Molecular Neurobiology (2019) 56:1096–1108
https://doi.org/10.1007/s12035-018-1161-2

Early Maternal Deprivation Induces Microglial Activation, Alters Glial Fibrillary Acidic Protein Immunoreactivity and Indoleamine 2,3-Dioxygenase during the Development of Offspring Rats

Gislaine Z. Réus[1] · Ritele H. Silva[1] · Airam B. de Moura[1] · Jaqueline F. Presa[1] · Helena M. Abelaira[1] · Mariane Abatti[2] · Andriele Vieira[2] · Bruna Pescador[2] · Monique Michels[2] · Zuleide M. Ignácio[1] · Felipe Dal-Pizzol[2,3] · João Quevedo[1,4,5,6]

Received: 20 December 2017 / Accepted: 29 May 2018 / Published online: 5 June 2018

Abstract

Maternal deprivation (MD) induces behavioral changes and impacts brain circuits that could be associated with the pathophysiology of depression. This study investigated the markers of microglia and astrocyte activation as well as indoleamine 2,3-dioxygenase (IDO) expression in developmental programming after early life MD (on postnatal days (PNDs) 20, 30, 40, and 60). On PND 60, the rats that were subjected to MD displayed depressive-like behavior. On PND 10, it was found that there was a decrease in the level of glial fibrillary acidic protein (GFAP) immunopositive cells, a decrease in the level of IDO expression, and an increase in the level of Iba-1 (microglial marker) in the hippocampus of rats that were subjected to MD. On PND 20, levels of GFAP were also found to have decreased in the hippocampus, and there was an increase in the level of Iba-1 in the hippocampus. AIF-1 (microglial marker) expression was observed in the PFC following MD. On PND 30, the levels of Iba-1 remained elevated. On PND 40, the levels of GFAP were found to have increased in the hippocampus of rats that were subjected to MD. On PND 60, the levels of GFAP and AIF-1 remained elevated following MD. These results suggest that early life stress induces negative developmental programming in rats, as demonstrated by depressive-like behavior in adult life. Moreover, MD increases microglial activation in both early and late developmental phases. The levels of GFAP and IDO decreased in the early stages but were found to be higher in later developmental periods. These findings suggest that MD could differentially affect the expression of the IDO enzyme, astrocytes, and microglial activation depending on the neurodevelopmental period. The onset of an inflammatory state from resident brain cells could be associated with the activation of the kynurenine pathway and the development of depressive behavior in adulthood.

Keywords Glial fibrillary acidic protein immunoreactivity · Neuroinflammation · Microglial activation · Indoleamine 2,3-dioxygenase · Maternal deprivation · Major depressive disorder

✉ Gislaine Z. Réus
gislainereus@unesc.net

[1] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, SC 88806-000, Brazil

[2] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, University of Southern Santa Catarina, Criciúma, SC, Brazil

[3] Center of Excellence in Applied Neurosciences of Santa Catarina (NENASC), Graduate Program in Medical Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil

[4] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

[5] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

[6] Neuroscience Graduate Program, Graduate School of Biomedical Sciences, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA

Introduction

Currently, mental disorders constitute one of the largest public health problems [1]. It is estimated that there are 450 million people suffering from a mental disorder [2]. Major depressive disorder (MDD) is one of the most common forms of mood disorders, affecting about 350 million people, with MDD being associated with considerable suffering for both the individuals and their families. Moreover, the degree of physical and social impairment, plus the substantial use of health care resources amongst patients with MDD are comparable only to figures associated with chronic cardiac disease.

It is well documented that the mother-baby interaction is equally as important as any other physiological need [3]. Each experience throughout development is important for the consolidation of emotional maturity. The early neonatal environment has profound impact on the development of the central nervous system (CNS). Environmental stimuli interact with the genetic blueprint by shaping individual phenotypes and their subsequent perception and reactivity to various challenges. Increasing amounts of basic, clinical, and epidemiological evidence supports the notion that exposure to an adverse early environment may underlie not only a vulnerability to, but the later expression of, MDD [4]. The onset of MDD tends to be earlier for those who experience childhood adversities [5].

Studies involving maternal deprivation in rats have provided direct evidence that exposure to adverse events in early development can result in exaggerated stress reactivity, and also leads to alterations in several neural circuits that persist into adulthood [6]. However, studies are still necessary to investigate the mechanisms by which early stress is crucial to developing psychiatric conditions. Recently, a study from our group demonstrated that rats subjected to maternal deprivation developed depressive-like behavior only during adult life; however, an inflammatory profile as well as increased levels of oxidative stress in the CNS and peripherally were revealed early in life, and persisted throughout different developmental stages [7].

A considerable amount of evidence suggests that dysregulation of the immune system, including inflammation, mitochondrial dysfunction, and oxidant-antioxidant imbalance may be associated with the pathophysiology, and could play a significant role in the development and progression of MDD [8, 9]. Proinflammatory cytokines increase the activity of the indoleamine 2,3-dioxygenase enzyme (IDO), leading to tryptophan degradation in the kynurenine pathway. This in turn causes the production of quinolinic acid that directly activates the *N*-methyl-D-aspartate (NMDA) receptors and increases glutamatergic excitotoxicity, leading to a decrease in the availability of central serotonin [10]. Interestingly, both the kynurenine pathway and glutamatergic system are involved in the pathophysiology of MDD [11, 12].

It is known that inflammatory cytokines can affect brain resident cells, including astrocytes and microglia [13, 14]. Microglial activation has been implicated in both neurodegenerative diseases and natural aging, and very recently, it was discovered that there were increased levels of microglia in the postmortem brain samples of individuals who suffered from MDD, as evidenced by increases in the levels of Iba-1 and CD45 gene expression [15]. Astrocyte atrophy or dysregulation have also been shown in experimental and clinical studies [16, 17]. Although evidence in the literature has highlighted the role of glial cells as well as the kynurenine pathway in the pathophysiology of MDD, the timeline involved with these changes after an early life stress remains to be discovered. Thus, this study was aimed at investigating if maternal deprivation in rats induces depressive behavior, microglial activation, astrocyte atrophy, and changes in IDO expression in the brain areas involved with MDD (PFC and hippocampus) during different phases of development (infancy, adolescence, and adult life).

Material and Methods

Animals

For this study, female Wistar rats (total = 27) (3 months of age, weighing 250–280 g) were obtained from the breeding colony of Universidade do Extremo Sul Catarinense (UNESC, Criciúma, SC, Brazil) and were housed for 1 week in the presence of males for mating purposes. At the end of 7 days, the pregnant rats were housed individually with ad libitum access to food and water. The pregnant rats remained individually housed for the birth of the pups and their sexual identification. All mothers and pups were kept on a 12-h light/dark cycle (06:00 a.m. to 06:00 p.m.), at a temperature of 23 ± 1 °C. Only the male pups were used during the maternal deprivation period. One day after birth, the maternal deprivation protocol was applied to 50% of the male pups from days 1–10 after birth; the other males were used as controls. All of the experimental procedures involving animals were performed in accordance with the NIH Guidelines for the Care and Usage of Laboratory Animals, within the Brazilian Society for Neuroscience and Behaviour recommendations for animal care, and with the approval of the local Ethics Committee under protocol number 058/2016-1.

Experimental Groups and Maternal Care Deprivation 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 a period of 3 h per day during the first 10 days. We prefer this MCD protocol because it does not require the manipulation of the pups [18]. At the end of each daily deprivation session, the mothers were returned to their home boxes; this procedure was carried out during the light part of the daily cycle, between 8:00 a.m. and 12:00 p.m. The control rats remained in their resident boxes together with their mothers throughout the experiment.

At the completion of the maternal deprivation protocol, different groups of animals (deprived and non-deprived (controls)) were subjected to behavioral tests and biochemical analysis [7]. At PND 10 (infancy), a group of rats ($N = 6$ deprived and $N = 6$ non-deprived; total = 12 rats) were killed and their brains were used for biochemical tests; during this period, we used a lower number of animals, because we were not evaluating depressive behavior. At PND 20 (adolescence), PND 30 (young adult), PND 40 (young adult), and PND 60 (adult), different animals ($N = 12$ deprived; $N = 12$ non-deprived for each phase; total = 96 rats) were subjected to the forced swimming test (FST). After the behavioral test, the rats were killed and brain samples were used for biochemical analysis. The PFC and hippocampus were used for gene expression analysis via real time RT-PCR (RT-qPCR), while the hippocampus was used for immunohistochemistry.

