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NEUROGENESIS IN ADULT MAMMALIAN HIPPOCAMPUS AFTER ISCHEMIA: RODENT *VERSUS* PRIMATE MODELS

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The adult mammalian hippocampus harbors neural precursor cells capable of generating glial and neuronal cells. Their existence was demonstrated in both non-primate mammals and primates including humans, and recently has attracted a significant scientific interest focused on the precursor cell potential to replace lost brain cells. Proliferation and differentiation of hippocampal progenitors are affected by brain injury and are regulated by a variety of molecular signals. Brain ischemia is a major activator of these precursors as demonstrated by experimental models in rodents and monkeys. In rodent hippocampal formation, ischemia increases neurogenesis and gliogenesis in both dentate gyrus and cornu Ammonis. In monkeys, however, ischemia was unable to trigger production of new neurons in cornu Ammonis, while in dentate gyrus postischemic progenitor cell activation was at lower levels than in rodents. Thus, rodents and primates appear to differ in their precursor cells' ability to perform neurogenesis. Unraveling the molecular machinery responsible for this interspecies discrepancy might reveal novel strategies to manipulate neural precursors for therapeutic purposes in humans. ***Biomed Rev 2005; 16: 1-11.***

Key words: neural progenitor, cell proliferation, cell differentiation, growth factor, transcription factor

INTRODUCTION

A central postulate in neuroscience has stated that the adult brain lacks an intrinsic ability to regenerate its neurons (1). This dogma has been challenged in the last few decades as mounting evidence demonstrated the existence of a phenomenon designated as **adult neurogenesis**: *de novo* generation of neurons by neural progenitor cells in the adult brain. At present, adult neurogenesis is widely recognized to occur in two regions of the mammalian brain, including the hippocampus and the subventricular zone (SVZ) along the walls of the lateral ventricle (2). The hippocampal dentate gyrus (DG) harbors progenitor cells located in its subgranular zone (SGZ), a thin band of tissue adjacent to the innermost layer of granule neurons (3). Hippocampal neurogenesis was first described four decades ago (4), but although these initial data were supported by other researchers in the 1980s (5), they were not widely accepted by the scientific community at the time. However, in the 1990s, the introduction of modern methodology led to accumulation of new data overturning the dogma that no new neurons are added to the adult brain (6). An important role in the process of recognition of adult neurogenesis had *in vivo* studies in adult monkeys (7,8) and

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humans (9) showing that hippocampal neurogenesis occurs not only in adult non-primate mammals, but also in the primate (including human) brain. The persistent neuronal production in the adult brain suggested a previously unrecognized potential for self-repair after injury, and this attracted a significant scientific interest on the molecular cues regulating neural progenitors and how these cells would respond to brain damage (reviewed in 10-13). The detailed knowledge on these two aspects of the precursor cell biology would allow their manipulation toward a desired phenotype as a means for treating human brain diseases. To accomplish this task effectively, the knowledge on the mechanisms regulating primate brain progenitor cells should be particularly expanded. However, at present, it is unclear to what extent the data from the widely used rodent animal models are translatable to the human brain, because the research on primate adult neurogenesis and neural precursor cells is less advanced than research on rodents. The present paper will review the current status of postischemic adult neurogenesis research in the rodent hippocampus, and will outline emerging interspecies differences between rodents and primates in respect to progenitor cell proliferation and capability to regenerate neurons after ischemia.

MOLECULAR REGULATION OF ADULT NEUROGENESIS

The process of generation of neurons by progenitor cells in adult brain can be classified for didactical purposes into three phases: (*i*) proliferation of precursor cells, (*ii*) their migration toward target areas, and (*iii*) gradual differentiation into mature neuronal phenotypes including integration into networks. In the case of DG, progenitors proliferate in SGZ, and migrate radially at a short distance to differentiate into projection neurons of the granule cell layer (2,3) (see Fig. 2). The two best recognized mitogens for neural stem/progenitor cells are basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) (14-17) (Fig. 1). A neural stem cell-derived molecule, the glycosylated form of cystatin C is necessary as a cofactor for bFGF-dependent proliferation (18). Vascular endothelial growth factor (VEGF) initially stimulates angiogenesis,

