the ICV infusions, OUA induced an increase in the levels of interleukin (IL)-1 β , IL-6, tumor necrosis factor α , and cytokine-induced neutrophil chemoattractant-1. In contrast, Li treatment decreased these parameters.

Conclusion: The animal model of BD induced by an OUA is able to induce neuroinflammation, which supports its construct validity for the BD research.

Introduction

Bipolar disorder (BD) is one of the most prevalent forms of mental illness comprising different mood shifts, ranging from episodes of mania or hypomania, depression, euthymia, and mixed states.¹ Euphoria, expansive mood, high energy, psychomotor agitation or hyperactivity, and increased risk-taking behavior characterize the manic episodes, while deep sadness and anhedonia usually represent the depressive phase.^{2,3} BD is a common and disabling condition associated with increased mortality in comparison to the general population, whose world prevalence rate stands at 1–2.5%.^{4,5}

Precise BD pathological mechanisms are not fully understood, but several biological systems were supposed to act perpetuating the condition.⁶ For example, oxidative stress, mitochondrial dysfunction, the release of inflammatory cytokines, and, consequently, the changes in ionic regulation channels can play a pivotal role in the pathophysiology.^{7–10} The advance in our understanding of BD pathophysiology can ultimately improve the therapeutic arsenal used for this condition.¹¹

In this regard, lithium (Li) is a mood stabilizer drug used for more than 60 years, able to reduce acute symptoms of mania and also used in bipolar depression and BD maintenance therapy, avoiding new mood episodes.^{8,12} Studies have shown that Li induces widespread effects on different cellular

pathways, with antioxidant, neuroprotective, and neurotrophic properties.¹³ Besides, the drug collaborates with the inflammatory system modulation in bipolar patients and animal models of mania.^{8,12}

Research using animal models of psychiatric disorders has been relevant to unravel the pathophysiology of these conditions.¹⁴ To illustrate, research conducted with animal models of BD has helped to discover the pathways involved in its development.⁹ In this context, the present study uses as a platform the animal model of BD induced by the intracerebroventricular (ICV) administration of ouabain (OUA), a well-known glycoside that inhibits the sodium/potassium adenosinetriphosphatase enzyme ($\text{Na}^+/\text{K}^+\text{-ATPase}$; EC 3.6.3.9).¹⁵ Recently, the Valvassori and coworkers provided all validities required for a proper animal model of BD using this same platform since it mimics manic and depression behaviors in the same animal, as well as the alterations concerning pathophysiology and remission of the symptoms through traditional pharmacotherapy.¹⁶

Indeed, evidence has shown a significantly impaired $\text{Na}^+/\text{K}^+\text{-ATPase}$ activity in bipolar patients in manic and depressive episodes.¹⁷ Additionally, recent research shows that this enzyme inhibition can lead to increased cytokine release, oxidative stress, immune response, and inflammation.¹⁷⁻²⁰ Cytokines are small peptides and proteins responsible for cell interactions and are included in this class of molecules the cytokine-induced neutrophil chemoattractant 1 (CINC-1), interleukin(IL)-1 β , IL-6, IL-10, and tumor necrosis factor α (TNF- α).²¹ IL-6 is a multifunctional cytokine and a trophic factor for hybridoma and myeloma cells that mediates the transformation of activated B cells into antibody-synthesizing ones.²² IL-10 limits neuroinflammation by altering the resident glia and infiltrating leukocytes' response and by decreasing the synthesis of mediators by such cells.²³ IL-1 β is a potent pro-inflammatory cytokine mostly synthesized macrophages and other innate immune system cells, usually in response to the recognition of pathogen-associated molecular patterns by these cells.²⁴ CINC-1 is a pro-inflammatory cytokine and a relevant chemoattractant to neutrophils, collaborating to infiltration of these cells in tissues.²⁵ TNF- α is a pro-inflammatory mediator that drives immune pathways to prevent infiltration or damage to tissues and rules the survival or apoptosis of its target cells.²⁶

Therefore, as an additional step towards a better insight on the proinflammatory imbalance of the disorder and validation of the BD model induced by OUA, the present study evaluates the behavior of rats and investigates the levels of important inflammatory mediators, i.e., IL-1 β , IL-6, IL-10, CINC-1, and TNF- α in the frontal cortex and hippocampus, seven and 14 days after a single ICV administration of the $\text{Na}^+/\text{K}^+\text{-ATPase}$ inhibitor. Besides, it is also presented the Li effect in this experimental scenario.

Material And Methods

Animals

Thirty-two adult male Wistar rats (*Rattus norvegicus*, heterogenic strain), with 250–350 g body weight, were obtained from the breeding colony at the University of Southern Santa Catarina (UNESC). Animals

were housed as five per cage, with ad libitum food and water and controlled conditions (12 h-light/dark cycle; lights on at 6:00 a.m.; $22 \pm 1^\circ\text{C}$). Experimental procedures started following approval by the UNESCO's Ethical Committee on Animal Use for Research (record 66/2010), under the guidelines from the National Institutes of Health (US) "Guide for the Care and Use of Laboratory Animals"²⁷ and the Brazilian Society for Neuroscience and Behavior.

Surgical procedure

Animals were submitted to anesthesia by intramuscular injection of ketamine (80 mg/kg body weight) and xylazine (10 mg/kg) and accommodated in a stereotaxic apparatus, followed by removing the skin and hair covering the head. After this, it was placed a 27-gauge guide cannula (9 mm) on the surface of the cranial bone, according to the following coordinates: 0.9 mm posterior to bregma; 1.5 mm right from the midline; and 1 mm above the lateral brain ventricle.²⁸ After marking, it was carrying out a 2 mm orifice on the animal skull, and a cannula was ventrally implanted 2.6 mm to the superior surface of the bone and fixed with dental acrylic cement.

