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Sex-related long-term behavioral and hippocampal cellular alterations after nociceptive stimulation throughout postnatal development in rats

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1. Introduction

Advances in early postnatal medical care have allowed substantial increase in the survival rate of prematurely-born infants (Stoll et al., 2010). However, stressful routine interventions in intensive care units result in excessive exposure of preterm neonates to multiple invasive procedures (Carabajal et al., 2008; Simons et al., 2003) that may cause long lasting alterations in somatosensory and cognitive processing (Anand, 2000; Grunau et al., 1998, 1994). The mechanism subjacent to such altered processing could be related to reduction of white matter and maturation of subcortical gray matter as has been recently described after procedural pain (Brummelte et al., 2012).

Recent clinical follow up studies have demonstrated that in adulthood, former preterm children display altered pain threshold (Hermann et al., 2006; Hohmeister et al., 2010; Walker et al., 2009).
and decreased modulatory pain mechanisms (Goffaux et al., 2008). Ten year-old former preterm infants rate medical-related pain pictures as more intense that other pain pictures, indicating that they may have more pain memories than age-matched full term infants (Grunau et al., 1998). However, one limitation of the clinical studies is the difficulty to distinguish between long-term effects of pain and confounding factors such as stress, neonatal morbidities and environmental influences (Brummette et al., 2012).

Newborn rats and humans display similar behavioral repertoire in response to noxious stimulation (Guy and Abbott, 1992; Johnston et al., 1993). Such parallel suggests that functional properties in the nociceptive processing may share similar mechanisms that can be investigated using animal models for nociception. As previously described (Anand et al., 1999), at birth the formation of central nervous system is incomplete in rat pups and corresponds to that of 24 week intra-uterine human preterm neonates, following similar patterns in the development of pain system (Fitzgerald and Anand, 1993). The first postnatal week in newborn rat pups corresponds to human premature infants from 24 to 36 weeks of gestation (Kim et al., 1996; Wilson, 1995), offering a suitable condition to model and compare preterm to full term infants subjected to noxious stimulation.

The involvement of hippocampal formation in cognitive tasks has been recognized for decades (Milner, 1972; Squire, 1982). It has been proposed that differences in dentate granule cell proliferation affect the formation of temporal associations during acquisition and retention (Aimone et al., 2006). In adult animals, while dentate neurogenesis rate is positively related to better performance in cognitive tasks, it is negatively related to stress and anxiety (for review, see Schoenfeld and Gould (2012)). However, there is limited information regarding the effects of neonatal nociception on neurogenesis and its role on subsequent pain threshold and anxiety-like behavior. Recently it was demonstrated that the natural ability of dentate granule cells to proliferate from birth until adulthood may be affected by early neonatal nociceptive stimulation. In response to inflammatory nociceptive stimulus applied on postnatal day 1 (P1), hippocampal dentate granule cell layer displays greater elevation in mitotic rate during adolescence (Leslie et al., 2011), which raises questions about the role of hippocampus in long-term nociceptive processing.

The sex of the patient and/or experimental animal may be an important modulator of the long term effects of neonatal pain and stress. Recent experimental studies have addressed these sex differences in behavioral expression after early postnatal noxious stimulation (LaPrairie and Murphy, 2007, 2010; Negrigo et al., 2011) and sex-specific behavioral alterations between preterm and age-matched infants (Guinsburg et al., 2000).

Considering the abovementioned evidence, the purpose of the present study was to investigate the consequences of nociceptive stimuli in three different phases of development on hippocampal cell proliferation and activation, hippocampal BDNF levels, anxiety-like behavior and baseline corticosterone plasma levels of male and female adolescent rats.

2. Methods

This was an experimental, randomized, controlled and blind study. Protocol approval was granted by the Independent Ethics Committee of the Universidade Federal de São Paulo (approval #1378/09). All efforts were made to minimize animal suffering and to reduce the number of animals to the minimum necessary.

2.1. Subjects

Time-pregnant Wistar rats were obtained on the 14th day of gestation from the Center for Development of Animal Models of this University. Dams were housed individually in plastic cages and placed in the animal facility, equipped with an automatic temperature control system (23 ± 2 °C), ventilation, and a 12-h light–dark cycle (lights on at 7:00 AM). All litters were studied from the first day of life (P1) on, reared identically, weaned at P21, and from then on, housed in same-sex groups of 8. All possible care was taken in order to avoid stress to the dams and litters during the experimental protocol.

