# Neural Regenerative Options in the Management of Ischemic Brain Injury

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*Ischemic brain injury is a difficult clinical scenario for which adequate treatment paradigms are lacking. The formation of ischemic scar tissue in the cerebral cortex creates a barrier to regenerative efforts and restoration of function. Various agents that regulate the cell cycle hold promise for modulating the naturally progressive formation of this astrocytic scar tissue. Use of these agents will involve the development of strategies that influence the regenerative potential of neural progenitor cells.*

**Key Words:** dentate gyrus, hippocampus, neural stem cells, neurogenesis

**Abbreviations Used:** BrdU, bromodeoxyuridine; CNS, central nervous system; DNA, deoxyribonucleic acid; G-CSF, granulocyte colony-stimulating factor; mRNA, messenger ribonucleic acid; NMDA, N-methyl-D-aspartate

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Until recently, it was widely believed that neuronal production in the mammalian brain occurred only during the prenatal period. Over the past 15 years, however, evidence has shown that neurogenesis continues in certain areas of the adult mammalian brain, namely, in the subventricular zone and hippocampal dentate gyrus (Fig. 1).[20] An obvious question is whether adult neurogenesis has the potential to replace dying neurons in the setting of brain injury. New evidence indicates that such neurogenesis is indeed possible. The logical clinical implication of this finding is the potential to augment the production of mature neurons in the injured brain. This ability would be of considerable value in victims of large strokes who seldom regain significant function in distributions where neural tissue has died.

In contrast to the recently discovered adult neurogenesis, hematopoietic stem cells have been studied extensively for a relatively long time. The hematopoietic system contains a pool of progenitor cells that differentiate into mature cells. Studying the molecular mechanisms governing the proliferation, differentiation, and apoptosis of the hematopoietic stem cell population provides valuable clues about the biology of neural progenitor cells.

This review examines neurogenesis in the normal brain and in response to ischemic insults. Factors that drive proliferation of stem cell populations into neural progenitor cells and those that drive differentiation of neural progenitor cells into neurons and, ultimately, integration into neural networks are discussed. Such therapeutic modalities can be broadly classified as using either an exogenous or an endogenous approach. These basic is-

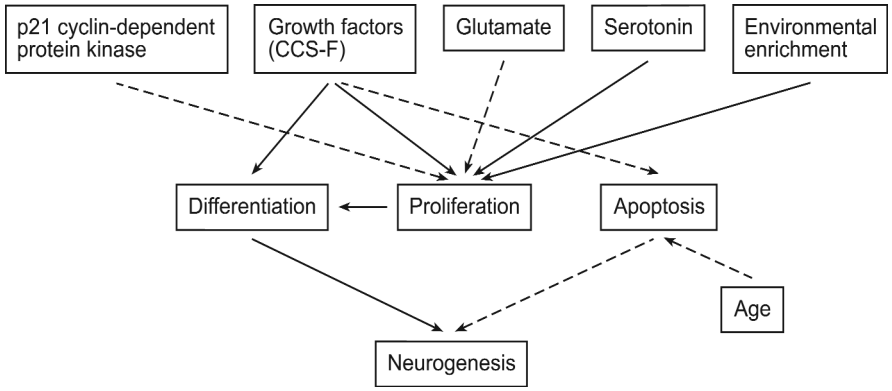


**Figure1.** Flow chart of the pathways involved in the differentiation of endogenous neural stem cells.

sues must be understood before strategies can be devised to improve outcomes based on repopulating neurons and achieving functional integration in the setting of acute ischemic injury.

## Hippocampal Dentate Gyrus

Neural stem cells are located in the subgranular zone of the adult mammalian hippocampal dentate gyrus.[15,19] The primary neural progenitor cells, bipotent radial glia-like stem cells with astrocytic properties, differentiate into lineage-determined progenitor cells and then to mature neurons.[6,16] The mature neurons migrate a short distance and populate the granular cell layer of the hippocampal dentate gyrus. The S-phase marker bromodeoxyuridine (BrdU) has been used to track dividing cells in the adult rat dentate gyrus. Based on such studies, young adult rats generate 9,000 new cells each day or more than 250,000 per month. The new granule neurons generated each month constitute 6% of the total size of the granular cell population as well as 30 to 60% of afferent and efferent cells.[5]

