Cell replacement therapies for central nervous system disorders

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In animal models, immature neural precursors can replace lost neurons, restore function and promote brain self-repair. Clinical trials in Parkinson's disease suggest that similar approaches may also work in the diseased human brain. But how realistic is it that cell replacement can be developed into effective clinical therapy?

The nervous system, unlike many other tissues, has a limited capacity for self-repair; mature nerve cells lack the ability to regenerate, and neural stem cells, although they exist even in the adult brain, have a limited ability to generate new functional neurons in response to injury. For this reason, there is great interest in the possibility of repairing the nervous system by transplanting new cells that can replace those lost through damage or disease.

Given the complexity of brain circuitry and function, this prospect may seem remote. There is, however, much evidence from animal studies showing that neuronal replacement and partial reconstruction of neuronal circuitry is possible. These results raise the hope of radical new therapies for hitherto intractable human neurodegenerative diseases, but they also raise both scientific and ethical concerns. From a scientific standpoint, the rationale for application of cell therapy may not be equally good for all types of brain damage, and for some CNS disorders the arguments remain highly speculative. The scientific uncertainties lead in turn to ethical concerns; in particular, there is a danger that the rush to apply stem-cell or fetal-cell therapies in patients may lead to scientifically ill-founded clinical trials that lack sufficient support from rigorous preclinical research.

In this commentary, we discuss the scientific basis for cell transplantation therapy in four major CNS disorders in which clinical trials have already begun: Parkinson's disease (PD), Huntington's disease (HD), epilepsy and stroke. We argue that application of fetal neural transplantation is well justified in patients with PD and HD because it has already been shown to work in relevant rodent and primate models, and because its efficacy in both cases can be attributed to specific biological mechanisms. In contrast, no such data are available for either epilepsy or stroke; in these disorders, therefore, we believe that clinical trials are still premature. We also discuss the emerging technology of neural stem cells. Although we agree that these may provide a new powerful tool for transplantation studies, we believe that any clinical application of this new technology must await convincing preclinical data, not only to demonstrate efficacy but also to reveal the mechanism underlying any observed functional recovery.

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New cellular elements in adult brain

In most cases, the therapeutic effects of implanted neurons or neuronal precursors are likely to depend on their becoming structurally and functionally integrated into the brain. Neurons survive and grow well after transplantation only if they are immature, that is, at a developmental stage when they are terminally differentiated but have not formed extensive axonal connections. Their ability to establish reciprocal connectivity with the host brain also depends on the age of the host: it is maximal during the fetal period and gradually diminishes during postnatal development. Nevertheless, grafted neurons can establish functionally appropriate connections even in the adult brain, and this capacity is much increased when the host circuitry is damaged, suggesting that mechanisms regulating neuronal differentiation and connectivity during development may be reactivated by lesions or degenerative changes[1–3]. For example, host cells that have lost their normal inputs may release factors that stimulate axon outgrowth, thereby promoting their reinnervation by grafted cells[4].

However, reconstruction of neural circuitry is not always a prerequisite for functional recovery[4]. Transplanted cells may release neurotransmitters (such as dopamine, acetylcholine or GABA) in a non-regulated tonic manner, or they may produce (or be engineered to produce) neurotrophic or neuroprotective factors that can counteract degeneration or promote regeneration. Moreover, transplanted glial cells may be used to modify the response to injury and assist in structural repair[5] or to promote remyelination in demyelinating disorders[6].

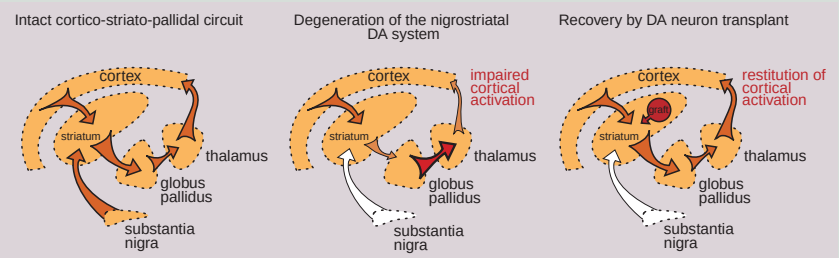
Thus, cell replacement can improve functional defitcits in the adult brain through different mechanisms. In the following sections, we will examine what has been demonstrated with respect to symptomatic relief after cell transplantation, and underlying mechanisms of improvement, in animal models of four major CNS disorders. In particular, we will examine the scientific bases for initiation of clinical trials in these diseases.

