



Published in final edited form as:

*Behav Brain Res.* 2019 December 30; 376: 112209. doi:10.1016/j.bbr.2019.112209.

## Functions of subventricular zone neural precursor cells in stroke recovery

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### Abstract

The proliferation and ectopic migration of neural precursor cells (NPCs) in response to ischemic brain injury was first reported two decades ago. Since then, studies of brain injury-induced subventricular zone cytotgenesis, primarily in rodent models, have provided insight into the cellular and molecular determinants of this phenomenon and its modulation by various factors. However, despite considerable correlational evidence—and some direct evidence—to support contributions of NPCs to behavioral recovery after stroke, the causal mechanisms have not been identified. Here we discuss the subventricular zone cytotgenic response and its possible roles in brain injury and disease, focusing on rodent models of stroke. Emerging evidence suggests that NPCs can modulate harmful responses and enhance reparative responses to neurologic diseases. We speculatively identify four broad functions of NPCs in the context of stroke: cell replacement, cytoprotection, remodeling of residual tissue, and immunomodulation. Thus, NPCs may have pleiotropic functions in supporting behavioral recovery after stroke.

### Keywords

Cytogenesis; neural precursor cell; neurogenesis; recovery; stem cell; stroke

## 1. Introduction

The discovery of cell genesis in the adult mammalian brain displaced the belief that lost brain cells—due to stroke, for example—were irreplaceable. Indeed, stroke and other brain injuries instigate a cytotgenic response characterized by increased cell proliferation and altered migration of new cells. While it is now appreciated that the subventricular zone (SVZ) cytotgenic response to stroke is massively insufficient for complete repair, that new

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cells are formed and travel long distances to remodeling brain regions after injury is consistent with SVZ cytogenesis being an important repair process. Here we discuss this process, including whether newborn cells might meaningfully contribute to behavioral recovery after stroke and other brain injuries, and, if so, how. We consider evidence primarily from rodent stroke models examining the SVZ but also include some discussion on studies of other nervous system injury models, neural precursor cell transplantation, and injury-induced cytogenesis in the subgranular zone. In addition, we discuss several factors that modify the SVZ cytogenic response to stroke. While there has been a strong research focus on post-stroke neurogenesis, we suggest that neurogenesis is only one aspect of a diverse SVZ cytogenic response. Based on available evidence, we propose that multiple cell types arising from the SVZ contribute to stroke recovery through several mechanisms.

## 2. Subventricular zone cytogenesis in the adult brain

The formation of new brain cells in adulthood is a process conserved among mammals [1-5]. Neuro- and gliogenesis in the adult brain are largely restricted to the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) within the hippocampal dentate gyrus. There is also evidence of comparably sparse cell genesis in other regions of the intact and injured central nervous system [6-8]. We focus on the SVZ niche and refer to a broad population of cells, neural precursor cells (NPCs), which encompasses cellular subpopulations along a spectrum of differentiation – from stem cells to lineage-defined progenitors. Because dividing NPCs give rise to neurons and glia, we use the term “cytogenesis” (rather than “neurogenesis”) to refer to the formation of new cells from NPCs in the adult brain. Figure 1 illustrates SVZ cytogenesis in the adult brain and the cellular subpopulations involved.

Neural stem cells (type B cells in the SVZ) are radial glia-like, GFAP<sup>+</sup> (glial fibrillary acidic protein), slowly dividing, multipotent cells [9,10]. They can divide symmetrically, forming two identical daughter cells, or asymmetrically, forming one stem cell and one differentiating cell. Symmetric division, the likely dominant type [11], includes self-renewal (two stem cell daughters) and differentiative (two non-stem cell daughters) division. Transit amplifying cells (type C cells) are rapidly dividing intermediates that give rise to immature glia and neurons (type A cells). In addition to these three subpopulations, recent evidence suggests that a scarce subpopulation of octamer-binding transcription factor 4<sup>+</sup> (Oct4<sup>+</sup>) cells, termed primitive neural stem cells, exists in the adult SVZ [12,13]. The function of these cells in the intact brain is unclear, but they appear to serve as a reserve pool for replacing or expanding the GFAP<sup>+</sup> type B cell population upon its depletion or after injury. Finally, while some have reported that SVZ ependymal cells may be a source of new cells after injury [14], there are reports to the contrary [15-17].

NPCs have a unique relationship with another cell population: blood vessels. In contrast to most of the brain, cytogenic niches have conspicuously bare vessels that are permissive to passage of small molecules [18]. Close association between NPCs and particularly permeable vessels positions nascent cells to integrate blood borne signals that regulate their behavior [19], and may allow NPCs to quickly identify and respond to distant or systemic stimuli, including noxious stimuli such as injury [20]. Close apposition to vasculature also

gives NPCs privileged access to endothelial cell-derived soluble factors [21]. Outside of cytogenic regions, neuroblasts rely on blood vessels as a scaffold during migration [22]. As we discuss later, the intimate relationship between NPCs and vessels endures and transforms in the injured brain.

### 3. The NPC cytogenic response to stroke

In the intact brain, newborn cells migrate from the SVZ to the olfactory bulb via the rostral migratory stream. Injury and disease modify this process by increasing NPC proliferation and inducing ectopic migration (Figure 1A and 2). In this section we discuss the NPC response to stroke and its behavioral significance in the context of rodent models of ischemic stroke.

#### 3.1 Stroke-induced SVZ NPC proliferation and migration

Stroke induces robust gene expression changes across cell types in surrounding regions beginning within hours [23,24]. Gene expression changes within SVZ cells recapitulate developmental programs that drive proliferation of neural stem cells and transit amplifying cells and migration of progenitors [25]. Within days after injury, cell proliferation is significantly increased within the SVZ across stroke models [26-32]. Stroke-induced NPC proliferation and migration has been observed in non-human primates [33] and humans [34-36] (cf. [37]). In rodent models, SVZ proliferation tends to peak within the first 2 weeks post-infarct [27]. Stroke-induced NPC proliferation is facilitated, at least in part, by enhanced vascular permeability within the SVZ (beyond the already unusually high permeability of SVZ vessels [18]), which allows endothelial cells and NPCs to respond to systemic signalling altered by stroke [20]. Indeed, stroke induces SVZ vessels to increase proliferation of closely associated stem cells via notch signalling [20]. The stroke-increased notch ligand expression in SVZ vessels is transient and coincides with the peak in NPC proliferation.

