Published in final edited form as:

Expert Opin Biol Ther. 2006 September ; 6(9): 879–890. doi:10.1517/14712598.6.9.879.

Neurogenesis as a potential therapeutic strategy for neurodegenerative diseases

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Abstract

In the adult mammalian brain, new neurons are continuously generated from a proliferating population of neural progenitor/stem cells and become incorporated into the existing neuronal circuitry via a process termed adult neurogenesis. The existence of active functional adult neurogenesis raises the exciting possibility that manipulating endogenous neural progenitors, or transplanting the progeny of exogenously expanded neural progenitors, may lead to successful cell replacement therapies for various degenerative neurological diseases. Significant effort is being made to decipher the mechanisms regulating adult neurogenesis, which may allow us to translate this endogenous neuronal replacement system into therapeutic interventions for neurodegenerative diseases. This review focuses on adult neurogenesis as a strategy to derive potential therapies, and discusses future directions in the field.

Keywords

adult neurogenesis; development; neurodegenerative disease; progenitor; regeneration; stem cell; transplantation

1. Introduction

A long-standing dogma in neuroscience, since the time of Ramon y Cajal, declares that the adult mammalian brain is unable to generate new neurons and is fated to degrade with time, incapable of regeneration after injury [1]. In the last decade, repeated demonstration of active adult neurogenesis in almost all mammals, including humans, has put this dogma to rest [2–4]. Now it is clear that a population of neural progenitor/stem cells (NSCs) exists in the mature central nervous system (CNS), and the adult CNS can integrate these differentiated NSCs as new neurons into the established circuitry. This striking regenerative capacity of the adult CNS holds significant promise for developing novel strategies to treat various neurodegenerative diseases.

NSCs have the basic properties of all stem cells, namely the capacity for extensive self-renewal and giving rise to differentiated cell types [5]. By manipulating the endogenous NSCs or by transplanting cells derived from embryonic or adult NSCs, it may be possible to

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repair degenerated populations of neurons in Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) [6,7]. A deep understanding of these disease mechanisms, in conjunction with deciphering the mechanisms regulating endogenous adult neurogenesis, will be necessary to develop effective neurodegenerative disease therapies that guide transplanted or endogenously coaxed new cells to migrate, differentiate, integrate and survive in the existing circuitry of the adult CNS to promote functional recovery.

This review summarises the existing body of research on the basic mechanisms of adult neurogenesis, and discusses recent studies aimed at enhancing endogenous populations of adult NSCs and investigating how they respond to various physiological and disease processes. The authors also review transplantation studies in both normal and diseased environments using various cell types, including neural progenitors and their progeny, and engineered cells designed to secrete various factors to enhance the survival of existing cell populations. Finally, the authors propose future directions for the advancement of NSC-based therapies for neurodegenerative diseases. Interested readers can consult several comprehensive reviews on a similar topic [6–10].

2. Neurodegenerative diseases as potential targets for neural stem cell therapy

Progressive degeneration of specific neuronal types and deterioration of local neuronal circuitry are the hallmarks of degenerative neurological diseases, such as PD, AD, HD and ALS. As these diseases are lethal with limited therapeutic options, replacement of degenerated populations with NSC therapy may be essential.

Some degenerative neurological diseases involve loss of specific neuronal subtypes within restricted regions, such as HD, ALS and PD. HD is an autosomal dominant inherited disease causing degeneration of striatal medium spiny neurons and cortical neurons, with symptoms of involuntary jerking movements, balance impairment, cognitive disability and a projected survivability of 15 – 30 years after diagnosis [11]. HD is caused by inherited CAG trinucleotide repeats that are amplified over generations [12]. There is no known cure for HD, with treatment limited to alleviating symptoms and providing genetic counselling for individuals and their family members. ALS is a neurodegenerative disease of the upper corticospinal tract and lower motor neurons, causing gradual weakness, muscle atrophy and eventual respiratory failure [13]. More than 90% of ALS patients have a sporadic form of the disease and the remaining 10% of the population have various autosomally inherited dominant mutations, with 20% being due to mutations in the gene for superoxide dismutase-1. The aetiology of ALS is still not fully understood. PD occurs when dopaminergic neurons in the substantia nigra degenerate, causing dopamine levels to drop significantly in their target area, the striatum [14]. PD occurs in > 1% of the population over the age of 65, with symptoms of muscular rigidity, resting tremor, gait abnormalities and slow, laboured movement. Existing treatments focus on sustaining dopamine levels or stimulating the striatum with surgically implanted deep brain electrodes [15]. Other degenerative neurological diseases, such as AD, involve degeneration of multiple neuronal