## Forebrain Subventricular Zone

Adult neurogenesis also occurs in the mammalian subventricular zone. Neurons are generated in the subependymal layer of the lateral ventricles and then migrate tangentially toward the olfactory bulb and striatum. In the olfactory bulb, these neurons climb radially into the granule and periglomerular cell layers. There, they assume the nuclear morphology of granule cells and express neuron-specific markers.[11] Most of these adult-generated granule neurons persist within the olfactory bulbs for at least 4 months.[10] Chains of neuroblasts also extend from the subventricular zone to the peri-infarct striatum. Many of these newly generated cells persist in the striatum and cortex adjacent to infarcts. However, 35 days after stroke, only the cells in the neostriatum express neuronal markers.[25] As with the hippocampal dentate gyrus, the persistent neurogenesis in the subventricular zone has been implicated in olfactory memory and odor discrimination.[1]

## Response to Ischemic Insults

### Stroke-Induced Neurogenesis

Recent evidence suggests that ischemia significantly induces neurogenesis in the brain of adult rodents.[20] Liu et al. first demonstrated a marked increase in cell birth in the dentate subgranular zone of gerbils in response to transient global ischemia.[21] Subsequent studies confirmed that focal[9,12,31,32] and global[30] cortical ischemia significantly increases the proliferation of neural stem cells in both the dentate gyrus and subventricular zone. This phenomenon peaks 7 to 10 days after ischemia is induced and returns to normal levels within a month.[20]

*Differentiation of Stem Cells to Mature Neurons.* Neural stem cells from adult rats co-cultured with primary neurons and astrocytes from neonatal hippocampus differentiate into electrically active neurons and integrate into neural networks with functional synaptic transmission (Fig. 2).[29] Preliminary evidence suggests that these cells play a role in hippocampal-dependent memory formation.[29] At 35 days after stroke, the chain of neuroblasts that migrates from the subventricular zone to the peri-infarct striatum also expresses neuronal markers.[25] As with the hippocampal dentate gyrus, the persistent neurogenesis in the subventricular zone has been implicated in olfactory memory and odor discrimination.[1]

*Migration of Neural Precursors.* Understanding the movement of neural precursors generated in the adult mammalian brain to their final destination has important implications for functional recovery after ischemic injury. Recall that normal neural stem cells in the hippocampal dentate gyrus differentiate into progenitor cells and then to mature neurons.[6,16] The latter migrate a short distance to populate the granular cell layer of the hippocampal dentate gyrus. Newly divided neuronal precursor cells also migrate from the subgranular zone into the granule cell layer and differentiate into neurons.[21] Migrating neuroblasts move as chains through a well-defined pathway, the rostral migratory stream. These chains contain only closely apposed neuroblasts. Another astrocytic cell type ensheathes the chains of migrating neuroblasts. Hence during chain migration, neural precursors move in association with each other; they are not guided by radial glial or axonal fibers.[22]

Parent et al. induced focal stroke in adult rats and assessed cellular proliferation and neurogenesis with BrdU labeling and immunostaining.[25] Brains examined 10 to 21 days after stroke showed chains of neuroblasts extending from the

subventricular zone to the peri-infarct striatum. These findings combined with those from several other studies indicate that focal and global ischemic injury directs the migration of the neuroblasts to the site of the infarct.[2,13]

*Long-Term Survival of New Adult Neurons.* An important question is how long the newly generated neurons in the ischemic adult brain persist. It is estimated that fewer than 1% of the ischemic and dying striatal neurons are replaced through adult neurogenesis.[2] This estimate suggests that most neurons generated in the adult brain in response to injury do not survive for a significant length of time. Understanding the mechanism of cell death in adult-born neurons is the first step in developing therapies intended to maximize functional recovery after stroke.

## Neurogenesis in the Aging Brain

Most of the new neurons generated in response to stroke in adult rats do not survive, and stroke most often occurs in older populations. Therefore, an important question is whether aging influences the survival of adult-born neurons. Yagita et al. used a four-vessel transient ischemia model in rats to investigate the role of aging on proliferation of neural progenitor cells.[34] In both young and old rats, neurogenesis significantly increased in the subventricular zone in response to transient global ischemia. Advanced age, however, profoundly influenced the survival of the newly generated neural precursor cells. One month after the ischemia was induced, the number of surviving neural progenitor cells decreased seven-fold in the older rats. To develop strategies to maximize functional recovery after stroke in the aging population, we must first understand the molecular mechanism by which senescence decreases the survival of neurons. Is it through programmed cell death or tissue necrosis? What signaling pathways are involved? How can this cell death mechanism be down-regulated?

