Stress
The International Journal on the Biology of Stress

ISSN: 1025-3890 (Print) 1607-8888 (Online) Journal homepage: http://www.tandfonline.com/loi/ists20

Maternal separation in early life modifies anxious behavior and Fos and glucocorticoid receptor expression in limbic neurons after chronic stress in rats: effects of tianeptine

Verónica Trujillo, Patricia E. Durando & Marta M. Suárez

To cite this article: Verónica Trujillo, Patricia E. Durando & Marta M. Suárez (2015): Maternal separation in early life modifies anxious behavior and Fos and glucocorticoid receptor expression in limbic neurons after chronic stress in rats: effects of tianeptine, Stress, DOI: 10.3109/10253890.2015.1105958

To link to this article: http://dx.doi.org/10.3109/10253890.2015.1105958

Accepted author version posted online: 09 Oct 2015.
Published online: 20 Nov 2015.

Submit your article to this journal

Article views: 28

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=ists20

Download by: [Verónica Trujillo] **Date:** 24 November 2015, At: 14:56

STRESS
The International Journal on the Biology of Stress

http://informahealthcare.com/sts
ISSN: 1025-3890 (print), 1607-8888 (electronic)

Stress, Early Online: 1–13
© 2015 Taylor & Francis. DOI: 10.3109/10253890.2015.1105958

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH REPORT

Maternal separation in early life modifies anxious behavior and Fos and glucocorticoid receptor expression in limbic neurons after chronic stress in rats: effects of tianeptine

Verónica Trujillo, Patricia E. Durando, and Marta M. Suárez

Laboratorio De Fisiología Animal, Facultad De Ciencias Exactas, Físicas Y Naturales, Universidad Nacional De Córdoba, Córdoba, Argentina

Abstract

Early-life adversity can lead to long-term consequence persisting into adulthood. Here, we assess the implications of an adverse early environment on vulnerability to stress during adulthood. We hypothesized that the interplay between early and late stress would result in a differential phenotype regarding the number of neurons immunoreactive for glucocorticoid receptor (GR-ir) and neuronal activity as assessed by Fos immunoreactivity (Fos-ir) in brain areas related to stress responses and anxiety-like behavior. We also expected that the antidepressant tianeptine could correct some of the alterations induced in our model. Male Wistar rats were subjected to daily maternal separation (MS) for 4.5 h during the first 3 weeks of life. As adults, the rats were exposed to chronic stress for 24 d and they were treated daily with tianeptine (10 mg/kg intraperitoneal) or vehicle (isotonic saline). Fos-ir was increased by MS in all structures analyzed. Chronic stress reduced Fos-ir in the hippocampus, but increased it in the paraventricular nucleus. Furthermore, chronic stress increased GR-ir in hippocampus (CA1) and amygdala in control non-MS rats. By contrast, when MS and chronic stress were combined, GR-ir was decreased in these structures. Additionally, whereas tianeptine did not affect Fos-ir, it regulated GR-ir in a region-dependent manner, in hippocampus and amygdala opposing in some cases the stress or MS effects. Furthermore, tianeptine reversed the MS- or stress-induced anxious behavior. The interplay between MS and chronic stress observed indicates that MS rats have a modified phenotype, which is expressed when they are challenged by stress in later life.

Keywords

Amygdala, antidepressant, elevated plus maze, Fos immunoreactivity, glucocorticoid receptor, hippocampus

History

Received 27 March 2015
Revised 5 October 2015
Accepted 6 October 2015
Published online 17 November 2015

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

Introduction

Clinical evidence links the onset of depressive episodes with stressful life events (Kendler et al., 1999, 2000; Kessler, 1997). In addition, both in depression and in chronic stress conditions, the hypothalamic-pituitary-adrenal (HPA) axis is dysregulated, as indicated by hypercortisolism and elevated levels of adrenocorticotrophic hormone (ACTH) (Wong et al., 2000; Young et al., 2004). This association suggests that, at least, some components of stress responses are altered during depression. The HPA axis is under positive or negative corticosterone feedback control. Glucocorticoids (cortisol in humans, corticosterone in rodents) exert positive feedback actions to increase corticotropin-releasing hormone (CRH) expression in the amygdala, mainly in the central and medial nuclei. The negative feedback control exerted by glucocorticoids acts on the hippocampus and HPA axis (de Kloet, 2013; Herman et al., 2005). In the brain, glucocorticoid effects are mediated by two types of receptors: the mineralocorticoid or type I receptor (MR) and the glucocorticoid or type II receptor (GR). The GR is ubiquitously located in the brain, particularly in the hippocampus, amygdala, paraventricular nucleus (PVN), prefrontal cortex, and the ascending aminergic neurons (de Kloet, 2013; Taylor et al., 2005). The MR is abundantly expressed in hippocampus, while MR is minimally expressed in the PVN and amygdala. The GR displays a 10-fold lower affinity for glucocorticoid than the MR (Reul & de Kloet, 1985); for this reason, GR is only activated by high levels of glucocorticoids, which are achieved following stress or at the circadian peak (de Kloet, 2013).

Adverse experiences in early life are a powerful risk factor for impairment of physical health and cognitive functions, drug susceptibility, and vulnerability to stress-related disorders such as depression and anxiety in later life (Claessens et al., 2011; Daskalakis et al., 2013; Heuser & Lammers, 2003). In rats, the neonatal period between postnatal days 3–14 is characterized as the stress hyporesponsive period (SHRP). This is a critical developmental stage for HPA maturation, in which stimulation of corticosterone secretion is attenuated following mild stressors, which otherwise would elicit a marked response in adults (Levine, 2001). It has been suggested that maternal care and feeding regulate the HPA

Correspondence: Marta Suárez and Verónica Trujillo, Laboratorio de Fisiología Animal, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299, Córdoba 5000, Argentina. Tel: +54 03514332100 ext 239. Fax: +54 03514332097. E-mail: msuarez@efn.uncor.edu (Marta M. Suárez); vero3802@gmail.com (Verónica Trujillo)

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

axis sensitivity of neonates during the SHRP (Claessens et al., 2011). Thus, HPA disinhibition induced by early maternal separation (MS) may affect brain development, leading to persistent long-term changes in the systems that regulate stress responses. In adults, such changes include basal or stress-induced sensitization of the HPA axis and the sympathetic-adrenal-medullary system, which results in increased adrenal sensitivity to ACTH and increased plasma norepinephrine concentration (Anisman et al., 1998; Ladd et al., 2005; Suárez et al., 2001, 2002).

Tianeptine is an antidepressant with a structure near to, but distinct from tricyclic antidepressants (TCAs), and with pharmacological and neurochemical properties different from those of TCAs. There have been reported actions of this antidepressant on the glutamatergic system (McEwen et al., 2010; Racagni & Popoli, 2010; Svenningsson et al., 2007). Tianeptine has demonstrated clinical antidepressant efficacy, at least equal to selective serotonin reuptake inhibitors (SSRIs) (Kasper & Olie, 2002; Lepine et al., 2001; Novotny & Faltus, 2002), and it is better tolerated and has less side effects than TCAs (Ridout & Hindmarch, 2001) and SSRIs (Atmaca et al., 2003; Lepine et al., 2001). Additionally, this antidepressant relieves anxiety symptoms that are often associated with depression (Lepine et al., 2001; Novotny & Faltus, 2002). In previous studies, it was shown that tianeptine attenuates the increase in ACTH and corticosterone secretion and Fos expression in the PVN that are induced by lipopolysaccharide or immobilization stress (Castanon et al., 2003; Delbende et al., 1991). Tianeptine also reduces levels of CRH mRNA in the dorsal and ventral bed nucleus of the stria terminalis and in the central amygdala in both naïve and chronic mild stressed rats (Kim et al., 2006). Our previous results showed that tianeptine decreases the chronic stress-induced increased of epinephrine and norepinephrine secretion and anxiety behavior (Trujillo et al., 2009).

The hypothesis for this study was that early MS produces lasting effects in terms of vulnerability to chronic stress in adulthood, and in an unpredictable chronic stress model would alter the immunoreactivity of neurons that express Fos and GR in key structures involved in stress response regulation. In order to investigate whether such central alterations are linked to behavioral changes, we evaluated anxiety indices in the elevated plus maze. Finally, we tested the hypothesis that chronic treatment with the antidepressant tianeptine would prevent or reverses the possible neuroendocrine and behavioral changes caused by MS and chronic stress.

We expected to find long-term effects of MS resulting in a modified phenotype of adult rats under chronic stress conditions concerning anxiety behavior, basal neuronal activity, and immunoreactivity of GR in hippocampus, amygdala, and hypothalamus. We also expected that the treatment with the antidepressant tianeptine could reverse some of these potential alterations.

