HHS Public Access

Author manuscript

Prog Neurobiol. Author manuscript; available in PMC 2020 February 01.

Published in final edited form as:

Prog Neurobiol. 2019 February ; 173: 1–17. doi:10.1016/j.pneurobio.2018.05.004.

Neural stem cell therapies and hypoxic-ischemic brain injury

Lei Huang and **Lubo Zhang**

Lawrence D. Longo, MD Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA

Abstract

Hypoxic-ischemic brain injury is a significant cause of morbidity and mortality in the adult as well as in the neonate. Extensive pre-clinical studies have shown promising therapeutic effects of neural stem cell-based treatments for hypoxic-ischemic brain injury. There are two major strategies of neural stem cell-based therapies: transplanting exogenous neural stem cells and boosting self-repair of endogenous neural stem cells. Neural stem cell transplantation has been proved to improve functional recovery after brain injury through multiple by-stander mechanisms (e.g., neuroprotection, immunomodulation), rather than simple cell-replacement. Endogenous neural stem cells reside in certain neurogenic niches of the brain and response to brain injury. Many molecules (*e.g.*, neurotrophic factors) can stimulate or enhance proliferation and differentiation of endogenous neural stem cells after injury. In this review, we first present an overview of neural stem cells during normal brain development and the effect of hypoxic-ischemic injury on the activation and function of endogenous neural stem cells in the brain. We then summarize and discuss the current knowledge of strategies and mechanisms for neural stem cell-based therapies on brain hypoxic-ischemic injury, including neonatal hypoxic-ischemic brain injury and adult ischemic stroke.

1. Introduction

Neural stem cells (NSCs) are self-renewing and multipotent cells. They hold the potential to differentiate into multiple cell lineages, such as the neuron, astrocyte, and oligodendrocyte. NSCs distribute throughout the developing brain and reside in two major neurogenic niches –subventricular zone (SVZ) and subgranular zone (SGZ) in the adult brain. After brain injury, the endogenous quiescent NSCs become active and participate in the process of brain repair. However, the self-repair process is usually inadequate and transient. Aiming to promote the neurorestorative process, the investigations of neural stem cell therapies on either enhancing endogenous neurogenesis or applying exogenous NSCs have remarkably

Corresponding author: Lei Huang, MD, PhD, Lawrence D. Longo, MD Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, Tel: 909-558-4325, Fax: 909-558-4029, lehuang@llu.edu.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this paper.

surged during the last decade. In this review, we summarize the current knowledge about the neural stem cell therapy on hypoxic-ischemic (HI) brain injury, specifically neonatal HI brain injury and adult ischemic stroke.

2. Brief overview of NSCs in the brain development

The journey of neural development starts from the neuroepithelial (NE) cells, a group of pseudostratified cells lined the cerebral ventricles of the neural tube. NE cells are multipotential NSCs with two end feet, touching both the pial surface and the ventricular surface (Figure 1). Initially, NE cells self-renew and symmetrically divide to increase the number (Haubensak *et al.*, 2004). This period is critical and affects the final thickness of the neocortex (Dehay *et al.*, 2015; Sun and Hevner, 2014). As neurogenesis progresses, NE cells transform into radial glial (RG) cells, starting to express glial markers, such as astrocyte-specific glutamate transporter (GLAST) and glial fibrillary acidic protein (GFAP) (Gotz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009). RG cells retain within the ventricular zone (VZ) and share some similar characteristics with NE cells, like long apical and basal processes. However, unlike NE cells, RG cells divide asymmetrically, producing one daughter RG cell and one intermediate neural progenitor cell (nIPC), which will differentiate into neurons. Some RG cells also become postmitotic neurons directly. Moreover, the process of RG cells like a scaffold guides the newly-born neurons to migrate out of the ventricular zone. Time-lapse imaging evidence has shown neurons move along the radial fiber of RG cells to their final location of the cortical plate (Noctor *et al.*, 2004; Noctor *et al.*, 2008). Another mitotic feature of RG cells, reserved from NE cells, is interkinetic nuclear migration (INM). In the cell cycle, the nuclear changes its position depending on the phase of cell cycle. During the G2 phase, nuclei move towards the apical surface, and on the opposite, they move basal ward in the period of G1 (Taverna and Huttner, 2010). INM has been proven an essential step to avoid overcrowd of RG cells and ensure normal brain histogenesis (Okamoto *et al.*, 2013). The regulation of symmetric and asymmetric division has been extensively reviewed elsewhere (Homem *et al.*, 2015; Jiang and Nardelli, 2016; Lui *et al.*, 2011), and we only briefly discuss it here. The Notch signaling pathway is a critical regulator to determine the proliferative or differentiative state of RG cells. Oscillatory expression of Notch effector gene *Hes1* exhibits in self-renewal RG cells, leading to maintenance of the progenitor identity (Shimojo *et al.*, 2008). Conversely, diminish of this oscillatory expression by upregulating proneural factors cause neuronal differentiation (Imayoshi *et al.*, 2013). Interestingly, intercellular communication between RG cells and differentiated daughter cells also participate in the regulation of cell fate, through the Notch signaling pathway. RG differentiated daughter cells express Notch ligands, e.g., Delta-like 1 (Dll1), which bind to Notch receptors of their neighbor RG cells and activate the Notch signal to maintain the undifferentiated status of RG cells (Dong *et al.*, 2012; Homem *et al.*, 2015; Nelson *et al.*, 2013). Other intrinsic factors include β-catenin (Draganova *et al.*, 2015; Masuda and Ishitani, 2017), Sox2 (Avilion *et al.*, 2003; Hutton and Pevny, 2011), glycogen synthase kinase 3 (GSK-3) (Kim *et al.*, 2009), etc. For example, conditional deletion of GSK-3 significantly increases proliferation of mouse neural progenitors, *via* dysregulation of the Notch/β-catenin signaling pathway.

Epigenetic modifications, including DNA methylation, histone modification, and non-coding RNAs are important mechanisms in the regulation of neural development (Yao *et al.*, 2016). An important class of non-coding regulatory RNAs, micro RNAs (miRs) dynamically express during neurogenesis with various functions. For instance, let-7 family continuously express, whereas miR-124 increases starting from the mid or late phase of development (Miska *et al.*, 2004; Yao *et al.*, 2012). Moreover, substantial evidence from loss- and gain- of function studies indicate that miRs play a critical role in both proliferation and differentiation of NSCs during the development. For example, downregulation of miR-145 decreased the expression of mature neuronal markers, suggesting a crucial role of miR-145 in neuronal differentiation of NSCs. This effect is mediated by the Sox2-Lin28/Let-7 pathway (Morgado *et al.*, 2016). Besides intrinsic regulators, the neurogenesis process is also modulated by many extrinsic factors, such as Colony Stimulating Factor-1 (CSF-1) (Nandi *et al.*, 2012), and fibroblast growth factor (FGF) (Dee *et al.*, 2016). At the end of cortical development, most RG cells translocate towards the cortical plate and transform into astrocytes. Meanwhile, some RG cells differentiate into intermediate progenitor cells that become oligodendrocytes.

In the adult brain, NSCs or neural progenitor cells mainly persist in the SVZ, known as B cells. B cells give rise to transient amplifying C cells that then produce immature neuroblasts (A cells). Under normal condition, neuroblasts migrate toward the olfactory bulb (OB) through the rostral migratory stream (RMS). In the OB, these cells differentiate into mature interneurons (Carleton *et al.*, 2003). Of note, B cells hold many properties of RG cells, like the process to the ventricular surface and expression of astroglial markers, e.g., GFAP and GLAST (Kriegstein and Alvarez-Buylla, 2009). However, different from RG cells, these NSCs in adult SVZ remain largely quiescent. Recent studies indicate that metabolic states are important cues to regulate adult NSC quiescent (Ito and Suda, 2014), including but not limited to hypoxia and glycolysis. For example, mitochondrial kinase mutation induces glycolysis and impedes neuronal differentiation of NSCs (Agnihotri *et al.*, 2017). Furthermore, abundant extrinsic and intrinsic signals are involved in regulating adult neurogenesis (reviewed by Faigle and Song, 2013).

Another major region that NSCs persist in the adult brain is the subgranular zone (SGZ) of the dentate gyrus (DG). The radial astrocytes, also referred to type I cells, serve as NSCs that generate granule neurons in the SGZ. These type I cells give rise to intermediate progenitor cells (type II cells), and then progressively differentiate into mature neurons through the neuroblast phase (Feliciano *et al.*, 2015; Kriegstein and Alvarez-Buylla, 2009).

3. Effect of hypoxic-ischemic brain injury on endogenous NSCs

3.1 Neurogenesis after neonatal hypoxic-ischemic brain injury

Hypoxic-ischemic (HI) injury remains one of most common causes of damage to the neonate’s brain. Neonatal HI brain injury occurs in 1–4 cases per 1,000 live births in the United States and accounts for about one-fourth neonatal deaths worldwide (Kurinczuk *et al.*, 2010; Lawn *et al.*, 2010). Of survived newborns, more than one million children develop severe and chronic neuropsychological impairments, including cerebral palsy and epilepsy, motor and cognitive deficits (Fernandez-Lopez *et al.*, 2014; Gonzales-Portillo *et al.*, 2014).

The common reason for perinatal hypoxic brain injury is intrauterine asphyxia due to circulatory problems, including placental abruption, placental arterial clotting, and inflammatory processes (Fatemi *et al.*, 2009). Moreover, it is worth to note that fetal stress that is a common consequence of gestational complications (e.g., hypoxia, diabetes, smoking, preeclampsia, infection) significantly increase the vulnerability of neonatal hypoxic-ischemic brain injury (Fajersztajn and Veras, 2017; Li *et al.*, 2012).

Upon HI attack, oxygen and glucose supplies to the neonatal brain are transiently depleted, which causes an energy failure or energy-inefficient state and is regarded as the primary insult. This attack initiates a cascade of deleterious cellular events involving dysfunction of transcellular ion pumping and accumulation of excitatory glutamate and oxygen free radicals (Perlman, 2006; Yildiz *et al.*, 2017). After transient resuscitation, secondary injuries may follow, which include inflammation, mitochondrial dysfunction, and cell death (Johnston *et al.*, 2001; Perlman, 2006; Vannucci, 2000). To explore the pathophysiology of neonatal HI brain injury, several animal models have been developed during last decade (Yager and Ashwal, 2009). Among them, Rice-Vannucci model (Levine, 1960) is the most well-accepted, including unilateral common carotid artery ligation and subsequent hypoxic (8–10% O_2) treatment. Using these models, studies have revealed several unique features of neonatal HI brain injury, which may relate to the immature nervous system. The immature brain has a limited activity of antioxidant enzymes (e.g., glutathione peroxidase, copper-zinc superoxide dismutase) around birth (Sheldon *et al.*, 2004). Thus, the neonatal brain is more susceptible to oxidative damage caused by HI injury (Sheldon et al., 2004). Moreover, a “continuum” phenotype of cell death, hybrid features of apoptosis and necrosis, exist in the injured neonatal brain (Northington *et al.*, 2007). Next, we will first focus on the response of endogenous NSCs to HI injury in the neonatal brain.

In the neonatal rat, severe brain HI insult was initially reported to delete 20% of total cells in SVZ within few hours, and the size of SVZ becomes smaller three weeks later (Levison *et al.*, 2001). However, following studies have shown that not all the types of cells are affected by acute HI injury. Only the cells localized in the lateral area of SVZ suffer apoptosis, while cells within the medial SVZ resist HI injury. Calpain and caspase 3 are reported to participate in this apoptosis process (Romanko *et al.*, 2007). The increased activity of Calpain occurs as early as four hours after HI, following by caspase 3 activation four hours later (Romanko *et al.*, 2007). In vitro study indicates that B-cell lymphoma 2 (BCL-2)/adenovirus E1B 19 kDa interacting protein-3 (BNIP3) is another pathway involved in hypoxia-induced NSC/NPC apoptosis and is independent of caspase activity (Walls *et al.*, 2009). Phenotype study showed that PSA-NCAM positive neuroblasts that locate in the lateral area of SVZ are vulnerable to HI; however, nestin-positive neural stem/progenitor cells resist to HI and close to the lateral ventricle (Romanko *et al.*, 2004). One of the possible reasons for this phenomenon is that medial SVZ NSCs reside in the neurogenic niche that is normally an area of physiologic hypoxia. And hypoxia is the factor to keep NSC proliferation. So NSCs in this area may adapt the hypoxic environment and are more resistant to HI insult.

Different from the previous report, Jennifer Plane *et al.* demonstrated that HI injury on postnatal day 10 (P10) mice obviously enlarged the ipsilateral SVZ and significantly

increased the cell proliferation three weeks after HI (Plane *et al.*, 2004). This study further indicates that ectopic striatal migration of neuroblasts and neurogenesis in the ipsilateral striatum post-HI injury. Similar results in rat neonatal HI model are reported by Takeshi Hayashi *et al.*(Hayashi *et al.*, 2005). Hypoxic-ischemic injury increases cell proliferation in both ipsilateral cortex and striatum, and phenotype study indicates these dividing cells are not only doublecortin (DCX) positive neuroblasts but also GFAP/Neural/glial antigen 2 (NG2) positive glial cells (Hayashi *et al.*, 2005). These studies suggest that HI injury-induced proliferation of SVZ NSCs participate in both neural replacement and gliosis. Furthermore, tissues isolated from ipsilateral SVZ suffered a moderate HI injury can generate more tripotential neurospheres *in vitro*, which prefer to differentiate into neurons and oligodendrocytes (Felling *et al.*, 2006; Yang and Levison, 2006). A more recent study, using novel multimarker flow cytometry, analyzed the population change of SVZ NSCs after HI injury *in vivo* (Buono *et al.*, 2015). Interestingly, multipotential progenitors (MPs) and glia-restricted progenitors (GRPs), instead of NSCs, remarkably increase 48 h after HI. This finding may partly explain the reason for gliogenesis after neonatal HI injury. However, some fundamental questions remain unanswered, such as the relationship between migrated neuroblasts and dividing glial progenitors on brain recovery after neonatal HI injury.

At present, it is clear that neuroblasts migrate to the striatum/cortex, and some glial progenitor cells surround the HI-affected brain. The next question is whether the migrated neuroblasts or newly born neurons survive in the HI-affected area. So far, it is still a controversy about the fate of newly born neurons. Jennifer Plane *et al.* have shown that newly generated striatum neurons cannot survive for two weeks (Plane *et al.*, 2004). While, others reported that such neurons, with 5-bromo-2′-deoxyuridine $(BrdU)^+$ and $Rbfox3(NeuN)^+$, still exist in striatum or cortex five weeks after the injury (Felling *et al.*, 2006; Yang *et al.*, 2007). However, most of the newly formed neurons (about 85%) induced by injury die before maturation, even though some of them could survive for five weeks (Yang *et al.*, 2007). Thus, understanding the reasons for the death of newly born neurons will be necessary for future studies.

One serious issue that needs to pay attention to in animal HI study or translational clinical study is the severity of HI injury. The disparate observations on NSC response to injury and cell survival between studies could be due to the differences in the severity of HI injury. For example, 45 mins 10% oxygen treatment was used to induce hypoxia in P10 CD-1 mice in Jennifer Plane *et al.* study, while 90 mins 8% oxygen was reported to apply on P6 rats in the study of Ryan Felling *et al.* Therefore, the relationship between the severity of HI and NSC response remains to be explored. And how to quantify the severity and to predict the NSC response based on the level of severity are the important questions that warrant the further investigation.

Beside NSCs in SVZ, NSCs within dentate gyrus subgranular zone (SGZ) also respond to neonatal HI injury. Similar to the studies of SVZ, committed type 2b and type 3 (DCX positive) neural progenitors in SGZ are vulnerable to HI injury, leading to cell apoptosis at 24 h after HI (Kwak *et al.*, 2015; Miles and Kernie, 2008). However, nestin-positive type 1 and type 2a neural stem/progenitors not only resist acute injury but also actively proliferate, following by long-term neuronal restore (Miles and Kernie, 2008).

3.2 Neurogenesis after adult ischemic stroke

Stroke is still the leading cause of adult chronic disability, and the fifth leading cause of death in the United States (U.S.), which kills more than 130,000 Americans each year (Benjamin *et al.*, 2017). Of the 6 million Americans who are stroke survivors, 71% are unable to return to work. About $36.5 billion are spent due to stroke every year (Benjamin *et al.*, 2017; Howard and Goff, 2012). Currently, the treatments for stroke are insufficient. Tissue plasminogen activator (tPA) is still the only FDA-approved drug for acute ischemic stroke. In addition to tPA, emerging evidence has shown that endovascular therapy is beneficial and promising (Cougo-Pinto *et al.*, 2015). However, only a small portion of stroke patients can benefit from these treatments due to the narrow therapeutic time window and the strict therapeutic criteria that are uneasy to fulfill (Cougo-Pinto *et al.*, 2015). Meanwhile, most of the survivors cannot fully complete neurological and functional recovery and have to face several obstacles to normal life, even after the utility of long-term rehabilitation (Qureshi *et al.*, 2013).

Like neonatal HI injury, lack of blood supply triggers a serial of pathophysiological events leading to neural cell death after ischemic stroke. The mechanism includes excitotoxicity, mitochondrial dysfunction, protein misfolding, oxidative stress and inflammatory response (George and Steinberg, 2015). Although these pathways are first recognized as the detrimental effects in the development of neural injury, some of the pathways have also been proven beneficial for brain recovery, such as neurogenesis (George and Steinberg, 2015; Hao *et al.*, 2014). Next, we will discuss the response of endogenous NSCs to ischemic stroke.

