Prenatal stress alters Fos protein expression in hippocampus and locus coeruleus stress-related brain structures

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\textbf{Summary}
Prenatal stress (PS) durably influences responses of rats from birth throughout life by inducing deficits of the hypothalamo-pituitary-adrenal (HPA) axis feedback. The neuronal mechanisms sustaining such alterations are still unknown. The purpose of the present study was to determine whether in PS and control rats, the exposure to a mild stressor differentially induces Fos protein in hippocampus and locus coeruleus, brain areas involved in the feedback control of the HPA axis. Moreover, Fos protein expression was also evaluated in the hypothalamic paraventricular nucleus (PVN) that reflect the magnitude of the hormonal response to stress. Basal plasma corticosterone levels were not different between the groups, while, PS rats exhibited higher number of Fos-immunoreactive neurons than controls, in the hippocampus and locus coeruleus in basal condition. A higher basal expression of a marker of GABAergic synapses, the vGAT, was also observed in the hypothalamus of PS rats. Fifteen minutes after the end of the exposure to the open arm of the elevated plus-maze (mild stress) a similar increased plasma corticosterone levels was observed in both groups in parallel with an increased number of Fos-immunoreactive neurons in the PVN. Return to basal plasma corticosterone values was delayed only in the PS rats. On the contrary, after stress, no changes in Fos-immunoreactivity were observed in the hippocampus and locus coeruleus of PS rats compared to basal.

\textbf{Abbreviations}
AP-1, transcription factor activator; AVP, arginin-vasopressin; CA3, amon horn 3; C, control; CRH, corticotropin-releasing hormone; DG, dentate gyrus; GR, glucocorticoid receptor; HPA axis, hypothalamo-pituitary-adrenal axis; MR, mineralocorticoid receptor; PaMP, PVN, medial parvocellular part; PaLM, PVN, lateral magnocellular part; PS, prenatal stress; PVN, paraventricular nucleus; vGAT, GABA vesicular transporter; vGlut1, vesicular glutamate transporter 1.

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1. Introduction

In rats, development of the central nervous system can be durably altered by deleterious perinatal environment, predisposing the organism to long-term behavioural abnormalities (for review, Chapillon et al., 2002; Maccari et al., 2003; Weinstock, 2005). Adult PS rats display an increased ‘anxiety’ (Vallée et al., 1997) and depressive-like behaviour (Morley-Fletcher et al., 2003, 2004). The HPA axis activity was associated with these behavioural alterations (Vallée et al., 1997, 1999). In fact, adult PS rats show increased stress-induced plasma concentrations of adrenocorticotropic hormone (ACTH) (Takahashi and Kalin, 1991; McCormick et al., 1995), prolonged stress-induced corticosterone secretion and decreased binding capacity of hippocampal corticosteroid receptors (Weinstock et al., 1992; Maccari et al., 1995) suggesting a reduced feedback mechanisms of HPA axis in PS rats. An increased basal corticosterone levels was observed in the evening before the light-off (Koehl et al., 1999). Efferent limbic fibres as well as ascendant brainstem inputs are known to modulate the HPA axis activity (for review Herman and Cullinan, 1997) and PS rats show reduced noradrenaline content and increased noradrenaline turnover in the hippocampus and neocortex (Takahashi et al., 1992; Muneoka et al., 1997). Moreover, PS rats exhibit alterations of the monoaminergic system and cholinergic neurones (Peters, 1986; Poland et al., 1995; Day et al., 1999; Morley-Fletcher et al., 2004). These data strengthens evidence of the existence of deficient HPA axis feedback mechanisms in PS rats. In this context, we examined whether PS induced differential expression of Fos protein in the PVN and in two structures principally involved in the feedback mechanisms of the HPA axis, the hippocampus and locus coeruleus, in both basal condition and after exposure to a mild stressor. We determined also whether PS affected catecholaminergic activated neurons located in the locus coeruleus. Finally, we evaluated the hypothalamic content in GABA and glutamate vesicular transporters that are directly correlated to GABA and glutamate concentrations (McIntire et al., 1997; Takamori et al., 2000) and reflects their involvement in the control of PVN neurons activity.

