Delayed Recovery of Movement-Related Cortical Function in Parkinson's Disease after Striatal Dopaminergic Grafts

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Intrastriatal transplantation of dopaminergic neurones aims to repair the selective loss of nigrostriatal projections and the consequent dysfunction of striatocortical circuitries in Parkinson's disease (PD). Here, we have studied the effects of bilateral human embryonic dopaminergic grafts on the movement-related activation of frontal cortical areas in 4 PD patients using $H_2{}^{15}O$ positron emission tomography and a joystick movement task. At 6.5 months after transplantation, mean striatal dopamine storage capacity as measured by ^{18}F-dopa positron emission tomography was already significantly elevated in these patients. This was associated with a modest clinical improvement on the Unified Parkinson's Disease Rating Scale, whereas the impaired cortical activation was unchanged. At 18 months after surgery, there was further significant clinical improvement in the absence of any additional increase in striatal ^{18}F-dopa uptake. Rostral supplementary motor and dorsal prefrontal cortical activation during performance of joystick movements had significantly improved, however. Our data suggest that the function of the graft goes beyond that of a simple dopamine delivery system and that functional integration of the grafted neurones within the host brain is necessary to produce substantial clinical recovery in PD.

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a lifetime risk of 1 in 40.[1,2] It is characterized by tremor, rigidity, and hypokinesia. Levodopa treatment is effective in the initial phase, but within 5 to 10 years, most patients develop fluctuating therapeutic responses and involuntary movements. The critical pathological change in PD is degeneration of the substantia nigra and a consequent loss of dopaminergic innervation of the striatum. Implantation of dopamine-producing cells within denervated striatal structures seems to be a rational restorative approach to treat PD.

Studies in animal models of PD have shown that intrastriatal grafts of embryonic ventral mesencephalic dopamine neurones reinnervate the striatum, form synaptic contacts with host neurones, release dopamine, and improve motor function.[3,4] Clinical and ^{18}F-dopa positron emission tomography (PET) studies have demonstrated survival of intrastriatal grafts of human embryonic mesencephalic tissue associated with therapeutically valuable improvements in parkinsonian patients.[5,6] Histopathological findings in 2 patients have provided definite evidence for graft survival and dopaminergic reinnervation of the host striatum.[7–9] To what extent the grafts become functionally integrated in a patient's brain and the mechanisms leading to the beneficial effects in PD have not been clarified, however.

The major output of the neostriatum via pallidothalamic connections is to premotor and prefrontal cortical areas. Regional cerebral blood flow (rCBF) studies in PD patients have shown relatively impaired activation of the supplementary motor area (SMA) and dorsolateral prefrontal cortex (DLPFC) during performance of volitional movements.[10,11] The impairment

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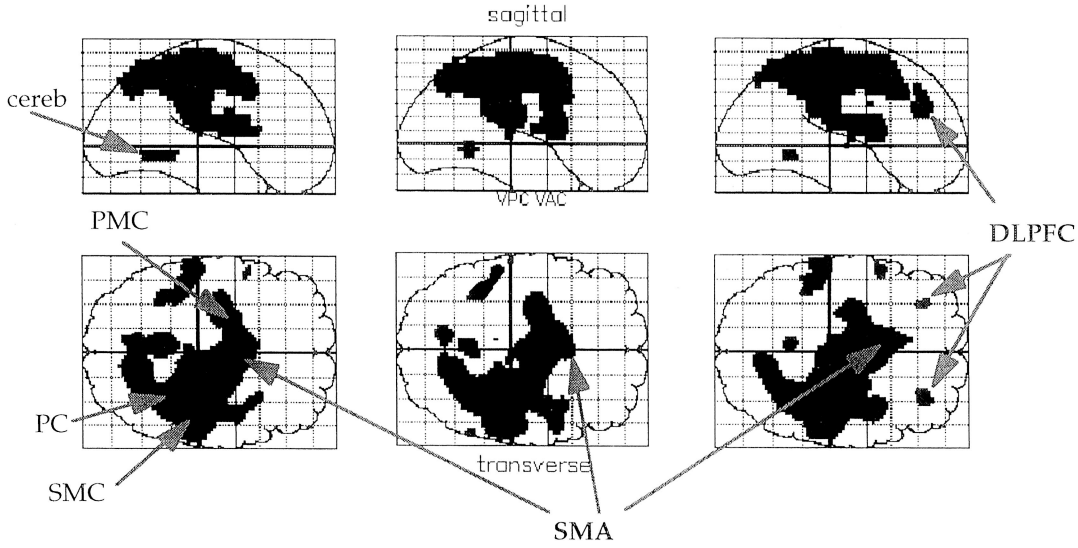

