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Serotonergic Neurons Mediate Dyskinesia Side Effects in Parkinson's Patients with Neural Transplants

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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.

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PARKINSON'S DISEASE

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-HT_{1A}) 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 reinnervate 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 insufficiently 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).

^{18}F-dopa, a marker for dopamine synthesis (converted by the aromatic amino acid decarboxylase), and ^{11}C-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 ^{11}C-3-amino-4-(2-dimethylaminomethylphenylthio)

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Table 1. Transplantation characteristics. L, left; R, right; Put, putamen; Caud, caudate nucleus; VM, ventral mesencephalon.

	Patient 7	Patient 15
Year of transplantation	1993 1994	1996
Location of implanted sites	L Put R Put	L Put + Caud/R Put + Caud
Number of implant sites	5 5	5 + 2/5 + 2
Number of donors	5 5	4/4
Size of donor tissue	L Put: mean, 22 mm (range, 14–26 mm) R Put: mean, 18 mm (range, 14–22 mm)	L Put and L Caud: mean, 20 mm (range,15–26 mm) R Put and R Caud: mean, 19 mm (range,13–23 mm)
Amount of implanted tissue	L Put: 5 VM R Put: 5 VM	L Put: 2.9 VM L Caud: 1.1 VM R Put: 2.9 VM R Caud: 1.1 VM
Time from abortion to implantation	L Put: mean, 7.25 hours (range, 5.5–9 hours) R Put: mean, 5.5 hours (range, 3.5–7.5 hours)	L Put: mean, 5 hours (range, 3.5–6.5 hours)* L Caud: mean, 6.75 hours (range, 6–7.5 hours)* R Put: mean, 4.25 hours (range, 2.5–6 hours)* R Caud: mean, 6 hours (range 5–7 hours)*

*Exposed to the lazaroid tirilazad mesylate during storage and dissociation; patient given lazaroid for 3 days (*11*).

Table 2. Participant characteristics. UPDRS part III (motor score) worst score is 108; UPDRS total worst score is 199. LED (L-dopa equivalent dose) was calculated as follows: LED (mg) = (1 × L-dopa) + (0.77 × L-dopa CR) + (1.43 × L-dopa + entacapone) + (1.11 × L-dopa CR + entacapone) + (20 × ropinirole) + (20 × ropinirole ER) + (100 × pramipexole) + (30 × rotigotine) + (10 × bromocriptine) + (8 × apomorphine) + (100 × pergolide) + (67 × cabergoline). CR, controlled release; ER, extended release; M, male; F, female; off, off-medication.

	Normal controls (*n* = 12)	Advanced PD (*n* = 12)	Patient 7	Patient 15
Sex	10 M/2 F	10 M/2 F	Male	Male
Age (years ± SD)	63.3 ± 7.0	67.1 ± 7.2	65	66
UPDRS part III off (mean ± SD)	—	44.5 ± 10.2	13	7
UPDRS total off (mean ± SD)	—	77.5 ± 16.8	37	17
PD duration (years ± SD)	—	12.5 ± 3.7	26	25
Daily LED (mean ± SD)	—	1087 ± 692	0	0
MMSE (mean ± SD)	29.42 ± 0.7	28.50 ± 2.0	29	29

benzonitrile (^{11}C-DASB) PET, which binds to the serotonin transporter and serves as a marker of serotonergic neuron density, we found excessive serotonergic innervation in the grafted putamen. ^{11}C-DASB binding was 172% and 285% higher than mean values in healthy normal individuals and advanced PD patients, respectively. In contrast, ^{11}C-DASB binding in the nongrafted caudate nucleus was reduced by 29.4% compared to that in normal individuals and was similar to the mean values in patients with advanced PD (Fig. 2, A to C and E to G). The ratio of serotonergic to dopaminergic innervation in the grafted putamen, estimated as the ^{11}C-DASB/^{18}F-dopa binding ratio, was increased by 230% compared to the ratio in normal people (356 versus 108).