From early 2000s, the increasing evidence indicates the existing of post-stroke cell proliferation in SVZ (Jin *et al.*, 2001; Parent *et al.*, 2002; Zhang *et al.*, 2001), SGZ(Jin *et al.*, 2001; Yagita *et al.*, 2001), heavily relied on the technique of 5 -bromo-2′-deoxyuridine (BrdU) staining. Moreover, this cell proliferation in SVZ and SGZ starts from 2–5 days after stroke and lasts for about 30 days, with a peak on day7–8 post-ischemia (Yagita *et al.*, 2001; Zhang *et al.*, 2001). Stroke also causes the changes of NSC dividing pattern from asymmetric to symmetric (Zhang *et al.*, 2004). Most of the BrdU-positive proliferating cells in SVZ are DCX-positive neuroblasts, none or few of them are mature neurons. Interestingly, DCX positive cells are also observed in the ipsilateral striatum (Arvidsson *et al.*, 2002; Jin *et al.*, 2003) and cortex (Jin *et al.*, 2003) after stroke. This phenomenon raises a new question about the resource of neuroblasts outside the neurogenic area. Do they generate locally or migrate from the existing neurogenic niche after stroke? Proof of evidence has been demonstrated that the dividing neuroblasts migrate out from SVZ to the ipsilateral striatum and peri-infarct area (Arvidsson *et al.*, 2002; Parent *et al.*, 2002, Yamashita *et al.*, 2006). More interestingly, the SVZ-derived neuroblasts could further differentiate into neurons and form synapses (Yamashita *et al.*, 2006). Taking advantage of *in vivo* tracking and transgenic mouse, we now know that DCX-positive cells can move at an incredible speed of 17.98 ± 0.57 μm/h out of SVZ following ischemia (Zhang *et al.*, 2009). Such migration mainly follows existed Rostral Migratory Stream (RMS) and is close to microvasculature (Thored *et al.*, 2007). And some of the migrating neuroblasts change their direction to ipsilateral striatum or cortex, rather than chain migration to the olfactory bulb in the normal brain. It is still debated whether such migration to stroke-affected area expenses

the cells whose original target of movement is OB. Studies have also shown that stromal cell-derived factor-1 (SDF-1) is the main regulator for neuroblast migration, through the chemokine(C-X-C motif) receptor 4 (CXCR4) (Kokovay *et al.*, 2010; Thored *et al.*, 2006). Even though dividing neuroblasts migrate to the stroke-affected area and try to replace the lost neurons, most of them die in about two weeks. The reason remains elusive and may be due to the un-health post-ischemic environment. Brain ischemia initiates an inflammatory cascade, such as microglial activation, immune cells infiltration and the release of toxic proinflammatory molecules (Tobin *et al.*, 2014). Inflammation not only leads to neuron death but also affects the neurogenesis and survival of newly born neurons. Several lines of evidence indicate that microglial activation associated with inflammation disrupts the neurogenesis in the hippocampus (Monje *et al.*, 2003), and proinflammatory factor interferon-γ (IFN-γ) increases apoptosis of cultured NSCs (Ben-Hur *et al.*, 2003). Moreover, a recent study demonstrates that deletion of circulating monocytes after stroke improves the short-term survival of newly formed neuroblasts in SVZ (Laterza *et al.*, 2017). Besides neuroblast, some studies indicate that many of the migrated neural progenitor cells give rise to reactive astrocytes and participate into the astrogliosis after stroke (Li *et al.*, 2010). It may be a good way to prevent the extension of inflammatory factors from infarction in the acute phase of stroke. While, the role of gliosis after stroke, especially its long-term effect, is still elusive and needs further investigation.

A clear conclusion about endogenous post-stroke neurogenesis is that although the period of SVZ cell proliferation seems short, this process is critical for stroke recovery. Transgenic ablation of SVZ neuroblasts increases brain infarction and worsens the stroke-caused behavioral deficits (Jin *et al.*, 2010b). In addition, conditional delete of neural progenitor cells impedes the cognitive function and reduces synaptic connectivity after stroke (Sun *et al.*, 2013a). Furthermore, it should be noted that most of the studies mentioned above used young adult animals. However, over 80% of strokes occur in the elderly. Thus, age should be an important factor to be considered when studying neurogenesis after stroke. Indeed, aging reduces SVZ cell proliferation and migration of neuroblasts after stroke, even though neurogenesis is still observed (Moraga *et al.*, 2015).

Post-stroke neurogenesis was also proved in human brains through the study of immunostaining on the brain specimens of stroke patients (Jin *et al.*, 2006; Nakayama *et al.*, 2010). Proliferated neuroblasts or newly-born neurons were found in the ischemic penumbra area of cortex, and some of them seem under migration 30 days after stroke (Jin *et al.*, 2006). In addition, Nakayama *et al.* (2010) have shown that nestin-positive cells exist in the post-stroke cortex from as early as one day after stroke, and disappear after three months. Nonetheless, many questions remain to be verified on human post-stroke neurogenesis, due to the limitation of patient samples and a shortage of available *in vivo* technologies. For example, the duration and location of post-stroke NSC proliferation remain unclear, as well as the resource of newly generated neurons in the cortex.

4. NSC-based therapy for hypoxic-ischemic brain injury

4.1 Exogenous NSC transplantation

4.1.1 NSC transplantation in neonatal HI brain injury—Although endogenous NSCs have the self-repair ability after brain injury, it is usually insufficient and needs time to proliferate and migrate to the lesion area. Therefore, transplantation of exogenous NSCs is probably a more efficient way to improve the brain restore after injury. Moreover, *in vitro* cultured NSCs have the potential of self-renewal and differentiation to neuronal or glial cells. No matter the neural stem cells are derived from embryonic stem cells or isolated from fetal brains, substantial pre-clinical evidence has indicated that neural stem cell transplantation is efficient and effective for treating neonatal hypoxic-ischemic brain injury (Table 1).

4.1.1.1 Effect of NSC transplantation: The first question that all the studies of stem cell transplantation is whether stem cell therapy can reduce brain infarction and behavioral deficits. Indeed, several studies have shown that NSC transplantation significantly reduces HI-induced lesion volume in the acute and subacute phase (Sato *et al.*, 2008) and the brain loss in the chronic phase of brain HI injury (Braccioli *et al.*, 2017). However, the change in brain lesion volume is not always correlated with the improvement of behavioral functions. For example, although there is no significant difference on the change of infarct size after NSC treatment, an apparent improvement in sensorimotor functions have been observed in the NSC treated group compared to the vehicle group (Daadi *et al.*, 2010). Thus, another important factor to evaluate the impact of NSC transplantation is the recovery of behavioral functions, such as sensorimotor, cognitive functions. The improvement of sensorimotor functions evaluated by cylinder test, beam walking test, and rotorad test has been shown 30 days after NSC transplantation treatment (Daadi *et al.*, 2010; Shinoyama *et al.*, 2013). Similarly, a delayed NSC treatment study has reported that this behavioral improvement can even last for about two months after NSC transplantation (Braccioli *et al.*, 2017). Using T-maze and Morris water maze tests, NSC transplantation has also indicated the better recovery in the capability of spatial memory in a neonatal cerebral HI model one month after the cell treatment (Ji *et al.*, 2015; Zheng *et al.*, 2012). In addition to the reduction of infarction and improvement of behavioral functions, NSC treatment also attenuates neuroinflammation after HI injury. The HI-induced activation of microglia (Braccioli *et al.*, 2017) and upregulation of pro-inflammatory factors (e.g., interleukin-1β (IL-1β) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)) (Ji *et al.*, 2015) are reduced after NSC transplantation. Furthermore, engrafted NSCs participate into the remodeling of neuroplasticity, as the evidence of axonal growth has been noticed after NSC transplantation (Daadi *et al.*, 2010; Shinoyama *et al.*, 2013).

4.1.1.2 Methodologies of NSC transplantation

Source of NSCs: Both human and animal NSCs have been evaluated in pre-clinical studies (Daadi *et al.*, 2010; Ji *et al.*, 2015; Lee *et al.*, 2010; Zheng *et al.*, 2012), aimed to test the efficacy of NSC therapy for neonatal brain HI injury. Mouse and rat NSCs can be generated from fetal brain tissues (E14-16) (Sato *et al.*, 2008) and grow as neurosphere. Another way to obtain animal NSCs is to derive them from animal embryonic stem cell (ESC) line with or

without genetic modification (Shinoyama *et al.*, 2013). Similarly, human NSCs can be derived from human ESC line (Daadi *et al.*, 2010). More importantly, these ESC-derived NSCs have been proved safe for transplantation, as they do not form tumors after transplanted into normal nude animals (Daadi *et al.*, 2008). Meanwhile, brain tissues of human fetal cadaver that involved neurogenic area (like SVZ) are another resource for acquiring hNSCs (Ashwal *et al.*, 2014). Although all of these NSCs have been reported the neuroprotective role for neonatal HI treatment, there is no direct comparison between the efficacies of NSCs generated from different sources yet. Therefore, it is still difficult to conclude which type (s) of NSCs are the most efficient for the NSC therapy. For better translating NSC therapy to clinical studies, many variables need to be compared with the two kinds of human NSCs, like the safety, the immune response in the host environment, the accessibility and the operability.

Route of NSC transplantation: Up to now, intracerebroventricular (i.c.v), intracerebral (cortex or hippocampus) and intranasal deliveries of NSCs have been reported in the pre-clinical studies (Ashwal *et al.*, 2014; Ji *et al.*, 2015; Sato *et al.*, 2008; Zheng *et al.*, 2012). Engrafted NSCs distribute around the lateral ventricle and are close to the lesion area 7 days after i.c.v transplantation (Sato *et al.*, 2008). Although such cell treatment reduces the lesion volume, it remains unclear that how many engrafted cells migrate to the lesion and the long-term effect of this therapy. Four weeks after intracerebral injection of NSCs, engrafted cells survive, and about 40% of them differentiate into mature neurons (Daadi *et al.*, 2010). Moreover, NSCs successfully distribute to the ipsilateral hemisphere and survive for 42 days after being intranasal administrated (Ji *et al.*, 2015). Due to lack of system comparison on the efficiency of delivery routes, we still do not know which method(s) is ideal for the clinical application. However, intranasal administration is less invasive than intracerebral and i.c.v injection. Comparing to the latter ones, if the intranasal treatment has proved the similar therapeutic effect, it will be a more acceptable and practical way to perform the cell therapy in the clinical study.

Timing of NSC transplantation: Timing is a critical factor in designing and performing stem cell therapy, as HI-induced brain injury is dynamically changed. However, it is never straightforward to find the optimized time point for cell transplantation, due to the complexity of the pathogenic process of the disease. Most reported pre-clinical studies selected 24 hours after HI injury as the time point to transplant NSCs (Daadi *et al.*, 2010; Ji *et al.*, 2015; Sato *et al.*, 2008). The possible reason is to avoid the acute excitotoxicity and inflammatory reaction after brain injury. However, human NSC transplantation 24 hours after HI increased microglial accumulation around the injection site in the previous study (Daadi *et al.*, 2010). But it is unclear whether or not these increased microglia cause death of engrafts. A recent study indicates that delayed transplantation of NSCs (10 days after HI) still improves long-term functional recovery and reduces brain loss (Braccioli *et al.*, 2017). This suggests that the therapeutic time window for NSC transplant may be wider than previously thought, probably due to the high plasticity of the neonatal brain. Further studies are needed to explore the optimized timing for NSC transplantation for neonatal HI brain injury.

Dosage of NSC transplantation: Dosage is another factor that should be considered to perform the cell transplantation. A low dose may be not effective to rescue the brain damage, whereas a too high dose may cause side effects, such as forming cell clots. Unfortunately, there is no study yet to test the dose-dependent effect of NSC transplantation on neonatal HI injury. The dosage selected in the reported pre-clinical studies probably based on their pilot studies, ranging from a total of 1×10^5 to 3×10^5 cells. Even though these dosages have been reported effective for neuroprotection, it is difficult to get an ideal dosage based on previous studies. Because various transplantation parameters, e.g., timing, delivery route, and animal model, will impact the effective dosage, it is necessary to optimize the dosage in certain scenario of cell transplantation.

4.1.2 NSC transplantation in adult ischemic stroke—NSC therapy for the treatment of stroke in the adult has been much more widely investigated, compared to NSC therapy in neonatal HI injury. More than two hundred publications were published in the past decades. NSC transplantation is no doubt beneficial for reducing infarct volume and behavioral deficits caused by stroke. Indeed, a recent meta-analysis and systematic review analyzes the effect of preclinical NSC transplantation on stroke and indicates favorable outcomes of NSC therapy in the stroke treatment (Chen *et al.*, 2016). In the following section, we will discuss the methodology and underlying mechanisms of NSC therapy for stroke (Table 2), providing a rationale for future translational studies.

4.1.2.1 Transplantation methodologies

Source of NSCs: Four primary sources of NSCs can be used in the treatment of stroke: 1) NSCs generated from fetal brains; 2) NSCs derived from ESCs (ESC-NSC); 3) immortalized neural precursor cells; 4) induced pluripotent stem cell-derived NSCs (iPSC-NSC). NSCs generated from fetal brains, and ESCs are the most widely used for pre-clinical studies. These NSCs have the potential for proliferation and neuronal differentiation *in vitro*. After being transplanted, they rapidly migrate to the infarcted area, leading to a reduction of neuronal apoptosis and improvement of behavioral function (Huang *et al.*, 2014; Jin *et al.*, 2010a). About 40 – 60% of engrafted NSCs differentiate into neurons (Takagi *et al.*, 2005). Immortalized neural precursor cells, like CTX0E03 (Pollock *et al.*, 2006) and HB2.F3 cells (Chang *et al.*, 2013), are neural precursor cells with an incorporated immortalizing oncogenes. Preclinical studies have shown the neuroprotective effect by transplanting these cells, and they have been used in ongoing type I clinical trial (Irion *et al.*, 2017). Compared to other types of NSCs, iPSC-NSCs have their unique advantage. Firstly, iPSC-NSC transplantation remarkably reduced behavioral deficits after stroke (Eckert *et al.*, 2015; Oki *et al.*, 2012). Moreover, these cells can be generated from stroke patients' own skin fibroblasts, which eliminate the immune rejection and ethical concern. Human iPSC-NSCs can differentiate into mature neurons with electrophysiological activity and can survive for at least four months in the brain of animal stroke models. In addition, there is no tumor formation during this period (Oki *et al.*, 2012). Although iPSC-NSCs are promising, one pitfall is that the process of iPSC-NSCs can be time-consuming. However, this could be overcome with the rapid technological development of iPSC culture.

Route of NSC transplantation: Intracerebral, intravascular and intracerebroventricular transplantation of NSCs have all been investigated and confirmed the positive functional improvement after stroke. However, it remains unclear which one is the optimal route for NSC transplantation. It may depend on the situation of stroke, like topology of brain lesion and stroke subtype. More NSCs are generally distributed into the infarcted area by intracerebral delivery, compared to intravascular injection. This is the reason why most of the pre-clinical studies have selected this delivery route. However, caution should be observed when translating this to clinical study because intracerebral delivery is invasive and raises the risk of several adverse events, *e.g.*, seizure (Savitz *et al.*, 2005). Considering intravascular delivery, intra-arterial has been proven more efficient than intravenous administration. A systematic comparison of intra-arterial and intravenous delivery has demonstrated that most of the NSCs are trapped in the lung after intravenous injection. In contrast, about 93% NSCs are distributed into the brain following intra-arterial injection (Pendharkar *et al.*, 2010). However, a safety issue of intra-arterial cell delivery is the possibility of forming microemboli, which could cause micro-strokes. But this issue could be avoided by optimizing injection paradigms, like speed of cell infusion and appropriate blood flow. For example, micro-stroke has not been observed with a microneedle injection technique without slowing down the anterograde blood flow (Chua *et al.*, 2011).

Timing of NSC transplantation: Selecting a reasonable time point is another essential step to design cell therapy. The timing chose in preclinical studies vary widely from 6 hours to 6 weeks. However, few studies have directly addressed the question of optimal transplantation timing with cell survival. Intra-arterial NSC transplantation 72 hours after stroke results in highest cell brain distribution and less host immune response, compared to the transplantation 7 or 14 days after stroke (Rosenblum *et al.*, 2012). Similarly, NSCs survive better with early (48 hours post stroke) intrastriatal transplantation than late (6 weeks) transplantation (Darsalia *et al.*, 2011). Thus, it appears that 2–3 days after stroke would be optimal for the NSC treatment, which should avoid post-stroke abundance of excitotoxic molecules and acute inflammatory response in the host environment. Moreover, timing of NSC transplantation has the distinct effects on the fate of engrafted NSCs. NSCs prefer to differentiate into glial cells if they are intravenously delivered in the acute phase (less than 24 hours after stroke); conversely, delayed transplantation enhances the neuronal differentiation (Doeppner *et al.*, 2014; Rosenblum *et al.*, 2012).

