Direct brain infusion of glial cell line–derived neurotrophic factor in Parkinson disease

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Glial cell line–derived neurotrophic factor (GDNF) is a potent neurotrophic factor with restorative effects in a wide variety of rodent and primate models of Parkinson disease, but penetration into brain tissue from either the blood or the cerebro-spinal fluid is limited. Here we delivered GDNF directly into the putamen of five Parkinson patients in a phase 1 safety trial. One catheter needed to be repositioned and there were changes in the magnetic resonance images that disappeared after lowering the concentration of GDNF. After one year, there were no serious clinical side effects, a 39% improvement in the off-medication motor sub-score of the Unified Parkinson's Disease Rating Scale (UPDRS) and a 61% improvement in the activities of daily living sub-score. Medication-induced dyskinesias were reduced by 64% and were not observed off medication during chronic GDNF delivery. Positron emission tomography (PET) scans of [^{18}F]dopamine uptake showed a significant 28% increase in putamen dopamine storage after 18 months, suggesting a direct effect of GDNF on dopamine function. This study warrants careful examination of GDNF as a treatment for Parkinson disease.

Parkinson disease is a neurodegenerative disorder characterized by impairment of motor function, due largely to a progressive loss of dopamine neurons in the substantia nigra. The mainstay of treatment is L-3,4-dihydroxyphenylalanine (L-dopa) or dopamine agonists. As the disease progresses, patients typically become less responsive to L-dopa and develop motor side effects. These patients may be helped with surgical therapies, including deep brain stimulation and transplantation of fetal dopamine neurons (reviewed in ref. 1). But neither of these approaches is considered neuroprotective and thus would not be expected to halt the continual loss of remaining dopamine neurons. GDNF is important for the development and maintenance of dopamine neurons[2]. In rodent and primate models of Parkinson disease involving selective degeneration of dopamine neurons, GDNF has been shown to be neuroprotective, to encourage fiber outgrowth and to improve motor function when delivered into the cerebral ventricles or directly into the striatum or substantia nigra[3–6]. Intraparenchymal delivery is effective whether by bolus injection, by chronic infusion using a pump or by infecting the brain with live replication-deficient viral particles engineered to deliver GDNF[7–9].

These encouraging results in animal models led to a human study in which GDNF was administered by monthly bolus injections into the cerebral ventricles of Parkinson disease patients. No beneficial clinical effects were seen using this route of delivery. Furthermore, side effects were reported and there was no evidence of restoration of dopamine fibers in the striatum in one subject post-mortem[10,11]. The reason for the poor efficacy of intraventricular delivery of GDNF in these patients is unclear, but is probably related to an insufficient concentration of GDNF in the relevant structures. In moving from an animal model to a human, there is substantial increase in the volume of tissue through which this relatively large protein must penetrate. The reported side effects may have resulted from its wide dispersal throughout the cerebrospinal fluid, allowing it to act on structures outside the motor pathways that also have GDNF receptors[12].

Of the alternative methods of GDNF delivery in humans, the use of viral vectors would be inappropriate at this stage because further development and safety studies are required. Bolus injection into the parenchyma exposes the patient to a higher risk of tissue trauma and denies the clinician the means to fine-tune and optimize dose delivery. In this phase 1 clinical trial, we delivered GDNF directly into the putamen of patients with Parkinson disease by chronic infusion using pumps. Infusion into the postero-dorsal putamen (its sensorimotor component) was chosen because in Parkinson disease this is the region that is most severely depleted of dopamine. We anticipated that any observed clinical benefits would be due to retrograde transport of GDNF down the surviving nigro-striatal axons, leading to

