Stroke-Induced Neurogenesis in Aged Brain

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Background and Purpose—Stroke induced by middle cerebral artery occlusion (MCAO) triggers increased neurogenesis in the damaged striatum and nondamaged hippocampus of young adult rodents. We explored whether stroke influences neurogenesis similarly in the aged brain.

Methods—Young adult (3 months) and old (15 months) rats were subjected to 1 hour of MCAO, and new cells were labeled by intraperitoneal injection of 5-bromo-2′-deoxyuridine 5′-monophosphate (BrdU), a marker for dividing cells, for 2 weeks thereafter. Animals were euthanized at 7 weeks after the insult, and neurogenesis was assessed immunocytochemically with antibodies against BrdU and neuronal markers with epifluorescence or confocal microscopy.

Results—Young and old rats exhibited the same increased numbers of new striatal neurons after stroke, despite basal cell proliferation in the subventricular zone being reduced in the aged brain. In contrast, both the number of stroke-generated granule cells and basal neurogenesis in the dentate subgranular zone were lower in old compared with young animals. Also, the ability of newly formed cells to differentiate into neurons was impaired in the aged dentate gyrus.

Conclusions—Basal neurogenesis is impaired in the subgranular and subventricular zones of aged animals, but both regions react to stroke with increased formation of new neurons. The magnitude of striatal neurogenesis after stroke is similar in young and old animals, indicating that this potential mechanism for self-repair also operates in the aged brain.

Key Words: cerebral arteries • hippocampus • ischemia • neuronal plasticity • stem cells • stroke, ischemic

The production of neurons in the mammalian brain continues throughout life. Neurons generated in the subventricular zone (SVZ) migrate to the olfactory bulb, and they differentiate into interneurons. Neurogenesis in the dentate subgranular zone (GCL) gives rise to neurons in the granule cell layer (GCL). Basal hippocampal and olfactory bulb neurogenesis declines in aged animals, but different treatments also increase the production of new neurons in old brains. Stroke induced by middle cerebral artery occlusion (MCAO) in young adult rodents triggers increased neurogenesis in the SGZ/GCL and SVZ. This insult causes neuronal loss in the striatum and cerebral cortex, whereas hippocampal formation is spared. The SVZ neuroblasts migrate into the damaged striatal area and adopt the phenotype of projection neurons. Stroke leads to deficits in hippocampus-associated spatial memory, and the increase in SGZ neurogenesis might aim to counteract cognitive impairments. Striatal neurogenesis, therefore, may contribute to recovery of stroke-impaired motor function.

Ischemic stroke occurs more often in aged humans. It is therefore of major clinical interest to explore whether the aged brain retains the capacity for stroke-induced neurogenesis. Here we show that both basal and stroke-induced hippocampal neurogenesis is attenuated in old animals. In contrast, despite reduced basal proliferation in the SVZ, the number of striatal neurons generated after stroke is similar in young and aged rats.

Materials and Methods

Animals and Experimental Groups

Female Wistar rats (Scanbur-BK AB, Sollentuna, Sweden), aged 3 (n = 8; body weight, 270 to 290g) or 15 (n = 11; 320 to 330g) months, were ovariectomized and after 5 weeks subjected to MCAO (n = 6 and n = 8 for young and old rats, respectively) or sham surgery (n = 2 and n = 3). Rats were allowed to survive for 7 weeks thereafter. Bromodeoxyuridine (BrdU; 50 mg/kg IP, Sigma) was given twice daily for 2 weeks from the day after MCAO or sham surgery. Experimental procedures were conducted according to the guidelines set by the Malmö-Lund Ethical Committee for the use and care of laboratory animals.

Middle Cerebral Artery Occlusion

Rats were anesthetized by spontaneous inhalation of N2O and O2 (70%:30%, vol/vol) with 1% halothane. Stroke was induced by the intraluminal filament technique. Common and external carotid arteries were ligated, and the internal carotid artery was temporarily closed. A monofilament was advanced through the internal carotid artery to the origin of the MCA. After 1 hour of occlusion, the animals were reanesthetized and the filament was withdrawn. Ani-
mals subjected to sham surgery were treated similarly, except that the filament was not advanced to the origin of the MCA.

**Immunohistochemistry**

Animals were anesthetized and transcardially perfused with saline followed by 4% paraformaldehyde. Thirty-micron coronal sections were stained as described previously. In brief, free-floating sections were first incubated in 1 mol/L HCl for 30 minutes at 65°C (only for BrdU immunostaining). Sections were then incubated at 4°C in potassium phosphate-buffered saline containing 5% appropriate serum, 0.25% Triton-X, and the following primary antibodies: mouse anti-NeuN (1:100, Chemicon) for 24 hours; rabbit anti-S100 (1:500, SWANT) for 24 hours; mouse anti-Ki67 (1:50, Novocasta Laboratories) for 48 hours; goat anti-DCX (1:400, Santa Cruz Biotechnology) for 24 hours; and mouse anti-ED1 (1:200, Serotec) for 24 hours.

