Imaging Cell Therapy in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting about 1 person in every 500 in the United States. A pioneering treatment involving transplantation of dopamine-rich fetal grafts in the brains of patients with PD was initiated nearly 2 decades ago. The goal was to restore dopaminergic neurons in the nigrostriatal pathway that are selectively lost in PD and hence improve motor performance. Although there have been inconsistent results among different trials, some PD patients showed encouraging clinical improvements in motor performance but no improvement in nonmotor symptoms. PD causes not only severe motor symptoms associated with dopamine loss but also nonmotor symptoms such as depression, fatigue, visual hallucinations, and sleep problems that have a significant negative impact on quality of life. Given the importance of nonmotor symptoms in PD, Politis et al. decided to use sophisticated brain imaging techniques to investigate why three PD patients transplanted with fetal grafts 13 to 16 years previously still exhibited nonmotor symptoms even as their motor symptoms improved. When these patients were imaged by positron emission tomography, radioactive tracers that tag dopamine neurons and receptors showed that dopamine neuronal function was restored by the fetal grafts. Also, the principal site of synthesis of another key neurotransmitter, called norepinephrine, was unaffected in these patients. But another scan with an agent that binds to the serotonin transporter and measures the integrity of serotonin-producing neurons showed that there were far fewer serotonin neurons than usual in brain areas related to the regulation of sleep, arousal, feeding, satiety, mood, and emotion. These findings indicate that for more complete, long-term symptomatic relief of both motor and nonmotor symptoms in PD, dopamine neuron replacement with fetal or stem cells will need to be combined with other therapeutic approaches such as additional grafts of serotonin neurons in specific brain areas to relieve nonmotor symptoms by restoring serotonin neurotransmission.
Serotonin Neuron Loss and Nonmotor Symptoms Continue in Parkinson’s Patients Treated with Dopamine Grafts

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Cell therapy studies in patients with Parkinson’s disease (PD) have been confined to intrastriatal transplantation of dopamine-rich fetal mesencephalic tissue in efforts to improve motor performance. Although some PD patients receiving the dopamine-rich grafts showed improvements in motor symptoms due to replacement of dopaminergic neurons, they still suffered from nonmotor symptoms including depression, fatigue, visual hallucinations, and sleep problems. Using functional imaging and clinical evaluation of motor and nonmotor symptoms in three PD patients transplanted with intrastriatal fetal grafts 13 to 16 years previously, we assessed whether reestablishment of dopaminergic neuronal networks is sufficient to improve a broad range of symptoms. At 13 to 16 years after transplantation, dopaminergic innervation was restored to normal levels in basal ganglia and preserved in a number of extrabasal ganglia areas. These changes were associated with long-lasting relief of motor symptoms. Then, we assessed the integrity of their serotoninergic and norepinephrine neuronal systems using [11C]DASB ([11C]3-amino-4-(2-dimethylaminomethylphenylthio) benzonitrile) positron emission tomography (PET) and [18F]-dopa PET, respectively. [18F]-Dopa uptake in the locus coeruleus was within the normal range. In contrast, [11C]DASB uptake in the raphe nuclei and regions receiving serotonergic projections was markedly reduced. These results indicate ongoing degeneration of serotonergic raphe nuclei and their projections to regions involved in the regulation of sleep, arousal, feeding, satiety, mood, and emotion. Our findings indicate that future cell-based therapies using fetal tissue or stem cells in PD patients may require additional grafts of serotoninergic neurons to relieve nonmotor symptoms by restoring serotoninergic neurotransmission in specific cerebral targets.

INTRODUCTION

Cell therapies with fetal tissue or stem cells to treat patients with Parkinson’s disease (PD) aim to provide long-lasting relief of disease symptoms. It is now widely acknowledged that the clinical spectrum of PD involves not only the classic motor symptoms of rigidity, bradykinesia and tremor, but also a number of nonmotor symptoms such as problems with mood, sleep, and gastrointestinal tract function (1, 2). These nonmotor symptoms are a source of increasing complaints by PD patients as the disease advances (2), have a significant negative impact on quality of life (3), and are difficult to treat (4).

On the basis of extensive pharmacological research and preclinical grafting experiments in animals, clinical trials using intrastriatal transplantation of fetal ventral mesencephalic (fVM) tissue in patients with PD have demonstrated that dopamine (DA)-rich grafts can reinnervate the striatum, release DA, and, in some cases, produce long-lasting relief of motor symptoms (5). Moreover, follow-up studies now suggest ways to avoid or treat the serious adverse effects of graft-induced dyskinesias that have hampered further development of cell therapy for PD (6–8). So far, clinical cell therapy research in PD has been restricted to the reestablishment of dopaminergic function in the striatum and the improvement of motor symptoms.

