Journal of Neuroimmunology 218 (2010) 12–20

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Stress induced cognitive deficit is differentially modulated in BALB/c and C57Bl/6 mice
Correlation with Th1/Th2 balance after stress exposure

M.L. Palumbo [a], M.C. Canzobre [b], C.G. Pascuan [a], H. Ríos [b], M. Wald [a], A.M. Genaro [a,*]

[a] CEFYBO-CONICET, 1ª. Cátedra de Farmacología, Facultad de Medicina, UBA, Argentina
[b] Instituto de Biología Celular y Neurociencias Prof. De Robertis, Facultad de Medicina, UBA, Argentina

ARTICLE INFO

Article history:
Received 27 July 2009
Received in revised form 25 October 2009
Accepted 9 November 2009

Keywords:
Chronic mild stress
Hippocampus
Learning and memory
Th1/Th2 balance
Reactive oxygen species
BALB/c and C57BL/6 inbred strains of mice

ABSTRACT

This work shows a comparative study on the effects of chronic mild stress upon learning and memory and immunity, in BALB/c and C57BL/6 mice. Stressed BALB/c, but not C57Bl/6 mice, showed a poor learning performance, morphological alterations in the hippocampus with an increase in oxidative stress. A correlation between poor memory performance and the increase of the Th2/Th1 balance was found. Our results suggest that vulnerability to cognitive deficit associated with stress exposition could be related to a differential regulation of Th1/Th2 cytokine balance, suggesting a better learning performance for individuals that produce Th1 type cytokine after stress exposition.

1. Introduction

Stress is defined as any situation capable of perturbing the physiological or psychological homeostasis. Exposure to adverse situations affects an important number of aspects of our daily life. While response to stress is a necessary survival mechanism, prolonged stress can have several repercussions affecting behavioral, endocrine and immunological parameters (McEwen, 1998). Recognition of these effects has led McEwen to develop a new terminology to link the protective and damaging effect of the biologic response to stressors, namely, allostasis and allostatic overload (McEwen, 1998). These two terms allow for a more restricted and precise definition of the overused word "stress", and provide a view of how the essential protective and adaptive effects of the physiological mediators that maintain homeostasis are also involved in the cumulative effects of daily life when they are mismanaged or overused. Allostasis refers to the adaptive processes that maintain homeostasis through the production of mediators such as adrenalin, cortisol and cytokines. 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 result from being "stressed out" (McEwen, 2008). The hippocampus, a limbic area involved in learning and memory, is particularly sensitive to the effects of stress (Kim and Yoon, 1998). In a previous report (Palumbo et al., 2007) we have shown that BALB/c mice exposed to chronic stress had a poor learning performance in both open field and passive avoidance inhibitory tasks with respect to control mice. Moreover, animals under stress showed a morphological alteration in the hippocampus related to a diminished nitric oxide production by neuronal nitric oxide synthase and an increment of oxidative stress. Also, we have found that chronic stress exposure induces a decrease in T-cell proliferative response in vitro and a lower T-cell dependent antibody production in vivo with changes in stress hormone regulation of T-lymphocyte reactivity (Silberman et al., 2003, 2004).

On the other hand, it has been observed that C57BL/6 and BALB/c, two genetically different inbred strains of mice, differ one from another in several behavioral responses and in neurodevelopment and neurochemistry parameters. Thus, BALB/c is considered an emotive and anxious strain with a low basal locomotor activity and increased stress reactivity; whereas C57BL/6 is considered a non-emotive, non-anxious and active strain in different experimental situations, such as the open-field test or the elevated plus-maze test (Belzung and Griebel, 2001; Marona-Lewicka and Vetulani, 1989; Tang et al., 2002; Dulawa et al., 2004). Concerning the neurochemical studies, BALB/c mice show approximately two fold lower levels of serotonin in the forebrain than C57BL/6 strain (Zhang et al., 2004). The concentration of 17-OH-pregnenolone, a neurosteroid related with behavior, e.g. aggression, adaptation to stress or learning in mice,

* Corresponding author. CEFYBO-CONICET, 1ª. Cát. de Farmacología, Facultad de Medicina, UBA, Paraguay 2155, Piso 15, 1121 Ciudad de Buenos Aires, Argentina. Tel.: +54 11 4962 4431x116; fax: +54 11 4962 4431x106.
E-mail address: amgenaro@yahoo.com.ar (A.M. Genaro).

0165-5728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2009.11.005

is significantly higher in the whole brain of C57BL/6 than in BALB/c animals (Tagawa et al., 2006). Moreover, significantly less maternal care and elevated stress-induced corticosterone levels were observed in BALB/c as compared to C57BL/6J mice (Priebe et al., 2005). In line with these findings, we have previously shown that stressed BALB/c, but not C57Bl/6 mice, showed a poor learning performance in both open field and passive avoidance inhibitory tasks (Palumbo et al, 2009).

On the other hand, genetic control of the Th1/Th2 balance has been related to differences in both innate (Watanabe et al., 2004) and acquired immunity (Guinazu et al., 2004). Deregulation of cytokines (Th1 versus Th2) has been reported to be involved in the pathogenesis of many human diseases such as autoimmune diseases, sleep disturbance, major depression and other disorders (Kaufmann et al., 2007; Schwarz et al., 2001). Cytokines have been shown to affect many behaviors, including sleep, appetite, sexual-behavior, memory and motor activity. In fact, cytokines are responsible for behavior displayed during infectious diseases, referred collectively as sickness behavior (Larson and Dunn, 2001; Dantzer and Kelley, 2007).

As mentioned above, dysregulated allostasis can lead to disease. The main mediators in the adaptive response to stressors involve glucocorticoids, catecholamines and the balance between pro-inflammatory (Th1) versus anti-inflammatory (Th2) cytokines (McEwen, 2008).

In this context, the aim of the present work was to investigate the possible role of altered Th1/Th2 balance in mediating differential cognitive response to chronic stress exposure in Th1-biased C57BL/6 versus Th2-biased BALB/c mice. As the adaptive response to stressors involves the activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS), the participation of these axes in the endocrine response to stress is also discussed. It was our interest to establish a correlation between peripheral mediators with brain and cognitive alterations, because this type of correlations may be of interest in human pathologies that course with cognitive deficits.

2. Materials and methods

2.1. Animals

BALB/c and C57Bl/6J inbred strains of mice from Instituto Nacional de Tecnología Agropecuaria (INTA) were used throughout the experiments. Animals were maintained on a 12-h light/dark cycle under controlled temperatures (18–22 °C). Food and water were freely available. Two-month-old animals were distributed into two groups; one group was housed in normal conditions (control) and the other group was subjected to chronic unpredictable mild stress (CMS-animals).

Forty animals of each condition (control and CMS-mice) and strain (BALB/c and C57BL/6) were used for behavioral, histological, neurochemical and immunological studies. Behavioral experiments were conducted before the dark phase (5 pm).

2.2. Chronic mild stress model

The stress scheme was slightly modified from that previously used in rats by Willner et al. (1992) and in mice by Monleon et al. (1995). The animals were housed individually and alternatively submitted to: one 16-h period of water deprivation; two periods of continuous overnight illumination; two periods (7 and 17 h) of 45° cage tilt; one 17-h period in a soiled cage (100 ml water in sawdust bedding); one period (8 h) of food deprivation; one 17-h period of paired housing (animals are always housed in the same pairs, but the location alternates between the home cages of each member of the pair). All the individual stressors used have been classified as “mild” according to the Animals (Scientific Procedures) Act of 1986 (UK legislation). The stressors were scheduled throughout the week, in a similar manner to that previously described (Palumbo et al., 2007) for 6 weeks. Animals were left undisturbed in their home cages 24 h prior to killing by decapitation at 10:00 AM.

