The cellular repair of the brain in Parkinson’s disease—past, present and future

Mark Sayles², Meena Jain³, Roger A. Barker a,b,*
²Cambridge Centre for Brain Repair, University of Cambridge, Forvie Site, Robinson Way, Cambridge, CB2 2PY, UK
³Department of Neurology, Addenbrooke’s Hospital, Hills Road, Cambridge, CB2 2QQ, UK

Abstract

Damage to the central nervous system was once considered irreparable. However, there is now growing optimism that neural transplant therapies may one day enable complete circuit reconstruction and thus functional benefit for patients with neurodegenerative conditions such as Parkinson’s disease (PD), and perhaps even those with more widespread damage such as stroke patients. Indeed, since the late 1980s hundreds of patients with Parkinson’s disease have received allografts of dopamine-rich embryonic human neural tissue. The grafted tissue has been shown to survive and ameliorate many of the symptoms of the disease, both in the clinical setting and in animal models of the disease. However, practical problems associated with tissue procurement and storage, and ethical concerns over using aborted human fetal tissue have fuelled a search for alternative sources of suitable material for grafting. In particular, stem cells and xenogeneic embryonic dopamine-rich neural tissue are being explored, both of which bring their own practical and ethical dilemmas. Here we review the progress made in neural transplantation, both in the laboratory and in the clinic with particular attention to the development of stem cell and xenogeneic tissue based therapy.

Keywords: Transplantation; Parkinson’s disease; Dopamine; Xenografts; Stem cells

1. The origins of neural transplantation

In the early 1970s, neural transplantation re-emerged after studies began to demonstrate clear principles necc-
essential for reliable and reproducible neural grafting to be achieved [4,5]. The suggestion of a potential use of the technique as a therapy for Parkinson’s disease, a common neurodegenerative disorder characterised by the degeneration of the dopaminergic neurons of the substantia nigra, only fuelled this enthusiasm. The main clinical features of PD are bradykinesia, tremor, rigidity and postural instability, although significant other deficits are seen in most patients including disturbances of mood, cognition and autonomic dysfunction reflecting the diffuse pathology of advanced PD. A wide range of pharmacological therapies exists for PD, which are especially effective in the early stages and include levodopa, dopamine agonists and MAO-B inhibitors. Levodopa is the most effective drug therapy and is ultimately used in most patients but is associated with problematic side effects such as motor fluctuations with long-term use. Surgical ablative and stimulating therapies, whilst being effective for these aspects of the condition in advanced PD, do not attempt to cure the patient. Neural transplantation however, aimed to do just this, by replacing the missing dopamine neurons and in so doing effectively cure the patient of their condition, or at least a substantial part of it [6].

2. Neural transplantation in Parkinson’s disease

2.1. Preclinical studies

The over-riding and main aim of neural transplantation in PD has been to simply provide dopaminergic neurons to replace the core loss of the dopamine-releasing nigrostriatal neurons that characterises this condition. This can either be achieved by direct dopamine release as with transplantation of dopamine-releasing tissue (e.g. carotid body, adrenal medulla), through synaptic dopamine replacement by the transplantation of embryonic dopamine neurons, or ideally by reconstructing damaged circuitry, possibly employing the use of stem cells or xenogenic tissue which may have a greater potential for more widespread fibre outgrowth. However, in the first instance we will concentrate on the use of allografts of embryonic nigral tissue. Two different groups simultaneously reported successful transplantation of neural tissue into the rat 6-OHDA model of PD in 1979. Perlow et al. placed grafts of embryonic ventral mesencephalon (VM) into the lateral ventricle [7], whilst Björklund and Stenevi placed the tissue in a dorsal cortical cavity [8]. Both groups described functional recovery on simple motor tasks, along with graft survival and dopaminergic fibre outgrowth on histological analysis [9]. The advent of cell suspension grafts however, allowed more flexible placing of grafts within the brain, and it was subsequently shown that functional effect was dependent on the age of embryo from which the tissue was harvested (reviewed in Ref. [10]), and the site of implantation within the striatum [11,12]. Grafts placed into the substantia nigra, the site of degenerating dopaminergic neuronal cell bodies, failed to extend axons to the striatum and so deliver functional benefit [12]. The ectopic placement of grafts within the striatum (Fig. 1) lead to the amelioration of a wide range of symptoms, for example, an embryonic VM dopaminergic graft into the dorsal striatum will ameliorate drug-induced rotation behaviour [13], whereas a more laterally placed graft in the striatum will alleviate sensory neglect, but not the rotation behaviour [14], and thus it follows that multiple grafts would yield an additive pattern of recovery (discussed in Ref. [10]). The possible mechanisms by which such cells may exert their effects are outlined in Table 1.

Transplanted embryonic nigral neurons are capable of survival and integration within the host brain, and also display many characteristics of intact nigrostriatal neurons such as the synthesis and release of dopamine, as well as showing similar firing rates. Thus, these early studies have demonstrated that nigral grafts are capable
of survival and integration within the host brain. However, with rat allografts, only approximately 1000–2500 TH-immunopositive dopamine neurons survive [10] and yet the intact rat VM contains approximately 50 000 dopamine neurons [16]. It follows then that only 2–5% of implanted cells actually survive the transplantation process, and thus an important topic of research concentrated on the optimisation of graft survival by improving the efficiency of tissue preparation [17,18]. A variety of different approaches have been investigated and those which seem to be the most effective are antioxidants, e.g. lazaroids [19,20]; caspase inhibitors [21–23]; calcium channel blockers [24], and neurotrophic factors, especially GDNF which also promotes fibre outgrowth of nigral grafts [25–27].

Despite these difficulties, the experimental studies in rodents have led to a series of non-human primate studies that have reproduced the positive findings [28–31], thus providing the rationale for grafting of this tissue in patients with PD.

### 2.2. Clinical studies

Initial clinical studies of transplantation in PD employed adrenal medulla (AM) tissue as the donor tissue. The rationale behind this was that this tissue can be autologously transplanted and is rich in catecholamines, including some dopamine. Studies in animal models of PD during the early 1980s had shown that AM grafts are able to survive transplantation, and that the number of surviving TH-positive cells resulting from transplantation appeared to relate to functional recovery. However, there was a large degree of variability between studies, which at best only provided a partial restoration of function. Furthermore, the mode of action of such transplants was not entirely clear and there were anxieties about how effective such a therapy would be in PD, especially given that the adrenal medulla appears to be affected by the primary disease process. Nevertheless, based on these experimental data, the first clinical trials were undertaken in patients with PD in the early 1980s in Sweden with only minimal benefit [32,33]. However, Madrazo et al., using an open neurosurgical approach into the caudate nucleus, reported dramatic improvement in motor and cognitive function [34,35], although there were some concerns regarding the interpretation of the data [36]. Nevertheless, many of these transplants were undertaken, and in general, patients were shown to have a mean increase in the percentage of time spent in the ‘on’ phase of L-dopa treatment from 47.6 to 75%, although the improvement was not permanent, usually disappearing by 18 months [37,38]. Despite these apparent benefits, the peri-operative morbidity was found to be high, due to the undertaking of an open neurosurgical procedure in combination with an abdominal operation to obtain the AM [39]. Thus, it was felt that the high level of mortality and morbidity associated with this procedure outweighed the modest and transient improvements that the AM graft offered the patient, and therefore it was no longer justified, especially given that post-mortem studies have shown minimal or no survival of the grafted AM tissue.

