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Maternal deprivation increases microglial activation and neuroinflammatory markers in the prefrontal cortex and hippocampus of infant rats

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ABSTRACT

A relationship between neuroinflammation and the development of psychiatric disorder have been revealed by many studies in the past decade. Although studies have shown that stressors can induce long-term changes that may be related to behavioral responses, these alterations have been poorly studied soon after a stressor, such as maternal deprivation (MD). Thus, this study was designed to investigate the acute effect of experimental induction of MD on inflammatory and microglial activation markers in the brain of infant rats. Early MD was induced from postnatal day (PND) 1–10. On PND 10 the prefrontal cortex (PFC) and hippocampus from MD and control groups were removed to investigate microglial activation and neuroinflammatory markers. In the PFC the expressions of cluster of differentiation molecule 11B (CD11B), toll-like receptor (TLR)-2, and TLR-4 were increased in rats subjected to MD. The arginase expression was elevated in the PFC and hippocampus of maternally deprived rats. The cytokines interleukin-5 (IL-5), −6, −7, −10, tumor necrosis factor (TNF-α), and interferon gamma (INF-γ) were increased in the PFC of MD rats group. In the PFC the macrophage inflammatory proteins (MIP)-1α levels were reduced in MD rats group. In the hippocampus only the levels of TNF-α and INF-γ were elevated in infant rats subjected to MD. In conclusion, our results support the hypothesis that neuroinflammation and microglial activation, mainly in the PFC, could be involved with changes in the brain resident cells following MD, and these alterations could be associated to the development of psychiatric conditions late in life.

1. Introduction

Studies have been revealed that children exposed to early life stress, such as abuse, negligence, and maternal separation have more cognitive impairment, and risk for the development of anxiety and depression (Chugani et al., 2001; Herringa et al., 2013). Comparable results were reported in rodent models of depression (Maciel et al., 2018; Réus et al., 2015a; Valvassori et al., 2014). Recent evidences expose that these behavioral influences are mediated by inflammatory processes (Delpech et al., 2016; Johnson and Kaffman, 2018), including increased proinflammatory cytokines levels (Réus et al., 2017) and microglial activation (Delpech et al., 2016; Roque et al., 2016; Reus et al., 2018b; 2019).

Microglial cells have been pointed out as key regulators of synaptic remodeling during development in the adult central nervous system (CNS), and these mechanisms are mediated by immune molecules which interact with microglia and synapses (Wu et al., 2015). Pro-inflammatory cytokines from periphery can lead to a microglial activation that in turn can release other neuroinflammatory markers and

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affect other resident cells in the CNS (Nakamura et al., 2005; Zhang et al., 2010). A neuroinflammatory status as well as microglial activation have been reported as key factors involved with the pathophysiology of neuropsychiatric conditions, including major depressive disorder (MDD), and schizophrenia (De Picker et al., 2017; Réus et al., 2015b, 2019).

Neuroinflammatory processes may directly influence the neurodevelopment. In fact, in preterm neonate's plasma increased levels of interleukin (IL)-6 were associated with development delay (Magalhães et al., 2018). In addition, a correlation between chemokine CXCL8/IL-8 plasma levels with cognitive and motor delay was observed (Magalhães et al., 2018). In rodents subjected to maternal separation it was found changes in the microglial cells and an increase in the expression of genes involved in inflammation between postnatal day (PND) 14 and 28 (Delpech et al., 2016). Previously, studies from our group demonstrated that rats exposed to maternal deprivation (MD) early in life displayed depressive-like behavior in adult life, and also an increase in the pro-inflammatory cytokines and in the microglial activation markers were evident during different developmental stages (Réus et al., 2017, 2019). Therefore, suggesting that an inflammatory process resulting from MD could be associated to the depressive symptoms that appear later in life. However, the neuroinflammatory and microglial markers changes resulting from MD in infant rats have been poorly investigated. Thus, the aim of this study was to investigate on neuroinflammatory status and microglial activation after the experimental induction of MD in infant rats.

2. Material and methods

2.1. Animals

For this study, female pregnant Wistar rats (E15) were obtained from Charles River. The pregnant rats were housed individually with *ad libitum* access to food and water. The pregnant rats were housed individually for the birth of the pups and their identification. All mothers and pups were kept on a 12-h light/dark cycle at a temperature of 23 ± 1 °C. Only male pups were subjected to the MD from PND 1–10. The control male pups were allowed to stay with the mother. All protocols were approved by the Institutional Animal Welfare Committee of the Center for Laboratory Animal Medicine and Care (CLAMC) for The University of Texas Health Science Center at Houston (UTHSC), TX, USA (AWC-15-0133). All possible efforts were made to reduce animal suffering and the number of animals used.

2.2. Experimental groups and maternal deprivation (MD) protocol

The deprivation protocol consisted of removing the mother from the residence box and taking her to another room. The pups were maintained in their home cage (grouped in the nest in the presence of maternal odor). The pups were deprived of the mother for 3 h per day during the PND 1–10. We prefer this MD protocol because it does not require the manipulation of the pups (Réus et al., 2017). At the end of each daily deprivation session, the mothers were returned to their home cages; this procedure was carried out during the light part of the cycle, between 9:00 a.m. and 12:00 p.m. The control rats remained in their resident boxes together with their mothers throughout the experiment. Pups from both control and MD groups were euthanized on PND 10.