Recovery of function in PD

The cell replacement strategy in Parkinson's disease is based on a well-defined biological mechanism: recovery of function by restoration of dopaminergic neurotransmission in the striatum (**Box 1**). This disorder is particularly suitable for testing neuronal replacement approaches, for several reasons. The neurons that degenerate in PD—the dopaminergic neurons of the substantia nigra—are clearly circumscribed, and their main target, the striatum, is anatomically well defined and easily accessible, Moreover, there are well-characterized rodent and primate models of PD, and although these have a different etiology from the human disease, they nevertheless mimic its cardinal features, and they have repeatedly proved to have good predictive value with respect to effects of therapeutic interventions on symptoms in PD patients.

The cells used for transplantation are taken from the ventral midbrain (which includes the substantia nigra) during fetal development, at the time when nigral dopaminergic neurons are undergoing terminal differentiation. The fetal

Box 1. Restoration of dopaminergic neurotransmission in Parkinson's disease.



The dopaminergic neurons in the substantia nigra are important regulators of corticostriatal neurotransmission (left). Their loss leads to increased threshold for activation of the striatopallidothalamic output pathway and impaired movement-related activation of the prefrontal motor cortical areas (middle). Restoration of striatal dopamine neurotransmission by grafted dopaminergic neurons (right) may improve striatal function by at least three different mechanisms, as summarized in the table. In patients with advanced PD, intrastriatal grafts of nigral dopaminergic neurons from 6–9 week-old human embryos can give long-lasting and therapeutically valuable symptomatic relief[16,17]. Survival of the grafted dopaminergic neurons, reinnervation of the striatum, and formation of synaptic connections have been demonstrated in two autopsy cases[19,20]. A recent study demonstrated, using positron emission tomography (PET), that intrastriatal transplants of nigral dopaminergic neurons can normalize both dopamine synthesis and storage (as assessed by striatal [^{18}F]fluorodopa uptake) as well as spontaneous and drug-induced dopamine release (measured as dopamine D_2 receptor occupancy in the grafted putamen)[55]. The gradual onset of substantial clinical improvement seen in many of the grafted PD patients correlates with the recovery of movement-related cortical activation (P. Piccini, O. Lindvall, A. Björklund *et al.*, unpublished data; right). This suggests that functional normalization of the host corticostriatal circuitry (mechanism C) may be important for full expression of the functional capacity of grafted dopaminergic neurons.

Mechanism	Meaning	Effect in animals	Clinical correlate
A. Non-regulated DA release	Normalization of DA receptor sensitivity	Reversal of drug-induced rotation	Partial symptomatic relief; reduced need for L-DOPA medication
B. Synaptic DA release	Physiologically regulated DA receptor activation	Improved initiation of movement	More pronounced improvement of mobility
C. Regulated DA neuron function	Reconstruction of both afferent and efferent connections	Improved skilled limb-use	Recovery of cortical activation; improved execution of complex motor programs

Based on data from refs. 7-20 and 55 and unpublished data (P. Piccini *et al.*). DA, dopamine.

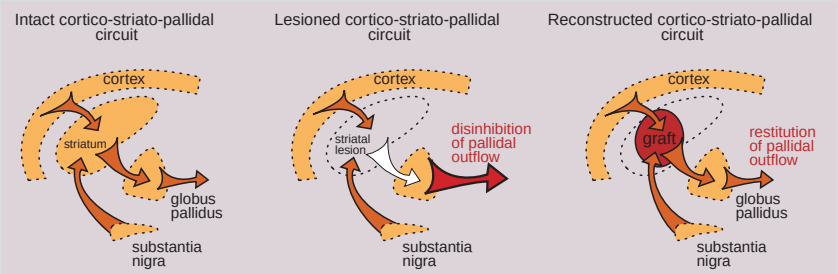
cells are grafted into the host striatum, allowing them to establish functional synaptic contacts with the denervated striatal neurons, their normal synaptic targets. *In vivo* data show that the grafted neurons are spontaneously active and that, in the areas reached by their outgrowing axons, dopamine synthesis, turnover and release are restored to near-normal levels. This is accompanied by significant amelioration of behavioral deficits in both rodent and monkey models[7–9]. Functional recovery clearly depends on a specific interaction with the host striatal target: nigral dopaminergic neurons grafted outside the basal ganglia, or non-dopaminergic neurons grafted to otherwise effective striatal sites, fail to induce improvement. Moreover, the recovered function depends on the continued survival of the grafted cells[7,8,10].