GFAP<sup>+</sup> neural stem cells are the primary upstream source of new neurons in the SVZ of the intact and injured brain [9,38]. The stroke-initiated SVZ cytogenic response includes multiple lineages. After stroke, SVZ NPCs produce immature neurons (DCX<sup>+</sup>) and, predominantly, glia (GFAP<sup>+</sup>, NG2<sup>+</sup>, or OLIG2<sup>+</sup>) [30,39] (Figure 1). Multipotent NPCs also migrate to peri-infarct areas where they can proliferate and give rise to new astrocyte-lineage cells [40].

SVZ-derived NPCs migrate long distances rather quickly. By 7 days after distant cortical stroke in mice, thousands of neuroblasts are seen in peri-infarct cortex 2-4 mm from the SVZ [38]. We [41] and others [38] have observed NPCs reaching peri-infarct cortex within 3 days post-infarct in mice. The process of NPC migration is coupled to remodeling vasculature. Blood vessels act as a scaffold to facilitate NPC migration towards the lesion [42]. Within the peri-infarct region, remodeling vasculature expresses molecular cues (e.g., SFD-1, Ang1, EPO) necessary for directed NPC migration [38,43-45]. Once they reach peri-infarct vessels, newborn cells closely associate with them in a manner reminiscent of the SVZ vascular niche where vessels are uniquely permissive to passage of small molecules [18]. Close association with vasculature positions NPCs to respond to both circulating and

local signals in the ectopic peri-infarct niche. Interestingly, migration of new cells towards cortical infarcts continues for at least many months, suggesting that injuries persistently alter the migratory goal of NPCs [27,46].

Studies on the survival of new neurons after experimental stroke suggest a bleak fate for newborn cells. Based on the number of immature neurons produced soon after stroke and thymidine analogue co-labeling at later survival times, it is estimated that <20% of newborn neurons survive to become mature neurons [28,29,38]. If the survival and integration of new neurons in peri-infarct regions supports post-stroke recovery, the failure for significant numbers of new neurons to survive could limit recovery of function in stroke survivors (for review, see [47]). Importantly, to our knowledge, no studies have assessed survival of new SVZ-derived glia in peri-infarct regions. Future studies of SVZ-derived cell survival could make use of indelible, inherited labeling systems [30] to track and manipulate both glial and neuronal lineages in peri-infarct tissue.

### 3.2 Behavioral significance of the SVZ cytogenic response to stroke

There is abundant correlational evidence to support a beneficial role for NPCs in stroke recovery. Generally, treatments or manipulations that enhance NPC proliferation or survival are associated with improved behavioral outcome, while manipulations with the opposite effects are typically associated with worsened outcome, as previously reviewed [48]. Here we focus on studies that have directly evaluated the contributions of NPCs to behavioral recovery in rodent models of cerebral ischemia (summarized in Table 1).

Several studies have directly examined contributions of SVZ NPCs to motor recovery in mouse models. In a series of studies, Jin and colleagues [49-51] found that ablation of neuroblasts in DCX-TK (doublecortin-thymidine kinase) mice prior to permanent or distal middle cerebral artery occlusion (MCAo) resulted in significantly larger lesion size and worse motor outcome on several tasks. However, Sun and colleagues [26] found no aggravation of lesion size or worsening of locomotor function in mice lacking NPCs (ablated with the Nestin-TK system) after distal MCAo. No studies have examined behavioral consequences of NPC ablation in focal stroke models that are most commonly used to model chronic post-stroke motor impairments, such as endothelin-1 or photothrombosis [52]. However, a recent study found that tetanus expression in new SVZ-derived neurons impaired motor recovery on the grid walking and pasta handling tasks following motor cortical photothrombotic lesions in mice [53]. Overall, stronger direct evidence that SVZ NPCs support motor behavioral recovery in stroke models is needed.

Together, these studies suggest that SVZ NPCs might support favorable outcomes from stroke; however, they have often used relatively insensitive tasks for probing motor function and have often not been able to discriminate between neuroprotective and other functions. In Section 5, we discuss possible causal mechanisms of SVZ NPC-mediated improvement of outcome after stroke.

### 3.3 Functional contributions of the SGZ cytogenic response to stroke

In addition to the SVZ, stroke potently stimulates neurogenesis within the SGZ. Studies on the functional impact of stroke-augmented SGZ cytotogenesis have shown conflicting results.

Two studies have assessed roles of SGZ NPCs in ischemia-induced cognitive impairment with NPC ablation methods. Raber and colleagues [54] subjected gerbils to x-ray irradiation to diminish SGZ proliferation two weeks prior to global ischemia induced by transient occlusion of both carotid arteries. This ischemia model most significantly damages vulnerable cell populations—including hippocampal CA1 pyramidal neurons—and produces memory deficits. Irradiation treatment exacerbated ischemia-induced spatial memory deficits as assayed with the Morris water maze [54]. Sun and colleagues [26] used Nestin-TK mice to ablate NPCs prior to distal MCAo. Mice lacking NPCs had impaired spatial learning and memory when tested on the Barnes maze beginning 8 weeks post-stroke [26]. Lastly, voluntary running was found to specifically increase SGZ neurogenesis and ameliorate cognitive deficits after transient MCAo in mice [55]. The number of new surviving dentate cells correlated with performance on the Morris water maze. These studies suggest that SGZ cytogenesis supports some aspects of cognitive recovery in models of stroke.

In contrast, some studies have reported morphological [56-58] and electrophysiological [59] abnormalities in new SGZ-derived neurons after stroke. These studies may suggest that stroke adversely impacts the integration of new neurons, at least within the hippocampus. Cuartero and colleagues [58] recently showed that post-MCAo increases in SGZ neurogenesis correlated with impairments in context and spatial memory. Further augmenting post-stroke SGZ neurogenesis with memantine or running exacerbated these deficits, whereas pharmacological or genetic inhibition of neurogenesis ameliorated them [58]. These studies provide a cautionary note for neuron replacement strategies: integration of new neurons into existing circuits can go awry.

In summary, the NPC cytogenic response is often—but not exclusively—associated with behavioral benefits. In the next section, we describe several modifiers of stroke-induced cytogenesis.

#### 4. Modifiers of stroke-induced SVZ cytogenesis

The unique microenvironment of the SVZ and the stroke-created niche in peri-infarct regions allows NPCs access to an assortment of regulatory signals that can modulate their behavior. In this section we discuss several factors that modify the SVZ cytogenic response to injury and the implications for stroke recovery and treatment. We direct readers to other works for reviews of molecular regulation of NPC fate and proliferation [19,21,60].