The experimental protocol was approved by the Internal Ethics Committee of the school of Medicine of the University of Buenos Aires, i.e., Comité Institucional para Cuidado y Uso de los Animales de Laboratorio (CICUAL).

2.3. Open field habituation

Open-field habituation was performed using a rectangular chamber (42 cm × 35 cm × 15 cm) made of gray polyvinylchloride (PVC) as described previously (Palumbo et al., 2007). The behavioral parameters registered during sessions of 5 min were: rearing (vertical activity), locomotion (horizontal activity) and corner time. Animals were re-exposed to the open field 24 h after the initial trial. The open field test was performed between 5:00 and 7:00 PM. Habituation is defined as the relative decrease in activity between a repeated open field trial and the first trial (Frisch et al., 2005).

2.4. Y-maze spontaneous alternation

Spontaneous alternation behavior in a Y-maze task was recorded and evaluated as a spatial memory task. The apparatus consisted of three identical black Plexiglas arms (l × w × h, 28 × 10 × 20 cm). Mice were acclimated to the testing room for at least 20 min prior to testing. At the beginning of the session, mice were placed at the end of one fixed arm of the Y-maze and allowed to explore freely for 6 min. The sequence of arm entries was recorded visually. Alternation refers to three successive visits to the three separate arms of the maze. The percent of alternation was calculated as the number of alternations divided by the number of total arm entries minus 2, multiplied by 100 (Dillon et al., 2008; Kim et al., 2008).

2.5. Histological procedure

Perfusion of the hippocampus was performed according to a modification of the original method (Gonzalez-Aguilar and De Robertis, 1963). Fixing, embedding in Paraplast, cutting and hematoxylin-eosin staining were performed as previously described (Palumbo et al., 2007). Thickness was determined by measuring the width of each hippocampal region in at least 10 points. To determine neuronal density, neuronal nuclei were counted from 10 μm thick sections in a high magnification microphotograph to avoid a double count of neurons. At least ten slices per mouse were used to quantify the number of neurons. In order to avoid differences due to the staining procedure between different experimental conditions, sections were stained simultaneously. Histological fields were randomly chosen and photographed using an Axiophot microscope (Zeiss) and a digital camera.

2.6. Analysis of reactive oxygen species

Determination of reactive oxygen species (ROS) was based on a method previously described (Keston and Brand, 1965; LeBel et al., 1989). The method relies on oxidation of the non-fluorescent probe, 2,7-dichlorofluorescin diacetate (DCFH), by reactive oxygen species, to form the highly fluorescent 2,7-dichlorofluorescein (DCF). Briefly, after sacrifice, the whole brain was immediately removed and, the hippocampus was dissected on ice and homogenized in ice-cold Locke's buffer (NaCl 154 mmol/L, KCl 5,6 mmol/L, NaHCO3 3,6 mmol/L, CaCl2 2,3 mmol/L, glucosa 5,6 mmol/L, HEPES 5 mmol/L). Then, the homogenate was diluted 1:10 in ice-cold Locke's buffer to obtain a concentration of 0.5 mg tissue/mL. Aliquots (1 mL) of the homogenate were incubated with 10 μL of 2,7-dichlorofluorescin diacetate (final concentration 5 μmol/L) at 37 °C for 15 min. Later, cells were incubated in the presence or the absence of 100 μM N-metil-D-aspartate (NMDA)

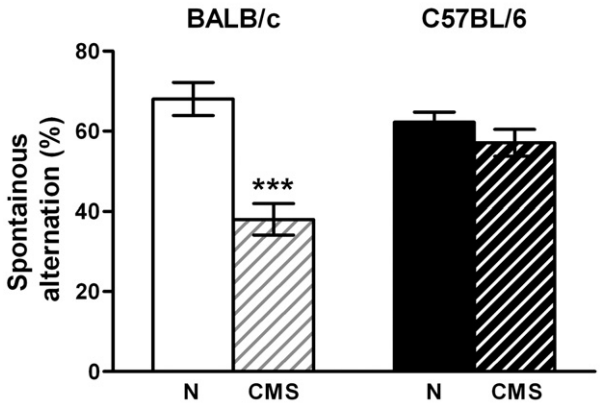


Fig. 1. Mice behavior in the Y-maze. The graphic show the spontaneous alternation behavior in percentage, in BALB/c (clear bars) and C57BL/6 (dark bars), in control (plain bars) and CMS (crossed bars) mice. Results represent the mean ± SEM of eight animals for each group. *** $p<.001$ respect to corresponding control.

to stimulate ROS production for 15 additional minutes as previously described (Palumbo et al., 2007). Results were expressed as pmol/mg tissue.

2.7. Corticosterone determination

To avoid fluctuations on plasma corticosterone levels due to circadian rhythms, animals were bled at 12:00 PM on the day of sacrifice. Blood from animals under different experimental conditions was collected on ice in 0.25 M EDTA and phases were separated in a refrigerated centrifuge. Plasma was stored at −80 °C until the assay was performed. Corticosterone levels were determined by high-pressure liquid chromatography (HPLC) as a protocol slightly modified from that previously used in rats by Sargent (1985).

2.8. Catecholamines assay

Catecholamines concentrations were determined in spleen samples by a fluorometric assay (Laverty and Taylor, 1968). Briefly, spleens were homogenized in 12.5% sodium sulfite, 10% EDTA in 0.4 N percloric acid. After 24 h at 4ªC the homogenate was centrifuged at 5000 rpm for 10 min. The supernatants were brought to pH 8.2 and seeded in pre-washed alumina columns. The eluate was oxidized with iodine in an alkaline medium. The fluorescence was recorded at 375 nm in a spectrofluorometer using an excitation source of 325 nm.

2.9. Cell suspensions and culture conditions

Lymphoid cell suspensions from control and CMS mice were obtained as previously described (Edgar et al., 2002). Briefly, lymph nodes (axilary, inguinal and mesenteric) and spleens were removed and disrupted through a 1 mm metal mesh, and the cell suspension was filtered through a 10 µm nylon mesh. The suspension was depleted of red bloods and dead cells by centrifugation over Ficoll/Hypaque (density 1,084 from SIGMA). After three washes in RPMI 1640, cells were re-suspended in RPMI 1640 supplemented with 10% of batched-tested non-stimulatory fetal calf serum, 2 mM glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 50 µM beta-mercaptoethanol.

2.10. Mitogen assay

Proliferation was determined by culturing 2×10^5 cells per well in 96-well plates in 100 µl triplicated aliquots in supplemented medium. Aliquots of 100 µl of mitogens were added to each microculture to yield the appropiate concentration: Concanavalin A (Con A; Sigma Aldrich, St Louis, Missouri) (0.5, 1 and 2 µg/ml) was used as a T-cell selective mitogens, and lipopolysaccharide (LPS; Sigma Aldrich, St Louis, Missouri) (30 µg/ml) as a B-cell selective mitogens, to induce proliferation. In control cultures, stimulants were replaced by 100 µl of culture medium. Then cells were cultured at 37 °C in a 5% CO_2 atmosphere for different periods. Mitogenic activity was measured by adding 1 µCi [^{3}H]thymidine per well for the last 18-h period of culture. The thymidine incorporation was measured by scintillation counting after retention over GF/C glass-fiber filters (Whatman) of the acid-insoluble macromolecular fraction. The means of the triplicated determinations were calculated for each mitogen concentration. Mitogen-stimulated cells displayed the expected kinetic proliferation, with a peak of proliferation at the third day of culture.