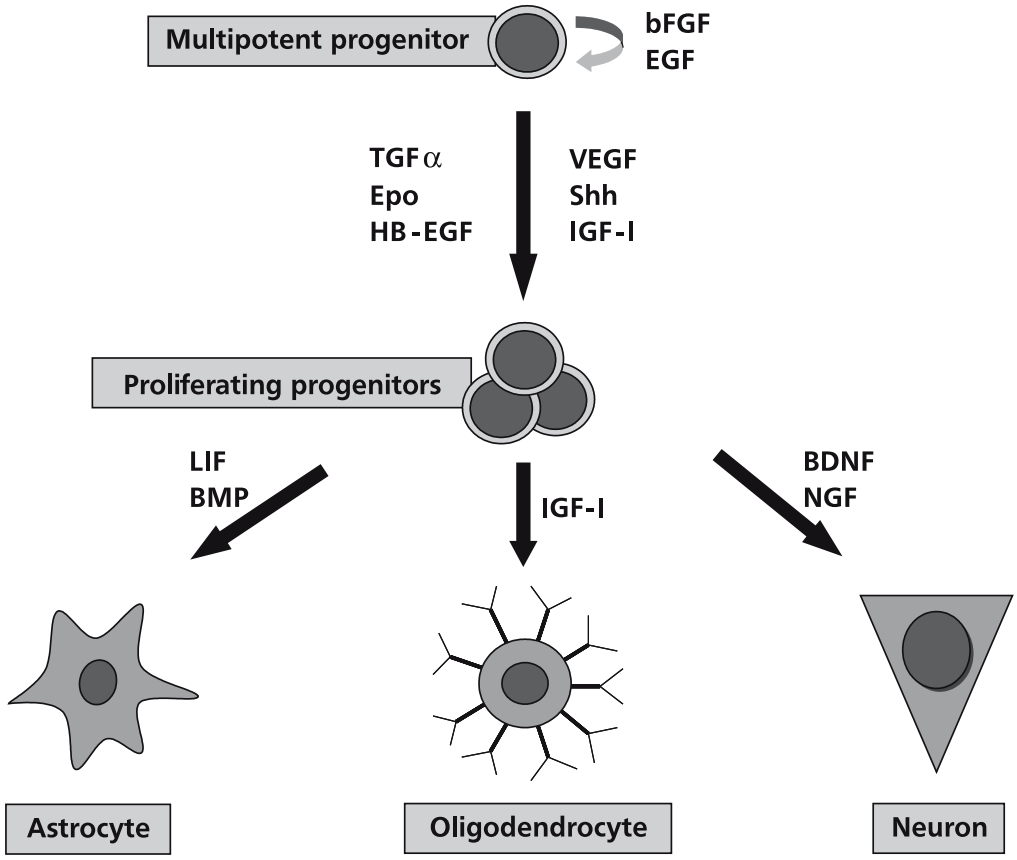

Figure 1. *Molecular signals regulating adult hippocampal progenitor cells act at different levels of differentiation of precursors. See text for abbreviations.*

and subsequently neurogenesis (19,20). The neurotrophins brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin (NT)-3 and glial cell line-derived neurotrophic factor (GDNF) enhance proliferation and/or neuronal differentiation of hippocampal progenitors (21-23). Other growth factors positively regulating progenitor cell proliferation or differentiation include transforming growth factor-α (TGFα) (24), insulin-like growth factor-I (IGF-I) (25), erythropoietin (26), as well as the developmental signals sonic hedgehog (Shh) (27) and Wnt (28). In contrast, the cytokine interleukin-6 reduced hippocampal neurogenesis (29).