Sections were incubated for 2 hours at room temperature with secondary antibodies: Cy3-conjugated donkey anti-rat (1:200, Jackson ImmunoResearch Laboratories) for BrdU, Cy3-conjugated donkey anti-mouse (1:200, Jackson ImmunoResearch Laboratories) for Ki67, biotinylated horse anti-mouse (1:200, Vector Laboratories) for NeuN and ED1, and biotinylated goat anti-rabbit (1:200, Vector Laboratories) for S100β. After biotinylated secondary antibodies were added, sections were treated with potassium phosphate-buffered saline containing Alexa488-conjugated streptavidin (1:200, Molecular Probes) for 2 hours at room temperature.

**Cell Counting and Volume Measurement**

Cell numbers were quantified in 3 representative evenly spaced sections per animal (from 2.8 to 3.8 mm posterior to bregma and from 1 mm anterior to 0.3 mm posterior to bregma for hippocampus and striatum, respectively). For quantification of colocalization of BrdU and NeuN in the SGZ/GCL, at least 50 BrdU-positive cells in each animal were analyzed for NeuN labeling. All BrdU/NeuN, BrdU/DCX-, BrdU/S100β-, and Ki67/DCX-double-labeled cells identified by epifluorescence microscopy were validated with a confocal laser scanning microscope. Infarct volume was measured with a computerized setup for stereology, driven by CAST 2 software (Olympus).

**Statistical Analysis**

Values are mean±SEM and represent the sum of counted cells in 3 sections. Comparisons were performed with Student’s paired or unpaired t test or 1-way ANOVA followed by a post hoc Bonferroni/Dunn test. Differences were considered significant at P<0.05. Because no differences in numbers of DCX-, BrdU-, and ED1-positive cells were observed between the left and right hippocampus of sham-operated young and old rats, data from the 2 sides were pooled.

**Results**

Irrespective of treatment, old rats showed a 20-fold higher number of lipofuscin-containing cells in the SGZ/GCL compared with young animals (Figure 1). Lipofuscin accumulates in some neurons with increasing age. Thus, the brains of the 15-month-old rats were functionally “aged.”

In all animals subjected to MCAO, there was almost complete neuronal loss in the dorsolateral striatum. The pattern of striatal and cortical damage was similar in aged and young rats. The volume of the lesioned striatum did not differ between young (18.1±3 mm³) and old (23.9±8 mm³) animals.

Old animals that had been subjected to stroke or sham surgery 7 weeks previously showed similar, markedly reduced cell proliferation in the SGZ, as judged by the number of Ki67-positive cells. This proliferation represented only 4% to 10% of that in young animals on the side ipsilateral to MCAO (6.6±1.4 and 0.4±0.2 cells per section in young and old rats, respectively).

The neuroblast marker DCX is expressed in neuronal progenitors and young neurons during stroke. Therefore, the DCX-positive cells in the SGZ/GCL at 7 weeks after surgery most likely represented neuroblasts and young neurons born during the preceding 2 weeks. In support of this idea, only single DCX-positive cells were colabeled with BrdU or Ki67. In the dentate gyrus ipsilateral to MCAO or sham procedure in old rats, there were 88% and 82%, respectively, lower numbers of DCX-positive neurons compared with those in young animals (Figure 2). We observed no differences in the number of DCX-positive cells between sham-operated and MCAO-subjected animals in either age group, indicating that at 7 weeks after stroke, the generation of hippocampal neuroblasts was at a basal level.

In young stroke animals, there was an ipsilateral (10.1-fold) as well as a contralateral (4.1-fold) increase in the number of BrdU-positive cells in the SGZ/GCL (Figure 3). Stroke also gave rise to more BrdU-positive cells in the SGZ/GCL of old animals. However, the increase was smaller (2.9-fold) and only occurred ipsilateral to the MCAO (Figure 3D). Also, BrdU/NeuN double-stained cells (Figure 3E–3G) were fewer in old (44.5±5.1% of BrdU-positive cells) compared with young (63.0±3.9%) animals. This finding indicates that the ability of new cells to develop along a neuronal lineage was impaired in the old hippocampus. Virtually none of the BrdU-positive cells in the SGZ/GCL of rats subjected to stroke were double-labeled for the astrocytic marker S100β.

**Figure 1.** A, Epifluorescence photomicrograph of the tip of the dentate GCL of 1 rat in the 15-month ("old") group. Arrows depict 2 cells with lipofuscin accumulation (autofluorescent white grains visible under UV light). B, Number of lipofuscin-containing cells in the SGZ/GCL of young and old rats, ipsilateral and contralateral to the MCAO. Values are mean±SEM. *P<0.05, Student’s unpaired t test.

Arguing against the possibility that the level of microglia activation was responsible for the age-related decrease in hippocampal neurogenesis, we observed no differences between young and old animals in the number of ED1-positive microglia in the SGZ/GCL after either stroke or sham surgery (Figure 4).

