Neurochem Res (2013) 38:2323–2335
DOI 10.1007/s11064-013-1143-8

ORIGINAL PAPER

Age-Dependent Effects of Prenatal Stress on the Corticolimbic Dopaminergic System Development in the Rat Male Offspring

María Eugenia Pallarés · Carlos Javier Baier · Ezequiela Adrover · Melisa Carolina Monteleone · Marcela Adriana Brocco · Marta Cristina Antonelli

Received: 24 July 2013 / Revised: 23 August 2013 / Accepted: 26 August 2013 / Published online: 8 September 2013

Abstract We have previously demonstrated that prenatal stress (PS) exerts an impairment of midbrain dopaminergic (DA) system metabolism especially after puberty, suggesting a particular sensitivity of DA development to variations in gonadal hormonal peaks. Furthermore we demonstrated that PS alters the long term androgens profile of the rat male offspring from prepubertal to adult stages. In this work we evaluated the sexual hormones activational effects on the DA system by analysing PS effects on the dopaminergic D2-like (D2R) and on the gonadal hormones receptor levels on cortical and hippocampal areas of prepubertal and adult male offspring. We further evaluated the dendritic arborization in the same areas by quantifying MAP2 immunoexpresion. Our results show that PS affected oestrogen receptor alpha (ERα) expression: mRNA *er1s* and ERα protein levels were decreased on prefrontal cortex and hippocampus of adult offspring. Moreover, PS reduced D2R protein levels in hippocampus of prepubertal rats. Morphological studies revealed that prepubertal PS rats presented decreased MAP2 immunoexpression in both areas suggesting that PS reduces the number of dendritic arborizations. Our findings suggest that PS exerts long-term effects on the DA system by altering the normal connectivity in the areas, and by modulating the expression of D2R and ERα in an age-related pattern. Since the developing forebrain DA system was shown to be influenced by androgen exposure, and PS was shown to disrupt perinatal testosterone surges, our results suggest that prenatal insults might be affecting the organizational role of androgens and differentially modulating their activational role on the DA development.

Keywords Prenatal restraint stress · D2R · MAP2 · Oestrogen receptor alpha

M. E. Pallarés · C. J. Baier · E. Adrover · M. C. Antonelli (✉)
Instituto de Química y Fisicoquímica Biologicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
e-mail: mca@fmed.uba.ar

Present Address:
M. E. Pallarés · M. C. Antonelli
Instituto de Biología Celular y Neurociencias "Prof. E. De Robertis" (IBCN), Paraguay 2155, 3°Piso, C1121ABG, Buenos Aires, Argentina

Present Address:
C. J. Baier
Instituto de Investigaciones Bioquímicas de Bahía Blanca, CC 857, B8000FWB Bahía Blanca, Argentina

M. C. Monteleone · M. A. Brocco
Instituto de Investigaciones Biotecnológicas, Universidad de San Martín, San Martín, Buenos Aires, Argentina

Introduction

It has been demonstrated that PS may cause structural and functional changes on the fetal central nervous system development, exerting long term consequences on the progeny [1, 2]. Research in this field showed that the offspring of stressed pregnant mothers displayed delays in motor development, impaired adaptation to stressful conditions and altered sexual behavior. Moreover, subsequent vulnerability to anxiety, enhanced propensity to self-administer drugs and learning deficits has also been reported [1, 3–5]. Most of these alterations have been attributed to changes in midbrain DA activity, suggesting that the development of the DA is sensitive to disruption by exposure to early stressors.

Dopamine regulates diverse behavioral and cognitive functions that are critical for integrating mammalian

responses and adaptations to the environment. The corticolimbic system is considered to be of particular interest for the pathophysiology of idiopathic psychiatric disorders including psychoses and mania, as well as in schizophrenia and attention-deficit hyperactivity disorder (ADHD) [6], which have been traditionally related to dopaminergic mesolimbic and mesocortical pathways. Our laboratory has a long standing interest in the effects of PS on development of the DA system [7]. We have demonstrated that PS induced impairments of DA metabolism that were differentially affected after puberty [8–12], suggesting that perinatal events might render the dopaminergic circuitry more vulnerable to puberty variation of the hormonal circulating levels. During this period, the gonadal hormones act on peripheral tissues where they induce the appearance of secondary sex characteristics, but they also act centrally to influence the remodeling of the adolescent brain, orchestrating brain plasticity, and behavioral maturation [13–15]. In addition, puberty is a pivotal time on the etiology of certain psychopathologies traditionally linked to dopaminergic pathways [16, 17]. In males, some disorders that have a childhood onset, such as ADHD, not only deteriorate during puberty but also wane while reaching to adulthood. Others like schizophrenia, substance abuse disorder, and depression typically emerges during late adolescence or early adulthood [17, 18]. The modulation of DA pathways by gonadal hormones has long been demonstrated by Yang and Shieh [19] who postulated that the nigrostriatal and mesolimbic systems are modulated by circulating gonadal steroids, oestradiol, and testosterone. Moreover, Creutz and Kritzer [20] reported that the soma projections of DA system contain high number of oestrogens and androgen receptors.

The effect of gonadal hormones on the brain maturation takes place at two different periods of life: (a) *the organizational phase* that takes place during critical periods of foetal development, sex steroids promote cellular and molecular events which will determine sexual differentiation of the brain; and (b) *the activational phase* during the onset of puberty, oscillations in their circulating levels affect a wide variety of neuronal phenomena, ranging from cyclic remodeling of synaptic circuitry to transynaptic modulation of neurotransmission [21]. In the rat, androgens-induced masculinization of the reproductive tract and the brain sexual behavior take places by the action of two perinatal testosterone surges: the first over gestational day 18.5, and the second, during the first 4 h after birth [22–24]. It was reported that PS interferes with androgens synthesis during the first surge of prenatal testosterone, affecting the male reproductive tract formation and inducing abnormal testosterone levels during the progeny lifespan, among other effects [25–29]. In our hands, PS induced long-term effects on the male offspring reproductive system and spermatogenesis development, particularly by inducing a long term imbalance of circulating sexual hormones levels [30].

Considering that PS interferes with the normal development of the DA pathway and that manipulation of the perinatal environment results in long-term changes at the hormonal levels, our aim was to assess if changes in gonadal steroid receptors could be temporally related to changes in the dopaminergic system of the corticolimbic system in the prenatally stressed male offspring. We employed prepubertal and adult male rats that were prenatally stressed to evaluate androgen and oestrogen receptors, as well as steroideogenic enzymes mRNA expression in the corticolimbic dopaminergic areas: prefrontal cortex (PFC) and hippocampus (HPC). Furthermore, we analysed PS effects on dopaminergic D2-like (D2R) expression as well as on MAP2 (a microtubule-associated protein present almost exclusively in dendrites) immunoreactivity at earlier stages. This work explore certain aspects of the sexual hormones and brain development interaction aiming to offer a better understanding to the pathophysiology of several psychopathologies that are turned on during adolescence in subjects with a PS history.

Materials and Methods

Animals

Virgin female Wistar rats weighing 250–280gand sexually experienced Wistar male rats weighing 400–450 g were obtained from highly inbred rats from the animal facility at the University of Buenos Aires. A maximum of five rats were housed per cage with ad libitum access to standard rat chow (*Asociación de CooperativasArgentinas*—Buenos Aires, Argentina) and water. A constant light/dark cycle, with lights on at 06:00 h and off at 18:00 h, and a room temperature of 21–25 °C were maintained. Females were individually mated with a male in a mating cage. Vaginal smears were taken on the following morning. The day on which spermatozoa were found in the smear was designated as day 1 of pregnancy. All procedures were in agreement with the standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (Facultad de Medicina, Universidad de Buenos Aires).

Prenatal Procedures and Experimental Design

Pregnant dams were randomly assigned to either the control or the prenatal stress group and were individually housed with ad libitum access to standard rat chow and water. A constant light/dark cycle (on at 06:00 h, off at

18:00 h) was maintained at a temperature of 21–25 °C. C rats (n = 7) were left undisturbed in the home cage, while PS dams (n = 7) were subjected to a restraint stress procedure, which involved rats being transferred to an experimental room where the stressor was applied. Pregnant females were individually placed into a transparent plastic restrainer fitted closely to body size for three 45-min periods per day (09:00, 12:00 and 16:00 h) between days 14 and 21 of pregnancy. The restrainer had ventilation holes, and dimensions appropriate for a pregnant rat of 350 g: internal diameter 64 mm, and an adjustable length of 149–208 mm. This type of stressor was chosen because it has an indirect influence on the foetuses via a direct stress on the mother [31, 32]. The sessions were performed in a lit environment. No other subjects were present in the experimental room during the stress exposure. At the end of the stress session, the rats were returned to the animal housing room and were then individually housed with free access to food and water.