Nuclear factors were found to affect hippocampal progenitors. Cyclin D2 (but not D1 or D3) selectively regulated adult neural progenitor cells as cyclin D2 deletion completely abolished proliferation of neuronal precursors and generation of new neurons in adult DG (30). Other DNA-binding molecules were also implicated in the regulation of progenitor cell proliferation and differentiation, including the transcription factors cAMP response element-binding protein (CREB) (31) and E2F1 (32). Interestingly, DNA-binding proteins functioning during normal development may continue to be expressed in neurogenic regions of the adult brain. Thus, the transcription factor Pax6, which is expressed in precursor cells during embryonic brain development, is also present in adult hippocampal dentate gyrus (33) and its inactivation leads to a marked decrease in progenitor cell proliferation (34). Similar findings were obtained for another developmental transcription factor, Emx2 (35).

The neurotransmitters GABA (36,37) and glutamate (38,39) reciprocally regulate hippocampal progenitor cells as GABA activates while glutamate inhibits their proliferation. Further, GABA enhances progenitor cell synaptic maturation and neuronal integration (37), thus acting as a differentiation signal as well. Other molecules regulating progenitor cell differentiation toward neuronal or glial phenotype include bone morphogenetic protein (BMP) family members and leukemia inhibition factor (LIF). BMPs instruct progenitors to adopt a glial cell fate (40,41), acting in cooperation with LIF (42). As mentioned above, neurotrophins were implicated in neural progenitor cell differentiation toward a neuronal phenotype (22), while insulin-like growth factor-I (IGF-I) instructs oligodendroglial fate (43). To date, numerous signals are known to affect progenitor cells, acting at different levels of lineage development (Fig. 1).

HIPPOCAMPAL NEUROGENESIS AFTER ISCHEMIA IN RODENTS

Two types of circulatory perturbations incur characteristic types of ischemic injury to the brain (44): (*i*) stroke (a complete occlusion of a cerebral artery) irreversibly kills the neurons in its core region and severely damages those in the penumbrial region; and (*ii*) reversible circulatory arrest, with a transient total stop of cerebral blood flow, selectively kills vulnerable cell populations. Animal models reflect these clinical conditions as focal ischemic models replicate stroke and global ischemic models replicate cardiac arrest.

Ischemia increases hippocampal neurogenesis in both global and focal models. First data in this respect came from a global ischemic model in gerbils (45). The authors demonstrated that ischemia increased nearly 12-fold cell proliferation in SGZ with a peak in the second postischemic week. Investigation of the long-term fate of *de novo* generated cells revealed that over half of them acquired neuronal phenotype in the granule cell layer of DG, while a smaller fraction had become astrocytes in CA4 sector (45). These observations were repeated in mice (46) and rats (47), confirming that postischemic neurogenic enhancement is a common phenomenon among various rodent species. The latter authors demonstrated that ischemia increases the quantity of adult-generated neurons in DGL, but does not affect neuronal differentiation (47). Importantly, the proliferating cells in SGZ were identified as neural progenitor cells (48), and the gradual changes in the expression of precursor cell markers indicates a stepwise cellular maturation before integration into the granule cell layer (49). This observation supports the conclusion that the adult-generated neurons in the granule layer are derived from proliferating progenitors in SGZ. Further confirmation for this lineage relationship has been obtained using a retroviral vector (50), which also demonstrated that *de novo* generated neurons after ischemia were able to extend dendrites into the molecular layer of DG similarly to normal state (51). Increase of DG neurogenesis was reported also in models of focal ischemic injury such as seen with ligation of the middle cerebral artery. Focal ischemic infarction in one hemisphere triggered progenitor cell proliferation in ipsilateral and/or contralateral SGZ (52), but only the ipsilateral progenitors survived in the long term (53).

Importantly, ischemia activated progenitor cells not only in DG, but also in the hippocampal CA1 sector – the most vulnerable to global ischemic injury brain region (44). Precursor cells residing in adjacent to CA1 periventricular region migrated toward cell-depleted pyramidal layer of CA1 where they differentiated into hippocampal pyramidal neurons (53). Subsequent experiments in adult (54,55) and neonatal (56) animals supported these observations, and suggested that the rodent brain possesses an endogenous ability to repair damaged hippocampal CA1 neurons. Further, postischemic

