Brain, Behavior, and Immunity 28 (2013) 196–206

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

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

Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice

Yolanda Diz-Chaves *, Mariana Astiz, Maria José Bellini, Luis M. Garcia-Segura

Instituto Cajal, Avenida Doctor Arce 37, E-28002 Madrid, Spain

ARTICLE INFO

Article history:
Received 6 August 2012
Received in revised form 8 November 2012
Accepted 26 November 2012
Available online 1 December 2012

Keywords:
Prenatal stress
Inflammation
Cytokines
Interleukin 1-beta (IL1-β)
Tumor necrosis factor-alpha (TNF-α)
Microglia
Astroglia
Hippocampus

ABSTRACT

Early life experiences, such as prenatal stress, may result in permanent alterations in the function of the nervous and immune systems. In this study we have assessed whether prenatal stress affects the inflammatory response of the hippocampal formation of male mice to an inflammatory challenge during adulthood. Pregnant C57BL/6 mice were randomly assigned to stress ($n = 10$) or non-stress ($n = 10$) groups. Animals of the stress group were placed in plastic transparent cylinders and exposed to bright light for 3 sessions of 45 min every day from gestational day 12 to parturition. Non-stressed pregnant mice were left undisturbed. At four months of age, non stressed and prenatally stressed male offspring were killed, 24 h after the systemic administration of lipopolysaccharide (LPS) or vehicle. Under basal conditions, prenatally stressed animals showed increased expression of interleukin 1β and tumor necrosis factor-α (TNF-α) in the hippocampus and an increased percentage of microglia cells with reactive morphology in CA1 compared to non-stressed males. Furthermore, prenatally stressed mice showed increased TNF-α immunoreactivity in CA1 and increased number of Iba-1 immunoreactive microglia and GFAP-immunoreactive astrocytes in the dentate gyrus after LPS administration. In contrast, LPS did not induce such changes in non-stressed animals. These findings indicate that prenatal stress induces a basal proinflammatory status in the hippocampal formation during adulthood that results in an enhanced activation of microglia and astrocytes in response to a proinflammatory insult.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

In fetal life the tissues and organs of the body go through what are called "critical" periods of development (Widdowson and McCance, 1975). It is known that development and plasticity of the neuroendocrine system can be affected by many factors, and that adverse events during the prenatal period can result in long-lasting changes in adulthood (Matthews, 2002; Ader et al., 1990, 1995; Darnaudéry and Maccari, 2008; de Kloet and Oitzl, 2003; Weinstock, 2005). The influence of stress during pregnancy has received an increase attention in the last decades. In humans, prenatal stress is considered a risk factor for development of behavioral alterations and it has been associated with aggression, hyperactivity, anxiety, attention-deficit disorders and cognitive problems in adolescence and adulthood (Meijer, 1985; Wadhwa et al., 2001; O'Connor et al., 2003; Huizink et al., 2003, 2004; Austin et al., 2005; Gutteling et al., 2005; Talge et al., 2007), including an increase in the risk of schizophrenia, which is considered to have a developmental component (Brixey et al., 1993; Howes et al., 2004). In rats, prenatal restraint stress can exert profound influences on offspring's development (Maccari et al., 2001), causing permanent neurobiological and behavioral alterations. Some paradigms of chronic stress during gestation reduce the ability to filter or discriminate relevant from irrelevant information in adult life. This is one major defect observed in schizophrenic subjects and it is assessed by measuring prepulse inhibition, a parameter that is disrupted both in schizophrenic patients and in prenatally stressed rats (Koenig et al., 2005). Prenatal stress also produces learning and memory deficits in young, adult and aged rats, such as impairments in spatial memory assessed in the Morris water maze (Smith et al., 1981; Lordi et al., 1997; Vallée et al., 1999; Lemaire et al., 2000; Gué et al., 2004; Darnaudéry et al., 2006). However, one of the most obvious effects of prenatal stress in rodents is an increase in affective disorders in adult life. Thus, prenatal stressed rodents show enhanced emotional reactivity (Thompson, 1957; Fride et al., 1986; Wakshlak and Weinstock, 1990; Vallée et al., 1997a; Weinstock, 1997), increased anxiety behaviors (Poltyrev et al., 1996; Vallée et al., 1997b; Morley-Fletcher et al., 2003), enhanced conditioned fear (Griffin et al., 2003) and an increase in depression-like behaviors (Alonso et al., 1991; Secoli and Teixeira, 1998; Morley-Fletcher et al., 2003; Louvart et al., 2005). The hippocampus is involved in

* Corresponding author. Tel.: +34 915854729; fax: +34 915854754.
E-mail addresses: yolanda_diz@hotmail.com, ydiz@cajal.csic.es (Y. Diz-Chaves).

0889-1591/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2012.11.013

most of the behaviors that are affected by prenatal stress and is, therefore, the brain region selected for the present study.

Studies in non-pregnant populations have shown that stressful experiences can elevate inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) even in the absence of infection (Maes et al., 1998; Rozlog, 1999). In addition, it has been hypothesized that maternal psychosocial stress affects pregnancy outcome by altering inflammatory markers via neurochemicals that are released as part of the physiological response to stress (Coussons-Read et al., 2007; Dunkel Schetter, 2011). Indeed, prenatal stress results in an enhancement of certain aspects of immune function, including elevated levels of inflammatory cytokines in the periphery and in the brain (Laviola et al., 2004; Vanbesien-Mailliot et al., 2007; Diz-Chaves et al., 2012). This pro-inflammatory status of prenatally stressed animals may affect normal brain function, since cytokines such as IL6, IL-1β and TNF-α have different physiological roles, including regulation of neuronal development, ionic homeostasis, neuropeptide release and synaptic plasticity (Raber et al., 1998; Albensi and Mattson, 2000; Skoff et al., 2009; Park and Bowers, 2010). In addition, cytokines could take part in the pathogenesis and progression of neurodegenerative diseases (Griffin, 2006; Perry et al., 2007).

Our hypothesis is that prenatal stress causes a proinflammatory status that may influence the response to inflammatory challenges during adult life, further contributing to behavioral alterations. The enhanced inflammatory response in prenatally stressed animals can increase the levels of peripheral cytokines, such as IL1, in response to an inflammatory challenge during adulthood. Peripheral cytokines are able to cross the blood brain barrier, activate microglia and astroglia and promote local inflammation in brain structures, such as the hippocampus, which is involved in cognitive and affective alterations associated with prenatal stress (Corcos et al., 2002; Merlot et al., 2008; Laviola et al., 2004; Vanbesien-Mailliot et al., 2007; Diz-Chaves et al., 2012; McEwen et al., 1992; McEwen, 2000). In addition, peripheral cytokines stimulate the hypothalamo–pituitary–adrenal (HPA) axis (Dunn, 2000; Schöbitz et al., 1994) and elevate plasma corticosterone levels (Hashimoto et al., 2001), which in turn may affect the stress response and contribute to behavioral impairment. Thus, it is important to determine whether prenatal stress affects the inflammatory response in the hippocampus and the levels of corticosterone in response to inflammatory challenges in adulthood.

To assess these possibilities, in this study we have determined the influence of prenatal stress on corticosterone levels and the response of the hippocampus to an inflammatory challenge in male mice during adulthood. Systemic administration of lipopolysaccharide (LPS) was used to induce an inflammatory response, since this procedure is known to increase plasma corticosterone levels (Hashimoto et al., 2001) and to alter the levels of different inflammatory molecules in the hippocampus (Dantzer, 2004; Kubera et al., 2011; Monje et al., 2003). The inflammatory response of the hippocampus was evaluated by assessing the mRNA levels of interleukin 1β (IL1β) and tumor necrosis factor-α (TNF-α) and TNF-α immunoreactivity. In addition, we also assessed immunoreactivity for Iba-1, a marker of microglia, and immunoreactivity for GFAP, a marker of astroglia. Both glial cell types contribute to the inflammatory response and changes in the morphology and/or number of these cells are indicative of an altered neuroinflammatory status (Dong and Benveniste, 2001; Griffin, 2006; Perry et al., 2007; Streit et al., 1999; Block and Hong, 2005; Aloisi, 1999).

2. Material and methods

Animals were handled in accordance with the guidelines presented in the UFAW Handbook on the Care and Management of Laboratory Animals and following the European Union guidelines (Council Directives 86/609/EEC and 2010/63/UE). Experimental procedures were approved by our institutional animal use and care committee. Special care was taken to minimize suffering and to reduce the number of animals used to the minimum required for statistical accuracy. Animals were maintained in a temperature controlled room, with 12:12 h light/dark schedule and received food and water *ad libitum*.