However, the emergence of robust experimental data on embryonic ventral mesencephalic (VM) tissue transplantation, leads to the use of this source of tissue in clinical trials, first carried out in Mexico and Sweden [40,41]. These patients showed little clinical benefit in these initial trials, but following refinement of the techniques better results were obtained. Indeed, now over 300 patients have been transplanted worldwide, and published studies have shown that grafted dopaminergic neurons derived from the embryonic VM can survive in the human parkinsonian brain and reinervate part of the host striatum, provided the trials are performed using well-validated procedural and assessment protocols such as the core assessment protocol for intracerebral transplantation (CAPIT) [42–47]. More recently, long-term graft survival has been shown despite progressive degeneration of the patient’s own dopamine neurons consistent with the normal progression of the

### Table 1

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Effect</th>
<th>Behavioural effect in animals</th>
<th>Clinical correlate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-regulated dopamine release</td>
<td>Normalisation of dopamine supersensitivity</td>
<td>Reversal of drug induced rotation</td>
<td>Partial symptomatic relief</td>
</tr>
<tr>
<td>Synaptic DA release</td>
<td>Physiologically regulated DA receptor activation</td>
<td>Improved initiation of movement</td>
<td>More pronounced improvement of mobility</td>
</tr>
<tr>
<td>Regulated DA neuron function</td>
<td>Reconstruction of both afferent and efferent connections</td>
<td>Improved skilled limb use</td>
<td>Recovery of cortical activation, improved execution of complex motor programs</td>
</tr>
</tbody>
</table>

M. Sayles et al. / Transplant Immunology 12 (2004) 321–342
disease [45]. Clinically, numerous open-label trials have also shown that grafts can give rise to long lasting relief of symptoms as measured using the UPDRS, such as increases in the percentage of time in the ‘on’ phase with a concomitant reduction in the percentage of time spent in the ‘off’ phase as well as improvements in ‘off’ phase rigidity, hypokinesia and dyskinesia [48]. However, not all of the symptoms of PD are responsive to this type of treatment, with tremor and gait being particularly resistant. However, these early trials were all open label with no placebo arm, and whilst many would argue that to include such a group would be unethical at this stage in the development of the technique, others have suggested that a double-blind placebo controlled trial is in fact necessary in order to support the efficacy of the procedure [49].

With this in mind, the first double-blind placebo controlled trial of transplantation in PD was published in 2001 [50]. This study showed a 28% improvement in the UPDRS total ‘off’ score, with the UPDRS motor ‘off’ component improving 34% in the younger group of patients (<60 years) at 1 year, although more recent evidence suggests that this is related more to L-dopa responsiveness prior to transplantation, rather than an effect of age per se [51]. There were no changes in the placebo group. By 3 years after transplantation, the total ‘off’ scores for the younger group showed further improvement (38%). The older patients did not improve as a group, although individuals did. Overall, the results seemed quite disappointing, but a comparison of the data with those from other published transplantation studies showed that the UPDRS motor scores improved to a similar extent in all studies, at least up to the first year [52]. Thus the results were more promising than initially thought, although much of the negative publicity that this trial attracted related to the fact that 15% of patients developed severe dyskinesias during ‘off’ phases, which were interpreted as ‘a relative excess of dopamine’ derived from ‘too many’ grafted neurons. However, this interpretation has been refuted, especially given the published post-mortem data with this study, which may also reflect the major methodological differences of this trial, including the mode of cell storage and preparation, the surgical procedures, and the lack of immunosuppression [53,54]. In addition, retrospective analysis of 14 patients who were followed up for up to 11 years after grafting in the Swedish cohort, found that dyskinesias increased during post-operative ‘off’ phases but were generally of mild to moderate severity. They were not related to the magnitude of graft-derived dopaminergic re-innervation as judged by \(^{18}\text{F}\)-dopa PET, and the increase did not correlate with ‘off’ phase global dyskinesia rating scale scores indicating that ‘off’ phase dyskinesias probably did not result from excessive growth of grafted dopaminergic neurons, but were more likely related to large dopamine asymmetries across the grafted putamen [55,56]. However, the exact mechanisms for ‘off’ phase dyskinesias must be understood in order to prevent this side-effect post-transplantation in future: a point which has been emphasised by the results of the most recent double-blind transplant trial in PD [54,57]. In this further trial, 34 patients with advanced PD were randomised into either a surgical placebo control group (11 patients) or groups that received bilateral transplants (23 patients) of either one or four donors per side using well-validated tissue preparation techniques. Limited benefit of the procedure on clinical outcomes was seen despite good graft survival, as demonstrated by PET scanning and post-mortem examination. Dyskinesias were again present in a significant number of the transplanted group, which the authors of this study suggest may be due to poorly functioning transplants as a result of their inactivation by immune rejection mechanisms. However, the selection of patient groups (that is, those with very advanced PD) may have had a critical influence on the outcome, and thus the procedure may be more suitable for particular subgroups of PD patients as is suggested in this study when mild PD cases (UPDRS ≤49) are compared to the more advanced cases (UPDRS ≥49).

Therefore, it would appear that neural transplantation is an effective therapy for certain aspects of PD, although it is not without side effects. However, irrespective of this, the widespread adoption of this technique is limited by the practical and ethical problems associated with tissue procurement. Firstly, there are strong ethical dilemmas associated with the use of tissue derived from human embryonic material, particularly given that it is acquired from elective termination of pregnancies (TOP). In addition, 3–5 fetuses per side per patient are required in order to achieve survival of adequate numbers of dopamine neurons (approx. 100 000) and these must be harvested at the optimal age of 6–8 weeks post-conception. Attempts to decrease the number by increasing the yield of dopaminergic cells obtained by the addition of compounds such as lazaroids have been evaluated, but still the number of fetuses required is high (2–3 per side) [58,59]. Tissue should ideally be implanted within hours of being harvested, although some methods for relatively short-term storage (days) have been evaluated [60]. Therefore, at the moment, the best results rely on the simultaneous collection of multiple donor embryos, all within the correct developmental age window, which logistically is very difficult to achieve. Further issues that also need to be taken into account include the accuracy of dissection. The tissue obtained from routine TOP, even using the low-pressure suction technique [61] is typically fragmented, making accurate dissection very difficult and thus the chances of transplanting non-neural tissue a real possibility. Furthermore, there are issues regarding the sterility and infective potential of the tissue, even
Table 2
Alternatives to primary fetal neuron transplantation

<table>
<thead>
<tr>
<th>Molecular replacement</th>
<th>Neuronal replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral neurons/ganglia</td>
<td>Peripheral neurons/ganglia</td>
</tr>
<tr>
<td>Secretory cell lines</td>
<td>Neural precursor cells</td>
</tr>
<tr>
<td>Encapsulated cell lines</td>
<td>Immortalised cell lines</td>
</tr>
<tr>
<td>Genetically engineered cells</td>
<td>Xenografts</td>
</tr>
<tr>
<td>Genetically modified cell lines</td>
<td></td>
</tr>
<tr>
<td>In vivo gene transfer</td>
<td></td>
</tr>
<tr>
<td>Slow release polymers/matrices</td>
<td></td>
</tr>
</tbody>
</table>

though it is screened for bacterial and viral infections prior to transplantation [62,63]. Thus, the reliance on human fetal donor tissue for the widespread adoption of clinical transplantation programmes in PD is hard to envisage, which has led to the search for an alternative tissue supply, which bypasses many of these obstacles.

3. Alternative cell sources

Alternative sources of cells for PD broadly divide into those that provide molecular replacement of dopamine (e.g. secretory cell lines, encapsulated cell lines, slow release polymers and genetically modified cells), and those that aim to replace dopaminergic neurons (and as such attempt to reconstruct the damaged circuitry), such as neural stem cells and xenogeneic tissue [6]. In the next sections, we will focus on those cell sources, which aim to replace dopamine neurons only, in particular, stem cells and xenografts. Other cell sources are summarised in Tables 2 and 3.

