Efficacy of Transplant and Endogenous Precursor and Stem Cell Interventions on Stroke Recovery: A Critical Assessment

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4.1 Introduction

In the last decade, there has been a growing realization that the injured brain has considerable capacity for reorganization and self-repair after injury. For example, brain damage results in increased dendritic growth and spine formation, synaptogenesis and upregulation of growth factors and reorganization of cortical sensorimotor maps (Jones and Schallert 1992; Cramer and Chopp 2000; Schallert et al.

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2000), processes implicated in brain repair and recovery. Importantly, similar forms of plasticity can be produced by behavioural experience, such as housing animals in enriched environments or exposing them to intensive rehabilitation, cortical stimulation or motor training (Ohlsson and Johansson 1995; Nudo et al. 1996; Kolb et al. 1998; Biernaskie and Corbett 2001; Kleim et al. 2003). Unfortunately, the extent of motor recovery after stroke remains limited with most patients experiencing debilitating deficits years after the injury (Cramer and Chopp 2000). Innovations in rehabilitation therapy suggested by clinical (e.g. constraint therapy). (Dromerick et al. 2000) and animal studies (Biernaskie and Corbett 2001) require intensive and prolonged periods of rehabilitation that may not be suitable for many stroke patients who may be constrained by other medical conditions (e.g. frailty), by motivational deficits or by cognitive dysfunction. While rehabilitation is helpful, many patients, particularly those with moderate to severe injury, are left with persistent impairments in daily living activities (Dobkin 2005). Clearly, additional interventions will be required to produce more complete recovery in most stroke patients.

One approach that has created a great deal of interest is to replace neurons lost as a result of stroke. The impetus for this notion is based on the discovery that the mammalian brain (including humans) produces new cells (neurogenesis) throughout life (Altman and Das 1965; Reynolds and Weiss 1992; Cameron et al. 1993). These newly generated cells proliferate in response to environmental stimuli and endogenous hormones (e.g. enriched environments, exercise) (Kempermann et al. 1997; van Praag et al. 1999; Shingo et al. 2003; Mak and Weiss 2010) and become functionally integrated into existing circuitry (Shors et al. 2001; van Praag et al. 2002), although evidence on this point is limited. Of particular relevance to stroke recovery is the observation that endogenous or transplanted stem cells *migrate* towards the site of damage thereby raising the exciting possibility that they may participate in functional recovery (Chen et al. 2001; Veizovic et al. 2001; Komitova et al. 2005). To date most studies have utilized exogenous delivery of neural precursor cells after focal ischaemia where early indications suggest that functional recovery is improved but the effects are small and limited in duration (Zhao et al. 2002; Bliss et al. 2006; Hicks et al. 2007; Hicks et al. 2009; Zhang et al. 2011); see Andrews et al. (2008). Similar results have been achieved in a few studies where growth factors or environmental enrichment has been used to encourage survival and migration of endogenous precursor cells to the peri-infarct cortex (Komitova et al. 2005; Tsai et al. 2006; Kolb et al. 2007). In these studies, it is unclear whether the newly formed cells are being incorporated into existing circuits or whether the cells are fully functional. It is possible that the observed benefits may be due to indirect effects of these cells (e.g. source of growth factors) on undamaged circuits that ultimately reorganize in response to the injury (Bliss et al. 2007).

In this chapter, we attempt to critically assess both endogenous and exogenous approaches to repair the stroke-damaged brain with special emphasis on additional factors including behavioural outcome measures, post-stroke environment, age and other variables that modulate “apparent efficacy” of stem cell treatments.

4.2 Mobilization of Endogenous Stem Cells and Stroke Recovery

The existence of neural stem cells in the adult brain holds great promise for the development of neural repair strategies (Reynolds and Weiss 1992; Weiss et al. 1996). Neural stem cells and their direct progeny (progenitor cells) – collectively known as neural precursor cells (NPCs) – possess the ability to generate all the neural cell types that comprise the central nervous system and are therefore thought to be good targets for the development of cell-based therapies to repair the injured CNS. Since their original isolation and characterization in vitro, much has been learned about the in vivo location (the neural stem cell niche) and the factors that regulate the behaviour and lineage dynamics of resident NPCs. The prospect of activating these endogenous NPCs using biologics and enticing them to contribute to repair of the injured brain has become a compelling prospect. There are a number of advantages of manipulating endogenous precursors in situ including the lack of immune rejection issues and the circumvention of concerns of altering the growth characteristics of cells (i.e. generating transformed cell lines) with prolonged culture prior to their use in transplantation paradigms (Morshead et al. 2002).