Methods

Animals

All experimental procedures were performed according to the NIH guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996) with protocols approved by the National University of Córdoba, Argentina.

Wistar derived rats ($n = 103$) were bred and rearing in our colony. Rats were housed in a temperature-controlled room ($21 \pm 1\,^{\circ}C$) under artificial illumination (12:12 h light/dark schedule; lights on at 07:00 h). Except when required by the stress paradigm the rats had *ad libitum* access to food (standard laboratory chow) and tap water. The day of birth was designated as postnatal day (PND) 0. On PND 1 litters were culled to 10 pups per dam (equal sex ratio, as near as possible). Whole litters were randomly assigned to one of two rearing conditions: MS or standard animal facility rearing (AFR).

Early maternal separation

For MS litters, pups were separated daily from their mother for 4.5 h between PND 1 and PND 21 (Ogawa et al., 1994). Separation consisted of removing the dam from the home cage and placing it alone into an adjacent cage while the litter was kept together in the nest. After the separation period, the dam was returned to the home cage. Separation were carried out between 08:00 h and 12:30 h. Control litters (AFR) remained undisturbed in the maternal cage except for a bedding change twice a week, until weaning at PND 22.

Post-weaning housing conditions

From weaning until PND 49, male offspring were group-housed with their respective littermates and handled daily by the same researcher to minimize stress reactions to manipulation at the time of treatment application. At PND 49, male offspring from both rearing conditions (MS and AFR) were randomly subdivided into four treatments: chronic stress with vehicle, or with tianeptine, and non-stressed with vehicle, or with tianeptine, yielding eight experimental groups: 1 – AFR control (non-stressed + vehicle) ($n = 15$), 2 – AFR + tianeptine ($n = 12$), 3 – AFR + chronic stress/vehicle ($n = 16$), 4 – AFR + chonic stress/tianeptine ($n = 13$), 5 – MS control (non-stressed + vehicle) ($n = 14$), 6 – MS + tianeptine ($n = 12$), 7 – MS + chronic stress/vehicle ($n = 12$), and 8 – MS + chronic stress/tianeptine ($n = 9$). To control for litter effects, each experimental group was made up of rats from different litters (one rat per litter per condition). Male offspring were housed in standard cages in groups of four until the end of the experimental period.

We thus used a factorial ($2 \times 2 \times 2$) experimental design, with *separation* (AFR or MS), *stress* (non-stressed or chronic stressed), and *drug* (vehicle or tianeptine) as factors.

Chronic stress

Rats in the stressed groups were exposed to 24 d of variable and unpredictable chronic stress started at PND 50 (200–250 g body weight). The chronic stress paradigm consisted of five stressors with varying intensities that were presented to the rats, during the light phase, randomly in time and order over the course of 24 d (Table 1). Only one stressor was applied per day. The following stressors were used: 4 h of noise produced by an alarm bell (85 dB; 2.5 Hz) (randomly applied on 6 of 24 d); loss of consciousness by ether anesthesia and subsequent exposure for 2 min (on 5 d);

Table 1. Chronic stress model.

Day	Stressor	Hour
1	Ether anesthesia	16:30 h
2	Two saline injections	10:00 and 14:00 h
3	Immobilization	11:30–12:30 h
4	Noise	12:00–16:00 h
5	Fasting	For 24 h
6	Rest day	–
7	Ether anesthesia	12:00 h
8	Noise	13:00–17:00 h
9	Immobilization	08:30–09:30 h
10	Ether anesthesia	16:30 h
11	Noise	10:00–14:00 h
12	Fasting	For 24 h
13	Rest day	–
14	Immobilization	11:30–12:30 h
15	Noise	13:00–17:00 h
16	Two saline injections	09:30 and 13:30 h
17	Ether anesthesia	15:00 h
18	Noise	08:30–12:30 h
19	Fasting	For 24 h
20	Rest day	–
21	Ether anesthesia	12:00 h
22	Two saline injections	10:00 and 14:00 h
23	Immobilization	16:00–17:00 h
24	Noise	08:00–12:00 h

Rats were exposed to the various stressors for 24 d.

two intraperitoneal (i.p.) injections of 0.5 ml isotonic (0.9%) saline solution at 4 h intervals (on 4 d); restraint for 1 h by placement inside a 6 cm diameter metal grid cylinder (on 4 d); and food deprivation for 24 h (on 3 d). There were also 3 d without stress. These stressors were applied randomly with the exception of the last day when noise was used as the last stressor. The stress paradigm used did not affect the body weight of the rats (Suárez et al., 1996). The non-stressed groups were not exposed to any stressor.

Tianeptine and vehicle administration

Starting at PND 50 rats were treated daily across 24 d with either tianeptine (commercial name: Stablon; from Servier Laboratories, Suresnes, France) or vehicle according to the assigned group. Tianeptine (10 mg/kg) was prepared diluted in 0.9% NaCl solution (vehicle). Both tianeptine and vehicle were administrated i.p. in a volume of 0.5 ml between 12:00 h and 13:00 h.

Anxiety-like behavior

Twenty-four hours after the last stressor (PND 74), between 10:00 h and 12:00 h, rats were tested in the elevated plus maze apparatus. At the same age, the non-stressed groups were also tested. The apparatus consisted of a plus-shaped platform elevated 50 cm from the floor. Two of the opposing arms (50×10 cm) were enclosed by 40-cm high side and end walls (closed arms), whereas the other two had no walls (open arms). In the middle of the four arms there was a central area (10×10 cm). The elevated plus maze test is based on creating a conflict between the rat's exploratory drive and its innate fear of open and exposed areas. Thus, decreased open-arm exploration was taken to indicate enhanced anxiety-related behavior (Pellow et al., 1985). Rats were transported to a quiet experimental room 2 h before the behavioral test to eliminate any stressor effects of the new environment. At the beginning of the test, each rat was placed singly onto the central area of the maze facing a closed arm and it was allowed to explore the plus maze freely over 5 min. Rat behavior on the elevated plus maze was video-recorded to later obtain the following parameters: number of entries into open arms, number of entries into closed arms, and time spent in the open arms. Two indices of anxiety were obtained: 1 – the number of entries into open arms expressed as a percentage of the total number of entries, and 2 – the amount of time spent in the open arms expressed as a percentage of the total time. Besides the total number of entries (entries into open arms + entries into closed arms) was used as a locomotion index (Dawson & Tricklebank, 1995). Between each session the maze was wiped with 20% ethanol to remove olfactory cues.

Immunohistochemical analyses

Immunohistochemical procedure

At PND 75, rats (300–350 g body weight) were perfuse-fixed between 09:00 h and 12:00 h. The rats were deeply anesthetized with 6% chloral hydrate (9 ml/kg i.p.) and transcardially perfused with heparinized 0.9% saline followed by 4% paraformaldehyde, alcoholic 0.2% picric acid saturated solution in 0.1 M phosphate buffer (PB); brains were removed, post-fixed in the perfusion solution for 24 h at 4 °C and then stored in 20% sucrose-PB solution at 4 °C. Brains were sectioned using a freezing microtome into coronal slices of 40 μm thickness containing the areas of interest, according to a stereotaxic atlas of the rat brain (Paxinos & Watson, 2007). Slices were subdivided into two sets, for subsequent determination of Fos and GR immunoreactivity by the free-floating method. The time line of experimental procedures is schematized in Figure 1.

After blocking endogenous peroxidase (10% methanol and 10% H_2O_2 in PB; for 1 h) and blocking nonspecific binding to serum constituents (10% normal horse serum in PB; for 1 h), slices were incubated over 48 h at 4 °C with the respective primary antibody (rabbit anti-Fos, Ab-5 Oncogene Science, Cambridge, MA, 1:10,000 or rabbit anti-GR, E-20 sc-1003 Santa Cruz Biotechnology, Inc., Dallas, TX, 1:250). Following this, the slices were incubated for 2 h at room temperature in the biotinylated secondary antibody (goat anti-rabbit; Jackson ImmunoResearch, Inc., West Grove, PA, 1:1000), then with avidin-biotin-peroxidase complex (ABC Elite kit; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. Immunoreactivity was detected using 3,3′-diaminobenzidine (DAB; Sigma-Aldrich, Corp., St. Louis, MO) as chromogen. Finally, free-floating sections were mounted on gelatinized glass slides using Albrecht gelatin, air-dried overnight, dehydrated in xylene, and cover-slipped with DPX mounting medium (Fluka, Buchs, Switzerland). Samples in which primary antibodies were excluded were processed in parallel and used as negative controls (not displayed).

Cell counting

Slices were examined under a light microscope (BX 41 Olympus). Magnification was $100 \times$ ($10 \times$ objective; $10 \times$ ocular).

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

Figure 1. Experimental procedures. Schematic representation showing the time line of experimental procedures of the study. PND, postnatal day; day of birth = day 0.

