Neuronal cell transplantation for Parkinson's and Huntington's diseases

Stephen B Dunnett, A Lisa Kendall, Colin Watts and Eduardo M Torres

MRC Cambridge Centre for Brain Repair and Department of Experimental Psychology, University of Cambridge, Cambridge, UK

The brain constitutes a privileged transplantation site. Under appropriate conditions neuronal tissues can survive transplantation into the damaged brain, integrate with the host, and alleviate functional impairments associated with neurological disease. The experimental techniques have been developed to the point of clinical application with demonstrable benefit in Parkinson's disease, and similar applications in Huntington's disease appear to be imminent. Nevertheless, present techniques require use of embryonic/fetal tissues which will limit the availability of donors for the foreseeable future. There is an active search for alternative sources of tissue that are equally effective but more readily available, including engineered cells, expanded stem/precursor cells, and xenografts.

Transplantation of neuronal cells

Transplantation of neural tissues (comprising both neurones and glial cells) offers considerable promise for providing radically new methods of treating a wide range of presently intractable neurodegenerative conditions of the central nervous system (CNS). The furthest progress has so far been achieved in Parkinson's disease (PD). However, the initial clinical trials are also now underway for pain and Huntington's disease (HD), with longer-term prospects of applications to motor neurones disease, epilepsy, multiple sclerosis and Alzheimer's disease all under active development. PD and HD are the two neurodegenerative diseases in which structural repair of neuronal circuits in the brain by cellular grafts is most advanced, and these will, therefore, provide the focus of the present review.

The techniques of transplantation in the CNS are not new, with the first experimental attempts undertaken in 1890, and the first demonstrable success in 1917. However, the modern era developed from a series of landmark publications in 1970 and 1971 that first outlined the technical conditions for achieving reliable and reproducible survival.
of neuronal tissues in a variety of different model systems in the rat brain.

Use of embryonic donors

CNS neurones only survive transplantation if taken from embryonic or neonatal donors. In particular, there is a narrow time window in development when the cells must be taken to ensure good survival. This critical time window corresponds to the period when the neurones undergo their final mitotic cell division and their neuronal fate and phenotype is now determined. At this stage, the cells are moving into an active growth phase, giving rise to vigorous outgrowth of neurites which seek appropriate targets under the control both of the cell’s intrinsic genetic programme and of the external substrate and growth factor environment of the host brain. If the cells are harvested earlier in development, their fate is not determined. If harvested later, their survival is compromised by the trauma involved in dissecting a fully ramified neurone, the greater susceptibility to anoxia, and the reduced growth potential of the cell.

Selection of appropriate target site

The second factor important in graft survival is the selection of an appropriate transplantation site. For solid grafts (i.e. implantation of ‘chunks’ of dissected tissue directly into the host brain), the requirement is for a richly vascularised site into which the graft can be placed for its nourishment and rapid incorporation into the host blood and cerebral spinal fluid circulation. There are a limited number of suitable sites in the brain, such as the choroid-plexus rich ventricles, the choroidal fissure overlying the diencephalon, or on the surface of the iris in the anterior eye-chamber. Alternatively, artificial cavities can be made by aspiration and new vascular beds created surgically, or by a delayed cavitation procedure in which a new highly vascular pia reforms over a period of weeks on the cavity floor and walls. These constraints on the location are for the implantation of solid tissue pieces into the CNS and do not apply to the same extent to cell suspension grafts (see below).
Cell suspension techniques

In the early 1980s, a major development of transplantation techniques came with the introduction of procedures for grafting embryonic tissue not as solid pieces into CNS cavities, but by stereotaxic injection of suspensions of dissociated cells (see Fig. 1). The age of donor embryos is again critical for graft viability, but the cell suspensions can be injected in small aliquots into any site within the host neuropil achieving good survival. The cell suspension method of grafting has a number of distinct advantages:

- there is complete flexibility allowing single or multiple deposits of a single cell type or different types of neurone, into one nucleus or distributed sites in the host brain
- cells can be labelled or manipulated in culture prior to implantation
- there is much reduced surgical trauma involved in a stereotaxic injection of cells and no need to create additional cavities or conditioning lesions
- cells can be grafted along tracks to provide a guidance substrate for axon growth between different parts of the brain.