treatment with bFGF and EGF was able to increase the number of progenitor-generated CA1 neurons to levels sufficient to ameliorate post-ischemic neurological deficits, as the new CA1 pyramidal cells integrated into circuitry and expressed functional synapses (53). Thus, bFGF and EGF exerted postischemic effects similar to their effects on normal subjects (14-17). A dozen of other signaling molecules were found to affect postischemic progenitor cell proliferation and/or fate (Table 1). These include heparin-binding EGF-like growth factor (HB-EGF) (57), VEGF (58), stem cell factor (SCF) (59), IGF-I (60), GDNF (60), granulocyte colony-stimulating factor (G-CSF) (61), and stromal cell-derived factor-1α (SDF-1α) (62). Surprisingly, the neurotrophin BDNF, known for its stimulatory effect on progenitor proliferation and neuronal differentiation under normal conditions (21,22) had an opposite effect after ischemia suppressing postischemic DG neurogenesis (63). Accordingly, blockade of BDNF receptor increased the number of adult-generated DG neurons after ischemia (64). These results are important as they suggest that the potential neuroprotective agents may have differential effects on progenitor cell proliferation and differentiation depending on the region and type of injury, and that ischemia-induced neurogenesis may be regulated differently than neurogenesis in non-ischemic brain.

Other factors affecting postischemic progenitor cells include neurotransmitters nitric oxide (NO) and glutamate, both of which increase postischemic neurogenesis (65,66) while inhibit DG precursors in intact brain (39,67). The effects of NO on progenitor cells after ischemia appear to depend on the source of the molecule: inhibitory effects appear to be due to neuronal NO synthase (NOS) (68), while activatory - to endothelial NOS (69) and/or inducible NOS (70). Conditions such as environmental enrichment (71), physical exercise (72), ionizing radiation (73) also affect DG neurogenesis. Finally, the preservation of stroke-activated neurogenesis in aged brain was demonstrated (74,75), which is clinically relevant as stroke occurs more frequently in aged humans. To date, over 100 papers have been published on postischemic neurogenesis in hippocampus and/or other brain regions, and the reader is referred to number of recent reviews for further reading (76-81).

HIPPOCAMPAL NEUROGENESIS AFTER ISCHEMIA IN MONKEYS

The studies investigating neurogenesis after cerebral ischemia have primarily used rodent animal models. In order to more closely inspect the correlation of the experimental data in rodents to the clinical conditions in the human brain, we utilized a primate model of global cerebral ischemia using adult macaque monkeys. In this model we completely but transiently block all blood flow to the brain structures, which causes a major neuronal injury to the hippocampal CA1

Table 1. *Effect of various signaling molecules on postischemic progenitor cell proliferation as compared to their effects on normal brains. Note that some factors have opposite effects in postischemic or normal brains. See text for abbreviations.*

	Effect	
	Postischemic brain	**Normal brain**
• EGF	↑	↑
• bFGF	↑	↑
• HB-EGF	↑	↑
• VEGF	↑	↑
• SCF	↑	↑
• IGF-I	↑	↑
• GDNF	↑	↑
• G-CSF	↑	↑
• SDF-1α	↑	↑
• BDNF	↓	↑
• NO	↑	↓
• Glutamate	↑	↓

sector, while DG remains intact (82,83). We then investigated the distribution and phenotype of *de novo* generated cells in the hippocampus focusing on DG and CA1. Similarly to the rodent brain after global ischemia, we observed a progenitor proliferation peak in monkey SGZ of DG in the second week after the insult (84). Early after ischemia, numerous proliferating cells in SGZ formed clusters and expressed markers of uncommitted neural progenitors (Fig. 2B). After the second postischemic week, we observed increased proportions of neuronal progenitors, many of which extended processes parallel to the granule cell layer of DG (Fig. 2C). Further, beyond the fourth postischemic week, some adult-generated cells in DG expressed markers of mature neurons (Fig. 2D) and appeared to establish morphological contacts with neighboring neuronal cells (85). Altogether, these results suggest gradual maturation of putative new neurons in postischemic monkey DG (Fig. 2A).