Images were captured using a high-resolution digital camera (Olympus Corporation, Tokyo, Japan). The numbers of Fos and of GR immunoreactive neurons were counted in the parvocellular portion of the paraventricular hypothalamic nucleus (PVN) (bregma: −1.08 to −1.56 mm), dorsal hippocampus (CA1–CA3 and dentate gyrus, bregma: −2.76 to −3.60 mm), and central (CeA) and medial (MeA) nuclei of amygdala (bregma: −2.16 to −2.76 mm). Between 4 and 7 sections per rat were analyzed depending on the size of the brain area. The number of positive cells was counted using a semi-automatized method with Fiji software (National Institutes of Health, Bethesda, MD; http://fiji.sc/Fiji). Investigators were blinded to the experimental grouping while taking photomicrographs and performing the image analysis. Once the number of positive Fos and GR nuclei was determined in each slice, the relative density of the population of cells immunopositive for each protein was calculated by dividing this number by the area measured in each case.

Statistical analysis

For the plus maze test, comparisons among experimental groups were made by using a three-way analysis of variance (ANOVA), considering *separation* (AFR × MS), *stress* (non-stressed × chronic stressed), and *drug* (vehicle × tianeptine) as independent variables. When variables did not meet the requirements of normality and homogeneity of variance, data were transformed to ranks.

Immunocytochemical data were evaluated using a linear mixed effect model with three fixed factors (*separation*, *stress*, and *drug*) and a random factor (*slices*).

Data are presented as mean ± standard error of the mean (SEM). Fisher's least significant difference (LSD) post hoc test was performed for further examination of group differences, in the case of significance for factors or interactions between factors from the ANOVA. In all comparisons, p values of less than 0.05 were considered to indicate statistical significance. All analyses were conducted by using Infostat software (Universidad Nacional de Córdoba, Córdoba, Argentina; www.infostat.com.ar).

Results

Anxiety-like behavior

The percentage of time spent in the open arms (%TOA) and the percentage of entries into open arms (%EOA) was obtained in the elevated plus maze test to assess the anxiety-like behavior of rats. An increase in these measures was taken to indicate a lower level of anxiety. For %TOA (Figure 2A), a significant interaction was found for *separation* × *drug* [$F_{(1,95)} = 4.29$, $p = 0.041$]. The post hoc analysis showed that MS, tianeptine-treated rats spent more time in the open arms than the corresponding vehicle-treated rats ($p < 0.05$), indicating an anxiolytic effect of tianeptine in MS rats. There was also a significant main effect of *stress* [$F_{(1,95)} = 6.85$, $p = 0.010$]. An anxiogenic effect of stress was found, since stressed rats spent less time exploring the open arms ($p < 0.05$). Analysis of the %EOA (Figure 2B) showed a main effect for every factor: *separation* [$F_{(1,95)} = 3.94$, $p = 0.050$], *stress* [$F_{(1,95)} = 5.48$, $p = 0.021$], and *drug* [$F_{(1,95)} = 4.25$, $p = 0.042$]. The LSD test revealed that, while both MS and stress decreased entries into open arms, tianeptine treatment increased open arm entries ($p < 0.05$). Lastly, there were no significant differences in the number of total entries, indicating no changes in locomotor activity among the different experimental groups (data not shown).

Fos immunoreactivity

Dorsal hippocampus

For Fos immunoreactivity (Fos-ir), there was a significant main effect of *separation* [$F_{(1,25)} = 5.18$, $p = 0.032$] and *stress* [$F_{(1,25)} = 4.36$, $p = 0.047$] in the CA1 subfield (Figure 3A). Post hoc analysis revealed that MS increased Fos-ir, while chronic stress decreased it ($p < 0.05$). Furthermore, in the CA2 subfield (Figure 3B), ANOVA showed a significant main effect of *stress* [$F_{(1,25)} = 4.53$, $p = 0.043$]. As in the CA1 subfield, stress decreased Fos-ir in CA2 ($p < 0.05$). In the CA3 subfield (Figure 3C) a significant interaction was observed for *separation* × *stress* [$F_{(1,25)} = 6.04$, $p = 0.021$]. In this region, stressed AFR rats showed higher Fos-ir levels than the corresponding non-stressed rats ($p < 0.05$). In the non-stressed groups, MS increased number of Fos-positive neurons ($p < 0.05$). Lastly, statistical analysis of Fos-ir in the dentate gyrus (DG) of the dorsal hippocampus revealed no significant differences among treatments (data not displayed).

Representative photomicrographs showing Fos-ir in the dorsal hippocampus are presented in Figure 4(A) and (B).

Amygdala

In the MeA (Figure 3D and 4C and D), there was a significant main effect of *separation* [$F_{(1,26)} = 5.20$, $p = 0.031$]. The post hoc analysis revealed that MS increased Fos-ir in the MeA ($p < 0.05$). In contrast, there were no significant differences for Fos-ir in the CeA (results not displayed).

Paraventricular nucleus

Regarding Fos-ir in the PVN (Figures 3E and 4E and F), there was a significant main effect of *separation* [$F_{(1,26)} = 7.15$,

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

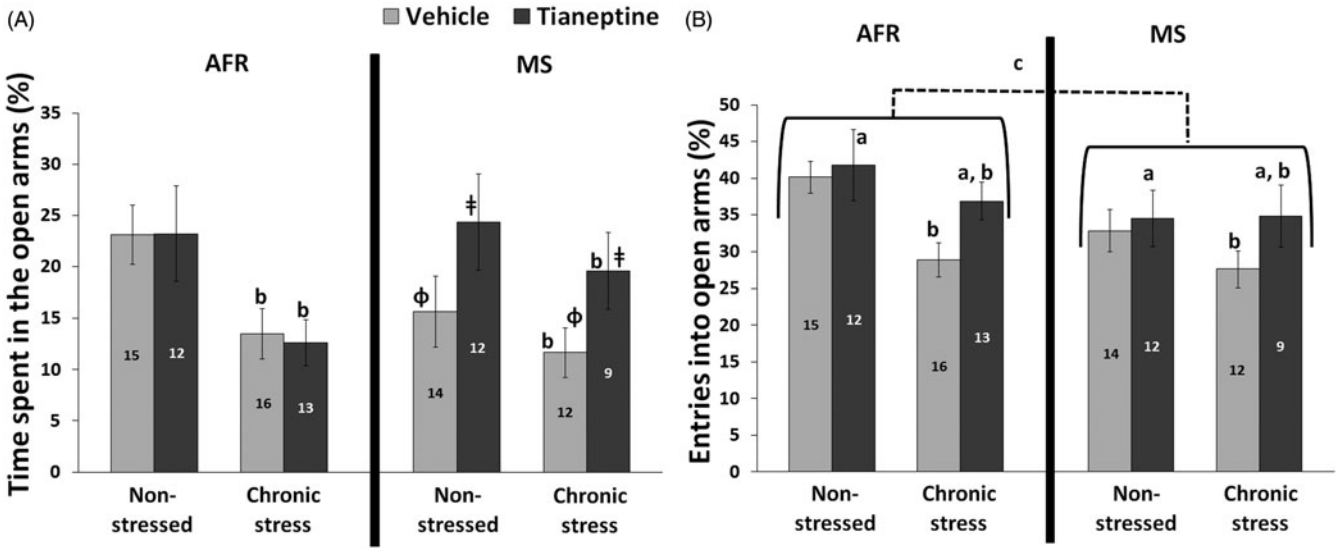

Figure 2. Performance on the elevated plus maze. (A) Percentage of time spent in the open arms (%TOA); (B) percentage of entries into the open arms (%EOA) during a 5-min exposure to the plus maze test. AFR, standard animal facility reared; MS, maternally separated rats. The rats were submitted to chronic stress or notstressed under tianeptine (dark gray bars) or vehicle (light gray bars) treatment. Values are mean ± SEM. The number of rats per group is indicated in each bar. Data were analyzed by a three-way ANOVA followed by Fisher's LSD post hoc test. For %TOA, there was a significant *separation* × *drug* interaction; and a significant *stress* main effect. For %EOA, there were significant main effects for each treatment. Letters indicate significant differences for main factors ($p < 0.05$): (a) *drug* treatment (the four tianeptine groups vs. the four vehicle groups); (b) *stress* treatment (the four chronic stress groups vs. the four non-stressed groups); and (c) *separation* treatment (the four MS groups vs. the four AFR groups). Symbols indicate significant interaction effects: The groups indicated with ''‡'' are significantly different from groups indicated with ''Φ'' ($p < 0.05$).

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

$p = 0.013$] and *stress* [$F_{(1,26)} = 4.48$, $p = 0.044$]. In the PVN both MS and stress increased the number of Fos-positive neurons ($p < 0.05$).

