Review article

Neural stem cell therapy for neuropsychiatric disorders

Valenzuela M, Sidhu K, Dean S, Sachdev P. Neural stem cell therapy for neuropsychiatric disorders.

Objective: To conduct a comprehensive literature review of the area of neural stem cells and neuropsychiatry.

Methods: 'Neural stem cells' (NSCs) and 'neurogenesis' were used as keywords in Medline (1966 – November 2006) to identify relevant papers in the areas of Alzheimer's disease (AD), depression, schizophrenia and Parkinson's disease (PD). This list was supplemented with papers from reference lists of seminal reviews.

Results: The concept of a 'stem cell' continues to evolve and is currently defined by operational criteria related to symmetrical renewal, multipotency and functional viability. *In vivo* adult mammalian neurogenesis occurs in discrete niches in the subventricular and subgranular zones – however, functional precursor cells can be generated *in vitro* from a wide variety of biological sources. Both artificial and physiological microenvironment is therefore critical to the characteristics and behaviour of neural precursors, and it is not straightforward how results from the laboratory can be extrapolated to the living organism. Transplant strategies in PD have shown that it is possible for primitive neural tissue to engraft into neuropathic brain areas, become biologically functional and lead to amelioration of clinical signs and symptoms. However, with long-term follow-up, significant problems related to intractable side-effects and potential neoplastic growth have been reported. These are therefore the potentials and pitfalls for NSC technology in neuropsychiatry. In AD, the physiology of amyloid precursor protein may directly interact with NSCs, and a role in memory function has been speculated. The role of endogenous neurogenesis has also been implicated in the etiology of depression. The significance of NSCs and neurogenesis for schizophrenia is still emerging.

Conclusions: There are a number of technical and conceptual challenges ahead before the promise of NSCs can be harnessed for the understanding and treatment of neuropsychiatric disorders. Further research into fundamental NSC biology and how this interacts with the neuropsychiatric disease processes is required.

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What are neural stem cells?

Neural stem cells (NSCs) are primitive cells that have an unrestricted ability to self-generate and then differentiate and function as one of three cell lineages, including neurons, oligodendrocytes and astrocytes.

A consensus biological definition for NSCs is elusive because there does not seem to be a fixed feature that is shared by all NSCs. In other words, no cellular characteristic is sufficient to prospectively guarantee 'stemness'. Rather, a stemness spectrum is more appropriate where cellular microenvironment appears to act as a major determinant: in one context, a cell may appear and behave as a regular cell, but in another gain stem-like qualities (1). In the brain, stem cells are therefore a fluid and dynamic entity. As we shall

see, exactly what one means by a 'neural stem cell' also depends on the ontogenic framework in which it is assumed to participate. Furthermore, *in vivo*, there is an important transition state between NSCs and their differentiated progeny, a type of cell loosely termed *neuroprogenitor*. These cells are capable of more limited self-renewal and can be biased to differentiate into one type of cell over another. The term neural precursor has therefore been introduced as an alternative and covers both NSCs and neuro-progenitors.

For practical purposes, it is therefore necessary for an NSC to meet a number of operational criteria. The first and most crucial is a capability for *symmetric division*, referring to continuing self-renewal of like-cells. This is best shown by testing for *clonogenicity* (2), the ability for a single isolated cell to generate an entire population of like-cells. Recently, it was suggested that NSCs and neuro-progenitors could be distinguished *in vitro* by their ability to symmetrically expand the original cell population beyond five passages (3). *Passaging*

refers to the process of splitting a population into two or more subpopulations, repopulating the culture vessel over time and then splitting it again and so forth. The second key criterion is *multi-potency*, assessed by changing cells from an expansion medium to a differentiation medium, and showing that they can differentiate into at least two different kinds of cells. Each of these criteria is typically assessed *in vitro*. The third, and often most challenging test for NSCs, is demonstration of *in vivo* survival, differentiation and viability.

Surface antigens and other markers have been used to characterize and purify NSC colonies. As mentioned, no single marker, or combination of markers, can unambiguously identify stem cells in the brain. Common usage is summarized in Table 1. Nestin is the most frequently used 'marker' for NSCs; however, it is also expressed by neuroprogenitors (1). Exclusion of markers can also be equally informative because NSCs should not express surface proteins found in mature differentiated neurons such as TH, NeuN or synaptophysin (see Fig. 1).

Table 1. Commonly used markers for NSCs, neuroprogenitors and neurons

Marker	Hypothesized role	Reference
NSCs		
Nestin	Class VI intermediate filament expressed in the developing central nervous system (CNS) in early embryonic neuroepithelial stem cells. Also expressed in some neuroprogenitors.	Hockfield et al. (7)
NCAM	Essential for the correct establishment of synaptic connectivity. Regulator of hippocampal plasticity.	Rutishauser et al. (8)
CD133/prominin-1	Expressed on the membrane protrusions of the apical surface of neuroepithelial cells.	Uchida et al. (9)
Neuroprogenitors		
GFAP	Class III intermediate filament protein. Has a cytoskeletal role and is also expressed in NSC and astrocytes. GFAP-expressing progenitor cells are a predominant source cell for adult neurogenesis.	Garcia et al. (10)
Mash1	Regulates neurogenesis in committed neuronal precursor cells, its early expression activates a subset of neuron-specific genes to promote differentiation.	McNay et al. (11)
Sox1, Sox2, Sox3	Earliest transcription factors expressed in the developing neural tube and also involved in neurogenesis. Maintains neural progenitor cells in an undifferentiated state. Also used as NSC markers.	Bylund et al. (12), Brazel et al. (13)
FGF-2 receptor	FGF-2 promotes proliferation of NSC. Its receptor has been identified in many early neural progenitors as well as oligodendrocyte and astrocyte lineages.	Reimers et al. (14)
Olig2	Implicated as a repressor of neurogenesis in cells reacting to brain injury.	Buffo et al. (15)
Immature neurons		
β-tubulin III/Tuj1	Abundant in the CNS and PNS. In adult tissues, the distribution of β-tubulin III is almost exclusively neuron specific. See Fig. 1 for example.	Fanarraga et al. (16)
Immature oligodendrocytes		
GalC	Galactocerebroside – a major glycolipid of myelin, which plays a role in myelination. It is used as a specific antigenic marker for myelin-forming cells such as oligodendrocytes.	Dupree and Popko (17)
Immature astrocytes		
GFAP	See above.	
Mature neurons		
MAP2	Essential for development and maintenance of neuronal morphology. Its association with neurofilaments and actin filament may guide its interaction among microtubules, other cytoskeletal elements and cytoplasmic organelles.	Binder et al. (18)
NeuN	Present in most CNS and PNS neuronal cell types. First appears at the developmental time points that correspond with the withdrawal of the neuron from the cell cycle and/or with the initiation of terminal differentiation of the neuron.	Mullen et al. (19)
TH	Involved in the conversion of phenylalanine to dopamine. Regularly used as a marker for DA neurons.	Pickel et al. (20)
Synaptophysin	Integral membrane protein of transmitter-containing vesicles found in synapses. May be involved in the regulation of exocytosis and/or endocytosis of neurotransmitters.	Wiedenmann and Franke (21)

