

Factors affecting the clinical outcome after neural transplantation in Parkinson's disease

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Intrastratial grafts of embryonic mesencephalic tissue can survive in the brains of patients with Parkinson's disease, but the degree of symptomatic relief is highly variable and some cases develop troublesome dyskinesias. Here we explored, using clinical assessment and ¹⁸F-dopa and ¹¹C-raclopride PET, factors which may influence the functional outcome after transplantation. We observed increased ¹⁸F-dopa uptake in the grafted putamen, signifying continued survival of the transplanted dopaminergic neurons, in parallel with a progressive reduction of ¹⁸F-dopa uptake in non-grafted regions for the whole patient group. The patients with the best functional outcome after transplantation exhibited no dopaminergic denervation in areas outside the grafted areas either preoperatively or at 1 or 2 years post-operatively. In contrast, patients with no or modest clinical benefit showed reduction of ¹⁸F-dopa in ventral striatum prior to or following transplantation, which may have limited graft-induced improvement. We obtained no evidence that dyskinesias were caused by abnormal dopamine (DA) release from the grafts. As has been observed for intrinsic dopaminergic neurons, there was a significant correlation between ¹⁸F-dopa uptake and methamphetamine-induced change of ¹¹C-raclopride binding (as a measure of DA release) in the putamen containing the graft. Furthermore, we observed no correlation between ¹¹C-raclopride binding in anterior, posterior or entire putamen under basal conditions or after methamphetamine, and dyskinesia severity scores in the contralateral side of the body. Withdrawal of immunosuppression at 29 months after transplantation caused no reduction of ¹⁸F-dopa uptake or worsening of UPDRS motor score, indicating continued survival and function of the graft. However, patients showed increased dyskinesia scores, which might have been caused either by growth of the graft or worsening of a low-grade inflammation around the graft. These findings indicate that poor outcome after transplantation is associated with progressive dopaminergic denervation in areas outside the grafts, a process which may have started already before surgery. Also, that the development of dyskinesias after transplantation is not associated with excessive DA release from the grafts. Finally, our data provide evidence that long-term immunosuppression can be withdrawn without interfering with graft survival or the motor recovery induced by transplantation.

Keywords: Parkinson's disease; neural transplantation; dopamine; dyskinesias; positron emission tomography

Abbreviations: CDRS = Clinical Dyskinesia Rating Scale; DA = dopamine; GID = graft-induced dyskinesias; SNpc = substantia nigra pars compacta; SPM = statistical parametric mapping; UPDRS = Unified Parkinson's Disease Rating Scale; VTA = ventral tegmental area

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Introduction

The clinical trials with transplantation of human embryonic mesencephalic tissue in patients with Parkinson's disease have demonstrated that grafted neurons can reinnervate the

denervated striatum (Kordower *et al.*, 1995, 1996, 1998), release dopamine (DA) (Piccini *et al.*, 1999) and become functionally integrated in host neural circuitries (Piccini

et al., 2000). However, the functional outcome after transplantation has been variable with some patients showing major improvement and others no or only modest clinical benefit (Lindvall and Hagell, 2000; Freed *et al.*, 2001; Olanow *et al.*, 2003; Polgar *et al.*, 2003). Similarly, the transplant-induced increases in ^{18}F -dopa uptake in the grafted striatum, as assessed with PET, have varied between patients, suggesting that the magnitude of survival and growth of the grafted DA neurons is one important factor in determining the degree of symptomatic relief. From the autopsy cases of Kordower *et al.* (1995, 1996, 1998), it seems that a good clinical response is associated with survival of at least 100 000 dopaminergic neurons in the putamen and reinnervation of 1/3 to 1/2 of putaminal volume. This level of graft survival and fibre outgrowth probably corresponds to a recovery of putaminal ^{18}F -dopa uptake to ~50% of normal (Hagell and Brundin, 2001). It is clear, though, that the magnitude of increase of putaminal ^{18}F -dopa uptake does not always match the level of improvement after transplantation. For example, in the series of Olanow *et al.* (2003), there was a significant one-third increase of ^{18}F -dopa uptake in the grafted posterior putamen but no clinical improvement compared with sham-operated patients at the end of the 2-year follow-up period. This poor response may in part be explained by more advanced pathology in these patients as evidenced by substantially higher doses of anti-parkinsonian medication as compared with patients in the Lund programme (Hagell *et al.*, 2002; Olanow *et al.*, 2003).

Although the loss of nigrostriatal DA neurons is regarded as the main cause underlying disease symptoms in idiopathic Parkinson's disease, it is well known that these patients display a range of other degenerative changes in dopaminergic and other neuron systems (Braak *et al.*, 2003). So far, neither the selection of patients for grafting nor the transplantation procedure has been based on the preoperative distribution and magnitude of the degenerative changes. It is also unknown to what extent degeneration continues in non-grafted areas following transplantation, and if the occurrence of a more extensive dopaminergic denervation is compatible with a good clinical response.

A major problem for dopaminergic cell replacement in Parkinson's disease is that ~15% of patients develop troublesome dyskinesias in the off-phase after transplantation of embryonic mesencephalic tissue (Freed *et al.*, 2001; Hagell *et al.*, 2002; Olanow *et al.*, 2003). This adverse event has been proposed to be the result of excess DA caused by continued outgrowth from the grafts (Freed *et al.*, 2001). However, the occurrence of graft-induced dyskinesias (GID) has not correlated with high post-operative striatal ^{18}F -dopa or with the most pronounced graft-induced increases in striatal ^{18}F -dopa. When comparing regional putaminal ^{18}F -dopa in dyskinetic and non-dyskinetic grafted patients, Ma *et al.* (2002) found evidence of an imbalance between the dopaminergic innervation in the ventral and dorsal putamen in the dyskinetic cases. In contrast, Olanow *et al.* (2003) reported no differences in either regional

or global levels of striatal ^{18}F -dopa between patients with and without GID. It must be emphasized, however, that even if the density of the transplant-derived dopaminergic reinnervation as evidenced with ^{18}F -dopa-PET was not pathological in dyskinetic patients, GID could still be caused by dopaminergic mechanisms, such as abnormal regulation of DA release from the grafts (Cenci and Hagell, 2005).