2.11. Flow cytometry

CD4 T-helper/inducer and CD8 T-cytotoxic/suppressor lymphocytes were determined in lymph node-cell suspensions by flow cytometry. Briefly, aliquots of cell suspensions were stained with fluorescein-conjugated anti-mouse CD4 or with phycoerythrin-conjugated anti-mouse CD8 monoclonal antibodies. Lymphocytes were identified by FACS analysis using a FACScan flow cytometer (Becton Dickinson) with logarithmic amplification, and FACScan research software, and the percentage of lymphocytes expressing CD4 and CD8 was determined. Isotype controls (IgG1-FITC/IgG2a-PE) were used for each assay to determine non-specific staining.

2.12. Cytokine release

Lymphoid cells (1×10^6/ml) were stimulated with Con A (1 µg/ml) for 24 h at 37 °C in a 5% CO_2 atmosphere in a Falcon 24-well plate. After incubation, culture supernatants were harvested and their IFN-γ, IL-2, IL-6, IL-10 and IL-4 activities were determined by ELISA kits (Amersham Biosciences, Little Chalfont, Buckinghamshire).

2.13. Statistical analysis

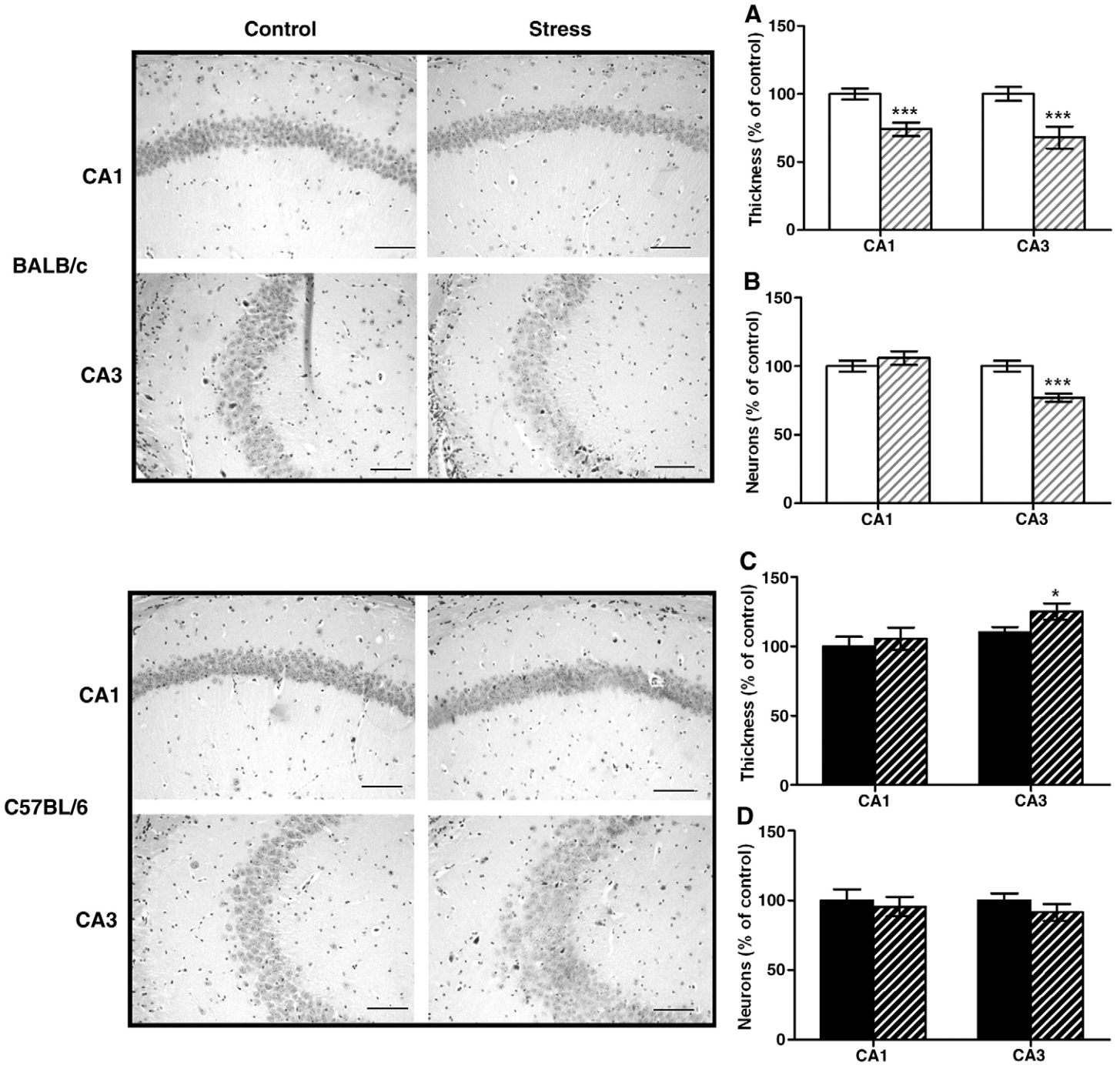
The results were analyzed by two-way analysis of variance (ANOVA) to examine significant main effects or interactions. When multiple comparisons were necessary after ANOVA, the Bonferroni test was applied. The open field results were analyzed by two-way ANOVA for repeated measures with the Bonferroni post-hoc test. When data was not normally distributed, non-parametric Friedman T^2-test was performed. Differences between means were considered significant if $p<.05$. Pearson's correlation test was used to examine the degree of correlation between these values.

3. Results

3.1. Memory-related behaviors

To analyze the effect of CMS on learning and memory we evaluated habituation to an open field and alternation in the Y maze. According our previous results (see Palumbo et al., 2009). BALB/c mice but not C57BL/6

Fig. 2. Hippocampal histology. The figure shows a representative photomicrographs of coronal sections of CA1 and CA3 hippocampus sub-fields from BALB/c (up panel) and C57BL/6 (down panel) mice in control and stressed mice. Bar: 100 µm. The structure of hippocampus was evaluated in control (plain bars) and CMS (crossed bars) mice in BALB/c (clear bars) and C57BL/6 (dark bars) animals. Graphics A and B show the thickness and the number of neurons of CA1 and CA3 hippocampus sub-fields in BALB/c mice respectively. Graphics C and D show the thickness and the number of neurons in both region of hippocampus in C57BL/6 mice respectively. The number of neurons and the thickness are represents as percentage respect to control. Results represent the mean ± SEM of five animals of each group. * $p<.05$, *** $p<.001$ respect to control.


Control
Stress
CA1
BALB/c
CA3
A
Thickness (% of control)
150
100
50
0
***
***
CA1
CA3
B
Neurons (% of control)
150
100
50
0
***
CA1
CA3
CA1
C57BL/6
CA3
C
Thickness (% of control)
150
100
50
0
*
CA1
CA3
D
Neurons (% of control)
150
100
50
0
CA1
CA3

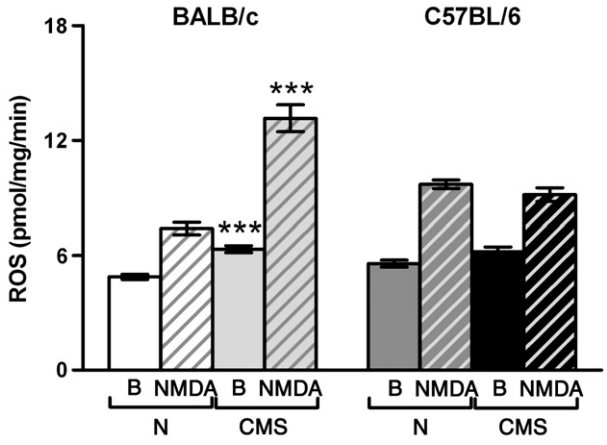


Fig. 3. Reactive oxygen species (ROS) levels. ROS production basal (B) (plain bars) and NMDA stimulated (striped bars) were determinate in hippocampus from BALB/c (clear bars) and C57BL/6 (dark bars) in control and CMS mice. Results represent the mean ± SEM of two independent experiments performed in duplicate with three animals of each group. *** $p<.001$ respect to the corresponding control.

submitted to CMS had a poor habituation in the open field test (data not shown). Alternation spontaneous behavior of stressed and control mice was examined in a Y-maze task. Two-way ANOVA showed significant changes in the percent of spontaneous alternation that depend on condition (control or CMS) and the strains of mice [interaction: strain x condition, $F(3,36)=11.71$, $p<.01$]. As illustrated in Fig. 1, simple effects analysis showed a significant decreased in CMS-BALB/c mice as compared to control [$F(3,36)=35.26$, $p<.001$]. Non-significant differences were found in the percent of spontaneous alternation between CMS and control C57BL/6 animals [$F(3,36)=0.97$, NS].

