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Stem cell therapy for human neurodegenerative disorders–how to make it work

Olle Lindvall[1,2], Zaal Kokaia[2,3,5] & Alberto Martinez-Serrano[4,5]

Recent progress shows that neurons suitable for transplantation can be generated from stem cells in culture, and that the adult brain produces new neurons from its own stem cells in response to injury. These findings raise hope for the development of stem cell therapies in human neurodegenerative disorders. Before clinical trials are initiated, we need to know much more about how to control stem cell proliferation and differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits, and optimize functional recovery in animal models closely resembling the human disease.

Stem cells are immature cells with prolonged self-renewal capacity and, depending on their origin, ability to differentiate into multiple cell types or all cells of the body. Transplantation of stem cells or their derivatives, and mobilization of endogenous stem cells within the adult brain, have been proposed as future therapies for neurodegenerative diseases. It may seem unrealistic, though, to induce functional recovery by replacing cells lost through disease, considering the complexity of human brain structure and function. Studies in animal models have nevertheless demonstrated that neuronal replacement and partial reconstruction of damaged neuronal circuitry is possible. There is also evidence from clinical trials that cell replacement in the diseased human brain can lead to symptomatic relief.

Here we review the scientific basis of stem cell therapies and discuss their prospects in Parkinson's disease, stroke, amyotrophic lateral sclerosis (ALS) and Huntington's disease. In each of these neurodegenerative diseases, a different spectrum of cell types is affected; therefore, different types of neurons are required for replacement. We argue that long-term survival of new, functionally integrated neurons is the main goal to achieve maximum symptomatic relief through stem cell therapy. Stem cell transplantation may also lead to clinically valuable improvements through other mechanisms as well (**Box 1**).

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Can cell therapy work in patients with Parkinson's disease?

The main pathology in Parkinson's disease (PD) is a degeneration of nigrostriatal dopaminergic neurons. Studies in patients with PD after intrastriatal transplantation of human fetal mesencephalic tissue, rich in postmitotic dopaminergic neurons, have provided proof of principle that neuronal replacement can work in the human brain. The grafted neurons survive and reinnervate the striatum for as long as 10 years despite an ongoing disease process, which destroys the patient's own dopaminergic neurons[1,2]. The grafts are able to normalize striatal dopamine release[2] and to reverse the impairment of cortical activation underlying AKINESIA[3]. Thus, grafted dopaminergic neurons can become functionally integrated into neuronal circuitries in the brain[3]. Several open-label trials have reported clinical benefit (see refs. 4,5). Some patients have been able to withdraw from L-dopa treatment for several years and resume an independent life[2].

Two recent sham surgery-controlled trials showed only modest improvement[6,7], which illustrates that present cell replacement procedures are far from optimal. The poor response in one study could be explained by markedly fewer surviving grafted dopaminergic neurons[6] as compared with that in open-label trials[1,8,9]. In the other study, patients were more severely disabled at the time of transplantation (compare refs. 7 and 10), indicating extensive degenerative changes. No or short-term immunosuppression was given in these studies[6,7], which may be necessary to avoid immune reactions causing dysfunctional grafts[7].

DYSKINESIAS can develop after transplantation[7,10] and become troublesome in 7–15% of grafted patients[6,7,10]. This adverse effect is not due to dopaminergic overgrowth[7,10,11]. It may be caused by uneven and patchy reinnervation[11], giving rise to low or intermediate amounts of striatal dopamine, or by chronic inflammatory and immune responses around the graft[7]. Alternatively, graft-induced

Box 1 Possible mechanisms underlying improvements after cell transplantation

Mechanism	Mode of action
Tissue damage	Inflammation-induced stimulation of host plastic responses
	Interference with host neural activity
Correction of biochemical deficit	Release of missing transmitter ('minipump')
Growth factor secretion	Stimulation of plastic responses and improved survival and function of host neurons
Local reinnervation	Restoration of synaptic transmitter release
Reconstruction of neural circuitries	Re-establishment of functional afferent and efferent connections

dyskinesias could be explained by unfavorable composition of the graft with respect to the predominant type of mesencephalic dopaminergic neurons from the substantia nigra or ventral tegmental area[12] and the proportion of cells that are not dopaminergic.

Neurons from stem cells for Parkinson's disease

It is improbable that transplantation of human fetal mesencephalic tissue will become routine treatment for persons with PD because of problems with tissue availability and too much variation in functional outcome. Stem cell technology (**Fig. 1**) has the potential to generate large numbers of dopaminergic neurons in standardized preparations. On the basis of results with fetal transplants in animals and humans, it is possible to identify a set of requirements that probably also need to be fulfilled by stem cell–derived cells to induce marked clinical improvement: (i) the cells should release dopamine in a regulated manner and should show the molecular, morphological and electrophysiological properties of substantia nigra neurons[12]; (ii) the cells must be able to reverse in animals those motor deficits that resemble the symptoms in persons with PD; (iii) the yield of cells should allow for at least 100,000 grafted dopaminergic neurons to survive over the long term in each human putamen[13]; (iv) the grafted dopaminergic neurons should re-establish a dense terminal network throughout the striatum; (v) the grafts must become functionally integrated into host neural circuitries[3].

Neurons with dopaminergic phenotype surviving transplantation have been generated in culture from mouse and monkey embryonic stem cells (ESCs) and from neural stem cells (NSCs) derived from the fetal rodent and human brain (**Fig. 2**). There is little evidence that dopaminergic neurons can be made from NSCs in adult brain or from stem cells in other tissues. In most cases (**Supplementary Table 1** online), it remains to be shown that the stem cell–derived neurons, after implantation in animal models, fulfill the requirements of clinically successful grafts—that is, to reinnervate most of the denervated striatum, restore dopamine release *in vivo* and substantially improve Parkinson's-like symptoms. The most promising results so far have been obtained using mouse ESCs (**Supplementary Table 1** online). Dopamine neurons can be generated also from human ESCs (A.L. Perrier *et al.*, personal communication; R.D.G. McKay *et al.*, personal communication), which is probably necessary for a clinical application. However, chromosomal aberrations have been observed in mid-term cultured human ESC lines[14]. Although genomic stability was previously reported[15], this issue should be explored before human ESCs are used for transplantation. Notably, genomic stability was found in human NSC and precursor lines from fetal forebrain and spinal cord, even after 4 years in culture[16,17].

Only 5–10% of cells in fetal mesencephalic grafts are dopaminergic neurons. It is not yet known whether it is favorable to implant a pure population of dopaminergic neurons or whether the graft should also contain a specific composition of other neuron types and glial cells to induce maximum symptomatic relief. Recent studies indicate a major role of astrocytes in specifying neuronal phenotypes during embryonic development, suggesting that glial cells are important for fate decision by NSCs and precursors before or after transplantation[18,19].

