



# Inflammation and neural repair after ischemic brain injury

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## ABSTRACT

Stroke causes neuronal cell death and destruction of neuronal circuits in the brain and spinal cord. Injury to the brain tissue induces sterile inflammation triggered by the extracellular release of endogenous molecules, but cerebral inflammation after stroke is gradually resolved within several days. In this pro-resolving process, inflammatory cells adopt a pro-resolving or repairing phenotype in the injured brain, activating endogenous repairing programs. Although the mechanisms involved in the transition from inflammation to neural repair after stroke remain largely unknown to date, some of the mechanisms for inflammation and neural repair have been clarified in detail. This review focuses on the molecular or cellular mechanisms involved in sterile inflammation and neural repair after stroke. This accumulation of evidence may be helpful for speculating about the endogenous repairing mechanisms in the brain and identifying therapeutic targets for improving the functional prognoses of stroke patients.

## 1. Introduction

Stroke is a major cause of death and disability worldwide (World Health Organization, 2018). Most patients surviving after stroke suffer from various neurological deficits and impaired activities of daily living over the long term. Since many additional countries are expected to join the extant super-aged societies (in which more than one in five persons is 65 or older) by 2030, the number of stroke patients is likewise expected to increase year by year. In order to prevent a rapid rise in social security expenses, therapeutic methods of improving the functional prognoses of stroke patients are emergently needed.

Stroke causes neuronal cell death and destruction of neural circuits, resulting in the impairment of motor, sensory or higher brain function. Approximately 70% of strokes consist of a cerebral infarction caused by the severe loss of cerebral blood flow (cerebral ischemia). Ischemic death of brain tissue induces inflammation and brain edema which worsen functional prognosis. Thrombolytic therapy by means of the intravenous administration of recombinant tissue-plasminogen activator (rt-PA) within 4.5 h after stroke onset reduces ischemic brain damage. Although thrombectomy has also been performed for patients with occlusion of the main cerebral or carotid artery, rehabilitation is still the main therapeutic method to promote functional recovery for most ischemic stroke patients. Normally, the neurological deficits in stroke patients will improve for up to six months after stroke with the appropriate rehabilitation programs. It has been thought that neurological function is restored by the reorganization of neuronal circuits in

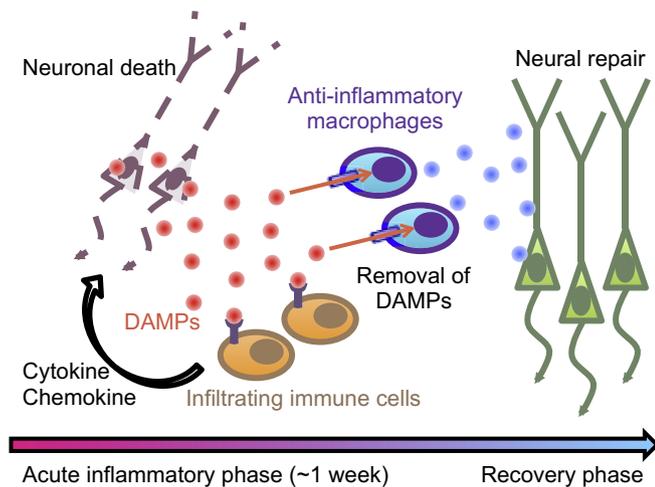
the remaining normal brain tissue after stroke (Murphy and Corbett, 2009). In order to develop a therapeutic method that will improve the functional prognosis of stroke patients, it is necessary to elucidate the mechanisms of neural repair after stroke.

## 2. Inflammation and neural repair after ischemic stroke

Severe loss of cerebral blood supply causes the expression of immediate early genes (c-fos, c-jun, Hsp70, etc.) and metabolic dysfunction in the neurons. These phenomena induce the influx of  $Ca^{2+}$  ions within the neurons, which then activate various intracellular proteases and phospholipases, resulting in the degradation of the cellular membrane and neuronal cell death. Ischemic necrosis of brain cells releases intracellular molecules into the extracellular space, including damage-associated molecular patterns (DAMPs) (Kono and Rock, 2008) (Fig. 1). High mobility group box 1 (HMGB1) and the peroxiredoxin family proteins (PRXs) are major DAMPs in the ischemic brain (Hayakawa et al., 2010; Shichita et al., 2012). These molecules are recognized by pattern recognition receptors (PRRs) in infiltrating immune cells. Toll-like receptors (TLRs), receptor for advanced glycation end product (RAGE), and macrophage-inducible C-type lectin (Mincle) are major PRRs that trigger post-ischemic inflammation in the brain (Arumugam et al., 2017; Muhammad et al., 2008).

