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Brain ischemia, neurogenesis, and neurotrophic receptor expression in primates

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ABSTRACT

Generation of new neurons persists in the normal adult mammalian brain, with neural stem/progenitor cells residing in at least two brain regions: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG). Adult neurogenesis is well documented in the rodent, and has also been demonstrated in vivo in non-human primates and humans. Brain injuries such as ischemia affect neurogenesis in adult rodents as both global and focal ischemic insults enhance the proliferation of progenitor cells residing in SGZ or SVZ. We addressed the issue whether an injury-triggered activation of endogenous neuronal precursors also takes place in the adult primate brain. We found that the ischemic insult increased the number of progenitor cells in monkey SGZ and SVZ, and caused gliogenesis in the ischemia-prone hippocampal CA1 sector. To better understand the mechanisms regulating precursor cell division and differentiation in the primate, we analyzed the expression at protein level of a panel of potential regulatory molecules, including neurotrophic factors and their receptors. We found that a fraction of mitotic progenitors were positive for the neurotrophin receptor TrkB, while immature neurons expressed the neurotrophin receptor TrkA. Astroglia, ependymal cells and blood vessels in SVZ were positive for distinctive sets of ligands/receptors, which we characterized. Thus, a network of neurotrophic signals operating in an autocrine or paracrine manner may regulate neurogenesis in adult primate SVZ. We also analyzed microglial and astroglial proliferation in postischemic hippocampal CA1 sector. We found that proliferating postischemic microglia in adult monkey CA1 sector express the neurotrophin receptor TrkA, while activated astrocytes were labeled for nerve growth factor (NGF), ligand for TrkA, and the tyrosine kinase TrkB, a receptor for brain-derived neurotrophic factor (BDNF). These results implicate NGF and BDNF as regulators of postischemic glial proliferation in adult primate hippocampus.

Key words

Neural progenitor • Primate • Brain ischemia • Neurotrophins

Introduction: the concept of adult neurogenesis

A central dogma in neuroscience has stated lack of ability of the adult brain to regenerate its neurons (Cajal, 1928). This postulate has been challenged in the recent 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, this process is accepted to occur in at least two regions of the mammalian brain: the hippocampus and the subventricular zone (SVZ) along the walls of the lateral ventricle (Gage, 2000). 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 (Gage et al., 1998). For the first time, hippocampal

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neurogenesis was described more than four decades ago (Altman and Das, 1965). At the time, however, these findings were not widely accepted by the scientific community, although supported by other researchers (Kaplan and Bell, 1984). In the last two decades, however, the introduction of modern techniques led to accumulation of new data overturning the dogma that no new neurons are added to the adult brain (Gross, 2000). An important role in the process of recognition of adult neurogenesis had *in vivo* studies in adult monkeys (Gould et al., 1999; Kornack and Rakic, 1999) and humans (Eriksson et al., 1998) 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 (Kuhn et al., 2001; Hallbergson et al., 2003; Parent, 2003; Lie et al., 2004). The elucidation of the molecular cues regulating neural progenitors and their response to brain damage would allow directing these cells toward a targeted phenotype needed for treating specific human neurological diseases. Most of the data on progenitor cell biology are derived from non-primate mammals, mainly rodents. Thus, advancing the knowledge on primate progenitor cell biology would have an important impact on their effective use for therapies in humans.