2. Methods

All experiments were conducted in accordance with the principles of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC).
2.1. Housing conditions

Adult virgin Sprague–Dawley female rats (n=10, Charles River, L’Arbresle, France) weighting 240 g were housed in groups of 5 per cage, for 2 weeks before mating, in order to coordinate their oestrus cycle. They were then housed overnight separately with a sexually experienced male (400 g). The following morning, a vaginal smear was performed on each female in order to determine their date of fecundation. Every negative female was then placed back in the common housing cage for the day and given back to the male the following night until sperm were seen in the vaginal smear. Pregnant rats were then randomly assigned to prenatal stress (PS) or control (C) groups, individually housed in plastic cages, allowed ad libitum access to food and water, and maintained on a 12 h light/dark cycle (light on at 0700 h am) with constant temperature and humidity.

2.2. Prenatal stress paradigm

The PS procedure was performed every day from day 11 of pregnancy until delivery. As previously described (Maccari et al., 1995; Morley-Fletcher et al., 2003), pregnant females were restrained for 45 min three times a day during the light phase, in a transparent plastic cylinder (7 cm in diameter and 19 cm long) exposed to a bright light. Control females were left undisturbed in their home cages. Biological mothers raised the offspring until weaning, 21 days after birth. Only litters of 8-13 pups with similar sex ratio were kept for the study (Chapman and Stern, 1979) to standardize postnatal conditions and adequate milk supply. A maximum of two male pups was used from each litter to prevent any 'litter effects'. Male rats from each experimental condition were housed in groups of five until they reached 2 months of age. They were then housed in groups of two until the experiments.

2.3. Experimental procedures

The mild stressor consisted of an exposure of adult PS and C rats to the open arm of the elevated plus maze for 5 min (Landgraf et al., 1999). Two weeks before the beginning of the experiment, rats had been manipulated every day in order to minimize any effect of handling.

Plasma corticosterone assay. Adult PS (n=27) and C (n=26) rats (4-month-old) were exposed or not to the open arm (open arm condition). They were subdivided in four groups corresponding to different times of blood samplings before and after the exposure to the mild stress of 5 min: (1) basal condition (PS rats, n=6; C rats, n=6); (2) 15 min after stress (T15; PS rats, n=7; C rats, n=6); (3) 60 min (T60; PS rats, n=7; C rats, n=7) and (4) 120 min after stress (T120; PS rats, n=7; C rats, n=7). The T120 group was used to perform the immunocytochemical study. In addition, 6 PS rats and 6 C rats were left undisturbed (basal condition). For each animal, blood samples were collected by the tail in tubes containing 5 ml EDTA (0.1% w/v). All experiments were performed between 0900 and 1200 h to avoid circadian variations of plasma corticosterone concentrations. Plasma corticosterone levels were measured with a radioimmunoassay kit (Kit ImmunChem TM, ICN Pharmaceuticals, Orsay, France) using a highly specific corticosterone antiserum. The minimum level of detection was 0.2 mg/100 ml and the intra- and interassay coefficients of variation were, respectively, 5 and 9%.

2.4. Immunohistochemical procedure

We used rats from basal condition (PS rats, n=6; C rats, n=6) and T120 group (PS rats, n=6; C rats, n=6). Since Fos protein expression reaches a maximum between 90 and 120 min after the stress (Krukoff, 1999), only the T120 after stress group was used to perform the immunocytochemical study related to the exposure to the mild stressor. After blood samplings, rats were immediately anaesthetized with sodium pentobarbital (60 mg/kg) and perfused with 200 ml saline (NaCl, 0.9%, w/v), followed by a cold solution of phosphate buffer (PB, 0.1 M, pH 7.4) containing paraformaldehyde (400 ml; 4%). Brain was removed, postfixed for two hours at 4 °C in the same fixative, then immersed overnight at 4 °C in PB containing 20% sucrose. Frontal sections (50 µm) of the brain and medulla were cut on a cryotome, rinsed in PB and processed for the immunocytochemical detection of Fos protein. Sections of each group were incubated for 48 h in PB containing 0.2% Triton X-100 and the primary Fos rabbit antiserum (Santa Cruz, USA; 1:10,000, sc-52). Then, the biotinylated donkey anti-rabbit serum (Jackson Immunoresearch, Immunotech, Marseille, France; 1:500) in PB containing 0.1% Triton X-100 was applied for 90 min. In a second step, for catecholaminergic staining, the catecholamine synthetic enzyme tyrosine hydroxylase (TH) (Chemicon Int., Temecula, USA) was immunocytochemically detected on the same sections. The primary TH sheep antiserum was used...
at the dilution of 1:2000 for 24 h at room temperature. The peroxidase activity was visualised using only 3,3′-diaminobenzidine. The specificity of the immunostaining was assessed by omission of the primary or secondary antibody from the protocol. After processing, tissue sections were mounted onto gelatine-alum-chrome coated slides, dehydrated, cleared in toluene and cover-slipped with Eukitt (Poly Labo, Strasbourg, France). Brain sections belonging to C and PS groups were treated in parallel to avoid differences due to the technical procedure.

