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Alteration of neurotrophin and cytokine expression in lymphocytes as novel peripheral markers of spatial memory deficits induced by prenatal stress

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HIGHLIGHTS

- Prenatal stress induced spatial memory impairment in adult female mice.
- Memory alteration was related to GR, BDNF and Th1/Th2 balance changes in the hippocampus.
- These changes were found in peripheral lymphocytes as well.
- Lymphocytes could be peripheral markers of susceptibility to behavioral alteration.

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ABSTRACT

Much evidence has suggested that early life adversity can have a lasting effect on behavior. The aim of this study was to explore the impact of prenatal exposure to stress on cognition in adult life and how it impacts chronic stress situations. In addition, we investigated the participation of glucocorticoids, neurotrophins and cytokines in prenatal stress effects. For this purpose, pregnant mice were placed in a cylindrical restraint tube for 2 h daily during the last week of pregnancy. Control pregnant females were left undisturbed during their entire pregnancy period. Object-in-place task results showed that adult female mice exposed to prenatal stress exhibited an impairment in spatial memory. However, in the alternation test this memory deficit was only found in prenatally stressed mice submitted to chronic stress. This alteration occurred in parallel with a decrease in BDNF, an increase in glucocorticoid receptors and an alteration of Th1/Th2 in the hippocampus. Interestingly, these changes were observed in peripheral lymph nodes as well. However, none of the mentioned changes were observed in adult male mice. These results indicate that lymphoid cells could be good candidates as peripheral markers of susceptibility to behavioral alterations associated with prenatal exposure to stress.

1. Introduction

Stress and adaptation to stress requires numerous homeostatic adjustments. Allostasis refers to the adaptive processes that maintain homeostasis through interrelations between the hypothalamus–pituitary–adrenal axis (HPA), sympathetic–parasympathetic efferent pathways and chemical messengers (hormones, neurotransmitters, interleukins, neurotrophins) [1]. These mediators of the stress response promote adaptation in the aftermath of acute stress, but they also contribute to allostatic overload: the wear and tear on the body and brain that results from being "stressed out" [2]. While a response to stress is a necessary survival mechanism, prolonged stress can produce severe consequences that affect behavioral, endocrine and immunological parameters [2]. Among these parameters, the hippocampus, which is a

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limbic area involved in learning and memory, is particularly sensitive to stress [3]. In particular, structural alterations of the hippocampal formation and reduction of neurogenesis in the adult dentate gyrus have been observed in different animal models of chronic stress [4,5].

Exposure to early life adversity may deeply affect brain development leading to long-lasting effects on neuronal structure and behavior playing a key role in the etiology of anxiety and mood disorders. Several evidence reveals that neuronal and synaptic changes induced by prenatal stress (PS) exposure are highly region-specific. On the structural level the most dramatic changes are found in limbic and prefrontal cortical areas, those regions which are involved in cognitive as well as emotional functions. In this context, the hippocampus has been the classically studied brain area to investigate PS-related effects (for a review see [6]).

Moreover, PS can result in stable long-term changes in central and peripheral stress response systems as well as influence the response to stress in adulthood [7,8]. It has also been found that prenatal stress (PS) induces an enhanced fear-like behavioral profile and dysregulation of brain noradrenergic and HPA activity after a stress during adulthood [8,9]. However, there are studies in animals [10–12] that suggest that PS has an adaptive effect that helps offspring respond appropriately to stressors in the environment. Genetic predisposition, including animal strain, polymorphism and gender, are factors that contribute to vulnerability/resilience against PS [13].

In addition, there are other factors, such as neurotrophins and cytokines, that have been shown to be involved in stress-related pathology [14,15], even though the alteration of their levels under PS has not been exhaustively studied.

Neurotrophins are a family of secreted growth factors that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and NT4. They play crucial roles in the formation and plasticity of neuronal networks and have been shown to be involved in the pathophysiology of suicide [16] and depression [17].

Several studies have demonstrated that immune system can signal the central nervous system through the action of cytokines (for a review see [18]). Among others, cytokines can be classified by their action: pro-inflammatory or anti-inflammatory; and by the type of T lymphocyte that produce them: T-helper 1 lymphocytes (Th1) or T-helper 2 lymphocytes (Th2). In general, Th-1 lymphocytes release cytokines enhancing the cell mediated immune response (i.e. IFN-γ, IL-2). Whereas, Th-2 cytokines (i.e. IL-4, IL-10) enhance the humoral response by activating cells to express antibodies. Th-1 cytokines are mainly pro-inflammatory, while Th-2 cytokines are mainly anti-inflammatory. Equilibrium between pro- and anti-inflammatory is essential to maintain the homeostasis in the immune system [15]. In addition, shifts in the Th1/Th2 balance have been involved in the pathogenesis of many human illnesses, such as autoimmune diseases, sleep disturbance, major depression and other disorders [15,19]. It has been proposed that immunity might play an important role in maintenance, protection and repair of both a healthy and diseased CNS [20]. Recently, we found a correlation between poor memory performance and a shift to Th2 responses [21].

In this context, the purpose of the present study was to analyze the impact of PS exposure on behavior and cognition in adult life as well as the impact of PS on chronic stress situations. In addition, we investigated if these long-lasting effects induced by prenatal stress exposure were related to alterations in stress reactivity and/or changes in cytokine and/or neurotrophin levels in hippocampus. Furthermore, we analyzed if the molecular changes in hippocampus are also found in lymphocytes in order to propose these cells as peripheral markers of susceptibility to behavioral alterations. Because several studies have reached a consensus that PS has sex-specific adverse effects on behavior [22], we compared the PS effects in males and females in this study.

2. Materials and methods

2.1. Animals

Inbred 60-day-old BALB/c mice were acquired from the Veterinary School of the University of Buenos Aires (Argentina). The mice were housed and maintained on a 12/12 light/dark cycle under a controlled temperature (18–22 °C). Animals were taken care of and sacrificed according to the rules of the "Guide for the Care and Use of Laboratory Animals" (NIH) (revision 2011) and to the EC Directive 86/609/EEC (revision 2010). The experimental protocol was also approved by the Institutional Committee for the Use and Care of Laboratory Animal rules (CICUAL, School of Medicine, University of Buenos Aires, Argentina) under resolution 2962/10 and 2947/13.

2.2. Experimental design

Fig. 1 shows a scheme of the experimental design used in the present work. Pregnant mice were divided in two groups, one left undisturbed and the other submitted to restraint stress. Both 60 day old prenatally unstressed and stressed female and male offspring were randomly assigned to the following groups: acute stress, chronic stress and undisturbed groups. The combination of prenatal and adult treatment resulted in the following six groups: mice that never were exposed to stress (offspring from unstressed females that were left undisturbed, CN), mice from unstressed females that received chronic stress as adults (CN-CS), mice exposed only to prenatal stress (PS), mice that received both prenatal stress and chronic stress as adults (PS-CS), mice from unstressed females that received acute stress as adults (CN-AS), and mice that received both prenatal stress and acute stress as adults (PS-AS). With the exception of animals used for determining plasma corticosterone levels, all animals were used for behavioral testing and several biochemical and molecular determinations. After behavioral testing, mice were left undisturbed in their home cages for 48 h prior to sacrifice.

2.3. Prenatal stress

The PS model was conducted as we previously described [23] according the protocol reported by Popova et al. [24]. Briefly, 40 pregnant mice were placed in a cylindrical restraint tube (4 cm diameter, 10 cm long) for 2 h daily (from 10 AM to 12) from day 15 of pregnancy until delivery (days 20–21). Non-exposed control pregnant female ($n = 40$) were left undisturbed during their entire pregnancy. Food intake and body weight were not different between the control and stressed pregnant mice. Pregnant females gave birth to about four or five pups per litter. No differences were found between the number and weight of alive pups between the control and stressed pregnant females, as previously reported [23]. In addition, no changes in maternal behavior were observed throughout lactation. Pups from both control and stressed mothers were separated at postnatal day 21 and placed in an identical environment up to an age of 60 days. To exclude possible litter effects, mice from different litters were randomly assigned to each group.

2.4. Stress in adult life

To determine behavioral changes induced by chronic stress exposure, offspring of 60 days of age were restrained by placing each animal in a well-ventilated polypropylene tube (2.8 cm diameter–11.5 cm long) for 2 h starting at 10:00 AM, and then the mice were returned to their cages. This procedure was repeated for 3 weeks and then the mice were left undisturbed for 1 day before behavioral tests or sacrifice.

In order to asses stress reactivity, corticosterone levels were determined in animals submitted or not to acute stress. Offspring of 60 days of age were restrained by placing each animal in a well-ventilated polypropylene tube (2.8 cm diameter–11.5 cm long) for 2 h