Adult neurogenesis after brain ischemia

Two major models of ischemic injury to the brain are known (Lipton, 1999): (*i*) focal ischemia (stroke) affects unilaterally the neurons within the territory of a given cerebral artery (usually the middle cerebral artery); and (*ii*) transient, but global (bilateral), cerebral hypoperfusion selectively kills vulnerable cell populations. Ischemia has been shown to increase hippocampal neurogenesis in both global and focal models. After transient global ischemia, the proliferation of progenitors was upregulated several fold, and roughly half of postischemic precursors acquired neuronal phenotype in the granule cell layer of DG, while a few became astrocytes in CA4 sector (Liu et al., 1998). Similar observations were reported across various rodent species (Takagi et al., 1999; Kee et al., 2001). Additional experiments revealed that the new neurons are derived from SGZ neural progenitor cells (Yagita et al., 2001), and that precursors maturate to DG neurons in a gradual stepwise manner (Iwai et al., 2002). Further, adult-generated neurons were able to extend dendrites into the molecular layer of DG establishing synapses with developmentally-generated neurons (Tanaka et al., 2004). Increase of DG neurogenesis was reported also after focal ischemic injury (Jin et al., 2001), but only the progenitors in the ipsilateral hemisphere survived in the long term (Takasawa et al., 2002).
Ischemia activated progenitor cells also in the hippocampal CA1 sector – the most vulnerable to global ischemic injury brain region (Lipton, 1999). Precursor cells residing in adjacent to CA1 periventricular region migrated toward the cell-depleted pyramidal layer of CA1 where some of them were able to differentiate into hippocampal pyramidal neurons (Nakatomi et al., 2002). Subsequent experiments in adult (Schmidt and Reymann, 2002; Bendel et al., 2005) and neonatal (Daval et al., 2004) animals supported these observations, and suggested that the rodent brain possesses an endogenous ability to repair damaged to hippocampal CA1 neurons. Further, postischemic treatment with beta fibroblast growth factor (bFGF) and epidermal growth factor (EGF) was able to increase in 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 (Nakatomi et al., 2002). In addition to bFGF and EGF, several other signaling molecules were found to affect postischemic progenitor cell proliferation and/or fate in rodents, but their relevance to primates remains to be studied (reviewed by Sharp et al., 2002; Kokaia and Lindvall, 2003; Felling and Levison, 2003; Ab0rahams et al., 2004; Zhang et al., 2005; Lichtenwalner and Parent, 2006).

Postischemic neurogenesis in monkeys

In order to investigate the effect of ischemia in environment closer to the human brain than the rodent brain, we used a primate model of global cerebral ischemia in adult macaque monkeys (Yamashima et al., 1998; Yamashima, 2000). In this model, we

investigated the distribution and phenotype of *de novo* generated cells in the hippocampus. As previously found in rodents, ischemia increased the progenitor cell proliferation in a delayed manner, in the second postischemic week (Tonchev at al., 2003a). Long-term analysis of the fate of the progenitor cells in DG revealed gradual maturation and establishment of contacts with neighboring neuronal cells (Tonchev at al., 2006). Thus, similarly to the rodent brain after ischemia, monkey DG precursors exhibit capability of gradually maturing into granule neurons (Fig. 1).

Unlike the situation in DG, no neurogenesis was observed in postischemic CA1, in which only glial cells were renewed (Tonchev et al., 2003b, 2006). In support of these results, neuronal progenitors in the periventricular area adjacent to CA1 were absent or were of transient existence (Tonchev et al., 2006). Therefore, progenitors of different hippocampal subregions apparently exhibit differential neurogenic potential.

Interestingly, the data from monkeys differ from the results in the commonly used rodent models, in three aspects (Fig. 2). First, the density of neural progenitor cells detected by us in the SGZ of monkey DG (Tonchev et al., 2003a) was several folds lower than the reported in rodents (Liu et al., 1998). Second, the percentage of adult-generated postischemic cells differentiating to immature neurons in monkeys was several times lower than in rodents (Kee et al., 2001). Consequently, the postischemic production of granule cells was much lower in monkey DG than in rodent DG (Liu et al., 1998; Kee et al., 2001). Such interspecies differences related to differences in the expression patterns of specific neurogenic transcription factors in DG and CA1 of monkey and rodent hippocampus (Tonchev et al., 2006). We suggested that this differential expression may be involved in the differential ability of DG or CA1 to generate new neurons (Tonchev et al., 2006).