Postmortem studies have shown that brain pathology in PD extends beyond the striatum (9) and the dopaminergic system, and studies in animal models of PD have demonstrated that dysfunction of the norepinephrine (NE) and serotoninergic neuronal systems could be implicated in the occurrence of nonmotor symptoms (10–13). Furthermore, there is now an established connection between extrastriatal serotoninergic dysfunction and the occurrence of nonmotor symptoms on the basis of in vivo positron emission tomography (PET) studies in PD patients (14–18).

Here, using in vivo PET and a battery of clinical assessments in three PD patients who had received neural transplants 13 to 16 years previously (patients 7, 13, and 15 in the Lund series) (19, 20), we tested the hypothesis that intrastriatal transplantation of DA-rich fVM tissue is insufficient to generate relief of nonmotor symptoms. Moreover, we assessed the integrity of extrastriatal dopaminergic, NE, and serotoninergic systems and hypothesized that non-DA monoaminergic dysfunction may be a major contributor to nonmotor symptoms. The results of PD patients receiving grafts were compared with data from 24 PD control patients who had not received grafts and 24 normal healthy controls matched for age and sex (Table 1).

RESULTS

Clinical status and nonmotor symptoms

The three transplanted PD patients studied here have been followed for 13 to 16 years after surgery (patient 7 = 16th year after transplantation;
patient 13 = 14th year; and patient 15 = 13th year). At their last assessment, they had significantly reduced unified PD rating scale (UPDRS) motor and total scores compared to ungrafted PD control patients in an “off” medication state ($P < 0.001$), even though the duration of their PD averaged 20 years longer than that of the ungrafted PD control patients (Table 1). Their marked improvements in UPDRS motor scores began during the first posttransplantation year for patient 13, the second year for patient 7, and from the third year for patient 15. By the end of the third posttransplantation year, their UPDRS motor scores were reduced from baseline by 40 to 56% (6, 8, 19, 20). As a consequence of improvements in motor performance, patient 13 was able to stop l-dopa medication during the first year, patient 7 by the fourth, and patient 15 by the fifth year after transplantation.

The three grafted patients were rated for quality of life measures and with a clinical battery of tests for screening nonmotor symptoms.

### Table 1. Clinical characteristics of PD patients and healthy controls. M, male; F, female.

<table>
<thead>
<tr>
<th></th>
<th>[^{11}C]DASB PET study</th>
<th>[^{18}F]-Dopa PET study</th>
<th>PD transplanted (n = 3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control (n = 12)</td>
<td>PD control (n = 12)</td>
<td>Normal control (n = 12)</td>
</tr>
<tr>
<td>Sex</td>
<td>10 M/2 F</td>
<td>10 M/2 F</td>
<td>10 M/2 F</td>
</tr>
<tr>
<td>Age</td>
<td>63.3 ± 7.0†</td>
<td>62.9 ± 8.9</td>
<td>63.3 ± 5.9</td>
</tr>
<tr>
<td>Parkinson’s disease duration</td>
<td>—</td>
<td>8.1 ± 1.1</td>
<td>—</td>
</tr>
<tr>
<td>Unified Parkinson’s Disease Rating Scale (UPDRS) Motor Part III OFF</td>
<td>—</td>
<td>43.2 ± 12.0</td>
<td>—</td>
</tr>
<tr>
<td>UPDRS OFF (total)</td>
<td>—</td>
<td>72.3 ± 21.9</td>
<td>—</td>
</tr>
<tr>
<td>Daily l-dopa equivalent dose</td>
<td>—</td>
<td>795 ± 312</td>
<td>—</td>
</tr>
<tr>
<td>Mini-Mental State Examination</td>
<td>29.4 ± 0.7</td>
<td>27.8 ± 2.9</td>
<td>29.5 ± 0.5</td>
</tr>
<tr>
<td>Beck Depression Inventory Second Edition</td>
<td>3.1 ± 2.6</td>
<td>10.3 ± 4.4</td>
<td>2.9 ± 2.8</td>
</tr>
<tr>
<td>Parkinson’s Disease Questionnaire–39§</td>
<td>—</td>
<td>63.2 ± 21.4</td>
<td>—</td>
</tr>
<tr>
<td>Nonmotor symptoms assessment scale for Parkinson’s disease</td>
<td>—</td>
<td>44.0 ± 33.1</td>
<td>—</td>
</tr>
</tbody>
</table>

*Values at the time of their last PET imaging assessment. †Mean ± SD. §These subjects did not receive any dopaminergic medication. §Worst score = 156 (never = 0; always = 4).