types with diffuse localisations. AD selectively causes neuronal degeneration in the cortex and hippocampus where β -amyloid plaques form intracellularly with neurofibrillary tangles [16]. AD afflicts 10% of persons over the age of 65 and presents with gradual, uncompromising decline in cognition and memory function until death.

In the late 1980s, human fetal mesencephalic tissue transplantation into the striatum of human PD patients led to significant functional recovery with evidence of increased dopamine production [15,17–19]. Similar recovery was seen with fetal tissue transplantation into HD patients [20,21]. These transplantations required a significant amount of fetal tissue, making it unfeasible as a high-capacity therapeutic option. Double-blind clinical trials later failed to show significant benefit with fetal grafts in PD, and dyskinesia side effects were observed in some patients [22]. Variability in the preparation of the tissue and the heterogeneous cell population of the grafts may account for the different outcomes. Despite these setbacks, successful cases of functional recovery after fetal transplants showed the promise of cell replacement therapy in degenerative neurological diseases.

NSCs have distinct advantages over primary tissue preparations as they can be expanded, genetically modified in culture, and enriched to a more distinct and pure neuronal type prior to transplantation [7]. For successful treatment of neurodegenerative diseases, the proper cell populations must be derived, including neuronal subtypes, such as dopaminergic neurons for PD and medium spiny neurons for HD. These neurons must survive and not succumb to the disease mechanism that destroys the degenerating population. Appropriate circuits must be restored, requiring nerve guidance and synapse formation mechanisms to be in place. Most importantly, cell replacement therapies must be superior to existing treatments without causing further harm.

3. Basic biology of neurogenesis in the adult CNS

Significant progress has been made in the last decade in our understanding of the basic processes of neurogenesis in the adult CNS, as well as its regulation under physiological and pathological conditions [4,23]. The prevailing view is that active neurogenesis in the intact adult mammalian CNS is limited to two specialised regions, the dentate gyrus of the hippocampus and the lateral ventricles extending to the olfactory bulb. Adult neurogenesis in these regions appears to recapitulate the embryonic neuronal developmental process, including proliferation and fate specification of neural progenitors, neuronal maturation, migration, targeting and synapse formation (Figure 1). Areas outside these two regions are generally considered ‘non-neurogenic’, where proliferating NSCs contribute largely to gliogenesis under normal conditions with minimal or no neurogenesis. However, under certain pathological conditions, or after isolation and culture, these NSCs seem capable of producing both neurons and glia, suggesting that NSCs with neurogenic potential may be present throughout the adult CNS [8,9].

3.1 Neurogenesis in neurogenic regions of the adult mammalian CNS

In the hippocampal system, a population of NSCs, localised in the subgranule zone (SGZ) between the hilus and the granule cell layer of the dentate gyrus (Figure 1, A1, B1), proliferate and give rise to neuroblasts, which then migrate a short distance into the inner

granule cell layer and differentiate into granule neurons [4,23]. These new neurons extend their axonal and dendritic projections, becoming synaptically integrated within 2 – 4 weeks after birth (Figure 1, D1) [24–27]. Approximately half of the newborn neurons survive for 1 month after birth and are maintained for an extended period of time [28].

In the lateral ventricles, adult NSCs in the subventricular zone (SVZ), a region beneath the ependymal cell layer (Figure 1, A1), proliferate to generate neuroblasts [29]. These neuroblasts migrate a significant distance anteriorly, via the rostral migratory stream (RMS), to the olfactory bulb [30] and differentiate into two types of olfactory interneurons: granule and glomerular neurons (Figure 1, C1). Interestingly, migration to the olfactory bulb has been observed in all mammalian species studied except humans, although humans have an actively dividing population of NSCs in the SVZ [31,32].