2.5. Morphological analysis

Neurons immunoreactive for Fos protein (Fos-IR) exhibit a dark-brown nucleus, TH-immunoreactive (TH-IR) neurons had a brown cytoplasm and double labelled (Fos-IR/TH-IR) neurons show a brown cytoplasm containing a dark-brown nucleus. Fos immunoreactive nuclei were manually counted under a light microscope (Leica, Germany) by an independent experimenter. The sections levels were standardised according to the atlas of Paxinos and Watson (1997). The PVN was counted in the midhypothalamus level from −1.8 to −2.12 mm posterior to bregma. The dorsal hippocampus (CA1, CA2, CA3, DG) was counted from level −2.13 to −3.80 mm. Finally, all Fos-IR nuclei, TH-IR and Fos-IR/TH-IR neurons were counted in the locus coeruleus from level −9.3 to level −10.30 mm. A first observation was done at ×10 objective to identify the structure. Then, neurones were counted at the ×20 and/or ×40 objective to check the Fos labelling. For all brain regions considered, the total number of Fos-IR neurons was obtained by counting them bilaterally on each slice. For some rats, a slight difference exists between the number of collected slices. Thus, to obtain homogeneous values between rats, the total number of Fos-IR neurons counted for one region was divided by the total number of sections used.

2.6. Western blotting

Ten adult rats (C rats n=5 and PS rats n=5) were used. The hypothalamic area surrounding and including the PVN (from level −1.3 to −2.30 mm posterior to Bregma, according to the atlas of Paxinos and Watson, 1997) was rapidly dissected on dry ice, immediately frozen in liquid nitrogen and stored at −80 °C until use. Hypothalamus were homogenised at 4 °C in the buffer (320 mM sucrose, 5 mM Heps-NaOH pH7.4 and 0.1 mM EDTA) containing a cocktail of protease inhibitors (0.2 mM phenylmethanesulfonyl fluoride, 1 mg/ml aprotinin, and 0.5 mg/ml leupeptin). Protein concentrations within homogenates were measured by BCA assay (Pierce, Germany) and adjusted to 2 mg/ml in Laemmli buffer (Laemmli, 1970) in order to load 40 mg/sample. Proteins were separated onto 12% polyacrylamide gel electrophoresis containing SDS (SDS-PAGE), and then transferred onto nitrocellulose membranes. In order to identify the proteins of interest, molecular size markers (110-21.4 kDa range, BioRad) were used in parallel. For the immunoblotting, membranes were blocked for 30 min at room temperature with 5% non-fat dried milk, 5% normal goat serum and 0.1% Tween 20 in Tris buffered saline, then incubated for 1 h at room temperature with one of the following primary antibodies: polyclonal vesicular glutamate transporter 1 (anti-vGlut1, 1:10,000 in blocking buffer, Synaptic System, Germany) or GABA vesicular transporter (polyclonal anti-vGAT, 1:2000 in blocking buffer, Chemicon, Germany). After, 5 min washes in blocking buffer, membranes were then incubated for 1 h at room temperature with an anti-rabbit HRP-conjugated antibody (1:5000 in blocking buffer, Bio-Rad, Germany). Finally, immunoreactivity was visualised with enhanced chemiluminescence (Amersham Biosciences, Germany) and was quantified by densitometry (Molecular Analyst Software; Bio-Rad, France). After each vGlut1 or vGAT immunodetection, tubulin was revealed on the same membrane with a monoclonal anti-tubulin primary antibody (1:20,000 in blocking buffer, Sigma, Germany) and an anti-mouse HRP-conjugated secondary antibody (1:5000 in blocking buffer, Biorad). Results were expressed as a ratio of optic densities (OD): OD vGlut1/OD tubulin or OD vGAT/OD tubulin.

2.7. Statistical analysis

Data are expressed as mean±SEM. The Shapiron-Wilk test was first used to evaluate the normality of the population distribution. Since the populations studied were not normal (P<0.05), values were analysed with the non-parametric statistical Mann-Whitney test (for within-group differences). Significance was set at P<0.05.