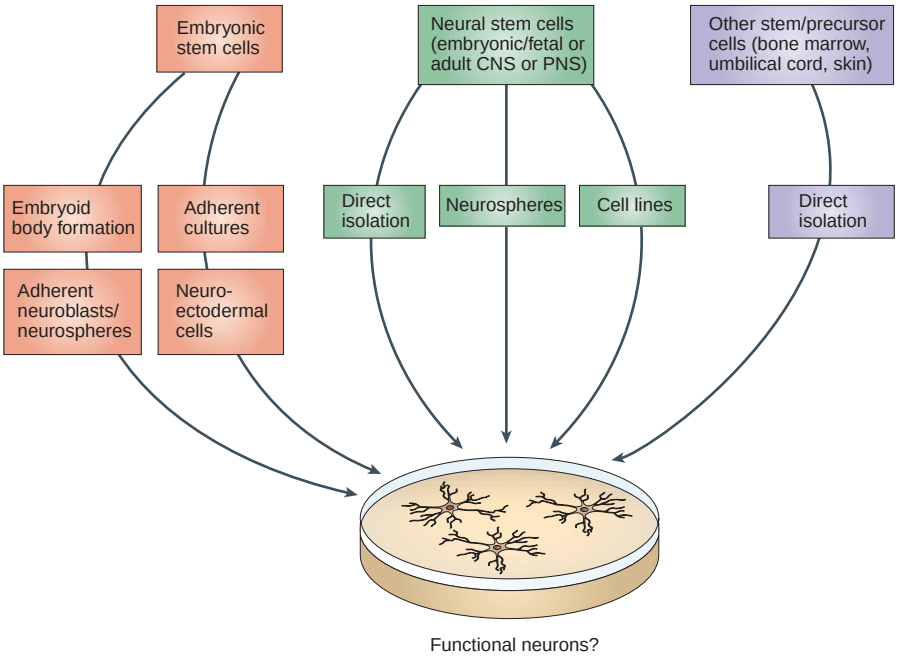

Figure 1 Generation of neurons *in vitro* from stem cells. In the case of ESCs, removal of leukemia inhibitory factor and addition of retinoic acid lead to formation of embryoid bodies. Neural fate can also be induced directly in adherent cultures. Embryoid bodies are treated with epidermal growth factor and fibroblast growth factor 2, or cultured with stromal cells to generate adherent neuroblasts or floating NSC aggregates. Similar treatment of adherent cultures generates neuroectodermal cells. Neuronal differentiation is induced by removal of mitogens. NSCs are isolated through FACS, on the basis of reporter genes driven by neural promoters, or by selective gating of cells showing defined antigenic properties. Alternatively, strains of NSCs are enriched using mitogens, or NSC lines are derived using mitogens and cell cycle–controlling genes. *In vitro* patterning with extracellular signals, or genetic modification, induces specific neuronal phenotypes. Stem cells can be isolated from other organs, expanded and differentiated into neural cells using, for example, FGF-2, retinoic acid or nerve growth factor.

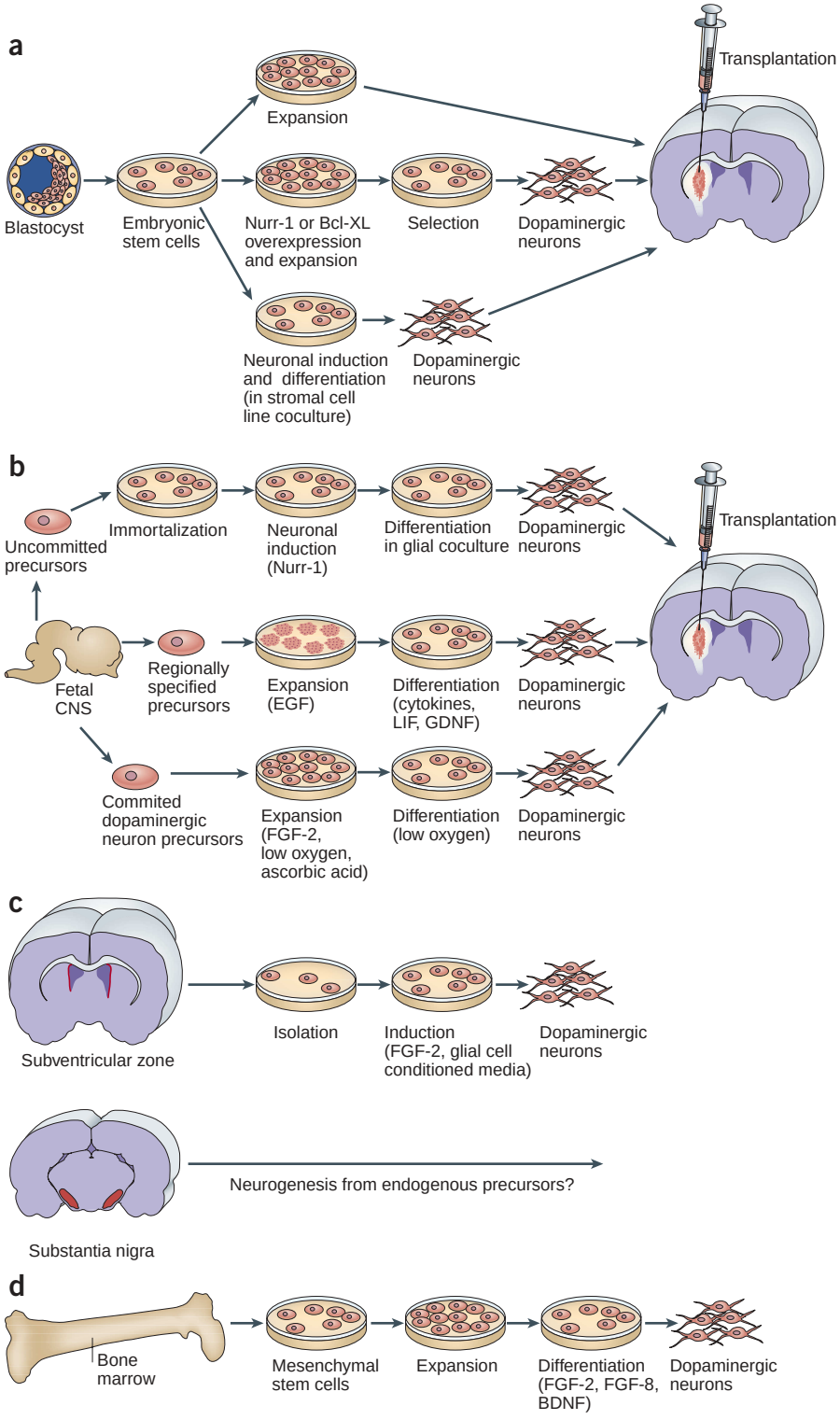

Figure 2 Generation of dopaminergic neurons for Parkinson's disease. (**a**) Embryonic stem cells. Mouse ESCs have been transplanted directly to the striatum[36] or differentiated beforehand to dopaminergic neurons after overexpression of the transcription factor, Nurr-1 (refs. 31, 32) or Bcl-XL (ref. 30), or culture with a stromal cell line[86,87] (also primate ESCs[88]). (**b**) Neural stem cells from fetal brain. Mouse cerebellar precursors have been immortalized, engineered to overexpress Nurr-1 and cultured with ventral mesencephalon astrocytes[19]. Regionally specified rat and human mesencephalic precursors have been grown as neurospheres[89,90]. Committed rat and human mesencephalic dopaminergic neuron precursors have been expanded[91–93]. GDNF, Glial cell line–derived neurotrophic factor; LIF, leukemia inhibitory factor. (**c**) Neural stem cells from adult brain. Tyrosine hydroxylase–expressing cells have been generated from mouse SVZ (ref. 94). Some controversial evidence suggests that neurogenesis occurs in adult mouse substantia nigra[21]. (**d**) Stem cells in other tissues. Cells expressing dopaminergic markers and with some neuronal properties have been generated from adult mouse mesenchymal stem cells[95–97].

The protocols for generation of dopaminergic neurons (**Fig. 2** and **Supplementary Table 1** online) give rise to mixed cell populations. FLUORESCENCE-ACTIVATED CELL SORTING (FACS) of mesencephalic dopaminergic precursors has had limited success because of difficulties in finding specific surface antigens. HOMOLOGOUS RECOMBINATION[20] may make it possible to generate transgenic human ESCs, expressing reporter genes that allow for purification of dopaminergic neuroblasts.