2.3. Western blot analysis

Western blot analysis was performed as per the protocols of previous studies (Dandekar et al., 2017; Zanos et al., 2016). Rats were deeply anesthetized and decapitated to remove the brain from the skull. Quickly the PFC and hippocampus were then dissected on ice. The isolated tissue samples were frozen immediately in liquid nitrogen and then stored at −80 °C until further processing. Each rat brain region was thawed and homogenized in complete protease inhibitor cocktail tablets (Roche, Diagnostics, IN, USA). The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C. Protein concentrations in tissue plasma were determined using the bovine serum albumin (BSA) method. For the Western blot run, equal amount of protein (25–50 μg as recommended for each antibody) for each sample was loaded in Mini-Protean TGX precast gels (Bio-Rad, California, USA). Proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes using a Trans-Blot® Turbo™ system (Bio-Rad, California, USA). These PVDF membranes were blocked with 5% nonfat dry milk (Bio-Rad, California, USA) in tris-buffered saline plus 0.1% tween 20 buffer (TBST, Bio-Rad, California, USA) for 1 h at room temperature (RT) and kept overnight in a cold room on a shaker with each of the following primary antibodies (separately processed): Ionized calcium binding adaptor molecule (Iba)-1 (Abcam, ab108539), cluster of differentiation (CD)11B (Abcam ab75476), toll-like receptor (TLR)-2 (LSBio, LS-3C393195), TLR-4 (Abcam, ab30667), nitric oxide synthases (NOS) (Origen, TA314266), arginase 1(Thermo Fisher, PA5-18684) (1:1000). On the next day, the blots were washed three times in TBST and incubated with a horseradish peroxidase conjugated secondary antibody (1:10,000) for 1 h, at RT. After three final washes for 10 min each with TBST, bands were detected using enhanced chemiluminescence (Clarity Western ECL Substrate; Bio-Rad, California, USA) with the ChemiDoc MP (Bio-Rad, California, USA) western blotting imaging system. After imaging, the blots were incubated in the stripping buffer (Thermo Fisher Scientific; 46430, IL, USA) for 10–15 min at RT followed by three washes with TBST. The stripped blots were incubated in blocking solution (5% nonfat dry milk in TBST) for 1 h and incubated with the primary antibody directed against the β-tubulin (1:5,000, Abcam, Cambridge, USA) or β-tubulin (Abcam, ab6046) for loading control. Densitometric analysis of each protein was conducted using Image Lab™ software (Bio-Rad, California, USA).

2.4. Inflammatory markers

The cytokines levels [IL1-α, IL1-β, IL-5, IL-6, IL-7, IL-10, IL-12, TNF-α, INF-γ, 6-CSF, M-CSF, VEGF, GRO-KC, MCP-1, and Rantes] were assayed using multiplex fluorescent immunoassay kits (Bio-Plex Pro™ Rat Cytokine 15-Plex Assay). The xMAP platform used here was based on the Rules-Based Medicine (RBM) fluorescent beads and antibody pairs. These are sensitive, specific and widely used reagents, sourced by numerous manufacturers, and data collected using xMAP multiplex beads are widely reported in the literature in studies in which multiple proteins are assayed simultaneously. Tissue lysate were preparing according to the instructions provided by Bio-Plex Cell Lysis kit (#171304011) with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), followed by centrifugation at 4 °C for 10 min at 10,000 × *g*.

The assays were conducted in 96-well polystyrene, round-bottom microplates. Initially, 50 μL aliquot of the working bead mixture was transferred into the wells, and the plate was washed 2 times by adding 100 μL of assay buffer into each well. After, 50 μL of the standard, control or total extracts were added to each well, as indicated. The plate was incubated on a plate shaker (850 rpm) in the dark at RT for 60 min. The plate was then placed in the magnetic separator and incubated for separation for 60 s. The supernatant was carefully removed from each well by manual inversion. Beads were washed 3 times by adding 100 μL of assay buffer into each well to ensure the absence of any undesirable or non-specifically bound antibodies. After this protocol, 25 μL of a detection antibody were added to each well. Incubation was again conducted in darkness and at RT on a plate shaker (850 rpm) for 30 min, and washing was performed as previously described. Finally, 50 μL of streptavidin-PE was added to each well. The plate was incubated on a plate shaker (850 rpm) in the dark at RT for 10 min. The supernatant was carefully removed after magnetic separation of the beads by manual inversion, and washing was performed as previously

described. Assay buffer (125 μl) was added into each well, and the plate was placed onto a plate shaker for approximately 30 s in order to achieve gentle agitation of the beads. Samples were run in duplicate using a Bioplex system (Bio-Plex 200 Systems, BioRad, Hercules, CA) and data analysis was conducted in Bio-Plex Manager 4.0 using a 5-parameter logistic regression model.

2.5. Statistical analysis

The data from western blotting analysis and inflammatory markers were examined by Student's t-test for unpaired data and are expressed as the mean ± standard error of the mean (S.E.M). p values of < 0.05 were considered to be statistically significant. The analyses were performed using the Statistical Package for the Social Science (SPSS) software, version 21.0.

