ORIGINAL PAPER

Maternal Separation Induced Alterations of Neurogenesis in the Rat Rostral Migratory Stream

Enikő Račeková · Kamila Lievajová · Ján Danko ·
Marcela Martončíková · Slávka Flešárová ·
Viera Almašiová · Judita Orendáčová

Abstract   1. The aim of our study was to investigate the possibility that maternal separation, an experimental model for studies of early environmental influences, has an effect on postnatal neurogenesis in neurogenic pathway—the rostral migratory stream (RMS). 2. Rat pups were subjected to maternal separation daily for 3 h, starting from the first postnatal day (P1) till P14 or P21. In the first two groups, brains were analyzed at the age of P14 and P21, respectively. In the third group, after 3 weeks of maternal separation, 1 week of normal rearing was allowed, and the brains were analyzed at P28. The controls matched the age of maternally separated animals. Dividing cells were labeled by bromodeoxyuridine; dying cells were visualized by Fluoro-Jade C and nitric oxide (NO) producing cells by NADPH-diaphorase histochemistry. 3. Quantitative analysis of proliferating cells in the RMS showed that maternal separation decreased the number of dividing cells in all experimental groups. This decrease was most prominent in the caudal part of the RMS. The amount of dying cells was increased at the end of 3 weeks of maternal separation as well as 1 week later. The number of differentiated nitrergic cells in the RMS was increased at the end of 2 or 3 weeks of maternal separation, respectively. Besides quantitative changes, maternally separated animals showed an accelerated maturation of nitrergic cells. 4. Our results indicate that an exposure of rats to adverse environmental factors in early postnatal periods may induce acute site-specific changes in the RMS neurogenesis.

Introduction

The production of neurons during adulthood has been demonstrated in the mammalian hippocampus and olfactory bulb (OB) (Gage et al. 1998; Alvarez-Buylla and Garcia-Verdugo 2002; Carleton et al. 2003). The OB, the first relay on the sensory pathway, is the target for neuronal progenitors generated in the subventricular zone (SVZ). Cells born in the SVZ proceed toward the OB along a concise pathway, up to 5 mm long in rodents, called the rostral migratory stream (RMS) (Luskin 1993; Lois and Alvarez-Buylla 1994). Within the OB the newborn cells mature into two main types of interneurons, periglomerular cells and granule cells (Betarbet et al. 1996; Doetsch and Scharff 2001) and establish functional circuits with the existing neurons (Carlén et al. 2002). It is well known that precursor cells of the SVZ maintain the ability to divide during their migration within the RMS (Menezes et al. 1995; Feng and Walsh 2001). The cellular composition of the RMS is not homogenous. In rodents it is composed of cells with neuronal and astrocytic identities. Neuronal cells in the RMS include neuronal progenitors, neuroblasts, and biochemically differentiated neurons can be also distinguished (Jankovski and Sotelo 1996; Doetsch et al. 1997).

   In recent years many molecules have been found to be expressed in the RMS and to directly affect processes of neurogenesis. Signaling molecules that control proliferation,

E. Račeková (✉) · K. Lievajová · M. Martončíková ·
J. Orendáčová
Institute of Neurobiology, Slovak Academy of Sciences,
Šoltésovej 4, 040 01 Kosice, Slovak Republic
e-mail: racekova@saske.sk

J. Danko · S. Flešárová · V. Almašiová
University of Veterinary Medicine, Komenského 73,
041 81 Kosice, Slovak Republic

migration, or differentiation of neural precursors in the adult brain, are presently intensively studied. We have previously identified one of the phenotypically determined cell types in the RMS, cells producing a versatile diffusible signaling molecule of nitric oxide (NO). Our morphological examination has revealed that certain number of the RMS cells contains the neuronal isoform of the NO synthase (nNOS) and produces this messenger (Račeková et al. 2005). We have also shown that the expression of nNOS is evident from the postnatal day 10 (Račeková et al. 2003) and persists throughout the entire life of the animal (Račeková et al. 2005). The recent study of Gutièrrez-Mecinas et al. (2007) has provided further anatomical evidence for the role of this messenger in adult neurogenesis, indicating that migrating neuroblasts of the RMS contain the transduction machinery required for supporting a regulatory action of NO.

It is well established that survival of newborn cells in the OB depends on sensory input (Biebl et al. 2000; Petreanu and Alvarez-Buylla 2002). For example, the dependence of the survival of newly generated neurons on sensory stimulation in the OB has been demonstrated by decreased neurogenesis due to increased cell death after naris closure (Corotto et al. 1994). In another study, using anosmic mice, it was found that sensory input is critical for the survival of young granule cells during maturation, and once synaptically connected, their survival depends on the level of activity that they receive (Petreanu and Alvarez-Buylla 2002). However, there are only few studies examining whether a similar environmental manipulation may influence the cells in the RMS, which are destined to become OB interneurons.

Maternal separation is a commonly used and well-described model of environmental influences on development and subsequent nervous system function and behavior. Several studies have clearly demonstrated that repeated maternal separation during the first 2–3 weeks of life have long-term consequences on endocrine, behavioral, and brain development later in life. For example, maternal separation in rats caused stress hyperactivity (Francis and Meaney 1999) and long-term effects induced by maternal separation appear to depend upon changes in the structure and function of medial prefrontal cortex neurons, which are involved in the regulation of the stress response (Spencer et al. 2005). In the first weeks of life, mother is the only source of information necessary for pups' survival and this information is mediated mainly by olfaction. Pups, separated from their mother are thus deprived from the strong source of olfactory stimuli.