Forced Swimming Test (FST)

The FST was conducted according to previous reports [19, 20]. The test involves two individual exposures to a cylindrical tank which is filled with water, where the rats cannot touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 80 cm tall, 30 cm in diameter, and is filled with water (22–23 °C) to a depth of 40 cm. On PNDs 20, 30, 40, and 60, the rats were individually placed in the cylinder containing water for a period of 15 min (pre-test session). On PNDs 21, 31, 41, and 61, the rats were again subjected to the FST for a 5-min session (test session), and the immobility, swimming, and climbing times of rats were recorded in seconds.

Biochemical Analysis

Analysis of Gene Expression by Real-Time PCR (RT-qPCR)

Total RNA was isolated with Trizol® reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The total RNA was quantified by spectrophotometry (A260/280 nm) and then treated with deoxyribonuclease I (Invitrogen) to eliminate genomic DNA contamination in accordance with the manufacturer's instructions. The cDNA was synthesized with the ImProm-II™ Reverse Transcription System (Promega) from 1 μg of total RNA, following the manufacturer's instructions. Quantitative PCR was performed using SYBR® Green I (Invitrogen) to detect double-strand cDNA synthesis. Reactions were undertaken in a volume of 25 μL using 12.5 μL of diluted cDNA, containing a final concentration of 0.2× SYBR® Green I (Invitrogen), 100 μM dNTP, 1× PCR Buffer, 3 mM MgCl2 0.25 U Platinum® Taq DNA Polymerase (Invitrogen), and 200 nM of each reverse and forward primers using GFAP, AIF-1, and IDO (Table 1). The PCR cycling conditions were: an initial polymerase activation step for a period of 5 min at 95 °C, 40 cycles of 15 s at 95 °C for denaturation, 35 s at 60 °C for annealing, and 15 s at 72 °C for elongation. At the end of cycling protocol, a melting-curve analysis was included and fluorescence was measured from 60 to 99 °C, showing one single peak in all cases. *Gapd* was used as reference genes for normalization. Relative expression levels were determined using the 7500 Fast Real-Time System Sequence Detection Software v.2.0.5 (Applied Biosystems). Relative mRNA expression levels were determined using the target/GAPDH method (primers are in Table 1).

Iba-1 and GFAP by Immunohistochemistry

To further characterize microglial and astrocyte activation, hippocampal immunohistochemistry was performed. Briefly, 40-μm sections from the hippocampus were incubated in 0.5% hydrogen peroxide with 0.1 M PBS (pH 7.4) containing 0.3% Triton-X100 (PBST) for a period of 30 min at room temperature to block endogenous peroxidase activity. After washing with PBST, the sections were incubated for 30 min with PBST containing 2% bovine serum albumin to block non-specific protein binding. The sections were then incubated overnight at 4 °C with a rabbit monoclonal IgG antibody against Iba-1 (1:100 dilution) or rabbit polyclonal antibody against GFAP (1:200 dilutions; Abcam, Cambridge, MA). After washing with PBST, the sections were incubated at room temperature for 1 h with biotinylated anti-rabbit IgG (1:100 dilution; Abcam). The sections were then incubated with 3,3′-diaminobenzidine (DAB) (Spring Bioscience). Sixteen random images per brain section were acquired at × 100 magnification, and immunopositive areas were expressed as a percentage of total area analyzed. Densitometry analysis of the films was performed using the Image Jv.1.34 image analysis software. Positive controls were used in all groups, according to the datasheet for each antibody.

Statistical Analysis

Data were analyzed using the Student's *t* test for unpaired data and are expressed as the mean ± standard error of the mean (SEM). 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.

Table 1 Primer sequences for RT-qPCR experiments included in the study

GAPDH	SENS: GAACATCATCCCTGCATCCA REVS: GCCAGTGAGCTTCCCGTTCA
GFAP	SENS: CAGACTTTCTCCAACCTCCAG REVS: CTCCTGCTTCGAGTCCTTAATG
AIF-1	SENS: GCCTCATCGTCATCTCCCCA REVS: AGGAAGTGCTTGTTGATCCCA
IDO	SENS: CCTGACTTATGAGAACATGGACGT REVS: ATACACCAGACCGTCTGATAGCTG

Results

The Effects of Maternal Deprivation on the Immobility Time in the Forced Swimming Test during Different Phases of Developmental Programming

The depressive-like behavior displayed by rats subjected to maternal deprivation is illustrated in Fig. 1. For rats that were subjected to maternal deprivation, the immobility time was not found to have altered at 20, 30, or 40 PND ($t = 1.077$; $df = 22$; $p = 0.293$; $t = 1.538$; $df = 18$; $p = 0.141$; $t = 0.734$; $df = 27$; $p = 0.469$, respectively). However, at 60 PND, the maternally deprived rats showed an increase in their immobility times ($t = -3.063$; $df = 16$; $p = 0.007$; Fig. 1).

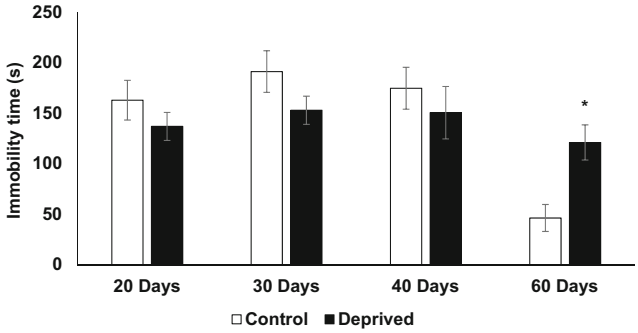

Fig. 1 The effects of maternal deprivation on the immobility time of rats that were subjected to the forced swimming test at 20, 30, 40, and 60 PNDs. Values are expressed as mean ± SEM ($n = 12$ per group). *$p < 0.05$ different from non-deprived, according to the Student's t test

The Effects of Maternal Deprivation on the Markers of Astrocyte (GFAP) and Microglial Activation (Iba-1) as Visualized by Immunohistochemistry

Figure 2 shows the levels of reactive astrocytes in the hippocampus as visualized by immunohistochemistry. No change in the levels of reactive astrocytes were identified by GFAP immunopositive cells at 10 and 30 PNDs following maternal deprivation ($t = 2.31$; $df = 8$; $p = 0.05$; Fig. 2a; $t = 1.22$; $df = 4$; $p = 0.288$; Fig. 2c, respectively). At 20 PND, a reduction in the levels of GFAP immunopositive cells was demonstrated in maternally deprived rats ($t = 2.85$; $df = 4$; $p = 0.046$; Fig. 2b). Conversely, at PNDs 40 and 60, it was demonstrated that there was an increase in the levels of reactive astrocytes identified by GFAP immunopositive cells in the hippocampus of rats that were subjected to maternal deprivation ($t = 5.51$; $df = 4$; $p = 0.005$; Fig. 2d; $t = 6.39$; $df = 4$; $p = 0.003$; Fig. 2e, respectively).

Microglial activation marked by Iba-1 is illustrated in Fig. 3. At 10, 20, and 30 PNDs, it was observed that there was an increase in the levels of Iba-1 immunopositive cells, indicating microglial activation in the hippocampus of rats that were subjected to maternal deprivation ($t = 6.95$; $df = 11$; $p < 0.0001$; Fig. 3a; $t = 6.57$; $df = 4$; $p = 0.003$; Fig. 3b; $t = 6.26$; $df = 3$; $p = 0.008$; Fig. 3c). No change was found in the levels of Iba-1 immunopositive cells at 40 and 60 PNDs following maternal deprivation ($t = 0.064$; $df = 6$; $p = 0.951$; Fig. 3d; $t = 0.921$; $df = 3$; $p = 0.425$; Fig. 3b).

The Effects of Maternal Deprivation on IDO, GFAP, and AIF-1 Expression in the PFC and Hippocampus

The effects of maternal deprivation on the levels of IDO expression are shown in Fig. 4. At 10 PND, the expression of IDO did not alter in the PFC ($t = 1.725$; $df = 6$; $p = 0.135$; Fig. 4a). However, in the hippocampus, IDO expression was reduced at 10 PND ($t = 2.488$; $df = 6$; $p = 0.0.047$; Fig. 4a). At 20 PND, IDO expression was not changed in the PFC ($t = 0.792$; $df = 4$; $p = 0.473$; Fig. 4b) nor in the hippocampus ($t = -0.917$; $df = 6$; $p = 0.394$; Fig. 4a). Similarly, at 30 and 40 PNDs, IDO expression did not alter (30 PND; PFC $t = -1.957$; $df = 7$; $p = 0.091$; hippocampus $t = -0.368$; $df = 7$; $p = 0.724$; Fig. 4c), and (40 PND; PFC $t = -0.544$; $df = 9$; $p = 0.599$; hippocampus $t = -0.773$; $df = 8$; $p = 0.462$; Fig. 4d). IDO expression was elevated in the PFC at 60 PND ($t = -3.34$; $df = 8$; $p = 0.010$; Fig. 4e), but in the hippocampus, IDO expression did not alter at 60 PND ($t = -0.94$; $df = 9$; $p = 0.927$; Fig. 4e).