Whether new dopaminergic neurons are generated from endogenous NSCs in the adult brain is controversial. Zhao *et al.*[21] reported continuous formation of dopaminergic neurons in the adult mouse substantia nigra (**Fig. 2**). The rate of neurogenesis doubled after a lesion of the dopamine system. In contrast, other investigators observed only a glial response and failed to detect any neurogenesis following dopaminergic lesions[22,23] (Y. Chen, Y. Ai and D.M. Gash, personal communication; D.K. Morris-Irvin *et al.*, personal communication). In the study of Zhao *et al.*[21], evidence for neurogenesis was mainly based on bromodeoxyuridine incorporation, which, however, may have also other explanations[24,25].

How to develop a stem-cell therapy for Parkinson's disease

A clinically competitive cell therapy must provide advantages over current treatments for PD. Cell-based approaches should induce long-lasting, major improvements of mobility and suppression of dyskinesias. Alternatively, the new cells should improve symptoms that are resistant to other treatments, such as balance problems. Improvements after fetal grafts[4,6,7] have not exceeded those found with deep brain stimulation[26], and there is no convincing evidence for reversal of drug-resistant symptoms[4]. Incomplete recovery could be due to only part of the striatum having been reinnervated[4,9]. Even in animals with good reinnervation, however, improvements are only partial[27], indicating that the ectopic graft placement in the striatum may be of crucial importance. Grafts implanted in the substantia nigra give some improvements in animals[27,28] and have been tested

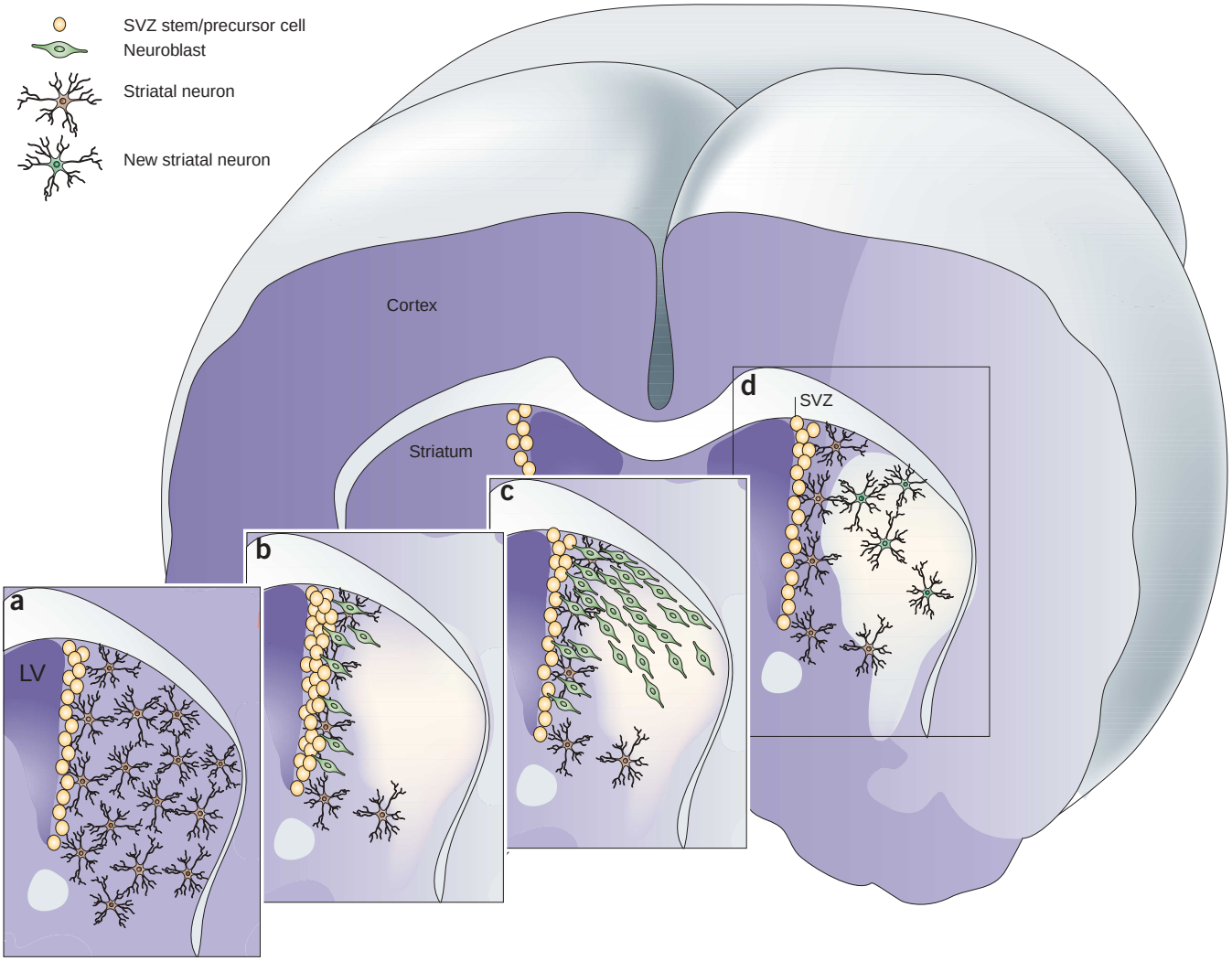

Figure 3 Generation of striatal neurons from endogenous stem cells after stroke. (**a**) Neural stem or precursor cells reside in the subventricular zone (SVZ). (**b**) Stroke induced by middle cerebral artery occlusion, which leads to death of striatal neurons (white area) and damage to overlying cortex, triggers increased proliferation of SVZ precursors. (**c**) Newly formed neurons migrate to the damaged part of the striatum. (**d**) After maturation, the new neurons express markers specific for striatal projection neurons or interneurons. Data from rat[43,44] and mouse[48]. LV, lateral ventricle.

clinically[29], but they are not able to reconstruct the nigrostriatal pathway[27].

Even if stem cell technology can generate large numbers of dopaminergic neurons, the development of effective cell therapy for PD will require three additional advances. First, better criteria for selecting the patients suitable for cell therapy have to be defined. Dopaminergic cell therapy will most likely be successful only in those affected individuals who show marked symptomatic benefit in response to L-dopa and in whom the main pathology is a loss of dopaminergic neurons. Debilitating symptoms in PD and related disorders are also caused by pathological changes in non-dopaminergic systems. Until we know how to repair these systems, enrollment of individuals with such symptoms in clinical trials with dopaminergic cell therapy should be carefully considered.

Second, the functional efficacy of grafts must be improved. On the basis of imaging before surgery, the transplantation procedure should be customized with respect to the dose and location of grafted cells so that the repair of the dopamine system will be as complete as possible in each patient's brain. There is so far no evidence that stem cell–derived dopaminergic neurons will induce more pronounced improvement as compared with primary neurons in fetal grafts. One advantage with stem cells is the possibility for controlled genetic modification, which, for example, could be used to increase survival, differentiation, migration and function of their progeny[19,30–32]. For more complete reversal of Parkinson's symptoms, it may be necessary to stimulate regrowth of axons from grafts in the substantia nigra to the striatum; this would probably require modulation of host growth-inhibitory mechanisms[33]. The ability of grafted NSCs to rescue dysfunctional dopaminergic neurons[34], through release of neurotrophic molecules, could also promote symptomatic relief (**Box 1**). It is unknown whether immunosuppressive treatment is needed in patients with human stem cell grafts. Results with ALLOGENEIC fetal grafts[7] suggest that effective immunosuppression, at least for 1 year after transplantation, is necessary to optimize functional outcome. If immune reactions constitute a substantial problem, alternative solutions could be to generate transgenic ESCs or NSCs. ISOGENIC stem cells seem ideal but require therapeutic cloning or the use of adult stem cells from the patient.