Animals recovered from the procedure within three days, time in which they subcutaneously received tramadol hydrochloride (10 mg/kg body weight) each 12 h to mitigate the postoperative pain, by following the manufacturer's instructions (União Química Farmacêutica Nacional S. A., São Paulo, Brazil).

Experimental design

On the fourth day after the surgical procedure, a 30-gauge cannula was placed inside the guide cannula and linked to a microsyringe by using a polyethylene tube. The tip of the infusion cannula extended 1.0 mm beyond the guide cannula to reach the right lateral brain ventricle. Each animal received a single ICV injection of artificial cerebrospinal fluid (aCSF, 5 μL) or OUA (10^{-3} M) dissolved in aCSF (5 μL). Each infusion lasted 30 seconds to prevent the liquid efflux.^{18,29} From the day following the aCSF or OUA injection, animals received intraperitoneal injections of saline (Sal, 1 mL/kg) or Li (47.5 mg/kg) twice a day, for 14 days.³⁰ Therefore, the rats were randomly assigned to four groups ($n = 8$ per group): 1) aCSF + Sal, 2) aCSF + Li, 3) OUA + Sal, and 4) OUA + Li (Figure 1).

Behavioral tests

Open field

The open field test occurred in two periods: seven and 14 days after ICV infusions, to provide insightful information on the locomotion, exploratory, risk-taking, and stereotypical behaviors of the animals. Briefly, the test ran in an apparatus with a 40 cm \times 60 cm floor surrounded by 50 cm-high walls made of brown plywood with a frontal wall made of glass. The floor was divided into nine equal rectangles by black lines and covered with a glass base. Each animal was gently placed in the left rear rectangle to explore the

arena for 5 minutes (min). The hyperlocomotion indicates a manic-like behavior through the total number of crossings and rearings during the entire test period.³¹

Forced swimming

The procedure was carried out 13 days following ICV infusions to investigate animal immobility (total immobility or movements to keep the heads out of the water), which is a depressive-like behavior. The test occurred in two days of procedures, where each rat is placed in a cylinder with water (23 °C) in sufficient quantity so that it cannot support its paws on the bottom. On the first day, animals swam inside the apparatus for 15 min (training session). Twenty-four hours following training, the test session took place in which was assessed the times of swimming, climbing, and immobility for 5 min.³²

Biochemical analysis

Brain samples

On the 14th day following OUA or aCSF infusion, after the behavioral tests, the rats were killed with a guillotine and the brain dissected to obtain the frontal cortex and hippocampus. Procedure occurred on a Petri dish placed on ice, followed by immersion of the samples in liquid nitrogen and subsequent storing at -80°C for subsequent biochemical analysis.

Neuroinflammation parameters

Levels of TNF- α , IL-1 β , IL-6, IL-10, and CINC-1

The hippocampus and frontal cortex were homogenized in an extraction solution containing aprotinin (100 mg tissue per 1 mL). The concentration of cytokines/chemokine was determined in the brain structures using commercially available ELISA assays, following instructions supplied by the manufacturer (DuoSet kits, R&D Systems, MN, United States). Data are expressed as pg/100 mg tissue.

Protein quantification

Total protein was measured by the Lowry and coworkers' method,³³ with slight modifications.³⁴ Bovine serum albumin was used as a standard.

Statistical analysis

All data are present as mean \pm standard error of the mean. Variables were analyzed according to their distribution, with the Shapiro-Wilk's test for normality used for this purpose. Differences between groups determined by two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The software used in the analyzes was Statistica 7 (StatSoft, Inc., Tulsa, OK, United States). Differences were statistically significant as $p \leq 0.05$.

Results

Behavioral analyses

The open field test was performed to evaluate the rat locomotion. Assessed parameters included the number of crossings and rearings. Seven days after the rats received a single ICV injection of OUA, they presented a marked pattern of locomotion in comparison to aCSF group animals (Figure 2). Li administration reversed the increase in the crossings and rearings induced by OUA. Two-way ANOVA revealed significant OUA effects in the following seven days after ICV infusions [Crossings: $F(1,36) = 143.90, p < 0.05$; Rearings: $F(1,36) = 71.61, p < 0.05$] and treatment [Crossings: $F(1,36) = 118.35, p < 0.05$; Rearings: $F(1,36) = 66.54, p < 0.05$].

On the other hand, no significant differences between groups were observed in these parameters in the rats evaluated 14 days following OUA infusion [Crossings: $F(1,36) = 10.02, p = 0.003$; Rearings: $F(1,36) = 0.243, p = 0.62$] and treatment [Crossings: $F(1,36) = 0.22, p = 0.640$; Rearings: $F(1,36) = 0.007, p = 0.93$]. Thus, the evaluation of this behavior on the 14th after OUA injection revealed that rats did not exhibit the manic-like behavior as observed on the 7th day.

The next step of the behavioral assessments was the forced swimming test. In this context, it was demonstrated a significant increase in the time of immobility (in seconds) at 14 days following OUA administration. Besides, there was a significant decrease in the time of swimming (seconds) in rats in that same test. Altogether, these results demonstrate that after 14 days of OUA administration, the animals presented hopelessness, which is a depressive-like behavior. On the other hand, Li administration reversed the increase in immobility induced by OUA (Figure 2) [Immobility: $F(1,36) = 301.06, p < 0.05$; Swimming: $F(1,36) = 301.06, p < 0.05$] and treatment [Immobility: $F(1,36) = 301.06, p < 0.05$; Swimming: $F(1,36) = 301.06, p < 0.05$].

Neuroinflammation parameters

On the 14th day after OUA injection, the neurochemical measurements were conducted, with investigation of the effect of Li treatment in this experimental scenario. Data depicted in the following figures show significant alterations in the levels of IL-1 β (Figure 3), IL-6 (Figure 4), IL-10 (Figure 5), TNF- α (Figure 6), and CINC-1 (Figure 7) in rats subjected to the animal model of BD induced by OUA. A single OUA administration elicited an increase in the levels of all inflammation parameters, regardless of cerebral structure, while Li administration prevented this alteration.

