Serotonin and noradrenaline content and release in the dorsal hippocampus during learning and spatial memory in prenatally stressed rats

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Prenatal stress causes learning and spatial memory deficits in adulthood by modifying hippocampal function. The dorsal hippocampus contains serotonergic and noradrenergic neuron terminals, which are related to cognitive processes. It is currently unknown whether prenatal stress modifies serotonin (5-HT) and noradrenaline (NA) content and their release in the hippocampus during cognitive performance. Therefore, we measured these variables in the dorsal hippocampus of prenatally stressed males during spatial learning and memory tests. Cognitive tests were performed in 3-month-old control and prenatally stressed male rats in the Morris water maze. After cognitive tests, the dorsal hippocampus was dissected to quantify 5-HT and NA content. In other males, 5-HT and NA release in the dorsal hippocampus was assessed by microdialysis, before and after cognitive tests. Prenatally stressed males showed longer latencies to reach the platform, compared to control animals. Hippocampal 5-HT content decreased during learning and memory tasks in both groups, while NA content was not modified in prenatally stressed males neither before, nor after learning and memory tests. 5-HT and NA release were significantly lower in prenatally stressed animals during spatial learning and memory tasks. Corticosterone response was greater in prenatally stressed animals compared to controls. These results show that cognitive disruption caused by prenatal stress is related to decreased 5-HT and NA release, and to higher adrenal axis response in prenatally stressed animals.

Key words: learning, spatial memory, prenatal stress, serotonin, noradrenaline, dorsal hippocampus

INTRODUCTION

In rodents, maternal stress during pregnancy can cause psychopathologies in the offspring, such as anxiety and depressive-like behaviors, as well as cognitive deficits (Weinstock, 2017). Also, alterations in development and maturation of brain structures, such as the hippocampus, have been reported (Fujioka et al., 2006). The dorsal hippocampus is involved in spatial learning and memory processes in rats and primates (Bannerman et al., 2004; Tanti and Belzung, 2013; Grigoryan and Segal, 2016), specifically, regions CA1, CA3 and the dentate gyrus (DG) (Griffin et al., 2007). Lesions of this brain structure (Lee and Kesner, 2003) or prenatal stress (Aleksandrov et al., 2001; Akatsu et al., 2015; Guerrero et al., 2016; Weinstock, 2017) cause deficits in learning and in short-term spatial memory in adult rodents. The hippocampus contains dense noradrenergic fibers (Vizi and Kiss, 1998) coming from the locus coeruleus (LC) which are located in the dentate gyrus (DG) of the hippocampus (Palacios-Filardo

and Mellor, 2019). Prenatal stress by exposure to a cold environment decreases tyrosine hydroxylase immunoreactivity in LC neurons of young rats, thus disturbing the development of noradrenergic neurons (Bock et al., 2015; Ikemoto et al., 2015). It has been reported that noradrenaline (NA) is important for hippocampus-dependent memory retrieval, but not for acquisition and short-term memory in the Morris water maze (MWM). These effects of NA are mediated by β_1-adrenergic receptors (Murchinson et al., 2004; Schimanski et al., 2007). Prenatal stress by malnutrition (Flores et al., 2011) or immobilization (Grigoryan and Segal, 2013) down regulates β-adrenergic receptors and decreases the affinity of β receptors to noradrenergic agonists in the dorsal hippocampus of rats (Grigoryan and Segal, 2013). Furthermore, NA content decreases in the hippocampus of offspring prenatally stressed by chronic unpredictable mild stress (Guan et al., 2017). Serotonin (5-HT) is another neurotransmitter involved in learning and memory processes (Killa and Manahan-Vaughan, 2002), and is found in large quantities in the hippocampus (Glikman-Johnston et al., 2015), mainly in CA1, CA2, CA3 and DG regions (Moore and Halaris, 1975). The serotonergic system also plays a role in learning and memory with four 5-HT receptor subtypes: 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_7 located along the dorsal-ventral axis of the hippocampus (Tanaka et al., 2012), and disruption of spatial learning in the offspring caused by prenatal stress is related to decreased mRNA expression of those same receptors in the hippocampus (Akastu et al., 2015). The increase of extracellular 5-HT concentration, either by pharmacologically stimulating its release or by blocking its reuptake, improves or maintains memory performance, while reduced 5HT levels impair spatial memory in rats (Glikman-Johnston et al., 2015). Optogenetic activation of serotonergic terminals in the hippocampal pyramidal neurons of the CA1 region potentiates excitatory transmission at CA3-to-CA1 synapses and enhances spatial memory. In contrast, optogenetic silencing of CA1 5-HT terminals inhibits spatial memory in mice (Texeira et al., 2018). Regarding hippocampal 5-HT content, the results are contradictory, for example, some studies report decreased 5-HT content in the offspring of dams exposed to stress by crowding (Hayashi et al, 1998) or chronic unpredictable mild stress (Guan et al., 2017) during pregnancy, with higher levels of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and increased metabolic rate (Hayashi et al, 1998). However, other studies did not find changes in hippocampal 5-HT content in offspring from mothers stressed by restraint (Gemmel et al., 2016). Another study, reports increased 5-HT content in the hippocampus of fetuses from dams stressed by chronic unpredictable stress,

decreased 5-HIAA, 5-HIAA-5-HT ratio, 5-HT transporter, and 5-HT_{1A} (Huang et al, 2012). Nonetheless, 5-HT and 5-HIAA content assessment does not necessarily reflect what is really happening with the release of neurotransmitters, and it is not clear whether the alterations in learning and memory processes in prenatally stressed animals are related to changes in the release of NA and 5-HT. Therefore, the objective of this study was to evaluate the content and release of 5-HT and NA in the dorsal hippocampus during learning and spatial memory in adult prenatally stressed rats. Baseline activity of the adrenal axis and its response to learning and spatial memory tests were also evaluated.