Fig 1. Areas of activation associated with freely selected joystick movements before (left), at 6.5 months (center), and at 18.3 months (right) after bilateral implantation of embryonic mesencephalic tissue within the caudate nucleus and putamen in 4 Parkinson's disease patients. Note that at a mean of 18.3 months after implantation, there is a further significant increase in activation of the rostral supplementary motor area and additional significant activation of the dorsolateral prefrontal cortex compared with preoperative values and values 6.5 months after surgery. The threshold value ($p < 0.01$) *was corrected for multiple nonindependent comparisons. SMC = sensorimotor cortex; PMC = lateral premotor cortex; PC = parietal cortex (Brodmann area 40); cereb = cerebellum; BG = basal ganglia; SMA = supplementary motor area; DLPFC = dorsolateral prefrontal cortex.*

of function in these association areas is postulated to underlie the akinesia of internally generated movements observed in PD patients.[10–12] Dopaminergic drugs such as apomorphine give marked symptomatic relief in PD and are able to restore activation of these cortical areas.[13]

The objective of this study was to explore whether dopaminergic grafts improve movement-related frontal cortical function in PD patients and whether this improvement occurs in parallel with an increase of striatal dopamine storage capacity and amelioration of parkinsonian symptoms.

Patients and Methods

Four patients with idiopathic PD (age range, 49.2 ± 5.9 years; disease duration, 12.8 ± 1.7 years) were implanted with human embryonic mesencephalic tissue bilaterally within the caudate nucleus and putamen. Clinical evaluations as well as ^{18}F-dopa PET and $H_2{}^{15}O$ PET activation studies were performed 1 to 5 months before transplantation and at 6.5 ± 2.7 and 18.3 ± 3.4 months after surgery.

Informed consent was obtained from the patients according to the Declaration of Helsinki, and the procedures were approved by the local Ethical Committees in Lund (Research Ethics Committee, Lund University) and London (Imperial College School of Medicine/Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals Research Ethics Committee).

Graft Preparation and Neurosurgical Procedure

Detailed descriptions are available elsewhere.[14–16] Briefly, dissociated ventral mesencephalic tissue from 3 to 5 aborted human embryos (aged 5–7 weeks after conception, length of 13–27 mm from crown to rump) was implanted on each side in the putamen and head of the caudate nucleus along five and two trajectories, respectively. Twenty microliters of cell suspension was deposited along each trajectory. To improve the survival of grafted dopamine neurones, the lazaroid tirilazad mesylate (3.0 μM) was added during each step of tissue preparation and was also administered to the patients (1.5 mg/kg intravenously four times per day) for 3 days after implantation. All patients were immunosuppressed with prednisolone, azathioprine, and cyclosporin. One patient was grafted bilaterally in one session, 2 patients were operated on with intervals of 2 and 4 weeks between surgery on each

Table 1. Locations and Peak z Scores of Areas in Which Regional Cerebral Blood Flow during Joystick Movements Was Significantly Greater 18.3 Months after Bilateral Implantation of Embryonic Mesencephalic Tissue into Caudate and Putamen Compared with Preoperative Status in 4 Parkinson's Disease Patients

Areas of Increased Activation	*x*	*y*	*z*	*z* Score
Rostral supplementary motor area	−1	1	54	4.31
Right dorsolateral prefrontal cortex	30	36	32	4.09