DA, dopamine; NCAM, neural cell adhesion molecule; GFAP, Gliol Fibrillary Acidic Protein; PNS, peripheral nervous system.

Fig. 1. Differentiated neurons derived from human embryonic stem cells. Blue staining (left) is for DAPI (a cell nucleus marker), red (middle) is for β-III tubulin and the far right figure shows the overlap. From Sidhu et al. (unpublished work).
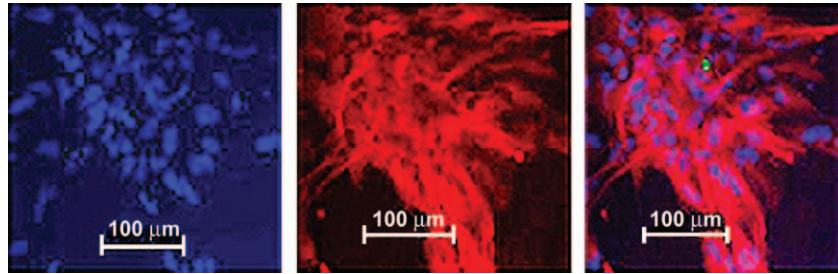
Neurogenesis and biology of endogenous NSCs

The human brain develops from the outermost ectodermal layer of the primordial inner cell mass of the blastocyst. During the first weeks of life, a number of important biosignals are known to influence neural development. Fibroblast growth factors (FGF) and Wnt play a role in the first step of neural induction, whereby nascent ectoderm is pushed down the neuroepithelial track (4). This step also appears to require suppression of bone morphogenetic protein (5). Once the neuroepithelial fate has been determined, the neural plate folds to form the neural tube. This temporary structure manifests strong rostrocaudal and dorsoventral axes, setting up a 'coordinate grid' of sorts for the establishment of the neonatal brain. Retinoic acid (RA) plays a major role in the 'caudalization' gradient and Sonic hedgehog is important for signaling the 'ventralization' gradient. These two factors combine, for example, to push developing neurons down the motor neuron pathway. As we shall see, in the laboratory, these signaling factors are recapitulated to artificially induce and elaborate the NSC population (6).

During infant and child development, the further ontogeny of NSCs remains controversial. Under the classical scheme, neurons mature from a single (as yet unidentified) stem cell, which then sequentially produce NCSs, neuroprogenitors and then immature neurons (Fig. 2). This kind of schema draws heavily from hematopoeitic ontogeny, where well-documented relationships exist between stem cells, progenitors and different blast cells, which persist into adulthood. More recently, researchers have theorized as to the role of radial glial-like cells as a quiescent (potential) stem cell, which under certain conditions can transform into a local neural precursor cell and also provide architectural guidance for their mobilization (for a review of this topic, see Kempermann (1).
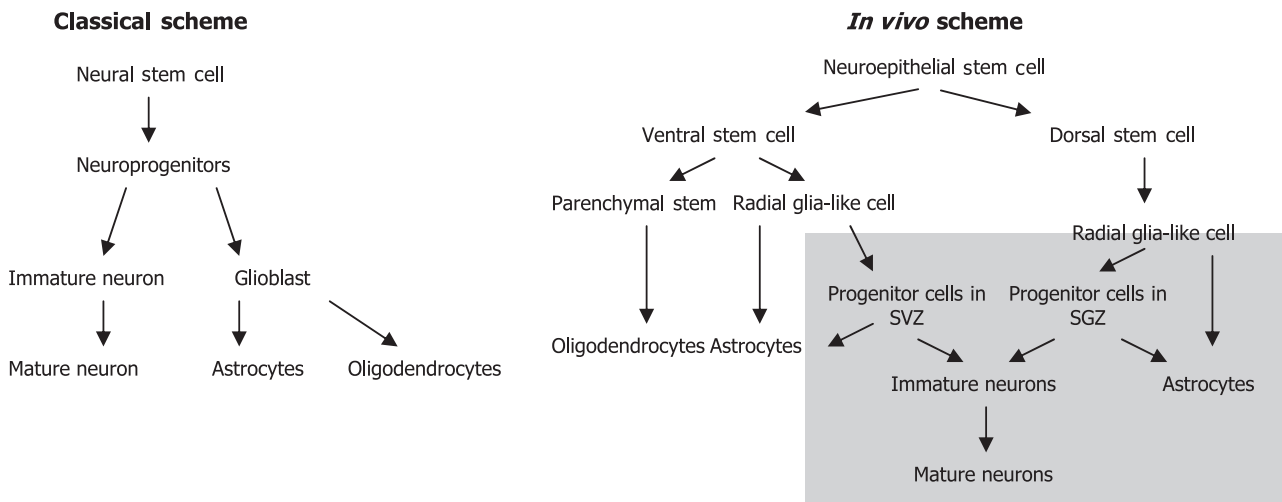

Fig. 2. Ontogenetic schemas for mammalian neurogenesis [adapted from Kempermann (1)]. The classical scheme draws heavily from parallels in hematology, which is unlikely to be reflected in the brain. The *in vivo* scheme is highly preliminary and is empirically challenging because *in vitro* development shares little resemblance to the course of events *in vivo*. The gray area represents the hypothetical steps relevant to adult neurogenesis. SGZ, subgranular zone of dentate gyrus; SVZ, subventricular zone.