Adult neurogenesis is a dynamic process influenced by environmental changes, such as various growth factors, pathological conditions, injuries and external stimuli [4,8]. *In vivo* studies have shown that NSCs are responsive to numerous physiological conditions, including seizures [33,34], ischaemia [35–37], depression [38], environmental enrichment and exercise [39].

The cellular and molecular mechanisms regulating adult neurogenesis are largely unknown [23]. Specific anatomical and cell type characteristics of the neurogenic niches seem to play essential roles for NSCs due to their close proximity with endothelial cells [40] of capillaries [41], astrocytes [42–44] and ependymal cells [45]. In addition to growth factors that serve as mitogens for NSCs, including epidermal growth factor (EGF), fibroblast growth factor (FGF)-2 and Sonic hedgehog (Shh) [9], molecules that regulate fate specification of adult NSCs are beginning to be identified. Bone morphogenic protein (BMP) was shown to promote glial differentiation of NSCs both *in vitro* and *in vivo* [45]. Secreted noggin in the SVZ and neurogenesis-1 in the SGZ act as BMP antagonists, causing these factors to shift the niche towards creating new neurons [45,46]. Wnt, expressed by local astrocytes in the adult neurogenic regions, was shown to promote neuroblast proliferation and neuronal fate specification [47]. Knocking down Wnt signalling significantly decreases hippocampal neurogenesis, whereas overexpression of Wnt3 causes an increase. Retinoic acid, a potent NSC neuronal differentiation factor *in vitro* that has connections to the Wnt signalling pathway, also plays essential roles in adult neurogenesis *in vivo* [48].

The cellular and molecular mechanisms regulating neuronal maturation, targeting and synaptic integration are less understood. Recent studies have revealed the essential role of GABA, a major inhibitory neurotransmitter, in multiple steps of adult neurogenesis, including proliferation of neural progenitors in the SVZ [49], migration of neuroblasts in the RMS [50], neuronal differentiation [51] and synaptic integration in the dentate gyrus [27].

A driving question of adult neurogenesis asks how this relic of development occurs in the adult brain: is it due to the nature of NSCs themselves, or the environment nurturing them? Most probably, it is a synergistic action with genetic instructions guiding the development of NSCs, the microenvironment providing cell–cell interactions and paracrine factors that control the proliferation rate, instructing the cells to navigate, eventually driving activity-

dependent incorporation and the survival or death of the new cells [27,29,43]. Adult neurogenesis demonstrates the extensive plasticity of the newborn neurons, while at the same time this phenomenon demonstrates the tremendous plasticity of the mature CNS to support and guide nerve growth, migration and synapse formation. Understanding this endogenous process of new neuron acceptance by the adult environment will be essential to developing successful NSC-based cell replacement therapies.

3.2 Neurogenesis in non-neurogenic regions of the adult mammalian CNS

Active gliogenesis has been observed throughout the CNS, while neuronal fate specification from NSCs in regions outside of neurogenic niches remains controversial [4,8,52]. There are both technical difficulties as well as theoretical issues in detecting new neurons, as new neuron incorporation could possibly cause destabilisation of the highly organised mature, adult connectivity [53]. The prevailing view is that adult neurogenesis is extremely limited in the non-neurogenic regions of the intact adult mammalian CNS. On the other hand, NSC populations with neurogenic potential appear to exist in these non-neurogenic regions, as their neuronal fate specification *in vitro* and *in vivo* has been demonstrated under various conditions [4,8,9].

Specific injury- or disease-induced changes in non-neurogenic regions have demonstrated proliferation and neuronal differentiation of local NSCs. Using a mild, site-specific induction of apoptosis in the cortex of adult mice, Magavi and colleagues demonstrated the activation of a small population of NSCs in the cortex, which eventually differentiated into neurons expressing mature neuronal markers with apparent incorporation [54]. In a later study, the same group induced targeted apoptosis of corticospinal motor neurons and, remarkably, neurogenesis was induced with the long-distance projection of newly formed corticospinal motor neurons to the spinal cord [55].