Because of their ectopic location (that is, in striatum rather than substantia nigra), the grafted dopaminergic neurons are unlikely to receive normal afferent inputs. Why, then, does the treatment work? The answer may be that in the intact brain, baseline striatal dopamine neurotransmission is normally maintained by tonic synaptic and nonsynaptic dopamine release, which is largely independent of changes in neuronal impulse flow in the nigrostriatal pathway. In grafted animals, the establishment of a new dopamine-storing terminal network in the host striatum is closely correlated with the onset of graft-derived dopamine release[11] and with the reversal of dopamine receptor supersensitivity[12,13], suggesting that the grafted neurons are spontaneously active and release dopamine at both synaptic and nonsynaptic sites at a near-normal rate. However, the grafted neurons may not entirely lack host afferent control. In one study[14], over 50% of grafted neurons responded to stimulation of either frontal cortex or striatum, and some also showed burst firing, which normally depends on a functional cortical input. This is also consistent with anatomical evidence[15], and these studies suggest that inputs from the host premotor cortex and/or striatum may contribute to the functional efficacy of intrastriatal dopaminergic neuron transplants.

The results of human patient trials are generally consistent with findings in experimental animals[16,17] (**Box 1**). Most of these clinical studies, however, have been small open trials without control groups[18], raising the possibility that the observed symptomatic relief might be due to investigator bias or placebo effects. The latter are known to occur in PD and can be long lasting (at least up to six months)[18]. However, several lines of evidence argue against these artifactual explanations. The clinical improvements developed gradually over the first 6–24 months after transplantation. They have been long lasting (at least 5-10 years). In unilaterally operated patients, the improvement has been predominantly unilateral, affecting the limbs contralateral to the grafted striatum even as the symptoms on the ipsilateral side continued to deteriorate. Finally, the degree of functional recovery has broadly corresponded to the magnitude of restoration of [^{18}F]fluorodopa uptake in the grafted striatum, as measured by positron emission tomography, and this uptake has in the best cases reached almost normal levels.

Recently, preliminary data were reported from the first double-blind, sham surgery-controlled study (C.R. Freed *et al.*, *Soc. Neurosci. Abstr.* **25,** 212, 1999). These data indicate a modest clinical response after grafting bilaterally in the putamen (part of the striatum), with significant improvement occurring only in patients aged 60 years or younger. This study is important because it provides the first direct evidence for a specific graft-derived improvement that can be distinguished from a placebo effect. However, in two cases who have come to autopsy, the number of surviving dopaminergic neurons per putamen was only

Box 2. Restoration of basal ganglia circuitry in Huntington's disease.



Huntington's disease represents a 'disconnection syndrome' that is distinctly different from PD. The cortico-striatal system is essential for the execution of movements (left). In patients with HD, progressive degeneration of neurons in the striatum, and later also in cerebral cortex, disrupts function in the cortico-striato-pallidal circuit and induces severe impairments in both motor and cognitive functions[56]. The striatal GABAergic projection neurons provide an inhibitory control of two major striatal output structures, globus pallidus and pars reticulata of the substantia nigra (not shown). Loss of these neurons, in animals with striatal lesions or in HD patients, results in disinhibition of pallidal outflow (middle). Striatal precursors, obtained from the ganglionic eminence of the early developing forebrain, can re-establish a new striatopallidal projection after implantation into the lesioned striatum (right). In rodent and primate HD models, the grafted cells reinstate inhibitory GABAergic control of the pallidal output neurons and normalize both cognitive and motor behavior[21–25]. As summarized in the table, recovery of different aspects of striatal function is likely to depend on different levels of striatal circuitry reconstruction.

Level of reconstruction	Observations in animals	Functional correlate
A. Establishment of efferent connectivity	Restoration of synapses and GABA release in globus pallidus	Disinhibition of pallidal output; reduced locomotor hyperactivity
B. Establishment of both afferent and efferent connectivity	Restoration of cortical, striatal and nigral afferents to grafted striatal neurons	Recovery of complex motor and cognitive behavior
C. Development of more complex integrated circuitry	Relearning of striatum-dependent motor skills	Restitution of a new habit-learning system in the lesioned striatum

Based on data from refs. 21–25 and 57.

7000–40,000 (as reported by the authors at the 1999 American Academy of Neurology meeting). This is considerably fewer than in previous cases, where the clinical response has been more pronounced (80,000–135,000 surviving dopaminergic neurons)[19,20], a difference that is readily explained because less tissue and longer tissue storage times (up to 4 weeks) were used in the double-blind study. It seems likely that the number of surviving graft neurons is a critical factor in determining the magnitude of symptomatic relief, and that a minimum of 80,000 dopaminergic neurons may be required to obtain a good therapeutic effect—about one fifth of the normal number of dopaminergic neurons in the human substantia nigra.