Although the evidence for continued cell production in the SVZ/RMS is now strong, the range of exogenous factors that influence this process remains largely unknown. The objective of our experiment was to investigate the possibility that odor deprivation induced by maternal separation has an effect on postnatal neurogenesis in the RMS.

Materials and Methods

Animals and Maternal Separation

Wistar albino rats were employed in this study. Experiments were approved by the Institutional Ethical Committee, in accordance with current national legislation.

Beginning from the first postnatal day (P1), pups were separated from the dam for a period of 3 h daily. Dams were first removed and placed in an adjacent cage. Litters were then transferred to a plastic container and placed into an incubator with the temperature set at approximately 34°C, the temperature consistent with nest measurements. After the isolation period, the pups were returned to their home cage, where they were reunited with the dam. The animals were divided into three groups based on the duration of maternal separation and survival periods: The 1st group—P14 rats (2 weeks of maternal separation; $n = 12$); The 2nd group—P21 rats (3 weeks of maternal separation; $n = 12$); The 3rd group—P28 rats (3 weeks of maternal separation followed by 1-week survival; $n = 12$). Control rats ($n = 36$) were reared under the same conditions except maternal separation, and sacrificed at postnatal days 14, 21, and 28 ($n = 12$ each survival period).

Tissue Processing

Both control and maternally separated rats were deeply anesthetized with xylazine/ketamine and intracardially perfused with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brain was dissected out, postfixed in the same fixative and cryoprotected with 30% sucrose in PB overnight. Sagittal serial sections (40 μm) were cut on a cryostat and processed either for immunohistochemical labeling or histochemical staining ($n = 6$ for each method of processing).

Bromodeoxyuridine Administration and Detection

To label proliferating cells, six animals from each experimental group received a single i.p. injection of 50 mg/kg of bromodeoxyuridine (BrdU; Sigma; dissolved in 0.9% NaCl with 0.007 M NaOH), under light Halothane anesthesia. Two hours after BrdU administration, the rats were perfused as described above.

To reveal the BrdU-immunoreactive cells, the sections were washed in 0.1 M PBS, treated with 2 N HCl at 60°C for 30 min to fragment DNA, and subsequently neutralized

in 0.1 M borate buffer, pH 8.4. To suppress endogenous peroxidase activity, the sections were incubated for 30 min in 20% methanol containing 0.3% H_2O_2. After blocking in normal serum the sections were exposed to 1:500 diluted BrdU primary monoclonal antibody (AbD Serotec, OBT0030CX, Oxford, UK), overnight at room temperature. The sections were then incubated with the secondary antibody (biotinylated goat anti-rat IgG, dilution 1:200), followed by standard ABC peroxidase solution (Vector, CA). DAB was used as a sensitive chromogen for visualization of BrdU-immunoreactive cells.

Fluoro-Jade C Histochemistry

In order to label dying cells in RMS, sections from one hemisphere from six animals in each experimental group were processed for Fluoro-Jade C (FJ-C; Histo-Chem Inc.) histochemistry. Sections were mounted on 2% gelatin-coated slides and air dried at 50°C for 30 min. Subsequently the slides were immersed in absolute alcohol for 3 min, for 1 min in 70% alcohol, for 1 min in distilled water, and then incubated in 0.06% potassium permanganate for 12 min. Then the slides were transferred for 1 h to FJ-C solution (1 μg/ml in 0.1% acetic acid). The proper dilution of staining solution was accomplished by first making a 0.01% stock solution of the dye in distilled water and then adding 1 ml of the stock solution to 99 ml of 0.1% acetic acid vehicle. The working solution was used within 2 h of preparation. The slides were then rinsed through three changes of distilled water for 1 min per change. The air-dried slides were cleared in xylene and cover slipped with DPX.

NADPH-d Histochemistry

NADPH-d reaction was performed on sections taken from one hemisphere from six animals in each experimental group. Free-floating sections were incubated in 0.1 M PB, pH 7.4 containing 0.4 mg/ml Nitroblue tetrazolium, 0.3% Triton X-100, 5 mg/ml malic acid, 4 mg/ml magnesium chloride and 0.8 mg/ml NADPH (ICN Biomedicals Inc.) at 37°C for 1 h (Scherer-Singler et al. 1983). The sections were then rinsed in 0.1 M PB, mounted on gelatin slides, air-dried overnight, cleared with xylene, and cover slipped with Entellan.

Quantitative Analysis

We have examined the number of BrdU, FJ-C, and NADPH-d-positive cells in the RMS of experimental as well as control rats. Only sections in which the entire RMS was visible were analyzed (six to eight sections for each

animal). The caudal limit of the RMS was defined in sagittal sections as the point at which the lumen of the lateral ventricle opens up at the interface of the corpus callosum and striatum. The rostral limit was defined as the point at which the OB begins.

Sections processed for BrdU-immunohistochemistry were examined by light microscope (Olympus BX-51) equipped with camera system DP50 (Olympus). To evaluate these sections, images were captured. BrdU-positive cells were counted separately in three anatomical parts of the RMS, i.e., in the vertical arm, the horizontal arm, and in the elbow (Fig. 1) with aid of Disector program (version 2.0) for stereometric unbiased estimation of particle number (Tomori et al. 2001). The counting was performed in three-dimensional space browsing through serial sections. We applied the point-counting method needed for estimation of the particle density. The outcomes were expressed as the average number of BrdU-positive cells per mm^3.