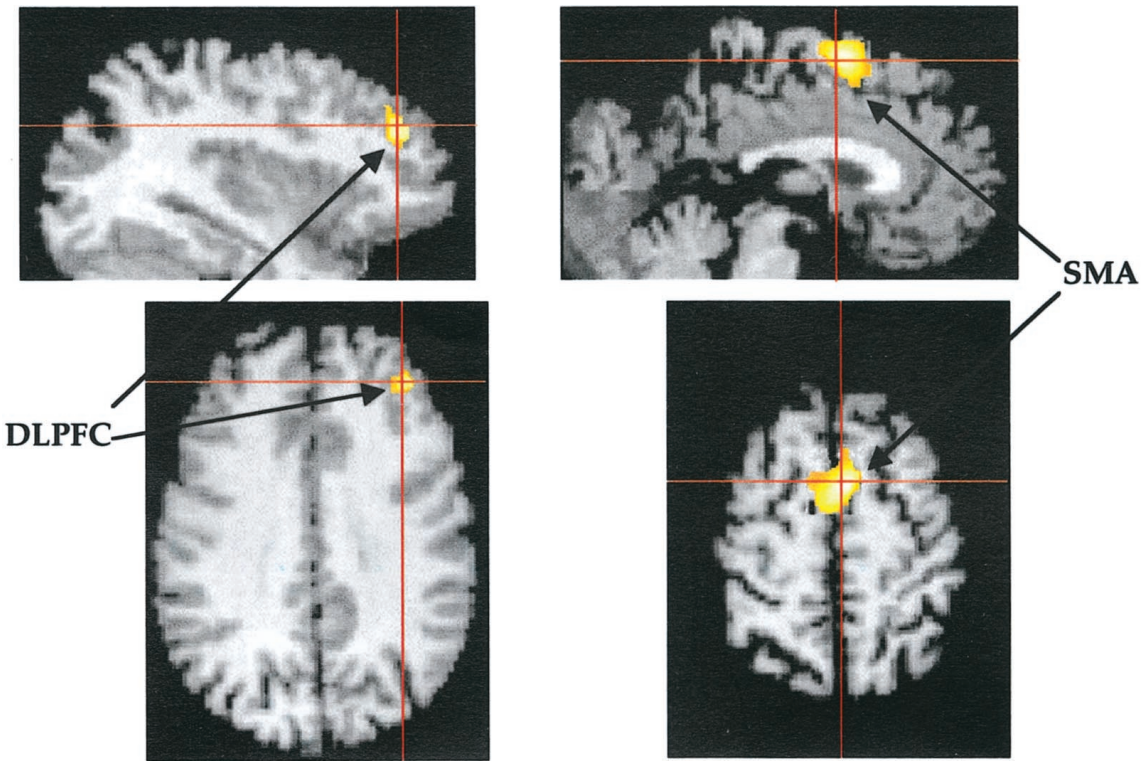

Fig 2. Areas of increased movement-related activation at a mean of 18.3 months after bilateral implantation of embryonic mesencephalic tissue within the caudate nucleus and putamen as compared with preoperative movement-related activation in 4 Parkinson's disease patients. Activated regions (threshold of $p < 0.001$*, with correction for multiple nonindependent comparisons) have been superimposed onto a normalized T1-weighted MRI scan. SMA = supplementary motor area; DLPFC = right dorsolateral prefrontal cortex.*

side, and 1 patient received the second graft 6 months after the first graft.

