Cell Survival and Clinical Outcome Following Intrastriatal Transplantation in Parkinson Disease

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Abstract. Intrastriatal transplantation of embryonic dopaminergic neurons is currently explored as a restorative cell therapy for Parkinson disease (PD). Clinical results have varied, probably due to differences in transplantation methodology and patient selection. In this review, we assess clinical trials and autopsy findings in grafted PD patients and suggest that a minimum number of surviving dopaminergic neurons is required for a favorable outcome. Restoration of [18F]-fluorodopa uptake in the putamen to about 50% of the normal mean seems necessary for moderate to marked clinical benefit to occur. Some studies indicate that this may require mesencephalic tissue from 3–5 human embryos implanted into each hemisphere. The volume, density and pattern of fiber outgrowth and reinnervation, as well as functional integration and dopamine release, are postulated as additional important factors for an optimal clinical outcome. For neural transplantation to become a feasible therapeutic alternative in PD, graft survival must be increased and the need for multiple donors of human embryonic tissue substantially decreased or alternate sources of donor tissue developed. Donor cells derived from alternative sources should demonstrate features comparable to those associated with successful implantation of human embryonic tissue before clinical trials are considered.

Key Words: Autopsy; Dopamine; Neural transplantation; Parkinson disease; Positron emission tomography.

INTRODUCTION

Implantation of human embryonic dopaminergic neurons into the striatum of patients with Parkinson disease (PD) has been investigated for over a decade (1). The magnitude and, to some extent, pattern of recovery vary between patients (1). In the best cases, marked symptomatic relief with reduced “off”-phase (periods of poor drug response with increased PD-related disability) severity, reduced time spent in “off” periods, and decreased drug requirements have been achieved for up to a decade. In addition, long-term improved health-related quality of life and an ability to resume full-time work have been evident (1–7). These findings demonstrate that compromised brain circuits can be repaired and lost neurological functions restored in humans, and suggest that cell-based restorative therapies for PD and other neurological disorders are possible. More recently, xenogeneic donor tissue, derived from porcine embryos, has also been used for neural grafts in PD patients (8). Recent discoveries related to human stem cells, for example, have further widened the horizon within this field (9). Despite the currently rapid scientific development, the mechanisms involved in functional recovery are only partly understood, even when grafting human embryonic mesencephalic tissue with encouraging results. To better understand when cell transplantation can lead to substantial clinical recovery, it is important to critically review the clinical grafting trials performed so far.

In order to induce substantial clinical recovery, survival of dopamine (DA)-rich cells in the grafted tissue is likely to be a key factor. Survival of nigral tissue transplanted in patients can be assessed in vivo by measuring striatal uptake of [18F]-fluorodopa (FD) using positron emission tomography (PET). The FD is taken up presynaptically, thus providing a measure of the number of viable striatal DA terminals. In patients with PD, putaminal FD uptake correlates inversely with the degree of motor impairment (10, 11), whereas the correlation between motor symptoms and decreases in FD uptake in the caudate nucleus is weaker (11). For measures of cognitive impairment in PD there is a stronger correlation between the severity of symptoms and decline in FD uptake in the caudate than putamen (12). Striatal FD uptake in vivo correlates strongly with postmortem findings, both regarding numbers of remaining substantia nigra DA neurons and striatal DA levels (13). Thus, there is a good rationale for using FD PET as a measure of dopaminergic denervation in PD patients and to assess restoration of reinnervation after intrastriatal grafting. Importantly, PET studies in rats indicate a correlation between striatal binding of [11C]RTI-121, another marker for DA terminals, and survival of intrastriatal nigral grafts (14), adding further support to the use of PET as an in vivo correlate of graft function.

Studies assessing graft survival by means of striatal FD PET before and after transplantation have indicated that the degree of clinical improvement is related to the level of increased striatal FD uptake (3, 4). Most probably, increases in FD PET are closely correlated to the density of graft-derived dopaminergic innervation in the patients’ striatum. In rats, the number of surviving grafted neurons

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and the density and extent of transplant-derived fiber outgrowth appear to be correlated (15, 16). Therefore, the number of surviving DA neurons in the graft still constitutes a highly relevant index of graft outcome. Observations of a correlation between increases in FD uptake and symptomatic relief in PD patients are in agreement with results in rats, which suggest a relationship between behavioral effects and survival of grafted DA neurons (17). It is possible that there is a stronger correlation between functional graft effects, i.e. symptomatic relief, and the number and distribution of graft-derived DA terminals in the host striatum than just the number of surviving neurons in the transplants. However, for practical reasons it is easier to assess the number of surviving neurons rather than the extent of fiber outgrowth, and therefore this is the most commonly used morphological outcome parameter in animal experiments.

The correlation between behavioral effects of grafts and the survival of transplanted DA neurons has been illustrated in several experiments. For example, there appears to be a minimal threshold of surviving grafted DA neurons needed to reverse amphetamine-induced motor asymmetry, so called “rotational behavior,” in hemiparkinsonian rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal system (15, 17, 18). To achieve a 50% reduction in rotational behavior 6 wk after surgery, approximately 1,000 rat tyrosine hydroxylase immunoreactive (TH-ir) neurons must survive in the graft (18). Complete reversal of motor asymmetry is achieved with 2,000–3,000 TH-ir neurons. Beyond this number of cells there does not appear to be any major additional graft-induced effect on amphetamine-induced rotational behavior (17, 18).