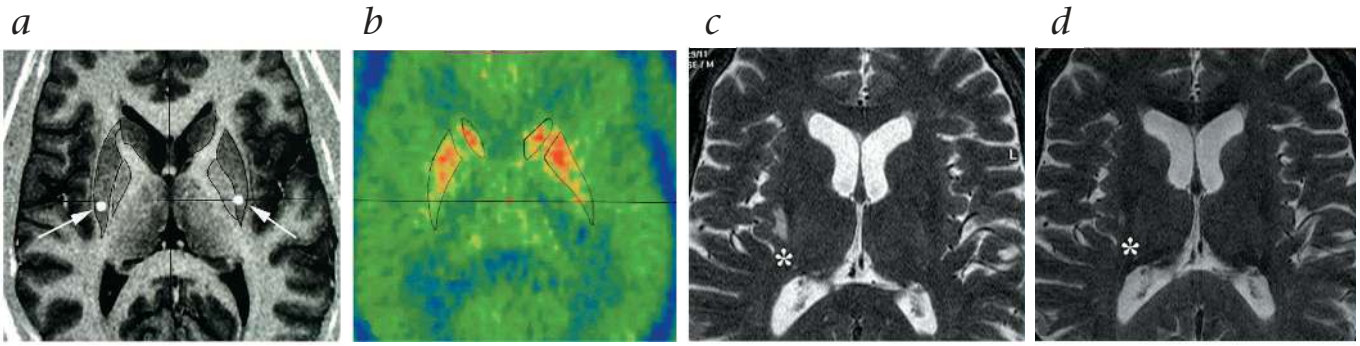

Fig. 1 Surgical targeting and changes in MRI after chronic GDNF delivery. ***a***, MRI scan showing targeted regions in the dorsal putamen of patient P2 (arrows and white circles). ***b***, The marked areas showed a low [^{18}F]dopa signal in an adjacent PET image. ***c***, High signal in the area around the catheter tip in patient P1 6 months after treatment with GDNF at a concentration of 43.2 μg/d at an infusion rate of 6 μl/h (region above asterisk). ***d***, Resolution in same patient after treatment with a lower GDNF concentration of 14.4 μg/d at a flow rate of 6 μl/h for 1 month (region above asterisk).

greater dopamine delivery and possibly local axonal sprouting in the putamen, as previously reported in primate models[8].

Study design and safety issues

We selected five patients with idiopathic, L-dopa–responsive Parkinson disease who were poorly controlled on optimal medical therapy. The first case (patient P1) treated in the trial had predominantly unilateral disease affecting the left side and underwent contralateral putamenal implantation of catheter and pump for GDNF delivery. The remaining patients (P2–P5) had bilateral disease and bilateral putamenal implantations of delivery systems. We determined the precise region of the dorsal putamen to be targeted for infusion by co-localization studies using PET scans of [^{18}F]dopamine uptake ([^{18}F]dopa PET) and magnetic resonance imaging (Figs. 1*a* and *b*). Pump placement and stereotactic surgery were tolerated well by all patients, but there were some complications. Patient P1 required that the catheter be repositioned to center it exactly in the dorsal putamen. This was achieved successfully on a second pass during the surgical procedure. Patient P4 developed a wound infection related to the pumps and connection tubing, which was successfully treated with explantation of the extracranial devices, antibiotics and re-implantation in four weeks.

The clinical side effects due to GDNF infusion itself were limited (Table 1). There was no nausea, anorexia, vomiting or weight loss reported, as in the previous intraventricular trial[10]. There were no hematological or blood-chemistry abnormalities. The only consistent finding was Lhermitte's phenomenon (tingling passing from the neck down the arms and sometimes onto the trunk and down the legs, provoked by neck flexion). This event was mild, intermittent, non-distressing and occurred most frequently at the higher dose; in fact, it was often described as "pleasurable". In all patients, however, T2 magnetic resonance images (MRIs) showed a region of high signal intensity around the tips of the catheters. This response varied between patients and even between the two hemispheres in bilaterally implanted cases. The signal change was most evident following the dose escalation of GDNF (Fig. 1*c*). The explanation for this signal change is unclear, but the high signal areas might be areas of vasogenic edema or protein buildup. Our uncertainty as to the relevance of these changes led us to reduce GDNF delivery between three and six months back to 14.4 μg per putamen per day for all patients, which resulted in a substantial reduction of the high signal (Fig. 1*d*).

Table 1 Patient data and overall effects of GDNF

	P1 (□)	P2 (△)	P3 (▽)	P4 (◇)	P5 (○)
Patient data					
Age (years)	62	46	56	56	51
Duration of Parkinson disease	6	13	30	27	19
Unilateral or bilateral pump (U/B)	U	B	B	B	B
L-dopa equivalents at 0 months	667	615	2154	680	762
Change in L-DOPA after 1 year	+10%	+6%	–54%	+17%	–40%
Side effects					
Hypersalivation			*		*
Taste abnormalities	*		*	*	*
Lhermitte's	*	*	*	*	*
Headaches				*	*
Vivid dreams	*	*			
Apthous mouth ulceration			*	*	*
MRI changes	*	*	*	*	*
Nausea or vomiting					
Weight loss					
Pump-related discomfort					*
Procedural adverse events					
Repositioning of catheter	*				
Pump infection				*	
Other clinical effects					
Recovery of taste and smell	*			*	*
Revival of sexual function			*	*	*
Improved bladder function					*
Reduction in tinnitis					*