Adult NPCs can be found along the entire neuraxis of the adult CNS (Reynolds and Weiss 1992; Weiss et al. 1996) and have been well studied in the two neurogenic regions that persist into adulthood: the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus (Morshead et al. 1994; Palmer et al. 1995). The SVZ lines the walls of forebrain lateral ventricles and is comprised of a single cell layer of ependymal cells and an adjacent 2–4-cell-layer-thick region called the subependyma (Morshead et al. 1994; Chiasson et al. 1999). Neural stem cells in the subependyma of the SVZ are slowly proliferating cells (Morshead et al. 1994) that express glial fibrillary acidic protein (GFAP) (Doetsch et al. 1999; Morshead et al. 2003; Garcia et al. 2004). The rapidly dividing progeny undergo cell death or migrate along the rostral migratory stream to the olfactory bulb, where they differentiate into interneurons and become functionally integrated into the neuronal network (Lois and Alvarez-Buylla 1994; Morshead et al. 1998; Carleton et al. 2003). Neurogenesis in the rodent olfactory bulb is thought to play a role in olfactory learning and memory (Krakauer et al. 2012). The adult human SVZ similarly contains a population of GFAP-expressing cells which are capable of forming self-renewing, multipotent colonies when isolated in vitro, thereby confirming the presence of NPCs in the adult human brain (Sanai et al. 2004; van den Berge et al. 2010). Furthermore, migrating neurogenic progeny have been observed in the human brain albeit in much smaller numbers than what is observed in rodents (Curtis et al. 2007; Wang et al. 2011).

Similarly, within the DG of the hippocampus, a subpopulation of GFAP-expressing cells with a radial morphology has been purported to act as multipotent self-renewing neural stem cells (Palmer et al. 1997). While the existence of neural stem cells within the hippocampus has been challenged on more than one occasion (Palmer et al. 1997; Seaberg and van der Kooy 2002; Suh et al. 2007), it is unrefuted that the hippocampus is a neurogenic region of the brain throughout life and the

newly born neurons generated within this region play a role in hippocampal plasticity and cognitive function (Jessberger and Gage 2008).

NPCs in the adult brain elicit the fundamental properties in vivo that would benefit the development of strategies to promote their contribution to neural repair, namely, proliferation, migration and differentiation into neural phenotypes. Indeed, it has been demonstrated that brain injury alone can activate endogenous NPCs (Liu et al. 1998; Jin et al. 2000; Parent et al. 2002; Zhang et al. 2004; Kernie and Parent 2010). The activation of endogenous NPCs in response to stroke has been demonstrated using proliferation assays (i.e. measuring the numbers of BrdU-labelled cells in the SVZ and DG) as well as in vitro colony-forming assays. These assays reveal that injury alone generates a larger pool of cells that can potentially be utilized for brain repair.

The first demonstration of the recruitment of NPCs directly to the stroke-injury site was in a focal model of transient middle cerebral artery occlusion (MCAo) in rats. Immunohistochemistry revealed a population of SVZ-derived NPCs proliferating, migrating to the site of infarct and generating new neurons in the striatum and parietal lobe (Arvidsson et al. 2002; Parent et al. 2002). The number of striatal neurons generated was small, and functional recovery was not assayed, but nonetheless, these studies demonstrated the brain's inherent, albeit limited, capacity for self-repair. Further studies suggested that this activation and recruitment process persists for several months after the ischaemic attack (Thored et al. 2006; Yamashita et al. 2006) and that newly formed neurons become synaptically integrated as determined by morphological and electrophysiological studies (Yamashita et al. 2006; Hou et al. 2008). These phenomena have more recently been examined in humans, where post-mortem biopsies of stroke patients have shown the presence of proliferating and differentiating cells in the ischaemic penumbra as well as the ipsilateral SVZ (Jin et al. 2006; Marti-Fabregas et al. 2010). However, the degree of endogenous activation following stroke is clearly not sufficient for functional recovery as demonstrated by the persistent functional impairments observed in patients following stroke.