Patient 15 reported PD-related symptoms since 1982 and was diagnosed in 1985. Preoperatively, after a 6-year period with good response to L-dopa treatment, he developed severe motor complications including diphasic and L-dopa–induced peak-dose dyskinesias, on-off fluctuations, and wearing-off phenomena without off-phase dyskinesias. He had received bilateral ventral mesencephalic tissue transplants in the caudate and putamen 13 years earlier (*11*) (Tables 1 and 2). For 3 years after surgery, he did not experience any improvement in his motor symptoms, although the amount of administered L-dopa was reduced and on-period dyskinesias were slightly reduced. From the fourth postoperative year, motor symptoms gradually improved and off periods faded out, and 8 years ago, all dopaminergic medications were stopped (Fig. 1B). Currently, he has no "off" periods but has developed GIDs, which are poorly controlled with amantadine. His dyskinesias are moderately disabling and are evident most of the time. His dyskinesias include involuntary movements mainly of the lower extremities, although trunk and upper extremity involuntary movements are also seen (movie S3).

^{18}F-dopa and ^{11}C-raclopride PET imaging in this patient has shown restoration of dopamine neurons and dopamine release to normal values in the grafted putamen (Fig. 1B). ^{11}C-DASB PET revealed excessive binding of the radiotracer in both grafted parts of the striatum (caudate nucleus: 123% and 252%; putamen: 77% and 150%; increased compared to normal and advanced PD mean values, respectively) (Fig. 2, A, B, and D to G). The ^{11}C-DASB/^{18}F-dopa binding ratio in the grafted putamen was 146% increased compared to normal (266 versus 108).

Our in vivo ^{11}C-DASB and ^{18}F-dopa PET imaging data indicate serotonergic hyperinnervation in the grafted caudate nucleus and putamen that gives rise to high serotonergic relative to dopaminergic innervation in these areas. We hypothesized that this excess of serotonergic innervation could contribute to GIDs by releasing dopamine as a false transmitter. Therefore, we investigated whether administering a 5-HT$_{1A}$ agonist in low, repeated doses would dampen the activity of serotonin neurons, inhibit their transmitter release, and thereby attenuate GIDs. Fifteen milligrams of the 5-HT$_{1A}$ agonist buspirone, given in three doses of 5 mg at 30-min intervals, significantly attenuated the severity of GIDs in both patients 7 and 15 for 4 hours when compared