Ischemic insults induce the expression of various inflammatory cytokines and chemokines. In the acute phase of post-ischemic inflammation, macrophages and neutrophils infiltrating into ischemic

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**Fig. 1.** Sterile inflammation after ischemic brain injury. In the injured brain, infiltrating immune cells are activated by DAMPs released from dead neurons. Various cytokines and chemokines produced from activated infiltrating macrophages and neutrophils promote ischemic brain damages. Several days after stroke onset, the immune cells adopt an anti-inflammatory phenotype that resolves post-ischemic inflammation and produces growth factors for neural repair.

brain region produce cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ), which directly injure neurons. IL-23 produced by macrophages activates IL-17 production in infiltrating  $\gamma\delta$ T lymphocytes and causes subacute phase (a few days after stroke onset) inflammation. Several studies have tried to demonstrate a therapeutic effect of T-cell-targeted therapy (Shichita et al., 2009; Fu et al., 2014; Elkins et al., 2017). The neurotoxic population of macrophages and T lymphocytes still remains to be clarified. However, therapeutic strategies targeting specific neurotoxic cytokines may be hopeful to promote neural repair after ischemic stroke.

Monocyte chemoattractant protein-1 (MCP-1) is an essential chemokine for the infiltration of circulating monocytes (Mildner et al., 2007). It has recently been clarified that circulating monocytes can be divided into two groups: Ly-6C<sup>hi</sup>CCR2<sup>+</sup> licensed monocytes and Ly-6C<sup>low</sup>CCR2<sup>-</sup> patrolling monocytes. CCR2 is the receptor for MCP-1, which enables CCR2<sup>+</sup> monocytes to infiltrate into the ischemic brain (Gliem et al., 2012); CCR2<sup>-</sup> monocytes, on the other hand, do not infiltrate but circulate in the blood, suggesting a role in peripheral immunity such as protection against bacterial infection. Neutrophils and CCR2<sup>+</sup> monocytes infiltrate into the ischemic brain and are then activated through the PRRs to produce inflammatory factors. The activation of TLR2, TLR4, RAGE, and Mincle triggers post-ischemic inflammation which exacerbates the pathology of ischemic stroke, given that the deficiency of these PRRs attenuates the inflammation and exerts neuroprotective effects in the murine model of ischemic stroke (Shichita et al., 2012).

Infiltrating immune cells and microglia are pivotal players in cerebral post-ischemic inflammation, though immune cells of both types gradually adopt anti-inflammatory and repairing phenotypes (Kim et al., 2016; Lelancette-Hebert et al., 2007). Various DAMPs are extracellularly released from dying brain cells to activate infiltrating immune cells in the acute or subacute phase, but thereafter DAMPs are removed from the ischemic brain, leading to the resolution of cerebral post-ischemic inflammation (Shichita et al., 2017). The scavenger receptors MSR1 and MARCO are important for the clearance of DAMPs. MSR1 and MARCO efficiently internalize DAMPs within macrophages and microglia for lysosomal degradation. Recently, the glymphatic system has been identified as an efficient drainage mechanism for scavenging wastes and pathogenic molecules from the brain (Albargothy et al., 2018). Although the role of the glymphatic system in the resolution of cerebral inflammation remains to be clarified, DAMPs

or inflammatory factors may be removed through a similar drainage system in the brain, perhaps through cerebrospinal fluid flow or cerebral blood flow.

