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Investigation of the Early Postnatal Neurogenesis in Rats

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After birth in mammals, neurogenesis continues in restricted regions throughout life. We used the thymidine analog, 5-bromo-2'-deoxyuridine (BrdU), that labels DNA, and cell-type-specific markers for neuronal cell lineage (nestin, Dcx, MAP(2) and NeuN). We statistically investigated two parameters of neurogenesis (cell proliferation and neuronal differentiation) in 10 different regions of the developing rat brain at various time points after birth from P1 to P51 (P, postnatal day). Both of these parameters showed regional distribution and age dependency during the postnatal period. Peak neurogenesis was found in the dentate gyrus during the first week of life with a progressive decline after P9. Since adult mammalian neurogenesis consists generally of the same processes as early postnatal neurogenesis, our investigaton might provide usefull information for studying normal and pathological neurogenesis.

Key words: Neurogenesis, BrdU, rat, dentate gyrus.

Introduction

In the mammalian central nervous system (CNS), neurogenesis 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 the subgranular zone (SGZ) of the dentate gyrus[1] (DG). Adult neurogenesis repeats the stages of generation and functional integration of new neurons similarly to the developing brain. Therefore, data on early postnatal neurogenensis might give a better understanding to adult neurogenesis. The goal of the present study was to provide additional data on basic neurogenic mechanisms of the developing and adult brain.

Material and Methods

All animal experiments were done in accordance to the institutional guidelines. Wistar rats (BgVV, Berlin) at different age between P0 and P30 were injected i.p. with BrdU (Sigma, St. Louis, MO) using two paradigms: 1) Type 1-100 mg/kg BrdU on P0, P3, P7, P10, P14, P21, P30; perfusion 24 h or 21 days later for each time point (14 groups, n=3

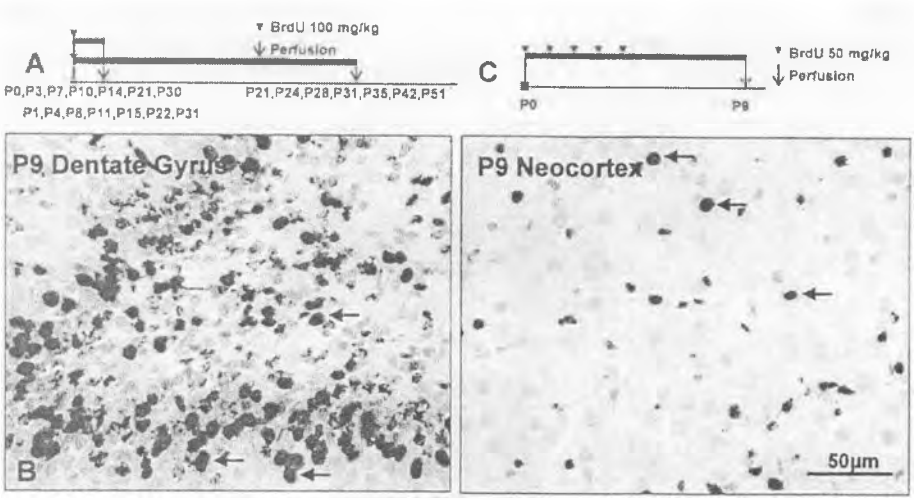

Fig. 1. **A** – Type 1 BrdU paradigm (1 × 100 mg); **B** – Light micrographs showing BrdU(+) cells (arrows) in dentate gyrus/neocortex on P9, VNR substrate, hematoxylin counterstaining; **C** – Type 2 BrdU paradigm (50 mg daily for 5 days) on P9 corresponding to the images in **B**

per group) (Fig. 1A); 2) Type 2-50 mg/kg BrdU daily for 5 days starting on P0, P3, P6 and P14; perfusion 9 days later. A similar group as P6 was designed with a survival time of 28 days (n=5) (Fig. 2A). 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 immunostaining for peroxidase or fluorescence labelling. First antibodies to detect BrdU, Nestin, Doublecortin (Dcx), NeuN and MAP(2) were used. Biotinylated second antibodies, ABC reagent and DAB/VNR substrate were then applied. By fluorescence labeling, Alexa Fluor 594 or 488 and Vectashield mounting medium with DAPI were used. Morphometric countings evaluating BrdU(+) nuclei on P9, P12, P15, P23 and P34 or Dcx(+) cells, BrdU(+) nuclei and Dcx(+)/BrdU(+) colocalization using confocal images were done in a blinded fashion. Values are presented as mean ± S.E.M.

Results

We first compared same age groups with different BrdU paradigms or survival timings between P1-P51 (Fig. 1A). We found that in both groups, 24 h and 21 days, older animals expressed progressively less BrdU and longer survival timings reduced singnificantly the number of labeled cells. At the end of the second postnatal week BrdU/neuronal marker expression was restricted mainly to two regions – SVZ of the lateral ventricle and SGZ of DG. BrdU staining in DG and cortex on P9 is shown in Fig. 1B; corresponding BrdU paradigm is indicated in Fig. 1C. Summarized total proliferative scores of BrdU(+) cells on P9, P12, P15, P23 and P34 (BrdU paradigm in Fig. 2A) showed statistical significance (Fig. 2B). With few exceptions, cell countings in ten brain regions have shown significant decrease in proliferative rate to the previous age group (Fig. 2C). Compared to P23, values for P34 were higher because of younger animals by P34. We quantified numbers of immuno(+) cells for Dcx(+) (77.12%), BrdU(+) (50.60%) and cells coexpressing both of these markers (39.48%) in the DG on P15 (Fig. 3).