Data from two-way ANOVA revealed significant effects of OUA administration on the IL-1 β levels [frontal cortex: $F(1,16) = 30.95, p = 0.002$; hippocampus: $F(1,16) = 70.55, p < 0.05$], as well treatment [frontal cortex: $F(1,16) = 71.96, p < 0.05$; hippocampus: $F(1,16) = 67.94, p < 0.05$], and a significant OUA administration \times Li interaction [frontal cortex: $F(1,48) = 45.24, p = 0.15$; hippocampus: $F(1,47) = 41.63, p < 0.05$]. The same pattern of alterations was observed on the IL-6 levels regarding effect of OUA [frontal cortex: $F(1,16) = 38.82, p = 0.002$; hippocampus: $F(1,16) = 32.12, p < 0.05$], treatment [frontal cortex: $F(1,16) = 69.34, p < 0.05$; hippocampus: $F(1,16) = 51.60, p < 0.05$], as well as a significant OUA

administration × Li interaction [frontal cortex: $F(1,48) = 49.31, p = 0.15$; hippocampus: $F(1,47) = 27.11, p < 0.05$].

Additionally, a similar set of alterations was observed on the IL-10 levels, concerning to the effect of OUA [frontal cortex: $F(1,16) = 15.09, p = 0.001$; hippocampus: $F(1,16) = 8.71, p = 0.009$], treatment [frontal cortex: $F(1,16) = 1.81, p = 0.197$; hippocampus: $F(1,16) = 17.17, p < 0.05$], as well as a OUA administration × Li interaction [frontal cortex: $F(1,48) = 9.26, p = 0.007$; hippocampus: $F(1,47) = 19.38, p < 0.05$]. The same pattern can be observed on the levels of TNF- α , regarding the effects of OUA [frontal cortex: $F(1,16) = 7.75, p < 0.05$; hippocampus: $F(1,16) = 33.73, p < 0.05$], treatment [frontal cortex: $F(1,16) = 1.81, p = 0.197$; hippocampus: $F(1,16) = 10.61, p < 0.05$], as well the OUA administration × Li interaction [frontal cortex: $F(1,48) = 19.06, p < 0.05$; hippocampus: $F(1,47) = 15.83, p < 0.05$]. Finally, the CINC-1 levels followed the same pattern as demonstrated above, considering the effects of OUA [frontal cortex: $F(1,16) = 22.75, p < 0.05$; hippocampus: $F(1,16) = 7.71, p = 0.013$], treatment [frontal cortex: $F(1,16) = 17.02, p < 0.05$; hippocampus: $F(1,16) = 6.95, p = 0.017$], and the OUA administration × Li interaction [frontal cortex: $F(1,48) = 15.73, p = 0.001$; hippocampus: $F(1,47) = 15.11, p = 0.001$].

Discussion

Inflammation has been increasingly reported in the research focusing on the BD pathophysiology and therapy, both in clinical trials^{35,36} and animal studies.³⁷ In bipolar patients, inflammatory mediators have been implicated in the disorder, potentially influencing the progression or severity.^{38,39} For instance, the cytokines whose levels are prone to an increase in BD patients, in comparison to healthy controls, include IL-4, IL-6, IL-10, and TNF- α .⁴⁰ Additionally, the use of anti-inflammatory drugs as adjunctive therapy to mood stabilizers in the context of bipolar mania has demonstrated promising outcomes.^{35,37,41} Accordingly, it will be of great interest that a BD animal model, besides mimic the behavioral alterations of the disorder, also recapitulates the inflammatory alterations that may occur in bipolar patients, which highlights the need for further research in this field.

In this regard, the present study sought a better insight into the OUA model of BD, which was recently validated by the paper of Valvassori and coworkers.¹⁶ By corroborating data from these authors, the present study demonstrated that OUA administration induces an increase in the number of crossings and rearings seven days after OUA administration in rats, and these alterations are preventable by Li. Additionally, such alterations do not perpetuate until 14 days following the ICV infusions, while the forced swimming test showed a depressive-like behavior (“hopelessness”) in the same animals at this period (also preventable by Li). Therefore, our behavioral findings reinforce the consistency of the OUA administration to model BD, which is a step forward to its validation. Indeed, in bipolar patients, manic episodes last at least seven days, while depressive symptoms are present within 14 days in the same subjects.² This pattern is accurately represented in the OUA model. Besides the study carried out by Valvassori et al.,¹⁶ our findings are similar to data provided by other papers, especially in the context of manic-like behaviors following injection of the Na⁺/K⁺-ATPase inhibitor.^{37,42,43}

Moreover, the present study focused on the need to model the pro-inflammatory perspective of BD.¹⁰ To this aim, we measured the levels of relevant inflammatory mediators (IL-1 β , IL-6, IL-10, and TNF α) 14 days after OUA ICV infusions. In all parameters evaluated, OUA elicited a significant increase, while the Li administration reversed these alterations. Intriguingly, a similar study performed by our research group measuring the same cytokines did not find any alterations in these parameters, except IL-6, whose levels were decreased in the striatum of rats receiving OUA.²¹ Nonetheless, that paper investigated the OUA effect in an acute context, and here the findings are presented in a subchronic/chronic perspective, with the inclusion of the Li treatment for validation purposes. Data provided by our study mimic the inflammatory pathophysiological alterations reported in patients,⁴⁴ which reinforces the validity of the model to simulate the pathophysiological aspects of the disorder. Further studies are required to unravel the precise mechanisms underlying these alterations in the inflammatory parameters. One possibility is that OUA can activate neuronal signaling pathways ruling the release of cytokines, such as the nuclear factor kappa B (NF- κ B), which is activated by the glycoside according to prior studies.^{45,46} Also, NF- κ B upregulation was detected in bipolar patients, potentially contributing to the pro-inflammatory balance of the disorder.^{47,48}

