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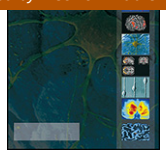

Neonatal inflammatory pain increases hippocampal neurogenesis in rat pups

Ana Teresa F.S. Leslie[a], Katherine G. Akers[c], Alonso Martinez-Canabal[c,d], Luís Eugênio de Araújo Mello[b], Luciene Covolan[b], Ruth Guinsburg[a,*]

[a] Departamento de Pediatria, Universidade Federal de São Paulo, São Paulo, Brazil
[b] Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, Brazil
[c] Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada
[d] Institute of Medical Science, University of Toronto, Canada

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ABSTRACT

Preterm infants undergo several painful procedures during their stay in neonatal intensive care units. Previous studies suggest that early painful experiences may have an impact on brain development. Here, we used an animal model to investigate the effect of neonatal pain on the generation of new neurons in the dentate gyrus region of the hippocampus. Rat pups received intraplantar injections of complete Freund's adjuvant (CFA), a painful inflammatory agent, on either P1 or P8 and were sacrificed on P22. We found that rat pups injected with CFA on P8 had more BrdU-labeled cells and a higher density of cells expressing doublecortin (DCX) in the subgranular zone of the dentate gyrus. No change in BrdU-labeling or DCX expression was observed in pups injected with CFA on P1. These findings indicate that neonatal pain can increase hippocampal neurogenesis, suggesting that early painful experiences may shape brain development and thereby influence behavioral outcome.

As the survival rate of prematurely born infants continues to rise due to advances in neonatology, greater numbers of babies are spending time in neonatal intensive care units, where they undergo several painful procedures such as heel pricks, endotracheal suction, and intravenous cannula insertion [5]. These painful experiences occur at a time when the brain is rapidly developing and therefore particularly susceptible to exogenous and endogenous insults [41]. Thus, by affecting brain development, early painful experiences may exert a profound and long-lasting influence on behavioral outcome. Studies of children born prematurely suggest that neonatal pain is linked to abnormalities in cognitive, emotional, and psychosocial function later in life [1,9,22]. Currently, however, the changes in brain function that underlie the effects of early painful experiences remain unclear.

The hippocampus is one brain region that is likely to be involved in the developmental abnormalities associated with neonatal pain given its vulnerability to early traumatic events [4,34] and its role in cognition and emotion [6,18]. A unique characteristic of the hippocampus is that, unlike most brain regions, it continues to generate new neurons after birth and into adulthood [14]. After their generation in the subgranular zone (SGZ) of the dentate gyrus, new neurons migrate into the granule cell layer and become structurally and functionally integrated into existing hippocampal circuitry [43]. The detection of new hippocampal neurons can be achieved using two complimentary approaches. First, new cells can incorporate synthetic nucleosides, such as the thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) [12], into their DNA during replication. Second, new neurons naturally express specific markers, such as doublecortin (DCX) [10], at certain stages in their maturation. In both approaches, the new cells (i.e., BrdU+ or DCX+ cells) can be identified in brain tissue using immunohistochemical techniques.

In this study, we used an animal model to investigate the effect of chronic neonatal pain on the generation of new hippocampal neurons. Rat pups received an intraplantar injection of complete Freund's adjuvant (CFA), an inflammatory agent used in experimental studies to induce pain that persists for several days [37]. To examine whether the effect of neonatal pain on hippocampal neurogenesis differs depending on the age at which it is experienced, injections of CFA occurred on either postnatal day (P) 1 or 8, the ages at which the rat brain is roughly equivalent to a preterm and term human brain, respectively [16].

All experimental procedures were approved by the Federal University of São Paulo Research Ethics Board. Wistar Han rats were bred at the Animal Resource Center of the Federal University of São Paulo and housed in plastic cages in a laboratory equipped with an automatic temperature control system (23 ± 2 °C), ventilation, a 12 h light-dark cycle (lights on at 07:00 h), and unrestricted access to food and water. The day of birth was designated as P0. Litters were culled to 8 pups within 24 h after birth, and body weights were measured on P1, P8, P15, and P22.