METHODS

Subjects

Three-month-old female Wistar rats, weighing 200-250 g obtained from the vivarium of the Autonomous Metropolitan University were kept under controlled temperature conditions (23±2°C) with an inverted light-dark cycle, 12/12 (lights off at 9:00 am), with water and food *ad libitum.* Pregnant females were randomly assigned to control (n=20) or maternal stress (n=20) groups (Fig. 1). Control females remained unaltered during pregnancy, except for daily weighing. The experimental procedures were carried out in accordance with Mexican Official Regulations (NOM-062-ZOO-1999) and the guidelines for ethical research, teaching and dissemination of the Biological Sciences Division of the Autonomous Metropolitan University, Iztapalapa. This study was approved by the Ethics Commission of the Biology and Health Sciences Division of the Autonomous Metropolitan University.

Stress procedure

Pregnant females assigned to maternal stress were exposed to stress by cold water immersion twice a day (9:00 a.m. and 15:00 p.m.), during the last week of gestation (day 15 to 21). Dams were placed in tanks filled with water at 15°C, 15 cm depth, for 15 min. Once this time elapsed, rats were picked out from water, dried with a towel and returned to their individual cages (Retana-Márquez et al, 2009). Exposure to the stressor was performed in a room other than the housing room.

After birth, litters from each experimental group were homogenized in number, sexed, and weighed. On postnatal day 22, offspring were weaned and males from both groups were separated from females. About 50 % of descendants in both groups were males, which were
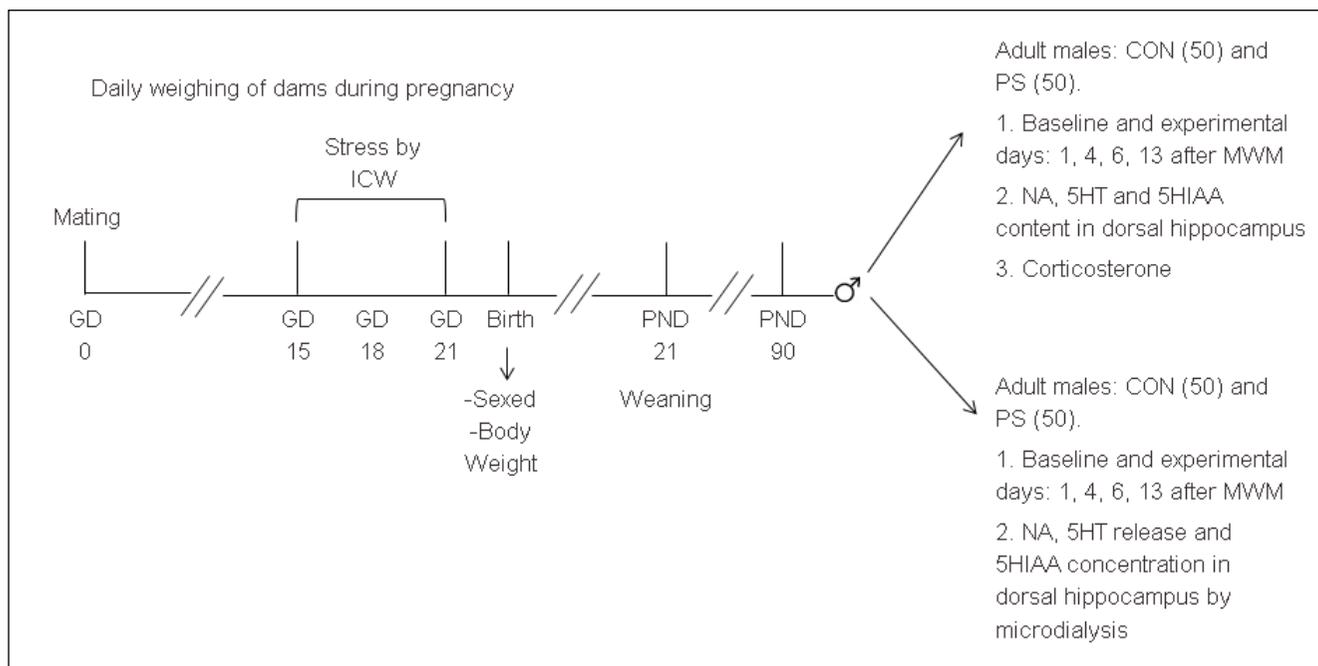
Fig. 1. Timeline of experimental design. Females were stressed by immersion in cold water (ICW) from gestational days (GD) 15-21. Weaning occurred at postnatal day (PND) 21. Serotonin (5-HT) and its metabolite, 5-Hidroxyindoleacetic acid (5-HIAA), as well as noradrenaline (NA) were assessed in control (CON) and prenatally stressed (PS) males at baseline and after the Morris water maze (MWM) in homogenates and dialysates on days 1, 4, 6, and 13.

used for this study. In order to avoid litter effects, one rat from each of twenty litters per group was tested in each experimental day, which is appropriate for studies using mammals that have litters (Holson and Pearce, 1992; Williams et al, 2017). Therefore, 10 (non-siblings) prenatally stressed or control males were used separately for each analyzed day.

Behavioral tests

Spatial learning and memory tests were performed using the MWM in adult (3 months) control and prenatally stressed (n=50; baseline and experimental days: 1, 4, 6, 13, n=10, each group) male offspring. A circular pool (170 cm in diameter and 70 cm in height) was used; water height 30 cm, temperature 22±2°C. Four imaginary quadrants (NE, NW, SE, and SW) divided the pool, and subjects were released from different starting points in each trial and each day. Three contrasting figures used as spatial cues were placed on the white walls surrounding the pool. A transparent acrylic platform (18 cm × 18 cm) was placed inside the pool, in one of the quadrants 2 cm below the surface of the water. The behavioral parameter analyzed was escape latency: time the rat takes to find the submerged platform in the learning phase (days 1-4), and in the memory phase (days 6 and 13) (Kapoor et al, 2009; Nazeri et al, 2015;

Guerrero et al, 2016). During learning sessions (days 1 - 4), four trials per session were performed. For the first trial on day 1, if a rat did not find the platform within a 60 s period, the experimenter guided the rat to the platform and left it on the platform for 30 s (Kapoor et al, 2009). When rats found the platform within the 60 s period, they were left there for 30 s. For memory sessions, a single trial was carried out and latencies were recorded (Guerrero et al, 2016).