One hundred and thirty four pups (67 males and 67 females) underwent noxious stimulation, whereas 46 pups were not submitted to any noxious manipulation. A subset of 96 animals was used both for the behavioral and MRI experiments and 36 animals were used in the study of hippocampal cell proliferation and differentiation. Additional 61 animals were used for determination of hippocampal BDNF (final N = 53) and to determine the brain corticosterone levels (final N = 61). Observers who were blind to the treatment of neonatal rats performed and evaluated the behavioral testing and other measurements. Weight gain was measured with an electronic scale (sensitivity 0.1 g) on P1, P21, P40 and no differences could be detected among groups, as previously reported by our group (Leslie et al., 2008). Vaginal smears were conducted in order to determine the phase of the estrous cycle, and all samples were taken between 08:00 and 09:00 h. All females that were already cycling were excluded from study.

2.2. Noxious stimulation

The protocol for noxious stimulation of the rats was previously described (Leslie et al., 2011). Briefly, on postnatal days 1, 8 or 21 rat pups received a single intraplantar injection of an inflammatory agent, the complete Freund's adjuvant (CFA, 25 μg, Sigma, Saint Louis, MI, US) into the left hindpaw and were returned to their home-cage. These groups were named P1, P8 and P21, respectively. Control (CTL) animals were not injected but were handled at the same time as experimental pups. These ages were chosen because P1 corresponds to 24 weeks of human gestation (extreme prematurity) (Anand et al., 1999), whereas P8 would correspond approximately to 38–40 weeks of intra-uterine age; finally, day 21 was included as a control group for painful stimulation during later development. The experimental design is summarized in Fig. 1. All animals in a single litter were assigned to a particular group (manipulated at P1, P8, P21 or not stimulated), to avoid the influence that handling of pups at different ages could have on maternal care towards the remainder of the litter. Moreover, within each litter, pups were randomly assigned to different experiments (behavior and MRI analysis or dentate cell proliferation/differentiation) to avoid litter effects.

2.3. Nociceptive and anxiety measurements

Behavioral tests (hot plate and elevated plus maze tests) results are based on data from 19 animals for P1 group (11 males, 8 females); 26 animals for P8 group (12 males, 14 females); 31 animals for P21 (13 males, 18 females) group and 20 CTL animals (10 males, 10 females). The nociceptive test consisted of placing the rat on a hot plate (52.5 °C) and measuring the latency for and the number of lickings of the hindpaw (either one). To avoid potential tissue damage, a 20 s automatic termination of the heat stimulus was established if no paw withdrawal occurred. This cut off time (20 s) was then considered as the maximal latency. The hot plate test data was averaged from three trials with 15 min intervals. The testing apparatus was cleaned between sessions. The observer was blind to treatment condition during testing.

The elevated plus maze apparatus consisted of a central platform (5 × 5 cm) with two open arms (50 cm long, 10 cm wide and 0.5 cm high borders) and two closed arms (same dimensions as the open arms with 40 cm high walls) elevated 50 cm above the ground. Rats were placed on the central platform facing the open arm and were observed during 5 min. The numbers of entries into the open and closed arms as well as the time spent in each arm were recorded.

2.4. Manganese-enhanced MRI (MEMRI) procedures

MEMRI acquisitions were performed in a subset of animals after the behavioral tests: 10 animals for P1 group (5 males, 5 females); 13 animals for P8 group (5 males, 8 females); 16 animals for P21 group (7 males, 9 females) and 10 animals for CTL (5 males and 5 females) group. Detailed MEMRI procedure has been previously published elsewhere (Malheiros et al., 2012). Briefly, MnCl₂ (60 mg/kg, i.p.) was administrated to all animals; the solution consisted of 100 mM MnCl₂-4H₂O (Sigma, Saint Louis, MI, US) in 400 mM bicarbonate buffer, pH 7.4. In order to investigate the pattern of cellular activation in the dorsal hippocampus following nociceptive stimulation, all animals including controls, received a single injection of CFA (25 μL) in the left hindpaw 12 h after manganese chloride injection and two hours before MRI brain scanning. MRIs were obtained in a 2 T 30 cm horizontal superconducting magnet (Biospect, Oxford, UK) interfaced to a Bruker AvIII console (Bruker-Biospin, Ettlingen, GE) running Paravision 5.1 software (Bruker, Ettlingen, GE). A Double Crossed Saddle radiofrequency coil (Papouts, 2006) was used as a head probe in animals anesthetized with ketamine/xylazine (95/12 mg/kg, i.p.). A T₁-weighted 3D FLASH (Fast Low Angle Shot) sequence (TR = 200 ms, TE = 5.8 ms, flip angle = 90°, 4 averages, 40 min/animal) was acquired. A volume of 40 × 40 × 12.2 mm³ was covered with 192 × 192 × 16 points, producing a spatial resolution of 208 × 208 × 700 μm³. All images were normalized to the same window and level settings.