3. Results

3.1. Plasma corticosterone levels after exposure to the open arm

In basal condition, no significant difference was found between C and PS rats. Exposure to the open
Furthermore, the number of activated neurons only in C rats after stress (rats; Fig. 2C). In the ventral part of the PaMP, neurons number after exposure to the open arm PS rats showed a similar increase of the Fos-IR values, declining to baseline only at T120 min after removing the rats from their home cage for the basal group (basal, PS rats, n=6; C rats, n=6). Data were compared using a Mann–Whitney U-test: **, P<0.01, basal vs open arm.

Arm induced a significant increase in the plasma corticosterone levels in both groups 15 min after the end of the exposure (U=0, P<0.01 for the C rats; U=1, P<0.01 for the PS rats; Fig. 1). This increase was followed by a delayed return to basal values in the PS rats: at T60 corticosterone level reached the baseline in C rats while in PS rats it remained elevated (U=4, P<0.01, T60 vs basal values), declining to baseline only at T120 min after stress (Fig. 1).

3.2. Expression of Fos protein in the PVN

Within the PVN, Fos-IR neurons were mostly found in the medial parvocellular region (PaMP) in basal and after stress conditions (Fig. 2A). In basal condition, no significant difference was noted between C and PS rats (Fig. 2B). Both in C and PS rats, exposure to the open arm significantly increased the number of Fos-IR neurons (respectively, U=0, P<0.005, U=2.5, P<0.01; Fig. 2B). Moreover, this stressor activated significantly more neurons in C than in PS rats (U=2, P<0.01; Fig. 2B).

Considering the dorsal part of the PaMP, both C and PS rats showed a similar increase of the Fos-IR neurons number after exposure to the open arm (U=5.5, P<0.05 for C rats, U=2, P<0.01 for PS rats; Fig. 2C). In the ventral part of the PaMP, increased number of Fos-IR neurons was observed only in C rats after stress (U=0, P<0.005; Fig. 2D). Furthermore, the number of activated neurons after the open arm exposure was higher in C rats compared to PS rats (U=3, P<0.05; Fig. 2D).

3.3. Western blot analysis in the hypothalamus

Since vGlut-1 and vGAT are responsible for uptake and storage of, respectively, glutamate and GABA by synaptic vesicles in the central nervous system, we measured their respective expressions in the hypothalamus by western blot. No significant changes were observed for vGlut1 in the PVN and the surrounding hypothalamic area between C and PS rats. However, PS rats displayed a higher expression of vGAT in the same region (U=0, P<0.01; Fig. 3).

3.4. Expression of Fos protein in the hippocampus

Within the hippocampus, Fos-IR neurons were located in the pyramidal layer of the Ammon horns CA1, CA2, and CA3 and in the granular layer of the dentate gyrus in basal and after stress conditions (Fig. 4A). In basal condition, considering the whole hippocampus, PS rats exhibited a higher number of Fos-IR neurons than C rats (U=0, P<0.005; Fig. 4B). The exposure to the open arm had no effect on the activation of PS hippocampal neurons. On the contrary, the C rats showed a significant increase in the number of Fos-IR neurons after such a stressor (U=0, P<0.005, Fig. 4B). More specifically, in basal condition, both in CA3 and dentate gyrus, PS rats exhibited a higher number of Fos-IR neurons than C rats (U=5, P<0.05 in CA3, U=2, P<0.01 in dentate gyrus; Fig. 4C and D). Exposure to the open arm induced an increase in the number of Fos-IR neurons only in the C rats (U=1, P<0.01 in CA3, U=0, P<0.005 in dentate gyrus; Fig. 4C and D). After this stress, the number of activated neurons tended to be higher in the CA3 of C rats compared to PS rats (U=7, P=0.07; Fig. 4C).

3.5. Expression of Fos protein in the locus coeruleus catecholaminergic neurons

Within the locus coeruleus, Fos-IR neurons were scattered throughout the nucleus in basal and after stress conditions (Fig. 5A). In basal condition, PS had more Fos-IR neurons compared to control (U=0, P<0.005; Fig. 5B). Exposure to the open arm did not modify the number of Fos-IR in the locus coeruleus of PS rats, whereas a significant increase was noted in C rats (U=0, P<0.005; Fig. 5B). Catecholaminergic neurons were globally found...
along the locus coeruleus, with a higher number in the dorsal than in the ventral region (data not shown). The number of TH-IR neurons was similar between the four groups of rats (Fig. 5C). Following exposure to the open arm, the percentage of the double-labelled neurons towards the TH-IR neurons population (Fos-IR/TH-IR) was higher in PS rats than in C rats \((U=4, P<0.05, \text{ C vs PS rats; Fig. 5D})\).