On the day of parturition, litter characteristics were recorded, and litters culled to 10 pups, maintaining similar number of males and females, whenever possible. Seven litters were maintained for each experimental group. Weaning was performed at postnatal day (PND) 21.The male and female offspring were housed in separate cages, with no more than five pups per cage, with standard rat chow and water ad libitum. In this study, only male offspring were used. Offspring with 28 days old were designated as *prepubertal* rats, while at PND 75 they were designated as *adult* rats. To avoid litter effects, one pup from each litter was tested for each experiment. The timeline in Fig. 1 illustrates the experimental design.

Tissue Collection

At PND 28 or PND 75 three to seven males from different litter, belonging to C or PS group, were rapidly euthanized by decapitation between 09:00 and 10:00 h. Brains were removed from the skull for further dissection. PFC and HPC were rapidly extracted on ice according to anatomical landmarks corresponding to Paxinos and Watson [33] rat brain atlas. Brain sections were weighted and differentially processed for Western blot or for quantitative real time reverse transcription polymerase chain reaction (RT-qPCR) analysis.

Western Blotting

Prefrontal cortex (PFC) and HPC extracts were homogenised in 5 ml/g (per gram of tissue) of ice-cold RIPA lyses buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 % Triton X-100; 1 % Sodium deoxycolate; 0.1 % SDS; 1 % protease inhibitors). Following centrifugation of the homogenate for 15 min at 16000×*g* at 4 °C, the protein was extracted from the supernatant and quantified by the Bradford method [34]. Equal amounts of protein (75 µg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) through either 10 % (D2R, ERα and ERβ) or 12 % (androgen receptor, AR) acrylamide [35] under reducing conditions and then blotted

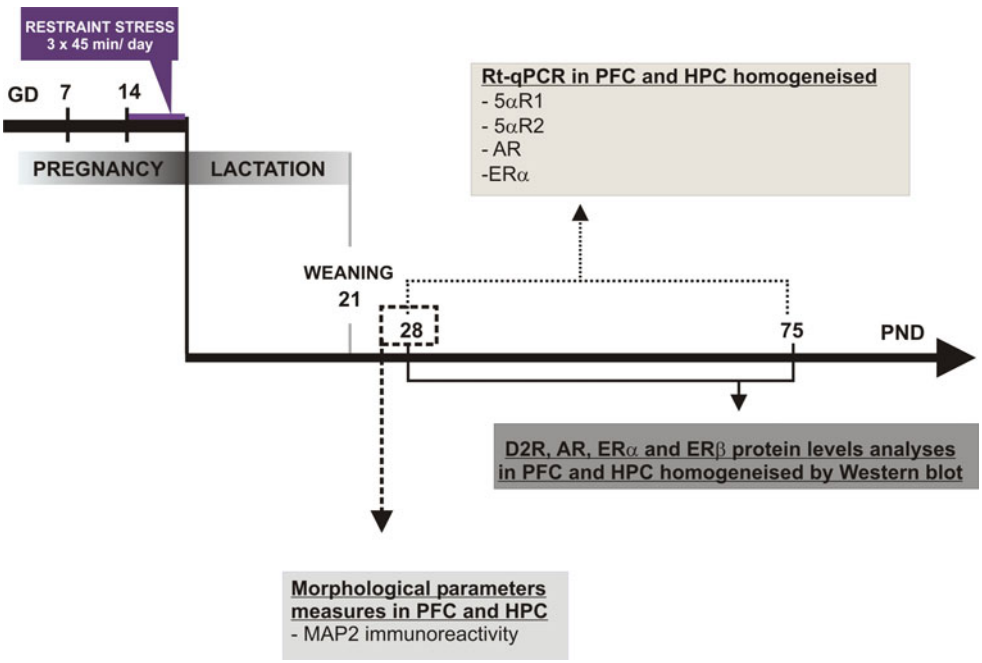

Fig. 1 Diagram of the experimental design. *GD* gestational day, *PND* postnatal day, *D2R* dopamine D2-like receptor, *AR* androgen receptor, *ERα* oestrogen receptor alpha, *ERβ* oestrogen receptor beta, RT-qPCR

onto polyvinylidene fluoride (PVNDF) membrane (Immobilon-P; Millipore, Watford, United Kingdom). The blot was blocked with a 5 % non-fat dry milk in TBST (10 mM tris-HCl pH 7.5, 150 mMNaCl, 0.1 % tween-20) for 150 min and subsequently incubated overnight at 4 °C with polyclonal anti-AR (Santa Cruz Biotechnology Inc, N-20 sc-816; 1:100), anti- ERα (Santa Cruz Biotechnology Inc, MC20 sc-542; 1:500), anti-Erβ (Thermo Scientific PA1-310B; 1:500) or anti- D2R (Chemicon International INC-Millipore; 1:1,000). After washing in TBST, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (1:5,000) for 1 h. The bands were visualized by enhanced chemiluminescence (ECL, AmershamBioscences). Each receptor expression was normalized to the β-ACTIN values.

mRNA Isolation and RT-qPCR

Prefrontal cortex (PFC) and HPC from PND 28 and PND 75 rats belonging to C or PS groups were immediately homogenised in TRIzol™ reagent (Life Technologies, Carlsbad, CA) to isolate total RNA, according to the manufacturer's instructions. PolyA mRNA was purified using the PolyA-Tract mRNA Isolation System (Promega, Madison, WI). Complementary DNA was synthesised using oligodT and SuperScriptTM II Reverse Transcriptase (Life Technologies, Carlsbad, CA). qPCRs were carried out in a 7,500 Real-Time PCR System (Applied Biosystems, Life Technologies, Foster City, CA). Quantification of each cDNA was achieved using SYBR Green Master Mix Reagent (Applied Biosystems) in triplicate. Primer sequences are detailed in Table 1. Normalisation was accomplished employing succinate dehydrogenase complex subunit A (*sdha*) and beta-2 microglobulin (*β2m*) as the reference geneS. Values shown in all figures were calculated using *β2m*. Normalisations to *sdha* resulted in almost identical patterns. Relative quantification was performed using a comparative CT method (guide to performing relative quantitation of gene expression using real-time quantitative PCR [36, 37]. Before each experiment, the calibration curves were validated. Samples with curves amplified out of the calibrated dynamic range were eliminated. All procedures followed the manufacturer's instructions. Values shown are relative to the control animals at PND 28.

Table 1 Sequences of primers used for RT-qPCR

Name	Primer sequence (5′–3′)
srd5a1	R: agcaatcagcagcccttacaa
NM_017070.3	F: ggatggcagactgtgcttagg
srd5a2	R: catatagttcattgatttggaaaggagat
NM_022711.4	F: gtagcctctctctgccacacaa
ar	R: tgcacctgacctggttttca
NM_012502.1	F: tcacgcactggctgtacattc
esr1	R: ttccccaacaccatctgagaa
NM_012689.1	F: cgtttcagggattcgcagaa
esr2	R: cactgcacttcccaggagtca
NM_012754.1	F: aacttggcattcggtggtaca
β2 m	R: tggcgtcttctgcccttct
NM_012512.2	F: gcgatggaggactgcatca
sdha	R: acaaggtgcggattgatgagt
NM_130428.1	F: gaacggatagcaggaggtaca

srd5a1 steroid 5α-reductase isoform 1, *srd5a2* steroid 5α-reductase isoform 2, *ar* androgen receptor, *esr1* oestrogen receptor isoform alpha, *esr2* oestrogen receptor beta, *β2m* β-2 microglobulin, *sdha* succinate dehydrogenase complex subunit A flavoprotein

Fixation and Tissue Processing

At PND 28, four control and four stressed rats were deeply anesthetized with xylazine/ketamine hydrochloride solution (Mallinckrodt, 10 and 75 mg/kg respectively). They were perfused through the cardiac left ventricle, initially with a cold saline solution containing 0.05 % w/v NaNO2 plus 50 IU of heparin and subsequently with a cold fixative solution containing 4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed, post-fixed in the same cold fixative solution for 4 h, subsequently cryoprotected by overnight inmersion in 15 and 30 % w/v sucrose and stored at −80° until processing for immunohistochemistry. Eight series of 25-μm-thick coronal sections for PFC and ten for the HPC were cut on a Leyca cryostat. The sections were stored at −20 °C in 0.1 M phosphate buffer, pH 7.4, with 50 % w/v glycerol added as a cryoprotector until their use in immunocytochemical studies.