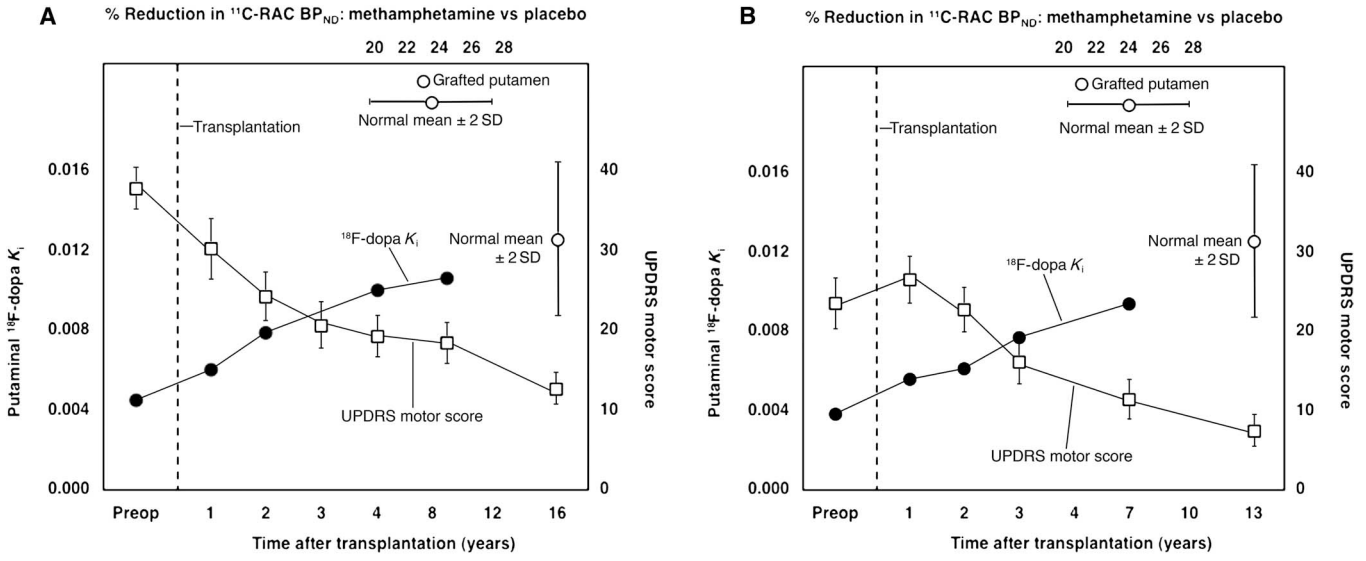

Fig. 1. Fetal mesencephalic grafts provide long-lasting major relief of motor symptoms and restore dopamine innervation and release in the grafted striatum. (**A** and **B**) Patients 7 (A) and 15 (B) from the Lund series. Motor examination scores of the UPDRS (worst possible score = 108) (mean ± 95% confidence interval) in the off phase (open squares) and ^{18}F-dopa K_i values in the grafted putamen (filled circles), preoperatively (Preop) and at various time points after transplantation. Comparative data on ^{18}F-dopa K_i values in the putamen (mean ± 2 SD) are given on the right for a group of 16 healthy volunteers. Percentage reduction of ^{11}C-raclopride binding values (BP$_{ND}$) (upper right corner) in the grafted putamen after methamphetamine administration at postoperative year 9 and 7 for patient 7 and 15, respectively, and in the putamen of a group of six healthy volunteers (mean ± 2 SD). ^{18}F-dopa K_i values and UPDRS scores up to 2.5 years postoperatively for patient 7 (*8*) and during the first year after transplantation for patient 15 (*10*) were reported previously.

to no-drug or to placebo administration (Fig. 2, H and I, and movies S1 to S4). The Unified Parkinson's Disease Rating Scale (UPDRS) motor (part III) scores remained unchanged after buspirone administration (13 for patient 7; 7 for patient 15), and there were no side effects.

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 ^{11}C-DASB signal 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 grafted putamen and the development of GIDs, patient 7, who had more severe GIDs than patient 15, showed greater ^{11}C-DASB binding and a higher serotonergic/dopaminergic innervation ratio in the grafted putamen. This finding is compatible with studies on 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$_{1A}$ 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$_{1A}$ 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 nonphysiological release of dopamine from serotonergic 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$_{1A}$ agonists to dampen serotonergic neurotransmission by activation of the inhibitory autoreceptors.

Whether GIDs and 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 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 L-dopa are administered orally and reach the extensively denervated striatum (as

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.