For these reasons dissociated cell suspensions are the likely method of choice for most (present or future) applications involving neuronal replacement in human neurodegenerative disease.

![Fig. 1 Schematic illustration of the procedures for grafting embryonic substantia nigra as a dissociated cell suspension into the rat neostriatum. Identical procedures are followed for other species, including man.](http://bmb.oxfordjournals.org/)

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Survival, connectivity, and mechanisms of action

The early development of clinical application of neural grafting in PD has largely developed empirically, i.e., on the basis of identifying transplantation strategies that can function effectively in animal models of the human disease. The empirical approach is a necessary component of an effective programme to identify novel therapeutic strategy, but is more likely to achieve rapid progress if combined with a theoretical analysis of the mechanisms by which a graft might influence host function, in combination with the type of structural repair or reconstruction that would be necessary to achieve recovery in a particular condition.

Early studies of nigral grafts in a simple rat model of PD showed that recovery of simple motor deficits was associated with survival of dopamine cells in embryonic nigral grafts and correlated with the extent of dopamine fibre reinnervation of the denervated neostriatum. It was, therefore, natural to conclude that the regeneration of a new nigral dopamine input from the grafts underlay the functional recovery. This may be true, but it has become apparent in the intervening years that grafts can induce functional recovery by a variety of different mechanisms, some of which are considerably less specific in nature (see Table 1). It, therefore, becomes necessary to analyse the minimum levels of reconstruction necessary to achieve functional recovery and design appropriate graft strategies according to rational criteria. For example, in amyotrophic lateral sclerosis, the primary pathology

<table>
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<td><strong>Pharmacological</strong></td>
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<td>Grafts provide synaptic reinnervation and tonic reactivation of host brain</td>
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<td><strong>Reconstruction</strong></td>
<td>Grafts establish input and output connections with host brain that restores functional neuronal circuits</td>
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Examples:
- Caudate lesions alleviate PD
- Vasopresun secretion by hypothalamic grafts in diabetic rats.
- Adrenal grafts secrete factors that stimulate sprouting in host brain
- Peripheral nerve segments allow regrowth of CNS axons in optic or spinal cord pathways
- Nigral grafts provide synaptic reinnervation of striatum in PD
- Sinatal grafts reconstructed cortico-striato-pallidal circuits for HD (9)
involves degeneration and loss of spinal motor neurones. Although it may prove prohibitively complex (although perhaps not impossible) to develop a transplantation strategy to graft replacement motor neurones and achieve their appropriate reinnervation of distal targets, grafts of encapsulated cells engineered to secrete ciliary neurotrophic factor (CNTF) are now being considered as a source for growth factor delivery into the CNS to provide trophic support and retard progression of the disease. Prospects for prophylactic use of grafts should be explored to the full. Nevertheless, once neurones are lost from the mature CNS they are not replaced, and ultimately the only prospects for repair may be neuronal replacement and reconstruction.

**Immunological factors**

Immunological factors turn out to be of less concern in the CNS than might have been expected from the long history of studies in the field of organ transplantation. The brain has long been known to be ‘immunologically privileged’ as a result of the ability of the blood–brain barrier to prevent many circulating immune cells and antibodies from penetrating the brain neuropil, the absence of expression of major histocompatibility antigens (HMC) by neurones, and the lack of a clearly defined lymphatic drainage system in the brain. As a consequence, allografts in the brain between donor embryos and mature hosts of the same species generally survive well in rats and monkeys without immunosuppression. Nevertheless, the privilege is only partial, and neuronal xenografts into mature hosts are rapidly rejected. Similarly, even if a graft does become accepted behind the blood–brain barrier, an immune response precipitated by a skin graft can lead to rejection of the graft in the brain also.