3. Results

3.1. Effects of MD on microglial markers in the PFC and hippocampus of infant rats

The expression of microglial activation markers by western blotting analysis are illustrated in Figs. 1–6. The Iba-1 expression was not altering in the PFC ($t = 0.222$, $df = 7$, $p = 0.831$, Fig. 1A) and hippocampus ($t = 0.788$, $df = 7$, $p = 0.456$, Fig. 1B). The expression of CD11B was increased in the PFC of MD rats ($t = -3.653$, $df = 4.268$, $p = 0.019$, Fig. 2A), however, in the hippocampus CD11B expression did not differ between deprived and control rats ($t = -1.391$, $df = 4.889$, $p = 0.224$, Fig. 1B). The TLR-2 and TLR-4 expression were elevated in the PFC of MD rats ($t = -3.108$, $df = 6$, $p = 0.021$, Fig. 3A, and $t = -2.550$, $df = 6$, $p = 0.044$, Fig. 4A, respectively). In the hippocampus we did not find changes in the TLR-2 or TLR-4 ($t = 0.234$, $df = 7$, $p = 0.822$, Fig. 3B, and $t = -.79$, $df = 7$, $p = 0.939$, Fig. 4B). In MD rats the expression of iNOS was not altered in both PFC ($t = -1.053$, $df = 7$, $p = 0.327$, Fig. 5A) and hippocampus ($t = -.99$, $df = 7$, $p = 0.924$, Fig. 5B). In MD rats it was found an increase in the arginase expression in the PFC ($t = -4.486$, $df = 7$, $p = 0.003$, Fig. 6A), and in the hippocampus ($t = -2.786$, $df = 7$, $p = 0.027$, Fig. 6B).

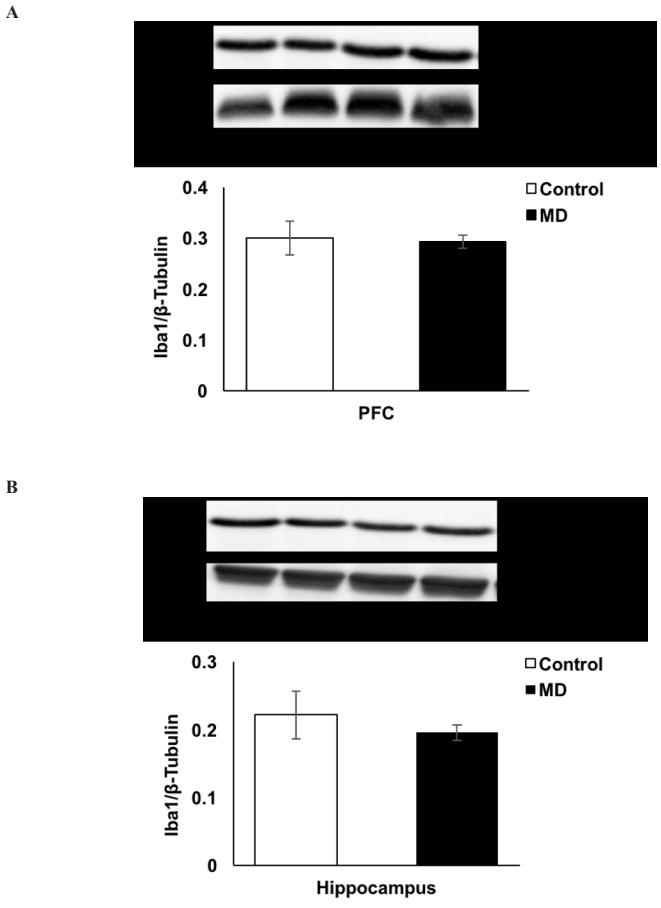

Fig. 1. The effects of maternal deprivation on Iba-1 expression in infant rats, PFC (A) and hippocampus (B). Representative blots of Iba-1 and β-tubulin protein expression are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 4–5).

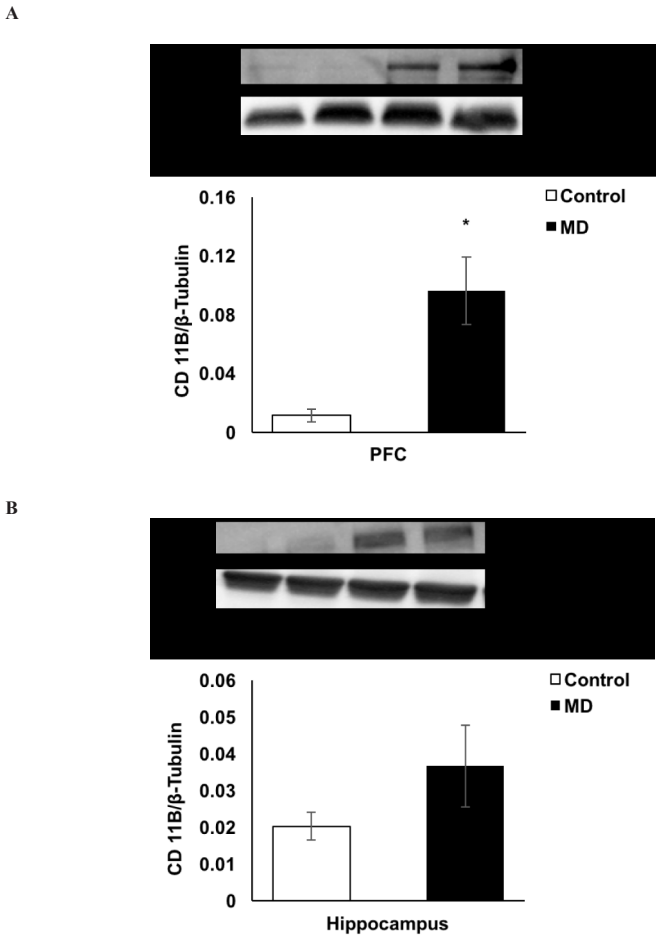

Fig. 2. The effects of maternal deprivation on CD11B expression in infant rats, PFC (A) and hippocampus (B). Representative blots of CD11B and β-tubulin protein expression are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 4–5). *p $<$ 0.05 different from non-deprived, according to Student t-test.