### Table 2. Nonmotor symptoms in transplanted PD patients.

<table>
<thead>
<tr>
<th>Patient 7</th>
<th>Patient 13</th>
<th>Patient 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD nonmotor symptoms (NMS) questionnaire</td>
<td>9 yes/21 no</td>
<td>4 yes/26 no</td>
</tr>
<tr>
<td>NMS assessment scale for PD</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Parkinson’s Disease Sleep Scale (worst = 150)</td>
<td>38</td>
<td>13</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary urgency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturia</td>
<td>Loss of concentration</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>Back pain</td>
<td></td>
</tr>
<tr>
<td>Loss of concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive daytime sleepiness</td>
<td>Excessive sweating</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>Hypersalivation</td>
<td></td>
</tr>
<tr>
<td>Paresthesias</td>
<td>Loss of smell</td>
<td></td>
</tr>
<tr>
<td>Joint pain</td>
<td>Weight loss</td>
<td></td>
</tr>
<tr>
<td>Leg cramps</td>
<td>Urinary frequency</td>
<td></td>
</tr>
<tr>
<td>Hypersalivation</td>
<td>Loss of concentration</td>
<td></td>
</tr>
<tr>
<td>Loss of smell</td>
<td>Back pain</td>
<td></td>
</tr>
</tbody>
</table>

*Hospital Anxiety and Depression Scale: (anxiety = 5/depression = 7).
Their 39-item PD questionnaire (PDQ-39) scores were significantly reduced compared to those of ungrafted PD control patients ($P < 0.001$) (Table 1). The Mini-Mental State Examination (MMSE) scores of the three transplanted PD patients, the PD control patients, and the normal healthy controls were all within the normal range (Table 1). The Beck Depression Inventory Second Edition (BDI-II) mean scores were higher in the PD transplanted and PD control patients compared to normal controls ($P < 0.01$) but were not raised above the discriminating threshold score for depression (>16) (21, 22) (Table 1).

The PD nonmotor symptoms questionnaire, the nonmotor symptoms assessment scale for PD, the Hospital Anxiety and Depression Scale (HADS), the PD Sleep Scale (PDSS), and body mass index (BMI) measures revealed the presence of a number of nonmotor symptoms in patients 7, 13, and 15 (Table 2). All patients reported significant bowel and bladder dysfunction. Patients 7 and 15 exhibited episodes of excessive daytime sleepiness and reduced concentration. Patients 13 and 15 had problems with their libido. Patient 13 experienced abnormal weight loss and excessive sweating, whereas patient 15 was subject to episodes of anxiety and mood swings and experienced visual hallucinations (Table 2).

**Dopaminergic and extrastratal monoaminergic innervation**

By measuring the activity of aromatic amino acid decarboxylase, $^{18}$F-dopa PET provides an indirect measure of presynaptic dopaminergic neurons. We showed previously that there was a gradual increase in uptake of $^{18}$F-dopa ($^{18}$F-dopa uptake is measured by the influx constant $K_i$) in the striatum that reached the normal range by the fourth posttransplantation year and has been sustained to date in the three grafted PD patients (6, 8, 19, 20). Here, we assessed $^{18}$F-dopa uptake in the basal ganglia (combined values for caudate, putamen, globus pallidus, subthalamic nucleus, and substantia nigra) 13 to 16 years after transplantation. We found that $^{18}$F-dopa uptake in the basal ganglia was restored to normal levels (mean ± 2 SD = 0.0078 to 0.0110): patient 7 (0.0084), patient 13 (0.0084), and patient 15 (0.0086) (Fig. 1 and Table 3).

In line with dopaminergic neuron restoration in the striatum and basal ganglia shown by longitudinal $^{18}$F-dopa PET studies, previous imaging studies using $[^{11}$C]raclopride PET have demonstrated restoration of DA release in the striatum (6, 8, 23, 24). $[^{11}$C]Raclopride is a marker of postsynaptic DA D2 receptor availability (double $[^{11}$C]raclopride PET scans with placebo and methamphetamine infusion challenges allow indirect measurements of DA release). In the current, more extended, analysis, we found restoration of DA release in the basal ganglia (8% reduction of $[^{11}$C]raclopride binding after methamphetamine; normal range: 6 to 13%) in the three transplanted PD patients.

We further examined $^{18}$F-dopa uptake in a number of extrabasal ganglia areas such as the hypothalamus, insula, prefrontal cortex (PFC), and thalamus in the 13 grafted PD patients 13 to 16 years after transplantation. We found that in the three grafted PD patients, $^{18}$F-dopa uptake was within normal levels in virtually all areas (Fig. 2 and Table 3).