GR immunoreactivity

Dorsal hippocampus

There was a significant interaction for *separation* × *stress* in GR immunoreactivity in the CA1 [$F_{(1,27)} = 7.82$, $p = 0.009$], CA2 [$F_{(1,27)} = 17.87$, $p = 0.0002$], CA3 [$F_{(1,27)} = 9.20$, $p = 0.005$] and the DG [$F_{(1,27)} = 4.81$, $p = 0.04$]. In all subfields (Figure 5A–D), the post hoc analysis in AFR rats revealed that stress increased glucocorticoid receptor (GR-ir) ($p < 0.05$), while this effect was not observed in MS rats. A significant main effect of *drug* was also found in all subfields [$F_{(1,27)} = 15.74$, $p = 0.0005$ for CA1; $F_{(1,27)} = 10.62$, $p = 0.003$ for CA2; $F_{(1,27)} = 19.80$, $p = 0.0001$ for CA3; and $F_{(1,27)} = 9.78$, $p = 0.004$ for DG], with tianeptine-treated rats showing more stained nuclei than vehicle-treated rats ($p < 0.05$). Furthermore, in the CA2 subfield (Figure 5B) of non-stressed rats, MS increased number of GR-positive cells, which was not further increased in stressed MS rats ($p < 0.05$). In this subfield, there was a significant main effect of *stress* [$F_{(1,27)} = 5.09$, $p = 0.03$], which increased GR-ir in the AFR group ($p < 0.05$). In the CA3 subfield (Figure 5C) of non-stressed rats, MS increased number of GR-positive neurons. The interaction for *separation* × *drug* [$F_{(1,27)} = 4.31$, $p = 0.048$] was also significant in this region. The LSD post hoc test revealed that MS, tianeptine-treated rats had higher GR-ir levels than the respective AFR groups, and also than the corresponding vehicle-treated groups ($p < 0.05$). Representative photomicrographs showing GR-ir in the dorsal hippocampus are presented in Figure 6(A) and (B).

Amygdala

In the MeA, a significant interaction was found for *separation* × *stress* × *drug* [$F_{(1,24)} = 5.94$, $p = 0.023$]. Figure 5(E) shows that the AFR+ chronic stress/vehicle and MS + chronic stress/tianeptine groups both had more GR-ir neurons than the respective non-stressed group ($p < 0.05$). Conversely, the MS + chronic stress/vehicle group had a lower density of GR-positive cells than the corresponding non-stressed group ($p < 0.05$). Furthermore, MS increased GR-ir in non-stressed, vehicle-treated rats, but decreased it in the non-stressed, tianeptine-treated and stressed, vehicle-treated groups ($p < 0.05$). In the AFR, non-stressed group and in the MS, stressed group, tianeptine administration increased number of stained nuclei. However, in the MS, non-stressed group, tianeptine decreased GR-ir. Representative photomicrographs of GR expression in the MeA are shown in Figure 6(C) and (D). There were no significant differences for GR-ir in CeA (results not shown).

Paraventricular nucleus

Statistical analysis of GR-ir in the hypothalamic PVN revealed no significant differences between treatments. These results are not displayed.

Discussion

To analyze whether MS produces a modified phenotype regarding stress response regulation in the adult rat, we determined GR and Fos-ir in key structures involved in controlling the stress response. We also evaluated anxiety indices. Furthermore, we analyzed whether chronic treatment with the antidepressant tianeptine prevents or reverses the possible neuroendocrine and behavioral changes

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

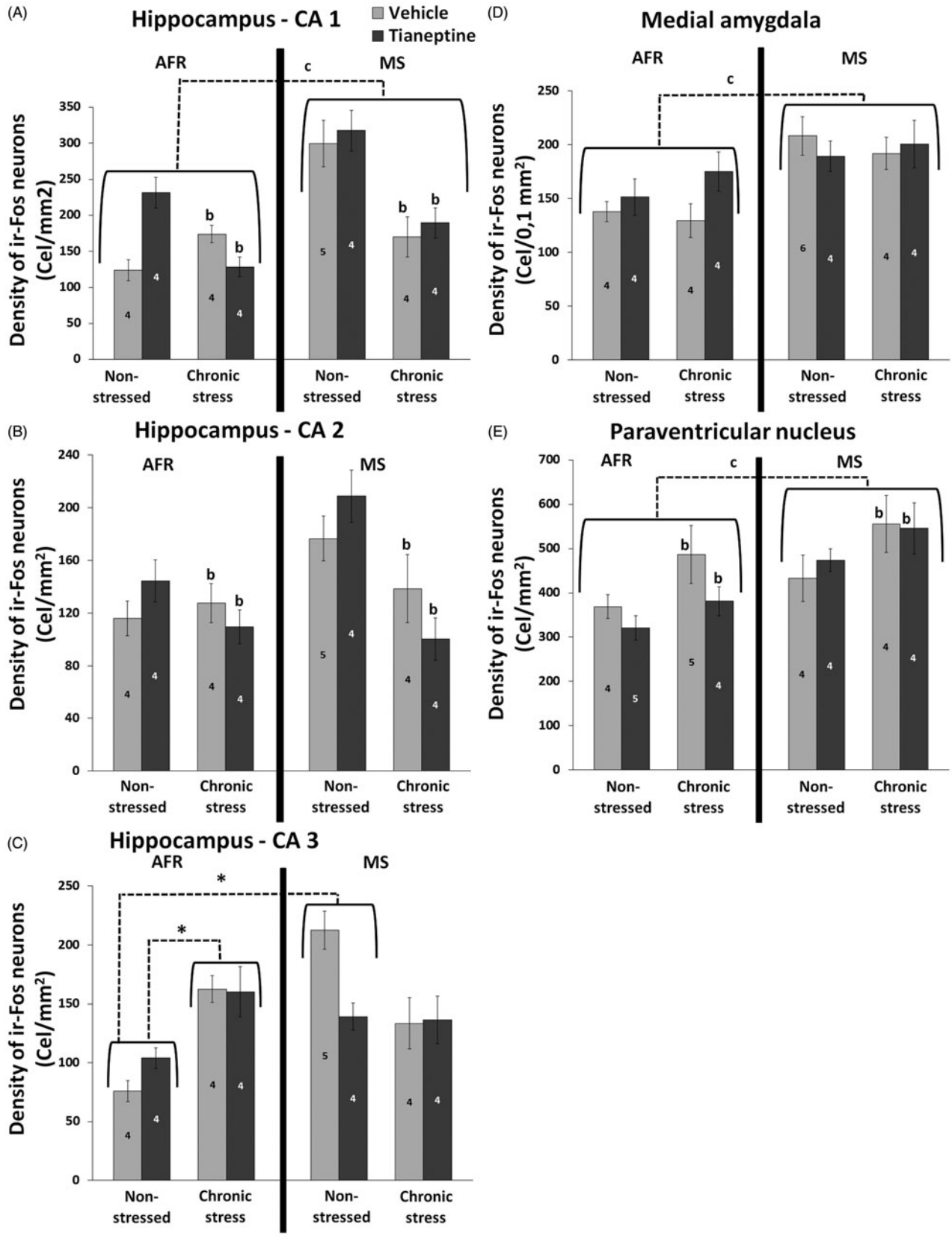

Figure 3. Fos immunoreactive neurons. Mean density of Fos positive neurons in the (A) CA1, (B) CA2, (C) CA3 subfield of dorsal hippocampus, and in the (D) medial amygdaloid nucleus (MeA) and (E) paraventricular nucleus (PVN). Values are mean ± SEM of standard animal facility reared (AFR) and maternally separated (MS) rats submitted to chronic stress or non-stressed under tianeptine (dark gray bars) or vehicle (light gray bars) treatment. The number of rats per group is included inside each bar. Data were analyzed by a linear mixed effect model followed by Fisher's LSD post hoc test. In CA1, there was a significant main effect of *separation* and also of *stress*. In CA2, there was a significant *stress* main effect. In CA3, there was a significant *separation* × *stress* interaction. In MeA there was a significant *separation* main effect. In PVN there was a significant main effect of *separation* and also of *stress*. Letters indicate significant differences for main factors ($p < 0.05$): (b) *stress* treatment (the four chronic stress groups vs. the four non-stressed groups); and (c) *separation* treatment (the four MS groups vs. the four AFR groups). (*) Denotes differences between groups indicated with the dotted line ($p < 0.05$).