With the goal of augmenting the self-repair mechanisms of the brain, a thorough understanding of mechanisms that underlie the activation of NPCs is needed. Various NPC "activation factors" have been examined for their potential role in modifying NPC proliferation kinetics, increasing NPC cell survival, enhancing cell migration to infarct site and/or promoting neurogenesis and angiogenesis. Factors such as vascular endothelial growth factor (VEGF) (Jin et al. 2000; Wittko et al. 2009; Shin et al. 2010), epidermal growth factor (EGF) (Craig et al. 1996), fibroblast growth factor (FGF) (Kuhn et al. 1997) (Leker et al. 2007), transforming growth factor alpha (TGF-α) (Guerra-Crespo et al. 2009) and erythropoietin (EPO) (Wang et al. 2012) have been shown to influence the proliferation and/or differentiation of endogenous NPCs in the adult brain under baseline conditions and following stroke. Craig and colleagues (1996) were the first to demonstrate that the intraventricular administration of exogenous EGF expanded the SVZ progenitor population in vivo and induced their migration away from the neurogenic niche lining the lateral ventricles and into the surrounding parenchyma in the intact adult brain.

This demonstration that endogenous NPCs could be modified by the application of exogenous factors led to a number of factors, alone and in combination, being utilized in stroke models. In addition to those mentioned above, glia-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF) and human chorionic gonadotropin (hCG) (Teramoto et al. 2003; Leker et al. 2007; Kobayashi et al. 2006; Schabitz et al. 2007; Kolb et al. 2007; Belayev et al. 2009) have been administered in stroke models.

With the goal of clinical application, enhancing NPC proliferation using mitogens may not be the most appropriate avenue for increasing the size of the NPC pool due to the risk of tumorigenesis that is implicit with enhanced proliferation. An alternative approach to expanding the NPC pool post-ischaemia is by promoting cell survival through inhibition of apoptosis. Recently it was reported that cyclosporin A (CsA), a commonly used immunosuppressive drug, acts directly on NPCs to increase their survival without affecting cell cycle kinetics (Hunt et al. 2010). While the pro-survival mechanism is not clear, the administration of CsA in vivo results in a >2-fold increase in the size of the NPC pool in control mice. Moreover, mice that received CsA following stroke showed an expansion of the NPC pool, migration of the NPCs to the site of injury, new tissue formation at the site of cortical ischaemia, as well as recovery of motor function (Erlandsson et al. 2011). Regulating the mode of division of NPCs to promote symmetry of division at the expense of asymmetric division will also result in increased numbers of NPCs in vivo. Signalling molecules such as Notch (Wang et al. 2009) and Wnt (Piccin and Morshead 2011) have been shown to increase in models of stroke and during tissue regeneration in the adult brain. Moreover, intraventricular infusion of Notch activators leads to improved motor function in stroke-injured mice (Androutsellis-Theotokis et al. 2006; Wang et al. 2009). These studies highlight the importance of considering a number of different targets to increase the size of the NPC pool for application in endogenous repair strategies.

The redirected cell migration towards the site of injury has been shown to be regulated by chemoattractrant molecules such as stromal cell-derived factor (SDF-1), monocyte chemoattractant protein (MCP-1), angiopoietin (Ang-1), slits, matrix metalloproteases, galectin-1 and osteopontin (Imitola et al. 2004; Thored et al. 2006; Yamashita et al. 2006; Langdon and Corbett 2012; Kempermann 2011). Thin astrocytic processes and blood vessels have been shown to serve as scaffolds for the migration (Yamashita et al. 2006). Understanding the factors that promote migration and the cells that respond to injury will facilitate the design of future therapies to enhance the regenerative process after stroke.

Functional benefits have been achieved by mobilizing endogenous stem cells with growth factors or combinations of growth factors, yet many basic issues remain to be resolved. The challenge of inducing effective functional integration of newly generated neurons into existing neural and synaptic networks is ongoing, and reports to this effect remain controversial. It has been suggested that substantial replacement of infarcted tissue by NPCs is unlikely and that NPC-mediated neurogenesis may be inconsequential in functional recovery (Kempermann 2011). Hence, it is not

clear that developing self-repair strategies following stroke should be restricted to neurogenesis as reconstruction of glial cells and the vascular system is also required. Notably, deleterious effects of enhanced neurogenesis have been reported (Scharfman and Hen 2007), highlighting the importance of balancing cell replacement with positive outcomes.