NSCs have been derived from the striatum, spinal cord and white matter tracts of both rodents and humans [56,57]. NSCs from these regions exhibit *in vitro* differentiation characteristics quite similar to neurogenic niche-derived cells. When transplanted back into the adult hippocampus, clones of spinal cord-derived NSCs were capable of differentiating into granule neurons in the dentate gyrus, demonstrating the role of environmental cues in fate specification [58]. These *in vitro* and *in vivo* studies suggest that NSCs with neurogenic potential are present throughout the adult CNS and their neuronal differentiation could be influenced by their local environment. Identification of these environmental signals could promote neurogenesis in different regions of the adult brain for cell replacement therapy in degenerative neurological diseases (Figure 1, A2 and B2).

4. Endogenous cell replacement in degenerative neurological diseases

A tempting strategy for neurodegenerative therapies involves recruiting endogenous NSC populations *in situ* to replace degenerating neuron populations. This would require increasing endogenous NSC proliferation, promoting neuronal fate specification and differentiation, luring the migration of new neurons to locations of degeneration and guiding their integration into the adult circuitry (Figure 1, A2, B2 and C2).

At a limited level, the adult brain appears to already have an injury-responsive neuronal replacement mechanism in place. Global or focal cerebral ischaemia causes a significant upregulation of neurogenesis in both the SVZ and dentate gyrus [35,59]. With focal ischaemia, NSC proliferation increases in the SVZ ipsilateral to the infarct, and within the first 2 weeks immature neurons redirect their migration laterally to implant into the damaged striatum (Figure 1, C2) [60,61]. Some of these cells mature and acquire morphological and immunohistochemical characteristics of medium spiny neurons [37]. Whether these neurons become electrically active and synaptically integrate into striatal circuits remains to be determined. Only 20% of the NSC-derived neurons survived beyond 6 weeks, most likely as a result of inadequate tropic or activity-dependent support for integration and survival. In another study, using a growth factor cocktail infusion, Nakatomi and colleagues were able to recruit resident NSCs of the posterior periventricular region, an extension of the SVZ, to migrate to the CA1 hippocampal cell layer, where they replaced lost CA1 pyramidal neurons after global ischaemia [62]. Notably, they observed significant cognitive recovery after 7 weeks.

After inducing temporal lobe epilepsy in rats, Parent and co-workers observed a 5-fold increase in adult NSC proliferation in the dentate gyrus of the hippocampus for the first 2 weeks, which returned to the control level by 4 weeks [33]. Seizures have also been shown to accelerate synaptic integration of newborn neurons [63]. In addition, some SGZ NSCs migrated into the hilus and differentiated into mature granule neurons [34,64]. Due to this aberrant synaptic integration, these ectopic granule neurons could potentially contribute to the recurrent seizures that occur chronically after the initial seizure induction [34]. These findings emphasise the importance of proper neuronal subtype differentiation and targeting (Figure 1, D1 and D2).

Taken together, these studies demonstrated that NSCs outside the SVZ and dentate gyrus, responding to a modified CNS environment, can be activated to migrate into the parenchyma, differentiate into neurons and integrate into the adult circuitry. Understanding the molecular mechanisms that allow the environment to sustain neurogenesis and new cell incorporation will be crucial for successful endogenous cell replacement therapies.

5. Transplantation therapy for degenerative neurological diseases

For maximal benefit in neurodegenerative therapies, it may be necessary to transplant exogenous cells due to the limited proliferation capacity of endogenous NSCs [10]. In addition, recruiting new neurons that are derived from endogenous neural progenitors in the diseased CNS, without neuroprotective therapies, makes these cells prone to the same degeneration mechanisms as the existing mature neurons. At present, there are two therapeutic transplantation strategies: to replace lost neurons or provide tropic support for dying neurons.