## Neurotransmitters

Glutamate, the main excitatory neurotransmitter in the brain, appears to play a role in adult neurogenesis. Activation of NMDA-glutamate receptors rapidly decreases proliferation of neuronal stem cells in rats.[4] In contrast, administration of NMDA-receptor antagonists rapidly increases the number of cells during the S phase.[4] Lesioning of the entorhinal cortex, the main excitatory (glutamate-mediated) afferent input to the hippocampal granule neurons, also increases neurogenesis in the dentate gyrus. This finding suggests that glutamate negatively affects adult neurogenesis.

Serotonin is another neurotransmitter implicated in adult neurogenesis. Chronic antidepressant therapy with serotonin-reuptake inhibitors significantly increases proliferation of adult neuronal precursor cells in the dentate gyrus.[23] Conversely, inhibiting the serotoninergic pathway decreases neuronal proliferation in the dentate gyrus and subventricular zones.[3] However, the effect of serotonin on ischemia-induced proliferation of neural progenitor cells has not yet been established.

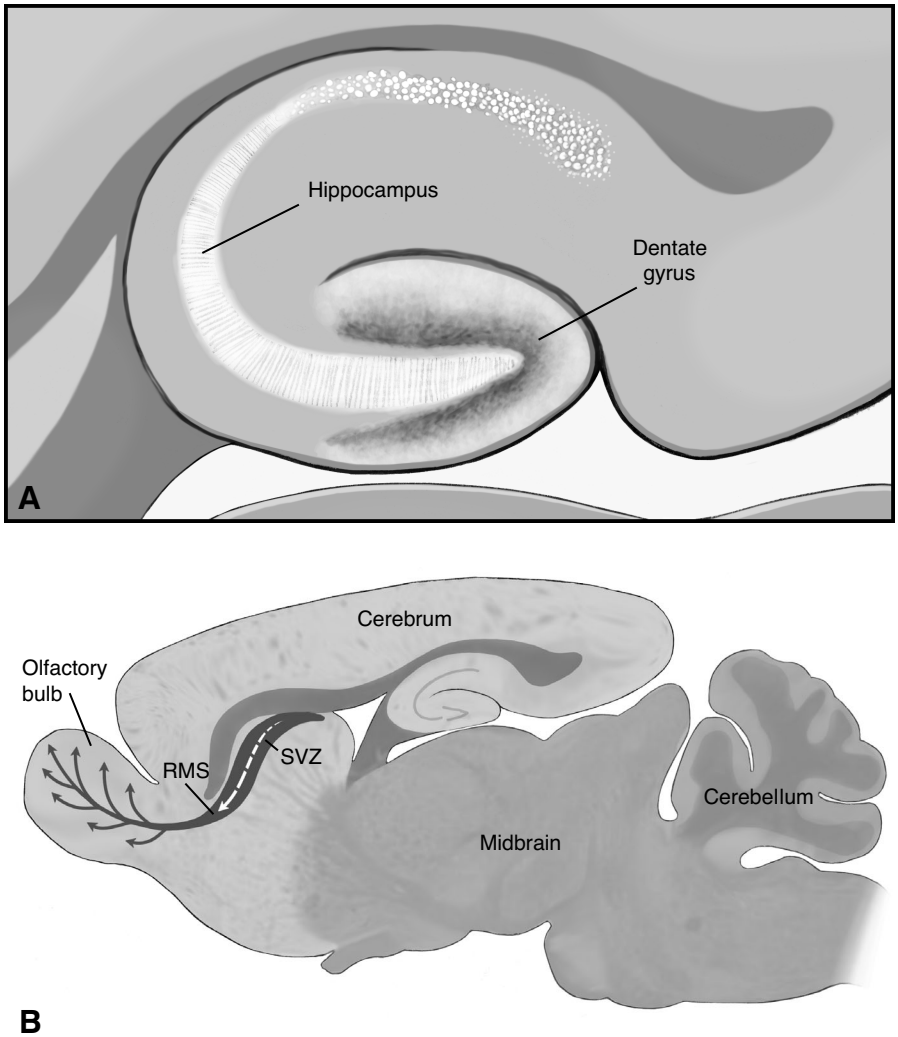


**Figure 2.** The two niches containing neural stem cells in the CNS of the adult mammal. (*A*) adult hippocampus. (*B*) Longitudinal section of the adult mouse brain in which the subventricular zone (SVZ) and the rostral migratory stream (RMS) are indicated. Neuronal precursors migrate tangetially along the rostral migratory stream from the SVZ to the olfactory bulb.

## Environmental Enrichment

Environmental enrichment, consisting of social interactions, various physical activities, and exposure to novel objects, enhances functional recovery after stroke.[17,33] At the cellular level, environmental enrichment has increased dendritic branching and dendritic spines in pyramidal neurons of layers II–III in the contralateral cortex.[14] At the molecular level, environmental enrichment induces differential gene regulation in the injured brain.