3.2. Histological studies

The effects of stress exposure on the morphology of the hippocampus were assessed by measuring the size and density of neurons throughout the dentate gyrus, CA3 and CA1 areas of the hippocampal formation. Histological modifications were evident in CA1 and CA3 subfields, as shown in Fig. 2. No changes were found in the dentate gyrus (data not shown). Two-way ANOVA showed that thickness of the sub-field CA1 and CA3 was significantly altered depending on strain and condition [interaction: strain x condition, CA1: $F(3,50)=11.60$, $p<.01$ and CA3: $F(3,48)=37.33$, $p<.001$]. Simple effects analysis revealed a significant decrease in CA1 thickness in CMS BALB/c mice [$F(1,47)=16.33$, $p<.001$] but not in CMS C57BL/6 [$F(1,47)=0.33$, NS] with respect to their corresponding controls. In the CA3 thickness, a significant decrease was observed in CMS BALB/c mice [$F(1,45)=21.52$, $p<.001$] and an increase in CMS C57BL/6 mice [$F(1,45)=9.96$, $p<.05$] with respect to corresponding control.

With regard to a number of neurons, two-way ANOVA revealed a significant interaction in CA3 but not in CA1 sub-field depending on strain and condition [interaction: strain x condition, CA3: $F(3,48)=8.34$, $p<.01$ and CA1: $F(3,50)=1.71$, NS]. Simple effects analysis only showed a significant reduction in neuronal density in CA3 sub-field in hippocampi from BALB/c mice exposed to CMS [$F(1,45)=32.55$, $p<.001$] but not in CMS C57BL/6 mice [$F(1,45)=2.90$, NS]. Concerning the number of neurons in CA1 sub-filed, post-hoc analysis indicated that neither CMS BALB/c nor CMS C57BL/6 animals had differences as compared to their corresponding controls.

3.3. ROS production

Basal and NMDA-stimulated ROS production were determined in the tissue homogenates of hippocampi from normal and CMS animals of both BALB/c and C57BL/6 mice (Fig. 3).

Two-way ANOVA revealed a significant interaction in the basal and NMDA-stimulated ROS productions depending on strain and condition [interaction: strain x condition, basal: $F(3,23)=4.43$, $p<.05$ and NMDA: $F(3,23)=51.99$, $p<.001$; respectively]. Simple effects analysis showed significant differences in both basal [$F(1,20)=28.00$, $p<.001$] and NMDA-stimulated [$F(1,20)=81.81$, $p<.001$] ROS production in the hippocampus from CMS BALB/c mice with respect to control. However, non significant differences were found for both basal [$F(1,20)=3.98$, NS] and NMDA-stimulated ROS production [$F(1,20)=.90$, NS] between normal and CMS C57BL/6 animals.

3.4. Serum corticosterone levels

Hormone levels of BALB/c and C57BL/6 mice were measured during CMS exposure (Table 1). Friedman analyses revealed significant differences in the corticosterone levels of BALB/c ($T^2=18.20$, $p<.001$) and C57BL/6 mice ($T^2=5.90$, $p<.001$). BALB/c animals subjected to the CMS procedure showed a significant increase in hormone levels during the first 2 weeks of exposure (CMS vs. control, $p<.01$ for 1 and 2 weeks of CMS exposure). After 3 weeks of stress, serum corticosterone levels returned to basal values and did not show any statistically significant differences when compared to controls (after 6 weeks, NS). Corticosterone of C57BL/6 mice exposed to the CMS model showed an increase in the 3rd and 4th weeks of CMS exposure ($p<.01$), returning to basal levels after 6 weeks of CMS (CMS vs. control, NS).

3.5. Spleen NE concentrations

Catecholamine levels of BALB/c and C57BL/6 were determined after CMS exposure (Table 1). Two-way ANOVA revealed an significative interaction between the strains and the different weeks of CMS exposure [$F(11,83)=7.01$, $p<.0001$]. As described in Table 1, splenic norepinephrine (NE) concentrations showed a pattern similar to serum corticosterone levels in both strains. Simple effects analysis showed that BALB/c mice presented an early increase of NE, which was lost after 3 weeks of CMS exposure [1st, 2nd and 3rd weeks of CMS vs. control; $p<.001$]. However, C57BL/6 mice showed an increase of NE only at 4th week ($p<.01$), which was lost after 6 weeks of CMS exposure.

3.6. Mitogen-induced proliferation

Lymphocytes from normal and CMS animals of BALB/c and C57BL/6 mice were stimulated with the optimal T or B mitogens concentrations. Due to the well-known lymphoid profile, lymph nodes cell suspensions were used for Con A-induced T selective mitogen proliferation, while spleen lymphocyte suspensions were used to evaluate lipopolysaccharides (LPS) (B-cell mitogen) effect (Fig. 4).

Table 1
Catecholamine concentrations in splenic samples and serum corticosterone levels from control and CMS-exposed animals.

Time of CMS Exposure	BALB/c		C57BL/6	
	[Corticosterone] (ng/ml)	[Norepinephrine] (pg/mg)	[Corticosterone] (ng/ml)	[Norepinephrine] (pg/mg)
Control	131 ± 5	760 ± 70	74 ± 6	405 ± 53
1 week	432 ± 28 **	1338 ± 91***	73 ± 3	548 ± 37
2 weeks	392 ± 21 **	1248 ± 101***	79 ± 6	464 ± 48
3 weeks	181 ± 18	1314 ± 117***	102 ± 6 **	335 ± 67
4 weeks	140 ± 19	812 ± 73	135 ± 17 **	726 ± 96 **
6 weeks	143 ± 9	889 ± 93	85 ± 10	448 ± 46

Catecholamines and corticosterone analyses were performed in control (non-exposed) and 1–6 weeks CMS-exposed animals. Results represent the mean ± SEM of the three independent experiments with three animals of each group. **$p<.01$, ***$p<.001$ respect to control.