Sections processed for FJ-C histochemistry were examined by the Olympus Reflected Fluorescence system U-RFL-T, the Olympus BX51 and the digital camera DP50. The amount of FJ-C and NADPH-d-positive cells was assessed semi-quantitatively by counting all the positive cells along the RMS manually with supporting Image Tool software. The number of NADPH-d and FJ-C positive cells was expressed as mean number of cells per section.

Statistical analyses were performed using the one-way ANOVA test, Tukey–Kramer and Student t-tests. The values were expressed as means ± S.E.M.
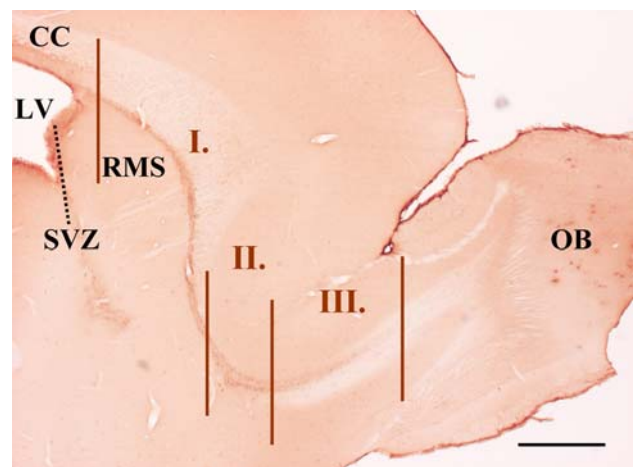
Fig. 1 Sagittal section of the rat forebrain demonstrating BrdU-labeled cells in the rostral migratory stream (RMS). Vertical lines separate the individual parts of the RMS: I.—the vertical arm, II.—the elbow, III.—the horizontal arm. CC—corpus callosum; LV—lateral ventricle; SVZ—subventricular zone; OB—olfactory bulb. Scale bar = 1 mm

Results

Proliferating Cells in the RMS

The results of BrdU-immunohistochemistry showed that maternal separation for 3 h daily induced obvious qualitative changes in the RMS (Fig. 2a–f). In P14 control rats (Fig. 2a) a very low density of BrdU-positive nuclei has been observed in comparison with the examined later postnatal ages (Fig. 2c and e). At maternally separated animals of the same age, i.e., at P14, low density of dividing cells was even more striking (Fig. 2b). At P21 maternally separated rats, rare occurrence of BrdU-positive cells was most apparent in the RMS vertical arm (Fig. 2d).

The density of dividing cells at P28 maternally separated rats lightly increased but it was still reduced when compared to the control animals.

