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Neuroinflammation induced by lipopolysaccharide leads to memory impairment and alterations in hippocampal leptin signaling

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ABSTRACT

Peripheral inflammation promotes immune-to-brain communication, mediated by cytokines that affect brain activity. Lipopolysaccharide (LPS) has been widely used to mimic systemic inflammation, and the adipokine leptin, released in this condition, modulates hypothalamic leptin receptors (ObR), contributing to sickness behavior. In this study, we used the intracerebroventricular (ICV) route for LPS administration in an attempt to evaluate an acute and direct of this pathogen-associated molecular pattern on leptin-mediated signaling in the hippocampus, where ObR has been implicated in modulating cognitive response. We used bilateral ICV injection of LPS (25 μg/ventricle) in 60-day-old male Wistar rats and the analysis were performed 48 h after surgery. Neuroinflammation was characterized in the LPS group by an increase in concentration of IL-1β, COX-2 and TLR4 in the hippocampus as well as glial fibrillary acidic protein (GFAP), indicating an astrocyte commitment. Cognitive damage was observed in the animals of the LPS group by an inability to increase the recognition index during the object recognition test. We observed an increase in the concentration of leptin receptors in the hippocampus, which was unaccompanied by changes in the proteins involved in leptin intracellular signaling (p-STAT3 and SOCS3). Moreover, we found a decrease in leptin concentration in the serum of the animals in the LPS group accompanied by an increase in TNF-α levels. Our results showed that neuroinflammation, even in an acute state, can lead to cognitive impairment and may be associated with leptin signaling disturbances in the hippocampus.

1. Introduction

Neuroinflammation has been well established as an important feature of various brain disorders. It can affect neural development, alter blood-brain barrier and lead to neurodegenerative diseases [1–5]. It has been suggested that neuroinflammation is involved in the progression of these disorders [2,6,7] and the cognitive impairment observed has been linked to proinflammatory cytokines [8–10]. In fact, IL-1β, IL-6, and TNF-α are able to modulate neuronal function, reducing hippocampal synaptic plasticity [11–14].

Behavioral and neurochemical changes, as a result of neuroinflammation, have been most frequently investigated by peripheral administration of lipopolysaccharide (LPS), which can directly and indirectly (via peripheral release of cytokines and hormones) affect the central nervous system (CNS). This leads to the establishing of immune-to-brain communication [15–17]. However, it is necessary, complementary and relevant to understand the direct and acute effect of LPS on CNS receptors for the molecular pattern associated with the pathogen, even to characterize bidirectional communication [18,19]. The brain circuitry involved in recognition memory includes the medial prefrontal cortex, the perirhinal cortex and the hippocampus, all of which play a critical role in object recognition (OR) task, in rodents (see [20] for a review). Cognitive deficits associated with hippocampal dysfunction assessed by OR have been used to study models of

Abbreviations: COX, cyclooxygenase; GFAP, Glial fibrillary acidic protein; HBSS, Hank's balanced salt solution; ICV, intracerebroventricular; IL, interleukin; LPS, lipopolysaccharide; OF, open field; OR, object recognition; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNF-α, tumor necrosis factor alpha

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neuroinflammation [21,22].

In the case of peripheral inflammation, leptin has been suggested as one of the adipose signals that modulates the inflammatory response in the brain. This hormone has a critical role in leukocyte recruitment in the brain following severe systemic inflammation [23]. Leptin is a peptide hormone secreted mainly by the adipose tissue and its action is mediated by the ObR receptor [24]. It regulates many physiological functions, including energy homeostasis and immunity [25–27]. It acts as an adipokine on many cells of the immune system, stimulating its activation and proliferation, as well as reducing its apoptosis [28,29]. However, the specific roles played by leptin in neuroinflammation are still under debate. There is evidence of leptin exerting pro-inflammatory action [30–32], as well as anti-inflammatory action in rats and mice [33–35]. More recently, leptin has been suggested as a licenser and enhancer of immune functions. In situations of low concentration or impaired signaling a deficit occurs in the immune response and in high pathological concentrations, such as obesity, it assists in a harmful inflammatory response [29].

In CNS, the most studied and known function of leptin is in the regulation of food intake, acting on the hypothalamus. Neuroinflammation in this tissue has been implicated in leptin resistance, which is one of the factors that leads to obesity [36]. This may occur in the hypothalamus through the LPS activation of inflammatory pathways, such as TLR4-IKKβ [37,38], which, in turn, lead to an increase in SOCS3 and PTP1B. These molecules inhibit JAK2-STAT3 signaling of leptin through binding to a specific tyrosine residue on ObR or dephosphorylating JAK2, respectively [36,39].

The presence of ObR in other brain regions, such as the hippocampus, has already been identified, where leptin may play a role in the regulation of memory and learning [40]. In support of this, db/db mice and Zucker rats, both of which have mutations in the leptin receptor gene, exhibit a cognitive deficit [41,42]. Furthermore, leptin has also been implicated as neuroprotective in certain neurodegenerative diseases associated with neuroinflammation, such as Alzheimer's and Parkinson's diseases [43]. On the other hand, in multiple sclerosis, high levels of serum leptin are related to episodes of exacerbation of the disease, while a reduction is associated with an improvement of symptomatology [44].