5-HT, 5-HIAA, and NA hippocampal content

Immediately after behavioral tests, control and prenatally stressed males were euthanized by decapitation on days 1, 4 (learning), 6, and 13 (memory). The right dorsal hippocampus was dissected and frozen (-83°C) until evaluation. 300 µL of perchloric acid (0.1 M) were added to thawed samples, homogenized with a plunger, kept on ice and covered, to prevent degradation due to light. Samples were centrifuged for 15 min at 8000 rpm at 4°C. The supernatant was filtered with MF™ nitrocellulose membranes (Millipore, Merck, Ireland), 0.45 µm pore, and 10 µL of the filtered sample were injected to a high-performance liquid chromatography system with electrochemical detection (HPLC-ED). Trunk blood was also collected for serum corticosterone quantification.

Microdialysis procedure

In other control and prenatally stressed males (n=50, each), stereotaxic surgeries were performed. Rats were anesthetized with ketamine (PiSA® S.A. DE C.V. Mexico, 8 mg/kg, i.p.) and xylazine (PiSA® S.A. DE C. V. Mexico, 20 mg/kg, i.m.). A guide cannula was directed to the right dorsal hippocampus: antero/posterior (A/P) -5.3 mm from bregma, medial/lateral to midline (M/L) -5.2 mm, and dorsal/ventral from dura (D/V) -3.0 mm (Paxinos and Watson, 2007). Four stainless steel screws were placed on the skull and the implant was fixed with dental acrylic. The animals were allowed to recover for 10 days after surgery. Immediately after behavioral tests on days 1, 4, 7 and 13, the microdialysis probe (polyacrylonitrile membrane, pore size: 40,000 D), was introduced, protruding 3 mm below the guide cannula, in the dorsal hippocampus, and fixed to the skull with dental acrylic. Ringer's solution was used (0.5 μL/min flow rate) and dialysates were collected every hour to obtain an adequate volume (30 μL, each). Two dialysates were collected from each rat. Samples (10 μL) were injected to the HPLC-ED. At the end of microdialysis, brains were obtained, and cannula position was verified. No misplaced cannula was found; therefore, no male was excluded.

Biochemical procedures

Amine detection

Samples were injected into the HPLC-DE chromatographic system with a Rheodyne (Waters Corp., Milford, MA, USA). A precolumn (Symmetry C18, particle size 3.5 μm), 2.1 × 10 mm Waters Corp., Milford, MA, USA) was used. Analytes were separated in a Symmetry C18 column (particle size 5 μm) 2.1 × 150 mm (Waters Corp., Milford, MA, USA). Phosphate mobile phase (pH=3.1) at a flow of 0.2 mL/min was delivered with a 515 pump (Waters Corp., Milford, MA, USA). Analytes were detected with an electrochemical detector 2465 (Waters Corp., Milford, MA, USA) at a sensitivity of 5 nA and +800 mV power. The results were analyzed with the Millennium 32 program (Waters Corp., Milford, MA, USA).

Statistical analysis

Data are shown as mean ± standard error of the mean (SEM). Body weight gain in pregnant females was analyzed by linear regression. Body weight of male pups, litter size and number of male descendants were ana-

lyzed with Student's t-test. Data from MWM, serum corticosterone levels, 5-HT, 5-HIAA, NA content and release were analyzed by two-way ANOVA (condition and days as factors) followed by Tukey's *post hoc* test. Correlations for arrival latencies in the MWM *vs.* the content, amine release and corticosterone were analyzed by Pearson's test. The differences between the groups were considered significant when $P<0.05$. GraphPad PRISM version 6.01 statistical software (GraphPad Software Inc., USA) was used for statistical analysis.

Corticosterone assessment

Trunk blood samples were taken from all the subjects for corticosterone liquid-liquid extraction (Woodward and Emery, 1987) and quantification at baseline and after behavioral tests. For steroid extraction, serum (1 mL) was mixed with 100 μL of a solution of 19-nortestosterone (5 μg/mL in water/methanol) as an internal standard. A mixture of diethyl ether-dichloromethane (5 mL, 60:40 v/v) was used for corticosterone extraction, vortexed for 1 min and centrifuged for 5 min at 800 rpm. The organic phase was obtained and mixed with 1 mL of HPLC-grade water, stirred for 1 min and centrifuged for 5 min. The organic phase was obtained (3 mL) and evaporated at room temperature. The residue was re-dissolved in 100 μL of methanol-water (60:40 v/v). Corticosterone was separated and detected in a HPLC-UV system with a precolumn (Symmetry C18, particle size 3.5 μm, 2.1 × 10 mm (Waters Corp., Milford, MA, USA). The separation was made at 40°C in a Waters Symmetry C18 column (particle size 5 μm) 2.0 × 150 mm; Waters Corp., Milford, MA, USA with a water-acetonitrile mixture, HPLC grade (65:35 v/v), at a flow rate of 0.4 mL/min. A 600-MS controller system was used for the mobile phase and the steroids were detected with a UV detector (model 486, Waters Corp., Milford MA at a 250 nm wavelength.) The results were analyzed using the Millennium 32 program (Waters Corp., Milford, MA, USA).