The poor outcome in the two clinical trials in which either no (Freed *et al.*, 2001) or only short-term, low-dose immunosuppression (Olanow *et al.*, 2003) was given has raised the possibility that immune reactions might compromise the survival and function of dopaminergic grafts in humans. Thus, the initial significant improvement in the grafted patients of Olanow *et al.* (2003) as compared with sham-operated cases was lost following withdrawal of immunosuppression after 6 months. Also, in two patients who came to autopsy, the grafts were surrounded by activated microglia suggesting an immune response (Olanow *et al.*, 2003). Such inflammatory reactions could lead to reduced graft survival, functional deterioration (Hudson *et al.*, 1994; Shinoda *et al.*, 1995), and possibly also the development of dyskinesias (Cenci and Hagell, 2005). However, it is not clear whether these consequences are observed if immunosuppression is withdrawn when the grafts are fully developed at 1–2 years.

Here we studied patients with idiopathic Parkinson's disease who had received intrastriatal grafts of human embryonic mesencephalic tissue within the Lund/London/Marburg transplantation programme. The objectives were 3-fold: first, to describe the patterns of dopaminergic innervation and denervation across the whole brain preoperatively and after transplantation, and to test the hypothesis that in cases with good outcome, the dopaminergic degeneration is minimal outside grafted areas; secondly, to explore whether there is an abnormal regulation of DA release from grafted dopaminergic terminals, and if the occurrence of GID is dependent on excessive DA release in putaminal subregions; thirdly, to investigate if withdrawal of long-term immunosuppression in transplanted patients compromises graft survival and is associated with development of dyskinesias and clinical deterioration.

Methods

Patients

Nine patients (numbers 4, 7 and 12–18) from our full series of 14 grafted idiopathic Parkinson's disease patients (Hagell *et al.*, 2002) were selected. For the remaining grafted patients (numbers 1–3, 9 and 10), PET and/or clinical data needed to meet study objectives were not available. The number of patients included in the various analyses is based on availability of required data. Patients' consent was obtained according to the Declaration of Helsinki and the procedures were approved by the local Ethical Committees in Lund, London and Munich.

Grafting procedures

Details have been described elsewhere (Wenning *et al.*, 1997; Hagell *et al.*, 1999, 2002; Piccini *et al.*, 1999; Brundin *et al.*, 2000). Briefly, all

patients received stereotaxic implantations of dissociated human embryonic ventral mesencephalic tissue. Patients 4 and 18 were grafted unilaterally in the right putamen (Wenning *et al.*, 1997; Piccini *et al.*, 1999; Hagell *et al.*, 2002), Patients 7 and 17 were grafted bilaterally in the putamen (Wenning *et al.*, 1997; Hagell *et al.*, 1999, 2002;) and Patients 12–16 were grafted bilaterally in the putamen and caudate nuclei (Brundin *et al.*, 2000). All patients received immunosuppressive therapy from 2 days before the first transplantation (Wenning *et al.*, 1997) using a standard regimen of cyclosporin, azathioprine and prednisolone (Lindvall *et al.*, 1989). Apart from Patient 14 (azathioprine discontinued during the first post-operative month owing to a liver reaction), this regimen was maintained for 16–64 (mean 29) months. Cyclosporin levels were carefully monitored and dose titrations were made accordingly throughout the treatment period. The maintenance level of cyclosporin was 100–150 ng/ml.

Clinical assessment

Patients were selected and followed according to the Core Assessment Program for Intracerebral Transplantations (Langston *et al.*, 1992). Clinical evaluations were performed in the practically defined 'off' phase (i.e. in the morning ≥ 12 h after the last dose of anti-parkinsonian medication) and following the intake of an individually standardized single dose of L-dopa, which was the same at each assessment. Dyskinesias were assessed retrospectively from blinded video recordings in practically defined 'off' and during the peak of the L-dopa-induced 'on' response using the Clinical Dyskinesia Rating Scale (CDRS) (Hagell and Widner, 1999; Hagell *et al.*, 2002). Patients' daily dopaminergic drug requirement was expressed as L-dopa equivalents (Hagell *et al.*, 2002).

Overall outcome was evaluated independently by two assessors according to the global ordered outcome score proposed by Schouten (2000). Such a score totals favourable and unfavourable results by taking aspects such as the underlying parkinsonism, non-motor features, complications of disease and therapy, daily functioning and drug requirement into consideration. A score of 5 = great beneficial effects and no side effects, or beneficial effects are much more important than side effects, a score of 4 = beneficial effects are slightly more important than side effects, a score of 3 = side effects and beneficial effects are equally important, or both are absent, a score of 2 = side effects are (slightly) more important than beneficial effects and a score of 1 = serious side effects and no beneficial effect, or side effects are much more important than beneficial effects. In this study, complications of disease were considered together with side effects.

