Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 19
Sofia • 2012

Proliferation and Apoptosis during Early Postnatal Neurogenesis in Rat Brain

V. Goranova[1], O. Uckermann[2] and C. Ikonomidou[3]

[1]Dept. of Anatomy, Histology and Embryology, Medical University, Varna, Bulgaria; [2]Department of Pediatric Neurology, Childrens' Hospital, Technical University, Dresden, Germany; [3]Waisman Center and Department of Neurology, University of Wisconsin, Madison, WI, USA

We studied neurogenesis in the developing brain using the thymidine analog, 5-bromo-2'-deoxyuridine (BrdU) to assess proliferation and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method to evaluate apoptosis.

Wistar rats between postnatal day 0 (P0) and P30 received single or multiple BrdU injections by various survival time points. Peroxidase or fluorescence immunohistochemistry were applied on paraffin sections to label BrdU+ cells, neuronal markers such as nestin, Dcx, MAP(2) and NeuN or for TUNEL. Cell proliferation and neuronal apoptosis were analyzed using standard or confocal microscopy and stereology. Peak neurogenesis was found in the dentate gyrus during the first postnatal week with a progressive decline after P9. Colocalization of BrdU with neuronal markers was found mainly within the dentate gyrus and cerebellum. Most of the newly generated cells became nonneuronal cells (endothelial and glial cells). Apoptosis did not contribute to cell death of the newly generated neuronal progenitors.

Key words: BrdU, TUNEL, neurogenesis, developing brain

Introduction

In the mammalian brain, prenatal (embryonal) neurogenesis continues after birth as early and adult postnatal events of generation and functional integration of new neurons. It occurs intensively during the first weeks after birth picking at P7-P9. Thereafter, it remains longlife in the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the dentate gyrus (DG)[4,8,9]. Neurogenesis includes several processes such as proliferation, migration, differentiation, maturation and apoptosis that play crucial roles in the final physiological outcome. Apoptosis, or programmed cell death, is the death of excess or unwanted cells that could be activated also in the presence of appropriate stimuli in time of detrimental stress[5,6,10]. Coordination between cell proliferation and apoptosis maintains tissue homeostasis in the brain. TUNEL method is widely used for demonstration of apoptotic cell death opposite to necrotic cell death. In this study, we investigated the rate of proliferation and apoptosis as well as if apoptosis contributes significantly to cell death of newly generated neuronal progenitors and mature neurons during the early postnatal period.

Material and Methods

Wistar rats (BgVV, Berlin) at different age between P0 and P30 received single or multiple 100 or 50 mg/kg BrdU (Sigma, St. Louis, MO) injections by various survival time points between P1 and P51. Animals were anesthetized and transcardially perfused with 0.01M PBS, followed by 4% paraformaldehyde in 0.1M PB. The brains were removed and postfixed in the same fixative. Paraffin sections were processed for single or double peroxidase or fluorescence immunostaining. TUNEL was performed using ApopTag® Peroxidase or Fluorescein *In Situ* Apoptosis Detection kit. First antibodies against BrdU, Nestin, Dcx, MAP(2) and NeuN were used followed by biotinylated second antibodies, ABC reagent and DAB or VNR substrate. Alexa Fluor 594 or 488 and Vectashield mounting medium with DAPI were used by fluorescence labeling. Morphometric countings evaluating BrdU+ nuclei on P9, P12, P15 and P23 were done in a blinded fashion. Confocal microscopy was applied for colocalization of BrdU with neuronal markers or TUNEL.