**Table 3. Regional $^{18}$F-dopa influx rate constant ($K_i$) values and between-group statistical comparisons (mean ± SD) for normal control (NC) individuals, Parkinson’s disease (PD) patients, and Parkinson’s disease patients with intrastral grafts (PDG). All $P$ values after analysis of variance (ANOVA) with Bonferroni post test. NS, nonsignificant.**

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>NC</th>
<th>PD</th>
<th>PDG</th>
<th>NC versus PD</th>
<th>NC versus PDG</th>
<th>PD versus PDG</th>
<th>ANOVA $P$ and $F$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>0.0051 ± 0.0005</td>
<td>0.0031 ± 0.0005</td>
<td>0.0038 ± 0.0008</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 42.44$</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>0.0033 ± 0.0004</td>
<td>0.0027 ± 0.0002</td>
<td>0.0030 ± 0.0001</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.01$; $F_{2,24} = 8.74$</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>0.0092 ± 0.0007</td>
<td>0.0057 ± 0.0009</td>
<td>0.0085 ± 0.0001</td>
<td>$P &lt; 0.0001$</td>
<td>NS</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 65.39$</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.0065 ± 0.0007</td>
<td>0.0053 ± 0.0010</td>
<td>0.0055 ± 0.0007</td>
<td>$P &lt; 0.05$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.05$; $F_{2,24} = 5.56$</td>
</tr>
<tr>
<td>Insula</td>
<td>0.0028 ± 0.0005</td>
<td>0.0022 ± 0.0002</td>
<td>0.0031 ± 0.0004</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P = 0.0007$; $F_{2,24} = 9.95$</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>0.0053 ± 0.0007</td>
<td>0.0042 ± 0.0010</td>
<td>0.0045 ± 0.0000</td>
<td>$P &lt; 0.05$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.05$; $F_{2,24} = 5.19$</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td>0.0025 ± 0.0004</td>
<td>0.0017 ± 0.0002</td>
<td>0.0025 ± 0.0005</td>
<td>$P &lt; 0.0001$</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 18.25$</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>0.0024 ± 0.0004</td>
<td>0.0015 ± 0.0003</td>
<td>0.0024 ± 0.0002</td>
<td>$P &lt; 0.0001$</td>
<td>NS</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 25.31$</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>0.0059 ± 0.0006</td>
<td>0.0046 ± 0.0010</td>
<td>0.0053 ± 0.0001</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.01$; $F_{2,24} = 6.55$</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.0032 ± 0.0004</td>
<td>0.0026 ± 0.0005</td>
<td>0.0030 ± 0.0005</td>
<td>$P &lt; 0.05$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.05$; $F_{2,24} = 4.67$</td>
</tr>
</tbody>
</table>
Noradrenergic innervation
The locus coeruleus is the principal origin of NE neurons and sends widespread NE projections to cortical and brainstem areas (25, 26). We measured 18F-dopa uptake in locus coeruleus as a marker of the functional integrity of NE neurons (27) 13 to 16 years after transplantation. We found that 18F-dopa uptake in the locus coeruleus was within normal levels (normal mean ± 2 SD = 0.0040 to 0.0066) for patient 7 (0.0045), patient 13 (0.0045), and patient 15 (0.0046) (Fig. 3 and Table 3).

Serotonergic innervation
The PET marker [11C]3-amino-4-(2-dimethylaminomethylphenylthio) benzonitrile [11C]DASB binds to the serotonin transporter and serves as a functional marker of serotonergic neuronal integrity. The raphe region contains a large population of serotonin neurons (28). The serotonergic raphe nuclei showed major reductions in [11C]DASB binding ([11C]DASB binding is measured by the binding potential BPND) in patient 7 (1.27), patient 13 (1.37), and patient 15 (1.27). These reductions fell below the range for both normal healthy individuals and PD controls in a number of extrastriatal regions that receive serotonergic innervation from raphe nuclei (Fig. 5). These reductions in [11C]DASB binding relative to normal healthy individuals ranged from 43.4% reduction in the amygdala (38.4% reduction relative to PD control patients) to 44.9% in thalamus (26.1% compared to PD controls), 46.3% in hypothalamus (30.7% compared to PD controls), 73.7% in anterior cingulate cortex (ACC) (61.2% compared to PD controls), 74.6% in the insula (60.2% compared to PD controls), 77.6% in posterior cingulate cortex (PCC) (65.5% compared to PD controls), and 80.9% in PFC (67.9% compared to PD controls) (Fig. 5 and Table 4).
DISCUSSION

Here, we show major loss of serotonergic neurons in raphe nuclei and widespread serotonergic denervation in extrastriatal forebrain areas in three PD patients who had received intrastriatal transplants of DA-rich fVM tissue 13 to 16 years earlier. In these patients, the storage and release of DA—measured by 18F-dopa and [11C]raclopride binding changes induced by methamphetamine—had been restored to normal levels in grafted striatal areas. In addition, DA storage was preserved at normal levels in basal ganglia and several limbic and cortical forebrain regions. These patients exhibited major motor symptom recovery after transplantation, allowing withdrawal of their L-dopa medication for several years. However, clinical assessments demonstrated the presence of a number of nonmotor symptoms diminishing quality of life for these patients. These results are consistent with ongoing pathology in nondopaminergic systems indicated by loss of [11C]DASB binding. This ongoing pathology affected serotonergic neurons in raphe nuclei and their projections to cortical, subcortical, and deep nuclei areas. Although the evidence presented here is circumstantial, a causative link is supported by data from biochemical (13), animal (11), and several in vivo PET studies demonstrating serotonergic system dysfunction in PD patients suffering from nonmotor symptoms (14–18).