PET

Study of patterns of dopaminergic innervation and denervation

We localized significant changes in dopaminergic innervation across the whole brain by applying statistical parametric mapping (SPM) to ^{18}F -dopa PET studies. Parametric images of ^{18}F -dopa K_i , generated as previously described (Whone *et al.*, 2004), were interrogated with SPM99 software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) implemented in Matlab5. ^{18}F -dopa parametric images were spatially normalized to a normal ^{18}F -dopa template created with SPM software as previously described (Whone *et al.*, 2004). Normalized ^{18}F -dopa parametric images were then spatially smoothed using a $6 \times 6 \times 6$ mm (full-width at half

maximum) isotropic Gaussian kernel. We performed two separate analyses:

- (i) Within-group analysis in the transplanted cohort. Preoperative ^{18}F -dopa scans were compared with scans obtained at 1 year, and ^{18}F -dopa scans obtained at 1 year were compared with the scans performed 2 years after transplantation. Six patients with bilateral grafts were included in the analysis (Patients 12–17).
- (ii) Between-group analysis. Preoperative and 1- and 2- year post-transplantation follow-up ^{18}F -dopa scans of each individual patient were compared with a group of normal subjects to evaluate the pattern of dopaminergic denervation outside the area of transplantation. Eight patients with uni- or bilateral grafts were included in the analysis (Patients 7, 12–18).

Appropriate weighted contrasts were performed to localize significant changes in mean voxel K_i values with SPM. The contrasts were used to generate Z scores on a voxel basis using the general linear model (Friston *et al.*, 1995a, b). Regional brain differences were considered significant when maps of Z scores exceeded a threshold of 2.33 ($P < 0.01$) after correction for cluster size ($P < 0.05$). No global normalization was applied.

Study of DA storage capacity and release in grafted striatum

^{18}F -Dopa studies were performed to evaluate storage capacity of DA, whereas changes in availability of ^{11}C -raclopride binding after an acute challenge with methamphetamine were measured to assess DA release in the grafted putamen.

Eight transplanted patients (numbers 4, 7, 12–15, 17 and 18) and six non-grafted Parkinson's disease patients were studied. Each subject was PET scanned with ^{11}C -raclopride twice, 2–3 days apart, and was assigned randomly to have an intravenous dose of normal saline in one scan and methamphetamine (0.3 mg/kg) in the other scan. Saline or methamphetamine was administered as a bolus over 30 s, 7 min before the injection of ^{11}C -raclopride. Subjects did not know whether they would receive placebo or methamphetamine. A dose of ~ 130 MBq (range 121–135) of ^{11}C -raclopride was then injected intravenously. The analysis of the scans was performed using a region of interest (ROI) approach (Piccini *et al.*, 2003). In brief, parametric images of ^{11}C -raclopride binding potential (BP) and relative delivery (R_t) were generated from the dynamic ^{11}C -raclopride scans using a basis function implementation of the simplified reference region compartmental model with the cerebellum as the reference tissue. MR images for each subject were anatomically co-registered with their respective parametric images of ^{11}C -raclopride BP using integral images of tracer activity. Values of BP and R_t for caudate and putaminal regions were obtained by defining on the co-registered MRIs ROIs that were subsequently applied to the parametric images.

All the Parkinson's disease patients also had ^{18}F -dopa PET 1–2 days after their two ^{11}C -raclopride studies. A dose of ~ 110 MBq (range 104–118) of ^{18}F -dopa was administered intravenously over 30 s. Scanning began at the start of tracer infusion generating 25 time frames of 30 s to 5-min epochs over 93 min. ^{18}F -dopa PET scans were analysed using a standard ROI approach and multiple time graphical analysis with an occipital reference tissue input function (Brooks *et al.*, 1990; Rakshi *et al.*, 1999). Normal values of ^{18}F -dopa were obtained by selecting from our database a group of 16 healthy subjects matched for age and sex with the group of Parkinson's disease patients and studied using the same scanner and protocol as the Parkinson's disease patients.

Study of the effects of withdrawal of immunosuppression

Six patients (numbers 4, 7 and 14–17) with available ^{18}F -dopa PET and clinical data (UPDRS motor score and global CDRS score during the practically defined off-phase) prior to and after withdrawal of immunosuppression were studied. Clinical evaluations and ^{18}F -dopa uptake were compared prior to and after withdrawal of immunosuppression.

Statistics

Variables violating assumptions underlying the use of parametric statistics are described as median and inter-quartile range and analysed by means of non-parametric statistics. Other data are described as mean \pm standard deviation (SD), and analysed using parametric statistics. Hence, the Wilcoxon signed rank test and paired *t*-test are used for comparisons between two related samples, and correlations among variables are explored by Spearman rank correlation (r_s) and Pearson's product-moment correlation (r). *P*-values are 2-tailed. Statistical analyses were performed using SPSS 11.5 for Windows.

Results

Dopaminergic denervation and reinnervation

We first confirmed using SPM, which localizes significant changes across the whole brain, that the grafts gave rise to increased ^{18}F -dopa uptake bilaterally in the putamen, and also demonstrated concomitant reductions in the substantia nigra and the median raphe during the first 2 years after transplantation (Table 1; Fig. 1). We then wanted to explore with SPM, in a group of eight patients, the possibility that the extent of dopaminergic denervation in non-grafted areas influenced the outcome after transplantation. As we have reported previously (Hagell *et al.*, 2002), all patients in this group showed increased putaminal ^{18}F -dopa uptake after transplantation indicating graft survival. Examination of ^{18}F -dopa uptake outside the transplanted striatum in individual patients revealed that the three patients who at follow-up had the best global outcome score of 5 (Patients 7, 13 and 15) did not show any decreases in ^{18}F -dopa uptake in these regions in comparison with a normal control group either preoperatively or post-operatively (Table 2). In the others, we found reductions in the ventral striatum (Table 2). Although Patients 14 and 12 (score of 4) did not show any decreases in the preoperative scans, they displayed decreases in the ventral

striatum in the first and second post-operative scans. The patients with the worst global outcome (Patients 16, 17 and 18) exhibited decreased ventral striatal ^{18}F -dopa uptake in comparison with normal controls both before and after transplantation (Table 2).