Information is limited on the effect of neuroinflammation *per se* on leptin signaling in the hippocampus, which could be associated with the cognitive impairment observed in neuroinflammatory conditions. Considering the involvement of leptin in neuroinflammatory diseases and its relation with memory and learning, we investigated acute neuroinflammation induced by a single intracerebroventricular (ICV) administration of LPS, hypothesizing a change in leptin signaling in the hippocampus and the subsequent cognitive response. In addition, we assessed the central and peripheral inflammatory parameters, as well as the astrocytic response to neuroinflammation.

2. Material and methods

2.1. Materials

Monoclonal anti-S100B (SH-B1) and LPS from *Escherichia coli* 055:B5 were purchased from Sigma [Missouri, USA]. Polyclonal anti-S100B was purchased from DAKO [São Paulo, Brazil], anti-rabbit, anti-mouse and anti-goat peroxidase-linked and ECL Western Blotting Detection Reagents were purchased from GE [Little Chalfont, United Kingdom]. Anti-ObR, anti-SOCS3, anti-p-STAT3 (Tyr705), anti-STAT3, anti-TLR4 and anti-COX-2 were purchased from Santa Cruz Biotechnology [Texas, USA] and anti-actin was purchased from Millipore [Massachusetts, USA].

2.2. Animals

All the procedures complied with the ARRIVE guidelines and were carried out in accordance with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the local authorities (Ethics Committee on the Use of Animals – UFRGS, nº 25337). Male adult Wistar rats (60 days old) were obtained from our breeding colony (Department of Biochemistry, UFRGS, Porto Alegre, Brazil). The animals were maintained under controlled light and environmental conditions (12 h light / 12 h dark cycle at a constant temperature of 22 ± 1 °C) and had free access to commercial chow and water.

The animals were weighed on digital scales before and 48 h after the surgical procedure to evaluate change in weight. The food and water consumption were evaluated during the 48 h after the surgery through weighing using digital scales. Different animals were used for behavioral tests and molecular measurements.

2.3. Surgical procedure

For ventricular access, the animals were anesthetized with ketamine/xylazine (75 and 10 mg/Kg, respectively, i.p.) and placed in a stereotaxic apparatus. A midline sagittal incision was made in the scalp and one burr hole was drilled in the skull over both ventricles. The following coordinates were used: 0.9 mm posterior to bregma; 1.5 mm lateral to sagittal suture; 3.6 mm beneath the brain surface [45]. The rats received an ICV injection of 5 µL of LPS 5 µg/µL or vehicle (*Hank's balanced salt solution* – HBSS, containing in mM: 137 NaCl, 5.36 KCl, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$, 0.63 $Na_2HPO_4{\cdot}7H_2O$, 0.44 KH_2PO_4, 4.17 $NaHCO_3$ and 5.6 glucose, adjusted to pH 7.4) in each ventricle [46]. Forty-eight hours after the surgical procedure, rats were anesthetized and the blood samples were collected by careful intracardiac puncture, using a 5-mL non-heparinized syringe to obtain 3 mL of blood. The hippocampi were dissected out and chopped in transverse slices of 0.3 mm obtained using a McIlwain Tissue Chopper, and slices were then separated and stored in a freezer at −80 °C until they were processed according to the procedure for each analysis. Blood samples were incubated at room temperature (25 °C) for 10 min and centrifuged at 1700 x g for 10 min to obtain serum, which was

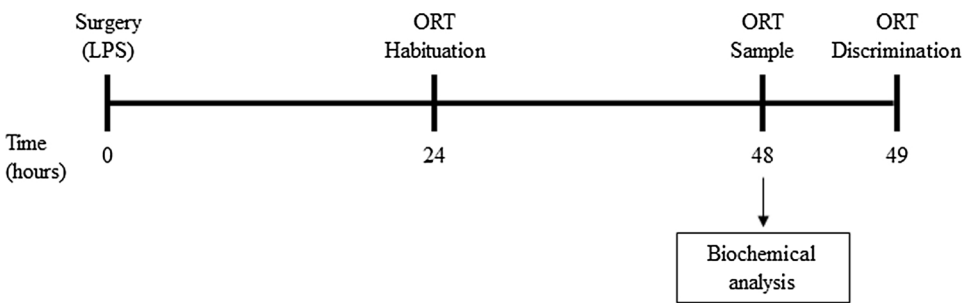

Fig. 1. Schematic representation of the experimental plan. Time is represented in hours. Cognitive evaluation is based on the Object Recognition Test (ORT) with three distinct phases: habituation, sample, and discrimination. Hippocampi and serum were harvested for biochemical evaluation. Different animals were used for behavioral tests and biochemical evaluation.

separated and stored at −80 °C until further analysis. A schematic representation of the experimental procedure is shown in Fig. 1, indicating times of surgery, cognitive behavior and biochemical analysis.