Animals used in our experiments were derived from four different reproductions performed at separated seasonal periods throughout the year. Adult virgin C57BL/6 female mice (2 months of age) from the Complutense University animal colony were group-housed (6/cage) to coordinate their estrous cycle. The stage of the estrous cycle was determined by the appearance of the vagina (Champlin et al., 1973). Females in estrous were individually housed for 24 h in the presence of a sexually experienced male C57BL/6 mouse. Females were then examined to detect for the possible presence of a vaginal plug, which was used to confirm mating. The day of plug detection was considered as gestational day 1. Pregnant mice were then randomly assigned to stress ($n = 10$) or non-stress ($n = 10$) groups and individually housed in plastic breeding cages. Stress was started from the gestational day 12 to parturition. Pregnant mice were individually placed in plastic transparent cylinders (3.5 cm diameter, 10 cm long) and exposed to bright light (2,400 lux) for 45 min. Animals were daily submitted to three stress sessions starting at 09:00, 12:00 and 16:00 h, whereas non-stress pregnant animals were left undisturbed in their home cages as previously described (Ward and Weisz, 1980). Male and female offspring were weaned 21 days after birth, and only offspring from litters containing 5–9 pups with similar numbers of males and females were used in the experiments. A maximum of two male pups were taken from each litter to control for litter effects.

Four month-old males, 11 non-stressed (NS) and 13 prenatally stressed (PS), were used in the study. A group of them received an i.p. injection of 5 mg/kg of LPS (from Escherichia coli 0111:B4, L2630 Sigma–Aldrich) dissolved in PBS or vehicle (PBS). Therefore, four groups of animals were generated: non-stressed injected with vehicle (NS-VEH = 5), non-stressed injected with LPS (NS-LPS = 6), prenatally stressed injected with vehicle (PS-VEH = 6) and prenatally stressed injected with LPS (PS-LPS = 7). The dosage of LPS used (5 mg/kg, i.p.) was based on our previous study realized in prenatal stressed female mice (Diz-Chaves et al., 2012). Mice were sacrificed 24 h later by decapitation, trunk blood was collected and the brain was removed. The left hemisphere was immersed in 4% paraformaldehyde (Sigma–Aldrich) in 0.1 M phosphate buffer, pH 7.4 during 72 h and then rinsed with phosphate buffer and stored at −20 °C in a cryoprotective solution. The hippocampus was dissected from the other half of the brain and stored at −80 °C for real time RT-PCR analysis.

2.1. Corticosterone radioimmunoassay

Plasma samples collected from trunk blood were assayed using a commercial ^{125}I RIA kit for corticosterone (ImmuChem Double Antibody, MP Biomedicals, Orangeburg, NY, USA). The minimum detection limit of the assay is 7.7 ng/ml and intraassay coefficient of variation is 7.1%.

2.2. Real time RT-PCR analysis

Interleukin 1β (IL1β) and tumor necrosis factor-α (TNF-α) levels were assessed in the hippocampus by quantitative real-time polymerase chain reaction. Tissue was homogenated and RNA was extracted using an illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Buckinghamshire, UK). First strand cDNA was prepared

from RNA using a RevertAidTM H Minus First Strand cDNA Synthesis Kit (MBI Fermentas, Bath, UK) following the manufacturer's instructions. After reverse transcription (RT), the cDNA was diluted 1:8 (TNF-α, IL1β) and 5 μl were amplified by real-time PCR in 15 μl using SYBR Green master mix or TaqMan Universal PCR Master Mix (Applied Biosystems, AB, Foster City, CA) in a ABI Prism 7500 Sequence Detector (AB), with conventional AB cycling parameters (40 cycles of 95 °C, 15 s; 60 °C, 1 min). Primer sequences were designed using Primer Express (AB): IL1β: Forward 5′-CGACAAAA-TACCTGTGGCCT-3′ Reverse 5′-TTCTTTGGGTATTGCTTGGG-3′; TNF-α: Forward, 5′-GAAAAGCAAGCAGCCAACCA-3′, Reverse, 5′-CGGAT-CATGCTTTCTGTGCTC-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as control housekeeping gene. GAPDH TaqMan probes and primers were the Assay-on-Demand gene expression products (AB). After amplification, a denaturing curve was performed to ensure the presence of unique amplification products. All reactions were performed in duplicate. Data was represented using the $2^{-\Delta\Delta Ct}$ method. The cycle threshold (Ct) was determined for each gene. ΔCt was calculated by the difference between the Ct of IL1β and TNF-α and the Ct of GAPDH. Then, ΔΔCt was calculated by normalizing the ΔCt of each sample to the mean ΔCt value of the NS-VEH group. Finally $2^{-\Delta\Delta Ct}$ was calculated and its mean value was represented as percentage of NS-VEH values in the figures.

2.3. Immunohistochemistry

Sagittal sections of the hippocampus, 50 μm thick, were obtained using a Vibratome (VT 1000S, Leica Microsystems, Wetzlar, Germany). Immunohistochemistry was carried out in free-floating sections under moderate shaking. To decrease variability, sections for all experimental groups were processed in parallel in each assay run. Endogenous peroxidase activity was quenched for 10 min at room temperature in a solution of 3% hydrogen peroxide in 30% methanol. After several washes in 0.1 M phosphate buffer (pH 7.4), containing 0.3% BSA, 0.3% TritonX-100 and 0.9% NaCl (washing buffer), sections were incubated overnight at 4 °C with one of the following primary antibodies: a rabbit polyclonal antibody to Iba1 (Ionized calcium binding adaptor molecule 1, a marker of microglia) corresponding to C-terminus (Wako Chemical Industries, Japan; diluted 1:2000), a rabbit polyclonal antibody to glial fibrillary acidic protein (GFAP, a marker of astroglia; DakoCytomation, DK-2600, Glostrup, Denmark Z-0334,diluted 1:1000) and a rabbit polyclonal antibody to TNF-α (ab6671, Abcam, Cambrige, UK, diluted 1:100). Primary antibody was diluted in washing buffer containing 3% normal goat serum. After incubation with the primary antibody, sections were rinsed in buffer and incubated for 2 h at room temperature with biotinylated goat anti-rabbit immunoglobulin G (Pierce Antibody; Rockford, IL 61101, USA; diluted 1:300 in washing buffer). After several washes in buffer, sections were incubated for 90 min at room temperature with avidin–biotin peroxidase complex (ImmunoPure ABC peroxidase staining kit, Pierce). The reaction product was revealed by incubating the sections with 2 μg/ml 3,3′-diaminobenzidine (Sigma–Aldrich) and 0.01% hydrogen peroxide in 0.1 M phosphate buffer. Then, sections were dehydrated, mounted on gelatinized slides and examined with a Leitz Laborlux microscope. For specificity controls, sections were processed in the same way but omitting the primary antibodies.

2.4. Morphometric analysis

The morphometric analysis was performed by an investigator that was unaware of the identity of the experimental groups. The number of Iba1- and GFAP- immunoreactive cells were estimated with the optical disector method in the hilus of dentate gyrus and CA1 of hippocampus, using total section thickness for disector height at 40× (Hatton and Von Bartheld, 1999; Howard and Reed, 1998) and a counting frame of 220 × 220 μm. Section thickness was measured using a digital length gauge device (Heidenhain-Metro MT 12/ND221; Traunreut, Germany) attached to the stage of a Leitz microscope. A total of 28 counting frames were assessed/animal. In addition, the percentage of Iba1 immunoreactive cells with different morphologies was also assessed. To this aim, for each animal 120 cells were analyzed in the hilus of the dentate gyrus and in CA1. Cells were classified in five morphological types as previously described (Diz-Chaves et al., 2012): Type I, cells with few cellular processes (two or less); Type II, cells showing 3–5 short branches; Type III, cells with numerous (>5) and longer cell

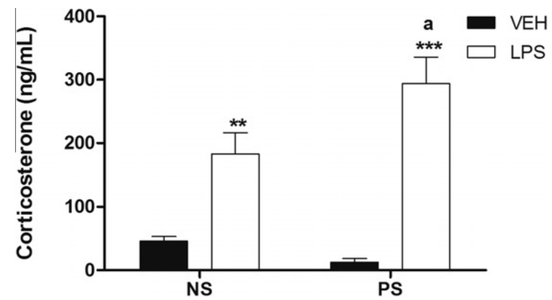

Fig. 1. Corticosterone plasma levels in non-stressed and prenatally-stressed male mice in baseline and after LPS insult. NS, non-stressed mice; PS, prenatally stressed mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean + SEM. a, Significant differences ($p < 0.05$) between PS-LPS and NS-LPS mice. **, Significant differences ($p < 0.01$) between NS-VEH and NS-LPS and ***, significant differences ($p < 0.001$) between PS-VEH and PS-LPS.