18F-Dopa PET measures the activity of the enzyme aromatic amino acid decarboxylase and reflects NE neuron density in the locus coeruleus (27). We found that 18F-dopa uptake in the locus coeruleus was normal in all three transplanted PD patients, indicating preserved function of this NE nucleus. Postmortem studies have demonstrated Lewy body pathology in the locus coeruleus of brains from PD patients (29–32), as well as marked reductions in tissue NE concentrations in forebrain regions innervated by the locus coeruleus (33, 34).

Our finding of normal 18F-dopa uptake by the locus coeruleus in the transplanted PD patients suggests that the cell bodies in the locus coeruleus remain relatively intact, although their axonal projections may be affected. Notably, the grafted PD patients had a significantly younger mean age of onset compared to the PD control patients, and this could result in a different form or evolution of the disease. Indi...
Table 4. Regional $[^{11}C]DASB$ binding potential ($BP_{ND}$) values and between-group statistical comparisons (mean ± SD) for normal control (NC) individuals, Parkinson's disease (PD) patients, and Parkinson's disease patients with intrastriatal grafts (PDG). All $P$ values after ANOVA with Bonferroni post test. NS, nonsignificant.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>NC</th>
<th>PD</th>
<th>PDG</th>
<th>NC versus PD</th>
<th>PD versus PDG</th>
<th>ANOVA $P$ and $F$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>1.14 ± 0.17</td>
<td>0.96 ± 0.16</td>
<td>0.66 ± 0.03</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.0003$; $F_{2,24} = 11.52$</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>0.38 ± 0.07</td>
<td>0.26 ± 0.08</td>
<td>0.15 ± 0.05</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.001$</td>
<td>NS</td>
</tr>
<tr>
<td>Raphe nuclei</td>
<td>2.19 ± 0.24</td>
<td>1.77 ± 0.19</td>
<td>1.31 ± 0.06</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$; $F_{2,24} = 25.09$</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.22 ± 0.13</td>
<td>0.95 ± 0.14</td>
<td>0.71 ± 0.07</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 24.88$</td>
</tr>
<tr>
<td>Insula</td>
<td>0.37 ± 0.04</td>
<td>0.22 ± 0.07</td>
<td>0.12 ± 0.04</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 36.16$</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td>0.37 ± 0.07</td>
<td>0.24 ± 0.07</td>
<td>0.09 ± 0.02</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 23.19$</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>0.20 ± 0.02</td>
<td>0.12 ± 0.04</td>
<td>0.04 ± 0.00</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 54.25$</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.47 ± 0.18</td>
<td>1.10 ± 0.16</td>
<td>0.89 ± 0.09</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>NS</td>
</tr>
<tr>
<td>Mid-pons area</td>
<td>0.89 ± 0.09</td>
<td>0.67 ± 0.12</td>
<td>0.56 ± 0.03</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$; $F_{2,24} = 21.29$</td>
</tr>
</tbody>
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Fig. 5. Serotonergic denervation in cortical and subcortical structures in transplanted PD patients. (A) Transverse summed $[^{11}C]DASB$ PET images co-registered and fused with 1.5-T MRI images at the cortical level for a 64-year-old healthy male showing normal cortical $[^{11}C]DASB$ uptake (normal brain); a 64-year-old male with PD for 8 years showing mild to moderate decreases in cortical $[^{11}C]DASB$ uptake (PD control); transplanted PD patient 7, a 65-year-old male diagnosed with PD 27 years ago, who received a bilateral intraputaminal transplant 16 years ago, showing severe decreases in cortical $[^{11}C]DASB$ uptake; transplanted PD patient 13, a 55-year-old male diagnosed with PD 28 years ago, who received bilateral intraputaminal and intracaudate grafts 14 years ago, showing severe decreases in cortical $[^{11}C]DASB$ uptake; and transplanted PD patient 15, a 66-year-old male diagnosed with PD 26 years ago, who received bilateral intraputaminal and intracaudate grafts 13 years ago, showing severe decreases in cortical $[^{11}C]DASB$ uptake. (B) Scatter plots showing marked reductions in $[^{11}C]DASB$ binding ($BP_{ND}$) values for transplanted PD patients 7, 13, and 15 compared to 12 normal controls (NC) and 12 PD patients matched for age and sex, who did not receive a transplant. $[^{11}C]DASB$ binding ($BP_{ND}$) values are shown for the following brain regions: amygdala, ACC, hypothalamus, insula, mid-pons, PCC, PFC, and thalamus. Scatter dot plot and error bars represent mean ± 95% confidence interval. Color bar reflects intensity of $[^{11}C]DASB$ binding ($BP_{ND}$ range; 0 to 3).
viduals who develop PD at a younger age are more likely to have a genetic cause for their disease, and it is known that carriers of recessive mutations in the gene encoding parkin (PARK2) do not have Lewy body pathology. Such a genetic cause means that the disease would not necessarily follow Braak staging related to idiopathic PD (9). However, we have not found any causative genetic mutations such as point mutations or exonic deletions/duplications in the coding region of the PARK2 gene in patients 7 and 15 (patient 13 has not been checked for genetic mutations).