In the mature mammal, the past 10 years has seen a revolution of sorts with recognition that adult, and even *late adult*, neurogenesis can occur (22–24). This research has thus seen the disposal of the 'no-new-neuron' dogma articulated by Ramon y Cajal almost 100 years ago. Interestingly, radio-labeling for newly divided cells enabled visualization of new neurons in the mammalian brain in the 1960s (25), but the results have only recently found a receptive audience.

There is now a growing understanding of two major endogenous neurogenetic streams in the adult mammal. The first involves neuroprogenitors in the subventricular zone, which produce neural precursors that mobilize along the rostral migratory stream to enmesh into olfactory neural circuits in the orbitofrontal cortex. A second neurogenetic stream is composed of cells in the subgranular layer of the hippocampus, which eventually differentiate into the granular layer and then integrate into the CA3 subfields of the dentate gyrus (26). The functional relevance for adult neurogenesis in the hippocampus is hotly debated, with some evidence for a role in memory function (1,27) and competing evidence for no mnemonic or cognitive significance (28).

Challenges for the generation of NSCs

Homogeneity and assay technique

Reynolds and Weiss (29) first reported on a suspension assay, which involved culturing adult mouse brain tissue in a flask with a high concentration of the neurotrophic agent epidermal growth factor (EGF) in the absence of serum. A number of days later, the majority of cells perished; however, spheres of several hundred cells had developed and floated to the surface of the suspension (see Fig. 3). These *neurospheres* were subsequently found to meet functional NSC criteria and have been used extensively in experimental transplant studies because they can amplify quickly and readily *in vitro*, which is of vital importance in the context of

disease states where several million neurons may be lost.

While the neurosphere assay has led to many important insights into NSC biology, it is limited by an inherent heterogeneity. Neurospheres are composed of an admixture of true NSCs, neuro-progenitors and immature neurons (30). Neuro-spheres are highly complex biological systems within themselves, with mitotic, apoptotic and even phagocytotic events occurring at any one time (31). Estimates of the 'true' frequency of NSCs within neurospheres range from 0.1% to 0.6% (3). The functional and morphological heterogeneity of neurospheres therefore probably makes their use as an assay for NSC *quantitation* invalid (2). These considerations mean that a transplant that utilizes an amplified population of neurospheres will each start with highly variable NSC numbers, leading to potentially unpredictable results and poor repeatability (32). There are, however, several technical strategies being developed to try to produce more uniform neurospheres (33).

Another technical option is to culture NSCs on a gelatin-coated surface, such that individual NSCs grow and divide as a monolayer (34). Using similar trophic factors, it has been reported that the adherent monolayer assay produces a more homogenous NSC population (35), with *in vivo* neural differentiation augmented from approximately 5% to over 40%. The adherent monolayer assay is also versatile, facilitating propagation from a number of sources (ie, human embryonic stem cells or adult stem cells) (36). The main disadvantage of the technique, however, is slower turn-around time (1).

Symmetry and senescence

A key goal of NSC culture is the ability to amplify vast numbers of cells. With sufficient resources, it would be, for example, theoretically possible to start with a single NSC and after 64 passages produce 10^{19} cells, more than the number of stars

Full thickness canine skin sample obtained post mortem.

Neurosphere derived from canine skin.

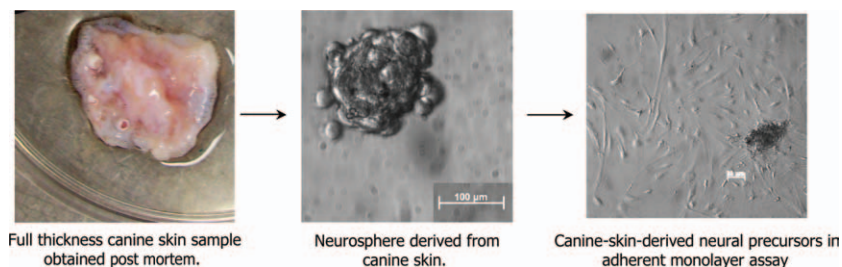Canine-skin-derived neural precursors in adherent monolayer assay

Fig. 3. Neural precursors derived from canine skin using the monolayer assay after induction of neurospheres.

in our galaxy! Yet, this of course relies on the assumption that the act of passaging does not itself affect the productive qualities of the cells. Unfortunately, this is not always the case. First, in some culture systems, extended passaging has been found to bias NSCs culture away from symmetrical division, with later passages tending to produce neuroprogenitors and immature neurons rather than self-copies (32). Second, evidence for chromosomal instability has been observed with extended passaging of adult NSCs (cellular senescence) (37). As we will review, these issues affect NSCs derived from different tissue origins selectively. From a clinical perspective, however, truly limitless self-replication is not required. Rather, what is more relevant is to establish a minimal level of symmetry and chromosomal stability to allow for the production of sufficiently viable NSCs for transplant.

Tissue source and autogenicity

One source of NSCs is mammalian embryonic tissue (38,39). Embryonic tissue is pluripotent, and researchers found that *in vitro* application of RA, which in normal development promotes fetal ectoderm down the neural pathway, led to development of a structure called embryoid bodies (6). In the neurogenic environment of high-concentration FGF-2 and EGF, embryoid bodies can be cultured to produce NSC lines.

NSCs derived from embryonic tissue have a number of advantages. First, they appear to be less prone to chromosomal senescence over extended passages (40) and exhibit symmetric division. However, society continues to debate the ethical and legal implications of using human embryonic tissue for medical treatment. Also, derivation of NSCs from embryonic tissue entails a heterologous graft and potential for tissue rejection and consequent immunosuppression. Even though embryonic tissue does not express the human leukocyte antigen (HLA) antigens that mediate self- and non-self-recognition, it is not clear whether subsequent differentiation into mature cell phenotypes would lead to expression of the donor or host HLA. Interestingly, some studies of allogenic NSC transplants in mice and humans do not report development of tissue rejection (41–43). Because of the strong pluripotency of embryonic tissue, a major drawback of NSC transplants derived from this tissue source remains the potential for teratoma formation (38,44).