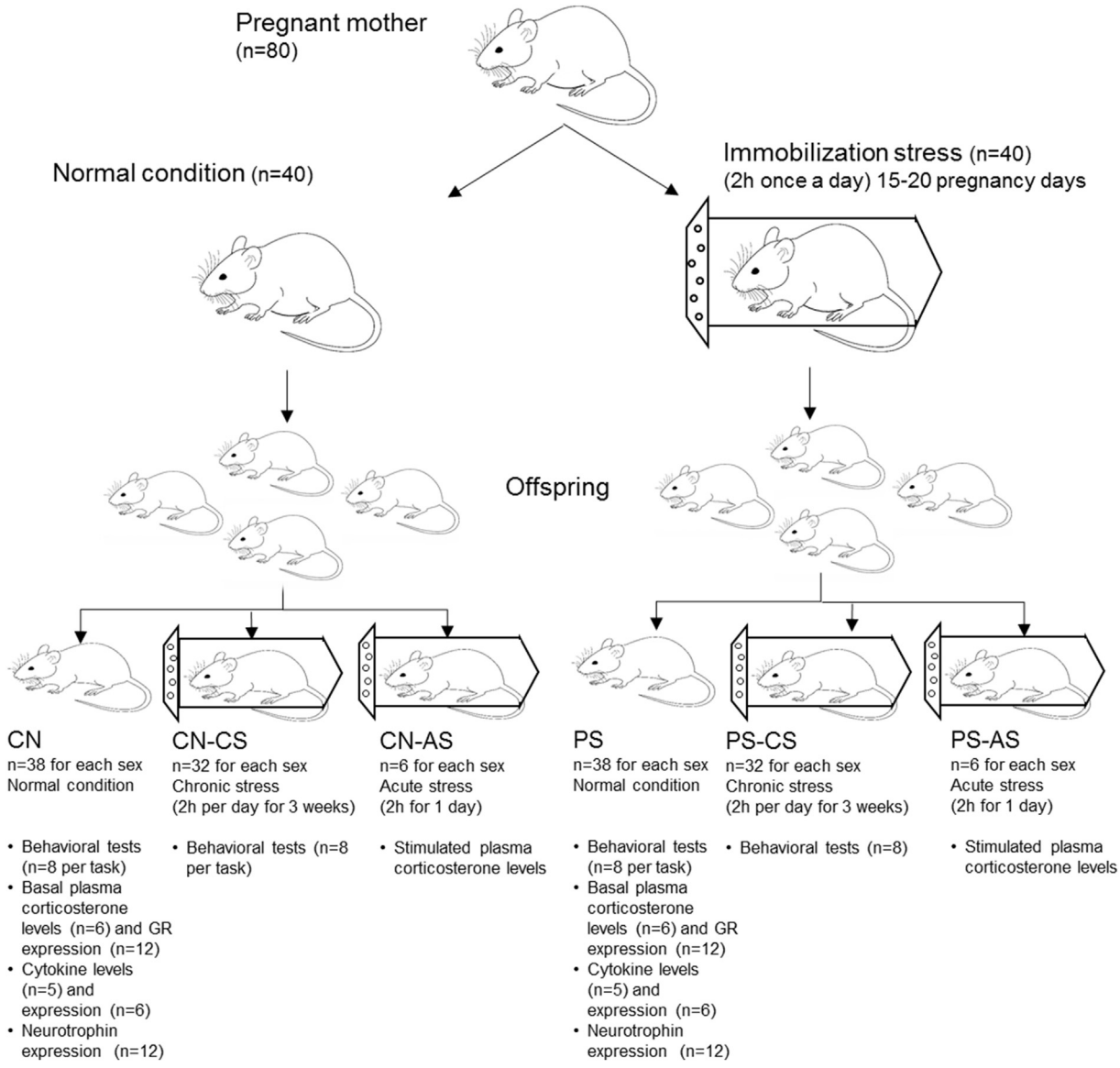

Fig. 1. Scheme diagram of the experimental groups used in the present work. Pregnant BALB/c mice (n = 80) were divided in two groups: one received daily immobilization stress for 2 h once a day from day 15 of pregnancy until delivery, and the other was left without any disturbance. The 60-day-old offspring of non-stressed mothers were distributed in the following subgroups: undisturbed (CN; n = 38), chronic restraint stress (2 h per day for 3 weeks; CN-CS; n = 32), and acute restraint stress (2 h only one day; CN-AS; n = 6). Similarly, the 60-day-old offspring of stressed mothers were divided in the following subgroups: undisturbed (PS; n = 38), chronic restraint stress (PS-CS; n = 32), and acute restraint stress (PS-AS; n = 6).

starting at 10:00 AM. Mice were returned to their cages, left undisturbed for 15 min, and then sacrificed by cervical dislocation.

It is important to note that animals were not physically compressed in the restraint procedure.

2.5. Behavioral tests

Behavioral tests were conducted between 5:00 and 7:00 pm and recorded using a video camera (Sony DCB-DVD810), as previously reported [5,21,25]. For each task, n = 8 per group were tested. Because behavioral assessments can influence the animals' behavior in subsequent tests, different mice were tested in each task.

2.5.1. Open-field habituation

An open-field test was performed to evaluate contextual non-associative learning and memory. For this purpose, a rectangular chamber (l × w × h, 42 × 35 × 15 cm) that was divided into 30 squares of 7 × 7 cm was used. On the first day, the animals were placed into the center of the open field and behavior was recorded for 5 min. The behavioral parameters that were determined included (1) crossings (horizontal activity), which were the number of horizontal lines crossed, and (2) rearing (vertical activity), which was the number of times a mouse stood on its hind legs. After 24 h, the mice were re-exposed to the arena and the parameters were measured again. The habituation was estimated as the relative decrease in activity between the first and second exposure to the open field.

2.5.2. Y-maze spontaneous alternation

Spontaneous alternation using a Y-maze was used to assess spatial working memory. The Y-maze used in this study consisted of three identical black plexiglass arms (l × w × h, 42 × 35 × 15 cm) linked by a common central platform. At the beginning of the session, the mice were put into the neutral zone of the Y-maze, and arm entries were registered for 6 min. Alternation behavior was defined as consecutive entries into all three arms without repeat entries and was expressed as the percentage of the total arm entries. An alternation was defined as three successive entries into the three separate arms of the maze without repeat entries. The percent of alternation was calculated as the

number of alternations divided by the total arm entries minus 2, multiplied by 100.

2.5.3. Object recognition task

The novel object recognition task was used to evaluate recognition memory in mice, and the test is based on the inherent tendency of rodents to explore a novel object longer than a familiar one. The object recognition task was performed according to the procedure described by Bevins and Besheer [26]. Before the trial, mice were habituated to an open field for a 10 min period for two consecutive days. During the training session, the mice were located in an open field containing two identical objects for 10 min. After 1 h, the mice were placed in the open field for 10 min with a familiar and a novel object. The discrimination ratio (DR) was calculated as the time spent exploring the novel object (TN) divided by the time spent exploring the familiar one (TF) plus the novel one in the test session [DR = TN / (TN + TF)].

2.5.4. Object-in-place task

The object-in-place task is a procedure that was developed to test rodent memory for the recognition of objects together with the spatial context in which they occur. The test was performed in an open-field arena with one wall covered in white opaque lucite that served as a spatial cue, as previously described [25]. Prior to testing object memory, the mice were acclimatized to an open field for 10 min each day for three or four consecutive days. This procedure comprised a familiarization phase and a test phase separated by a 1 h delay. During the familiarization phase, each mouse was placed in the middle of the arena and was allowed 5 min to investigate four different objects located equidistantly from the center. During the delay period, objects were cleaned with 80% ethanol to remove odors. For the test phase, two of the original four objects, those on either the right or the left of the open field, were switched. Mice were allowed to explore the objects for 3 min. The results were expressed as the discrimination ratio, which was the difference between the time spent exploring the moved objects (Mt) and the unmoved objects (Ut) divided by the total time spent exploring all the objects [DR = (Mt − Ut) / (Mt + Ut)].

2.6. Corticosterone determination

To evaluate HPA axis activity, corticosterone was determined in the plasma of animals that were either submitted or not submitted to acute stress. Blood from the animals was collected on ice in 0.25 M EDTA and separated in a refrigerated centrifuge. Plasma was stored at −80 °C until the assay was performed. For this purpose, 30 μl of plasma was extracted with 3 ml dichloromethane and dried in a SpeedVac (Thermo Scientific). Corticosterone levels were determined using a standard radioimmunoassay as previously described [23]. The [1,2,6,7-3H(N)-corticosterone] (20 Ci/mmol) came from Perkin Elmer Inc. (Waltham, MA, USA). The antibody (Sigma-Aldrich, St. Louis, MO, USA) cross-reactivity with other relevant steroids was 4.5% (cortisol), 20% (deoxycorticosterone) and 7.9% (testosterone).

2.7. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Lymph nodes (axillary, inguinal and mesenteric) and brain were removed from CN and PS sacrificed mice. Hippocampi were dissected on ice according Mouse Brain Anatomy Atlas. Tissues were stored at −80 °C until use.

RT-PCR was performed as previously described [23]. Total RNA was isolated from tissues by homogenization in Trizol Reagent (Invitrogen, Life technologies, California, USA) following the manufacturer's instructions. The total RNA was used as a template to generate first-strand cDNA synthesis using the M-MLV Reverse Transcriptase (Invitrogen, Life technologies, California, USA), random primers (Invitrogen, Life technologies, California, USA) and dNTPs (Invitrogen, Life technologies, California, USA). The cDNA amounts present in each sample were determined by a 7500 Real-Time PCR System (Applied Biosystems, Massachusetts, USA) using the KAPA SYBR® FAST qPCR Kit Master Mix (2×) Universal (Kapa Biosystems, Massachusetts, USA) and following the manufacturer's instructions. The sequences of mouse-specific primers, the annealing temperature and the amplicon size are provided in Table 1. Each RT-PCR quantification experiment was performed in duplicate. To verify that the SYBR Green dye detected only one PCR product, all the reactions were subjected to a heat dissociation protocol following the final cycle of PCR. Polymerase chain reaction product detection was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green dye to double-stranded DNA. Quantification of the target gene expression was performed using the comparative cycle threshold (Ct) method [27]. An average Ct value was calculated from the duplicate reactions and normalized to the expression of β-actin, and the $2^{(-\Delta\Delta Ct)}$ value was calculated. It is important to note that similar results were obtained using cyclophilin or glucose-6-phosphate-dehydrogenase (G6PDH) mRNA expression levels as housekeeping (data not shown).