GFAP expression was decreased at 10 PND in the PFC ($t = 2.845$; $df = 5$; $p = 0.036$; Fig. 5a), but it did not alter in the hippocampus ($t = 0.460$; $df = 8$; $p = 0.658$; Fig. 5a). Neither the PFC nor the hippocampus showed any changes in GFAP expression at 20, 30, and 40 PNDs after maternal deprivation (20 PND; PFC $t = -0.773$; $df = 4$; $p = 0.483$; hippocampus $t = -0.453$; $df = 6$; $p = 0.679$; Fig. 5b), (30 PND; PFC $t = 1.723$; $df = 6$; $p = 0.136$; hippocampus $t = -0.772$; $df = 7$; $p = 0.465$; Fig. 5c), and (40 PND; PFC $t = 1.323$; $df = 9$; $p = 0.219$; hippocampus $t = 0.334$; $df = 9$; $p = 0.094$; Fig. 5d). At 60 PND, GFAP expression was not changed in the PFC ($t = -1.647$; $df = 8$; $p = 0.138$; Fig. 5e); however, in the hippocampus, GFAP expression was found to have increased at 60 PND ($t = -4.599$; $df = 6$; $p = 0.004$; Fig. 5e).

The expression of AIF-1, a marker of microglial activation, is illustrated in Fig. 6. At 10 PND following maternal deprivation, AIF-1 expression was not found to have altered in the

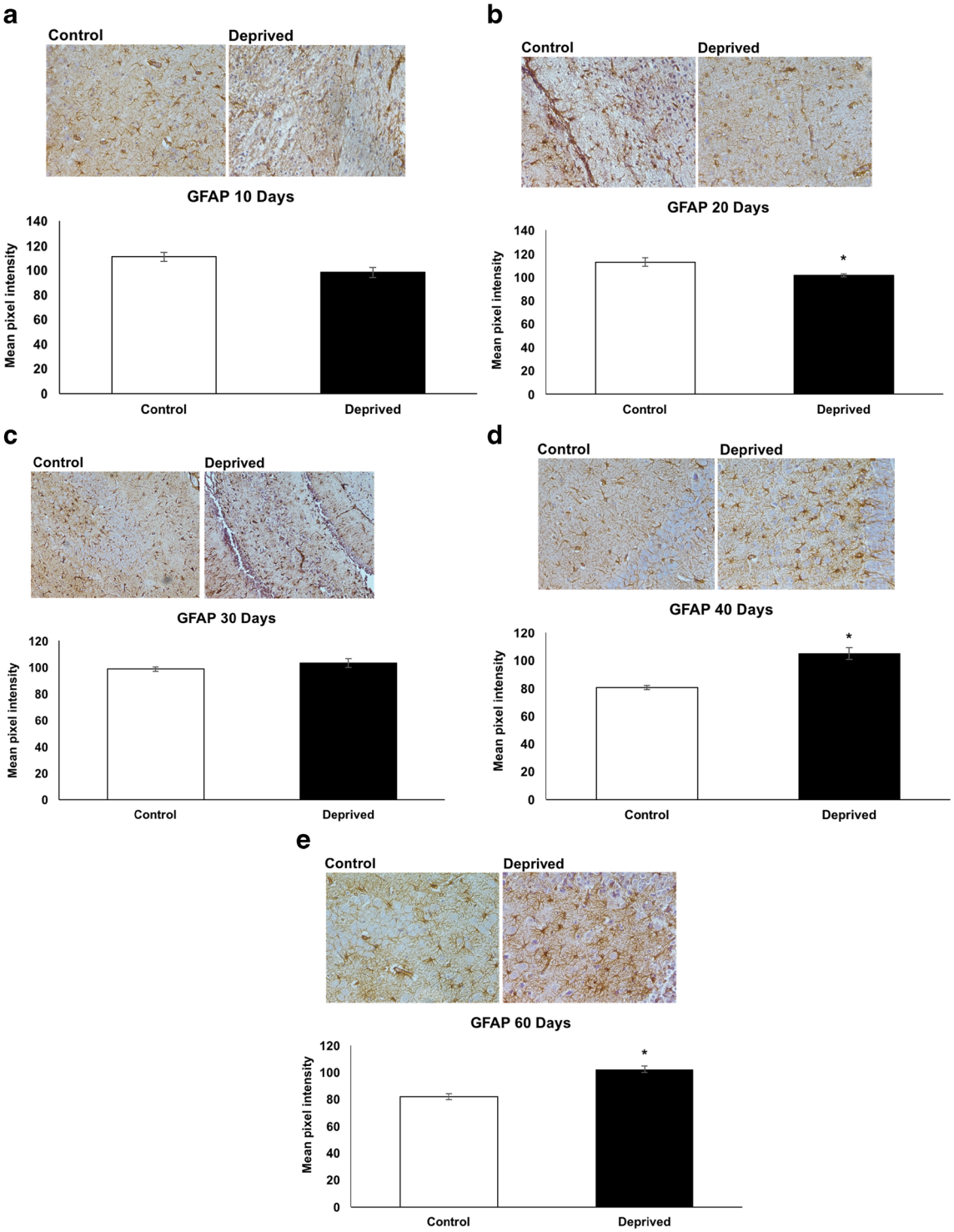

Fig. 2 The effects of maternal deprivation on hippocampal GFAP immunopositive cells as visualized by immunohistochemistry at 10 (**a**), 20 (**b**), 30 (**c**), 40 (**d**), and 60 (**e**) PNDs. Values are expressed as mean ± SEM ($n = 3$ per group). *$p < 0.05$, different from non-deprived, according to the Student's t test

PFC ($t = 0.952$; $df = 2.147$; $p = 0.435$; Fig. 6a), nor in the hippocampus ($t = -2.433$; $df = 3.055$; $p = 0.092$; Fig. 6a). At 20 PND, the expression of AIF-1 was increased in the PFC ($t = -4.667$; $df = 4$; $p = 0.010$; Fig. 6b), but there was no change in the expression of AIF-1 within the hippocampus on PND 20 ($t = 1.856$; $df = 4.174$; $p = 0.134$; Fig. 6b). The expression of AIF-1 did not change at either 30 or 40 PND (30 PND; PFC $t = 1.009$; $df = 6$; $p = 0.352$; hippocampus $t = -$