RESULTS

Body weight gain in pregnant females

Average body weight gain in stressed mothers was lower than in control dams (t_{38}=4.59, P=0.0059). The correlation coefficient (r^2) for the control curve was 0.9970, and r^2 for stressed dams was 0.9898. The slopes of the curves were different (P=0.01891) (Fig. 2). Birth weights of prenatally stressed male offspring were lower than those of control offspring (t_{98}=3.628; P=0.0005).
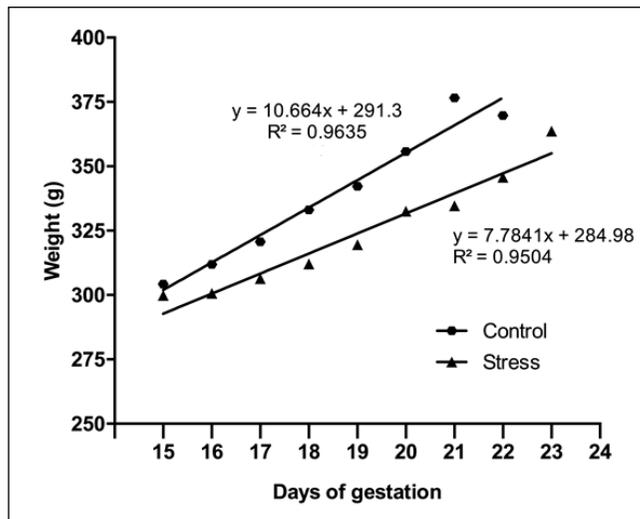
Fig. 2. Body weight of pregnant females on gestational days 15 to 24. The linear regression analysis shows differences between the slopes of the control group *vs.* the stress group p=0.01891. Correlation coefficient for control curve: 0.9970. Correlation coefficient for PS curve: 0.9898. The slopes indicate the average increase in body weight per day; the weight of the stressed dams was significantly lower than that of control dams (p=0.01), n=10 per group.

No differences were observed in litter size or in average number of females and males in both groups.

Spatial learning and memory

Arrival latencies to the platform in MWM decreased progressively during learning days (1-4) in control animals, and remained low on days 6 and 13 of memory evaluation. In contrast, prenatally stressed subjects showed higher arrival latencies than those in the control group on all test days [$F_{4,90}$=21.244; P=0.007], (Fig. 3A). The average escape latencies in prenatally stressed males were significantly higher than those in the control group [t_{98}=10.90; P=0.0001] (Fig. 3B).

5-HT, 5HIAA content and release in the dorsal hippocampus

Baseline content of 5-HT in the dorsal hippocampus of prenatally stressed animals was similar to control, and decreased in both groups during learning (1-4) and memory (7 and 13) days [$F_{4,90}$=28.25; P=0.0001] (Fig. 4A). Baseline 5-HIAA content and after learning and memory tests were also similar in both groups, and it also decreased in both groups during learning and memory days [$F_{4,90}$=16.85; P=0.0001] (Fig. 4B).

Baseline 5-HT release in the dorsal hippocampus of prenatally stressed rats was lower than in control males. On learning days (1 and 4), 5-HT release in the dorsal hippocampus of control animals increased about 6.5 times over its baseline and remained high after memory tests. In contrast, during learning and memory tests, 5-HT release increased slightly in prenatally stressed males, but to a lesser degree than in control males [$F_{4,90}$=434.8 P=0.0001] (Fig. 5A). With respect to extracellular 5-HIAA, baseline levels were similar in both groups. After learning and
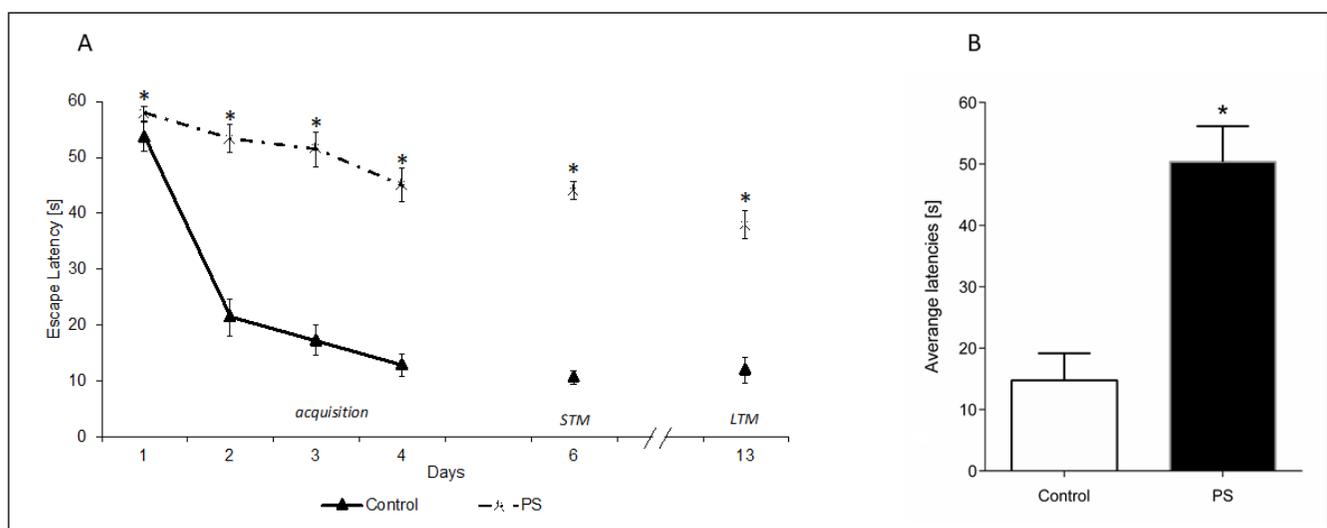