In order to obtain detailed picture on the extent of proliferation, the number of BrdU-positive cells was counted in the RMS individual anatomical parts—the vertical and horizontal arms joined by the elbow. In our previous study, we have shown that the number of proliferating cells in the RMS gradually increases in the postnatal stage P14–P28 (Martončíková et al. 2006). In maternally separated rats, this pattern of increasing proliferation activity remained similar to that in the corresponding control groups; however, the number of proliferating cells in their RMS was decreased at all related
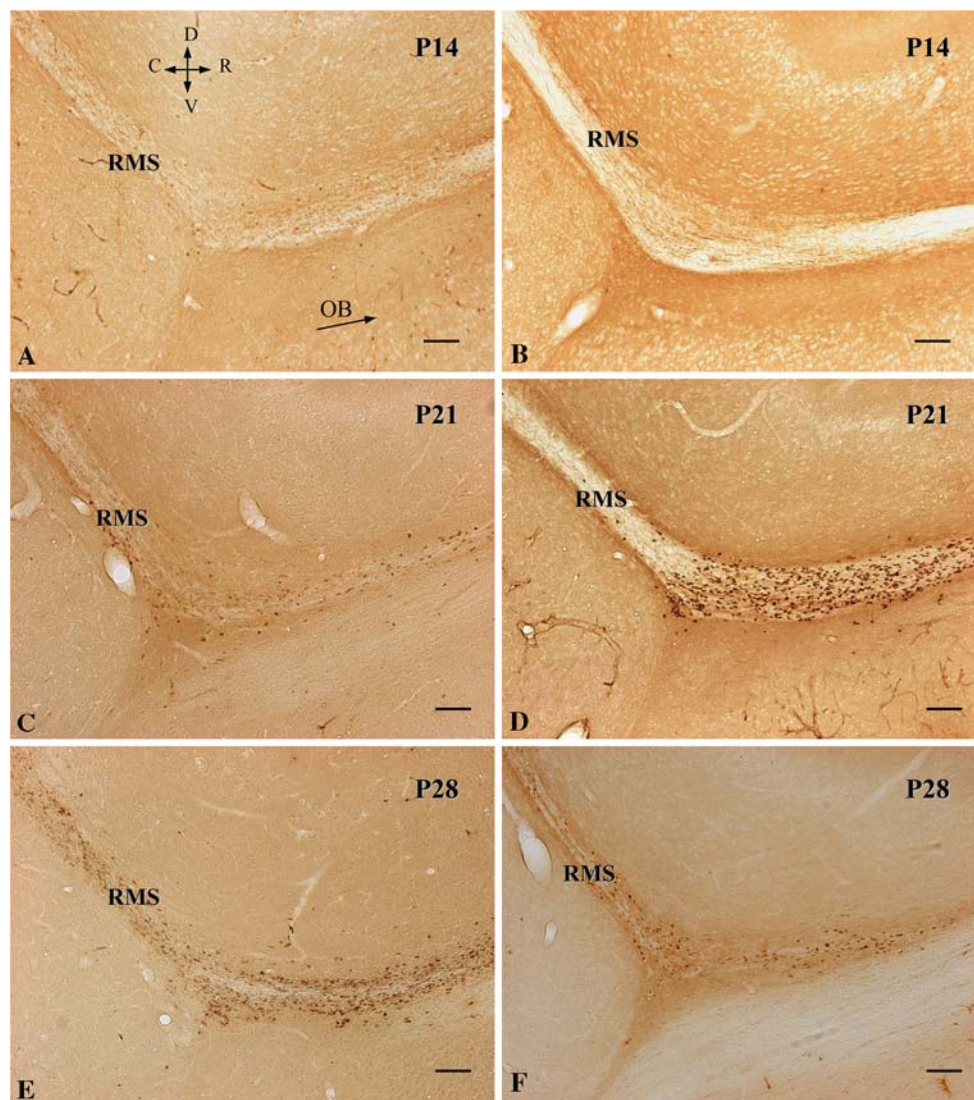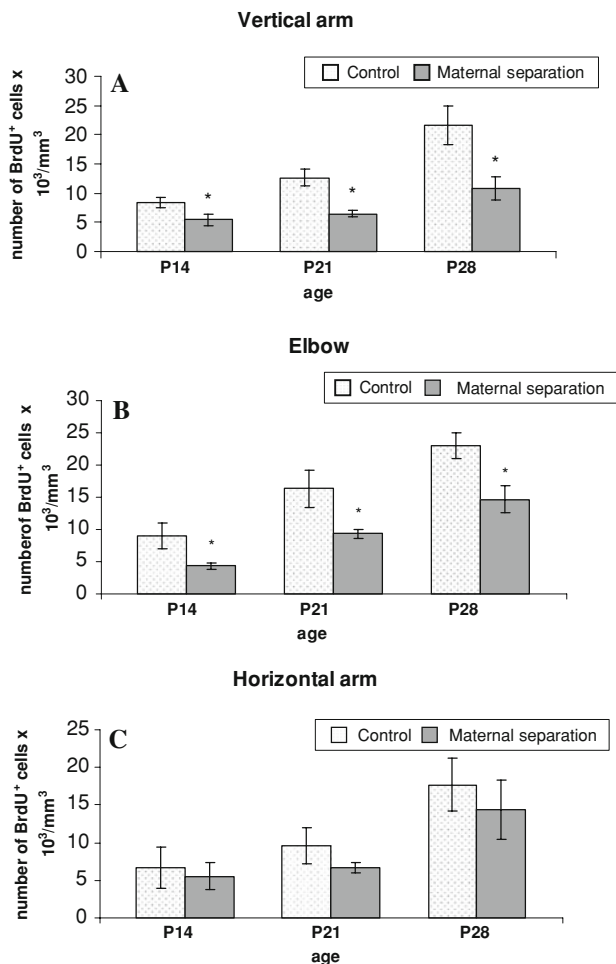

Fig. 2 Immunohistochemical staining for BrdU in the RMS of P14, P21, P28 control (left column) and maternally separated (right column) rats. The BrdU-positive cells are visible by brown stained nuclei. Note the low density of labeled cells both at P14 control (a) and experimental rats (b). Marked reduction of BrdU-positive cells in the RMS vertical arm of P21 maternally separated rat (d) in comparison with control animal of the same age (c). Different density of proliferating cells of P28 control (e) and maternally separated rats (f). OB-olfactory bulb. Scale bar = 100 μm

intervals of investigation (Fig. 3a–c). Concerning the RMS single parts, this decrease was statistically significant in its caudal parts—the vertical arm and the elbow (Fig. 3a and b). These findings are inversely proportional to the control rats
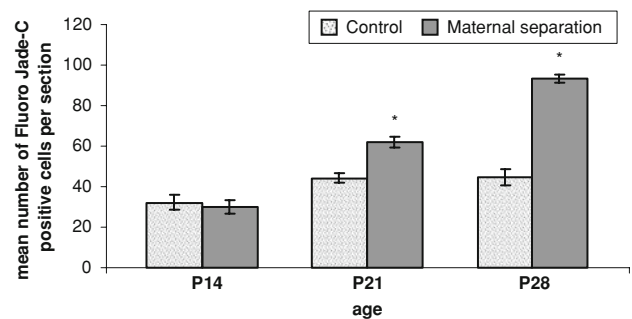
of the same ages as in these parts of the migratory stream the number of proliferating cells is the highest under physiological conditions throughout the life. Maternal separation induced decrease in proliferation was found also in the RMS horizontal arm, but this did not reach statistical significance in any experimental group (Fig. 3c).