##### 4.1 Age

Cytogenesis declines with age. Histological analysis of the mouse SVZ throughout aging reveals a progressive thinning of the structure, reduction in proliferation, and accumulation of astrocytes [61]. While the number of neural stem cells is stable with age (cf. [62]), beginning in middle age [63], neural stem cells adopt a quiescent state associated with changes in gene expression [64] and morphology [63], and a smaller progenitor pool. NPCs integrate many signals that control their behavior, including systemic, local, and intrinsic signals [65]. Remarkably, transplanting “old” neural stem cells into a young niche, or into an environment resembling a young niche, restores proliferation kinetics comparable to young stem cells in a young milieu [66,67]. Conversely, placing young stem cells in an “old”

environment precipitates quiescence [66]. The role of systemic factors in regulating NPC behavior with aging is also elegantly demonstrated in the heterochronic parabiont model system in which the vascular systems of young and old mice are joined, thereby causing the exchange of circulating factors. Age-related diminishment of NPCs in the SVZ [68] and SGZ [69] is rescued in the old parabiont. Similarly, age-related vascular rarefaction can be reversed [68]. These effects can be mimicked by injection of young plasma [69,70], or even a single “youthful” factor, GFD11 [68]. Correspondingly, exposure to an “aging” factor, CCL11, reduces neurogenesis in young mice [69]. These data implicate circulating and environmental factors, rather than cell-intrinsic signals, as the dominant signal encouraging age-related neural stem cell quiescence.

Cytogenesis is influenced by the interaction of age and injury. Although cell proliferation is reduced in the aged brain, its enhancement by ischemia is maintained [71]. Adamczak and colleagues [72] used longitudinal *in vivo* bioluminescence imaging to track neurogenesis in DCX-luciferase mice. They observed a ~50% decline in SVZ neurogenesis from 2 to 12 months of age. Following transient MCAo, neurogenesis increased ipsilaterally to lesions regardless of age, but the magnitude of the neurogenic response in 12 month-old mice was lower than in 3 month-old mice. Age-related blunting of the cytogenic response to ischemia has also been reported in the rat SVZ [71] and SGZ [73]. The Wnt signalling pathway is one regulator of cytogenesis that varies with age. Stroke increases local Wnt signalling that is paralleled by SVZ stem cell activation [66]. However, in the aged SVZ, Wnt signalling is reduced and not amplified by stroke, consistent with the relatively diminished cytogenic response. Therefore, both local and systemic signals likely play a role in age- and injury-dependent regulation of cytogenesis.

Aging has a marked dampening effect on cytogenesis. Beyond reducing the absolute number of NPCs, does aging affect the ability of NPCs to beneficially support function in the context of disease and injury? For example, might NPCs in the old brain secrete a different amount or assortment of intercellular signaling factors [74]? Given that stroke disproportionately affects older adults, understanding effects of aging on NPCs may have major clinical relevance.

## 4.2 Experience

The enhancement of SGZ neurogenesis by environmental enrichment and physical activity is well described [75,76]. In contrast, experience effects on SVZ cytogenesis in the intact and stroke brain are less clear. In one study, enriched environment housing of mice subjected to transient MCAo attenuated stroke-induced proliferation in the SVZ, as measured by BrdU (bromodeoxyuridine, administered daily during the first week post-infarct) and DCX immunolabeling at 4 weeks [77]. Moreover, there were half as many immature neurons and glia migrating towards the infarct in enriched mice [77]. In contrast, two studies showed that rats in enriched housing after cortical infarcts had increased proliferation in the SVZ 1 and 5 weeks post-stroke, as measured by DCX, BrdU (administered daily during the first week post-infarct), and Ki67 immunolabeling [78,79]. This increased proliferation was paralleled by improved motor function. These studies indicate that post-stroke environmental enrichment can have varying effects on SVZ cytogenesis. Several differences between

studies could explain their conflicting results. The study finding reduced SVZ proliferation subjected mice to rather short (3 hours per day) bouts of enriched housing with 3-4 mice per cage in a stroke model that caused cortical and striatal damage [77]. The studies finding increased SVZ proliferation continuously housed 9-10 rats in enriched housing after infarcts restricted to cortex [78,79]. Therefore, the parameters of enrichment, species, and location of damage may influence the effects of environmental enrichment on post-stroke SVZ cytogenesis.

Several studies have assessed the effects of use-dependent behavioral manipulations on cytogenesis. Four studies found that constraint-induced forced use of the stroke-impaired limb ameliorated functional deficits and increased SVZ neurogenesis in various rat models [80-83]. In two studies, this was coupled with increased angiogenesis [81,83]. Recently, Liang and colleagues [53] showed that overuse of the impaired forelimb induced by Botox injections in the less-impaired limb after photothrombosis in the forelimb area of motor cortex increased the number and survival of SVZ-derived cells in peri-infarct cortex, promoted their neuronal differentiation, and augmented synaptogenesis. Importantly, chemogenetically increasing activity in peri-infarct forelimb motor cortex (mimicking forelimb overuse) also increased the density of SVZ-derived cells, whereas chemogenetic inhibition of the same region had an opposite effect [53]. Interestingly, Liang and colleagues [53] also found that this activity-dependent modulation of post-stroke cytogenesis was region-specific: while impaired forelimb overuse increased the density of DCX<sup>+</sup> cells in peri-infarct cortex, hindlimb overuse decreased peri-infarct DCX<sup>+</sup> cell density. These studies demonstrate the sensitivity of post-stroke SVZ cytogenesis to activity-dependent modulation.

Voluntary wheel running induces changes in protein expression in peri-infarct cortex, including upregulation of proteins associated with cytogenesis [84]. Surprisingly, Komitova and colleagues [79] found that voluntary running in the first week post-infarct attenuated injury-induced SVZ stem cell and neuroblast proliferation in a rat cortical infarct model. Behavioral outcome was not affected. Interestingly, there was no effect of running on the total number of proliferating cells (Ki67<sup>+</sup> or BrdU<sup>+</sup>) within the SVZ, perhaps indicating a shift towards gliogenesis. Another study found that treadmill running beginning two days after MCAo in rats increased the number of BrdU<sup>+</sup> cells in peri-infarct regions relative to non-runners [85]. Together, these studies show that stroke and behavioral experience interact to modify SVZ cytogenesis.