4.3 Transplant Approaches to Stroke Recovery

A variety of different cell types (hematopoietic, immortalized cell lines, neural stem/precursor cells.) and routes of delivery (e.g. intravenous, intracerebral) have been used in transplant studies. Since these topics are covered in detail by other authors in this book, they will not be the focus here. Instead, we will direct discussion towards the efficacy of different stem cell approaches to improving functional recovery following stroke with a critical assessment of the associated outcome measures employed. This is predicated on the widespread failure of stroke neuroprotective strategies in which preclinical studies failed to adequately incorporate fundamentally important aspects of human stroke (Corbett and Nurse 1998; Endres et al. 2008) thereby resulting in what has been termed translational roadblock (Endres et al. 2008). Further, we will not consider studies where the goal was to achieve neuroprotection for the obvious reason that the time window, like for tissue plasminogen factor, is very narrow with the result that only a minority of stroke patients (~10 %) would derive benefit.

A number of studies have used systemic delivery of stem cells with the intravenous route offering the least risk for ultimate use in humans. In one early study, Chopp and colleagues subjected rats to 2 h of transient MCAo using the intraluminal suture method (Li et al. 2001). Ischaemia was followed 24 h later by intracarotid delivery of mesenchymal stem and progenitor cells (MSC). Animals were then tested several times over the 14-day post-stroke survival period using a modified neurological test score and an adhesive tape removal test (Schallert et al. 1982). Rats receiving MSC recovered more rapidly compared to ischaemic controls, but both groups improved by the final day 14 behavioural test. Results were similar using an intravenous delivery route 24 h after MCAo (Chen et al. 2001) with marginally faster recovery in neurological deficit scores and tape removal tasks. Interestingly, transplants of conditionally immortalized neuroepithelial cells into the *undamaged* hemisphere several weeks after 60 min of MCAo improved performance on the tape removal test and mitigated drug-induced rotational asymmetry (Veizovic et al. 2001). A minority of the transplanted cells crossed the midline and migrated to the peri-infarct zone suggesting that the benefit was perhaps due to secondary effects (e.g. reduction in atrophy) in the undamaged hemisphere. Complicating the interpretation is the finding that infarct volumes were reduced in transplanted animals suggesting these animals may have had smaller infarcts to start with, which would account for the perceived modest functional benefits of the transplants. This possibility is further supported by the fact that there is a sensitive time window of approximately 1 month following stroke when recovery-promoting interventions are most

effective (Biernaskie et al. 2004; Murphy and Corbett 2009) and the transplants in this study were administered when this window is closing. Using human hNT cells (derived from embryonic carcinoma cells) transplanted 1 week after a permanent distal MCAo, Bliss and colleagues found good survival (~40 %) after 4 weeks, but this did not translate into very robust post-stroke behavioural recovery (Bliss et al. 2006). Indeed, the only improvement was on a ledged beam test with no effect of transplantation on forelimb asymmetry (i.e. cylinder and tape removal tests). In a subsequent study, this group determined that transplanted embryonic human neural stem cells in nude, T-cell-deficient rats produced neurological improvement in a limb-placing task. This effect was attributed to cell-induced improvement of blood-brain barrier integrity, reduced inflammation and enhanced vascularization that was shown to be VEGF dependent (Horie et al. 2011). Surprisingly, recovery of limb placing varied considerably among cell-treated animals, with some showing marked improvement in limb placing 1 week after transplantation, while remaining rats took several weeks longer to recover. It is unclear what accounts for differences in recovery (e.g. variation in stroke size, location). The effects of human NPCs on stroke recovery were examined in another series of experiments that included additional behavioural tests (postural reflexes, cylinder test and body swing test); however, in this study, there was no early improvement in recovery even on the limb-placing task with significant benefits only becoming apparent 4 or more weeks after stroke depending on the test (Andres et al. 2011). In contrast, administration of human umbilical tissue-derived cells early (1 day) or up to 30 days post-stroke improved neurological deficit scores and decreased tape removal latencies in rats subjected to 2 h of MCAo (Zhang et al. 2011). This recovery was associated with enhanced synaptogenesis, neurogenesis and angiogenesis.

In summary, a variety of stem cell types have been used in efforts to promote functional recovery after stroke. Results appear encouraging particularly since efficacy has been demonstrated in different species (rat, mouse) and stroke models (e.g. transient and permanent MCAo); however, the magnitude of the enhanced recovery remains rather modest in all of these studies, suggesting that additional ways to enhance the activity and survival of transplanted and resident stem and NPCs may be required.