MRI data were analyzed using the Paravision 5.1 software. One author (JMM), blind to the group's identity, manually outlined the region of interest (ROI)
representing the dorsal hippocampus. This was bilaterally drawn from a single coronal section of the 3D T1-weighted volume located 3.6 mm caudal to the bregma (Paxinos and Watson, 1998), with anatomic boundaries defined as illustrated in Fig. 4A–B. The relative MEMRI signal enhancement in the DG, CA1 and CA3 areas was calculated as the ratio of the mean signal intensity in the ROIs to the mean signal intensity of the adjacent corpus callosum. MEMRI hyperintensity was considered when a relative increase in signal intensity was observed when compared to controls (Malheiro et al., 2012).

2.5. BrdU and NeuN immunohistochemistry

On P21, a subset of animals from all groups received a 5-Bromo-2’-deoxyuridine injection (BrdU; Sigma); four doses of 50 μg/g of body mass, dissolved in 0.9% 0.007 N sodium hydroxide), via intraperitoneal route, every six hours. On P40, the rats were weighed, injected with a lethal dose of thionembutal (50 mg/kg, Cristalia, São Paulo, BR) just prior to the transcardiac perfusion through the left ventricle. This interval between BrdU administration and the animals’ sacrifice allowed not only to access mitotic rate, but also the differentiation of newly generated cells into neurons in the dentate granule cell layer. The brain was then removed and kept in 30% sucrose solution. Thirty micrometer thick coronal brain sections along the hippocampal septo-temporal axis could be obtained with the aid of a cryostat. Sections from each animal were collected serially in 24 well plates, cryopreserved in anti-freezing storage at −20 °C.

The immunohistochemistry procedure for BrdU was performed on all sections from two equidistant wells. With this distribution of sections, we were able to run experiments on 1 section out of 12 representative sections along the rostro-caudal extension of the dentate gyrus. After extensive washing, sections were incubated for 10 min with 3% H2O2 in phosphate buffer solution (PBS), washed in PBS, followed by 10 min in 1 N HCl. After treatment with blocking buffer solution (BB), sections were incubated with biotinylated anti-rat secondary antibody (1:1,500, Vector Laboratories, Burlingame, CA, US) and incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:10,000, Molecular Probes, Eugene, OR, US). NeuN-positive cells were detected in a plate reader at 450 nm (Quick Elisa). Values were reported in pg/mL.

2.6. BDNF immunodetection

Hippocampal formation from the same animals used for corticosterone plasma levels was removed immediately after decapitation and homogenized in 0.01 M Tris hydrochloride (pH 7.6) containing 5.8% of sodium chloride, 10% of glycerol, 1% of Nonidet P40 (NP-40), 0.4% of ethylenediamine tetraacetic acid (EDTA) and protease inhibitors. Samples were sonicated and stored at −80 °C. Baseline levels of hippocampal BDNF expression was obtained from 16 non-stimulated animals (8 males and 8 females).

Hippocampal BDNF expression assays were performed using the ELISA kit E-max® (Promega) according to manufacturer’s recommendations. Samples from studied groups previously stored at −80 °C were centrifuged for 5 min at 14,000 rpm at 4 °C and the supernatant transferred to a 96-well plate (Corning Costar) coated with anti-BDNF (1:1000) then incubated for 2 h at room temperature. After this period, the plate was washed with Tris-buffered saline Tween-20 (TBS-T) and incubated with the following antibodies: anti-human (1:500) for 2 h, and conjugate anti-Ig Y HRP® (1:2000) for 1 h. After these procedures, color reaction with tetramethyl benzidine was quantified in a plate reader at 450 nm (Quick Elisa). Values were reported in pg/mL.