Dying Cells in RMS

For detection of dying cells we have used a fluorochrome, FJ-C, which has been confirmed to detect dying neurons regardless of the cause of cell death (Schmued 2003; Mitrušková et al. 2005). Although FJ-C stained cells were present in the RMS both of control and experimental animals, the maternally separated rats showed higher density of labeled cells (Fig. 4a and b). Evaluation of the number of FJ-C-positive cells revealed that 2 weeks of maternal separation did not cause significant changes in dying cells number (Fig. 5) However, after 3 weeks lasting maternal separation (P21 rats) and at P28 animals, which survived 1 week after 3 weeks of maternal separation, the number of these cells was markedly increased in comparison with control rats (Fig. 5).

Fig. 3 Graphs show the number of BrdU-positive cells in the RMS vertical arm (**a**), elbow (**b**), and horizontal arm (**c**). Maternal separation decreased the number of proliferating cells at all intervals of investigation. Asterisk (*) indicates significant difference, $p < 0.05$

Fig. 5 Quantification of FJ-C positive cells in the RMS of control and maternally separated rats shows significant increase of labeled cells at the end of 3 weeks of maternal separation (P21 rats) and 1 week later (P28 rats). Asterisk (*) indicates significant difference, $p < 0.05$
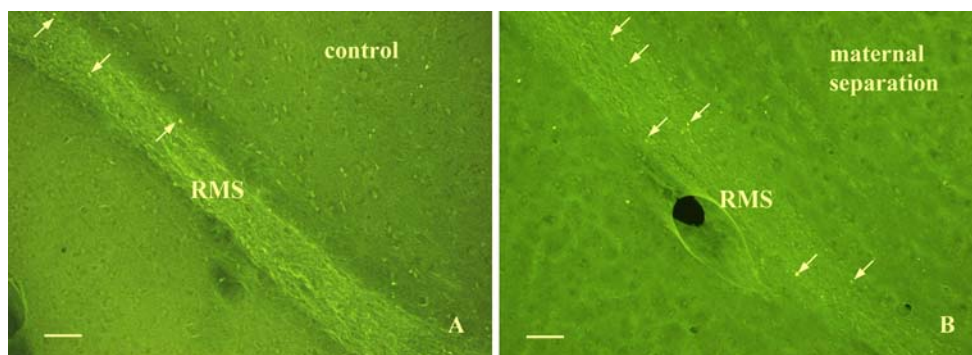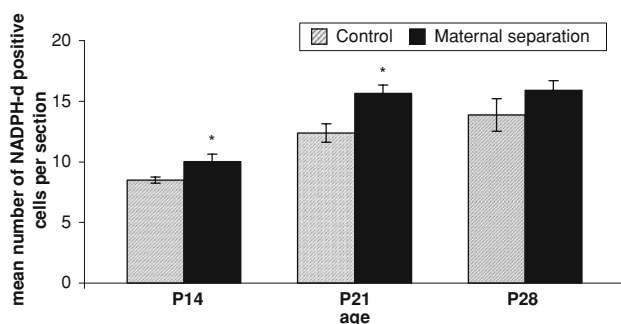

Fig. 4 Representative photomicrographs of FJ-C positive cells in the RMS of P21 rats. Low number of positive cells (arrows) in the RMS of control animal (**a**). Increased number of FJ-C positive cells (arrows) in the RMS after 3 weeks of maternal separation (**b**). Scale bar = 50 μm
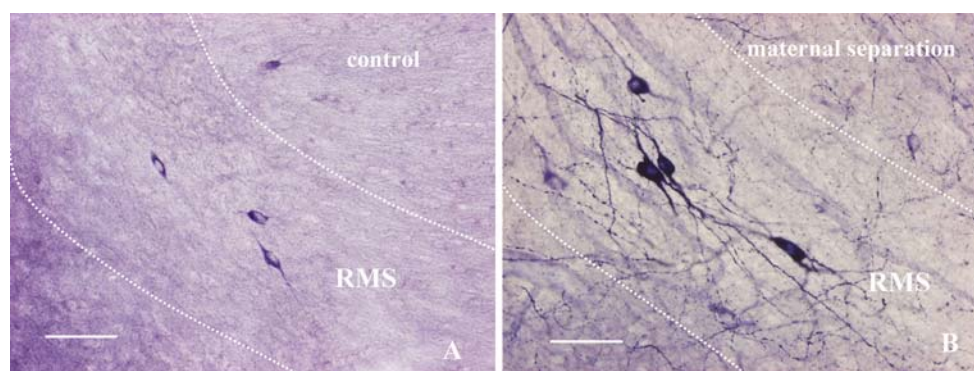
Cell Differentiation

The results of quantitative analysis of NADPH-d-positive cells showed that the number of these cells in the RMS of maternally separated rats was increased in comparison with corresponding control animals in all experimental groups (Fig. 6). This increase was statistically significant at P14 and P21 rats, i.e., immediately after the end of 2 or 3 weeks of maternal separation, respectively. In the RMS of rats surviving 1 week after 3 weeks of maternal separation, the number of nitrergic cells was still increased but this increase was not significant.

In addition to quantitative changes, maternally separated animals displayed an accelerated maturation of NO producing cells. NADPH-d-positive cells in P14 and P21 control rats had bipolar spindle-shaped cell body and at these ages only short fibers were visible (Fig. 7a). However, nitrergic cells at P14 and P21 maternally separated rats showed morphological characteristics that are typical for NADPH-d-positive cells of adult rats (Fig. 7b). In the RMS of these animals besides bipolar cells, triangular and

Fig. 6 Quantification of NADPH-d positive cells in the RMS. The differences between control and maternally separated rats are significant (*p < 0.05) at the end of 2 weeks as well as 3 weeks of maternal separation (P14 and P21 rats)

multipolar cells were also present like in adult RMS. Typically, these cells had well-developed abundant varicose processes, which are normally detected only in adult animals.