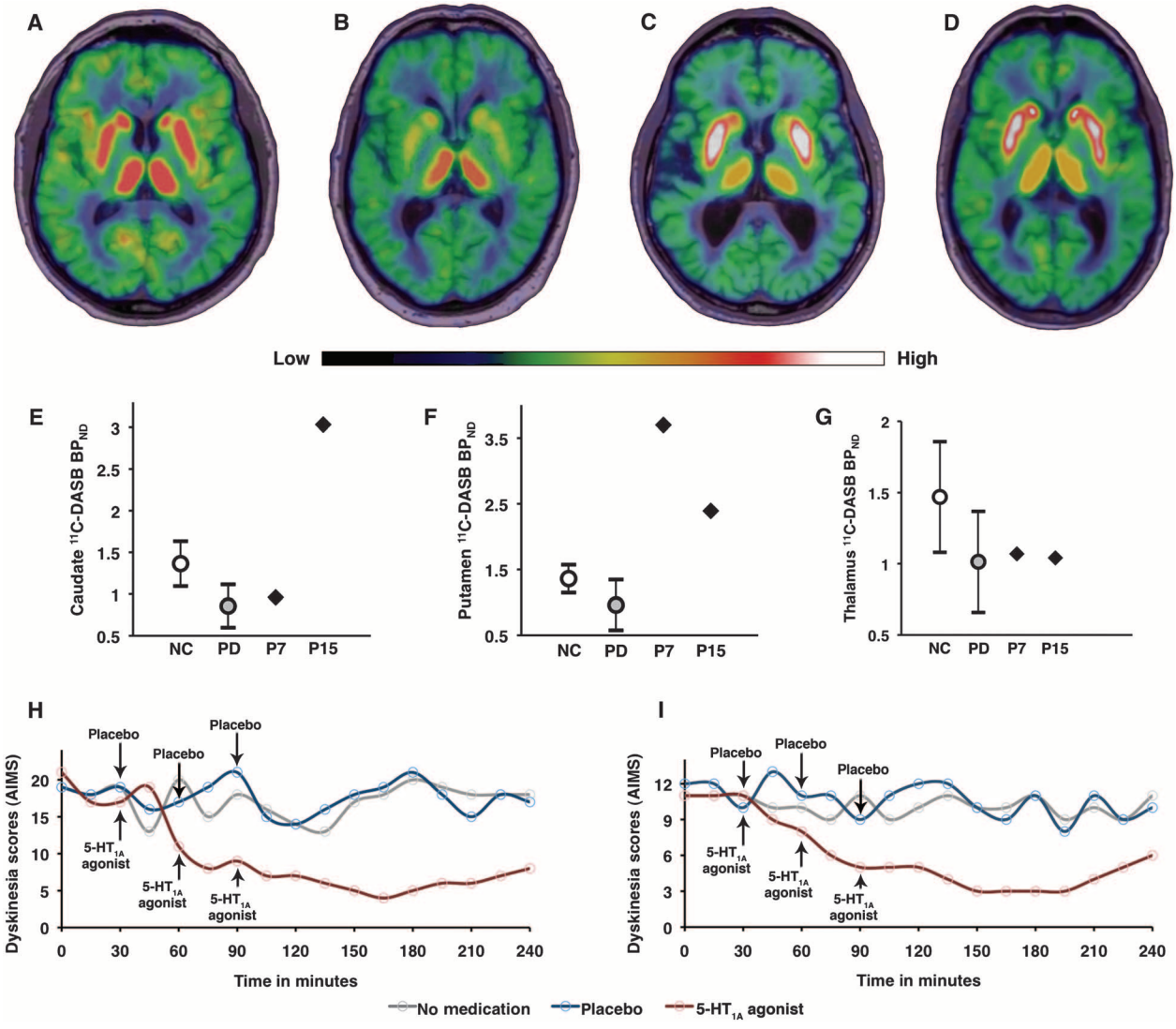

Fig. 2. Serotonergic hyperinnervation in the grafted striatum causes dyskinesias, which are effectively suppressed by a 5-HT_{1A} agonist. (**A** to **D**) Summed ^{11}C-DASB PET images coregistered and fused with 1.5-T MRI images at the level of the dorsal basal ganglia for (A) a 64-year-old healthy 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 (± 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 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 cells (*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-HT_{1A} 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}C-DASB, ^{18}F-dopa, and ^{11}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_{1A} agonist trial

Patients 7 and 15 underwent a double-blind acute oral challenge with the 5-HT_{1A} 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_{max} = 0.89 ± 0.15 (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}C-DASB scans were performed with an ECAT HR^{+} (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 with localizing the regions of interest (ROIs). The PD patients stopped medication for at least 18 hours before scanning.

^{18}F-dopa and ^{11}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}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}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-

tribution ratios (VDRs) were computed for ROI TACs with the graphical analysis method of Logan *et al.* (*35*), and the binding potential of the specifically bound radioligand relative to the nondisplaceable radioligand in tissue (BP_{ND}) was calculated as VDR-1 (*36*, *37*).

^{18}F-dopa data analysis

Protocols are as described previously (*6*, *38*). Briefly, regional ^{18}F-dopa influx rate constant (K_i) values for caudate nucleus and putamen were defined with the multiple time graphical analysis method with the occipital reference tissue as the input function.

^{11}C-raclopride data analysis

Protocols are as described previously (*6*). Briefly, patients 7 and 15 and six healthy male normal controls undertook two ^{11}C-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 ^{11}C-raclopride binding (BP_{ND}) 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 as the reference tissue (*39*).

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 two-tailed test. The α level was set at $P < 0.05$.

SUPPLEMENTARY MATERIAL

www.sciencetranslationalmedicine.org/cgi/content/full/2/38/38ra46/DC1
Imaging data
Movie S1. Patient 7 after administration of placebo.
Movie S2. Patient 7 after administration of 5-HT$_{1A}$ agonist.
Movie S3. Patient 15 after administration of placebo.
Movie S4. Patient 15 after administration of 5-HT$_{1A}$ agonist.

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