DA release and occurrence of GID

We first explored whether there was a correlation between DA storage capacity as measured by ^{18}F -dopa uptake and drug-induced endogenous DA release quantified with ^{11}C -raclopride-binding in the grafted putamen. Data from 14 grafted putamina were included in the analysis (from eight patients, six with bilateral and two with unilateral grafts; Table 3). There was about a 10% reduction of putaminal ^{11}C -raclopride BP after methamphetamine in comparison with saline (Table 4). This reduction is substantially less than the 25% reported for the putamen in a group of normal volunteers (Piccini *et al.*, 2003).

Next we plotted the ^{18}F -dopa K_i values and the percentage reduction of ^{11}C -raclopride BP induced by methamphetamine for each patient's grafted putamen (Fig. 2). There was a significant positive correlation between levels of DA storage and drug-induced DA release (Fig. 2; $r = 0.7$, $P = 0.01$). We compared these findings with those from a previously reported correlation analysis using data from non-grafted Parkinson's disease patients (Piccini *et al.*, 2003). Interestingly, there was a trend that for a similar level of putamen ^{18}F -dopa uptake in grafted and non-grafted patients, the percentage change in ^{11}C -raclopride BP induced by methamphetamine was less pronounced in the transplanted cases (Fig. 2). Taken together, these findings do not support the hypothesis of excessive DA release from the terminals of grafted neurons.

We finally explored the possibility of a relationship between levels of basal and/or drug-induced DA release from grafted neurons and the severity of GID. The global off-phase CDRS scores for each side of the body were plotted against the ^{11}C -raclopride BP in the entire contralateral putamen or in its anterior and posterior parts separately. No significant correlations were detected in any of these analyses (Fig. 3A–F). Thus, we found no evidence of an association between GID and abnormally high regional putaminal DA release either under basal conditions or after methamphetamine administration.

Table 1 Clinical characteristics of patients ($n = 6$) subjected to repeated ^{18}F -dopa-PET scans prior to and at 1 and 2 years after bilateral transplantation to monitor pattern of dopaminergic denervation and reinnervation

	At first transplantation	1 year after transplantation	2 years after transplantation ^a
Age/duration of Parkinson's disease (years) ^b	54.1 \pm 9.2/13.1 \pm 1.9		
Hoehn and Yahr stage ^c	3 (3–3.9)		
Daily dose of L-dopa equivalents (mg) ^b	908.3 \pm 432.9	535.8 \pm 424	460 \pm 329.3
UPDRS motor score in 'off' ^c	40.5 (29.8–58)	20 (17.5–27.9)	23.3 (20.5–32.3)
Time spent in 'off' (%) ^b	25.2 \pm 20.4	13.4 \pm 15.5 ^d	21.3 \pm 21.6 ^d
Global CDRS dyskinesia score in 'off' ^c	0 (0–1.5) ^e	0.8 (0–6.8) ^f	2 (1.3–10.9) ^f

^aOne patient was scanned 4 years after transplantation. ^bMean \pm SD. ^cMedian (interquartile range). ^d $n = 4$. ^e $n = 3$. ^f $n = 6$.