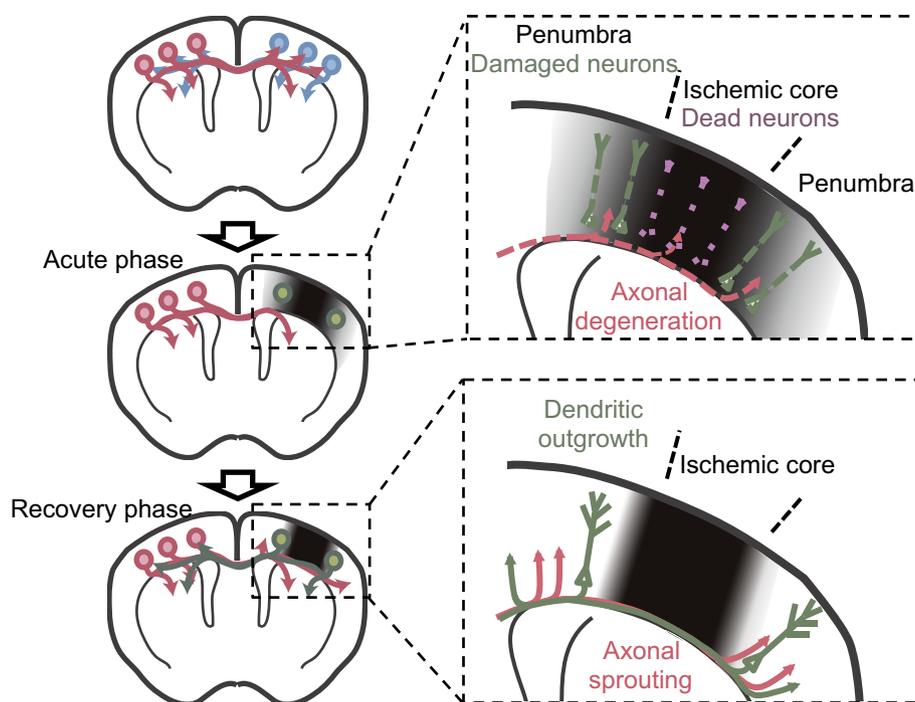
Inflammation is believed to induce tissue repair after injury. Indeed, inflammatory immune cells adopt a pro-resolving phenotype that also produces anti-inflammatory or repairing molecules such as arginase or neurotrophic factor. The strong anti-inflammatory agents known as steroids have not been approved as therapeutic drugs which improve functional prognosis after ischemic stroke. This indicates that cerebral post-ischemic inflammation may have beneficial effects on neural repair. Although it has been believed for many years that damaged brain tissue could never be regenerated, many recent reports have clarified that the brain can in fact be repaired after injury.

### 3. Neuronal circuit remodeling after stroke

#### 3.1. Circuit remodeling in the cerebral cortex and the subcerebral regions

After focal ischemic injury, it has been reported that neuronal circuits in the cortical area around the injured site are reorganized in rodents, non-human primates, and human stroke patients to compensate for lost neurological functions. Spatial mapping of the primary motor cortex (M1) by intracortical microstimulation (ICMS) has revealed the apparent functional reorganization of movement representations in M1 (Nudo and Milliken, 1996). Rehabilitation training of skilled hand use in squirrel monkeys can prevent reduction of the hand territory around the infarct area and expand hand representation into regions formerly occupied by elbow or shoulder representations (Nudo et al., 1996). This reorganization of an undamaged motor-output area is shown to be accompanied by the recovery of motor performance. Similar changes in motor-output area have been observed using focal transcranial magnetic stimulation (TMS) in human stroke patients (Liepert et al., 1998). Stroke patients who receive constraint-induced movement therapy (CIMT) in the training of an impaired limb show increased neuronal excitability in the motor-output area for that limb. Focal ischemic injury of the cerebral cortex also increases neuronal activity in cortical areas adjacent to the lesion. Coagulation of the blood vessels supplying the hand area of M1 in squirrel monkeys induces a substantial enlargement of hand representation in the ventral premotor cortex (PMv), a remote region (Frost et al., 2003). When ibotenic acid is injected, neuronal activity in the PMv increases within two months after focal injury of M1. This increase is important for the recovery of motor performance in this period (Murata et al., 2015).