Fig. 3. (A) Escape latencies in the Morris water maze (MWM). Prenatally stressed animals showed higher arrival latencies than control animals on all days of evaluation of the spatial learning and memory tests. Repeated measures ANOVA, * p=0.007 compared to control group. (B) Average escape latencies in control and prenatally stressed animals. PS subjects had higher latencies than control males. Data shown as Mean ± S.E.M. t-student * p=0.04 compared with control. STM: short-term memory; LTM: long term memory.

memory tests, extracellular 5-HIAA increased in the dorsal hippocampus of control rats, but there were no changes in prenatally stressed rats [$F_{4,90}=64.06$; $P=0.0091$] (Fig. 5B). 5-HT release during learning correlated negatively with arrival latencies in the control rats in the MWM ($r=-0.9789$, $P=0.001$). In prenatal-
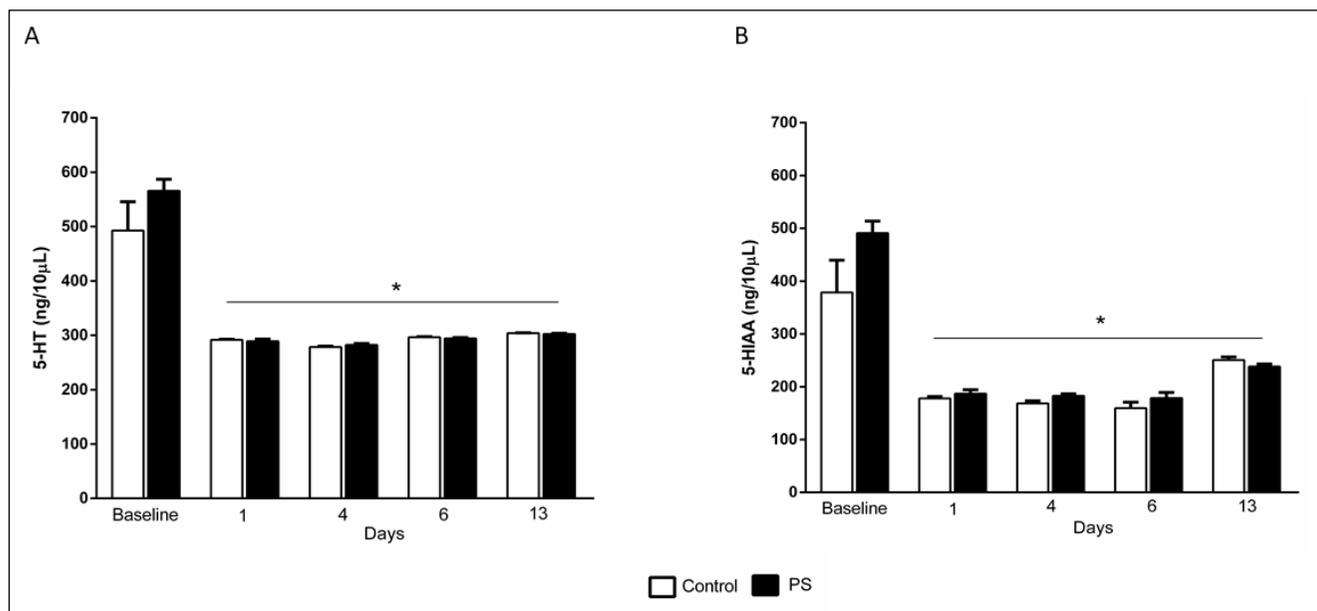

Fig. 4. (A) 5-HT content in the dorsal hippocampus in control and prenatally stressed males. (A) Baseline 5-HT concentrations were similar in both groups and decreased similarly after learning (days 1-4), short (day 7), and long term (day 13) memory tests. (B) 5-HIAA content was also similar in both groups and decreased in the same way on the days of learning and memory assessment. Data shown as Mean ± S.E.M. Two-way ANOVA (p=0.0001). * p=0.0003 compared with baseline content from both groups. Control group: n=10, each day. Prenatally stressed males: n=10 each day.
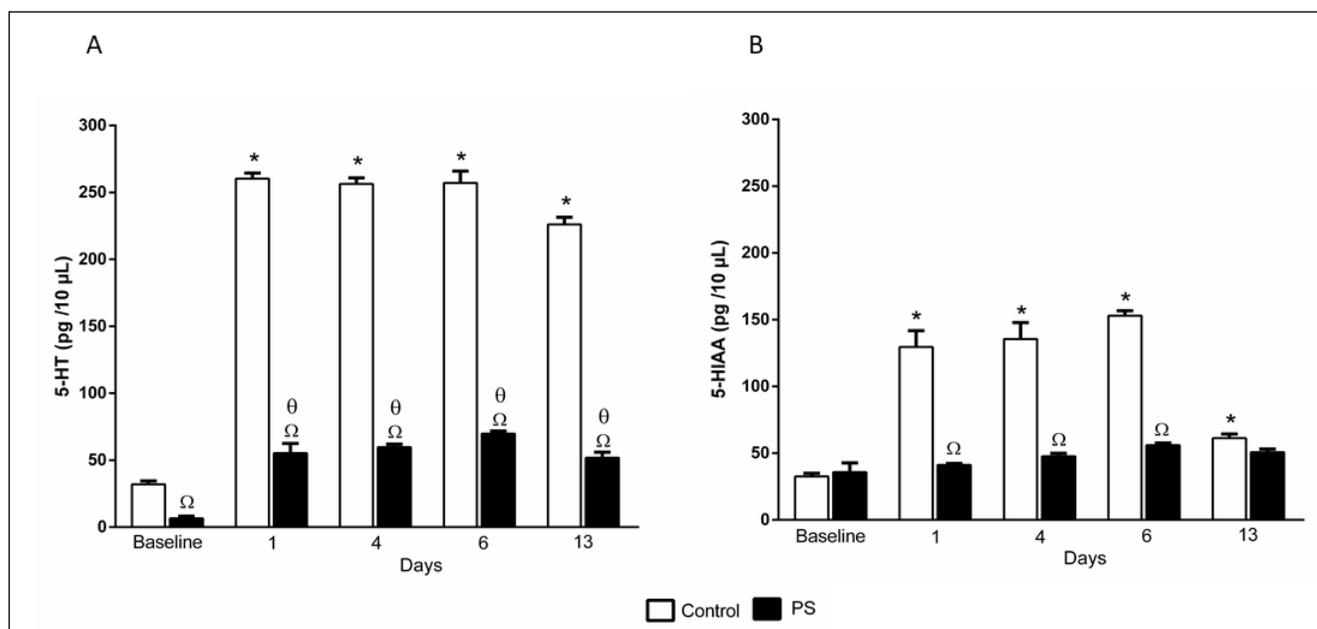