5.1 Available cell types for transplantation

Embryonic stem (ES) cells are pluripotent stem cells derived from the inner cell mass of a blastocyst with the capacity to differentiate into all cell types of an organism [65,66]. Newly developed techniques allow long-term expansion of pluripotent stem cells, including human

ES cells and embryonic germ (EG) cells (Figure 1, A3) [67,68]. With various *in vitro* manipulations, the cells can be induced to differentiate into distinct cell types, including specific neuronal subtypes such as dopaminergic neurons (Figure 1, B3) [69,70]. Despite the potential for ES cells, their application still has ethical and technical issues to overcome, such as immune complications and the possibility that undifferentiated ES cells may form teratomas after transplantation [10].

Somatic stem cells, such as mesenchymal and neural stem cells, are multipotent and can give rise to cell types of a particular organ. NSCs can be derived from various CNS regions of fetal and adult mammalian tissue, including adult human resected surgical specimens [71] and postmortem tissue [72]. These multipotent stem cells can be expanded in defined culture media for numerous passages and differentiated into the different neural cell types, including neurons, astrocytes and oligodendrocytes [8,9]. Other types of somatic stem cells, such as mesenchymal stem cells, could potentially provide tropic support for degenerating cells after transplantation, although their ability to give rise to functional neurons remains controversial. Non-stem cells, such as genetically modified fibroblasts, can also be used for this purpose [73,74].

5.2 Transplanting NSCs and their progeny into the normal CNS

Numerous studies have been performed to transplant embryonic, fetal or adult-derived NSCs into various regions of the normal CNS. These studies are essential to understand how the NSCs differentiate, migrate and incorporate into the adult environment both inside and outside the neurogenic niches [29].

Adult rat hippocampal NSCs expanded *in vitro* with media containing FGF-2 were transplanted into the adult rat hippocampus [75]. These NSCs differentiated into astrocytes as well as mature neurons with phenotypes identical to adjacent mature granule neurons, demonstrating the ability of these cells to retain their characteristics even after *in vitro* expansion. When these hippocampal NSCs were transplanted into the RMS, the cells migrated like SVZ-derived cells and incorporated into the olfactory bulb as interneurons [76]. This study demonstrates that regional cues drive adult NSC neuronal subtype differentiation and adult NSCs exhibit plasticity to differentiate into neuronal phenotypes other than their normal fate *in situ*.

Zhang and colleagues differentiated human ES cells into neuronal precursors *in vitro* and transplanted these cells into the lateral ventricles of newborn mice [77]. The transplanted cells incorporated into the hippocampus and cortex as neurons and astrocytes without the formation of teratomas, a common complication of undifferentiated ES cell transplantation. Lepore and colleagues performed a similar series of experiments in adult rats where undifferentiated neuroepithelial stem cells, differentiated lineage-restricted neuron or glial precursors were isolated and transplanted into the hippocampus, spinal cord and striatum [78]. Interestingly, the undifferentiated, immature neuroepithelial cells did not survive, whereas the predifferentiated glial and neuronal precursors survived, integrated and differentiated into mature neurons and astrocytes in all the transplanted regions. When the authors repeated the transplantation of neuroepithelial cells, but instead predifferentiated the

cells to a precursor state, they observed similar survival and integration as seen in the lineage-restricted precursors.

Human-derived NSCs from fetal and adult tissue are also capable of differentiating into neurons and glia *in vitro* and after transplantation. Human fetal-derived NSCs were enriched by cell sorting, expanded in culture and transplanted into the lateral ventricle of neonatal mice [79]. Seven months later, the cells were found to incorporate into the SVZ and SGZ, where some of the cells differentiated into neurons and glia, while some transplanted cells were still dividing in these germinal niches. Similar results were seen from adult human subcortical white matter-derived progenitor cells after transplantation into rat fetuses [57].

These studies show that the brain can incorporate new populations of cells, even NSCs derived from human tissue, although the germinal niches are more receptive by guiding the fate and incorporation of the new cells compared with other brain regions. In principle, expanding NSCs in culture does not irreversibly alter them to a non-neural progenitor state, but proper pretransplantation differentiation seems essential to ensure the fate of transplanted NSCs.