Third, strategies to avoid adverse effects must be developed. New animal models are needed to reveal the pathophysiological mechanisms of graft-induced dyskinesias[35]. The risk for teratoma from ESCs as well as the consequences of introducing new genes in stem cell–derived neurons should be carefully evaluated. Implantation of mouse ESCs into rat striatum caused teratomas in 20% of the animals[36]. However, the risk is reduced if the cells are differentiated beforehand *in vitro*. Importantly, ESCs seem more prone to generate tumors when implanted into the same species from which they were derived[37]. Thus, an absence of tumors after implantation of human ESCs into rodents does not exclude their occurrence in the human brain. To improve safety it may be necessary to engineer ESCs with regulatable suicide genes.

Can cell therapy work in stroke?

In stroke, occlusion of a cerebral artery leads to focal ischemia in a restricted CNS region. Many different types of neurons and glial cells degenerate in stroke. It has not yet been convincingly demonstrated that neuronal replacement induces symptomatic relief in individuals who have suffered strokes. In the only reported clinical trial, persons with stroke affecting basal ganglia received implants of neurons generated from the human NT-2 teratocarcinoma cell line into the infarcted area[38]. Improvements in some affected individuals correlated with increased metabolic activity at the graft site[39]. This finding could be interpreted as graft function but might as well reflect inflammation or increased activity in host neurons. Autopsy in one individual who had suffered a stroke revealed a population of grafted cells expressing a neuronal marker 2 years after surgery[40].

Neurons from stem cells for stroke

Cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke (**Supplementary Table 2** online). Although the transplanted cells can survive and partly reverse some behavioral deficits, the mechanisms underlying the observed improvements are unclear and there is little evidence for neuronal replacement. In most cases, only a few grafted cells survived, and these did not show the phenotype of the dead neurons. Moreover, it is unknown whether these cells are functional neurons and establish connections with host neurons. Bone marrow–derived cells were also described to give rise to neurons in the

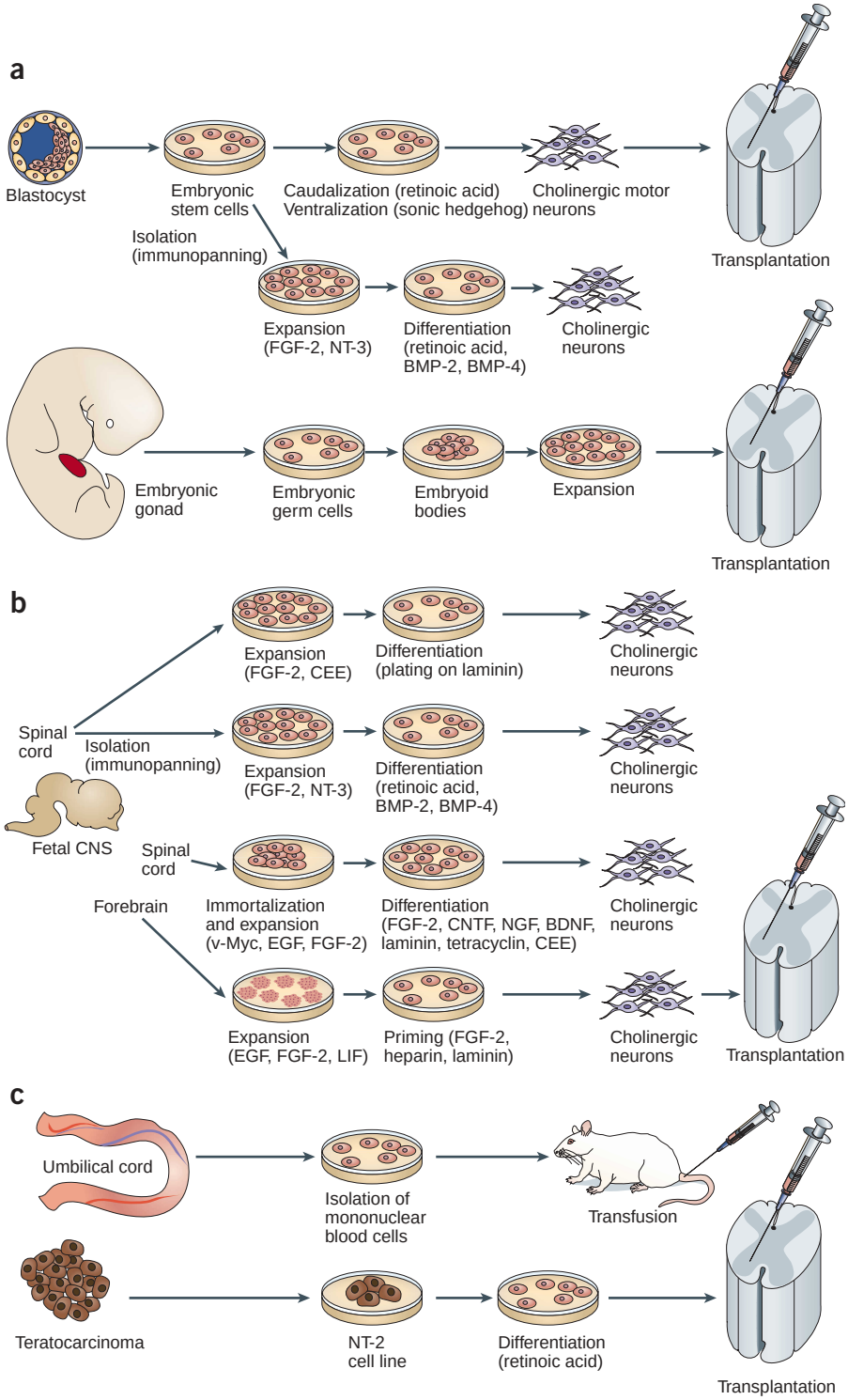

Figure 4 Generation of cholinergic motor neurons for ALS. (**a**) Mouse ESCs[98–100] can give rise to cholinergic motor neurons through embryoid body formation, and caudalization by retinoic acid and possibly ventralization by sonic hedgehog[101]. Embryonic neural cell adhesion molecule (E-NCAM) immunopanning can isolate neuron-restricted progenitors[99], which are expanded and differentiated to cholinergic neurons. Human embryonic germ cells generate very low numbers of cholinergic neurons in spinal cord after delivery into the CSF[73]. (**b**) Cholinergic motor neuron precursors can be isolated from rodent fetal spinal cord, through generation of multipotent neuroepithelial precursors or using E-NCAM immunopanning[102–104]. Cell lines from human fetal spinal cord[105], and predifferentiated human NSCs, derived as neurospheres from fetal cortex[106], also generate cholinergic neurons. (**c**) Following transfusion of umbilical cord blood[72], or transplantation of a human teratocarcinoma cell line[71,107,108], cells expressing neuronal but not cholinergic markers are found in spinal cord. BMP, Bone morphogenetic protein; CEE, chicken embryo extract.

stroke-damaged brain (**Supplementary Table 2** online). However, two recent reports challenge this interpretation by demonstrating that fusion is responsible for the appearance of donor-derived neurons after systemic administration of bone marrow cells[41,42].