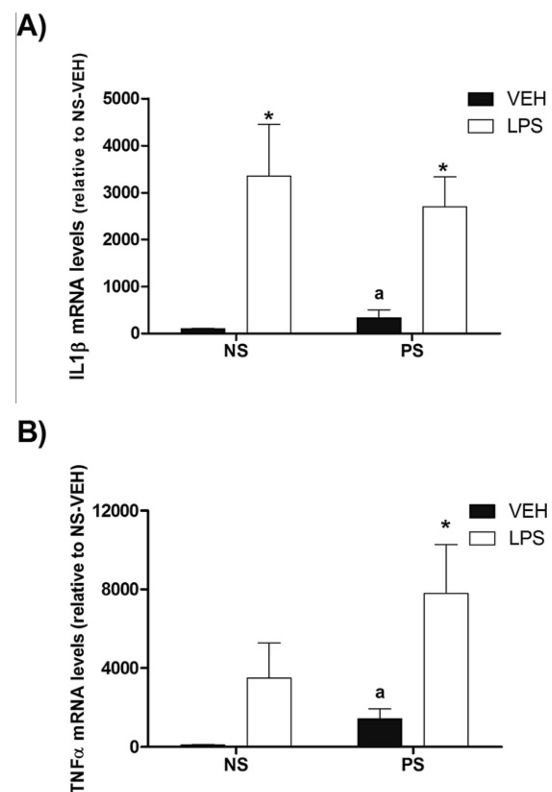

Fig. 2. (A), Interleukin-1β (IL1β) and (B) tumor necrosis factor α (TNF-α) mRNA levels in the hippocampus. NS, non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean + SEM. *Significant differences ($p < 0.05$) between LPS groups and their respective VEH groups. a, Significant differences ($p < 0.05$) between PS-VEH and NS-VEH group.

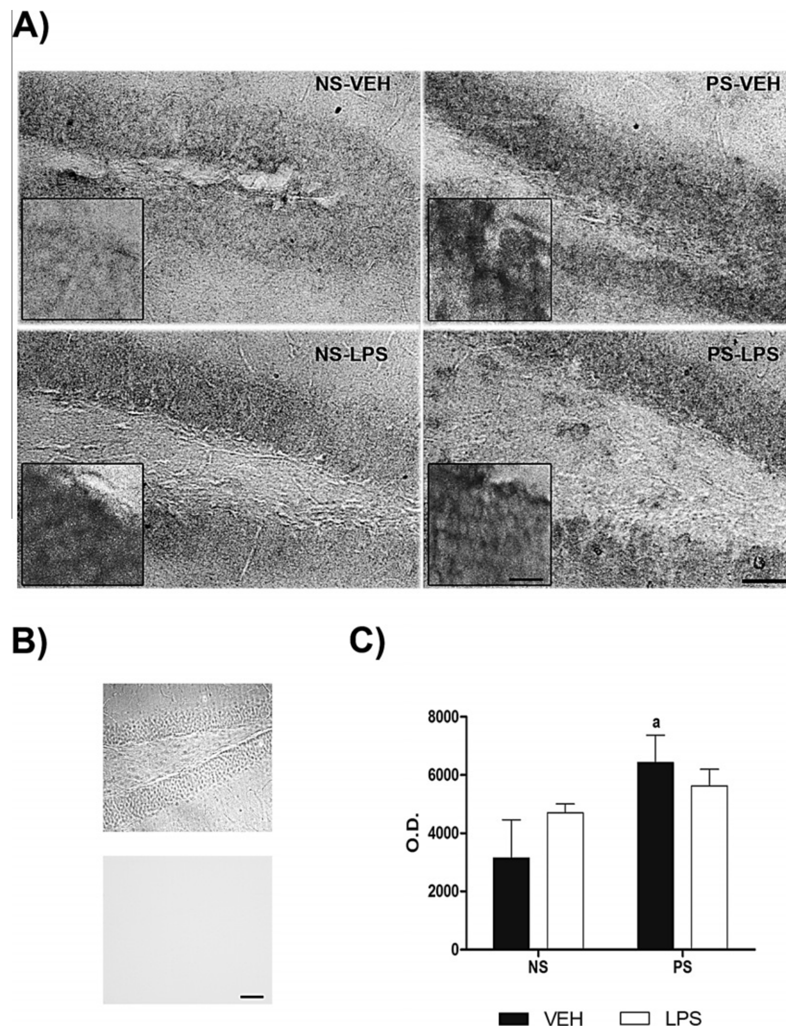

Fig. 3. (A) Representative images of TNF-alpha immunoreactivity in the dentate gyrus. NS-VEH, non-stressed male mouse treated with vehicle; PS-VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS; PS-LPS, prenatally stressed male mouse treated with LPS. Scale bar: 45 μm; scale bar in insets represents 15 μm. (B) Example of a section of the dentate gyrus processed for immunohistochemistry in absence of the primary antibody. Upper panel, phase contrast microscopy. Bottom panel, bright field microscopy. Scale bar: 75 μm. (C) Quantification of TNF-alpha immunoreactivity in the dentate gyrus. NS, non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are arbitrary optical density values and are represented as mean + SEM. a, significant differences ($p < 0.05$) between PS-VEH and NS-VEH group.

processes and a small cell body; Type IV, cells with large somas and retracted and thicker processes and Type V, cells with amoeboid cell body, numerous short processes and intense Iba1 immunostaining. Types IV and V were considered as reactive morphologies and their sum was represented as the percentage of total cells.

For TNF-α, optical density (OD) was calculated by the image processing program, image J (http://rsbweb.nih.gov/ij/). Briefly, images were captured on a Leitz microscope. All color JPEG images were digitally converted into a grey scale image before the initiation of the analysis. First, a calibration of the image was performed by the program. Then, an area in the zone of study was delimited in the dentate gyrus (including part of the granular layers and the hilus), and CA1. This area was of the same size for all analyzed images. In addition, a blank area with no immunoreactive cells was delimited in the section to normalize OD values.

2.5. Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism5 software (GraphPad Software, San Diego, CA, USA). Main and interactive effects were analyzed by two-way analysis of variance (ANOVA) for factorial measures. The Student's *t*-test for one-to-one comparisons was used when appropriate. When justified by the ANOVA analysis, differences between individual group means were analyzed by the Bonferroni posthoc test. Differences were considered statistically significant at $p \leqslant 0.05$.

3. Results

3.1. Prenatal stress enhances plasma corticosterone response to LPS

LPS treatment resulted in a significant increase in plasma corticosterone levels (Fig. 1; $F_{(1,19)} = 41.10$ $p < 0.0001$; two-way ANOVA) in both NS and PS mice ($p < 0.01$ and $p < 0.001$, respectively; Bonferroni's posttest). PS did not significantly affect corticosterone levels ($F_{(1,19)} = 1.443$ $p = 0.24$), but there was an interaction between PS and LPS ($F_{(1,19)} = 4.89$ $p = 0.03$), having LPS a higher effect in PS mice than in NS mice ($p < 0.05$; Bonferroni's posttest).