2.8. Western blot

Lymph nodes (axillary, inguinal and mesenteric) and brain were removed from CN and PS sacrificed mice. Hippocampi were dissected on ice according Mouse Brain Anatomy Atlas. Tissues were homogenized in buffer (20 mM HEPES, 1 mM DTT, 1 μM leupeptin, 1 μM PSMF, 0.2 μM L-valine). After centrifugation at 10,000 rpm for 10 min, 3× sodium dodecyl sulfate (SDS) sample buffer (2% SDS, 10% (v/v) glycerol, 62.5 mmol/l Tris–HCl, pH 6.8, 0.2% bromophenol blue, 10 mmol/l 2-mercaptoethanol) were added to the supernatants. Proteins were separated by SDS-PAGE on 12% polyacrylamide gels and transferred to nitrocellulose membranes blocked with Tris buffered saline (TBS) containing 0.05% Tween 20 (TBST) and 5% non-fat dry milk for 2 h [23]. Membranes were individually incubated with one of the following antibodies: rabbit anti-mouse glucocorticoid receptor, rabbit anti-mouse BDNF (Santa Cruz Biotechnology, Santa Cruz, Texas, USA), rabbit anti-mouse NT3 or rabbit anti-mouse NGF (Abcam, Cambridge Science, Cambridge, UK) for 24 h. Rabbit anti-mouse β-actin (Santa Cruz Biotechnology, Santa Cruz, Texas, USA) was used as a loading and transference control. Then, membranes were incubated for 1 h with HRP-conjugated goat anti-rabbit immunoglobulin (Ig)G as a secondary antibody (1:2000; Santa Cruz Biotechnology, Santa Cruz, Texas, USA). The membranes were analyzed by chemiluminescence (Amersham Biosciences, UK), as previously described [23].

2.9. Cytokine release

Cytokine production in lymphoid cells was evaluated as previously reported [21]. Briefly, lymph nodes (axillary, inguinal and mesenteric) were removed from CN and PS sacrificed mice and were disrupted through a 1 mm metal mesh, and the cell suspension was filtered through a 10 μm nylon mesh. Cells (1×10^6) were stimulated with concanavalin A (1 μg/ml) for 24 h at 37 °C in a 5% CO_2 atmosphere in a Falcon 24-well plate. Culture supernatants were collected and IFN-γ, IL-2, IL-10 and IL-4 levels were determined by ELISA kits (Affymetrix eBioscience).

2.10. Statistical analysis

Data were expressed as the mean ± standard error of the mean (SEM) for each group. All the data were processed using STATISTICA software (StatSoft, Inc., Tulsa, Oklahoma, USA). The normality and homogeneity of variance for the dataset were tested using the Shapiro-Wilk test and Levene's test, respectively. Open field data were analyzed with the General Linear Model (GLM) repeated measures analysis with sex (female and male), prenatal treatment (normal or PS), postnatal

Table 1
Primers used for the mRNA gene expression by RT-PCR.

Gene	Primers sequence	Amplicon size (bp)	Annealing T°
BDNF	Fw: 5′CTGAGCGTGTGTGACAGTATTA3′ Rv: 5′CTTTGGATACCGGGACTTTCTC3′	112	60
NT3	Fw: 5′CCTGGAAATAGTCACACGGATG3′ Rv: 5′CTTGGATGCCACGGAGATAAG3′	115	60
NGF	Fw: 5′CAGTGAGGTGCATAGCGTAAT3′ Rv: 5′CTCCTTCTGGGACATTGCTATC3′	107	60
IL-2	Fw: 5′TGAGCAGGATGGAGAATTACAG3′ Rv: 5′GAGGTCCAAGTTCATCTTCTAGG3′	123	60
IL-4	Fw: 5′TTCATCGATAAGCTGCACCA3′ Rv: 5′GCATGATGCTCTTTAGGCTTTC3′	80	62
IL-10	Fw: 5′GGACTTTAAGGGTTACTTGGGT3′ Rv: 5′ATTTCTGGGCCATGCTTCT3′	99	60
IFN-Y'	Fw: 5′TGCTGATGGGAGGAGATGTCTAC3′ Rv: 5′ACCTGACACATTCGAGTGCTGT3′	76	58
GR	Fw: 5′CCAAGGGTCTGGAGAGGAC3′ Rv: 5′CTGGACGGAGGAGAACTCAC3′	123	60
β-Actin	Fw: 5′CAACTTGATGTATGAAGGCTTTGGT3′ Rv:5′ACTTTTATTGGTCTCAAGTCAGTGTACAG3′	97	61
G6PDH	Fw: 5′GAAGCTGCCAATGGATACTTAGA3′ Rv: 5′CCACCGTTCATTCTCCACATAG3′	99	60
Cyclophilin B	Fw: 5′CGAGTCGTCTTTGGACTCTTT3′ Rv: 5′GCCAAATCCTTTCTCTCCTGTA3′	87	60

treatment (no stress or chronic stress in the adult life) and time as factors. The other behavioral test and corticosterone data were analyzed using GLM to examine the significance of the main effects and their interactions. qRT-PCR, WB and cytokine production data were evaluated by two-way ANOVA with sex and prenatal treatment as factors. In all cases, if ANOVA showed significant differences between groups, Fisher's Least Significant Difference (LSD) post-hoc test was performed to determine significance level. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Effect of prenatal and/or postnatal stress in learning and memory

To analyze the effects of PS on learning and memory, we performed different tests in no-PS and PS female and male mice that were either chronically stressed or not in their adult life. See Table S1 for complete ANOVA results.

Fig. 2A shows the open field results. GLM repeated measure analysis indicated a non-significant effect of interaction time × gender [crossings, $F_{(1,56)} = 0.29$, NS; rearing, $F_{(1,56)} = 0.38$, NS] and time × prenatal [crossings, $F_{(1,56)} = 3.82$, NS; rearing, $F_{(1,56)} = 0.05$, NS]. These results indicate that female and male had similar open field performance and that prenatal exposition did not modify it. However, a significant effect of interaction time × postnatal treatment on crossing [$F_{(1,56)} = 21.08$, $P < 0.001$] and rearing [$F_{(1,56)} = 16.45$, $P < 0.001$] was observed, which means that postnatal chronic stress exposure altered habituation ability. In addition, post-hoc analyses showed a significant decrease in both horizontal activity and in rearing activity 24 h post-training for both CN and PS female and male mice. In summary, these results indicated that CN and PS female and male groups were able to habituate to the environment after repeated testing. In contrast, female and male mice revealed poor habituation after chronic stress exposure in their adult life (CN-CS and PS-CS) (Fig. 2A).

Fig. 2B shows the object recognition task results. No significant differences were found for different experimental groups in the time exploring a novel object. [GLM factorial ANOVA; interaction prenatal × postnatal × gender: $F_{(1,56)} = 0.015$, NS; main effect gender: $F_{(1,56)} = 3.09$, NS, main effect prenatal: $F_{(1,56)} = 0.44$, NS, postnatal: $F_{(1,56)} = 0.02$, NS].

Spontaneous alternation behavior of control and PS mice submitted or not submitted to chronic stress in their adult life was examined in a Y-maze task (Fig. 2C). GLM factorial ANOVA showed a non-significant interaction for gender × prenatal [$F_{(1,56)} = 1.31$, NS] indicating that PS exposure effect was not different between genders. However, there was a significant interaction gender × postnatal [$F_{(1,56)} = 4.24$, $P < 0.05$], which means that postnatal stress exposure exerted a differential effect depending on the gender. Moreover, post-hoc analyses revealed there was no effect of prenatal stress for females, but PS females showed significantly reduced alternation compared to CN mice after chronic stress as adults. Non-significant differences were found in the percent of spontaneous alternation for males in any experimental group (Fig. 2C).

Finally, GLM factorial ANOVA for object-in-place task results exhibited a significant interaction for gender × prenatal × postnatal [$F_{(1,56)} = 5.73$, $P < 0.05$] which means that all factors had a differential effect in the memory for the recognition of objects together with the spatial context. As displayed in Fig. 2D, post-hoc analyses revealed that PS exposure induced a significant decrease in the discrimination index in females. In addition, exposure to chronic stress in their adult life provoked an impairment in the discrimination capacity for control females. However, both prenatal and postnatal stress exposure was not able to modify the discrimination index in male mice (Fig. 2D).

3.2. Participation of corticosterone in the impaired spatial memory performance of PS females

Taking into account that alterations in HPA system is the most commonly proposed mechanism related to behavior disorders [8,28,29], we evaluated basal and acute stress-induced plasma levels of corticosterone. Moreover, the expression of the glucocorticoid receptor on the hippocampus and lymph nodes from normal and PS adult mice was analyzed. See Table S2 for complete ANOVA results.

Table 2 shows plasma corticosterone level of CN and PS without (basal) or with acute stress. GLM factorial ANOVA revealed a significant interaction prenatal × postnatal [$F_{(1,40)} = 4.36$, $P < 0.05$] and gender × postnatal [$F_{(1,40)} = 5.90$, $P < 0.05$] indicating that acute stress response is different depending on gender and prenatal treatment. Post-hoc analyses showed a significant increase of corticosterone after acute stress exposure for both CN and PS females and males. In addition, prenatal treatment did not modify basal corticosterone levels in both male and female. However, prenatal exposure led to a lower increase of corticosterone levels after acute stress in both female ($P < 0.05$) and male ($P < 0.01$) (Table 2).