2.4. Object recognition task (OR task)

All experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). All apparatuses were cleaned with 10 % ethanol solution and then dried with a paper towel after each trial. The OR task was conducted in the open field (OF), as previously described [47] and adapted by Hansen [48]. The test consisted of three distinct phases: habituation, sample, and discrimination. The habituation phase was performed 24 h after the ICV injection of LPS, during which time the rats were allowed to explore the OF for 10 min, 24 h before the next phase, thus totaling 48 h after surgery, as well as molecular measurements. In the sample phase, two identical objects (C1 and C2 cubes) were placed in opposite corners of the OF, 20 cm distant from the walls and ~60 cm apart from each other, and the rats were allowed to explore them for 5 min. After the end of the sample phase, the rats were removed from the OF and kept in the home cage for 1 h. After that, in the discrimination phase, an identical copy of the familiar object (C3) and a novel T-shaped object (T) were placed in the locations previously occupied by C1 and C2, and the rats were allowed to explore the objects for 5 min. The locations of the objects were counterbalanced in each session. The time spent by the rats exploring each object was monitored with a video system placed in an adjacent room and manually measured by a blinded researcher for group distribution. Exploration of an object was defined as sniffing or touching the object with the nose and/or forepaws. To analyze the cognitive performance of rats, a recognition index was calculated in each session, as follows; time exploring novel object/time exploring both objects. Recordings and mobility analyses were performed using ANY-maze behavioral tracking software version 6.17.

2.5. S100B and GFAP measurement

Hippocampal slices were homogenized in PBS (50 mM NaCl, 80 mM Na_2HPO_4, 20 mM $NaH_2PO_4{\cdot}H_2O$, pH 7.4), containing 1 mM EGTA and 1 mM phenylmethyl-sulphonyl fluoride (PMSF). S100B was measured by ELISA, as previously described [49]. Briefly, 50 µl of sample plus 50 µl of Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B. For serum samples, polyclonal anti-S100 was incubated for 30 min and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. For the hippocampal slices, both antibodies were incubated together for 1 h. Color reaction with o-phenylenediamine was measured at 492 nm. The standard S100B curve ranged from 0.002 to 1 ng/mL for serum samples and 0.02–10 ng/ml for the hippocampal slices. ELISA for GFAP was carried out, as previously described [50], by coating microtiter plates with 100 µL of samples for 24 h at 4 °C. Incubation with a polyclonal anti-GFAP from rabbit for 1 h was followed by incubation with an anti-rabbit conjugated with peroxidase for 1 h, at room temperature. A colorimetric reaction with o-phenylenediamine was measured at 492 nm. The standard human GFAP (from Calbiochem) curve ranged from 0.1–5 ng/mL. Data from hippocampal samples were normalized against total protein, which was measured by Lowry's method, modified by Peterson, using bovine serum albumin as a standard [51].

2.6. Quantification of IL-1β, TNF-α and leptin

Hippocampal slices, for IL-1β and leptin quantification, were homogenized in diluent buffer followed by centrifugation at 1000 × g for 5 min at 4 °C and the assay was carried out in 100 µL of supernatant. Serum samples, for TNF-α and leptin quantification, were collected and processed as indicated by the manufacturer. The IL-1β, TNF-α and leptin content were measured using a rat ELISA from eBioscience (Ref. 88-6010-22, San Diego, USA), Peprotech (Ref. 900-M54, Rocky Hill, NJ, USA) and Sigma (Ref. 900-M54, Rocky Hill, NJ, USA), respectively. Leptin data are expressed as arbitrary units that represent the sample absorbance corrected for the absorbance value of the blank. Data from hippocampal samples were normalized against total protein.

2.7. Western blot analysis

Equal amounts (30 µg) of proteins from each sample were boiled in sample buffer (0.0625 M Tris–HCl, pH 6.8, 2 % (w/v) SDS, 5 % (w/v) β-mercaptoethanol, 10 % (v/v) glycerol, 0.002 % (w/v) bromophenol blue, 1 mM sodium orthovanadate) and electrophoresed in 12 % (w/v) SDS-polyacrylamide gel. The separated proteins were blotted onto a nitrocellulose membrane. Antibodies anti-ObR, anti-SOCS3, anti-pSTAT3, anti-STAT3, anti-COX2, anti-TLR4 or anti-actin were used at a dilution of 1:5000. After incubating with the primary antibody overnight at 4 °C, membranes were washed and incubated with peroxidase-conjugated antibody immunoglobulin (IgG) at a dilution of 1:10,000 for 1 h at 4 °C. Actin expression was used as a control of the total amount of protein. The chemiluminescent reactions were developed using luminol as the substrate (ECL Western Blotting Analysis System, GE Healthcare) and evaluated in the luminescence image analyzer (Image Quant LAS4000 from GE). The immunocontent of all proteins was determined for optical density using ImageJ software.

2.8. Statistical analysis

Statistical analysis was performed with SPSS software version 20.0. We used the Student's *t*-test for the evaluation of all the results. For the behavioral experiments where we used the one-sample *t*-test to determine whether the recognition index was different from a chance performance (0.5). For the analysis of body weight variation we used repeated measures ANOVA followed by Sidak. We considered $p < 0.05$ as statistically significant.