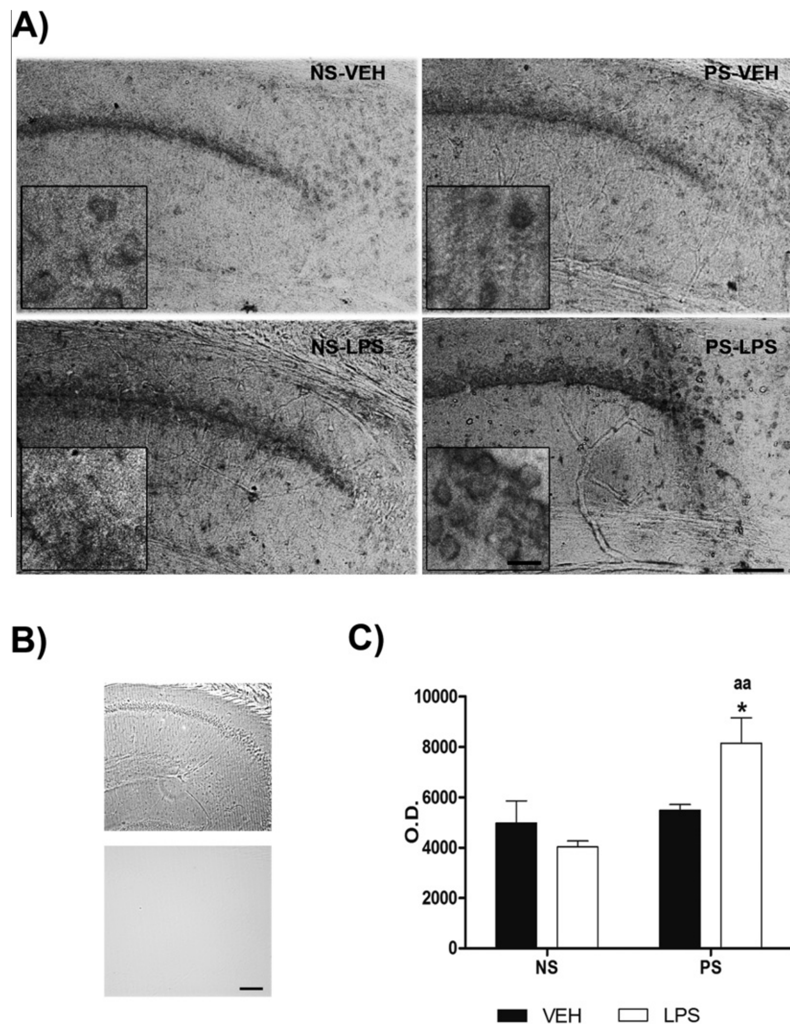

Fig. 4. (A) Representative images of TNF-alpha immunoreactive cells in CA1. NS/VEH, non-stressed male mouse treated with vehicle; PS/VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS; PS-LPS, prenatally stressed male mouse treated with LPS. Scale bar: 90 μm; Scale bar in insets: 15 μm. (B) Example of a section of CA1 processed for immunohistochemistry in absence of the primary antibody. Upper panel, phase contrast microscopy. Bottom panel, bright field microscopy. Scale bar: 75 μm. (C) Quantification of TNF-alpha immunoreactivity in CA1. S, non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are arbitrary optical density values and are represented as mean + SEM. *Significant differences ($p < 0.05$) between PS-VEH and PS-LPS; aa, significant differences ($p < 0.01$) between PS-LPS and NS-LPS group.

3.2. Prenatal stress increased the expression of IL1β and TNF-α under basal conditions and the expression of TNF-α after LPS treatment in the hippocampus

Prenatal stress increased the mRNA levels of IL1β and TNF-α under basal conditions (NS-VEH vs PS-VEH; T-test; $p < 0.05$; Fig. 2A and B). The administration of LPS increased the mRNA levels of IL1β, both in NS and PS mice ($F_{(1,19)} = 15.23$; $p < 0.001$; two way ANOVA; $p < 0.05$; Bonferroni's posttest) and there was not an interaction effect between PS and LPS (Fig. 2A). Moreover, LPS also increased the mRNA levels of TNF-α, but this increase was statistically significant only in PS mice ($F_{(1,18)} = 6.31$; $p = 0.02$; two-way ANOVA; $p < 0.05$; Bonferroni's posttest; Fig. 2B).

3.3. Prenatal stress increased TNF-α immunoreactivity in the dentate gyrus

Under basal conditions, PS mice displayed an increased TNF-α immunoreactive signal in the granular cell layer and the hilus of dentate gyrus compared to NS animals (Fig. 3; PS-VEH vs NS-VEH; two-way ANOVA; $F_{(1,11)} = 8.14$, $p = 0.015$; Bonferroni's posttest). LPS did not significantly affect TNF-α immunoreactivity and there was not a significant interaction between PS and LPS. No staining was detected in control sections.

3.4. LPS increased TNF-α immunoreactivity in CA1 of prenatally stressed mice

LPS increased TNF-α immunoreactive levels in CA1, but only in PS animals (Fig. 4; $F_{(1,9)} = 18.17$; $p = 0.002$; two-way ANOVA; PS-LPS vs NS-LPS $p < 0.01$; PS-LPS vs PS-VEH $p < 0.05$; Bonferroni's posttest). The statistical analysis revealed an interaction of PS and LPS ($F_{(1,9)} = 11.06$; $p = 0.008$). No staining was detected in control sections.

3.5. LPS increased the number of Iba-1 immunoreactive cells in the dentate gyrus of prenatally stressed mice

The number of Iba1-immunoreactive cells in the hilus of the dentate gyrus was not significantly different between NS and PS mice under basal conditions (NS-VEH vs PS-VEH; Fig. 5A and B). The administration of LPS significantly increased the number of

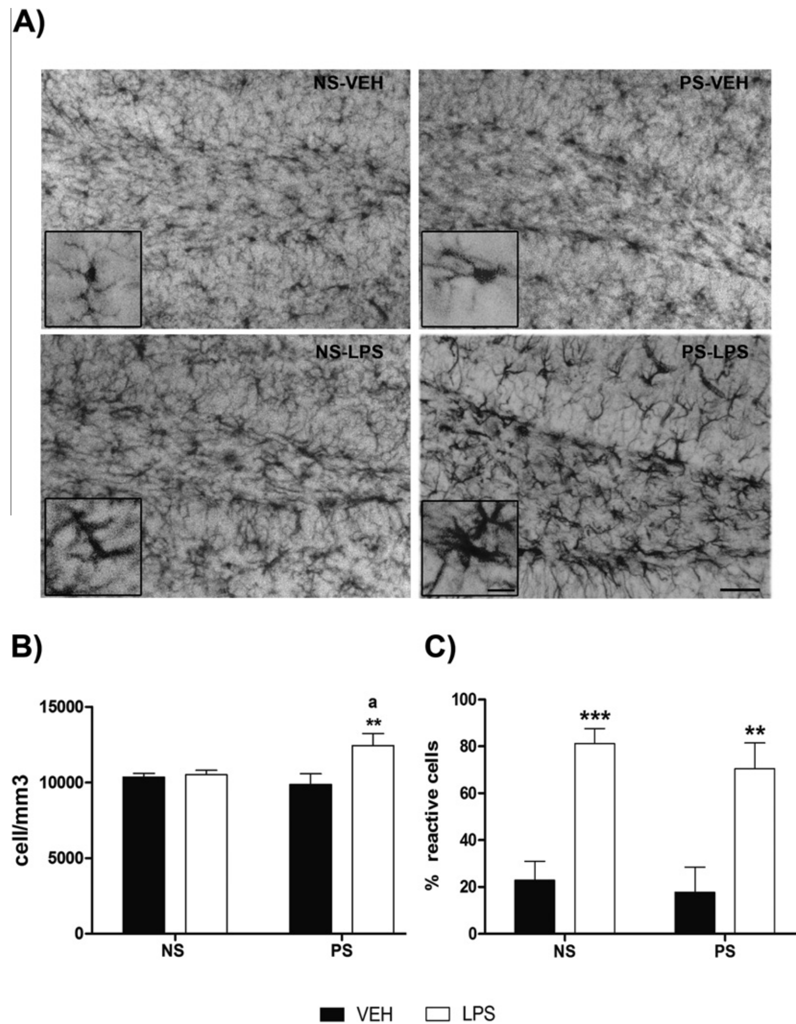

Fig. 5. (A) Representative images of the dentate gyrus of the hippocampus showing immunoreactivity for Iba1. NS-VEH, non-stressed male mouse treated with vehicle; PS-VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS and PS-LPS, prenatally stressed male mouse treated with LPS. Scale bar, 45 µm. In the insets, the scale bar represents 15 µm. (B) Number of Iba1 immunoreactive cells/mm^3 in the hilus of the dentate gyrus. NS, Non-stressed male mice; PS, Prenatally stressed males. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean + SEM. **Significant differences ($p < 0.01$) between PS-VEH and PS-LPS. a, significant differences ($p < 0.05$) between PS-LPS and NS-LPS. (C) Percentage of Iba1 immunoreactive cells with reactive morphology in the hilus of the dentate gyrus. NS, non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean + SEM. **Significant differences ($p < 0.01$) between PS-VEH and PS-LPS. ***Significant differences ($p < 0.001$) between NS-VEH and NS-LPS.

Iba1-immunoreactive cells in the hilus of the dentate gyrus, but only in PS mice (Fig. 5A and B; $F_{(1,19)} = 6.16$; $p = 0.02$; two-way ANOVA; PS-LPS vs PS-VEH $p < 0.01$; PS-LPS vs NS-LPS $p < 0.05$; Bonferroni's posttest). There was a significant interaction between PS and LPS ($F_{(1,19)} = 4.87$; $p = 0.03$).