Table 2 GDNF improves UPDRS and CAPIT clinical rating scores off and on medication

			Time after GDNF treatment		
	Medication	Baseline	3 months	6 months	12 months
UPDRS I (total)	Off	66 ± 15	46 ± 8.9 (–30%)	44 ± 6.5 (–33%)	35 ± 11 (–48%)
	On	28 ± 3.7	15 ± 3.2 (–48%)	20 ± 2.4 (–28%)	15 ± 3.4 (–45%)
UPDRS II (activities of daily living)	Off	21 ± 3.7	15 ± 3.0 (–30%)	13 ± 2.7 (–37%)	8.2 ± 3.3 (–61%)
	On	5.2 ± 2.2	2.0 ± 1.6 (–62%)	3.4 ± 0.9 (–35%)	2.6 ± 2.3 (–50%)
UPDRS III (motor examination)	Off	33 ± 6.9	25 ± 4.8 (–24%)	23 ± 2.9 (–32%)	20 ± 7.5 (–39%)
	On	10 ± 2.8	6.6 ± 3.6 (–39%)	9.0 ± 4.1 (–17%)	6.8 ± 4.2 (–37%)
UPDRS IVa (dyskinesias)	On	5.0 ± 2.6	1.8 ± 1.1 (–64%)	3.0 ± 1.5 (–40%)	1.8 ± 1.1 (–64%)
UPDRS IVb (fluctuations)	On	4.8 ± 2.6	3.2 ± 1.9 (–33%)	3.8 ± 1.6 (–21%)	3.4 ± 1.9 (–29%)
CAPIT (pronation/supination)	Off	38.4 ± 23	16.8 ± 4.9 (–56%)	16.0 ± 4.0 (–58%)	14.1 ± 4.4 (–63%)
	On	14.0 ± 3.2	11.6 ± 2.1 (–17%)	11.2 ± 1.9 (–20%)	10.9 ± 2.3 (–20%)
CAPIT (hand/arm movements)	Off	18.4 ± 5.6	10.4 ± 2.9 (–43%)	9.3 ± 1.9 (–50%)	8.6 ± 3.2 (–53%)
	On	7.0 ± 1.8	5.6 ± 1.0 (–20%)	5.5 ± 1.1 (–22%)	5.6 ± 1.5 (–28%)
CAPIT (finger dexterity)	Off	64.8 ± 45	27.7 ± 8.8 (–57%)	28.2 ± 7.3 (–56%)	24.5 ± 5.1 (–62%)
	On	27.4 ± 9.4	20.9 ± 5.3 (–24%)	21.6 ± 3.9 (–21%)	19.8 ± 3.5 (–28%)
CAPIT (leg movements)	Off	17.1 ± 6.7	8.4 ± 1.8 (–51%)	7.4 ± 2.1 (–56%)	7.5 ± 1.3 (–56%)
	On	6.6 ± 0.5	5.7 ± 0.6 (–14%)	5.7 ± 0.4 (–14%)	5.5 ± 0.4 (–17%)

UPDRS scores represent the mean ± s.d. for 5 patients. CAPIT scores represent the mean time ± s.d., in seconds, to complete task for the left limb.

Efficacy of GDNF infusion

In all patients, the symptoms of Parkinson disease improved after three months of GNDF infusion. Periods of severe immobility, one of the cardinal features of Parkinson disease that occupied approximately 20% of the waking day before surgery, were eliminated completely after six months of GDNF infusion. These changes were not due to increases in medication. The study protocol aimed to maintain medication at the same dosages throughout the first year of GDNF treatment. But patient P3 had been taking medication on demand owing to frequent periods of akinesia at the onset of the study and needed to reduce his medication as his symptoms improved (Table 1). Patient P5 had increased sensitivity to L-dopa after GDNF infusion and also needed to reduce his dosage.

