Are Both Embryonic Migratory Pathways Preserved in the Adult Brain Cerebral Cortex?

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Abstract: Cell migration in the adult brain is discussed. We compared our studies on cell densities after cortical injury with our study on hippocampal proliferation and neurogenesis. We have shown that postnatal hypoxia increases cell density in cortical layer II of the somatosensory, motor and auditory cortices and in layer V of the motor cortex. Moreover, we have shown that a photochemical lesion through the entire cortical thickness increases the number of newly generated cells. The number of newly generated cells was enhanced by beam walking pre-treatment and substantially enhanced by fluoxetine pre-treatment; following fluoxetine pre-treatment, a large number of newly generated cells were observed in the auditory cortex. Subsequently, we studied the generation of new cells in the hippocampal dentate gyrus. After Morris water maze training, in comparison with an untrained group, proliferation in the granular cell layer was suppressed. That suppression was compensated for by fluoxetine administration during the period of learning. We observed different results in the hilus of the dentate gyrus, where suppression was observed after combined Morris water maze and fluoxetine treatment. We hypothesize that cell migration in the brain cortex persists in adulthood and that this migration is stimulated by both physiological and pathological conditions. Appropriate stimulation of the neurogenetic system is a possible promising therapy for brain diseases.

Introduction

Neuronal cell migration in the brain is a widely studied subject. During embryogenesis, migration into the cortex is realized in two different ways. From the ventricular zone future principal cells migrate to the cortex radially; support for this migration is provided by radial glia [1]. Interneurons reach their final positions from the ganglionic eminence on the ventral telencephalon to the cortex and hippocampus through tangential migration [2]. Interneurons are an important part of the newly generated neuronal tissue, because they act before principal neurones [3]. The number of cortical neurones is determined by the timing and mode of cell division as well as by the magnitude of cell death. For migration, the presence of signalling molecules, the activation of channels and receptors, the extension of a leading process, and the translocation of the nucleus and cytoplasm are essential [4].

Neurogenesis in the cerebral cortex of adult mammals is a widely discussed topic. The majority of neurones in the adult neocortex are produced embryonically during a brief but intense period of neuronal proliferation [5]. Recently, two neurogenic zones were described in the adult brain [6], the subventricular zone (SVZ) and the subgranular layer of the hippocampal dentate gyrus, where stem cells are present throughout the entire lifespan.

When studying proliferation in cortical regions, differing results have been

reported. Experiments with behavioural enrichment, which usually increases the generation of new neurones in the hippocampus [7], have shown no changes in the number of newly born neurones in the cerebral cortex [8]. Other experiments have shown the presence of new neurones in the cortex after cortical injury [9]. Experiments on brain cell extracts in culture have shown that a neurogenic effect could introduce growth factors such as fibroblast growth factor [10].

Enhanced numbers of neuronal cell bodies in cortical layers II and V after intermittent postnatal hypoxia in young rats

Studying post-hypoxic changes in rats has a long scientific tradition. In young animals, the results of such studies mimic the possible changes after prenatal exposure to hypoxia in man. We studied changes in glial cells after hypoxia and found delayed development of astrocytes in the hippocampus after hypoxic injury [11]. Usually, there is after injury the opposite situation, astrogliosis [12]. To compare our results with the previous study of Langmeier and Marešová [13], we counted neuronal cell bodies in some cortical regions in which, in that earlier study, the results were different from those seen in controls. We determined the neuronal cell body density in predefined regions; coronal sections were from the coronal plane AP –3 to AP –4 from bregma [14], and neuronal cell bodies were