4.4 Use of Biotechnology to Enhance Stem Cell Therapies

Stem cell therapies for stroke have been pursued by two fundamentally different approaches: (1) stem cell transplantation and (2) endogenous stem cell stimulation. The former includes multiple different types of stem cells, such as adult NPCs, embryonic stem cells and mesenchymal stem cells, among others. The sites for transplantation include direct injection into the brain (Daadi et al. 2008) or systemic injection into the blood (Wang et al. 2008). These strategies have met with some success (Bliss et al. 2007) and are discussed here and in other chapters. Herein, our focus is on endogenous stem cell stimulation although these technologies can also be utilized in transplant studies with or without mobilization of endogenous cells.

The challenge in endogenous stimulation is how to stimulate NPCs specifically without stimulating other cells types. One way to achieve this is with a local or targeted delivery strategy of activation factors (Lanfranconi et al. 2011).

The blood-brain barrier makes local delivery strategies difficult because it significantly reduces (or inhibits) the diffusion of biomolecules across the vasculature into the brain (Pardridge et al. 1992). Thus, the blood-brain barrier (BBB) makes normal routes of delivery, such as oral or intravenous (i.v.), ineffective (Ferber 2007). While some molecules, such as erythropoietin (EPO), can cross the BBB (Hanson and Frey 2008), large systemic doses are required to achieve the local concentration in the brain tissue required for efficacy, resulting in blood thickening systemically which is undesirable, especially in cases of stroke (Torup 2007).

There are some strategies that are being investigated to promote greater accumulation in the brain after systemic i.v. delivery. For example, there are some formulations that have either the biomolecule modified with poly(ethylene glycol) (PEG) (Meinel et al. 2004) or nano-/microparticle (in which the biomolecule is encapsulated) modified with PEG (Li et al. 2011). Modification with PEG prolongs blood circulation time and increased accumulation in the brain; however, the penetration distance into the brain, the accumulated dose in the brain and the associated systemic toxicity can be limiting.

Alternative strategies include local delivery strategies, such as focal opening of the BBB with, for example, ultrasound (de Boer and Gaillard 2007). In this strategy, ultrasound is focused in a specific region of the brain and opens the BBB for a defined period of time. The advantage of this technique is the precise location of the BBB opening. The disadvantage is that all circulating molecules and cells can now cross the BBB in that defined volume of tissue.

Another strategy includes use of intraventricular infusion of biomolecules (Jonhagen et al. 1998). Here, a catheter/minipump system is employed where a cannula is inserted through the brain into the ventricle. Since the endogenous stem cells line the lateral ventricles, this strategy is effective for their stimulation (Kolb et al. 2007). A potential limitation of this strategy is the brain tissue damage associated with cannula insertion. Moreover, the biomolecules delivered to the cerebrospinal fluid in the ventricles are dispersed throughout the brain and spinal cord. Consequently, limited amounts of these factors diffuse into the brain tissue.

A new strategy has recently been proposed wherein biomolecules are dispersed within a hydrogel that is injected directly on the brain tissue (Cooke et al. 2011; Wang et al. 2012) In this example, a small burr hole is made in the skull and the dura is pierced, into which a small volume of hydrogel is injected, thereby releasing biomolecules directly to the brain. In this strategy, the blood-brain barrier is circumvented and the biomolecules are released into the tissue. The advantage of this technique is the local and targeted strategy achieves high local concentration of biomolecules directly into brain tissue, for endogenous tissue stimulation. The limitation of this technique is that biomolecules have to diffuse through brain tissue in order to reach the endogenous cells. While the distance required to reach the NPCs lining the lateral ventricles has been achieved in a mouse brain, it is not clear whether the same success will be realized in larger animal models, including in the human brain.

4.5 Is Stem Cell Therapy for Stroke Ready for Prime Time?

In the 1990s, it appeared that there were an abundance of new drugs, all with the potential to markedly reduce stroke damage with a resultant reduction in functional impairments. Dozens of putative neuroprotective agents were rushed into clinical trials but all failed (O'Collins et al. 2006). What went wrong? Consensus was that both preclinical (i.e. animal models) and clinical studies were seriously flawed (Endres et al. 2008). In order to not repeat the same mistakes, there have been two round-table meetings of scientists, clinicians and industry representatives to establish guidelines and recommendations for Stem Cell Therapies as an Emerging Paradigm for Stroke (STEPS I and II) (Wechsler 2009; Savitz et al. 2011). One issue is whether existing stem cell studies measure up to exiting STEPS guidelines and a second is whether the STEPS II guidelines go far enough to ensure that we do not generate another translational roadblock as with neuroprotection.