Table 3
Other sources of cells entering clinical transplantation trials in PD patients

<table>
<thead>
<tr>
<th>Other cell sources</th>
<th>Physiological role</th>
<th>Pre-clinical trials</th>
<th>Clinical TRIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal pigment epithelium</td>
<td>Dopamine-producing support cells found in the inner layer of the retina</td>
<td>Long term survival in animal models of PD and amelioration of motor symptoms [64,65]</td>
<td>Phase I clinical trials under way with the cells attached to cross-linked gelatin microcarriers (Spheramine® Titan Pharmaceuticals Inc.). At 12 months there was 48% improvement in UPDRS score in the ‘off’ phase [66]</td>
</tr>
<tr>
<td>Carotid body cells</td>
<td>Physiological arterial sensors. Release dopamine in response to low oxygen tension.</td>
<td>Transplantation into animal models of PD has resulted in amelioration of motor symptoms but the mechanism behind the functional recovery remains unknown [67,68].</td>
<td>An attractive source of cells as they could be used in autologous transplantation paradigms, therefore eliminating the need for immunosuppression [69].</td>
</tr>
</tbody>
</table>

3.1. Stem cells

The existence of stem cells was originally defined in the haematological system [70,71], but the last decade has seen the discovery of cells with stem-cell properties (namely, self-renewal and multipotentiality [72]), from many other tissues including the brain. These key properties have therefore made such cells very attractive alternative cell sources for neural transplantation. However, in order for these cells to be useful in such a context, they must fulfil certain criteria as outlined in Table 4.

3.1.1. Fetal-derived neural stem cells

In a series of studies, Svendsen et al. transplanted human fetal expanded neural precursor cells (ENPs) into the striatum of rats lesioned with 6-OHDA and examined both the phenotypic differentiation using some human-specific markers and the ability of the cells to ameliorate asymmetry in amphetamine-induced rotation.
tests ([73–75]; reviewed in Ref. [76]). These studies are difficult to compare directly due to the different ways in which the ENPs were isolated, grown and transplanted, but some useful general points can be discerned: (1) ENPs survive implantation and differentiate into neurons and glia, with astrocytes being the most common cell type to emerge, but with the proportion of neurons being enhanced when FGF-2 is used as the mitogen. (2) Functionally relevant dopaminergic neurons (as assessed by immunohistochemistry for TH) emerge variably and in small numbers; moreover expression of TH, and inference dopamine synthesis by these cells may be transient. (3) In a few animals these neurons may be sufficient to cause a partial reduction in amphetamine-induced rotation, but this is inconsistent, and the magnitude of the effect is small and does not approach that obtained with primary grafts. The generation of some TH-positive neurones in the above studies encourages the belief that ENP populations are competent to generate dopaminergic cells, an assertion that is supported by observations that larger numbers of TH-positive cells are seen when transplanted ENPs differentiate in the olfactory bulb—a neurogenic region of the adult brain where TH-neurones normally reside [77,78]. However, it is also clear that the extent of dopaminergic differentiation is very small and must be increased in a controllable fashion for these cells to be of clinical value in PD. The cited studies all transplanted undifferentiated stem cell populations directly into the striatum and it is known from work in vitro that precursor cells isolated from the VM quickly lose the ability to spontaneously differentiate into dopaminergic cells, and thus extrinsic signals are required to induce a dopaminergic fate [79–82] and the adult striatum does not provide these signals [83]. Thus ‘pre-differentiation’ of the ENPs prior to implantation would seem logical.

The potential of this approach has been suggested by the work of Studer et al., who expanded precursors from the rat VM with FGF-2 for 8 days and then allowed them to differentiate in roller-tube cultures with serum-containing medium for several days prior to implantation [84]. The yield of TH-positive neurons was greater than that reported in studies where no pre-differentiation step was employed, and was sufficient to produce a partial amelioration of drug-induced rotation, although it should be borne in mind that the degree of expansion was small. It is further uncertain whether this method would be effective following longer expansion periods as the addition of serum to human fetal telencephalon-derived EGF and FGF-2 expanded ENPs prior to grafting in the same model did not result in any TH-positive cells differentiating [85].

An alternative approach that has been employed in an attempt to increase dopamine release from ENP derived cells has been to employ ex vivo genetic techniques to modify cells prior to implantation to express TH [86,87]. Ex vivo techniques are preferable to direct in vivo gene transfer as they allow for the exact insertion site of genes to be regulated, and thus protein delivery rates can be established prior to patient use. Corti et al. infected human FGF-2 expanded fetal precursors with an adenovirus vector encoding human TH under the negative control of a tetracycline-based gene regulatory system. On transplantation into the rat striatum, regulatable TH expression was demonstrated. Whether such neurons produce a functional benefit akin to that induced by true nigral dopaminergic neurons remains to be assessed along with clinical issues of safety and problems with downregulation of the transgene after long-term transplantation needs to be addressed.

3.1.2. Embryonic stem cells

Embryonic stem cells (ES cells) are derived from the inner cell mass of the embryonic blastula. They are pluripotent and so have the capability to produce derivatives of all three primary germ layers, that is, the endoderm, mesoderm and ectoderm. To date, ES cells have been isolated from mice [88,89], primates [90–92] and humans [93–97], although much of the work has focussed on mouse-derived cells. The production of neurons positive for tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, and thus a good marker for dopaminergic neurons, was first demonstrated in vitro from mouse ES cells by Lee et al. [89]. This has now been extended to in vivo studies in the 6-OHDA model of PD in which transplanted murine ES cells in low numbers gave rise to dopaminergic neurons which in turn produce a gradual and sustained decrease in amphetamine-induced rotation from 5 to 9 weeks after grafting [98]. This behavioural recovery was accompanied by PET and functional magnetic resonance imaging (fMRI) scans that suggested dopamine-mediated hemodynamic changes in the striatum and associated brain circuitry (Fig. 2). This was therefore the first convincing demonstration that ES cells could differentiate into mature and functional dopamine neurons that were able to integrate appropriately within the circuitry of the basal ganglia to give stable functional recovery. However, despite the small numbers of cells injected, 20% of rats still developed teratomas at the transplant site within 9 weeks of implantation. Thus, strategies to eliminate all proliferating and non-neuronal cells from the grafts must be developed before this technique can be considered safe for use clinically. Around the same time, a study was published in which ES cells were transfected with Nurr1 (a transcription factor involved in the differentiation of dopaminergic cells), to create a stable ES cell line [99]. Cells were then differentiated into dopamine-producing cells by a multi-step process and grafted into 6-OHDA lesioned rats. Tyrosine hydroxylase-positive cells from the grafts showed complex
Fig. 2. Sources of stem cells under consideration for grafting in PD.

morphologies, as well as electrophysiological and behavioural properties expected of neurons from the midbrain. In unilaterally lesioned animals, turning bias with amphetamine stimulation was ameliorated in the Nurrl-grafted animals, and remained so for up to 8 weeks following the graft. Significant improvement was also seen in other tests of motor deficits. This study provides further encouraging evidence for the use of ES cells for the treatment of PD, particularly as the cells in this study did not show any immunoreactivity to Ki-67 (a protein expressed throughout the division cycle of a cell), and no teratomas were observed in contrast to the study by Björklund et al. described above. However, the long-term effects of these grafts must be established, in particular with respect to tumour formation.

Advances in technology has led to the isolation of ES cells from human embryos (HES cells), either by using embryos at the blastocyst stage, donated by individuals undergoing fertility treatment or fetal gonadal tissue obtained after elective termination of pregnancy [95,100]. These human-derived cells require much characterisation, for example, in terms of tissues derived on differentiation, expansion rates, and confirmation of pluripotency by clonal analysis before they can be useful in transplantation paradigms [101].