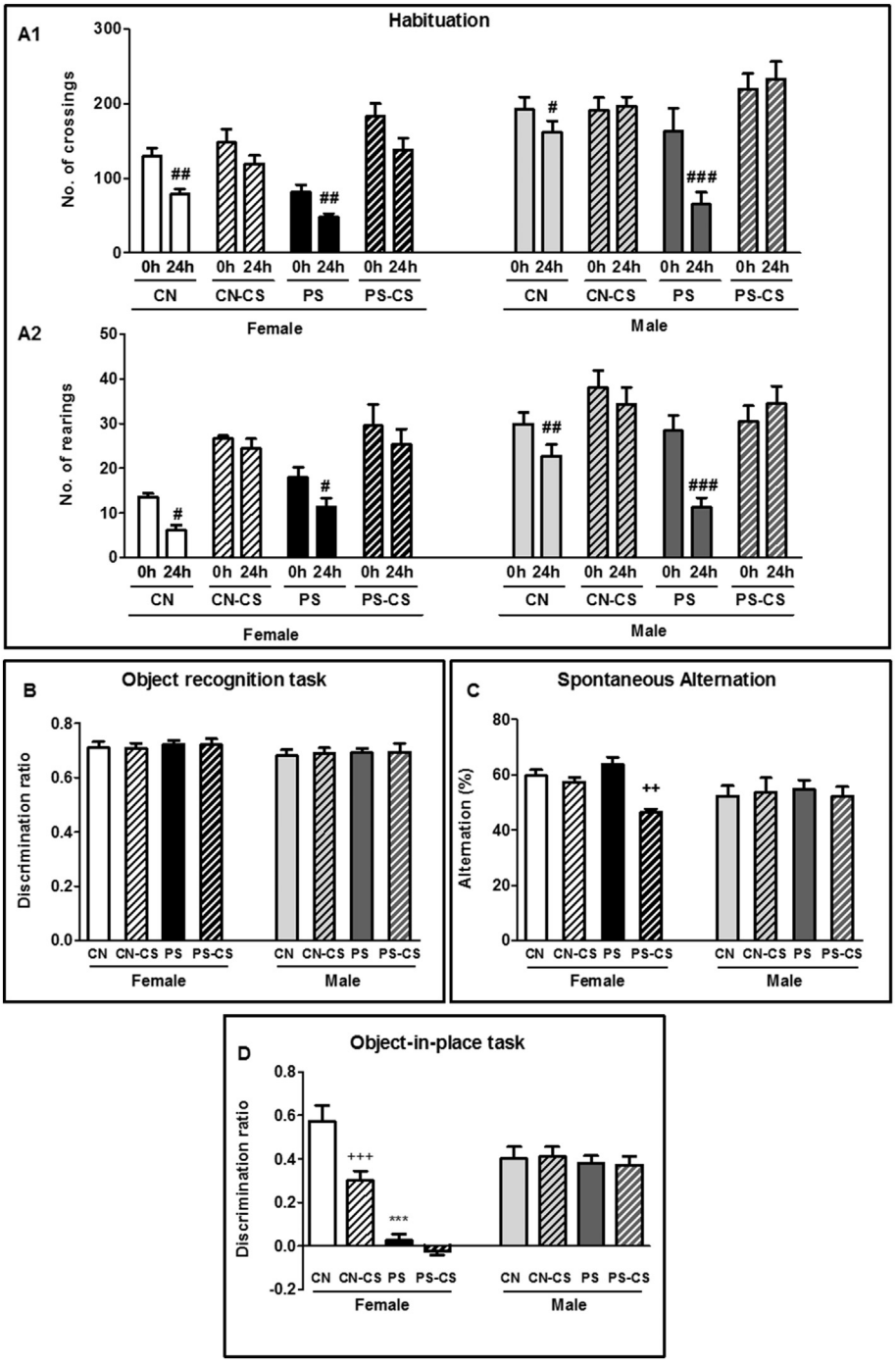

Fig. 2. Prenatal stress induces impairment of spatial memory in female, but not in male mice. Prenatally unstressed females (white bars) or prenatally stressed females (dark bars) that were either not exposed (plain bars) or exposed to chronic stress in adult life (crossed bars) and prenatally unstressed males (light grey bars) or prenatally stressed males (dark grey bars) that were not exposed (plain bars) or exposed to chronic stress in adult life (crossed bars) were assessed in the following tests: (A) Habituation. The graphic shows locomotion (A1) and rearing (A2) for the first day (0 h) and second day (24 h), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus training. (B) Object recognition task. The panel presents values for the discrimination ratio calculated as (TN / TN + TF), where TN is the time exploring a new object and TF is the time exploring a familiar object. (C) Spontaneous alternation. The results shown are the % of alternation with respect to total arm entries in a Y-maze, $^{++}P < 0.01$ compared to mice that were not exposed to chronic stress in their adult life. (D) Object-in-place. The panel displays the discrimination ratio calculated as (Mt) / (Mt + Ut)] where MT is the time exploring the moved object and UT is the time exploring an unmoved object, $^{+++}P < 0.001$ compared to mice that were not exposed to chronic stress in their adult life. $^{***}P < 0.001$ compared to unstressed mice. The results are shown as the mean ± SEM of 8 mice for each group per task.

With respect to the corticoid receptor, both the mRNA expression (Fig. 3A and C) and protein levels (Fig. 3B and D) in the hippocampus (Fig. 3A and B) and lymph nodes (Fig. 3C and D) were analyzed in both female and male mice. Two-way ANOVA for GR mRNA expression levels showed a significant effect of prenatal treatment [$F_{(1,20)} = 78.12$, $P < 0.001$] without significant differences between male and female [interaction gender × prenatal; $F_{(1,20)} = 0.85$, NS] in the hippocampus. Similar results were observed in the lymph nodes [Two-way ANOVA; interaction prenatal × gender: $F_{(1,20)} = 0.50$, NS; main effect prenatal: $F_{(1,20)} = 13.83$, $P < 0.01$]. Post-hoc analyses indicated a significant increase in the corticoid receptor for both prenatal-treated males and females in the hippocampus (Fig. 3A) and lymph nodes (Fig. 3C).

Table 2
Plasma corticosterone levels.

Corticosterone concentration (ng/ml)				
	Female		Male	
	Basal	Acute stress	Basal	Acute stress
CN	120 ± 12	1271 ± 192^{+++}	57 ± 8	499 ± 54^{+++}
PS	83 ± 12	785 ± 78^{+++}	62 ± 8	274 ± 48^{+++}

The results represent the mean ± SEM of six animals per group.
$^{+++}$ $P < 0.001$ with respect to the corresponding postnatally unstressed mice.

Western blot GR analyses displayed comparable results in both the hippocampus [interaction gender × prenatal: $F_{(1,20)} = 14.63$, $P < 0.01$] and lymph nodes [prenatal treatment: $F_{(1,20)} = 43.90$, $P < 0.001$]. Post-hoc analyses showed a significant increase in the corticoid receptor for both prenatal-treated males and females in the hippocampus (Fig. 3B) and lymph nodes (Fig. 3D).

3.3. Cytokine profile alteration in stressed animals

To evaluate if PS exposure induced some alteration of the cytokine profile, we determined the expression of INF-γ, IL-2 (Th1 cytokines), IL-4, and IL-10 (Th2 cytokines) in both the hippocampus and lymphocytes. See Table S3 for complete ANOVA results.

Fig. 4 displays the cytokine mRNA expression for the hippocampus (A–D) and lymph nodes (E–H) from mice submitted or not submitted to PS. For INF-γ, two-way ANOVA indicated a different response to prenatal exposition depending on gender in both hippocampus [interaction prenatal × gender: $F_{(1,20)} = 28.42$, $P < 0.001$] and lymph nodes [$F_{(1,20)} = 6.02$, $P < 0.05$]. In fact, a post-hoc test showed that PS females had a reduced expression of INF-γ compared to the control group for both the hippocampus and lymph nodes. For males, an increase was observed in both tissues but only was significant in the hippocampus (Fig. 4A and E). For IL-4, a significant effect was observed depending on prenatal treatment either in the lymph nodes [interaction prenatal × gender: $F_{(1,20)} = 7.57$, $P < 0.05$] and in the hippocampus [main effect prenatal: $F_{(1,20)} = 10.04$, $P < 0.01$]. Post-hoc tests revealed a significant increase in IL-4 in females for both the hippocampus and lymph nodes, but not in males (Fig. 4C and G). No differences were found for IL-2 and IL-10 expression for both the hippocampus and lymph nodes (Fig. 4B and F; Fig. 4D and H, respectively).

In addition, cytokine production in the lymphocytes after concanavalin-A stimulation showed similar results [INF-γ: interaction prenatal × gender: $F_{(1,20)} = 22.13$, $P < 0.001$; IL-4: interaction prenatal × gender: $F_{(1,20)} = 17.40$, $P < 0.001$]. Post-hoc tests indicated that PS induced a significant decrease in IFN-γ and a significant increase in IL-4 in the lymph nodes (Table 3).

3.4. Neurotrophin expression in the hippocampus and lymph nodes

We analyzed whether the mRNA expression and protein levels of BDNF, NT-3 and NGF were altered by PS exposure in both females and males. See Table S4 for complete ANOVA results.

In the hippocampus, two-way ANOVA revealed a significant effect of prenatal stress on BDNF mRNA values depending on gender [interaction prenatal × gender: $F_{(1,20)} = 7.00$, $P < 0.05$] and protein levels [$F_{(1,20)} = 13.75$, $P < 0.01$]. Post-hoc analyses indicated a significant decrease for mRNA and protein levels in prenatal-treated females, but not for