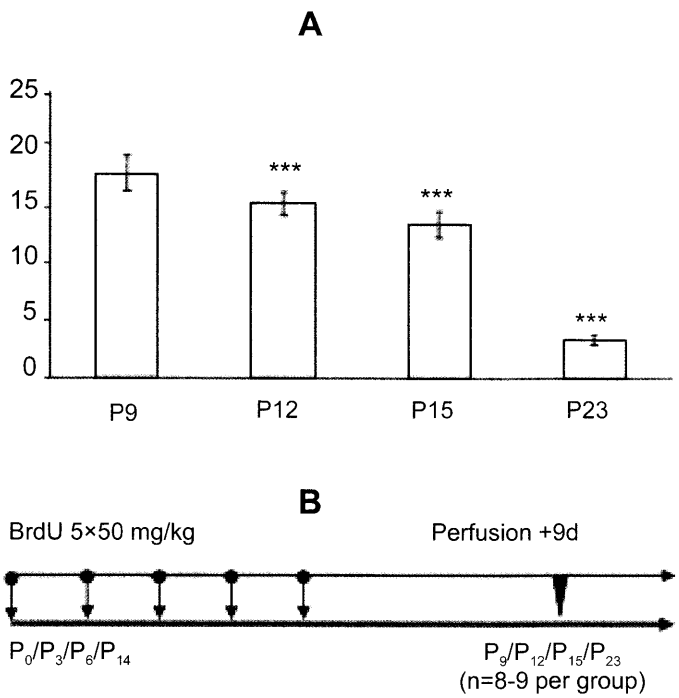

Fig. 1. (A) Summarized total proliferative scores of BrdU+ cell counts by peroxidase immunohistochemistry on P9, P12, P15 and P23; ten brain regions are included (frontal cortex layer I, frontal cortex layer II, parietal cortex layer I, parietal cortex layer II, caudate nucleus, mediodorsal thalamus, corpus callosum, molecular layer of cerebellum, subgranular zone of the dentate gyrus, granular cell layer of the dentate gyrus). Cell countings have shown a significant decrease in the proliferative rate to each previous age group. Student't *t*-test; ***P<0.001, values are presented as mean ± S.E.M. **(B)** BrdU paradigm: 50 mg daily for 5 days starting on P0, P3, P6 and P14; perfusion is performed 9 days later on P9, P12, P15 and P23 respectively.

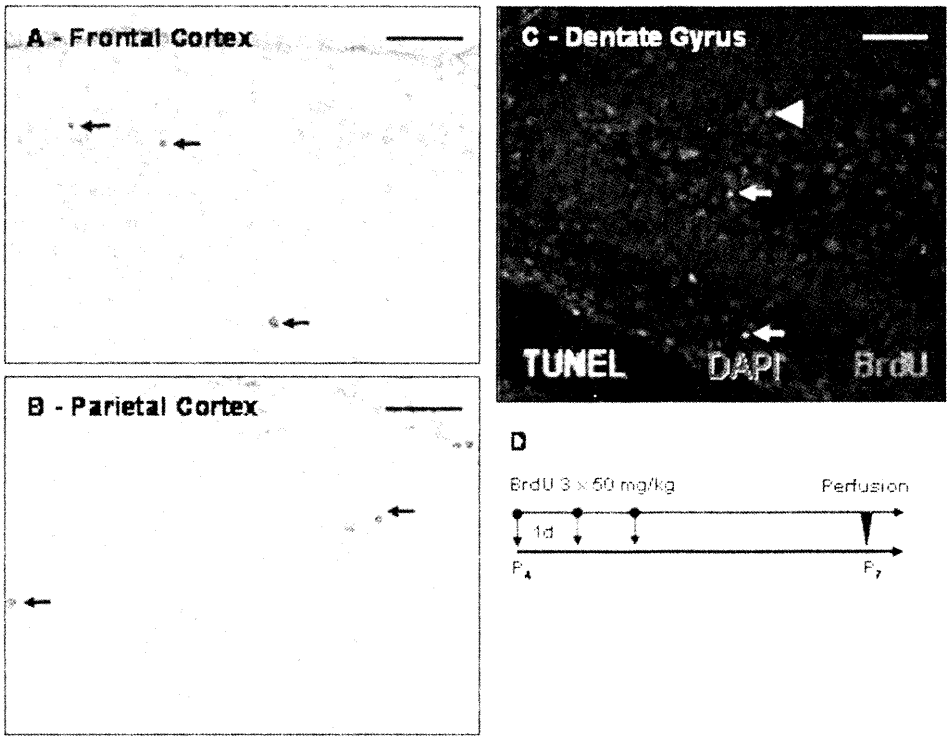

Fig. 2. (A, B) Single TUNEL+ nuclei detected by peroxidase immunostaining with DAB located in the brain cortex; **(C)** confocal image after fluorescein immunohistochemistry for TUNEL (green), BrdU (red) and DAPI (blue) in DG **(D)** BrdU paradigm applied in C; arrow = TUNEL+ cell, arrowhead = BrdU+ cell, bar = 50μm.