3.6. LPS treatment altered the morphology of Iba1 cells in the dentate gyrus of the hippocampus

In addition to the differences in number, an obvious qualitative difference in the morphology of Iba-1 immunoreactive cells was observed after LPS treatment (Fig. 5A and C). Mice treated with LPS showed an increased percentage of Iba1-immunoreactive cells with larger somas and retracted and thicker processes, a phenotype characteristic of reactive microglia (Fig. 5A and C; $F_{(1,21)} = 35.67$; $p < 0.0001$; two-way ANOVA; NS-LPS vs NS-VEH $p < 0.001$; PS-LPS vs PS-VEH $p < 0.01$; Bonferroni's posttest). There was not a significant interaction between PS and LPS on the proportion of Iba-1 immunoreactive cells with reactive phenotype ($F_{(1,21)} = 0.086$; $p = 0.77$).

3.7. Prenatally stressed mice displayed increased percentage of Iba1-immunopositive cells with reactive morphology in CA1

A similar number of Iba-1 immunoreactive cells was observed in the CA1 region in all the experimental groups (Fig. 6A and B). However, prenatal stress under basal conditions increased the proportion of Iba1-immunoreactive cells with larger somas and retracted and thicker processes (Fig. 6A and C; $F_{(1,16)} = 87.7$; $p < 0.0001$; two-way ANOVA; $F_{(1,16)} = 6.8$; $p = 0.02$, effect of PS; PS-VEH vs NS-VEH $p < 0.05$; Bonferroni's posttest). In addition, LPS increased the proportion of Iba1-immunoreactive cells with reactive phenotype ($F_{(1,16)} = 6.8$; $p = 0.02$) in both NS and PS animals ($p < 0.001$; Bonferroni's posttest; Fig. 5A and C). No interaction between PS and LPS was detected in this parameter ($F_{(1,16)} = 0.01$; $p = 0.9$).

3.8. LPS treatment significantly increased the number of GFAP-immunoreactive cells in the dentate gyrus of prenatally stressed mice

Under basal conditions, no difference was observed in the number of GFAP-immunoreactive cells in the hilus of the dentate gyrus

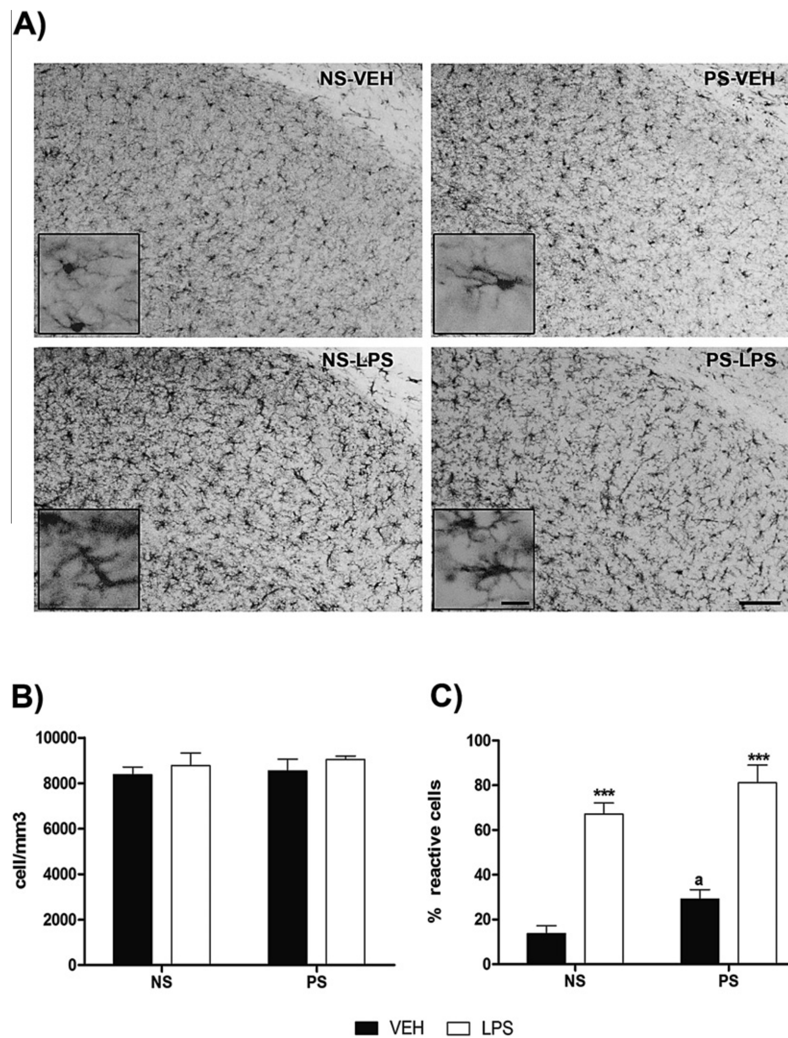

Fig. 6. (A) Representative images of CA1 showing immunoreactivity for Iba1. NS-VEH, non-stressed male mouse treated with vehicle; PS-VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS; PS-LPS, prenatally stressed male mouse treated with LPS. Insets show details of the morphology of Iba1 immunoreactive cells at high magnification. Scale bar, 90 μm. In the insets, the scale bar represents 15 μm. (B) Number of Iba1 immunoreactive cells/mm^3 in CA1. NS, non-stressed mice; PS, prenatally stressed mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean ± SEM. (C) Percentage of Iba1 immunoreactive cells with reactive morphology in CA1. NS, non-stressed mice; PS, prenatally stressed mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean ± SEM. ***Significant differences ($p < 0.001$) between NS-VEH and NS-LPS, and PS-VEH and PS-LPS. a, significant differences ($p < 0.05$) between PS-VEH and NS-VEH.

between NS and PS mice ($F_{(1,20)} = 0.93$; $p = 0.34$; Fig. 7A and B). LPS increased the number of GFAP-immunoreactive cells, but only in PS mice ($F_{(1,20)} = 7.85$; $p < 0.01$; two-way ANOVA; $p < 0.05$; Bonferroni's posttest; Fig. 7A and B). However, no significant interaction between PS and LPS was detected ($F_{(1,20)} = 1.54$; $p = 0.22$). In addition, the qualitative inspection of astrocytes showed that some of them displayed increased hypertrophic processes and soma in PS mice treated with LPS (Fig. 7A). This morphological change is characteristic of reactive astrogliosis.

The number of GFAP-immunoreactive cells was also estimated in CA1 (Fig. 8). The statistical analysis revealed that there was an effect of LPS (two-way ANOVA, $F_{(1,20)} = 8.8$; $p = 0.007$; Fig. 8A and B), but not an interaction of PS and LPS ($F_{(1,20)} = 0.08$; $p = 0.7$) and not an effect of PS ($F_{(1,20)} = 0.1$; $p = 0.74$; Fig. 8A and B). No posthoc differences were observed.

4. Discussion

As expected, our present results show that the administration of LPS caused an increase in plasma corticosterone levels in both prenatally stressed and non-stressed animals. However, the corticosterone response to LPS was enhanced in prenatally stressed animals. This finding is in agreement with previous studies in male rats, showing that prenatal stress increases the HPA axis response to different stimuli, including LPS (Henry et al., 1994; Vallée et al., 1999; Viltart et al., 2006; Hashimoto et al., 2001; Kohman et al., 2008). By the modification of the response of HPA to inflammation, prenatal stress may contribute to functional brain impairment, since alterations in the HPA axis are associated with behavioral pathologies and early aging of cognitive performances (de Kloet et al., 2005; Maccari et al., 2001; Maccari et al., 1995).