So called 'adult NSCs' are derived from post-pubertal mammalian tissue, with brain isolates the most common source. During this procedure, extracts from mouse or human brain are dissociated and NSCs isolated by culture in media favoring neuronal development. Presumably, the richest source for adult neural precursors are the subventricular and hippocampal zones as these are the sites of known neurogenesis; yet, cultures of neural precursors have been reported from the cortex, white-matter and other brain areas as well (45). The significance and identity of the originating cells in these contexts is unknown.

Bone marrow is another source of adult NSCs, a region known to be rich in mesenchymal stem cells. This strategy remains controversial because of the requirement to show transdifferentiation, that is, for a cell to cross from one development cell lineage (endoderm) to another (ectoderm). Whether true transdifferentiation actually happens *in vivo* has been questioned because of reports of bone-marrow-derived NSC transplants exhibiting cell fusion rather than division and neural differentiation (46). Cells thought to be newly differentiated neurons actually contained three or more chromosomal copies, suggesting the transplants had become endocytosed into a preexisting neuron. The matter is far from settled, however, with other groups reporting authentic pluripotency for cells derived from bone marrow (47).

As alluded to earlier, adult NSCs may not allow for such extended passaging as embryonically derived NSCs and may also exhibit chromosomal instability. Adult NSCs, however, also benefit from a number of practical and clinically relevant advantages. Ethical issues surrounding embryonic tissue are, for example, not pertinent. Access to source tissue from the same patient means that at least in principle, autologous transplant is feasible. This would in turn obviate any requirement for immunosuppressive therapy. Finally, adult NSCs derived from the brain are already 'lineage restricted' and so benefit from less potential for uncontrolled teratoma formation than tissue derived from embryonic stem cells.

An autologous, homogenous adult NSC technology that allows for extended passaging without loss of symmetric division or chromosomal stability is therefore the ideal starting point for any neurorestorative treatment. Stem cells resident in mammalian skin may provide a promising initial step in this direction. As reviewed earlier, neurons originate from the same ectodermal lineage as the epidermis. Stem cells from this region could therefore make an attractive adult NSC source as transdifferentiation would not be required. Toma et al. were one of the first groups to report on the appearance of neurosphere-like aggregates of cells within days after rodent skin cells had been

cultured in a suspension assay (48,49). These spheres were found to be positive for nestin and negative for mesenchymal markers or immature neural and glial markers. They also showed symmetrical division for greater than 30 passages, clonogenicity, and differentiated into neurons and glia in equal proportions after removal of mitogens. Significantly, these cells also differentiated *in vivo* into viable neurons after transplantation into the chicken ovum. Subsequent studies have shown that adult human skin can also readily be used to derive adult neural precursors (50).

We have adapted a technique whereby skin-derived neurospheres are transferred into an adherent monolayer assay. This technique appears to produce a homogenous culture of neural precursors, which are stable for a number of passages (see Fig. 3). This technique may be useful for future clinical research because as little as 2–6 cm^2 of skin is required as an initial tissue source, a procedure with minimal invasiveness and morbidity.

Survival: does *in vitro* predict *in vivo*?

One of the more sobering statistics in stem cell research is that after careful *in vitro* laboratory work over many months, having generated perhaps 10^6 NSCs for transplant, only a small fraction of these appear to survive for any length of time *in vivo*. Survival is therefore one of the major challenges faced by stem cell therapy. Moreover, what determines why some NSCs will survive, differentiate and integrate into the host's neural architecture, and others will not, is unclear. The gliogenic environment produced by inflammation and injury may, for example, be a critical factor in determining cell fate of transplanted NSCs (51). This brings up a fundamental and still open question for the area: do *in vitro* results have sufficient predictive validity when translated into the live organism?

Transplanted cells also enter into 'hostile territory' where pathology has altered the microenvironment considerably; whether such changes may benefit or detract from NSC survival and integration is simply not known for many of the neuropsychiatric diseases discussed below. These considerations suggest that *quality* may be more important than *quantity* when attempting NSC transplantation, but exactly what qualities are salient is undetermined. Brain-derived neurotrophic factor (BDNF) may be one humoral factor that could benefit transplanted NSCs as it has been implicated in stimulation of endogenous neuro-

genesis after both exercise (52) and complex mental activity (53).

NSC treatment strategies

Exogenous NSC transplant

Transplantation of neural precursors is one of two major neurorestorative strategies being pursued. These studies show considerable variability in terms of the cell type being transplanted. NSCs, neuroprogenitors or immature neurons have all been used. Important issues to consider include the condition of interest and theorized role for the transplant. NSCs are highly mobile and when, for example, injected into the contralateral cortical area in a stroke model, they translocate across the breadth of the brain to engraft into ischemic area (54). Chemotaxis presumably guides NSCs to the area of brain damage. The precise signal of interest in unknown, with secreted amyloid precursor protein (APPs) one potential mediator as it is induced by a range of noxious stimuli (55) and also stimulates endogenous neurogenesis (56).

Transplant of NSCs (and to a lesser extent neuroprogenitors) is therefore more likely to be useful if the nature of the brain lesion is diffuse or difficult to access stereotactically. Transplants of large quantities of immature neurons, on the other hand, which show very little mobilization beyond the region of delivery, are likely to be more suitable if the lesion is very discrete, like in acute spinal injury, and is relatively easy to access surgically.

Stimulation of endogenous NSCs

Another treatment strategy being explored is artificial stimulation of endogenous neurogenesis. These studies are based on an emerging understanding of regulatory factors relevant to physiological neurogenesis. One-to-one relationships between experimental manipulations and altered neurogenesis are, however, likely to be oversimplifications. The *in vivo* regulatory system appears to be highly multidimensional and interdependent (1).

Environmental factors found to increase neurogenesis include enrichment (57) and physical activity (52). Chronic stress, on the other hand, decreases neurogenesis (58). Signaling molecules involved in regulation include vascular endothelial growth factor (59), FGF-2 (45) and BDNF (60), among others. The outcomes of clinical trials of exogenous growth factor therapy in the context of

neurodegenerative diseases have been reviewed concisely elsewhere (61). Many of these studies have examined the role of glial cell-line-derived neurotrophic factor, a trophic factor implicated in improving survival of developing neurons (62). In general, outcomes have so far been disappointing, with the suggestion that neuroprotection may be a more realistic ambition than neurorestoration for this NSC strategy.