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

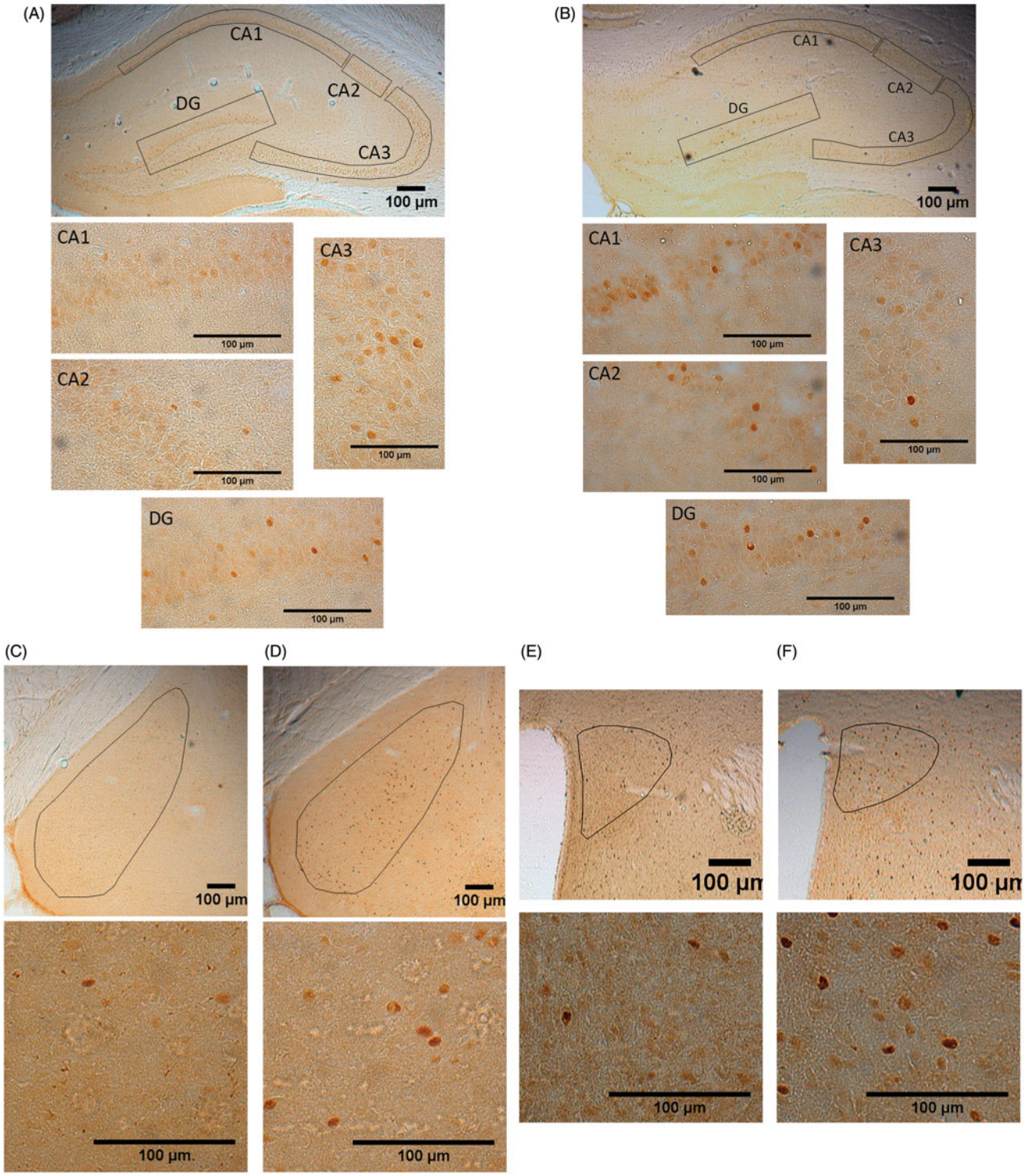

Figure 4. Representative photomicrographs showing the pattern of Fos-immunoreactivity. Fos detected immunohistochemically in coronal sections is evident as dark nuclei of neurons. (A) hippocampus from AFR + chronic stress/vehicle group; (B) hippocampus from MS-control group; (C) medial amygdala from AFR + chronic stress/vehicle group; (D) medial amygdala from MS-control group; (E) paraventricular nucleus from AFR-control group; and (F) paraventricular nucleus from MS + chronic stress/vehicle group. AFR, standard animal facility reared; MS, maternally separated. Scale bars: 100 µm.

caused by chronic stress and MS. According to the well-documented influence of early experience on the functioning of specific circuits that underlie adult stress responses, we expected to observe a different phenotype in each experimental condition. As some antidepressants can correct neuroendocrine features, we also expected that tianeptine would reverse or attenuate the alterations resulting from MS and/or the chronic stress model. Our results showed that MS increased Fos-ir in the brain regions examined, and it also increased anxiety-like behavior. Chronic stress decreased Fos-ir in the hippocampal CA1 and CA2 sublayers, but increased Fos-ir in the PVN. Chronic stress

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

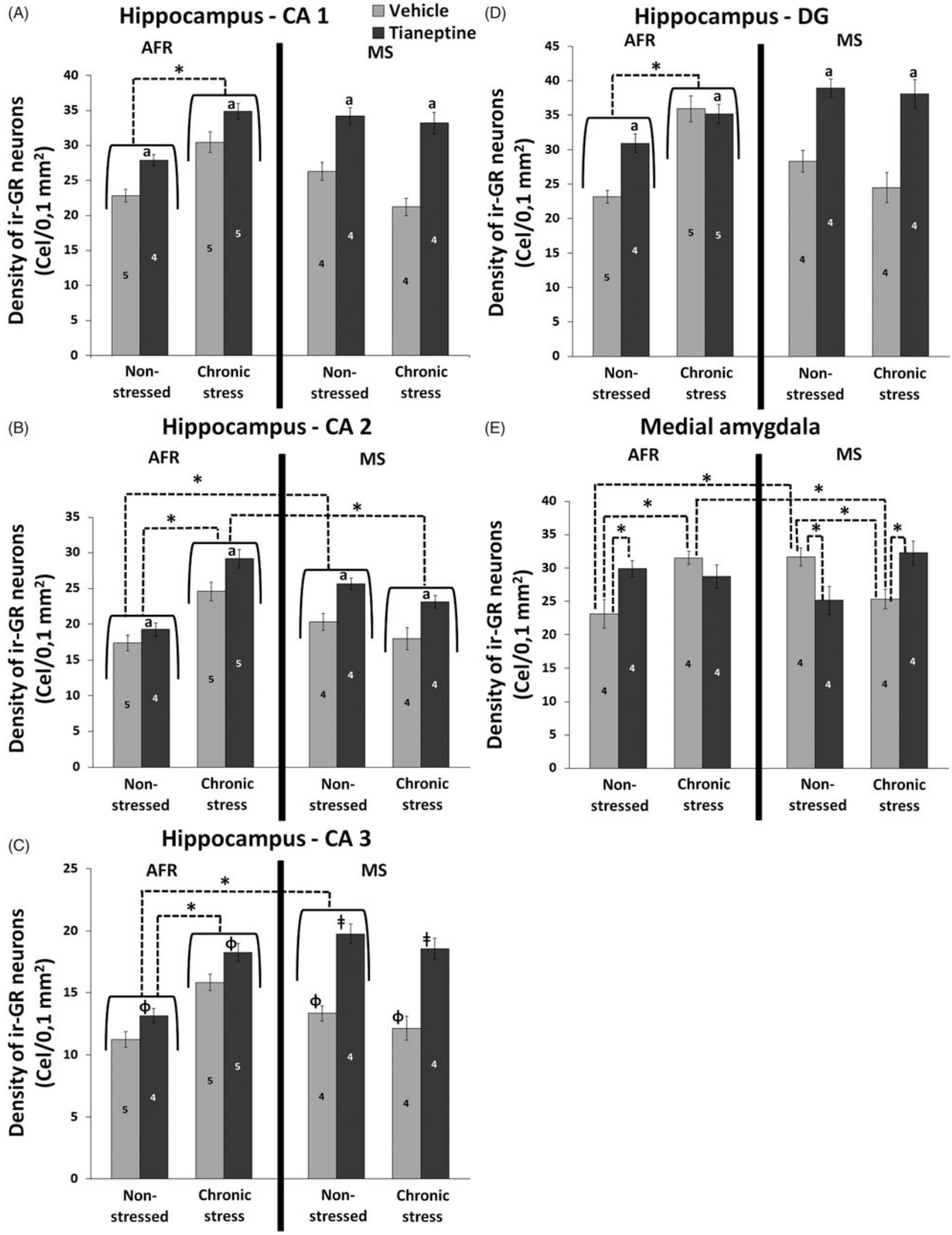

Figure 5. Glucocorticoid receptor (GR) immunoreactive neurons. Mean density of GR immunoreactive neurons in (A) CA1, (B) CA2, (C) CA3 subfield, (D) DG (dentate gyrus) of dorsal hippocampus; (E) medial amygdaloid nucleus (MeA). Values are mean ± SEM of standard animal facility reared (AFR) and maternally separated (MS) rats submitted to chronic stress or non-stressed under tianeptine (dark gray bars) or vehicle (light gray bars) treatment. The number of rats per group is included inside each bar. Data were analyzed by a linear mixed effect model followed by Fisher's LSD post hoc test. In all hippocampal sublayers there was a significant *separation* × *stress* interaction. In CA3, there was a significant *separation* × *drug* interaction. In CA1, CA2, and DG there was a significant *drug* main effect. In MeA there was a significant *separation* × *stress* × *drug* interaction. (a) Indicates significant differences of *drug* main factor (the four tianeptine groups vs. the four vehicle groups) ($p < 0.05$). Symbols indicate significant interaction effects: the groups indicated with ''‡'' are significantly different from groups indicated with ''Φ'' ($p < 0.05$). (*) Denotes differences between groups indicated with the dotted line ($p < 0.05$).