When immunological factors are of concern, standard immunosuppression therapies are effective. Thus daily cyclosporin allows survival of mouse, human and porcine tissues in the rat brain, which has proved important in empirical studies of human embryonic age and cell viability in preparation for clinical trials of human fetal allografts in PD. Triple therapy has been used as a cautionary procedure in most clinical trials undertaken so far in PD patients, although the necessity of prolonged immunosuppression for allografts in man is not yet resolved. Whereas the efficacy of cyclosporin A and triple therapy across major species barriers suggests that cell-mediated rejection is an important consideration even in the relatively privileged brain. The presence of a complement-mediated hyperacute mechanism in this organ has not been fully evaluated and the presence of pre-formed pig antibodies in human brain is not yet known (see White, this issue).
Nigral grafts in ‘PD’ rats

Animals do not suffer from idiopathic PD. Nevertheless, lesions of forebrain dopamine systems, made using selective catecholamine neurotoxins, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produce marked Parkinson-like motor symptoms in mice, rats and monkeys (and, indeed, following accidental self-administration in man). These models of the human disease have been extensively investigated in their own right, in the search for new, more effective, and selective pharmaceutical agents and, indeed, as the basis for evaluating novel surgical treatments, such as pallidotomy, subthalamic stimulation or cell transplantation. In view of the number of up-to-date reviews of the functional effects of nigral transplants\textsuperscript{13,14}, suffice to summarise this large literature as follows:

- dopamine cells of the embryonic nigra survive transplantation into the lateral ventricles, into cortical cavities overlying the striatum, or as dissociated cell suspensions into the striatum itself (see Fig. 2)
- grafted dopamine cells give rise to a rich axonal plexus that grows into the host striatum (see Fig. 2) and makes morphologically appropriate synaptic connections onto the appropriate populations of host medium spiny neurones
- the grafts restore regulated dopamine release at physiological levels close to the grafts and, typically, at a declining level at greater distances. Turnover and post-synaptic receptor supersensitivity are both regulated to maintain appropriate levels of dopaminergic activation in the host striatum
- nigral grafts alleviate many of the motor deficits induced by dopamine depleting lesions, including spontaneous and drug induced rotation, side bias and neglect of contralateral space in animals with unilateral lesions, and the akinesia and rigidity characteristic of profound bilateral lesions. Even some more complex learned behaviours such as conditioned rotation and self-stimulation can be alleviated by the nigral grafts
- there remain a core of deficits that are not affected by the grafts. These include precise motor control of the hands/paws for coordinated manipulation skills, and the profound regulatory deficits in eating and drinking induced by bilateral lesions.

Note that in all cases the grafts are positioned in an ectopic site (i.e. in the striatum itself). It is hypothesised that the deficits that recover relate to functions that are regulated by (and can be restored by) a diffuse local dopamine activation of the striatum, whereas those that do not recover involve functions which are normally mediated by patterned information relayed via the nigrostriatal bundle, which is not restored by the ectopic
placement of the grafts. When taking these experimental studies to the clinic, we should anticipate that not all symptoms will be equally amenable to alleviation by transplants. Moreover, further improvements may depend not only on quantitative factors (such as more surviving cells or better integration of the grafts), but rather on the adoption of quite different grafting strategies, such as the development of ways not only to reinnervate the striatum at a terminal level but also to reconstruct the full nigrostriatal circuitry (see Fig. 2).

Fig. 2 Nigral grafts providing a dopaminergic reinnervation of the dopamine depleted rat striatum. The intact nigrostriatal system is seen on the right side of the brain. Visualised by tyrosine hydroxylase immunohistochemistry (From a study by Fricker RA and Annett LE, unpublished data, with thanks.)