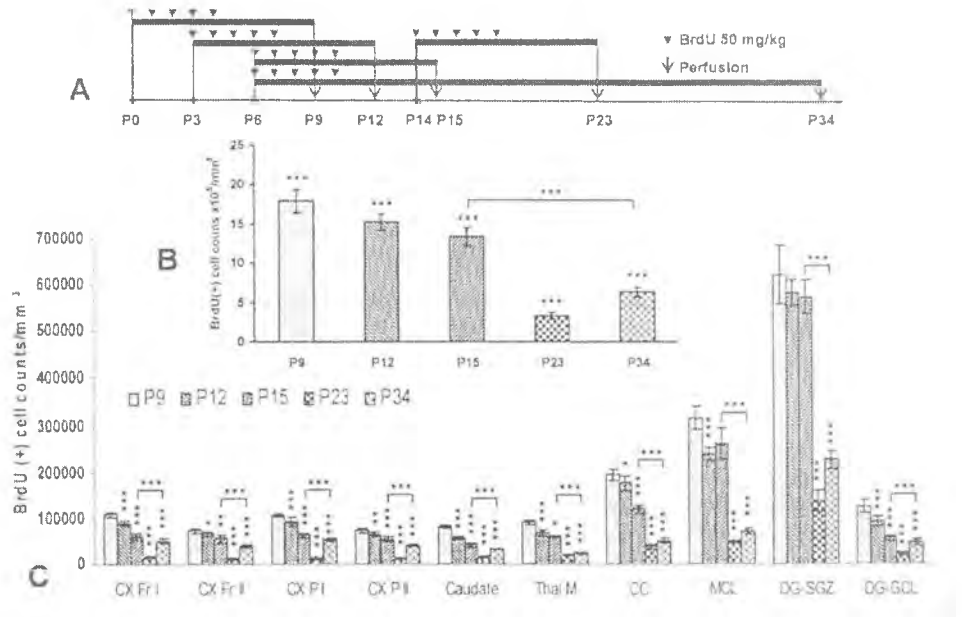

Fig. 2. **A** – Type 2 BrdU paradigm (50 mg daily for 5 days); **B** – Summarized total proliferative scores of BrdU(+) cell counts on P9 (n=9), P12 (n=9), P15 (n=8), P23 (n=8) and P34 (n=5); **C** – BrdU(+) cell counts in ten individual brain regions (frontal cortex layer I = CX Fr I, frontal cortex layer II = CX Fr II, parietal cortex layer I = CX P I, parietal cortex layer II = CX P II, caudate nucleus = Caudate, mediodorsal thalamus = Thal M, corpus callosum = CC, molecular layer of cerebellum = MCL, subgranular zone of the dentate gyrus = DG-SGZ, granular cell layer of the dentate gyrus = DG-SGL on P9, P12, P15, P23 and P34. Student't t-test; ***P<0.001, **P<0.01 or *P<0.05, when compared with total or individual score of the previous time point or between P15 and P34 as indicated

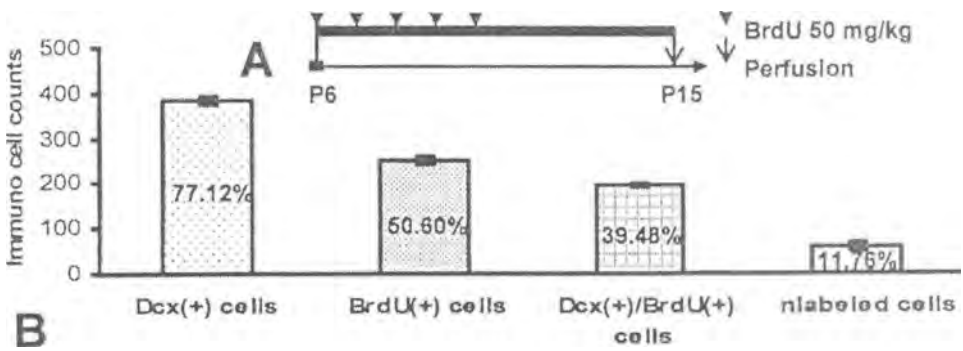

Fig. 3. **A** – Type 2 BrdU paradigm (50 mg daily for 5 days) on P15; **B** – Percentage of Dcx(+) cells, BrdU(+) cells, Dcx(+)/BrdU(+) cells and unlabeled cells in the dentate gyrus on P15 (n=5)

During the first two postnatal weeks, Nestin(+) radial glia and Dcx(+) cells underwent a considerable reduction. MAP(2) and NeuN immunoexpression increased after P9 and remained constant by older animals. Frequent colocalization for BrdU(+)/Nestin(+) or BrdU(+)/Dcx(+) was found in specific regions such as the SVZ, cerebellum, corpus callosum and DG-SGZ. BrdU(+)/MAP(+) or BrdU(+)/NeuN(+) colocalization was seen rarely by later timings.

Discussion

Cell proliferation and neuronal differentiation in the developing rat brain display regional distribution and age dependency. BrdU labeling shows dose, age and survival time dependency. Expressions of Nestin and Dcx decrease, especially for nestin(+) radial glia in neocortex. Radial glia are known not only to guide migrating neurons to outer cortical layers but also to generate neurons [3]. We registered also an increasing expression for MAP(2) and NeuN showing that neuronal migration and differentiation are still going on during the early postnatal period. This is one of the vulnerable periods to pathological factors leading to disruption [2].

We found that after the first three weeks, postnatal neurogenesis in the rat is considerably reduced and restricted to two brain regions: the SVZ of the lateral ventricle and SGZ of the DG. In both zones multipotent neural precursors are preserved lifelong [1] and may contribute to repair following CNS diseases [4]. Our data on early postnatal neurogenesis might contribute to a better understanding of basic neurogenic events in the mammalian brain.

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