Recent findings in rodents suggest an alternative approach to cell therapy in stroke based on self-repair (**Fig. 3**). Stroke leads to increased generation of neurons from NSCs in the subventricular zone (SVZ)[43–45]. These immature neurons migrate into the damaged striatum, where they express markers of striatal medium spiny projection neurons. Thus, the new neurons seem to differentiate into the phenotype of most neurons destroyed by the ischemic lesion. However, because >80% of the new neurons die during the first weeks after stroke, they only replace a small fraction (~0.2%) of the mature striatal neurons that have died[44]. Several factors can increase adult neurogenesis by stimulating formation and/or improving survival of new neurons, including fibroblast growth factor 2 (FGF-2)[46,47], epidermal growth factor (EGF)[48], stem cell factor[46], erythropoietin[49], brain-derived neurotrophic factor[50,51], caspase inhibitors[52] and anti-inflammatory drugs[53,54].

Whether the new neurons formed after stroke are functional is unknown. Evidence for functional neuronal replacement in the ischemically damaged brain has been reported from a model of global forebrain ischemia. Intraventricular infusion of FGF-2 and EGF gave rise to regeneration of hippocampal CA1 pyramidal neurons from NSCs around the posterior periventricle adjacent to the hippocampus[55]. Whereas the new neurons seemed to form afferent and efferent connections and to reverse some functional deficits, it cannot be excluded that the functional improvement was caused by the growth factors themselves and not by regeneration of CA1 neurons.

There is no substantial formation of new neurons in the cerebral cortex after stroke[43,44,56]. Notably, targeted apoptotic degeneration of cortical neurons in mice, leaving tissue architecture intact, leads to formation of new cortical neurons extending axons to the thalamus[57]. Thus, restricted self-repair capacity in ischemically damaged cortex is probably due to lack of cues necessary to trigger neurogenesis from putative local parenchymal NSCs or migration of neuroblasts from the SVZ.

GLOSSARY

Akinesia Lack or paucity of movement.

Dyskinesias Hyperkinetic and dystonic abnormal involuntary movements and postures.

Fluorescence-activated cell sorting A method that allows the separation of cells expressing fluorescent marker proteins or other surface proteins that can be detected using fluorescent antibodies. A laser beam excites the fluorescent tag, and the emission of light triggers the cell sorting.

Homologous recombination The substitution of a segment of DNA with one that is identical or almost identical to it. It occurs naturally during meiosis, but can also be used experimentally for gene targeting to modify the sequence of a gene.

Allogeneic A term that denotes subjects, tissues or cells that are of the same species, but are antigenically distinct.

Isogenic A term that denotes subjects, tissues or cells that have identical genotypes. It is synonymous with syngeneic.

Neurosphere Free-floating, spherical aggregates of neural stem cells. Cells in the neurospheres can proliferate in culture while retaining the potency to differentiate into neurons and glia.

How to develop a stem cell therapy for stroke

To repair the stroke-damaged brain may seem unrealistic because of atrophy and loss of many cell types. However, even re-establishment of only a fraction of damaged neuronal circuitries could have important implications. In the ideal scenario, NSCs implanted in the damaged area will differentiate *in situ* into those cells that have died. This strategy requires that the largely unknown developmental mechanisms instructing stem cells to differentiate into specific cell types will work also in the brain of the affected individual. For maximum functional recovery, transplantation should probably be combined with stimulation of neurogenesis from endogenous NSCs. Neurogenesis occurs from NSCs in the human SVZ[58,59], and neuronal precursors are found in human subcortical white matter[60].

Adequate blood supply will be crucial for survival and development of the new neurons. Neurogenesis is closely associated with angiogenesis from endothelial precursors[61]. Angiogenesis occurs in the human brain after stroke but may have to be further stimulated to increase the yield of surviving new neurons. Administration of vascular endothelial growth factor (VEGF) promotes SVZ neurogenesis and angiogenesis in the penumbra region (region at risk) after stroke[62]. VEGF can also guide directed migration of undifferentiated SVZ neural progenitors[63]. For efficient repair it may be necessary to provide NSCs with a platform so that they can re-form appropriate brain structure. In neonatal mice[64], NSCs seeded on synthetic extracellular matrix and implanted into the ischemia-damaged area generated new vascularized parenchyma comprising neurons and glia.

Research should now aim to identify and improve efficacy of different mechanisms, which may underlie the benefit of stem cells after stroke (**Box 1**). For developing the neuronal replacement strategy toward clinical application, three different tasks can be distinguished: (i) Proof of principle should be obtained that neurons generated from NSCs can survive in large numbers in animals subjected to stroke, migrate to appropriate locations, show morphological and functional properties of those neurons that have died and establish afferent and efferent synaptic interactions with neurons that survived the insult. Magnetic resonance imaging seems ideal for noninvasive imaging at high spatial and temporal resolution of the survival, migration and differentiation of grafted cells[65]. (ii) Behavioral recovery must be optimized in animal models. Strategies to improve survival, differentiation and integration of NSCs will require detailed knowledge of the regulation of these processes. The time window after the insult when the generation of new neurons will lead to maximum restitution of neuronal circuitries and functional recovery should be determined. (iii) There is a need to define which patients are suitable for stem cell therapy. The occurrence of striatal neurogenesis after stroke[43,44,48] focuses the interest on individuals with basal ganglia infarcts. If stem cells can also generate cortical neurons and repair axonal damage, individuals with lesions in the cerebral cortex may be included. A strategy for repair of infarcted white matter was suggested recently by the observation that NEUROSPHERES derived from adult tissue injected intravenously or intraventricularly in mice[66] gave rise to cells that migrated to demyelinated areas and remyelinated axons.

Stem cell therapy for amyotrophic lateral sclerosis?

In its common form, ALS is characterized by progressive dysfunction and degeneration of motor neurons in cerebral cortex, brain stem and spinal cord. Muscle weakness progresses rapidly and causes death within

a few years. To have long-term value, stem cell therapy must restore function of both upper and lower motor neurons. Successful replacement of cortical motor neurons requires not only re-establishment of spinal cord connections but also precise functional integration of the new neurons into cortical circuitries. Corticospinal or corticobulbar systems are not reconstructed after implantation of fetal cortical tissue into adult neocortex[67]. Late-stage fetal cortical neurons, however, replace apoptotic neurons when grafted into adult mouse neocortex, receive afferents from host brain and project to the contralateral hemisphere[68]. This finding supports the strategy of differentiating stem cells along specific cortical neuronal lineages *in vitro* and transplanting them so as to reconstruct cortical circuitry. It is unknown, though, if such cortical neuronal replacement will work in the brains of individuals with ALS.

Is it then possible to replace spinal motor neurons in ALS? Fetal motor neurons grafted to the adult rat spinal cord lacking motor neurons migrate to the ventral horn and make functional connections with skeletal muscle[69,70]. Whether these neurons are integrated in neuronal circuits and restore reflexes and voluntary movements is unclear.

Functional benefits have been detected after cell implantation in ALS models, although it is unlikely that the observed effects were due to neuronal replacement and re-establishment of connectivity. Spinal grafts of neurons generated from the human NT-2 cell line[71] and intravenous administration of human umbilical cord blood cells[72] delayed disease progression in mice. Human embryonic germ cell derivatives delivered into the CSF of rats with motor neuron injury were distributed over the spinal cord and migrated into the parenchyma[73]. The paralyzed rats showed partial motor recovery, probably because the grafted cells protected host neurons and facilitated their reafferentation by secreting growth factors.

A great deal of basic research should be done before persons with ALS should be considered for clinical trials. Cells with characteristics of cholinergic neurons have been generated from stem cells of various sources (**Fig. 4**), but their functional properties and ability to repair the spinal cord in ALS models are unknown. In the shorter term, strategies to retard disease progression seem to be a more realistic clinical approach as compared with neuronal replacement.