One potential limitation of the present study is that Na⁺/K⁺-ATPase activity was not measured to confirm that OUA effects are related to an inhibition of this enzyme. However, this parameter was not altered by the glycoside in the Valvassori and coworkers' paper,¹⁶ which provided the experimental platform for the present study, although OUA is a well-known inhibitor. That study showed no inhibition on the 14th day after ICV infusion of the drug, time in which were carried out the measurements here. By regarding that the enzyme activity is pivotal for many neuronal roles, a stable decrease in this parameter might generate neurological disturbances,^{49,50} so that the protein can conform to the high OUA concentration to prevent disruption of its functions. Another possibility is the fact glycoside effect on the Na⁺/K⁺-ATPase may be independent on the ion transport activity of the protein; instead, the drug can activate a signaling complex comprising the enzyme, cluster determinant 36 (CD36), and Toll-like receptor 4, subsequently triggering inflammation driven by NF- κ B.⁴⁶

It is worthy to note that experiments from the present study timely mimicked the behavioral alterations of the disorder (face validity), as well as its pathophysiological inflammatory alterations (construct validity). Additionally, Li, a standard drug in BD therapy, was able to mitigate the behavioral and pathophysiological alterations induced in the model (predictive validity), which is the final criterion to validate OUA administration as a BD model.

In summary, OUA elicited a manic-like behavior seven days after its ICV administration, which was followed by depressive-like alterations several days later. Additionally, this drug induced an increase in all measured inflammatory mediators (IL-1 β , IL-6, IL-10, and TNF α) 14 days after ICV infusions. Li administration alleviated or reversed all these disturbances in behavior and neurochemistry, indicating that OUA administration is a valid model to study neuroinflammation in the context of BD. Since some

cytokines are also significantly altered in bipolar patients, this experimental model might be useful to the screening of drug candidates for the therapy of this condition.

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Declarations

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Author Contributions

All authors contributed to the study conception and design. Samira S. Valvassori and João Quevedo contributed to design and development; methodological design; supervision (responsible for organizing and executing the project and writing the manuscript); analysis/interpretation; literature survey; writing and critical review. Jorge M. Aguiar-Geraldo, Taise Possamai-Della, Dayane D. da-Rosa, Samira Menegas, Gustavo C. Dal-Pont and José H. Cararo participated data collection and processing; biochemical analyzes of the samples as well as performed statistical analyzes; analysis / interpretation; literature survey and writing.

Data availability

All data generated or analysed during this study are included in this published article.

Ethics Approval

Experimental procedures started following approval by the UNESCO's Ethical Committee on Animal Use for Research (record 66/2010), under the guidelines from the National Institutes of Health (US) "Guide for the Care and Use of Laboratory Animals" and the Brazilian Society for Neuroscience and Behavior.

Conflicts of Interests

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

Consent to Participate

Not applicable

Consent for Publication

All authors have agreed to the submission to Molecular Neurobiology.

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Figures

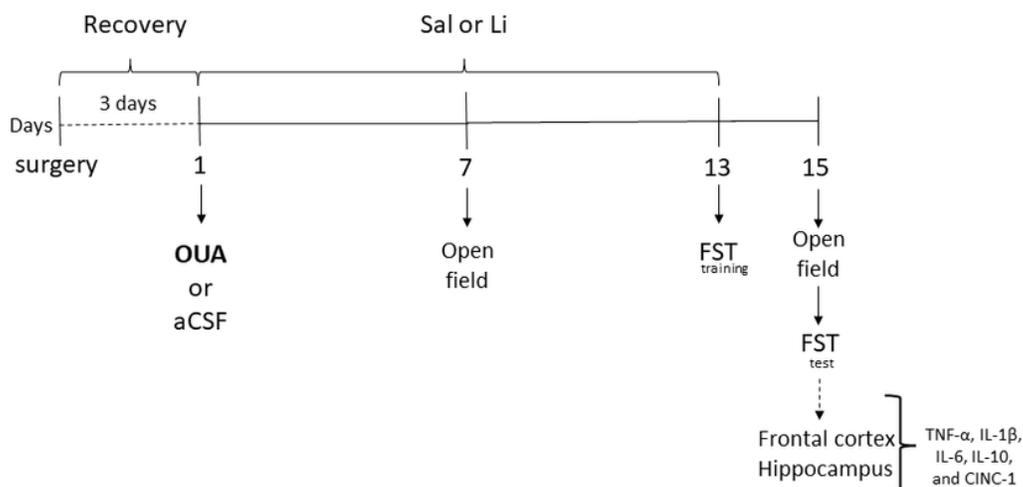


Figure 1

Schematic representation of the experimental design of present study. (Abbreviations: aCSF: artificial cerebrospinal fluid; CINC-1: cytokine-induced neutrophil chemoattractant 1; ICV: intracerebroventricular; IL-1 β : interleukin 1 β ; IL-6: interleukin 6; IL-10: interleukin 10; Li: lithium; OUA: ouabain; Sal: saline; TNF- α : tumor necrosis factor α)