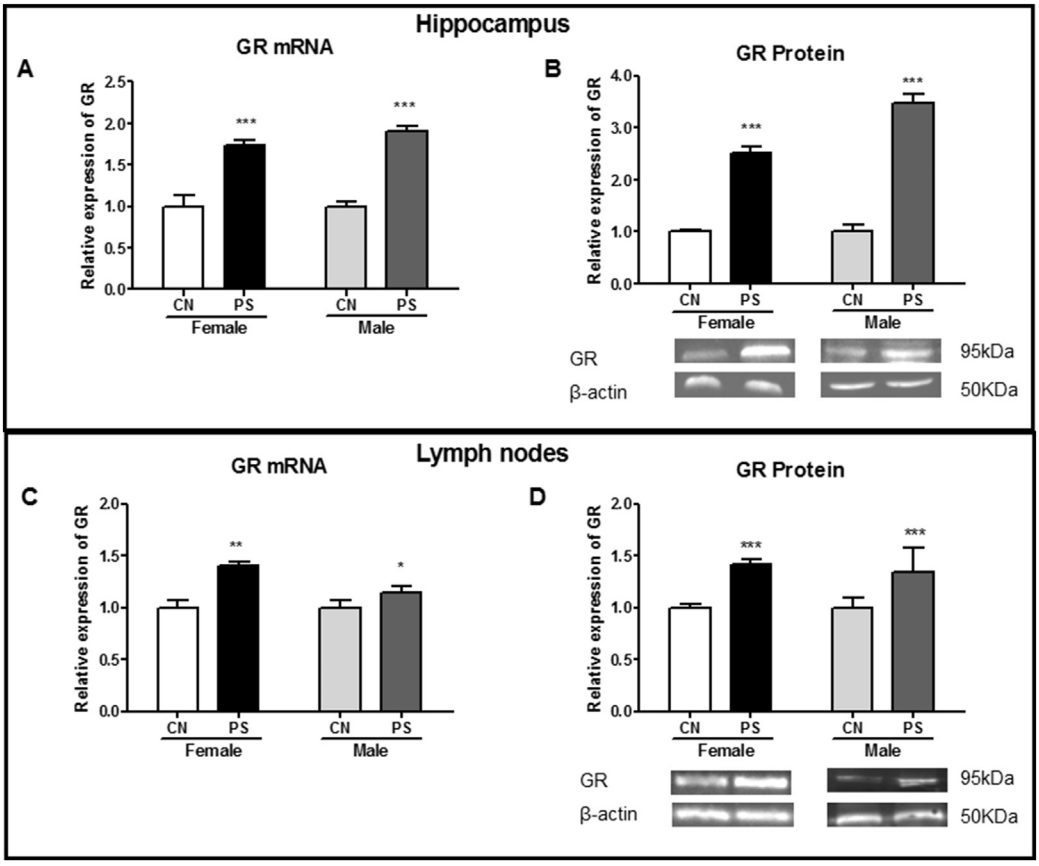

Fig. 3. Prenatal stress induces an increment of corticosterone receptor mRNA and protein expression in both hippocampus and lymph nodes either in female or male mice. qRT-PCR (A and C) and Western blot (B and D) analyses were performed in the hippocampus (A and B) and in lymph node homogenates (C and D) from control (CN, white or light grey bars) and prenatally stressed (PS, black or dark grey bars) female or male mice. qRT-PCR data were calculated with the $2^{-\Delta\Delta Ct}$ method. The graphics indicate the mean ± SEM of six independent experiments. For Western blots, the bands shown are representative of six independent experiments. The graphics indicate the mean ± SEM of the relative intensity (RI) for the receptor with respect to β-actin. The control group was normalized to a mean value of 1. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ with respect to the prenatally unstressed control mice.

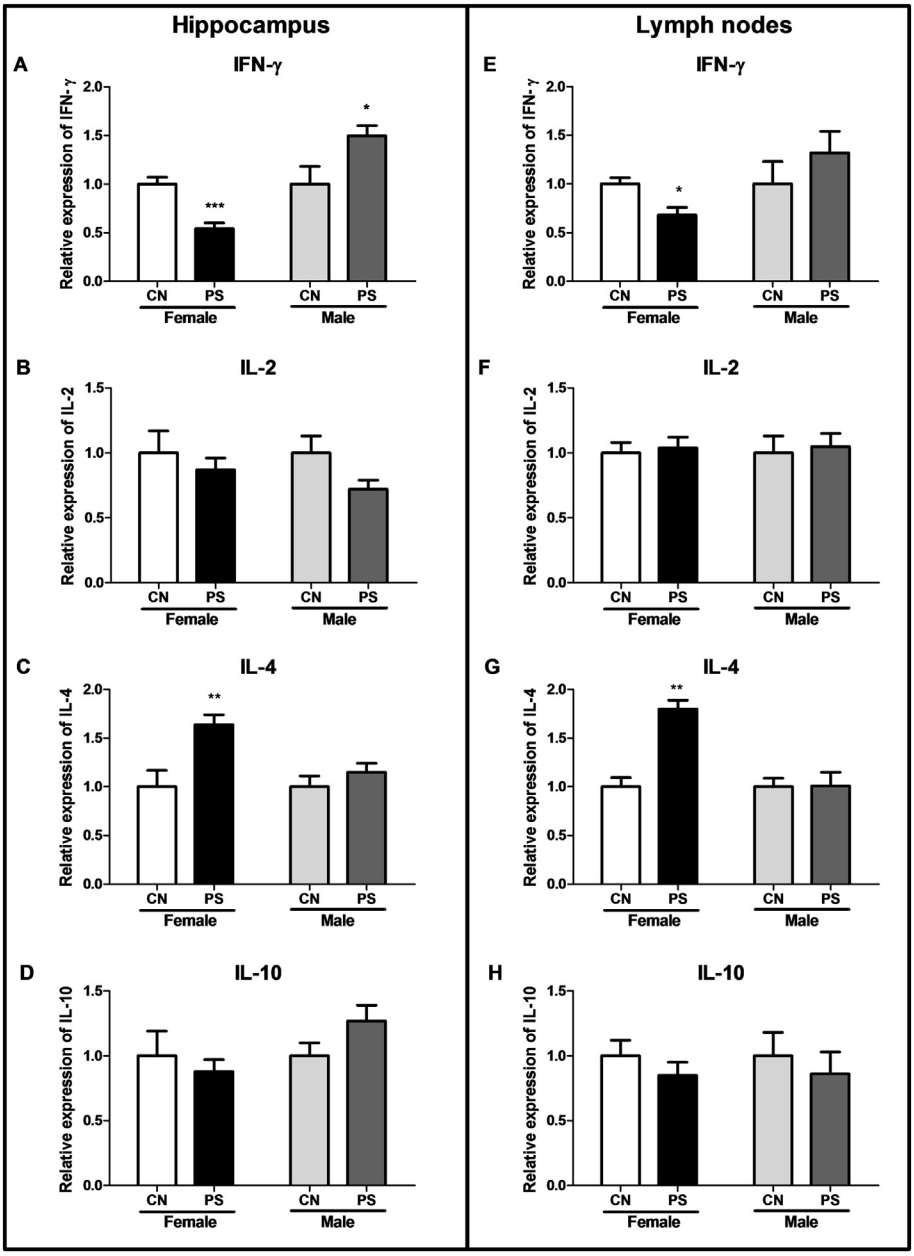

Fig. 4. Prenatal stress provokes a decrease in IFN-γ and an increase in IL-4 mRNA expression in both hippocampus and lymph nodes in female, but not in male mice. qRT-PCR analyses were performed in the hippocampus (A–D) and lymph node homogenates (E–H) from control (CN, white or light grey bars) and prenatally stressed (PS, black or dark grey bars) female and male mice. The expression levels for INF-γ (A and E), IL-2 (B and F), IL-4 (C and G) and IL-10 (D and H) were calculated with the $2^{-\Delta\Delta Ct}$ method. The graphics indicate the mean ± SEM of six independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ with respect to the control.

males (Fig. 5A and D, respectively). Two-way ANOVA showed no significant differences in the mRNA values and protein levels for NT3 (Fig. 5B and E) and NGF (Fig. 5C and F).

Table 3
Cytokine production in stimulated lymphocytes.

Cytokine production in lymphocytes (pg/ml)				
	Female		Male	
	CN	PS	CN	PS
IFN-Ƴ	2919 ± 188	1091 ± 154**	1224 ± 245	1290 ± 206
IL-2	1169 ± 219	992 ± 101	1877 ± 186	2211 ± 323
IL-4	17 ± 2	41 ± 2***	35 ± 4	31 ± 5
IL-10	126 ± 41	249 ± 19*	23 ± 5	24 ± 4

The results represent the mean ± SEM of five animals per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ with respect to the control.

In the lymph nodes, two-way ANOVA displayed similar effects of PS on BDNF mRNA than in the hippocampus [interaction gender × prenatal: $F_{(1,20)} = 64.80$, $P < 0.001$] and protein levels [$F_{(1,20)} = 13.07$, $P < 0.01$]. Moreover, a significant interaction for gender × prenatal for NT3 mRNA values [$F_{(1,20)} = 14.59$, $P < 0.01$] and protein levels [$F_{(1,20)} = 26.97$, $P < 0.001$] was found. Post-hoc analyses indicated a significant decrease for mRNA and protein levels in prenatal-treated females, but not for males, for both BDNF (Fig. 6A and D) and NT3 (Fig. 6B and E). No significant differences were detected for NGF mRNA or protein values in the lymph nodes (Fig. 6C and F).

4. Discussion

In this study, we show that prenatal restraint stress during late gestation induced long-lasting deficits in spatial memory performance in female, but not male, mice. In addition, this alteration accompanied a