Results

We analyzed cell proliferation comparing same age groups with different BrdU paradigms, i.e., ages, dosages or survival timings. By same ages, higher dosages of BrdU produced a more intensive immunostaining when survival times were equal. By same dosage, younger animals showed a more expressed staining, whereas longer survival times showed reduced labeling. This was demonstrated by summarized total proliferative scores of BrdU+ cell counts in ten brain regions on P9, P12, P15 and P23 (Fig. 1). At the end of the second postnatal week, frequent colocalization for BrdU+/Nestin+ and BrdU+/Dcx+ was mainly found in specific regions such as the SVZ, cerebellum, corpus callosum and DG-SGZ. BrdU+/MAP+ and BrdU+/NeuN+ colocalization was occasionally seen by later timings (data not shown). Many of the newly generated cells were negative for the neuronal markers applied.
TUNEL method showed a relatively low level of cell death in the early postnatal period except for the first week. Single TUNEL+ nuclei were found maily in the gray matter areas such as thalamus, caudate and cortex (Fig. 2A,B). No colocalization of BrdU or any of the neuronal markers with TUNEL was found in the brain regions analyzed (Fig. 2C,D).

Discussion

During the early postnatal period, cell proliferation and neuronal differentiation in the rat brain are very intensive. Together with apoptosis, they play an important role in shaping the developing nervous system. Not all BrdU labeled cells observed in our study belong to the neuronal lineage. Colocalization of BrdU with some of the neuronal markers applied is an indice for neuronal differentiation. The newly generated cells that were negative for neuronal markers become mainly endothelial and glial cells. After the first two to three postnatal weeks, neurogenesis in the rat brain is attenuated but remains lifelong in the SVZ of the lateral ventricle and SGZ of the DG. In both zones multipotent neural precursors are preserved and may serve to repair injuries to the brain[1-3,7].

Apoptosis does not contribute significantly to cell death of the newly generated neuronal progenitors and mature neurons during the early postnatal period. Our data on early postnatal neurogenenic processes might be helpful for a better understanding of the adult neurogenesis in the mammalian brain.

Acknowledgements: Supported by BMBF grants 01GZ0305 and PBZ-MN-001/P05/2002/25-28.

References

1. Danzer, S. C., 2008. Postnatal and adult neurogenesis in the development of human disease. – Neuroscientist, 14(5), 446-458.
2. DeCarolis, N. A., A. J. Eisch, 2010. Hippocampal neurogenesis as a target for the treatment of mental illness: a critical evaluation. – Neuropharmacology, 58(6), 884-893.
3. Eisch, A. J., H. A. Cameron, J. M. Encinas, L. A. Meltzer, G. L. Ming, L. S. Overstreet-Wadiche, 2008. Adult neurogenesis, mental health, and mental illness: hope or hype? – J. Neurosci., 28(46), 11785-91.
4. Gage, F. H., 2002. Neurogenesis in the adult brain. – J. Neurosci., 22(3), 612–613.
5. Liou, A. K., R. S. Clark, D. C. Henshall, X. M. Yin, J. Chen, 2003. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. – Prog. Neurobiol., 69(2), 103-142.
6. Raff, M., 1998. Cell suicide for beginners. – Nature, 396, 119-122.
7. Snyder, J. S., A. Soumier, M. Brewer, J. Pickel, H. A. Cameron, 2011. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. – Nature, 476(7361), 458-461.
8. Toni, N., E. M. Teng, E. A. Bushong, J. B. Aimone, C. Zhao, A. Consiglio, H. van Praag, M. E. Martone, M. H. Ellisman, F. H. Gage, 2007. Synapse formation on neurons born in the adult hippocampus. – Nat. Neurosci., 10(6), 727-734.
9. van Praag, H., A. F. Schinder, B. R. Christie, N. Toni, T. D. Palmer, F. H. Gage, 2002. Functional neurogenesis in the adult hippocampus. – Nature, 415(6875), 1030-1034.
10. Yuan, J., B. A. Yankner, 2000. Apoptosis in the nervous system. – Nature, 407, 802-807.