### 4.3 Inflammation

Stroke triggers a significant inflammatory response involving innate and adaptive immunity [86,87]. Activated microglia expressing pro-cytogenic factors (e.g., IGF-1) accumulate in the SVZ after stroke, implying that they facilitate the NPC response [88]. Notably, microglia in the SVZ are morphologically and molecularly distinct from those in peri-infarct striatum [88]. This observation might reflect functionally separate subpopulations of microglia after stroke whereby a predominantly pro-cytogenic subpopulation is spatially and temporally restricted in the SVZ. To examine the role of inflammation in the cytogenic response, Chapman and colleagues [89] showed that striatal LPS injection induced an inflammatory

response comparable to MCAo, but without overt cell death. The authors identified a distinct transcriptional program shared by microglia activated by LPS and ischemia. Critically, intrastriatal LPS caused a neurogenic response resembling that following focal ischemia, despite inflammation being the only noxious cue. These findings position acute inflammation as a contributing stimulus for the cytogenic response to stroke.

The poor survival of newborn cells after stroke has often been attributed to the harsh inflammatory environment within peri-infarct regions [28,43]. The inflammatory response to stroke is critical to limiting the spread of cytotoxic debris within the infarct [90]. However, properly timed anti-inflammatory treatment could be an effective strategy for limiting death of new cells. In support of this idea, post-infarct anti-inflammatory treatments promote the survival of new neurons in peri-infarct cortex [91] and the SGZ [92], and post-stroke NPC proliferation and survival are enhanced in immunodeficient mice [93].

Inflammation can positively and negatively regulate cytogenesis. A more refined understanding of the role of the inflammatory response in directing NPC behavior might uncover novel treatment approaches. We will discuss in the next section that the relationship between inflammation and SVZ cytogenesis is likely bidirectional; NPCs may have immunomodulatory functions.

The factors we have described, as well as others, that modify SVZ cytogenesis most certainly interact. For example, aging is associated with chronic, mild inflammation and a concomitant blunting of the acute inflammatory response [94]. Such interactions must be considered in studies examining modifiers of SVZ cytogenesis and during development of new therapies involving cytogenesis. Relative contributions of various cytogenesis-altering factors will be difficult to disentangle. Moreover, most studies focus on modification of neurogenesis, and some factors might differentially modify gliogenesis or bias cell fate decisions. Studies on modifiers of SVZ cytogenesis could benefit from a view that is less neuron-centric.

## 5. Possible functional contributions of neural precursor cells after stroke

A mechanistic understanding of the roles of NPCs in neurologic injury and disease is crucial to the development of novel treatment strategies aimed at exploiting the endogenous cytogenic response. In this section we discuss evidence from several models and manipulations, including NPC ablation and transplantation, which provide insight into potential functions of NPCs after stroke. While the pathobiology of stroke shares many commonalities with other forms of injury and disease, there are unique aspects for each condition that should be considered. Furthermore, because NPCs are modified by their environment and other factors, there are likely differences in the functions of transplanted versus endogenous NPCs. Nonetheless, NPCs appear to possess pleiotropic functions that render them beneficial across diverse disease states (Figure 3).

### 5.1 Cell replacement

Perhaps the most obvious potential role of NPCs after stroke is cell replacement. As we discussed, the number of new cells produced in response to experimental stroke is



considerably lower than the number killed, and relatively few new neurons appear to survive long-term [28,29]. However, a relatively small population of new cells could still have powerful effects. Although stroke kills neurons and glia, and both populations are produced from the SVZ, most studies of endogenous cell replacement after experimental ischemic injury have focused on new neurons. Young hippocampal neurons are unique in that they are highly excitable and plastic [95]. New neurons in peri-infarct cortex of mice are similarly excitable [39]. Of particular interest in the abnormal context of peri-infarct regions, where excessive GABA signalling is rampant [96], is that young neurons (in both the SVZ and SGZ) are initially excited by GABA, and GABA-induced depolarization promotes differentiation and survival of newborn neurons in the dentate gyrus and olfactory bulb [19,95]. Whether GABA similarly affects differentiation and survival of new neurons in peri-infarct regions is unclear, but new neurons in peri-infarct cortex receive largely GABAergic input 4 weeks after cortical infarcts in mice [39]. Intriguingly, there is some evidence that new hippocampal neurons are preferentially recruited into behaviorally-relevant circuits relative to older neurons [19,97]. After sensorimotor cortical infarcts in mice, conferring similar properties (preferential recruitment into circuits) to a small pool of residual neurons in peri-infarct cortex by virus-mediated CREB overexpression improves functional remapping and behavioral recovery [98]. Therefore, newborn neurons could disproportionately influence plasticity of the residual neural circuits that contribute to behavioral recovery [99]. However, the extent to which new peri-infarct neurons share the unique properties of new neurons in the intact brain is uncertain.

Some neuroblasts become mature neurons in the vicinity of the infarct [28,29,31,38] and can display ultrastructural features of synapses [100]. One study found that SVZ-derived neurons in peri-infarct striatum exhibited post-synaptic currents and action potentials 8 weeks after MCAo [101]. Similarly, Hou and colleagues [102] showed that SVZ-derived neuroblasts in peri-infarct regions developed increasingly complex morphology over time after MCAo. At 8 weeks post-infarct, new neurons in peri-infarct striatum were largely cholinergic or GABAergic, fired action potentials, and received mixed synaptic inputs. These studies indicate that new neurons can functionally integrate into peri-infarct circuits. A recent study by Kannangara and colleagues [39] found that new neurons in peri-infarct cortex 4 weeks after photothrombotic cortical infarcts were predominantly GABAergic and fired action potentials, but were quite sparsely innervated relative to typical cortical GABAergic neurons. This finding suggests somewhat poor integration of new neurons into residual circuits, at least at the 4-week time point. Furthermore, this study raises the question of whether improving integration of new neurons would enhance behavioral recovery. A recent study by Liang and colleagues [53] showed that new SVZ-derived neurons express synaptic markers in peri-infarct cortex after photothrombosis. In addition, they found that overuse of the impaired forelimb greatly increased synaptogenesis and connectivity in new neurons measured two months post-stroke [53]. Importantly, blocking neurotransmitter release specifically from new neurons with tetanus toxin impaired long-term motor function [53].