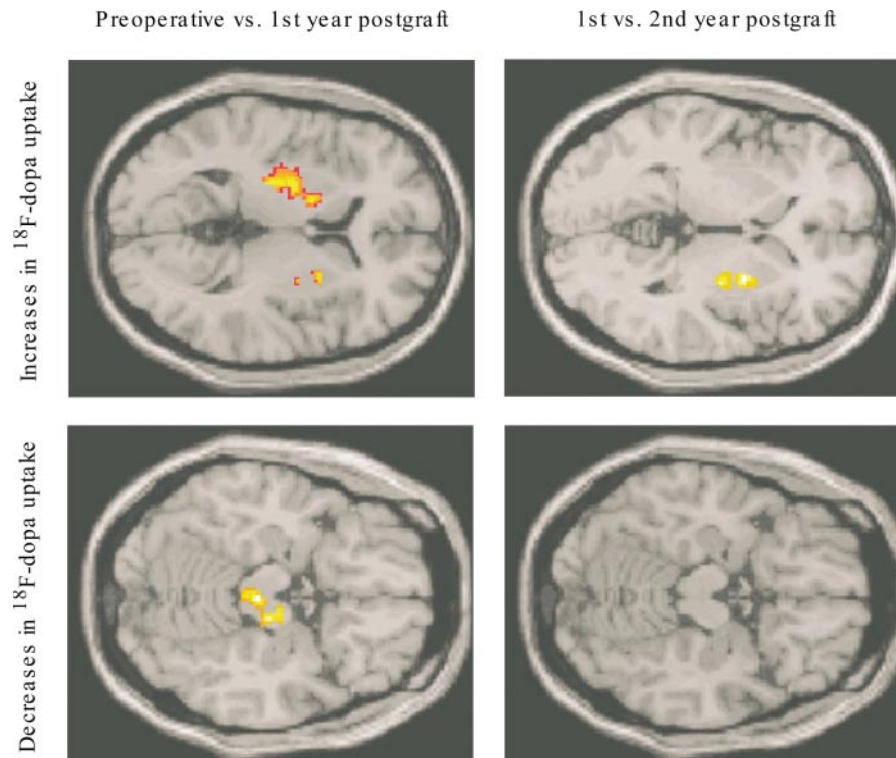


Fig. 1 Transaxial projections of statistical parametric maps showing areas of significant increases (top) and decreases (bottom) in ^{18}F -dopa uptake in six patients with Parkinson's disease subjected to bilateral intrastriatal transplantation of embryonic mesencephalic tissue. Left: preoperative versus first year post-graft comparison. Right: first year versus second year post-graft comparison (one patient was scanned 4 years after transplantation). The areas are superimposed on a standard MRI template. Clinical characteristics are summarized in Table 1. Scans performed 1 year after transplantation showed foci of significantly increased putaminal uptake bilaterally compared with preoperatively [right putamen 22, 0, 2 (x, y, z) $Z = 3.08$; left putamen $-22, -6, -2$ (x, y, z) $Z = 3.56$]. The increase was greater for the left putamen (right putamen, cluster size = 104; left putamen, cluster size 535). Between the first and second post-operative ^{18}F -dopa scans there was a further increase in ^{18}F -dopa uptake in the right putamen [28, 2, 2 (x, y, z), $Z = 2.53$, cluster size = 153], whereas ^{18}F -dopa uptake did not change on the left side. Possible explanations for the differences between the sides in this patient group are discussed in Brundin *et al.* (2000). Decreases in ^{18}F -dopa uptake were only found in the right substantia nigra [14, $-18, -16$ (x, y, z) $Z = 2.90$] and in the median raphe [4, $-24, -16$ (x, y, z) $Z = 3.04$] at the time of the first follow-up, whereas no further significant decreases were found at the time of the second follow-up.

Effects of withdrawal of long-term immunosuppression

We identified six patients (numbers 4, 7 and 14–17) who had been subjected to ^{18}F -dopa-PET prior to and after withdrawal of immunosuppression (Table 5). One patient was grafted unilaterally and five patients bilaterally in the putamen with a mean interval of 4 months between the two surgeries. In these patients, immunosuppression was completely withdrawn at a mean of 29 months after the last transplantation. We compared clinical evaluations of parkinsonism (UPDRS motor score) and dyskinesias (global CDRS score) prior to and after stopping immunosuppression at time points as close as possible to when ^{18}F -dopa-PET had been performed. Clinical data were collected at two time points prior to withdrawal of immunosuppression (at a mean of 26 and 9 months before withdrawal) and at a mean of 23 months after immunosuppression had been completely stopped. ^{18}F -dopa-PET scans were carried out at a mean of 9 months before withdrawal was started and 21 months after it had been completed.

The ^{18}F -dopa K_i values in the grafted striatum (expressed as percentage of the normal mean in healthy controls), showed no evidence that the withdrawal of immunosuppression compromised the survival of the grafts. In fact, the putaminal K_i value for the whole group increased from an average of $\sim 46\%$ before to 65% of normal mean after stopping immunosuppression ($P = 0.03$; Fig. 4A and Table 5). We did not observe any significant clinical deterioration as evaluated using the UPDRS motor score prior to and after drug withdrawal (22 and 21, respectively; $P = 0.22$; Fig. 4B and Table 5). In contrast, dyskinesias in the 'off' phase had increased in all six patients at the assessment post-immunosuppression (Fig. 4C). The global CDRS score was significantly higher ($P = 0.03$) in the whole patient group after as compared with before stopping immunosuppression (Table 5).

Discussion

We demonstrate here that during the first 2 years after transplantation of human embryonic mesencephalic tissue,

degenerative and regenerative changes in the dopaminergic system occur simultaneously in grafted patients. Applying SPM in our group of patients, we have shown for the first time, that increases in ^{18}F -dopa uptake in the grafted putamen were accompanied by decreases in substantia nigra and median raphe region. Thus, we found no evidence indicating that the grafts protect intrinsic dopaminergic neurons in the

Table 2 Global outcome score and occurrence of decreases in ^{18}F -dopa uptake in ventral striatum of individual patients before and after transplantation

Patient number	Global outcome score	Decreases in ventral striatal ^{18}F -dopa uptake*		Co-ordinates MNI space [†]			Z score
				x	y	z	
7	5	Pre	No				
		1st post	No				
		2nd post	No				
13	5	Pre	No				
		1st post	No				
		2nd post	No				
15	5	Pre	No				
		1st post	No				
		2nd post	No				
12	4	Pre	No				
		1st post	Yes	24	12	-4	2.98
		2nd post	Yes	12	12	-6	2.99
14	4	Pre	No				
		1st post	Yes	-18	8	-4	2.94
		2nd post	Yes	-18	10	-6	4.25
16	3	Pre	Yes	10	-20	-6	3.53
		1st post	Yes	8	-20	-6	3.87
		2nd post	Yes	16	-16	-6	4.58
18	3	Pre	Yes	22	20	-4	3.48
		Post	Yes	-18	8	-6	4.13
17	1	Pre	Yes	16	-2	-4	3.45
		Post	Yes	14	12	-6	3.74

*SPM comparison: each subject's ^{18}F -dopa scan has been compared with scans from a group of normal volunteers. [†]Montreal Neurological Institute.

Table 3 Clinical characteristics of patients ($n = 8$) subjected to both ^{11}C -raclopride- and ^{18}F -dopa-PET

	At first transplantation	At time of PET scans
Age/duration of Parkinson's disease (years)*	51.9 ± 5.9/12 ± 2.5	
Hoehn and Yahr stage [†]	3 (3–4.6)	
Daily dose of L-dopa equivalents (mg)*	911.9 ± 605.9	490.1 ± 617.4
UPDRS motor score in 'off' [†]	42.5 (38.8–55.6)	22.5 (17.0–22.1)
Time spent in 'off' (%)*	28.3 ± 21.7	11.2 ± 15.4
Global CDRS dyskinesia score in 'off' [†]	0 (0–3)	3.5 (1.9–7.9)

*Mean ± SD. [†]Median (interquartile range).

substantia nigra against degeneration. It is conceivable that the presumed continuing loss of dopaminergic cell bodies and dendrites in substantia nigra, despite a functioning graft, could affect the evolution of symptoms after transplantation. Thus, animal experiments have indicated that DA in the substantia nigra can influence both locomotor activity and complex motor behaviours (Robertson, 1992; Bergquist *et al.*, 2003). Interestingly, our data suggest that those patients who showed the best functional response over the first 2 years after transplantation exhibited very little dopaminergic denervation outside the grafted areas. In contrast, we detected that in patients with moderate or poor overall outcome, a significant degeneration in the ventral striatum developed during the follow-up period or was present already prior to surgery.