5.3 Transplanting NSCs and their progeny into the diseased CNS

Studies have also been performed to determine the behaviour of exogenously derived cells when transplanted into various disease models. With endogenous populations of NSCs becoming activated in various neurodegenerative diseases, as outlined above, it seems that the adult CNS may transform and become more receptive to cell replacement for transplanted exogenous NSCs.

In an animal model of PD, Björklund and co-workers injected undifferentiated blastocyst-derived ES cells into the striatum of rats and observed dopaminergic-like tyrosine hydroxylase-positive neurons after 14 – 16 weeks [80]. Within 7 weeks they observed significant behavioural recovery, but 20% of the animals formed lethal teratomas. Instead of undifferentiated ES cells, Kim and colleagues first expressed a transcription factor, nuclear receptor related-1, in mouse ES cells to promote differentiation into tyrosine hydroxylase-positive precursor neurons in culture with the addition of various growth factors [81]. When these immature neurons were injected into the striatum of PD rats, they differentiated into electrically active dopaminergic neurons receiving synaptic inputs within 4 weeks and survived up to 8 weeks without forming teratomas. This study demonstrates the importance of proper differentiation of NSCs before transplantation in order to insure the proper cell fate without forming tumours.

In work concentrating on ALS therapies, adult rats that had an experimental paralysis model were transplanted with human EG cells into the CSF at the lumbar region of the spinal cord [82]. The cells migrated and incorporated into the parenchyma of injured, but not uninjured, animals, and a small population of cells appeared to differentiate into neurons and astrocytes. The behavioural recovery in the animals was probably from tropic effects of transplanted cells rescuing host neurons instead of functional cell replacement. When mouse ES cells that were first partially differentiated into spinal motor neurons *in vitro* were transplanted into the same paralysis model, ~ 25% survived for > 1 month [83]. After

infusing a Rho kinase inhibitor to block the inhibitory effect of myelin on axonal growth, extensive neurite outgrowth was observed compared with rats not receiving the inhibitor, where no neurite outgrowth into the white matter was observed.

More recently, the same group used a multistep treatment to address proper differentiation, neurite outgrowth, survival and synapse formation [84]. Mouse ES cells were differentiated into committed motor neuron progenitors, mixed with a drug to block myelin inhibition, and were transplanted into the spinal cord of injured rats. In addition, glial-derived neurotrophic factor (GDNF)-secreting fetal NSCs were injected into the sciatic nerves, as GDNF has been shown to be an effective motor neuron outgrowth factor, known to attract axons. There was significant behavioural recovery with the multistep treatment, and a portion of the ES-derived cells differentiated into neurons that integrated into the host circuitry, having intact neuron–muscular junctions at skeletal muscles. This elegant study illustrates how complicated future cell therapies may need to be in order to address all the steps of new neuron incorporation.

Human ES cells have also been induced to differentiate into dopaminergic neurons or motor neurons in culture [69,70,85,86]. Dopaminergic differentiation of human ES cells can be induced by either coculture with PA6 cells [69] or a combination of FGF-8 and Shh [86]. When these human dopaminergic neurons were transplanted into animal models of PD, they did not survive and could not maintain their dopaminergic phenotype in the adult brain [87,88], suggesting that additional strategies are needed for human cells. In other studies, McBride and co-workers treated human fetal cortex stem cells *in vitro* with ciliary neurotrophic factor, which aided in predifferentiating the cells into neurons, and injected the cells into the striatum of HD adult rats [89]. Although the animals had limited behavioural recovery as compared with vehicle-injected animals, the stem cell-transplanted animals had a significant increase in striatal volume, with the transplanted cells differentiating into neurons and astrocytes.