Nigral (and adrenal) grafts in PD patients

Adrenal grafts in PD

The experimental studies in animals have clearly demonstrated that implants of embryonic dopamine cells can alleviate many of the
symptoms associated with experimental dopamine deficiency, leading to the natural consideration of whether a similar strategy might be effective in human disease, in particular PD. However, both the research and therapeutic use of human embryonic and fetal tissues is ethically controversial in most Western societies. Consequently, the first clinical trials were undertaken in Sweden in 1982 using an alternative catecholamine secreting tissue, the chromaffin cells of the adrenal medulla. Unlike CNS tissues, peripheral nervous tissue may survive transplantation even in adulthood, and so single adrenal glands could be collected for autografts from the patients themselves, bypassing both the ethical and immunological concerns associated with fetal allografts. Unfortunately, this procedure was without great success. The claim in 1986 of dramatic alleviation in several further patients in Mexico by an open ventricular adrenal autograft procedure, initially stimulated many further trials in many centres, in particular in the US. However, the several American series provided the foundation for systematic multi-centre trials coordinated by the American Association of Neurological Surgeons and the United Parkinson Foundation, which failed to replicate any dramatic benefit of adrenal autograft procedures. Although some statistical benefit could be identified in a minority of patients, it was not of substantial clinical benefit and was associated with considerable morbidity and mortality.

Moreover, as a series of patients came to post mortem, it became apparent that any functional benefit from adrenal grafts was typically associated with either poor or no detectable graft survival. Indeed, it turned out that adrenal grafts probably function not by replacement of lost dopamine but by a trophic mechanism involving the variable induction of a sprouting response in spared host nigrostriatal fibres. The use of adrenal autografts has now largely fallen into disrepute, but provides a lesson of the need for rigorous experimental evaluation rather than uncritical enthusiastic adoption of new therapies.

**Ethical guidelines for use of fetal tissues in neural transplantation**

Following rejection of the adrenal autograft alternative, the development of human embryonic tissue transplantation was also pioneered in Sweden. An extensive and detailed consideration of the ethical issues by the Swedish Society for Medicine led to a set of guidelines for the ethical use of human embryonic tissue for neural tissue transplantation. These have become the foundation of discussions and guidelines in other countries in Europe and North America. In particular, the European centres involved in the practice and development of neural
Neuronal cells for PD and HD

Table 2  NECTAR ethical guidelines for the use of human embryonic or fetal tissue for experimental and clinical neurotransplantation and research

1. Tissue for transplantation or research may be obtained from dead embryos or fetuses, their death resulting from legally induced or spontaneous abortion. Death of an intact embryo or fetus is defined as absence of respiration and heart beats.

2. It is not allowed to keep intact embryos or fetuses alive artificially for the purpose of removing usable material.

3. The decision to terminate pregnancy must under no circumstances be influenced by the possible or desired subsequent use of the embryo or fetus and must therefore precede any introduction of the possible use of the embryonic or fetal tissue. There should be no link between the donor and the recipient, nor designation of the recipient by the donor.

4. The procedure of abortion, or the timing, must not be influenced by the requirements of the transplantation activity when this would be in conflict with the woman’s interests or would increase embryonic or fetal distress.

5. No material can be used without informed consent of the woman involved. This informed consent should, whenever possible, be obtained prior to abortion.

6. Screening of the woman for transmissible diseases requires informed consent.

7. Nervous tissue may be used for transplantation as suspended: cell preparations or tissue fragments.

8. All members of the hospital or research staff directly involved in any of the procedures must be fully informed.

9. The procurement of embryos, fetuses or their tissue must not involve profit or remuneration.

transplantation, through the European network for neural transplantation (NECTAR), have adopted a consensus position, summarised in Table 2. Following the first cases of neural transplantation in the UK (in Birmingham in 1978), the Royal Society of Medicine produced an interim set of guidelines, which were formally developed in the Polkinghorne report of 1989, and current UK research is governed by these as interpreted by an additional memorandum of guidance on the supply of fetal tissue for research, diagnosis and therapy from the Department of Health in 1995.

Nigral grafts in PD

The first Swedish trial in two patients was without great success. The grafts did not survive well in the PD brain, as evidenced by a very poor signal in fluorodopa PET scan, and the patients showed little sustained benefit in timed neurological tests. This led to revision of several aspects of the transplantation protocol, most notably a substantial modification of the implantation instrument to reduce surgical trauma. Subsequent patients have shown dramatically improved benefit of the transplants. This is evidenced in three main realms:
• fluorodopa PET scans have indicated good survival of the grafts and restoration of uptake signal in the grafted striatum rising to an asymptotic level restoring the uptake constant into the normal range over a 2–3 year period. Restitution is seen in the unilaterally grafted putamen, whereas a progressive deterioration in the signal is seen in the non-grafted putamen associated with the underlying progression of the disease itself.