Time is an important factor to consider in the potential contributions of cell replacement to behavioral recovery. Neuroblasts migrate from the SVZ towards the infarct, which can take over one week (and likely longer in humans) [38]. Then, they develop progressively more



## 5.2 Cytoprotection

Stroke creates a region of irreversible cell death—the ischemic core—surrounded by a region of ischemic tissue at risk, the penumbra. Significant effort has been directed at reducing the extent of the ischemic core and salvaging the penumbra. Considerable evidence that NPCs have cytoprotective functions comes from NPC transplantation studies. For example, Bacigallupi and colleagues [109] administered neural stem cells (from cultures of isolated adult mouse SVZ NPCs) intravenously 72 hours after transient MCAo in young adult mice. This treatment improved behavioral function, decreased peri-infarct apoptosis, and modestly reduced lesion size measured one month later. At the transcriptome level, stem cell transplantation regulated gene expression in a manner indicative of reduced inflammation and astrogliosis. These transcriptomic changes were corroborated by reduced numbers of activated microglia and infiltrated leukocytes in peri-infarct striatum. Given the changes in the brain microenvironment with age that we discussed earlier, a clinically important question is whether such a treatment would translate to the aged brain. Another study examined effects of intraparenchymal neural stem cell (from cultures of neural stem cells isolated from the telencephalon of embryonic mice) administration in young and aged rats after transient MCAo [114]. The results were auspicious: while there was an age-related aggravation of injury, stem cell transplantation was cytoprotective regardless of age. These studies suggest that, at least in some cases, transplanted NPCs exert protective effects; however, exogenously administered cells have experienced a vastly different environment than NPCs in vivo and therefore likely behave quite differently. The functions of transplanted NPCs may differ from the endogenous NPC response.

Evidence supporting cytoprotection by endogenous NPCs comes from NPC ablation experiments. In a series of studies, Jin and colleagues [49-51] used DCX-TK mice and large vessel occlusion stroke models to study contributions of neuroblasts to stroke outcome. They consistently found that neuroblast ablation prior to ischemia exacerbated infarct severity. However, it should be noted that no cytoprotective effect was found in a study that ablated NPCs in Nestin-TK mice (which ablates considerably more NPCs than the DCX-TK system [26,49,51,115]) prior to distal MCAo [26]. The basis for these inconsistent findings is unclear. The studies by Jin and colleagues [49-51] identify protective effects of neuroblasts, but they do not identify mechanisms underlying cytoprotection. A study by Butti and colleagues [116] showed that excitotoxic damage from 4-aminopyridine-induced seizures or transient MCAo was worsened in animals lacking NPCs (ablated by ganciclovir administration to nestin-TK mice or with the antimetabolic agent ara-C). Importantly, the authors found that NPCs protect nearby striatal neurons through secretion of the endocannabinoid arachidonoyl ethanolamide, which regulates glutamatergic tone: production of arachidonoyl ethanolamide was increased in response to 4-aminopyridine, but this effect was abolished in mice lacking NPCs [116]. Pharmacologically restoring cannabinoid levels reversed the exacerbation of excitotoxic damage in mice lacking NPCs [116]. It is unclear whether this protective mechanism is generalized to more distant insults (e.g. cortical infarcts). Nonetheless, factors secreted by NPCs may have potent protective effects. As noted earlier, administration of NPC-derived extracellular vesicles reduces infarct size in murine and porcine stroke models [111,112]. Identification and administration of the specific factors mediating these effects is a promising treatment avenue.

### 5.3 Repair and remodeling of residual tissue

Functional improvements in animal stroke models have been linked with remodeling of peri-infarct tissue [117]. The remodeling process involves coordinated reorganization among many cell types, including neurons, blood vessels, and astrocytes [99,103]. NPCs migrate to areas of active remodeling after stroke, which positions them well to influence remodeling processes. NPCs could modify repair processes through cell-cell contact or through release of soluble or vesicle-contained factors (e.g. growth factors, miRNAs, cytokines) [43,118-120]. Hypoxia induces changes in the extracellular vesicle content of cultured neural stem cells [121], which suggests that the NPC secretome is adaptively varied. Characterization of factors transmitted by NPCs and how they vary in the context of neurologic disease and injury would provide substantial insight into NPC functions.

NPCs secrete neurotrophic factors that could facilitate remodelling of peri-infarct tissue [122,123]. However, the types and amounts of factors produced by NPCs varies greatly with the source and experiences of cells (e.g. immortalized cell lines vs. isolated NPCs vs. NPCs *in vivo* [124,125]). Nonetheless, neural stem cell transplantation studies support significant roles of transplanted cell-derived factors such as vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) in ameliorating injury and disease [110,126]. Andres and colleagues [110] transplanted human neural stem cells into peri-infarct cortex one week after distal MCAo in rats. NPC transplantation improved behavioral outcome without affecting lesion size, suggesting that behavioral benefits were not attributable to cytoprotection. Indeed, NPC transplantation bilaterally increased dendritic complexity and promoted axonal sprouting throughout the motor system. In addition, infarct-caused deficits in axonal transport were rescued by transplanted NPCs. *In vitro* experiments revealed several factors implicated in these effects on neural remodeling, including VEGF and thrombospondins. The majority of transplanted NPCs remained undifferentiated. This study provides strong support for the idea that NPC-secreted factors can facilitate reparative processes after stroke. Additionally, neural stem cell transplantation has been associated with increased angiogenesis in peri-infarct cortex, along with augmented expression of VEGF in resident astrocytes and vasculature [114]. Cell transplantation in models of Alzheimer's [127] and Huntington's [128] diseases have shown a functional role for NPC-derived BDNF. Whether the endogenous cytogetic response has comparable capabilities remains to be determined. Notably, in the intact SGZ, but not SVZ, NPCs secrete large amounts of VEGF [74].

These studies provide evidence that plastic responses to disease and injury might be beneficially supported by NPCs and identify mechanisms through which NPC might contribute to remodeling processes after stroke. However, to our knowledge, the contributions of endogenous cytogenesis to remodeling processes after stroke have not yet been addressed experimentally.