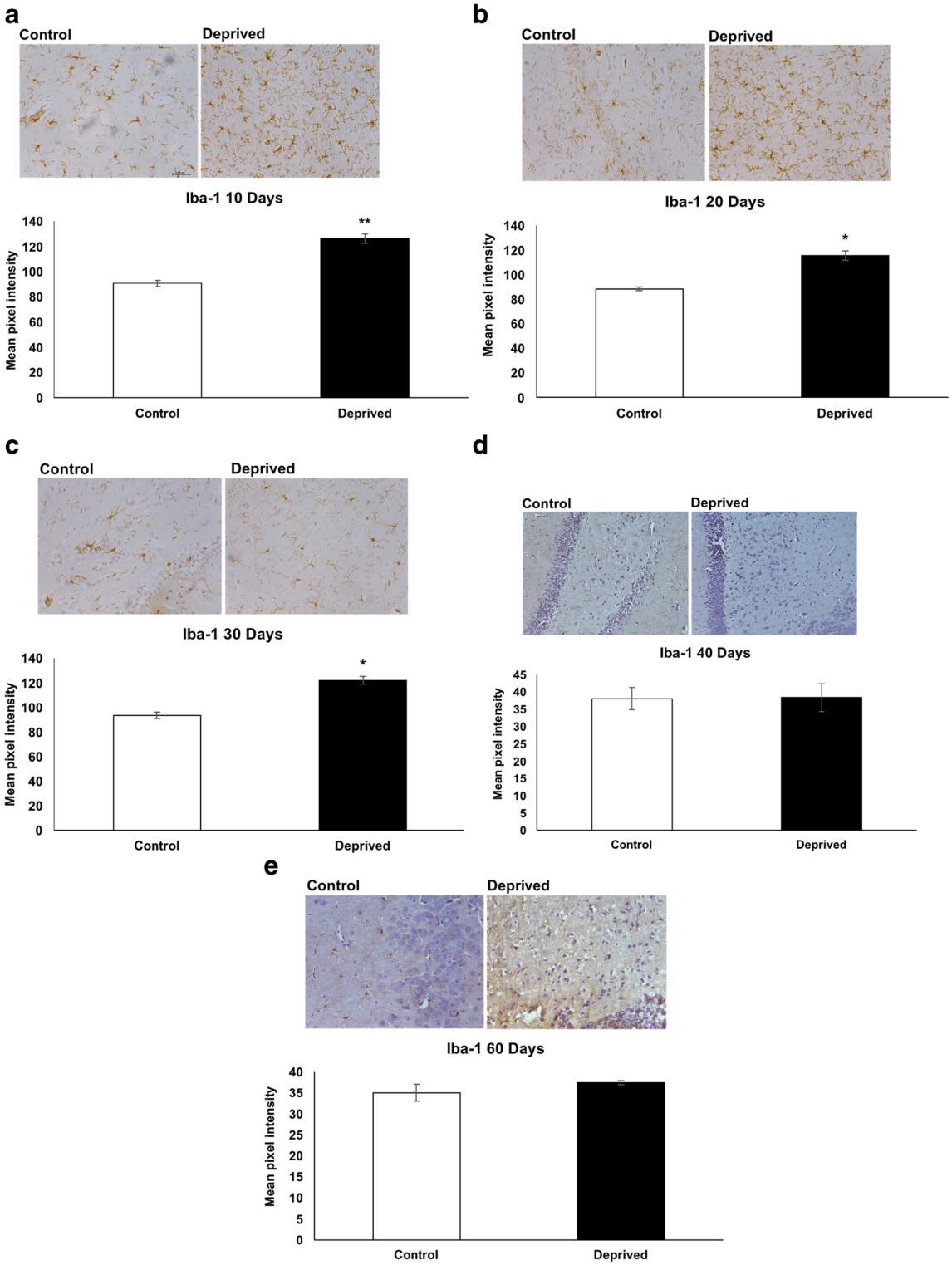

Fig. 3 The effects of maternal deprivation on hippocampal Iba-1 immunopositive cells as visualized by immunohistochemistry at 10 (**a**), 20 (**b**), 30 (**c**), 40 (**d**), and 60 (**e**) PNDs. Values are expressed as mean ± SEM ($n = 3$ per group). *$p < 0.05$ and **$p < 0.0001$, different from non-deprived, according to the Student's t test

1.079; $df = 7$; $p = 0.316$; Fig. 6c), and (40 PND; PFC $t = 1.216$; $df = 9$; $p = 0.255$; hippocampus $t = 1.100$; $df = 7$; $p = 0.316$; Fig. 6d). At 60 PND, AIF-1 expression was increased in the PFC ($t = -5.089$; $df = 7$; $p = 0.001$; Fig. 6e), but AIF-1 expression was not changed within the hippocampus at 60 PND ($t = -0.137$; $df = 3.759$; $p = 0.898$; Fig. 6e).

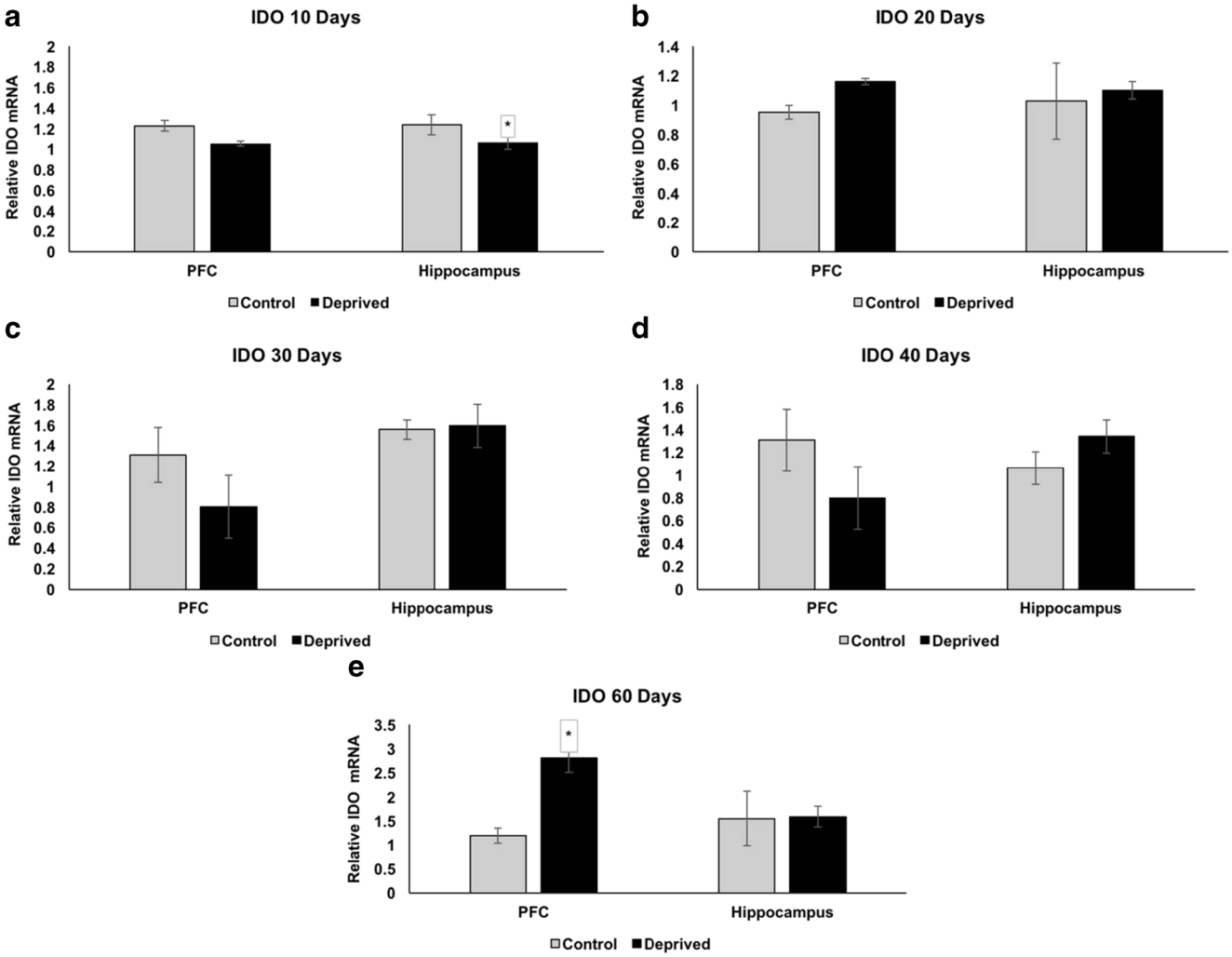

Fig. 4 The effects of maternal deprivation on PFC and hippocampal IDO mRNA at 10 (**a**), 20 (**b**), 30 (**c**), 40 (**d**), and 60 (**e**) PNDs. Values are expressed as mean ± SEM ($n = 3$ per group). *$p < 0.05$ and *$p < 0.0001$, different from non-deprived, according to the Student's t test

Discussion

The development of mood disorders, including MDD, could be associated with adverse experiences in early life [5]. In the present study, it was demonstrated that maternal deprivation induces depressive-like behaviour in adulthood (60 PND). However, changes in glial cells, astrocytes and microglia, as well as in levels of IDO expression were observed during different phases of development. Accordingly, previous studies have also demonstrated that early maternal deprivation was able to induce depressive-like behavior in adult rats, as demonstrated by anhedonic behavior in the splash test and increased immobility times in the forced swimming test [6, 7, 20, 21]. Recently, we showed that rats subjected to maternal deprivation displayed depressive-like behavior in the forced swimming test at 60 PND; however, increases in the levels of neuroinflammation and oxidative stress were evident in other developmental periods [7], suggesting that maternal deprivation leads to inflammatory alterations that could influence late behavioral responses.

Studies have suggested that an inflammatory status could affect brain resident cells, such as neurons, astrocytes and microglia. In fact, IL-18 (−/−) knockout mice have microglial activation induced by stress [22], suggesting that cytokines play an important role within the microglial regulation of the stress response. Moreover, mice subjected to stress in the prenatal period have increases in their levels of IL-1β mRNA and total number of activated microglia, marked by Iba-1 in the hippocampus [23]. Another study revealed that maternal immune activation induced by lipopolysaccharide (LPS) in mice leads to an increase in the expression of proinflammatory cytokines during fetal brain development, and this persisted throughout adult life [24]. Also, the inflammation induced by maternal immune activation triggered brain resident cells and altered astrocytes and microglia in the amygdala during the prenatal period [24].

