Editor's Summary

The Two Faces of Fetal Grafts

Before stem cells, there were fetal grafts. Pioneering treatments performed in the 1990s in patients with Parkinson's disease proved that the diseased brain could be repaired, at least for a while. Two of these patients received grafts, one in the putamen and the other in both the caudate and the putamen, of fetal midbrain tissue. For several years, the patients showed mild improvement but eventually were able to function well with no drugs. Recently, however, both have started to experience abnormal uncontrolled movements, which Politis and colleagues have determined are a result of an overabundance of serotonin-using neurons that developed from the graft. A serotonin agonist eliminates these dyskinesias.

Brain imaging exposed what was happening in these patients' brains. When imaged by positron emission tomography, radioactive tracers that tag dopaminergic neurons and that bind to the dopamine receptor showed that the dopamine neurons that decay during Parkinson's disease were restored by the grafts. Another scan with an agent that binds to the serotonin transporter showed an abnormality; there seemed to be more serotonin neurons than usual. This presented a conundrum because dyskinesias in Parkinson's disease are thought to be a result of dopamine, not serotonin, stimulation.

The authors hypothesized that the explanation lies in the ability of the serotonin neurons to switch to a different neurotransmitter to adopt dopamine as a so-called false transmitter, releasing it to cause dyskinesias. If this were the case, then desensitizing these serotonin neurons, and so inhibiting their activity, would reduce the dyskinesias. They tested this idea by giving the patients low doses of a serotonin receptor agonist called buspirone. Both patients responded by a sudden and almost complete resolution of the troublesome abnormal movements, suggesting that the excess serotonergic neurons had in fact been pumping out dopamine, causing the dyskinesias.

The patients described here are only two of a larger number who received fetal neural tissue implants years ago. In some patients, the grafted cells survived, possibly as a result of stem cells within the graft, and were able to replace the function of the diseased dopamine cells, forming connections with the existing brain cells. Exploration of the long-term consequences of such replacement tissue, such as the atypical movements and their inhibition reported here, is important in that it will inform future treatments with grafts that consist of cells from other sources, such as bioengineered or stem cells.
Serotonergic Neurons Mediate Dyskinesia Side Effects in Parkinson’s Patients with Neural Transplants

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Troublesome involuntary movements in the absence of dopaminergic medication, so-called off-medication dyskinesias, are a serious adverse effect of fetal neural grafts that hinders the development of cell-based therapies for Parkinson’s disease. The mechanisms underlying these dyskinesias are not well understood, and it is not known whether they are the same as in the dyskinesias induced by l-dopa treatment. Using in vivo brain imaging, we show excessive serotonergic innervation in the grafted striatum of two patients with Parkinson’s disease, who had exhibited major motor recovery after transplantation with dopamine-rich fetal mesencephalic tissue but had later developed off-medication dyskinesias. The dyskinesias were markedly attenuated by systemic administration of a serotonin [5-hydroxytryptamine (5-HT)] receptor (5-HT1A) agonist, which dampens transmitter release from serotonergic neurons, indicating that the dyskinesias were caused by the serotonergic hyperinnervation. Our observations suggest strategies for avoiding and treating graft-induced dyskinesias that result from cell therapies for Parkinson’s disease with fetal tissue or stem cells.

INTRODUCTION

Clinical trials assessing the efficacy of intrastriatal transplantation of fetal ventral mesencephalic tissue in patients with Parkinson’s disease (PD) have shown that grafted dopaminergic neurons can reinervate the striatum, release dopamine, and, in some cases, produce long-lasting symptomatic relief (1). However, further development of dopaminergic cell replacement therapy is hampered by the occurrence of off-phase graft-induced dyskinesias (GIDs) in most recipients (2–5). These dyskinesias are involuntary movements in the absence of dopaminergic medication and differ from on-phase peak-dose dyskinesias, which appear when brain and plasma concentrations of l-dopa and dopamine are high.

The results from a number of positron emission tomography (PET) studies are inconsistent with GIDs being the consequence of graft-derived dopaminergic overgrowth or excessive release of dopamine (3–6). Animal models of PD indicate that serotonergic neurons may contribute to the dyskinesias induced by l-dopa treatment by converting l-dopa to dopamine, which is stored and then released from the serotonergic terminals in a dysregulated manner (7). Serotonergic neurons have been found at postmortem in the grafted tissue of PD patients (8). Whether these serotonergic neurons contribute to GIDs in humans is unknown.