Neuronal circuit remodeling occurs not only around the injured area but also in the contralateral cortex and distal ipsilateral cortical areas to the lesion site, leading to a global reorganization of the neuronal network in the cerebral cortex. In response to sensory stimulation of an impaired limb, neuronal activity in the somatosensory cortex contralateral to the injured hemisphere has been observed in rodents through electrophysiological experiments and functional magnetic resonance imaging (fMRI) (Dijkhuizen et al., 2001, 2003; Taketsuru et al., 2009; Taketsuru et al., 2013). The change in functional connectivity between the cortical areas after focal thrombosis has been analyzed through voltage sensitive dye (VSD) imaging with channelrhodopsin-2 (ChR2)-mediated local photostimulation of the cortex (Lim et al., 2014). Functional connectivity between cortical areas is globally depressed one week after stroke onset but is partially recovered by eight weeks after stroke onset. In human stroke patients, the ability to modulate the reorganization of neuronal networks after stroke through rehabilitation would be useful as it would enable us to minimize neurological deficits (Grefkes and Fink, 2014). Neuronal activity in response to the sensory stimulation of an impaired hand has been observed in bilateral somatosensory cortex through fMRI (Grefkes and Fink, 2011). Many reports using fMRI also reveal changes in functional connectivity between cortical areas in human stroke patients (Carter et al., 2010).



**Fig. 2.** Neuronal circuit remodeling after stroke. Ischemic brain injury causes neuronal cell death and axonal degeneration in the ischemic core. In the recovery phase, dendritic outgrowth and axonal sprouting projected to the contralateral cortex are observed in the damaged neurons around the peri-infarct area (penumbral area).

Remodeling of cortical activities is accompanied by morphological changes in the dendrites and axons of the neurons (Fig. 2). It has been reported that dendritic outgrowth of layer 5 pyramidal cells in the rat motor cortex contralateral to a lesion induced by middle cerebral artery occlusion (MCAO) is enhanced by rehabilitation (Jones and Schallert, 1994) or by being housed in an enriched-environment cage in which many objects for exploration are placed (Biernaskie and Corbett, 2001). Ischemic injury induces a high level of dendritic spine turnover around the focal ischemic lesion and also in the contralateral cortex to the injured site (Brown et al., 2009; Taketsuru et al., 2009). Axons sprout to the peri-injured region, contralateral cortex, and striatum after ischemic brain injury. Focal ischemic injury in barrel cortex induces axonal sprouting around the injured site in rats (Thomas et al., 2001). Axonal sprouting from the somatosensory cortex contralateral to the injured site is observed four weeks after thermocoagulatory injury (Carmichael and Chesselet, 2002). Change in axonal projection has been also reported in macaque monkeys. Ischemic injury in the M1 hand area increases reciprocal axonal projections between the PMv and the primary somatosensory cortex (Dancause et al., 2005).

Stroke also induces circuit remodeling of the motor-output pathway in the subcerebral regions such as the brain stem and spinal cord. Forced limb use after intracerebral hemorrhage in internal capsule increases axonal sprouting from the cortical motor area to the red nucleus (Ishida et al., 2016). This is important for the recovery of motor performance of the impaired limb, given that performance recovery is abolished by inhibition of the cortico-rubral pathway. CIMT increases the number of contralateral cortico-spinal axons to the ischemic cortical lesion which cross the midline to the cervical spinal cord and project to the ipsilateral spinal motor neurons (Zhao et al., 2013).

Ischemic injury thus induces remodeling of the cortical map and synaptic connections between the peri-infarcted region and distal cortical areas. Remodeling of the cortical circuit is enhanced by rehabilitation and is thought to be important for recovery from neurological dysfunctions. However, the functional significance of the reconstructed circuits has not been elucidated. Recently emerged techniques will allow detailed analysis of the circuit remodeling that

occurs after stroke and the molecular mechanisms involved in this process. Tissue clearing methods enable us to analyze the axonal projection of whole-brain circuits in three dimensions (Ke et al., 2013; Tomer et al., 2014; Hama et al., 2015; Susaki et al., 2015). Imaging of in vivo neuronal activity with optogenetic manipulation reveals the functional connectivity between cortical areas (Lim et al., 2013, 2014). Optogenetics is also useful to investigate the functional significance of reorganized circuits. Molecular mechanisms and cell type identities in the reorganized circuits will be clarified by the method of cell sorting from brain tissue and transcriptome analysis (Tasic et al., 2016). Clarifying the function of reorganized neuronal circuits will enable the development of novel therapeutic methods that will improve the functional prognosis of stroke patients.