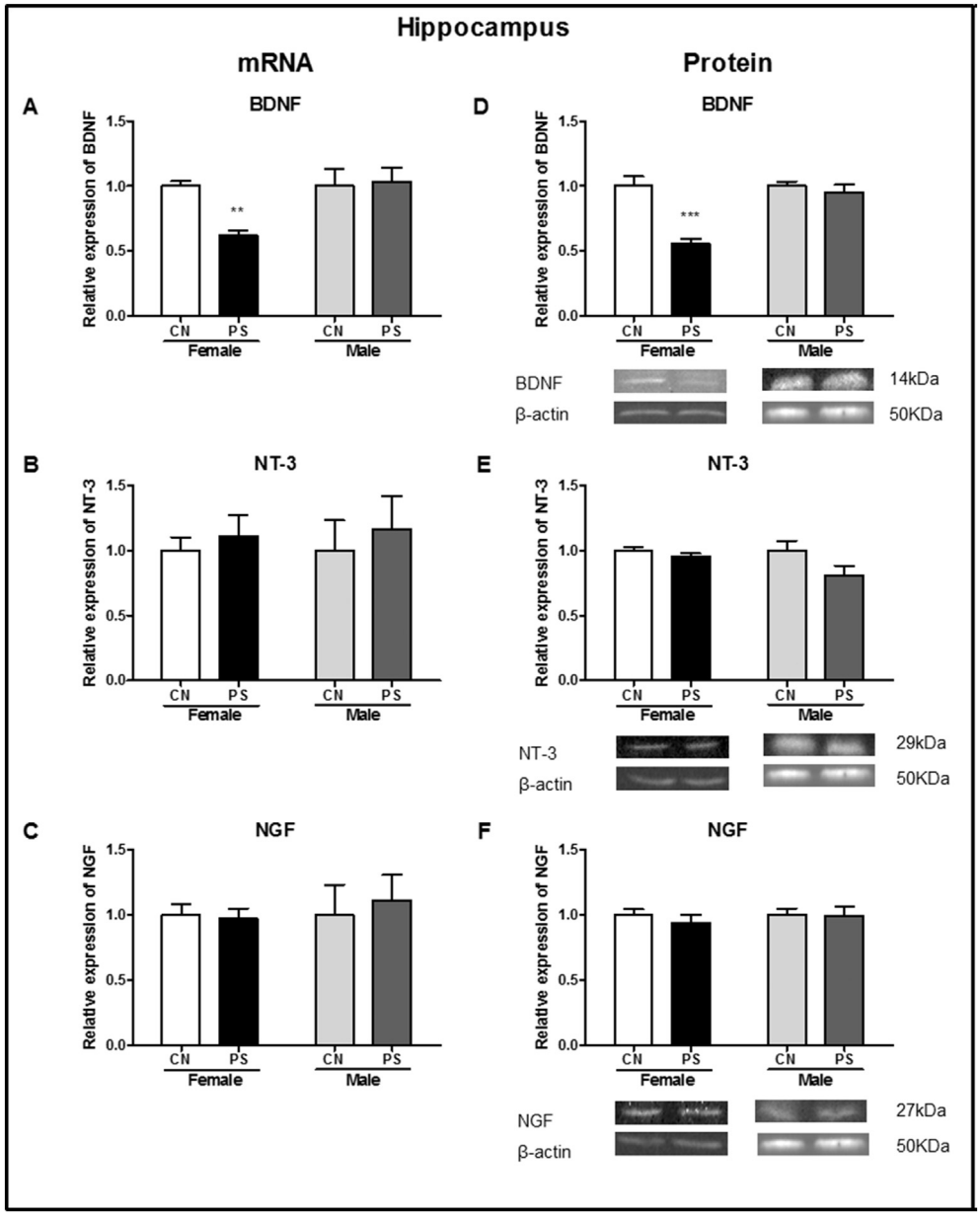

Fig. 5. Prenatal stress induces a decrease in BDNF mRNA and protein expression in hippocampus in female, but not in male mice. qRT-PCR (A, B and C) and Western blots (D, E and F) were performed in hippocampus homogenates from control (CN, white or light grey bars) and prenatally stressed (PS, black or dark grey bars) female or male mice. qRT-PCR data were calculated with the $2^{-\Delta\Delta Ct}$ method using β-actin. The graphics indicate the mean ± SEM of six independent experiments. For Western blots, the bands shown are representative of six independent experiments. The graphics indicate the mean ± SEM of the relative intensity (RI) for BDNF (A and D), NT3 (B and E), and NG*F* (C and F) respect to β-actin. The control group was normalized to a mean value of 1. $^{**}P < 0.01$, $^{***}P < 0.001$ with respect to the control.

decrease in BDNF, an increase in the GR receptor and Th1/Th2 alterations in the hippocampus. Interestingly, these latter changes were observed in peripheral lymph nodes too.

It has been suggested that prenatal stress may result in psychopathology through both specific effects and a general susceptibility to behavioral alterations in postnatal life [30]. In agreement with this suggestion, our results indicated that adult females exposed to PS had a spatial memory impairment that was more pronounced after chronic stress exposure. Thus, PS resulted in a deficit for recognizing objects in place but not in spontaneous alternation with spatial cues. Object-in-place task showed that adult female mice exposed to prenatal stress exhibited an impairment in spatial memory. In addition, after chronic stress exposure, females exposed to PS showed a poorer spontaneous alternation than control mice. However, other memories, such as habituation and object recognition without spatial cues were not affected. By contrast, PS had no effect on male offspring. The differential effect of PS on the learning and memory abilities of males and females probably has a physiological basis that is determined in intrauterine development. However, the sex-bias is not consistent in terms of the direction and predictability of the outcomes in the offspring. Thus, on the behavioral level, some studies reported changes in offspring emotionality that occurred predominantly in males [31]. By contrast, other studies have reported opposite findings, such as increased anxiety and depression-like behavior that predominantly occurred in prenatally stressed female [32, 33]. Wu et al. [34] found that 1-month-old prenatally stressed female and male rats showed a memory disability in the water maze task and in passive avoidance training compared to control rats. Nevertheless, in 3-month-old offspring, this effect was observed only in females, which suggests that memory impairment may be reversible in male offspring. Additionally, the influence of sex on the effect of PS at the behavioral level has been reported in the literature with different results. Some studies found changes predominantly in males and others in

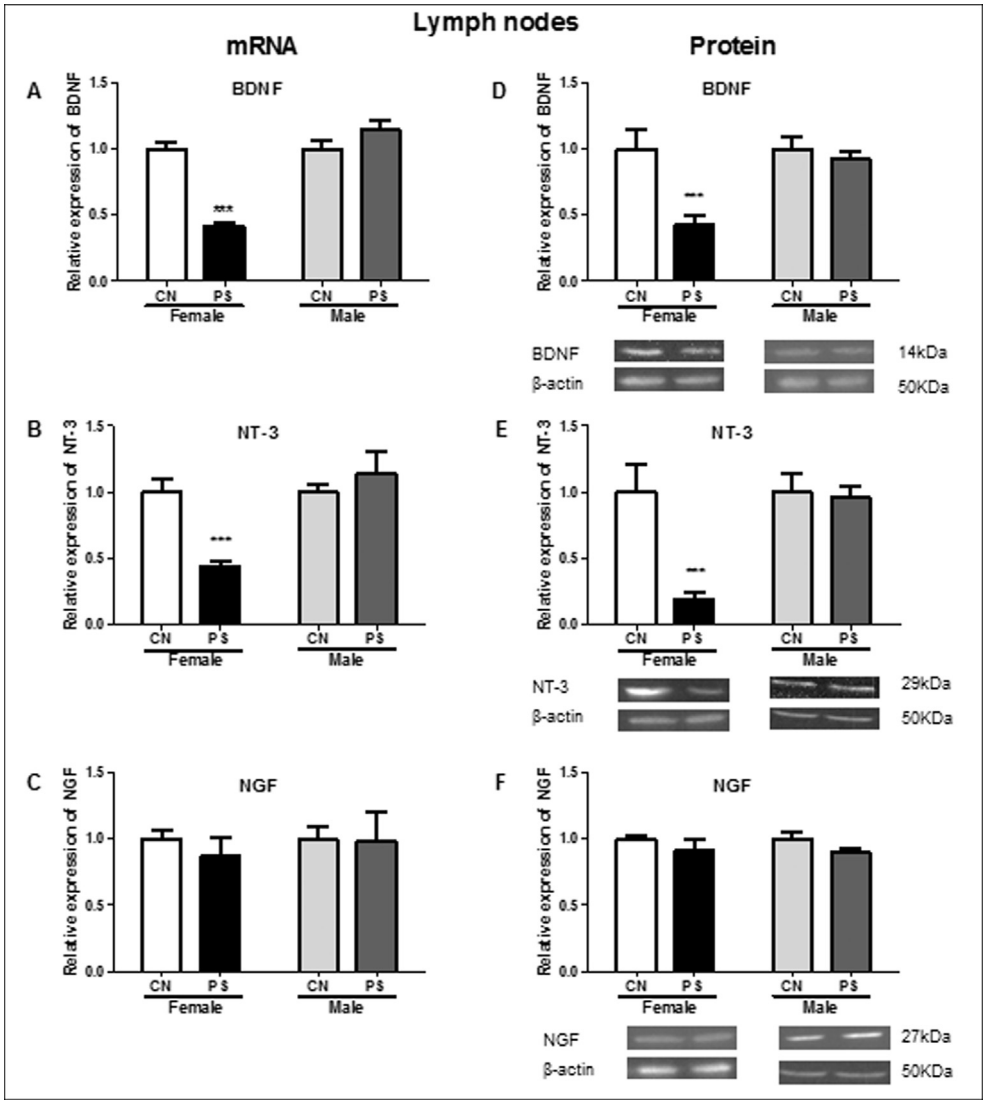

Fig. 6. Prenatal stress induces a decrease in BDNF and NT-3 mRNA and protein expression in lymph nodes in female, but not in male mice. qRT-PCR (A, B and C) and Western blots (D, E and F) were performed in lymph node homogenates from control (CN, white or light grey bars) and prenatally stressed (PS, black or dark grey bars) female or male mice as indicated in Fig. 5. ***$P < 0.001$ with respect to the control.

females [35]. Finally, PS can differentially affect males and females depending on the gestation period when PS is exerted because during intrauterine development, the brain and neuronal differentiation are different for each sex. In general, it was found that if stress occurred during early gestation, it mainly affects the behavior of males, whereas stress during the late gestational period predominantly influence the behavior of female offspring [for a review see 35]. Nevertheless, the reason why males or females are more vulnerable or resilient, and the contribution of hormone-mediated mechanisms, has not been clarified [22].

It was proposed that PS may produce stable changes in central and peripheral stress response systems that represent a potential vulnerability to subsequent adult stress [7,8]. In particular, HPA hyperactivity has been described in the offspring of mother rats submitted to stress in the third week of gestation [28] and has been the most commonly proposed mechanism related to behavior alterations [29]. Learning and memory of spatial and contextual information depends on the hippocampus. In general, data from several studies seem to have established a link between poor behavioral performance and altered hippocampal morphology and biochemistry in PS animals [35]. In addition, elevated glucocorticoid levels have been shown to be a remarkable mediator of these effects [29,36]. It has been proposed that elevated maternal corticoids levels induce HPA hyperactivity due to a reduced negative feedback by decrease of hippocampal GR-expressing neurons [37]. However, it has been recently found that prenatal exposure to glucocorticoids has no observable programming effects on stress reactivity in adult offspring, but results in long-lasting alterations in cognition [38]. In fact it has been proposed that despite the important role of cortisol in fetal programming, there is much evidence indicating that associations between maternal psychosocial stress and offspring outcome may involve other mechanisms than cortisol physiology [29]. Moreover, our results showed that prenatal stress did not affect basal levels of glucocorticoids levels but led to a lower increase after acute stress in both female and male suggesting a hypoactive HPA axis. However, we found greater glucocorticoid receptor mRNA expression and protein levels in the hippocampus for both females and males. It might be, that an increased glucocorticoid receptor level mimics the effect of elevated glucocorticoids. However, PS in males did not induce spatial memory impairment as it did in females. We can postulate that in the presence of less protective mechanisms, the increase in glucocorticoid receptors could be a harmful factor. Additionally, it was described that glucocorticoid administration decreased BDNF levels in the hippocampus [39]. Interestingly, our results indicated that PS females had a reduction in BDNF mRNA expression and protein levels in the hippocampus.