* Corresponding author at: Rua Vicente Felix 77, Apt 09, São Paulo 01410-020, SP, Brazil. Tel.: +55 11 30642663; fax: +55 11 50840535.
E-mail address: ruthgbr@netpoint.com.br (R. Guinsburg).

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On either P1 or P8, rat pups received a single subcutaneous injection of CFA (25 μl; Sigma) or 0.9% saline (25 μl) into the plantar surface of the left hind paw and were returned to their home cages (P1 CFA $n = 27$, P1 saline $n = 23$, P8 CFA $n = 18$, P8 saline $n = 17$). Each experimental group contained a maximum of one male and one female pup from each litter. Pups were identified using marks from permanent marker pens of different colours.

For pups injected with CFA or saline on P1, BrdU (100 mg/kg, i.p., Sigma) dissolved in 0.007 N NaOH/0.9% saline was injected on either P8 (CFA $n = 6$, saline $n = 5$) or P15 (CFA $n = 21$, saline $n = 18$). For pups injected with CFA or saline on P8, BrdU was injected on P15. A total of 4 injections were given to each pup, with 6 h between each injection.

On P22, pups were deeply anesthetised with sodium pentobarbital (150 mg/kg; i.p.) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Brains were removed, post-fixed overnight in 4% paraformaldehyde, and embedded in paraffin. Coronal sections (10 μm) were obtained across the entire dorsal-ventral extension of the dentate gyrus using a microtome. After sectioning, slides underwent deparaffinization. Six sections at regular intervals of approximately 1 mm were selected from each animal for BrdU and DCX immunohistochemistry. BrdU labeling was performed for all animals, and DCX labeling was performed for a random subset of animals from each experimental group (P1 CFA $n = 6$, P1 saline $n = 5$, P8 CFA $n = 5$, P8 saline $n = 5$).

For BrdU labeling, slides were treated with 2 N HCl at 37 °C for 20 min to expose the BrdU antigen. Slides were then incubated with the primary antibody (monoclonal rat anti-BrdU, 1:200, Accurate Chemicals) at room temperature overnight and the secondary antibody (biotinylated goat anti-rat; 1:200, Jackson Immuno-research) at room temperature for 2 h. Antibodies were diluted in blocking solution containing 0.05% goat serum and 0.1% Triton X-100 dissolved in PBS. BrdU+ cells were visualized using avidin–biotin-peroxidase complex (ABC, Vector Laboratories) followed by diaminobenzidine (DAB, Sigma). Slides were counterstained with Harris Hematoxylin (Sigma), dehydrated, and coverslipped with Permount (Sigma). BrdU+ cells were manually counted from the SGZ (at the junction between the granule cell layer and the hilus) using a microscope (Nikon Eclipse) with a 40× objective.

For DCX labeling, slides were treated with 0.01 M citrate buffer (6.0 pH) in a 97 °C steamer for 1 h. Slides were then incubated with the primary antibody (rabbit anti-DCX, 1:1000, Cell Signaling) at room temperature overnight and the secondary antibody (biotinylated goat anti-rabbit, 1:2000, Jackson Laboratories) at room temperature for 2 h. DCX+ cells were visualized using ABC followed by DAB. Slides were counterstained with Harris Hematoxylin, dehydrated, and coverslipped with Permount. Quantification of DCX+ cells was performed using a microscope (Olympus BX61) attached to a digital camera (Retiga 11.0) and motorized stage (MBF Bioscience). Using Stereo Investigator software (MBF Bioscience), images of 10 uniformly distributed areas (160 μm × 200 μm) from each dentate gyrus were captured using a 40× objective. DCX+ cells were manually counted from the images using Image J software.