3. Results

3.1. LPS induces neuroinflammation in hippocampus

The intracellular content of the proinflammatory cytokine IL-1β, COX-2 and TLR4 in the hippocampus of rats was assessed 48 h after a single ICV injection of LPS or vehicle in order to determine the presence of an inflammatory response in this tissue. Intracellular levels of these inflammatory markers were significantly higher in the LPS group when compared to sham rats (Fig. 2) ($p < 0.001$ for IL-1β; $p = 0.034$ for COX-2; and $p = 0.037$ for TLR4), demonstrating the presence of neuroinflammation in this tissue.

3.2. Acute LPS ICV causes memory impairment

The object recognition task was used in order to verify memory impairment. We observed, in the discrimination phase, that acute LPS ICV injection cause memory impairment. The recognition index did not increase in the LPS group when compared to chance performance ($p = 0.438$), whereas, in the sham group, there was an increase in the recognition index ($p = 0.005$). It is important to notice, that in the sample phase, neither group differed significantly when comparing chance performance, indicating similar exploration of both familiar objects (Fig. 3) ($p = 0.225$ for LPS group; $p = 0.160$ for sham group). There was no significant difference between the groups in the total time of exploration of both objects during the sample and the discrimination phases ($p = 0.575$ and $p = 0.670$, respectively). Besides, there was no difference between total distance travelled, average speed, total time mobile and total time immobile during the habituation phase (Table 1) ($p = 0.969$, $p = 0.969$, $p = 0.932$, $p = 0.932$, respectively), demonstrating a similar exploratory capacity among the groups.

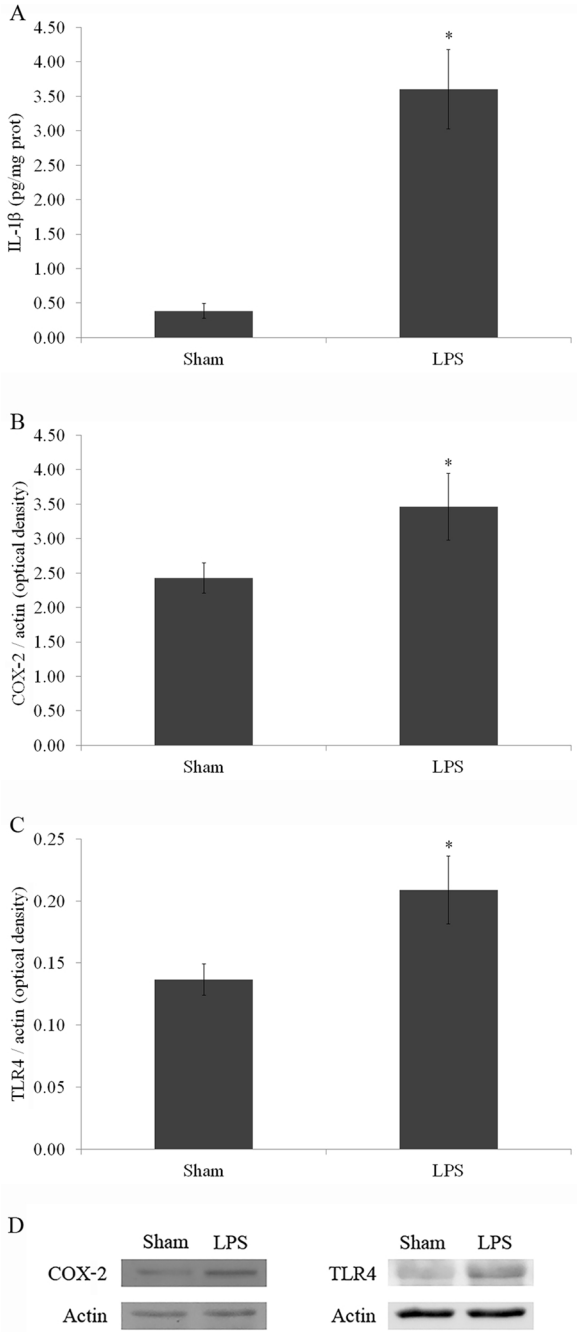

Fig. 2. LPS induces neuroinflammation in the hippocampus. ICV LPS or saline solution injection was carried out on adult Wistar rats under anaesthesia. After 48 h, the hippocampus was dissected and the IL-1β (A), COX-2 (B) or TLR4 (C) content measured by immunoblot. Representative images are shown in D. Data are expressed as means ± S.E.M (N = 5–8 animals per group). * Significantly different from sham group (Student *t*-test, $p < 0.05$).

3.3. Acute LPS ICV causes an increase in GFAP, but not in S100B content in the hippocampus

The intracellular content of GFAP and S100B was analyzed in order to assess a possible astrocytic response in the hippocampus of rats subjected to acute LPS ICV. We observed a significant increase of GFAP in the hippocampus during the neuroinflammatory response (Fig. 4A) ($p = 0.007$), without any alteration in S100B immunocontent (Fig. 4B) ($p = 0.100$).