Immunohistochemistry

25-μm-thick brain sections of both C and PS rats were selected according to anatomical landmarks corresponding to the Paxinos and Watson [33] rat brain atlas. The following areas were analysed for immunohistochemistry: (1) layers 2 and 3 of the frontal cortex, and (2) the stratum radiatum of the hippocampal CA1 area. The sections were simultaneously processed in the free-floating state. To inhibit endogenous peroxidase activity, tissue sections were previously dehydrated, treated with 0.5 % v/v H_2O_2 in methanol for 30 min at room temperature, and rehydrated. Brain sections were treated for 1 h with 3 % v/v normal goat serum in phosphate-buffered saline (PBS) to block nonspecific binding sites. After two rinses in PBS plus 0.025 % v/v Triton X-100 (PBS-X), sections were

incubated for 48 h at 4 °C with primary antibodies to MAP2 (Synaptic System 188002; 1:1,000). After five rinses in PBS-X, sections were incubated for 1 h at room temperature with biotinylated secondary antibodies diluted 1:200. After further washing in PBS-X, sections were incubated for 1 h with streptavidin-peroxidase complex diluted 1:200. Sections were then washed five times in PBS and twice in 0.1 M acetate buffer, pH 6 (AcB), and development of peroxidase activity was carried out with 0.035 % w/v 3,3-diaminobenzidine hydrochloride (DAB) plus 2.5 % w/v nickel ammonium sulfate and 0.1 % v/v H_2O_2 dissolved in AcB. After the enzymatic reaction step, sections were washed three times in AcB and once in distilled water. Finally, sections were mounted on gelatin-coated slides, air dried, and coverslipped using Permount for light microscopic observation.

Image Analysis of Morphometric Studies

To ensure objectivity, all measurements were performed on coded slides, under blind conditions, by two observers for each experiment, carrying out the measurements of immunolabelled brain sections of both controls and prenatally stressed rats with the same standardized observation schedule. Five serial tissue sections were selected according to anatomical landmarks corresponding to the Paxinos and Watson [33] rat brain atlas, for each area and for each animal. All morphometric parameters (mean gray, cell area, and relative areas) were obtained with an Axiophot Zeiss light microscope equipped with a video camera on line with a Zeiss-Kontron VIDAS image analyser. The images were digitized and processed in a resolution of 256 gray levels for each pixel. The morphometric studies using immunolabelled structures for cytoskeletal proteins were performed as previously described [38]. To evaluate the MAP2 immunoreactivity fibers, the total area of the immunolabelled fibers was related to the total area of the corresponding microscopic field in a grid of 400 × 300 μm for PFC or 200 × 200 μm for HPC, thus rendering a relative area parameter.

Statistical Analysis

Litter characteristics analyses, Western blot and MAP2 immunoreactivity results were analysed by Student-t test. RT-qPCR analysis were analysed by two-way ANOVA in order to evaluate the differences between prenatal treatment, age and possible interactions between both factors. When interactions were found, simple effects ANOVA analyses were done followed by Tukey pos-hoc test for multiple comparison. Visual inspection of histograms, qq plots and random distribution of fitted values were checked after data transformation. All results are presented as mean ± SEM. The observed differences were considered to be statistically significant when $p < 0.05$. n values reported in figures represent number of litters. Analysis of data was performed by using SPSS 13.0 version and Infostat 2013.

Results

Effects of Gestational Stress on Litter Parameters

Litter parameters analysis on the day of birth, revealed that PS did not interfere with the length of gestation, number of pups or sex ratio (Table 2). Offspring analyses of body weight gain demonstrated that PS did not affect the weight of the pups at birth.

PS Effects on D2R Protein Levels and on MAP2 Immunoreactivity on Offspring DA System

Western blot data analysis from PS effects on the D2R protein expression on PFC and HPC is shown in Fig. 2. At PND 28, PS induced a decrease of 51 % on the receptor protein concentrations only in HPC in comparison of the values of receptor obtained in control group ($T_{(4)} = -2.98$, $p = 0.0406$ for HPC PND 28 control vs PS). Non changes on D2R expression were found at PND 75.

MAP2 immuno-density of each area analysed was calculated by carefully matching control and PS sections in the same antero-posterior plane to avoid differences in cell distribution along the rostro-caudal axis. A decrease in relative area of the MAP2-immunostained processes were found in PS rats compared with control. The immunolabelled processes were observed as round stains or longitudinal tracts, according to whether dendrites were transversally or tangentially sectioned. In control rats, dendrites were observed to be abundant in all areas studied,

Table 2 Litter characteristics

Evaluated parameter	Control	Prenatal stress
Lenght of gestations (days)	22.6 ± 0.4 (5)	22.6 ± 0.37 (5)
Litter lenght		
N° PUPS	11.6 ± 0.81 (5)	12.2 ± 1.07 (5)
N° MALE PUPS	6.6 ± 0.68 (5)	6 ± 0.71 (5)
N° FEMALE PUPS	4.8 ± 0.73 (5)	6.2 ± 0.7 (5)
Pups body weight at birth (gr)	6.83 ± 0.16 (5)	6.33 ± 0.09 (5)

The number between parentheses depicts the number of litter used for each evaluated parameter. (t-student statistical test)

PND postnatal day

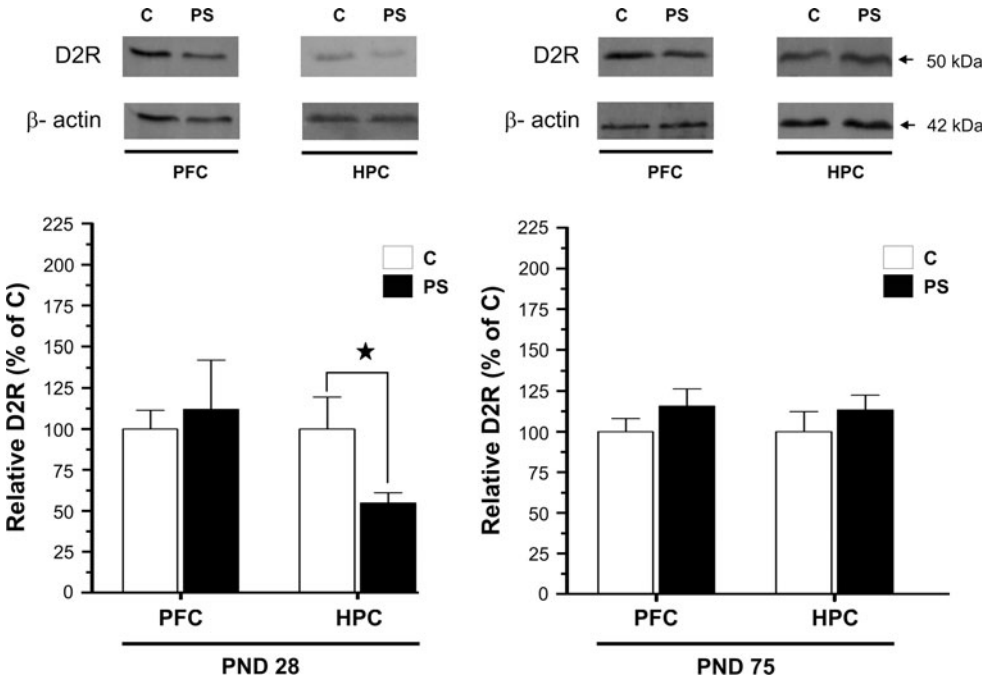

Fig. 2 Effects of prenatal stress (PS) on D2R protein levels in 28 and 75 days-old rats. *C* control, *PFC* prefrontal cortex, *HPC* hippocampus, *PND* postnatal day. Images belong to representative D2R immunoblot for each brain area belonging to C or PS rats. The immunoreactive band for the D2R migrated to 50 kDa. ACTIN expression indicates uniform loading in each lane. Bars represent the mean ± SEM of the density belonging to the corresponding protein bands, expressed as the relative D2R levels in percentage of the control values. *Star* demonstrate the presence of statistical differences between experimental group for each brain area and age (n = 3–4; $^*p < 0.05$; t-student statistical test)