The most widely used and validated scale for assessing functional changes in Parkinson disease is the UPDRS. The total UPDRS scores in the clinically defined 'off' phase, when assessed 12 months after commencement of GDNF infusion was 48% lower than the baseline value (Table 2 and Fig. 2*a*). Although this was a small group of patients, we carried out a non-parametric significance test, which showed that this reduction was highly significant across the three time points ($P < 0.005$; Friedman test). We observed the largest effects over the first three months, but the effects persisted throughout the trial. There was also a 45% reduction in total UPDRS scores in the clinically defined 'on' phase after 12 months, which followed a similar pattern over time ($P < 0.002$; Friedman test; Table 2 and Fig. 2*a*). Although patient P4's final score was still below baseline (Fig. 2*a*), his symptoms were worse at the 12-month assessment. This may have been due to an unrelated inter-current infection.

When we analyzed the results, it was clear that these effects on total UPDRS scores during the off phase were reflected by improvements in the UPDRS sub-scores for activities of daily living (ADL), sub-scale II ($P < 0.002$; Friedman test; Table 2 and Fig. 2*b*), and motor, sub-scale III ($P < 0.002$; Friedman test; Table 2 and Fig. 2*c*). Dyskinesias (involuntary movements) are a common problem in Parkinson disease and were suffered by all but one of the patients at the start of the trial. The overall dyskinesia scores (UPDRS sub-scale IVa) were significantly reduced on medication after GDNF infusion for 12 months ($P < 0.01$; Friedman test; Table 2). We observed no dyskinesias in these patients when they were off medication. We assessed timed motor tests, which followed the protocol outlined by the Core Assessment Program for Intracerebral Transplantation (CAPIT)[13]. These were also improved in both the off and on medication states (Table 2). We as-

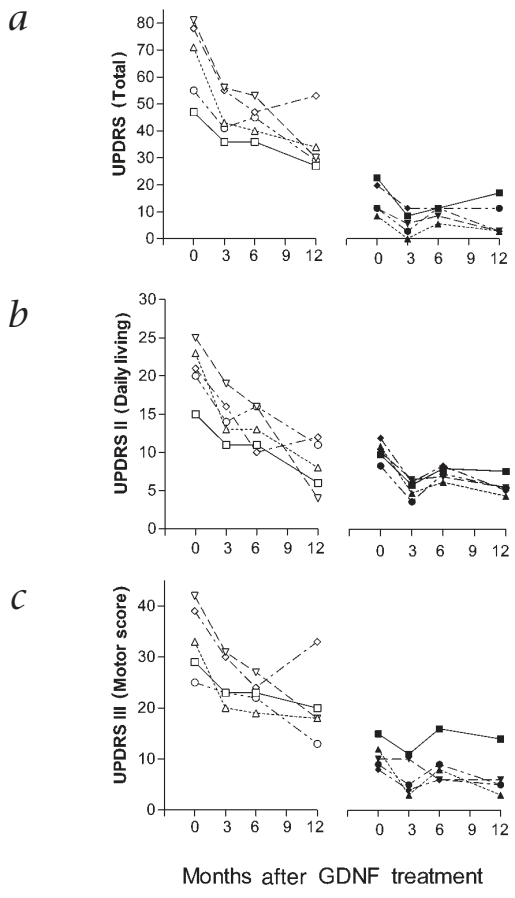

Fig. 2 UPDRS scores for patients, 0, 3, 6 and 12 months after GDNF infusion. Open symbols, off medication; closed symbols, on medication. Symbols for patients are designated in Table 1.