At the same time, the proportion of *de novo* generated cells expressing neuronal phenotype in the granule cell layer was about 15%, while nearly about 50% of the adult-generated

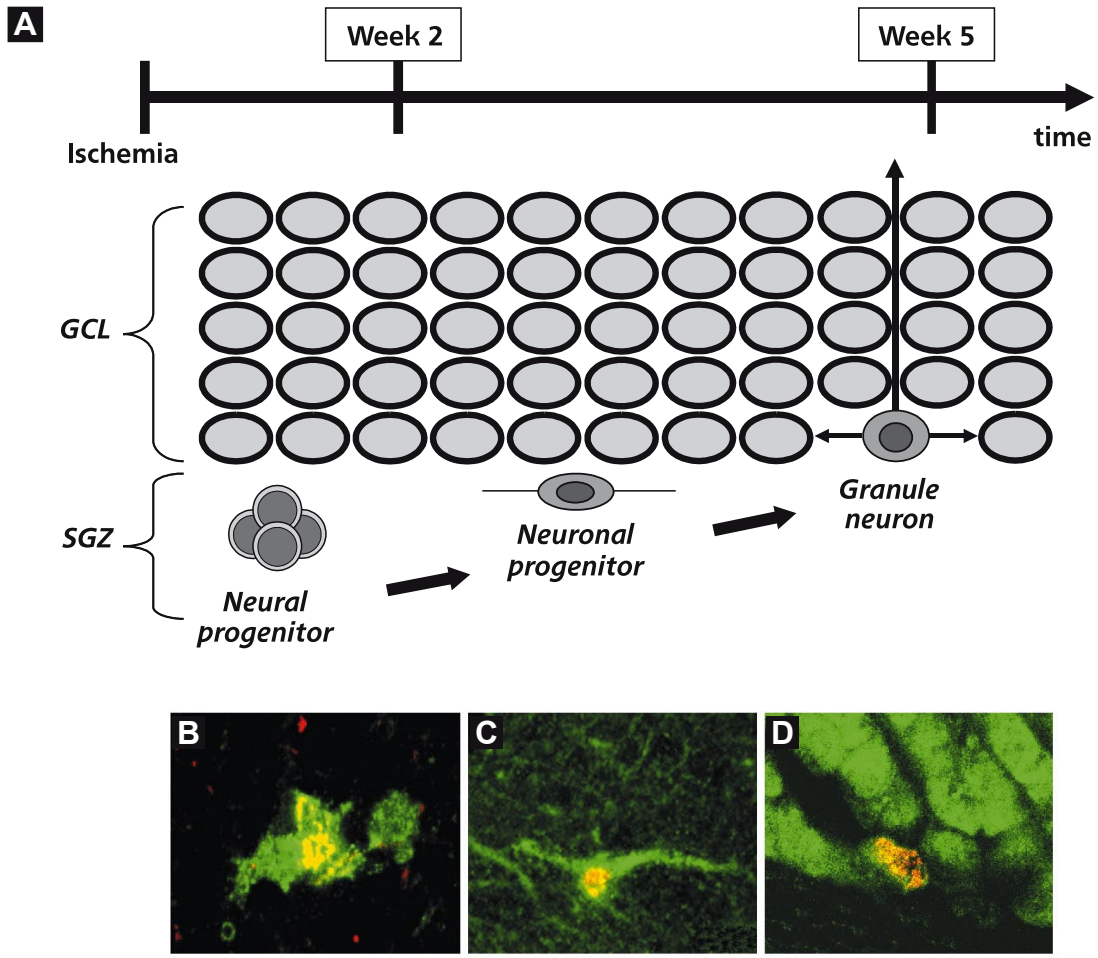

Figure 2. *Phenotypes of adult-generated cells in monkey DG.* ***A:*** *Schematic presentation of putative major steps in neuronal generation by progenitor cells, including cluster formation by neural progenitors in SGZ, followed by bipolar morphology of neuronal progenitors extending processes along the granule cell layer (GCL) of DG, and finally integration of mature granule cells into the granule layer.* ***B:*** *A cluster of neural progenitors (green), some of which exhibit features of proliferation (yellow).* ***C:*** *A neuronal progenitor cell (yellow nucleus indicates preceding cell division) extends processes in SGZ.* ***D:*** *A neuron in the innermost layer of GCL (yellow) was* de novo *generated after ischemia. Note that its morphology is similar to adjacent developmentally generated neurons (green).*