Recovery of function in HD

Huntington's disease is an inherited fatal disorder in which the abnormal processing of the defective Huntingtin protein results in progressive neurodegeneration, particularly in striatum and cortex. In the rat and primate models that have been used in transplantation studies to date, the neuropathological, neurochemical and behavioral features of the disease are reproduced by intrastriatal injections of excitotoxins or systemic injections of metabolic toxins. As with PD, the acute damage in these animal models is different from the slow pathogenetic process of the human disease; nevertheless, toxin-induced lesions have proved very useful for investigating the restorative capacity of neural grafts and the mechanisms underlying the observed functional improvements.

Many of the symptoms of HD result from the loss of inhibitory connections from the striatum to other structures such as the globus pallidus (**Box 2**). Cell replacement therapy seeks to restore these inhibitory connections. Because the lost neurons are part of an intricately organized corticostriatopallidal circuit, proper functioning of the grafted neurons is likely to depend not only on their forming connections with appropriate postsynaptic targets but also on their receiving appropriate synaptic inputs. Grafted progenitor cells from the striatal primordium of the developing forebrain form a striatum-like structure at the site of implantation. Some of these cells differentiate into mature striatal projection neurons, which establish functional GABAergic innervation in the denervated globus pallidus and receive synaptic inputs from cortex, thalamus and substantia nigra[21,22]. This level of circuitry reconstruction is sufficient to reverse lesion-induced deficits in both motor and cognitive behavior, not only in rats but also in the larger and more complex striatal system of monkeys[23,24]. Indeed, in the rat HD model, grafted cells can even rescue the ability to form and maintain motor habits[25], a type of procedural learning that depends on the striatum in both animals and man[26,27]. Thus, well-learned motor habits, which are disrupted by lesions of the striatum, can be relearned in the presence of a functional graft. This suggests that the implanted striatal primordia may have the capacity to reconstitute a new habit-learning system that becomes integrated within, and functionally connected to, the host corticostriatal circuitry.

These encouraging results in our view justify the initiation of clinical trials in human HD patients, particularly given the lack of other treatment options, and given that no major risks have emerged from cell transplantation studies in PD patients. The main objective of the first studies should be to demonstrate unequivocally that striatal grafts can survive and induce a measurable functional effect in human patients; even if the effects are initially too small to be therapeutically useful, they will form the basis for further refinements to the procedure.

Clinical trials using intrastriatal implantation of fetal striatal tissue are now underway in several centers[28,29]. In one patient who died 18 months after surgery, autopsy revealed not only that the grafted cells had survived and extended neurites into the host tissue, but that dopaminergic fibers from the host had also innervated the grafted tissue (T.B. Freeman *et al. Novartis Found. Symp.* **231**, 10, 1999). Similarly, of five HD patients who had received transplants of human fetal striatal neuroblasts bilaterally in the caudate nucleus and putamen, three had metabolically active grafts, as detected by

Box 3. Seizure suppression in epilepsy.

The effects of cell transplantation have been investigated in a variety of epilepsy models, for example, genetically epilepsy-prone rats, subcortically denervated hippocampus and administration of kainic acid or pilocarpine[31]. Although seizure suppression has been reported after transplantation in several of these models, the underlying mechanisms of graft action are unclear in most cases. The majority of studies have been done in the rat kindling model, which resembles the most common type of epilepsy in adult humans (temporal lobe epilepsy or complex partial seizures). In kindling, repeated electrical stimulation of limbic areas leads to progressive intensification of stimulus-induced seizure activity, culminating in generalized seizures. Once established, the increased seizure susceptibility is permanent. Neurotoxin-induced lesions of forebrain noradrenergic, serotonergic or cholinergic neurons have been used to make animals more epileptogenic, that is, to facilitate the development of kindling. In such animals, transplants of embryonic noradrenergic, serotoninergic or cholinergic neurons are efficient in retarding seizure development induced by kindling stimulations[31,58,59] (see table). This antiepileptogenic effect is well correlated with the extent of graft-derived noradrenergic or cholinergic innervation of the stimulated brain structure. However, the clinical relevance of these findings is unclear, because lesions of these neuromodulatory systems are not characteristic features of human epilepsy. Grafts of fetal striatal GABAergic neurons or GABA-releasing polymers have only induced short-lasting anticonvulsant effects, that is, suppression of the spread and generalization of seizure activity in already kindled animals[32,33] (see table). In an alternative approach, cells engineered to secrete GABA have been used to influence epileptogenesis in the rat kindling model[60].