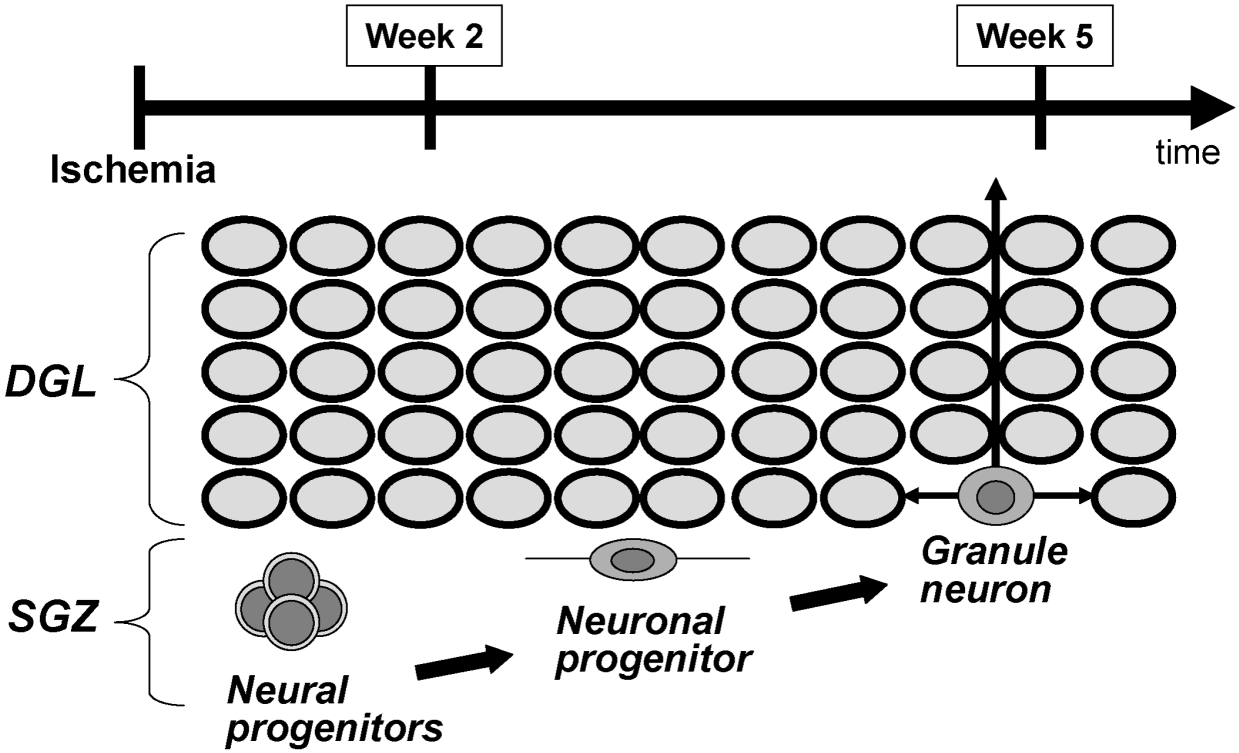
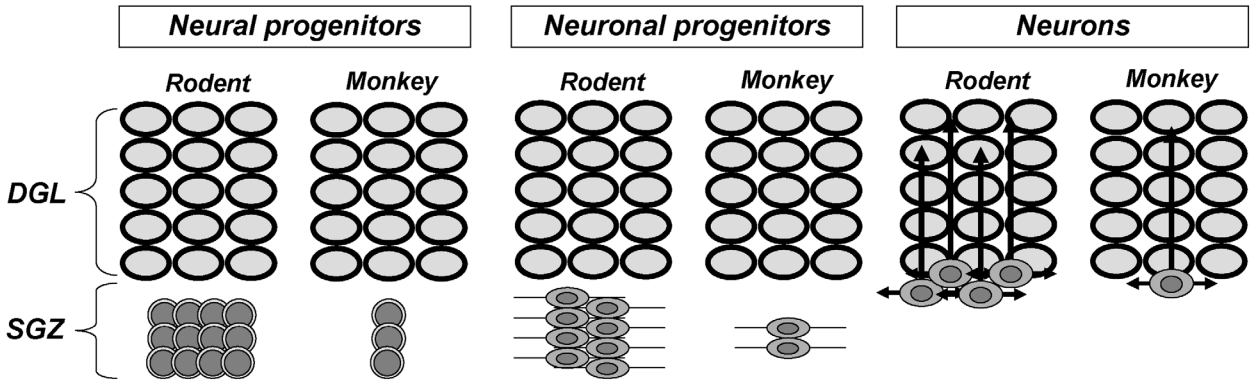


Fig. 1. - Step-wise maturation of progenitor cells in adult monkey dentate gyrus. Immature neural progenitors form cellular clusters in the subgranular zone (SGZ). This is followed by bipolar morphology of neuronal progenitors (immature neurons) which extending processes along the granule cell layer (GCL). Finally, integration of maturating granule cells into the GCL takes place.