For co-localization of BrdU and DCX, we used Tyramide Signal Amplification (TSA) protocol. Sections were incubated with a mixture of primary antibodies, anti BrdU 1:500 (Accurate Chemical) and anti DCX 1:100 (Santa Cruz) at room temperature overnight, and a mixture of secondary fluorescence-tagged antibodies, anti rat Horseradish Peroxidase 1:500 (Cell Signaling) and Alexa Fluor® 568 conjugated 1:300 (Invitrogen) at room temperature for 2 h. Rinsed sections were mounted onto gelatine-subbed slides and coverslipped with Vector Vectashield. All double stained cells for DCX and BrdU were quantified manually in a series of coronal sections covering the anterior–posterior extent of the hippocampus using an Olympus microscope BX61 attached to a digital camera Retiga 11.0 and a MBF motorized stage. The percentage of co-localization was calculated by dividing the number of double-labeled cells by the number of BrdU+ cells.

Table 1
Injection of CFA did not affect pup body weight (mean ± SEM).

Group	Body weight (g)			
	P1	P8	P15	P22
P1 saline	6.64 ± .17	17.72 ± .45	32.19 ± .84	47.07 ± 1.53
P1 CFA	6.80 ± .16	17.34 ± .42	31.30 ± .78	45.99 ± 1.41
P8 saline	6.65 ± .17	17.79 ± .46	31.39 ± .87	46.82 ± 1.57
P8 CFA	6.83 ± .21	17.65 ± .55	30.38 ± 1.03	45.77 ± 1.87

Body weights were analyzed using three-way ANOVA (SPSS) with treatment (CFA or saline) and age at treatment (P1 or P8) as between-subject factors and age at weighing (P1, P8, P15, or P22) as a within-subject factor. BrdU data were analyzed using two-way ANOVA with treatment and age at BrdU injection (P8 or P15) as between-subject factors and one-way ANOVA with treatment as a between-subject factor. DCX data were analyzed using one-way ANOVA with treatment as a between-subject factor.

Rat pups treated with CFA exhibited inflammation around the injection site with oedema and redness lasting 2–4 days. Pups treated with saline showed no signs of inflammation. All pups gained weight across the duration of the experiment (Table 1) [$F_{(3, 192)} = 2372.80$, $p < .001$], with no significant differences in weight gain among treatments ($p > .05$).

Among pups that were treated on P1, we found more BrdU+ cells in the SGZ when BrdU was injected on P8 compared to when BrdU was injected on P15 [$F_{(1, 40)} = 59.31$, $p < .001$]. However, regardless of when BrdU was injected, there were no differences in BrdU+ cell number between pups treated with CFA and pups treated with saline (Fig. 1A) [$p > .05$]. In contrast, among pups that were treated on P8, we found that pups treated with CFA had more BrdU+ cells in the SGZ (Fig. 1B and C) compared to pups treated with saline when BrdU was injected on P15 (Fig. 1B and D) [$F_{(1, 33)} = 6.48$, $p = .016$]. The proportion of BrdU+ cells that were also DCX+ was approximately 25% for both groups (Fig. 1B and E).

Similarly, there was no difference in DCX+ cell density in the SGZ between pups treated with CFA on P1 and pups treated with saline (Fig. 2A) [$p > .05$]. However, pups treated with CFA on P8 had higher densities of DCX+ cells compared to pups treated with saline (Fig. 2B–D) [$F_{(1, 8)} = 12.82$, $p = .007$].

We examined the effect of chronic neonatal pain on the generation of new neurons in the dentate gyrus region of the hippocampus. We found that intraplantar injection of rat pups with the inflammatory agent CFA on P8 increased the number of BrdU+ cells present in the SGZ two weeks after the painful event. We also found an increase in the density of cells that expressed DCX, a marker expressed by young neurons predominately within the first two weeks following cell division [10]. We observed no change in BrdU+ cell number or DCX+ cell density when CFA was injected on P1. Together, these findings provide converging evidence that early painful experiences can increase in hippocampal neurogenesis.