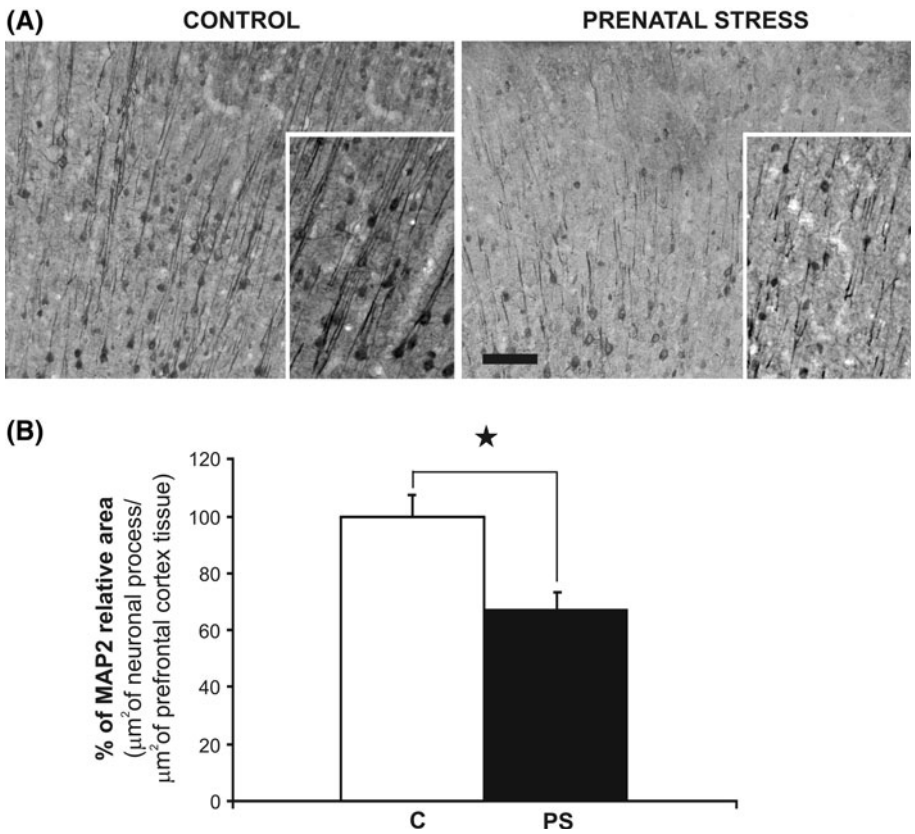

Fig. 3 Effects of prenatal stress (PS) on MAP2 immunoreactivity at layers II and III of the PFC in prepubertal rats.*C* control. **a** Micrograph shows an example of immunostaining with an anti-MAP2 antibody in prefrontal cortex for each experimental group (20× objective magnification; 40× objective magnification for *insets*. *Scale bar* 100 μm. **b** Values are reported as mean ± SEM of the MAP2 immunoreactivity in PFC (μm^2 neuronal processes/ μm^2 tissue). *Star* demonstrate the presence of statistical differences between experimental group (n = 4; $^*p < 0.05$; t-student statistical test)

but were scarce in prenatally stressed rats (Figs. 3a and 4a). Semiquantitative analysis of these results showed that the decrease was statistically significant in PFC (31 %) and CA1 hippocampal (27 %) areas (Figs. 3b, 4b; $T_{(6)} = 3.45$, $p = 0.0136$ for control vs PS in PFC; $T_{(6)} = 2.85$, $p = 0.029$ for control vs PS in HPC).

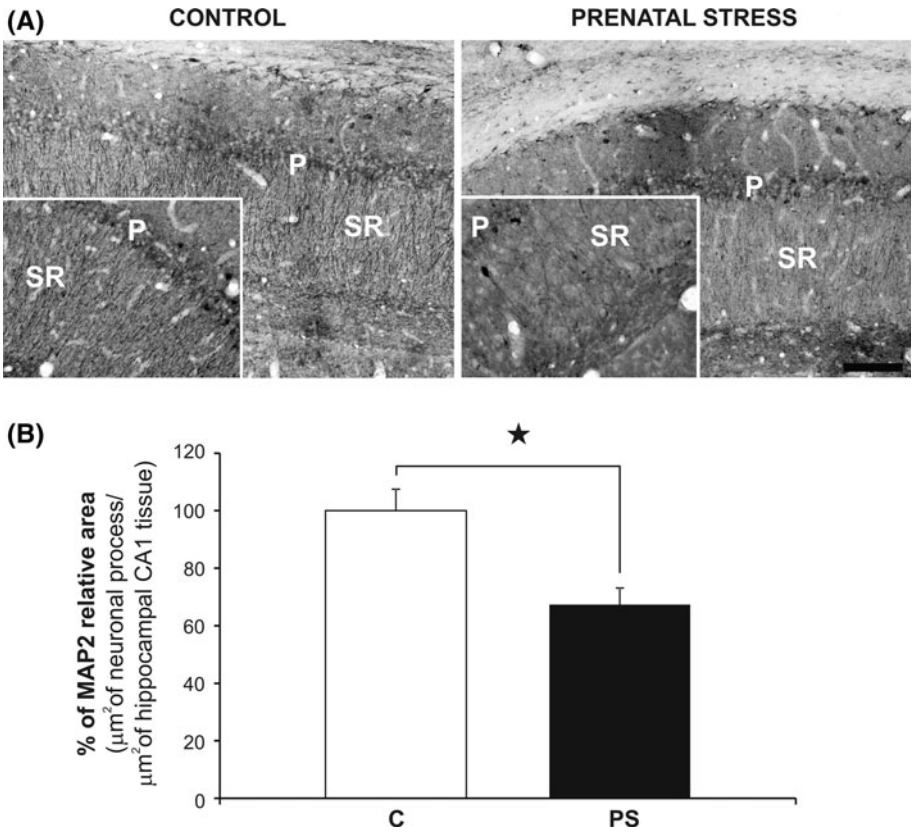

Fig. 4 Effects of prenatal stress (PS) on MAP2 immunoreactivity at hippocampal CA1 area in prepubertal rats.*C* control, *P* pyramidal neuronal layer, *SR* stratum radiatum. **a** *Micrograph* shows an example of immunostaining with an anti-MAP2 antibody in hippocampal CA1 area for each experimental group (20× objective magnification; 40× objective magnification for *insets*). *Scale bar* 100 µm. **b** Values are reported as mean ± SEM of the MAP2 immunoreactivity in HPC (μm^2 neuronal processes/ μm^2 tissue). *Star* demonstrate the presence of statistical differences between experimental group (n = 4; $^*p < 0.05$; t-student statistical test)

PS Effects on Sexual Hormone Receptors and Steroidogenic Enzymes in Prepubertal and Adult Offspring DA System

Androgen Receptor

Prenatal stress effects on the androgen receptor expression on PFC and HPC are shown in Fig. 5. Western blot data analysis revealed no statistical differences on AR protein measured at PND 28 or PND 75, in control and PS rats (Fig. 5a). Statistical analyses of PFC revealed no effects due to age or PS factors on *ar* mRNA levels (Fig. 5b). In HPC, a significant age-dependent increase was observed in *ar* expression between PND 28 and 75 only in control rats ($F_{(1,16)} = 8$, $p = 0.0121$ PND 28 vs PND 75, control group) (Fig. 5c): control adult rats presented an increase of 118 % on *ar* mRNA levels in comparison of prepubertal control rats. However in PS rats hippocampal *ar* mRNA levels were found similar at both prepubertal an adult ages.

Oestrogen Receptor Alpha

Prenatal stress effects on oestrogen alpha receptor expression on PFC and HPC are shown in Fig. 6. Similar ERα protein levels were found in brain areas of prepubertal offspring belonging to control and PS groups. However at PND 75, PS induced a decrease on the receptor protein concentrations both in PFC (31 %) and HPC (57 %) (Fig. 6a; $T_{(4)} = 7.15$, $p = 0.002$ for PFC- PND 75 control vs PS; $T_{(4)} = 4.09$, $p = 0.0095$ for HPC–PND 75 control vs PS). *esr1* mRNA was expressed in both brain tested areas. An age-factor effect on the receptor gene expression was found on PFC: at PND 75 *esr1* mRNA levels were diminished in comparison with PND 28, both in control and PS offspring (Fig. 6b; $F_{(1,13)} = 6.52$, $p = 0.0268$ PND 28 vs 75). On the other hand, an age x prenatal treatment interaction was found on the *esr1* expression in HPC: while adult control rats presented an increase of 110 % in the receptor mRNA levels in comparison to PND 28 control rats, such increase was not found in PS rats (Fig. 6c; $F_{(1,14)} = 6.87$, $p = 0.02$ control vs PS, PND 75).

Oestrogen Receptor Beta

Prenatal stress effects on the ERβ protein expression on PFC and HPC, of prepubertal and adult offspring, is shown in Fig. 7. Western blot data analysis revealed that ERβ protein measured at PND 28 or PND 75 show no statistical differences in either control or PS offspring. *esr2* mRNA expression could not be detected neither in PFC nor in HPC.