Relevance to neuropsychiatric disorders

What are we aiming for?

The first and perhaps most important question to pose when considering NSC therapy for a neuropsychiatric disorder is what is the indication and what precisely is the desired outcome? Simply transplanting NSCs into the area of disease and hoping for the best is clearly unsatisfactory. There are a number of therapeutic mechanisms of potential relevance, and each needs careful consideration when assessing efficacy.

One mechanism of action may be a transient humoral effect. In this case, the NSC graft survives for a short period of time and during this period is able to secrete neurotransmitters, growth factors or other signaling molecules, which increase the functional effectiveness of local established neuronal circuits. Under these circumstances, the observed behavioural or clinical benefit may be short lived.

Another mechanistic action for the transplant is that cells migrate to the lesion of pathology and then rather than becoming functionally integrated, either differentiate into glia or into electrophysiologically inert neurons. In this instance, the effect is thus mainly supportive in a metabolic, regulatory or anatomical sense. This may nevertheless result in correlated clinical improvement. The distinction between a supportive vs functional graft is hence important.

In general, researchers hope that some degree of functional integration will occur. There are a number of substantial challenges for this to proceed. First, the transplanted cells need to arrive at the right location. Chemotaxis presumably helps the cells migrate to the pathological locus; yet, this is not necessarily the best destination if the local microenvironment is not permissive to the growth, differentiation and integration of NSCs. Second, there needs to be the right number of cells present. In Parkinson's disease (PD), 80% of substantia nigra neurons are typically lost before clinical onset, representing a potential deficit of 10^7 neurons. Next, enough cells need to not only

survive within an excitotoxic and apoptotic environment but also begin to differentiate into the local class of specialist neuron. Finally, as the cells mature, they need to make sufficient synaptic connections within the local circuit, become electrophysiologically functional and begin to participate in the local and distributed dynamical information processes that have been disrupted. Moreover, all of this needs to occur basically on its own! These are challenging tests for any therapeutic strategy. In the following section, we will briefly review those studies that have explored this approach across a number of neuropsychiatric models and assess if NSC therapeutics represents a realistic goal.

Alzheimer's disease

Alzheimer's disease (AD) begins principally in the medial temporal lobe (MTL), including the entorhinal cortex and hippocampus, before spreading to multimodal neocortex, and eventually almost the entire brain (63). Precisely, how the pathological features of AD, which include senile plaques composed of extracellular aggregates of beta-amyloid, and hyperphosphorylated tau in the form of intracellular neurofibrillary tangles, cause the initial amnestic syndrome of early dementia remains unclear. Loss of synapses and neurons in the hippocampus are profound, exceeding the degree of local neuropathology (64). On one level, it is unsurprising that memory problems occur given the critical role these regions play in memory function. Yet, in clinicopathological studies, the most robust ultrastructural correlates of cognitive status in dementia are not the neuropathological lesions, or MTL synaptic numbers, but synaptic density in the frontal lobe (65,66). Individual differences in compensatory functional reorganization in the frontal lobe in response to disruption to MTL memory circuits may therefore be important to the expression of clinical symptoms in AD (67,68).

How does incipient AD neuropathology in the MTL affect neurogenesis and NSC biology? These are fundamental questions for the area and definitive answers are yet to emerge. Molecular studies of familial early-onset AD, which remains exceedingly rare, have focused research on pathways involved in the production of beta-amyloid. In these individuals, mutations to genes on chromosomes 21 (APP) and 14 (presenilins) have been found. APP is a naturally occurring transmembrane protein, which is sequentially cleaved to produce both intracellular and extracellular beta-amyloid and secreted APP (APP_s). Activation of

this pathway can occur in response to a range of noxious stimuli, including ischemia, seizures and brain injury (55). *In vitro* studies suggest that APP_s appears to have a neurotrophic effect, with reports of upregulation of neurite outgrowth and cellular proliferation (56). This may, however, exhibit a nonlinear dose-dependent relationship because at lower doses APP_s promotes *both* neuronal and glial differentiation of NSCs *in vitro*, but at higher doses (>25 ng/ml), preferential astrocytes differentiation is observed (69). Transgenic mice that lack APP show significantly degraded migration of NSCs after transplantation, suggesting a tropic signaling role as well (69). The possibility that exogenous transplants of NSCs may preferentially differentiate into glia rather than NSCs in the AD microenvironment hence needs careful consideration.

The impact of beta-amyloid on endogenous neurogenesis is unclear. Haughey et al. (70) conducted a systematic analysis of the effects of this protein on NSC proliferation and differentiation. Transgenic mice, which overexpressed APP, had significantly lower neural proliferation in the dentate gyrus when compared with control mice. When human-fetus-derived neurospheres were treated with concentrations of beta-amyloid over the 1 μM range *in vitro*, proliferation was also significantly decreased. Neuroprogenitor apoptosis also increased at these levels, possibly through disruption of proper cellular calcium homeostasis. However, these results were based on beta-amyloid concentrations 1000 times those seen in clinical AD, which are in the nanomolar range (55). At these levels, no discernible effects on NSC biology were noted. This may explain why in the single reported analysis of neurogenesis in postmortem AD, a significant *increase* in neural precursor levels was seen in the dentate gyrus compared with age- and sex-matched controls (71). The large amount of atrophy that occurs in the MTL in AD risks artifactual elevation of protein concentrations because of volume packing; yet, in this report, no such change was noted in control neural proteins.

In AD, we suggest that neurotoxic pathological processes may signal compensatory neurogenesis by means of the APP pathway; yet, this ultimately fails to preserve mnemonic function because of the profound extent of neural and synaptic loss. So could augmentation of neurogenesis through exogenous supplementation of NSCs be of any use? The few transplant studies to date are encouraging. One study of aging rats found a significant amelioration of behavioural decline after transplantation of human-derived NSCs into the lateral ventricle, in conjunction with evidence of engraftment in the MTL and cortical areas (41). Another study used cholinergic denervation of the MTL through excitotoxic lesion of the nucleus basalis of Meynert (NBM) to mimic some of the neurotoxic and behavioural features of AD (72). They compared the effect of transplantation of neurospheres directly into the frontal cortex in mice with NBM lesions with results from healthy control mice; a significant rescue of cognitive performance was seen in the NBM lesion group, accompanied by differentiation of choline-positive and serotonin-positive neurons around the graft site. Interestingly, control mice developed teratomas and showed even worse memory performance than untreated NBM mice.