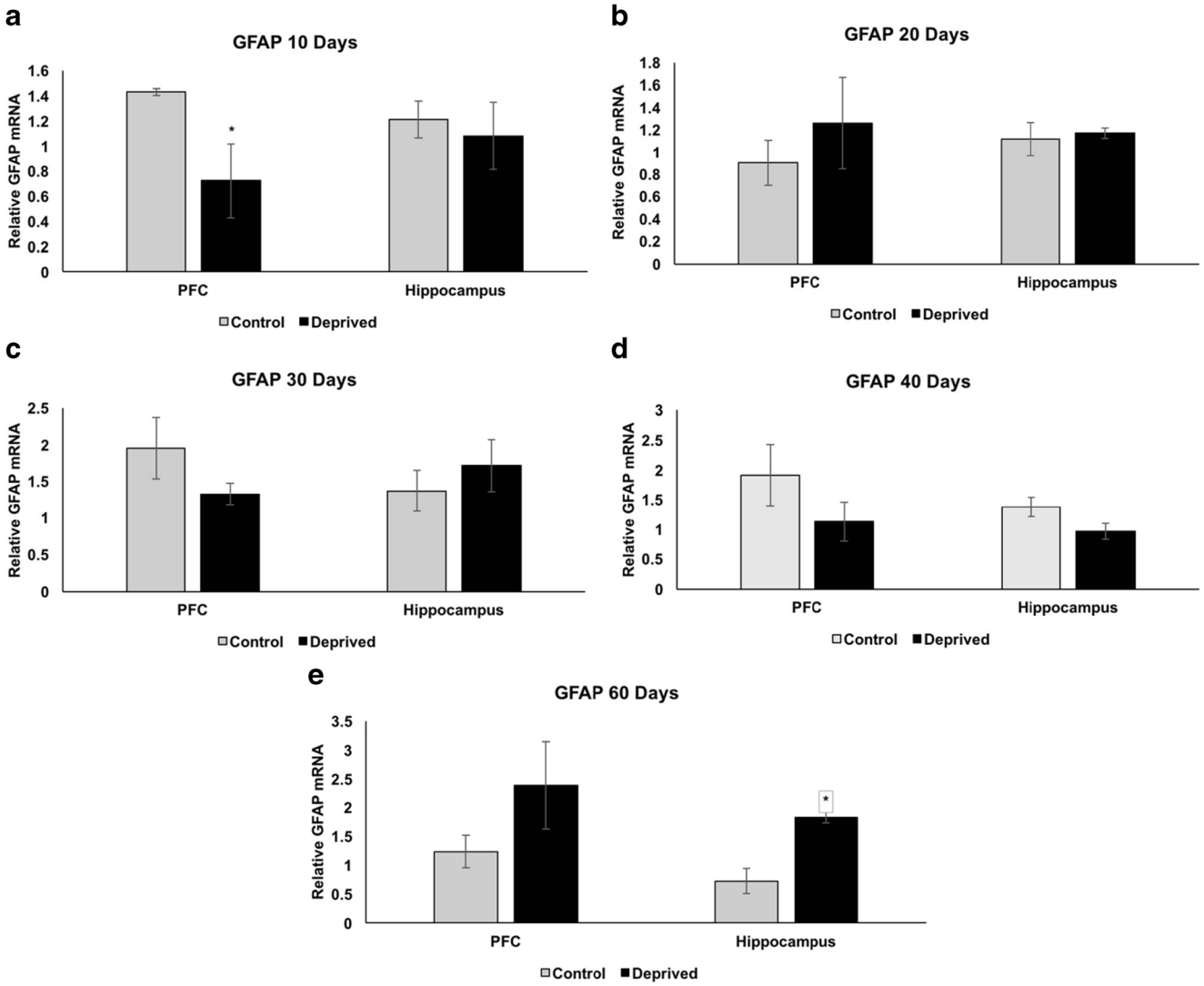

Fig. 5 The effects of maternal deprivation on PFC and hippocampal GFAP mRNA at 10 (**a**), 20 (**b**), 30 (**c**), 40 (**d**), and 60 (**e**) PNDs. Values are expressed as mean ± SEM ($n = 3$ per group). *$p < 0.05$ and *$p < 0.0001$, different from non-deprived, according to the Student's t test

Astrocytes are important glial cells that are involved in maintaining metabolic homeostasis, and neurotransmitters functions within the brain. Moreover, studies have reported that astrocytes play a pivotal role in various psychiatric disease conditions, including MDD [16, 25, 26]. GFAP is a cytoskeletal protein that is expressed by astrocytes, and displays an important function in cell structure and movement, besides having a role in neuron-astrocyte communication [27]. Higher levels of GFAP expression are associated with astrocyte activation [28]. Though there are studies documenting the role of astrocytes in MDD, little attention has been paid to the effects of maternal deprivation during the different stages of development. In the present study, we showed that rats subjected to maternal deprivation had a reduction in the levels of GFAP immunopositive cells in the hippocampus at 20 PND, and mRNA GFAP at 10 PND in the prefrontal cortex. However, GFAP was found to have increased at 40 and 60 PND in the hippocampus following maternal deprivation, suggesting that astrocyte cells have different types of activation during phases of development. In fact, astrocytes play an important role in synaptic function, formation, and remodeling during development [29]. Moreover, the function of astrocyte synapses could alter during development due to lesions or changes in physiological conditions [30]. Clinical studies have revealed that both mRNA and the number of GFAP immunopositive cells are decreased in the cerebral cortex and hippocampus of patients with MDD [31, 32]. Experimental studies have also reported a decrease in the expression of GFAP immunopositive cells in the cerebral cortex and hippocampus of rodents that were subjected to chronic stress [33, 34]. In accordance with the present study, where it was found that there was a reduction of GFAP in early life (10 and 20

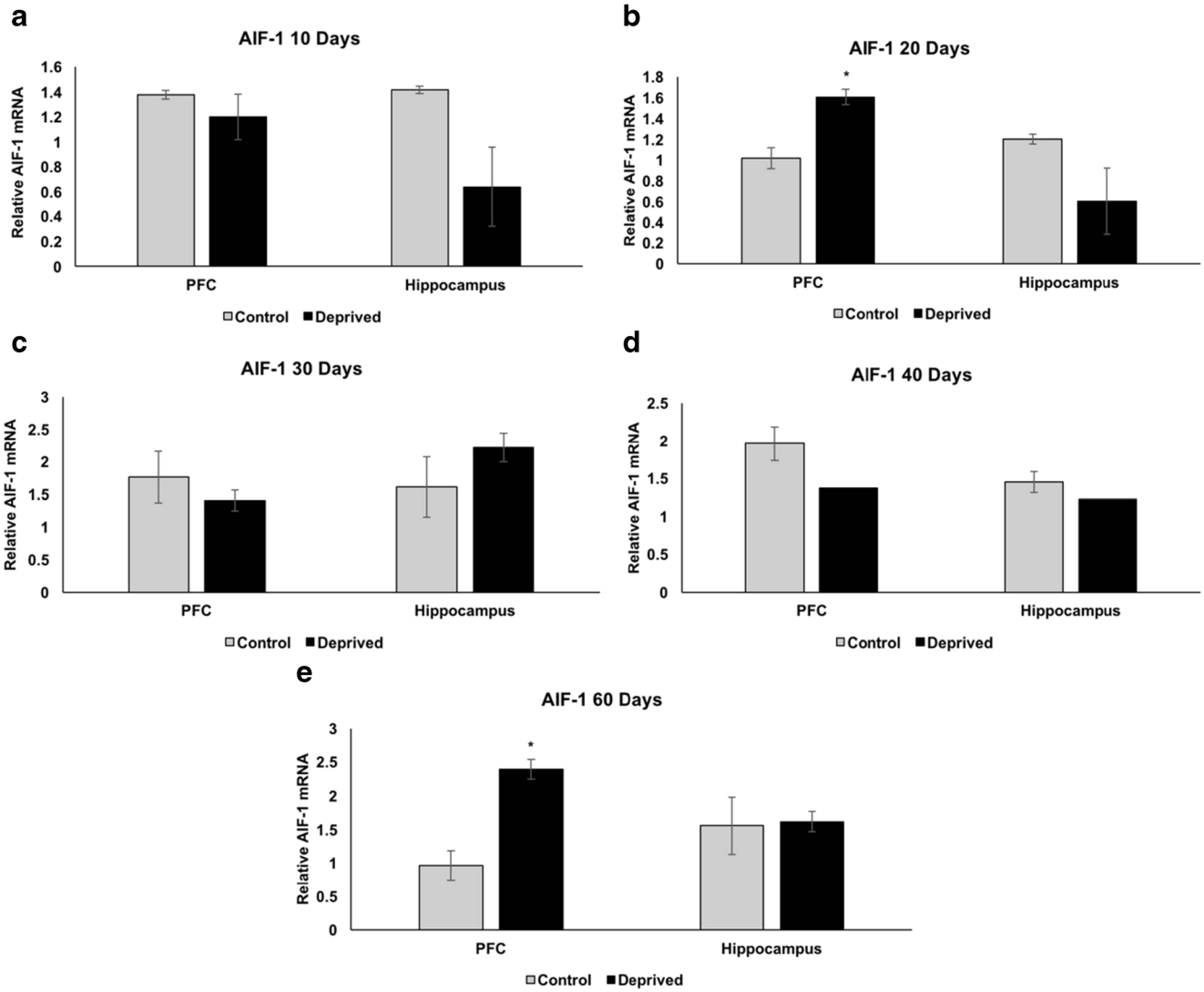

Fig. 6 The effects of maternal deprivation on PFC and hippocampal AIF-1 mRNA at 10 (**a**), 20 (**b**), 30 (**c**), 40 (**d**), and 60 (**e**) PNDs. Values are expressed as mean ± SEM ($n = 3$ per group). *$p < 0.05$ and *$p < 0.0001$, different from non-deprived, according to the Student's t test