Serotonergic neurons in the dorsal, medial, and ventral raphe nuclei send axonal projections to widespread areas of the brain (35, 36). In the three transplanted PD patients, we observed a marked decrease in \(^{11}C\)DASB binding in the raphe nuclei (more than 40%) and associated extrastriatal target areas (up to 80%) including the amygdala, hypothalamus, insula, cingulate, and prefrontal cortices. The loss of extrastriatal serotonergic innervation is most likely due to degeneration of raphe nuclei projections, consistent with previous findings that both Lewy bodies and cell loss have been observed in these nuclei at early stages of PD (9, 37–39). We have previously shown in patients 7, 13, and 15 that in parallel with this degeneration, fVM tissue grafts gave rise to serotonergic hyperinnervation locally in the striatum (increased striatal \(^{11}C\)DASB binding), which was associated with graft-induced dyskinasias (6, 8). The new data show that the serotonergic neurons within striatal grafts were not able to restore serotonergic innervation or compensate for its loss outside of the striatum.

According to Braak staging, early Lewy body pathology targets the caudal nuclei of raphe (raphe magnus; stage 2), which predominantly project to the brainstem and spinal cord and later targets the rostral nuclei (raphe dorsalis and medialis; stage 3), which project to the thalamus, limbic, and cortical regions (9, 40). Previous in vivo PET data on patients with early, established, and advanced PD (average of 14 years after the appearance of symptoms) have demonstrated marked serotonergic denervation starting in striatal and extrastriatal regions, whereas medio-dorsal and medio-ventral raphe nuclei are affected only in the advanced stages of the disease (41). The unique \(^{11}C\)DASB PET data we present here from transplanted PD patients with 27 to 29 years of disease duration indicate a progressive and continuous degeneration of serotonergic raphe neurons affecting widespread areas of the forebrain.

Gifted PD patients were not receiving dopaminergic medication, whereas PD control patients were receiving both DA agonists and \(\text{L-dopa}\). An influence of DA agonists and \(\text{L-dopa}\) on serotonergic transmission could be a confounding factor in the PET analysis. However, a recent study indicated that chronic exposure to dopaminergic therapy did not alter \(^{11}C\)DASB binding in a set of brain regions similar to those investigated here (41).

The nonmotor symptoms in the grafted PD patients included excessive daytime sleepiness, constipation, reduced concentration, weight loss, anxiety and depression, and visual hallucinations. Several lines of evidence indicate that some of these symptoms are associated with the marked widespread decrease in brain serotonergic innervation. The serotonergic system is involved in the regulation of both basic and complex physiological functions such as sleep, arousal, feeding, satiety, mood, and emotion (42). Accordingly, several in vivo PET studies have demonstrated serotonergic system dysfunction in PD patients suffering from nonmotor symptoms such as depression (14, 17), fatigue (16), abnormal changes in BMI (18), and visual hallucinations (15).

We demonstrate here in three PD patients the occurrence of nonmotor symptoms associated with a widespread loss of serotonergic innervation despite successful intrastriatal dopaminergic grafts and major recovery of motor function. Our findings show that implantation into striatum of dopaminergic neuroblasts or precursors from fetal brain tissue or derived from stem cells will not be able to ameliorate the entire clinical spectrum of PD symptoms. For more complete, long-term symptomatic relief in PD patients, intrastriatal DA cell replacement will need to be combined with other therapeutic approaches targeting extrastriatal nondopaminergic systems. Our findings raise the possibility that in the future, suitably targeted implantation of serotonergic neuroblasts or precursors in raphe nuclei or forebrain areas may counteract nonmotor symptoms in PD. However, as a first step, before clinical application is considered, the ability of serotonin grafts to reverse nonmotor deficits in PD must be demonstrated in appropriate animal models.