The DA neurons projecting to the putamen, located in the ventral tier of the substantia nigra pars compacta (SNpc) (Lynd-Balta and Haber, 1994a, b; Lewis and Sesack, 1997), are the ones most consistently and severely affected in Parkinson's disease patients. The loss of these cells is prominent early in the disease and becomes more pronounced over time (Fearnley and Lees, 1991; Damier *et al.*, 1999). The DA neurons innervating ventral striatal regions, including nucleus accumbens and adjacent parts of the caudate nucleus, are located in the dorsal tier of the SNpc and the ventral tegmental area (VTA) (Lynd-Balta and Haber, 1994a, b; Lewis and Sesack, 1997). In moderate-to-severe Parkinson's disease cases, the loss of DA neurons in parts of the dorsal tier and the VTA may vary, irrespective of disease duration,

Table 4 Mean ^{18}F -dopa uptake (K_i) and ^{11}C -raclopride BP in the grafted putamen of eight Parkinson's disease patients implanted with embryonic mesencephalic tissue

	Right putamen (n = 8)	Left putamen (n = 6)
^{18}F -dopa uptake (K_i)*	0.0072 ± 0.0027	0.0076 ± 0.0025
^{11}C -Raclopride BP		
Saline*	2.69 ± 0.45	2.88 ± 0.47
Methamphetamine*	2.39 ± 0.46	2.65 ± 0.56
$\Delta\%$ *	-10.58 ± 7.76	-8.52 ± 7.34
$\Delta\%$ for left + right putamen		-9.55%

*Mean ± SD. $\Delta\%$, percent change in ^{11}C -raclopride BP following administration of methamphetamine as compared with saline.

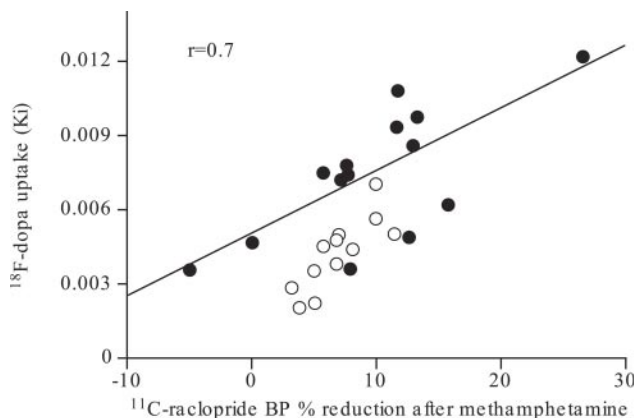


Fig. 2 Correlation between ^{18}F -dopa uptake (K_i) and percentage reduction of ^{11}C -raclopride BP after methamphetamine in the grafted putamen (data from left and right side were pooled) of six patients with bilateral and two patients with unilateral transplants (filled circles and line). For comparison, values from left and right putamen in a group of non-grafted Parkinson's patients are given (open circles; Piccini *et al.*, 2003).

from <30–40% to 80–90% in some cases (Fearnley and Lees, 1991; Damier *et al.*, 1999). Animal studies have shown that the DA neurons innervating ventral striatal regions play an important role in motor behaviour, such as response selection, behavioural switching and incentive-motivational processes, and that the execution of coherent behavioural responses requires coordinated DA release in both dorsal and ventral striatal areas (Dunnett and Robbins, 1992). Lesions involving the DA innervation of the dorsal striatum are known to result in motor initiation impairments (i.e. deficits in stepping and reaction time tasks, and impaired sensorimotor orienting responses), whereas DA-denervating lesions that involve also the ventral striatum and associated frontal and limbic cortical areas induce more profound impairments in the execution of goal-directed purposeful complex movements (Kirik *et al.*, 1998; Barneoud *et al.*, 2000; Winkler *et al.*, 2002). Transplantation studies in 6-hydroxydopamine-lesioned rats have shown that the functional recovery induced by embryonic DA neuron grafts,