Clinical Evaluation

Patients were assessed clinically according to the Unified Parkinson's Disease Rating Scale (UPDRS)[17] in the practically defined "off" phase[18] at the time of PET scanning. In addition, they underwent clinical assessment according to the Core Assessment Program for Intracerebral Transplantation[18]; a detailed description of these clinical observations is published separately.[16]

PET Scanning Procedures and Data Analysis

Patients were scanned in the practically defined "off" condition after overnight withdrawal of anti-parkinsonian medication. $H_2{}^{15}O$ PET activation studies comprised 12 measurements of rCBF: 6 at rest and 6 while patients were performing paced joystick movements in freely selected directions using the left hand. The motor paradigm performed during PET thus consisted of two tasks: "rest" and "movement." The order of the task was balanced so as to eliminate the effects of time and habituation. During the "movement" task, the patients were instructed to move a joystick with their left hand in one of four freely chosen directions: left, right, forward, or backward. They were instructed to make a single movement as soon as possible after a pacing tone. The pacing frequency was set to one tone every 4 seconds. The "rest" condition involved the patients holding the joystick loosely with their right hand. They were instructed not to move and to ignore the pacing tones.

Scans were performed using a CTI 953 PET camera (CTI, Knoxville, TN. MRC, Cyclotron Building, Imperial College School of Medicine, Hammersmith Hospital, London, UK) and data acquired in a three-dimensional mode. An intravenous bolus of 3 ml of saline containing 11.5 mCi of $H_2{}^{15}O$ was injected for each measurement of rCBF. Scanning commenced 30 seconds after the start of tracer infusion, and there was a 10-minute interscan interval. ^{18}F-dopa PET was performed 1 to 2 days after the $H_2{}^{15}O$ studies. A dose of approximately 80 MBq of ^{18}F-dopa was administered intravenously over 30 seconds. Scanning began at the start of tracer infusion, with 25 time frames over 93 minutes. ^{18}F-dopa PET scans were analyzed using a standard region of interest approach and multiple time graphics analysis with an occipital reference tissue input function.[19]

$H_2{}^{15}O$ PET activation data were interrogated with statistical parametric mapping (SPM) to localize significant movement-related blood flow increases in brain areas. Realignment and normalization were performed using the SPM98 package (Wellcome Department of Cognitive Neurology, London, UK), and statistical analysis was performed with the SPM95 package (Wellcome Department of Cognitive Neurology, London, UK).[20] The images were realigned using the mean of all 12 scans as a reference and were transformed to standard stereotaxic space to allow for comparison of scan data in identical voxels across different subjects and

Table 2. Mean Striatal ^{18}F-dopa Uptake in 4 Parkinson's Disease Patients before and after Bilateral Implantation of Embryonic Mesencephalic Tissue in the Caudate Nucleus and Putamen

	Before Surgery		First Postoperative Evaluation (6.5 months)[a]		Second Postoperative Evaluation (18.3 months)[a]	
^{18}F-dopa K_i	Putamen	Caudate	Putamen	Caudate	Putamen	Caudate
Mean	0.0043	0.0086	0.0076[b]	0.0109[b]	0.0077[b]	0.0111[b]
SD	0.0010	0.0013	0.0016	0.0013	0.0014	0.0012

[a]Months after surgery.
[b]$p < 0.001$ compared with preoperative status (paired t test).

K_i = ^{18}F-dopa influx rate constant. The K_i values for each subject were given as the average of the left and right side values on three consecutive planes (x, y, z).

scans. The images were then smoothed using a Gaussian filter of 12-mm FWHM to remove high-frequency noise and to accommodate differences in gyral anatomy between subjects. Global blood flow was normalized by ANCOVA across the entire data set to a mean of 50 ml per 100 ml per minute. The normalization process adjusted the rCBF values for each voxel to take account of variations in global activity across subjects before and after surgery. The adjusted rCBF voxel values were used for the statistical analysis.