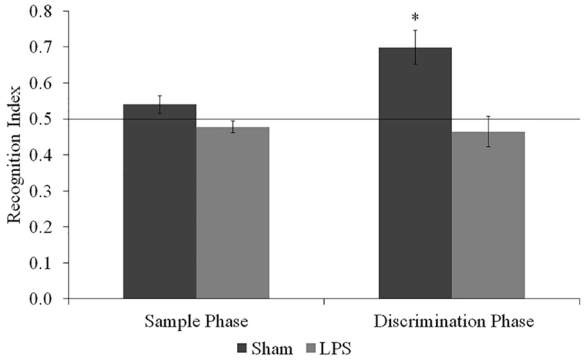

Fig. 3. Neuroinflammation causes memory impairment. The recognition index was evaluated 48 h after the ICV LPS or saline solution injection. The line on the graph indicates recognition index 0.5. Data are expressed as means ± S.E.M (N = 5–8 animals per group) and were analyzed by one-sample *t*-test. * indicates $p < 0.005$ versus chance level (indicated by the line).

Table 1
Mobility data in the behavioral habituation phase.

	Total distance travelled (m)	Average speed (mm/s)	Total time mobile (s)	Total time immobile (s)
Sham	9.56 ± 1.68	32.0 ± 5.5	121.0 ± 19.1	171.9 ± 19.1
LPS	9.44 ± 2.49	31.6 ± 8.3	111,3 ± 23.0	181.62 ± 23.0

All data are expressed as means ± standard error (N = 5–8 animals per group) and were considered statistically different when $p < 0.05$ (Student's t-test).

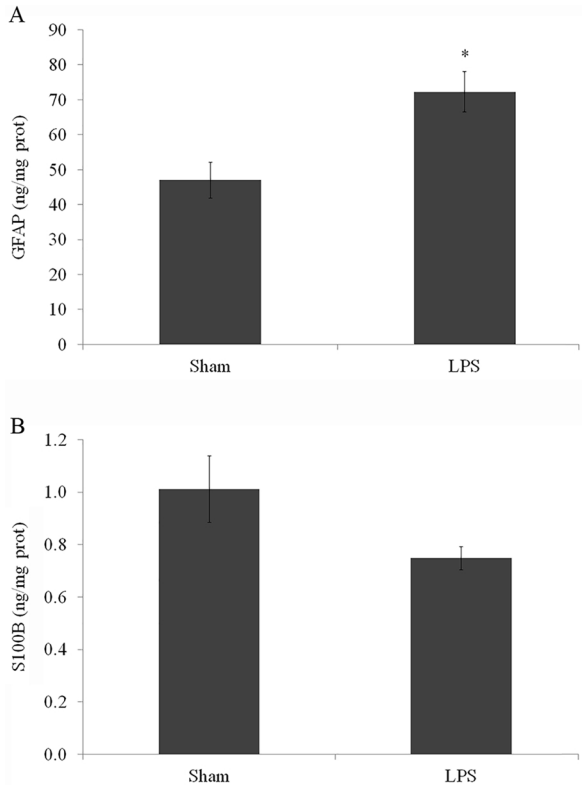

Fig. 4. Neuroinflammation causes an increase in GFAP, but does not change S100B content in the hippocampus. ICV LPS or saline solution injection was carried out on adult Wistar rats under anaesthesia. After 48 h, the hippocampus was dissected and the GFAP (A) and S100B (B) content measured by ELISA. Data are expressed as means ± S.E.M (N = 5–8 animals per group). * Significantly different from sham group (Student *t*-test, $p < 0.05$).