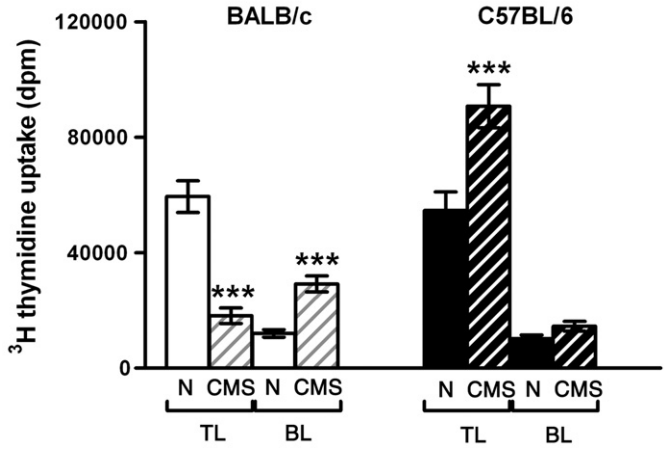


Fig. 4. Cellular proliferation assay. Proliferation of B and T-lymphocytes (BL and TL, respectively) were determinate by titrated thymidine uptake from BALB/c (clear bars) and C57BL/6 (dark bars) in control (plain bars) and CMS (crossed bars) mice. Results represent the mean ± SEM of four independent experiments with three animals for each group. *** $p<.001$ respect to the corresponding control. ^{3}H-thymidine uptake for non-stimulated cells was: BALB/c, control: 2194.7 ± 442.3 and CMS: 2677.1 ± 546.8 and C57BL/6, control: 3515.0 ± 742.5 and CMS: 3251.3 ± 546.4.

Thymidine uptake of unstimulated control lymphocytes was similar for both strain (see legend Fig. 4). Two-way ANOVA revealed that T- and B-cell proliferations were significantly different depending on both strain and condition [interaction: strain x condition, T-cell: F (3,47) = 55.09, $p<.001$ and B-cell: F (3,47) = 7.44, $p<.01$; respectively]. Simple effects analysis revealed that lymphoid cells from CMS BALB/c animals had a lower T cell response to the mitogen ConA [F (1,44) = 41.18, $p<.001$] and a higher B cell response to LPS [F (1,44) = 32.83, $p<.001$] than cells from normal animals. On the contrary, T cells from CMS C57Bl/6 mice [F (1,44) = 16.64, $p<.001$] give a higher proliferative response than normal T cells. However, no differences were found between normal and CMS B cell proliferation [F (1,44) = 3.50, NS] (Fig. 4).

3.7. Lymphocytes subsets distribution

To determine if the alterations of mitogen-induced T- and B-cell proliferation were due to changes in the immune distribution, percentage of $CD4^+$, $CD8^+$, $CD4CD8^+$ and $CD4CD8^-$ were determined on lymph nodes in non-exposed and 6 weeks CMS BALB/c and C57BL/6 mice. As seen in Table 2 no differences were observed between controls and CMS-exposed animals in both strains of mice [interaction: strain x condition, $CD4^+$: F (3,17) = .001, NS; $CD8^+$: F (3,17) = .01, NS; $CD4^+$ $CD8^+$: F (3,17) = .001, NS and $CD4^-$–$CD8^-$: F (3,17) = .001, NS].

3.8. Cytokine release

To evaluate the Th1/Th2 balance in both strains of mice, INF-γ and IL-2 (Th1 cytokines) and IL-4, IL-10 and IL-6 (Th2 cytokines) were determined in supernatants from Con A-stimulated lymphocytes. Two-way ANOVA revealed that significant changes in INF-γ, IL-2, IL-10, IL-4 and IL-6 production were observed depending on the strain of mice and the condition [interaction: strain x condition, INF-γ: F (3,23) = 45.77, $p<.001$; IL-2: F (3,23) = 4.81, $p<.05$; IL-10: F (3,23) = 129.20, $p<.001$; IL-4: F (3,23) = 84.73, $p<.001$ and IL-6: F (3,23) = 134.64, $p<.001$]. As seen in Fig. 5, CMS BALB/c mice showed a lower production of INF-γ [F(1,20) = 22.89, $p<.001$] and a higher release of IL-4 [F(1,20) = 46.45, $p<.001$], IL-10 [F(1,20) = 73.53, $p<.001$] and IL-6 [F(1,20) = 287.26, $p<.001$] than control animals. No changes were found in IL-2 levels between control and CMS-animals [F(1,20) = 2.50, NS]. In contrast, C57BL/6 data shows that CMS-animals had, as compared to controls, a higher release of INF-γ [F(1,20) = 22.89, $p<.001$] and IL-2 [F(1,20) = 24.00, $p<.001$], a lower release of IL-4 [F(1,20) = 37.73, $p<.001$] and IL-10 [F(1,20) = 56.25, $p<.001$], but no changes were found in the IL-6 production [F(1,20) = .29, NS].

Table 2
Percentage of lymphocyte subsets from control and CMS animals.

Lymphocyte subset	BALB/c		C57BL/6	
	Control	CMS	Control	CMS
CD4+	57 ± 4	62 ± 3	42 ± 5	47 ± 4
CD8+	13 ± 2	10 ± 2	26 ± 5	23 ± 4
CD4+ CD8+	0.27 ± 0.04	0.28 ± 0.04	0.05 ± 0.01	0.08 ± 0.01
CD4− CD8−	30 ± 2	28 ± 2	32 ± 4	30 ± 3

FACS analysis was performed on lymph nodes cell suspensions from control and CMS animals. Results are expressed as percentage of total lymphocytes and represent the mean ± S.D. of two experiments using two animals of each group.

3.9. Correlation analysis

As shown in Fig. 6, the Pearson's correlation test showed a negative correlation between the percentage of spontaneous alternation and the relation Th2/Th1 (defined as IL-10/INFγ ratio) in BALB/c mice taking both control and CMS ($r = -.8657$, $p = .0012$). When analysis is performed in CMS BALB/c mice, the correlation remain significant ($r = -.9729$, $p = .0053$). However, no correlation was found in C57BL/6 mice ($r = -.01271$, NS).

4. Discussion

Herein, we studied the effects of stress on learning and memory and their relationship with peripheral Th1/Th2 cytokines production. For this purpose we used two genetically different inbred strains of mice, C57BL/6 (Th1) and BALB/c (Th2).

We previously showed (Palumbo et al., 2007) that chronic stress exposure induces a reduced habituation to the open field and poor memory retention in the passive avoidance inhibitory task in BALB/c mice. However, we found that chronic stress does not induce any alterations in learning and memory in C57BL/6 mice (Palumbo et al., 2009). In order to check that CMS model induces similar changes, we performed open field test and a Y-maze task, which has been widely used to assess spatial memory. Results show an impairment memory in CMS BALB/c mice but not in stressed C57Bl/6 mice with respect to control animals.

Moreover, we reported that CMS BALB/c mice have a lower number of neurons in CA3 area than control mice as well as a decreased thickness in CA1 and CA3 sub-field (Palumbo et al., 2007). Nevertheless, neither the number of neurons nor the thickness of the CA1 and CA3 hippocampal areas, were found to be significant decreased in CMS C57BL/6 mice with respect to control. Indeed, surprisingly, an increase in CA3 thickness was found in C57BL/6 stressed mice. Similar findings were reported by Duric and McCarson (2005) in a model of stress and pain in rats. These authors found edematous changes in the hippocampal tissue. Edema was more robust in the acute model of stress and appears to decrease with time. The authors propose that overt atrophy may be more robust in a further stage, and in concert with edema recovery processes stimulated by unknown compensatory cellular mechanisms, which may be responsible for lesser changes in the hippocampal volume after chronic stress. Taking into account these findings and our results it is possible suggest that C57Bl/6 mice are more resistant to the effects of stress and that changes in the hippocampus occur in an earlier stage. Many studies have demonstrated the participation of hippocampus in learning and memory processes. It is important to point out that particularly CA1 and CA3 constitute important areas involved in the processes of learning and memory (Daumas et al., 2005). On the other hand, oxidative stress has been implicated in the pathogenesis of many neurodegenerative and