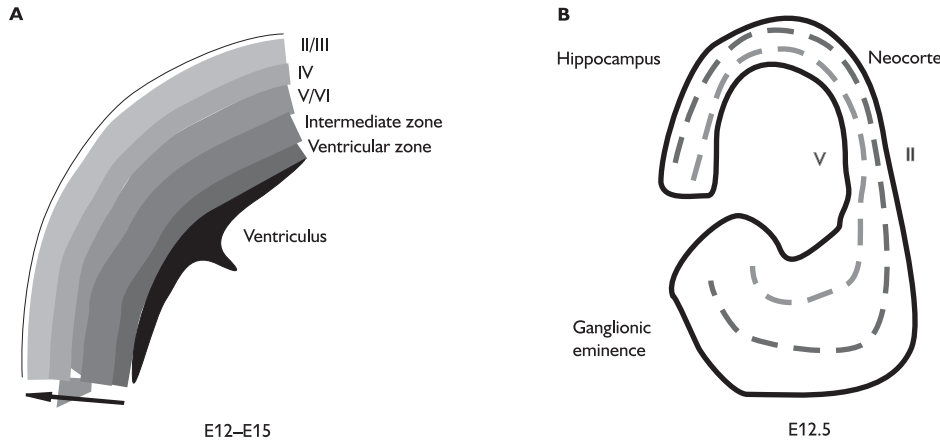

Figure 1 – Models of embryonic migration into the cortex in the rat. A. Radial migration, in which migratory cells from the ventricular zone fill the cortical layers in successive steps from the bottom to the top, adopted from Cavinnes et al. [1]. B. Tangential migration, GABAergic interneurons migrate from the ganglionic eminence through the cortex to the hippocampus, adopted from Pleasure et al. [2]. Both processes take place during approximately the same time period, days 12 to 15 of embryogenesis.

counted in layers II and V of the motor, somatosensory and auditory areas, in the pyramidal layer of the hippocampal CA1 and CA3 regions and in the hilus of the dentate gyrus (Fig. 2) in animals 24 days old. For counting, a special program for the Zeiss KS 400 image analysis system was used [15]. The density was determined per 0.01 mm^2 [11]. We showed [11] that postnatal hypoxia enhances the cell density in cortical layer II of the somatosensory, motor and auditory areas and in layer V of the motor cortex. In each counting, we also measured the mean cell size, and we found smaller neuronal cell bodies in layer II of the motor cortex and in the hilus of the dentate gyrus (Figure 2). This phenomenon could have