PNDs), another study discovered that 14 day old Fischer rats that were subjected to maternal deprivation had a reduction in the density of astrocytes reactive for GFAP in the brain areas implicated in behavior associated with stress [35]. Nevertheless, male rats that were subjected to maternal deprivation had an increase in the numbers of GFAP immunopositive cells in the hippocampus and cortex. Female rats did not show this alteration [36]. These findings indicate that maternal deprivation could induce different responses in the astrocytes during development, and these responses may well be gender dependent. In fact, lower levels of GFAP are most prominent in the prefrontal cortex of young individuals with MDD, indicating that changes to astrocytes could be an early contributor to the pathophysiology of MDD [37, 38]. Data from the present study revealed that rats subjected to maternal deprivation had an increase in the numbers of mRNA GFAP and GFAP immunopositive cells in adult life, indicating an increase in astrocyte activation. These results could be linked to a decrease in microglial activation marked by Iba-1 during this developmental period. In fact, a study demonstrated that cultures of neuron and glial cells damaged by a dopaminergic neurotoxin had astrocyte activation following microglial activation, showing that microglia and astrocyte reactions occur due to neural death in distinct periods [13]. Our results showed a microglial activation marked by Iba-1 at 10, 20, and 30 PNDs, and AIF-1 at 20 and 60 PNDs. Iba-1 is expressed in the macrophages of brain meninges, supraependymal macrophages, superficial and stromal cells of the choroid plexus, as well as in microglia [39]. Iba-1 expression is enhanced under pathological conditions [40–42]. In addition, chronic stress increases the number of microglia Iba-1-positive cells in the prefrontal cortex of mice susceptible to anhedonia [43]. Some studies have suggested that AIF-1 should be considered identical to Iba-1. However,

not all of the commonly used antibodies against Iba-1/AIF-1 demonstrate the same products [39]. AIF-1 is a 143 amino acid cytoplasmic protein that is primarily identified in humans and rats. Evidence suggests that AIF-1 is involved in inflammatory processes [44]. In fact, AIF-1 is expressed by activated T cells and macrophage, and regulates the expression of inflammatory mediators, such as cytokines [45]. Iba-1 is a 17-kDa actin-binding protein that is specifically and constitutively expressed in all microglia [46]. It is widely employed as an immunohistochemical marker for both ramified and activated microglia [47]. Iba-1 is shown to have a function in the actin-crosslinking involved in the membrane ruffling of microglia [48]. Since membrane ruffling is essential for the morphological changes from quiescent ramified microglia to activated amoeboid microglia, microglial activation is associated with increased expression of Iba-1 [48]. Densitometry can therefore be utilized for measuring microglial activation, especially when microglia are strongly activated and/or the numbers of microglia are substantially increased.

Studies of the literature have shown that proinflammatory cytokines produced by microglia could induce astrocyte hypertrophy [13]. The levels of cytokines produced by microglia are elevated in response to damage, and some cytokine receptors, such as IL-1, IL-6, and TNF-α are also found in astrocytes [14, 17, 49]. Microglial cells are resident in the central nervous system and are able to become activated in response to physiological changes via alterations in morphology and function. Studies have related that microglial activation is implicated with the pathophysiology of MDD [26, 50]. Several chronic stressors are able to induce microglial proliferation in the brain of rodents, and these effects are associated with depressive behaviour [51]. The present study revealed that the behavioral changes that occurred in adult life were quite different to the astrocyte hypertrophy and microglial activation that were more evident during early life following maternal deprivation. These results could be explained, at least in part, by alterations in neuronal cells that were initially induced by glial changes. It is also possible that higher levels of glutamate over long periods, atrophy of astrocytes or inflammatory mediators from microglia could all be contributing factors for synaptic and dendritic cytoarchitectural loss [52, 53], leading to an impairment of neuronal function. Indeed, microglial cells play an important role in neurogenesis, synaptogenesis, and the remodeling of axons and synapses. To our knowledge, this is the first study to demonstrate the effects of maternal deprivation in microglial activation marked by Iba-1 and AIF-1 during development. Zhao et al. [54] revealed that maternal sleep deprivation during the 4th, 9th, and 18th gestational days induced cognitive impairment, anhedonic behavior, and increased inflammatory mediators and microglial activation in the hippocampus of offspring at 20 PND. Additionally, the microglial markers for the M1 phenotype, IL-1β, IL-6, TNF-α, CD68, and nitric oxide synthase were increased, while treatment with minocycline, a drug with anti-inflammatory properties, reduced the levels of Iba-1 immunopositive cells and cytokine production in the offspring rats [55]. These findings indicate that stressors during pre and postnatal days could induce microglial changes that persist during development.

Inflammatory cytokines from microglial activation could activate the enzyme IDO, which is responsible for metabolizing tryptophan into kynurenine [12, 56, 57]. In microglial cells, kynurenine is preferentially converted into quinolenic acid, a neurotoxic metabolic or kynurenic acid, which has neuroprotective effects mediated by NMDA receptors [58, 59]. Thus, the kynurenine pathway may play a double role, depending on the balance of the pathway and the factors influencing it. The kynurenine pathway has been implicated in the pathophysiology of a number of psychiatric conditions, including schizophrenia [60] and MDD [57]. In the present study, we revealed that mRNA IDO expression was decreased in the hippocampus at 10 PND (infancy) and increased in the PFC at 60 PND (adulthood). The expression of enzymes and key components of the kynurenine pathway reflects the fact that this pathway is present during embryonic development [61, 62]. Furthermore, the kynurenine pathway is seen to play an important role in cerebral development. In fact, it was demonstrated that there were changes in synaptic transmission and the expression of proteins involved with neuroplasticity in the brains of adult offspring Wistar rats after prenatal inhibition of the kynurenine pathway [63]. Thus, we suggest that the lower levels of IDO mRNA expression at 10 PND found in maternally deprived rats in the present study could be associated with later behavioral phenotypes. Another hypothesis is that IDO-mediated changes in the kynurenine pathway may be attributed to an inflammatory state, primarily via microglial activation induced by maternal deprivation and later by promoting the induction of IDO activity and the synthesis of neurotoxic metabolites [64]. In fact, the onset and severity of depressive symptoms in patients under immunotherapy is strongly associated with IDO activity [65]. Many proinflammatory cytokines may be inductors of IDO expression, including TNFα, and IL-1β [66]. Higher levels of proinflammatory cytokines were reported in the periphery and in the brain areas involved with MDD in maternally deprived adult rats (60 PND) [7, 67]. However, ketamine, an antagonist of the NMDA receptor, was able to reduce the levels of inflammatory cytokines and displays antidepressant-like effects in adult rats following maternal deprivation [67], thus we suggest that the increased levels of IDO mRNA expression in adult rats that were subjected to maternal deprivation could be associated, at least in part, with the elevated levels of pro-inflammatory cytokines that were stimulated by microglial activation in early life stages.

Actually, depressive-like behavior induced by LPS in genetically obese mice was associated with IDO activation following elevated levels of TNFα, and IL-1β in the hippocampus [68]. On the other hand, the lower levels of IDO mRNA expression at 10 PND found in the present study could be attributed to anti-inflammatory cytokines, such as IL-4 and IL-10. In fact, these cytokines may inhibit IDO [66]. Results from our group (no published data) revealed an increase in the levels of IL-10 in the brain of rats at 10 PND following maternal deprivation, thus reduced levels of IDO expression could be associated with elevated levels of IL-10. Contrarily, IL-10 was found to be reduced throughout developmental programming (PNDs 20, 30, 40, and 60) [7], according to elevated levels of IDO mRNA expression as reported in adult life. A considered limitation of the present study is the fact that metabolites of the kynurenine pathway were not evaluated. The other changes found in the present study are manifestations due to the activation of neurotoxic pathway metabolites.