A key consideration in stroke recovery is the outcome measures used. As outlined in STEPS II (Savitz et al. 2011), a battery of tests, sensitive to the deficits, carried out at intervals over at least a month should be employed, preferably in multiple laboratories. Only some of these criteria have been met. Most studies use several behavioural tests conducted for 2 or more weeks after the stem cell interventions (Li et al. 2001; Veizovic et al. 2001; Modo et al. 2002; Zhao et al. 2002; Bliss et al. 2006; Andres et al. 2011; Zhang et al. 2011). However, the behavioural tests are often based on subjective neurological deficit scores, with some using tape removal and/or cylinder tests to gauge sensory-motor impairments (Bliss et al. 2006; Andres et al. 2011; Zhang et al. 2011). The problem with such tests is that they reveal deficits early after stroke, but often, there is near complete spontaneous recovery after several weeks (Murphy and Corbett 2009). Indeed, it appears that in many cases, the stem cell treatments are accelerating recovery since the control animals are showing the same recovery profiles albeit at a slightly slower rate. These findings are reminiscent of early work with amphetamine (Feeney et al. 1982) where this drug sped up recovery on simple beam-traversing tasks and attempts to demonstrate clinical efficacy have failed (Gladstone et al. 2006). Because of the inherent risks and invasive nature of stem cell therapies, they would only be used on patients with chronic, debilitating deficits that accompany moderate to severe stroke. Accordingly, animal models investigating the efficacy of endogenous or transplant stem cell approaches need to incorporate impairments that are not only chronic but that target common clinical disabilities such as the inability to use the fingers. Only a few stem cell studies have used more sensitive sensory-motor reaching tasks such as the staircase test or single pellet reaching tests that assess skilled use of the digits (Kolb et al. 2007; Andrews et al. 2008; Liu et al. 2011). Using human adult bone marrow-derived cells, Andrews and colleagues (Andrews et al. 2008) reported substantial recovery in a single pellet reaching task compared to control rats that correlated with sprouting of the corticorubral tract into the damaged hemisphere. In our own studies, using cortical and striatal transplants of mouse SVZ cells into rats with forelimb motor cortex stroke, we detected a small (~10 %), nonsignificant improvement in staircase skilled reaching compared to controls. In another study, transplants of human NPCs

in rats exposed to enriched environments produced improvement in spontaneous limb use in the cylinder test, but there was no benefit in resolving reaching impairments in the staircase test (Hicks et al. 2009). The general limited recovery of skilled reaching compared to the uniform benefit in resolving simple neurological deficits highlights the need to use more sensitive and clinically relevant outcome measures in animal studies.

Another important consideration is that stroke patients typically receive rehabilitation that exerts its recovery-enhancing effects by promoting the same neuroplasticity processes (e.g. upregulation of growth factors, sprouting of dendritic spines and new connections) (Murphy and Corbett 2009) that are activated by stem cell interventions (Andres et al. 2011). Thus, it is important that preclinical studies incorporate rehabilitation, along with other key attributes of human stroke (e.g. older animals, disease co-morbidity) since these variables can markedly alter the efficacy of stem cell treatments. For example, rehabilitation affects the behaviour of transplanted stem cells. Hicks showed that transplanted mouse NPCs derived from SVZ migrated greater distances and showed a trend towards increased survival in rats that were exposed to a combination of enriched environments and exercise following focal ischaemic stroke (Hicks et al. 2007). In more recent work, we found that EGF- and EPO-induced mobilization of the endogenous NPC pool resulted in significantly faster recovery of skilled reaching after cortical stroke in rats provided it was *combined* with enriched rehabilitation. This is the first demonstration that stem cell therapy produces an additive benefit in stroke recovery *above* what can be achieved with an optimized rehabilitation paradigm. In the absence of such demonstration, stem cell therapies should not be advanced to clinical trials due to the inherent risk and invasiveness to patients that are predominantly elderly and frail.

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