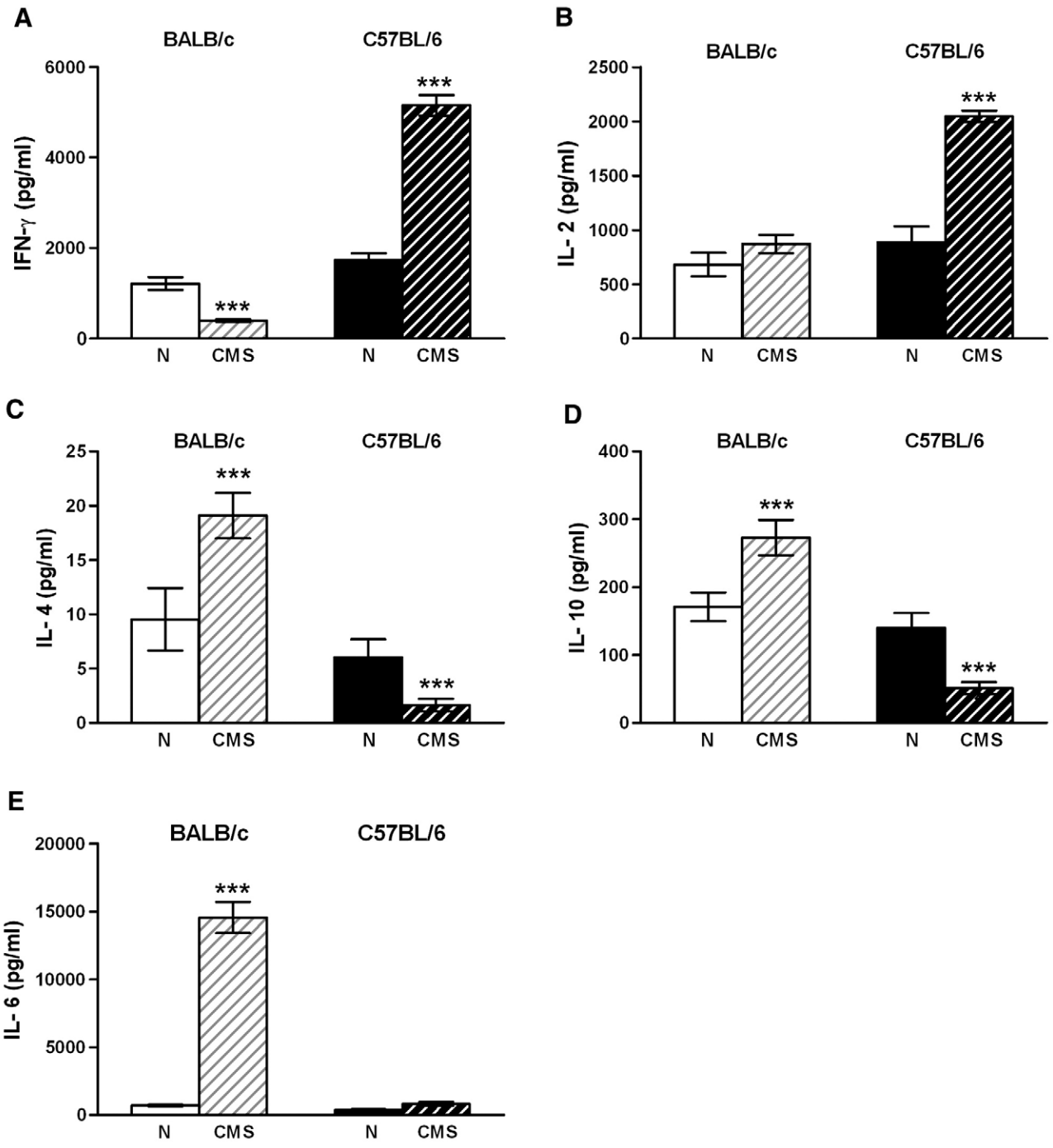


Fig. 5. Cytokine levels. The concentration of INF-γ (panel A), IL-2 (panel B), IL-4 (panel C), IL-10 (panel D) and IL-6 (panel E) was determinate in supernatants of lymphocytes from lymph node stimulated by Con A for 24 h from BALB/c (clear bars) and C57BL/6 (dark bars) in control (plain bars) and CMS (crossed bars) mice. Results represent the mean ± SEM of three independent experiments with two animals for each group. ** $p<.01$, *** $p<.001$ respect to the corresponding control.

neurological disorders (Ischiropoulos and Beckman, 2003) with ROS as part of the intracellular effectors of damage. Under normal conditions, these toxic species are produced by cellular metabolism and neutralized by endogenous antioxidant defenses. However, in adverse conditions, cellular defenses might result insufficient to counteract ROS attack, leading to an increased vulnerability and, eventually, to cell death (Ischiropoulos and Beckman, 2003). Previous studies have suggested that the formation of ROS during NMDA exposure is involved in triggering the excitotoxic cascade (Kishida and Klann, 2007). According to our previous results (Palumbo et al., 2007), a higher basal and NMDA stimulated ROS production was found in CMS BALB/c mice hippocampus with respect to control. However, non-significant effects of stress exposure on ROS production were observed in C57BL/6 mice hippocampus. These results could be indicating that stress induces an increased vulnerability to neurotoxic mechanisms in the hippocampus of BALB/c mice, but not in that of C57BL/6 mice.

Studies on brain-immune interactions have revealed bidirectional connections between the neural and neuroendocrine systems and the immune system (Sternberg, 2000). The immune system can signal the central nerve system through the action of cytokines (Eskandari et al.,

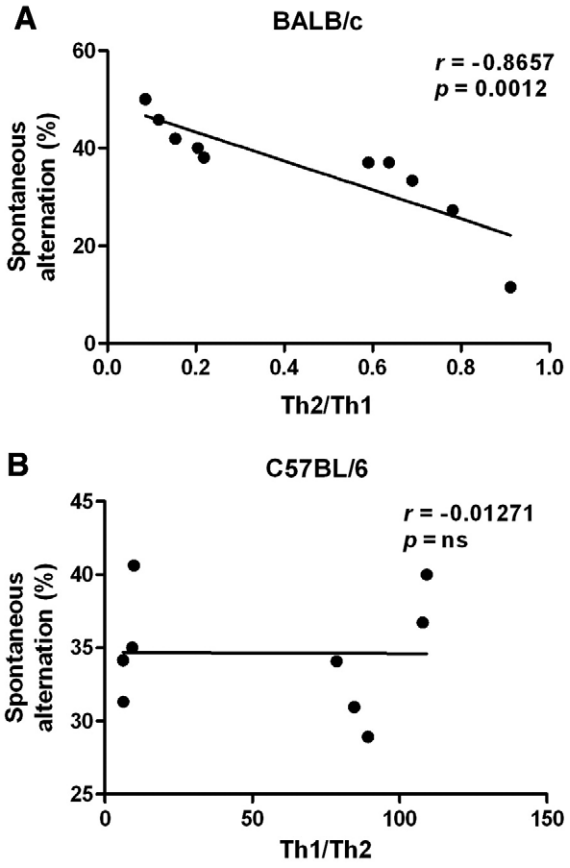


Fig. 6. Correlation between spontaneous alternation percentage and the relation Th2/Th1 in BALB/c mice (A), $r = -0.8657$, $p = 0.0012$. Correlation between spontaneous alternation percentage and the relation Th1/Th2 in C57BL/6 mice (B), $r = -0.01271$, $p = \text{ns}$.