Other ES-like cells within human bone marrow mesenchymal stem cell cultures, known as multipotent adult progenitor cells (MAPC), hold great potential, as at the single cell level, these cells can differentiate and express markers of the mesodermal, endodermal and neuroectodermal lineages in vitro [102]. These cells have caused great excitement due to them possibly having the same developmental potential as ES cells along with the practical and ethical advantage that they can be isolated from the patients themselves. So far the expansion in FGF for 14 days of MAPCs leads to them expressing neurofilament-200, a neuronal marker, glial fibrillary acidic protein (GFAP), an astrocytic marker, and galac-
tocerebroside (GalC), an oligodendrocyte marker. Following treatment with basic fibroblast growth factor (bFGF), FGF8 and brain-derived neurotrophic factor (BDNF), 30% of cells expressed markers of dopamine containing cells [dopa decarboxylase (DDC) and TH], 20% expressed markers of serotonergic neurons and 50% were GABAergic. However, much more work needs to be done with these cells before they can be considered clinically for PD.

### 3.1.3. Stem cells derived from adult tissues

#### 3.1.3.1. Adult neural stem cells

For many years it was believed that neurogenesis in the adult mammalian central nervous system was not possible. However, work using tritiated thymidine labelling showing the presence of active DNA synthesis in cells with neuronal morphology, provided the first evidence against this long held dogma [103]. The lack of cell type specific markers at that time made it difficult to conclude unequivocally that these cells were neurons. It was not until recently, using bromodeoxyuridine (BrdU) to label proliferating cells and their progeny that the presence of active neurogenesis in certain brain regions of several mammalian species such as rodents (Fig. 3) and non-human primates has been confirmed. These neurons are thought to derive from a population of neural precursor cells (NPCs), and it has previously been shown that NPCs taken from the adult brain can be propagated in vitro and do indeed show these characteristics [104]. The primary areas of the brain in which this phenomenon is found include the subependymal layer of the ventricular zone [105–107] which gives rise to neurons of the olfactory bulb [108] and the subgranular zone of the dentate gyrus of the hippocampus [109–111], although recent evidence has emerged suggesting that the dentate gyrus in fact contains only restricted progenitors and not true neural stem cells [112]. Further sources of multipotent and self-renewing cells have been discovered from regions previously thought to be non-neurogenic, that is, the adult rat spinal cord, the primate neocortex, and even the substantia nigra (SN) [113–115].

The existence of neurogenesis in the adult human hippocampus has been confirmed [116], and progenitor cells from this area of the brain have been isolated [117]. Cultures derived from postnatal human post-mortem brains have been established and shown to yield viable progenitor cells, with the longest interval between death and harvesting being approximately 20 h [118]. Thus, this promises to provide yet another source of cells for cell replacement therapy in which autologous graft paradigms could be envisaged, as it bypasses the need for embryonic tissue, assuming of course that the cells themselves are not affected by the disease process [119].

At the current time, there is limited pre-clinical evidence to support the effectiveness of this technique in animal models of PD. Nevertheless, autologous transplantation of adult neural stem cells has been attempted clinically in at least one patient with PD. In this case, a cerebral cortical biopsy was performed, and the patient’s neural stem cells were isolated and expanded in vitro for several months using ‘epigenetic factors’ which resulted in 15% of the neurons being dopaminergic [120]. Cells were transplanted unilaterally into the putamen, resulting in an 88% improvement in motor ‘off’ phase at 12 months and a 55.6% increase in dopamine uptake in the left putamen using 18F-dopa PET. Whilst the results presented were encouraging, clearly further work needs to be done, and in particular, a better description of this case in the form of a peer-reviewed published account is needed.

#### 3.1.3.2. Adult-derived stem cells from other tissues – transdifferentiation

It has for a long time been thought that tissue-specific stem cells could only differentiate into cells of the tissue of origin, but there have been several papers in recent years describing the plasticity of stem cells in different experimental situations [121]. Murine NPCs have shown conversion in vivo into haemopoietic cells [122], although this has recently been questioned and is thought to be due to transformation of NPCs by excessive passaging in vitro [123], as well as into skeletal muscle cells in vitro and in vivo under defined conditions [124]. In addition, murine adult NPCs have been shown to contribute to all three germ layers of chimeric chick-mouse embryos [125], although the exact identity of the cells in these different layers was not determined. Whether these apparent transformations observed in mice translate to humans is yet to be discovered. Such studies suggest that stem cells may
in fact have similar phenotypic potential irrespective of where they reside, an assertion supported by similarities in the cDNA microarray analyses of haematopoietic and neural stem cells [126–128]. Therefore it is theoretically possible that stem cells derived from other systems, such as the skin and bone marrow may be used for neural cell therapy. The bone marrow contains a host of different stem cells, including pluripotent cells that are able to give rise to cells of the haematopoietic lineage. Transplantation of bone marrow cells into irradiated recipients has been shown to be an effective way of repopulating blood cells both in animal models and in patients as a therapy for lympho-proliferative disorders. When transplanted into irradiated recipients, rodent bone marrow cells have been shown to migrate into the brain, and differentiate into microglia and astrocytes although no neuronal differentiation has been seen [129]. However, two independent groups have now shown that bone marrow cells, when taken from adult mice and injected into normal neonatal or adult mice that had previously been irradiated, migrate into the brain and express neuronal-specific proteins including 200-kD neurofilament and β-III-tubulin [130,131]. Although the number of bone marrow derived cells was small (0.2% of the total neurons) [130], this still provides proof of concept that cells derived from the bone marrow can express neuron-specific genes and assume a neuronal phenotype in the CNS, although replication of the results has proven difficult and questions about cell fusion as opposed to transdifferentiation have arisen.

In addition to these haematopoietic precursors, bone marrow also appears to contain a population of non-haematopoietic stem cells, which are referred to as mesenchymal stem cells or bone marrow stromal cells (BMSCs), because they arise from stromal structures of the marrow [132]. Human BMSCs from adult bone marrow, engrafted into the rodent brain are capable of migration and survival without inducing a vigorous immune reaction by the host [133]. Following grafting, these cells lost markers typical of marrow stromal cells in culture such as immunoreactivity to antibodies against collagen and fibronectin, and developed many of the characteristics of astrocytes [133]. Recently, these cells have been reported to express markers of astrocytes, oligodendroglia and neurons in vitro [134], and when transplanted into a rodent model of PD, following transfection with the human TH in order to produce dopamine, were able to produce significant functional recovery [135].

3.2. Xenogeneic tissue

A different approach to stem cells for repairing the CNS is the use of embryonic neural tissue from another species (xenografts). Historically, xenografts were the first neural transplants to be undertaken, but in the modern era experimental work on neural xenotransplantation began as early as the 1980s when neural cell replacement therapies were in their infancy [136,137]. Embryonic pig neural tissue (at stage E26–27) is held to be the most suitable material for transplantation into humans, since pig brains are of a similar size to ours and porcine tissue is preferred to that of non-human primates because they have large litters and are amenable to genetic modification, making them attractive sources of whole organ grafts as well as neural cell grafts. There has recently been a resurgence in interest surrounding porcine neural xenografts after it was shown that xenografted tissue appears to extend axonal and dendritic outgrowths over larger distances than equivalent allografted tissue [138–141]. There is also evidence to suggest that xenografted cells migrate much more than allografted cells in the host CNS and may even migrate preferentially to regions of pathology [142]. Despite these recent encouraging findings there remains two key obstacles to the widespread clinical implementation of porcine neural xenografts, immune-mediated rejection, and the potential risk of infection with porcine endogenous retroviruses (PERVs).