The inflammatory response of the hippocampus in response to LPS was also potentiated by prenatal stress. Although LPS increased the mRNA levels of IL1β and TNF-α in the hippocampus of both prenatally stressed and non-stressed mice, the effect for TNF-α was statistically significant in prenatally stressed animals only. LPS also resulted in a significant increase in TNF-α immunoreactivity in CA1 and this effect was also restricted to prenatally stressed mice. The number of microglia and astrocytes, two cell types that contribute to the inflammatory response of the brain (Monje et al., 2003; Dong and Benveniste, 2001; Griffin, 2006; Perry

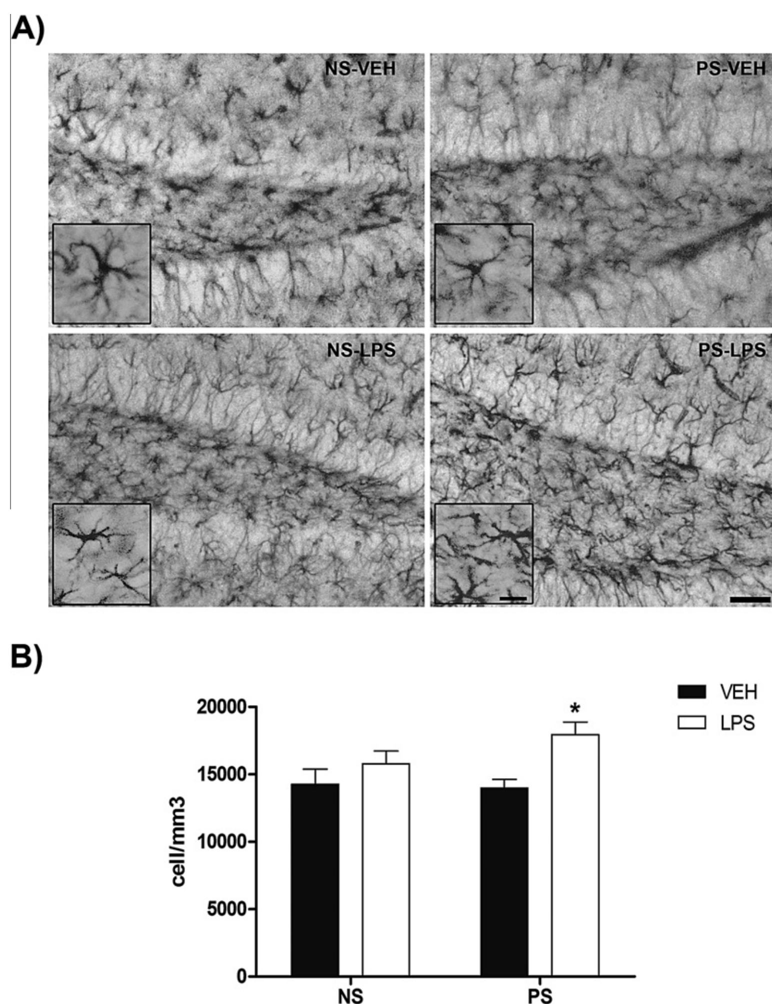

Fig. 7. (A) Representative images of the dentate gyrus of the hippocampus showing immunoreactivity for GFAP. NS-VEH, non-stressed male mouse treated with vehicle; PS-VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS and PS-LPS, prenatally stressed male mouse treated with LPS. Scale bar, 45 μm. In the inserts, the scale bar represents 15 μm. (B) Number of GFAP immunoreactive cells/mm^3 in the hilus of the dentate gyrus of the hippocampus. NS, Non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean ± SEM. *Significant differences ($p < 0.05$) versus PS-VEH.

et al., 2007; Streit et al., 1999; Jurgens and Johnson, 2012; McEwen, 2000; Santos-Galindo et al., 2011; Espinosa-Oliva et al., 2011), were also increased by LPS in the hippocampus, but only in the animals submitted to prenatal stress. In all these cases, ANOVA analysis revealed a significant interaction between prenatal stress and LPS treatment, with the exception of GFAP immunoreactive astrocytes. This exception may be the consequence of insufficient statistical power. Furthermore, some effects of LPS were not altered by prenatal stress, such as the increase in the levels of IL1β in the hippocampus and the increase in the proportion of microglia with a reactive phenotype in the dentate gyrus. Therefore, prenatal stress seems to affect specific aspects of the neuroinflammatory response.

In addition, the influence of prenatal stress on the response to LPS showed a regional specificity. For instance, prenatal stress enhanced the effect of LPS on TNFα immunoreactivity in CA1, but not in the dentate gyrus. In contrast, prenatal stress enhanced the effect of LPS on the number of Iba-1 immunoreactive cells in the dentate gyrus, but not in CA1. These regional differences may be in part due to the different expression of glucocorticoid and mineralocorticoid receptors in CA1 and the dentate gyrus (Joëls, 2008). In addition, prenatal stress is known to have regional effects on neuronal morphology (Barros et al., 2006; Mychasiuk et al., 2012; Tamura et al., 2011; Weinstock, 2011). For instance, in prepubertal male rats, prenatal stress causes an increase in spine density and dendritic complexity in granule cells of the dentate gyrus, while it reduces these parameters in CA3 pyramidal neurons (Bock et al., 2011). These regional effects of prenatal stress on neuronal and glial morphology may probably have an impact on regional neuro-glia communication and neuronal function.

Our findings suggest that prenatal stress predisposes for increased hippocampal immune response and enhanced microglia and astroglia activation when the organism is exposed to a proinflamatory challenge in adult life. The enhanced neuroinflammatory response to LPS in the animals submitted to prenatal stress may be the consequence of an increased peripheral inflammatory response (Espinosa-Oliva et al., 2011; Laviola et al., 2004; Vanbesien-Mailliot et al., 2007; Collier et al., 2011), which may cause enhanced glial activation and the consequent increased production of inflammatory molecules in the hippocampus. In addition, prenatal stress may increase the responsiveness of glial cells to peripheral and/or locally produced cytokines. Further studies should determine the precise mechanisms involved in the enhanced neuroimmune and glial response to LPS of prenatally stressed animals. In addition, the consequences of this enhanced activation of the immune and glial responses in the hippocampus are also unknown.

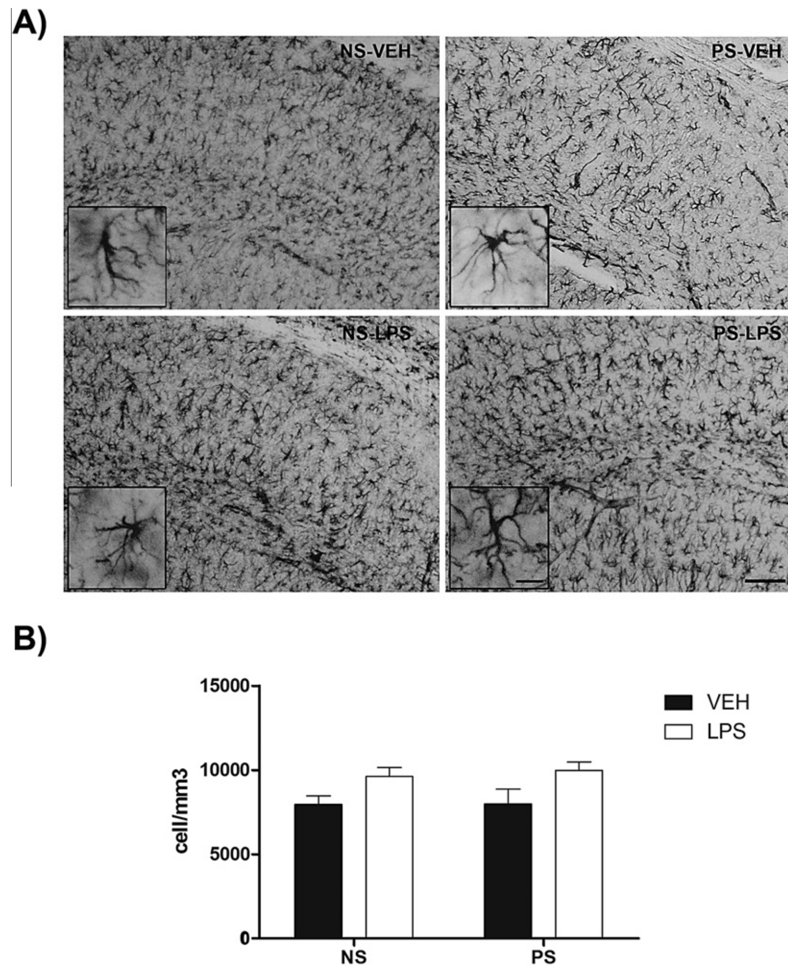

Fig. 8. (A) Representative images of CA1 showing immunoreactivity for GFAP. NS-VEH, non-stressed male mouse treated with vehicle; PS-VEH, prenatally stressed male mouse treated with vehicle; NS-LPS, non-stressed male mouse treated with LPS and PS-LPS, prenatally stressed male mouse treated with LPS. Scale bar, 90 μm. In the insets, the scale bar represents 15 μm. (B) Number of GFAP immunoreactive cells/mm^3 in CA1. NS, non-stressed male mice; PS, prenatally stressed male mice. Filled bars, animals treated with vehicle (VEH). Empty bars, animals treated with LPS. Data are mean ± SEM.