**MATERIALS AND METHODS**

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

**Subjects**

We studied three PD patients with bilateral intrastral transplantation of fVM tissue (patients 7, 13, and 15 in the Lund series) with PET imaging and a battery of clinical assessments. Their results were compared with a group of 24 nondemented, nondepressed patients with idiopathic PD fulfilling the UK Brain Bank clinical criteria for PD (43) with no other history of neurological or psychiatric disorder, and a group of 24 normal controls, all in good health with no past medical history (Table 1).

**Transplantation procedures**

Details for the tissue preparation and neurosurgical procedures have been described previously (6, 8, 19, 20, 44–46). In brief, dissociated fVM tissue was implanted with computed tomography (CT)– and magnetic resonance imaging (MRI)–guided stereotactic neurosurgery along five trajectories bilaterally in the putamen (all patients) and two trajectories in the head of the caudate nucleus (patients 13 and 15). The tissue was procured from dead human fetuses aged 6 to 8 weeks after conception and was obtained from routine suction abortions. All patients received immunosuppressive treatment starting 2 days before transplantation and continuing for a mean of 29 months (44).

**Clinical assessments**

All subjects underwent a detailed clinical interview. General clinical assessments included the UPDRS and the 32-item Wearing-off Questionnaire (WOQ-32). UPDRS motor scores from previous assessments were retrospectively analyzed. The battery of nonmotor clinical assessments included the PD nonmotor symptoms questionnaire, the nonmotor symptom assessment scale for PD, and the MMSE. Anxiety and depression levels were evaluated with BDI-II, the Hamilton Rating Scale for Depression (HRSD), and HADS. Sleep was evaluated with the PDSS and quality of life with PDQ-39. Changes in BMI were evaluated as shown elsewhere (18). \(\text{L-dopa}\) equivalent dose (LED) was calculated with a formula previously described (41).
Scanning procedures

All PD patients and healthy volunteers were scanned at the Cyclotron Building, Hammersmith Hospital (London, UK). All patients had their l-dopa medication stopped for at least 18 hours before PET, and DA agonists were stopped 3 days before scanning. Subjects were scanned after fasting and in a resting state with low light and no noise in the room. Smoking and consumption of alcohol, coffee, and other caffeinated beverages were not allowed at least 12 hours before scanning. All subjects had a volumetric 1.5-T MRI (Picker Eclipse) for the purposes of image registration to facilitate localization of the regions of interest (ROIs) and for volume analysis. Subjects were placed in the scanners with transaxial planes oriented parallel to the orbitomeatal line, and head positioning was monitored throughout the scan.

$^{18}$F-Dopa

$^{18}$F-Dopa PET scans were performed with an ECAT HR+ (CTI/Siemens 962) PET tomography scanner that has 15.5-cm total axial field of view (FOV) and provides 63 transaxial planes. This camera has a mean reconstructed image transaxial resolution over a 10-cm radius FOV (from the center) of 6.0 + 0.5 mm and an axial resolution of 5.0 + 0.8 mm (47). To improve $^{18}$F-dopa availability to the brain, we pretreated subjects with 150 mg of carbidopa 1 hour before radioisotope injection (48). A mean dose of 185 MBq of $^{18}$F-dopa in 10 ml of normal saline was infused intravenously over 30 s. Scanning began at the start of tracer infusion generating 26 time frames over 94 min and 30 s.

$[^{11}]$C-Raclopride

All $[^{11}]$C-raclopride scans were performed with the ECAT EXACT HR$^{+}$ PET tomography scanner as previously described (23, 24). Briefly, each subject received two $[^{11}]$C-raclopride PET scans, 2 to 3 days apart, and was assigned randomly to have an intravenous dose of normal saline before one scan and methamphetamine (0.3 mg/kg) before the other scan. Saline or methamphetamine was administered as a bolus over 30 s, 7 min before the injection of 140-MBq intravenous bolus dose of $[^{11}]$C-raclopride. Subjects did not know whether they would receive saline or methamphetamine. Scanning began 30 s before tracer infusion, generating 20 time frames over 60 min.

$[^{11}]$C-DASB

The $[^{11}]$C-DASB scans were performed with the ECAT EXACT HR$^{+}$ PET tomography scanner as previously described (17, 41). A mean $[^{11}]$C-DASB dose of 450 MBq was administered as a bolus intravenous injection 30 s after the initiation of scanning.

PET data analysis

The investigators analyzing the scans were blinded to the clinical characteristics of the participants. ROI image analysis was used.