Here, we have examined the role of serotonergic neurons within intrastriatal grafts in the development of GIDs in two PD patients who had shown recovery of motor function after intrastriatal fetal ventral mesencephalic tissue transplantation (patients 7 and 15 in the Lund series) (9–11).

RESULTS

Patient 7 reported PD-related symptoms since 1980 and was diagnosed in 1984. Preoperatively, after a 5-year “honeymoon period” in which he experienced a good response to l-dopa treatment, he developed severe motor complications including diphasic (appear when brain and plasma concentrations of l-dopa and dopamine are rising or falling, that is, at the beginning and the end of the l-dopa action cycle) and l-dopa–induced peak-dose dyskinesias, on-off fluctuations (diurnal fluctuations in the psychomotor state), and wearing-off phenomena (shorter duration of the beneficial effect of l-dopa) without any off-phase dyskinesias. He had bilateral intraputaminal transplantation of fetal ventral mesencephalic tissue 16 years ago (9, 10) (Tables 1 and 2). After transplantation and for the following 3 years, his motor symptoms were moderately improved and off periods were shorter and less severe, whereas on-period dyskinesias were reduced. From the fourth postoperative year, his parkinsonism was significantly improved, and he no longer required dopaminergic medication (Fig. 1A). Currently, he has no off periods but experiences GIDs, which are insufficently relieved by amantadine (a glutamate N-methyl-d-aspartic acid receptor antagonist used for treating dyskinesias). GIDs are present almost constantly, causing disability. His dyskinesia phenotype includes involuntary movements, mainly of the trunk and lower and upper extremities, although facial and oral involuntary movements are also detected (movie S1).

11C-l-dopa, a marker for dopamine synthesis (converted by the aromatic amino acid decarboxylase), and 11C-raclopride, a marker of postsynaptic dopamine D2 receptor availability (double scan with placebo and methamphetamine infusion allows indirect measurements of dopamine release), PET showed dopaminergic neuron restoration and dopamine release after transplantation, which rose to normal values in the grafted putamen (Fig. 1A). Using 11C-3-amino-4-(2-dimethylaminomethylphenylthio)
Table 1. Transplantation characteristics. L, left; R, right; Put, putamen; Caud, caudate nucleus; VM, ventral mesencephalon.

<table>
<thead>
<tr>
<th>Patient 7</th>
<th>Patient 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of transplantation</td>
<td>1993</td>
</tr>
<tr>
<td>Location of implanted sites</td>
<td>L Put</td>
</tr>
<tr>
<td>Number of implant sites</td>
<td>5</td>
</tr>
<tr>
<td>Number of donors</td>
<td>5</td>
</tr>
<tr>
<td>Size of donor tissue</td>
<td>L Put: mean, 22 mm (range, 14–26 mm); R Put: mean, 18 mm (range, 14–22 mm)</td>
</tr>
<tr>
<td>Amount of implanted tissue</td>
<td>L Put: 5 VM; R Put: 5 VM</td>
</tr>
<tr>
<td>Time from abortion to implantation</td>
<td>L Put: mean, 7.25 hours (range, 5.5–9 hours); R Put: mean, 5.5 hours (range, 3.5–7.5 hours)</td>
</tr>
</tbody>
</table>

*Exposed to the lazaroid trilazad mesylate during storage and dissociation; patient given lazaroid for 3 days (11).
The crucial role of serotonergic neurons in causing GIDs was confirmed when administration of repeated doses of the 5-HT\textsubscript{1A} agonist buspirone markedly attenuated dyskinesia severity in both transplanted patients. Serotonin neurons can take up dopamine from the extracellular space via serotonin transporters, where it competes with serotonin for vesicular storage and release (14–16). Also, 5-HT\textsubscript{1A} receptor agonists (including buspirone) can block the false release of dopamine from the serotonergic neurons by activating the inhibitory serotonin autoreceptors (7, 17). We propose that GIDs are caused by dysregulated release of dopamine from the dense graft-derived serotonergic innervation. The high serotonin-to-dopamine transporter ratio in the grafted putamen [a result of serotonergic hyperinnervation and reduced expression or loss of function of dopamine transporters, as seen in vivo (18) and at postmortem (19, 20)] would exacerbate this situation. Owing to a lack of normal autoregulatory feedback, the nonphysiologic release of dopamine from sermonergic terminals is dysregulated, and abnormal swings will result in GIDs. In addition, excess serotonin release can act directly on the dopamine terminals to induce an activity-independent, amphetamine-like release of dopamine, probably via reversal of the dopamine transporter (21–23), and may further enhance the dysregulation of dopamine release, worsening GIDs. The attenuation of GIDs that we observed is thus readily explained by the ability of 5-HT\textsubscript{1A} agonists to dampen serotonergic neurotransmission by activation of the inhibitory autoreceptors.