Can stem cell therapy be developed for Huntington's disease?

Huntington's disease is a fatal disorder characterized by chorea and progressive dementia. The main pathology is a loss of medium spiny projection neurons in the striatum due to a mutation in the huntingtin gene. Cell therapy in Huntington's disease aims at restoring brain function by replacing these neurons. In animal models, intrastriatal grafts of fetal striatal tissue containing projection neurons re-establish connections with the globus pallidus and receive inputs from host cerebral cortex[74]. This level of reconstruction of corticostriatopallidal circuitry is sufficient to reverse motor and cognitive deficits in rats and monkeys[74–76].

Clinical trials with intrastriatal transplantation of human fetal striatal tissue support the cell replacement strategy in Huntington's disease. The grafts survived without typical pathology, contained striatal projection neurons and interneurons, and received afferents from the patient's brain[77]. However, the extent of clinical benefit was unclear. One open-label trial indicated cognitive and motor improvements[78], whereas outcome was unchanged in the other[79]. Clinical improvement was associated with reduction of striatal and cortical hypometabolism, suggesting that the grafts had restored function in striato-cortical neural loops[80].

Substantial benefit following cell therapy will require that many more grafted striatal neurons survive than the low numbers achieved in the trials with fetal tissue[77]. The stem cell technology could markedly increase the availability of such cells. It is notable that most stem cell sources generate cells *in vitro* that stain for γ-aminobutyric acid (GABA). The mechanisms governing this default GABAergic differentiation are unknown, and there is no evidence that other striatal neuronal markers are expressed by these cells. Mouse ESC derivatives[81], autologous bone marrow cells[82] and human forebrain neurosphere cells[83] have been transplanted into rat striatum. Expression of markers for striatal projection neurons was described[83] but could not be confirmed by others[84].

Basic research should now explore how to generate and select striatal projection neurons from stem cells. Subsequent studies should be able to show that these neurons survive transplantation, become anatomically and functionally integrated, and improve motor and cognitive function in Huntington's models. Recently, neural proliferation was reported to be increased in the subependymal layer adjacent to the caudate nucleus in patients with Huntington's disease[85]. This finding suggests that in the brain of someone affected with Huntington's, there is an ineffective neuroregenerative response that, if the generated neurons can survive over the long term and are functional despite carrying the mutation, may become a future therapeutic target.

The ability of stem cell–derived striatal neurons to maintain a stable clinical condition over a long period of time will be essential for their therapeutic value in Huntington's disease. Reconstruction of striatal circuitry alone may be insufficient, because the progressive neocortical degeneration in Huntington's disease is probably not secondary to neuronal loss in the striatum. Functional restoration through neuronal replacement probably has to be combined with neuroprotective strategies for optimum clinical benefit.

Perspectives

The development of stem cell–based therapies for neurodegenerative disorders is still at an early stage. Many basic issues remain to be resolved, and we need to move forward with caution and avoid scientifically ill-founded trials in affected individuals. One challenge now is to identify molecular determinants of stem cell proliferation so as to control undesired growth and genetic alterations of ESCs, as well as to better manage the expansion of NSCs. We also need to know how to pattern stem cells to obtain a more complete repertoire of various types of cells for replacement, and how to induce effective functional integration of stem cell–derived neurons into existing neural and synaptic networks. Technological advances will be needed to make precise genetic modifications of stem cells or their progeny that will enhance their capacity for migration, integration and pathway reconstruction.

The potential of the brain's self-repair mechanisms is virtually unexplored. We need to develop technologies for genetic labeling of stem cell progeny so that we can firmly establish where neurogenesis occurs and which cell types are generated following damage. The functional properties of the new neurons and their ability to form appropriate afferent and efferent connections should be determined. We also need to identify, with the aid of genomic and proteomic approaches, the cellular and molecular players that, in a concerted action, regulate different steps of neurogenesis. On the basis of this knowledge, we should design strategies to deliver molecules that improve the yield of new functional neurons and other cells in the damaged area.

To aid in further progress toward the clinic, we also need to develop animal models that closely mimic the human disease. Such models will allow us to assess and balance potential risks and benefits of stem cell therapies before their application in humans. Likewise, we need to

improve noninvasive imaging technologies so that we can monitor regenerative processes subsequent to stem cell–based approaches in animals and humans.

The time and the scientific effort required should not dampen our enthusiasm for developing stem cell therapies. For the first time, there is real hope that in the future we will be able to offer persons with currently intractable neurodegenerative diseases effective cell-based treatments to restore brain function.

Note: Supplementary information is available on the Nature Medicine website.