Direct studies of postmortem material from patients grafted with embryonic neurons can provide us with a detailed understanding of the relationship between the number of surviving grafted DA neurons and the postoperative clinical outcome. In this context, the best marker for surviving DA neurons is TH-immunoreactivity. If postmortem brain tissue fixation parameters are not optimal, TH immunostaining may not be possible and neuromelanin becomes an alternative marker for identifying grafted dopaminergic neurons. The shortcoming of this marker is that it is not present in all mesencephalic DA neurons that survive in transplants of embryonic mesencephalon. First, neuromelanin is not present when the DA neurons are immature (19) and second, it only appears in neurons of the substantia nigra, not the adjacent ventral tegmental area (20). Embryonic mesencephalic grafts contain both nigral and ventral tegmental DA neurons (21) and therefore melanin will fail to detect a significant portion of the grafted cells.

In this paper we address the importance of graft survival for a positive clinical outcome following intrastriatal neural transplantation in patients with PD by reviewing recent results from bilaterally grafted PD patients and available autopsy data. Other factors likely to contribute to the outcome are also explored.

Observations in Clinical Transplantation Trials

There is still no published prospective study that examines the impact of varying the amount of grafted donor tissue on clinical recovery in PD patients. However, the amount of tissue implanted into each patient differs among, and in some cases within, surgical centers. Thus, it is possible to obtain some, albeit not systematic, information from the literature regarding the impact of varying the amount of transplanted tissue on the functional outcome in patients.

In Table 1, we summarize results regarding postoperative changes in the motor examination section of the Unified Parkinson’s Disease Rating Scale (UPDRS) and time spent “off” from 6 independent clinical series of PD patients grafted bilaterally in the putamen using different amounts of donor tissue (3, 4, 6, 7, 22, 23). In 5 groups of grafted patients, the amount of tissue implanted into each putamen corresponds to 3–5 human embryonic ventral mesencephalon (3, 4, 6, 7, 22). As detailed in Table 1, some of these studies employed short-term tissue

<table>
<thead>
<tr>
<th>Postoperative change</th>
<th>No. of grafted human embryonic ventral mesencephalon per patient putamen and mean percent change in putaminal [18F]-fluorodopa uptake, motor impairment and time spent in the “off” phase at 10–23 months after transplantation; from 4 recent series of PD patients receiving bilateral putaminal grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ref.</strong></td>
<td><strong>No. of VM/putamen</strong></td>
</tr>
<tr>
<td>Ref. 4 (n = 6)</td>
<td>3–4</td>
</tr>
<tr>
<td>Ref. 3 (n = 4)</td>
<td>4.9</td>
</tr>
<tr>
<td>Ref. 23 (n = 19)</td>
<td>2.0</td>
</tr>
<tr>
<td>Ref. 6 (n = 5)</td>
<td>2.8</td>
</tr>
<tr>
<td>Ref. 7 (n = 5)</td>
<td>3.25*</td>
</tr>
<tr>
<td>Ref. 22 (n = 3)</td>
<td>1–1.5</td>
</tr>
<tr>
<td>Ref. 22 (n = 6)</td>
<td>3</td>
</tr>
</tbody>
</table>

* As assessed during practically defined “off” phase, i.e. in the morning, ≥12 hours following the last dose of antiparkinsonian medication and ≥1 hour after arising. † Excluding 1 patient with possible multiple system atrophy. ‡ The graft tissue was treated with the lazaroid tirilazad mesylate. This can be estimated to yield an increase in cell survival by about 1.5–2-fold, thus corresponding to 4.2–5.6 untreated donors per putamen. § The graft tissue was stored for 6 days in the presence of GDNF before implantation. ‡ Total UPDRS score. Abbreviations: VM, ventral mesencephalon; FD, [18F]-fluorodopa; PET, positron emission tomography; UPDRS, Unified Parkinson Disease Rating Scale; n.r., not reported.
storage and treatment of the donor tissue with neuroprotective or neurotrophic agents. PET data is currently available in 5 patient groups (3, 4, 6, 7, 23). In patients receiving tissue from 3–5 donors, the FD uptake in the putamen exhibited a 55%–107% mean increase at 10–23 months following transplantation, compared to preoperative values. Clinically, these patients displayed a reduction of symptoms corresponding to an overall 30%–40% improvement of the UPDRS motor score in the drug-free, practically defined “off” phase. Moreover, there was a 43%–59% reduction of time spent “off,” and, in the majority of cases, a reduced need for antiparkinsonian drugs. The clinical data from 9 bilaterally grafted patients reported by Nguyen and collaborators (22) reveal superior benefits in patients receiving more embryonic donor tissue. Two years following bilateral transplantations, 3 patients each receiving cells from 2–3 donors (i.e. 1–1.5 per putamen) displayed only mild, if any, benefits. This was illustrated by a mere 6% improvement of the UPDRS motor score during “off,” and even a 15% increase in motor score during “off” at 12 months postoperatively (26). At autopsy, no surviving TH-ir neurons could be identified, but neuromelanin was found in the graft. Autopsy also revealed that the patient did not have idiopathic PD, but striatonigral degeneration.