Moreover, only PS rats tended to present an increase in the percentage of double-labelled neurons after stress compared to basal condition \((U=6, P=0.06, \text{ basal vs open arm; Fig. 5D})\).

4. Discussion

The present work provided evidence for the first time that PS has an impact on neuronal activation of
the hippocampus and locus coeruleus, stress-related brain structures, implicated in the feedback mechanisms of the HPA axis. Our results give neuroanatomical basis to explain the neuroendocrine deficit of HPA axis feedback reported in PS rats by previous studies (Maccari et al., 1995; Barbazanges et al., 1996; Vallée et al., 1997; Dugovic et al., 1999). Moreover, the alteration of the plasma corticosterone levels found in our study reinforces these previous data underlying the high sensitivity of PS rats to stressors whatever their strength or duration. In fact, the mild stress, open arm exposure used here, was shorter (5 compared to 30 min) and was less stressful than the restraint stress used in the previous published experiments.

In basal condition, both PS and C rats exhibited similar plasma levels of corticosterone associated with a similar level of the Fos protein immunoreactivity in the parvocellular PVN. Furthermore, the hypothalamic area surrounding and including the PVN showed a significant increase of the hypothalamic vGAT and no change in the density of vGlut-1 in PS rats, suggesting a higher GABAergic inhibition in this area, even if an increased vGAT not always means a more release of GABA. Interestingly, PS rats presented a higher number of Fos-IR neurons in the hippocampus and locus coeruleus in basal condition. Given that we found an increased vGAT in the PVN and an increased hippocampal Fos immunoreactivity in PS rats, we would have expected a decreased Fos protein in the PVN of PS rats. We can explain the surprising absence of change in the PVN Fos immunoreactivity considering the increased locus coeruleus neuronal activation in PS rats. Locus coeruleus neurons provide stimulatory noradrenergic input to the PVN (Plotsky, 1987; Daftary et al., 2000). Indeed, this locus coeruleus excitatory effect at the PVN level (Saphier, 1989; Han et al., 2002) could be contrasted by the local vGAT inhibitory effect, in order to maintain in PS rats a basal activation of the CRH PVN neurons similar to C rats. Furthermore, an increased noradrenergic signal from the locus coeruleus to hippocampus reduces hippocampal

**Figure 3** Western immunoblot of vesicular transporters for GABA (vGAT) and type-1 glutamate (vGlut1) in the hypothalamic area surrounding the hypothalamic paraventricular nucleus of control (C) and prenatally stressed (PS) rats. Tubulin was detected after vGlut1 or vGAT on the same membranes. Results are expressed as a ratio of optic densities (OD): OD vglut1/OD tubulin or OD vGAT/OD tubulin. PS rats displayed a higher expression of vGAT in the same region and no significant changes were observed for vGlut1 in the PVN and the surrounding hypothalamic area between control and PS rats. *, $P<0.05$. 
glucocorticoid and mineralocorticoid receptors (GR/MR) (Maccari et al., 1992; Kabbaj et al., 1995). Adult PS rats present a deficit in the hippocampal GR/MR at rest (Maccari et al., 1995). Thus, we can suggest that, in basal condition, the higher neuronal activity in the hippocampus of PS rats could be a physiological response to compensate the decreased hippocampal MR/GR in order to maintain in PS rats basal plasma corticosterone levels similar to the C rats.

After the exposure to open arm, plasma corticosterone secretion was similar in both PS and control groups 15 min after stress. Interestingly, in the whole parvocellular PVN, the exposure to open arm increased the number of Fos-IR neurons in both PS and control rats, even if the reactivity in the PS was lower than in C rats. The parvocellular PVN neurons counted included CRH neurons driving the HPA axis in the medial dorsal part of the PVN and the autonomic related neurons in the medial