2003). Deregulation of cytokines (Th1 versus Th2) has been reported to be involved in the pathogenesis of many human diseases such as autoimmune diseases, sleep disturbance, major depression and other disorders (Kaufmann et al., 2007; Schwarz et al., 2001). Therefore, we evaluated the effect of stress on immune functions by the ability of lymphoid cells to proliferate and produce Th1 and Th2 cytokines. According to our previous results (Silberman et al., 2003, 2004), mitogen-induced T-cell proliferation is decreased, whereas B-cell proliferation is increased, in BALB/c exposed to stress. On the contrary, an increase in the proliferative response of T-cell without changes in B-cell proliferation was observed in CMS C57BL/6. No change in CD4/CD8 relationship was observed in these strains of mice. In order to determine the Th1/Th2 balance, INF-γ and IL-2 as Th1 and IL-6, IL-10 and IL-4 as Th2 cytokine production were measured in stimulated lymphocytes from BALB/c and C57BL/6. We found that stress exposure induced a decrease in INF-γ and an increase in IL-4, IL-10 and IL-6, in BALB/c mice. In CMS C57BL/6 mice, we found a significant increase in INF-γ and IL-2 and a decrease in IL-4 and IL-10. These results suggest that stress induces a Th2 response in BALB/c and a Th1 response in C57BL/6 mice. It is important to point out that significant changes in the IL-6 production by macrophages were not found under these conditions (data not shown).

It was suggested that INF-γ may enhance neurodegeneration in a number of chronic neuroinflamatory diseases, including multiple sclerosis and HIV-1 infection (Benveniste, 1997; Popko et al., 1997). However, Koustova et al. (2000) reported in a model of retroviral encephalopathy that immune processes regulated by INF-γ may be required to suppress neurodegeneration associated with chronic inflammatory states. Loewenbrueck et al. (2008) showed for the first time that Aβ1-42-specific Th1-type T-cell memory is present in young humans, producing high levels of IFN-γ and IL-2. In contrast, individuals with Alzheimer's disease produce IL-10 only in the absence of any effector cytokine. The authors postulated that this Aβ1-42-specific Th1 response has a beneficial role in clearing Aβ1-42 in humans. Besides, an association between perceived social support and Th1 dominance was described (Miyazaki et al., 2005).

Considering these findings it is possible suggest that the INF-γ increase, observed in C57BL/6 mice after stress exposition, could constitute a protective mechanism against the deleterious effect of stress. In BALB/c mice, the decrease of IFN-γ with the important IL-6 increased production could constitute a mechanism that contributes to neurodegeneration processes. In fact, blockade of endogenous IL-6 after hippocampus-dependent spatial alternation learning resulted in significant improvement of long-term memory (Balschun et al., 2004). Furthermore, IL-6 KO mice exhibited a facilitation of radial maze learning over 30 days, in terms of lower number of working memory errors (Braida et al., 2004). Recent evidences have suggested that hippocampal interleukin-1 (IL-1) beta is also required in the brain for the physiological regulation in memory processes (Goshen et al., 2007; Labrousse et al., 2009). However, high levels of this cytokine produce detrimental effects on memory functioning (Goshen et al., 2007). Probably, hippocampal levels of IL-1 beta and other cytokines could be altered in animals exposed to CMS, as previously described (Koo and Duman, 2008). However, it seems unlikely that the increase in IL-1 beta is involved, since it has been shown that IL-1 mediates its effect via adrenocortical activation (Goshen et al., 2008) and in our CMS model memory impairment is not correlated with corticosterone increase, as we discuss below. We are performing studies to clarify this point. It was demonstrated that stress involves the activation of the HPA axis and the SNS which, in turn, modulate the immune response. Moreover, it was reported that corticosterone administration induces neuronal atrophy in the hippocampal CA3 subfield which, in turn, induces memory impairment (McEwen, 2001). In this context, the participation of activation of HPA and SNS systems in immunological and behavioral effects induced by CMS exposure was investigated. Results of BALB/c animals subjected to CMS show a moderate increment of corticosterone and catecholamine levels by the first three weeks, returning to basal levels after 4 weeks of stress exposure. However, alterations in immune response appear after 4 weeks of CMS exposure (Silberman et al., 2002). Moreover, memory impairment appears after 4 weeks of CMS exposure (Palumbo et al., 2007). In this way, there were not a temporal relationship between corticosterone increase (1 and 2 weeks of CMS exposure) and behavioral alteration (4–6 weeks of CMS exposure). It is important to note that it is not possible to rule out that the early hormone increase could be inducing late changes in immune and behavioral alteration. However there is an increase of corticosterone levels but not memory impairment in C57Bl/6 mice. These findings support the hypothesis that there is no correlation between corticosterone increase and cognitive deficits.

These results show, for the first time, that BALB/c mice are more vulnerable to the effects of stress than C57BL/6 mice, and that this correlates with a differential regulation of the Th1/Th2 cytokine balance. In C57BL/6 mice, stress induces a Th1 response with an increase of IFN-γ production that in turn could be a protective mechanism against to the neurodegenerative processes. On the other hand, an increase in Th2 cytokines and a decrease in IFN-γ correlate with a poor memory performance in CMS BALB/c mice. In fact, C57Bl/6 mice showed no significant ROS production or changes in the structure of the hippocampus. On contrary, tissue toxicity together with increases in oxidative stress and neuronal loss were induced CMS BALB/c mice.

To conclude, the present results could be useful to generate new strategies to the treatment of the adverse consequences of stress and aging memory impairments taking actions mediated by cytokines into account.

Acknowledgments

The authors thank Maria Rosa Gonzalez Murano for her technical assistance, Daniel Gonzalez for his invaluable help in the animal stress model and Mrs. Patricia Fernandez for the secretarial assistance. This work was supported by PIP 6049 and PIP 00281 from CONICET and UBACyT M024 from University of Buenos Aires.