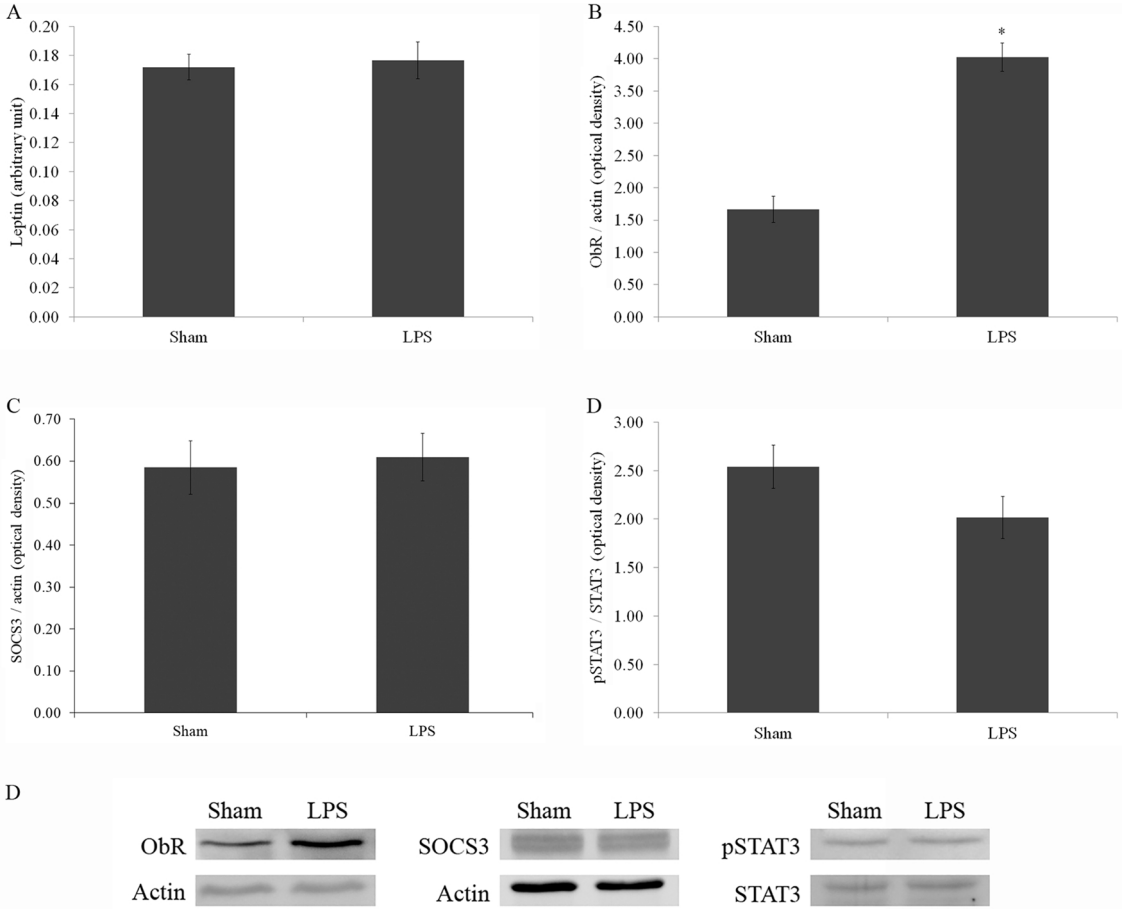

Fig. 5. Neuroinflammation increases leptin signaling in the hippocampus. ICV LPS or saline solution injection was carried out on adult Wistar rats under anaesthesia. After 48 h, the hippocampus was dissected and the leptin (A), ObR (B), SOCS3 (C) and STAT3 (D) content measured by western blot. Representative images are shown in D. Data are expressed as means ± S.E.M (N = 5–8 animals per group). * Significantly different from sham group (Student *t*-test, $p < 0.05$).

3.4. Neuroinflammation increases leptin signaling in the hippocampus

We evaluated the effect of neuroinflammation induced by acute LPS ICV injection on leptin expression in the hippocampus. Although neuroinflammation was not able to induce any significant change on intracellular levels of leptin in the hippocampus compared to sham rats (Fig. 5A) ($p = 0.766$), we observed a significantly higher immunocontent of ObR in the hippocampus of LPS-treated rats (Fig. 5B) ($p < 0.001$). No alteration in SOCS3 immunocontent or in p-STAT3 / STAT3 ratio were observed (Fig. 5C, D) ($p = 0.775$ and $p = 0.107$, respectively).

3.5. Neuroinflammation causes changes in serum TNF-α, leptin and S100B

In order to investigate if neuroinflammation induced by LPS could lead to a systemic inflammatory response we measured serum TNF-α levels. LPS-treated rats showed a slight increase in serum TNF-α (Fig. 6A) ($p = 0.020$). On the other hand, leptin and S100B in the serum were significantly decreased (Fig. 6B, C) ($p = 0.023$ and $p = 0.001$, respectively).

3.6. Neuroinflammation causes weight loss and reduces food intake

There was no significant difference in animal weights between the groups before or after ICV injection ($p = 0.188$ and $p = 0.135$, respectively). However, the LPS group showed a significant reduction in weight, forty-eight hours after surgery ($p < 0.001$), while the sham group maintained its weight ($p = 0.104$). Accordingly, the food consumption in the LPS group was lower ($p = 0.020$). There was no difference in water consumption over the 48 h (Table 2) ($p = 0.698$).

4. Discussion

Cognitive deficits observed in tasks dependent on hippocampal integrity have been induced by neuroinflammation as a result of both peripheral and central LPS administration [18,52]. However, research has concentrated more on memory impairment caused by inflammation in response to chronic LPS exposure [5,10,53,54]. In the present study, we induced an acute neuroinflammation through a single ICV injection of LPS. Inflammation was demonstrated by an increase in IL-1β, COX-2 and TLR4 expression found in the hippocampus, an important brain region for learning and memory [55,56]. We found object recognition task impairment in animals subjected to acute ICV-LPS inflammation.