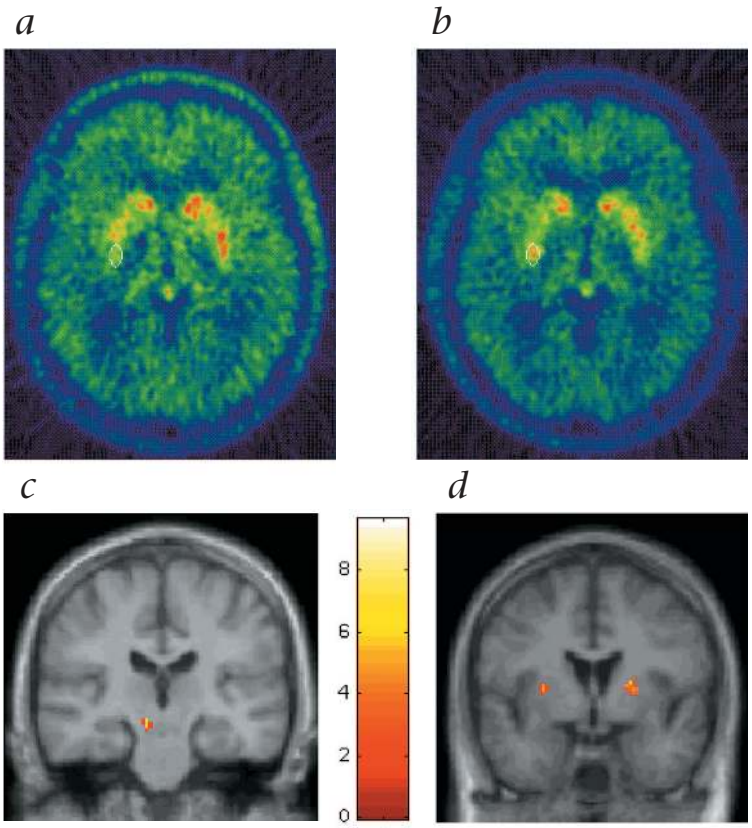

Fig. 3 GDNF increases [^{18}F]dopa influx. ***a***, PET image of patient P1, who presented with unilateral Parkinson disease before GDNF infusion and showed reduced dopamine storage on left side of brain. ***b***, The same patient 12 months after unilateral GDNF infusion to the putamen. Circle represents region of interest around the catheter tip used in the analysis. ***c*** and ***d***, Coronal MRI slices showing increased [^{18}F]dopa uptake (colored regions) in the region of the substantia nigra (*c*) and putamen (*d*). Pre-operative [^{18}F]dopa PET images from the 5 patients were compared with their 6-month follow-up scans using a paired Student's *t*-test in SPM99. The images are thresholded at $P = 0.05$ and uncorrected at cluster level. Color scale represents *z* value scores for *c* and *d*.

sessed the functional status of the patients using the Parkinson Disease Questionnaire (PDQ-39)[14]. This test also showed general improvement over time, with the overall scores tending towards levels expected in a control population (see Supplementary Table 1 online).

Three patients had long-standing loss of sensation of smell and taste, as is often the case in Parkinson disease. These symptoms greatly improved or resolved completely between three and six weeks of GDNF infusion (Table 1). With this recovery, however, patients intermittently experienced abnormal sensations of taste, reporting 'metallic' or 'soapy' tastes. At the highest dose of GDNF, three patients reported recovery of normal sexual function, both in terms of interest and potency. This recovery subsided as the dose was reduced.

We evaluated four of the five patients for both a pretreatment cognitive assessment and a 12-month review assessment. With the exception of impaired verbal memory in one patient, all patients had normal cognitive function with average or better scores on the cognitive test battery. The cognitive assessment battery has been previously described[15] and contained tests of verbal intellect, verbal and visual memory, attention and executive function. There were no significant changes in any of these tests after GDNF delivery (data not shown). Furthermore, none of the four patients were clinically depressed or anxious at follow-up.

[^{18}F]dopa PET scan changes

[^{18}F]dopa PET scans give a direct indication of dopamine storage capacity in the brain and have been used extensively to assess dopamine changes in Parkinson disease[16]. Baseline scans indicated that the posterior segment of the putamen in all patients had low [^{18}F]dopa uptake (Table 3). These regions of reduced dopamine storage were used to establish the optimal site for placing the catheters for GDNF delivery (Fig. 1*b*). After six months, treatment with GDNF increased [^{18}F]dopa uptake by 24.5% (0–49%) in a 0.36-cc ovoid volume around the tip of each catheter (Figs. 3*a* and *b* and Table 3), whereas regions away from the catheter continued to show signal decline (Fig. 3*a*). Twelve months after GDNF infusion, the same analysis also indicated increases in [^{18}F]dopa uptake. But this was complicated by the fact that patient P2 moved considerably during the third scan at 12 months, which may have resulted in an underestimate of his true [^{18}F]dopa uptake and reduced the power of the statistical analysis, which did not reach significance ($P = 0.096$; Student's two-tailed *t*-test; Table 3). Although full clinical testing had not been completed at the time of writing,the patients were reassessed for dopamine uptake after 18 months of GDNF infusion. At this time, there were further increases in [^{18}F]dopa uptake and no movement in the scanner. The increases were now significantly different from their pre-operative values ($P = 0.021$ using Student's two-tailed *t*-test; Table 3).