### 3.2. Therapeutic targets in circuit remodeling

Knowledge of the molecular mechanisms underlying circuit remodeling will allow us to target molecules that promote the reorganization of neuronal circuits after stroke. Transcriptome analysis of cortical neurons that project axons to the peri-injured area after ischemic injury has revealed enhanced expression levels of growth factors, cell adhesion, axonal guidance and cytoskeletal modifying molecules in mice (Li et al., 2010). Molecules that inhibit axon growth, such as Nogo-A/Nogo receptor and RhoA/Rho-associated kinase, are also increased around the injured site in rats (Zhao et al., 2013). CIMT after stroke decreases the expression of these axonal outgrowth inhibitors. Chondroitinase ABC or anti-Nogo-A antibody injection to the spinal cord increases the number of axons sprouting from the uninjured cortex to the spinal cord in rats after stroke (Soleman et al., 2012; Bachmann et al., 2014; Lindau et al., 2014; Wahl et al., 2014). Chondroitinase ABC injection into the penumbral region around the infarcted area increases synaptic density and improves the recovery of motor performance (Gharbawie et al., 2005).

Inosine, a purine nucleoside, promotes the expression of growth factors and axonal growth in neurons (Benowitz et al., 1998). It has been reported that inosine stimulation of the cortex contralateral to the

injured site increases axonal projections to the red nucleus and spinal cord (Chen et al., 2002). Treatment with inosine alters the expression levels of certain genes that are implicated in complement cascade or mitochondrial function in the brain tissue contralateral to the infarct in rats (Zai et al., 2009).

Signaling by ephrin and its receptor is important for growth cone activity and axon guidance (Klein, 2012). Ephrin-A5, which inhibits axonal sprouting and recovery of motor function after stroke, is upregulated in astrocytes around the infarcted area (Overman et al., 2012). Blockage of ephrin-A5 signaling promotes axonal sprouting around the infarcted region and improves the recovery of motor performance.

The synaptic delivery of AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptors is required for experience-dependent neuronal plasticity (Lee et al., 2000). Recently, edonergic maleate, which binds to collapsing response mediator protein 2 (CRMP2), has been demonstrated as the drug most promising for clinical application for stroke (Abe et al., 2018). Edonergic maleate promotes the activity-dependent delivery of synaptic AMPA receptors and the recovery of motor performance by rehabilitation in mice and macaque monkeys.

Mesenchymal stem cells (MSCs) have been well known to produce various neurotrophic factors, which promote neuroprotection, anti-inflammation, and angiogenesis in the animal models of ischemic stroke (Nomura et al., 2005; Liu et al., 2006; Horita et al., 2008; Onda et al., 2008; Toyama et al., 2009). These neurotrophic factors have beneficial effects on recovery process after ischemic stroke. Several clinical studies have been tried to investigate the therapeutic effect of MSC transplantation in human ischemic stroke patients (Honmou et al., 2011).

Some therapeutic methods have worked well in animal models of stroke and are now in the second or third phase of clinical trials. To date, however, few therapeutics have been applied to clinical treatment. In order to accelerate the development of effective therapy, it is necessary to elucidate the detailed molecular mechanisms involved in neuronal circuit remodeling.

#### 4. Neurogenesis after stroke

Adult neurogenesis occurs only in limited niches, such as the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle in the cerebellum (Zhao et al., 2008; Kempermann et al., 2018). Newly generated neuroblasts in the SVZ migrate to the olfactory bulb and join the olfactory circuit (Fig. 3, left). It has been thought that neurogenesis in the neocortex occurs only rarely in normal healthy conditions. After ischemic stroke, however, the neuroblasts generated in the SVZ migrate to the injured region where they are thought to be involved in the reorganization of neuronal circuits (Kojima et al., 2010) (Fig. 3, right). Cortical neurogenesis after brain injury has been reported in rodents, macaques and human stroke patients. Cortical neurogenesis after ischemic injury was first reported in rats (Gu et al., 2000; Magavi et al., 2000; Jiang et al., 2001). Newly generated neurons in the injured cortical region are labeled by the cell proliferation specific marker 5-bromodeoxyuridine