Our results are in line with experimental evidence that has noted the crucial role of BDNF in the growth, survival and differentiation of neurons. Moreover, BDNF has been implicated in learning and memory formation [40].

It is known that the immune system can signal the central nervous system through immune mediators [for a review see 18]. It was reported that cognitive deficits were related to a systemic Th1/Th2 imbalance and could be reversed when balance was restored [5,41,42]. In addition, experiments demonstrated that influenza vaccines administered during early pregnancy induced a systemic Th1 bias and increased hippocampal neurogenesis and neurotrophin levels in offspring [43]. The central nervous system expresses low levels cytokines and their receptors physiologically in a constitutive way. Infiltrating immune cells and resident glial cells, such as astrocytes and microglia, are the main sources of these factors [44]. Cytokines exert actions on proliferation, migration and survival of neural stem/progenitor cells (NPCs). It is known that activated microglia have generally been viewed as a uniformly hostile cell population that causes inflammation, interferes with cell survival and blocks neurogenesis. However, Schwartz and their group [20] have demonstrated that a properly controlled local immune response can support cell survival and promote recovery after central nervous system injury. In particular, Butovsky et al. [45] showed that when activated by IL-4, microglia can induce a bias towards oligodendrogenesis, whereas IFN-γ activated microglia promotes neuronal differentiation.

In this study, we found that PS induced in females led to a decrease in INF-γ and an increase in IL-4 mRNA levels in both the hippocampus and peripheral immune system. No alterations in the hippocampus and peripheral cytokine profile were found in males. IFN-γ has been implicated in both neural damage and repair. High levels of IFN-γ in the brain were found to lead to reactive gliosis, hypomyelination, and defective cerebellar development and function associated with high mortality at 2–4 weeks of age [46]. However, it was suggested that low levels of IFN- γ in the brain could have a neuroprotective role through modulation of glial capacity to buffer excess glutamate release, which would promote the protection of hippocampal neurons from excitotoxicity [47,48]. In addition, Koustova et al. [49] demonstrated in mice homozygous for a germ line deletion of the interferon-gamma gene that the presence of IFN-γ is necessary at some point in the inflammatory process to protect against neurodegeneration. These findings suggest that IFN-γ could play an important role in neuronal protection and repair. Moreover, it has been proposed that T cell-derived IFN-γ may play a key role in promoting neurogenesis via induction of neurotrophic factors, such as BDNF and IGF-1 [50].

The mechanisms underlying the link between PS and their behavioral consequences cannot be explained by a single pathway. Certainly, there are several developmental pathways affected by PS in the hippocampus with consequences that remain into adulthood. Emerging evidences indicate that epigenetic regulation prior to birth (DNA methylation, histone modification and microRNA) represent a form of molecular memory that may modify brain function over extended periods of time [51]. Early-life stress in mice was described to cause sustained DNA hypomethylation of an important regulatory region of the arginine vasopressin gene [52]. Furthermore, Tsankova et al. [53] found that repeated stress in mice could increase histone H3K27 methylation in the hippocampus with suppressive effects upon the BDNF gene promoter region.

Our results showed that glucocorticoid receptors, BDNF expression and a shift to Th2 dominated immune response in the hippocampus were related to spatial memory impairment in adult female mice. These sex specific responses could be due to epigenetic mechanisms and need to be explored in a more detailed research. However, the most important finding was that these alterations were found in peripheral lymphocytes, which suggests that these cells could be good candidates as peripheral markers of susceptibility to cognitive deficits associated to mood disorders. Further investigations are necessary to confirm this approach and for the evaluation of early therapeutic guidelines for alleviating behavioral alterations associated with prenatal exposure to stress.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2017.01.045.

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References

[1] B.S. McEwen, Physiology and neurobiology of stress and adaptation: central role of the brain, Physiol. Rev. 87 (2007) 873–904, http://dx.doi.org/10.1152/physrev.00041.2006.
[2] B.S. McEwen, Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators, Eur. J. Pharmacol. 583 (2008) 174–185, http://dx.doi.org/10.1016/j.ejphar.2007.11.071.
[3] J.J. Kim, K.S. Yoon, Stress: metaplastic effects in the hippocampus, Trends Neurosci. 21 (1998) 505–509http://dx.doi.org/10.1016/S0166-2236(98)01322-8.
[4] B.S. McEwen, Plasticity of the hippocampus: adaptation to chronic stress and allostatic load, Ann. N. Y. Acad. Sci. 933 (2001) 265–277, http://dx.doi.org/10.1111/j.1749-6632.2001.tb05830.x.
[5] M.L. Palumbo, M.F. Trinchero, M.A. Zorrilla-Zubilete, A.F. Schinder, A.M. Genaro, Glatiramer acetate reverts stress-induced alterations on adult neurogenesis and behavior. Involvement of Th1/Th2 balance, Brain Behav. Immun. 26 (2012) 429–438, http://dx.doi.org/10.1016/j.bbi.2011.12.006.
[6] J. Bock, T. Wainstock, K. Braun, M. Segal, Stress in utero: prenatal programming of brain plasticity and cognition, Biol. Psychiatry 78 (2015) 315–326, http://dx.doi.org/10.1016/j.biopsych.2015.02.036.
[7] S. Chung, G.H. Son, S.H. Park, E. Park, K.H. Lee, D. Geum, K. Kim, Differential adaptive responses to chronic stress of maternally stressed male mice offspring, Endocrinology 146 (2005) 3202–3210, http://dx.doi.org/10.1210/en.2004-1458.
[8] M.K. Green, C.S.S. Rani, A. Joshi, A.E. Soto-Piña, P.A. Martinez, A. Frazer, R. Strong, D.A. Morilak, Prenatal stress induces long term stress vulnerability, compromising stress response systems in the brain and impairing extinction, Neuroscience 192 (2011) 438–445, http://dx.doi.org/10.1016/j.neuroscience.2011.06.041.
[9] W. Otten, E. Kanitz, D. Couret, I. Veissier, A. Prunier, E. Merlot, Maternal social stress during late pregnancy affects hypothalamic-pituitary-adrenal function and brain neurotransmitter systems in pig offspring, Domest. Anim. Endocrinol. 38 (2010) 146–156, http://dx.doi.org/10.1016/j.domaniend.2009.09.002.
[10] S. Cabezas, J. Blas, T.A. Marchant, S. Moreno, Physiological stress levels predict survival probabilities in wild rabbits, Horm. Behav. 51 (2007) 313–320, http://dx.doi.org/10.1016/j.yhbeh.2006.11.004.
[11] D.C. Lay Jr., H.G. Kattesh, J.E. Cunnick, M.J. Daniels, G. Kranendonk, K.A. McMunn, M.J. Toscano, M.P. Roberts, Effect of prenatal stress on subsequent response to mixing stress and a lipopolysaccharide challenge in pigs, J. Anim. Sci. 89 (2011) 1787–1794, http://dx.doi.org/10.2527/jas.2010-3612.
[12] Y.A. Lee, Y.J. Kim, Y. Goto, Cognitive and affective alterations by prenatal and postnatal stress interaction, Physiol. Behav. 165 (2016) 146–153, http://dx.doi.org/10.1016/j.physbeh.2016.07.014.
[13] E.W. Neeley, R. Berger, J.I. Koenig, S. Leonard, Strain dependent effects of prenatal stress on gene expression in the rat hippocampus, Physiol. Behav. 104 (2011) 334–339, http://dx.doi.org/10.1016/j.physbeh.2011.02.032.
[14] S. Capoccia, A. Berry, V. Bellisario, D. Vacirca, E. Ortona, E. Alleva, F. Cirulli, Quality and timing of stressors differentially impact on brain plasticity and neuroendocrine-immune function in mice, Neural Plasticity. 971817 (2013)http://dx.doi.org/10.1155/2013/971817.
[15] I. Kaufmann, C. Eisner, P. Richter, V. Huge, A. Beyer, A. Chouker, G. Schelling, M. Thiel, Lymphocyte subsets and the role of TH1/TH2 balance in stressed chronic pain patients, Neuroimmunomodulation 14 (2007) 272–280, http://dx.doi.org/10.1159/000115041.
[16] Y. Dwivedi, Brain-derived neurotrophic factor in suicide pathophysiology, in: Y. Dwivedi (Ed.), The Neurobiological Basis of Suicide. Chapter 8, CRC Press, Boca Raton, 2012 Available from: http://www.ncbi.nlm.nih.gov/books/NBK107216/.
[17] C. Jiang, S.R. Salton, The role of neurotrophins in major depressive disorder, Transl. Neurosci. 4 (2013) 46–58, http://dx.doi.org/10.2478/s13380-013-0103-8.
[18] F. Eskandari, J.I. Webster, E.M. Sternberg, Neural immune pathways and their connection to inflammatory diseases, Arthritis Research & Therapy 5 (2003) 251–265, http://dx.doi.org/10.1186/ar1002.
[19] M.J. Schwarz, S. Chiang, N. Müller, M. Ackenheil, T-helper-1 and T-helper-2 responses in psychiatric disorders, Brain Behav. Immun. 15 (2001) 340–370, http://dx.doi.org/10.1006/brbi.2001.0647.
[20] M. Schwartz, A. London, R. Shechter, Boosting T-cell immunity as a therapeutic approach for neurodegenerative conditions: the role of innate immunity, Neuroscience 158 (2009) 1133–1142, http://dx.doi.org/10.1016/j.neuroscience.2008.12.013.