Fig. 5. 5-HT release at baseline and after learning tests (Days 1 and 4) and after short- (day 7) and long-term (day 13) memory tests. 5-HT release in the hippocampus of prenatally stressed rats was lower than in the controls both at baseline and during learning and memory tests. (B) Baseline extracellular 5-HIAA and after learning and memory tests in the dorsal hippocampus of control and prenatally stressed rats. The baseline concentrations were similar in both groups, but after the learning and memory tests, the metabolite concentrations increased in the control group only. Data shown as Mean ± S.E.M. Two-way ANOVA, * p=0.005 compared with control baseline; Ω p=0.046 compared with control of each day; θ p=0.001 compared with the PS baseline. Control group: n=10, each day. Prenatally stressed males: n=10 each day.

ly stressed rats, a negative correlation was observed between arrival latencies in the MWM during learning and 5-HT release (r=-0.8940, P=0.01). In addition, baseline 5-HT release correlated negatively with baseline corticosterone concentrations in prenatally stressed rats (r=-0.9884, P=0.001). Similarly, the increase in corticosterone during learning (r=-0.7576, P=0.05) and memory (r=-0.9487, P=0.001) in the MWM correlated negatively with low 5-HT release in prenatally stressed rats.

NA content and release in the dorsal hippocampus

Baseline NA content was significantly lower in the dorsal hippocampus of prenatally stressed animals, compared to controls. During learning and memory tests, NA content decreased significantly in the control subjects, with respect to baseline levels ($F_{4,90}$=32.44; P=0.0010). In contrast, no changes in NA content were observed in prenatally stressed males after learning, short- and long-term memory (Fig. 6).

Baseline release of NA in the dorsal hippocampus was lower in prenatally stressed rats compared to controls. After learning and memory tests, NA release increased significantly in control rats with respect to baseline levels ($F_{4,90}$=60.88; P=0.0001), but not in prenatally stressed rats, in which no change in NA release was observed, neither after learning nor after memory tests (Fig. 7).

The release of NA in the control group correlated negatively with arrival latencies in the long-term memory test (r=-0.9121, P=0.01). On the other hand, arrival latencies in prenatally stressed rats in the MWM correlated negatively with the release of NA (r=-0.9361, P=0.01). In a similar way to 5-HT, corticosterone concentrations correlated negatively with NA release, both on learning days (r=-0.9961, P=0.001) and on long-term memory days (r=-0.9999, P=0.001).

Corticosterone levels

Baseline serum corticosterone concentrations in prenatally stressed animals were significantly higher than those in the control animals. After learning and memory tests in the MWM, corticosterone levels increased significantly in both groups, but were higher in prenatally stressed animals [$F_{4,90}$=19.48; P=0.0001] (Fig. 8). High corticosterone levels in prenatally stressed males correlated positively with their arrival latencies in the MWM on days 1 and 13 (r=0.9754, P=0.01; r=0.8904, P=0.001, respectively).
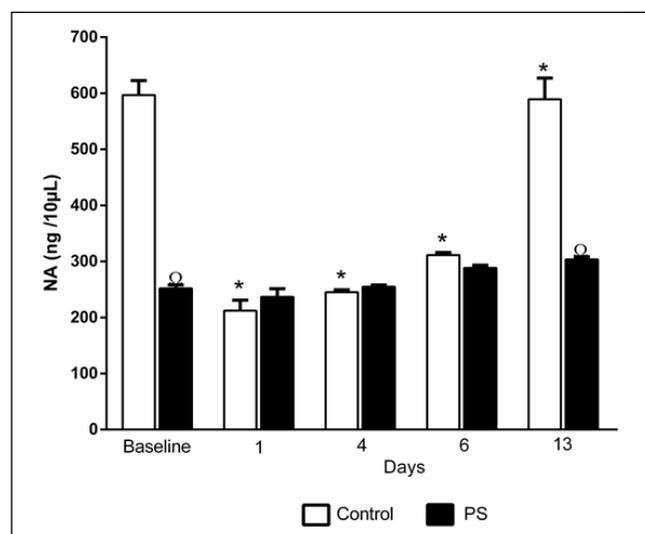

Fig. 6. NA content in the dorsal hippocampus of control and prenatally stressed animals. NA content was significantly lower in prenatally stressed males compared with control in baseline conditions and on day 13 (long-term memory), when NA content increased in control rats. No changes in dorsal hippocampus NA were observed in prenatally stressed rats. Data shown as Mean ± S.E.M. Two-way ANOVA, p=0.0004. * p=0.003 compared to baseline control; Ω p=0.003 compared to each day control. Control group: n=10, each day. Prenatally stressed males: n=10 each day.
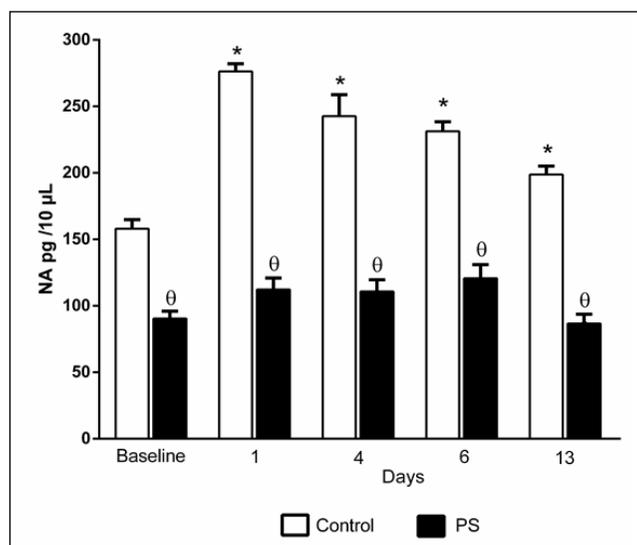