Recent evidence suggests that postischemic environmental enrichment also enhances neurogenesis in the adult germinal zones. Komitova et al. induced stroke by ligating the middle cerebral artery of spontaneously hypertensive rats, which were then exposed to a standard or enriched environmental condition.[18] One and 7 days after stroke, rats exposed to the enriched environment performed significantly better than rats exposed to the standard environment. Five weeks after stroke, there was less cellular proliferation in the subventricular zone of the stroke-lesioned rats in the standard environment compared to intact animals.

Postischemic environmental enrichment counteracts the decreased level of cellular proliferation in the subventricular zone while increasing the pool of neural progenitor cells in the subventricular zone. The generation of neural precursor cells capable of recruitment to the stroke site also increases. The same investigators demonstrated similar findings in the hippocampi of rats with strokes in response to environmental enrichment.[24] One month after injury, these rats demonstrated improved spatial learning compared to control animals and a net increase in hippocampal neurogenesis.

## Factors (G-CSF)

G-CSF, a 19.6 kDa growth factor, exerts neuroprotective effects after focal cerebral ischemia in the rat model.[27] It counteracts programmed cell death and enhances functional recovery. G-CSF is widely expressed in neurons in the CNS, and it is upregulated in response to cerebral ischemia, suggesting an autocrine neuroprotective signaling cascade.

In myeloid lineage cells, G-CSF induces enhanced survival and differentiation into mature neutrophilic granulocytes.[28] It appears that G-CSF exerts a similar effect on survival and differentiation of neural stem cells.[28] Adult neural stem cells isolated from the subventricular zone and hippocampus of rats express G-CSF in vitro.[28] G-CSF greatly induces promoter activity of the mature neuronal marker gene III-tubulin. In culture it also induces dose-dependent increases in levels of the mRNA of several differentiation markers. It does not, however, deplete the pool of undifferentiated cells.

## Endogenous Suppression of Neural Regeneration (p21 Cyclin-Dependent Kinase Inhibitor)

The efficacy of neural stem cell expansion in response to injury depends on the proliferative capacity of the cells. Understanding the molecular kinetics of the cell cycle is the key to inducting the proliferation of stem cells for therapeutic purposes. In the hematopoietic stem cell model, there are two types of cellular proliferation: high-capacity proliferation of progenitor cells in response to cytokines and low-proliferation capacity quiescent stem cells. The latter are insensitive to cytokines but intermittently supply the proliferative pool. Likewise in the CNS, quiescent multipotent precursor cells or neural stem cells give rise to the proliferative adult neural progenitor cells. The inherently lower rate of turnover in the CNS may likewise be related to quiescence in neural progenitor cells.[26]

This possibility could explain the mild proliferation and migration of neural precursor cells and the subsequent minimal functional improvement in response to brain injury. Proteins such as cyclin-dependent kinase inhibitors, which down-regulate the cell cycle, mediate cellular quiescence. The cyclin-dependent kinase inhibitor p21cip1/waf1 specifically downregulates the cell cycle in hematopoietic stem cells (p21).[7,8] The p21 cyclin-dependent kinase inhibitor also prevents quiescent progenitor cells in the brain from entering the cell cycle.[26] Qiu and colleagues demonstrated that ischemic brain injury induces proliferation of quiescent neural progenitor cells in the hippocampus and in the subventricular zone of p21 knockout mice.[26] The increased proliferation of stem cells, however, did not occur in nonlesioned p21 knockout mice.

## Conclusion

Future efforts aimed at investigating other regulators of the cell cycle, including the other members of the cyclin-dependent kinase-inhibitor family, will improve understanding of the relative quiescence of neural progenitor cells. Eventually, such studies may lead to improved functional recovery of the CNS in response to ischemic injury by spurring the development of strategies to enhance proliferative potential of neural progenitor cells.