Epilepsy model	Transplantation approach	Observations in animals	Mechanism	Clinical correlate
Kindling in NE-, 5-HT- or ACh-depleted rats	Embryonic neurons into hippocampus (NE or ACh) or olfactory bulb (5-HT)	Retardation of seizure development correlated to extent of graft-derived reinnervation (NE or ACh)	Restoration of synaptic NE, 5-HT or ACh release	Antiepileptogenic effect
Kindling in non-lesioned, epileptic rats	Embryonic striatal GABA neurons or polymers releasing GABA close to substantia nigra	Transient reduction of seizure generalization	Transient increase of local GABA levels (?)	Anticonvulsant effect

Based on data from refs. 31–33, 58–60. ACh, acetylcholine; 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine.

[^{18}F]fluorodeoxyglucose PET (M. Peschanski, personal communication). Interestingly, these three cases showed clinical benefits in motor and cognitive functions, whereas the two other patients continued to deteriorate. Thus fetal striatal tissue can be implanted without adverse effects and can survive despite an ongoing disease process; moveover, it seems that this can lead to symptomatic relief. It remains to be determined, however, whether striatal transplants can lead to a more complete and long-lasting functional recovery in this complex degenerative disease.

Seizure suppression in epilepsy

Epilepsy comprises a heterogeneous group of disorders, characterized by recurrent seizures due to hyperactivity and synchronization of activity within populations of neurons. Although the causes of epilepsy are diverse, they all seem to lead to changes in synaptic functions and intrinsic properties of neurons[30]. Unlike PD or HD, no specific pathology has been identified that might serve as a target for correction by cell therapy. Nevertheless, the proposed imbalance between excitatory and inhibitory neurotransmission has raised the possibility that seizures could be suppressed by transplanting cells that release inhibitory transmitters.

From a clinical perspective, there are two problems with the grafting studies done so far in animal models of epilepsy. First, most of these models are created by administration of proconvulsant drugs or electrical stimulation, often combined with neurotoxin-induced lesions; thus, although these models mimic some characteristic features of epileptic seizures, the basis of the abnormal excitability may be different from that of human epilepsy, a possibility that is difficult to evaluate in the absence of a recognizable cellular pathology. Second, the effect of grafted inhibitory neurons is mainly antiepileptogenic; in other words, the implants are able to partly prevent development of the permanent increase of neuronal excitability characteristic of epilepsy[31] (**Box 3**). In most cases, cell implantation has been performed before the induction of the epileptic syndrome. Such a treatment, however, would only be useful as a prophylactic. To be therapeutically useful, cell implants should preferably be anticonvulsant; that is, they should suppress seizure activity in already epileptic animals. In theory, seizures might be suppressed by transplanting GABA-releasing cells either into the epileptic focus (where there may be a deficit of GABAergic transmission) or into sites that contribute to the spread and generalization of seizures (such as the pars reticulata of the substantia nigra). In practice, however, it remains to be shown that GABA-releasing implants can induce increased and long-lasting inhibition in an epileptic brain region. The effects observed so far have been transient, either because GABA release from the grafts declined over time, or because the host target neurons downregulate their own GABA receptors following transplantation[32,33].

Thus, the scientific basis for clinical trials with transplantation of GABA-producing cells as anticonvulsant therapy is currently very weak. Nevertheless, intracerebral implantation of porcine fetal striatal GABA-rich tissue has been performed in a group of epileptic patients in whom surgical removal of the brain region receiving the transplant was planned (http://www.diacrin.com).

Recovery of function after stroke

Two different types of insults can cause ischemic damage to the brain. Cardiac arrest or coronary artery occlusion, which leads to abrupt and near-total interruption of cerebral blood flow, causes selective neuronal death to certain vulnerable neuronal populations such as the pyramidal neurons of hippocampal CA1. In contrast, occlusion of a cerebral artery—that is, stroke—gives rise to irreversible damage in a core region and a partially reversible injury in the surrounding penumbra zone. In animals, so-called global ischemia models mimic the effects of cardiac arrest or coronary artery occlusion, whereas focal ischemia models replicate the consequences of stroke. These models are useful to explore various restorative strategies.

Can cell transplants reconstruct neural circuits that have been damaged by ischemic insults and thereby lead to functional recovery? In the case of global ischemia, this has been achieved at least to some extent by transplantation of fetal

Box 4. Functional recovery following cerebral ischemic insults.

Animal models of focal ischemia (stroke) and global ischemia closely resemble the human disease. Stroke induced by occlusion of the middle cerebral artery leads to neuronal death in cortical and striatal areas, whereas brief periods of global ischemia, induced by cardiac arrest or coronary artery occlusion, cause selective loss of CA1 pyramidal cells in the hippocampus. Implantation of cells into the damaged areas may lead to functional recovery, but there is little direct evidence for restoration of connectivity or functional integration of the grafted neurons into host neural circuitries.