### 5.4 Immunomodulation

Inflammation after stroke is both beneficial and detrimental. As described earlier, inflammation regulates cytogenesis. However, the relationship is bidirectional [129]. In the SGZ, NPCs secrete several factors that regulate microglial behavior, including their

proliferation, proclivity for phagocytosis, and secretion of cytokines and chemokines [130]. Substantial evidence, primarily from NPC transplantation studies (reviewed by [131,132]), supports immunomodulatory roles of NPCs in the context of acute and/or chronic inflammation. For example, intrastriatal transplantation of human NPCs reduced peri-infarct microglial accumulation at 6 and 14 weeks post transient MCAo in rats [133]. Bacigaluppi and colleagues [109] showed that intravenous delivery of adult mouse SVZ-derived NPCs three days after transient MCAo in mice reduced numbers of microglia and infiltrated leukocytes in peri-infarct regions. In a mouse model of spinal cord injury, Cusimano and colleagues [119] showed that NPC transplantation improved locomotor recovery and decreased reactive astrogliosis and the number of microglia at the site of injury. Importantly, the treatment markedly affected expression of genes implicated in inflammation and reduced the proportion of M1-like (generally pro-inflammatory) macrophages. Transplanted cells accumulated at the lesion border, largely undifferentiated, in close association with resident and infiltrated immune cells, commonly forming intercellular junctions. The latter observation might suggest direct cell-cell modulation. Another study by the same group revealed similar immunomodulatory mechanisms by endogenous spinal cord NPCs [134]. Mice with NPCs ablated prior to spinal cord injury had more severe lesions, worse locomotor recovery, decreased expression of trophic factors, and increased M1-like myeloid cells. The difference in lesion severity could be a contributing factor to the latter observations. Nonetheless, these studies suggest potent immunomodulatory effects of NPCs in acute and chronic inflammatory settings.

In summary, transplanted NPCs have an immunomodulatory role in stroke and nervous system injury models—for example, by suppressing T-cell infiltration and promoting M2-like (generally protective) microglia/macrophage phenotypes over M1-like phenotypes [132]. These effects limit damage and may support reparative processes. Notably, secreted factors likely mediate these immunomodulatory effects because neural stem cell extracellular vesicles alone have a modulating effect on inflammation in a rodent stroke model [111]. At least in the spinal cord, endogenous NPCs regulate the inflammatory response to injury, but there is no direct evidence of the same effect of SVZ NPCs after stroke. Future work should directly examine whether endogenous NPCs have immunomodulatory functions in stroke models.

An important caveat of the studies we have discussed is that much of the data supporting various functions of NPCs arises from NPC transplantation studies. This gain-of-function strategy for probing NPC roles is imperfect because transplanted cells come from many sources, each a drastically different environment from the normal SVZ niche. Given, among other factors, the predilection of NPCs to adjust their behavior based on environmental cues, transplanted cells likely behave quite differently from endogenous NPCs. Additionally, these studies encompass diverse contexts (disease models), many of which are significantly different from human stroke. However, these studies provide an indispensable perspective on the flexibility of NPC functions. Moreover, data on endogenous NPC functions, although relatively scarce, seem to corroborate the pleiotropic effects of NPCs revealed in transplant studies.

## 6. Concluding remarks

Stroke prompts profound changes in the behavior of SVZ NPCs. The proliferation and migration of NPCs towards the insult makes it tempting to view NPC behavior as regenerative. However, only a meagre proportion of new cells appear to survive in the inhospitable milieu surrounding ischemic lesions. While the process of SVZ cytogenesis is demonstrably amenable to modification—for example by age, experience, and inflammation—functions of new cells have remained largely a mystery. In this review we have summarized data supporting several potential roles of NPCs.

We speculatively identified four functions of NPCs after stroke and possibly in other disease contexts: cell replacement, cytoprotection, remodeling of residual tissue, and immunomodulation (Figure 3). Although there is evidence that endogenous NPCs replace some neurons, the functional significance of this replacement may be minor because 1) few new neurons survive, 2) new neurons seemingly mature and integrate in peri-infarct regions over a time course delayed relative to behavioral recovery, and 3) some evidence suggests that integration of new neurons is poor [39]. However, the integration process could meaningfully influence plasticity of surviving neurons. Replacement of glia has been less studied. There is direct evidence that NPCs are cytoprotective in the context of excitotoxicity [116], including after infarcts encroaching upon the SVZ [49-51], but whether this protective mechanism functions over long distances (i.e. in a human brain) is unclear. Stem and progenitor cell transplantation studies support roles of NPCs in remodeling the injured brain and modulating the immune response, but evidence for these same functions in SVZ NPCs is needed.

Many questions remain surrounding the cytogenic response to injury. The rerouting of NPCs towards a lesion is long-lasting [27,46]. However, due to the time-variant nature of reparative and deleterious processes after stroke [103], is there a critical window during which NPCs are beneficial? Does this time window depend on factors intrinsic (e.g. location, severity) or extrinsic (e.g. age) to the cytogenic stimulus? Other uncertainties concern the types of cells involved in the cytogenic response. The NPC population that responds to stroke is heterogeneous. While most studies focus on the neurogenic response to stroke, there is a significantly larger glial component [30,39]. Furthermore, undifferentiated cells migrate into peri-infarct tissue [40]. Do the functions of these nascent cells vary depending on their phenotype? If so, could we regulate NPC fate to promote phenotype-specific functions that may be differentially favorable depending on the disease context? Ultimately, many of the beneficial functions of NPCs likely depend on their ability to interact with other cell types, including through secreted factors [108,111-113,122,131]. Therefore, characterizing the secretome of NPCs could yield significant insights into their functions. In particular, we need to understand how the NPC secretome varies with injury and disease, and whether it can be manipulated as a therapeutic avenue. The answers to these questions could have significant clinical implications. The emergence of tools that, for example, allow *in vivo* cell-type specific, temporally controllable manipulation and labelling (e.g. inducible cre/loxP [135]) of NPCs and large scale “omic” profiling will allow the behavioral contributions and underlying molecular mechanisms of NPCs in neurologic disease to be elucidated.

A variety of neurologic diseases and injuries induce NPC proliferation and migration, and this response is typically associated with repair and recovery. The cytogenic response is discernibly modified by many factors—including age, experience, and inflammation—that interact complexly with one another as well as with disease states. Here, we described four potential mechanisms by which NPCs may contribute to repair and recovery from stroke and other diseases: cell replacement, cytoprotection, remodeling of residual tissue, and immunomodulation. Significant experimental effort will be needed to clarify the functions of the cytogenic response, and to therapeutically leverage it, but we believe that the clinical impact will justify these efforts.

## Acknowledgements

This work was supported by Canadian Institutes of Health Research Doctoral Award DFS-157838 to MRW, National Institutes of Health R37 NS056839, R21 NS101564 to TAJ, and R01 MH102595, R01 MH117426 to MRD. We thank two anonymous reviewers for their constructive feedback.