Conclusions

In conclusion, our findings propose that the stress induced by maternal deprivation in early life has a negative effect on later behavior, besides causing changes to glial cells and the pathways involved with neuroplasticity and neuronal survival. Although behavioral changes only occurred in adult life, the changes in markers associated with microglial and astrocyte activation, as well as alterations in the levels of IDO enzymes were detected in early life. These changes could, at least in part, be involved with the long-term behavioral phenotype. More studies are needed to better investigate the kynurenine pathway, and to discover how it is connected to glial and neuron cell damage caused during development following the trauma induced by maternal deprivation. In addition, therapeutic approaches targeting these cells and pathways require more investigation.

Acknowledgements The Translational Psychiatry Program (USA) is funded by the Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth). The Laboratory of Neurosciences (Brazil) is one of the members of the Center of Excellence in Applied Neurosciences of Santa Catarina (NENASC). This research is supported by grants from CNPq (JQ), FAPESC (JQ, GZR, and ZMI); Instituto Cérebro e Mente (JQ and GZR), and UNESC (JQ, and GZR). JQ is a 1A CNPq Research Fellow.

Authors' Contributions GZR, RHS, HMA, ZMI, and JQ performed the research. ABM, HMA, and JFP worked on the maternal deprivation procedures and the removal of brain structures. MA, BP, AV, MM, and FDP worked on the immunohistochemistry and PCR analysis. GZR, ABM, and RHS analyzed the data and wrote the paper. All authors approved the final version of the manuscript.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no conflict of interest.

References

1. Ménard C, Pfau ML, Hodes GE, Russo SJ (2017) Immune and neuroendocrine mechanisms of stress vulnerability and resilience. Neuropsychopharmacology 42(1):62–80. https://doi.org/10.1038/npp.2016.90
2. GBD 2015 (2016) Disease and Injury Incidence and Prevalence Collaborators Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: A systematic analysis for the global burden of disease study 2015. Lancet 388(10053):1545–1602. https://doi.org/10.1016/S0140-6736(16)31678-6
3. Winnicott DW (1941) On influencing and being influenced. New Era in Home and School, 22. In: 1957b (35–39), 1964a (199–204)
4. Ignácio ZM, Réus GZ, Quevedo J, Kalinichev M, Francis D (2017) Maternal deprivation. In: Reference module in neuroscience and biobehavioral psychology. Elsevier. ISBN 9780128093245
5. Widom CS, DuMont K, Czaja SJ (2007) A prospective investigation of major depressive disorder and comorbidity in abused and neglected children grown up. Arch Gen Psychiatry 64(1):49–56
6. Ignácio ZM, Réus GZ, Abelaira HM, Maciel AL, de Moura AB, Matos D, Demo JP, da Silva JB et al (2017) Quetiapine treatment reverses depressive-like behavior and reduces DNA methyltransferase activity induced by maternal deprivation. Behav Brain Res 320:225–232. https://doi.org/10.1016/j.bbr.2016.11.044
7. Réus GZ, Fernandes GC, de Moura AB, Silva RH, Darabas AC, de Souza TG, Abelaira HM, Carneiro C et al (2017) Early life experience contributes to the developmental programming of depressive-like behaviour, neuroinflammation and oxidative stress. J Psychiatr Res 95:196–207. https://doi.org/10.1016/j.jpsychires.2017.08.020
8. Schiepers OJ, Wichers MC, Maes M (2005) Cytokines and major depression. Prog Neuro-Psychopharmacol Biol Psychiatry 29(2):201–217
9. Czarny P, Wigner P, Galecki P, Sliwinski T (2017) The interplay between inflammation, oxidative stress, DNA damage, DNA repair and mitochondrial dysfunction in depression. Prog Neuro-Psychopharmacol Biol Psychiatry 80(Pt C):309–321. https://doi.org/10.1016/j.pnpbp.2017.06.036
10. Miller AJ, Zégre Z (2016) Landscape-scale disturbance: insights into the complexity of catchment hydrology in the mountaintop removal mining region of the eastern United States. Land 5(3):22. https://doi.org/10.3390/land5030022
11. Réus GZ, Abelaira HM, Tuon T, Titus SE, Ignácio ZM, Rodrigues AL, Quevedo J (2016) Glutamatergic NMDA receptor as therapeutic target for depression. Adv Protein Chem Struct Biol 103:169–202. https://doi.org/10.1016/bs.apcsb.2015.10.003
12. Cervenka I, Agudelo LZ, Ruas JL (2017) Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. Science 357(6349). https://doi.org/10.1126/science.aaf9794
13. Zhang D, Hu X, Qian L, O'Callaghan JP, Hong JS (2010) Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol 41(2–3):232–241. https://doi.org/10.1007/s12035-010-8098-4
14. Nakamura M, Okada S, Toyama Y, Okano H (2005) Role of IL-6 in spinal cord injury in a mouse model. Clin Rev Allergy Immunol 28(3):197–204
15. Torres-Platas SG, Cruceanu C, Chen GG, Turecki G, Mechawar N (2014) Evidence for increased microglial priming and macrophage

recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain Behav Immun 42:50–59. https://doi.org/10.1016/j.bbi.2014.05.007
16. Monai H, Hirase H (2017) Astrocytes as a target of transcranial direct current stimulation (tDCS) to treat depression. Neurosci Res S0168-0102(17):30532–30531. https://doi.org/10.1016/j.neures.2017.08.012
17. Norris JG, Tang LP, Sparacio SM, Benveniste EM (1994) Signal transduction pathways mediating astrocyte IL-6 induction by IL-1 beta and tumor necrosis factor-alpha. J Immunol 152(2):841–850
18. Mello PB (2009) Physical exercise can reverse the deficit in fear memory induced by maternal deprivation. Neurobiol Learn Mem 92(3):364–369. https://doi.org/10.1016/j.nlm.2009.04.004
19. Porsolt RD (1979) Animal model of depression. Biomedicine 30(3):139–140
20. Réus GZ, Stringari RB, Ribeiro KF, Cipriano AL, Panizzutti BS, Stertz L, Lersch C, Kapczinski F et al (2011) Maternal deprivation induces depressive-like behaviour and alters neurotrophin levels in the rat brain. Neurochem Res 36(3):460–466. https://doi.org/10.1007/s11064-010-0364-3
21. Réus GZ, Carlessi AS, Titus SE, Abelaira HM, Ignácio ZM, da Luz JR, Matias BI, Bruchchen L et al (2015) A single dose of S-ketamine induces long-term antidepressant effects and decreases oxidative stress in adulthood rats following maternal deprivation. Dev Neurobiol 75(11):1268–1281. https://doi.org/10.1002/dneu.22283
22. Sugama S, Fujita M, Hashimoto M, Conti B (2007) Stress induced morphological microglial activation in the rodent brain: Involvement of interleukin-18. Neuroscience 146(3):1388–1399
23. Diz-Chaves Y, Pernía O, Carrero P, Garcia-Segura LM (2012) Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. J Neuroinflammation 9:71. https://doi.org/10.1186/1742-2094-9-71
24. O'Loughlin E, Pakan JMP, Yilmazer-Hanke D, McDermott KW (2017) Acute in utero exposure to lipopolysaccharide induces inflammation in the pre- and postnatal brain and alters the glial cytoarchitecture in the developing amygdala. J Neuroinflammation 14(1):212. https://doi.org/10.1186/s12974-017-0981-8
25. Kim R, Healey KL, Sepulveda-Orengo MT, Reissner KJ (2017) Astroglial correlates of neuropsychiatric disease: from astrocytopathy to astrogliosis. Prog Neuro-Psychopharmacol Biol Psychiatry S0278-5846(17):30485–30482. https://doi.org/10.1016/j.pnpbp.2017.10.002
26. Sild M, Ruthazer ES, Booij L (2017) Major depressive disorder and anxiety disorders from the glial perspective: etiological mechanisms, intervention and monitoring. Neurosci Biobehav Rev 83:474–488. https://doi.org/10.1016/j.neubiorev.2017.09.014
27. Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26(10):523–530
28. Middeldorp J, Hol EM (2011) GFAP in health and disease. Prog Neurobiol 93(3):421–443. https://doi.org/10.1016/j.pneurobio.2011.01.005
29. Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, Horvath S, Geschwind DH (2013) Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. Cell 155(5):1008–1021. https://doi.org/10.1016/j.cell.2013.10.031
30. Procko C, Lu Y, Shaham S (2011) Glia delimit shape changes of sensory neuron receptive endings in C. elegans. Development 138(7):1371–1381. https://doi.org/10.1242/dev.058305
31. Si X, Miguel-Hidalgo JJ, O'Dwyer G, Stockmeier CA, Rajkowska G (2004) Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. Neuropsychopharmacology 29(11):2088–2096
32. Webster MJ, O'Grady J, Kleinman JE, Weickert CS (2005) Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. Neuroscience 133(2):453–461
33. Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31(8):1616–1626
34. Banasr M, Chowdhury GM, Terwilliger R, Newton SS, Duman RS, Behar KL, Sanacora G (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry 15(5):501–511. https://doi.org/10.1038/mp.2008.106
35. Leventopoulos M, Rüedi-Bettschen D, Knuesel I, Feldon J, Pryce CR, Opacka-Juffry J (2007) Long-term effects of early life deprivation on brain glia in Fischer rats. Brain Res 1142:119–126
36. Llorente R, Gallardo ML, Berzal AL, Prada C, Garcia-Segura LM, Viveros MP (2009) Early maternal deprivation in rats induces gender-dependent effects on developing hippocampal and cerebellar cells. Int J Dev Neurosci 27(3):233–241. https://doi.org/10.1016/j.ijdevneu.2009.01.002
37. Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity inthe prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48(8):861–873
38. Sanacora G, Banasr M (2013) From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. Biol Psychiatry 73(12):1172–1179. https://doi.org/10.1016/j.biopsych.2013.03.032
39. Kirik OV, Sukhorukova EG, Korzhevskiĭ DE (2010) Calcium-binding Iba-1/AIF-1 protein in rat brain cells. Morfologiia 137(2):5–8
40. Biesmans S, Meert TF, Bouwknecht JA, Acton PD, Davoodi N, De Haes P, Kuijlaars J, Langlois X et al (2013) Systemic immune activation leads to neuroinflammation and sickness behavior in mice. Mediat Inflamm 2013:271359. https://doi.org/10.1155/2013/271359
41. Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S (1998) Microglia-specific localisation of a novel calcium binding protein, Iba1. Mol Brain Res 57(1):1–9
42. Ito D, Tanaka K, Suzuki S, Dembo T, Fukuuchi Y (2001) Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. Stroke 32(5):1208–1215
43. Couch Y, Anthony DC, Dolgov O, Revischin A, Festoff B, Santos AI, Steinbusch HW, Strekalova T (2013) Microglial activation, increased TNF and SERT expression in the prefrontal cortex define stress-altered behaviour in mice susceptible to anhedonia. Brain Behav Immun 29:136–146. https://doi.org/10.1016/j.bbi.2012.12.017
44. Autieri MV, Kelemen S, Thomas BA, Feller ED, Goldman BI, Eisen HJ (2002) Allograft inflammatory factor-1 expression correlates with cardiac rejection and development of cardiac allograft vasculopathy. Circulation 106(17):2218–2223
45. Zhao YY, Yan DJ, Chen ZW (2013) Role of AIF-1 in the regulation of inflammatory activation and diverse disease processes. Cell Immunol 284(1–2):75–83. https://doi.org/10.1016/j.cellimm.2013.07.008
46. Ahmed Z, Shaw G, Sharma VP, Yang C, McGowan E, Dickson DW (2007) Actin-binding proteins coronin-1a and IBA-1 are effective microglial markers for immunohistochemistry. J Histochem Cytochem 55(7):687–700