The number of new cells in the dentate gyrus at any given moment is the result of multiple processes including the proliferation of cells from a progenitor population and the survival of those cells as they mature and integrate into the existing neural circuitry [14]. In this initial study, we chose to investigate whether neonatal pain affects the early stages of hippocampal neurogenesis by injecting BrdU one week after initiation of the painful stimulus on P8 and quantifying the number of BrdU+ cells one week after BrdU injection. The observed increase in BrdU+ cells in pups that experienced

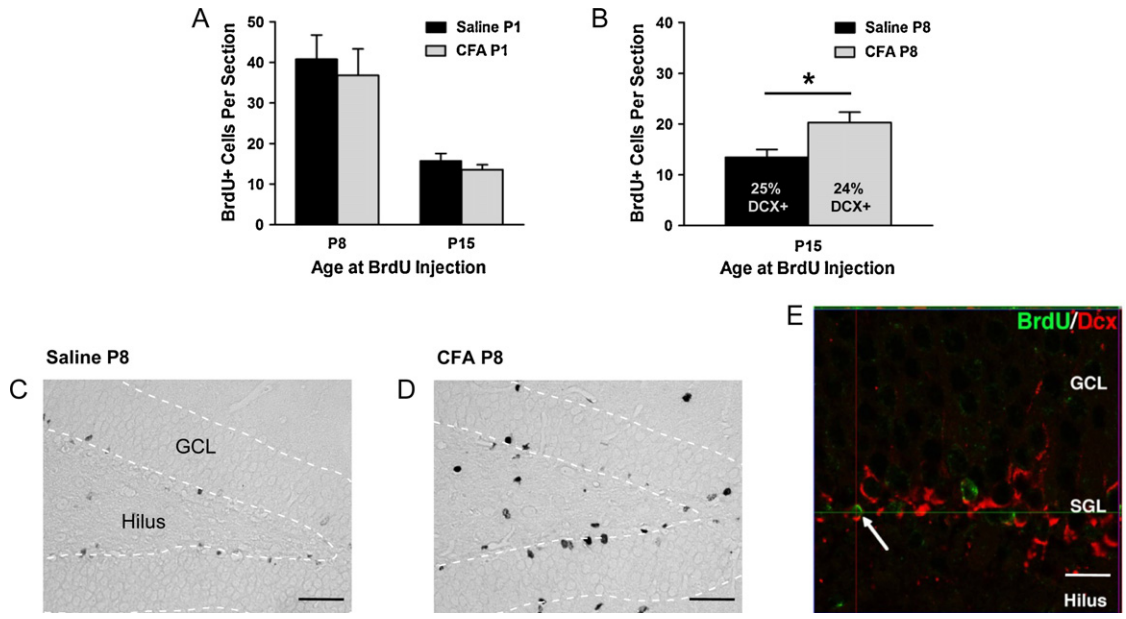

Fig. 1. Effect of neonatal pain on BrdU labeling in the SGZ of the dentate gyrus. (A) There were no differences in the number of BrdU+ cells between pups injected with CFA on P1 and pups injected with saline on P1. (B) Pups injected with CFA on P8 had significantly more BrdU+ cells than pups injected with saline on P8. Both groups had similar numbers of BrdU+ cells that were also DCX+. Representative images BrdU+ cells in P8 saline pups, magnification 20× (C), BrdU+ cells in P8 CFA pups, magnification 20× (D), and a BrdU+/DCX+ cell in P8 pups (white arrow in E), magnification 100×. $^*p < .05$. GCL = granule cell layer, SGZ = subgranular zone. Scale bars = 40 μm in CD, 100 μm in (E).

pain starting on P8 could therefore be due to an enhancement in either the proliferation or the short-term (i.e., 7-day) survival of new cells. However, we observed no change in the proportion of BrdU+ cells that were also DCX+, suggesting that neonatal pain has no effect on the differentiation of new cells into neurons. To further examine the effect of neonatal pain on different stages of hippocampal neurogenesis, future studies could assess the number of BrdU+ cells in the hours immediately following BrdU injection (i.e., proliferation) and after a delay of several days or weeks (i.e., survival), as well as their morphological development (i.e., dendritic braching, spines).