The SVZ is probably the major source for the stroke-generated striatal neurons. At 7 weeks after MCAO, the number of Ki67-positive cells in the SVZ of old animals was lower (59%) compared with that in young animals (Figure 5) with no side differences in either group. A similar proportion of Ki67-positive cells was double-labeled for
DCX (Figure 5H–5J), irrespective of age or brain side (31%/110061% and 35%/110062%, and 39%/110068% and 37%/110062% on ipsilateral and contralateral sides of old and young animals, respectively). High fiber density did not allow us to quantify DCX-positive neuroblasts in the SVZ itself. In both young and old animals, substantially more DCX-positive cells were observed in the striatum ipsilateral to the MCAO compared with the contralateral side or in sham-operated animals (Figure 5B and 5C). Only single, striatal DCX-positive cells were colabeled with BrdU or Ki67. There were no differences between young and old animals in the number of DCX-positive cells in the striatum ipsilateral to the stroke. These data indicate that the insult-induced production of neuroblasts at 7 weeks after stroke is independent of age.

We observed no differences between young and old animals in the number or distribution of new, mature striatal neurons formed after stroke. At 7 weeks after MCAO, significant increases in the number of BrdU/NeuN double-positive cells were observed in the stroke-damaged striatum in both age groups (Figure 5K–5N). Stroke markedly increased the number of BrdU/S100β double-labeled astrocytes in the ipsilateral striatum. Gliogenesis was more pronounced in young compared with old animals (Figure 5O). Because we did not observe any differences between young and old animals in the number of new striatal neurons, our data argue against a major role for gliogenesis in the regulation of stroke-induced neurogenesis.

Discussion

Previous studies have shown that stroke gives rise to increased cell proliferation and generation of neuroblasts in the SVZ of both young and aged rats. We demonstrate for the first time production of mature striatal neurons after stroke in old animals. Despite an age-related reduction of basal SVZ proliferation, the number of stroke-generated new striatal neurons was similar in young and aged rats. In the dentate gyrus, on the other hand, both basal and stroke-induced formation of new granule cells was significantly reduced in old rats.
The decrease of basal hippocampal neurogenesis in aged animals is in accordance with previous studies. It is uncertain, but elevation of glucocorticoid levels could be a contributing factor. However, Heine and coworkers found no correlation between corticosterone levels and decreased hippocampal proliferation in aged rats. It is also inconceivable that age-related alterations in levels of ovarian hormones were responsible for the differences in hippocampal neurogenesis, because all of our animals were ovariectomized.

We found that at 7 weeks, stroke had increased the number of new neurons in the SGZ/GCL in both age groups, though with smaller magnitude in old rats. A previous study showed that at 5 weeks after global forebrain ischemia, new cells in the SVZ/GCL were present in increased numbers in young animals, whereas their survival in old animals was poor. The discrepancy compared with our findings is most readily explained by differences between insults and the degree of accompanying hippocampal inflammation.

Recently, it was demonstrated that MCAO leads to activation of cAMP-response element–binding protein (CREB) and that inhibition of CREB blocks, whereas CREB overexpression increases, MCAO-induced hippocampal neurogenesis. We have previously shown that MCAO promotes hippocampal neurogenesis through N-methyl-D-aspartate receptor–mediated glutamatergic mechanisms, probably by altering the levels of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). BDNF stimulates phosphorylation of CREB in hippocampal neurons and increases the survival of the newly generated neurons. There is marked reduction of both CRE-binding activity and injury-induced BDNF levels in the aged hippocampus. We hypothesize that the attenuation of stroke-induced hippocampal neurogenesis in old animals could be due to the diminished CREB activity, partly occurring as a consequence of impaired BDNF signaling.

The formation of mature striatal neurons after stroke was similar in young and old rats. We could not determine whether the stroke-induced increase of SVZ proliferation, shown previously in young animals, was attenuated in the aged animals. Sato et al found that stroke led to similarly increased numbers of polysialylated neutral cell adhesion molecule (PSA-NCAM)–positive neuroblasts in the SVZ of young and old rats but with a peak at 1 and 3 days, respectively. In contrast, Jin et al found a lower increase in the proliferation and number of DCX-positive cells in the SVZ during the first 24 hours after stroke in old animals. Our findings suggest that the mechanisms involved in different...
stages of stroke-induced neurogenesis, such as migration and survival of the newly formed neurons, are able to compensate for the age-related reduction of basal proliferation.

We observed no differences between young and old rats in the distributional pattern of the new, DCX-positive neuroblasts and mature BrdU/NeuN double-labeled neurons within the damaged striatum. This is in agreement with the observation that SVZ progenitors isolated from young and old mice exhibit similar migratory capacity in vitro and provides the first evidence that the stroke-damaged aged brain is permissive for neuroblast migration.

### Summary

Our data indicate that the stroke-induced increase of neurogenesis in the dentate gyrus is attenuated in old rats, suggesting that this presumed mechanism to reverse cognitive alterations declines with increasing age. In contrast, striatal neurogenesis is induced to the same level in young and old animals. Whether the new striatal neurons contribute to behavioral improvement after stroke is unknown. However, we show here that the various steps of striatal neurogenesis also operate in animals at an age corresponding to that when stroke is frequent in humans, which supports the idea that stimulation of the brain’s self-repair mechanisms may become a future therapeutic target to promote functional recovery after stroke in patients.

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### References


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