References

Balschun, D., Wetzel, W., Del Rey, A., Pitossi, F., Schneider, H., Zuschratter, W., Besedovsky, H.O., 2004. Interleukin-6: a cytokine to forget. FASEB J. 18, 1788–1790.
Belzung, C., Griebel, G., 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav. Brain Res. 125, 141–149.
Benveniste, E.N., 1997. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J. Mol. Med. 75, 165–173.
Braida, D., Sacerdote, P., Panerai, A.E., Bianchi, M., Aloisi, A.M., Iosue, S., Sala, M., 2004. Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav. Brain Res. 153, 423–429.
Dantzer, R., Kelley, K.W., 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21, 153–160.
Daumas, S., Halley, H., Francés, B., Lassalle, J.M., 2005. Encoding, consolidation, and retrieval of contextual memory: differential involvement of dorsal CA3 and CA1 hippocampal subregions. Learn. Mem. 12, 375–382.
Dillon, G.M., Qu, X., Marcus, J.N., Dodart, J.C., 2008. Excitotoxic lesions restricted to the dorsal CA1 field of the hippocampus impair spatial memory and extinction learning in C57BL/6 mice. Neurobiol. Learn. Mem. 90, 426–433.
Dulawa, S.C., Holick, K.A., Gundersen, B., Hen, R., 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29, 1321–1330.
Duric, V., McCarson, K.E., 2005. Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat model of pain and stress. Neuroscience 133, 999–1006.
Edgar, V.A., Cremaschi, G.A., Sterin-Borda, L., Genaro, A.M., 2002. Altered expression of autonomic neurotransmitter receptors and proliferative responses in lymphocytes from a chronic mild stress model of depression: effects of fluoxetine. Brain Behav. Immun. 16, 333–350.
Eskandari, F., Webster, J.I., Sternberg, E.M., 2003. Neural immune pathways and their connection to inflammatory diseases. Arthritis Res. Ther. 5, 251–265.
Frisch, C., De Souza-Silva, M., Söhl, G., Güldenagel, M., Willecke, K., Huston, J.P., Dere, E., 2005. Stimulus complexity dependent memory impairment and changes in motor performance after deletion of the neuronal gap junction protein connexin36 in mice. Behav. Brain Res. 157, 177–185.
Gonzalez-Aguilar, F., De Robertis, E., 1963. A formalin-perfusion method for histophysiological study of the central nervous system with the electron microscopy. Neurology 13, 758–763.
Goshen, I., Kreisel, T., Ounallah-Saad, H., Renbaum, P., Zalzstein, Y., Ben-Hur, T., Levy-Lahad, E., Yirmiya, R., 2007. A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32, 1106–1115.
Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., Yirmiya, R., 2008. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol. Psychiatry 13, 717–728.
Guinazu, N., Pellegrini, A., Giordanengo, L., Auki, M.P., Rivarola, H.W., Cano, R., Rodrigues, M.M., Gea, S., 2004. Immune response to a major Trypanosoma cruzi antigen, cruzipain, is differentially modulated in C57BL/6BL/6 and BALB/c mice. Microbes Infect. 6, 1250–1258.
Ischiropoulos, H., Beckman, J.S., 2003. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J. Clin. Invest. 111, 163–169.
Kaufmann, I., Eisner, C., Richter, P., Huge, V., Beyer, A., Chouker, A., Schelling, G., Thiel, M., 2007. Lymphocyte subsets and the role of TH1/TH2 balance in stressed chronic pain patients. Neuroimmunomodulation 14, 272–280.
Keston, A.S., Brand, T.R., 1965. The fluorometric analysis of ultramicro quantities of hydrogen peroxide. Anal. Biochem. 11, 1–5.
Kim, J.J., Yoon, K.S., 1998. Stress: metaplastic effects in the hippocampus. Trends Neurosci. 21, 505–509.
Kim, M.J., Choi, S.J., Lim, S.T., Kim, H.K., Kim, Y.J., Yoon, H.G., Shin, D.H., 2008. Zeatin supplement improves scopolamine-induced memory impairment in mice. Biosci. Biotechnol. Biochem. 72, 577–581.
Kishida, K.T., Klann, E., 2007. Sources and targets of reactive oxygen species in synaptic plasticity and memory. Antioxid. Redox Signal. 9, 233–244.
Koo, J.W., Duman, R.S., 2008. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc. Natl. Acad. Sci. U. S. A. 105, 751–756.
Koustova, E., Sei, Y., McCarty, T., Espey, M.G., Ming, R., Morse III, H.C., Basile, A.S., 2000. Accelerated development of neurochemical and behavioral deficits in LP-BM5 infected mice with targeted deletions of the IFN-gamma gene. J. Neuroimmunol. 108, 112–121.
Labrousse, V.F., Costes, L., Aubert, A., Darnaudéry, M., Ferreira, G., Amédée, T., Layé, S., 2009. Impaired interleukin-1beta and c-Fos expression in the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-deficient mice. PLoS ONE 4, e6006.
Larson, S.J., Dunn, A.J., 2001. Behavioral effects of cytokines. Brain Behav. Immun. 15, 371–387.
Laverty, R., Taylor, K.M., 1968. The fluorometric assay of catecholamines and related compounds: improvements and extensions to the hydroxyindole technique. Anal. Biochem. 22, 269–279.
LeBel, C.P., Odunze, I.N., Adams Jr, J.D., Bondy, S.C., 1989. Perturbations in cerebral oxygen radical formation and membrane order following vitamin E deficiency. Biochem. Biophys. Res. Commun. 163, 860–866.
Loewenbrueck, K.F., Tigno-Aranjuez, J.T., Boehm, B.O., Lehmann, P.V., Tary-Lehmann, M., 2008. Th1 responses to beta-amyloid in young humans convert to regulatory IL-10 responses in Down syndrome and Alzheimer's disease. Neurobiol. Aging. doi: 10.1016/j.neurobiolaging.2008.09.007.
Marona-Lewicka, D., Vetulani, J., 1989. Neurochemical correlates of differences in responses to psychotropic drugs. I. Apomorphine and morphine effects on locomotor activity of C57/BL and Balb/C mice. Pol. J. Pharmacol. Pharm. 41, 431–438.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators: allostasis and allostatic load. N. Engl. J. Med. 338, 171–179.
McEwen, B.S., 2001. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann. N. Y. Acad. Sci. 933, 265–277.
McEwen, B.S., 2008. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur. J. Pharmacol. 583, 174–185.
Miyazaki, T., Ishikawa, T., Nakata, A., Sakurai, T., Miki, A., Fujita, O., Kobayashi, F., Haratani, T., Iimori, H., Sakami, S., Fujioka, Y., Kawamura, N., 2005. Association between perceived social support and Th1 dominance. Biol. Psychol. 70, 30–37.
Monleon, S., D'Aquila, P., Parra, A., Simon, V.M., Brain, P.F., Willner, P., 1995. Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. Psychopharmacology 117, 453–457.
Palumbo, M.L., Fosser, N.S., Rios, H., Zorrilla Zubilete, M.A., Guelman, L.R., Cremaschi, G.A., Genaro, A.M., 2007. Loss of hippocampal neuronal nitric oxide synthase contributes to the stress-related deficit in learning and memory. J. Neurochem. 102, 261–274.
Palumbo, M.L., Zorrilla-Zubilete, M.A., Cremaschi, G.A., Genaro, A.M., 2009. Different effect of chronic stress on learning and memory in BALB/c and C57BL/6 inbred mice: Involvement of hippocampal NO production and PKC activity. Stress 4, 350–361.
Popko, B., Corbin, J.G., Baerwald, K.D., Dupree, J., Garcia, A.M., 1997. The effects of interferon-gamma on the central nervous system. Mol. Neurobiol. 14, 19–35.
Priebe, K., Romeo, R.D., Francis, D.D., Sisti, H.M., Mueller, A., McEwen, B.S., Brake, W.G., 2005. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: a cross-fostering study. Dev. Psychobiol. 48, 95–96.
Sargent, R.N., 1985. Determination of corticosterone in rat plasma by HPLC. J. Anal. Toxicol. 9, 20–23.
Schwarz, M.J., Chiang, S., Müller, N., Ackenheil, M., 2001. T-helper-1 and T-helper-2 responses in psychiatric disorders. Brain Behav. Immun. 15, 340–370.
Silberman, D.M., Wald, M., Genaro, A.M., 2002. Effects of chronic mild stress on lymphocyte proliferative response. Participation of serum thyroid hormones and corticosterone. Int. Immunopharmacol. 2, 487–497.
Silberman, D.M., Wald, M.R., Genaro, A.M., 2003. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. J. Neuroimmunol. 144, 53–60.
Silberman, D.M., Ayelli-Edgar, V., Zorrilla-Zubilete, M., Zieher, L.M., Genaro, A.M., 2004. Impaired T-cell dependent humoral response and its relationship with T lymphocyte sensitivity to stress hormones in a chronic mild stress model of depression. Brain Behav. Immun. 18, 81–90.
Sternberg, E.M., 2000. Interactions between the immune and neuroendocrine systems. Prog. Brain Res. 122, 35–42.
Tagawa, N., Sugimoto, Y., Yamada, J., Kobayashi, Y., 2006. Strain differences of neurosteroid levels in mouse brain. Steroids 71, 776–784.
Tang, X., Orchard, S.M., Sanford, L.D., 2002. Home cage activity and behavioral performance in inbred and hybrid mice. Behav. Brain Res. 136, 555–569.
Watanabe, H., Numata, K., Ito, T., Takagi, K., Matsukawa, A., 2004. Innate immune response in Th1- and Th2-dominant mouse strains. Shock 22, 460–466.
Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci. Biobehav. Rev. 16, 525–534.
Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., 2004. Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305, 217.