• in all but one patient, the proportion of the day spent in the ‘on’ state has improved considerably, with a decline in both the number and duration of ‘off’ episodes. Attempts were initially made to titrate an optimal L-dopa dose prior to transplantation and maintain this at a constant level following surgery, so as to provide a stable baseline for assessment. However, as the grafts have become established the patients’ L-dopa requirements have declined, to the point that at least one of the patients reached a point 3 years after transplantation of being in continuous ‘on’ with complete withdrawal of L-dopa.

• in all but one patient, neurological function has been seen to undergo dramatic and sustained improvement in timed tests of movement. Recovery is most marked on the side of the body contralateral to the implants, and is more marked in distal motor control (e.g. the pronation-supination test), than in axial movement, gait and balance. The tremor does not benefit from grafting. On this basis, several of the patients initially receiving a unilateral implant have now been scheduled also to receive implantation on the contralateral side.

The results from the Swedish series have now been replicated in several other centres including Paris, Tampa, Denver and Los Angeles. In addition, there have been several other centres world-wide that also report clear benefit but where the experimental evidence to support those claims is sparser. In comparing studies, the use of controlled timed neurological tests and the independent validation of graft survival by in vivo scanning are critical parameters in determining graft efficacy. Although there remain aspects of the syndrome that are less well alleviated, clearly the grafts can induce a dramatic and sustained clinical improvement in some patients. Conversely, the basic experimental studies of cell transplantation indicate clear principles for effective graft survival. Without identifying culprits, it is apparent that centres that pay less rigorous attention to issues such as the age of donor embryos, tissue dissection, implantation site, surgical trauma or immunological factors generally achieve less convincing positive outcome than those centres whose clinical practice is closely rooted in experimental biology.

Two further developments have been important in bridging the gaps between experimental studies in animals and the clinical trials in patients. First, there has been concern that a cell transplantation strategy
Neuronal cells for PD and HD may not be viable if the grafts are immediately subject to assault by an ongoing disease process in idiopathic PD. This concern has been invalidated by the fact that embryonic nigral grafts have a similar time course of benefit in patients with PD due to acute exposure to the toxin MPTP\textsuperscript{21}. Secondly, there has been concern with whether restitution of the fluorodopa PET signal actually represents a tissue reaction in host striatum rather than survival and growth of the grafts. The use of \textit{in vivo} imaging as a reliable marker of graft survival has recently been corroborated by \textit{post mortem} evidence of survival of large numbers of dopamine cells in nigral grafts and extensive dopaminergic reinnervation in the Parkinson brain that correlated closely with the PET image\textsuperscript{22}.

**Striatal grafts in ‘HD’ rats**

Striatal lesions in rats, whether made with excitotoxic amino acids (ibotenic acid, quinolinic acid) or the newer metabolic toxins (malonate, 3-nitropropionic acid) can reproduce many of the key pathological and behavioural symptoms of HD. Thus, the lesions induce selective destruction of the medium spiny neurones of the striatum, with long-term secondary atrophy in afferent and efferent nuclei in the cortex, thalamus and substantia nigra, and these lesions produce clear deficits in both motor function and in cognitive tests, in particular those sensitive to frontal cortical damage.

Striatal grafts not only survive transplantation to the excitototically lesioned striatum, they also develop extensive afferent and efferent connections with the host brain and restore deficits in both motor and cognitive tasks\textsuperscript{23}. The particular interest in this as a model system is that it appears to be the clearest example yet available of transplant-derived recovery through a mechanism of reconstruction (Table 1)\textsuperscript{24}:

