Dopamine release from nigral transplants visualized *in vivo* in a Parkinson's patient

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Synaptic dopamine release from embryonic nigral transplants has been monitored in the striatum of a patient with Parkinson's disease using [^{11}C]-raclopride positron emission tomography to measure dopamine D_2 receptor occupancy by the endogenous transmitter. In this patient, who had received a transplant in the right putamen 10 years earlier, grafts had restored both basal and drug-induced dopamine release to normal levels. This was associated with sustained, marked clinical benefit and normalized levels of dopamine storage in the grafted putamen. Despite an ongoing disease process, grafted neurons can thus continue for a decade to store and release dopamine and give rise to substantial symptomatic relief.

Cell transplantation in Parkinson's disease (PD) aims at correcting the impairment of striatal dopaminergic neurotransmission caused by degeneration of substantia nigra neurons. In rodents and nonhuman primates with experimental parkinsonism, intrastriatal transplants of embryonic nigral neurons reinnervate the striatum, form synaptic connections, release dopamine and improve motor deficits[1,2]. Clinical trials have demonstrated that grafts of human embryonic nigral neurons can give rise to long-lasting symptomatic relief in patients with PD[3,4]. In the most successful cases, improvement after transplantation has been dramatic, and patients in an advanced stage of the disorder have been able to stop L-dopa treatment and resume an independent life. The gradual onset of clinical improvement over the first 6–24 months after transplantation is compatible with the protracted development, growth and maturation of new striatal dopaminergic innervation by the grafted neurons. Thus the magnitude of the transplant-induced effect probably reflects the capacity of the grafted neurons for regulated dopamine release at regenerated synaptic sites. Survival of the grafted dopamine neurons, reinnervation of the host striatum and formation of synaptic connections were shown in two autopsy cases with PD[5,6]. However, it has not yet been possible to demonstrate dopamine release from transplants *in vivo* in humans. Here we monitored synaptic dopamine release from intraputaminal nigral grafts in a PD patient using binding of the dopamine D_2 receptor antagonist [^{11}C]-raclopride (RAC) and positron emission tomography (PET)[7,8].

Results

Our 69-year-old patient had typical PD, which started with rigidity and tremor in the left arm in 1980. L-dopa treatment was successful until 1986, when he developed 'on-off' motor fluctuations (see ref. 9). In 1989, he was grafted with human embryonic mesencephalic tissue in the right putamen[9]. During the first three postoperative years, the patient showed gradual, major clinical improvement (**Fig. 1a**). Rigidity and hypokinesia were markedly reduced bilaterally but predominantly contralaterally to the graft, 'on-off' fluctuations disappeared, and L-dopa could be withdrawn after 32 months[10]. Immunosuppressive treatment was stopped at 64 months[11]. Low-dose L-dopa (1/3 of preoperative dose) was reintroduced at 74 months due to progression of symptoms axially and in the limbs ipsilateral to the graft. He responded well to this medication, and motor function and L-dopa dose remained unchanged thereafter. Now, ten years after transplantation, he shows continuous marked benefit with virtually no rigidity, minor hypokinesia, intermittent, mild resting tremor and no 'on-off' fluctuations (**Fig. 1a**).

The regional [^{18}F]-dopa influx constant (K_i^o) values in the grafted putamen increased up to 3 years post-surgery, by which time [^{18}F]-dopa uptake had reached normal levels. There were only minor additional changes by 6 and 10 years after transplantation (**Fig. 1b**). In contrast, [^{18}F]-dopa uptake values in the caudate nuclei and in the non-grafted putamen (**Fig. 1b**) decreased gradually.