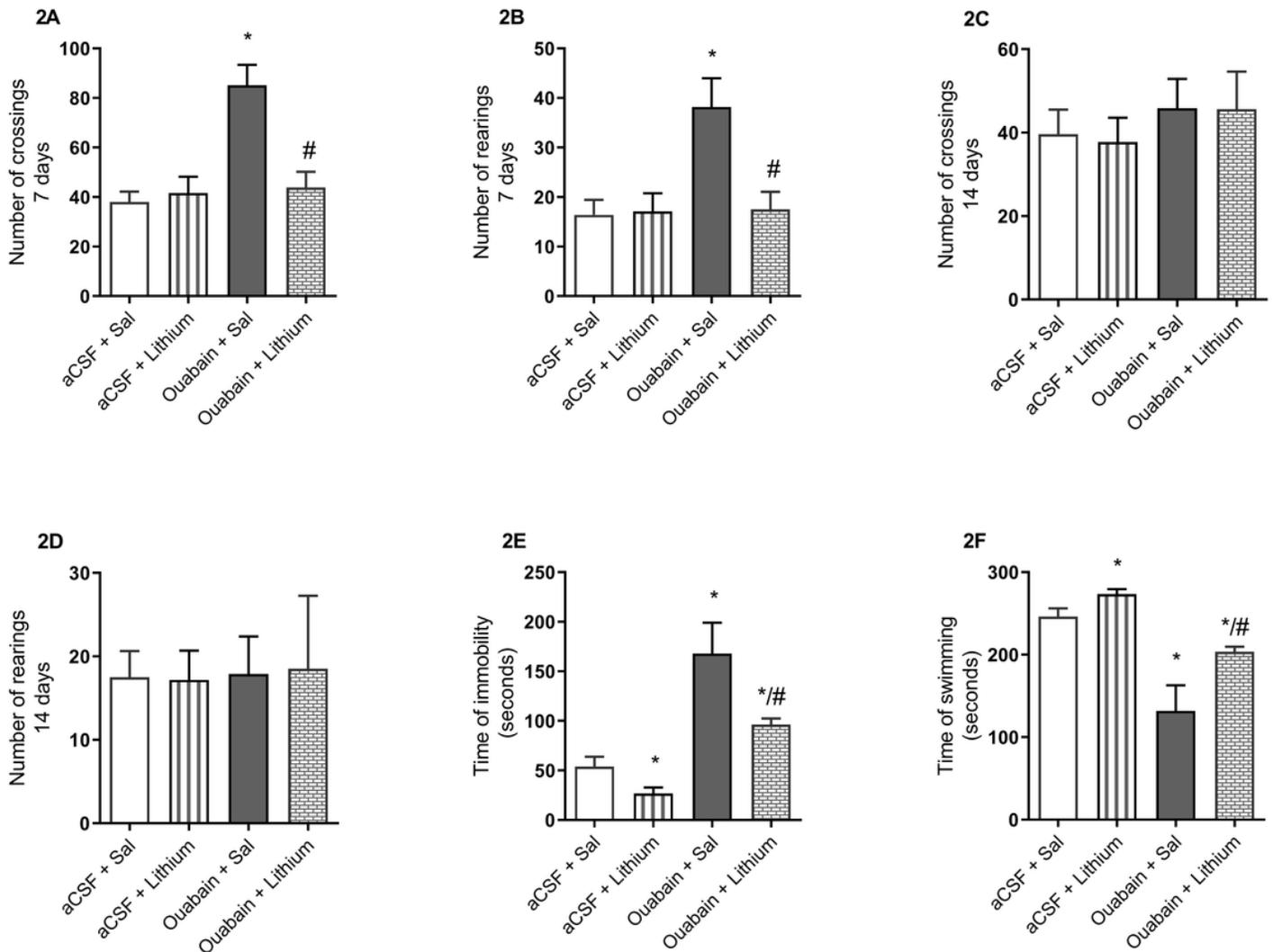


Figure 2

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the behavioral parameters of the open field (2A–2D) and forced swimming (2E–2F) tests in adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments seven and 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (arbitrary units or time, in seconds [s]). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group