- the grafts are located in a homotopic site (i.e. the graft tissue replaces lesioned striatum in the place from where the intrinsic neurones had been lost)
- the grafts reconstruct the lost circuitry at an anatomical level (restoring appropriate patterns of cortico-striatal and nigro-striatal inputs to the grafts and striato-pallidal and striato-nigral outputs from the grafts)
- the grafts reconstruct the lost circuitry at an ultrastructural level. Electron microscopic analysis indicates that both the cortical and dopaminergic inputs make synaptic connections onto Golgi impregnated GABAergic medium spiny neurones in the grafts identified by retrograde tracing as projecting to the globus pallidus.
electrophysiological recordings have demonstrated functional relay of physiological information into the grafts
- *in vivo* neurochemistry of GABA turnover in the globus pallidus indicates relay of nigro-striato-pallidal information within integrated graft-host circuitry
- the grafts restore function on tests sensitive to the integrity of cortico-striato-pallidal loops. Thus, for example, in the 1930s Jacobsen introduced the delayed alternation test as the prototype for assessing prefrontal deficits, and it is now known that lesions anywhere in the relevant cortico-subcortical prefrontal system disrupt performance in this task. Striatal grafts restore the ability of striatal lesioned rats to learn the delayed alternation task, suggesting functional reconnection of the disconnected circuit.

On the basis of these results, it is now a matter of active consideration whether a similar transplantation strategy may be relevant for neurodegenerative diseases of the neostriatum such as HD.

**Preclinical studies towards clinical trials in HD**

Experience in taking neural transplantation to clinical application in PD has emphasised the likelihood that clinical trials of striatal transplantation are most likely to prove successful if built upon solid experimental foundations, and if rigorous attention is paid to assessment of functional outcome.

**Dissection and internal organisation of striatal grafts**

Striatal grafts in rats develop a patchy internal organisation, comprising striatal-like patches (designated the ‘P zones’) that stain with a variety of markers characteristic of striatal cells, interspersed by ‘NP zones’ that stain with markers characteristic of other non-striatal nuclei, such as cortex or globus pallidus. The problem arises because the embryonic ganglionic eminence from which striatal neurones originate and which is dissected in ‘striatal’ grafts in fact includes germinal and sub-germinal cell layers that give rise to a diverse range of cortical as well as subcortical neurones. Isacson and colleagues have argued that a higher percentage of P zones is obtained in striatal grafts by restricting the dissection to the lateral ridge of the ganglionic eminence (LGE). Although it is clear from both *in vitro* and graft studies, the LGE dissection yields much higher proportions of striatal-like cells than a
dissection restricted to the medial ridge, it is not yet demonstrated that the restricted dissection is preferable either in terms of total cell number, differentiation of different cell types or functional efficacy over the classical dissection.

The issue of dissection has become more complicated since several groups have used xenotransplantation of human tissues into immuno-suppressed rats in order to determine optimal ages of human embryos and to validate the dissection of human embryonic striatum for grafting. Two problems have come to light. Firstly, even when using the restricted LGE dissection, human striatal xenografts have been seen to yield remarkably low proportions of P zones, or to be comprised entirely of non-striatal tissue. This may simply be due to the longer time course for human striatal development or to the absence of appropriate differentiating factors in the brain of a different species. However, the fact that human xenografts were so influential in determining the optimal age and dissection of human nigral grafts gives cause for caution when a similar strategy is not successful for striatal tissues. Of perhaps greater concern has been the report from two labs of apparent overgrowth of a small proportion of human striatal xenografts in rats. Again, the problem may be one of absence of species-specific inhibitory factors regulating growth and differentiation of graft tissues in the xenograft environment, but the issue should give cause for concern until resolved.

The strategy we are adopting is to compare striatal grafts within and between primate as well as rodent species. As illustrated in Figure 3, striatal grafts survive well in primates, develop a similar pattern of internal organisation to that seen in rat allografts, and do not indicate the overgrowth that has been observed in some reports of human-to-rat striatal xenografts. Moreover, in a study still in progress, the striatal grafts appear, partially, to alleviate deficits in reaching for food by the monkeys (Kendall AL, unpublished data). Conversely, we have seen that primate-to-rat and porcine-to-rat xenografts show poor striatal development, similar to that previously described for human-to-rat xenografts. However, the allograft studies in primates have so far been conducted over a 6–9 months’ survival period whereas most xenografts have been restricted to shorter survival times because of the adverse side-effects of chronic immunosuppression. Consequently, we cannot yet resolve whether the relatively poor results so far reported with human striatal tissues implanted in rats is due to the protracted striatal development in large species or to the xenograft environment. However, we can now conclude that the positive results on functional reconstruction by striatal allografts are not restricted to rodent species—they appear to apply equally in the primate realm, which improves our optimism for a positive functional outcome for striatal allografts in man.
Transplantation