2.7. Corticosterone plasma levels

Blood samples from 13 animals from P1 group (4 males; 9 females); 16 animals from P8 group (8 males; 8 females); 16 animals from P21 group (8 males; 8 females) were included. In order to establish non-stimulated corticosterone levels, an additional group of 16 animals (CTL group; 8 males; 8 females) was included in the analysis. Trunk blood was obtained in pre-cooled vials containing EDTA (1 drop of a 6% solution), and each sampling yielded 150–300 μl of blood. Samples were centrifuged at 2300 rpm, for 20 min at 4 °C and plasma was stored at −20 °C until determination of hormone levels. Corticosterone levels were determined in

![Fig. 1. Schematic representation of the experimental design. Rat pups received CFA (intraplantar injection, 25 μl) on P1 or P8 or P21. On the left side, the subset of animals was submitted to behavioral and MRI studies (n = 96); behavioral tests started on P40. On P45, these animals received another injection of CFA (as above) 2 h before MRI scan. The right side shows the subset of animals used for BrdU and BrdU/NeuN immunohistochemical procedures to assess dentate granule cell proliferation and differentiation (n = 36). These animals received BrdU (intraperitoneal, 200 μg/kg) on P21 and were perfused on P40.](Image 119x541 to 466x727)
duplicate by a double antibody radioimmunoassay method, specific for rats and mice, using a commercial kit (MP Biomedicals, Costa Mesa, CA). The sensitivity of the assay is 25 ng/ml, and intra- and inter-assay variations are, respectively, 7.1% and 10.3%.

2.8. Statistical analysis

Results are presented as mean ± SEM. BrdU labeled cells, double labeled cells, behavioral parameters, MEMRI signal, BDNF hippocampal levels and corticosterone plasma levels were analyzed by a two-way analysis of variance (ANOVA) with group (CTL, P1, P8, P21) and sex (male, female) as variables, followed by Tukey-Kramer post hoc test, using the Statistica 7 software. Significance was set at \( P < 0.05 \).

3. Results

3.1. Nociceptive and anxiety measurements

3.1.1. Latency to lick the paw (Fig. 2A)

Main effects of sex \( [F_{(1,88)} = 6.404; P < 0.02] \) and group \( [F_{(3,88)} = 12.8312; P < 0.00001] \) were revealed. Stimulated females took longer than males to lick the paws \( (P < 0.02) \) and all stimulated adolescents, regardless of the age when the noxious stimulus was applied, displayed greater latencies to lick their paws than CTL animals \( (P's < 0.001) \).

3.1.2. Frequency of licking (Fig. 2B)

A main effect of group was detected \( [F_{(3,88)} = 20.3364; P < 0.00001] \), with a similar profile to the latency to lick the paw, e.g., all stimulated animals licked the paws less than CTL ones \( (P's < 0.001) \).

3.1.3. Elevated plus maze

There were no differences among the groups as to the number of entries into the open arms, but main effects of group \( [F_{(3,88)} = 5.3617; P < 0.002] \) and sex \( [F_{(1,88)} = 5.5853; P < 0.02] \) were shown for the time spent in the open arms. Again, all stimulated groups spent more time in the open arms than CTL rats \( (P's < 0.01) \) and females spent more time than males (Fig. 3B; \( P < 0.01 \)). P1 females had more number of entries than P1 males \( (P < 0.01) \).

3.2. MEMRI (Fig. 4)

The MEMRI signal was analyzed in 49 animals. A main effect of sex was detected \( [F_{(1, 48)} = 6.32; P < 0.01] \) with the post-hoc test indicating significantly higher signal intensity for females in CA3 \( [F_{(1, 48)} = 7.76; P < 0.01] \) and DG \( [F_{(1, 48)} = 17.43; P < 0.01] \). In the hippocampus higher manganese signal was obtained in dentate gyrus \( (1.058667 ± 0.006866) \) and CA3 \( (1.050880 ± 0.007549) \) compared to CA1 region \( (0.938122 ± 0.004908) \); \( P < 0.01 \). The manganese enhanced signal was higher in the dentate gyrus of P1 females \( (1.101780 ± 0.020813) \) than in the male counterparts \( (1.002537 ± 0.006199) \); \( P < 0.01 \). No specific differences could be detected in the pyramidal fields of hippocampus in the P1 group.

Fig. 2. Effects of intraplantar CFA injection on P1, P8 or P21 on the hot plate test. In A, graphical representation of the latency to lick the paws. All noxious-stimulated groups took longer to lick their paws than CTL groups \( (*P < 0.001) \). Females took longer than males to lick the paws \( (zP < 0.02) \). In B, representation of the frequency of paw lickings. Noxious-stimulated groups lick less the paws than CTL ones \( (*P < 0.001) \). Data is presented as mean ± S.E.M.