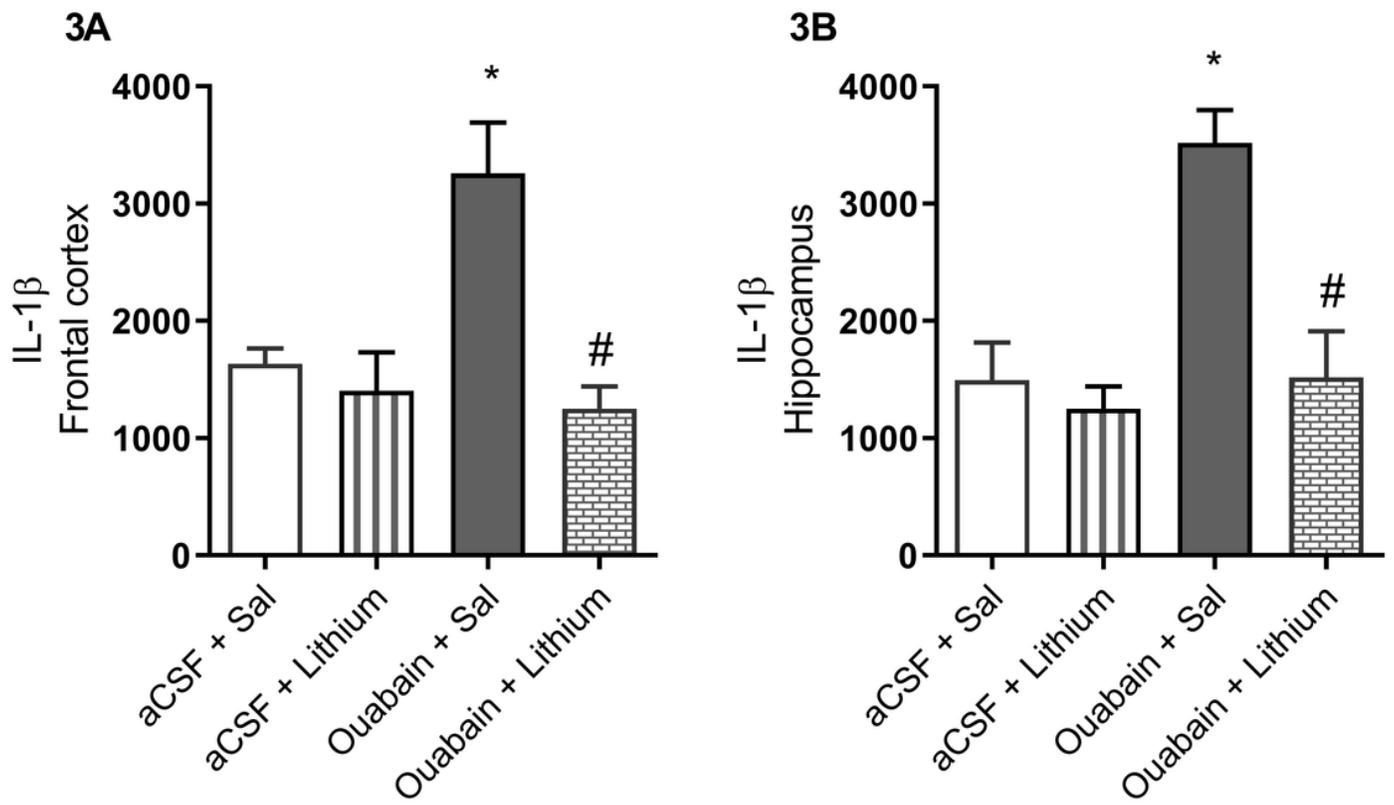


Figure 3

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the levels of interleukin 1 β (IL-1 β) in the frontal cortex (**3A**) and hippocampus (**3B**) of adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (picograms per 100 milligrams [pg/100 mg] tissue). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group

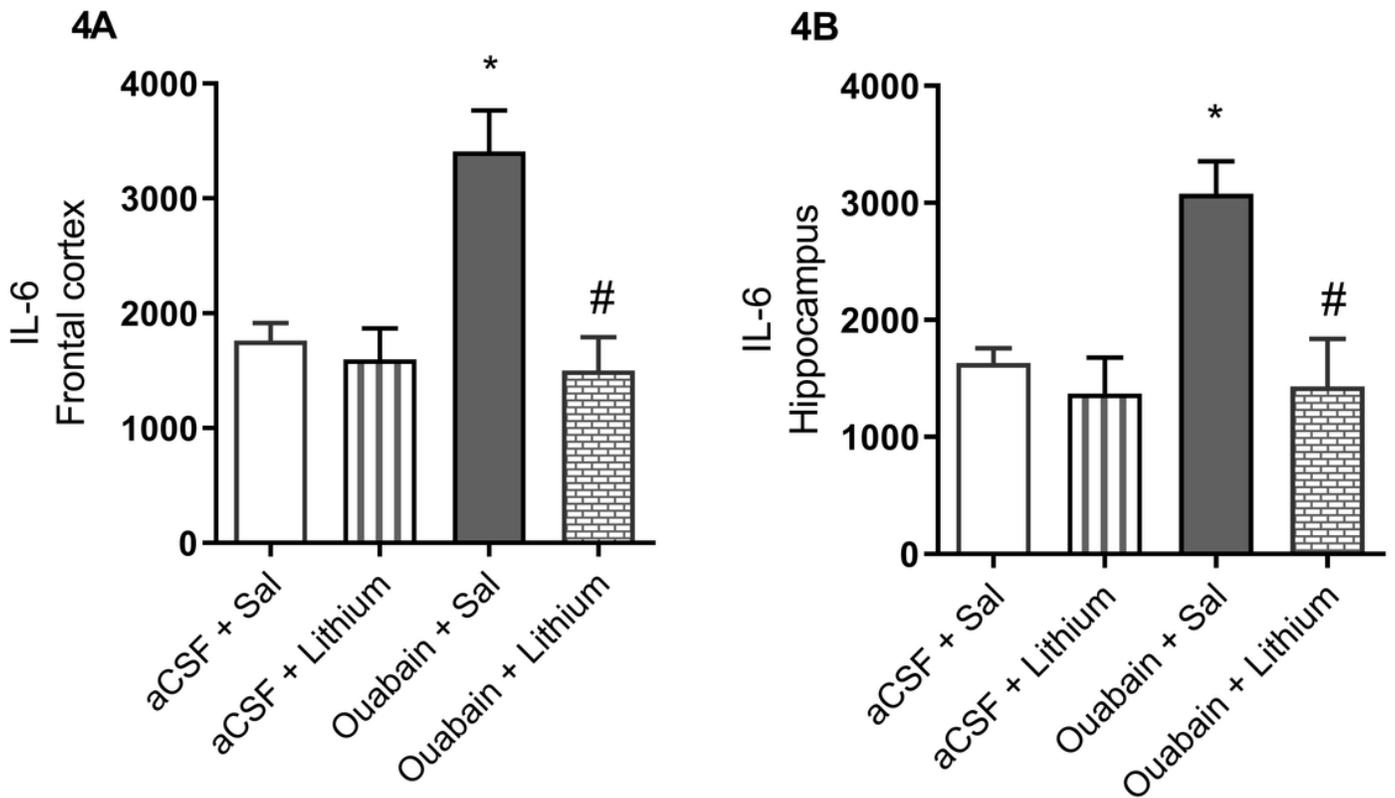


Figure 4

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the levels of interleukin 6 (IL-6) in the frontal cortex (**4A**) and hippocampus (**4B**) of adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (picograms per 100 milligrams [pg/100 mg] tissue). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group