The impact of traumatic events on hippocampal neurogenesis depends on the nature of the insult and the developmental stage at which it occurs. For instance, in rodents, hippocampal neurogenesis is decreased after prenatal stress [28], postnatal lead poisoning [40], and adult social isolation [31], whereas it

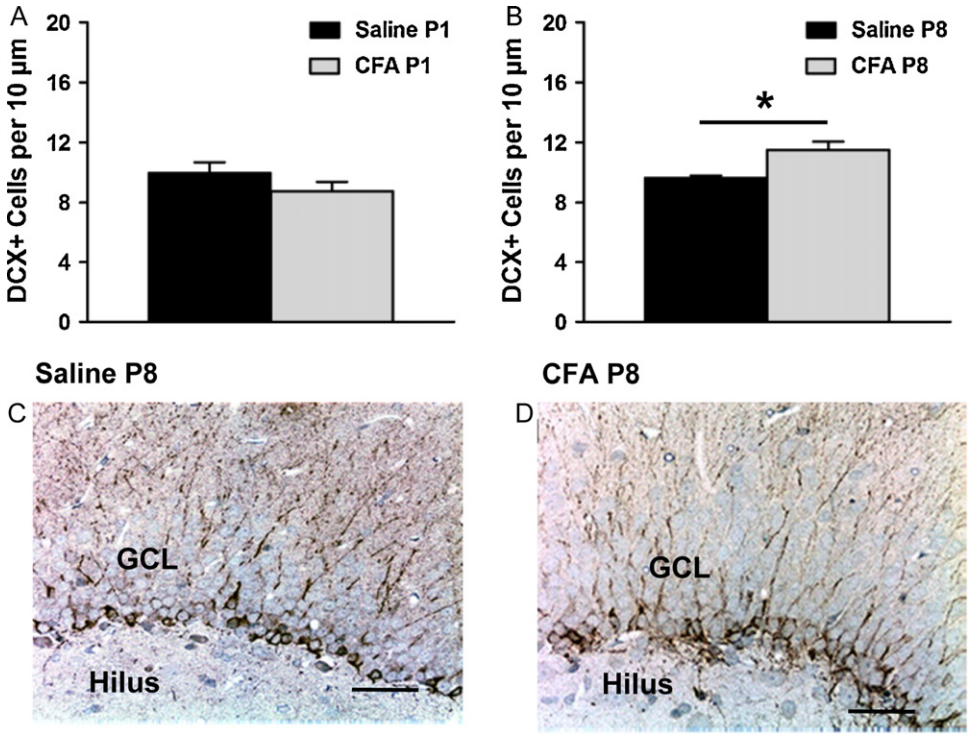

Fig. 2. Effect of neonatal pain on DCX expression in the SGZ of the dentate gyrus. (A) There were no differences in the number of DCX+ cells between pups injected with CFA on P1 and pups injected with saline on P1. (B) Pups injected with CFA on P8 had significantly more DCX+ cells than pups injected with saline on P8. Representative images of DCX+ cells in P8 saline pups, magnification 40× (C) and DCX+ cells in P8 CFA pups, magnification 40× (D). $^*p < .05$. GCL = granule cell layer. Scale bars = 100 μm in CD.

is increased after birth asphyxia [38], juvenile malnutrition [26], and adult electroconvulsive seizures [33]. Little is known, however, about how the generation of new hippocampal neurons is affected by pain. In contrast to our present finding that persistant inflammatory pain induced by CFA injection on P8 increased hippocampal neurogenesis, we previously found that acute pain induced by multiple needle pricks between P1 and P7 had no effect [29], suggesting that only certain types of painful experiences alter hippocampal neurogenesis during the neonatal period. Moreover, Duric and McCarson [17] found that three CFA injections during young adulthood decreased the generation of new hippocampal neurons, suggesting that the direction of the effect of pain on hippocampal neurogenesis depends on developmental stage.