Whether GIDs and \textit{L}-dopa–induced dyskinesias in nongrafted PD patients share the same pattern of dopamine release from the serotonergic terminals is unknown. Both our patients showed viable dopaminergic grafts, and in the absence of administration of exogenous \textit{L}-dopa, the serotonergic neuron–derived irregular swings in synaptic dopamine concentrations could be a phenomenon with prolonged duration and lower intensity compared to the sharp, short-term increase in synaptic dopamine concentrations when large doses of \textit{L}-dopa are administered orally and reach the extensively denervated striatum (as

**DISCUSSION**

We observed striatal serotonergic hyperinnervation in two patients with PD who had received fetal neural transplants. This excess of serotonin neurons was visualized with noninvasive PET imaging of serotonergic neurons and is most likely derived from the graft, because PD patients lose ~30% of their endogenous striatal serotonergic innervation (see Supplementary Material for imaging data), and the excess of \textsuperscript{11}C-DASB binding was confined to those areas implanted with ventral mesencephalic tissue (putamen in patient 7; caudate and putamen in patient 15). Supporting a causative role between the serotonergic hyperinnervation in the graft putamen and the development of GIDs, patient 7, who had more severe GIDs than patient 15, showed greater \textsuperscript{11}C-DASB binding and a higher serotonergic/dopaminergic innervation ratio in the grafted putamen. This finding is compatible with studies on \textit{L}-dopa–induced dyskinesias in animal models of PD (12, 13), which suggest that a high, graft-derived serotonergic/dopaminergic innervation ratio is associated with the development of dyskinesias. The most likely explanation for the higher putaminal serotonergic innervation in patient 7 than in patient 15 is that he received 42% more ventral mesencephalic tissue in each putamen (Table 1).

The crucial role of serotonergic neurons in causing GIDs was confirmed when administration of repeated doses of the 5-HT\textsubscript{1A} agonist buspirone markedly attenuated dyskinesia severity in both transplanted patients.
observed in l-dopa–induced dyskinesia). In our patients, GIDs were attenuated after small, repeated doses of the 5-HT$_{1A}$ agonist, which supports this scenario.

The limited availability of PD patients with neural transplants influenced patient selection for this study. For example, of five transplanted patients in the UK, two have died and one is bedridden and unable to participate in research. However, on the basis of the data reported here, it is conceivable that other grafted PD patients with GIDs showing a high serotonergic/dopaminergic innervation ratio in their putamen (compared to normal controls) should respond to 5-HT$_{1A}$ agonist treatment. We have not tested other 5-HT$_{1A}$ agonists because buspirone was the only one licensed for human use in the UK. The action of buspirone may be limited by its short half-life, and diurnal, repeated administration of this drug may give rise to adverse effects. Our results support the use and development of 5-HT$_{1A}$ agonists with prolonged duration for the treatment of GIDs.

![Image](image.png)