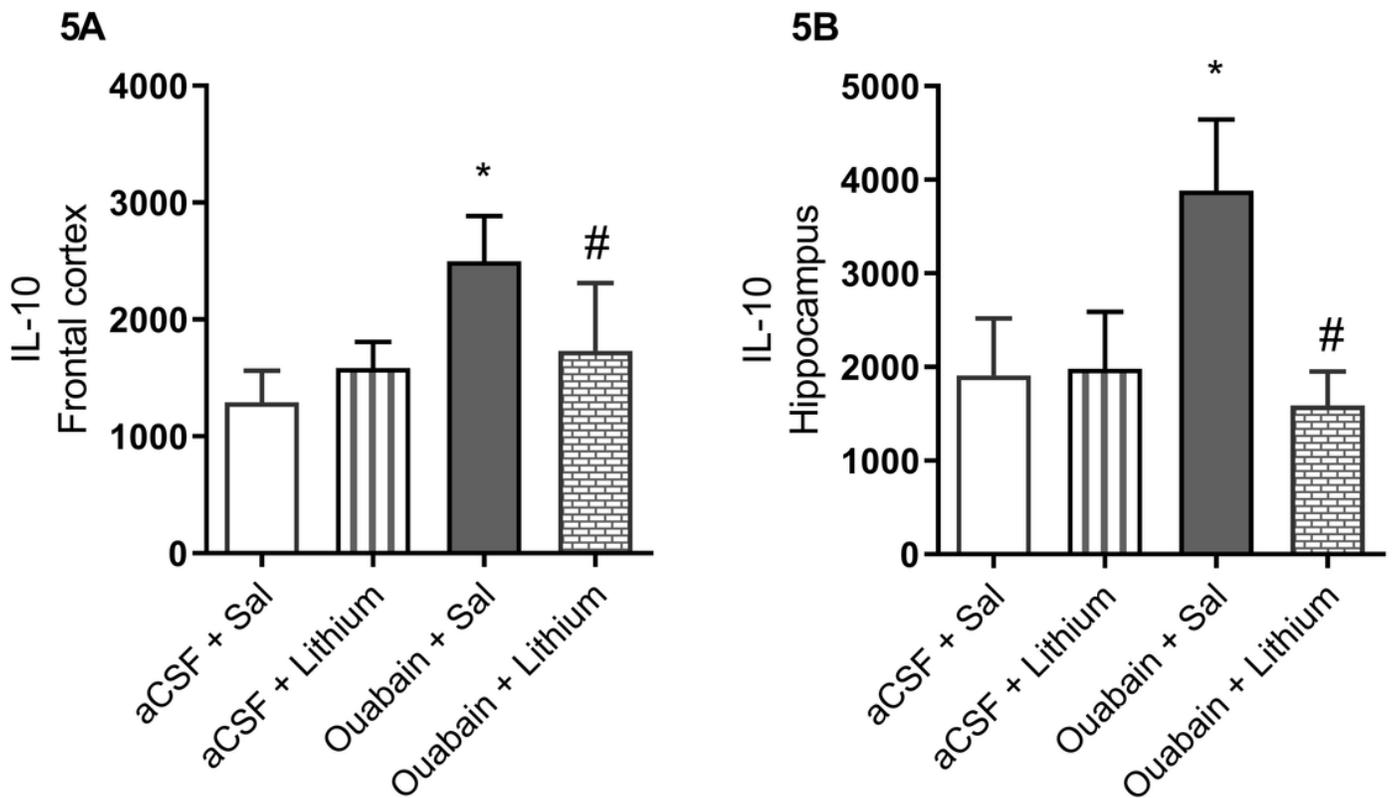


Figure 5

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the levels of interleukin 10 (IL-10) in the frontal cortex (**5A**) and hippocampus (**5B**) of adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (picograms per 100 milligrams [pg/100 mg] tissue). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group