Fig. 3 Striatal grafts in a primate (marmoset) model of HD (A) Caudate nucleus and putamen on the left side of the brain in the intact striatum (midline is to the right). (B) A quinolinic acid injection in a lesioned animal has completely destroyed the putamen but leaves caudate intact. (C) Striatal transplant replaces the lost putamen in a grafted animal and expresses a typical patchy appearance, with approximately 40% P zone and 60% NP zone (see text for explanation). Visualised with acetylcholinesterase histochemistry.

Clinical assessment protocols

A second factor that has distinguished clinical trials in PD has been the need for objective neurological test protocols, able to assess the patients in well defined stages of the drug on-off cycle, controlling for placebo and selection effects on the outcome, and backed up by quantitative measurement of graft survival using PET imaging. In order to achieve an objective longitudinal assessment of patient progress and comparability between European and US transplantation centres, a core assessment protocol for intracerebral transplantation (CAPIT) was developed to provide a standardised timing and selection of tests\textsuperscript{27}. This process was much aided by the long tradition and availability of standardised test batteries for PD (e.g. the United Parkinson's Disease Rating Scale, UPDRS) which had been developed over two decades in support of drug trials of novel dopaminergic agents.

A similar protocol for assessing the longitudinal progress for HD is more difficult for at least three reasons:

- although there are a large number of cross-sectional studies of the psychopathology of HD, there is no comparable background
literature on the progression of the disease. This is, in large part, because of the lack of available drug therapies, and there is only a limited range of available standardised tests available

- developing a new standardised test battery has been complicated by the need for longitudinal retests at set intervals. This has not proved problematic for the motor tests that predominate in PD batteries. However, HD is marked by cognitive and psychiatric features that are at least as debilitating as the motor symptoms. There are severe problems with test-retest savings on tests of cognition, learning and memory, which are only partly addressed by having a limited number of parallel forms

- fluorodopa PET scans have provided a selective visualisation of graft-derived dopaminergic terminals in the PD brain. However, most PET studies on HD have used fluorodeoxyglucose for metabolic mapping which will not distinguish striatal from non striatal tissues in the grafts. Recent studies in rats have indicated that other ligands (such as the D2 agonist raclopride) are more effective in detecting the striatal-like neurones selectively in striatal grafts and that these (rather than total metabolic signal) correlate better with both the functional efficacy of the grafts and with post mortem indices of P zone survival.

In spite of the difficulties, European centres have now agreed on a core assessment protocol for assessing striatal transplantation in HD, which involves regular tests of neurological, neuropsychological and psychiatric function at 6 monthly and yearly intervals over at least 1 year prior to transplantation and 2 years following transplantation, and using a dopamine receptor ligand in PET as well as MRI scans prior to and following surgery.

First clinical trials

The first clinical trials of striatal cell transplantation have now been undertaken. The only published studies involve 4 patients transplanted in Czechoslovakia and Cuba and 2 more from Mexico, but these reports are so brief as to be useless. More details are now becoming available of a series of 12 cases operated in 1995 and 1996 at the Good Samaritan Hospital in Los Angeles, apparently without complications, and with promising initial results in reducing both motor and cognitive symptoms, although the rapidity of recovery raises questions about the mechanisms of graft action and the long-term stability of recovery is not yet demonstrated. Further clinical trials are known to be in the planning
stage for the near future in Paris, Boston, Tampa and Cambridge, and no doubt elsewhere also.