4.1.2.2 Potential mechanisms of brain repair

Cell replacement: The initial goal of NSC therapy for stroke was to replace the dying neurons. Although the engrafted NSCs can differentiate into mature neurons and some of them have electrophysiological property (Daadi *et al.*, 2009; Oki *et al.*, 2012), the overall number of survived engrafts are far less than the number of lost neurons caused by stroke. Mounting evidence indicates that host immune response and inflammation status influence the survival of grafts. In vitro studies have shown that exposure of NPC to IFN-γ upregulates its expression of major histocompatibility complex (MHC) antigen (Hori *et al.*, 2003; Kim *et al.*, 2006). In addition, inflammatory cells (e.g., macrophage, microglia) cluster around the implantation site of exogenous NSCs in mouse brain (Buhnemann *et al.*, 2006; Kim *et al.*, 2006), while such cluster is not observed in the brain of

immunocompromised nude mouse (Kim *et al.*, 2006). Beside immune response, there are probably other mechanisms that cause apoptosis/cell death of grafts, as the application of immunosuppression agents could not completely reverse the death of grafts (Buhnemann *et al.*, 2006). For example, the abundance of reactive oxygen species after stroke may be another threaten, as genetically modifying NSC to overexpress antioxidant enzyme, Cu/Zn-superoxide dismutase(SOD1), increases graft survival in the ischemic brain (Sakata *et al.*, 2012a). Another possible reason is the loss of cell-cell connection, as single cells are usually prepared for transplantation. A recent study demonstrates that cell-cell contact enhances survival and neuronal differentiation of cultured NSCs (Jiao *et al.*, 2017). Such interaction increases the expression of gap junction and, more importantly, enhances the support of neurotrophic factors from each other (Jiao *et al.*, 2017). However, NSC treatment induced beneficial impact of behavioral function lasts for an extended period, even when the engrafted NSCs were insufficient to replace cell loss or most of them disappeared (Rosenblum *et al.*, 2015). This suggests that cell replacement only takes a small part of the therapeutic mechanism of NSC transplantation. Indeed, the previous study by Borlogan *et al.* provided a new notion that neuroprotective effect of stem cell therapy does not require the engrafted cells to physically pass the blood-brain-barrier and enter the brain (Borlongan *et al.*, 2004). Consistent with this concept, a recent study has shown that only conditioned medium from cultured neural progenitor cells is sufficient to reduce the infarct volume and improve behavioral recovery after stroke (Doeppner *et al.*, 2017).

Moreover, the neuronal differentiation rate of engrafted NSC after stroke varies from 10% to 60% (Buhnemann et al., 2006; Daadi *et al.*, 2009; Sakata *et al.*, 2012a). In addition to neurons, grafts are able to differentiate into glial cells, including astrocyte, microglia, oligodendrocytes in vivo (Rosenblum *et al.*, 2012; Sakata *et al.*, 2012b). Both cell-intrinsic factors and extrinsic factors (e.g., host environment, transplantation strategy, cell-cell interaction) could affect the fate of grafts. For example, early transplantation of NSC is prone to astrocytic differentiation but later transplantation is prone to neuronal differentiation (Rosenblum *et al.*, 2012). Furthermore, evidence shows that astrocytes negatively regulate hippocampus neurogenesis in an entorhinal cortex injury model (Wilhelmsson *et al.*, 2012). Thus, it is highly possible that the neuronal differentiation of grafts is hindered by reactive astrocytes after stroke. However, the underlying mechanism of grafted NSC differentiation in ischemic brain and how to precisely guide the differentiation are still elusive, which warrant further investigation.

Immunomodulation: We have shown that post-stroke inflammatory response is significantly reduced after NSC transplantation, and the activation of microglia is also suppressed by engrafted NSCs (Huang *et al.*, 2014). In line with our findings, several studies have confirmed the role of NSCs in immunomodulation after stroke, with downregulation of pro-inflammatory factors and activity of immune cells (Doeppner *et al.*, 2013; Doeppner *et al.*, 2014).

Bystander effect: The bystander effect is a currently well-accepted concept for explaining the neuroprotective effect of stem cell transplantation. It means that engrafted NSCs can either release growth and neurotrophic factors by themselves or stimulate host cells

upregulating expression of such factors. There is substantial evidence that cultured NSCs can secrete multiple trophic factors, such as BDNF, VEGF, and EGF (Hicks *et al.*, 2013). Moreover, human iPSC-NSCs increase VEGF expression of host astrocytes and promote angiogenesis after being transplanted into striatum of stroke mouse (Oki *et al.*, 2012).

Accelerating endogenous recovery: Although the spontaneous post-stroke neurogenesis is inadequate to repair the injured brain, the natural self-repair activity occurs as early as few days after stroke. The possibility of enhancing endogenous neurogenesis has been the focus of pre-clinical stroke studies. Expectedly, several NSC translational studies observed that exogenous cell administration has a consistent impact on the endogenous neurogenesis (Jin *et al.*, 2011; Park *et al.*, 2010; Zhang *et al.*, 2011). One possible explanation for the effect of stimulating endogenous neurogenesis is the bystander effect of cell treatment. However, it seems that the delivery route also has an effect. Intracerebral, instead of intravascular, injection of NSCs promote host cell proliferation in the SVZ (Minnerup *et al.*, 2011). Except for neurogenesis, NSC transplantation has been proven to secrete angiogenic factors, thereby promoting host angiogenesis or vasculogenesis (Hicks *et al.*, 2013; Zhang *et al.*, 2011). Meanwhile, stroke mostly occurs in the elderly, so the effect of NSC treatment in aged stroke animals may be more clinically relevant. Some studies have shown that NSC transplantation greatly enhances the neurogenesis and angiogenesis in both young and aged animals (24-month old) (Jin *et al.*, 2011; Tang *et al.*, 2014). Moreover, equal survived engrafted cells distribute in the host post-stroke environment, regardless of host's age (Tang *et al.*, 2014). This suggests that the age may not be a concern affecting the therapeutic effect of engrafted NSCs.

4.2 Enhancing neurogenic potential of endogenous NSCs

In this section, we will cover most of the main factors that are known to be involved in post-injury neurogenesis following the neonatal HI injury and adult ischemic stroke. We will not undertake a detailed review of factors that regulating neurogenesis in the normal adult brain or during central nervous system development, as several comprehensive reviews are discussing these topics (Faigle and Song, 2013; Semple *et al.*, 2013). Neurogenesis and involved factors within the ipsilateral brain after HI injury are illustrated in Figure 2.

4.2.1 Factors involved in the regulation of neurogenesis after neonatal HI brain injury

Erythropoietin (EPO): EPO, a hypoxia-inducible factor, is critical for erythropoiesis (Beleslin-Cokic *et al.*, 2004). Interestingly, studies also indicate that EPO can stimulate proliferation of NSCs through its specific membrane receptor – EPOR (Chen *et al.*, 2007; Shingo *et al.*, 2001). Consistent evidence has shown that EPO treatment not only reduces acute brain damage and behavioral deficits (Iwai *et al.*, 2007; Iwai *et al.*, 2010), but also increases SVZ neurogenesis and migration of neural progenitors after neonatal HI brain injury (Iwai *et al.*, 2007). Moreover, EPO administration attenuates white matter injury (Iwai *et al.*, 2010), which is a detrimental pathophysiology of neonatal HI injury and leads to dysfunction of the brain. In addition, oligodendrogenesis is significantly improved after a delayed EPO treatment (Iwai *et al.*, 2010). However, like every coin has two sides, long-term EPO administration could increase the risk of polycythemia and vascular thrombosis

(Coleman *et al.*, 2006). Fortunately, a recent study using asialo-EPO (AEPO) that is a non-erythropoietic derivative of EPO, demonstrates the similar beneficial effect on proliferation of oligodendrocyte progenitor cells (OPCs) without side effect of EPO injection (Kako *et al.*, 2012). Consecutive two weeks AEPO treatment increases mature of OPCs in corpus callosum (CC) of HI-insulted neonatal mice and improves myelin formation in the CC area two months after HI insult (Kako *et al.*, 2012).

Neurotrophic factors and growth factors: Brain-derived neurotrophic factor (BDNF) is a key element to keep neuronal survival. A delayed chronic combination treatment of BDNF and epidermal growth factor (EGF) significantly increases cell proliferation in the SVZ and ipsilateral striatum after HI insult in mouse (Im *et al.*, 2010), leading to improvement of behavioral functions. Meanwhile, the proliferation of newly generated neurons in the striatum is increased by the BDNF+EGF treatment (Im *et al.*, 2010). Even though the therapeutic effect of BDNF treatment is promising, the delivery of BDNF is a concern for clinical study, as BDNF may be difficult to pass the blood-brain-barrier (BBB). Vascular endothelial growth factor (VEGF) promotes angiogenesis, which is an important event to maintain the neurogenic niche. There are seven members of the VEGF family and the function of VEGF isoforms on neurogenesis is different. VEGF-A and VEGF-C at the SVZ are transiently induced after neonatal HI injury (Bain *et al.*, 2013). VEGF-A has been shown to enhance the differentiation of SVZ glial progenitors to astrocytes, and VEGF-C prefers to stimulate proliferation and differentiation of late oligodendrocyte progenitors through its receptor – VEFGR-3 (Bain *et al.*, 2013).

Leukemia inhibitory factor (LIF): LIF, a cytokine from the family of interleukin-6 (IL-6), is an important factor for maintenance of NSCs (Pitman *et al.*, 2004; Shimazaki *et al.*, 2001). HI injury transiently upregulates the mRNA levels of LIF in the SVZ, with a peak at 24 hours after injury (Buono *et al.*, 2015; Covey and Levison, 2007). In addition, LIF treatment increases proliferation of neurosphere generated from the SVZ *in vitro* (Covey and Levison, 2007). This effect of LIF is through the Notch signal pathway (Covey and Levison, 2007; Felling *et al.*, 2016). In a LIF heterozygotes transgenic mouse, HI-induced expansion of neural progenitors in the SVZ is inhibited, suggesting that LIF signal is required for NSCs responding to HI injury (Buono *et al.*, 2015). Furthermore, study by Ryan Felling *et al.* shows for the first time that LIF is released by the SVZ astrocytes responding to HI injury (Felling *et al.*, 2016). These studies have shed light on the potential of LIF as a treatment avenue to boost NSC neurogenesis after neonatal HI injury.

Hyperbaric oxygen (HBO): HBO therapy, using 100% oxygen at 2.4–3.0 absolute atmosphere (ATA), has been reported neuroprotective for many neurological diseases (Deng *et al.*, 2014). Surprisingly, brief application of HBO during reperfusion of HI injury causes cell death of oligodendrocyte glial progenitors in the cortex and leads to deficit of motor function 2–4 weeks after injury (Koch *et al.*, 2008). In contrast, consecutive perform HBO treatment for one week, starting from 3 hours after HI injury, significantly increases NSC proliferation in the SVZ, and improves behavioral recovery (Wang *et al.*, 2008). The paradigm of HBO treatment may explain the difference between these two studies. HBO cannot be administrated in the hyperacute phase of HI injury, as it could cause more severe

ischemic-reperfusion damage. The treatment time window is 3 hours to 24 hours after HI injury, based on the previous report (Wang *et al.*, 2008). Daily HBO treatment for one week may compensate the adverse aspect of toxic oxygen. Moreover, many other key parameters, like the severity of injury and dose of HBO treatment, also need to be considered in performing HBO therapy for clinical studies in the future.

Hypothermia: Growing evidence indicates that mild hypothermia (33–35 °C) is an effective management for neonatal HI encephalopathy. However, it is still uncertain that hypothermia is beneficial for neurogenesis after HI insult. The previous study has shown that a severe hypothermia (30 °C for 24 hours) reduces cell proliferation in the SGZ, but not in the SVZ (Kanagawa *et al.*, 2006). In contrast, a short period of hypothermia (28.5 °C for 4 hours) partially rescues the SGZ cells from apoptosis and increases the proliferation of neural progenitors in the SGZ (Kwak *et al.*, 2015). Consistent with this report, hypothermia (32–33 °C for 24 hours) attenuates HI-induced white matter damage and elevates proliferation of early oligodendrocyte progenitors in the hippocampus (Xiong *et al.*, 2013). A possible explanation for the controversial reports is the differences in the parameter of hypothermia. It appears that mild hypothermia with short period is neuroprotective and beneficial for neurogenesis after neonatal HI injury, while severe hypothermia may be not. To make future hypothermia studies more comparable, parameters used for hypothermia should be standardized. For instance, rectal temperature should be reported in all the studies, instead of only report water bath temperature. Besides, the severity of injury should always be considered, as it affects the evaluation of the efficacy of hypothermia therapy.

4.2.2 Factors involved in the regulation of neurogenesis after adult stroke

Growth factors

Epidermal growth factor (EGF): EGF is a critical mitogen to regulate NSC growth and maintenance *in vivo* and *in vitro* (Kuhn *et al.*, 1997; Reynolds *et al.*, 1992). The receptor of EGF, EGFR, is widely expressed on the SVZ NSCs (Seroogy *et al.*, 1995). The expression of EGFR in the SVZ area responds to cerebral ischemia (Ninomiya *et al.*, 2006). Elevated SVZ EGFR positive staining is observed from 7 days after stroke (Ninomiya *et al.*, 2006), which is coincident with the pattern of proliferation of SVZ NSCs. In addition, lateral ventricular infusion of Heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) increases cell proliferation in the SVZ and DG in normal animals (Jin *et al.*, 2002). The neurogenesis effect of EGF has also been confirmed by *in vitro* neurospheres study under the normoxic condition, and this effect takes place through EGFR (Jin *et al.*, 2002). In stroke animals, intraventricular administration of EGF not only stimulates proliferation of DCX positive neuroblasts in the SVZ (Ninomiya *et al.*, 2006), but also increases neuroblast migration out of the SVZ (Teramoto *et al.*, 2003). Furthermore, EGF treatment enhances the neuronal differentiation in the striatum after stroke, and newly generated neurons are spiny projection interneurons (parvalbumin+) (Teramoto *et al.*, 2003), which is the type of subpopulation affected by stroke. It is worthy of noting that EGF treatment can replace the lost neuronal cells caused by stroke, and enhance self-repair ability of brain post injury.

Fibroblast growth factor (FGF) + EGF: Like EGF, FGF-2 also can stimulate proliferation of cultured NSCs *in vitro*. However, the function of FGF-2 is distinct with EGF on the proliferation and migration of neural progenitor cells *in vivo*. FGF-2 increases newly born neurons in the olfactory bulb, while EGF increases striatum newly born cells through stimulating SVZ cell migration or proliferation of local neural progenitor cells (Kuhn *et al.*, 1997). In addition, expression of FGF-2 in the cortex is upregulated after stroke (Kumon *et al.*, 1993; Lin *et al.*, 1997). Endogenous FGF-2 is an essential regulator of cell proliferation after brain injury, as dividing cells in DG after stroke are significantly reduced in FGF-2 knockout mice compared to wild-type mice (Yoshimura *et al.*, 2001). In order to maximize the effect of growth factors on neurogenesis, many studies combine EGF with FGF-2 (Baldauf and Reymann, 2005; Oya *et al.*, 2008; Tureyen *et al.*, 2005). Three days infusion of EGF + FGF-2 increases cell proliferation in the SVZ and DG 7 days after stroke and improves the survival of newly born neurons at 21 days (Tureyen *et al.*, 2005). Also, the mix of EGF with FGF-2 has been reported the most efficient way of growth factor combination for boosting neurogenesis after stroke (Oya *et al.*, 2008). However, study by Baldauf *et al.* reemphasizes the importance of time frame for such combination therapy. A two weeks treatment with EGF + bFGF unexpectedly enlarges the size of stroke-induced brain injury, even though cell proliferation is increased in the SVZ two weeks after stroke (Baldauf and Reymann, 2005). It seems that long-term application of growth factor combination therapy may have detrimental effects on neuronal death.

Vascular endothelial growth factor (VEGF): In addition to the angiogenetic function, VEGF has been demonstrated a powerful neurogenic effect after stroke (Greenberg and Jin, 2013), including both VEGF-A and VEGF-B. A short period of VEGF-A intracerebroventricular administration increases cell proliferation in the SVZ and DG 28 days after stroke (Sun *et al.*, 2003). This neurogenesis effect is also accompanied with angiogenesis and acute neuroprotection (Sun *et al.*, 2003). Furthermore, SVZ neurogenesis is markedly enhanced in VEGF-overexpress transgenic mice after cerebral ischemia, as well as migration of neuroblasts and generation of newly born neurons in the cortex (Wang *et al.*, 2007). Similarly, VEGF-B knockout reduces the spontaneous post-stroke cell proliferation in the SVZ and DG (Sun *et al.*, 2006). Moreover, exogenous administration of VEGF-B successfully restores such proliferation (Sun *et al.*, 2006). This suggests that the VEGF treatment has therapeutic implications for stroke recovery by increasing angiogenesis and neurogenesis. However, the dosage should be considered carefully when applying VEGF therapy for the neurological disease. A study has shown that a high dose (500 ng/ml) and a low dose (50 ng/ml) of VEGF have distinct effects on proliferation and differentiation of neural progenitors in the SVZ (Meng *et al.*, 2006). The high dose significantly inhibits the proliferation and enhances neuronal differentiation, whereas the low dose does not have these effects (Meng *et al.*, 2006). A recent study indicates that the neurogenesis effect of VEGF after stroke is partly due to reactive astrocyte transdifferentiation into newly generated neurons (Shen *et al.*, 2016).