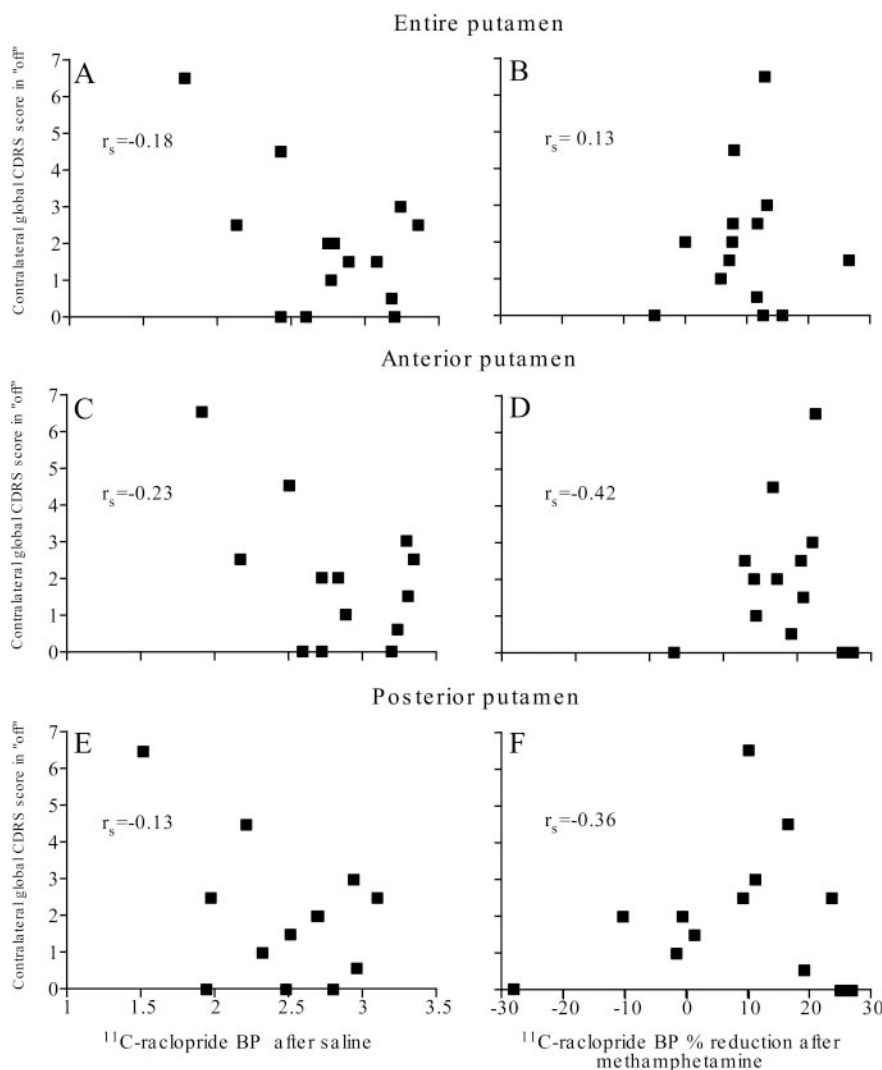


Fig. 3 Correlation between ^{11}C -raclopride BP after saline (A, C, E) or ^{11}C -raclopride BP percentage reduction after methamphetamine (B, D, F) in the entire (A, B) or anterior (C, D) or posterior (E, F) part of the putamen and the contralateral global CDRS score in 'off'.

Table 5 Clinical characteristics of patients ($n = 6$) subjected to ^{18}F -dopa-PET and neurological assessment before and after withdrawal of immunosuppression

	At first transplantation	Prior to withdrawal of immunosuppression	After withdrawal of immunosuppression
Age/duration of Parkinson's disease (years) ^a	56.3 ± 7.3/11.7 ± 2.4		
Hoehn and Yahr stage ^b	3 (2.75–3)		
Daily dose of L-dopa equivalents (mg) ^a	726.5 ± 310.9	416.7 ± 181.4	190.8 ± 211
UPDRS motor score in 'off' ^b	39.5 (29.8–42.3)	21.5 (19.4–37.2)	21.0 (17.8–24.3)
Global CDRS dyskinesia score in 'off' ^b	0 (0–1) ^c	0.8 (0–6.8)	4.3 (2.6–9.8)
^{18}F -dopa uptake in putamen ^{a,d}	28 ± 6.6	45.9 ± 9.9	64.8 ± 15.5

^aMean ± SD. ^bMedian (interquartile range). ^c $n = 5$. ^dPercentage of normal mean.

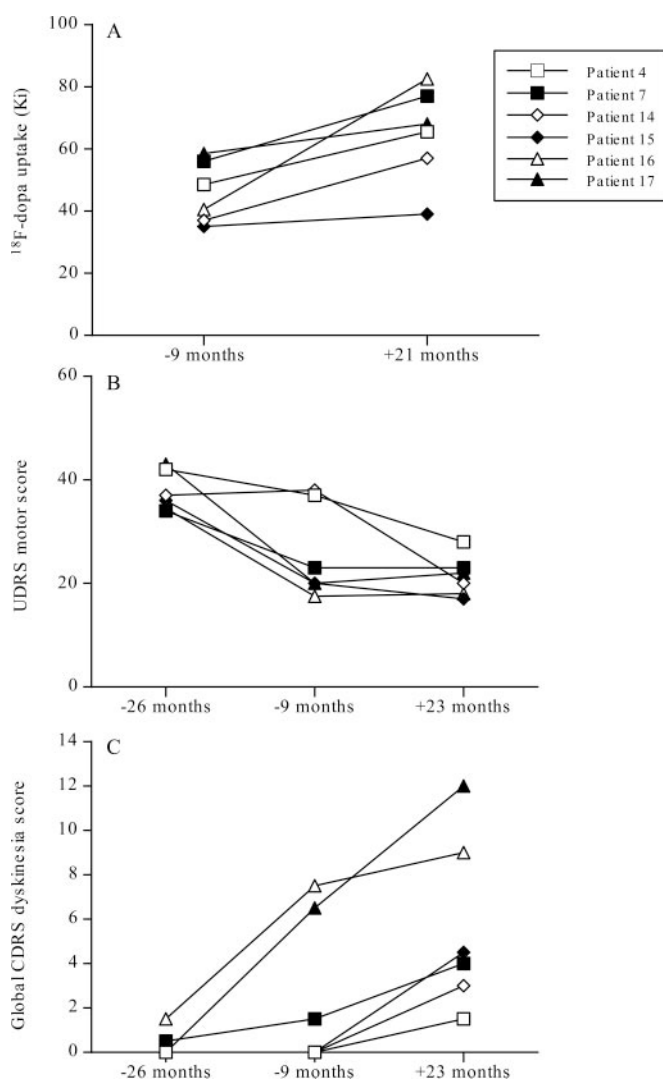


Fig. 4 Effects of withdrawal of immunosuppression in six Parkinson's patients on (A) ^{18}F -dopa uptake in grafted putamen (expressed as K_i in percentage of normal mean), and (B) UPDRS motor score and (C) global CDRS dyskinesia score in 'off'. Clinical data were collected at a mean of 26 and 9 months and PET scan data at a mean of 9 months before withdrawal and at 23 and 21 months, respectively, after complete withdrawal of immunosuppression.