Several studies have shown that induction of neuroinflammation following intraperitoneal injections of LPS leads to cognitive impairment in rodents. This was found in recognition memory, contextual fear conditioning and spatial memory [52,54,57–59]. Even though there are variations in doses and times used between studies, it is clear that peripheral inflammation can lead to a brain inflammatory response with consequent memory impairment. In addition, other neuroinflammation studies using LPS ICV injections have also shown cognitive impairment in rodents. Although most of these studies use multiple LPS injections to induce chronic neuroinflammation, a few studies have also shown cognitive impairment in models of acute neuroinflammation, such as the present study. Iloun et al. [60] and Wu et al. [18] using a single ICV LPS injection of similar doses, demonstrated cognitive

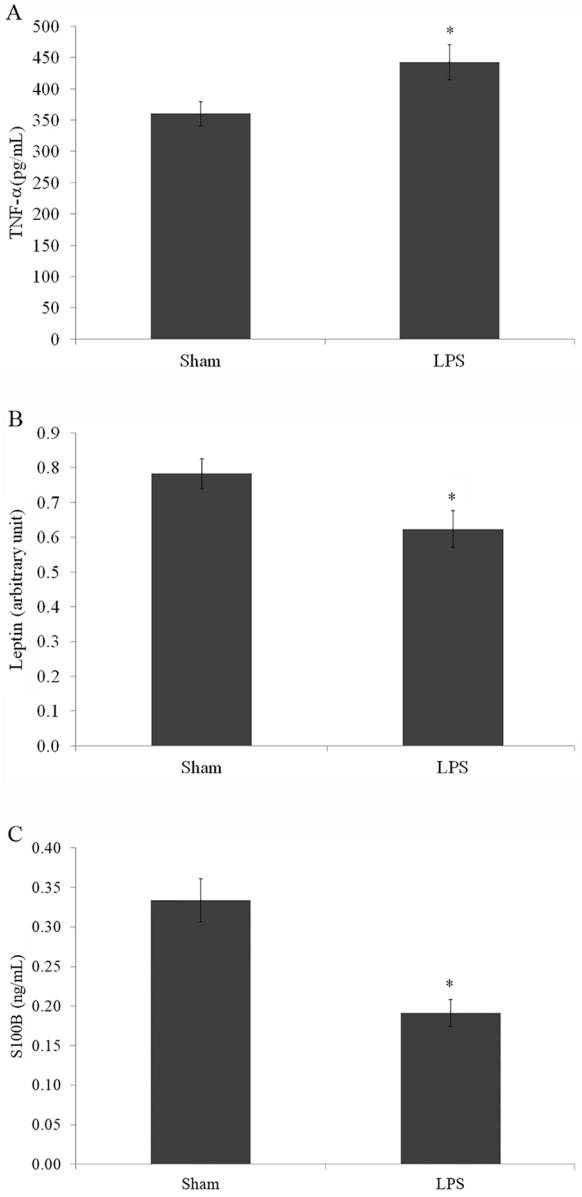

Fig. 6. Neuroinflammation causes changes in serum TNF-α, leptin and S100B. ICV LPS or saline solution injection was carried out on adult Wistar rats under anaesthesia. After 48 h, blood was collected by intracardiac puncture, and TNF-α (A), leptin (B) and S100B (C) were measured by ELISA from the serum obtained. Data are expressed as means ± S.E.M (N = 5–8 animals per group). * Significantly different from Sham group (Student *t*-test, $p < 0.05$).

Table 2
Body weight, food intake and water consumption in response to neuroinflammation.

	Body weight before LPS (g)	Body weight 48 h after LPS (g)	Food intake (g)	Water consumption (mL)
Sham	273.8 ± 8.7	269.6 ± 7.2	22.0 ± 1.4	32.0 ± 1.6
LPS	289.3 ± 6.5	254.7 ± 5.5#	10.7 ± 0.6*	32.9 ± 0.4

All data are expressed as means ± standard error (N = 5–8 animals per group) and were considered statistically different when $p < 0.05$. # indicates significantly different from body weight before LPS injection (repeated measures ANOVA) * indicates significantly different from sham group (Student's t-test).

impairment in spatial and recognition memory. However, despite being considered a model of acute neuroinflammation, the analyses were performed six days after the ICV injection. Our study may show that cognitive impairment has already begun prior to this, being seen 48 h after the ICV LPS injection. These effects of LPS have been related to the binding of LPS to TLR4 which activates the nuclear factor-κB pathway in microglia and astrocytes [59,61]. This inflammatory activation of glial cells leads to the release of neurotoxic substances such as nitric oxide, glutamate, cytotoxic cytokines, and superoxide radicals that leading to memory impairment [52,62,63].

In a harmful CNS situation, the first cell type to respond is the microglia. These cells are recruited and act by phagocyting cell pathogens or debris and releasing pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, as well as chemokines [64]. These inflammatory molecules activate other cell types involved in neuroinflammation, such as astrocytes. Moreover, astrocytes also exhibit TLR and are able to respond directly to LPS [17]. Therefore, the production of cytokines, in part, may be due to astroglial reactivity.