Neurotrophic factors: Neurotrophic factors, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), are crucial for maintaining neural stem cell/neuronal survival and promoting neuronal differentiation during brain development or after injury (Barde,

1989). Ischemic stroke elevates mRNA levels of both BDNF and NGF in stroke-affected cortex area (Dmitrieva *et al.*, 2016). The brain appears to initiate endogenous neuroprotective mechanisms against the brain injury after stroke. However, intranasal administration of NGF does not further increase SVZ cell proliferation after stroke (Zhu *et al.*, 2011). Instead, it improves the survival of newly born neurons in the striatum (Zhu *et al.*, 2011). Different from NGF, intravenous BDNF injection for five days increases neurogenesis and neural progenitor cell migration from the SVZ, leading to long-term improvement of sensorimotor functional recovery after stroke (Schabitz *et al.*, 2007). Interestingly, it has been reported that upregulation of BDNF *via* recombinant adeno-associated virus (rAAV) inhibits the formation of newborn dentate granule cells in a global ischemia rat model (Larsson *et al.*, 2002). The reason may lay on the methodology, as the rAAV-transducted cells may not release or secrete BDNF into the surrounding environment.

Erythropoietin (EPO): In addition to regulating hematopoiesis, EPO has been reported various other essential functions, including angiogenesis and neuroprotection (Ribatti *et al.*, 1999; Wang *et al.*, 2004). EPO is also critical for neural development. EPO and EPO receptor (EPOR) express in the developing neural tube of embryo, and Epo or EpoR knockout mice cannot survive due to incomplete neural tube closure (Tsai *et al.*, 2006). Intraperitoneal administration of EPO increases SVZ cell proliferation and angiogenesis around ischemic lesion after stroke (Wang *et al.*, 2004). In line with this, the SVZ size and cell proliferation in the SVZ are reduced in EpoR conditional knockdown mice (Tsai *et al.*, 2006). In addition, post-stroke neurogenesis is hindered in these transgenic mice (Tsai *et al.*, 2006). Furthermore, EPO, administrated daily for seven days, augments the proliferation of oligodendrocyte progenitor cells and improves white matter remodeling after embolic cerebral ischemia (Zhang *et al.*, 2010). Meanwhile, gene-expression profile study has shown that EPO treatment significantly upregulates neuronal plasticity-related genes (Egr1 and Egr2) after stroke (Mengozzi *et al.*, 2012). Despite a possible adverse effect of increasing hematocrit and thrombosis, EPO has a high potential for further investigation of clinical application as a stroke management strategy.

Transcriptional factors

Notch pathway: The Notch signaling pathway is a conserved and fundamental pathway critical for maintaining NSC proliferation and differentiation during neural development. Function as a receptor, Notch is a transmembrane protein, including extracellular domain, transmembrane domain and internal cellular domain (NICD). Notch has many ligands, like jagged 1 (JAG1), JAG2 and delta-like (Dll) protein (Ables *et al.*, 2011). Except for the developing brain, components of the notch signaling are cell-based throughout the adult brain, especially neurogenic area (Givogri *et al.*, 2006; Mizutani *et al.*, 2007; Stump *et al.*, 2002). DCX positive neuroblasts in the SVZ highly express Notch and cerebral ischemia increases the expression of the Notch pathway components (NICD, and Notch downstream target-Hes1 and Sonic Hedgehog) in SVZ cells (Wang *et al.*, 2009b). Importantly, SVZ cell proliferation is increased with Notch activation, whereas blocking the Notch signal with soluble Jagged1-Fc reduces the proliferation (Wang *et al.*, 2009b). Moreover, knocking down Notch by RNAi reverses post-stroke proliferation of neural progenitor cells isolated from the SVZ of stroke rats (Wang *et al.*, 2009a). Recent study also confirms the same

phenomena in aged animals. Post-stroke neurogenesis in the SVZ is enhanced by Notch activation and reduced by blocking the Notch signal in aged (20–24 months old) rats (Sun *et al.*, 2013b).

Sonic hedgehog (Shh): Shh is another well-studied morphogen, and the Shh pathway is crucial for dorso-ventral patterning process of developing CNS (Marti and Bovolenta, 2002). Moreover, it has an important role in the adult brain for stem cell maintenance (Ahn and Joyner, 2005; Palma *et al.*, 2005). Under the hypoxic condition, cultured neurons and neural progenitor cells increase Shh expression (Sims *et al.*, 2009). In vivo studies also demonstrate that cerebral ischemia upregulates the expression of Shh signal in the ipsilateral hippocampus (Sims *et al.*, 2009), cortex and striatum (Jin *et al.*, 2015) after injury. These findings suggest that the Shh pathway plays an important role in the neuronal cell response to hypoxia. The further study has shown that inhibition of Shh pathway abolishes the post-stroke cell proliferation in the hippocampus (Sims *et al.*, 2009). Shh-induced cell proliferation is vital for post-stroke behavioral recovery, as conditional knockout Shh gene in NSCs (Nestin positive) worsens the neurological function. Conversely, treatment with Shh signaling agonist (SAG) improves behavioral recovery after stroke (Jin *et al.*, 2015). Consistently, intrathecal administration of Shh protein in stroke animals also has the beneficial effect of SVZ neurogenesis and functional improvement (Bambakidis *et al.*, 2012).

Wingless-type MMTV integration site family (Wnt): The Wnt pathway is a conserved signaling pathway, regulating multiple cellular processes during the development of CNS, including neuronal migration and synaptic differentiation (Fradkin *et al.*, 2005). Accumulating evidence indicates that Wnt protein also participates in the adult neurogenesis under normal and pathophysiologic conditions (Hirota *et al.*, 2016). One major Wnt signaling pathway is the β-caternin-dependent canonical pathway. In the absence of Wnt, β-caternin degrades under basal situation. On the opposite, this pathway is initiated, and transcription factor β-caternin is preserved when Wnt binds to its receptor (Hirota *et al.*, 2016). Upregulation of Wnt *via* lentivirus intrastriatal injection increases the number of BrdU-positive cells in the striatum after endothelin-1-induced focal ischemic injury (Shruster *et al.*, 2012). In addition, stroke increases symmetric division of SVZ NSCs accompanying with upregulated Wnt signal in the same area (Piccin and Morshead, 2011). Similarly, declining expression of β-caternin, a downstream transcriptional factor of Wnt, decreases SVZ expansion and striatal neurogenesis after stroke (Lei *et al.*, 2008).

Epigenetic regulators

MicroRNAs: MicroRNAs (miRs) are a class of non-coding RNAs, participating in post-transcriptional gene regulation (Boyd, 2008). Mature miRs are single-stranded with ~21–22 nucleotides in length and can bind to 3′-untranslated region (3′UTR) of target mRNAs, which leads to translation repression and mRNA degradation (Boyd, 2008). MiRs have been shown to involve in the regulation of neural development and pathophysiology of various neurological disease including stroke. Studies about miRs and post-stroke neurogenesis are emerging. Four miRs related to neurogenesis have been widely investigated.

MiR-9: The expression of miR-9 starts during early neurosphere formation and promotes NSC proliferation (Delaloy *et al.*, 2010). Moreover, miR-9 participates in the migration of NSCs under brain injury. For example, miR-9 inhibits the migration of engrafted NSCs after being transplanted into ipsilateral striatum of stroke mice (Delaloy *et al.*, 2010). In addition, overexpression of miR-9 and miR-200 suppress the differentiation of oligodendrocyte precursor cells through downregulating the expression of serum response factor (SRF) (Buller *et al.*, 2012).

MiR-124: MiR-124 is specifically expressed in DCX-positive neuroblasts in adult SVZ (Cheng *et al.*, 2009). Knockdown of miR-124 enhances the division of SVZ neuroblasts. In contrast, overexpression of miR-124 increases the neuronal differentiation of SVZ cells *in vivo*, and this effect takes place through one of miR-124 direct target, Sox9 (Cheng *et al.*, 2009). In line with these findings, focal cerebral ischemia substantially reduces the expression of miR-124a in SVZ cells (Liu *et al.*, 2011), which may explain the post-stroke proliferation of SVZ cells. In addition, miR-124a transfection in neural progenitors promotes their differentiation to neurons. Furthermore, Jagged-1, a ligand of the Notch signal, has been demonstrated as a direct target of miR-124a (Liu *et al.*, 2011). Thus, stroke downregulates miR-124a with an increase of JAG1, thereby enhances the activity of Notch signal and improves neurogenesis. However, the upstream mechanisms in regulating miR-124 after stroke remain to be explored. More excitingly, a recent study has shown that administration of synthesized exosomes carrying miR-124 promotes neuronal differentiation of cortical NSCs after stroke (Yang *et al.*, 2017a). This suggests that miRs can be carried within exosomes and used as a therapeutic drug to treat neural diseases.

MiR-17-92 cluster: The miR-17-92 cluster includes six miRs (miR-17, miR-18a, miR-19a, miR-20a, miR19b, and miR-92) on human chromosome 13q31.3 (Xiao *et al.*, 2008; Yang *et al.*, 2017b). Stroke upregulates expression of miR-17-92 cluster in SVZ cells, especially miR-18a, miR-19a and miR-19b (Liu *et al.*, 2013). Moreover, miR-18 and miR-19a mimics significantly increase the SCZ cell proliferation after stroke. A study has also shown that the phosphatase and tensin homolog (PTEN) is a direct target of miR-17-92 cluster, and a decrease in PTEN protein levels elevate cell proliferation (Liu *et al.*, 2013). Furthermore, intravenous infusion of miR17-92 cluster-enriched exosomes enhances neurogenesis and oligodendrogenesis after stroke, as well as improvement of neurological behaviors (Xin *et al.*, 2017).

MiR-210: MiR-210 is so far the only miR that has been reported robustly induced by hypoxia in all cell types studied (Chan and Loscalzo, 2010). Brain miR-210 levels increase 24 hours after ischemic stroke. However, the role of miR-210 in post-stroke neurogenesis is still elusive. Using lentiviral vector to overexpress miR-210 in normal mice brain for 28 days, Zeng *et al.* reported that cell proliferation in the SVZ was significantly increased (Zeng *et al.*, 2014). A recent study by Volobouéva *et al.* found that reducing cellular miR-210 dramatically attenuated proliferation of cultured neural progenitor cells under inflammatory condition (Volobouéva *et al.*, 2017). Currently, there is no solid evidence yet to show that miR-210 promotes post-stroke neurogenesis, thus whether it could be a potential neurogenic candidate remains to be determined.

DNA methylation and histone modification: Although little is known about the relationship between these two types of epigenetic regulation with post-stroke neurogenesis, DNA methylation and histone modifications are indeed involved in stroke development and neurogenesis under normal condition. For example, levels of DNA methylation are elevated in ischemic striatum and cortex 24 hours after cerebral ischemia (Endres *et al.*, 2000), and the activity of DNA methyltransferase (DNMT) is negatively correlated with the stroke outcome. Inhibition of DNMT reduces ischemia-induced lesion volume (Endres *et al.*, 2000). Furthermore, neuronal differentiation of NSCs is decreased after genetic deleting of Methyl-CpG binding protein 1 (MBD1), a protein participating in DNA methylation-mediated gene repression (Zhao *et al.*, 2003). Recently, the epigenetic mechanisms on neuroplasticity after stroke has been reviewed (Felling and Song, 2015), providing further detailed discussion on this topic.

4.3 Direct neuronal reprogramming in vivo

Beside enhancing endogenous NSC to neurogenesis after injury, another emerging strategy is in vivo direct neuronal reprogramming. Although this technology is still in its infancy, it has already shed light and shown the promise on the brain repair after injury. The concept of direct reprogramming is partially inspired by the breakthrough of induced pluripotent stem cells technique, which demonstrates that pluripotent stem cells can be induced from skin fibroblast cells by only four transcription factors (Oct4, Sox2, Klf4, and c-Myc) (Takahashi and Yamanaka, 2006). Moreover, the induced pluripotent stem cells (iPSCs) are able to differentiate into various cell types, including neurons (Hu et al., 2010; Kim et al., 2011). The direct reprogramming could be regarded as a shortcut to directly acquire terminally differentiated cells from fibroblasts or other cell types bypassing the stage of PSC. Indeed, several pioneering studies have provided proof of evidence that cultured fibroblast and glial cells can be converted into neurons under neurogenetic transcription factors, like Achaete-scute complex homolog 1 (Ascl1) (Vierbuchen et al., 2010), paired box gene 6 (Pax6) (Heins et al., 2002), and neurogenin2 (Neurog2) (Berninger et al., 2007; Heinrich et al., 2010). Remarkably, the induced neuron not only is neural markers (e.g., MAP-2, Tuj-1) positive but also has the electrophysiological function (Berninger *et al.*, 2007; Vierbuchen *et al.*, 2010), as well as the synaptic connection with other cultured neurons (Vierbuchen *et al.*, 2010).

The great success of in vitro direct neuronal reprogramming encourages the transfer of this technique to in vivo studies, as the in vivo direct reprogramming has several unique advantages for brain repair. Firstly, this method avoids the ethical and immune-rejection problem associated with exogenous cell transplantation. Secondly, endogenous glial cells provide abundant cells to target, unlike cell graft that are limited by the cell resource and inadequacy of available cell numbers. However, the transfer of in vitro reprogramming to in vivo application is not straightforward. The efficacy of same transcriptional factors for direct reprogramming is quite different in vitro and in vivo. For example, Pax6 sufficiently converts astrocytes into neurons in vitro (Heins *et al.*, 2002), while very few induced neurons are observed after Pax6 is transduced into glial cells in mouse cortex and striatum (Grande *et al.*, 2013). Moreover, the brain region is another factor to affect the reprogramming efficacy. Striatum and neocortex resident cells show distinct responses for the combination of growth factors (GFs) and Neurog2 transfection; more NeuN positive neurons are induced in the

striatum after infection (Grande *et al.*, 2013). Furthermore, the environment of the brain is also critical. It has been reported that the brain with ischemic injury promotes the generation of induced neurons, as compared to the normal brain under the same treatment with GFs and Neurog2 (Grande *et al.*, 2013). Meanwhile, another big concern is the virus vector used for direct reprogramming. Currently, most of the studies, including both in vitro and in vivo experiments, rely on the virus vectors to express the neurogenic transcriptional factors. This may increase safety concerns when translating the technique into the clinical study. An alternate method is using small molecular compounds, which could avoid cerebral injection of the virus and easily offer widespread reprogramming in the injured brain. Substantial evidence indicates that sequentially applying a cocktail of chemical compounds, including nine small molecules (LDN193189, SB431542, TTNPB, Tzv, CHIR99021, VPA, DAPT, SAG, and Purmo), are able to reprogram the cultured astrocytes into neurons with fully electrophysiological function (Zhang *et al.*, 2015). However, the combination of multiple molecules may increase the complexity of this technique and affect the feasibility to transfer it to the clinical application. Thus, several obstacles still need to overcome for using molecules to reprogram glia in vivo, like how to optimize the combination of chemical compounds, and how to efficiently deliver these molecules to the target brain area. Even though many technical questions still need to be answered, in vivo direct reprogramming glial cells into neurons open a novel and attractive avenue for brain repair, as emerging studies demonstrate that in vivo induced neurons functionally mature and form synaptic connection to other existing neurons (Pereira et al., 2017; Zhang *et al.*, 2015).

Although applying in vivo glia-neuron reprogramming to repair brain damage after stroke is still in the early stage, other brain injury studies using the brain stab wound model have already demonstrated the feasibility of direct neuronal reprogramming for brain repair (Grande *et al.*, 2013). Although direct neuronal reprogramming has made a significant progress in the last decade, some major challenges still lay ahead, in order to make this approach more efficient and practical for the future. Firstly, the average neuronal converting and survival rate is low under previously reported protocols. Surprisingly, less than 25% resident cortex cells have been reprogramed to neurons in the stab wound brain injury model 7 days after infection, and the estimated neuronal replacement rate is only 3.2% one month later (Grande *et al.*, 2013). Recent studies provide the evidence that these challenges could be overcome by combining neurotrophic factors or anti-apoptotic factors with transfection of the neurogenic transcriptional factor. For example, co-expression of Bcl-2 facilitates the astrocyte-neuron conversation and increases the number of final survived neurons (Gascon *et al.*, 2016). To mend the injured brain, we still need to figure out the approach that guides the endogenous glial cells to reprogram into specific neuron subtypes and to form functional neuronal circuits between different subtypes of neurons. This is especially important for the neurological disease like stroke, which affects a wide range of the brain and damages multiple kinds of neurons. Last but not least, the functional recovery is the final goal of neuronal replacement/regeneration therapy, including in vivo direct reprogramming. Without evidence of functional improvement, it will be difficult to translate this therapy to the clinical application.