Surprisingly, although there was an increase in hippocampal neurogenesis after CFA injection on P8, we observed no change after CFA injection on P1. One possible reason is that there may be maturational differences in the brain systems that respond to neonatal pain. Particularly relevant to the present findings are potential age-related differences in corticosteroid or opioid neurotransmission, as both hormonal systems are altered by neonatal pain [21,27] and influence the generation of new hippocampal neurons [11,23]. For instance, in a study on the ontogeny of hypothalamic-pituitary–adrenal (HPA) axis function, P7 was the earliest age at which rat pups mounted a hormonal response to a stressor [39]. Thus, neonatal pain might activate the HPA axis at P8 but not P1, leading to a change in hippocampal neurogenesis only in P8 pups. Evidence from human studies indicates that neonatal pain produces a short-term decrease in basal and evoked stress hormone levels [19,20], which may thereby promote the generation of new hippocampal neurons shortly after the painful experience [25].

A second possible reason for why pain increased hippocampal neurogenesis among P8 but not P1 pups is that there may be age-related differences in maternal care, which has been shown to influence hippocampal development [30]. Rat pups subjected to daily needle pricks from P2 to 14 were found to receive more licking and grooming from their mother on P6 compared to pups that did not experience pain [42]. However, it is not known whether an increase in maternal care toward injured pups is of the same magnitude on different days of development. The frequency of maternal care behaviors such as licking and active nursing is highest during the first couple days after birth and then gradually decreases across subsequent days [13,32]. Therefore, the increase in maternal care toward injured pups may be more dramatic at P8 when the background level of care is relatively low, compared to at P1 when maternal care is already at peak levels.

In conclusion, we found that, although no difference on the proportion of double labeled cells was identified, chronic neonatal inflammatory pain can increase in the generation of new neurons in the dentate gyrus region of the hippocampus, indicating that early painful experiences may have a significant impact on brain development. Because the first few postnatal weeks are a period of rapid hippocampal growth in the rat, with total hippocampal volume increasing 26% daily from P1 to P7 and 12% daily from P7 to P21 [7], even a brief increase in the generation of neurons during this period could have a significant impact on brain development. Interestingly, children born preterm, and hence subjected to repeated painful procedures, have been found to have smaller hippocampi than children born at term [24,36], raising the possibility that a brief increase in neurogenesis induced by neonatal pain could be followed by a compensatory decline in subsequent hippocampal growth. These results suggest that altered hippocampal growth could underlie the cognitive, emotional, and social abnormalities observed in rodents [2,3,8] and humans [22] who experienced pain early in life.