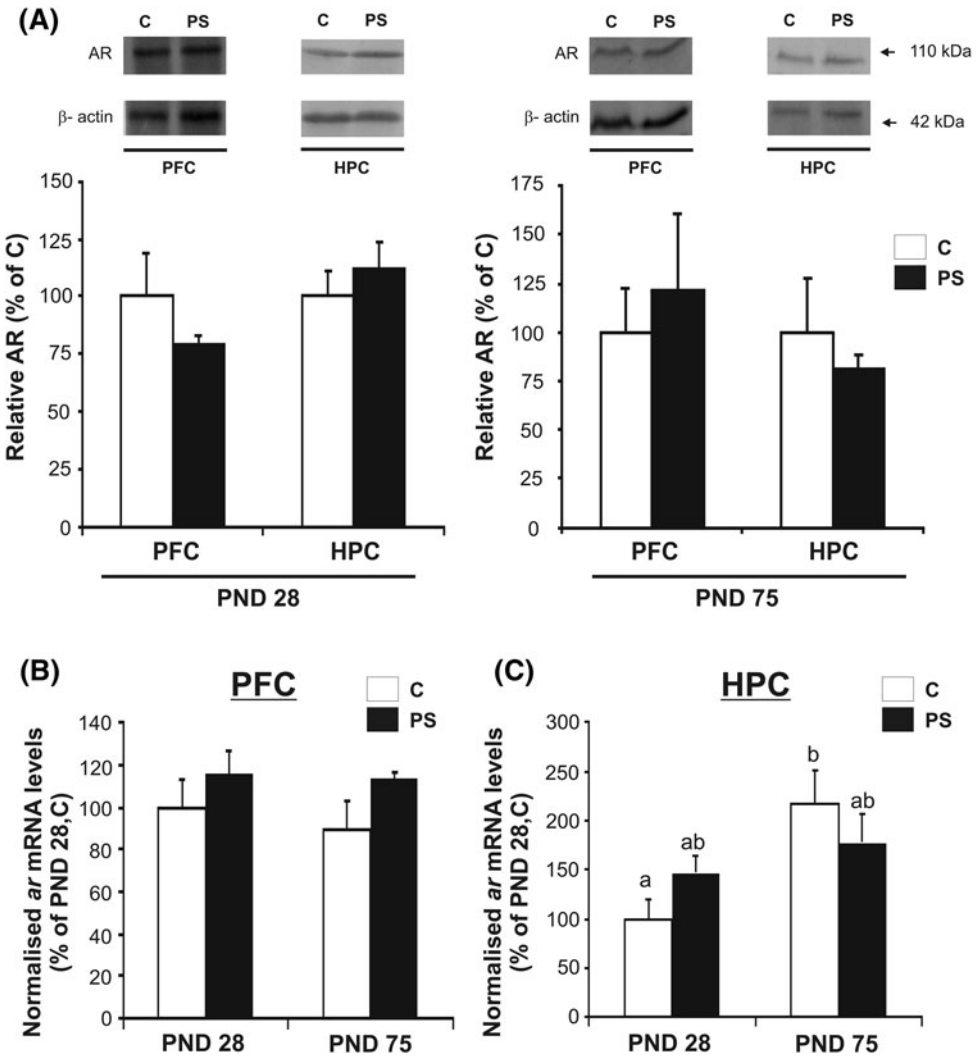

Fig. 5 Effects of prenatal stress (PS) on AR expression in 28 and 75 days-old rats. *C* control, *PFC* prefrontal cortex, *HPC* hippocampus, *PND* postnatal day. **a** Images belong to representative AR immunoblot for each brain area belonging to C or PS rats. The immunoreactive band for the AR migrated to 110 kDa. ACTIN expression indicates uniform loading in each lane. *Bars* represent the mean ± SEM of the density belonging to the corresponding protein bands, expressed as the relative AR levels in percentage of the control values. (n = 3–4; t-student statistical test). **b**–**c** The mRNA levels determined by real-time PCR in PFC (**b**) or HPC (**c**) are expressed relative to the housekeeping gene *beta-2 microglobulin* mRNA expression as arbitrary units and as % of PND 28 control rats. Values are reported as mean normalized *ar* mRNA levels ± SEM. *Different letters* depict significant differences between groups with $p < 0.05$. (n = 5–6 two-way ANOVA)

Steroidogenic Enzymes mRNA Expression

5αR isoenzyme and aromatase mRNA expression were evaluated in PFC and HPC, at PND 28 and PND 75, of control and PS rats. *cyp19* mRNA expression could not be detected in either brain areas. Data analysis from *srd5a1* and *srd5a2* mRNA levels revealed not statistical differences between experimental groups in all the evaluated brain areas (Fig. 8).

Discussion

In the present work, PS consequences on main areas belonging to the corticolimbic DA system of the male offspring were investigated at prepubertal and adult ages. Main findings of this study show that immobilization of the pregnant dam during the last week of gestation markedly modulates the development of the offspring DA system by inducing long-term changes on brain morphology as well as alterations on D2R and ERα receptor expression that are age and area dependent.

Effects of Prenatal Stress on Dopamine Type-2 Receptor Expression and on Prepubertal Dopaminergic System Morphology

In the mammalian brain, synapses and receptors from most brain regions are overproduced and eliminated by as much as 50 % immediately before birth, but also during the transitions from childhood to adulthood [39, 40]. The fluctuations on brain dopaminergic receptors during male puberty parallel the development and the wane of ADHD, depression, substance of abuse disorders and schizophrenia symptoms [41]. Due to the magnitude of the changes that takes place on the brain architecture during both temporal windows, higher sensitivity and increased vulnerability of perturbations were reported [17]. In this sense, the normal expression of dopaminergic receptors was shown to be influenced by in utero experience and to environmental

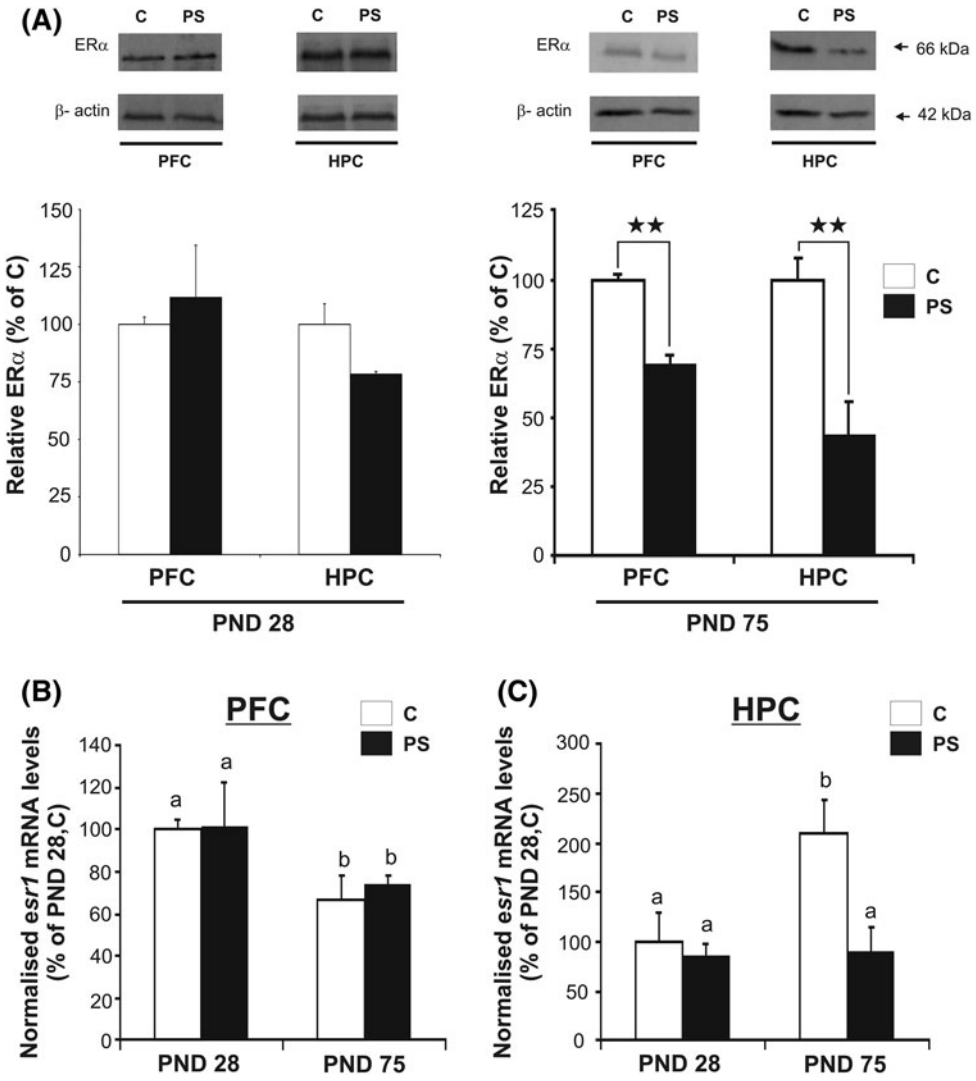

Fig. 6 Effects of prenatal stress (PS) on ERα expression in 28 and 75 days-old rats. *C* control, *PFC* prefrontal cortex, *HPC* hippocampus, *PND* postnatal day. **a** Images belong to representative ERα immunoblot for each brain area belonging to C or PS rats. The immunoreactive band for the ERα migrated to 66 kDa. ACTIN expression indicates uniform loading in each lane. *Bars* represent the mean ± SEM of the density belonging to the corresponding protein bands, expressed as the relative ERα levels in percentage of the control values. (n = 3-4; t-student statistical test). **b–c** The mRNA levels determined by real-time PCR in PFC (**b**) or HPC (**c**) are expressed relative to the housekeeping gene *beta-2 microglobulin* mRNA expression as arbitrary units and as % of PND 28 control rats. Values are reported as mean normalised*Esr1*mRNA levels ± SEM. *Different letters* depict significant differences between groups with $p < 0.05$. (n = 5–6 two-way ANOVA)