5. Conclusion remarks

Considering the limited treatment option for both neonatal HI brain injury and stroke in the adult, there is an urgent need to explore new effective treatment strategies to protect the brain and promote the neurological recovery after injury. Although we have witnessed the failure in development of thousands neuroprotective drugs, we cannot simply conclude that neuroprotection is unattainable in stroke patients. The reality may suggest that regulating single treatment target could be insufficient to achieve clinical relevant neuroprotection. In contrast, the NSC therapy is multi-targeting, and NSCs have several unique beneficial characteristics, including migration to the brain lesion and secretion of angiogenic and neurotrophic factors. All of these cannot be achieved at the same time by traditional single drug administration. Furthermore, solid evidence from pre-clinical studies has proven that the NSC therapy holds the positive therapeutic potential, with both strategies - enhancing endogenous neurogenesis and transplanting NSCs. However, several basic questions remain to be elucidated, in order to better understand the NSC therapy. For example, it is unclear at present that why post-injury neurogenesis is a transient process. What are the factor(s) or mechanism(s) that inhibit the survival of most engrafted NSCs? How do we promote engrafted NSCs integrating into the host neural network? In addition, there are many other questions related to cell transplantation methodologies. The optimal route/dosage/timing merits further investigations in order to better translate the promising cell therapy to the clinical setting. Of importance, as the personalized treatment becomes the direction of modern medicine, understanding the strategy how to set up specific NSC therapy for individual stroke patient will be necessary for the future investigation.

Acknowledgments

This work was supported in part by National Institutes of Health Grants HL083966 (LZ), HL118861 (LZ) and NS103017 (LZ). We apologize to those authors whose excellent studies covered by the scope of this review were unable to be cited due to space restriction.

Abbreviations

NSCs	neural stem cells
HI	hypoxic-ischemic
SVZ	subventricular zone
SGZ	subgranular zone
NE	neuroepithelial
RG	radial glial
GLAST	glutamate transporter
GFAP	glial fibrillary acidic protein
VZ	ventricular zone
nIPC	intermediate neural progenitor cell

INM	interkinetic nuclear migration
GSK-3	glycogen synthase kinase 3
miRs	micro RNAs
CSF-1	Colony Stimulating Factor-1
FGF	fibroblast growth factor
OB	olfactory bulb
RMS	rostral migratory stream
BNIP3	bcl-2/adenovirus E1B 19 kDa interacting protein-3
MP2s	multipotential progenitors
GRPs	glia-restricted progenitors
tPA	tissue plasminogen activator
SDF-1	stromal cell-derived factor-1
ESC	embryonic stem cell
iPSC-NSC	induced pluripotent stem cell-derived NSC
HBO	hyperbaric oxygen
EPO	erythropoietin
AEPO	asialo-EPO
OPCs	oligodendrocyte progenitor cells
CC	corpus callosum
BDNF	brain-derived neurotrophic factor
EGF	epidermal growth factor
BBB	blood-brain-barrier
VEGF	vascular endothelial growth factor
NGF	nerve growth factor
rAAV	recombinant adeno-associated virus
JAG1	jagged 1
Dll	delta-like
Shh	Sonic hedgehog
SAG	Shh signaling agonist

Wnt	Wingless-type
SRF	serum response factor
PTEN	phosphatase and tensin homolog
DNMT	DNA methyltransferase
MBD1	Methyl-CpG binding protein 1

References

Ables JL, Breunig JJ, Eisch AJ, Rakic P. 2011; Not(ch) just development: Notch signalling in the adult brain. Nature reviews. Neuroscience. 12:269–283. [PubMed: 21505516]

Agnihotri SK, Shen R, Li J, Gao X, Bueler H. 2017Loss of PINK1 leads to metabolic deficits in adult neural stem cells and impedes differentiation of newborn neurons in the mouse hippocampus. FASEB journal: official publication of the Federation of American Societies for Experimental Biology.

Ahn S, Joyner AL. 2005; In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. Nature. 437:894–897. [PubMed: 16208373]

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. 2002; Neuronal replacement from endogenous precursors in the adult brain after stroke. Nature medicine. 8:963–970.

Ashwal S, Ghosh N, Turenius CI, Dulcich M, Denham CM, Tone B, Hartman R, Snyder EY, Obenaus A. 2014; Reparative effects of neural stem cells in neonatal rats with hypoxic-ischemic injury are not influenced by host sex. Pediatric research. 75:603–611. [PubMed: 24463490]

Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. 2003; Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 17:126–140. [PubMed: 12514105]

Bacigaluppi M, Russo GL, Peruzzotti-Jametti L, Rossi S, Sandrone S, Butti E, De Ceglia R, Bergamaschi A, Motta C, Gallizioli M, Studer V, Colombo E, Farina C, Comi G, Politi LS, Muzio L, Villani C, Invernizzi RW, Hermann DM, Centonze D, Martino G. 2016; Neural Stem Cell Transplantation Induces Stroke Recovery by Upregulating Glutamate Transporter GLT-1 in Astrocytes. Journal of Neuroscience. 36:10529–10544. [PubMed: 27733606]

Bain JM, Moore L, Ren Z, Simonishvili S, Levison SW. 2013; Vascular endothelial growth factors A and C are induced in the SVZ following neonatal hypoxia-ischemia and exert different effects on neonatal glial progenitors. Translational stroke research. 4:158–170. [PubMed: 23565129]

Baldauf K, Reymann KG. 2005; Influence of EGF/bFGF treatment on proliferation, early neurogenesis and infarct volume after transient focal ischemia. Brain research. 1056:158–167. [PubMed: 16125154]

Bambakidis NC, Petrullis M, Kui X, Rothstein B, Karampelas I, Kuang YZ, Selman WR, LaManna JC, Miller RH. 2012; Improvement of neurological recovery and stimulation of neural progenitor cell proliferation by intrathecal administration of Sonic hedgehog. Journal of neurosurgery. 116:1114–1120. [PubMed: 22324418]

Barde YA. 1989; Trophic factors and neuronal survival. Neuron. 2:1525–1534. [PubMed: 2697237]

Beleslin-Cokic BB, Cokic VP, Yu X, Weksler BB, Schechter AN, Noguchi CT. 2004; Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. Blood. 104:2073–2080. [PubMed: 15205261]

Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jimenez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P. 2017; Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation. 135:e146–e603. [PubMed: 28122885]

Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N. 2003; Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Molecular and cellular neurosciences. 24:623–631. [PubMed: 14664813]

Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, Gotz M. 2007; Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. The Journal of neuroscience: the official journal of the Society for Neuroscience. 27:8654–8664. [PubMed: 17687043]

Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. 2004; Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke; a journal of cerebral circulation. 35:2385–2389.

Boyd SD. 2008; Everything you wanted to know about small RNA but were afraid to ask. Lab Invest. 88:569–578. [PubMed: 18427554]

Braccioli L, Heijnen CJ, Coffer PJ, Nijboer CH. 2017; Delayed administration of neural stem cells after hypoxia-ischemia reduces sensorimotor deficits, cerebral lesion size, and neuroinflammation in neonatal mice. Pediatric research. 81:127–135. [PubMed: 27632779]

Buhnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, Reymann KG, Dihne M. 2006; Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. Brain: a journal of neurology. 129:3238–3248. [PubMed: 17018551]

Buller B, Chopp M, Ueno Y, Zhang L, Zhang RL, Morris D, Zhang Y, Zhang ZG. 2012; Regulation of serum response factor by miRNA-200 and miRNA-9 modulates oligodendrocyte progenitor cell differentiation. Glia. 60:1906–1914. [PubMed: 22907787]

Buono KD, Goodus MT, Guardia Clausi M, Jiang Y, Loporchio D, Levison SW. 2015; Mechanisms of mouse neural precursor expansion after neonatal hypoxia-ischemia. The Journal of neuroscience: the official journal of the Society for Neuroscience. 35:8855–8865. [PubMed: 26063918]

Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM. 2003; Becoming a new neuron in the adult olfactory bulb. Nature neuroscience. 6:507–518. [PubMed: 12704391]

Chan SY, Loscalzo J. 2010; MicroRNA-210 A unique and pleiotropic hypoxamir. Cell Cycle. 9:1072–1083. [PubMed: 20237418]

Chang DJ, Lee N, Choi C, Jeon I, Oh SH, Shin DA, Hwang TS, Lee HJ, Kim SU, Moon H, Hong KS, Kang KS, Song J. 2013; Therapeutic Effect of BDNF- Overexpressing Human Neural Stem Cells (HB1.F3.BDNF) in a Rodent Model of Middle Cerebral Artery Occlusion. Cell transplantation. 22:1441–1452. [PubMed: 23044072]

Chen LK, Zhang GL, Gu YC, Guo XY. 2016Meta-Analysis and Systematic Review of Neural Stem Cells therapy for experimental ischemia stroke in preclinical studies. Scientific Reports. :6. [PubMed: 28442741]

Chen ZY, Asavaritikrai P, Prchal JT, Noguchi CT. 2007; Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. The Journal of biological chemistry. 282:25875–25883. [PubMed: 17604282]

Cheng LC, Pastrana E, Tavazoie M, Doetsch F. 2009; miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. Nature neuroscience. 12:399–408. [PubMed: 19287386]

Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X, Zhang J, Moseley ME, Guzman R. 2011; Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. Journal of Cerebral Blood Flow and Metabolism. 31:1263–1271. [PubMed: 21157474]

Coleman TR, Westenfelder C, Togel FE, Yang Y, Hu Z, Swenson L, Leuvenink HG, Ploeg RJ, d'Uscio LV, Katusic ZS, Ghezzi P, Zanetti A, Kaushansky K, Fox NE, Cerami A, Brines M. 2006; Cytoprotective doses of erythropoietin or carbamylated erythropoietin have markedly different procoagulant and vasoactive activities. Proceedings of the National Academy of Sciences of the United States of America. 103:5965–5970. [PubMed: 16585502]

Cougo-Pinto PT, Chandra RV, Simonsen CZ, Hirsch JA, Leslie-Mazwi T. 2015Intra-Arterial Therapy for Acute Ischemic Stroke: a Golden Age. Curr Treat Option Ne. :17.

Covey MV, Levison SW. 2007; Leukemia inhibitory factor participates in the expansion of neural stem/progenitors after perinatal hypoxia/ischemia. Neuroscience. 148:501–509. [PubMed: 17664044]

Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, Jiang K, Sun G, Wu JC, Steinberg GK. 2010; Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. Stroke; a journal of cerebral circulation. 41:516–523.

Daadi MM, Li ZJ, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK. 2009; Molecular and Magnetic Resonance Imaging of Human Embryonic Stem Cell-Derived Neural Stem Cell Grafts in Ischemic Rat Brain. Molecular Therapy. 17:1282–1291. [PubMed: 19436269]

Daadi MM, Maag AL, Steinberg GK. 2008; Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PloS one. 3:e1644. [PubMed: 18286199]

Darsalia V, Allison SJ, Cusulin C, Monni E, Kuzdas D, Kallur T, Lindvall O, Kokaia Z. 2011; Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 31:235–242.

Dee A, Li K, Heng X, Guo Q, Li JY. 2016; Regulation of self-renewing neural progenitors by FGF/ERK signaling controls formation of the inferior colliculus. Development. 143:3661–3673. [PubMed: 27578777]

Dehay C, Kennedy H, Kosik KS. 2015; The outer subventricular zone and primate-specific cortical complexification. Neuron. 85:683–694. [PubMed: 25695268]

Delaloy C, Liu L, Lee JA, Su H, Shen FX, Yang GY, Young WL, Ivey KN, Gao FB. 2010; MicroRNA-9 Coordinates Proliferation and Migration of Human Embryonic Stem Cell-Derived Neural Progenitors. Cell stem cell. 6:323–335. [PubMed: 20362537]

Deng J, Lei C, Chen Y, Fang Z, Yang Q, Zhang H, Cai M, Shi L, Dong H, Xiong L. 2014; Neuroprotective gases--fantasy or reality for clinical use? Progress in neurobiology. 115:210–245. [PubMed: 24440817]

Dmitrieva VG, Stavchansky VV, Povarova OV, Skvortsova VI, Limborska SA, Dergunova LV. 2016; Effects of ischemia on the expression of neurotrophins and their receptors in rat brain structures outside the lesion site, including on the opposite hemisphere. Molecular Biology. 50:684–692.

Doeppner TR, Kaltwasser B, Fengyan J, Hermann DM, Bahr M. 2013; TAT-Hsp70 induces neuroprotection against stroke via anti-inflammatory actions providing appropriate cellular microenvironment for transplantation of neural precursor cells. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 33:1778–1788.

Doeppner TR, Kaltwasser B, Teli MK, Bretschneider E, Bahr M, Hermann DM. 2014Effects of acute versus post-acute systemic delivery of neural progenitor cells on neurological recovery and brain remodeling after focal cerebral ischemia in mice. Cell death & disease. :5.

Doeppner TR, Traut V, Heidenreich A, Kaltwasser B, Bosche B, Bahr M, Hermann DM. 2017; Conditioned Medium Derived from Neural Progenitor Cells Induces Long-term Post-ischemic Neuroprotection, Sustained Neurological Recovery, Neurogenesis, and Angiogenesis. Molecular neurobiology. 54:1531–1540. [PubMed: 26860410]

Dong Z, Yang N, Yeo SY, Chitnis A, Guo S. 2012; Intralineage directional Notch signaling regulates self-renewal and differentiation of asymmetrically dividing radial glia. Neuron. 74:65–78. [PubMed: 22500631]

Draganova K, Zemke M, Zurkirchen L, Valenta T, Cantu C, Okoniewski M, Schmid MT, Hoffmans R, Gotz M, Basler K, Sommer L. 2015; Wnt/beta-catenin signaling regulates sequential fate decisions of murine cortical precursor cells. Stem cells (Dayton, Ohio). 33:170–182.

Eckert A, Huang L, Gonzalez R, Kim HS, Hamblin MH, Lee JP. 2015; Bystander Effect Fuels Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells to Quickly Attenuate Early Stage Neurological Deficits After Stroke. Stem cells translational medicine. 4:841–851. [PubMed: 26025980]

Endres M, Meisel A, Biniszkiewicz D, Namura S, Prass K, Ruscher K, Lipski A, Jaenisch R, Moskowitz MA, Dirnagl U. 2000; DNA methyltransferase contributes to delayed ischemic brain injury. The Journal of neuroscience: the official journal of the Society for Neuroscience. 20:3175–3181. [PubMed: 10777781]

Fajersztajn L, Veras MM. 2017; Hypoxia: From Placental Development to Fetal Programming. Birth Defects Res. 109:1377–1385. [PubMed: 29105382]

Faigle R, Song H. 2013; Signaling mechanisms regulating adult neural stem cells and neurogenesis. Biochimica et biophysica acta. 1830:2435–2448. [PubMed: 22982587]

Fatemi A, Wilson MA, Johnston MV. 2009; Hypoxic-ischemic encephalopathy in the term infant. Clinics in perinatology. 36:835–858. vii. [PubMed: 19944838]

Feliciano DM, Bordey A, Bonfanti L. 2015; Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain. Cold Spring Harb Perspect Biol. 7:a018846. [PubMed: 26384869]

Felling RJ, Covey MV, Wolujewicz P, Batish M, Levison SW. 2016; Astrocyte-produced leukemia inhibitory factor expands the neural stem/progenitor pool following perinatal hypoxia-ischemia. Journal of neuroscience research. 94:1531–1545. [PubMed: 27661001]

Felling RJ, Snyder MJ, Romanko MJ, Rothstein RP, Ziegler AN, Yang Z, Givogri MI, Bongarzone ER, Levison SW. 2006; Neural stem/progenitor cells participate in the regenerative response to perinatal hypoxia/ischemia. The Journal of neuroscience: the official journal of the Society for Neuroscience. 26:4359–4369. [PubMed: 16624956]

Felling RJ, Song H. 2015; Epigenetic mechanisms of neuroplasticity and the implications for stroke recovery. Experimental neurology. 268:37–45. [PubMed: 25263580]

Fernandez-Lopez D, Natarajan N, Ashwal S, Vexler ZS. 2014; Mechanisms of perinatal arterial ischemic stroke. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 34:921–932.

Fradkin LG, Garriga G, Salinas PC, Thomas JB, Yu X, Zou Y. 2005; Wnt signaling in neural circuit development. The Journal of neuroscience: the official journal of the Society for Neuroscience. 25:10376–10378. [PubMed: 16280576]

Gascon S, Murenu E, Masserdotti G, Ortega F, Russo GL, Petrik D, Deshpande A, Heinrich C, Karow M, Robertson SP, Schroeder T, Beckers J, Irmler M, Berndt C, Angeli JP, Conrad M, Berninger B, Gotz M. 2016; Identification and Successful Negotiation of a Metabolic Checkpoint in Direct Neuronal Reprogramming. Cell stem cell. 18:396–409. [PubMed: 26748418]

George PM, Steinberg GK. 2015; Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. Neuron. 87:297–309. [PubMed: 26182415]

Givogri MI, de Planell M, Galbiati F, Superchi D, Gritti A, Vescovi A, de Vellis J, Bongarzone ER. 2006; Notch signaling in astrocytes and neuroblasts of the adult subventricular zone in health and after cortical injury. Developmental neuroscience. 28:81–91. [PubMed: 16508306]

Gonzales-Portillo GS, Reyes S, Aguirre D, Pabon MM, Borlongan CV. 2014; Stem cell therapy for neonatal hypoxic-ischemic encephalopathy. Frontiers in neurology. 5:147. [PubMed: 25161645]

Gotz M, Huttner WB. 2005; The cell biology of neurogenesis. Nat Rev Mol Cell Biol. 6:777–788. [PubMed: 16314867]

Grande A, Sumiyoshi K, Lopez-Juarez A, Howard J, Sakthivel B, Aronow B, Campbell K, Nakafuku M. 2013; Environmental impact on direct neuronal reprogramming in vivo in the adult brain. Nat Commun. 4:2373. [PubMed: 23974433]

Greenberg DA, Jin K. 2013; Vascular endothelial growth factors (VEGFs) and stroke. Cellular and molecular life sciences: CMLS. 70:1753–1761. [PubMed: 23475070]

Hao L, Zou Z, Tian H, Zhang Y, Zhou H, Liu L. 2014; Stem cell-based therapies for ischemic stroke. BioMed research international. 2014:468748. [PubMed: 24719869]

Haubensak W, Attardo A, Denk W, Huttner WB. 2004; Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. Proceedings of the National Academy of Sciences of the United States of America. 101:3196–3201. [PubMed: 14963232]

Hayashi T, Iwai M, Ikeda T, Jin G, Deguchi K, Nagotani S, Zhang H, Sehara Y, Nagano I, Shoji M, Ikenoue T, Abe K. 2005; Neural precursor cells division and migration in neonatal rat brain after ischemic/hypoxic injury. Brain research. 1038:41–49. [PubMed: 15748871]

Heinrich C, Blum R, Gascon S, Masserdotti G, Tripathi P, Sanchez R, Tiedt S, Schroeder T, Gotz M, Berninger B. 2010Directing Astroglia from the Cerebral Cortex into Subtype Specific Functional Neurons. Plos Biology. :8.

Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M. 2002; Glial cells generate neurons: the role of the transcription factor Pax6. Nature neuroscience. 5:308–315. [PubMed: 11896398]

Hicks C, Stevanato L, Stroemer RP, Tang E, Richardson S, Sinden JD. 2013; In Vivo and In Vitro Characterization of the Angiogenic Effect of CTX0E03 Human Neural Stem Cells. Cell transplantation. 22:1541–1552. [PubMed: 23067568]

Hirota Y, Sawada M, Huang SH, Ogino T, Ohata S, Kubo A, Sawamoto K. 2016; Roles of Wnt Signaling in the Neurogenic Niche of the Adult Mouse Ventricular-Subventricular Zone. Neurochemical research. 41:222–230. [PubMed: 26572545]

Homem CC, Repic M, Knoblich JA. 2015; Proliferation control in neural stem and progenitor cells. Nature reviews. Neuroscience. 16:647–659. [PubMed: 26420377]

Hori J, Ng TF, Shatos M, Klassen H, Streilein JW, Young MJ. 2003; Neural progenitor cells lack immunogenicity and resist destruction as allografts. Stem cells (Dayton, Ohio). 21:405–416.

Howard G, Goff DC. 2012; Population shifts and the future of stroke: forecasts of the future burden of stroke. Annals of the New York Academy of Sciences. 1268:14–20. [PubMed: 22994216]

Hu BY, Weick JP, Yu J, Ma LX, Zhang XQ, Thomson JA, Zhang SC. 2010; Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proceedings of the National Academy of Sciences of the United States of America. 107:4335–4340. [PubMed: 20160098]

Huang L, Wong S, Snyder EY, Hamblin MH, Lee JP. 2014; Human neural stem cells rapidly ameliorate symptomatic inflammation in early-stage ischemic-reperfusion cerebral injury. Stem cell research & therapy. 5:129. [PubMed: 25418536]

Hutton SR, Pevny LH. 2011; SOX2 expression levels distinguish between neural progenitor populations of the developing dorsal telencephalon. Dev Biol. 352:40–47. [PubMed: 21256837]

Im SH, Yu JH, Park ES, Lee JE, Kim HO, Park KI, Kim GW, Park CI, Cho SR. 2010; Induction of striatal neurogenesis enhances functional recovery in an adult animal model of neonatal hypoxic-ischemic brain injury. Neuroscience. 169:259–268. [PubMed: 20610036]

Imayoshi I, Isomura A, Harima Y, Kawaguchi K, Kori H, Miyachi H, Fujiwara T, Ishidate F, Kageyama R. 2013; Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. Science. 342:1203–1208. [PubMed: 24179156]

Irion S, Zabierowski SE, Tomishima MJ. 2017; Bringing Neural Cell Therapies to the Clinic: Past and Future Strategies. Mol Ther Methods Clin Dev. 4:72–82. [PubMed: 28344993]

Ito K, Suda T. 2014; Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol. 15:243–256. [PubMed: 24651542]

Iwai M, Cao G, Yin W, Stetler RA, Liu J, Chen J. 2007; Erythropoietin promotes neuronal replacement through revascularization and neurogenesis after neonatal hypoxia/ischemia in rats. Stroke; a journal of cerebral circulation. 38:2795–2803.

Iwai M, Stetler RA, Xing J, Hu X, Gao Y, Zhang W, Chen J, Cao G. 2010; Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. Stroke; a journal of cerebral circulation. 41:1032–1037.

Ji G, Liu M, Zhao XF, Liu XY, Guo QL, Guan ZF, Zhou HG, Guo JC. 2015; NF-kappaB Signaling is Involved in the Effects of Intranasally Engrafted Human Neural Stem Cells on Neurofunctional Improvements in Neonatal Rat Hypoxic-Ischemic Encephalopathy. CNS neuroscience & therapeutics. 21:926–935. [PubMed: 26255634]

Jiao Q, Li X, An J, Zhang Z, Chen X, Tan J, Zhang P, Lu H, Liu Y. 2017; Cell-Cell Connection Enhances Proliferation and Neuronal Differentiation of Rat Embryonic Neural Stem/Progenitor Cells. Frontiers in cellular neuroscience. 11:200. [PubMed: 28785204]

Jiang XN, Nardelli J. 2016; Cellular and molecular introduction to brain development. Neurobiology of disease. 92:3–17. [PubMed: 26184894]

Jin K, Mao X, Xie L, Galvan V, Lai B, Wang Y, Gorostiza O, Wang X, Greenberg DA. 2010a; Transplantation of human neural precursor cells in Matrigel scaffolding improves outcome from focal cerebral ischemia after delayed postischemic treatment in rats. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 30:534–544.

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA. 2001; Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proceedings of the National Academy of Sciences of the United States of America. 98:4710–4715. [PubMed: 11296300]

Jin K, Sun Y, Xie L, Mao XO, Childs J, Peel A, Logvinova A, Banwait S, Greenberg DA. 2005; Comparison of ischemia-directed migration of neural precursor cells after intrastriatal, intraventricular, or intravenous transplantation in the rat. Neurobiology of disease. 18:366–374. [PubMed: 15686965]

Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, Greenberg DA. 2003; Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Molecular and cellular neurosciences. 24:171–189. [PubMed: 14550778]

Jin K, Wang X, Xie L, Mao XO, Greenberg DA. 2010b; Transgenic ablation of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. Proceedings of the National Academy of Sciences of the United States of America. 107:7993–7998. [PubMed: 20385829]

Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, Shen J, Mao Y, Banwait S, Greenberg DA. 2006; Evidence for stroke-induced neurogenesis in the human brain. Proceedings of the National Academy of Sciences of the United States of America. 103:13198–13202. [PubMed: 16924107]

Jin K, Xie L, Mao X, Greenberg MB, Moore A, Peng B, Greenberg RB, Greenberg DA. 2011; Effect of human neural precursor cell transplantation on endogenous neurogenesis after focal cerebral ischemia in the rat. Brain research. 1374:56–62. [PubMed: 21167824]

Jin KL, Mao XO, Sun YJ, Xie L, Jin L, Nishi E, Klagsburn M, Greenberg DA. 2002; Heparin-binding epidermal growth factor-like growth factor: Hypoxia-inducible expression in vitro and stimulation of neurogenesis in vitro and in vivo. Journal of Neuroscience. 22:5365–5373. [PubMed: 12097488]

Jin YM, Raviv N, Barnett A, Bambakidis NC, Filichia E, Luo Y. 2015The Shh Signaling Pathway Is Upregulated in Multiple Cell Types in Cortical Ischemia and Influences the Outcome of Stroke in an Animal Model. PloS one. :10.

Johnston MV, Trescher WH, Ishida A, Nakajima W. 2001; Neurobiology of hypoxic-ischemic injury in the developing brain. Pediatric research. 49:735–741. [PubMed: 11385130]

Kako E, Kaneko N, Aoyama M, Hida H, Takebayashi H, Ikenaka K, Asai K, Togari H, Sobue K, Sawamoto K. 2012; Subventricular zone-derived oligodendrogenesis in injured neonatal white matter in mice enhanced by a nonerythropoietic erythropoietin derivative. Stem cells (Dayton, Ohio). 30:2234–2247.

Kanagawa T, Fukuda H, Tsubouchi H, Komoto Y, Hayashi S, Fukui O, Shimoya K, Murata Y. 2006; A decrease of cell proliferation by hypothermia in the hippocampus of the neonatal rat. Brain research. 1111:36–40. [PubMed: 16904084]

Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK. 2004; Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proceedings of the National Academy of Sciences of the United States of America. 101:11839–11844. [PubMed: 15280535]

Kim DE, Tsuji K, Kim YR, Mueller FJ, Eom HS, Snyder EY, Lo EH, Weissleder R, Schellingerhout D. 2006; Neural stem cell transplant survival in brains of mice: Assessing the effect of immunity and ischemia by using real-time bioluminescent imaging. Radiology. 241:822–830. [PubMed: 17114629]

Kim J, Efe JA, Zhu S, Talantova M, Yuan X, Wang S, Lipton SA, Zhang K, Ding S. 2011; Direct reprogramming of mouse fibroblasts to neural progenitors. Proceedings of the National Academy of Sciences of the United States of America. 108:7838–7843. [PubMed: 21521790]

Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR, Snider WD. 2009; GSK-3 is a master regulator of neural progenitor homeostasis. Nature neuroscience. 12:1390–1397. [PubMed: 19801986]

Koch JD, Miles DK, Gilley JA, Yang CP, Kernie SG. 2008; Brief exposure to hyperoxia depletes the glial progenitor pool and impairs functional recovery after hypoxic-ischemic brain injury. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 28:1294–1306.

Kokovay E, Goderie S, Wang Y, Lotz S, Lin G, Sun Y, Roysam B, Shen Q, Temple S. 2010; Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. Cell stem cell. 7:163–173. [PubMed: 20682445]

Kriegstein A, Alvarez-Buylla A. 2009; The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci. 32:149–184. [PubMed: 19555289]

Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH. 1997; Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. The Journal of neuroscience: the official journal of the Society for Neuroscience. 17:5820–5829. [PubMed: 9221780]

Kumon Y, Sakaki S, Kadota O, Matsuda S, Fujita H, Yoshimura H, Sakanaka M. 1993; Transient increase in endogenous basic fibroblast growth factor in neurons of ischemic rat brains. Brain research. 605:169–174. [PubMed: 8467385]

Kurinczuk JJ, White-Koning M, Badawi N. 2010; Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev. 86:329–338. [PubMed: 20554402]

Kwak M, Lim S, Kang E, Furmanski O, Song H, Ryu YK, Mintz CD. 2015; Effects of Neonatal Hypoxic-Ischemic Injury and Hypothermic Neuroprotection on Neural Progenitor Cells in the Mouse Hippocampus. Developmental neuroscience. 37:428–439. [PubMed: 26087836]

Larsson E, Mandel RJ, Klein RL, Muzyczha N, Lindvall O, Kokaia Z. 2002; Suppression of insult-induced neurogenesis in adult rat brain by brain-derived neurotrophic factor. Experimental neurology. 177:1–8. [PubMed: 12429205]

Laterza C, Wattananit S, Uoshima N, Ge R, Pekny R, Tornero D, Monni E, Lindvall O, Kokaia Z. 2017; Monocyte depletion early after stroke promotes neurogenesis from endogenous neural stem cells in adult brain. Experimental neurology. 297:129–137. [PubMed: 28746827]

Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S. 2010; 3.6 million neonatal deaths--what is progressing and what is not? Semin Perinatol. 34:371–386. [PubMed: 21094412]

Lee IS, Jung K, Kim M, Park KI. 2010; Neural stem cells: properties and therapeutic potentials for hypoxic-ischemic brain injury in newborn infants. Pediatrics international: official journal of the Japan Pediatric Society. 52:855–865. [PubMed: 21029253]

Lei ZN, Zhang LM, Sun FY. 2008; Beta-catenin siRNA inhibits ischemia-induced striatal neurogenesis in adult rat brain following a transient middle cerebral artery occlusion. Neuroscience letters. 435:108–112. [PubMed: 18343578]

Levine S. 1960; Anoxic-ischemic encephalopathy in rats. The American journal of pathology. 36:1–17. [PubMed: 14416289]

Levison SW, Rothstein RP, Romanko MJ, Snyder MJ, Meyers RL, Vannucci SJ. 2001; Hypoxia/ischemia depletes the rat perinatal subventricular zone of oligodendrocyte progenitors and neural stem cells. Developmental neuroscience. 23:234–247. [PubMed: 11598326]

Li L, Harms KM, Ventura PB, Lagace DC, Eisch AJ, Cunningham LA. 2010; Focal cerebral ischemia induces a multilineage cytogenic response from adult subventricular zone that is predominantly gliogenic. Glia. 58:1610–1619. [PubMed: 20578055]

Li Y, Gonzalez P, Zhang L. 2012; Fetal stress and programming of hypoxic/ischemic-sensitive phenotype in the neonatal brain: mechanisms and possible interventions. Progress in neurobiology. 98:145–165. [PubMed: 22627492]

Lin TN, Te J, Lee M, Sun GY, Hsu CY. 1997; Induction of basic fibroblast growth factor (bFGF) expression following focal cerebral ischemia. Brain research. Molecular brain research. 49:255–265. [PubMed: 9387885]

Liu XS, Chopp M, Wang XL, Zhang L, Hozeska-Solgot A, Tang T, Kassis H, Zhang RL, Chen C, Xu J, Zhang ZG. 2013; MicroRNA-17-92 Cluster Mediates the Proliferation and Survival of Neural Progenitor Cells after Stroke. Journal of Biological Chemistry. 288:12478–12488. [PubMed: 23511639]

Liu XS, Chopp M, Zhang RL, Tao T, Wang XL, Kassis H, Hozeska-Solgot A, Zhang L, Chen C, Zhang ZG. 2011MicroRNA Profiling in Subventricular Zone after Stroke: MiR-124a Regulates Proliferation of Neural Progenitor Cells through Notch Signaling Pathway. PloS one. :6.

Lui JH, Hansen DV, Kriegstein AR. 2011; Development and evolution of the human neocortex. Cell. 146:18–36. [PubMed: 21729779]

Marti E, Bovolenta P. 2002; Sonic hedgehog in CNS development: one signal, multiple outputs. Trends in neurosciences. 25:89–96. [PubMed: 11814561]

Masuda T, Ishitani T. 2017; Context-dependent regulation of the beta-catenin transcriptional complex supports diverse functions of Wnt/beta-catenin signaling. J Biochem. 161:9–17. [PubMed: 28013224]

Meng H, Zhang Z, Zhang R, Liu X, Wang L, Robin AM, Chopp M. 2006; Biphasic effects of exogenous VEGF on VEGF expression of adult neural progenitors. Neuroscience letters. 393:97–101. [PubMed: 16269210]

Mengozzi M, Cervellini I, Villa P, Erbayraktar Z, Gokmen N, Yilmaz O, Erbayraktar S, Manohasandra M, Van Hummelen P, Vandenabeele P, Chernajovsky Y, Annenkov A, Ghezzi P. 2012; Erythropoietin-induced changes in brain gene expression reveal induction of synaptic plasticity genes in experimental stroke. Proceedings of the National Academy of Sciences of the United States of America. 109:9617–9622. [PubMed: 22645329]

Miles DK, Kernie SG. 2008; Hypoxic-ischemic brain injury activates early hippocampal stem/progenitor cells to replace vulnerable neuroblasts. Hippocampus. 18:793–806. [PubMed: 18446826]

Minnerup J, Kim JB, Schmidt A, Diederich K, Bauer H, Schilling M, Strecker JK, Ringelstein EB, Sommer C, Scholer HR, Schabitz WR. 2011; Effects of neural progenitor cells on sensorimotor recovery and endogenous repair mechanisms after photothrombotic stroke. Stroke; a journal of cerebral circulation. 42:1757–1763.

Miska EA, Alvarez-Saavedra E, Townsend M, Yoshii A, Sestan N, Rakic P, Constantine-Paton M, Horvitz HR. 2004; Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol. 5:R68. [PubMed: 15345052]

Mizutani K, Yoon K, Dang L, Tokunaga A, Gaiano N. 2007; Differential Notch signalling distinguishes neural stem cells from intermediate progenitors. Nature. 449:351–355. [PubMed: 17721509]

Monje ML, Toda H, Palmer TD. 2003; Inflammatory blockade restores adult hippocampal neurogenesis. Science. 302:1760–1765. [PubMed: 14615545]

Moraga A, Pradillo JM, Garcia-Culebras A, Palma-Tortosa S, Ballesteros I, Hernandez-Jimenez M, Moro MA, Lizasoain I. 2015Aging increases microglial proliferation, delays cell migration, and decreases cortical neurogenesis after focal cerebral ischemia. Journal of Neuroinflammation. :12. [PubMed: 25608762]

Morgado AL, Rodrigues CM, Sola S. 2016; MicroRNA-145 Regulates Neural Stem Cell Differentiation Through the Sox2-Lin28/let-7 Signaling Pathway. Stem cells (Dayton, Ohio). 34:1386–1395.