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References

[1] K.J. Anand, Effects of perinatal pain and stress , Prog. Brain Res. 122 (2000) 117–129.
[2] K.J. Anand, V. Coskun, K.V. Thrivikraman, C.B. Nemeroff, P.M. Plotsky, Long-term behavioral effects of repetitive pain in neonatal rat pups , Physiol. Behav. 66 (1999) 627–637.
[3] V.C. Anseloni, F. He, S.I. Novikova, M. Turnbach Robbins, I.A. Lidow, M. Ennis, M.S. Lidow, Alterations in stress-associated behaviors and neurochemical markers in adult rats after neonatal short-lasting local inflammatory insult , Neuroscience 131 (2005) 635–645.
[4] S. Avishai-Eliner, K.L. Brunson, C.A. Sandman, T.Z. Baram, Stressed-out, or in (utero)? Trends Neurosci. 25 (2002) 518–524.
[5] D.P. Barker, N. Rutter, Exposure to invasive procedures in neonatal intensive care unit admissions , Arch. Dis. Child. Fetal Neonatal Ed. 72 (1995) F47–F48.
[6] T. Bast, Toward an integrative perspective on hippocampal function: from the rapid encoding of experience to adaptive behavior , Rev. Neurosci. 18 (2007) 253–281.
[7] S.A. Bayer, Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life , J. Comp. Neurol. 190 (1980) 115–134.
[8] M. Bernardi, S. Genedani, A. Bertolini, Behavioral activity and active avoidance learning and retention in rats neonatally exposed to painful stimuli , Physiol. Behav. 36 (1986) 553–555.
[9] H. Bouza, The impact of pain in the immature brain , J. Matern. Fetal Neonatal Med. 22 (2009) 722–732.
[10] J.P. Brown, S. Couillard-Despres, C.A. Cooper-Kuhn, J. Winkler, L. Aigner, H.G. Kuhn, Transient expression of doublecortin during adult neurogenesis , J. Comp. Neurol. 467 (2003) 1–10.
[11] H.A. Cameron, E. Gould, Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus , Neuroscience 61 (1994) 203–209.
[12] H.A. Cameron, R.D.G. McKay, Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus , J. Comp. Neurol. 435 (2001) 406–417.
[13] F.A. Champagne, D.D. Francis, A. Mar, M.J. Meaney, Variations in maternal care in the rat as a mediating influence for the effects of environment on development , Physiol. Behav. 79 (2003) 359–371.
[14] B.R. Christie, H.A. Cameron, Neurogenesis in the adult hippocampus , Hippocampus 16 (2006) 199–207.
[16] J. Dobbing, J. Sands, Comparative aspects of the brain growth spurt , Early Hum. Dev. 311 (1979) 79–83.
[17] V. Duric, K.E. McCarson, Persistent pain produces stress-like alterations in hippocampal neurogenesis and gene expression , J. Pain 7 (2006) 544–555.
[18] H. Eichenbaum, Hippocampus: cognitive processes and neural representations that underlie declarative memory , Neuron 44 (2004) 109–120.
[19] R.E. Grunau, D.W. Haley, M.F. Whitfield, J. Weinberg, W. Yu, P. Thiessen, Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age , J. Pediatr. 150 (2007) 151–156.
[20] R.E. Grunau, L. Holsti, D.W. Haley, T. Oberlander, J. Weinberg, A. Solimano, M.F. Whitfield, C. Fitzgerald, W. Yu, Neonatal procedural pain exposure predicts lower cortisol and behavioral reactivity in preterm infants in the NICU , Pain 113 (2005) 293–300.
[21] R.E. Grunau, L. Holsti, J.W.B. Peters, Long-term consequences of pain in human neonates , Sem. Fetal Neonat. Med. 11 (2006) 268–275.
[22] R.E. Grunau, M.F. Whitfield, J. Petrie-Thomas, A.R. Synnes, I.L. Cepeda, A. Keidar, M. Rogers, M. MacKay, P. Hubber-Richard, D. Johannesen, Neonatal pain, parenting stress and interaction, in relation to cognitive and motor development at 8 and 18 months in preterm infants , Pain 143 (2009) 138–146.
[23] M.M. Holmes, L.A.M. Galea, Defensive behavior and hippocampal cell proliferation: differential modulation by naltrexone during stress , Behav. Neurosci. 116 (2002) 160–168.
[24] E.B. Isaacs, A. Lucas, W.K. Chong, S.J. Wood, C.L. Johnson, C. Marshall, F. Vargha-Khadem, D.G. Gadian, Hippocampal volume and everyday memory in children of very low birth weight , Pediatr. Res. 47 (2000) 713–720.
[25] M. Joels, Role of corticosteroid hormones in the dentate gyrus , Prog. Brain Res. 163 (2007) 355–370.
[26] R.S. King, W.A. DeBassio, T.L. Kemper, D.L. Rosene, J. Tonkiss, J.R. Galler, G.J. Blatt, Effects of prenatal protein malnutrition and acute postnatal stress on granule cell genesis in the fascia dentata of neonatal and juvenile rats , Dev. Brain Res. 150 (2004) 9–15.
[27] J.L. LaPrairie, A.Z. Murphy, Long-term impact of neonatal injury in male and female rats: sex differences, mechanisms and clinical implications , Front. Neuroendocrinol. 31 (2010) 193–202.
[28] V. Lemaire, M. Koehl, M. Le Moal, D.N. Abrous, Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus , Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11032–11037.
[29] A.T. Leslie, R. Guinsburg, L.E. Mello, L. Covolan, Repetitive nociceptive stimuli in newborn rats do not alter the hippocampal neurogenesis , Pediatr. Res. 63 (2008) 154–157.