Discussion

Continuing neurogenesis in adult central nervous system is a phenomenon that might be of specific importance for neuroplasticity and regeneration in CNS. The mechanisms involved in the regulation of adult neurogenesis are therefore intensively studied. It has been confirmed that survival of newly generated neurons in the OB is dependent on their being incorporated into an active circuit. For example, exposure to specific odors increases the number of adult-born granule cells within the OB (Rochefort et al. 2002). Conversely, olfactory deprivation enhances OB granule cell apoptosis (Najbauer et al. 2002) and olfactory discrimination is decreased by genetic manipulations aimed at reducing the number of new cells integrated into the adult OB (Enwere et al. 2004).

Although mitotically active cells are present in the RMS of the adult rodent (Altman 1969; Lois and Alvarez-Buylla 1993), little is known about the factors that influence the ongoing neurogenesis specifically in this region. The present study demonstrates that early maternal separation strongly influences the process of neurogenesis in the RMS. Our quantitative analysis showed that the animals after MS had significantly reduced number of proliferating cells and increased cell dying in the neurogenic pathway. Previous studies investigated the effects of stressful experiences on adult neurogenis mainly in the hippocampus (Gould et al. 1998; Tanapat et al. 2001). In the similar maternal separation paradigm, Mirescu et al. (2004) investigated the effect of maternal separation on

Fig. 7 Comparison of representative photomicrographs of NADPH-d staining in the RMS of P21 control (a) and maternally separated rats (b). The labeled cells in control animal have bipolar spindle-shaped labeled cell body with short fibers (a). The NADPH-d positive cells, in the RMS of maternally separated rat of the same age, are rather multipolar with well-developed varicose processes (b). The RMS is demarcated by dotted lines. Scale bar = 50 µm

neurogenesis in the hippocampus and have reported that rat pups, subjected to daily maternal separation between P1 and P14, showed decreased proliferation of granule cell precursors in adulthood. The authors suggested that the observed downregulation of neurogenesis in the hippocampus is mediated through a corticosteroid-dependent mechanism. Although many evidence confirm the possibility that glucocorticoids are involved in the suppression of cell proliferation, this influence is not detectable by merely examining total peripheral levels of glucocorticoids (Mirescu and Gould 2006). Maternally separated rats by Mirescu et al. (2004) showed normal basal levels of plasma corticosterone. The authors explained the reduction in cell proliferation associated with maternal separation as a result of hypersensitivity to normal levels of corticosterone.

Similar to our findings, altered cell proliferation induced by olfactory cues has been demonstrated in the RMS of prairie voles (Smith et al. 2001). In female prairie voles, male exposure induced an increase in cell proliferation within the migratory pathway, possibly via an estrogen-mediated process. In the RMS of female prairie vole, enhanced cell proliferation has been also shown following social isolation (Fowler et al. 2001). Interestingly, this increase was significantly higher in comparison to male exposure. The authors explain these differences by the fact that prairie voles are typical glucocorticoid-resistant animal. In both experiments, changes in proliferation are supposed to be influenced by hormonal regulation. Glucocorticoids are known to negatively influence cell proliferation in the adult hippocampus (Tanapat et al. 2001; Yu et al. 2004). However, a role of these hormones in the regulation of RMS neurogenesis has not been confirmed and thus their contribution to the altered cell proliferation in the migratory stream is questionable.

In this study, quantitative analysis of proliferating cells number showed that the decrease of proliferation was not uniform in individual anatomical parts of the RMS. The most prominent decrease was observed in the caudal parts, i.e., in the vertical arm and elbow. It is known that the density of dividing cells decreases following a caudorostral gradient, with a maximum around the lateral ventricle and minimum in the subependymal layer of the OB (Altman 1969). We suppose that regional differences in response of the RMS anatomical parts to maternal separation is due to the higher vulnerability of those parts of the RMS, where the proliferation is highest under physiological conditions.

Changes in the number of BrdU-positive cells may have resulted not only from altered cell proliferation but also from their survival. Along the route of migration a considerable number of SVZ-generated cells undergo cell death in the rat (Brunjes and Armstrong 1996; Morshead et al. 1998). Here, we showed increased number of dying cells at the end of 3 weeks lasting maternal separation and

1 week later. Interestingly, the number of dying cells was not changed by 2 weeks of maternal separation. Considering the deepest decrease of BrdU-positive cells amount at this time point, the role of cell death on altered cell proliferation appears to be subtle.

Besides changes in cell proliferation and cell dying, our results indicate that stressful experiences caused by maternal separation during early life can alter the pattern of NO producing cells within the RMS. The observed increase of the NADPH-d-positive cells number and their early maturation raises a question whether NO could contribute to the altered proliferation or cell dying.