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2. Piccini, P. *et al.* Dopamine release from nigral transplants visualized *in vivo* in a Parkinson's patient. *Nat. Neurosci.* **2**, 1137–1140 (1999).
3. Piccini, P. *et al.* Delayed recovery of movement-related cortical function in Parkinson's disease after striatal dopaminergic grafts. *Ann. Neurol.* **48**, 689–695 (2000).
4. Lindvall, O. & Hagell, P. Clinical observations after neural transplantation in Parkinson's disease. *Prog. Brain Res.* **127**, 299–320 (2000).
5. Polgar, S., Morris, M.E., Reilly, S., Bilney, B. & Sanberg, P.R. Reconstructive neurosurgery for Parkinson's disease: a systematic review and preliminary meta-analysis. *Brain Res. Bull.* **60**, 1–24 (2003).
6. Freed, C.R. *et al.* Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719 (2001).
7. Olanow, C.W. *et al.* A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* **54**, 403–414 (2003).
8. Kordower, J.H. *et al.* Fetal nigral grafts survive and mediate clinical benefit in a patient with Parkinson's disease. *Mov. Disord.* **13**, 383–393 (1998).
9. Kordower, J.H. *et al.* Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. *J. Comp. Neurol.* **370**, 203–230 (1996).
10. Hagell, P. *et al.* Dyskinesias following neural transplantation in Parkinson's disease. *Nat. Neurosci.* **5**, 627–628 (2002).
11. Ma, Y. *et al.* Dyskinesia after fetal cell transplantation for parkinsonism: a PET study. *Ann. Neurol.* **52**, 628–634 (2002).
12. Isacson, O., Björklund, L.M. & Schumacher, J.M. Toward full restoration of synaptic and terminal function of the dopaminergic system in Parkinson's disease by stem cells. *Ann. Neurol.* **53**, S135–S146 (2003).
13. Hagell, P. & Brundin, P. Cell survival and clinical outcome following intrastriatal transplantation in Parkinson disease. *J. Neuropathol. Exp. Neurol.* **60**, 741–752 (2001).
14. Draper, J.S. *et al.* Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat. Biotechnol.* **22**, 53–54 (2004).
15. Amit, M. *et al.* Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev. Biol.* **227**, 271–278 (2000).
16. Villa, A. *et al.* Long-term molecular and cellular stability of human neural stem cell lines. *Exp. Cell. Res.* **294**, 559–570 (2004).
17. Roy, N.S. *et al.* Telomerase immortalization of neuronally restricted progenitor cells derived from the human fetal spinal cord. *Nat. Biotechnol.* **22**, 297–305 (2004).
18. Song, H., Stevens, C.F. & Gage, F.H. Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39–44 (2002).
19. Wagner, J. *et al.* Induction of a midbrain dopaminergic phenotype in Nurr1-overexpressing neural stem cells by type 1 astrocytes. *Nat. Biotechnol.* **17**, 653–659 (1999).
20. Zwaka, T.P. & Thomson, J.A. Homologous recombination in human embryonic stem cells. *Nat. Biotechnol.* **21**, 319–321 (2003).
21. Zhao, M. *et al.* Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl. Acad. Sci. USA* **100**, 7925–7930 (2003).
22. Lie, D.C. *et al.* The adult substantia nigra contains progenitor cells with neurogenic potential. *J. Neurosci.* **22**, 6639–6649 (2002).
23. Mao, L., Lau, Y.S., Petroske, E. & Wang, J.Q. Profound astrogenesis in the striatum of adult mice following nigrostriatal dopaminergic lesion by repeated MPTP administration. *Dev. Brain Res.* **131**, 57–65 (2001).
24. Nowakowski, R.S. & Hayes, N.L. Stem cells: the promises and pitfalls. *Neuropsychopharmacology* **25**, 799–804 (2001).
25. El-Khodor, B.F., Oo, T.F., Kholodilov, N. & Burke, R.E. Ectopic expression of cell cycle markers in models of induced programmed cell death in dopamine neurons of the rat substantia nigra pars compacta. *Exp. Neurol.* **179**, 17–27 (2003).
26. Vitek, J.L. Deep brain stimulation for Parkinson's disease. A critical re-evaluation of STN versus GPi DBS. *Stereotact. Funct. Neurosurg.* **78**, 119–131 (2002).
27. Winkler, C., Kirik, D., Björklund, A. & Dunnett, S.B. Transplantation in the rat model of Parkinson's disease: ectopic versus homotopic graft placement. *Prog. Brain Res.* **127**, 233–265 (2000).
28. Mukhida, K., Baker, K.A., Sadi, D. & Mendez, I. Enhancement of sensorimotor behavioral recovery in hemiparkinsonian rats with intrastriatal, intranigral, and intrasubthalamic nucleus dopaminergic transplants. *J. Neurosci.* **21**, 3521–3530 (2001).
29. Mendez, I. *et al.* Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study. Report of three cases. *J. Neurosurg.* **96**, 589–596 (2002).
30. Shim, J.W. *et al.* Enhanced *in vitro* midbrain dopamine neuron differentiation, dopaminergic function, neurite outgrowth, and 1-methyl-4-phenylpyridium resistance in mouse embryonic stem cells overexpressing Bcl-XL. *J. Neurosci.* **24**, 843–852 (2004).
31. Kim, J.H. *et al.* Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* **418**, 50–56 (2002).
32. Chung, S. *et al.* Genetic engineering of mouse embryonic stem cells by Nurr1 enhances differentiation and maturation into dopaminergic neurons. *Eur. J. Neurosci.* **16**, 1829–1838 (2002).
33. Moon, L.D., Asher, R.A., Rhodes, K.E. & Fawcett, J.W. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. *Nat. Neurosci.* **4**, 465–466 (2001).
34. Ourednik, J., Ourednik, V., Lynch, W.P., Schachner, M. & Snyder, E.Y. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nat. Biotechnol.* **20**, 1103–1110 (2002).
35. Steece-Collier, K. *et al.* Embryonic mesencephalic grafts increase levodopa-induced forelimb hyperkinesia in parkinsonian rats. *Mov. Disord.* **18**, 1442–1454 (2003).
36. Björklund, L.M. *et al.* Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc. Natl. Acad. Sci. USA* **99**, 2344–2349 (2002).
37. Erdö, F. *et al.* Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. *J. Cereb. Blood Flow Metab.* **23**, 780–785 (2003).
38. Kondziolka, D. *et al.* Transplantation of cultured human neuronal cells for patients with stroke. *Neurology* **55**, 565–569 (2000).
39. Meltzer, C.C. *et al.* Serial [^{18}F]fluorodeoxyglucose positron emission tomography after human neuronal implantation for stroke. *Neurosurgery* **49**, 586–591 (2001).
40. Nelson, P.T. *et al.* Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. *Am. J. Pathol.* **160**, 1201–1206 (2002).
41. Alvarez-Dolado, M. *et al.* Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* **425**, 968–973 (2003).
42. Weimann, J.M., Johansson, C.B., Trejo, A. & Blau, H.M. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat. Cell Biol.* **5**, 959–966 (2003).
43. Parent, J.M., Vexler, Z.S., Gong, C., Derugin, N. & Ferriero, D.M. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann. Neurol.* **52**, 802–813 (2002).
44. Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. & Lindvall, O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat. Med.* **8**, 963–970 (2002).
45. Jin, K. *et al.* Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. *Mol. Cell Neurosci.* **24**, 171–189 (2003).
46. Jin, K., Mao, X.O., Sun, Y., Xie, L. & Greenberg, D.A. Stem cell factor stimulates neurogenesis *in vitro* and *in vivo*. *J. Clin. Invest.* **110**, 311–319 (2002).
47. Yoshimura, S. *et al.* FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proc. Natl. Acad. Sci. USA* **98**, 5874–5879 (2001).
48. Teramoto, T., Qiu, J., Plumier, J.C. & Moskowitz, M.A. EGF amplifies the replacement of parvalbumin-expressing striatal interneurons after ischemia. *J. Clin. Invest.* **111**, 1125–1132 (2003).
49. Shingo, T., Sorokan, S.T., Shimazaki, T. & Weiss, S. Erythropoietin regulates the *in vitro* and *in vivo* production of neuronal progenitors by mammalian forebrain neural stem cells. *J. Neurosci.* **21**, 9733–9743 (2001).
50. Gustafsson, E. *et al.* Anterograde delivery of brain-derived neurotrophic factor to striatum via nigral transduction of recombinant adeno-associated virus increases neuronal death but promotes neurogenic response following stroke. *Eur. J. Neurosci.* **17**, 2667–2678 (2003).
51. Chmielnicki, E., Benraiss, A., Economides, A.N. & Goldman, S.A. Adenovirally expressed noggin and brain-derived neurotrophic factor cooperate to induce new medium spiny neurons from resident progenitor cells in the adult striatal ventricular zone. *J. Neurosci.* **24**, 2133–2142 (2004).