Figure 4  (A) Bright-field photomicrographs showing the distribution of Fos-immunoreactive (Fos-IR) neurons in the hippocampus in control rats in basal condition and after exposure to the open arm. Within the hippocampus, Fos-IR neurons were located in the pyramidal layer of the Ammon horns CA1, CA2, and CA3 and in the granular layer of the dentate gyrus (DG). Only CA3 and DG were illustrated. (B) Mean number of Fos-IR neurons in basal condition and after exposure to the open arm for control (C) and prenatal stressed rats (PS) in the hippocampus and in detail for CA3 (C) and DG (D). Bar: 100 μm. CA3: Ammon horn 3; DG: dentate gyrus. Open bars: basal condition; full bars: stress condition. oo, P<0.01, ooo, P<0.005, basal vs open arm; * P<0.05, ** P<0.01, *** P<0.005, C vs PS.
ventral part of the PVN. Both PS and C rats showed a similar neuronal activation in the dorsal of the medial parvocellular region (PaMP), while in the ventral part of PaMP Fos protein was more increased after stress in C compared to PS rats. These data underline that, consistently with the corticosterone levels at T15, activation of the HPA axis mediated by CRH neurons of medial dorsal part of the PVN, did not differ between PS and C rats. In PS rats, the reduced parvocellular PVN neuronal activation is due to a reduction of neuronal activity only in the ventral part of the parvocellular PVN. Nevertheless, we can suggest that the lesser neuronal reactivity observed in the ventral PaMP of PS rats may be explained by an increased catecholaminergic activity in the locus coeruleus which acts on autonomic neurons (Han et al., 2002).

After exposure to the open arm, PS and C rats differed in the time necessary to return to basal values, given that it took 60 min after stress for C and 120 min for PS rats. Since this stressor produced a similar increase in the HPA activation,
the difference between PS and C rats was related to the feedback mechanisms of the HPA axis. In fact, PS rats showed similar number of Fos-IR neurons in the hippocampus (mainly in CA3 and dentate gyrus) and locus coeruleus after stress. This suggests no reactivity to stress of these brain structures in PS rats, structures involved in the feedback mechanisms. Thus, the absence of neuronal reactivity in the PS rats hippocampus after stress could explain this deficit in the HPA axis feedback mechanisms leading to a delay to return to basal corticosterone values. We also showed an increased basal levels of Fos in the hippocampus and LC of PS rats meaning a chronic and ‘ceiling’ activation of these neurons in the rest condition that could be explain at least in part the absence of hippocampus and LC reactivity. Furthermore, the absence of neuronal reactivity in the PS rats hippocampus can be in relation with the reduced number of hippocampal GR/MR in PS rats at rest (Maccari et al., 1995) and due to: (1) the increased percentage of the catecholaminergic neurons activated in the locus coeruleus in PS rats after stress, that in turn can act on hippocampal GR/MR number (Maccari et al., 1992; Kabbaj et al., 1995; Lai et al., 2003); (2) the already increased hippocampal neuronal activation in basal condition in PS rats (perhaps to compensate the decreased sensibibility to corticosterone), which could prevent supplementary hippocampal activation increase after stress. This absence of hippocampal reactivity can contribute to the PS rats allostatic overload (McEwens and Wingfield, 2003): the unproductive try to restore homeostasis in a good time, as for a prolonged HPA response, resulting in the incapacity to cope efficiently with the challenge and finally in a disease state.

Finally, another possible mechanism explaining the absence of neuronal reactivity in the hippocampus and locus coeruleus of PS rats in response to stress could be the inhibition of the transcription factor activator protein-1 (AP-1). The AP-1 regulates the transcription of late genes when activated by the heterodimer c-Fos/c-Jun (as a result of the immediate early genes transcription, c-fos and c-jun) (Herdegen and Leah, 1998). The prolonged corticosterone secretion after stress observed in PS rats (Maccari et al., 1995) could repress the AP-1 given that glucocorticoids regulate AP-1 (Diamond et al., 1990; Yang-Yen et al., 1990; Unlap and Jope, 1994), and consequently the heterodimer c-Fos/c-Jun activity, resulting in the absence of increase in the number of Fos-IR neurons in the hippocampus and locus coeruleus after stress.

In conclusion, our work raises new anatomical arguments suggesting that PS can have long-term neuronal effects within hippocampus and locus coeruleus brain structures involved in the feedback mechanisms of the HPA axis. The PS may affect the organisation of pathways and/or neuronal circuitry responsible to maintain a basal tonic regulation of the HPA axis similar to controls. These neuronal ‘reorganisation’ could affect the PS rats reactivity to stress leading to alterations in behaviour related to HPA axis response to stress.

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