3.2. Effects of MD on inflammatory markers in the PFC of infant rats

The levels of inflammatory markers in the PFC are represented in Fig. 7. The levels of IL1-α and IL1-β were not altering in infant rats subjected to MD ($t = -0.518$, $df = 9$, $p = 0.617$, Fig. 7A, and $t = 0.929$, $df = 9$, $p = 0.377$, Fig. 7B, respectively). The levels of IL-5, -6, -7, and -10 were increased in the PFC of MD rats (IL-5 → $t = -3.055$, $df = 4.119$, $p = 0.036$, IL-6 → $t = -3.837$, $df = 5.22$, $p = 0.011$, IL-7 → $t = -3.813$, $df = 5.098$, $p = 0.012$, IL-10 → $t = -3.463$, $df = 9$, $p = 0.007$, Fig. 7A). The IL-12 levels did not change in the PFC of MD infant rats ($t = -0.708$, $df = 9$, $p = 0.495$, Fig. 7A).

In the PFC a reduction of MIP-1α was observed in MD infant rats

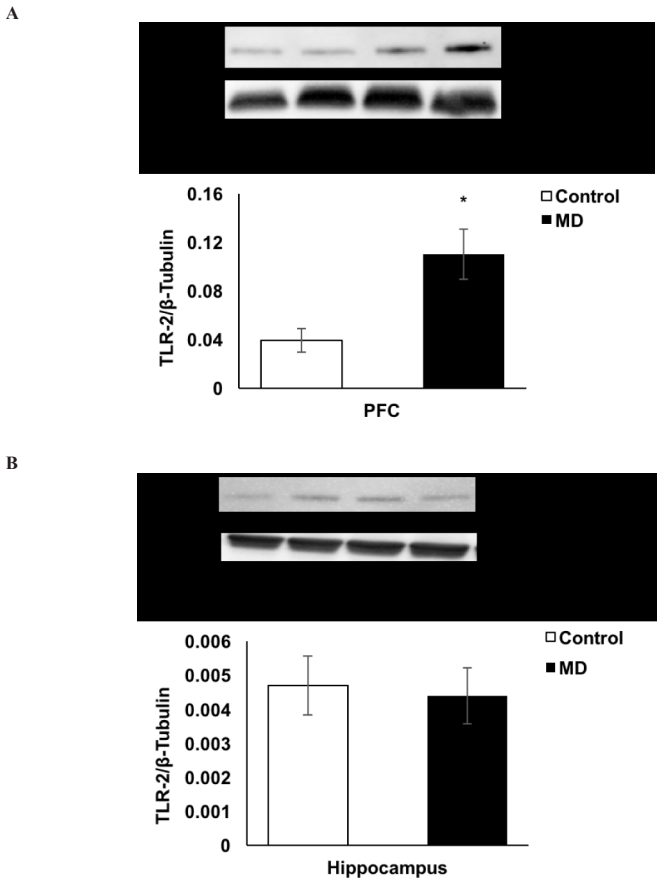

Fig. 3. The effects of maternal deprivation on TLR-2 expression in infant rats, PFC (A) and hippocampus (B). Representative blots of TLR-2 and β-tubulin protein expression are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 4–5). *p < 0.05 different from non-deprived, according to Student t-test.

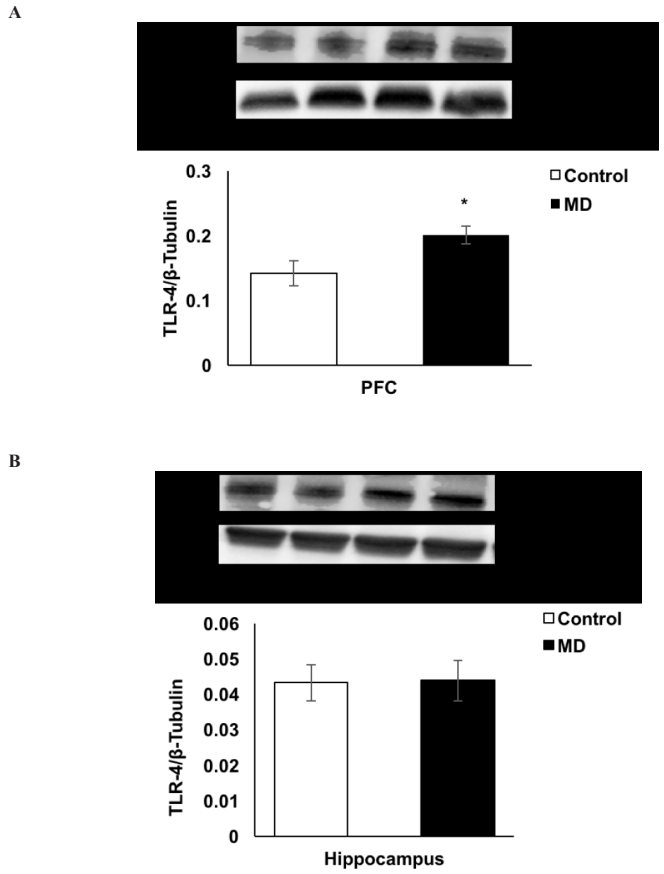

Fig. 4. The effects of maternal deprivation on TLR-4 expression in infant rats, PFC (A) and hippocampus (B). Representative blots of TLR-4 and β-tubulin protein expression respectively are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 3–5). *p < 0.05 different from non-deprived, according to Student t-test.