cells in monkeys surviving long-term after ischemia sustained their location in SGZ, clustering and expression of immature progenitor markers (85). Such distribution and phenotype are typical for early postischemic time points, when most proliferating cells are still immature (45-51). The lack of migration to the granule cell layer and/or differentiation of a large proportion of precursors raises the issue of which are the molecular signals responsible for this phenomenon. These could be extrinsic signals from the environment surrounding the progenitor cells (e.g. growth factors and/or cytokines) or intrinsic molecular switches such as transcription factors. Their identification will help to manipulate more efficiently adult primate brain progenitor cells (see next sections).

In contrast to DG, we detected no signs of neuronal production in postischemic CA1, where despite the marked cell loss followed by striking increase of proliferating cells, the latter were invariably of glial phenotype (85,86). Most proliferating cells in postischemic monkey CA1 were microglia, astrocytes were the second most common cell type, while only a few adult-generated oligodendrocytes were seen (85,86). We investigated the periventricular area adjacent to CA1, that is, SVZ of inferior horn of the lateral ventricle. While this zone did contain progenitor cells whose proliferation was upregulated after ischemia similarly to SGZ, they did not sustain their existence over time, and more importantly, these precursors did not exhibit an ability to differentiate into neuronal cells (85). Thus, progenitors from different hippocampal subregions appear to have differential ability to produce neurons.

HIPPOCAMPAL NEUROGENESIS: RODENT *VERSUS* MONKEY

Our findings in monkeys differ from studies in rodents in both quantitave and qualitative aspects. In DG, the peak postischemic quantity of proliferating cells in monkey SGZ (84) was a magnitude lower than the peak postischemic precursor cell quantity in rodent SGZ (45). A similar difference was observed between monkeys (~15%; ref. 85) and rodents (~80%; ref. 47) in respect to proportions of neuronal cells generated by DG precursors after ischemia. Even more striking was the discrepancy between monkeys and rodents in respect to neuronal generation in CA1 sector after transient global ischemia. In rodent CA1, a considerable proportion of pyramidal CA1 neurons, ranging from 10% at one month after ischemia (53) to 40% at 3 months (55), was regenerated by endogenous progenitors derived from adjacent periventricular area. In monkey CA1 sector, no signs of *de novo* neuronal generation were observed (85,86).

Certain molecular differences between monkeys and non-primate mammals in the regulation of hippocampal progenitors are emerging, and these could be involved in the interspecies differences mentioned above. Such molecular differences could be linked to intrinsic to progenitors cell fate determinants such as transcription factors. Thus, the precursor cells regenerating CA1 neurons in rodent hippocampus expressed the transcription factors Pax6 and Emx2 (53), both of which also regulate proliferation of hippocampal SGZ progenitors under normal conditions (34,35). In contrast, in monkey hippocampus, only SGZ precursors expressed Pax6 and Emx2 proteins while precursors in SVZ adjacent to CA1 were negative for these two transcription factors (85). This differential expression might be involved in the differential ability of DG or CA1 to generate new neurons, and may explain the differences in performing neurogenesis observed in rodent or primate CA1 sector (Fig. 3).

IMPLICATIONS OF FINDINGS IN MONKEYS FOR THERAPIES IN HUMANS

Currently, two major therapeutic approaches for brain repair by stem/progenitor cells have emerged (13): (*i*) endogenous progenitors might be recruited to sites of injury; (*ii*) *in vitro* expanded stem/progenitor cells might be transplanted in sites of injury. In cases of endogenous progenitor activation, the apparent sites of origin of such cells seem to be the germinative centers, such as SGZ and SVZ. The possibility of endogenous brain progenitor cell activation has been addressed mainly using rodent models. Therefore, studies in monkey disease models were necessary as monkeys represent the mammals that are evolutionally closest to humans. Significant variability exists between primates and non-primate mammals in respect to certain proliferation parameters, such as the timing and number of cell divisions during cortical development, resulting in interspecies variability in cortical size and organization (87). Further, in normal adult monkey hippocampus, the fraction of newly generated neurons was smaller than the calculated fraction for mice by an order of a magnitude (8). Our results demonstrated that these differences apply also for the postischemic hippocampus, and we have proposed some molecular differences between rodent and monkey hippocampal progenitors that could be involved in the interspecies discrepancies concerning neurogenesis.