[21] M.L. Palumbo, M.C. Canzobre, C.G. Pascuan, H. Ríos, M. Wald, A.M. Genaro, Stress induced cognitive deficit is differentially modulated in BALB/c and C57Bl/6 mice. Correlation with Th1/Th2 balance after stress exposure, J. Neuroimmunol. 218 (2010) 12–20, http://dx.doi.org/10.1016/j.jneuroim.2009.11.005.
[22] T.G. O'Connor, E.S. Barrett, Mechanisms of prenatal programing: identifying and distinguishing the impact of steroid hormones, Front. Endocrinol. 5 (2014) 52, http://dx.doi.org/10.3389/fendo.2014.00052.
[23] C.G. Pascuan, M.R. Rubinstein, M.L. Palumbo, A.M. Genaro, Prenatal stress induces up-regulation of glucocorticoid receptors on lymphoid cells modifying the T-cell response after acute stress exposure in the adult life, Physiol. Behav. 128 (2014) 141–147, http://dx.doi.org/10.1016/j.physbeh.2014.01.040.
[24] N.K. Popova, M.V. Morozova, T.G. Amstislavskaya, Prenatal stress and ethanol exposure produces inversion of sexual partner preference in mice, Neurosci. Lett. 489 (2011) 48–52, http://dx.doi.org/10.1016/j.neulet.2010.11.064.
[25] C.G. Pascuan, E.H. Simon, A.M. Genaro, M.L. Palumbo, Involvement of nitric oxide in improving stress-induced behavioural alteration by glatiramer acetate treatment in female BALB/c mice, Psychopharmacology 232 (2015) 1595–1605, http://dx.doi.org/10.1007/s00213-014-3791-z.
[26] R.A. Bevins, J. Besheer, Object recognition in rats and mice: a one-trial nonmatching-to-sample learning task to study 'recognition memory', Nat. Protoc. 1 (2006) 1306–1311, http://dx.doi.org/10.1038/nprot.2006.205.
[27] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2KDDCT method, Methods 25 (2001) 402–408, http://dx.doi.org/10.1006/meth.2001.1262.
[28] J.I. Koenig, G.I. Elmer, P.D. Shepard, P.R. Lee, C. Mayo, B. Joy, E. Hercher, D.L. Brady, 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 (2005) 251–261, http://dx.doi.org/10.1016/j.bbr.2004.05.030.
[29] R. Beijers, J.K. Buitelaar, deWeerth C. Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis, Eur. Child Adolesc. Psychiatry 10 (2014) 943–956, http://dx.doi.org/10.1007/s00787-014-0566-3.
[30] A.C. Huizink, E.J.H. Mulder, J.K. Buitelaar, Prenatal stress and risk for psychopathology: specific effects of induction of general susceptibility? Psychol. Bull. 130 (2004) 115–142, http://dx.doi.org/10.1037/0033-2909.130.1.115.
[31] B.R. Mueller, T.L. Bale, Sex-specific programming of offspring emotionality after stress early in pregnancy, J. Neurosci. 28 (2008) 9055–9065, http://dx.doi.org/10.1523/JNEUROSCI.1424-08.2008.
[32] A.T. Behan, D.L. van den Hove, L. Mueller, M.J. Jetten, H.W. Steinbusch, D.R. Cotter, J. Prickaerts, Evidence of female-specific glial deficits in the hippocampus in a mouse model of prenatal stress, Eur. Neuropsychopharmacol. 21 (2011) 71–79, http://dx.doi.org/10.1016/j.euroneuro.2010.07.004.
[33] M. Schroeder, T. Sultany, A. Weller, Prenatal stress effects on emotion regulation differ by genotype and sex in prepubertal rats, Dev. Psychobiol. 55 (2013) 176–192, http://dx.doi.org/10.1002/dev.21010.
[34] J. Wu, T.B. Song, Y.J. Li, K.S. He, L. Ge, L.R. Wang, Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats, Brain Res. 13 (2007) 205–213, http://dx.doi.org/10.1016/j.brainres.2007.01.024.
[35] J. Yang, H. Han, J. Cao, L. Li, L. Xu, Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring, Hippocampus 16 (2006) 431–436, http://dx.doi.org/10.1002/hipo.20181.
[36] M.D. Kvarta, K.E. Bradbrook, H.M. Dantrassy, A.M. Bailey, S.M. Thompson, Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporo ammonic synapses, J. Neurophysiol. 114 (2015) 1713–1724, http://dx.doi.org/10.1152/jn.00359.2015.
[37] E.C. Cottrell, J.R. Seckl, Prenatal stress, glucocorticoids and the programming of adult disease, Front. Behav. Neurosci. 3 (2009) 19, http://dx.doi.org/10.3389/neuro.08.019.2009 (eCollection 2009).
[38] Y. Zeng, N.M. Brydges, E.R. Wood, A.J. Drake, J. Hall, Prenatal glucocorticoid exposure in rats: programming effects on stress reactivity and cognition in adult offspring, Stress 18 (2015) 353–361, http://dx.doi.org/10.3109/10253890.2015.1055725.
[39] K.H. Choy, Y. de Visser, N.R. Nichols, M. van den Buuse, Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce BDNF expression in hippocampus: effects on learning and memory, Hippocampus 18 (2008) 655–667, http://dx.doi.org/10.1002/hipo.20425.
[40] P. Bekinschtein, M. Cammarota, L.M. Igaz, L.R. Bevilaqua, I. Izquierdo, J.H. Medina, Persistence of long-term memory storage requires a late protein synthesis-and BDNF-dependent phase in the hippocampus, Neuron 53 (2007) 261–277, http://dx.doi.org/10.1016/j.neuron.2006.11.025.
[41] K. Baruch, N. Ron-Harel, H. Gal, A. Deczkowska, E. Shifrut, W. Ndifon, N. Mirlas-Neisberg, M. Cardon, I. Vaknin, L. Cahalon, T. Berkutzki, M.P. Mattson, F. Gomez-Pinilla, N. Friedman, M. Schwartz, CNS-specific immunity at the choroid plexus shifts toward destructive Th2 inflammation in brain aging, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 2264–2269, http://dx.doi.org/10.1073/pnas.1211270110.
[42] F. He, J.T. Zou, Q.F. Zhou, D.L. Niu, W.H. Jia, Glatiramer acetate reverses cognitive deficits from cranial-irradiated rat by inducing hippocampal neurogenesis, J. Neuroimmunol. 271 (2014) 1–7, http://dx.doi.org/10.1016/j.jneuroim.2014.03.015.
[43] Y. Xia, F. Qi, J. Zou, J. Yang, Z. Yao, Influenza vaccination during early pregnancy contributes to neurogenesis and behavioral function in offspring, Brain Behav. Immun. 42 (2014) 212–221, http://dx.doi.org/10.1016/j.bbi.2014.06.202.
[44] T. Schmitz, L.J. Chew, Cytokines and myelination in the central nervous system, Sci. World J. 8 (2008) 1119–1147, http://dx.doi.org/10.1100/tsw.2008.140.
[45] O. Butovsky, Y. Ziv, A. Schwartz, G. Landa, A.E. Talpalar, S. Pluchino, G. Martino, M. Schwartz, Microglia activated by IL-4 or IFN-γ differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells, Mol. Cell. Neurosci. 31 (2006) 149–160, http://dx.doi.org/10.1016/j.mcn.2005.10.006.
[46] J. Lee, S.J. Kim, T.G. Son, S.L. Chan, M.P. Mattson, Interferon-γ is up-regulated in the hippocampus in response to intermittent fasting and protects hippocampal neurons against excitotoxicity, J. Neurosci. Res. 83 (2006) 1552–1557, http://dx.doi.org/10.1002/jnr.20831.
[47] J.G. Corbin, D. Kelly, E.M. Rath, K.D. Baerwald, K. Suzuki, B. Popko, Targeted CNS expression of interferon-gamma in transgenic mice leads to hypomyelination, reactive gliosis, and abnormal cerebellar development, Mol. Cell. Neurosci. 7 (1996) 354–370, http://dx.doi.org/10.1006/mcne.1996.0026.
[48] I. Shaked, D. Tchoresh, R. Gersner, G. Meiri, S. Mordechai, X. Xiao, R.P. Hart, M. Schwartz, Protective autoimmunity: interferon-γ enables microglia to remove glutamate without evoking inflammatory mediators, J. Neurochem. 92 (2005) 997–1009, http://dx.doi.org/10.1111/j.1471-4159.2004.02954.x.
[49] E. Koustova, Y. Sei, T. McCarty, M.G. Espey, R. Ming, H.C. Morse, A.S. Basile, Accelerated development of neurochemical and behavioral deficits in LP-BM5 infected mice with targeted deletions of the IFN-gamma gene, J. Neuroimmunol. 108 (2000) 112–121, http://dx.doi.org/10.1016/S0165-5728(00)00258-7.
[50] R. Baron, A. Nemirovsky, I. Harpaz, H. Cohe, T. Owens, A. Monsonego, IFNgamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease, FASEB J. 22 (2008) 2843–2852, http://dx.doi.org/10.1096/fj.08-105866.
[51] D.M. Silberman, G.B. Acosta, M.A. Zorrilla Zubilete, Long-term effects of early life stress exposure: role of epigenetic mechanisms, Pharmacol. Res. 109 (2016) 64–73, http://dx.doi.org/10.1016/j.phrs.2015.12.033.
[52] C. Murgatroyd, A.V. Patchev, Y. Wu, V. Micale, Y. Bockmuhl, D. Fischer, F. Holsboer, C.T. Wotjak, Almeida OF, D. Spengler, Dynamic DNA methylation programs persistent adverse effects of early-life stress, Nat. Neurosci. 12 (2009) 1559–1566, http://dx.doi.org/10.1038/nn.2436.
[53] N.M. Tsankova, O. Berton, W. Renthal, A. Kumar, R.L. Neve, E.J. Nestler, Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action, Nat. Neurosci. 9 (2006) 519–525, http://dx.doi.org/10.1038/nn1659.