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

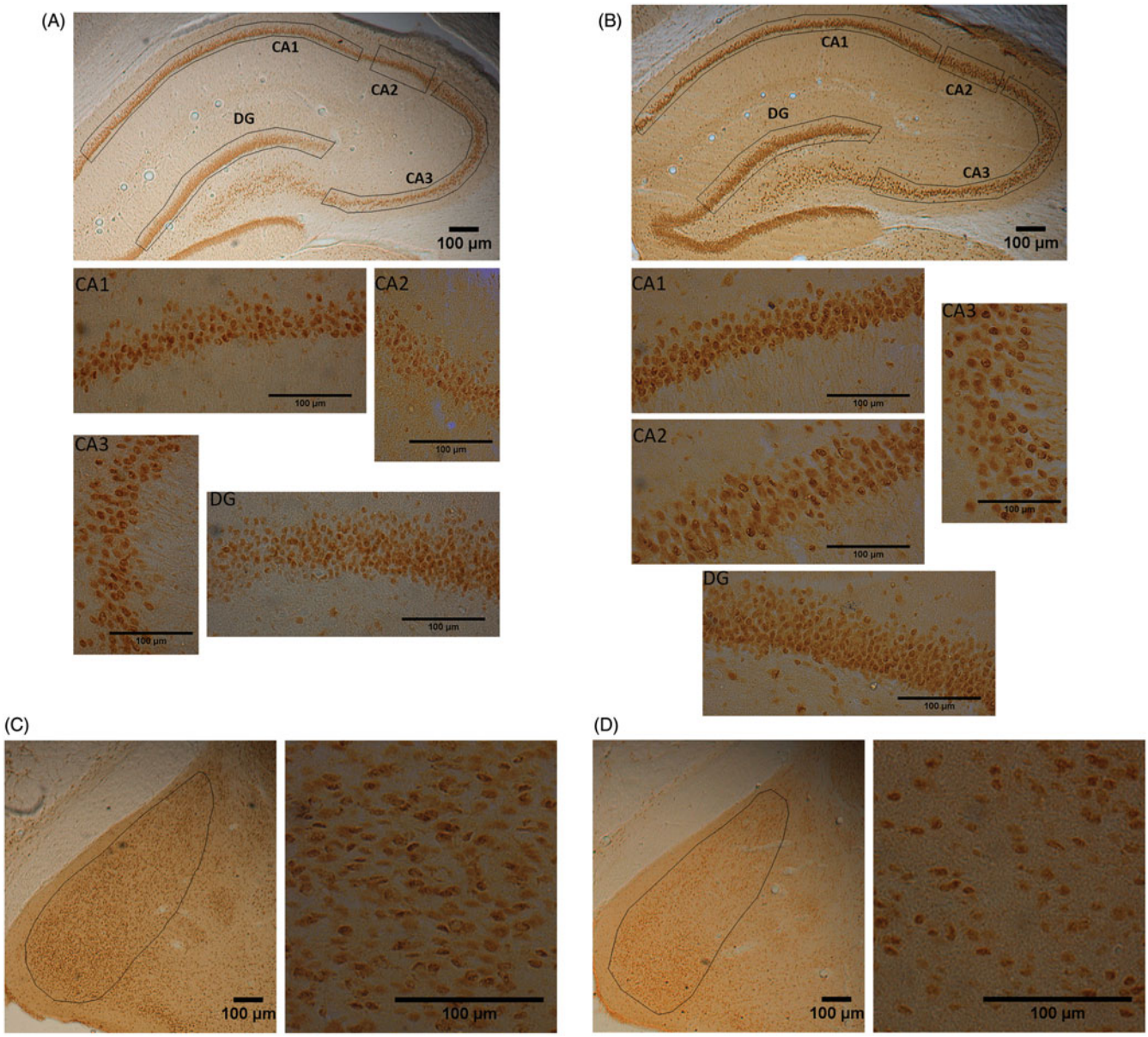

Figure 6. Representative photomicrographs showing the pattern of GR-immunoreactivity. (A) hippocampus from standard animal facility reared (AFR)-control group; (B) hippocampus from maternally separated (MS) + chronic stress/tianeptine group; (C) medial amygdala from MS-control group; and (D) medial amygdala from MS + tianeptine group. GR detected immunohistochemically in coronal sections is evident as dark nuclei of neurons. Scale bars: 100 µm.

also increased anxiety-like behavior. Tianeptine reversed the increased anxiety produced by chronic stress and MS treatments, but it had no significant effects on Fos-ir. However, the changes in GR immunoreactivity were highly dependent on the interaction of treatments. Thus, chronic stress increased GR-ir in the dorsal hippocampus and medial amygdala in AFR rats, but not in the MS groups. In the non-stressed groups, MS increased number of GR-ir nuclei in the hippocampal CA2 and CA3 areas and in medial amygdala. By contrast, in the stressed groups, MS decreased GR-ir in those structures. Tianeptine effects on GR in the amygdala also were context-dependent. Hence, tianeptine increased GR-ir in both the AFR non-stressed and MS + chronic stress groups, but decreased it in the MS non-stressed rats. Overall, our results support the view that early life stress induces long-term changes in phenotype, which in turn may underlie altered stress response regulation in adults, depending on the environment.

Anxiety-like behavior

We found an anxiogenic effect of chronic stress, since it decreased the %TOA and the %EOA (Figure 2). This confirms previous work from our laboratory (Cotella et al., 2009). In addition, acute stress was also shown to increase anxiety in rats (Han et al., 2014). Furthermore, the MS group spent less time in the open arms of the plus maze compared to the AFR group, indicating higher anxiety. Similar results have been obtained by other researchers (Aisa et al., 2007; Doron et al., 2014; Troakes & Ingram, 2009).

In contrast, tianeptine had an anxiolytic effect, as evidenced by the increase of the %EOA in the chronic stress groups and by the increase of the %TOA in the stressed

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

MS groups. Our results agree with other studies in which tianeptine showed an anxiolytic effect in rats exposed to acute psychosocial stress (Venzala et al., 2012; Zoladz et al., 2013), prenatal stress (Szymanska et al., 2009), and chronic immobilization stress (Pillai et al., 2012). Finally, there was no difference in the total number of entries among any of the experimental groups (results not displayed), indicating that none of the applied treatments produced changes in rat locomotion.

Fos immunoreactivity

Dorsal hippocampus

The chronic stress protocol used in this study had the overall effect of reducing Fos expression in the CA1 and CA2 sublayers of the dorsal hippocampus (Figure 3A and B), indicating a lower neuronal activity in this inhibitory structure of the HPA axis. Others recently have found similar effects (i.e. Fos expression in hippocampus was lower in the chronic stress group than in non-stressed controls) (Yazir et al., 2015).

However, in the CA1 sublayer, MS had a general effect increasing Fos activity as assessed by Fos immunocytochemistry (Figure 3A), while in the CA3 separation increased Fos only in non-stressed rats (Figure 3C). This effect would have an inhibitory consequence in the HPA axis. Moreover, other studies have found that prenatal stress resulted in increased Fos-ir in the hippocampus, but also increased neuronal activity in the locus coeruleus, a noradrenergic nucleus activator of the HPA axis (Viltart et al., 2006).

Amygdala

In the central amygdala, chronic stress did not produce significant changes in the number of GR or Fos-stained nuclei (results not displayed). It has been suggested that the MeA has a key role in HPA axis responses to emotional stress (i.e. stimuli indicating a possible threat but not implying an immediate challenge to survival), while the CeA would be less affected by this type of stress (Dayas et al., 1999). Instead, the CeA may have a greater influence on physiological stress, such as cardiovascular changes (Li & Dampney, 1994) or immunological processes (Ericsson et al., 1994). The moderate stressors used in the present work could explain a larger contribution of the MeA to stress-induced HPA axis activation.