## Abbreviations:

<b>ara-C</b>	cytosine arabinoside
<b>BrdU</b>	bromodeoxyuridine
<b>DCX</b>	doublecortin
<b>GABA</b>	gamma-aminobutyric acid
<b>GFAP</b>	glial fibrillary acidic protein
<b>LPS</b>	lipopolysaccharide
<b>MCAo</b>	middle cerebral artery occlusion
<b>NPCs</b>	neural precursor cells
<b>Oct4</b>	octamer-binding transcription factor 4
<b>SGZ</b>	subgranular zone
<b>SVZ</b>	subventricular zone
<b>TK</b>	thymidine kinase
<b>VEGF</b>	vascular endothelial growth factor

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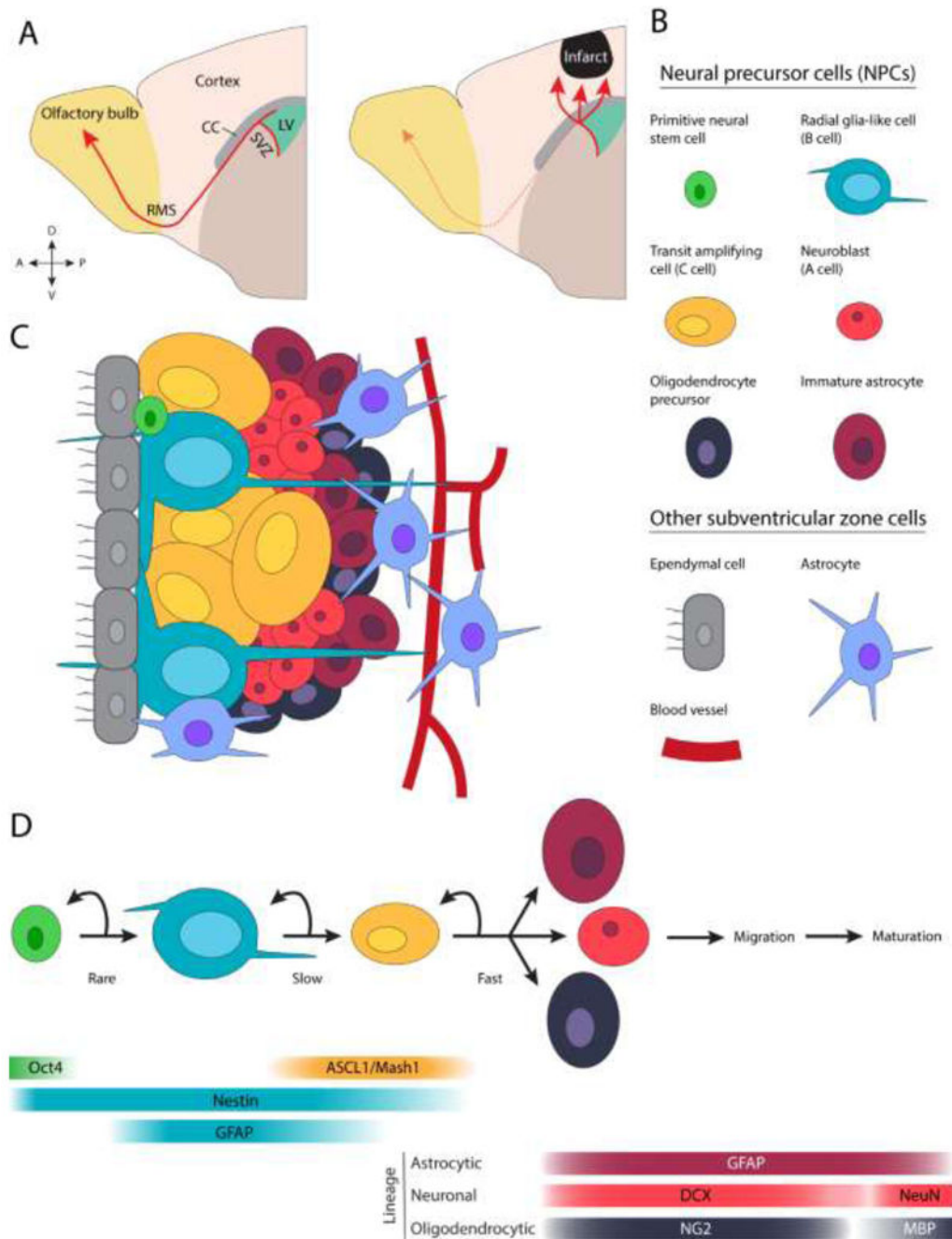
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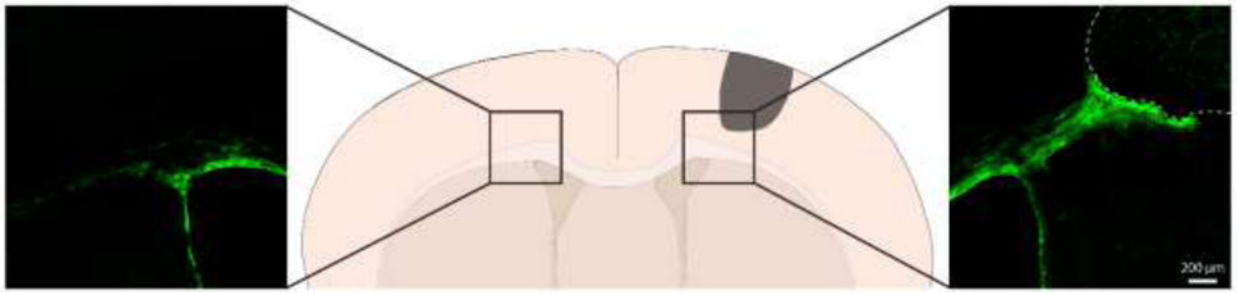
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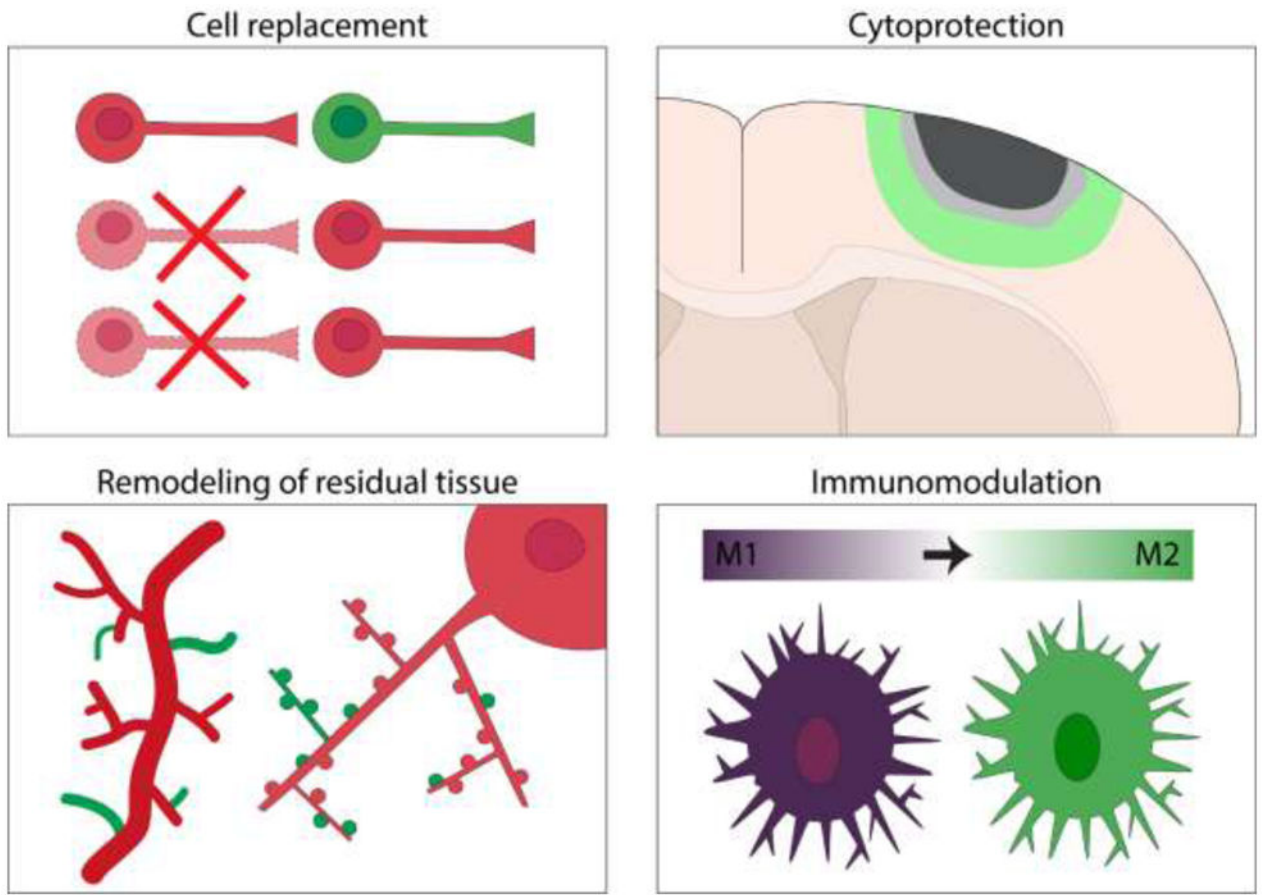
**Figure 1.**