The first serious attempts at experimental neural xenografting were performed by Björklund et al. [136] with the grafting of embryonic mouse ventral mesencephalon (VM) tissue into a cavity in the rat striatum. Grafts were seen to survive in 55% of cases and produced functional benefit in the absence of immunosuppression, although at 6 months post-grafting very few cells remained, with much of the graft mass being resorbed. The same group then demonstrated enhanced survival of embryonic mouse-to-rat neural xenografts treated with the immunosuppressive drug cyclosporin A (CsA) relative to non-immunosuppressed controls [137]. These early studies demonstrating unpredictable graft survival, which could be enhanced by immunosuppressive drug therapies, paved the way for investigations into the exact mechanisms of xenograft rejection in the immuno-competent host with a view to developing strategies to circumvent this immune reaction.

3.2.1. Rejection

Immune-mediated graft rejection is a major hurdle to overcome before large-scale clinical trials using pig-derived xenogeneic tissue can be entertained. It is clear from studies in rats that xenografted tissue placed in the relatively immune-privileged CNS is rapidly rejected over a period of days to weeks through a combination of cellular and humoral immune processes. Specifically, porcine neural xenograft rejection has been shown to fundamentally involve T-lymphocytes, although there is clear evidence in support of a role for antibodies and components of the complement cascade in the rejection process, e.g. [143,144]. However, the relative contribution of T-cells and humoral mediators to actual tissue
### Advantages and disadvantages of using porcine donor tissue for neural transplantation

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>The size and development of the porcine brain is similar to that seen in man</td>
<td>The risk of zoonotic infection, especially with PERVs</td>
</tr>
<tr>
<td>Easy to breed large titters</td>
<td>Immunological rejection</td>
</tr>
<tr>
<td>Can be transgenically modified</td>
<td>Different to cells originally lost in disease state</td>
</tr>
<tr>
<td>Long history of use for whole organ peripheral grafts</td>
<td></td>
</tr>
<tr>
<td>Xenotransplanted, over allografted, tissue may have the primary advantage of a greater potential for sending fibres into the host brain – possibly through the avoidance of species specific barriers to axonal growth</td>
<td></td>
</tr>
</tbody>
</table>

Loss is at present unknown. Experiments to unravel the extent to which each arm of the immune system is involved in xenograft rejection need to take into account differences in the pattern of rejection seen with concordant (between closely related species) and discordant (between two unrelated species) grafts.

#### 3.2.1.1. Mechanisms of rejection: cellular responses

The brain was once considered an ‘immunologically privileged site’ for transplantation [145,146]. However, it is now known that this privilege is not absolute since histoincompatible tissue grafted into the CNS is rejected by the host immune system albeit more slowly than if the tissue were placed peripherally [147]. It has been demonstrated that the speed and vigour of this rejection process is directly related to the phylogenetic distance between donor and host [148,149] with the key cellular mediators of graft rejection thought to be T cells (CD4+ and CD8+) and microglial cells [150].

The mammalian brain is devoid of any true lymphatic vessels and is isolated from the circulation by the blood–brain barrier (BBB). However, perivascular spaces along larger blood vessels are thought to allow lymphatic drainage of the brain. Arguably, this would allow graft-derived antigen access to the cerebral lymph nodes where it would be expected to activate naïve T-lymphocytes through indirect antigen presentation. Activated T-lymphocytes can cross the BBB to gain access to the brain parenchyma. Perivascular microglia acting as antigen-presenting cells (APCs) might process and present graft antigen to T-lymphocytes originally activated in lymphoid tissue for local maintenance of the immune response. The necessary disruption of the BBB at the time of grafting would clearly aid the recruitment of peripheral components of the immune system, both cellular and humoral, and once an immune response has been generated this will also serve to keep the BBB open. Much evidence implicates T-lymphocytes as the main culprits of cell loss from xenografted tissue, and indeed with nude athymic rats neural xenografts survive indefinitely, e.g. [142]. In vitro studies by Brevig et al. [151] demonstrated that human T cell (CD4+ and CD8+) proliferation is induced by porcine embryonic neurons when grown together in culture. This study also showed that pre-treatment of the porcine neurons with human serum reduced the proliferative response of human T cells, suggesting that such an approach might be useful clinically to improve graft survival, possibly by removing the more immunogenic cells. Antibodies against T-cells have also been employed to enhance the survival of intracerebral neural xenografts in rats [152,153]. Okura et al. [152] successfully used monoclonal antibodies (MAbs) generated against the αβ fragment of the rat T-cell receptor (anti-TCRαβ) and against the T-cell surface molecule CD2 (anti-CD2) to prolong the survival of mouse ventral mesencephalon tissue grafted into the rat lateral ventricle. MAbs were administered either alone or in combination on 3 consecutive days (days -2, -1 and 0 relative to the transplantation). The authors showed that transplantation of mouse VM with no immunosuppressive treatment results in complete graft rejection at 15 days post-transplantation. Administration of anti-CD2 alone gave no significant enhancement of graft survival, whereas in rats treated with the anti-TCRαβ MAb graft survival was seen until day 35 post-transplantation, with T cell infiltration being evident from day 15 onwards. Both antibodies used together prolonged graft survival until at least the longest time period examined in this study (20 weeks post-transplantation) with no evidence of T-cell infiltration at any time point in this group. Furthermore the authors showed that the induced tolerance is antigen-specific; a second graft from the same donor strain was well tolerated whereas a second graft from a different strain of donor mouse was rapidly rejected. In addition to the histological evidence showing graft survival in the 6-OHDA-lesioned rats treated with both MAbs, there was also significantly reduced drug-induced rotational behaviour, indicating that the grafted tissue produced a functional effect in the host. However,
encouraging as these results are, it must be borne in mind that concordant grafts were used in this study (mouse-to-rat). More recent work indicates that the situation is rather more complex for discordant grafts such as from pigs to humans, in which host antibodies and complement appear to contribute to rejection of the grafted cells. For example, Lena Larsson et al. [154] grafted embryonic mouse and rat VM tissue into the striatum of the phylogenetically distant guinea pig [155] to examine the mechanisms involved in the rejection of discordant neural xenografts. Results demonstrated that mouse-to-guinea pig grafts were rapidly rejected, with no TH-positive cells remaining at 2 weeks post-transplantation. Rat-to-guinea pig grafts, however, survived well, with a high density of TH-positive neurons. Indeed, there were no statistically significant differences in graft volume or TH immunoreactivity between the rat-to-rat allotransplant control group and the rat-to-guinea pig xenograft group. Therefore, although some evidence would appear to suggest discordant graft rejection proceeds by a mechanism distinct from that involved in concordant graft rejection, these results support the view that the situation is rather more complicated, with the immune reaction dependant on the exact species combination.

3.2.1.2. Mechanisms of rejection: humoral response. A so-called ‘hyperacute’ antibody-directed complement-mediated process results in the rejection of vascularised whole organ peripheral xenografts, (e.g. pig-to-human) in a matter of minutes [156]. Non-primate mammals and New-world primates produce large amounts of α-1,3-galactosyl terminated cell surface glycoproteins and glycolipids. In contrast, humans and other Old-world primates produce antibodies against these sugars [157–159] possibly due to their presence in bacteria colonising the gut in the first few years of life. These anti-Gal antibodies are thought to bind their target epitopes on the surface of donor endothelial cells, recruit complement and thereby initiate endothelial cell lysis in vascularised whole organ xenografts. Platelet aggregation and formation of micro-thrombi would quickly ensue, resulting in ischaemia and rejection of the grafted organ within minutes to hours [150].