Five patients from another early clinical series of grafted PD patients died between 18–40 months after grafting. Each patient received tissue from 1 fetal donor (11- to 19-wk old) unilaterally into the caudate nucleus (n = 3) or putamen (n = 2) (26–29). No immunosuppression was given. Neither FD PET data nor detailed information on clinical outcome are available for the individual patients. However, the reported outcome from the whole series of patients transplanted unilaterally in the caudate nucleus by the same group revealed a 13% improvement using a modified Webster score and 12% decreased time spent “off” at 12 months postoperatively (26). At autopsy, a few surviving TH-ir neurons were found in 3 cases, whereas none were found in the other 2 cases (27–29). In one of the cases with graft survival, 5,430 DA cells were found in a 5-mm³ volume of graft tissue (28). Of the cells defined as dopaminergic, 62% expressed TH-ir only; 16% contained TH and neuromelanin, and 22% of the cells had only neuromelanin granules. In addition, significant gliosis was found in all 5 cases, mainly at the graft/host interface.

From a different transplantation center, 3 postmortem cases have been described. These patients received grafts in the putamen (6- to 8-wk old donors, stored in culture for 1–4 wk prior to implantation) without postoperative immunosuppression. The first patient was grafted with tissue along multiple stereotactic trajectories in the right putamen, had a hemorrhagic stroke at the time of implantation, and died 30 days later. At autopsy, the graft sites were reported to contain numerous TH-ir neurons (30). The other 2 patients died at 7 months and 3 yr, respectively, following bilateral implantation of tissue from 2 donors into each putamen (23). No clinical or PET data on the patient who died 4 months after unilateral grafting of mesencephalic tissue obtained from 1 human embryonic donor. The tissue was cryopreserved for 79 days prior to implantation and the patient was immunosuppressed with cyclosporin A (CyA) for 7 wk postoperatively (24, 25). Brain imaging using FD PET was not performed and no clinical benefits were observed. At autopsy, no surviving TH-ir neurons could be identified, but neuromelanin was found in the graft. Autopsy also revealed that the patient did not have idiopathic PD, but striatonigral degeneration.

Autopsy Findings

We have been able to identify 14 PD patients who have died and undergone autopsy after transplantation of human or porcine embryonic mesencephalic DA-rich tissue. The results from these autopsies are summarized in Table 2 and described in more detail below.

The first case reported in the literature was that of a patient who died 4 months after unilateral grafting of mesencephalic tissue obtained from 1 human embryonic donor. The tissue was cryopreserved for 79 days prior to implantation and the patient was immunosuppressed with cyclosporin A (CyA) for 7 wk postoperatively (24, 25). Brain imaging using FD PET was not performed and no clinical benefits were observed. At autopsy, no surviving TH-ir neurons could be identified, but neuromelanin was found in the graft. Autopsy also revealed that the patient did not have idiopathic PD, but striatonigral degeneration.

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the implants. No Lewy bodies or neuromelanin were detected in the grafts. The patient who died 3 yr after transplantation exhibited a 33% improvement in total UPDRS score shortly before he passed away. His putaminal FD PET at 24 months postgrafting increased by 100%, but the preoperative level of FD uptake was not reported (23). Histopathological examination of this patient’s brain revealed 36,796 and 6,840 surviving TH-ir neurons in the right and left putamen, respectively. Fiber outgrowth was reported to extend the full width of the putamen. In addition, neuromelanin was found in all grafts in this patient. Inflammatory cells were found around the transplant tracts of both patients (23).

Between 1995 and 1998, Kordower et al (31–34) reported important autopsy results together with clinical data and PET findings in 3 striata from 2 patients. These patients died 18 and 19 months, respectively, after bilateral implantation of tissue from 3–4 donors (aged 6.5–9 wk) into each postcommissural putamen. The patients were immunsuppressed with CyA for 6 months postoperatively. In the first patient (31, 32), 126,162 and 81,905 surviving TH-ir neurons were found in the right and left putamen, respectively. Twelve months postoperatively, FD uptake had increased from approximately 35% to 71%, and 43% to 69% of normal values in the right and left putamen, respectively. In 1 putamen from the other patient (contralateral side not analyzed), about 138,000 surviving TH-ir neurons were found (34). FD uptake in this structure had shown an increase from 25% of normal preoperatively to 62% at 12 months after grafting. In both cases, extensive reinnervation of the host striatum was observed and there were abundant synapses made by grafted TH-ir fibers. Within the grafted postcommissural putamen, graft-derived reinnervation of 24% to 78% of the designated target area was obtained. Clinically, these patients...
### TABLE 2
Surviving Dopaminergic Tissue, Clinical Outcome, and PET Findings in Autopsy Reports of PD Patients Receiving Intrastriatal Implantations of Human Embryonic Mesencephalic Tissue