**Alternative sources of tissue in PD and HD**

The combination of the ethical controversies that surround the research and therapeutic use of embryonic tissues in most Western societies and the practical need to obtain tissue that is precisely staged, accurately dissected, and freshly collected together imply that availability of fetal tissues for cell transplantation in the brain is likely to prove extremely limited for the foreseeable future. Consequently, there is an urgent and active search for alternative sources of tissue.

**Engineered non-neuronal cells**

One strategy that is gaining widespread attention at present is to engineer cells that are ethically neutral and more readily available to express the particular phenotype required for transplantation. There have been rapid advances in recent years in a variety of techniques for cell manipulation and gene transfer in neurones\(^33\). This may prove suitable for grafts whose proposed mechanism of action is the secretion of defined gene products, such as deficient neurohormones, neurotransmitters or growth factors, and variations on this theme are in active development for PD\(^34\). However, this strategy is unlikely to prove effective where the goal is to implant neurones that can become integrated into the host neuronal circuitry, as will most probably need to apply in HD.

**Expanded stem and precursor cells**

A second strategy is to identify, harvest and grow early stem cells from the developing nervous system. Reynolds and Weiss\(^35\) introduced the techniques for large scale expansion of ‘neurospheres’ derived from multipotential stem cells from the embryonic mouse brain. Specifically, when treated with high doses of EGF, stem and precursor cells from the brain can be selectively expanded *in vitro*, and grown exponentially through multiple passages. These expanded cells can be grown from both rat and human as well as mouse embryonic tissues, they will yield both neuronal and glial phenotypes when allowed to differentiate *in vitro*, and they survive transplantation back into the lesioned rat CNS\(^36\).
However, although these studies have now demonstrated the essential feasibility of expanding and transplanting stem/precursor cells in principle, the present techniques are still constrained by the relatively small numbers of neurones which express specific neuronal phenotypes when grafted into the CNS.

**Xenografts**

A third strategy, the stimulation for which came from the limited availability of tissues for organ transplantation, is xenotransplantation (see White, this issue). The relative immunological privilege of the brain may mean that obtaining good xenograft survival in the brain will prove to be an easier immunological problem to solve than elsewhere in the body. This is further suggested by the success that has been achieved with survival of human fetal nigral and striatal tissues in the rat brain with only cyclosporin immunosuppression.

The identification of a suitable source of xenografts for neural transplantation in man depends on similar principles as relate to other organs. A variety of factors converge on promoting porcine donors as the most likely candidates in the first instance, not least because of the long-standing domestic relationships between pigs and man in agriculture, the similar size and development, the ease of breeding and production of large numbers of embryos per litter, and an optimal balance between immunological similarities but sufficient differences to alleviate safety concerns about transfer of infections between species.

Pigs have now been used as a source of neural transplantation into the striatum of both rats and primates for both nigral neurones in animal models of PD and of striatal neurones in models of HD. For example, in our own studies, we have grafted porcine nigra and striatum into rats and observed good survival of approximately 50% of grafts in each group (Fig. 4). Although these studies are still in progress, there are clear differences in graft growth and morphology between grafts derived from different donor ages (Watts C., unpublished data).

Nevertheless, in both our own studies and those of others, the rates of survival of xenografts (typically 50–70% of grafts) are typically poorer than the close to 100% survival observed with allografts in the brain, at least in rats, even without immunosuppression. Although the first clinical trials of porcine xenotransplantation with cyclosporin immunosuppression have already been undertaken in both PD and HD patients, it is not clear that the immunological problems are yet resolved or that the rates of graft survival are sufficiently close to that obtainable by human fetal allotransplantation to yet offer an acceptable alternative.
Key points for clinical practice

All the procedures discussed in this chapter are still experimental rather than representing clinical therapies. There is now clear evidence that embryonic neuronal allografts can alleviate deficits in PD, and that similar procedures may well be applicable in a range of other neurological diseases including HD. Nevertheless, the use of fetal tissues for cell transplantation remains ethically controversial and practically difficult. Consequently, although a limited number of cases may be treated this way, these trials should be taken as a proof of concept, pending development of suitable alternative sources of cells that can render the techniques more widely available. Several strategies are under active investigation, but none are yet of demonstrated viability.

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