[30] D. Liu, J. Diorio, J.C. Day, D.D. Francis, M.J. Meaney, Maternal care, hippocampal synaptogenesis and cognitive development in rats , Nat. Neurosci. 3 (2000) 799–806.
[31] L. Lu, G. Bao, H. Chen, P. Xia, X. Fan, J. Zhang, G. Pei, L. Ma, Modification of hippocampal neurogenesis and neuroplasticity by social environments , Exp. Neurol. 183 (2003) 600–609.
[32] S. Macri, G.J. Mason, H. Wurbel, Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats , Eur. J. Neurosci. 20 (2004) 1017–1024.
[33] T.M. Madsen, A. Treschow, J. Bengzon, T.G. Bolwig, O. Lindvall, A. Tingstrom, Increased neurogenesis in a model of electroconvulsive therapy , Biol. Psychiatry 47 (2000) 1043–1049.
[34] B.S. McEwen, Early life influences on life-long patterns of behavior and health , Ment. Retard. Dev. Disabil. Res. Rev. 9 (2003) 149–154.
[36] B.S Peterson, B. Vohr, L.H. Staib, C.J. Cannistraci, A. Dolberg, K.C. Schneider, K.H. Katz, M. Westerveld, S. Sparrow, A.W. Anderson, C.C. Duncan, R.W. Makuch, J.C. Gore, L.R. Ment, Regional brain volume abnormalities and long-term cognitive outcomes in preterm infants , JAMA 284 (2000) 1929–1947.
[37] M.A. Ruda, Q.-D. Ling, A.G. Hohmann, Y.B. Peng, T. Tachibana, Altered nociceptive neuronal circuits after neonatal peripheral inflammation , Science 289 (2000) 628–630.
[38] A. Scheepens, G. Wassink, M.J. Piersma, W.D.J. Van de Berg, C.E. Blanco, A delayed increase in hippocampal proliferation following global asphyxia in the neonatal rat , Dev. Brain Res. 142 (2003) 67–76.
[39] D.M. Vazquez, Stress and the developing limbic-hypothalamic-pituitary–adrenal axis , Psychoneuroendocrinology 23 (1998) 663–700.
[40] T. Verina, C.A. Rohde, T.R. Guilarte, Environmental lead exposure during early life alters granule cell neurogenesis and morphology in the hippocampus of young adult rats , Neuroscience 145 (2007) 1037–1047.
[41] J.J. Volpe, Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances , Lancet Neurol. 8 (2009) 110–124.
[42] C.-D. Walker, K. Kudreikis, A. Sherrard, C.C. Johnston, Repeated neonatal pain influences maternal behavior, but not stress responsiveness in rat offspring , Dev. Brain Res. 140 (2003) 253–261.
[43] C. Zhao, W. Deng, F.H. Gage, Mechanisms and functional implications of adult neurogenesis , Cell 132 (2008) 645–660.