changes. It was demonstrated that PS can impair dopamine neurotransmission and metabolism [9, 42]. In this work we show that PS effects on protein D2R levels in HPC were present as early as prepubertal age where reduced receptor levels were found in prenatally stressed offspring, suggesting that hippocampal D2R expression were altered at early developmental stages. Previous work from our laboratory showed increases of DA D2R in the PFC, nucleus accumbens, caudate putamen and HPC of adult PND 90 rats subjected to prenatal stress [43]. In this study D2R protein levels were similar in control and PS adult rats (PND 75). This discrepancy with the increase found by Berger et al. [43] can be attributed to the different age and methods employed to evaluate the receptor: in this study receptor levels were quantified by Western blot, while Berger used quantitative receptor autoradiography (QAR) technique [43]. The QAR technique allows precise quantification in discrete areas with tritiated ligands as opposed to whole area homogenates with site directed antibodies. In any case, our point is that the dopaminergic impairment exerted by PS is observed even before puberty.

Dopaminergic D2-like (D2R) role in HPC was related to the formation of memory processes and to mediate the stress response [44]. Diminished D2R were found on the HPC of patients with Alzheimer disease, and such reduction was shown to be related to the memory impairments related to the disease. The reduction in the hippocampal D2R levels found in our work is in coincidence with the dysfunctional behaviours reported in prenatally stressed animals [45, 46]. Moreover, and in agreement with our findings, Bowman et al. [47] reported a reduction on the number of dopaminergic metabolites in the HPC of prenatally stressed males.

Previous work from our laboratory reported that PS induced a reduction on MAP2 immunoreactivity in PFC and HPC of 90 days old male offspring, suggesting that PS should be inducing a reduction of dendritic arborizations in such areas [28]. In addition, Martinez-Tellez et al. [48]

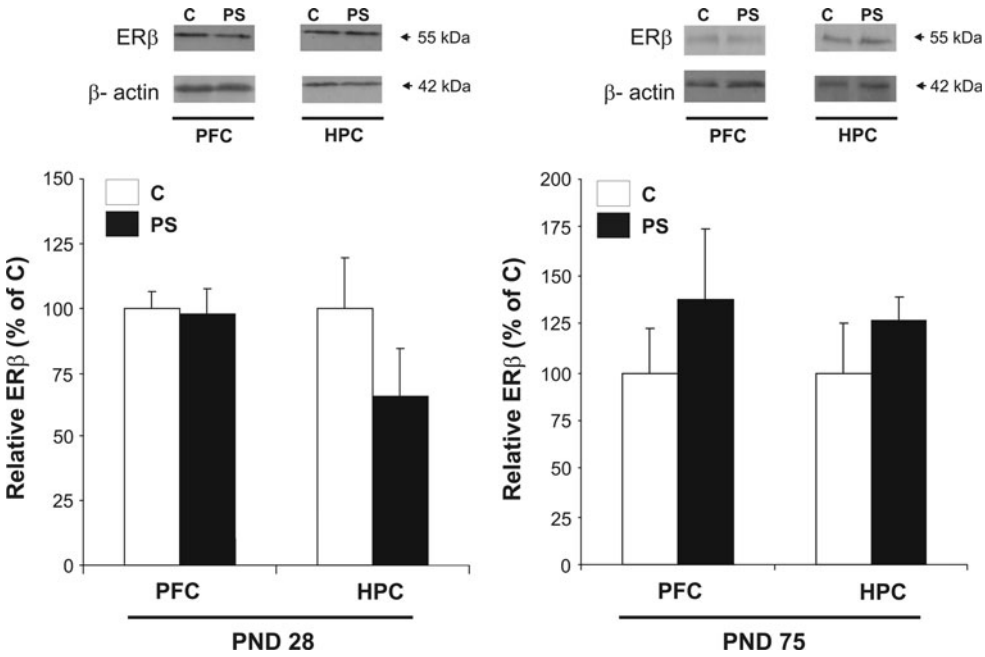

Fig. 7 Effects of prenatal stress (PS) on ERβ expression in 28 and 75 days-old rats. *C* control, *PFC* prefrontal cortex, *HPC* hippocampus, *PND* postnatal day. Images belong to representative ERβ immunoblot for each brain area belonging to C or PS rats. The immunoreactive band for the ERβ migrated to 55 kDa. ACTIN expression indicates uniform loading in each lane. *Bars* represent the mean ± SEM of the density belonging to the corresponding protein bands, expressed as the relative ERβ levels in percentage of the control values. (n = 4; t-student statistical test)

demonstrated that PS diminished the length and the complexity of the dendritic spines on the CA1 of PND 65 offspring. Therefore, it was hypothesized that prenatal stress could be an agent capable of disrupting the normal neuronal processes development that normally takes place from puberty to adult life [49], as well as altering the regulation of neuronal protein synthesis suppressing the number of synapses formation by reducing brain MAP2 synthesis. In order to extend those findings we evaluated the neural processes status of PS prepubertal offspring. The results demonstrated that PS reduced the immunoreactivity for MAP2 both in PFC and HPC suggesting that the reduction on neuronal processes previously reported by our group was present even at earlier prepubertal stages. Prenatal insults seem to be affecting the neural morphology of the mentioned areas very early in development. Moreover androgens activational role during puberty seems to be insufficient to restore such impairment.

The maturation of synaptic circuits is an essential process for normal brain functions development. Since synaptic transmission takes place in dendritic spines, changes on dendritic density or morphology could induce functional differences at synaptic levels [50]. In fact, a reduction in the length and in the complexity of the dendritic spines of the CA1 area was also associated to learning dysfunctions and a reduction on the long term potentiation on the HPC of prenatally stressed offspring [51]. On the other hand, and in coincidence with our results, it was reported that PS reduced the densities of basal and apical dendritic spines of the layer II and III on the PFC. Such reduction was associated with an increased anxious-like behaviour in prepubertal rats at both sexes [51].

Long Term Effects of Prenatal Stress on Sexual Hormones Receptor and Steroidogenic Enzymes Expresion in Dopaminergic System

Prenatal stress consequences on the morphology of sexually dimorphic brain areas, the distribution of oestrogen receptors in the hypothalamus and the sexual behaviour of the male offspring were extensively described in the literature [1, 26, 52]. However, gestational stress consequences on the sexual hormones receptor expression in extra-hypothalamic brain areas were poorly explored. Previous research from our group demonstrated that maternal stress during the last week of gestation in the rats induced long-term consequences in male offspring reproductive hormone profile by increasing total androgens concentrations in serum from prepubertal to adult ages, and by reducing testosterone concentrations in serum from adult rats [53]. Since the modulation of dopaminergic pathways by gonadal hormones has long been demonstrated, the aim of this work was to analyse the sexual hormones receptors as well as the steroidogenic enzymes expression in those corticolimbic dopaminergic areas. We found that the only isoform of the oestrogen receptor affected by PS was the alpha isoform : we observed reductions on the protein receptor levels both in PFC and HPC, and diminished mRNA receptor levels in HPC. Nevertheless, PS effects on the receptor were present only at adult stages (nor at PND 28), suggesting that in PS rats, oestrogens receptor availability should be modulated by activational hormone surges during puberty.