The patterns of cerebral activation associated with joystick movements before and after surgery were determined by comparing the adjusted mean rCBF values during the movement task with those during the rest task. To investigate the effects of transplantation on movement-associated activation, we conservatively considered the preoperative scans and the two sets of postoperative scans (at 6.5 and 18.3 months) as originating from 3 separate patient groups. We then tested for relative increases and decreases by comparing the postoperative movement-associated rCBF increases at 6.5 months with the preoperative movement-associated rCBF, the postoperative movement-associated rCBF increases at 18.3 months with the preoperative movement-associated rCBF, and the postoperative movement-associated rCBF increases at 18.3 months with those at 6.5 months. All comparisons were specified by appropriately weighted categorical contrast and performed on a voxel-by-voxel basis using ANOVA. This generates SPM t maps for the activation associated with each comparison. The SPM t maps were subsequently transformed to SPM z maps, and the levels of significance of areas of activation were assessed by the peak height of their foci using estimations based on the theory of random Gaussian fields. Significance was accepted if voxels survived a threshold value ($p < 0.01$) with correction for multiple nonindependent comparisons.

Results

Before transplantation, the brain areas where there was significant activation associated with performance of joystick movements in freely chosen directions included the right sensorimotor cortex, bilateral lateral premotor areas and SMA, inferior parietal association area (Brodmann area 40), medial parietal cortex (Brodmann area 7), and left cerebellum (Fig 1, left). The pattern of activation inducing increases in rCBF was similar to that described in previous studies on PD patients "off" medication[21]; in contrast to normal subjects performing this motor task, no significant activation in DLPFC was evident in PD patients.[10,11] No significant differences in cortical activation were observed in our PD patients at 6.5 months compared with preoperative cortical activation (see Fig 1, center), although $H_2{}^{15}O$ PET performed 18.3 months after grafting showed significant increases in rostral SMA and bilateral DLPFC activation during the task compared with during rest (see Fig 1, right). The comparison between increases in movement-associated activation before transplantation and at 18.3 months after grafting confirmed a significant increase in activation in rostral SMA and the right DLPFC at 18.3 months after surgery (Table 1, Fig 2).

In contrast, mean ^{18}F-dopa uptake values in the striatum were already significantly elevated at 6.5 months (+78% in the putamen and +27% in the caudate compared with preoperative values) with no further change at 18.3 months after grafting (Table 2; Fig 3). The increases in putaminal ^{18}F-dopa uptake were more pronounced than those in the head of the caudate.

The time course of clinical improvement paralleled that of the increases in task-induced activation of rostral SMA and DLPFC. Although symptomatic improvement was observed in the 4 patients at 6.5 months (25% reduction in UPDRS motor score), the patients continued to improve over the subsequent year, and by 18.3 months after surgery, the reduction in UPDRS motor score (50%) was highly significant (see Fig 3). Mean response times (time from the pacing tone to the completion of the joystick movement during scans) were faster at both 6.5 months (10%; $p = 0.06$) and 18.3 months (19%; $p = 0.02$) after grafting in comparison to the baseline times.

At 18.3 months, daily levodopa intake was reduced by a mean of 60%, and 1 patient was able to withdraw from levodopa treatment completely.

Discussion

This study demonstrates that intrastriatal grafts of human embryonic mesencephalic tissue in PD patients