(t = 3.251, df = 4.985, p = 0.023, Fig. 7B). There was an increase in the levels of TNF-α and INF-γ in the PFC of infant rats subjected to MD (t = −5.849, df = 5.829, p = 0.001, and t = −3.157, df = 9, p = 0.012, Fig. 7B, respectively). The levels of 6-CSF, M-CSF, VEGF, GRO-KC, MCP-1, and Rantes were did not different comparing MD and control infant rats (p > 0.05, Fig. 7B).

3.3. Effects of MD on inflammatory markers in the hippocampus of infant rats

In the hippocampus the inflammatory markers: IL1-α, IL1-β, IL-5, -6, -7, -10, and −12 were did not change in MD infant rats (p > 0.05, Fig. 8A).

The infant rats subjected to MD demonstrated increased levels of TNF-α and INF-γ in the hippocampus (TNF-α → t = −2.569, df = 8, p = 0.033, and INF-γ → t = −3.659, df = 4.071, p = 0.021, Fig. 8B, respectively). However, we did not find changes in the inflammatory markers 6-CSF, M-CSF, MIP-1α, VEGF, GRO-KC, MCP-1, and Rantes (p > 0.05, Fig. 8B).

4. Discussion

The current study revealed that rats exposed to MD early in life had changes in the microglial and neuroinflammatory markers in the brain areas involved with mood disorders, mainly in the PFC and these changes were found shortly after stress induction. In the PFC elevated expression of microglial activation markers such as CD11B, TLR-2, and TLR-4, and inflammatory markers such as IL-5, IL-6, and IL-7 was observed. In addition, in the hippocampus and PFC there was an increased expression of alternatively activated microglia arginase expression and inflammatory markers TNF-α and INF-γ levels was found. However, MIP-1α levels were decreased in the PFC.

The hippocampus and PFC are among the most studied structures in the MDD. A reduction in the synaptic plasticity and volumetric changes in the hippocampus are reported in patients with depression (Liu et al., 2017). In both, hippocampus and PFC, a volume reduction could be resulted from atrophy of glia and neurons in depression (Liu et al., 2017). In the present study changes associated to microglial activation and neuroinflammation were more evident in the PFC. The PFC when in situations of stress is seeming to have alterations in glutathione antioxidant enzyme and glutamatergic excitoxicity mediated by N-methyl-D-aspartate (NMDA) receptors (Walker et al., 2015; Yang et al., 2013), which are pathways involved indirectly with an exacerbated response to neuroinflammation. Furthermore, the PFC is an important area involved in the antidepressant response using different therapeutic strategies, such as ketamine (an antagonist of NMDA receptor), repetitive transcranial magnetic stimulation, and electroconvulsive therapy for treatment-resistant depression (Liu et al., 2017). Also, glutamate released from M1 microglia leads to a hypoactivation in the PFC and neural circuits associated to this area (Nakagawa and Chiba, 2014). Thus, we suggest that neuroinflammation and microglial activation in the PFC induced by MD could be involved with behavioral changes later in life, as previously demonstrated (Ignácio et al., 2017; Réus et al., 2015a, 2017). However, other systems and pathways could be involved. In fact, the exposure to MD is able to induce many consequences in the

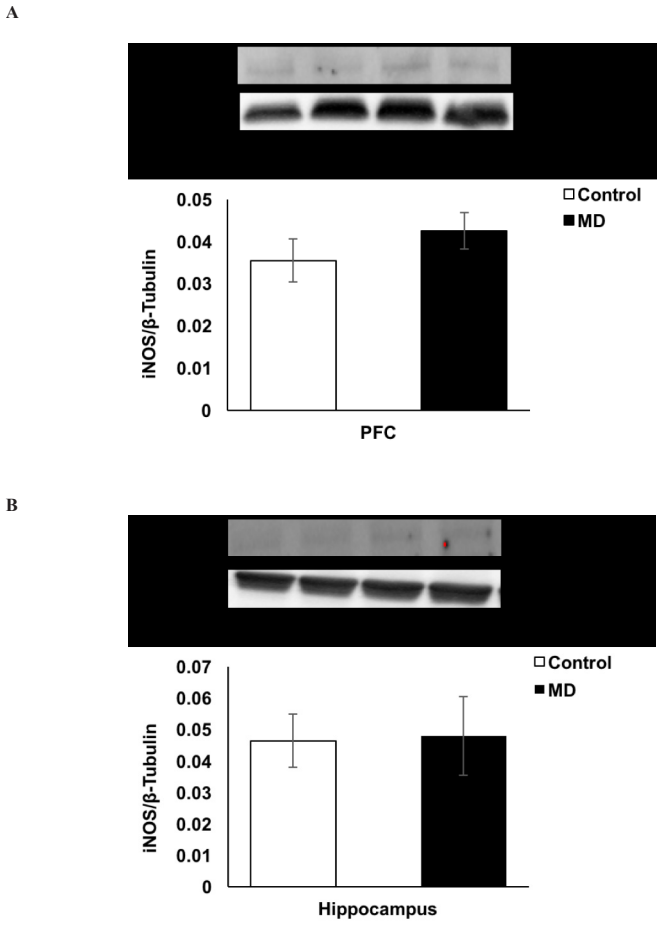

Fig. 5. The effects of maternal deprivation on iNOS expression in infant rats, PFC (A) and hippocampus (B). Representative blots of iNOS and β-tubulin protein expression respectively are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 4–5).