In fact, the very identity of hippocampal progenitors could be different between rodents and primates as in mice the primary progenitors of DG neurons appear to be cells expressing the astroglial marker glial fibrillary acidic protein (GFAP) (88,89), while in monkey SGZ we did not identify GFAP labeled cells expressing proliferation markers (84,90).

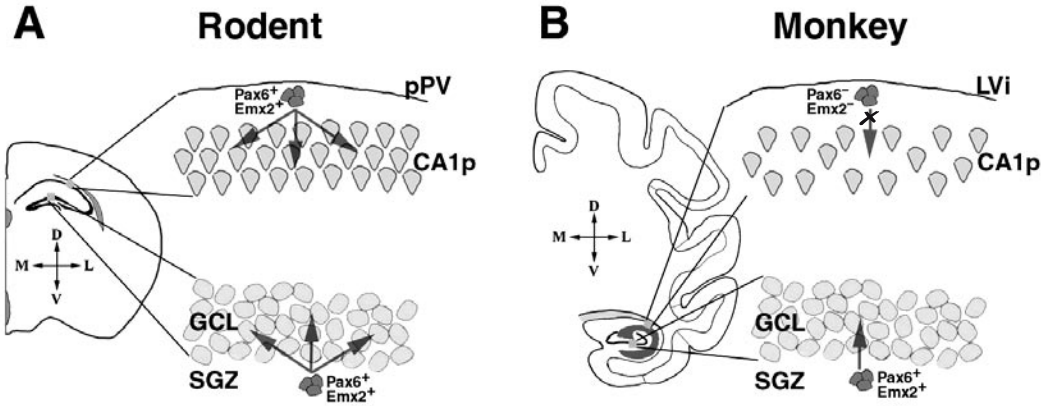

Figure 3. *Response of hippocampal progenitor cells to global cerebral ischemia in rodents (A) or monkeys (B).* ***A, bottom****: In the postischemic rodent DG, progenitor cells residing in SGZ increase their proliferation and subsequently migrate (arrows) to the granule cell layer (GCL) of DG to become granule neurons.* ***A, top****: In the postischemic rodent hippocampus proper, precursors residing around the walls of the posterior periventricle (pPV) replace neurons in the pyramidal cell layer of the hippocampal CA1 sector (CA1p).* ***B, bottom****: In monkey DG, the proliferative response is similarly increased after ischemia, but significantly reduced in terms of progenitor quantity and neuronal differentiation (exemplified by a single arrow* versus *three arrows in rodent SGZ).* ***B, top****: In the postischemic monkey CA1, precursors residing around the walls of the inferior horn of the lateral ventricle (LVi) increase their proliferation after ischemia, but do not replace neurons in CA1p. Note that while in monkeys SGZ progenitor cells are positive for the transcription factors Pax6 and Emx2, in SVZ along LVi they are negative for these proteins. Directions on the maps: D, dorsal; V, ventral; M, medial; L, lateral.*

By advancing our knowledge of how the fate of monkey endogenous progenitor cells is controlled, we shall more efficiently construct strategies for repair of the human brain. Such strategies should also take into account the age-related changes in the capacity of the brain to generate and support stem/progenitor cells as it is known that stroke is much more common in older patients.

SUMMARY

The generation of new neurons in adult hippocampus has been demonstrated in most mammalian species, including primates. Cerebral injuries such as ischemia mobilize endogenous progenitor cells and enhance neuronal generation. In the widely used rodent models this enhancement takes place in both CA1 sector and DG. In monkeys, postischemic neuronal production occurs in DG but not in CA1. These interspecies discrepancies could be a result of differential precursor cell modulation by intrinsic and/or extrinsic to the cell molecular signals. Their identification may open new possibilities for therapeutic manipulation of primate progenitor cells in the treatment of human neurological diseases.

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