<table>
<thead>
<tr>
<th>n</th>
<th>Target site(s)</th>
<th>No. of donors</th>
<th>Time of death</th>
<th>TH-ir cell count</th>
<th>FD-PET (Δ%)</th>
<th>Clin. Score (Δ%)</th>
<th>Time in “off”</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R CN</td>
<td>1</td>
<td>4 mo</td>
<td>0</td>
<td>n.r.</td>
<td>±0%</td>
<td>±0%</td>
<td>24, 25</td>
</tr>
<tr>
<td>5</td>
<td>R CN (n = 3)</td>
<td>1</td>
<td>18–40 mo</td>
<td>“Few” Th-ir (n = 3)</td>
<td>n.r.</td>
<td>−13%</td>
<td>−12%</td>
<td>26–29</td>
</tr>
<tr>
<td>5</td>
<td>R put (n = 2)</td>
<td></td>
<td></td>
<td>5,430 cells/5 mm³ in 1 of these</td>
<td>n.r.</td>
<td>(grp)³</td>
<td>(grp)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R put</td>
<td>1–2³</td>
<td>30 days</td>
<td>“Numerous”</td>
<td>n.r.</td>
<td>±0%</td>
<td>n.r.</td>
<td>23, 30</td>
</tr>
<tr>
<td></td>
<td>R + L put</td>
<td>4</td>
<td>3 yrs</td>
<td>36,796/6,840⁵</td>
<td>+100% (24 mo)</td>
<td>−33%</td>
<td>n.r.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R + L put</td>
<td>4</td>
<td>7 mo</td>
<td>24,115/38,392⁵</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>R + L put</td>
<td>7</td>
<td>18 mo</td>
<td>126,162/81,905⁶</td>
<td>+103%/+61%⁴</td>
<td>−37%</td>
<td>−100%</td>
<td>31, 32, 34</td>
</tr>
<tr>
<td>1</td>
<td>R CN + L put</td>
<td>2 + 1¹</td>
<td>23 mo</td>
<td>0</td>
<td>n.r.</td>
<td>±152%</td>
<td>n.r.</td>
<td>35, 36</td>
</tr>
<tr>
<td>1</td>
<td>R + L put</td>
<td>4</td>
<td>24 mo</td>
<td>n.r.</td>
<td>n.r.</td>
<td>−31%</td>
<td>n.r.</td>
<td>37, 38</td>
</tr>
<tr>
<td>1</td>
<td>R CN + put</td>
<td>n.r.¹</td>
<td>7 mo</td>
<td>~640</td>
<td>±0 (grp)</td>
<td>−19% (grp)⁵</td>
<td>n.r.</td>
<td>8, 40</td>
</tr>
</tbody>
</table>

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* Time elapsed between grafting and death; † During “off” phase; ‡ Clinical case history; § Modified Webster score; ‖ Not clear whether 1 or 2 donors were used; ¶ For right and left putamen, respectively; ‡ UPDRS total score; † Only 1 putamen analyzed histopathologically; § UPDRS motor score; ¶ Implantation of tissue from 2 donors plus intraventricular injection of a cell suspension from a third embryo; ‰ Patient/family report; ¶ Grafted with approximately 12 million mesencephalic cells from a nondefined number of porcine embryos. Abbreviations: Postop = postoperative; TH-ir = tyrosine hydroxylase immunoreactive; FD = [¹⁸F]-fluorodopa; PET = positron emission tomography; Δ% = mean postoperative percent change from baseline; R = right; L = left; CN = caudate nucleus; put = putamen; mo = months; n.r. = not reported; grp = for the whole group of patients; + = improved; yrs = years; p.c. = postcommissural.
showed 37% and 26% postoperative improvements in UPDRS score and 100% and 62% decreases in the daily amount of time spent in the “off” phase, respectively. Despite good survival of TH-ir grafted neurons and clear evidence of functional effects, there were numerous immune cells infiltrating the grafts in both patients (33).

A patient with advanced PD operated on in a different center died 23 months following transplantation. This patient had received tissue from two 16-wk-old donors into the right caudate nucleus and left putamen, with subsequent intraventricular injection of a cell suspension derived from a third (5- to 6-wk old) embryo. Immunosuppression with CyA and prednisolone had been given throughout the postoperative course. No surviving TH-ir neurons were found at autopsy. Instead, pieces of bone, hair, cartilage, and squamous epithelium were evident at multiple sites in the ventricles. Death was probably caused by obstruction of the fourth ventricle with secondary brainstem compression due to growth from the cell suspension implant (35). Although some postoperative benefit was reported in this patient (36), no clinical data have been provided in this tragic case. Furthermore, several manipulations with the antiparkinsonian medications took place postoperatively. In the absence of FD-PET data, this renders any claims of postoperative change of parkinsonian symptoms impossible to relate to graft function (35). In summary, both the surgical procedure and the clinical follow-up of this patient were clearly suboptimal.