47. Vinet J, Weering HR, Heinrich A, Kälin RE, Wegner A, Brouwer N, Heppner FL, Rooijen N et al (2012) Neuroprotective function for ramified microglia in hippocampal excitotoxicity. J Neuroinflammation 9:27. https://doi.org/10.1186/1742-2094-9-27
48. Sasaki Y, Ohsawa K, Kanazawa H, Kohsaka S, Imai Y (2001) Iba1 is an actin-cross-linking protein in macrophages/microglia. Biochem Biophys Res Commun 286(2):292–297
49. Benveniste EN, Benos DJ (1995) TNF-alpha- and IFN-gamma-mediated signal transduction pathways: Effects on glial cell gene expression and function. FASEB J 9(15):1577–1584
50. Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, Kapczinski F, Quevedo J (2015) The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience 300:141–154. https://doi.org/10.1016/j.neuroscience.2015.05.018
51. Nair A, Bonneau RH (2006) Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. J Neuroimmunol 171(1–2):72–85
52. Kang HJ, Voleti B, Hajszan T, Rajkowska G, Stockmeier CA, Licznerski P, Lepack A, Majik MS et al (2012) Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat Med 18(9):1413–1417
53. Hercher C, Canetti L, Turecki G, Mechawar N (2010) Anterior cingulate pyramidal neurons display altered dendritic branching in depressed suicides. J Psychiatr Res 44(5):286–293. https://doi.org/10.1016/j.jpsychires.2009.08.011
54. Zhao Q, Peng C, Wu X, Chen Y, Wang C, You Z (2014) Maternal sleep deprivation inhibits hippocampal neurogenesis associated with inflammatory response in young offspring rats. Neurobiol Dis 68:57–65. https://doi.org/10.1016/j.nbd.2014.04.008
55. Zhao Q, Xie X, Fan Y, Zhang J, Jiang W, Wu X, Yan S, Chen Y et al (2015) Phenotypic dysregulation of microglial activation in young offspring rats with maternal sleep deprivation-induced cognitive impairment. Sci Rep 5:9513. https://doi.org/10.1038/srep09513
56. Leklem JE (1971) Quantitative aspects of tryptophan metabolism in humans and other species: a review. Am J Clin Nutr 24(6):659–672
57. Réus GZ, Jansen K, Titus S, Carvalho AF, Gabbay V, Quevedo J (2015) Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidences from animal and human studies. J Psychiatr Res 68:316–328. https://doi.org/10.1016/j.jpsychires.2015.05.007
58. McNally L, Bhagwagar Z, Hannestad J (2008) Inflammation, glutamate, and glia in depression: a literature review. CNS Spectr 13(6):501–510
59. Müller N, Schwarz MJ (2007) The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Mol Psychiatry 12(11):988–1000
60. Réus GZ, Becker IRT, Scaini G, Petronilho F, Oses JP, Kaddurah-Daouk R, Ceretta LB, Zugno AI et al (2018) The inhibition of the kynurenine pathway prevents behavioral disturbances and oxidative stress in the brain of adult rats subjected to an animal model of schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 81:55–63. https://doi.org/10.1016/j.pnpbp.2017.10.009
61. Beal MF, Swartz KJ, Isacson O (1992) Developmental changes in brain kynurenic acid concentrations. Brain Res Dev Brain Res 68(1):136–139
62. Saito K, Nowak TS Jr, Suyama K, Quearry BJ, Saito M, Crowley JS, Markey SP, Heyes MP (1993) Kynurenine pathway enzymes in brain: responses to ischemic brain injury versus systemic immune activation. J Neurochem 61(6):2061–2070
63. Forrest CM, Khalil OS, Pisar M, McNair K, Kornisiuk E, Snitcofsky M, Gonzalez N, Jerusalinsky D et al (2013) Changes in synaptic transmission and protein expression in the brains of adult offspring afterprenatal inhibition of the kynurenine pathway. Neuroscience 254:241–259. https://doi.org/10.1016/j.neuroscience.2013.09.034
64. Chaves Filho AJM, Lima CNC, Vasconcelos SMM, de Lucena DF, Maes M, Macedo D (2018) IDO chronic immune activation and tryptophan metabolic pathway: A potential pathophysiologicallink between depression and obesity. Prog Neuro-Psychopharmacol Biol Psychiatry 80(Pt C):234–249. https://doi.org/10.1016/j.pnpbp.2017.04.035
65. Vignau J, Costisella O, Canva V, Imbenotte M, Duhamel A, Lhermitte M (2009) Impact of interferon alpha immunotherapy on tryptophan metabolism in patients with chronic hepatitis C. Results of a pilot studies on ten patients. L'Encéphale 35(5):477–483. https://doi.org/10.1016/j.encep.2007.09.007.
66. Oxenkrug GF (2010) Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan-kynurenine metabolism. Ann N Y Acad Sci 1199:1–14. https://doi.org/10.1111/j.1749-6632.2009.05356.x.
67. Réus GZ, Nacif MP, Abelaira HM, Tomaz DB, dos Santos MA, Carlessi AS, da Luz JR, Gonçalves RC et al (2015) Ketamine ameliorates depressive-like behaviors and immune alterations in adult rats following maternal deprivation. Neurosci Lett 584:83–87. https://doi.org/10.1016/j.neulet.2014.10.022
68. Dinel AL, André C, Aubert A, Ferreira G, Layé S, Castanon N (2014) Lipopolysaccharide-induced brain activation of the indoleamine 2,3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome. Psychoneuroendocrinology 40:48–59. https://doi.org/10.1016/j.psyneuen.2013.10.014