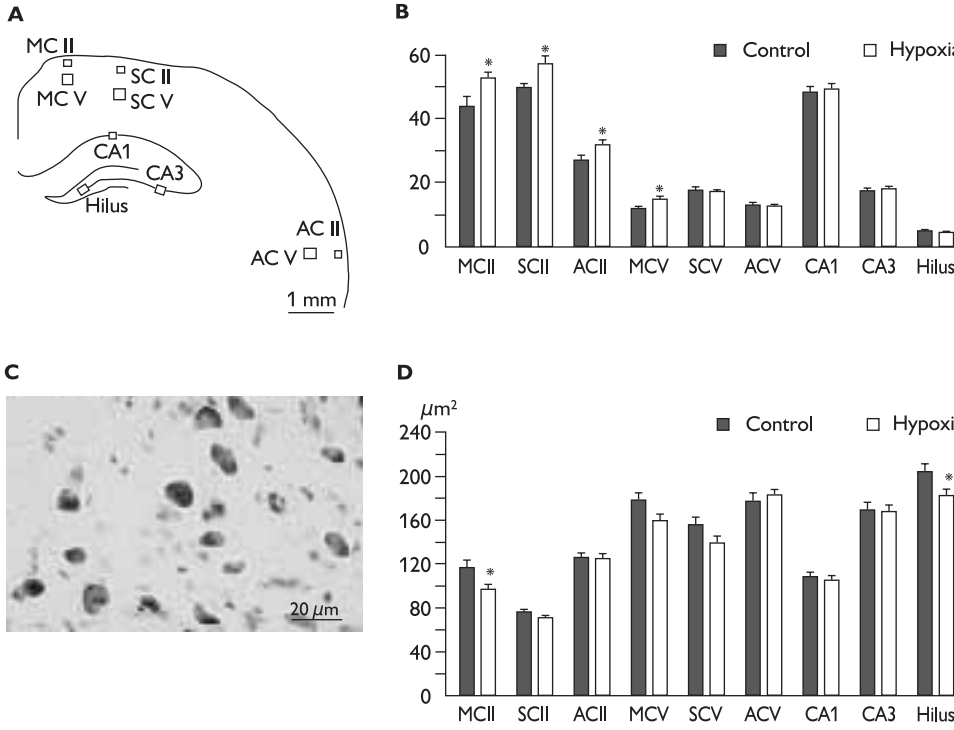

*Figure 2 – The density of neuronal bodies per 0.01 mm^2 and mean cell size in μm^2 in different brain regions at age of 24 days in control animals and in animals after exposure to intermittent hypobaric hypoxia from birth until the age of 19 days. A. Measured fields. Measurements were done in layer II of the motor cortex (MC II), layer V of the motor cortex (MC V), layer II of the somatosensory cortex (SC II), layer V of the somatosensory cortex (SC V), layer II of the primary auditory cortex (AC II), layer V of the primary auditory cortex (AC V), the hippocampal regions CA1 (CA1), CA3 (CA3), and the hilus of the dentate gyrus (Hilus). B. Cell densities in the areas described in A. C. Example of a density measurement in the hippocampal hilus of the dentate gyrus. White surrounds the measured cells in the predefined region. Tissue stained with cresyl violet according to Nissl. D. Mean cell size in the regions defined in A. *Significant difference from control ($p < 0.05$). Figures A, B and D modified from Šimonová et al. [11], figure C from Šimonová [15].*

several explanations, for example that some of these cells were smaller because they had divided more recently than cells in the control brains. The observed enhanced numerical density in cortical layers II and V was only transient; in adult rats, the final cell densities in the measured areas were similar in control and hypoxic brains [11].

Migrating neuronal progenitor cells around a cortical photochemical lesion are widespread on the lesion side throughout the entire layer II of the cerebral cortex after pre-treatment with fluoxetine

In our experiments, after an injury, (a photochemical lesion through the entire thickness of the cortex in one hemisphere), we showed that the number of newborn cells was substantially increased [16]. Some of the newly generated cells also expressed the neuronal marker NeuN, suggesting that they had differentiated into cortical neurones. In the study of Gu *et al*., where a similar model of a photochemical lesion was used, cortical neurogenesis in layers II–III was already observed at 72 hours after stroke induction [9]. In our study, 5 days after lesioning, the highest number of newborn cells (marked by bromodeoxyuridine) was present in the subcortical white matter and sensorimotor cortex around the wound. These structures are close to the lateral ventricle, so we believe that these results indicate a migration from this structure. We also employed 14 days beam walking and fluoxetine pre-treatment before lesioning, and after fluoxetine pre-treatment (total dose 50 mg per animal

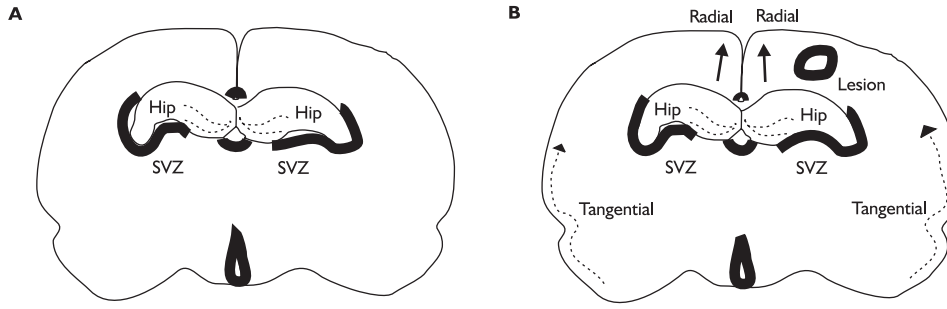

Figure 3 – Scheme of proliferating zones, radial and tangential neuronal migration in the adult rat brain before and after injury. A. Two main regions of progeny persist in the adult brain: the subventricular zone (SVZ) and the subgranular layer of the hippocampal dentate gyrus Hip. B. Adult brain after cortical injury. During embryogenesis, there are two main migratory pathways to the cortex; radial migration from the subventricular zone brings projection neurones (principal cells) and tangential migration from the ganglionic eminence brings local circuitry neurones (interneurons). Our results indicate that both pathways are conserved in adulthood and are activated after cortical injury. Arrows represent radial migration, dashed lines represent tangential migration in the lesioned as well as in the contralateral hemisphere.

administered on days 1, 5 and 14) the numbers were also substantially increased in the auditory cortex, with the majority of newly generated cells found in the superficial layers. A combination of pre-treatments enhanced the number of newly generated cells more than beam walking itself, but less than fluoxetine alone, and mostly around the lesion.