To monitor basal and drug-induced transmitter release from grafted dopaminergic neurons, the patient underwent two [^{11}C]-RAC PET scans ten years after grafting, one after a saline infusion and the other after a methamphetamine infusion. Binding of [^{11}C]-RAC to dopamine D_2 receptors was measured in the putamen and caudate nucleus on each side. [^{11}C]-RAC binding was upregulated by 43.7% compared with normal subjects in the non-grafted putamen but was normal in the grafted putamen (**Table 1** and **Fig. 2**). In the non-transplanted caudate nuclei of the PD patient, [^{11}C]-RAC binding was bilaterally increased with respect to binding in normal subjects. Following methampheta-

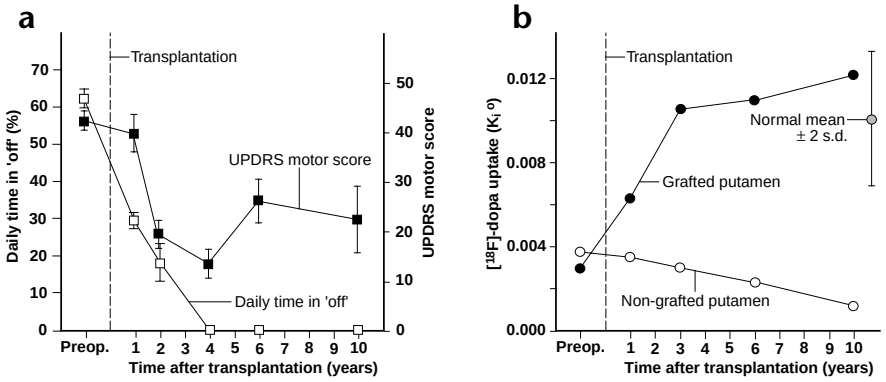

Fig. 1. Nigral transplants can give rise to long-lasting, major clinical improvement and restore dopamine storage in the striatum to normal levels despite an ongoing disease process. (**a**) Percentage of the day spent in the 'off'-phase and motor examination score of the Unified Parkinson's Disease Rating Scale (UPDRS; maximum score, 108) in the 'practically defined off' phase preoperatively and at various time points after implantation of ventral mesencephalic tissue from four human embryos into the anterior, middle and posterior portions of the right putamen in a patient with PD. Mean ± 95% confidence interval. (**b**) [^{18}F]-dopa K_i^o values in the grafted and non-grafted putamen of the same patient. Comparative data on [^{18}F]-dopa uptake in the putamen (mean ± 2 s.d.) are given for a group of 16 healthy volunteers. Data up to six years were reported previously[11].

mine administration, there was only a 4.5% reduction of [^{11}C]-RAC binding in the non-grafted putamen (**Table 1** and **Fig. 2**). In contrast, the binding reduction was much more pronounced (26.6%) in the grafted putamen and similar to the methamphetamine-induced decrease of [^{11}C]-RAC binding in the putamen of normal controls (24.2% and 23.6% in the left and right putamen, respectively; **Table 1**). We observed no difference between the right and left caudate nuclei in the decrease of [^{11}C]-RAC binding induced by methamphetamine.

Discussion

By use of noninvasive imaging of D_2 receptor occupancy, we have demonstrated that dopamine release from intrastriatal grafts of human embryonic mesencephalic tissue can be visualized *in vivo* in patients with PD. Our findings indicate that well-developed nigral grafts can restore both basal and drug-induced dopamine release to normal levels.