**Fig. 2.** Serotonergic hyperinnervation in the grafted striatum causes dyskinesias, which are effectively suppressed by a 5-HT$_{1A}$ agonist. (A to D) Summed $^{11}$C-DASB PET images coregistered and fused with 1.5-T MRI images at the level of the dorsal basal ganglia for (A) a 64-year-old healthy male ($^{11}$C-DASB binding (BP$_{ND}$) mean values: 1.27 for caudate nucleus, 1.42 for putamen, and 1.31 for thalamus); (B) a 65-year-old male with advanced PD since 16 years experiencing motor and nonmotor complications (BP$_{ND}$ mean values: 0.82 for caudate, 0.82 for putamen, and 1.21 for thalamus); (C) patient 7, a 65-year-old male who received bilateral intraputaminal transplantation 16 years ago showing bilateral increases in putaminal $^{11}$C-DASB binding (BP$_{ND}$ mean values: 0.96 for caudate, 3.70 for putamen, and 1.07 for thalamus); and (D) patient 15, a 66-year-old male with bilateral intraputaminal and intracaudate grafts for 13 years, showing bilateral increases in putaminal and caudate $^{11}$C-DASB binding (BP$_{ND}$ mean values: 3.03 for caudate, 2.39 for putamen, and 1.04 for thalamus). (E to G) Caudate nucleus (E), putamen (F), and thalamus (G) mean $^{11}$C-DASB BP$_{ND}$ values for patients 7 (P7) and 15 (P15) and mean $^{11}$C-DASB BP$_{ND}$ values ($\pm$ 2 SD) for a group of 12 normal controls (NC) and a group of 12 age- and sex-matched, advanced PD patients (see also Table 2 and Supplementary Material). (H and I) Dyskinesia scores, rated with the AIMS, for patients 7 (H) and 15 (I) after a double-blind acute challenge with three repeated 5-mg doses of the 5-HT$_{1A}$ agonist buspirone or three repeated doses of placebo. Comparative AIMS scores in the absence of a drug challenge are also shown.
Our findings have direct implications for the development of a clinically competitive cell replacement therapy in PD by indicating how the occurrence of GIDs might be prevented or minimized. First, the dissection of ventral mesencephalic tissue, which contains both dopaminergic and serotonergic progenitors (24), could be performed in such a way as to minimize the serotonergic component in the graft tissue. In rodents, this strategy leads to maximum functional restoration with minimum \( \text{l-dopa} \)-induced dyskinesias (12). Second, it needs to be determined that storage of tissue before implantation does not change the proportion of serotonergic and dopaminergic components. Storage and culture of the tissue are expected to alter its composition in favor of nondopaminergic and serotonergic components. Storage and culture of the tissue before implantation (25), and it has been reported that patients who received tissue that had been stored for long periods developed more pronounced GIDs than patients implanted with fresh tissue (2, 3). Third, serotonergic neurons contaminating dopaminergic neuron populations generated from stem cells should be kept to a minimum or removed by cell sorting. If, however, GIDs develop in future neural transplantation trials despite these preventive measures, we show here that they can be effectively treated with systemic administration of \( 5\text{-HT}_{1\text{A}} \) agonists.

**MATERIALS AND METHODS**

Ethical permission was obtained from the Hammersmith and Queen Charlotte’s and Chelsea Hospitals Research Ethics Committee. Permission to administer \( ^{11}\text{C-DASB} \), \( ^{18}\text{F-dopa} \), and \( ^{11}\text{C-raclopride} \) was obtained from the Administration of Radioactive Substances Advisory Committee of the UK. Written consent was obtained from all subjects in accordance with the Declaration of Helsinki.

**Subjects**

Twelve nondemented, nondepressed patients with advanced idiopathic PD fulfilling the UK Brain Bank clinical criteria for PD (26) and experiencing motor and nonmotor complications, 12 normal controls matched for age and sex, and patients 7 and 15 from the Lund series with fetal mesencephalic grafts were studied (Tables 1 and 2).

**Transplantation procedure**

Details for the tissue preparation and neurosurgical procedure are described elsewhere (9, 11, 27) (Table 1). Briefly, dissociated ventral mesencephalic tissue was implanted with computed tomography– and magnetic resonance imaging (MRI)–guided stereotactic neurosurgery along five trajectories in the putamen (patients 7 and 15) and two trajectories in the head of the caudate nucleus (patient 15). The tissue was procured from dead human fetuses aged 6 to 8 weeks after conception and was obtained from routine suction abortions. Both patients were given immunosuppressive treatment (28).

**Clinical evaluation**

The test battery included the UPDRS, the abnormal involuntary movement scale (AIMS), and the mini-mental state examination (MMSE). UPDRS motor score evaluations were carried out on eight occasions on three different days, whereas scores from previous assessments were retrospectively analyzed (Fig. 1 and Table 2).