However, sustained overactivation of microglia can induce highly and detrimental neurotoxic effects by the excess production of a large array of cytotoxic factors such as superoxide nitric oxide and TNF-α (Block and Hong, 2005; Qin et al., 2007).

The enhanced inflammatory and glial response to LPS of the hippocampus of prenatally stressed animals suggests that prenatal stress induces a proinflammatory status in adult male mice. This possibility is further supported by the fact that prenatal stress, under basal conditions, increased the mRNA levels of IL1β and TNF-α in the hippocampus, increased TNF-α immunoreactivity in the dentate gyrus, and increased the proportion of Iba1-immunoreactive cells with reactive phenotype in CA1. The increased expression of IL1β and TNF-α in the hippocampus of prenatally stressed mice may be related to depressive-like behavior characteristic of this animal model (Maccari et al., 2001), since IL1β and TNF-α are critical mediators of the depressive-like behavior caused by acute and chronic stress (Koo and Dumanm, 2008; Kubera et al., 2011). The changes in IL1β and TNF-α mRNA levels and in TNF-α and Iba1 immunoreactivity in the hippocampus of prenatally streesed mice occurred in absence of increased corticosterone response, suggesting a central effect. This central effect of prenatal stress may be mediated by alterations in the development of microglia (Gómez-González and Escobar, 2010), which in turn may affect the development and activity of astrocytes and neurons and the local production of cytokines.

In conclusion, our findings indicate that prenatal stress enhances, in adult male mice, the hippocampal response to an inflammatory challenge. In addition, the hippocampal formation of prenatally stressed male mice displays, under basal conditions, a proinflammatory status characterized by enhanced expression of IL1β and TNF-α and increased activation of microglia. The altered phenotype of microglia under basal conditions and the altered response of microglia and astroglia after an inflammatory challenge may contribute to the behavioral and learning impairments previously observed in prenatally stressed animals.

Acknowledgments

Authors acknowledge Dr. M. López-Gallardo and Dr. R. Llorente for their help during corticosterone radioimmunoassay determinations. This work was supported by Grants BFU2008-02950-C03-01 and BFU2011-30217-C03-01 from the Ministerio de Economía y Competitividad, Spain.

References

Ader, R., Felten, D., Cohen, N., 1990. Interactions between the brain and the immune system. Annu. Rev. Pharmacol. Toxicol. 30, 561–602.
Ader, R., Cohen, N., Felten, D., 1995. Psychoneuroimmunology: interactions between the nervous system and the immune system. Lancet 345, 99–103.

Albensi, B.C., Mattson, M.P., 2000. Evidence for the involvement of TNF and NF-κB in hippocampal synaptic plasticity. Synapse 35, 151–159.
Aloisi, F., 1999. The role of microglia and astrocytes in CNS immune surveillance and immunopathology. Adv. Exp. Med. Biol. 468, 123–133.
Alonso, S.J., Arevalo, R., Afonso, D., Rodríguez, M., 1991. Effects of maternal stress during pregnancy on forced swimming test behavior of the offspring. Physiol. Behav. 50, 511–517.
Austin, M.P., Hadzi-Pavlovic, D., Leader, L., Saint, K., Parker, G., 2005. Maternal trait anxiety, depression and life event stress in pregnancy: relationships with infant temperament. Early Hum Dev. 81, 183–190.
Barros, V.G., Duhalde-Vega, M., Caltana, L., Brusco, A., Antonelli, M.C., 2006. Astrocyte–neuron vulnerability to prenatal stress in the adult rat brain. J. Neurosci. Res. 83, 787–800.
Block, M.L., Hong, J.S., 2005. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog. Neurobiol. 76, 77–98.
Bock, J., Murmu, M.S., Biala, Y., Weinstock, M., Braun, K., 2011. Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats. Neuroscience 193, 34–43.
Brixey, S.N., Gallagher, B.J., McFalls, J.A., Parmelee, L.F., 1993. Gestational and neonatal factors in the etiology of schizophrenia. J. Clin. Psychol. 49, 447–456.
Champlin, A.K., Dorr, D.L., Gates, A.H., 1973. Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. Biol. Reprod. 8, 491–494.
Collier, C.T., Williams, P.N., Carroll, J.A., Welsh, T.H., Laurenz, J.C., 2011. Effect of maternal restraint stress during gestation on temporal lipopolysaccharide-induced neuroendocrine and immune responses of progeny. Domest. Anim. Endocrinol. 40, 40–50.
Corcos, M., Guilbaud, O., Hjalmarsson, L., Chambry, J., Jeammet, P., 2002. Cytokines and depression: an analogic approach. Biomed. Pharmacother. 56, 105–110.
Coussons-Read, M.E., Okun, M.L., Nettles, C.D., 2007. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav. Immun. 21, 343–350.
Dantzer, R., 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur. J. Pharmacol. 500, 399–411.
Darnaudéry, M., Maccari, S., 2008. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res. Rev. 57, 571–585.
Darnaudéry, M., Perez-Martin, M., Bélizaire, G., Maccari, S., Garcia-Segura, L.M., 2006. Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats. Neurobiol. Aging 27, 119–127.
de Kloet, E.R., Oitzl, M.S., 2003. Who cares for a stressed brain? The mother, the kid or both? Neurobiol. Aging 1, S61–S65.
de Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475.
Diz-Chaves, Y., Pernía, O., Carrero, P., Garcia-Segura, L.M., 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.
Dong, Y., Benveniste, E.N., 2001. Immune function of astrocytes. Glia 36, 180–190.
Dunkel Schetter, C., 2011. Psychological science on pregnancy: stress processes, biopsychosocial models, and emerging research issues. Ann. Rev. Psychol. 62, 531–558.
Dunn, A.J., 2000. Cytokine activation of the HPA Axis. Ann. N. Y. Acad. Sci. 917, 608–617.
Espinosa-Oliva, A.M., de Pablos, R.M., Villarán, R.F., Argüelles, S., Venero, J.L., Machado, A., Cano, J., 2011. Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. Neurobiol. Aging 32, 85–102.
Fride, E., Dan, Y., Feldon, J., Halevy, G., Weinstock, M., 1986. Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. Physiol. Behav. 37, 681–687.
Gómez-González, B., Escobar, A., 2010. Prenatal stress alters microglial development and distribution in postnatal rat brain. Acta Neuropathol. 119, 303–315.
Griffin, W.S., 2006. Inflammation and neurodegenerative diseases. Am. J. Clin. Nutr. 83, 470S–474S.
Griffin, W.C., Skinner, H.D., Salm, A.K., Birkle, D.L., 2003. Mild prenatal stress in rats is associated with enhanced conditioned fear. Physiol. Behav. 79, 209–215.
Gué, M., Bravard, A., Meunier, J., Veyrier, R., Gaillet, S., Recasens, M., Maurice, T., 2004. Sex differences in learning deficits induced by prenatal stress in juvenile rats. Behav. Brain Res. 150, 149–157.
Gutteling, B.M., de Weerth, C., Willemsen-Swinkels, S.H., Huizink, A.C., Mulder, E.J., Visser, G.H., Buitelaar, J.K., 2005. The effects of prenatal stress on temperament and problem behavior of 27-month-old toddlers. Eur. Child. Adolesc. Psychiatry 14, 41–51.
Hashimoto, M., Watanabe, T., Fujioka, T., Tan, N., Yamashita, H., Nakamura, S., 2001. Modulating effects of prenatal stress on hyperthermia induced in adult rat offspring by restraint or LPS-induced stress. Physiol. Behav. 73, 125–132.
Hatton, W.J., Von Bartheld, C.S., 1999. Analysis of cell death in the trochlear nucleus of the chick embryo: calibration of the optical disector counting method reveals systematic bias. J. Comp. Neurol. 409, 169–186.
Henry, C., Kabbaj, M., Simon, H., Le Moal, M., Maccari, S., 1994. Prenatal stress increases the hypothalamo–pituitary–adrenal axis response in young and adult rats. J. Neuroendocrinol. 6, 341–345.
Howard, C.V., Reed, M.G., 1998. Unbiased Stereology. Three-dimensional Measurement in Microscopy. BIOS Scientific Publishers, Oxford.
Howes, O.D., McDonald, C., Cannon, M., Arseneault, L., Boydell, J., Murray, R.M., 2004. Pathways to schizophrenia: the impact of environmental factors. Int. J. Neuropsychopharmacol. Suppl. 1, S7–S13.
Huizink, A.C., Robles de Medina, P.G., Mulder, E.J., Visser, G.H., Buitelaar, J.K., 2003. Stress during pregnancy is associated with developmental outcome in infancy. J. Child Psychol. Psychiatry 44, 810–818.
Huizink, A.C., Mulder, E.J., Buitelaar, J.K., 2004. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychol. Bull. 130, 115–142.
Joëls, M., 2008. Functional actions of corticosteroids in the hippocampus. Eur. J. Pharmacol. 583, 312–321.
Jurgens, H.A., Johnson, R.W., 2012. Dysregulated neuronal-microglial cross-talk during aging, stress and inflammation. Exp. Neurol. 233, 40–48.
Koenig, J.I., Elmer, G.I., Shepard, P.D., Lee, P.R., Mayo, C., Joy, B., Hercher, E., Brady, D.L., 2005. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav. Brain Res. 156, 251–261.
Kohman, R.A., Tarr, A.J., Day, C.E., McLinden, K.A., Boehm, G.W., 2008. Influence of prenatal stress on behavioral, endocrine, and cytokine responses to adulthood bacterial endotoxin exposure. Behav. Brain Res. 193, 257–268.
Koo, J.W., Dumanm, R.S., 2008. IL-1β is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc. Nat. Ac. Sci. USA 105, 751–756.
Kubera, M., Obuchowicz, E., Goehler, L., Brzeszcz, J., Maes, M., 2011. In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 744–759.
Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R., Bertini, M., Pacifici, R., 2004. Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. Eur. J. Neurosci. 20, 1655–1664.
Lemaire, V., Koehl, M., Le Moal, M., Abrous, D.N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc. Natl. Acad. Sci. USA 97, 11032–11037.
Lordi, B., Protais, P., Mellier, D., Caston, J., 1997. Acute stress in pregnant rats: effects on growth rate, learning, and memory capabilities of the offspring. Physiol. Behav. 62, 1087–1092.
Louvart, H., Maccari, S., Darnaudéry, M., 2005. Prenatal stress affects behavioral reactivity to an intense stress in adult female rats. Brain Res. 1031, 67–73.
Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H., Le Moal, M., 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J. Neurosci. 15, 110–116.
Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A.R., Cinque, C., Van Reeth, O., 2001. Prenatal stress and long-term consequences: implications of glucocorticoid hormones. Neurosci. Biobehav. Rev. 27, 119–127.
Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., Bosmans, E., De Meester, I., Benoy, I., Neels, H., Demedts, P., Janca, A., Scharpé, S., Smith, R.S., 1998. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and th1-like response in stress-induced anxiety. Cytokine 10, 313–318.
Matthews, S.G., 2002. Early programming of the hypothalamo–pituitary–adrenal axis. Trends Endocrinol. Metab. 13, 373–380.
McEwen, B.S., 2000. Effects of adverse experiences for brain structure and function. Biol. Psychiatry 48, 721–731.
McEwen, B.S., Gould, E., Sakai, R., 1992. The vulnerability of the hippocampus to protective and destructive effects of glucocorticoids in relation to stress. Br. J. Psychiatry Suppl. 15, 18–23.
Meijer, A., 1985. Child psychiatric sequelae of maternal war stress. Acta Psychiatr. Scand. 72, 505–511.
Merlot, E., Couret, D., Otten, W., 2008. Prenatal stress, fetal imprinting and immunity. Brain, Behav. Immun. 22, 42–51.
Monje, M.L., Toda, H., Palmer, T.D., 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302, 1760–1765.
Morley-Fletcher, S., Rea, M., Maccari, S., Laviola, G., 2003. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur. J. Neurosci. 18, 3367–3374.
Mychasiuk, R., Gibb, R., Kolb, B., 2012. Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. Synapse 66, 308–314.
O'Connor, T.G., Heron, J., Golding, J., Glover, V., ALSPAC Study Team, 2003. Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. J. Child. Psychol. Psychiatry 44, 1025–1036.
Park, K.M., Bowers, W.J., 2010. Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. Cellular Signalling 22, 977–983.
Perry, V.H., Cunningham, C., Holmes, C., 2007. Systemic infections and inflammation affect chronic neurodegeneration. Nat. Rev. Immunol. 7, 161–167.
Poltyrev, T., Keshet, G.I., Kay, G., Weinstock, M., 1996. Role of experimental conditions in determining differences in exploratory behavior of prenatally stressed rats. Dev. Psychobiol. 29, 453–462.
Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., Crews, F.T., 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55, 453–462.
Raber, J., Sorg, O., Horn, T.F., Yu, N., Koob, G.F., Campbell, I.L., Bloom, F.E., 1998. Inflammatory cytokines: putative regulators of neuronal and neuro-endocrine function. Brain Res. Brain Res. Rev. 26, 320–326.
Rozlog, L., 1999. Stress and immunity: implications for viral disease and wound healing. J. Periodontol. 70, 786–792.