On the basis of neuroprotective effects of nerve growth factor (NGF) in animal models of aging and neurotoxicity, a single cell-based clinical trial has been undertaken in AD, in the form of NGF gene therapy (73). Autologous fibroblasts were genetically engineered *in vitro* to secrete NGF and transplanted directly into the NBM of eight patients. One patient died as a result of a complication from the surgery, another suffered significant morbidity. Of the six remaining patients, the main effect was a slowing of the rate of cognitive decline over the 2-year follow-up: MMSE decline slowed from 6 points in the year preceding the treatment to 3-points/year thereafter. Annualized rates of changes on the ADAS-Cog did not change. Interestingly, serial positron emission tomography (PET) scans in four patients showed increased fluorodeoxy glucose (FDG) uptake in the cholinergic forebrain. The single patient to come to autopsy 4 weeks posttransplant showed robust survival of the graft and extensive penetration by cholinergic axons.

One of the main limitations to progress in cell-based therapeutics in AD has been the lack of an appropriate animal model. Over 50 transgenic mice strains are in use, each expressing a different combination of mutations related to beta-amyloid (Aβ) protein processing or tau regulation (74). These models therefore recapitulate processes presumed relevant to the inherited, early-onset version of AD, but the relevance of these mechanisms to sporadic AD, which comprises more than 95% of all AD, is yet to be established. For example, a model was recently introduced for AD that features both the cognitive, neuropathological and neurotoxic features of the disease, yet relies solely on knockout of NGF (75), and so calls into question whether beta-amyloid processing defects are a necessary and sufficient precipitating factor.

Current transgenic AD mice models are therefore highly dependent on theory-driven assumptions concerning the neurotoxic role of beta-amyloid and have so far shown poor ecological validity. For example, amyloid vaccination of transgenic AD mice improved cognitive deficits and decreased amyloid burden at autopsy (76), but clinical trials were forced to be prematurely abandoned because of four near-deaths from encephalitis. Amyloid-based transgenic mice also appear to show inconsistent neuron loss and synaptic drop out (74), which are microstructural hallmarks of clinical progression of AD (77). Moreover, as mentioned in relation to NSC biology, transgenic AD mice show impaired neurogenesis (70); yet, heightened neurogenesis has been found in patients with AD (71).

These considerations underline our poor understanding of amyloid physiology (55,78). As briefly reviewed, the APP-beta amyloid pathway may in fact have an important role in the brain's plastic response to injury of diverse origins, including the induction and mobilization of NSCs. Progress in cell-based therapy of AD will therefore require development of new models with improved ecological validity.

Depression

Depression is often, but not always, precipitated by a major stressor. Common stressors include problems at work, within relationships or unrealistic personal expectations or negative self-perceptions. Stress-induced glucocorticoids have been known for many years to be toxic to hippocampal neurons (79). Given that the hippocampus is one of the major zones for endogenous neurogenesis, these observations stimulated thinking about the link between stress-hormones, depression and native NSCs (80,81).

Behavioural models of depression generally involve exposure of rodents to unavoidable unpleasant situations and have uncovered a number of consistent findings. First, animal-modeled depression is associated with a reduced level of endogenous neurogenesis (82). Increased cortisol production may be one mediator of this link, because inhibition of cortisol synthesis rescues the production and survival of new neurons in the dentate gyrus in the face of stress (83). Strong acute stress or prolonged chronic stress leads to a rise in systemic cortisol, which in turn produces an excess of glutamate in the extracellular space that is both neurotoxic and unfavorable to neurogenesis (84).

Another key finding has been the link between a number of antidepressant medications and de novo neural production. Serotonin has widespread mitogenic effects throughout the body and during neural development (85). More recently, serotonin agonists have been shown to robustly increase adult neurogenesis (80). Two weeks of fluoxetine treatment (5 mg/kg), for example, increased proliferation of new neurons by more than 35% in adult rats (86) and reversed the deleterious effects on cellular proliferation caused by inescapable stress (87). The 5-HT1A receptor appears to be particularly involved as blockade decreases cellular proliferation in the dentate gyrus (88). Furthermore, it has been suggested that neurogenesis is a necessary process for the action of antidepressant medication, with the demonstration that irradiative abolishment of neurogenesis also neutralized the therapeutic effect of fluoxetine in a depression model (89). Intriguingly, noradrenaline, classical tricyclics (86), and even electro convulsive therapy (ECT) (90) have all been reported to increase neurogenesis. Treatment of animal-modeled depression with neurotrophic factors such as BDNF has also been found to be effective with as little as a single dose (91).

But how could turnover of new neurons in the hippocampus affect an individual's mood? This remains the main challenge for a rational NSC therapy of major depression. One hypothesis suggests that entorhinal input into the dentate gyrus comes in part from anterior cingulate, known to participate in mood perception and regulation. The hippocampus is also connected to the amygdala, an aversive emotion-processing region. Beyond this, the hippocampus is known to play a critical role in memory formation and retention. Thus, disruption to NSC biology in individuals with depression is suggested to 'lock-in' unpleasant memories in a type of reentrant cycle, further increasing stress-related cortisol levels and reducing neurogenic potential, '… patients cannot "escape" the psychological impact of the initial precipitating events and remain mired in a chronic depressive state' (Jacobs et al., p. 264 (80). Restoration of normal neurogenesis is therefore suggested to be a critical factor in recovery from depression. The 4- to 6-week lag in antidepressant effect is proposed as further supportive evidence as it parallels the time course for new neurons to divide, differentiate and then incorporate into the functional circuitry of the MTL.

Human data are, however, less consistent and more difficult to interpret given the lack of any consensus regarding postmortem immunohistochemical

markers of neurogenesis. A single postmortem study of individuals with major depression has shown no difference in measures of immature proliferating cells in the hippocampus compared with controls matched for age, postmortem interval, race and sex (92). Whether these individuals were on antidepressant medication at the time of death was also unrelated to cellular proliferation in this region, with trends toward lower proliferation in those on treatment (ie, contrary to predictions).