**5-HT\( _{1\text{A}} \) agonist trial**

Patients 7 and 15 underwent a double-blind acute oral challenge with the \( 5\text{-HT}_{1\text{A}} \) agonist buspirone and with the administration of placebo on two different days, close apart. Patients stopped any other medication (patient 7, amantadine; patient 15, amantadine and trihexyphenidyl) 72 hours before each challenge. Buspirone is rapidly and almost completely absorbed after oral administration (\( t_{\text{max}} = 0.89 \pm 0.15 \text{ (SD) hour} \) (29)). Neither the patients nor the investigator knew whether buspirone or placebo was administered, and the day for giving one or the other was selected randomly.

A total of 15 mg of buspirone or similar dose of placebo was administered, divided in three 5-mg doses at 30, 60, and 90 min after the beginning of the AIMS assessments. Comparable assessments on another day without buspirone, placebo, or amantadine were also performed. AIMS and UPDRS motor score assessments were carried out throughout the trial. The total duration of assessments lasted for 4 hours, and videos were recorded (Fig. 2, H and I, and movies S1 to S4).

**Scanning procedures**

\( ^{11}\text{C-DASB} \) scans were performed with an ECAT HR\( ^{7} \) (CTI/Siemens 962) three-dimensional (3D) PET (30) after intravenous injection of a 450-megabecquerel (MBq) mean tracer dose (range, 439 to 461). Scanning began 30 s before tracer infusion (bolus intravenous), generating 28 time frames of tissue data over 90 min. Subjects also underwent a volumetric T1 MRI that was obtained with a 1.5-T MRI (Picker Eclipse) scanner for the purposes of image registration and to facilitate localization of the regions of interest (ROIs). The PD patients stopped medication for at least 18 hours before scanning.

\( ^{18}\text{F-dopa} \) and \( ^{11}\text{C-raclopride} \) scans, retrospectively analyzed, were performed with an ECAT EXACT HR\( ^{++} \) (CTI/Siemens 966) PET scanner that has a 23.4-cm total axial field of view (FOV). The camera has a reconstructed (image) transaxial spatial resolution of 5.1 ± 0.6 mm and an axial resolution of 5.9 ± 0.6 mm over a 10-cm-radius FOV from the center (31). The scanning protocols have been previously described (6).

**\( ^{11}\text{C-DASB} \) data analysis**

The input function was derived from the nonspecific tracer binding signal in the posterior cerebellar gray matter cortex, excluding the vermis (32). After reconstruction of the dynamic \( ^{11}\text{C-DASB} \) image volume, a summed image volume was created from the entire dynamic data set with in-house software. Standardized samples of high-contrast ROIs were defined directly on the summed image, and these ROIs were applied to the dynamic data set. By obtaining the regional concentrations of radioactivity (kilobecquerels per milliliter) from the full dynamic scan, the decay-corrected time-activity curves (TACs) were computed and movement during the scan was assessed. The movement was corrected with a frame-by-frame realignment procedure, as previously described (33). Each subject’s MRI volume was then co-registered to the summed PET volume with the Mutual Information Registration algorithm in the SPM2 software package (Wellcome Department of Cognitive Neuroscience, Institute of Neurology) implemented in Matlab 6.5 (The MathWorks Inc.). After co-registration, the definition of ROIs was performed on the co-registered MRI with the help of Duvernoy 3D sectional atlas (34) with the Analyze (version 8.1, Mayo Foundation) medical imaging software package. ROIs were standardized for volume throughout subjects and manually defined on both hemispheres. The transformation parameters generated from the co-registration were applied to the dynamic PET data set and the ROI was projected onto the image volume. ROIs were then sampled to give new TACs that were checked against intrascan notes for movement correction improvement. Volume of dis-
F-dopa data analysis

Protocols are as described previously (6, 38). Briefly, regional F-dopa influx rate constant (K

11C-raclopride data analysis

Protocols are as described previously (6). Briefly, patients 7 and 15 and six healthy male normal controls undertook two 11C-raclopride scans after a bolus intravenous injection of methamphetamine (0.3 mg/kg) and after a bolus intravenous injection of saline. Subjects did not know whether they would receive placebo or methamphetamine. Parametric images of 11C-raclopride binding (BP

Statistical analysis

Statistical analyses were performed with GraphPad InStat (version 3.1a for Macintosh, GraphPad Software Inc.). Between-group comparisons were carried out with the nonparametric Mann-Whitney twotailed test. The α level was set at P < 0.05.

REFERENCES AND NOTES

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