Fig. 7. NA release in the dorsal hippocampus, baseline and after the MWM. NA release in control males was higher both in baseline conditions and after learning (days 1 and 4) and memory (days 7 and 13) tests. NA release in prenatally stressed rats did not change with spatial learning and memory tests. Data shown as Mean ± S.E.M. Two-way ANOVA. * p=0.019 compared to baseline control; θ p=0.006 compared to baseline of prenatal stress group. Control group: n=10, each day. Prenatally stressed males: n=10 each day.
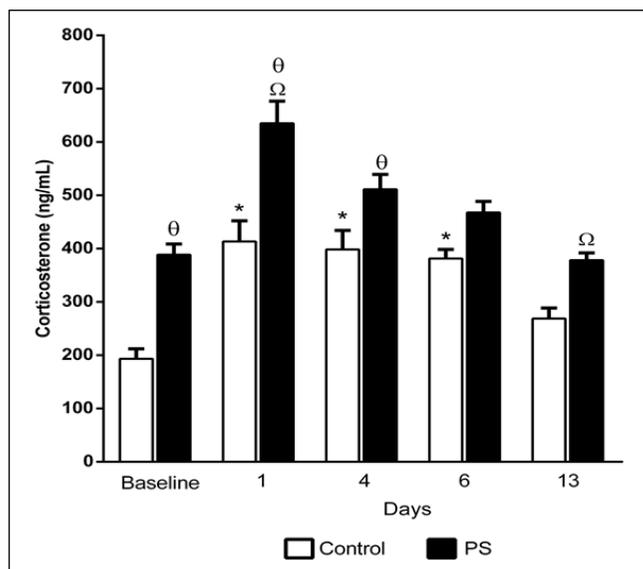
Fig. 8. Serum corticosterone concentrations in control and prenatally stressed (PS) male offspring. Baseline corticosterone concentrations in the prenatally stressed group were higher than those in the control animals. Higher corticosterone levels were observed before and after learning (days 1 and 4) and long-term memory (day 13) in prenatally stressed males. Data shown as Mean ± S.E.M. Two-way ANOVA. * $p=0.0003$ compared with baseline control group; $^{\Omega}$ $p=0.002$ compared with the control group of each day; $^{\theta}$ $p=0.001$ compared to baseline of prenatal stress group. Control group: n=10, each day. Prenatally stressed males: n=10 each day.

DISCUSSION

The results of this study show that cognitive deficiencies in spatial learning and memory, caused by prenatal stress, are related to a decrease in 5-HT and NA release.

Low weight gain observed in pregnant stressed females is consistent with other reports, despite differences in the stress method used (Amugongo and Hlusko, 2014; Van den Hove et al., 2014; Franko et al, 2017). This can be explained by CRH release during the stress response, which also has anorexic effects, decreasing food intake in stressed rats (Carr, 2002; González-Jiménez and Schmidt Río-Valle, 2012). CRH mediates the effects of leptin, which also increases by cold water immersion stress (Konishi et al., 2006) and, together with insulin, they reduce food intake and increase energy expenditure (González-Jiménez and Schmidt Río-Valle, 2012).

The decreased body weight at birth observed in prenatally stressed rats differs from other studies (Abe et al, 2007; Zohar and Weinstock, 2011; Amugongo and Hlusko, 2014), which did not find changes in offspring weight. The differences could be explained by the type of stressor used. Low birth weight can be due to maternal glucocorticoids released during stress, as was demonstrated by the administration of dexamethasone during the last third of gestation (Welberg et al., 2001). The increased concentration of maternal glucocorticoids could alter glucose transport to fetuses, as prenatal stress causes a decrease in the expression of placental GLUT1, GLUT3 and GLUT4 glucose transporters (Knipp et al., 1999; Mairesse et al., 2007; O'Donnel et al., 2009), thus resulting in low birth weight.

Concerning spatial learning and memory, the results of this study confirm that prenatal stress has deleterious effects, shown by longer arrival latencies. These results are consistent with other studies in which higher arrival latencies are observed in prenatally stressed male rats (Szuran et al., 2000; Weinstock, 2008; Modir et al., 2014). Other studies however, report decreased escape latencies in the MWM (Aleksandrov et al, 2001; Zuena et al., 2008) or no differences in spatial learning performance (Hayashi et al, 1998). The different results can be explained by the type of stressor used during pregnancy. In this study, cold water immersion stress was used, which has been proven to cause a more intense hypothalamic-pituitary-adrenal (HPA) axis response when applied acute or chronically, inducing prolonged activation of the adrenal axis in comparison with other stressors (Retana-Márquez et al, 2003). Immersion in cold water induces very high (more than 200%) concentrations of maternal glucocorticoids (Guerrero et al, 2016; García-Vargas et al., 2019). High concentrations of maternal corticosterone cause neuronal degeneration in the hippocampus (Sousa et al., 2000) and induce the loss of hippocampal neurons (Zhu et al., 2004), disrupt fetal hippocampus development as well as neurogenesis in adult life in males and females (Guerrero et al., 2016), retraction and atrophy of dendrites in the CA3 pyramidal neurons of the hippocampus (Conrad, 2006; Hosseini-Sharifabad and Hadinedoushan, 2007), leading to cognitive deficiencies in adulthood (Luine et al., 1994; Sunanda et al, 2000). In addition to the above evidence, the results of the present study show that prenatal stress also alters 5-HT and NA during cognitive processes.