Fig, 2. - Effects of global ischemia on various populations of adult-generated cells in SGZ of rodents and monkeys. In both rodent and monkey, the postischemic proliferative response is increased after ischemia, but in the monkey it is at a significantly lower level than in rodents in terms of progenitor quantity and neuronal differentiation, exemplified by lesser numbers of respective cellular populations in the monkey.

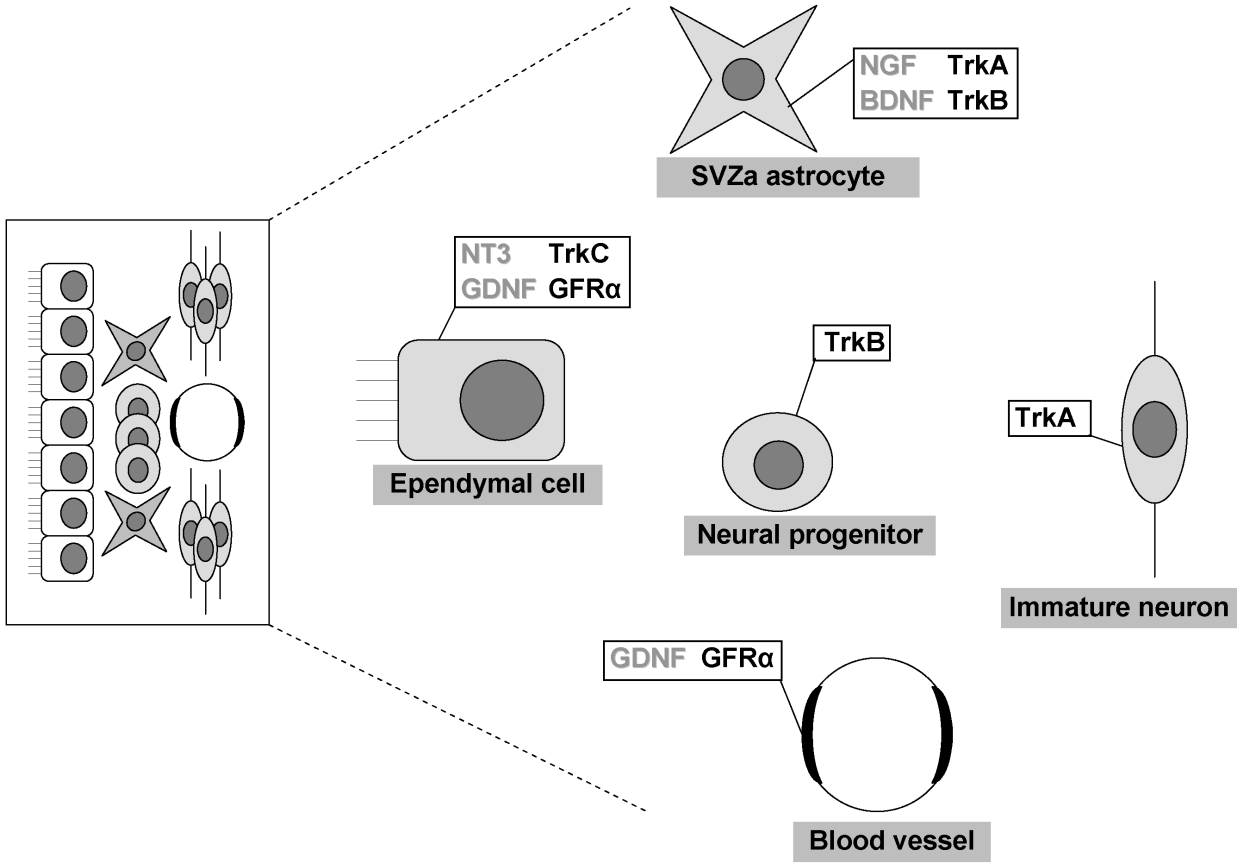


Fig. 3. - Expression patterns of selected neurotrophins and their receptors in monkey SVZ. Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT3, neurotrophin-3; Trk, tropomyosin-related kinase; GDNF, glial cell line-derived neurotrophic factor; GFRα1, GDNF family receptor α1.