The next case is a woman who received bilateral putaminal grafts from four 6.4- to 8.6-wk-old donors and was immunosuppressed with CyA for 6 months thereafter (37). The postoperative follow-up showed an approximate 30% reduction in UPDRS score at 9 months after surgery (38). Twenty-three months postoperatively she became comatose. Computerized tomography and magnetic resonance imaging scans showed a nodule and an associated large cyst arising from the left putamen, causing brainstem compression. Despite surgical decompression, the patient never regained consciousness but died within a few weeks. The published autopsy report describes inflammatory cells, macrophages, astrocytosis, and rests of choroid plexus (37). The cyst contained cerebrospinal fluid and genotyping indicated that the nodule contained tissue that was not derived from the host. In the postmortem report it was suggested that tissue located in the cyst nodule in the right hemisphere was not integrated into the host brain and was derived from choroid plexus of graft origin (37). However, subsequently this conclusion has been challenged by the neurosurgical team (38). No detectable levels of DA precursors or breakdown products were identified in this tissue. Unfortunately there was no description of the morphology of the contralateral striatum (37), which had also received nigral grafts, and as a whole this patient provides us with no useful information regarding the importance of graft survival for functional efficacy.

Xenografting using embryonic porcine tissue has recently been suggested as another alternative to human embryonic tissue (39) and clinical trials in PD have been initiated. In a recent phase I clinical safety trial, 12 patients underwent unilateral implantation of mesencephalic porcine tissue into the striatum and were followed clinically and by FD PET for 12 months thereafter. During the seventh month after grafting, 1 patient died and was autopsied. The patient had been grafted with a total of 12 million embryonic mesencephalic porcine cells into 1 site in the head of the caudate and 3 sites in the putamen. Histopathological examination revealed approximately 640 viable TH-ir DA neurons in the grafts (40). Postoperatively, the authors described this patient’s performance as “encouraging,” but no clinical follow-up data are available for this particular patient (8, 40). From the whole series of 12 patients, 10 patients were followed for 12 months. They showed mild clinical recovery (a mean 19% improved UPDRS motor score in “off” phase) and no evidence for graft survival could be detected by FD PET (8).

DISCUSSION

What characterizes grafts that elicit clinically valuable benefits in PD patients?

Available clinical data show that the outcome following transplantation is dependent on restoration of the number of viable dopaminergic neurons innervating the striatum as assessed by FD PET. Typically, PD patients considered suitable for transplantation trials exhibit a FD uptake in the putamen of approximately 30%–35% of normal values (Fig. 1). When reviewing the literature, it appears that an increase in FD uptake to a level corresponding to about 50%–60% of the normal mean is necessary to achieve clinically valuable antiparkinsonian effects (Fig. 1).

Available autopsy and clinical data indicate that a minimum number of neurons need to survive after grafting. This is illustrated by an absence of meaningful clinical improvements in cases with no, or virtually no, surviving grafts. However, the relationship between the amount of grafted tissue, surviving DA neurons, and degree of symptomatic relief is less clear in the few patients with surviving transplants that have come to autopsy. This is illustrated by the cases reported by Kordower et al (31, 32, 34) and Freed et al (23). In the former patients, tissue from a total of 7 donors was implanted bilaterally in the putamen. This yielded approximately 100,000 surviving TH-ir neurons per putamen and about 25%–40% symptomatic improvement in the “off” phase (Table 2). In contrast, the case reported by Freed et al (23) was grafted with tissue from 4 donors, yielding no more than about 7,000 and 37,000 surviving graft cells in each putamen.
In spite of these low cell numbers, a 33% postoperative reduction of parkinsonian symptomatology during “off” phase and a 100% increase in FD uptake were reported. It is also remarkable that in this patient, the postoperative PET signal did not differ between the sides (23), despite a more than 5-fold difference in the number of surviving DA graft neurons between the 2 sides (23). Since only 6,840 cells survived on the left side, a 100% increase in the FD PET uptake suggests that the preoperative value was relatively low. Because the Kordower cases (31, 34) appear less denervated preoperatively, any comparisons between the patients reported by Kordower et al and Freed et al should be made with caution. In contrast to the Kordower cases, no baseline values regarding either UPDRS score or FD PET uptake were provided for the patient described by Freed et al, only percent changes (23). Because small absolute changes may yield large relative changes from a low baseline, it is very difficult to interpret any data expressed solely in terms of percent changes.

Results obtained with nigral transplants in rats with partial lesions of the nigrostriatal pathway resembling PD have provided further insight into the relationship between cell numbers and graft efficacy. Grafts containing on average 7,500 TH-ir neurons and giving rise to a striatal density of TH-ir fibers nearing 50% of normal, cause a partial reduction of deficits in non-drug-induced forelimb function in the so-called stepping test (41). It is interesting to compare these numbers of neurons with the numbers believed to be present in the normal human and rat substantia nigra. For humans it has been estimated that 1 putamen is innervated by approximately 250,000 DA neurons (42) and for rats the caudate-putamen is innervated by approximately 15,000 DA neurons (43). It thus appears that grafts containing approximately half the number of DA neurons normally innervating the striatal target are needed for major graft effects to develop in both humans and rats. In the case of patients, the number of neurons needed in the transplants is obviously also related to the number of DA neurons still remaining in the intrinsic substantia nigra affected by the disease process. Nonetheless, based on animal experiments and available clinical data, we propose that approximately 100,000 surviving grafted DA neurons per putamen are sufficient to produce significant clinical benefit, while the consequences of smaller implants are less predictable.