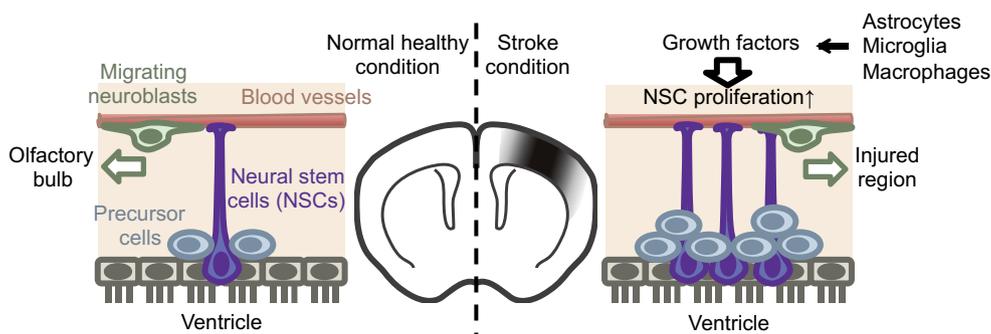
(BrdU) and the mature neuron specific marker NeuN. Neuroblasts labeled by immunostaining for the immature neuron marker doublecortin (DCX) migrate from the SVZ to the injured cortical region along the corpus callosum (Magavi et al., 2000; Jin et al., 2003; Sundholm-Peters et al., 2005; Yang et al., 2007). It has been reported that neural stem cells (NSCs) also exist in cortical layer 1 of adult rats where they generate GABAergic neurons of layer 2–6 after ischemic brain injury (Ohhira et al., 2010). These neurons generated after brain injury project axons to neighboring neurons or other brain regions and are integrated into the existing cortical circuits (Magavi et al., 2000; Ohira et al., 2010).

Adult neurogenesis after stroke is also reported in macaques and humans. The number of newly generated cells (BrdU-positive cells) is increased after ischemic brain injury in the cortex and striatum of macaques (Tonchev et al., 2005), though most of these are astrocytes and microglia. A few populations of BrdU-positive cells in the cortex and striatum show neuronal morphology and NeuN expression. Dcx-positive cells are also found in the penumbral region of stroke patients (Jin et al., 2006). Vascular endothelial cells may be involved in the promotion of neurogenesis after stroke since Dcx-positive cells are localized in the vicinity of the blood vessels.

Proliferation of NSCs in the SVZ is enhanced after ischemic brain injury. However, the integration of these newly generated neurons into the cortical circuits is limited due to tissue damage at the injured site and in the penumbral region. If the neurogenesis and integration of newly generated neurons into the circuits could be accelerated, the functional prognosis of stroke patients would be improved. In fact, neurogenesis can be enhanced by treatment with growth factors such as BDNF (Benraiss et al., 2001), FGF-2 (Leker et al., 2007), EPO (Kolb et al., 2007) and IGF-1 (Zhu et al., 2009). In addition to treatment for neural repair by transplantation of embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, next-generation therapy for stroke might include methods to promote endogenous neurogenesis.

#### 5. Concluding remarks

There is accumulated evidence about molecular or cellular mechanisms underlying cerebral inflammation and neuronal repair after stroke. However, effective therapeutic method which accelerates the resolution of inflammation and tissue repairing process has not been developed. This may be caused by that transition mechanisms from injury-induced inflammation to tissue repair largely remain to be clarified. Contribution of immune system to the reorganization of neuronal circuits or the adult neurogenesis has been recently elucidated. Indeed, neuronal circuits in the brain and the spinal cord are reorganized after stroke, and this circuit remodeling is thought to be a neural substrate of functional recovery. The brain is thus gifted with certain endogenous mechanisms triggering neuronal repair after stroke. It would be important to integrate the knowledge of immune system into the emerging evidence about the neuronal connection in the global neural network and molecular or cellular mechanisms of neuronal circuit function.



**Fig. 3.** Neurogenesis after ischemic brain injury. In the normal healthy condition, the newly generated neuroblasts in the subventricular zone of the lateral ventricle migrate to the olfactory bulb. After ischemic brain injury, neural stem cell (NSC) proliferation is promoted by growth factors released from astrocytes, microglia, macrophages and endothelial cells. The neuroblasts generated after stroke migrate to the injured brain region.

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