Ischemia model	Transplantation approach	Effects on behavioral deficits	Mechanism
Focal ischemia (stroke)	a. Embryonic cortex in cortex[61]	No improvement	–
	b. Embryonic cortex in cortex + enriched environment[37]	Improvement of posture and rotational asymmetry	Trophic action?
	c. Embryonic striatum in striatum[34]	Improvement of passive avoidance and motor asymmetry	Reestablishment of some efferent connections or trophic action?
	d. Human teratocarcinoma cell line in striatum[38,39]	Improvement of passive avoidance and motor asymmetry	Unknown
Global ischemia	a. Embryonic CA1 subfield to the CA1 region[34,35]	Improvement of spatial learning and memory	Reestablishment of some afferent and efferent connections?
	b. Conditionally immortalized neuroepithelial cell line to hippocampus[40]	Improvement of spatial learning	Unknown

hippocampal tissue into the damaged hippocampal CA1 area in rats[34,35] (**Box 4**). Significant improvement in this model requires homotypic replacement of the degenerated CA1 cells and establishment of reciprocal graft–host connectivity[35]. In animals subjected to focal ischemia, fetal cortical grafts placed in the infarcted cortical area receive afferent connections from cortex, thalamus and subcortical nuclei of the host, whereas efferent projections from the graft to the host brain are sparse[36]. In this model, the grafts were able to promote functional recovery only if the rats were housed in an enriched environment, for reasons that are unclear[37].

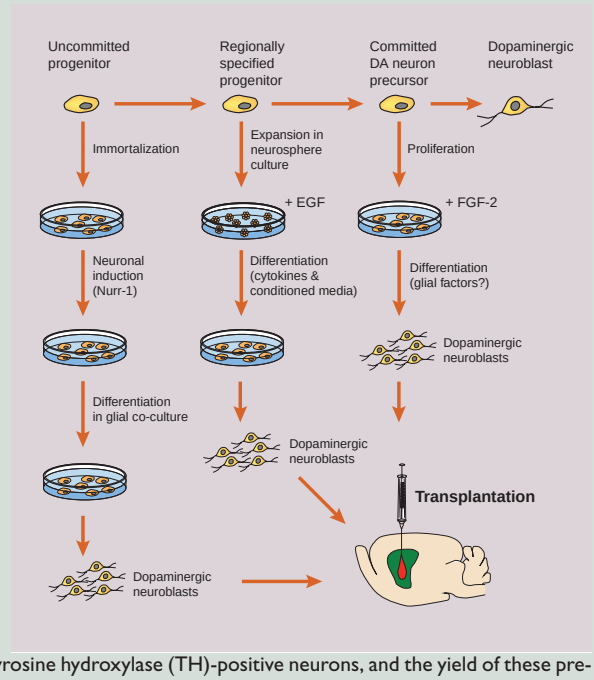
To avoid the use of human embryonic tissue, other sources of cells have been tested in ischemia models. In rats with focal ischemia, functional improvement was reported after intrastriatal implantation of neurons derived from a human teratocarcinoma cell line[38,39]. However, although cells expressing neuronal markers are detected within the grafts, there is no evidence that these cells develop into appropriate striatal neurons in this model. Functional recovery was also observed after global ischemia in rats, following implantation of a mouse hippocampal neuroepithelial cell line within the damaged hippocampus[40] (**Box 4**). In this case, grafted cells were demonstrated in the CA1 region, and a minority of them were identified either as astrocytes or neurons, mostly with a pyramidal-like morphology. In none of these studies, however, is it clear how the transplanted cells exerted their effects. It remains possible, for example, that the observed behavioral improvements may be due to secretion of trophic substances rather than the re-establishment of functional circuitry. Without better knowledge of the biological mechanisms of improvement, and optimization of the functional outcome in animal models, cell therapy for patients with ischemic damage is unlikely to develop to the point of therapeutic value. Nevertheless, this has not prevented the commencement of clinical trials in which either teratocarcinoma cells (same as above) or porcine fetal brain cells were transplanted into the brains of stroke patients (http://www.laytonbio.com; http://www.diacrin.com). Recently, however, the porcine cell trial was put on hold because of adverse events in two of their patients (http://www.diacrin.com/stroke1.htm).

Are we ready for clinical trials?