Despite their organizational role on the brain during early development, it was demonstrated that oestrogens

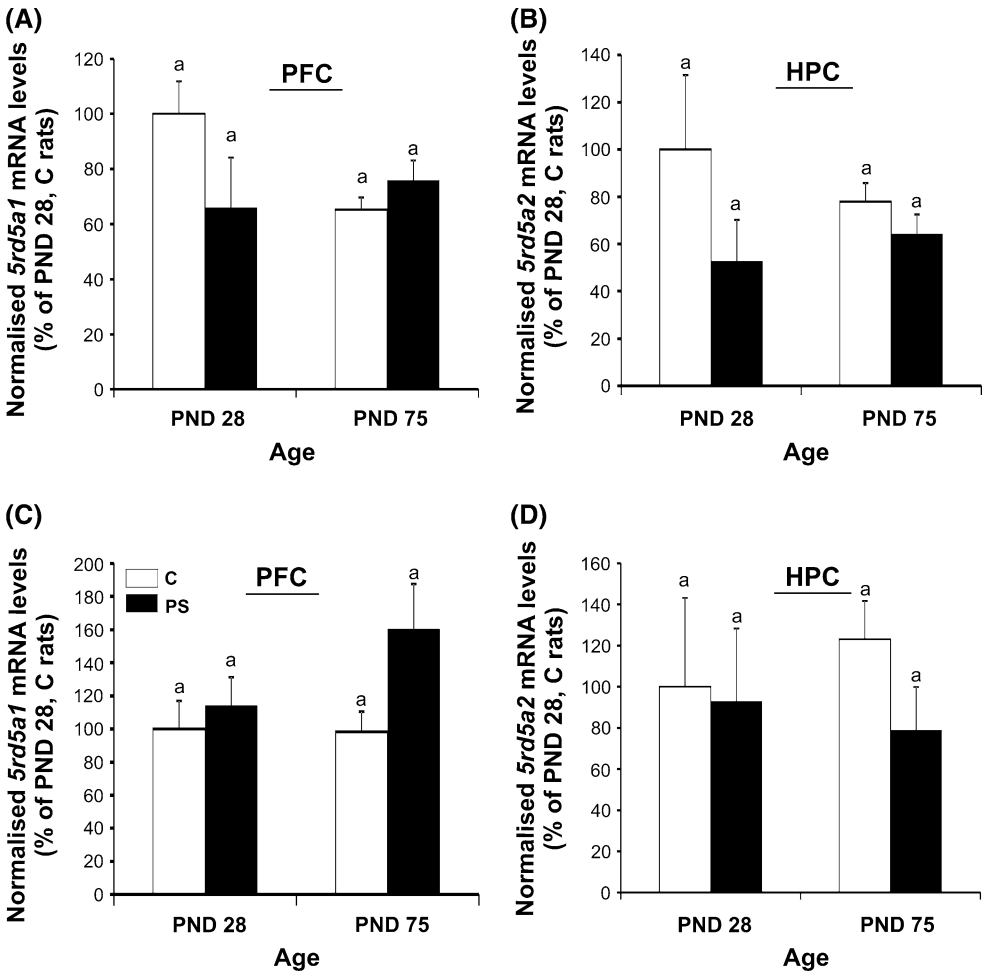

Fig. 8 Effect of prenatal stress on the steroid 5a-reductase isoform 1 (**a**–**b**) and 2 (**c**–**d**) mRNA expression in 28 and 75 days-old rats. *C* control, *PS* prenatal stress, *PND* postnatal day, *PFC* prefrontal cortex, *HPC* hippocampus. The mRNA levels determined by real-time PCR are expressed relative to the housekeeping gene *beta-2 microglobulin* mRNA expression as arbitrary units and as % of PND 28 control rats. Values are reported as mean ± SEM of the *5rd5a1* or *5rd5a2* mRNA levels in PFC (A,C) or HPC (D,E). No statistical differences were found between age, prenatal treatment or interaction between both factors. (n = 5–6; two-way ANOVA)

protect neural structures by preventing cellular death and by inducing antioxidants effects. It was also shown that oestrogens promote dendritic arborizations development and synaptogenesis processes. In this sense, during the last years their role as possible therapeutic agent on mental diseases was also explored [54–57]. Both in PFC and in HPC, oestrogen actions on cognitive modulation and neural process promotion were shown to be mediated by its interaction with the oestrogen receptor alpha isoform, whose presence were reported on neural pyramidal somas from cortical and hippocampal areas [54, 56, 57].

Abnormal oestrogen levels in brain were associated to reduced long term potentiation process and to deficient spatial and work memories on behavioural animal test [54]. It was reported that in rats, oestrogens could influence neural process development is by stimulating the expression of the brain-derived neurotrophic factor (BDNF) during brain development. BDNF is an important modulator of axonal and dendritic branches growth, as well as it influence synaptic transmission [56].

In addition to the PS consequences on learning and memory process on the progeny, and the induced alterations on the brain morphology, it was reported that PS induce a reduction on BDNF expression in PFC and HPC of the offspring [58, 59].

Therefore, beneficial oestrogens action on cognitive processes and neural morphology modulation could be lost in prenatally stressed adult offspring, since PS induce a reduction on the receptor available for hormonal action. We could hypothesised that morphological alterations observed in MAP2 processes in the PFC and HPC of PS rats could have a relationship with the altered expression of the oestrogen alpha receptor and likely, its signaling in both areas.

Finally mRNA levels of steroidogenic enzymes expression analyses revealed that only both isoform of the enzyme 5α-reductase, responsible for the metabolization of

testosterone to dihydrotestosterone, could be found on prepubertal and adult brain homogenised of control and PS rats. Prenatal stress did not induce changes on mRNA enzymes expression, suggesting that the sexual hormones capable to modulate PS effects on dopaminergic areas should be from gonadal origin and not produced by local brain synthesis.

Conclusions

The last week of gestation in rat constitute a critical period of DA development. The results obtained in this work demonstrates that stress exerted on the pregnant dam during this vulnerable period produces long-lasting consequences on offspring corticolimbic DA that might be an important morphological and neurochemical basis for the development of the behavioral and learning impairment previously reported in prenatally stressed rats. PS rats show decreased MAP2 immunoreactivity at prepubertal ages, suggesting that some plastic processes should be programmed prenatally and the increase in sexual hormones during puberty did not induce significant changes over the altered neural morphology found on the prenatally stressed progeny. On the other hand, D2R and ERα receptor turn-over presented variations whether if they were evaluated before or after puberty. Even though receptor availability is programmed during foetal stages, prenatal stress can modify this programme that can be further altered under the influence of the hormonal pattern in turn modified by the prenatal insult.

Acknowledgments We are grateful to Dr. Trinidad Saez for her help in image analysis of the data and to Dr. Sandra Zárate for her skillful technical assistance in the Western blot studies. We are also indebted to Martin Brahamian and Mercedes Imsem for their help and supervision of the rats management and care. The skillful technical assistance and bibliographical managements of Mrs Susana Buglione is greatly appreciated. This research was supported by grants from CONICET (PIP 2065) and ANPCYT (PICT 31981 and PICT 0040). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Weinstock M (2001) Alterations induced by gestational stress in brain morphology and behaviour of the offspring. Prog Neurobiol 65(5):427–451
2. Mastorci F, Vicentini M, Viltart O, Manghi M, Graiani G, Quaini F, Meerlo P, Nalivaiko E, Maccari S, Sgoifo A (2009) Long-term effects of prenatal stress: changes in adult cardiovascular regulation and sensitivity to stress. Neurosci Biobehav Rev 33(2): 191–203. doi:10.1016/j.neubiorev.2008.08.001
3. Weinstock M (2008) The long-term behavioural consequences of prenatal stress. Neurosci Biobehav Rev 32(6):1073–1086
4. Huizink AC, Mulder EJ, Buitelaar JK (2004) Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychol Bull 130(1):115–142
5. Darnaudery M, Maccari S (2008) Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Res Rev 57(2):571–585
6. Biederman J (2005) Attention-deficit/hyperactivity disorder: a selective overview. Biol Psychiatry 57(11):1215–1220. doi:10.1016/j.biopsych.2004.10.020
7. Baier CJ, Katunar MR, Adrover E, Pallares ME, Antonelli MC (2012) Gestational restraint stress and the developing dopaminergic system: an overview. Neurotox Res. doi:10.1007/s12640-011-9305-4
8. Henry C, Guegant G, Cador M, Arnauld E, Arsaut J, Le Moal M, Demotes-Mainard J (1995) Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. Brain Res 685(1–2):179–186
9. Diaz R, Fuxe K, Ogren SO (1997) Prenatal corticosterone treatment induces long-term changes in spontaneous and apomorphine-mediated motor activity in male and female rats. Neuroscience 81(1):129–140
10. Silvagni A, Barros VG, Mura C, Antonelli MC, Carboni E (2008) Prenatal restraint stress differentially modifies basal and stimulated dopamine and noradrenaline release in the nucleus accumbens shell: an 'in vivo' microdialysis study in adolescent and young adult rats. Eur J Neurosci 28(4):744–758. doi:10.1111/j.1460-9568.2008.06364.x
11. Carboni E, Barros VG, Ibba M, Silvagni A, Mura C, Antonelli MC (2010) Prenatal restraint stress: an in vivo microdialysis study on catecholamine release in the rat prefrontal cortex. Neuroscience 168(1):156–166. doi:10.1016/j.neuroscience.2010.03.046
12. Katunar M, Saez T, Brusco A, Antonelli M (2010) Ontogenetic expression of dopamine-related transcription factors and tyrosine hydroxylase in prenatally stressed rats. Neurotox Res 18(1):69–81
13. Nussey S, Whitehead S (2001) Endocrinology, an integrated approach. Oxford Bios Scientific Pulishers, Oxford
14. MacLusky NJ, Hajszan T, Prange-Kiel J, Leranth C (2006) Androgen modulation of hippocampal synaptic plasticity. Neuroscience 138(3):957–965. doi:10.1016/j.neuroscience.2005.12.054
15. Sato SM, Schulz KM, Sisk CL, Wood RI (2008) Adolescents and androgens, receptors and rewards. Horm Behav 53(5):647–658. doi:10.1016/j.yhbeh.2008.01.010
16. Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24(4):417–463
17. Andersen SL (2003) Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev 27(1–2):3–18
18. Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9(12): 947–957. doi:10.1038/nrn2513
19. Yang SC, Shieh KR (2007) Gonadal hormones-mediated effects on the stimulation of dopamine turnover in mesolimbic and nigrostriatal systems by cocaine- and amphetamine-regulated transcript (CART) peptide in male rats. Neuropharmacology 53(7):801–809. doi:10.1016/j.neuropharm.2007.08.007
20. Creutz LM, Kritzer MF (2004) Mesostriatal and mesolimbic projections of midbrain neurons immunoreactive for estrogen receptor beta or androgen receptors in rats. J Comp Neurol 476(4):348–362. doi:10.1002/cne.20229
21. Alonso R, Lopez-Coviella I (1998) Gonadal steroids and neuronal function. Neurochem Res 23(5):675–688
22. Weisz J, Ward IL (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106(1):306–316
23. Ward OB, Ward IL, Denning JH, Hendricks SE, French JA (2002) Hormonal mechanisms underlying aberrant sexual differentiation