$^{18}$F-Dopa data analysis

$^{18}$F-Dopa is taken up by monoaminergic neurons that contain aromatic amino acid decarboxylase. This makes $^{18}$F-dopa PET a marker of the pattern of monoaminergic neuron innervation in areas in which such innervation predominates—the dopaminergic neurons in the striatum and the NE neurons of the locus coeruleus (25). Here, we used a centralized analysis technique to implement quality control as has been described elsewhere (50). $^{18}$F-Dopa influx rate constant ($K_i$) values for each ROI were computed with the multiple-time linear graphical analysis method (51) with occipital cortex activity providing a reference input function representing nonspecific tissue tracer uptake (52). Summed images of the 26 frames of the dynamic $^{18}$F-dopa time series reflecting both tracer delivery and specific uptake of $^{18}$F-dopa collected over 30 to 90 min after intravenous infusion were created for spatial normalization purposes (52). The summed images were spatially normalized to a smoothed $^{18}$F-dopa template in Montreal Neurological Institute (MINI) stereotactic space (created in-house from healthy control subjects) with SPM5 (http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB7 (The Mathworks Inc.). Then, the transformation parameters were applied to the respective $^{18}$F-dopa $K_i$ maps. This technique standardizes brain position and shape, allowing the use of standard object maps in MINI space to quantify tracer uptake in preselected ROIs. To optimize image quality, we audited the quality of the reference region input function time-activity curves, the alignment of $K_i$ maps, and the corresponding summed images. The ROIs sampled included amygdala, ACC, basal ganglia (caudate, putamen, globus pallidus, subthalamic nucleus, and substantia nigra), hypothalamus, insula, locus coeruleus, PCC, PFC, red nucleus, and thalamus. Their anatomical definition has been previously described (27, 53) and was crosschecked with the Talairach and Tournoux (54) stereotactic atlas in combination with the Duvernoy (55) three-dimensional sectional atlas. The final object map was applied to the spatially normalized $K_i$ maps, and the values for specific $^{18}$F-dopa uptake of individual regions were extracted with ANALYZE8.1 medical imaging software (Mayo Foundation). To ensure correct placement of the ROIs, we inspected each plane of the normalized $K_i$ maps and summed images.

$[^{11}]$C-Raclopride data analysis

The $[^{11}]$C-raclopride ROI analytical approach has been described previously (23, 24). Briefly, parametric images of $[^{11}]$C-raclopride binding potential ($B_{PND}$) were generated from the dynamic $[^{11}]$C-raclopride PET scans with a basis function implementation of the simplified reference region compartmental model with the cerebellum providing the reference nonspecific tissue input function (56). The ROI analysis was performed with ANALYZE8.1, and ROIs sampled included caudate, putamen, total striatum, and basal ganglia. For each subject, the right to left averaged ROI binding potentials were calculated.

$[^{11}]$C-DASB data analysis

The $[^{11}]$C-DASB ROI analysis has been previously described (17, 41). Briefly, the input function was derived from the activity in the posterior cerebellar gray matter cortex, avoiding inclusion of the vermis (57). After reconstruction of the dynamic $[^{11}]$C-DASB image volume, a summed image volume was created from the entire dynamic data set. The MRIs of subjects were co-registered to the summed PET volume with the Mutual Information Registration algorithm in the SPM5. After co-registration, the definition of ROIs was performed on the co-registered MRIs with ANALYZE8.1. ROIs were manually defined on both hemispheres for amygdala, ACC, hypothalamus, insula, mid-pons area, PCC, PFC, raphe nuclei, and thalamus. The transformation characteristics generated from the co-registration were applied to the dynamic PET data set and the ROIs projected onto the image volume. By obtaining the regional concentrations of radioactivity (kBq/ml) from the full dynamic scan, the decay-corrected time-activity curves were computed and movement during the scan was assessed. Any movement detected was corrected with a frame-by-frame realignment procedure as previously described (58). Volume of...
distribution ratios (VDRs) were computed for ROI time-activity curves with the graphical analysis method of Logan et al. (59), and the BPND was calculated as VDR – 1 (60, 61). Right to left averaged BPND values were computed for all ROIs.

Statistical analysis (for Tables 3 and 4) Statistical analysis was performed with GraphPad Prism (version 5.0d for Macintosh, GraphPad Software Inc.). For all comparisons, variance homogeneity and Gaussianity were tested with Bartlett and Kolmogorov-Smirnov tests. Depending on the success of the normality tests and the differences on SDs between samples, parametric or nonparametric tests with or without corrections were used as appropriate.

Percentage decreases of regional $[11C]$raclopride BPND reflecting increases of DA release after a methamphetamine challenge compared to the practically defined OFF medication condition were calculated according to the following formula:

$$
\% \Delta_{[11C]} \text{Raclopride} = \frac{[11C] \text{Raclopride BPND}_{\text{OFF-Methamphetamine}}}{[11C] \text{Raclopride BPND}_{\text{OFF}}} \times 100
$$

REFERENCES AND NOTES


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