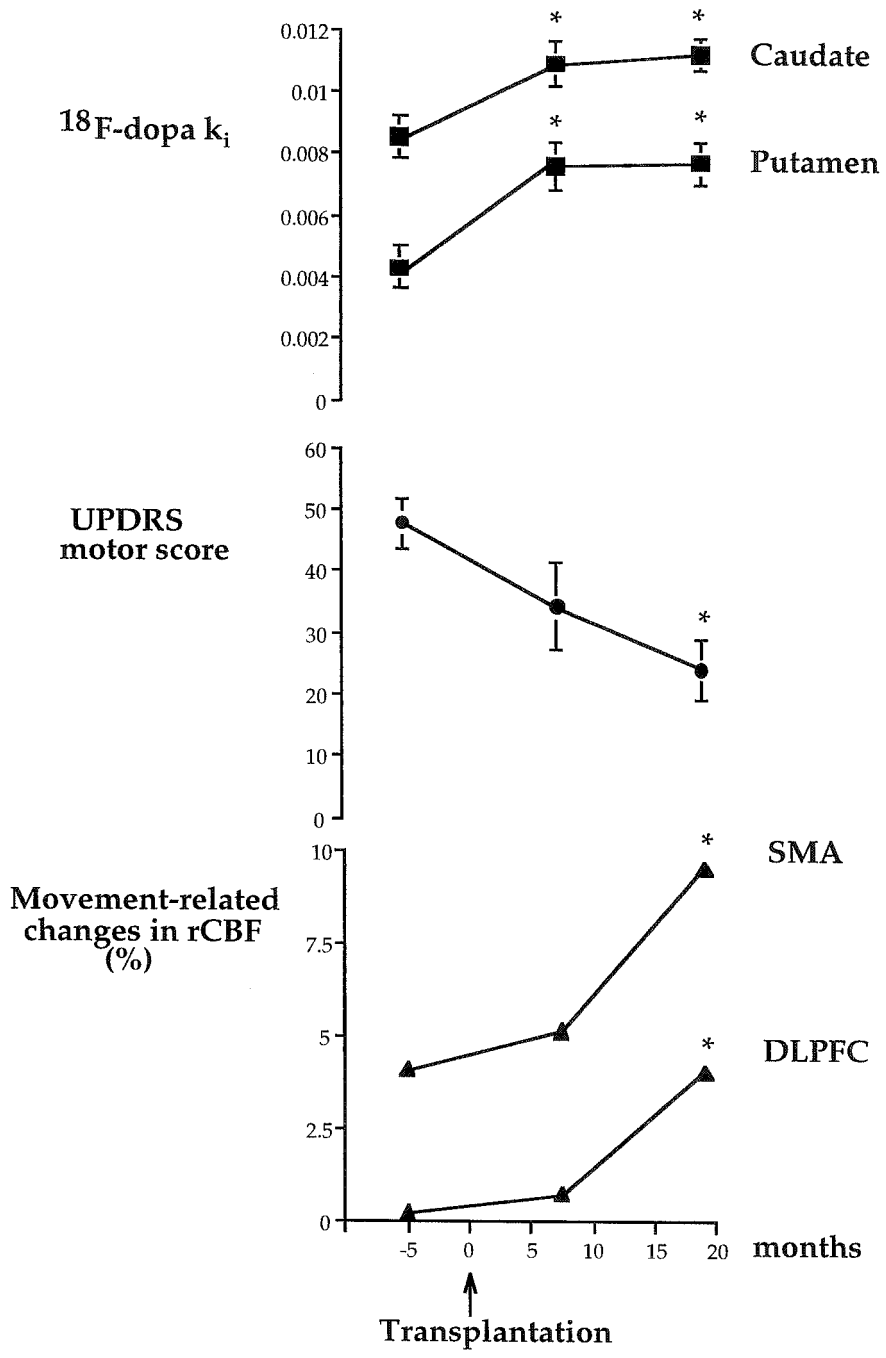

*Fig 3. Caudate and putaminal ^{18}F-dopa influx rate constant (k_i) values, United Parkinson's Disease Rating Scale (UPDRS) motor score, and percentage of movement-associated levels of regional cerebral blood flow in comparison to rest in the supplementary motor area (SMA) and dorsolateral prefrontal cortex (DLPFC) before surgery and at 6.5 and 18.3 months after bilateral implantation of embryonic mesencephalic tissue within the caudate nucleus and putamen. There was already a significant increase in ^{18}F-dopa uptake at 6.5 months after grafting, without any changes thereafter. Although modest symptomatic relief is observed at 6.5 months, the development of significant clinical improvement is delayed and coincides with the restoration of movement-related activation. The voxels selected for these results (SMA = −1, 1, 54; right DLPFC = 30, 36, 32 [x, y, z]) were those showing peak z scores (see Table 1). k_i values and UPDRS motor scores are mean ± SE. Months = months after surgery; ^{18}F-dopa k_i = ^{18}F-dopa influx rate constant. *p < 0.001 compared with preoperative value by Student's unpaired t test.*

can reverse specific deficits in brain function associated with degeneration of the nigrostriatal dopaminergic system. The grafts restored DLPFC activation and increased activation of rostral SMA during arm movements in freely selected directions. These frontal areas are known to be important in the preparation and selection of voluntary movements,[22,23] their function is influenced by basal ganglia-thalamocortical circuitries,[24] and their impairment is believed to underlie parkinsonian akinesia. Improvements in frontal activation and UPDRS motor scores occurred in parallel in our patients and continued over 18 months of follow-up despite no further significant increase in striatal ^{18}F-dopa uptake after 6 months. These data indicate that the significant clinical improvements in our patients were associated with more efficient cortical motor programming.