Fig. 3. Effects of plantar noxious stimulation on P1, P8 or P21 on anxiety-like behavior in male and female adolescent rats. In A: Number of entries in the open arms of the elevated plus maze. P1 females had more number of entries than P1 males \( (IP < 0.01) \). In B: Time spent in the open arms of the elevated plus maze. All stimulated animals spent more time in the open arms than CTL animals \( (P < 0.01) \) and females remained longer in the open arms than males, except CTL females, which showed an opposite response \( (zP < 0.01) \). Data is presented as mean ± S.E.M.
3.3. BrdU and NeuN immunohistochemistry

Proliferating BrdU-labeled cells were preferentially located in the subgranular zone of the dentate gyrus, but many cells were distributed along the entire width of granule cell layer; Fig. 5A.

Analysis of the number of BrdU immunolabeled cells in the dentate gyrus showed main effects of sex \( F(1,33) = 4.24; P < 0.05 \) and group \( F(3,33) = 10.36; P < 0.0001 \), but no interaction \( (P = 0.35) \), Fig. 5C. Females had a lower neurogenesis rate than males \((30.3 \pm 9.6 \text{ and } 68.4 \pm 19.8, \text{ respectively; } P < 0.05)\), and proliferation rates in P1 animals \((70.11 \pm 20.49)\) were greater than in controls \((4.72 \pm 1.71; P < 0.03)\), as well as proliferation rate in P8 stimulated \((129.79 \pm 32.75)\) rats was higher than in P21 \((16.31 \pm 2.37; P = 0.001)\) and CTL rats \((4.72 \pm 1.71; P < 0.001)\).

A main effect of group was detected for double labeled dentate cells \( F(3,48) = 5.38; P < 0.05 \). Pairwise comparisons indicated significant differences between controls \((89.89 \pm 5.02)\) and P1 \((63.02 \pm 5.14)\) groups; \( P < 0.001, P8 (83.02 \pm 4.51; P = 0.01)\); Fig. 5B and D.

3.4. BDNF immunodetection (Fig. 6A)

Hippocampal BDNF level was analyzed in 53 animals. Main effects were detected of sex \( F(1, 45) = 130.43; P = 0.000 \) and group \( F(3,45) = 106.88; P = 0.000 \) and an interaction between these factors \( F(3,45) = 48; P = 0.000 \). Higher BDNF level was observed in P8 males which differed from P1, P21 and CTL males \((P < 0.001)\) besides its female partner \((P < 0.001)\). P1 males also had higher hippocampal BDNF level than CTL and P21 \((P < 0.001)\) males and P1 female \((P < 0.001)\). Males and females of P1 and CTL groups were similar.

3.5. Corticosterone plasma levels (Fig. 6B)

There were main effects of group \( F(3,53) = 6.487; P < 0.001 \) and sex \( F(1,53) = 9.23; P < 0.005 \), but no interaction between these factors \( F(1,53) = 1.852; P = 0.15 \) on corticosterone plasma levels. Post-hoc analysis indicated that levels were lower in P1 rats than CTL and P21 ones \((P < 0.03 \text{ and } 0.01, \text{ respectively})\) and in P8 than in...
P21 rats ($P < 0.03$). Females displayed higher levels than their male counterparts, irrespective of the neonatal treatment ($P < 0.03$).

4. Discussion

This study investigated the effects of noxious stimulation applied in early and late post-natal ages on behavior, hippocampal cell activation and proliferation in rats and in hippocampal BDNF of adolescent rats. Our results indicated that nociceptive threshold was increased in P1, P8 and P21 stimulated groups, more in females than in males. In addition, all stimulated females spent more time in the open arms of the elevated plus maze, meaning that they were less anxious than age-matched males. Hippocampal dentate granule cell proliferation was greater in P1 and P8 groups, with more pronounced results in males than females. No sex differences were found in neurogenesis, but under a new challenge with CFA intraplantar application of CFA in all ages (P1, P8 or P21) suggest that throughout ontogeny differences in the behavioral repertoire might be subtle. Based on the literature from human (Hermann et al., 2006) and animal models (Benatti et al., 2009; LaPrairie and Murphy, 2007), one would expect that the earlier the noxious stimulation the more pronounced the changes in later nociception. The fact that P21 rats also showed high nociception thresholds may indicate that the critical period for establishment of the nociceptive circuitry is susceptible to additional factors not, as yet, well understood. Regarding sex, our results are similar to previous findings (LaPrairie and Murphy, 2007) being the thermal threshold significantly higher in neonatally injured females than males and controls. This has been supported by findings that ovarian sex hormones modulate dynorphin/kappa-opioid receptor pathway which is a prerequisite for spinal morphine antinociception (Liu et al., 2007).