Although interrogating a single volume around the tip of each catheter may identify local changes in [^{18}F]dopa uptake, changes elsewhere in the putamen or in the midbrain would be missed using this technique. Statistical parametric mapping (SPM) localizes significant changes in [^{18}F]dopa storage between scans throughout the brain at a voxel level and has recently been shown to be a useful method for detecting changes in dopaminergic function[17,18] and for following the progression of Parkinson disease[19]. When the preoperative and six-month images of [^{18}F]dopa influx constants (K_i values) were investigated with SPM, three regions showed significant focal increases in [^{18}F]dopa uptake: (i) the right posterior dorsal putamen (+17.9%), (ii) the left medial dorsal putamen (+25.3%) and (iii) the right substantia nigra (+16%). The exact locations of the regions with increased ^{18}F-dopa uptake were superimposed on a mean MRI template constructed from the individual T1-weighted MRIs of the five patients (Fig. 3*c* and *d*). The movement of patient P2 during the 12-month scan again made interpretation of this small sample very difficult. Despite this, the patients as a group continued to show a significant increase in [^{18}F]dopa uptake in the right substantia nigra region (+26%; paired *t*-test; $P < 0.05$ uncorrected at cluster level).

Discussion

This study shows for the first time that direct intra-putamenal GDNF infusion in patients with Parkinson disease can be tolerated for one year and leads to significant increases in dopamine storage in the putamen. The precise functional effects of GDNF delivery have not yet been determined owing to the open nature of the trial and the small number of patients. But the tests that

were done showed reductions in many of the rating scores for Parkinson disease. Lhermitte's phenomenon was the only consistent side effect reported. There were high-signal changes on T2-weighted MRIs, which were most prominent at higher concentrations of GDNF. The meaning of these MRI changes is not clear, but they might reflect protein deposition or local vasogenic edema. Because the phenomenon was reversible with dose reduction and because these areas corresponded with those showing increased [^{18}F]dopa uptake, it is unlikely that these signal changes indicate cell toxicity. It does suggest, however, that careful monitoring of the region around the catheter tip is essential in this type of clinical trial involving direct infusion of growth factors.

Table 3 GDNF increases dopamine storage in the region around the catheter

	Pre-operative	6 months	12 months	18 months
P1 (□)	0.0061	0.0091 (+49.2%)	0.0068 (+11.5%)	0.0065 (+6.6%)
P2 (△)	0.0045	0.0052 (+15.6%)	0.0040 (–11.1%)[a]	0.0053 (+17.7%)
P3 (▽)	0.0041	0.0054 (+31.7%)	0.0051 (+24.4%)	0.0061 (+48.8%)
P4 (◇)	0.0051	0.0051 (0%)	0.0071 (+39.2%)	0.0074 (+45.1%)
P5 (○)	0.0039	0.0047 (+20.5%)	0.0051 (+30.8%)	0.0050 (+28.2%)
Mean	0.00474	0.0059 (+24.5%)	0.00562 (+18.6%)	0.00606 (+27.9%)
s.d.	0.00089	0.0018	0.0013	0.00096
P value		0.083	0.096	0.021

Values of the [^{18}F]dopa influx constants (K_i) for the 5 patients preoperatively and at 6, 12 and 18 months after operation. The values for patient P1 are for the right side only. The remaining values are an average of left and right sides. The percent change from baseline is represented in brackets. *P* values based on two-tailed Student's *t*-test compared with pre-operative value. [a]Patient moved in scanner.

Although L-dopa equivalents were maintained in three of the five patients throughout this study, there was a significant reduction in dyskinetic movements by over 60% while on medication and no dyskinetic movements off medication. Reduction of L-dopa–induced dyskinesias has also been reported after intracerebral infusion of GDNF in monkeys[20]. This result is in contrast to recent fetal transplant trials in which some patients experienced dyskinesias of unknown origin when off medication[21,22]. We feel this finding is important for these patients and suggests that GDNF might regulate dopamine production, release and metabolism in the striatum, thus improving the processing of motor output. This may explain why our patients experienced better quality of life when on medication.