placed in the dorsal striatum, is more pronounced in animals with lesions confined to the caudate-putamen than in animals with complete lesions of the entire mesencephalic DA fore-brain projection (Kirik *et al.*, 2001). In line with these experimental data, the present findings may be taken to indicate that the overall functional impact of DA neuron replacement in the putamen is less pronounced in patients with more widespread forebrain DA denervations, i.e. in patients where mesencephalic DA neuron cell loss involves also portions of the dorsal tier of the SNpc and the VTA. Spared portions of the host DA system, particularly those innervating ventral striatal and cortical areas, may thus be necessary for intraputamenal DA cell replacement to exert optimal functional effects.

The data presented here argue against the hypothesis that GID are caused by excessive release of DA. The reduction of raclopride BP after methamphetamine was similar in grafted putamina (~10%) to that which has previously been found in putamina of non-grafted patients (~7–8%) (Piccini *et al.*, 2003), and markedly lower than that in putamina of healthy volunteers (~25%; Piccini *et al.*, 2003). Thus, even though we did not measure DA release preoperatively in our patients, it is conceivable that the grafts had only partially restored levels of DA release in putamen. This observation is in contrast to our previous finding in one grafted Parkinson's disease patient in whom both basal and amphetamine-induced DA release was restored to normal levels (Piccini *et al.*, 1999).

Similar to that which has been observed in striata of non-transplanted Parkinson's disease patients (Piccini *et al.*, 2003), we found that the levels of drug-induced DA release in grafted putamina were related to the levels of DA storage capacity, reflecting the number of functioning nerve terminals. In fact, DA release tended to be relatively lower from terminals of grafted neurons compared with intrinsic innervation (Piccini *et al.*, 2003). In accordance, the methamphetamine-induced DA release from grafts implanted in the rat Parkinson's disease model was found to be lower as compared with that from host dopaminergic terminals (Brundin *et al.*, 1988). Finally, there was no correlation between the levels of basal or methamphetamine-evoked DA release in the putamen and the severity of 'off' phase dyskinesias in the contralateral side of the body for the individual patients. Other possible

explanations for GID could be, e.g. uneven or patchy reinnervation (Ma *et al.*, 2002) giving rise to low or intermediate levels of striatal DA. Alternatively, GID could be explained by unfavourable composition of the graft with respect to the predominant type of mesencephalic dopaminergic neurons from the substantia nigra or VTA (Isacson *et al.*, 2003) and the proportion of non-dopaminergic cells (Cenci and Hagell, 2005).

The withdrawal of long-term immunosuppression was not followed by reduction of putaminal ^{18}F -dopa uptake in any patient. Because UPDRS motor scores were unaltered, our findings indicate that immunosuppression can be stopped, at least between 2 and 3 years after transplantation, without compromising the survival and function of the graft. In fact, as judged by the increase of ^{18}F -dopa uptake, it seems that the grafts can continue to grow after withdrawal of immunosuppression. However, dyskinesia scores were significantly higher after the withdrawal of immunosuppression. The most straightforward explanation to the worsening of 'off' phase dyskinesias in these patients is the continuous growth of the grafts, although there is so far no evidence from the clinical trials that extensive dopaminergic growth is responsible for this adverse event (Hagell *et al.*, 2002; Ma *et al.*, 2002; Olanow *et al.*, 2003). Hypothetically, an inflammatory response after the withdrawal of immunosuppression may have promoted the development of dyskinesias without causing rejection of the graft (for discussion, see Cenci and Hagell, 2005). Inflammatory cells have been observed in and around the embryonic mesencephalic grafts in other clinical trials following discontinuation of cyclosporin treatment (Kordower *et al.*, 1997; Olanow *et al.*, 2003). Arguing against this possibility, the development of GID in three of six patients had started already prior to the withdrawal of immunosuppression.

The present data have several implications for the development of a cell replacement therapy for Parkinson's disease: First, the best long-term results after transplantation were observed in those patients in whom the degeneration of the intrinsic dopaminergic system continued to be confined to areas reached by the grafts. Thus, optimum symptomatic relief will most likely require tailor-made grafting procedures and multiple implantation sites based on detailed imaging of the denervation patterns in the individual patient. Secondly, no support was obtained for the possibility that GID were caused by excessive DA release from grafted neurons. Therefore, other underlying mechanisms have to be explored. Thirdly, long-term immunosuppression could be stopped without compromising the survival of the graft or the associated symptomatic improvement. However, withdrawal may be associated with worsening of GID. This could be owing to continued growth of the graft or, possibly, a low-grade inflammation around the graft, or to a combination of these two factors. In the coming years, transplantation of human embryonic tissue will probably remain an important research tool to explore how best to repair the Parkinson's disease patient's brain. However, it is unlikely, even if

dyskinesias can be avoided, that the current procedure will become routine treatment because of problems with, e.g. tissue availability. Stem cell technology has the potential to generate large numbers of dopaminergic neurons in standardized preparations (Lindvall *et al.*, 2004). Our study has provided new insight into factors which will be important for the success of stem cell-based transplantation approaches.

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