In an ideal world, the development of cell therapies toward the clinic would be guided by scientific progress made in adequate animal models of human CNS disorders, based on fundamental insights into the underlying disease mechanisms. Clinical application should ideally not be undertaken until the optimal procedure and unequivocal proof of efficacy have been well established in animals, preferably in primate models closely mimicking the human disease, so that potential risks and benefits can be adequately assessed and balanced. However, the initiation of clinical trials is determined not only by scientific considerations but also by commercially guided decisions of biotechnology companies, and by the demands of severely disabled patients and their doctors for novel therapeutic strategies. In these circumstances, it is not always realistic to delay clinical trials until all the details have been worked out in animal models.

Nevertheless, the experience from PD patients indicates that clinical and experimental studies must develop hand-in-hand if the field is to progress. Development of radical new treatments such as cell transplantation is possible only through close interaction between well-designed clinical trials and animal experiments designed to clarify issues raised by observations in patients. This means that progress towards clinical trials must be made carefully and that human trials should not be undertaken until there is convincing preclinical data, that is, experimental data demonstrating not only functional efficacy in relevant animal models, but also a defined biological mechanism for the proposed therapeutic effect. In PD, for example, there is a defined therapeutic goal—dopaminergic neuron replacement—which helps to guide efforts toward more effective restoration of striatal dopamine neurotransmission as a means to further increase symptomatic relief. Moreover, it is possible to measure not only the clinical outcome but also (using neuroimaging techniques) dopamine storage and release in the striatum. The danger of pushing ahead with clinical trials without a defined and proven therapeutic mechanism is illustrated by the experience of adrenal medulla autotransplantation in PD. The rationale for this approach was that the patient's own adrenal chromaffin

Box 5. Generation of fate-specific neurons from *in vitro* expanded progenitors.



The use of stem cells or immature progenitors for transplantation may require that the cells are predifferentiated *in vitro* toward the desired fate, starting from either an uncommitted neural progenitor (left), a lineage-restricted and regionally specified progenitor (middle) or an already committed neuronal precursor (right). These alternative approaches have been explored to obtain dopaminergic neurons for transplantation in animal models of PD. In one approach, already committed E12 rat midbrain dopaminergic neuron precursors were expanded 10-fold in culture in a predifferentiated state[41] (right). On removal of the mitogen (FGF-2), 18% of the cells differentiated into tyrosine hydroxylase (TH)-positive neurons, and the yield of these presumed dopaminergic cells was increased 30-fold over the non-expanded controls. The expanded cells survived transplantation to the rat striatum, but the survival of the grafted TH-positive neurons was poor. In a second approach (middle), rat midbrain progenitors were expanded for weeks or months under EGF stimulation, in so-called neurosphere cultures, and subsequently differentiated into a dopaminergic neuronal fate by a combination of cytokines, midbrain membrane fragments and striatum-conditioned media[42,43]. About 50% of all neurons, and 20–25% of all cells, expressed the TH marker. The cytokine effect was observed with midbrain but not striatal progenitors, suggesting that the TH-positive cells obtained in this procedure were derived from a lineage-restricted midbrain precursor. In a third approach (left), a dopaminergic neuronal phenotype was induced in an immortalized mouse neural stem cell line (C17-2) by overexpression of Nurr-1 (a transcription factor that is likely to be critical in the development of midbrain dopaminergic neurons), in combination with as-yet unidentified factors derived from type-1 astrocytes[44]. Over 80% of the Nurr-1-transduced cells expressed the TH enzyme, as well as two other phenotypic markers, ADH-2 and c-Ret, characteristic of ventral midbrain dopaminergic neurons. The engineered neurons survived transplantation to the mouse striatum, but the yield was very low. These data suggest that neurons and glia may cooperate in both regional specification and induction of specific neuronal identities. If so, the ideal cell preparation for transplantation in PD may consist of a mixture of committed neuronal precursors and regionally specified glial cells.

cells could be used as a source of dopamine release in the striatum. Although some modest improvements were reported, it subsequently became clear that survival of the grafted chromaffin cells was very poor, and the results were reinterpreted as being due to some poorly defined 'trophic' mechanism that did not require dopamine secretion or even graft survival. Nevertheless, several hundred patients received adrenal transplants before the procedure was finally abandoned.

Sources of cells

As the above examples illustrate, the most promising results have been obtained with neurons derived from human fetuses. This is clearly unsatisfactory, for several reasons. Only limited amounts of human fetal tissue are available for grafting purposes. The use of human fetal tissue also raises questions regarding the standardization, viability and purity of the cell material. Finally, the use of aborted fetuses for medical purposes is ethically controversial and, in some countries, has led to intense political debate. Therefore, neural transplantation therapy is likely to remain highly experimental unless we can develop alternative sources of cells that are efficient, safe and ethically acceptable to most people.