Another speculative aspect of the NSC hypothesis for depression is that it relies on a highly nonlinear 'tipping point' phenomena occurring in the subgranular layer of the dentate gyrus. This is because normal production of neurons in this area is in the order of 2000–3000 cells/day, a vastly insufficient number to counteract the neurotoxic effects of high levels of cortisone. Thus, for the 'waxing and waning' of endogenous neurogenesis to have a functional impact within a much larger, complex and distributed mood state network, involving both limbic and cortical areas, the impact of these new neurons would need to be considerable. Interestingly, while no empirical data are available to support such an assertion, computational models for nonlinear 'butterfly effects' of this type are available from dynamical systems (93) and complexity theory (94).

Schizophrenia

The neurodevelopment hypothesis for the etiology of schizophrenia suggests that *in utero* and perinatal insults may affect proper brain development. In particular, the hippocampus has been implicated because of consistent clinical findings of localized atrophy (95). At some level, stem cells are likely to be implicated in this theory because by definition all neurons originate from NSCs during development. Interestingly, the extracellular matrix protein reelin, which has been found to be deficient in the brains of individuals with schizophrenia and is thought to play a role in neural migration during development (96), has been specifically implicated in signaling migration of transplanted NSCs (97). The role of *adult* NSC biology in schizophrenia, however, is only beginning to be explored.

Subanesthetic infusion of ketamine has been suggested as a rodent model for schizophrenia on the basis of altered social behaviour, disruption of latent inhibition and changes to dopamine, glutamate and serotonin binding and metabolism (98). Using this model, one group has reported increased neurogenesis in the subgranular zone of the hippocampus, contrary to expectations given the findings of atrophy in human clinical samples. Interestingly, in the only postmortem study of neurogenesis in schizophrenics to date, a similar increase in neurogenesis was found (92). Haloperidol treatment appears to increase neurogenesis in rodent studies by specific blockade of dopamine receptors on neural precursors (99). This finding indicates that postmortem results could be potentially explained by treatment rather than pathophysiological effects.

Parkinson's disease

Ambitions for NSC therapeutics in neuropsychiatry can learn much from transplant studies in PD because of a decade of experience with fetal tissue engraftment. At first glance, PD appears to be the ideal neurological candidate for a cell-based therapy: it is a highly prevalent and debilitating condition, symptomatic treatments are available but their effectiveness wanes with time, the pathogenic mechanisms are well understood and the neuropathological lesion and neural cell deficits relatively circumscribed. The best outcome of cell-based PD trials is perhaps the most that can be reasonably expected in neuropsychiatric disorders.

Early clinical transplant studies injected intact sections of fetal mesencephalon into the striatum of treatment-resistant patients with PD (100). Results were encouraging, with dramatic reductions in motor symptoms in some patients; however, these were difficult to interpret because of the absence of a control group. Freed et al. (42) reported on the results of the first randomised controlled trial (RCT) of fetal tissue transplant for the treatment of severe PD in 2001. They dissociated brain cells from an embryo and cultured it in the laboratory for 4 weeks before transplantation into the bilateral putamen using stereotactic surgery under local anesthesia. In the sham surgery group, burr holes were drilled through the skull but did not penetrate the dura. Patients below the age of 60 appeared to benefit from the treatment at the end of the first year of follow-up, with a significant improvement in clinical scores when patients were tested in the morning (ie, trough levels of levodopa). Furthermore, in 17/20 of the transplanted patients, ^{18}F-fluorodopa was significantly augmented in the basal ganglia when PET scans before and after treatment were compared. However, these promising initial results became negative on more extended follow-up. About 15% of transplanted patients, including those with significant 1-year

improvement, developed severe dystonia or dyskinesia in the 1- to 3-year follow-up period. More worryingly, these abnormal movements seemed to persist despite cessation of all antiparkinsonian medication.

So what can we learn from the fetal transplant literature in PD? First, it appears that precursor cells can survive and engraft into the human brain, including in those areas where neuropathology may be affecting the microenvironment. Second, at least some of these cells appear to become functional, as evident by increased dopaminergic transmission. Third, the potential for functional improvement is clearly possible, given reports of clinical improvement in a RCT. Fourth, there is a high level of unpredictability in identifying respondents from nonrespondents. Finally, it seems that precursor cells from fetal transplants may also inappropriately engraft into basal-ganglia-related motor networks, causing more problems than benefits in the long term. Thus, evaluation of any NSC treatment will need to have extended follow-up beyond 1 or 2 years.

One reason that fetal grafts in PD may have resulted in such variable results in many patients, and devastating side-effects in some, could be attributed to the highly heterogenous composition of the tissue. Transplant of more homogenous NSCs should predict better results. There have now been a number of trials of NSC transplant in animal models of PD (see Table 2). In general, these have found amelioration of motor signs in conjunction with the successful engraftment of transplants in varying degrees. Yet, the issue of sufficient NSC quantity and quality remains a big challenge because PD treatment demands not only a significant quantum of NSCs, but these also need to be able to differentiate into highly specialized dopaminergic neurons.

The recent trial by Roy et al. (44) is therefore highly informative. They tackled the volume issue by commencing with human embryonic stem cells and recreating the dopaminergic environment of the developing fetus by coculturing these cells alongside immortalized astrocytes derived from the fetal ventral midbrain. Using this technique, they were able to increase the *in vitro* yield of dopaminergic neurons from less than 12% to greater than 65%. Transplantation of these cells in a putamen-lesion model, which faithfully mimics many of the behavioural features of PD, found almost complete reversal of motor deficits. However, postmortem analysis at the 10-week recovery stage showed that *in situ* grafts contained less than 20% dopaminergic neurons, and most of these at the periphery of the graft (ie, near the host interface), where many activated glia were also seen. Hence, there was no discernible correlation between dopaminergic transplant survival and behavioural improvement. However, the most troublesome finding was that in the core of the transplant many neurons remained in an undifferentiated precursor state despite being 10-weeks postgrafting and moreover appeared to be aggressively expanding. Thus, while no evidence of teratoma or anaplastic changes were seen, there remains the possibility that the core of the transplant could have transformed into a neoplastic mass with the passage of time.