Baseline 5-HT and its metabolite (5-HIAA) content in the dorsal hippocampus were similar in control and in prenatally stressed rats. These results differ from other studies, in which prenatally stressed rats had lower 5-HT content compared to control animals (Hayashi et al, 1998; Soares-Cunha et al., 2018). This might be due to differences in the type of stressor used (crowding and saline injection, chronic unpredictable mild stress *vs.* immersion in cold water in the present study), activating the HPA axis differently, causing different maternal corticosteroid release, 60 ng/mL (Soares-Cunha et al., 2018) *vs.* 600 ng/mL by immersion in cold water (Guerrero et al, 2016) in the dark phase of cycle. Another important difference is

the age of rats at which 5-HT was evaluated: 3 weeks or 2 months in those studies, 3 months in the present study. Possibly, 5-HT turnover rate differs with age, which remains to be proven. The results of this work agree with those observed in studies in which prenatal dexamethasone exposure did not modiy 5-HT content with respect to controls at 3 months of age (Muneoka et al., 1997), despite the fact that 5-HT and its metabolite were quantified in the whole hippocampus, and not in the dorsal hippocampus, which has been directly related to cognitive processes (Bannerman et al., 2004; Tanti and Belzung, 2013; Lee and Kessner, 2003; Eichenbaum, 2000). This evidence supports the idea that increased glucocorticoid levels during pregnancy could directly influence the development of central noradrenergic and serotonergic systems (Muneoka et al., 1997). Despite similarities in baseline content of 5-HT and 5-HIAA in both groups, baseline 5-HT release in the dorsal hippocampus of prenatally stressed rats was significantly lower than in control rats. In addition, baseline extracellular 5-HIAA was higher than 5-HT in prenatally stressed rats, indicating that 5-HT is metabolized to a greater degree in prenatally stressed animals. The decrease of 5-HT and its metabolite content in hippocampal homogenates of control and prenatally stressed males suggests the release of 5-HT in the dorsal hippocampus of both groups in response to the cognitive tests. This was confirmed by microdialysis data. During learning, short- and long-term memory, 5-HT release and its metabolite increased in control rats. In comparison, 5-HT was released in the dorsal hippocampus of prenatally stressed males, although to a lesser degree than in controls. The low extracellular concentrations of the metabolite in dialysates of prenatally stressed males suggest that 5-HT could have been recaptured, instead of metabolized. Probably, the hippocampal serotonin transporter is increased in prenatally stressed in rats, as has been reported for prenatally stressed rats (Belay et al., 2011) and mice (Bielas et al., 2014). These data support the notion that the release of 5-HT in the dorsal hippocampus is important for learning processes and spatial memory. In prenatally stressed rats, low 5-HT release at baseline and during learning and memory tests, indicates that deficiencies in spatial cognitive performance are related to low 5-HT release, as indicated by correlation tests. These results are consistent with those reported in other studies, in which pharmacologically induced 5-HT release improves spatial memory (Glikmann-Johnston et al., 2015), while decreased release impairs it (Kuypers and Ramaekers, 2015).

Regarding NA, lower baseline content in the dorsal hippocampus of prenatally stressed rats was observed.

These results are similar to those obtained in rats from mothers in which dexamethasone was administered during pregnancy, showing lower levels of NA in the hippocampus (Muenoka et al., 1997). These alterations can be related to high maternal corticosterone release during maternal stress (Guerrero et al., 2016; García-Vargas et al., 2019), which can disrupt noradrenergic neurons in the dorsal hippocampus of fetal brains, leading to cognitive deficits in adulthood (Muneoka et al., 1997). The low content of NA observed in prenatally stressed rats at baseline, as well as during learning and spatial memory tests was related to its low release in the dorsal hippocampus, as microdialysis results showed. The increase in NA content on day 13 aside from its high release in control males could be due to the fact that NA facilitates long term potentiation (LTP) modulating synaptic plasticity (Palacios-Filardo and Mellor, 2019) and memory retrieval (Murchinson et al., 2004), mainly through the signaling of the adrenergic receptor β1 (Schimanski et al., 2007; Grigoryan and Segal, 2016). NA enhances NMDA and AMPA glutamatergic receptor phosphorylation by PKA, thus modulating LTP (Palacios-Filardo and Mellor, 2019) .

NA release correlated negatively with corticosterone during learning and memory processes. These data indicate that, in addition to 5-HT, NA release is also important for learning and memory processes, as the correlation between NA release and escape latencies during learning and memory tests shows. As far as we know, this is the first study showing that cognitive spatial deficiencies caused by prenatal stress are related to low 5-HT and NA release.

The higher baseline levels of corticosterone, as well as the higher response of HPA axis to MWM observed in prenatally stressed rats could contribute to alterations in the serotonergic and noradrenergic systems in the dorsal hippocampus, since both the MR, involved in baseline regulation of glucocorticoids (de Kloet et al., 2005; Seckl, 2007; Lupien et al., 2009) and the GR, related to the attenuation of the stress response (Groeneweg et al., 2012) are present in the hippocampus, which may interfere with acquisition and consolidation of information, reflecting low cognitive performance (Ishiwata et al, 2005; Guerrero et al, 2016).

CONCLUSIONS

Deficiencies in learning, short- and long-term spatial memory in adult prenatally stressed males are related to decreased 5-HT and NA release in the dorsal hippocampus. The high baseline levels of corticosterone, and the higher response of adrenal axis to MWM can contribute to cognitive deficits caused by prenatal stress.

ACKNOWLEDGEMENTS

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