[^{18}F]-dopa uptake, a good measure of dopamine storage capacity of dopaminergic terminals in the striatum, was restored to normal levels in the grafted putamen compared with 12% of normal on the non-grafted side. The grafted putamen had normal [^{11}C]-RAC binding, whereas D_2 site availability was upregulated by 43.7% in the non-grafted putamen. Taken together, these data indicate that the new transplant-derived innervation tonically releases sufficient dopamine to restore D_2 receptor occupancy to normal levels in the previously denervated striatum. In untreated PD patients, PET and post-mortem autoradiographic studies have shown upregulation of dopamine D_2 receptor binding in the putamen[12–14], which reverses after L-dopa treatment[15]. The elevated D_2 receptor binding in the non-grafted putamen of this patient was not reversed by his recent treatment with low-dose L-dopa. The normal level of D_2 receptor binding in the grafted putamen (contralateral to the limbs that preoperatively showed the most severe parkinsonian symptoms) is consistent with studies after transplantation in animal models of PD. In rats and monkeys with lesions of the nigrostriatal system, dopaminergic grafts that reinnervate the striatum normalize the upregulated D_2 receptor binding[1,16], most probably as a result of continuous stimulation of postsynaptic sites by dopamine released from the graft-derived axon terminals. Indeed, *in-vivo* monitoring of dopamine release in the rat PD model demonstrates that grafted nigral neurons can restore baseline striatal dopamine levels to control values[1].

The pronounced difference between the right and left putamen of our PD patient in amphetamine-induced decrease in [^{11}C]-RAC binding is compatible with enhanced extracellular dopamine levels and, hence, fewer D_2 receptor sites available for [^{11}C]-RAC binding in the grafted striatum. Microdialysis studies in nonhuman primates indicate that a 25–30% decrease in striatal D_2 receptor binding after administration of ampheta-

Table 1. Values of striatal [^{11}C]-RAC binding potential (BP) and relative tracer delivery (R_I) from [^{11}C]-RAC parametric images after intravenous infusion of methamphetamine or saline in five normal subjects and in a PD patient ten years after transplantation of embryonic mesencephalic tissue into the right putamen.

	Normal subjects (n = 5)								PD patient							
	R caudate		L caudate		R putamen		L putamen		R caudate		L caudate		R putamen (grafted)		L putamen	
	BP	R_I	BP	R_I	BP	R_I	BP	R_I	BP	R_I	BP	R_I	BP	R_I	BP	R_I
Saline	2.82	0.96	2.68	0.91	3.01	1.03	2.93	0.97	3.22	0.99	3.28	1.02	2.89	1.01	4.21	1.04
(± s.d.)	(0.10)	(0.11)	(0.16)	(0.07)	(0.13)	(0.09)	(0.19)	(0.11)								
methamphetamine	2.36*	0.91	2.32*	0.82	2.30*	0.95	2.22*	0.91	2.9	0.96	2.99	0.98	2.12	0.97	4.02	1
(± s.d.)	(0.11)	(0.04)	(0.22)	(0.12)	(0.11)	(0.08)	(0.14)	(0.12)								
%Δ	–16.3%	–5.2%	–13.4%	–9.9%	–23.6%	–7.8%	–24.2%	–6.2%	–9.9%	–3.0%	–8.8%	–3.9%	–26.6%	–4.0%	–4.5%	–3.8%
(± s.d.)	(2.1)	(4.9)	(3.9)	(6.1)	(2.1)	(3.5)	(1.4)	(3.8)								

R, right; L, left; s.d., standard deviation; %Δ, percentage difference (methamphetamine – saline/saline ×100). *$p < 0.05$ compared with saline (paired *t*-test). Reductions in R_I were not correlated to reductions in BP ($r^2 = 0.03$; $p = 0.45$).