Postmortem studies in grafted PD patients have provided additional important information. The tragic cases reported by Folkerth and Raymon (35) and Mamelak et al (37) illustrate the importance of careful tissue dissection. From animal experiments, it is clear that if mesenchymal tissue from, e.g. the meninges are included in the dissection, it can give rise to a variety of tissues such as skin, hair, and cartilage (44, 45; R. Ridley personal communication; L. Annett personal communication). As suggested from the 2 reported autopsy cases (35, 37), inclusion of proliferating mesenchymal tissue is not only neurologically ineffective, but also provides a major risk by uncontrolled growth with potentially catastrophic consequences for the patient.

Freed et al described another undesirable outcome in 5 of the 33 patients in their double-blind controlled study (23). These patients were reported to exhibit dyskinesias and dystonia postoperatively, even in the absence of medication. Post-grafting dyskinesias have also been reported by other investigators (2, 3, 6), but the magnitude of these adverse events appear clearly more pronounced in the cases from the Freed series. Although these adverse events have been suggested to depend upon a relative excess of dopamine emerging from the graft (23), there is presently little, if any, data in support of this notion. First, none of the information provided suggests that there is an excessive FD uptake in the grafted striatum of the patients reported by Freed et al (23). Second, transplant-induced dyskinesias of similar severity have not been reported in patients that were grafted with larger amounts of tissue and exhibited more pronounced postoperative symptomatic relief and restoration of FD uptake on PET (3–6). Third, in the patient with the most extensive dopaminergic graft-induced restoration of FD uptake reported to date, dyskinesias have been virtually absent (5, 46). In addition, clear improvements of levodopa-induced dyskinesias have been observed in patients with large surviving grafts (3, 4, 47). Interestingly, Lee and collaborators demonstrated that grafts ameliorate levodopa-induced dyskinesias in rats if >10%–20% restoration of striatal dopaminergic terminal density is achieved, but not if the extent of reinnervation is smaller (48). Available observations thus imply that the mechanisms underlying graft-induced dyskinesias go beyond that of a simple dopaminergic hyperinnervation and need to be carefully investigated before it is possible to firmly discuss their relationship with graft-derived reinnervation in PD. Moreover, it should be noted that the grafting protocol differs in several important respects between the Freed et al study and most of the other clinical transplantation programs. These differences may have been important for the development of dyskinesias. For example, Freed et al prepared the tissue into thin strands that were stored in explant culture for 1–4 wk before surgery (23). In addition, the graft tissue was injected along 2 tracts in the axial plane that were accessed by a transfrontal stereotactic approach. Finally, no immunosuppression was given to the patients.

What factors in addition to the number of graft cells are likely to govern functional efficacy?

Although survival of sufficient numbers of grafted dopaminergic neurons most certainly is a prerequisite for
substantial graft-induced postoperative clinical recovery, several other parameters are also likely to impact graft outcome.

For nigral grafts to be clinically effective, it is important that the patients primarily suffer from a nigrostriatal DA depletion, as is present in idiopathic PD and MPTP-induced parkinsonism, and not additional widespread brain pathology. This is illustrated by case number 8 in the Lund transplant series. This patient was initially diagnosed with idiopathic PD, but later it became evident that he suffered from an atypical form of PD, possibly multiple system atrophy (3). Despite having large grafts bilaterally, increasing the FD uptake to approximately 40%–55% of the normal mean in the putamen on both sides, this patient displayed little, if any, clinical benefit.

Also the stage of the disease, and thereby the extent of DA denervation in the striatum, is likely to influence graft outcome. It has been suggested in preclinical experiments that significant behavioral effects are more likely to develop in rats with extensive lesions of the nigrostriatal system than with partial denervation of the striatum (41). However, in patients with very advanced disease with marked and widespread DA depletion in the forebrain, it may be difficult to attain a clinically effective DA neurotransmission through grafting a limited amount of tissue to the striatum only.

In addition, it has been proposed that older graft recipients provide a less favorable striatal environment for nigral transplants. Thus, implants placed in old rats have been reported to be smaller and less effective than identical grafts placed in young animals (49). Whether this is also true for PD patients remains to be fully established. Freed et al (23) have suggested based on the 1-yr follow up of their series that human nigral grafts give rise to significant symptomatic relief only in patients younger than 60 yr. Subsequent observations in these patients have, however, suggested that also the older group develops functional benefits from the grafts, but that this occurs with a longer delay after surgery (23). This may also be reflected in a patient from the Lund series, grafted unilaterally in the putamen at the age of 59. His highly significant symptomatic relief mainly developed between 1 and 3 yr after surgery and was associated with a normalization of striatal FD uptake and striatal DA release (5), whereas several younger patients in the same series have exhibited beneficial effects sooner after surgery (3, 6).

The precise graft placement within mesotelencephalic DA target regions is an additional crucial factor. From rat studies it is clear that DA in the striatal complex and nucleus accumbens subserves many different types of behavior, and that there is a strong regional specificity of function of nigral transplants placed in various parts of the forebrain (17, 50). Observations in humans suggest that the putamen is the most important striatal region for motor functions (11, 12) and this region also displays the most extensive DA depletion in PD (51). Also within the putamen there are regional differences and it has been proposed that the postcommissural putamen is the most important target for nigral grafts (52). Several patients have been grafted also in the caudate nucleus. Despite evidence of clear graft survival, it remains unclear if nigral implants in the caudate can give rise to clinical benefit, or provide additional symptomatic benefits in patients with transplants in the putamen (6). Rat studies show that DA in the nucleus accumbens plays a vital role in locomotion, and that nigral transplants in this region increase the level of locomotor behavior in rats with local DA depletion in this area (50, 53). It remains to be established what the human equivalent role of the nucleus accumbens is, and if grafts of mesencephalic tissue in this target can elicit amelioration of specific symptoms in PD.