However, MS increased neuronal activity in the MeA as assessed by Fos immunocytochemistry (Figure 3D) both in basal conditions (non-stressed groups) and in a stressful environment (stressed groups). Conversely, in the CeA, MS had no effect on Fos-ir (data not shown). This is consistent with another study in which MS of Sprague Dawley offspring produced no increase in activity in this nucleus (Felice et al., 2014).

Paraventricular nucleus

Our results showed that both MS and chronic stress increase neuronal activity in the hypothalamic PVN (Figure 3E), suggesting greater activity of the HPA axis. Moreover, PVN activity does not depend on the interplay between these two treatments. A similar increase of PVN activity was found in other research following the application of acute restraint stress (Ma & Morilak, 2004) and following early MS in borderline hypertensive rats (Sanders & Anticevic, 2007).

GR immunoreactivity

Dorsal hippocampus

In all hippocampal sublayers, stress increased number of GR immunoreactive cells in the AFR but not in maternally separated rats (Figure 5A–D), indicating an effect of stress that is dependent on the early experience of rats. Other authors have found that chronic stress induced by water immersion and restraint increased nuclear GR but decreased the cytosolic fraction, suggesting greater receptor translocation to the nucleus (Mizoguchi et al., 2003). Similarly, acute stress exposure produced an increase in nuclear GR (Caudal et al., 2014). Taking into account that previous studies from our laboratory showed the chronic stress paradigm increases plasma corticosterone concentration (Suárez et al., 1996, 1999), the issues discussed above lead us to consider that the stress-induced increase of GR-ir in hippocampus, found here may be due to an increase in translocation of these receptors to the nucleus after binding to corticosterone. However, this proposal needs to be interpreted with caution as we used a semiquantitative technique that determined the number of positive neurons for the receptor but not the overall amount of receptor in a region.

Although we cannot entirely discard the possibility that the stress-induced increase in number of GR-immunoreactive neurons found in our study was due to a difference in the total number of cells we consider this possibility unlikely, since stress has been associated with a reduction in cell proliferation and with hippocampal volume loss (Czéh et al., 2001).

Moreover, MS increased GR-ir in the hippocampal CA2 and CA3 sublayers of non-stressed rats (Figure 5B and C), thus MS would promote negative feedback of the HPA axis when the stress level is low in adulthood. In contrast, in stressed rats MS decreased GR-ir in the CA2 (Figure 5B). In this case in separated and stressed rats the modulation of the HPA axis would be malfunctioning as a result of fewer hippocampal GR receptors. Conversely, in previous studies MS had no effect on hippocampal GR-ir (Ladd et al., 2005; Renard et al., 2010). This difference could be caused by the tianeptine administration in the present study, which was seen to affect hippocampal GR-ir.

Concerning pharmacologic treatment, the overall effect of tianeptine administration was to increase the level of GR-ir in the dorsal hippocampus (Figure 5A–D). This would contribute to shutting off the stress response, by increasing negative feedback exerted by corticosterone on the hippocampus, and thus the effect of tianeptine could attenuate HPA axis activity. Conversely, tianeptine treatment normalized the increase in hippocampal GR caused by prenatal stress (Szymanska et al., 2009).

Amygdala

In the medial amygdala, GR-ir levels were found to depend on the interaction between MS, chronic stress and tianeptine treatment. Figure 5(E) shows that in vehicle-treated groups

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

the stress protocol produced an increase of GR-ir cells in AFR rats, but a decrease in MS rats. In contrast, Han et al. (2014) observed no changes of MR-ir, GR-ir or MR/GR balance in the amygdala in rats following exposure to acute stress (Han et al., 2014); however, the subdivisions within the amygdala were not distinguished in that study. It is well known that the amygdala is not a homogeneous structure and even belongs to different functional systems (Swanson & Petrovich, 1998), thus an overall analysis of this structure may lead to changes in a particular nucleus being masked.

Additionally, in vehicle-treated groups, both stress (in AFR rats) and MS (in non-stressed rats) increased GR-ir in the MeA, which is expected to facilitate positive feedback exerted by glucocorticoid in this structure (de Kloet, 2013). However, the combination of both treatments (MS + chronic stress) decreased levels of GR-ir to values in control rats (AFR + non-stressed) (Figure 5E). This indicates that the final phenotype of the individual depends on the interaction of early life experiences and the adult environment.

Concerning tianeptine treatment, we found that while MS increased GR-ir in the MeA in non-stressed rats, tianeptine administration decreased it. Conversely, in stressed rats, MS decreased GR-ir, while tianeptine increased it (Figure 5E). These different effects of this drug demonstrate that tianeptine action on GR regulation in the amygdala is dependent on the state of the rat and that this antidepressant opposes the effects of MS on amygdala GR levels.

Paraventricular nucleus

We did not find significant differences in the number of GR-positive neurons after exposure to chronic stress or MS (data not shown). The present study confirm our previous results in which chronic stress did not alter the number of GR-ir neurons in the medial parvocellular portion of the paraventricular hypothalamic nucleus (Renard et al., 2010). Similarly, Zavala et al. (2011) demonstrated that repeated restraint stress produced no effects on the number of GR/Fos doubly stained neurons, but acute restraint stress did increase these (Zavala et al., 2011). Therefore, GR in this nucleus may be more affected by acute stress that by chronic stress. Tianeptine treatment had no effect on GR immunoreactivity in the PVN.

Conclusions

Our results support the idea that disturbance of maternal care generates a particular phenotype in the pups, which produces alterations in the regulation of stress responses in adulthood. In our study, this altered phenotype was evidenced by the pattern of GR-ir observed in the rats subjected to both stress protocols (in early and adult life) compared with rats subjected to only one of the two stress protocols. This is consistent with the cumulative stress hypothesis (McEwen & Wingfield, 2003), which postulates that the effects of stress are cumulative and hence harmful experiences in early life predispose the individual to be more vulnerable to adverse challenges in adulthood, with increased vulnerability for the development of psychiatric disorders. We also found similar differential responses to tianeptine between the groups; moreover, tianeptine affected expression of GR in limbic structures related to HPA axis regulation in a region-specific manner. However, contrary to expectations this drug did not affect Fos activity of the structures analyzed. In addition, chronic stress as well as MS increased anxiety-like behavior, which was corrected by chronic administration of tianeptine. Future studies in this model of MS and chronic stress should consider assessing additional parameters that allow quantification of the corticosteroid receptors in limbic regions such as mRNA measures by RT-PCR or protein levels by western blotting analyses.

Acknowledgements

We are grateful to Servier Laboratories S.A. for kindly providing tianeptine sodium salt.

Declaration of interest

This research was supported by the National University of Córdoba (SECyT Grants 114/10 and 162/12) and the National Council of Scientific and Technical Research, Argentina (PIP CONICET 2013-2015 GI 0597). Doctoral Fellowship CONICET PhD College of Biological Sciences, Faculty of Exact, Physical and Natural Sciences, National University of Córdoba, Argentina. The authors report no conflicts of interest and alone are responsible for the content and writing of this article and the funding sources had no influence in the content of this article.

References

Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology 32:256–66.

Anisman H, Zaharia MD, Meaney MJ, Merali Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 16:149–64.

Atmaca M, Kuloglu M, Tezcan E, Buyukbayram A. (2003). Switching to tianeptine in patients with antidepressant-induced sexual dysfunction. Hum Psychopharmacol 18:277–80.

Castanon N, Konsman JP, Medina C, Chauvet N, Dantzer R. (2003). Chronic treatment with the antidepressant tianeptine attenuates lipopolysaccharide-induced Fos expression in the rat paraventricular nucleus and HPA axis activation. Psychoneuroendocrinology 28: 19–34.

Caudal D, Jay TM, Godsil BP. (2014). Behavioral stress induces regionally-distinct shifts of brain mineralocorticoid and glucocorticoid receptor levels. Front Behav Neurosci 8:19.

Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL. (2011). Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. Psychopharmacology (Berl) 214:141–54.

Cotella EM, Lascano IM, Levin GM, Suarez MM. (2009). Amitriptyline treatment under chronic stress conditions: effect on circulating catecholamines and anxiety in early maternally separated rats. Int J Neurosci 119:664–80.

Czéh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, Bartolomucci A, Fuchs E. (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci USA 98:12796–801.

Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. (2013). The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38:1858–73.

Dawson GR, Tricklebank MD. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol Sci 16:33–6.

Dayas CV, Buller KM, Day TA. (1999). Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur J Neurosci 11:2312–22.

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015

de Kloet ER. (2013). Functional profile of the binary brain corticosteroid receptor system: mediating, multitasking, coordinating, integrating. Eur J Pharmacol 719:53–62.

Delbende C, Contesse V, Mocaer E, Kamoun A, Vaudry H. (1991). The novel antidepressant, tianeptine, reduces stress-evoked stimulation of the hypothalamo-pituitary-adrenal axis. Eur J Pharmacol 202:391–6.