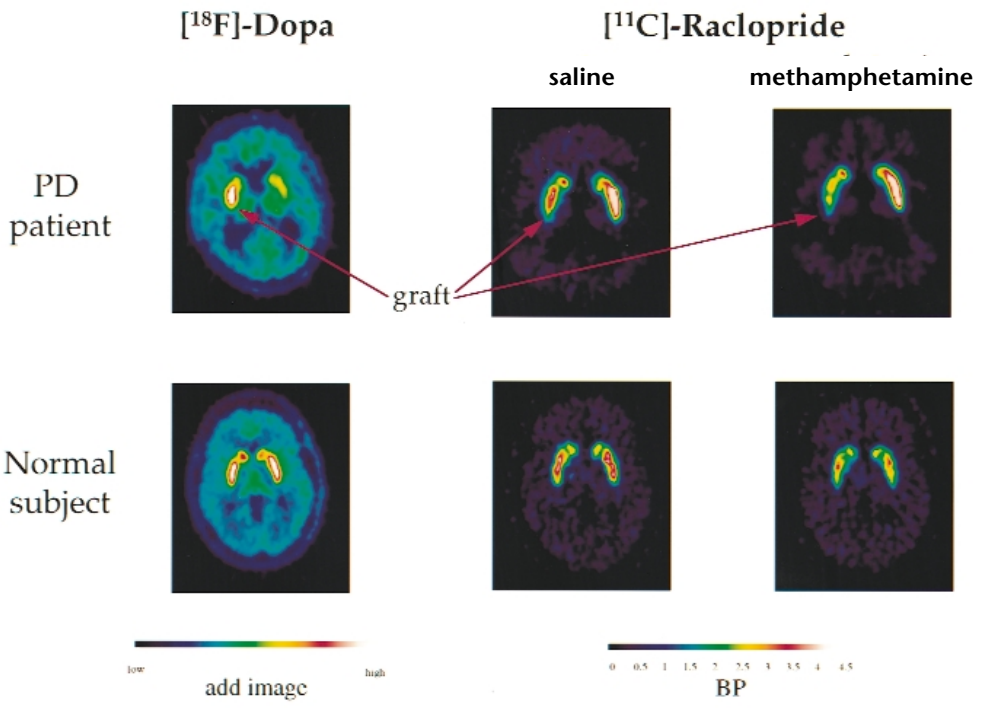

Fig. 2. Well-developed nigral grafts restore dopamine storage and basal and drug-induced dopamine release to normal levels in the striatum. Integral images of [^{18}F]-dopa (frames 1–26) and parametric images of [^{11}C]-RAC binding potential (BP) at the basal ganglia level for the PD patient ten years after transplantation of embryonic mesencephalic tissue to the right putamen (top) and for a normal subject (bottom). In the baseline condition (saline infusion, middle images), [^{11}C]-RAC binding is increased in the non-grafted putamen in the patient, whereas it is normal on the grafted side. After methamphetamine administration (right images), the binding reduction in the grafted putamen is similar to that seen in the putamen of normal subjects, whereas it is negligible in the non-grafted putamen. Arrows indicate the side of the putaminal implant in the PD patient.

mine (measured by single-photon emission computed tomography) reflects a 2- to 10-fold peak increase in extracellular dopamine levels[17]. Therefore, the 26.6% reduction in [^{11}C]-RAC binding detected in the grafted putamen of this patient following methamphetamine is likely to reflect a similar level of increase in extracellular dopamine derived from the transplanted neurons. The small, 4.5% reduction of [^{11}C]-RAC binding in the non-grafted putamen confirms the low capacity for dopamine release in this severely denervated structure. The blunted response to methamphetamine in the non-grafted putamen is similar to that found bilaterally in the putamen of two unoperated PD patients in an advanced stage of the disorder (patient 1, –4.5% and –6.7%; patient 2, –7.1% and –5.6%; right and left side, respectively; P.P. *et al.*, unpublished data).

Histopathological observations in another two patients with nigral transplants[5,6] confirm that increased striatal fluorodopa uptake is well correlated with dopaminergic neuron survival and extent of reinnervation of the host striatum. Our data in this patient suggest that in the best cases, nigral transplants reinnervate the putamen extensively, to a level sufficient to normalize both [^{18}F]-dopa uptake and D_2 receptor occupancy throughout the grafted putamen. The unilateral restoration of dopamine neurotransmission within the putamen was reflected by a sustained improvement of UPDRS motor score by about 50%. The level of motor impairment in this patient 10 years after transplantation and nearly 20 years after the onset of the disease was similar to that observed in early stages of PD. We found that grafts can survive for at least 10 years without any evidence of destruction by the disease process. The decrease of [^{18}F]-dopa uptake values in non-grafted striatal regions most likely reflects the progressive degeneration of the patient's own dopamine neurons. The long-term stability of the grafted neurons suggests that PD is caused not by an endogenous neurotoxin, but rather, by an intrinsic deficit in nigral dopaminergic neurons. There were no signs of rejection even 7.5 years after cessation of cyclosporine treatment and 4.5 years following complete withdrawal of immunosuppression.