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB. American Heart Association Statistics C, Stroke Statistics S. 2015; Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation. 131:e29–322. [PubMed: 25520374]

Nakayama D, Matsuyama T, Ishibashi-Ueda H, Nakagomi T, Kasahara Y, Hirose H, Kikuchi-Taura A, Stern DM, Mori H, Taguchi A. 2010; Injury-induced neural stem/progenitor cells in post-stroke human cerebral cortex. European Journal of Neuroscience. 31:90–98. [PubMed: 20104652]

Nandi S, Gokhan S, Dai XM, Wei S, Enikolopov G, Lin H, Mehler MF, Stanley ER. 2012; The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. Dev Biol. 367:100–113. [PubMed: 22542597]

Nelson BR, Hodge RD, Bedogni F, Hevner RF. 2013; Dynamic interactions between intermediate neurogenic progenitors and radial glia in embryonic mouse neocortex: potential role in Dll1-Notch signaling. The Journal of neuroscience: the official journal of the Society for Neuroscience. 33:9122–9139. [PubMed: 23699523]

Ninomiya M, Yamashita T, Hideyuki OE, Okano H, Sawamoto K. 2006; Enhanced neurogenesis in the ischemic striatum following EGF-induced expansion of transit-amplifying cells in the subventricular zone. Neuroscience letters. 403:63–67. [PubMed: 16701951]

Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. 2004; Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nature neuroscience. 7:136–144. [PubMed: 14703572]

Noctor SC, Martinez-Cerdeno V, Kriegstein AR. 2008; Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. The Journal of comparative neurology. 508:28–44. [PubMed: 18288691]

Northington FJ, Zelaya ME, O'Riordan DP, Blomgren K, Flock DL, Hagberg H, Ferriero DM, Martin LJ. 2007; Failure to complete apoptosis following neonatal hypoxia-ischemia manifests as "continuum" phenotype of cell death and occurs with multiple manifestations of mitochondrial dysfunction in rodent forebrain. Neuroscience. 149:822–833. [PubMed: 17961929]

Okamoto M, Namba T, Shinoda T, Kondo T, Watanabe T, Inoue Y, Takeuchi K, Enomoto Y, Ota K, Oda K, Wada Y, Sagou K, Saito K, Sakakibara A, Kawaguchi A, Nakajima K, Adachi T, Fujimori T, Ueda M, Hayashi S, Kaibuchi K, Miyata T. 2013; TAG-1-assisted progenitor elongation streamlines nuclear migration to optimize subapical crowding. Nature neuroscience. 16:1556–1566. [PubMed: 24056697]

Oki K, Tatarishvili J, Wood J, Koch P, Wattananit S, Mine Y, Monni E, Tornero D, Ahlenius H, Ladewig J, Brustle O, Lindvall O, Kokaia Z. 2012; Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. Stem cells (Dayton, Ohio). 30:1120–1133.

Oya S, Yoshikawa G, Takai K, Tanaka JI, Higashiyama S, Saito N, Kirino T, Kawahara N. 2008; Region-specific proliferative response of neural progenitors to exogenous stimulation by growth factors following ischemia. Neuroreport. 19:805–810. [PubMed: 18463491]

Palma V, Lim DA, Dahmane N, Sanchez P, Brionne TC, Herzberg CD, Gitton Y, Carleton A, Alvarez-Buylla A, Ruiz i Altaba A. 2005; Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. Development. 132:335–344. [PubMed: 15604099]

Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. 2002; Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Annals of neurology. 52:802–813. [PubMed: 12447935]

Park DH, Eve DJ, Sanberg PR, Musso J 3rd, Bachstetter AD, Wolfson A, Schlunk A, Baradez MO, Sinden JD, Gemma C. 2010; Increased neuronal proliferation in the dentate gyrus of aged rats following neural stem cell implantation. Stem cells and development. 19:175–180. [PubMed: 19569872]

Pendharkar AV, Chua JY, Andres RH, Wang N, Gaeta X, Wang H, De A, Choi R, Chen S, Rutt BK, Gambhir SS, Guzman R. 2010; Biodistribution of neural stem cells after intravascular therapy for hypoxic-ischemia. Stroke; a journal of cerebral circulation. 41:2064–2070.

Pereira M, Birtele M, Shrigley S, Benitez JA, Hedlund E, Parmar M, Ottosson DR. 2017; Direct Reprogramming of Resident NG2 Glia into Neurons with Properties of Fast-Spiking Parvalbumin-Containing Interneurons. Stem cell reports. 9:742–751. [PubMed: 28844658]

Perlman JM. 2006; Intervention strategies for neonatal hypoxic-ischemic cerebral injury. Clin Ther. 28:1353–1365. [PubMed: 17062309]

Piccin D, Morshead CM. 2011; Wnt signaling regulates symmetry of division of neural stem cells in the adult brain and in response to injury. Stem cells (Dayton, Ohio). 29:528–538.

Pitman M, Emery B, Binder M, Wang S, Butzkueven H, Kilpatrick TJ. 2004; LIF receptor signaling modulates neural stem cell renewal. Molecular and cellular neurosciences. 27:255–266. [PubMed: 15519241]

Plane JM, Liu R, Wang TW, Silverstein FS, Parent JM. 2004; Neonatal hypoxic-ischemic injury increases forebrain subventricular zone neurogenesis in the mouse. Neurobiology of disease. 16:585–595. [PubMed: 15262271]

Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, Dong Z, Hodges H, Price J, Sinden JD. 2006; A conditionally immortal clonal. stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Experimental neurology. 199:143–155. [PubMed: 16464451]

Qureshi AI, Adil MM, Zacharatos H, Suri MF. 2013; Factors associated with length of hospitalization in patients admitted with transient ischemic attack in United States. Stroke; a journal of cerebral circulation. 44:1601–1605.

Reynolds BA, Tetzlaff W, Weiss S. 1992; A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. The Journal of neuroscience: the official journal of the Society for Neuroscience. 12:4565–4574. [PubMed: 1432110]

Ribatti D, Presta M, Vacca A, Ria R, Giuliani R, Dell'Era P, Nico B, Roncali L, Dammacco F. 1999; Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood. 93:2627–2636. [PubMed: 10194442]

Romanko MJ, Rothstein RP, Levison SW. 2004; Neural stem cells in the subventricular zone are resilient to hypoxia/ischemia whereas progenitors are vulnerable. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 24:814–825.

Romanko MJ, Zhu C, Bahr BA, Blomgren K, Levison SW. 2007; Death effector activation in the subventricular zone subsequent to perinatal hypoxia/ischemia. Journal of neurochemistry. 103:1121–1131. [PubMed: 17711427]

Rosenblum S, Smith TN, Wang N, Chua JY, Westbroek E, Wang K, Guzman R. 2015; BDNF Pretreatment of Human Embryonic-Derived Neural Stem Cells Improves Cell Survival and Functional Recovery After Transplantation in Hypoxic-Ischemic Stroke. Cell transplantation. 24:2449–2461. [PubMed: 24594369]

Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, Guzman R. 2012; Timing of intra-arterial neural stem cell transplantation after hypoxia-ischemia influences cell engraftment, survival, and differentiation. Stroke; a journal of cerebral circulation. 43:1624–1631.

Sakata H, Niizuma K, Wakai T, Narasimhan P, Maier CM, Chan PH. 2012a; Neural stem cells genetically modified to overexpress cu/zn-superoxide dismutase enhance amelioration of ischemic stroke in mice. Stroke; a journal of cerebral circulation. 43:2423–2429.

Sakata H, Niizuma K, Yoshioka H, Kim GS, Jung JE, Katsu M, Narasimhan P, Maier CM, Nishiyama Y, Chan PH. 2012b; Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. The Journal of neuroscience: the official journal of the Society for Neuroscience. 32:3462–3473. [PubMed: 22399769]

Sato Y, Nakanishi K, Hayakawa M, Kakizawa H, Saito A, Kuroda Y, Ida M, Tokita Y, Aono S, Matsui F, Kojima S, Oohira A. 2008; Reduction of brain injury in neonatal hypoxic-ischemic rats by intracerebroventricular injection of neural stem/progenitor cells together with chondroitinase ABC. Reproductive sciences (Thousand Oaks, Calif). 15:613–620.

Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. 2005; Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. Cerebrovascular diseases (Basel, Switzerland). 20:101–107.

Schabitz WR, Steigleder T, Cooper-Kuhn CM, Schwab S, Sommer C, Schneider A, Kuhn HG. 2007; Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. Stroke; a journal of cerebral circulation. 38:2165–2172.

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. 2013; Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Progress in neurobiology. 106–107:1–16.

Seroogy KB, Gall CM, Lee DC, Kornblum HI. 1995; Proliferative zones of postnatal rat brain express epidermal growth factor receptor mRNA. Brain research. 670:157–164. [PubMed: 7719717]

Shen SW, Duan CL, Chen XH, Wang YQ, Sun X, Zhang QW, Cui HR, Sun FY. 2016; Neurogenic effect of VEGF is related to increase of astrocytes transdifferentiation into new mature neurons in rat brains after stroke. Neuropharmacology. 108:451–461. [PubMed: 26603138]

Sheldon RA, Jiang X, Francisco C, Christen S, Vexler ZS, Tauber MG, Ferriero DM. 2004; Manipulation of antioxidant pathways in neonatal murine brain. Pediatric research. 56:656–662. [PubMed: 15295091]

Shimazaki T, Shingo T, Weiss S. 2001; The ciliary neurotrophic factor/leukemia inhibitory factor/gp130 receptor complex operates in the maintenance of mammalian forebrain neural stem cells.

The Journal of neuroscience: the official journal of the Society for Neuroscience. 21:7642–7653. [PubMed: 11567054]

Shimojo H, Ohtsuka T, Kageyama R. 2008; Oscillations in notch signaling regulate maintenance of neural progenitors. Neuron. 58:52–64. [PubMed: 18400163]

Shingo T, Sorokan ST, Shimazaki T, Weiss S. 2001; Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. The Journal of neuroscience: the official journal of the Society for Neuroscience. 21:9733–9743. [PubMed: 11739582]

Shinoyama M, Ideguchi M, Kida H, Kajiwara K, Kagawa Y, Maeda Y, Nomura S, Suzuki M. 2013; Cortical region-specific engraftment of embryonic stem cell-derived neural progenitor cells restores axonal sprouting to a subcortical target and achieves motor functional recovery in a mouse model of neonatal hypoxic-ischemic brain injury. Frontiers in cellular neuroscience. 7:128. [PubMed: 23970853]

Shruster A, Ben-Zur T, Melamed E, Offen D. 2012; Wnt signaling enhances neurogenesis and improves neurological function after focal ischemic injury. PloS one. 7:e40843. [PubMed: 22815838]

Sims JR, Lee SW, Topalkara K, Qiu JH, Xu J, Zhou ZP, Moskowitz MA. 2009; Sonic Hedgehog Regulates Ischemia/Hypoxia-Induced Neural Progenitor Proliferation. Stroke; a journal of cerebral circulation. 40:3618–3626.

Stump G, Durrer A, Klein AL, Lutolf S, Suter U, Taylor V. 2002; Notch1 and its ligands Delta-like and Jagged are expressed and active in distinct cell populations in the postnatal mouse brain. Mech Dev. 114:153–159. [PubMed: 12175503]

Sun C, Sun H, Wu S, Lee CC, Akamatsu Y, Wang RK, Kernie SG, Liu J. 2013a; Conditional ablation of neuroprogenitor cells in adult mice impedes recovery of poststroke cognitive function and reduces synaptic connectivity in the perforant pathway. The Journal of neuroscience: the official journal of the Society for Neuroscience. 33:17314–17325. [PubMed: 24174664]

Sun F, Mao XO, Xie L, Ding MP, Shao B, Jin KL. 2013b; Notch1 signaling modulates neuronal progenitor activity in the subventricular zone in response to aging and focal ischemia. Aging cell. 12:978–987. [PubMed: 23834718]

Sun T, Hevner RF. 2014; Growth and folding of the mammalian cerebral cortex: from molecules to malformations. Nature reviews. Neuroscience. 15:217–232. [PubMed: 24646670]

Sun Y, Jin K, Childs JT, Xie L, Mao XO, Greenberg DA. 2006; Vascular endothelial growth factor-B (VEGFB) stimulates neurogenesis: evidence from knockout mice and growth factor administration. Dev Biol. 289:329–335. [PubMed: 16337622]

Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA. 2003; VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. The Journal of clinical investigation. 111:1843–1851. [PubMed: 12813020]

Takagi Y, Nishimura M, Morizane A, Takahashi J, Nozaki K, Hayashi J, Hashimoto N. 2005; Survival and differentiation of neural progenitor cells derived from embryonic stem cells and transplanted into ischemic brain. Journal of neurosurgery. 103:304–310. [PubMed: 16175861]

Takahashi K, Yamanaka S. 2006; Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 126:663–676. [PubMed: 16904174]

Tang YH, Wang JX, Lin XJ, Wang LQ, Shao B, Jin KL, Wang YT, Yang GY. 2014; Neural stem cell protects aged rat brain from ischemia-reperfusion injury through neurogenesis and angiogenesis. Journal of Cerebral Blood Flow and Metabolism. 34:1138–1147. [PubMed: 24714034]

Taverna E, Huttner WB. 2010; Neural progenitor nuclei IN motion. Neuron. 67:906–914. [PubMed: 20869589]

Teramoto T, Qu JH, Plumier JC, Moskowitz MA. 2003; EGF amplifies the replacement of parvalbumin-expressing striatal interneurons after ischemia. Journal of Clinical Investigation. 111:1125–1132. [PubMed: 12697732]

Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, Darsalia V, Ekdahl CT, Kokaia Z, Lindvall O. 2006; Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem cells (Dayton, Ohio). 24:739–747.

Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O. 2007; Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke; a journal of cerebral circulation. 38:3032–3039.

Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O. 2014; Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. Journal of Cerebral Blood Flow and Metabolism. 34:1573–1584. [PubMed: 25074747]

Tsai PT, Ohab JJ, Kertesz N, Groszer M, Matter C, Gao J, Liu X, Wu H, Carmichael ST. 2006; A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. Journal of Neuroscience. 26:1269–1274. [PubMed: 16436614]

Tureyen K, Vemuganti R, Bowen KK, Sailor KA, Dempsey RJ. 2005; EGF and FGF-2 infusion increases post-ischemic neural progenitor cell proliferation in the adult rat brain. Neurosurgery. 57:1254–1262. [PubMed: 16331174]

Vannucci RC. 2000; Hypoxic-ischemic encephalopathy. Am J Perinatol. 17:113–120. [PubMed: 11012134]

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. 2010; Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 463:1035–1041. [PubMed: 20107439]

Voloboueva LA, Sun XY, Xu LJ, Ouyang YB, Giffard RG. 2017; Distinct Effects of miR-210 Reduction on Neurogenesis: Increased Neuronal Survival of Inflammation But Reduced Proliferation Associated with Mitochondrial Enhancement. Journal of Neuroscience. 37:3072–3084. [PubMed: 28188219]

Walls KC, Ghosh AP, Ballestas ME, Klocke BJ, Roth KA. 2009; bcl-2/Adenovirus E1B 19-kd interacting protein 3 (BNIP3) regulates hypoxia-induced neural precursor cell death. Journal of neuropathology and experimental neurology. 68:1326–1338. [PubMed: 19915483]

Walton NM, Sutter BM, Laywell ED, Levkoff LH, Kearns SM, Marshall GP 2nd, Scheffler B, Steindler DA. 2006; Microglia instruct subventricular zone neurogenesis. Glia. 54:815–825. [PubMed: 16977605]

Wang L, Chopp M, Zhang RL, Zhang L, LeTourneau Y, Feng YF, Jiang A, Morris DC, Zhang ZG. 2009a; THE Notch PATHWAY MEDIATES EXPANSION OF A PROGENITOR POOL AND NEURONAL DIFFERENTIATION IN ADULT NEURAL PROGENITOR CELLS AFTER STROKE. Neuroscience. 158:1356–1363. [PubMed: 19059466]

Wang L, Zhang ZG, Wang Y, Zhang RL, Chopp M. 2004; Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. Stroke; a journal of cerebral circulation. 35:1732–1737.

Wang XL, Zhao YS, Yang YJ, Xie M, Yu XH. 2008; Therapeutic window of hyperbaric oxygen therapy for hypoxic-ischemic brain damage in newborn rats. Brain research. 1222:87–94. [PubMed: 18582850]

Wang XM, Mao XO, Xie L, Greenberg DA, Jin KL. 2009b; Involvement of Notch1 signaling in neurogenesis in the subventricular zone of normal and ischemic rat brain in vivo. Journal of Cerebral Blood Flow and Metabolism. 29:1644–1654. [PubMed: 19536070]

Wang Y, Jin K, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA. 2007; VEGF-overexpressing transgenic mice show enhanced post-ischemic neurogenesis and neuromigration. Journal of neuroscience research. 85:740–747. [PubMed: 17243175]

Wilhelmsson U, Faiz M, de Pablo Y, Sjoqvist M, Andersson D, Widestrand A, Potokar M, Stenovec M, Smith PL, Shinjyo N, Pekny T, Zorec R, Stahlberg A, Pekna M, Sahlgren C, Pekny M. 2012; Astrocytes negatively regulate neurogenesis through the Jagged1-mediated Notch pathway. Stem cells (Dayton, Ohio). 30:2320–2329.

Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kutok JL, Rajewsky K. 2008; Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 9:405–414. [PubMed: 18327259]

Xin HQ, Katakowski M, Wang FJ, Qian JY, Liu XS, Ali MM, Buller B, Zhang ZG, Chopp M. 2017; MicroRNA cluster miR-17-92 Cluster in Exosomes Enhance Neuroplasticity and Functional Recovery After Stroke in Rats. Stroke; a journal of cerebral circulation. 48:747–753.

Xiong M, Chen LX, Ma SM, Yang Y, Zhou WH. 2013; Short-term effects of hypothermia on axonal injury, preoligodendrocyte accumulation and oligodendrocyte myelination after hypoxia-ischemia in the hippocampus of immature rat brain. Developmental neuroscience. 35:17–27. [PubMed: 23446161]

Yager JY, Ashwal S. 2009; Animal models of perinatal hypoxic-ischemic brain damage. Pediatric neurology. 40:156–167. [PubMed: 19218028]

Yagita Y, Kitagawa K, Ohtsuki T, Takasawa K, Miyata T, Okano H, Hori M, Matsumoto M. 2001; Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. Stroke; a journal of cerebral circulation. 32:1890–1896.

Yamashita T, Ninomiya M, Acosta PH, Garcia-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K. 2006; Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. Journal of Neuroscience. 26:6627–6636. [PubMed: 16775151]

Yang JL, Zhang XF, Chen XJ, Wang L, Yang GD. 2017a; Exosome Mediated Delivery of miR-124 Promotes Neurogenesis after Ischemia. Molecular Therapy-Nucleic Acids. 7:278–287. [PubMed: 28624203]

Yang P, Cai L, Zhang G, Bian Z, Han G. 2017b; The role of the miR-17-92 cluster in neurogenesis and angiogenesis in the central nervous system of adults. Journal of neuroscience research. 95:1574–1581. [PubMed: 27869313]

Yang Z, Covey MV, Bitel CL, Ni L, Jonakait GM, Levison SW. 2007; Sustained neocortical neurogenesis after neonatal hypoxic/ischemic injury. Annals of neurology. 61:199–208. [PubMed: 17286251]

Yang Z, Levison SW. 2006; Hypoxia/ischemia expands the regenerative capacity of progenitors in the perinatal subventricular zone. Neuroscience. 139:555–564. [PubMed: 16500031]

Yao B, Christian KM, He C, Jin P, Ming GL, Song H. 2016; Epigenetic mechanisms in neurogenesis. Nature reviews. Neuroscience. 17:537–549. [PubMed: 27334043]

Yao MJ, Chen G, Zhao PP, Lu MH, Jian J, Liu MF, Yuan XB. 2012; Transcriptome analysis of microRNAs in developing cerebral cortex of rat. BMC Genomics. 13:232. [PubMed: 22691069]

Yildiz EP, Ekici B, Tatli B. 2017; Neonatal hypoxic ischemic encephalopathy: an update on disease pathogenesis and treatment. Expert review of neurotherapeutics. 17:449–459. [PubMed: 27830959]

Yoshimura S, Takagi Y, Harada J, Teramoto T, Thomas SS, Waeber C, Bakowska JC, Breakefield XO, Moskowitz MA. 2001; FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. Proceedings of the National Academy of Sciences of the United States of America. 98:5874–5879. [PubMed: 11320217]

Yuan T, Liao W, Feng NH, Lou YL, Niu X, Zhang AJ, Wang Y, Deng ZF. 2013; Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. Stem cell research & therapy. 4:73. [PubMed: 23769173]

Zeng L, He X, Wang Y, Tang Y, Zheng C, Cai H, Liu J, Wang Y, Fu Y, Yang GY. 2014; MicroRNA-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. Gene therapy. 21:37–43. [PubMed: 24152581]

Zhang L, Chopp M, Zhang RL, Wang L, Zhang J, Wang Y, Toh Y, Santra M, Lu M, Zhang ZG. 2010; Erythropoietin amplifies stroke-induced oligodendrogenesis in the rat. PloS one. 5:e11016. [PubMed: 20552017]

Zhang L, Yin JC, Yeh H, Ma NX, Lee G, Chen XA, Wang YM, Lin L, Chen L, Jin P, Wu GY, Chen G. 2015; Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons. Cell stem cell. 17:735–747. [PubMed: 26481520]

Zhang PB, Li J, Liu Y, Chen XL, Lu HX, Kang QY, Li WS, Gao M. 2011; Human embryonic neural stem cell transplantation increases subventricular zone cell proliferation and promotes peri-infarct angiogenesis after focal cerebral ischemia. Neuropathology: official journal of the Japanese Society of Neuropathology. 31:384–391. [PubMed: 21175862]

Zhang R, Zhang Z, Zhang C, Zhang L, Robin A, Wang Y, Lu M, Chopp M. 2004; Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal

differentiation in the adult rat. The Journal of neuroscience: the official journal of the Society for Neuroscience. 24:5810–5815. [PubMed: 15215303]

Zhang RL, Chopp M, Gregg SR, Toh Y, Roberts C, Letourneau Y, Buller B, Jia L, SPND, Zhang ZG. 2009; Patterns and dynamics of subventricular zone neuroblast migration in the ischemic striatum of the adult mouse. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 29:1240–1250.

Zhang RL, Zhang ZG, Zhang L, Chopp M. 2001; Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience. 105:33–41. [PubMed: 11483298]

Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K, Lein ES, Eadie BD, Willhoite AR, Muotri AR, Summers RG, Chun J, Lee KF, Gage FH. 2003; Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. Proceedings of the National Academy of Sciences of the United States of America. 100:6777–6782. [PubMed: 12748381]

Zheng XR, Zhang SS, Yin F, Tang JL, Yang YJ, Wang X, Zhong L. 2012; Neuroprotection of VEGF-expression neural stem cells in neonatal cerebral palsy rats. Behavioural brain research. 230:108–115. [PubMed: 22342488]

Zhu WS, Cheng SM, Xu GL, Ma MM, Zhou ZM, Liu DZ, Liu XF. 2011; Intranasal nerve growth factor enhances striatal neurogenesis in adult rats with focal cerebral ischemia. Drug Delivery. 18:338–343. [PubMed: 21348576]

Highlights

- This review provides a comprehensive overview of neural stem cell therapy for both neonatal and adult hypoxic-ischemic brain injury.
- Endogenous neural stem cells rapidly react to the injury and initiate a limited self-repair process.
- The strategies and mechanisms of exogenous neural stem cell transplantation for neonatal and adult hypoxic-ischemic brain are discussed.
- The modulators that facilitate or enhance the endogenous repair process are summarized.
- Direct in vivo neuronal reprogramming is promising for brain repair after ischemic stroke.

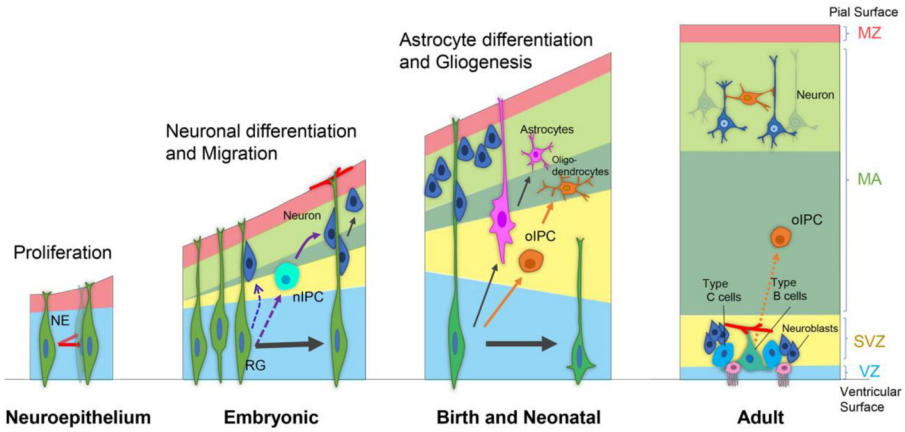

Figure 1. Neural stem cells in the neural development

During early development, Neuroepithelial (NE) cells divide symmetrically to expand the number of NE cells. This is followed by transform of NE cells into radial glial (RG) cells, which divide asymmetrically producing one daughter RG cell and one intermediate neural progenitor cell (nIPC). nIPCs differentiate into neurons, which then migrate along the radial processes of RG cells to the cortical plate. Some RG cells also directly generate neurons. Around birth, large parts of RG cells change its morphology, detach from the VZ, and finally convert to astrocyte. RG-derived oIPCs generate oligodendrocytes to participate in oligogenesis. In the adult brain, NSCs reside in SVZ, known as Type B cells. These cells generate intermediate progenitor cells (Type C cells) and then become neuroblasts (Type A cells). MZ, marginal zone; MA, mantle; SVZ, subventricular zone; VZ, ventricular zone; oIPC, oligodendrocytic progenitor cell

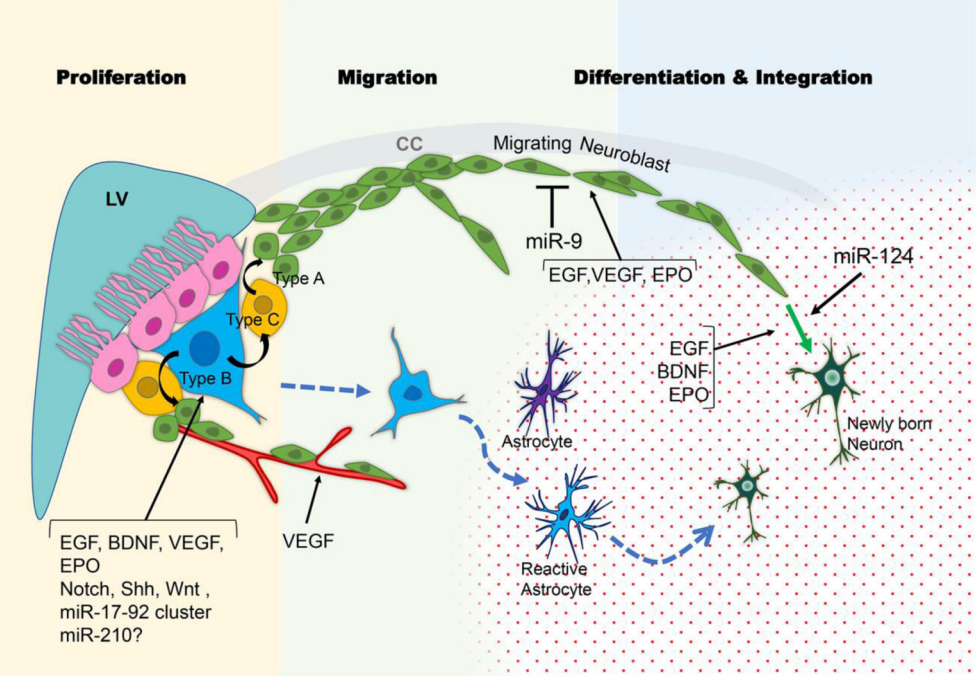

Figure 2. Endogenous neurogenesis after stroke and regulating factors

Neurogenesis in the SVZ is stimulated after stroke, leading to NSCs proliferation and migration of dividing neuroblasts. Neuroblasts move to the infarcted area following chain migration and along the vasculature. A small part of neuroblasts survive and become mature neurons to replace dying neurons. Many factors (Morphogens, Growth factors, Neurotrophic factors, EPO, MicroRNAs) regulate the process of neurogenesis, including proliferation, migration, and differentiation. Red dots indicate the stroke-affected area. BDNF, brain-derived neurotrophic factor; CC, corpus callosum; EGF, epidermal growth factor; EPO, erythropoietin; LV, lateral ventricle; miR, microRNA; Shh, Sonic hedgehog; Wnt, Wingless-type MMTV integration site family; VEGF, vascular endothelial growth factor.

Table 1

Neural stem cell transplantation in neonatal H/I injury model

Route	Timing of transplantation	Cell Type/ Modification Human NSCs	Dose	Species	Model	Major finding	Reference
Intracerebral	24h		3.0×10^5	SD rat (P7)	RVM (8%O_2) 90mins	Improving motor function recovery; Axonal sprouting enhancement	(Daadi *et al.*, 2010)
	48h	NPC(from ESC)	2.5×10^5	ICR mouse (P2)	RVM (8%O_2) 20mins	Improving motor function recovery; Enhancing axonal outgrowth	(Shinoyama *et al.*, 2013)
	72h	Mouse NSCs	5.0×10^5	SD rat (P10)	RVM (8%O_2) 90mins	NSC migration to lesion; Survived for 58weeks after HI	(Obenaus *et al.*, 2011)
	72h	Rat NSCs (from fetal brain) +enhanced VEGF expression	1.0×10^5	SD rat (P7)	RVM (8%O_2) 120mins	Reducing neuronal apoptosis; Increasing angiogenesis	(Zheng *et al.*, 2012)
	7 d	Human NSCs (fetal brain) +enhanced Neurog2 expression	9.6×10^5	ICR mouse (P7)	RVM (8%O_2) 90mins	Improving sensorimotor function recovery; Increasing neural plasticity	(Lee *et al.*, 2017)
	10 d	Mouse NSCs (from fetal brain)	1.0×10^5	C57Bl/6 mouse (P9)	RVM (10%O_2) 45mins	Reducing infarct volume; Improving motor function recovery	(Braccioli *et al.*, 2017)
Intracerebro- ventricular (i.c.v.)	2h	Mouse OPCs (Derived from mESCs)	2.5×10^5	Rat (P3)	RVM (6%O_2) 150mins	Improving motor function recovery;	(Chen *et al.*, 2015)
	24h	Rat NSC (from fetal brain)/+ChABC	2.5×10^5	SD rat (P7)	RVM (8%O_2) 120mins	Reducing infarct volume	(Sato *et al.*, 2008)
Intranasal	24h	Human NSCs	3.0×10^5	SD rat (P7)	RVM (7.8%O_2) 120mins	Reducing infarct volume; Improving sensorimotor function recovery; Suppressing inflammation	(Ji *et al.*, 2015)

Abbreviation in the Table: RVM: Rice-Vannucci model; NSCs: Neural Stem Cells; ESCs: Embryonic Stem Cells; OPCs: Oligodendrocyte progenitor cells

Table 2

Representative experimental studies of neural stem cell transplantation for adult ischemic stroke

Route	Timing of transplantation	Cell Type/ Modification NSCs derived from human iPSCs	Dose	Species	Model	Major finding	Reference
Intracerebral	0h		1.0×10^6	SD rat	tMCAO 120mins	Improving motor function recovery;	(Yuan *et al.*, 2013)
	24h	Mouse NSCs (from fetal brain)	1.0×10^6	SD rat	tMCAO 120mins	Improving motor function recovery; Increasing neurogenesis and angiogenesis	(Tang *et al.*, 2014)
	24h	Human CTX0E03 cell line	8.0×10^5	SD rat	tMCAO 70mins	Improving sensorimotor function; engrafts are not tumorigenic	(Pollock *et al.*, 2006)
	24h	Human NSCs (derived from ESCs)	1.0×10^6	C57BL6 Mouse	tMCAO 60mins	Engrafted NSC rapidly migrate to infarct area, reducing inflammation	(Huang *et al.*, 2014)
	7d	NSC derived from human iPSCs	1.0×10^5	C57BL6 Mouse	tMCAO 30mins	Improving function recovery; generating mature neuron	(Oki *et al.*, 2012)
	7d	Human NSCs (derived from ESCs)	1.0×10^5	SD rat	tMCAO 90mins	Grafts differentiate into neuron, oligodendrocytes, and form neural connectivity	(Daadi *et al.*, 2009)
	7d	Human NSCs (Isolated from fetal brain tissue)	1.0×10^5	SD rat	dMCAO	Engrafts survive, migrate to the lesion, and express the immature neuronal marker	(Kelly *et al.*, 2004)
Intracerebro ventricular (i.c.v.)	24h	mouse NPCs (Isolated from fetal brain tissue)	3.6×10^5	SD rat	tMCAO 60mins	Engrafts migrate towards the lesion tissue and express the immature neuronal marker	(Jin *et al.*, 2005)
Intraarterial	6h, 24h, 72h,	Mouse NSCs (from fetal brain)	5.0×10^5	C57BL6 mouse	Hypoxia-ischemia stroke (8%O_2) 20mins	Transplantation at 72h yield highest cell survival	(Rosenblum *et al.*, 2012)
	24h	Mouse NSCs (from fetal brain)	5.0×10^5	Nude mouse	Hypoxia-ischemia stroke (8%O_2) 30mins	IA is a superior delivery route vs. IV infusion.	(Pendharkar *et al.*, 2010)
Intravenous	72h	Mouse NPCs	1.0×10^6	C57B L6 mouse	tMCAO 45mins	NPC engrafts modulating axonal rewiring and dendritic plasticity; and improving long-term functional recovery.	(Bacigaluppi *et al.*, 2016)
	24h, 28d	Mouse NPCs (Isolated from adult SVZ)	1.0×10^6	C57B L6 mouse	tMCAO 30mins	Cell delivery at acute phage stabilizes BBB and modulate immune response; at chronic phage stimulates neurogenesis	(Doeppner *et al.*, 2014)

Abbreviation in the Table: NSCs: Neural Stem Cells; NPCs: neural progenitor cells; ESCs: Embryonic Stem Cells; iPSCs: induced pluripotent stem cells; tMCAO: transient middle cerebral artery occlusion; IA: intra-arterial; IV: intravenous; SD: Sprague Dawley; SVZ: subventricular zone