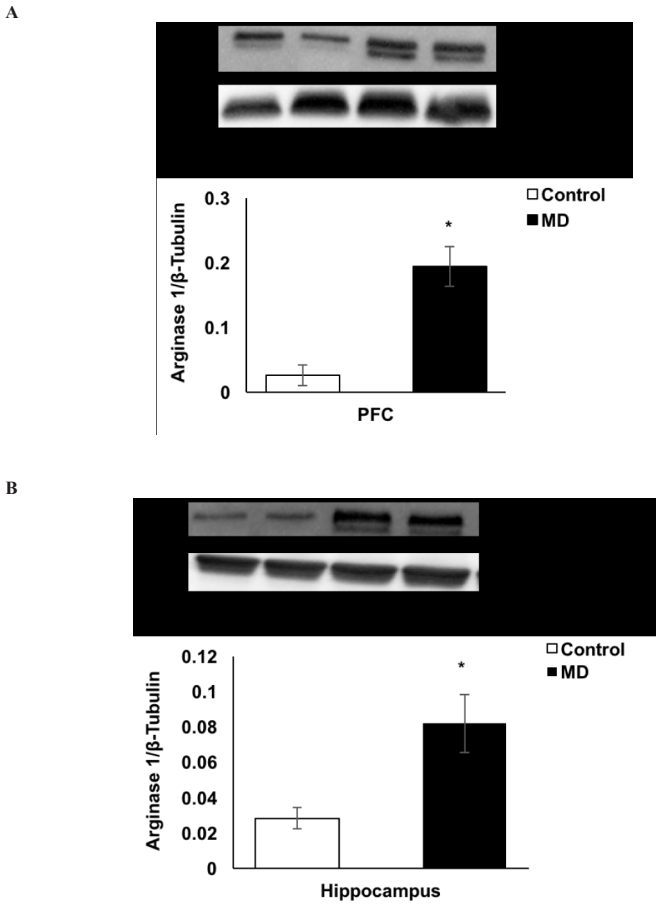

Fig. 6. The effects of maternal deprivation on arginase expression in infant rats, PFC (A) and hippocampus (B). Representative blots of arginase and β-tubulin protein expression are shown in the upper panels. Values are expressed as mean ± S.E.M. (n = 4–5). *p < 0.05 different from non-deprived, according to Student t-test.

offspring, including behavioral, epigenetic, and hypothalamic–pituitary–adrenal (HPA) axis changes (Ignácio et al., 2017; Réus et al., 2013a). Moreover, in rodents exposed to MD an increase in the oxidative stress, inflammation and energy metabolism dysregulation are found in the PFC, amygdala, hippocampus, and nucleus accumbens of adult rats (Della et al., 2013; Réus et al., 2013b).

Microglial cells are very crucial in the development of CNS. The primary function involved in the development include, axonal growth, neuronal survival and apoptosis, migration of neurons, and pruning and maturation synapses (Mosser et al., 2017). A very recently study from our group demonstrated that following MD in periods corresponding to childhood and adolescence microglia was activated, whereas in later periods the microglia was unchanged, but a reactivity of astrocytes was observed (Réus et al., 2019). The main activation states of microglial cells are M1 and M2. M1 state is characterized by the production of inflammatory mediators such as TNF-α, INF-γ, and M2 activation markers include mainly arginase and IL-10 (Franco and Fernandez-Suarez, 2015), suggesting that MD in the present study induced an activation of both M1 and M2 states due increased expression of arginase and higher levels of IL-10, TNF-α and INF-γ. Also, we found elevated expression of arginase 1 in both, PFC and hippocampus following MD. Arginase is an important protein in the modulation of nitric oxide generation; however, here iNOS expression was not changed. Although, in patients with MDD were found higher serum arginase activity when compared to health controls (Elgün and Kumbasar, 2000). Some findings suggest that different types of NOS have complex and inconsistent role in the brain when associated to the pathophysiology of depression (Zhang et al., 2013).

Microglia are also able to express all members of TLR family (Hanke and Kielian, 2011), including TLR2, that are constitutively expressed by this cell (Laflamme et al., 2003; Zekki et al., 2022). In the current study, we demonstrated that following MD offspring male rats had an increase of CD11B, TLR-2, and TLR-4 expressions in the PFC, but not in the hippocampus. Similar with our study Franklin et al. (2018) demonstrated no changes in the TLR4 in enriched hippocampal microglia after chronic unpredictable stress, but they found an increased levels of mRNA receptor for advanced glycation end products (RAGE) and high mobility group box 1 protein (HMGB1), which are involved in inflammatory responses. Burke et al. (2014) demonstrated in their study that rats subjects to an animal model of depression induced by olfactory bulbectomy had an increase in the CD11B in the PFC. On the other hand, the treatment with minocycline, an antibiotic drug, with antidepressant effects, reduced CD11B (Burke et al., 2014; Maciel et al., 2018).