Cytogenesis in the adult subventricular zone. A) Depiction of NPC migration from the SVZ in the intact and injured brain. B and C) Selected cellular subpopulations of the SVZ. D) A lineage model of SVZ cytotogenesis and molecular markers associated with each cellular subpopulation [12,19,30]. Note that other lineage models have been proposed [19,136]. ASCL1: Achaete-scute homolog 1, CC: corpus callosum, DCX: doublecortin, GFAP: glial fibrillary acidic protein, LV: lateral ventricle, MBP: myelin basic protein, NG2: neural/glia antigen 2, Oct4: octamer-binding transcription factor 4, RMS: rostral migratory stream.



**Figure 2.**

Migration of SVZ NPCs towards a unilateral cortical ischemic lesion. Expression of membrane-targeted GFP was induced in NPCs by administration of tamoxifen to an adult Nestin-CreER<sup>T2</sup>:: Ai35D (cre-dependent archaerhodopsin/GFP fusion protein) mouse. Expression of this membrane-targeted fusion protein allows visualization of the full morphology of labeled NPCs. A photothrombotic infarct was induced in motor cortex two weeks later. The mouse was euthanized 8 weeks post-infarct. Note the significantly fewer NPCs in white matter and absence of NPCs in cortical grey matter in the contralesional hemisphere.



**Figure 3.**

Summary of hypothesized beneficial functions of NPCs after stroke. NPCs might replace some cells lost to stroke (cell replacement), protect cells in peri-infarct regions from cell death (cytoprotection), support remodeling of residual tissue (e.g. by enhancing neuroplasticity of remaining neural circuits and angiogenesis of blood vessels), and modulate the immune response to stroke (immunomodulation).

**Table 1**

Chronological summary of studies that assessed behavioral outcome after NPC-specific manipulations in rodent models of stroke.

Model	Species	Method	Region	Tests and outcome	Ref.
<b>bCCAO</b>	Gerbil	Irradiation	SGZ	↓MWM	[54]
<b>pMCAo</b>	Mouse	DCX-TK	SGZ, SVZ	↓Rotarod ↓Limb placing ↓Elevated swing ↓Lesion size	[51]
<b>dMCAo, pMCAo</b>	Mouse	DCX-TK	SGZ, SVZ	↓Beam walking ↓Corner turn ↓Rotarod ↓Limb placing ↓Elevated swing ↓Lesion size	[50]
<b>dMCAo</b>	Mouse	DCX-TK	SGZ, SVZ	↓Beam walking ~Corner turn ~Limb placing ↓Elevated swing ↓Lesion size	[49]
<b>dMCAo</b>	Mouse	Nestin-TK	SGZ, SVZ	~Ladder ~Catwalk ↓Barnes ~Lesion size	[26]
<b>pMCAo</b>	Mouse	Nestin-CreERT <sup>2</sup> ::NSE-DTA	SGZ	↑MWM ↑Context memory	[58]
<b>Cortical PT</b>	Mouse	AAV-hSyn-TeNT	SVZ	↓Grid walking ↓Pasta handling	[53]

↑ = improved, ↓ = worsened, ~ = no change; abbreviations: AAV-hSyn-TeNT: adeno-associated virus – human synapsin – tetanus toxin, bCCAO: bilateral common carotid artery occlusion, dMCAo: distal middle cerebral artery occlusion, MWM: Morris water maze, NSE-DTA: neuron specific enolase – diphtheria toxin A, pMCAo: permanent middle cerebral artery occlusion, PT photothrombosis, tMCAo: transient middle cerebral artery occlusion