These studies make evident the abilities of NSCs with some preliminary results demonstrating incorporation, albeit at a very limited level. Carefully predifferentiating cells prior to transplantation, purifying the cell population to exclusively contain the correct cellular type(s) and modifying the transplanted region to allow for new neuron incorporation appear to be essential for successful functional recovery. The fate of transplanted cells may be enhanced with environmental stimulation, as seen in animal models [90,91], and for human transplantation, postoperative therapy may be essential to stimulate the cells to incorporate into the adult environment. Even though the diseased environment appears somewhat receptive to new cell populations, it still remains a diseased environment, and NSCs that are transplanted may also be prone to the same fate as the populations they are replacing. Thus, it may be logical to enhance the survival of transplanted NSCs with various tropic factors.

5.4 Transplantation of genetically engineered cells into the diseased CNS

Another strategy for stem cell transplantation involves engineering cells that secrete certain tropic factors and transplanting them into regions early in the disease process to enhance the survival of dysfunctional or degenerating cell populations. These approaches can be

described as *ex vivo* gene therapies that are localised to transplanted regions, minimising the risk of complications associated with virus-mediated gene therapies [73,92].

GDNF has been shown to enhance the survival of dopaminergic neurons in animal models of PD and a few clinical trials [93]. Direct infusion of GDNF into the putamen of human PD patients demonstrated significant recovery of coordination-related activities, stimulated sprouting of tyrosine hydroxylase-positive neurons and increased dopamine storage even 2 years after the start of infusion [94,95]. Rats with experimentally induced PD that were transplanted with normal or encapsulated GDNF-secreting fibroblasts into their striatum showed significant improvement in motor behaviour associated with slower dopaminergic neuron degeneration [96,97].

In a Phase I clinical trial, AD patients had autologous transplantations of fibroblasts that were transduced *in vitro* to express nerve growth factor (NGF), a growth factor supporting cholinergic neurons that are specifically degenerated in AD [74]. Twenty-two months after transplantation, a large population of cells appeared to be intact and secreting NGF. The mature neurons of the host also appeared to sprout cholinergic axons and there was a 51% improvement in cognitive function. This study demonstrates some significant recovery with tropic enhancement in addition to the ability to self-transplant and safely deliver neuroprotective genes in humans.

6. Expert opinion and conclusion

In summary, the reality of NSC therapy for the treatment of neurodegenerative diseases is becoming feasible, although a significant amount of research needs to be completed before successful human therapies are developed. The adult brain is a staggeringly complex organ with trillions of neuronal connections. Extrinsic manipulation to re-establish the correct circuitry lost in degenerative diseases, without any form of intrinsic guidance, would be hopeless. Fortunately, the adult brain still retains the ability to guide the incorporation of new cells in specific regions via the process of adult neurogenesis. Deciphering the mechanisms of this endogenous system can serve as a blueprint to translate into effective endogenous or exogenous NSC therapies for neurodegenerative diseases.

During the last few years, significant progress has been made in understanding the factors regulating fate specification in the neurogenic niche and how NSCs are affected by various physiological and disease stimuli. On the other hand, we are just beginning to understand how these cells incorporate into the adult circuitry. More research is needed to discover the intrinsic mechanisms that regulate self-renewal of embryonic and adult NSCs, efficient fate determination and, more particularly, specific neuronal subtype differentiation. Conversely, discovering the extrinsic factors and cell–cell interactions of the adult neurogenic niches that cause these microenvironments to nurture NSCs, and discovering how the new cells are accepted into the existing adult circuitry, both with proper neurite targeting and correct synapse formation, seem essential for developing therapies.

Injury-induced migration of NSCs from the ventricular regions of the brain to damaged areas indicates that a cell replacement system or, conversely, an acceptance mechanism can

be potentially activated in non-neurogenic regions of the adult CNS. What injury-induced factors are being secreted to cause this migration and lure the cells to the damaged tissue? Stromal cell-derived factor-1 α is a potential candidate as it may have a role in luring NSCs from the SVZ to the infarct in stroke-induced neurogenesis [98]. Other factors, such as vascular endothelial growth factor [99] and stem cell factor [100], have also been shown to enhance migration. By discovering more of these motility factors and further enhancing their release with infusions or engineered cell transplants over the course of the disease progression, it may be possible to recruit more endogenous NSCs to fill and incorporate into degenerating regions. These factors are also crucial for transplantation therapies as extensive migration from the injection sites is required to fill the diseased region with new cells (Figure 1, C3).