For a more detailed discussion of the relationships between nigral dopaminergic cell number, graft-derived innervation, and functional outcome it is useful to consider how these parameters interact in a patient with transplants only in the putamen. The relationship between volume of reinnervated striatum and behavioral graft effects may be complex and is likely to depend also on the density of the graft-derived terminal network. Detailed studies, using DA autoradiography, of the reinnervation of the rat striatum from a single intrastriatal mesencephalic implant have shown that the density of innervation declines in a linear fashion with distance from the graft-host border (54). The relationship between the size of a nigral transplant (measured as tissue volume) and the total number of dopaminergic terminals originating from the graft has been reported to show a “plateau” effect. Thus, when the graft increases beyond a certain size, few additional dopaminergic terminals are formed in the host striatum (54).

A positive linear relationship has been reported between graft TH-ir cell number and the volume of rat striatum reached by a significant transplant-derived TH-ir innervation (15). Furthermore, studies in hemiparkinsonian rats with virtually no residual endogenous DA have indicated a positive correlation between the volume of reinnervated striatum and functional efficacy of the grafts in the amphetamine-induced rotation test (15). Therefore, we propose that functional graft efficacy is a composite function dependent upon both density and volume of graft-derived fiber network (Fig. 2). According to this model, for any functional effect to occur a critical threshold of innervation (both regarding density and volume) of the host striatum has to be exceeded, and beyond a certain level of innervation there will be little or no additional benefit (Fig. 2).

Notably, the time interval between graft surgery and behavioral assessment also plays a role. In 2 separate studies, Nakao et al found a stronger correlation between graft cell number and reductions in amphetamine-induced
motor asymmetry in rats at 2 wk after surgery than at 5–6 wk (15, 18). Data from one of these experiments were later reanalyzed and the extent of reduction (compared to pretransplant) in rotational asymmetry in individual rats at 2 wk postgrafting was expressed as a percentage of the reduction at 6 wk (called “percent achievement”). A strong correlation between the percent achievement and the number of surviving TH-ir neurons in the graft at 2 wk following implantation was evident (55). Taken together, these observations suggest that graft TH-ir innervation, at least beyond a threshold density, is needed in a certain volume of striatum to elicit the behavioral effect, and the higher the number of DA neurons, the faster these crucial limits of DA neurotransmission are attained. According to this model, smaller grafts may eventually, to some extent, “catch up” and reinstate DA neurotransmission in significant portions of the striatum. However, if they are too small they will never be able to completely do so and therefore not provide quite the same degree of functional efficacy at the end-point (Fig. 2).

The ability of grafts to integrate functionally as well as structurally with the intrinsic host brain circuits is also of paramount importance. This is likely to require establishment of both efferent and afferent synaptic contacts with the host (17, 56). In rats receiving human xenografts, there appears to be a temporal correlation between graft-to-host synapse formation and onset of functional recovery (56). Preclinical observations have also shown that development of DA synthesis and storage capacity in the grafts precedes the more gradual improvements in complex sensorimotor behavior (45, 57). Thus, although expression of TH in grafted neurons are evident at least as early as 3 wk after grafting (58), behavioral effects are not observed until at least 11 wk after implantation, coinciding with formation of graft-to-host synapses (56). Recent clinical data suggest that a delayed functional integration also occurs in grafted PD patients. In a study of movement-related cortical activation in bilaterally grafted PD patients using \textsuperscript{H}_{2}\textsuperscript{15}O PET, Piccini et al (59) found that striatal DA storage capacity, as measured by FD PET, was significantly restored as early as 6.5 months after transplantation and did not change thereafter. At this time, symptomatic relief was only modest, as indicated by a mean 25% improved UPDRS motor score. Restoration of movement-related activation in prefrontal cortical areas did not occur until the second postoperative year, which coincided with a continued, substantial clinical improvement (50% improved UPDRS motor score, as compared to preoperatively). This suggests that not only graft survival, but also functional integration is required for the development of substantial clinical recovery in patients.

Finally, it also seems feasible to presume that in order to yield substantial functional effects, grafts need to be able to release DA in a regulated manner within the striatum. Recent observations by Piccini et al (5) support this notion. A patient grafted unilaterally in the putamen using tissue from 4 donors showed increasing FD uptake in the grafted putamen up to 3 yr postoperatively, when it reached normal levels. Only minor additional changes occurred up to 10 yr postoperatively. In contrast, FD uptake in nongrafted striatal regions decreased gradually.
Ten years after grafting, DA release was assessed in vivo by measuring DA D2 receptor $[^{11}C]$ raclopride binding in the presence and absence of amphetamine stimulation. There was markedly reduced transmitter release from nongrafted structures, whereas it was completely normalized in the grafted putamen, indicative of regulated DA release from the graft.