Since cellular neural xenografts are not dependant on intact donor-derived vasculature for survival, it is traditionally thought that their rejection is entirely T-cell mediated [149]. However, there is evidence to suggest that this is not the whole story. Immunosuppression using cyclosporin A (CsA) in experimental animal models of neural xenotransplantation [160,161] and in human PD patients receiving porcine VM grafts [162] does not consistently prevent rejection, although it does appear to prolong graft survival before the donor cells eventually succumb to the host immune system. MAbs against the T-cell antigens CD4 and CD8 have been used to deplete recipient mice of either CD4+, CD8+ or both populations of T-lymphocytes in order to determine the relative involvement of these T-cell subtypes in the rejection of both concordant (rat-to-mouse) and discordant (human-to-mouse) transplants [153]. Indefinite survival of discordant xenografts was achieved with administration of anti-CD4 MAbs whilst depletion of CD8+ T-cells had no significant effect on graft survival. It was not possible to induce indefinite survival of discordant grafts using either anti-CD4 or anti-CD8, although CD4+ cell depletion prolonged graft survival for up to 60 days. Thus, it would appear that mechanisms other than the T-lymphocyte response are at least partially responsible for the rejection of discordant xenografts. Although the blood–brain barrier normally prevents immunoglobulins from the systemic circulation entering the CNS, at the time of surgery, the necessary disruption to this barrier may allow preformed antibody to enter the graft. At a later stage, antibodies may be generated in response to donor-derived antigen draining to the cervical lymph nodes, although quite how these would cross the BBB to enter the brain parenchyma is not clear.

The xenograft study by Larsson et al. using immunoglobulin-deficient recipient mice [144] also provides clear evidence for a role of antibody-directed complement-mediated rejection of xenografts; embryonic porcine VM cells survived better in immunoglobulin-depleted mice than in control mice. Work in our own laboratory further supported an antibody-directed complement-mediated process in the rejection of embryonic porcine VM tissue in 6-OHDA-lesioned rats [143]. Xenografts were seen to be rejected over a period of 35 days with the graft site becoming infiltrated with CD8+ cells and staining positive for IgM and complement component (C3) deposition. Transient depletion of complement components in recipient rats using cobra venom factor (CVF) prolonged graft survival, although it did not prevent rejection, by delaying the onset of the cellular immune response.

However, the view that the anti-pig antibodies target only the α-1,3-Gal epitope has been recently questioned. Sumitran et al. [163], have demonstrated in vitro very low levels of the α-1,3-Gal epitope on embryonic porcine VM tissue with much of this being restricted to endothelial cells and microglia. The same group went on to show that anti-Gal depleted and non-depleted human sera have an equal cytotoxic effect on porcine embryonic VM cells in the presence of complement. Furthermore, this group identified three novel non-α-1,3-Gal epitopes on VM cells reactive with IgM present in the human serum, and suggest that modification of discordant donor tissue may prove necessary if it is to survive in a host. This observation has now been validated and extended to apply to porcine neural stem cells [164]. Other groups have also identified non-anti-Gal human anti-pig antibodies in normal healthy serum
Although humans possess several anti-pig antibodies in addition to anti-Gal, the Gal-reactive antibodies are by far the most important [166].

Given that the α-1,3-Gal epitope is the major xenoreactive carbohydrate resulting in pig-to-primate hyperacute graft rejection [159] it would seem a logical step to develop transgenic pig lines deficient in this antigen. Two independent groups reported successful cloning of heterozygous α-galactosyltransferase knockout piglets in 2001 [167,168] and now, using a second-round knockout and cloning strategy, four healthy female α-galactosyltransferase double knockout piglets have been produced [169].

In addition to xenografted cells expressing antigenic surface molecules such as α-1,3-galactosyl residues they are made much more susceptible to antibody-directed complement-mediated attack through their lack of human-specific complement inhibitor expression. A range of transgenic pig lines expressing human complement inhibitors such as DAF and CD59; and α-1,2-fucosyltransferase (H transferase; which modifies the cell surface carbohydrate phenotype, resulting in reduced α-1,3-Gal expression and decreased antibody binding) have been bred and characterised [170–174], although in general the expression of these human complement regulatory proteins on embryonic neural tissue is low. Nevertheless, using tissue from such embryonic transgenic pigs does not appear to adversely affect their viability, as evidenced by dopaminergic neuron xenograft survival in rats immunosuppressed with CsA [175]. Very recently the same group led by Ole Isacson has shown survival, for up to 12 weeks post-transplantation, of transgenic porcine VM tissue, expressing either the human complement inhibitor CD59 or human α-1,2-fucosyltransferase (H transferase), in primates rendered parkinsonian by MPTP administration by MPTP [176]. The grafted tissue was given in combination with a novel immunosuppressive regime involving administration of murine monoclonal anti-C5 antibodies, designed to halt the complement cascade at C5, preventing formation of the membrane attack complex (C5b-9), and triple drug therapy with cyclosporine A, methylprednisolone and azathioprine. These results are clearly relevant to the pig-to-human xenograft situation given the significant complement mediated lysis of porcine neurons caused by human serum [163].

Thus, it is probable that combining transgenic porcine tissue designed to interfere with humoral mediators of xenograft rejection with inhibitors of the T-cell mediated rejection process will be required for successful xenotransplantation, but how exactly that will be achieved remains to be seen.

3.2.2. Clinical trials: porcine neural xenografts

Despite strong evidence suggesting that xenografts would, at present, be of little clinical benefit due to their rejection, small-scale clinical trials have been carried out in the USA in patients with idiopathic PD and Huntington’s disease (HD) [177,162].

Schumacher et al. [162] reported the results of a 1-year follow up assessment of 12 PD patients transplanted unilaterally with suspensions of E25–E28 porcine VM tissue into the striatum Table 5. These patients received either CsA monotherapy or porcine tissue treated with a monoclonal antibody against MHC-I. One patient involved in this study died from a pulmonary embolism 7.5 months after transplantation, and at post-mortem had only 638 surviving porcine-derived dopaminergic cells in the host striatum out of the 12 million transplanted. Despite the use of immuno-suppression with cyclosporin A (CsA) [178], lymphocyte infiltration was also seen in the graft region. This extremely low survival rate agrees with the 18F-dopa PET data from the same study, which failed to demonstrate any significant increase in 18F-dopa uptake on the transplanted side. Taken together, these two pieces of evidence cast doubt on the inference that the significant decrease in UPDRS scores reported by Schumacher et al. (average improvement 19%, P = 0.01) are due to dopamine release at newly formed synapses from surviving graft tissue; somewhat reminiscent of the mystery surrounding the origin of functional benefit from adrenal medulla grafts. A second clinical trial of embryonic porcine VM xenotransplantation for PD, which remains unpublished, was reported in a press-release in 2001 (genzyme press-release 16th March 2001). In total, 18 patients underwent surgery, 10 receiving embryonic porcine VM tissue and 8 sham surgery. At the 18-month follow-up assessment both groups showed similar modest (20–25% UPDRS) improvement; the effects of grafting thus being equal to the placebo effect. However, a full account of this study in a peer-reviewed journal is required before any further comment can be made.

These results serve to emphasise the need for further experimental work aimed at understanding the process of rejection of discordant xenografts (e.g. pig-to-human) with a view to increasing xenograft survival before such techniques for nervous system repair are taken out of the laboratory and into the clinic.

3.2.3. Risk of infection

The use of pigs as a source of donor tissue, not only for neural and islet cell therapies but also whole organ xenografts (e.g. liver, heart), has raised concerns over the possibility of infectious diseases crossing the species barrier. Animal endogenous retroviruses (e.g. porcine endogenous retrovirus – PERV) are proviral DNA sequences integrated into the host genome, with pig chromosomes harbouring at least 50 copies of PERV [179]. Unlike most bacterial and viral pathogens, endogenous retroviruses cannot be eliminated from potential donor animals by simple pathogen-free, closed breeding
regimes. Perhaps the most ominous argument against the introduction of large-scale clinical trials of porcine xenograft therapies rests on the now widely accepted conclusion that both HIV-1 and HIV-2 represent zoonotic infections [180]. The vigour with which such viruses mutate adds further concern that PERVs could potentially infect human cells in vivo and mutate or combine with other human-specific proviral sequences to create a lethal viral infection akin to HIV with the potential for human-to-human spread. Ironically, the development of immunosuppressive therapies to enable xenograft survival could potentially increase the host susceptibility to infection by graft-derived retroviruses, especially with respect to the complement cascade.