### References


1. Alvarez-Buylla A, Garcia-Verdugo JM: Neurogenesis in adult subventricular zone. **J Neurosci 22:**629-634, 2002
2. Arvidsson A, Collin T, Kirik D, et al: Neuronal replacement from endogenous precursors in the adult brain after stroke. **Nat Med 8:**963-970, 2002
3. Brezun JM, Daszuta A: Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. **Neuroscience 89:**999-1002, 1999
4. Cameron HA, McEwen BS, Gould E: Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. **J Neurosci 15:**4687-4692, 1995
5. Cameron HA, McKay RD: Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. **J Comp Neurol 435:**406-417, 2001
6. Cameron HA, Woolley CS, McEwen BS, et al: Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. **Neuroscience 56:**337-344, 1993
7. Cheng T, Rodrigues N, Dombkowski D, et al: Stem cell repopulation efficiency but not pool size is governed by p27(kip1). **Nat Med 6:**1235-1240, 2000
8. Cheng T, Rodrigues N, Shen H, et al: Hematopoietic stem cell quiescence maintained by p21cip1/waf1. **Science 287:**1804-1808, 2000
9. Choi YS, Lee MY, Sung KW, et al: Regional differences in enhanced neurogenesis in the dentate gyrus of adult rats after transient forebrain ischemia. **Mol Cells 16:**232-238, 2003

10. Corotto FS, Henegar JA, Maruniak JA: Neurogenesis persists in the subependymal layer of the adult mouse brain. **Neurosci Lett 149:**111-114, 1993
11. Gheusi G, Cremer H, McLean H, et al: Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. **Proc Natl Acad Sci USA 97:**1823-1828, 2000
12. Jin K, Minami M, Lan JQ, et al: Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. **Proc Natl Acad Sci USA 98:**4710-4715, 2001
13. Jin K, Sun Y, Xie L, et al: Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. **Aging Cell 2:**175-183, 2003
14. Johansson BB: Functional and cellular effects of environmental enrichment after experimental brain infarcts. **Restor Neurol Neurosci 22:**163-174, 2004
15. Kaplan MS, Hinds JW: Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. **Science 197:**1092-1094, 1977
16. Kempermann G, Jessberger S, Steiner B, et al: Milestones of neuronal development in the adult hippocampus. **Trends Neurosci 27:**447-452, 2004
17. Keyvani K, Sachser N, Witte OW, et al: Gene expression profiling in the intact and injured brain following environmental enrichment. **J Neuropathol Exp Neurol 63:**598-609, 2004
18. Komitova M, Mattsson B, Johansson BB, et al: Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats. **Stroke 36:**1278-1282, 2005
19. Kuhn HG, Dickinson-Anson H, Gage FH: Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. **J Neurosci 16:**2027-2033, 1996
20. Lichtenwalner RJ, Parent JM: Adult neurogenesis and the ischemic forebrain. **J Cereb Blood Flow Metab 26:**1-20, 2006
21. Liu J, Solway K, Messing RO, et al: Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. **J Neurosci 18:**7768-7778, 1998
22. Lois C, Garcia-Verdugo JM, Alvarez-Buylla A: Chain migration of neuronal precursors. **Science 271:**978-981, 1996
23. Malberg JE, Eisch AJ, Nestler EJ, et al: Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. **J Neurosci 20:**9104-9110, 2000
24. Nilsson M, Perfilieva E, Johansson U, et al: Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. **J Neurobiol 39:**569-578, 1999
25. Parent JM, Vexler ZS, Gong C, et al: Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. **Ann Neurol 52:**802-813, 2002
26. Qiu J, Takagi Y, Harada J, et al: Regenerative response in ischemic brain restricted by p21cip1/waf1. **J Exp Med 199:**937-945, 2004
27. Schabitz WR, Kollmar R, Schwaninger M, et al: Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. **Stroke 34:**745-751, 2003
28. Schneider A, Kruger C, Steigleder T, et al: The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. **J Clin Invest 115:**2083-2098, 2005
29. Shors TJ, Miesegaes G, Beylin A, et al: Neurogenesis in the adult is involved in the formation of trace memories. **Nature 410:**372-376, 2001
30. Takagi Y, Nozaki K, Takahashi J, et al: Proliferation of neuronal precursor cells in the dentate gyrus is accelerated after transient forebrain ischemia in mice. **Brain Res 831:**283-287, 1999
31. Takasawa K, Kitagawa K, Yagita Y, et al: Increased proliferation of neural progenitor cells but reduced survival of newborn cells in the contralateral hippocampus after focal cerebral ischemia in rats. **J Cereb Blood Flow Metab 22:**299-307, 2002
32. Tureyen K, Vemuganti R, Sailor KA, et al: Transient focal cerebral ischemia-induced neurogenesis in the dentate gyrus of the adult mouse. **J Neurosurg 101:**799-805, 2004
33. Will B, Galani R, Kelche C, et al: Recovery from brain injury in animals: Relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). **Prog Neurobiol 72:**167-182, 2004
34. Yagita Y, Kitagawa K, Ohtsuki T, et al: Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. **Stroke 32:**1890-1896, 2001