In accordance with our results patients with MDD showed an upregulation of TLR, including TLRs 2, 3, 4, 5, 7 and 9 (Hung et al., 2016; Pandey et al., 2014). Jizhong et al. (2016) also reported an association with TLR-2 and -4 with depression. In fact, in depressive patients with irritable bowel syndrome the expression TLR were higher than in control group (Jizhong et al., 2016). In addition, in the PFC of patients with MDD were demonstrated an enhanced expression of TLR-4 (Garate et al., 2014). In other line, the treatment with antidepressants reduced depressive symptoms and the levels of TLR-1, -2, -4, and -6 (Hung et al., 2016). It was showed an upregulation of TIR-domain-containing

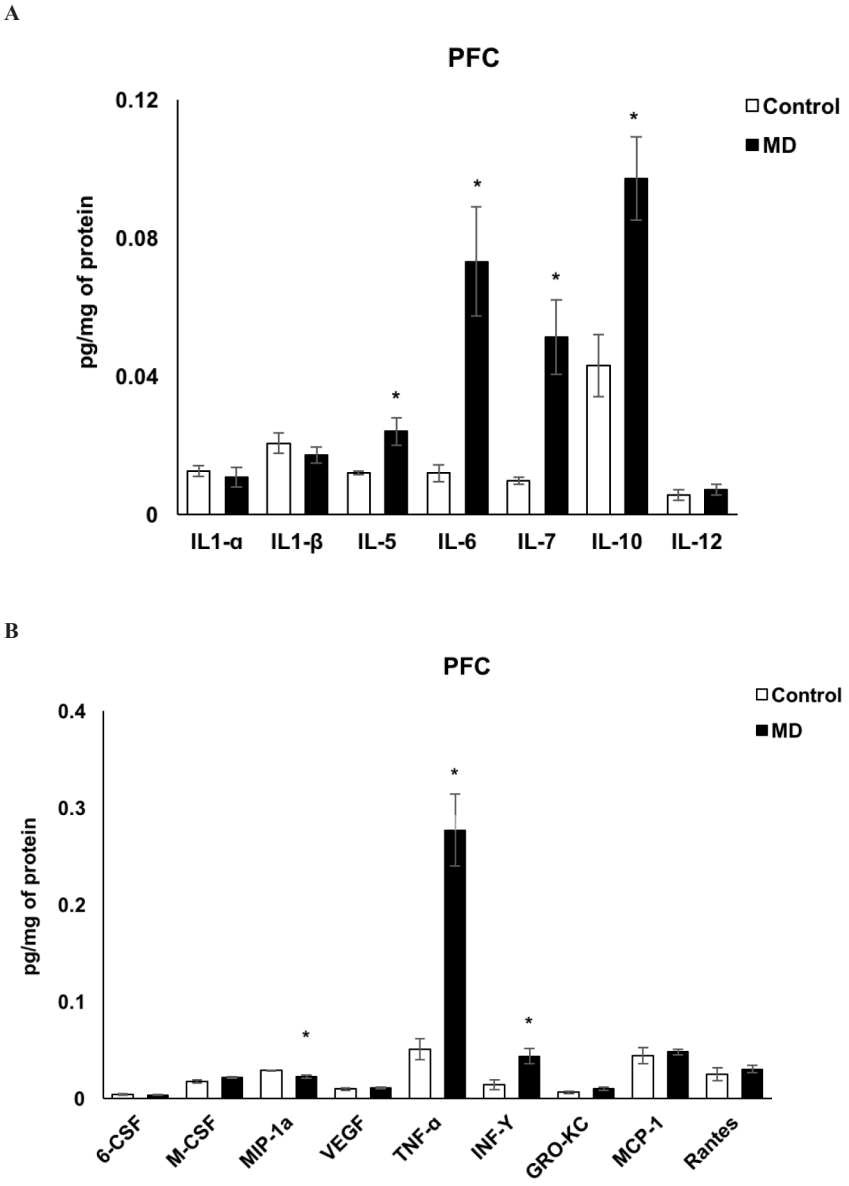

Fig. 7. The effects of maternal deprivation in the levels of IL1-α, IL1-β, IL-5, IL-6, IL-7, IL-10, IL-12 (A), and TNF-α, INF-γ, 6-CSF, M-CSF, VEGF, GRO-KC, MCP-1, and Rantes (B) in the PFC. Values are expressed as mean ± S.E.M. (n = 5–6). *p < 0.05 different from non-deprived, according to Student t-test.

adapter-inducing interferon-β (TRIF) and myeloid differentiation primary response (MyD) 88 (elements of the TLR-4 signaling pathways) in peripheral blood mononuclear cells of individuals with depression (Hajebrahimi et al., 2014). In addition, TLR-4 signaling pathways were up regulated in the dorsolateral PFC from suicidal depressed patients (Pandey et al., 2014). Garate et al. (2014) also revealed that mice exposed to repeated restraint/acoustic stress had an upregulation in the TLR-4 pathway in the brain PFC. Moreover, the authors used antibiotic intestinal decontamination and demonstrated an association with gut barrier dysfunction, bacterial translocation and activation of TLR-4 signaling pathway in the PFC following stress (Garate et al., 2014). In depression associated to neuropathic pain also was demonstrated elevated expressions of Iba1, CD11B and M1 markers (CD68, iNOS, IL-1β, TNF-α, and 8-OH-dG) in the PFC. On the other hand, minocycline administration was able to reverse these abnormalities (Xu et al., 2017).