With a view to answering the questions surrounding xenografts and PERV infection, attempts have been made to establish the extent to which PERV shows human cell-specific tropism in culture. However, it must be borne in mind that cultured cells and a whole animal are very different environments and although PERV might infect human cells in culture the host immune system may prevent such infection in vivo, although in any successful xenotransplant situation a degree of immunosuppression will undoubtedly be necessary. Wilson et al. [181] examined the ability of PERV to infect human cells in culture. Mitogenic activation of porcine peripheral blood monocytes was shown to initiate production of infectious retrovirus, which was capable of infecting co-cultured human cell lines. Although this confirms that in principle at least the porcine virus can infect human cells, whether or not this would occur in vivo is a different matter.

An in vivo study by van der Laan et al. [182] provided the first direct evidence that porcine-derived tissue transplanted into an immunocompromised mouse can result in cross-species infection. Non-obese diabetic, severe combined immunodeficiency (NOD/SCID) mice received grafts of pig pancreatic islet cells and were shown to have become infected with PERV in several tissue compartments including the spleen, liver, kidney, pancreas and intestine. Interestingly, infection was only seen in those tissue compartments in which there was tissue chimaerism, which may indicate that cell–cell contact is important in transmission of PERV from donor to host cells. In addition, the mice were severely immunodeficient and even though they had become infected with PERV, none of them became ill or showed tissue pathology.

Crucially, there has been no report of any human becoming infected with PERV following exposure to porcine tissue. However, most studies of this nature have been retrospective with limited porcine tissue survival and as such provide no evidence for a risk of infection, which is rather different from evidence for no risk of infection. A recent large-scale retrospective study of 160 patients treated with living pig tissue up to 12 years previously concluded that there was no evidence for persistent PERV infection in any of the patients [183]. Patients were exposed to living pig tissue through either: (1) extra-corporeal perfusion through pig liver; kidney or spleen for various reasons; (2) pancreatic islet transplantation for diabetes mellitus; or (3) pig skin grafts for burns. Using PCR (for detection of PERV DNA), RT-PCR (for detection of PERV RNA, a marker for virions) and protein immunoblot antibody assays (for detection of exposure to PERV antigen) Paradis et al. [183] demonstrated that in samples of peripheral blood lymphocytes and serum there was no evidence for persistent PERV infection in any patient examined. A group of 30 Russian patients treated with extra-corporeal splenic perfusion (ECSP) tested positive for PERV DNA sequences, but this was shown to be due to the persistent presence of pig cells in the patients circulation, presumably being flushed from the pig spleen during treatment. Thus, in this particular group of 30 patients, long-term (up to 8.5 years) survival of xenogeneic tissue was observed without immunosuppression. Although the conclusions of Paradis et al. are based on a large number of patients it is worth considering that the only cells tested for infection were peripheral blood lymphocytes, which cannot be productively infected with PERV in vitro [184]. Indeed, it would appear from in vitro work that human epithelial cell lines are much more susceptible to PERV infection than are human B and T cells [185].

Therefore the evidence in support of concerns over PERV transmission remains equivocal. It is important to understand that the introduction of any new therapy must be subject to cost-benefit analysis and that the small but significant risk of infection with PERV may be acceptable if the predicted benefits of using porcine tissue are borne out. The UK Xenotransplantation Interim Regulatory Authority (UKXIRA) was set up in 1997 to advise the government on issues of safety and efficacy related to xenotransplantation and has been commissioned to report on the risk of disease transmission and the practicalities of transplanting pig organs. The most recent report on the legality and ethics of xenotransplantation has sparked controversy after it was reported in the UK press that the government had refused to publish the report because of concerns expressed therein over the possibility of the UK government being sued for breaching international law if a HIV-like virus was to result and spread across the globe [186].

Work to circumvent the potential problem of PERV transmission continues and may go some way toward allaying public concern. The generation of PERV knockout pigs may prove more difficult than originally thought, since multiple copies of PERV genomes are present in normal pig genomes [187]. Very recently antibodies against a PERV transmembrane envelope protein (p15E) were shown to neutralise PERV infectiv-
ity and might therefore be useful to create an antiretroviral vaccine [188]. With the development of novel methods of preventing or at least reducing the risk of PERV infection the potential benefits of xenografted over allografted tissue discussed below makes porcine neural xenografts increasingly attractive as a treatment for conditions such as PD.

3.2.4. Does using xenografted tissue offer a primary advantage to allografted tissue?

In both animal models of PD and in PD patients undergoing neural transplantation, grafted neurons (of ventral mesencephalic origin) are usually placed ectopically in the striatum of the recipient [6,10]. This is thought to impose an intrinsic limit to the potential benefit attainable from current transplantation regimes, since the original neural circuitry involving projections to and from the substantia nigra (SN) and striatum is not reconstructed. The ectopic graft placement and resulting incomplete repair of host neural circuitry is thought to partially account for the variable and imperfect alleviation of parkinsonian symptoms, and possibly some of the serious side effects seen in PD patients undergoing allotransplantation with human embryonic VM tissue [50,51]. It has become clear through experimental work that allografted tissue must be placed ectopically in the striatum since the inhibitory nature of the host white matter prevents long-distance circuit reconstruction by intranigral grafts, restricting axonal outgrowth to the graft mass itself, with no reinnervation of the striatal neuropil [11,12,189]. However, it has been argued that much more complete functional recovery with fewer side effects may result from placement of grafts in the SN and subsequent circuit reconstruction [190]. Xenografted tissue appears somewhat better at projecting long distances in host white matter, allowing xenografted neurons (e.g. pig-to-rat; human-to-rat) to extend axonal outgrowths over large distances to target sites specific for their region of origin [120,139,140,191–193]. Both pig-to-rat and human-to-rat intranigral primary ventral mesencephalic xenografts have shown remarkable ability to project axons along the nigrostriatal tract and reinnervate the host striatum, although the functional efficacy of these grafts is unknown [140,193].

There are two possible explanations for these differences in axonal outgrowth between allografted and xenografted neurons. It may be that pig and human neurons have a greater intrinsic growth capacity than rat neurons, arguably due to the longer gestational period of humans and pigs compared to that of rats, allowing genes needed for embryonic axonal growth to be expressed over a much longer time period in order to form long-distance synaptic connections in a much larger CNS [194]. However, there is preliminary evidence in favour of an alternative explanation; species differences in CNS growth inhibitory molecules and/or their receptors may render xenografted axons less responsive to host-specific inhibitory cues ([195]; C. Hurelbrink and R.A. Barker, unpublished observations). There is much interest in this latter possibility since it has sparked speculation that the use of xenogeneic tissue as opposed to allogeneic tissue in a clinical setting may prove advantageous in terms of circuit repair and, therefore long-term alleviation of symptoms for PD patients.