Doron R, Lotan D, Versano Z, Benatav L, Franko M, Armoza S, Kately N, Rehavi M. (2014). Escitalopram or novel herbal mixture treatments during or following exposure to stress reduce anxiety-like behavior through corticosterone and BDNF modifications. PLoS One 9:e91455.

Ericsson A, Kovacs KJ, Sawchenko PE. (1994). A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J Neurosci 14:897–913.

Felice VD, Gibney SM, Gosselin RD, Dinan TG, O'Mahony SM, Cryan JF. (2014). Differential activation of the prefrontal cortex and amygdala following psychological stress and colorectal distension in the maternally separated rat. Neuroscience 267:252–62.

Han F, Ding J, Shi Y. (2014). Expression of amygdala mineralocorticoid receptor and glucocorticoid receptor in the single-prolonged stress rats. BMC Neurosci 15:77.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog Neuropsychopharmacol Biol Psychiatry 29:1201–13.

Heuser I, Lammers CH. (2003). Stress and the brain. Neurobiol Aging 24:S69–76. discussion S81-62.

Kasper S, Olie JP. (2002). A meta-analysis of randomized controlled trials of tianeptine versus SSRI in the short-term treatment of depression. Eur Psychiatry 17:331–40.

Kendler KS, Karkowski LM, Prescott CA. (1999). Causal relationship between stressful life events and the onset of major depression. Am J Psychiatry 156:837–41.

Kendler KS, Thornton LM, Gardner CO. (2000). Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the ''kindling'' hypothesis. Am J Psychiatry 157: 1243–51.

Kessler RC. (1997). The effects of stressful life events on depression. Annu Rev Psychol 48:191–214.

Kim SJ, Park SH, Choi SH, Moon BH, Lee KJ, Kang SW, Lee MS, et al. (2006). Effects of repeated tianeptine treatment on CRF mRNA expression in non-stressed and chronic mild stress-exposed rats. Neuropharmacology 50:824–33.

Ladd CO, Thrivikraman KV, Huot RL, Plotsky PM. (2005). Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. Psychoneuroendocrinology 30:520–33.

Lepine JP, Altamura C, Ansseau M, Gutierrez JL, Bitter I, Lader M, Waintraub L. (2001). Tianeptine and paroxetine in major depressive disorder, with a special focus on the anxious component in depression: an international, 6-week double-blind study dagger. Hum Psychopharmacol 16:219–27.

Levine S. (2001). Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. Physiol Behav 73:255–60.

Li YW, Dampney RA. (1994). Expression of Fos-like protein in brain following sustained hypertension and hypotension in conscious rabbits. Neuroscience 61:613–34.

Ma S, Morilak DA. (2004). Induction of FOS expression by acute immobilization stress is reduced in locus coeruleus and medial amygdala of Wistar-Kyoto rats compared to Sprague-Dawley rats. Neuroscience 124:963–72.

McEwen BS, Chattarji S, Diamond DM, Jay TM, Reagan LP, Svenningsson P, Fuchs E. (2010). The neurobiological properties of tianeptine (Stablon): from monoamine hypothesis to glutamatergic modulation. Mol Psychiatry 15:237–49.

McEwen BS, Wingfield JC. (2003). The concept of allostasis in biology and biomedicine. Horm Behav 43:2–15.

Mizoguchi K, Ishige A, Aburada M, Tabira T. (2003). Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. Neuroscience 119:887–97.

National Institutes of Health (1996). Guide for the Care and Use of Laboratory Animals. 1st ed. Washington, D.C.: National Academy Press.

Novotny V, Faltus F. (2002). Tianeptine and fluoxetine in major depression: a 6-week randomised double-blind study. Hum Psychopharmacol 17:299–303.

Ogawa T, Mikuni M, Kuroda Y, Muneoka K, Mori KJ, Takahashi K. (1994). Periodic maternal deprivation alters stress response in adult offspring: potentiates the negative feedback regulation of restraint stress-induced adrenocortical response and reduces the frequencies of open field-induced behaviors. Pharmacol Biochem Behav 49: 961–7.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 6th ed. San Diego, CA: Elsevier Academic Press. 2007.

Pellow S, Chopin P, File SE, Briley M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–67.

Pillai AG, Anilkumar S, Chattarji S. (2012). The same antidepressant elicits contrasting patterns of synaptic changes in the amygdala vs hippocampus. Neuropsychopharmacology 37:2702–11.

Racagni G, Popoli M. (2010). The pharmacological properties of antidepressants. Int Clin Psychopharmacol 25:117–31.

Renard GM, Rivarola MA, Suarez MM. (2010). Gender-dependent effects of early maternal separation and variable chronic stress on vasopressinergic activity and glucocorticoid receptor expression in adult rats. Dev Neurosci 32:71–80.

Reul JM, de Kloet ER. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117:2505–11.

Ridout F, Hindmarch I. (2001). Effects of tianeptine and mianserin on car driving skills. Psychopharmacology (Berl) 154:356–61.

Sanders BJ, Anticevic A. (2007). Maternal separation enhances neuronal activation and cardiovascular responses to acute stress in borderline hypertensive rats. Behav Brain Res 183:25–30.

Suárez MM, Fiol de Cuneo M, Vincenti L, Ruiz RD. (1996). Changes in corticosterone levels and sperm functional activity by chronic stress in rats. Arch Physiol Biochem 104:351–6.

Suárez MM, Molina S, Rivarola MA, Perassi NI. (2002). Effects of maternal deprivation on adrenal and behavioural responses in rats with anterodorsal thalami nuclei lesions. Life Sci 71:1125–37.

Suárez MM, Paglini P, Fernández R, Enders J, Maglianesi M, Perassi NI, Palma J. (1999). Influence of anterodorsal thalamic nuclei on the hypophyseal-adrenal axis and cardiac beta receptors in rats submitted to variable chronic stress. Acta Physiol Pharmacol Ther Latinoam 49: 71–8.

Suárez MM, Rivarola MA, Molina SM, Perassi NI, Levin GM, Cabrera R. (2001). Periodic maternal deprivation and lesion of anterodorsal thalami nuclei induce alteration on hypophyso adrenal system activity in adult rats. Life Sci 69:803–13.

Svenningsson P, Bateup H, Qi H, Takamiya K, Huganir RL, Spedding M, Roth BL, et al. (2007). Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine. Eur J Neurosci 26:3509–17.

Swanson LW, Petrovich GD. (1998). What is the amygdala? Trends Neurosci 21:323–31.

Szymanska M, Budziszewska B, Jaworska-Feil L, Basta-Kaim A, Kubera M, Leskiewicz M, Regulska M, Lason W. (2009). The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats. Psychoneuroendocrinology 34:822–32.

Taylor C, Fricker AD, Devi LA, Gomes I. (2005). Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways. Cell Signal 17:549–57.

Troakes C, Ingram CD. (2009). Anxiety behaviour of the male rat on the elevated plus maze: associated regional increase in c-fos mRNA expression and modulation by early maternal separation. Stress 12:362–9.

Trujillo V, Masseroni ML, Levin G, Suárez MM. (2009). Tianeptine influence on plasmatic catecholamine levels and anxiety index in rats under variable chronic stress after early maternal separation. Int J Neurosci 119:1210–27.

Venzala E, Garcia-Garcia AL, Elizalde N, Delagrange P, Tordera RM. (2012). Chronic social defeat stress model: behavioral features, antidepressant action, and interaction with biological risk factors. Psychopharmacology (Berl) 224:313–25.

Viltart O, Mairesse J, Darnaudery M, Louvart H, Vanbesien-Mailliot C, Catalani A, Maccari S. (2006). Prenatal stress alters Fos protein expression in hippocampus and locus coeruleus stress-related brain structures. Psychoneuroendocrinology 31:769–80.

Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, et al. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc Natl Acad Sci USA 97:325–30.

Yazir Y, Utkan T, Gacar N, Aricioglu F. (2015). Resveratrol exerts anti-inflammatory and neuroprotective effects to prevent memory deficits in rats exposed to chronic unpredictable mild stress. Physiol Behav 138:297–304.

Young EA, Abelson JL, Cameron OG. (2004). Effect of comorbid anxiety disorders on the hypothalamic-pituitary-adrenal axis response to a social stressor in major depression. Biol Psychiatry 56:113–20.

Zavala JK, Fernandez AA, Gosselink KL. (2011). Female responses to acute and repeated restraint stress differ from those in males. Physiol Behav 104:215–21.

Zoladz PR, Fleshner M, Diamond DM. (2013). Differential effectiveness of tianeptine, clonidine and amitriptyline in blocking traumatic memory expression, anxiety and hypertension in an animal model of PTSD. Prog Neuropsychopharmacol Biol Psychiatry 44:1–16.

Downloaded by [Verónica Trujillo] at 14:56 24 November 2015