The important question is from where do these cells migrate? Based on our results, we hypothesize that radial and tangential migration can appear during adulthood after cortical injury (Figure 3).

Morris water maze training suppresses, while fluoxetine enhances, proliferation in the granular layer of the dentate gyrus but not in the hilus

In our recent paper [17], we investigated whether physical and cognitive stimulation accompanied by stress in the Morris water maze affects the rate of proliferation in the hippocampus and whether the induced changes can be influenced by antidepressant treatment with fluoxetine. Learning in the water maze for 15 days caused a decrease in granular cell proliferation in the granular cell layer of the hippocampus. In the granular layer, the decrease in the number of newborn cells was reversed to control levels by the use of fluoxetine during training, but in the hilus this was not the case; see Figure 4 and Náměstková et al. [17]. We also investigated the group of cells that differentiated into neurones by using an antibody against doublecortin (DCX). DCX is a marker for newly generated neurones. This protein is essential for migration and persists in new neurones for about one month [17].

A new connection from the olfactory tubercle into the hilus was recently described by Kunzle [18]. This connection can presumably represent a possible migratory pathway. Migration from the hilus into the granular layer was already described by Cameron *et al.* in 1993 [19]. These authors suggest that the hilus of the dentate gyrus serves as a secondary proliferative zone. We suggest that this is a part of the newly recognised migratory pathway from the olfactory tubercle to the granular cell layer.

Fluoxetine can produce a substantial increase in the number of newly generated cells. As a selective serotonin re-uptake inhibitor, fluoxetine increases the amount of serotonin and, in humans, is effective in the treatment of depression from the first week of therapy [20]. The paracrine secretion of glutamate and GABA has been described in the embryonic and newborn hippocampus; these mediators act as diffusible signalling molecules prior to synapse formation [21]. We hypothesize that an increased concentration of serotonin in the extracellular space could mimic paracrine secretion during embryogenesis and, similarly as during embryogenesis, guide newborn cells to migrate to locations where they are needed. Fluoxetine usually increases the rate of proliferation, but in our experiments, and combined with water maze training, fluoxetine decreased the number of newly generated

cells in the hilus (Figure 4A). Because the number of newly generated neurones in the granular layer was not reduced, this means that the numbers of newborn glia were reduced. Because astrocytes can give rise not only to new glia but also to new neurones, the number of granular neurones coming to the granular layer from the hilus [19] could also be reduced at later time points. Newborn glial cells are important for the fate of neuronal tissue after injury and during learning [12, 22].