In vitro work by van den Pol and Spencer [195] demonstrated enhanced survival and axonal outgrowth from human (cortical) and rat (hippocampal and hypothalamic) neurons when co-cultured with heterospecific astrocytes (e.g. rat neurons with human astrocytes) rather than homospecific astrocytes (e.g. human neurons with human astrocytes). A recent in vivo study by Armstrong et al. [188] provided evidence that xenografted expanded neural precursor cells (ENPs) may be an even more flexible source of cells for use in neural transplant therapies where circuit reconstruction is required. Taking advantage of both the enhanced axonal outgrowth thought to be characteristic of xenografted neural tissue and the plastic nature of multipotential neural stem cells, this study aimed to examine the ability of porcine ENPs to differentiate into projection neurons and thereby reconstruct degenerate neural circuitry in the CsA-immunosuppressed 6-OHDA-lesioned rat model of PD. Embryonic (E26–27) porcine cortical neurons expanded in culture with FGF-2 and EGF were grafted into either the rat ventral mesencephalon or striatum. Although very few grafted ENPs differentiated into tyrosine-hydroxylase positive neurons in both graft locations and no functional recovery was seen, there was extensive axonal outgrowth across the graft–host interface with projections along host fibre tracts to targets appropriate for the graft site. Intramesencephalic ENPs projected to the mediodorsal thalamus, caudate-putamen, ventral striatum, and the amygdaloid nuclei along the internal capsule and the medial forebrain bundle. Similarly, those ENPs implanted in the striatum were shown to make local connections in the caudate-putamen and long-distance connections to the globus pallidus and substantia nigra. Crucially, staining for pig-specific synaptobrevin revealed synapse formation in the regions of grey matter in which ENP-derived axons were seen to ramify suggesting functional integration of grafted neurons with existing host circuitry. Interestingly, the pattern of striatal reinnervation was significantly different between the two graft sites. Intramesencephalic grafts resulted in an even distribution of re-innervation throughout the host striatum, whereas intrastriatal grafts were shown to reinnervate the dorsal striatum at a much higher density than the ventral striatum. Similar uneven
reinnervation has been suggested in the past as a possible cause of the rather severe dyskinetic side effects seen in several PD transplant patients receiving human embryonic tissue directly into the striatum [50,55,56]. The more even distribution of new synapses (with reduced risk of side effects) might have been seen as a further advantage of using xenogeneic tissue grafted into the SN in a clinical setting.

Although the results of Armstrong et al. show very few dopaminergic neurons developing from the grafted ENPs (of cortical origin) and no functional recovery on amphetamine-induced rotation behavioural testing, they do serve to demonstrate that a combination of increased axonal outgrowth, as a consequence of the xenograft paradigm, and possible future manipulations of neural stem cells to induce dopaminergic differentiation may provide the ideal cell source for repairing degenerating neural circuitry [120,139]. Similar experiments using porcine embryonic VM-derived ENPs has shown recently that these cells too have the capacity for long-range circuit reconstruction [196].

In addition to enhanced axonal outgrowth there is evidence to suggest that xenografted neurons have an increased propensity for migration within the adult host CNS compared to allografted cells [142]. Indeed, migration is a desirable property of transplanted neurones since the incomplete amelioration of symptoms seen with current neural replacement therapies for PD may be attributable to distant sites of pathology well outside of the primary graft region, for example, in the cortex and other basal ganglia and brainstem nuclei. If grafted cells possessed the ability to migrate to and fully integrate with these distant sites of pathology, symptomatic improvement would likely be considerably enhanced. Migration is a well-documented characteristic of ENPs [77,197] but has not been as extensively studied in primary neural graft-derived cells. Most studies examining cell migration from primary neural grafts have been in neonatal hosts, e.g. [198]. It is widely thought that the reduced capacity for neuronal migration seen when the same tissue is transplanted into an adult animal represents the absence of developmental guidance cues to which the grafted cells can respond. Some studies have reported much greater migratory capacity of graft-derived glial cells than neuronal cell types, e.g. [199].

Hurelbrink et al. grafted human fetal striatal tissue intrastratally in quinolinic acid-lesioned athymic rats in order to follow the migration of grafted cells through staining for human-specific cellular markers (HuNu, Tau, GFAP, Ki67) [142]. At 6 weeks, very few grafted cells had migrated away from the graft core, and most differentiated cells were of a neuronal phenotype. At 6 months, the graft core was much less dense, with many cells having migrated throughout the forebrain as far rostral as the olfactory bulb and caudal as the substantia nigra (Fig. 4). Graft-derived cells differentiated into both neurons and glial cells; something that would be expected from a tissue such as human fetal striatum containing neural precursor cells. Hurelbrink et al. offer two alternative explanations for the extensive migratory capacity of the transplanted human cells. It remains possible that the longer gestational period and larger brain size of humans relative to rats may enable the human-derived cells to respond to guidance cues for a more extended period. However, a rather more attractive proposition is that the xenotransplantation paradigm itself is responsible for the enhanced migratory capacity. An inability to respond to species-specific inhibitory cues may allow greater migration of xenografted cells than equivalent allografted cells and, therefore enhance integration of grafted neurons with host circuitry and enable migration of cells to sites of pathology well outside the graft mass itself. It remains to be seen whether this extensive migratory potential is a feature of all xenografted neural tissues, and whether it can target preferentially sites of pathology. However, the possible advantages of such a characteristic are obvious in terms of brain repair for patients with conditions such as PD.

Despite the issues of immune mediated rejection and the theoretical risk of infection with porcine endogenous retroviruses, xenogeneic neural tissues nevertheless remain an attractive source of material for use in brain repair surgery. Development of transgenic pigs in order to reduce antigenicity whilst limiting the risk of infection with PERVs may allow the exploitation of the unique properties of the xenograft situation discussed above to more completely reverse CNS circuit degeneration.

4. Conclusions

These are exciting times for the field of reparative neuroscience. Clinical trials using human embryonic nigral tissue have provided proof of the principle that neural cell replacement therapies can at least go some way toward curing PD. The challenge now is to find the most suitable material for transplantation that will allow widespread application of the technique and to then optimise the preparation of that tissue along with the surgical procedures in order to maximise the functional benefits and minimise the adverse side effects for the patient. The appearance of severe dyskinetic side effects in a significant number of transplant recipients in recent placebo controlled clinical trials [50,54] serves to emphasise the need to fully understand the mechanisms by which the grafted cells function within the host brain and to validate all techniques in the laboratory before transferring them to the clinic. This should be borne in mind for the many alternatives to human embryonic nigral tissue currently under consideration, of which stem cells and xenogeneic tissue hold particular
Fig. 4. Human fetal striatal tissue grafted in quinolinic acid-lesioned striatum of an athymic rat (Huntington’s disease model). The animal was killed 6 months post grafting and sections stained for human-specific nestin. Cells can be seen to have migrated throughout many brain regions. (a) Low power view of the graft mass. (b) High power view of the graft mass. (c) Higher power view of the edge of the graft, showing cells migrating from the graft mass. (d) Contralateral corpus callosum. (e) Ipsilateral corpus callosum. (f) High power view of the ipsilateral corpus callosum. (g) Ipsilateral frontal cortex. (h) Ipsilateral caudal striatum and thalamus. (i) Brainstem at the level of the substantia nigra. (Figure courtesy of Dr Carrie Hurelbrink).

promise but are both far from large-scale clinical trials. The transition of these alternative cell sources from laboratory to clinic should ideally be driven at a pace dictated by scientific understanding rather than personal or corporate aspiration. There is indeed a real danger of premature clinical trials with these tissues attracting negative publicity, which could significantly reduce support for these politically controversial therapies and ultimately have profound consequences for PD patients and their hope for a cure.
Acknowledgments

Dedicated to the memory of Dr. Anthony Vaughan Edwards of Fitzwilliams College, Cambridge.

References


Defer GL, Widner H, Marie RM, Remy P, Levivier M. Core


Freed CR, Patients transplanted with human embryonic dopamine neurons under double-blind design show stable clinical


Costa C, Zhao L, Burton WV, Rosas C, Bondioli KR, Williams BL, Hoagland TA, Dalmasso AP, Fodor WL. Transgenic pigs designed to express human CD59 and H-transferase to avoid humoral xenograft rejection.