Neurotrophic factor expression in monkey neurogenic niches

To better understand the mechanisms controlling cell proliferation and differentiation in monkey neurogenic niches, DG and SVZ, we evaluated the expression in these niches of neurotrophic factors, known regulators of neural development and progenitors in rodents. In SVZ, we constructed a molecular "map" of the cellular types expressing several families of neuropoietic and angiopoietic regulatory molecules (Fig. 3). For example, the nerve growth factor (NGF) was expressed by astrocytes in SVZ, while its high-affinity tyrosine kinase receptor TrkA was present in both astrocytes and immature neurons (Tonchev et al., 2007; Fig. 3). A related member of the neurotrophins family, brain-derived neurotrophic factor (BDNF), was expressed by SVZ astrocytes, while its high-affinity tyrosine kinase receptor TrkB was present in astrocytes and neural progenitors. Neurotrophin-3 (NT3), a third member of the same family, and its respective high-affinity receptor TrkC were both found in ependymal cells (Tonchev et al., 2007; Fig. 3).

In DG, BDNF was expressed by neurons and blood vessels, while TrkB – by progenitor cells (Yamashima et al., 2004). The expression patters on NGF and TrkA were less clear (data not shown). More intriguing was the situation in the ischemia-prone hippocampal CA1 sector. In control CA1, the only cellular population expressing neurotrophins and their high-affinity receptors were the pyramidal neurons (Tonchev et al., 2008; Fig. 4). However, after ischemia, reactive astrocytes start to express NGF and TrkB (BDNF receptor), while reactive microglia begin to express TrkA (NGF receptor). This opens a possibility for a trilateral neuron-astrocyte-microglial interaction mediated by NGF and BDNF (Fig. 4, arrows), which possibly regulates postischemic glial proliferation in CA1.

Conclusion

The knowledge of the constellation of factors acting in monkey SVZ and SGZ niches may assist their clinically relevant manipulation for therapeutic purposes. Although the mere expression analysis of a given molecule, such as a neurotrophin, in the progenitor cell niche does not provide information on the function of this factor, studying the expression patterns as summarized here represents a step in determining the molecular signature of the cell types comprising the niche in primates. It may thus lead to designing neurotrophic factor-based strategies for enhancement of endogenous precursor cells in monkey models, and eventually in the diseased human brain.

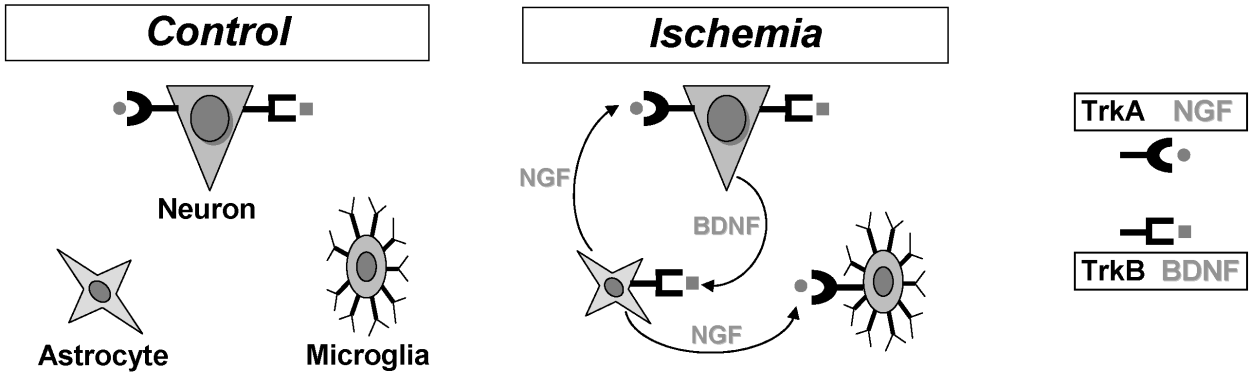


Fig. 4. - Expression of neurotrophins and their receptors in control and ischemic CA1 sector. In control CA1, only neurons express the molecules of interest. However, after ischemia, reactive astrocytes start to express NGF and TrkB (BDNF receptor), while reactive microglia begin to express TrkA (NGF receptor). This opens a possibility for a trilateral neuron-astrocyte-microglial network mediated by NGF and BDNF (arrows).

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