Cytokines in the brain displayed an important role in the cell proliferation and differentiation. Nevertheless, high or low levels of cytokines can induce deleterious effects (Schmitz and Chew, 2008). Numerous studies have suggested that some pro-inflammatory cytokines are strongly associated to the pathophysiology of MDD (Abelaira et al., 2014; Jeon and Kim, 2016; Réus et al., 2013c, 2015a). Higher cytokines levels can decrease neurotransmitters involved with mood regulation, including dopamine, noradrenaline and serotonin, beyond that, they can stimulate HPA axis to release glucocorticoids (Leonard, 2006). In the current study, we showed elevated levels of IL-5, -6, -7, and -10 in the PFC, and TNF-α and INF-γ in the PFC and hippocampus. Previously, we demonstrated higher levels of IL-1β, IL-6, and TNF-α, and lower levels of IL-10 in serum and brain of rats subjected to MD evaluated at PND 20, 30, 40, and 60 (Réus et al., 2017). Microglial activation in the aged mice was associated with higher induction of inflammatory IL-1β and anti-inflammatory IL-10 (Henry et al., 2009). In addition, in rats maternally deprived microglial activation was found in both early and late developmental periods (Réus et al., 2019). The elevated levels of IL-10 right after MD could be associated to a mechanism to promote anti-inflammatory effects following early stress. In fact, M2-activated microglia play an important role in repair, triggering anti-inflammatory responses by IL-10 release (Hanisch and Kettenmann, 2007). MIP play critical role in immune responses, and they are involved in the synthesis

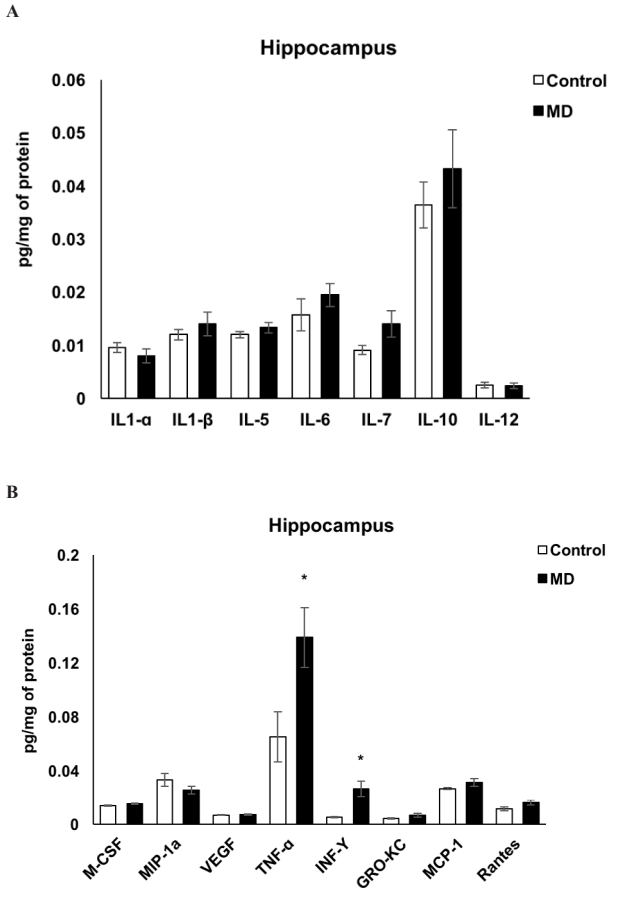

Fig. 8. The effects of maternal deprivation in the levels of IL1-α, IL1-β, IL-5, IL-6, IL-7, IL-10, IL-12 (A), and TNF-α, INF-γ, 6-CSF, M-CSF, VEGF, GRO-KC, MCP-1, and Rantes (B) in the hippocampus. Values are expressed as mean ± S.E.M. (n = 5–6). *p < 0.05 different from non-deprived, according to Student t-test.

and release of other pro-inflammatory cytokines, such as IL-6 and TNF-α. Here, we reported a reduction of MIP1α in the PFC. Although, in a more inflammatory state such as the one found here, one would expect that the levels of MIP were higher. In fact, in mice chronically stressed as well human with depression associated to post-traumatic stress elevated levels of MIP were reported (Cheng et al., 2015; Ogłodek and Just, 2018). However, no studies have been performed on MIP following MD.

5. Conclusions

In conclusion, our results showed that infant rats, following MD, had an increase in the markers associated to microglial activation, mainly in the PFC. The perturbation in the inflammatory status could be involved to the development of psychiatric disorders during the development. Hence, treatments targeting microglia and neuroinflammation could be considered as an alternative to be explored.

Author contributions

Réus, Barichello and Quevedo, designed the study and wrote the protocol. Giridharan, Selvaraj, and Scaini managed the literature searches and analyses and participated in experiments. Réus and Vijayasree undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflicts of interest

I. Clinical Research Support:
Janssen Pharmaceutical (Clinical Trial), Allergan (Clinical Trial).
II. Advisory Boards, Speaker Bureaus, Expert Witness, or Consultant
Daiichi Sankyo (Speaker Bureau).
III. Patent, Equity, or Royalty
Instituto de Neurociencias Dr. Joao Quevedo (Stockholder).
IV. Other
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