As this was a phase 1 safety trial with no control group, it is possible to overemphasize the significant reductions in UPDRS and timed motor scores. Clearly, a phase 2 trial with full blinding is now required. But the substantial changes in the clinical status of the patients in this trial warrant further discussion. It is unlikely that the effects could be related to lesioning or inflammatory changes from the catheter and GDNF infusion because a putamenal lesion would probably exacerbate a patient's condition[23] and because the PET data showed increased [^{18}F]dopa uptake in a region around the catheter, making tissue toxicity unlikely. Although placebo effects are known to occur in drug treatments for Parkinson disease, patients generally improve 30% at most and this is rarely sustained on repeated testing over six months[24]. Furthermore, no placebo effect has been seen in two double-blind controlled neurosurgical interventions for Parkinson disease[21,25]. Our overall 48% reductions in UPDRS scores during the off phase are higher than might be expected from placebo. The improvements were progressive, with the off period scores at 12 months tending closely towards the baseline best on period scores. A decline in most CAPIT timed tests adds further evidence substantiating an overall subjective improvement.

Given caveats about lack of blinding, small patient numbers and the need for further double-blinded randomized placebo controlled trials, we feel that GDNF is probably responsible for a substantial part of this improvement in clinical status. The finding of significant reductions in the UPDRS scores in the on clinical phase to 45% of baseline was particularly interesting. No such improvement in the on state has previously been reported after surgical treatment for Parkinson disease[25] or transplantation of fetal dopamine neurons[26]. Parkinson disease is often associated with impaired olfaction[27], assumed to be the result of Lewy bodies in the olfactory bulb and cortex[28]. It is interesting that three patients reported a return of sense of smell after GDNF infusion. The reason for this is unclear and merits testing in future trials.

The continuous infusion of GDNF was also associated with a 28% increase in [^{18}F]dopa uptake around the tip of the infusion catheter after 18 months. This was in contrast to the predicted decline of up to 20% over this period for Parkinson disease[16,29]. The region-of-interest approach, centered around the tip of the catheter defined by MRI, proved more sensitive than SPM analysis in measuring the increase in [^{18}F]dopa uptake in the putamen. This is presumably because the catheter tips were not in the same spatial location in all five patients. As a result, the localized increases in [^{18}F]dopa uptake may not have overlain each other on the normalized images, therefore reducing the power of SPM99. These increases in the putamen were accompanied by 18–26% increases in right nigral [^{18}F]dopa uptake. The SPM analysis was unable to detect any changes in the left substantia nigra as only four patients received GDNF in the left putamen (patient P1 received a unilateral right-sided infusion). The four patients probably provided insufficient power for SPM99 to detect a left-sided nigral increase. Increased dopamine storage at the level of the substantia nigra suggest that either local nigral dopamine terminals or neuron cell bodies were also responding to the GDNF delivered to putamen nerve terminals, possibly through its retrograde transport.

The exact mechanism by which GDNF works has yet to be established, but the early changes in sense of smell and the overall reductions in UPDRS at three months suggest at least an initial pharmacological action of GDNF in the putamen. This probably involves, in part, a direct stimulatory effect on dopamine release as shown in rodent models[30]. Whether GDNF is protecting against the ongoing dopamine neuronal cell death or inducing new fiber outgrowth from remaining dopamine neurons has yet to be established. Continued administration of GDNF, accompanied by monitoring of disease progression and further PET scans, will help resolve this issue. In the human brain, it is not clear how far this protein will diffuse away from the catheter tip, and it is possible that more rostral portions of the putamen will continue to degenerate if the GDNF does not diffuse this far. Although the use of a mechanical system to deliver GDNF is constrained by potential risks at implantation, risks of infection and limited diffusion of the drug from the tip of the catheter in the desired target, this study is clearly a first step in proving effectiveness of intraparenchymal delivery. The development of viral vectors or encapsulated cells releasing GDNF may provide alter-

native strategies in the future[31]. Furthermore, *ex vivo* modification and transplantation of neural stem cells secreting GDNF that can migrate and differentiate in the striatum and protect dopamine neurons is another therapeutic option[32,33]. Finally, this trial may stimulate further related studies in other neurodegenerative diseases such as Alzheimer disease, amyotrophic lateral sclerosis and Huntington disease, in which various neurotrophic factors have also been shown to have beneficial effects in animal models[34–36].

Methods

Patients. This pilot study included 5 Parkinson disease patients (Table 1). We obtained full consent from all patients in accordance with local ethics committees at both the Frenchay Hospital and the Hammersmith Hospital Trusts. All patients were diagnosed with idiopathic Parkinson disease according to standard criteria and selected for surgery when they were suffering substantial functional impairment despite optimal medical therapy. Exclusion criteria included women of child-bearing age, age over 65, the presence of clinically significant depression, systemic disease and inability or unwillingness to comply with long-term follow-up.