In the case of dopaminergic neurons, three different approaches based on the use of immature progenitors have been explored (Box 5). Dopaminergic neurons can be obtained by expanding cultures of embryonic rat midbrain that contain committed dopaminergic neuron precursors[41]. Another method is to grow up progenitor cells from the midbrain, and then induce them to adopt a dopaminergic fate before transplantation[42,43]. A third method is to induce immortalized progenitors to adopt a dopaminergic fate by introducing a gene that regulates their differentiation *in vitro*[44]. All three procedures have been successful in generating large numbers of neurons with the characteristics of a mature dopaminergic phenotype, which can survive and differentiate, at least to a degree, after transplantation to the striatum in rats or mice. However, it is not clear whether these predifferentiated cells develop all characteristics of fully differentiated nigral dopaminergic neurons after implantation. Indeed, transplantation studies in rats indicate that different types of dopaminergic neurons vary greatly in their capacity to reinnervate the striatum[7,45,46], suggesting that the usefulness of *in vitro* generated neurons will depend not only on their ability to adopt a dopaminergic fate, but also on their capacity to establish synaptic connections with striatal target neurons. Moreover, other types of neurons and/or glial cells in the graft may contribute to both differentiation and function of transplanted dopaminergic neurons. If so, an enriched population of predifferentiated dopaminergic neurons may not be the optimal cell preparation for treating PD patients.

Each brain disease affects a different spectrum of cell types, and so if neural transplantation is to succeed as a general therapeutic strategy, different types of cells will be required for each particular condition. Although multipotent stem cells might become an unlimited and self-renewing source of material for transplantation, the problems involved in turning them into the desired cell types are not trivial. Neuronal development depends on the interaction between extrinsic signals in the local environment and intrinsic mechanisms operating in a cell-autonomous manner. As cells become more restricted in their developmental potential, they become independent of extrinsic signals, and cell-autonomous programs take over[47]. Transplantation experiments show that the capacity of the host brain to direct the development of uncommitted progenitors in a region-specific manner is lost or downregulated during postnatal development, except in two areas, the dentate

gyrus and the anterior subventricular zone, where neurogenesis persists even in the adult[48,49]. *In vitro* expanded neural progenitors transplanted into either of these sites, but not outside the neurogenic zones, migrate and integrate along with the endogenous cells, and can differentiate into both neurons and glia[50–52].

Although other regions of the adult brain do not normally support region-specific differentiation of transplanted progenitor cells, there is some evidence to suggest that this ability may reappear after injury. Experiments with multipotent neural progenitors implanted into partially lesioned neocortex[3], hippocampus[53] or striatum[54] in adult rodents show that a fraction of the implanted cells (up to about 15%) are able to differentiate into region-specific neuronal cell types. It therefore seems that even regions outside the neurogenic zones can retain the capacity to direct the differentiation of neural precursors, and that these mechanisms may be reactivated by types of damage that leave tissue architecture and some of the intrinsic neurons intact.

Such findings are encouraging, but it is essential that we learn much more about the basic biology of stem and progenitor cells before their usefulness is tested clinically. We do not yet know to what extent immature progenitors can be made to replace lost neurons and restore functional connectivity in the adult mammalian brain. The use of immature progenitors for repair may require that the cells are predifferentiated *in vitro* toward the desired fate. Whether this is a realistic option depends on the difficulties in reproducing the timing and sequence of extrinsic signaling, the number of steps through which a progenitor cell has to pass, and the requirements for the cells (or mixture of cells) to be functional *in vivo*. Indeed, the cells may have to be genetically engineered to induce their differentiation toward specific lineages and, most likely, they will have to be tailored individually for each type of application.

Perspectives

Not all CNS diseases are equally suitable targets for cell replacement therapy. The best chances for success may be in those applications where clinical efficacy is determined by a single, defined biological mechanism, such as the restoration of striatal dopaminergic neurotransmission in PD by replacement of degenerated dopaminergic neurons. Given time and effort, stem cell technology holds the promise to turn cell therapy from a highly experimental procedure into a clinically useful treatment for large numbers of patients with hitherto intractable neurodegenerative diseases. It should be emphasized, though, that we need to learn much more about the mechanisms involved in the control of cell differentiation, regeneration and functional recovery in the damaged CNS, if we are to develop rational and efficient approaches to cell-based therapies. The complexity of the biological problems involved should not be underestimated, and progress should be made with great care. We would do well to learn the lesson from the troubled path of the gene therapy field: not to promise too much too early.

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