The inter-relationship between emotional and nociceptive outcomes is an issue still under investigation (Wilson et al., 2007), persistent alterations in somatosensory structure and function (Ren et al., 2004; Ruda et al., 2000; Torsney and Fitzgerald, 2003). The behavioral consequences, however, seem to vary according to duration (minutes to hours or days to weeks) and type (superficial or deep) of the noxious stimulation, with some inconsistency among the studies (for review, see LaPrairie and Murphy (2010)).
Reduced anxiety on the elevated plus maze test following neonatal nociceptive stimulus could be associated with increased expression of serotonin receptors, involved in the anxiolytic and analgesic response to beta-endorphin with maximal effect between P4 and P8. It is interesting to note that cholecystokinin receptors interact with the opioid system in brain nociceptive pathways and that beta-endorphin-mediated inhibition of DNA synthesis is antagonized by a sulfated fragment of cholecystokinin (Bartolome et al., 1994). These data are in agreement with our previous (Leslie et al., 2011) and current findings of higher proliferative rate of dentate granule cells mainly in P8, followed by P1 group, when compared to P21 and CTL groups. An alternative explanation for the increase level of neurogenesis in P8 male and female rats involves the peculiar profile of corticosterone response to stress in rat pups. In the first two weeks of life, rat pups undergo a developmental period known as the stress hyporesponsive period, during which most stressors fail to induce corticosterone secretion (Rosenfeld et al., 1992; Walker et al., 1986; Witek-Janusek, 1988). Because corticosterone has been implicated in impairment of dentate gyrus neurogenesis (reviewed in Schoenfeld and Gould (2012)) and of normal development of the hippocampus (reviewed in Pawluski et al. (2009)), we hypothesized that failure to increase secretion of this hormone might have protected neurogenesis in the dentate gyrus in P1 and, especially, P8 adolescents. In fact, the results of corticosterone and BDNF levels are in consonance with this hypothesis, and they showed almost an inverse specular profile; adolescents that exhibited lower corticosterone levels, displayed higher hippocampal BDNF levels. This pattern reflected well on the number of BrdU stained cells in the hippocampus, i.e., neurogenesis rate. Similar results were reported recently after anti-depressant treatment (Branchi et al., 2013; Capocchia et al., 2013).

The abovementioned studies strongly suggest that persistent alterations in baseline nociceptive threshold and anxiety levels associated to neonatal noxious stimulus are modulated in several structures along the central nervous system. One resultant, as
indicated by this study, is the increase in dentate granule cell proliferation and differentiation. Nineteen days after BrdU administration (on P21 and evaluation on P40), newly born granule cells are expected to extend their dendritic branches toward the molecular layer (Ribak et al., 2004), project axons toward the CA3 region of the hippocampus (Hastings and Gould, 1999; Zhao et al., 2006) and generate action potentials (van Praag et al., 2002). Altogether, these features could indicate high levels of dentate gyrus activity, as measured by MEMRI. As the resultant increase of adulthood hippocampal cell proliferation is more pronounced in P8 than P1 group, it could be hypothesized that measurement of hippocampal cell activity would provide more direct evidence of the roles of this brain structure in the determination of a complex behavior on P8 stimulated animals. Having this in mind, we focused our attention in the quantification of hippocampal cell activity during re-exposure of the animals to the same noxious stimulus. It was very interesting that among all hippocampal fields studied, the only area differentially activated on P40 was the dentate gyrus of P1 females. To the best of our knowledge, we report for the first time that adolescent animals that underwent neonatal noxious stimulation exhibit sexual dimorphism in hippocampal cell proliferation and activation, in addition to the previously reported behavioral data (LaPrairie and Murphy, 2007). Taken together, the present findings suggest that new neurons may play an important role not only in the cognitive functions of hippocampus, but also, in its nociceptive-related functions. Variations of hippocampal cell activity in response to long-term effects of acute noxious stimulation during ontogeny should be further investigated.

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**References**


**Conclusions**