52. Ekdahl, C.T. *et al.* Caspase-mediated death of newly formed neurons in the adult rat dentate gyrus following status epilepticus. *Eur. J. Neurosci.* **16**, 1463–1471 (2002).
53. Ekdahl, C.T., Claasen, J.-H., Bonde, S., Kokaia, Z. & Lindvall, O. Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. USA* **203**, 13632–13637 (2003).
54. Monje, M.L., Toda, H. & Palmer, T.D. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760–1765 (2003).
55. Nakatomi, H. *et al.* Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* **110**, 429–441 (2002).
56. Zhang, R.L., Zhang, Z.G., Zhang, L. & Chopp, M. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience* **105**, 33–41 (2001).
57. Magavi, S.S., Leavitt, B.R. & Macklis, J.D. Induction of neurogenesis in the neocortex of adult mice. *Nature* **405**, 951–955 (2000).
58. Eriksson, P.S. *et al.* Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313–1317 (1998).
59. Sanai, N. *et al.* Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740–744 (2004).
60. Nunes, M.C. *et al.* Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat. Med.* **9**, 439–447 (2003).
61. Palmer, T.D., Willhoite, A.R. & Gage, F.H. Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol.* **425**, 479–494 (2000).
62. Sun, Y. *et al.* VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J. Clin. Invest.* **111**, 1843–1851 (2003).
63. Zhang, H., Vutskits, L., Pepper, M.S. & Kiss, J.Z. VEGF is a chemoattractant for FGF-2-stimulated neural progenitors. *J. Cell Biol.* **163**, 1375–1384 (2003).
64. Park, K.I., Teng, Y.D. & Snyder, E.Y. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat. Biotechnol.* **20**, 1111–1117 (2002).
65. Hoehn, M. *et al.* Monitoring of implanted stem cell migration *in vivo*: a highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. *Proc. Natl. Acad. Sci. USA* **99**, 16267–16272 (2002).
66. Pluchino, S. *et al.* Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* **422**, 688–694 (2003).
67. Gates, M.A., Fricker-Gates, R.A. & Macklis, J.D. Reconstruction of cortical circuitry. *Prog. Brain Res.* **127**, 115–156 (2000).
68. Fricker-Gates, R.A., Shin, J.J., Tai, C.C., Catapano, L.A. & Macklis, J.D. Late-stage immature neocortical neurons reconstruct interhemispheric connections and form synaptic contacts with increased efficiency in adult mouse cortex undergoing targeted neurodegeneration. *J. Neurosci.* **22**, 4045–4056 (2002).
69. Nógrádi, A. & Vrbová, G. Improved motor function of denervated rat hindlimb muscles induced by embryonic spinal cord grafts. *Eur. J. Neurosci.* **8**, 2198–2203 (1996).
70. Clowry, G., Sieradzan, K. & Vrbová, G. Transplants of embryonic motoneurones to adult spinal cord: survival and innervation abilities. *Trends Neurosci.* **14**, 355–357 (1991).
71. Garbuzova-Davis, S. *et al.* Positive effect of transplantation of hNT neurons (NTera 2/D1 cell-line) in a model of familial amyotrophic lateral sclerosis. *Exp. Neurol.* **174**, 169–180 (2002).
72. Garbuzova-Davis, S. *et al.* Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J. Hematother. Stem Cell Res.* **12**, 255–270 (2003).
73. Kerr, D.A. *et al.* Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. *J. Neurosci.* **23**, 5131–5140 (2003).
74. Dunnett, S.B., Nathwani, F. & Björklund, A. The integration and function of striatal grafts. *Prog. Brain Res.* **127**, 345–380 (2000).
75. Kendall, A.L. *et al.* Functional integration of striatal allografts in a primate model of Huntington's disease. *Nat. Med.* **4**, 727–729 (1998).
76. Palfi, S. *et al.* Fetal striatal allografts reverse cognitive deficits in a primate model of Huntington disease. *Nat. Med.* **4**, 963–966 (1998).
77. Freeman, T.B. *et al.* Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. *Proc. Natl. Acad. Sci. USA* **97**, 13877–13882 (2000).
78. Bachoud-Levi, A.C. *et al.* Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. *Lancet* **356**, 1975–1979 (2000).
79. Hauser, R.A. *et al.* Bilateral human fetal striatal transplantation in Huntington's disease. *Neurology* **58**, 687–695 (2002).
80. Gaura, V. *et al.* Striatal neural grafting improves cortical metabolism in Huntington's disease patients. *Brain* **127**, 65–72 (2004).
81. Dinsmore, J. *et al.* Embryonic stem cells differentiated *in vitro* as a novel source of cells for transplantation. *Cell Transplant.* **5**, 131–143 (1996).
82. Lescaudron, L., Unni, D. & Dunbar, G.L. Autologous adult bone marrow stem cell transplantation in an animal model of Huntington's disease: behavioral and morphological outcomes. *Int. J. Neurosci.* **113**, 945–956 (2003).
83. Fricker, R.A. *et al.* Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. *J. Neurosci.* **19**, 5990–6005 (1999).
84. Englund, U., Björklund, A. & Wictorin, K. Migration patterns and phenotypic differentiation of long-term expanded human neural progenitor cells after transplantation into the adult rat brain. *Dev. Brain Res.* **134**, 123–141 (2002).
85. Curtis, M.A. *et al.* Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proc. Natl. Acad. Sci. USA* **100**, 9023–9027 (2003).
86. Kawasaki, H. *et al.* Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron* **28**, 31–40 (2000).
87. Barberi, T. *et al.* Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nat. Biotechnol.* **21**, 1200–1207 (2003).
88. Kawasaki, H. *et al.* Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity. *Proc. Natl. Acad. Sci. USA* **99**, 1580–1585 (2002).
89. Carvey, P.M. *et al.* A clonal line of mesencephalic progenitor cells converted to dopamine neurons by hematopoietic cytokines: a source of cells for transplantation in Parkinson's disease. *Exp. Neurol.* **171**, 98–108 (2001).
90. Storch, A. *et al.* Long-term proliferation and dopaminergic differentiation of human mesencephalic neural precursor cells. *Exp. Neurol.* **170**, 317–325 (2001).
91. Studer, L. *et al.* Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. *J. Neurosci.* **20**, 7377–7383 (2000).
92. Studer, L., Tabar, V. & McKay, R.D. Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nat. Neurosci.* **1**, 290–295 (1998).
93. Yan, J., Studer, L. & McKay, R.D. Ascorbic acid increases the yield of dopaminergic neurons derived from basic fibroblast growth factor expanded mesencephalic precursors. *J. Neurochem.* **76**, 307–311 (2001).
94. Daadi, M.M. & Weiss, S. Generation of tyrosine hydroxylase-producing neurons from precursors of the embryonic and adult forebrain. *J. Neurosci.* **19**, 4484–4497 (1999).
95. Jiang, Y. *et al.* Neuroectodermal differentiation from mouse multipotent adult progenitor cells. *Proc. Natl. Acad. Sci. USA* **100**, 11854–11860 (2003).
96. Jiang, Y. *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**, 41–49 (2002).
97. Li, Y. *et al.* Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neurosci. Lett.* **316**, 67–70 (2001).
98. Chiba, S., Iwasaki, Y., Sekino, H. & Suzuki, N. Transplantation of motoneuron-enriched neural cells derived from mouse embryonic stem cells improves motor function of hemiplegic mice. *Cell Transplant.* **12**, 457–468 (2003).
99. Mujtaba, T. *et al.* Lineage-restricted neural precursors can be isolated from both the mouse neural tube and cultured ES cells. *Dev. Biol.* **214**, 113–127 (1999).
100. Renoncourt, Y., Carroll, P., Filippi, P., Arce, V. & Alonso, S. Neurons derived *in vitro* from ES cells express homeoproteins characteristic of motoneurons and interneurons. *Mech. Dev.* **79**, 185–197 (1998).
101. Wichterle, H., Lieberam, I., Porter, J.A. & Jessell, T.M. Directed differentiation of embryonic stem cells into motor neurons. *Cell* **110**, 385–397 (2002).
102. Kalyani, A., Hobson, K. & Rao, M.S. Neuroepithelial stem cells from the embryonic spinal cord: isolation, characterization, and clonal analysis. *Dev. Biol.* **186**, 202–223 (1997).
103. Kalyani, A.J., Piper, D., Mujtaba, T., Lucero, M.T. & Rao, M.S. Spinal cord neuronal precursors generate multiple neuronal phenotypes in culture. *J. Neurosci.* **18**, 7856–7868 (1998).
104. Mayer-Proschel, M., Kalyani, A.J., Mujtaba, T. & Rao, M.S. Isolation of lineage-restricted neuronal precursors from multipotent neuroepithelial stem cells. *Neuron* **19**, 773–785 (1997).
105. Li, R. *et al.* Motoneuron differentiation of immortalized human spinal cord cell lines. *J. Neurosci. Res.* **59**, 342–352 (2000).
106. Wu, P. *et al.* Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. *Nat. Neurosci.* **5**, 1271–1278 (2002).
107. Willing, A.E. *et al.* hNT neurons delay onset of motor deficits in a model of amyotrophic lateral sclerosis. *Brain Res. Bull.* **56**, 525–530 (2001).
108. Garbuzova-Davis, S. *et al.* Intraspinal implantation of hNT neurons into SOD1 mice with apparent motor deficit. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* **2**, 175–180 (2001).