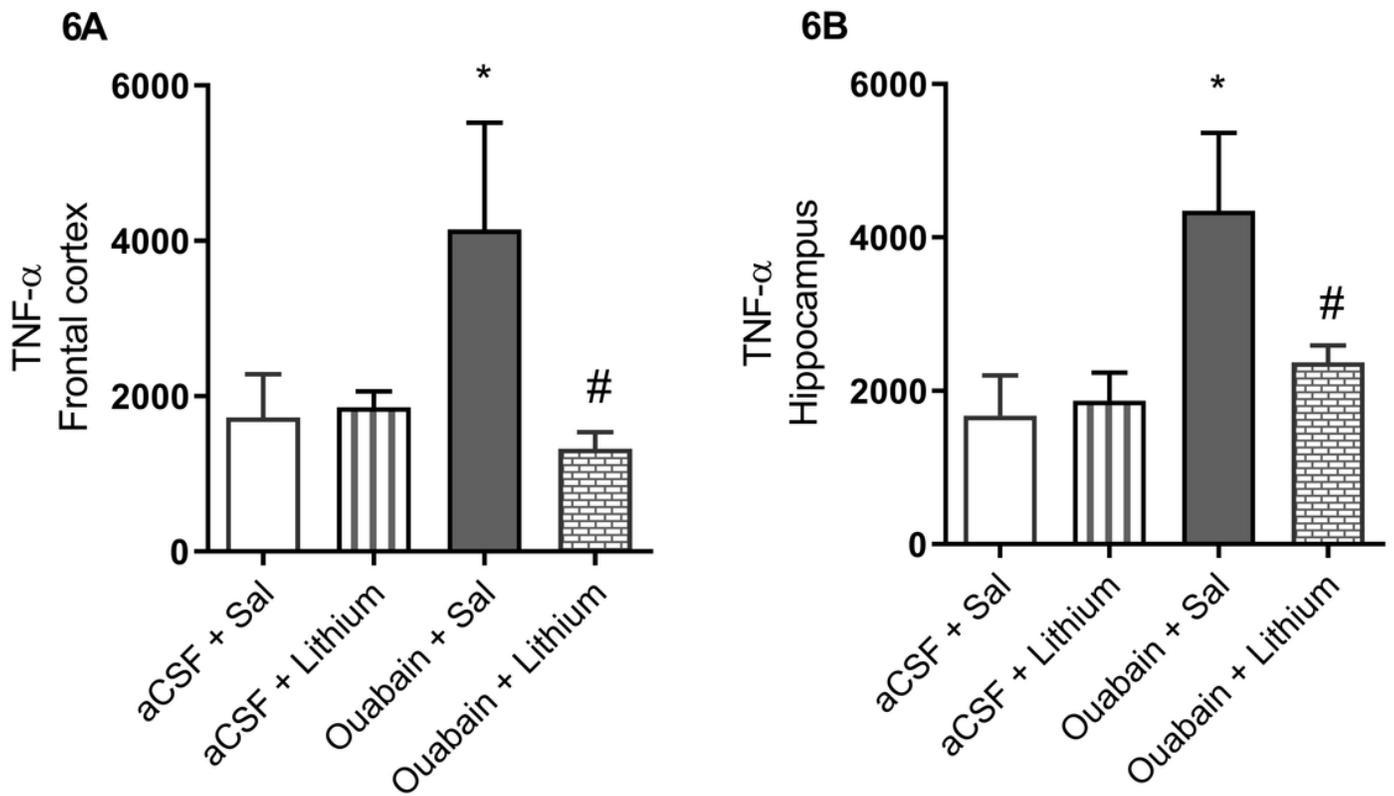


Figure 6

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the levels of the tumor necrosis factor α (TNF- α) in the frontal cortex (**6A**) and hippocampus (**6B**) of adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (picograms per 100 milligrams [pg/100 mg] tissue). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group

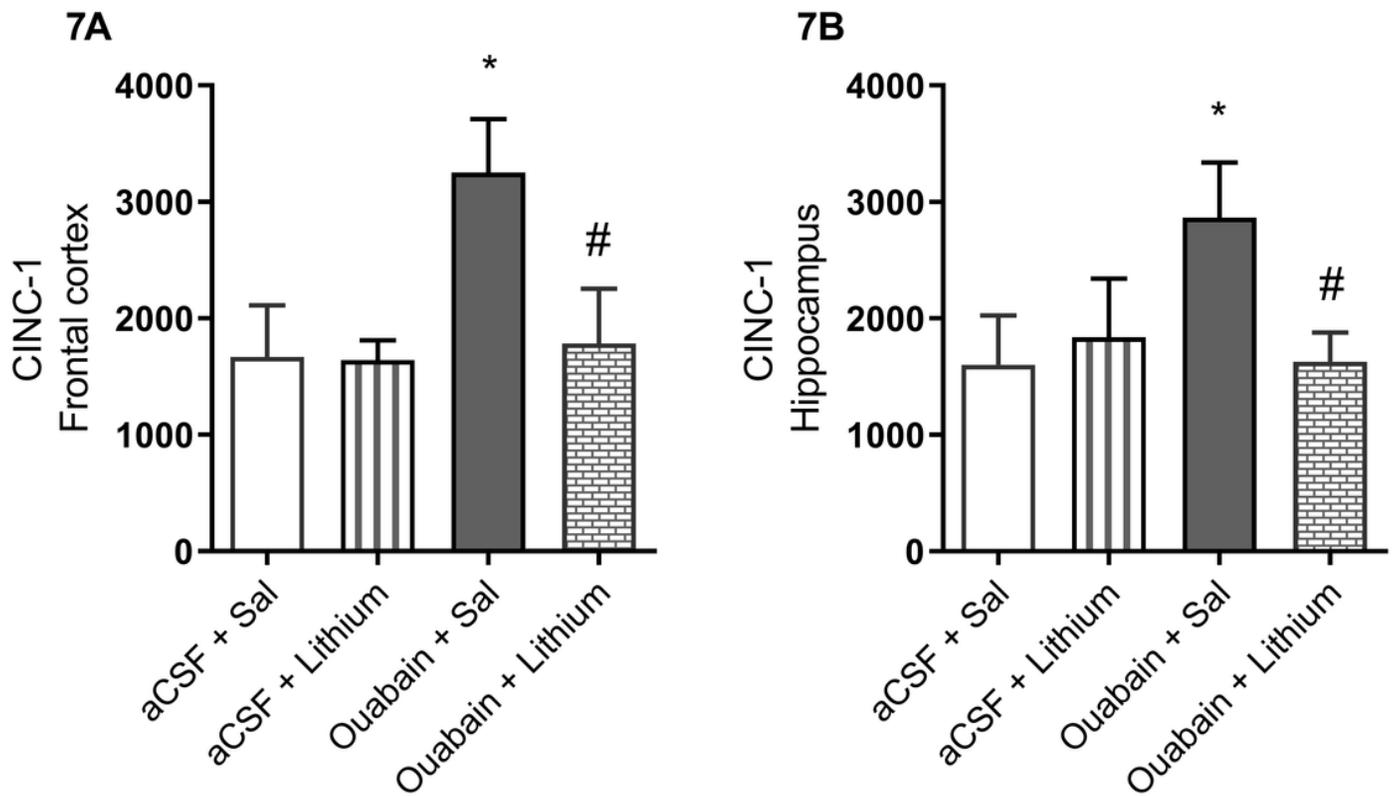


Figure 7

Effect of the intracerebroventricular (ICV) administration of ouabain (OUA) on the levels of the cytokine-induced neutrophil chemoattractant 1 (CINC-1) in the frontal cortex (**7A**) and hippocampus (**7B**) of adult male Wistar rats ($n = 8$ per group). Animals were subjected to the assessments 14 days following OUA infusion. Data were analyzed by two-way analysis of variance followed by Tukey's test when p was significant. Values are expressed as mean \pm standard error of the mean (picograms per 100 milligrams [pg/100 mg] tissue). * $p \leq 0.05$, as compared to aCSF + saline (Sal) group; # $p \leq 0.05$, as compared to OUA + Sal group