*How can the outcome of human embryonic nigral grafts be improved?*

Data from both animal and clinical studies suggest that only between 1% and 20% of grafted cells survive the procedure. Approaches to improve graft cell survival are thus needed. Therefore, it is important to understand both when and how grafted neurons die. Several studies from recent years have shown that the majority of grafted DA neurons die within the first wk after surgery, that apoptosis is prevalent in the grafted tissue, and that free radical stress plays a role in the demise of the cells (60). Therefore, treatment of the embryonic graft tissue immediately after retrieval and in conjunction with implantation surgery is important. By treating the graft tissue with different cytoprotective agents during these initial phases of the transplantation procedure or by administering growth factors to the graft and host, it has been possible to increase the survival of the transplanted DA neurons by a factor of 2 to 3 (60). Initial clinical observations (Table 1) suggest that treatment of human embryonic mesencephalic tissue with the lazaroid tirilazad mesylate (6) or GDNF (7) can improve graft survival also in PD patients. However, even with the most elaborate protocols employed in experimental animals, the cell survival does not seem to exceed a rate of approximately 50% of the implanted DA neurons (60). This means that if the aim is to use as little donor tissue as possible per patient, it may be necessary to further enhance graft function by taking measures to improve fiber outgrowth. In this context it appears that postsurgery administration of growth factors, e.g. GDNF, by local injection or gene transfer into the host brain effectively can enhance fiber outgrowth. Consequently, if mechanisms promoting graft efficacy, i.e. density of fiber outgrowth, could be sufficiently controlled and enhanced, the demand for large numbers of graft cells could be decreased.

*What are the alternative approaches to grafting human embryonic tissue in PD?*

There are logistical problems with obtaining sufficient donor tissue for each patient on a regular basis and some countries express serious ethical concerns with the use of human tissue from aborted embryos. Consequently, the numbers of trials conducted and clinical centers involved are small. Even for those centers with a functioning neural transplantation program, it is difficult to operate large numbers of patients using human embryonic tissue as the primary source of donor cells. Recent advances in our understanding of graft function in the human parkinsonian brain should be considered in the development of new alternative cell types. Regardless of source, any candidate cell type should not only demonstrate the ability to survive grafting in animal models, but also release DA and integrate in the host brain circuitry before being considered for clinical trials.

So far, autografts (e.g. adrenal medulla, carotid body, superior cervical ganglion), neural stem cells, and porcine xenografts have all been suggested as alternatives to human primary embryonic tissue. Adrenal medulla autografts are no longer considered a feasible choice because the clinical results and graft survival have been discouraging (29, 61). Regarding autografts of sympathetic ganglia and carotid bodies, there is still no published evidence to suggest that these types of cells can provide benefit of real therapeutic value or yield significant numbers of surviving TH-ir cells. Neural “stem cells,” either derived from neural precursors or from embryonic stem cells, are a promising alternative for the future, but still do not provide a viable clinical alternative (62). Xenografting using embryonic mesencephalic porcine tissue has recently been explored for clinical transplantation in PD patients. As discussed earlier, results so far have not provided any firm evidence of either graft survival or meaningful clinical benefits (8, 40). In addition to concerns regarding graft survival and axonal growth capacity, the potential risks for immunological reactions and virus transfer associated with cerebral implantation of xenogeneic porcine tissue have not yet been fully clarified (39).

**General Conclusions**

Clinical grafting trials in PD provide an important basis for the development of restorative, cell-based therapies for neurodegenerative disorders. Patients displaying functional recovery suggest that such a therapeutic principle is possible. Equally important for our understanding of the requirements for brain repair are the less successful cases. Clinical observations, in vivo monitoring of graft function, and autopsy findings from grafted PD patients suggest that the requirements for successful reconstruction of intrastriatal DA deficiency go beyond that of reintroducing a certain number of DA-producing cells. Important for the clinical response are the actual number of surviving grafted DA neurons coupled to the density of fiber outgrowth from the grafted cells and the volume of graft-derived reinnervation. Furthermore, synaptic connectivity between the graft and the host and the extent to which grafts become functionally integrated within the host brain circuitry are likely to be crucial factors. As mentioned above, several of these properties can now be assessed in vivo in grafted patients.
Clearly, a variety of factors may influence long-term outcome following transplantation. For neural transplantation to become a clinically feasible and available treatment alternative, graft survival must be maximized and the need for human embryonic tissue has to be minimized. Great efforts are being made to minimize and, ultimately, eliminate the need for multiple donors of human embryonic tissue and there are now several potentially interesting strategies under investigation. Before new sources of cells are tested clinically, not only graft survival, but also fiber outgrowth and function should be clearly demonstrated in experimental animals. Because of the availability of accumulated experience from 2 decades of research on grafts of primary embryonic DA neurons in animals and humans, there is now great hope that novel sources of donor tissue can be developed in a goal-directed fashion.

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REFERENCES

43. Janson AM, Möller A. Chronic nicotine treatment counteracts nigral cell loss induced by a partial mesodiencephalic hemitransection: An analysis of the total number and mean volume of neurons and glia in substantia nigra of the male rat. Neuroscience 1993;57:931–41