Conclusions

Cell-based therapies in PD show that in principle, it is possible to reverse some of the behavioural effects of neurodegeneration by transplantation of primitive neural tissue into the affected area. The potential to treat neuropsychiatric disorders with identifiable neural lesions is therefore realistic. However, much more technical and conceptual innovation remains. At present, we are like Odysseus navigating a passage between the Scylla and Charybdis: on the one hand, we risk deficient cell survival and integration into extant neural circuits and so negligible clinical effect; on the other, excess cell division and differentiation threatens development of severe side-effects or teratoma. It would be premature to trial NSC transplantation in patients before these issues have been resolved in animal models. Much of this work will focus on further characterizing and purifying the enigmatic neural precursor cell.

It is also evident that progress has been most rapid in those neuropsychiatric areas with appropriate animal models. PD and depression benefit from models with a satisfactory level of ecological validity and thus transplantation research has made some important advances in the former, and the role of neurogenesis in the etiology of the latter continues to develop. On the other hand, transgenic models for AD have limited ecological validity and the translation of results to clinical trial will be contentious. Models for schizophrenia are only beginning to emerge and will need to continue to improve for further understanding of the role of adult NSCs in the pathophysiology and treatment of this disorder.

For NSC-based treatments to become 'prime-time' clinical options, further research of native NSC biology is therefore required, alongside a more sophisticated understanding of the interaction of these processes with the major neuropsychiatric diseases.

Table 2. Results from transplant studies of neural precursors in models of aging, AD and PD

Condition and authors	Model	Cellular strategy	Behavioural effect	Follow-up time	Histology
Alzheimer's and aging					
Wang et al. (93)	C57BL/6 mice with ibotenic acid NBM lesion	Tx of mouse ES-cell-derived neurospheres ($1-5 \times 10^4$ cells/μl) into regions of the frontal association cortex and barrel field of S1 cortex. Control condition comprised of Tx into nonlesioned animal.	Working memory disruption was alleviated using an eight-arm radial test at 8 weeks post-Tx	12 weeks	Acetylcholine-positive and serotonin-positive neurons in and around the grafts. Tx into control animals caused teratoma formation and significant impairment in memory performance.
Kwak et al. (90)	APP-knockout mouse	Tx of 10^5 BrdU-labelled human NSCs (hNSCs) into the right ventricle. hNSCs were obtained from human fetal cortex.	Not assessed	4 weeks	hNSC grafts exhibited glial rather than neural differentiation. hNSC migration and differentiation was also reduced in APP-knockout mice, which suggests that APP regulates NSC biology in the adult brain. Altered APP metabolism in AD may have implications for its pathophysiology.
Qu et al. (41)	6-month-old vs. 24-month-old Fisher 344 rats	Tx of 10^5 BrdU-labelled hNSCs into right ventricle. Control condition included saline ventricular injection.	Significant improvement in Morris water maze in both younger and older memory-impaired animals. No improvement in older animals that were performing at level of younger animals before transplant.	30 days	Colocalization of βIII-tubulin and BrdU neurons, indicative of differentiated hNSCs, in the bilateral cingulate, parietal cortex and hippocampus (CA1, CA3 and dentate gyrus). Donor-derived astrocytes were also found in the hippocampal fields.
Parkinson's					
Harrower et al. (101)	Sprague-Dawley rats with unilateral 6-OHDA lesion of the right medial forebrain tract	Tx of primary porcine neural tissue (PPNT) and expanded porcine neural precursor cells (PNPCs $\sim10^6$) into the striatum of rats. Immunosuppression with cyclosporine.	No significant change in the amphetamine-induced body rotation in either animal groups over time.	19 weeks	Good PNPC graft integration with fibers extending into the surrounding host tissue including white matter with synapse formation. PPNT grafts had significantly poorer survival. Those grafts that did survive were infiltrated by CD8+ lymphocytes and macrophages. Despite the longer survival times in animals with graft integration and maturation, there was no functional efficacy as no DA neurons were seen in the transplants.
Takagi et al. (102)	MPTP-induced putamen lesions in cynomolgus monkeys	Tx of induced dopaminergic neurons from monkey ES cell-derived neurospheres (300 000–600 000 cells) directly into the putamen. Immunosuppression with cyclosporine.	Significant improvement in the neurological scores at 10 weeks post-Tx. Posture recovery and motility was significantly improved. No significant changes in head-checking movement. None of the treated animals developed dyskinesia	14 weeks	GABA-positive cells were detected in the graft at a slightly higher frequency than TH-positive cells, while few serotonin-positive cells were present. However, number of surviving DA neurons was very low, with only 1%–3% of the cells surviving. PET at 14 weeks post-Tx showed increases in fluoro-dopa uptake at the putamen of the recipient animals, indicating that the grafted cells functioned as DA neurons.

Table 2. Continued

Condition and authors	Model	Cellular strategy	Behavioural effect	Follow-up time	Histology
Roy et al. (44)	6-OHDA-induced striatal lesion in Sprague-Dawley rats	Tx of 500 000 immature dopaminergic neurons derived from hESCs that were cocultured alongside immortalized astrocytes from human fetal midbrain (ie, conditioned medium) to increase dopaminergic yield. Transplanted directly into neostriatum. Control groups included saline vehicle and a group that received hESCs transplant without conditioned medium. Immunosuppression with cyclosporine	Apomorphine-induced body rotations became more severe during 8 weeks of follow-up in control vehicle group. Abolishment of apomorphine-induced body rotation was seen in conditioned medium hESC-derived Tx group. Unconditioned hESC-derived Tx group deteriorated so precipitously that behavioural testing was not completed.	10 weeks	Conditioned medium protocol increased *in vitro* dopaminergic yield from 12% to 65%. Postmortem analysis showed that conditioned medium hESC-derived graft sites contained less than 20% dopaminergic neurons – most of these near the host/graft interface where many activated astrocytes were also seen. No correlation between functional recovery and level of dopaminergic engraftment in this group. Also, core of graft dominated by undifferentiated neural precursors, which appeared to be expanding aggressively, indicative of potential tumor formation. Unconditioned hESC-derived grafts were distinct: transformation into anaplastic tumors in each animal.

BrdU, bromodeoxyuridine; DA, dopamine; ES, embryonic stem cell; GABA, gamma-aminobutyric acid; hESC, human embryonic stem cell; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OHDA, 6-hydroxydopamine; Tx, transplant.

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