Previous clinical trials in parkinsonian patients have shown that the dopamine storage capacity in the striatum as evaluated by ^{18}F-dopa PET increases during the

first few months after transplantation and reaches maximal values within the first postoperative year in most cases.[25–28] Postoperative clinical recovery typically becomes evident 2 to 6 months after transplantation.[29–31] Some patients already exhibited maximum symptomatic relief at 12 months,[28] whereas others continued to improve beyond this time point.[27,31–33] Interestingly, the clinical improvement after grafting seems to show varying time courses for different symptoms in individual patients. Thus, rigidity has been observed to improve significantly as early as 4 to 7 months after surgery in contrast to the slowness of simple and complex arm and hand movements, which show a slower and continued improvement well beyond the first postoperative year in the same patient.[32,34]

Similar observations have been reported in animal experiments. In both the rat and marmoset PD models, the development of the capacity for dopamine synthesis and storage in grafts precedes the more gradual improvements in complex sensorimotor behavior by several months.[35,36] The establishment of a new dopamine-storing terminal network in the host striatum is closely correlated to the onset of graft-derived dopamine release[37] and reversal of dopamine receptor supersensitivity in the rat PD model.[36,38] These early effects may all be mediated by tonic nonregulated release of dopamine from spontaneously active grafted neurones. The later development of more pronounced functional effects suggests, however, a continued further maturation of the transplants and/or their synaptic connections with neuronal elements in the host. Fisher and colleagues[39] have shown that embryonic dopamine neurones grafted to the striatum in rats still retain immature electrophysiological features at 4 to 5 months after surgery and continue to mature over the subsequent months. This indicates that graft maturation may be a slow and protracted process, which continues many months after initial formation of the graft-derived dopamine-containing fiber projections.[40] Substantia nigra neurones in situ exert their functions in the striatum not only through tonic dopamine release but also by phasic changes in activity controlled by regulatory afferent inputs. Interestingly, in the study of Fisher and colleagues,[39] a large proportion (approximately 50%) of the grafted dopamine neurones received functional afferent inputs from the host cortex and thalamus. Burst firing is a critical feature of the phasic type of dopamine release and depends, at least in part, on cortical afferents developed in some of the grafted neurones.[41] This suggests that functional integration within the host corticostriatal circuitry may be important for full expression of the functional capacity of the grafted dopamine neurones. On the basis of these animal data, it seems likely that the protracted functional improvement seen in transplanted patients depends not only on graft survival and striatal reinnervation but also on the degree of graft–host integration.

In conclusion, our results show that embryonic dopaminergic transplants can restore cortical activation during freely selected movements in severely affected PD patients and provide new evidence that the functional effects of the grafted neurones go beyond those of a simple dopamine delivery system. We propose that restoration of nonregulated dopamine release such as that occurring in the early stages of maturation of the transplants is not sufficient to restore movement-related cortical activation and therefore only induces partial symptomatic relief. The ability of the grafted dopamine neurones to increase striatocortical neurotransmission and movement-related cortical activation and to give rise to substantial clinical improvement is likely to require the establishment of both afferent and efferent synaptic contacts with the host. Differences between patients in the degree and pattern of symptomatic recovery after transplantation could then be partly explained by variability in the magnitude of functional integration of the grafts in the host brain. These findings have important implications for our understanding of the mechanisms of action of neural transplants in PD and provide a critical test for the efficiency of cell replacement therapies in PD patients.

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