Modifying the environment to accept new cells, in addition to enhancing targeting and survival, is essential in the diseased environment. Successful cell therapy with unmodified NSCs would depend on the assumption that the newly incorporated cell population from either endogenous NSC recruitment or transplantation will be immune to the disease process of the degenerated population that is being replaced. With the promising results of *ex vivo* gene therapy, with tropic factor-secreting cell transplantation in humans [74] and combination therapies [84], it seems feasible that the transplanted NSCs themselves could be modified to secrete factors for autocrine and paracrine support. Another potential strategy would be to prime the region with an initial transplantation of support cells, either glial progenitors or fibroblasts, that replicate the germinal niches prior to transplanting NSCs, which could help in the survival and integration of the new cells.

In conclusion, in order for true NSC-based therapies to be successful, the functional incorporation of new cells replacing the degenerated population needs to occur. Differentiating into the correct subcellular phenotype, establishing the correct migration to the region, forming the correct dendritic and axonal connections, in addition to true functional electrophysiological integration into the existing circuitry with observable recovery in the patient, are all requirements for stem cell therapy. Although this seems daunting, taken together with the existing mass of encouraging research and foreseeable work to be performed, NSC-based treatments have high potential for future neurodegenerative disease therapies.

Acknowledgments

The authors thank J Sailor for help with editing the manuscript. The research in the authors' laboratories is supported by funding from the Packard Center for ALS at Johns Hopkins University, McKnight Scholar Award, NINDS and NIA (to HS), and Klingenstein Fellowship Award, Charles E. Culpeper Scholarship in Medical Science, March of Dimes, Whitehall Foundation, Alfred P. Sloan Foundation and NINDS (to G-LM).

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Figure 1. Outline of how adult neurogenesis mechanisms can be translated into endogenous and exogenous NSC replacement therapies for neurodegenerative diseases

A1. Neurogenesis occurs in the SVZ (1) of the lateral ventricles where the new neurons migrate via the RMS (2) to the olfactory bulb (3). Neurogenesis also occurs in the dentate gyrus (4) of the hippocampus (5) where it is confined to the SGZ of the GCL. **A2.** Infusion of various growth factors into the parenchyma or ventricles can activate existing NSCs within neurogenic regions and quiescent NSCs in the adult environment. In addition, disease mechanisms may affect proliferation of NSCs. **A3.** NSCs can be expanded in defined culture conditions for multiple passages before eventually being used for transplantation. **B1.** Fate specification is regulated by the local environment (the niche). In this example, NSCs in the

dentate gyrus differentiate into a restricted neural progenitor that will eventually become a granule neuron, or a restricted glial progenitor that will become an astrocyte. **B2.** In certain disease states, the existing NSCs generate new neurons in response to changes in the local environment. Infusing differentiation factors may enhance this response. **B3.** In culture, cells can be induced to differentiate into different cell types, including subtypes, prior to transplantation to insure proper *in vivo* differentiation without tumour formation. **C1.** In the SVZ, adjacent to the LV, new neurons migrate a significant distance anteriorly to the olfactory bulb via the RMS. In the hippocampus, the granule cells extend dendrites toward the molecular layer and axons to the CA3 in response to positive and negative guidance cues in the local environment. **C2.** Certain injuries and diseases induce migration of NSCs or their neuronal progeny to the injury site. **C3.** Transplanted cells need to migrate away from the injection site, finding the degenerated population and eventually form a three-dimensional network. **D1.** In the hippocampus, NSCs differentiate into functional granule neurons receiving input via their dendrites from the entorhinal cortex, and relay the signal down their axons to downstream targets in the hilus and CA3 region. **D2.** Both endogenous and exogenous NSC therapies require the new cells to appropriately incorporate into existing circuits.

DG: Dentate gyrus; GCL: Granule cell layer; LV: Lateral ventricles; NSC: Neural stem cell; RMS: Rostral migratory stream; SGZ: Subgranule zone; SVZ: Subventricular zone.