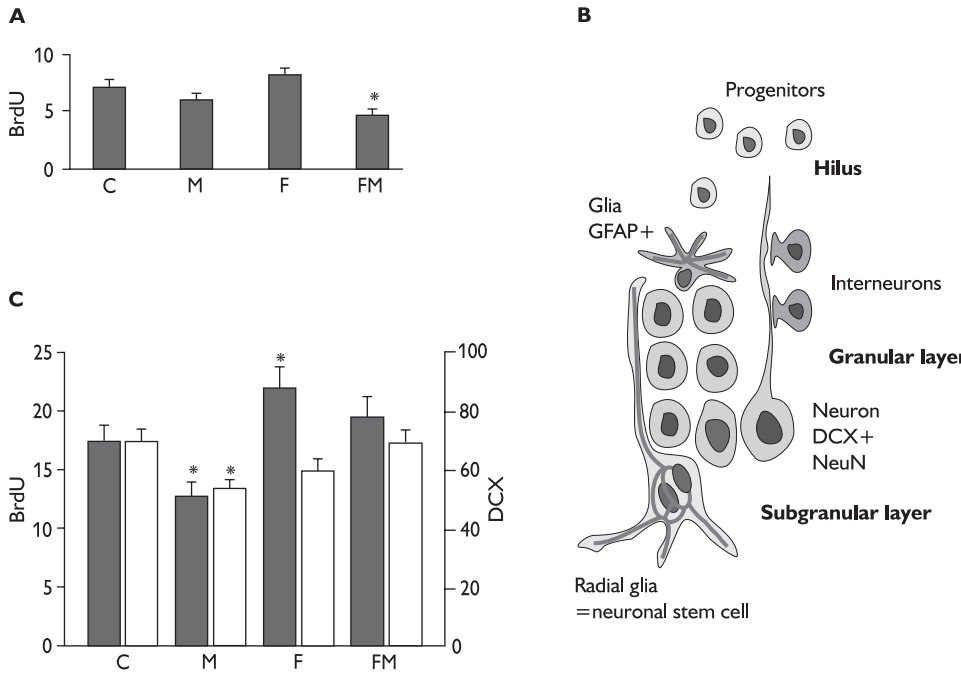

*Figure 4 – Hippocampal dentate gyrus – one of the proliferative zones in the adult rodent brain – after 15 days of Morris water maze training and fluoxetine administration. A. Proliferation in the hilus of the dentate gyrus in a single hippocampal slice, numbers of BrdU-positive cells in control animals (C), animals after water maze training (M), animals treated with fluoxetine (F, dose 5 mg/kg) and animals after fluoxetine treatment followed by water maze training (FM). B. Scheme of proliferation in the dentate gyrus of the rat. GFAP-positive radial glia gives rise to neuronal progenitors which are transiently DCX- and permanently NeuN-positive. Progenitors migrating from the hilus give rise to granular cells. In radial glia division, GFAP is involved, creating a structure similar to a basket. C. Proliferation in the granular cell layer after treatments in a single hippocampal slice. The left columns show the numbers of bromodeoxyuridine (BrdU)-positive cells, the right columns show the numbers of doublecortin (DCX)-positive cells. In the hilus, no DCX-positive cells were present. *Significant difference from control ($p < 0.05$). Graphs modified from Náměstková et al. 2005 [17].*

Based on the results presented here, we have generated a new model of cortical migration in adult animals (Figure 5). There is not only the rostral migratory stream itself [23], but two branches of this stream, one bringing new neurones to the cortex through layer II, the other bringing progenitors into the hilus of the dentate gyrus.

Conclusion

Currently, it is generally accepted that during embryogenesis, there are two main migratory pathways to the cortex: radial migration from the subventricular zone brings projection neurones (principal cells) and tangential migration from the ganglionic eminence brings local circuitry neurones (interneurons) [24]. We suggest that these two migratory pathways are conserved into adulthood. We conclude that endogenous stem cells and progenitors travelling along migratory pathways together represent a great hope for the treatment of brain injury.

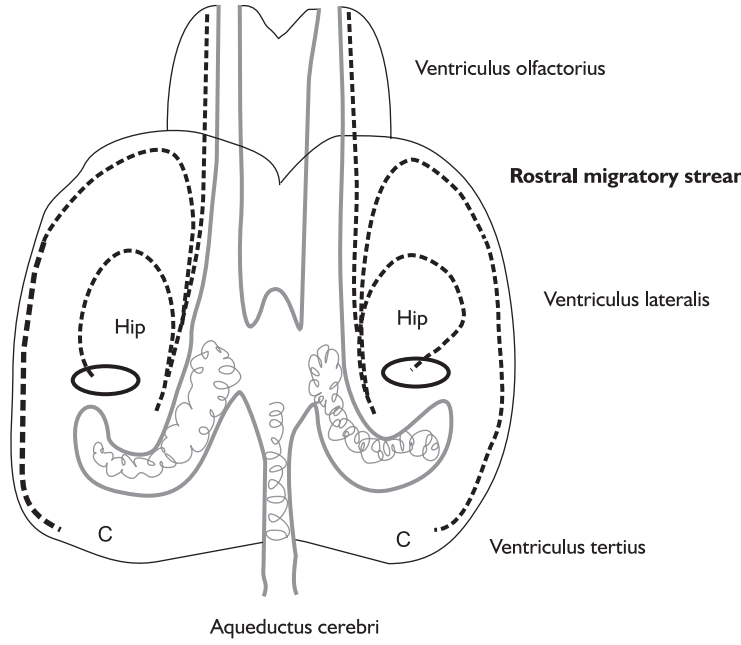

Figure 5 – Ventricular system of the cerebral cortex and the rostral migratory stream. The subventricular zone is, in the adult brain, the main source of new progenitors. Cells are distributed to the olphactory bulbs by the rostral migratory stream. Based on our results, we hypothesize that the stream has two branches, one bringing proliferating cells to the cerebral cortex (C), the other going back to the hilus of the hippocampal dentate gyrus (Hip).

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