Santos-Galindo, M., Acaz-Fonseca, E., Bellini, M.J., Garcia-Segura, L.M., 2011. Sex differences in the inflammatory response of primary astrocytes to lipopolysaccharide. Biol. Sex Differences 2, 1–7.

Schöbitz, B., Sutanto, W., Carey, M.P., Holsboer, F., de Kloet, E.R., 1994. Endotoxin and interleukin 1 decrease the affinity of hippocampal mineralocorticoid (type I) receptor in parallel to activation of the hypothalamic–pituitary–adrenal axis. Neuroendocrinology 60, 124–133.

Secoli, S.R., Teixeira, N.A., 1998. Chronic prenatal stress affects development and behavioral depression in rats. Stress 2, 273–280.

Skoff, A.M., Zhao, C., Adler, J.E., 2009. Interleukin-1alpha regulates substance P expression and release in adult sensory neurons. Exp. Neurol. 217, 395–400.

Smith, B.L., Wills, G., Naylor, D., 1981. The effects of prenatal stress on rat offsprings' learning ability. J. Psychol. 107, 45–51.

Streit, W.J., Walter, S.A., Pennell, N.A., 1999. Reactive microgliosis. Prog. Neurobiol. 57, 563–581.

Talge, N.M., Neal, C., Glover, V., 2007. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J. Child. Psychol. Psychiatry 48, 245–261.

Tamura, M., Sajo, M., Kakita, A., Matsuki, N., Koyama, R., 2011. Prenatal stress inhibits neuronal maturation through downregulation of mineralocorticoid receptors. J. Neurosci. 31, 11505–11514.

Thompson, W.R., 1957. Influence of prenatal maternal anxiety on emotionality in young rats. Science 125, 698–699.

Vallée, M., Mayo, W., Darnaudéry, M., Corpéchot, C., Young, J., Koehl, M., Le Moal, M., Baulieu, E.E., Robel, P., Simon, H., 1997a. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. Proc. Natl. Acad. Sci. USA 94, 14865–14870.

Vallée, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., Maccari, S., 1997b. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J. Neurosci. 17, 2626–2636.

Vallée, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M., Mayo, W., 1999. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance. a longitudinal study in the rat. Eur. J. Neurosci. 11, 2906–2916.

Vanbesien-Mailliot, C.C., Wolowczuk, I., Mairesse, J., Viltart, O., Delacre, M., Khalife, J., Chartier-Harlin, M.C., Maccari, S., 2007. Prenatal stress has pro-inflammatory consequences on the immune system in adult rats. Psychoneuroendocrinology 32, 114–124.

Viltart, O., Mairesse, J., Darnaudéry, M., Louvart, H., Vanbesien-Mailliot, C., Catalani, A., Maccari, S., 2006. Prenatal stress alters Fos protein expression in hippocampus and locus coeruleus stress-related brain structures. Psychoneuroendocrinology 31, 769–780.

Wadhwa, P.D., Sandman, C.A., Garite, T.J., 2001. The neurobiology of stress in human pregnancy: implications for prematurity and development of the fetal central nervous system. Prog. Brain Res. 133, 131–142.

Wakshlak, A., Weinstock, M., 1990. Neonatal handling reverses behavioral abnormalities induced in rats by prenatal stress. Physiol. Behav. 48, 289–292.

Ward, I.L., Weisz, J., 1980. Maternal stress alters plasma testosterone in fetal males. Science 207, 328–329.

Weinstock, M., 1997. Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? Neurosci. Biobehav. Rev. 21, 1–10.

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav. Immun. 19, 296–308.

Weinstock, M., 2011. Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. Stress 14, 604–613.

Widdowson, E.M., McCance, R.A., 1975. A review: new thoughts on growth. Pediatr. Res. 9, 154–156.