In conclusion, this study provides evidence that well-developed and clinically efficacious nigral transplants restore both basal and drug-induced dopamine release in the striatum of PD patients. Moreover, this capacity can be maintained for at least a decade, despite exposure of the grafted neurons to an ongoing disease process. Although the present observations are based on a single and particularly successful case, the results support the view that efficient restoration of dopamine release throughout the grafted putamen can offer major clinical improvement in transplanted PD patients. Our data also demonstrate the usefulness of the [^{11}C]-RAC binding method for *in-vivo* analysis of dopamine release from grafted dopaminergic cells after transplantation. This imaging technique is highly needed because, in addition to embryonic mesencephalic tissue, other sources of dopamine-producing cells have been considered potentially useful for grafting in patients; these include adrenal medulla, sympathetic ganglia, carotid body and genetically engineered fibroblasts or stem cells[4]. The [^{11}C]-RAC PET method is a powerful tool in the further development of cell replacement strategies in PD.

Methods

Ethical permissions were obtained from the Imperial College School of Medicine, Hammersmith Hospital Trust Research Ethics Committee and the Research Ethical Committee of the Medical Faculty at the University of Lund, Sweden. Approval to administer radiolabel ligands was obtained from the Administration of Radioactive Substances Advisory Committee of the United Kingdom. Written consent was obtained from all subjects.

Transplantation procedure. Details are described elsewhere[9]. Briefly, dissociated ventral mesencephalic tissue was implanted stereotaxically along three trajectories in the anterior, middle and posterior part of the right putamen. The tissue was procured from four human embryos (aged six

to seven weeks postconception) obtained at routine suction abortions. The patient was given immunosuppressive treatment[9].

Clinical evaluation. Motor fluctuations ('on-off' phenomena) were recorded continuously by the patient, who scored his symptoms every 30 min. Clinical assessments were performed in the morning after a drug-free period overnight ('practically defined off'). The test battery included Unified Parkinson's Disease Rating Scale (UPDRS) motor examination score, timed motor tasks and a single-dose L-dopa test[9]. Clinical evaluations were carried out on six occasions during the year before surgery, monthly during the first postoperative year, and four to five times annually thereafter.

Scanning procedures. The PD patient and five healthy male right-handed subjects (45 ± 8 years of age) were scanned using an ECAT EXACT3D (CTI/Siemens 966) 3D-only PET tomograph after intravenous injection of 129.5–166.5 MBq of [^{11}C]-RAC. Each subject was scanned twice and was given an intravenous dose of saline in one scan and methamphetamine (0.3 mg per kg) in the other scan. Saline or methamphetamine was administered as a bolus over 30 s, 7 min before the injection of [^{11}C]-RAC. Subjects did not know whether they would receive saline or methamphetamine. The PD patient stopped medication for at least 12 h before scanning. Parametric images of [^{11}C]-RAC binding potential (BP) and relative delivery (R_I) were generated from the dynamic [^{11}C]-RAC scans using a basis function implementation of the simplified reference region compartmental model with the cerebellum as the reference tissue[18]. MR images for each of the healthy subjects were coregistered to their respective parametric images of [^{11}C]-RAC BP using the integral images[19]. Values of BP and R_I for caudate and putaminal regions were obtained by defining on the coregistered MR images the regions of interest (ROIs) that were subsequently applied to the parametric images. [^{18}F]-dopa PET scans were performed as previously described[20]. All scans were analyzed by a single observer (P.P.).

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