Recently NO has been shown to affect adult neurogenesis in different ways. The role of this gaseous messenger in adult neurogenesis has been demonstrated as antiproliferative effect described both in the SVZ and dentate gyrus of the hippocampus in physiological conditions. For example, Packer et al. (2003) found that the number of newly generated cells in the neurogenic regions of nNOS knockout mouse strain is dramatically increased. Moreover it has been demonstrated that the systematic or pharmacological inhibition of NO synthesis causes an increase of neuronal precursor proliferation in the SVZ (Matarredona et al. 2004; Moreno-López et al. 2004; Romero-Grimaldi et al. 2006; Torroglosa et al. 2007). The increase of the NADPH-d-positive cells number and coincident decrease of proliferating cells number in the RMS of maternally separated rats seems to support the antiproliferative effect of NO.

Another reported action of NO is the regulation of cell migration in the CNS. Although the role of NO in the migration of cells of the RMS is not fully understood, some facts indicate that NO produced from the blood vessels can influence cell migration within this neurogenic region. The investigations of Chen et al. (2005) using endothelial NOS (eNOS) knockout mice showed that the absence of eNOS impairs the migration of cells from SVZ explants. Moreover, migrating neuroblasts containing the machinery to respond to NO signaling are clustered together in small groups adjacent to blood vessels and can therefore be exposed to the NO produced by the eNOS from these vessels (Gutièrrez-Mecinas et al. 2007). We can speculate that the enhanced NADPH-d-positivity observed in blood vessels within the RMS of maternally separated rats (data not shown) could also influence the migration of the RMS precursor cells. Further studies are needed to identify the mechanisms by which environmental factors regulate the dynamic changes of cellular proliferation and migration in the RMS.

In conclusion, our findings have revealed that manipulation of olfactory environment by maternal separation exerts strong acute effect on neurogenesis in the rat RMS. Determining the mechanisms involved in regulation of

neurogenesis by exogenous factors would help to better understand the functional relevance of postnatal neurogenesis.

References

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–458. doi:10.1002/cne.901370404

Alvarez-Buylla A, Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. J Neurosci 22:629–634

Betarbet R, Žigová T, Bakay RAE, Luskin MB (1996) Dopaminergic and GABAergic interneurons of the olfactory bulb are derived from the neonatal subventricular zone. Int J Dev Neurosci 14:921–930. doi:10.1016/S0736-5748(96)00066-4

Biebl M, Cooper CM, Winkler J, Kuhn HG (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. Neurosci Lett 291:17–20. doi:10.1016/S0304-3940(00)01368-9

Brunjes PC, Armstrong AM (1996) Apoptosis in the rostral migratory stream of the developing rat. Dev Brain Res 92:219–222. doi:10.1016/0165-3806(96)00006-5

Carlén M, Cassidy RM, Brismar H, Smith GA, Enquist LW, Frisen J (2002) Functional integration of adult-born neurons. Curr Biol 12:606–608. doi:10.1016/S0960-9822(02)00771-6

Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM (2003) Becoming a new neuron in the adult olfactory bulb. Nat Neurosci 6:507–518

Chen J, Zacharek A, Zhang C, Jiang H, Li Y, Roberts C, Lu M, Kapke A, Chopp M (2005) Endothelial nitric oxide synthase regulates brain derived neurothropic factor expression and neurogenesis after stroke in mice. J Neurosci 25:2366–2375. doi:10.1523/JNEUROSCI.5071-04.2005

Corotto FS, Henegar JR, Maruniak JA (1994) Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse. Neuroscience 61:739–744. doi:10.1016/0306-4522(94)90397-2

Doetsch F, Scharff C (2001) Challenges for brain repair: insights from adult neurogenesis in birds and animals. Brain Behav Evol 58:306–322. doi:10.1159/000057572

Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A (1997) Cellular composition and three dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046–5061

Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S (2004) Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J Neurosci 24:8354–8365. doi:10.1523/JNEUROSCI.2751-04.2004

Feng Y, Walsh CA (2001) Protein-protein interactions, cytoskeletal regulation and neuronal migration. Nat Rev Neurosci 2:408–416. doi:10.1038/35077559

Fowler CD, Liu Y, Ouimet C, Wang Z (2001) The effects of social enviroment on adult neurogenesis in the female prairie vole. J Neurobiol 51:115–128. doi:10.1002/neu.10042

Francis DD, Meaney MJ (1999) Maternal care and the development of stress responses. Curr Opin Neurobiol 9:128–134. doi:10.1016/S0959-4388(99)80016-6

Gage FH, Kempermann G, Palmer T, Peterson DA, Ray J (1998) Multipotent progenitor cells in the adult dentate gyrus. J Neurobiol 36:249–266. doi:10.1002/(SICI)1097-4695(199808)36:2<249::AID-NEU11>3.0.CO;2-9

Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 95:3168–3171. doi:10.1073/pnas.95.6.3168

Gutièrrez-Mecinas M, Crespo C, Blasco-Ibáñez JM, Nácher J, Varea E, Martínez-Guijarro FJ (2007) Migrating neuroblasts of the rostral migratory stream are putative targets for the action of nitric oxide. Eur J NeuroSci 26:392–402. doi:10.1111/j.1460-9568.2007.05672.x

Jankovski A, Sotelo C (1996) Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specifity as determined by heterochromic and heterotopic transplantation. J Comp Neurol 371:376–396. doi:10.1002/(SICI)1096-9861(19960729)371:3<376::AID-CNE3>3.0.CO;2-#

Lois C, Alvarez-Buylla A (1993) Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc Natl Acad Sci USA 90:2074–2077. doi:10.1073/pnas.90.5.2074

Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. Science 264:1145–1148. doi:10.1126/science.8178174

Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173–189. doi:10.1016/0896-6273(93)90281-U

Martončíková M, Račeková E, Orendáčová J (2006) The number of proliferating cells in the rostral migratory stream of rat during the first postnatal month. Cell Mol Neurobiol 26:1453–1461

Matarredona ER, Murillo-Carretero M, Moreno-López B, Estrada C (2004) Nitric oxide synthesis inhibition increases proliferation of neural precursors isolated from the postnatal mouse subventricular zone. Brain Res 995:274–284. doi:10.1016/j.brainres.2003.10.010

Menezes JR, Smith CM, Nelson KC, Luskin MB (1995) The division of neuronal progenitor cells during migration in the neonatal mammalian forebrain. Mol Cell Neurosci 6:496–508. doi:10.1006/mcne.1995.0002

Mirescu C, Gould E (2006) Stress and adult neurogenesis. Hippocampus 16:233–238. doi:10.1002/hipo.20155

Mirescu C, Peters JD, Gould E (2004) Early life experience alter response of adult neurogenesis to stress. Nat Neurosci 7:841–846. doi:10.1038/nn1290

Mitrušková B, Orednáčová J, Račeková E (2005) Fluoro Jade-B detection of dying cells in the SVZ and RMS of adult rats after bilateral olfactory bulbectomy. Cell Mol Neurobiol 25:1255–1264. doi:10.1007/s10571-005-8502-1

Moreno-López B, Romero-Grimaldi C, Noval JA, Murillo-Carretero M, Matarredona ER, Estrada C (2004) Nitric oxide is a physiological inhibitor of neurogenesis in the adult mouse subventricular zone and olfactory bulb. J Neurosci 24:85–95. doi:10.1523/JNEUROSCI.1574-03.2004

Morshead CM, Craig CG, van der Kooy D (1998) In vivo clonal analyses reveal the properties of endogenous neural stem cell proliferation in the adult mammalian forebrain. Development 125:2251–2261

Najbauer J, Yan XX, Leon M (2002) Internucleosomal DNA fragmentation during deprived and non-deprived olfactory development. Brain Res 926:118–125. doi:10.1016/S0006-8993(01)03313-3

Packer MA, Stasiv Y, Benraiss A, Chmielnicki E, Grinberg A, Westphal H, Goldman SA, Enikolopov G (2003) Nitric oxide negatively regulates mammalian adult neurogenesis. Proc Natl Acad Sci USA 100:9566–9571. doi:10.1073/pnas.1633579100

Petreanu LT, Alvarez-Buylla A (2002) Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. J Neurosci 22:6106–6113

Račeková E, Orendáčová J, Martončíková M, Vanický I (2003) NADPH-diaphorase positivity in the rostral migratory stream of the developing rat. Dev Brain Res 146:131–134. doi:10.1016/j.devbrainres.2003.09.014

Račeková E, Martončíková M, Mitrusková B, Čížková D, Orendáčová J (2005) Age-related changes of NADPH-diaphorase positivity in the rat rostral migratory stream. Cell Mol Neurobiol 25:1093–1105. doi:10.1007/s10571-005-8191-9

Rochefort C, Gheusi G, Vincent JD, Lledo PM (2002) Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci 22:2679–2689

Romero-Grimaldi C, Gheusi G, Lledo PM, Estrada C (2006) Chronic inhibition of nitric oxide synthesis enhances both subventricular zone neurogenesis and olfactory learning in adult mice. Eur J NeuroSci 24:2461–2470. doi:10.1111/j.1460-9568.2006.05127.x

Scherer-Singler U, Vincent SR, Kimura H, McGeer EG (1983) Demonstration of a unique population of neurons with NADPH diaphorase histochemistry. J Neurosci Methods 8:229–234. doi:10.1016/0165-0270(83)90085-7

Schmued LC (2003) Demonstration and localization on neuronal degeneration in the rat forebrain following a single exposure to MDMA. Brain Res 974:127–133. doi:10.1016/S0006-8993(03)02563-0

Smith MT, Pencea V, Wang Z, Luskin MB, Insel TR (2001) Increased number of BrdU-labeled neurons in the rostral migratory stream of the estrous prairie vole. Horm Behav 39:11–21. doi:10.1006/hbeh.2000.1630

Spencer SJ, Buller KM, Day TA (2005) Medial prefrontal cortex control of the paraventricular hypothalamic nucleus response to psychological stress: possible role of the bed nucleus of the stria terminalis. J Comp Neurol 481:363–376. doi:10.1002/cne.20376

Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E (2001) Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. J Comp Neurol 437:496–504. doi:10.1002/cne.1297

Tomori Z, Krekule I, Kubínová L (2001) Disector program for unbiased estimation of particle number, numerical density and mean volume. Image Anal Stereol 20:119–130

Torroglosa A, Murillo-Carretero M, Romero-Grimaldi C, Matarredona ER, Campos-Caro A, Estrada C (2007) Nitric oxide decreases subventricular zone stem cell prolioferation by inhibition of epidermal growth factor receptor and phosphoinositide-3-kinase/Akt pathway. Stem Cells 25:88–97. doi:10.1634/stemcells.2006-0131

Yu IT, Lee SH, Lee YS, Son H (2004) Differential effects of corticosterone and dexamethasone on hippocampal neurogenesis in vitro. Biochem Biophys Res Commun 317:484–490. doi:10.1016/j.bbrc.2004.03.071