Several studies have shown that changes in astrocytic reactivity can lead to memory impairment and this has been associated with their ability to regulate synaptic formation, transmission and plasticity [65]. Astrocytes have been shown to play an important role in the immune surveillance of the CNS. These cells are able to modulate the expression and secretion of various proteins in response to injury in a process called glial reactivity [66]. The increase in GFAP expression, a cytoskeleton protein characteristic of astrocytes, is considered an indicator of this reactivity [67]. Moreover, an increase in cytokines and S100B secretion may occur in an inflammatory astroglial response [17]. In our study, neuroinflammation caused an increase in hippocampal GFAP expression, confirming the astroglial reactivity of this tissue. However, the increase in GFAP alone is not sufficient to identify an effect on astrocyte functionality (eg, a decrease in glutamate uptake), whose impairment may affect synaptic plasticity and contribute to cognitive impairment. Although S100B content was not significantly altered in the hippocampus of these animals, it should be noted that intracellular content and secretion of S100B are not necessarily related, as was observed in *in vitro* studies [17,68,69]. Furthermore, we previously observed that ICV LPS is able to increase cerebrospinal fluid S100B, which would also indicate astroglial reactivity [17].

Besides its role in the control of food intake, leptin has been shown to be an important factor in memory formation, since animals with a deficiency in leptin receptor or in the production of this hormone, develop cognitive impairment [41]. In support of this, intrahippocampal administration of leptin improved memory in animal models of neurodegenerative diseases [43,70,71]. Leptin plays an important role in the regulation of the immune system and may play a protective role in exacerbated inflammation. On the other hand, a pro-inflammatory effect of leptin has been associated with the progression of multiple sclerosis. In fact, an increase in the signaling of this adipokine in animal models with this disease has been identified [44]. In our study, we observed an increase in the immunocontent of leptin receptor in the hippocampus in response to acute neuroinflammation. A similar response was found in the immune cells of animal models with multiple sclerosis [72]. This suggests a possible increase in the signaling of this adipokine in this tissue. However, this change is not related to the JAK / STAT signaling pathway, evidenced by the fact that we did not find alteration in STAT3 phosphorylation (Tyr705) nor in the content of SOCS3 in the hippocampus. Possibly, the increase in the immunocontent of the leptin receptor may be leading to changes in other leptin-activated signaling pathways. It is important to mention that although we investigated only leptin signaling, there are many other hippocampal alterations which may be occurring, such as BDNF signaling [73] or glucocorticoid signaling [74]. These may contribute to explain the cognitive deficit found in LPS-treated group.

It is known that leptin plays an important role in neuroinflammation induced by a peripheral inflammation, serving as an adipokine from the

periphery to the brain [75,76]. Peripheral inflammation induced by LPS, using different signals including leptin, affect hippocampal plasticity in rodents [77–79]. On the other hand, reduced levels of leptin were found in survivor mice subjected to sepsis, cecal ligation and puncture [80]. Herein, we investigated serum leptin levels in response to a central inflammation induced by a single ICV administration of LPS. In contrast to peripheral inflammation, we found a reduction in serum leptin. Although we found an increase in serum TNF-α, suggesting a possible peripheral inflammation, we observed a decrease in the quantity of leptin released by adipose tissue. This indicates that the hormone/adipokine has its profile of secretion altered by inflammation depending on how it was initiated.

In addition, a decrease in serum S100B was found. S100B is an astrocyte marker in the CNS, but is also expressed in adipocytes [81]. The concentration of S100B in the serum can be attributed to the secretion of this protein by the adipocytes and also, in part, to astrocyte secretion [81]. This protein has been proposed as a peripheral marker of central damage, since in this situation BBB permeability may increase, leading to an increase in the passage of this protein to the bloodstream [82]. Conversely, S100B serum levels do not always directly reflect the concentration of this protein in the CNS [69]. In light of this, the decrease in serum S100B observed in this study should be carefully interpreted. It could be better attributed to alteration in peripheral release than to release in the CNS, since acute LPS induced an increase of cerebral spinal fluid S100B [17].

Moreover, a correlation between circulating levels of S100B and leptin with altered BMI in humans [83] has been shown. Obese individuals have higher levels of S100B and leptin, whereas in patients with anorexia nervosa, the lower levels of S100B and leptin observed return to normal with weight gain [84]. Therefore, we could consider that the decrease in the levels of these two compounds in the serum of animals exposed to LPS ICV injection may be reflect the fast weight loss, which was statistically significant in LPS-treated animals. However, it has been reported that single intraperitoneal administration of LPS, but not the repeated, decreased body weight [85]. It is also important to note that the present study was performed only on males, so research with females should be done in the future.

5. Conclusions

Our results demonstrate that acute central neuroinflammation induced by a single ICV administration of LPS, characterized IL-1β, COX-2 and TLR4 in the hippocampus as well as of glial fibrillary acidic protein (GFAP), leads to cognitive impairment and may be associated with altered leptin signaling in the hippocampus. These data reinforce and broaden knowledge relative to the consequences of neuroinflammation in chronic inflammatory disorders. Acute central neuroinflammation also caused reduced serum leptin, suggesting that an additional peripheral alteration. This may help in the understanding of the role played by this hormone/adipokine during these neuroinflammatory events, as well as its role in neurodegenerative diseases.

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Declaration of Competing Interest

None.

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