in male rats prenatally exposed to alcohol, stress, or both. Arch Sex Behav 31(1):9–16
24. Scott HM, Mason JI, Sharpe RM (2009) Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev 30(7):883–925. doi:10.1210/er.2009-0016
25. Shono T, Suita S (2003) Disturbed pituitary-testicular axis inhibits testicular descent in the prenatal rat. BJU Int 92(6): 641–643
26. Gerardin DC, Pereira OC, Kempinas WG, Florio JC, Moreira EG, Bernardi MM (2005) Sexual behavior, neuroendocrine, and neurochemical aspects in male rats exposed prenatally to stress. Physiol Behav 84(1):97–104. doi:10.1016/j.physbeh.2004.10.014
27. Pereira OC, Bernardi MM, Gerardin DC (2006) Could neonatal testosterone replacement prevent alterations induced by prenatal stress in male rats? Life Sci 78(24):2767–2771. doi:10.1016/j.lfs.2005.10.035
28. Barros VG, Rodriguez P, Martijena ID, Perez A, Molina VA, Antonelli MC (2006) Prenatal stress and early adoption effects on benzodiazepine receptors and anxiogenic behavior in the adult rat brain. Synapse 60(8):609–618
29. Rodriguez N, Mayer N, Gauna HF (2007) Effects of prenatal stress on male offspring sexual maturity. Biocell 31(1):67–74
30. Pallares ME, Adrover E, Baier CJ, Bourguignon NS, Monteleone MC, Brocco MA, Gonzalez-Calvar SI, Antonelli MC (2013) Prenatal maternal restraint stress exposure alters the reproductive hormone profile and testis development of the rat male offspring. Stress 16(4):429–440. doi:10.3109/10253890.2012.761195
31. Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M (1995) Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J Neurosci 15(1 Pt 1):110–116
32. Ward IL, Weisz J (1984) Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology 114(5):1635–1644
33. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Harcourt Brace Jovanovich Publishers, San Diego
34. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
35. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680–685
36. Biosystems A (2004) Guide to performing relative quantitation of gene expression using real-time quantitative PCR
37. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29(9):e45
38. Ramos AJ, Tagliaferro P, Lopez EM, Pecci Saavedra J, Brusco A (2000) Neuroglial interactions in a model of para-chlorophenylalanine-induced serotonin depletion. Brain Res 883(1):1–14
39. Seeman P, Bzowej NH, Guan HC, Bergeron C, Becker LE, Reynolds GP, Bird ED, Riederer P, Jellinger K, Watanabe S et al (1987) Human brain dopamine receptors in children and aging adults. Synapse 1(5):399–404. doi:10.1002/syn.890010503
40. Teicher MH, Andersen SL, Hostetter JC Jr (1995) Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Brain Res Dev Brain Res 89(2):167–172
41. Andersen SL, Thompson AP, Krenzel E, Teicher MH (2002) Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. Psychoneuroendocrinology 27(6):683–691
42. Fride E, Weinstock M (1989) Alterations in behavioral and striatal dopamine asymmetries induced by prenatal stress. Pharmacol Biochem Behav 32(2):425–430
43. Berger MA, Barros VG, Sarchi MI, Tarazi FI, Antonelli MC (2002) Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. Neurochem Res 27(11): 1525–1533
44. Kamei H, Kameyama T, Nabeshima T (1995) Activation of both dopamine D1 and D2 receptors necessary for amelioration of conditioned fear stress. Eur J Pharmacol 273(3):229–233
45. Laloux C, Mairesse J, Van Camp G, Giovine A, Branchi I, Bouret S, Morley-Fletcher S, Bergonzelli G, Malagodi M, Gradini R, Nicoletti F, Darnaudery M, Maccari S (2012) Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. Psychoneuroendocrinology 37(10):1646–1658. doi:10.1016/j.psyneuen.2012.02.010
46. Morley-Fletcher S, Rea M, Maccari S, Laviola G (2003) Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur J Neurosci 18(12):3367–3374
47. Bowman RE, MacLusky NJ, Sarmiento Y, Frankfurt M, Gordon M, Luine VN (2004) Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology 145(8):3778–3787
48. Martinez-Tellez RI, Hernandez-Torres E, Gamboa C, Flores G (2009) Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring. Synapse 63(9):794–804. doi:10.1002/syn.20664
49. Huttenlocher PR (1979) Synaptic density in human frontal cortex—developmental changes and effects of aging. Brain Res 163(2):195–205
50. Segal M (2010) Dendritic spines, synaptic plasticity and neuronal survival: activity shapes dendritic spines to enhance neuronal viability. Eur J Neurosci 31(12):2178–2184. doi:10.1111/j.1460-9568.2010.07270.x
51. Weinstock M (2011) Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. Stress 14(6):604–613. doi:10.3109/10253890.2011.588294
52. Henry C, Arsaut J, Arnauld E, Demotes-Mainard J (1996) Transient neonatal elevation in hypothalamic estrogen receptor mRNA in prenatally-stressed male rats. Neurosci Lett 216(2): 141–145
53. Pallares ME, Adrover E, Baier CJ, Bourguignon NS, Monteleone MC, Brocco MA, Gonzalez-Calvar SI, Antonelli MC (2012) Prenatal maternal restraint stress exposure alters the reproductive hormone profile and testis development of the rat male offspring. Stress. doi:10.3109/10253890.2012.761195
54. McEwen BS, Alves SE (1999) Estrogen actions in the central nervous system. Endocr Rev 20(3):279–307
55. Kuppers E, Ivanova T, Karolczak M, Beyer C (2000) Estrogen: a multifunctional messenger to nigrostriatal dopaminergic neurons. J Neurocytol 29(5–6):375–385
56. Solum DT, Handa RJ (2002) Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus. J Neurosci 22(7):2650–2659
57. Wilson ME, Westberry JM, Trout AL (2011) Estrogen receptor-alpha gene expression in the cortex: sex differences during development and in adulthood. Horm Behav 59(3):353–357. doi:10.1016/j.yhbeh.2010.08.004
58. Van den Hove DL, Steinbusch HW, Scheepens A, Van de Berg WD, Kooiman LA, Boosten BJ, Prickaerts J, Blanco CE (2006) Prenatal stress and neonatal rat brain development. Neuroscience 137(1):145–155. doi:10.1016/j.neuroscience.2005.08.060
59. Fumagalli F, Bedogni F, Perez J, Racagni G, Riva MA (2004) Corticostriatal brain-derived neurotrophic factor dysregulation in adult rats following prenatal stress. Eur J Neurosci 20(5): 1348–1354