Surgery. With patients under general anesthesia, we affixed a stereotactic frame parallel to the orbito-meatal plane. The anterior and posterior commissures were identified in a mid-sagittal planning scan. We acquired axial images 2 mm thick parallel to the anterior–posterior plane and then obtained coronal images orthogonal to these. Using magnified hard copies of the MRI scans, we overlaid the inversion recovery scans with the inverted T2 images to enhance the definition of the putamenal boundaries in both planes. Using the PET images, we targeted the area of the postero-dorsal putamen with the lowest [^{18}F]dopa uptake for infusion (Fig. 1*b*). We implanted guide tubes of 1 mm diameter under stereotaxic conditions to a point above the putamen target over a guide rod. A 0.6-mm guide wire was introduced down the guide tube to target, and the patients then underwent repeat magnetic resonance and computed tomography imaging to verify target localization. The guide wire was then replaced with a 0.6-mm diameter catheter. We implanted GDNF-primed SynchroMed pumps (Medtronic, Minneapolis, Minnesota) in the upper abdominal region, subcutaneously in patient P1 and subfascially (beneath the anterior rectus sheath) in patients P2–P5; subfascial placement reduced the pump profile in the abdomen and improved cosmetic appearance. Catheters were tunneled connecting the pumps to the indwelling 0.6-mm intraparenchymal brain catheters. The SynchroMed pump is conformité Européene–marked and the intraparenchymal catheter is investigational.

GDNF production and infusion details. Recombinant-methionyl human glial cell line–derived neurotrophic factor (r-metHuGDNF) was prepared by Amgen (Thousand Oaks, California). This protein was produced in *Escherichia coli* cells that contain an expression plasmid with a DNA insert encoding mature human GDNF, with an addition of an N-terminal methionine. The r-metHuGDNF was made up in single-use vials in a buffer of 10 nM citrate and 150 mM sodium chloride at pH 5.0. After implantation, the SynchroMed pumps were programmed to deliver a continuous infusion of 14.4 μg of r-metHuGDNF per putamen per day at a rate of 6 μl/h. The pumps were refilled monthly with fresh solution. The low concentration of r-metHuGDNF was maintained for a period of 8 weeks. After 2 months the pumps were refilled and programmed to deliver 43.2 μg of r-metHuGDNF per putamen per day at a rate of 6 μl/h. Owing to the development of high-signal MRI (Fig. 1*c*), the infusion parameters were altered to deliver lower doses (10.8–14.4 μg of r-metHuGDNF) at lower rates (2–6 μl/h) in an attempt to establish safe and clinically effective parameters, with repeat MRI monitoring at regular intervals.

Clinical evaluation and follow-up. Clinical evaluations were based on CAPIT[13], a validated protocol for evaluating surgical treatments of idiopathic Parkinson disease. All patients were evaluated on the UPDRS scale and underwent timed motor tests at baseline and after 3, 6 and 12 months. We assessed patients in both off- and on-medication states. Before they were assessed off medication, patients fasted and medications were withdrawn overnight. We then repeated the same assessments after administration of L-dopa (on). In addition, we assessed patients for improvement in quality of life measures (PDQ-39 and SF-36), changes in medication (L-dopa equivalents) requirement and neuropsychology, which included tests of verbal intellect, verbal and visual memory, attention, executive function, anxiety and depression[15]. We used Friedman's related samples test to evaluate the significance of change over time in the rating scores. We did all analyses with SPSS Inc. (Chicago, Illinois).

Scanning procedures and image analysis. The patients had [^{18}F]dopa PET on 4 occasions, pre-operatively and at 6, 12 and 18 months postoperatively, using an ECAT EXACT HR++ camera (CTI/Siemens 966; Knoxville, Tennessee) after withdrawal from medication for at least 12 h. Patients received 150 mg of carbidopa and 400 mg of entacapone. 111 MBq of [^{18}F]dopa in normal saline was administered 1 h later as an intravenous bolus at the start of scanning. We acquired the images in 3D mode as 26 time-frames over 94.5 min (1×30 s, 4×1 min, 3×2 min, 3×3 min and 15×5 min). We generated parametric images of [^{18}F]dopa influx constants (K_i) from time-frames 25.5–94.5 min after injection, using in-house software[17,18] using the multiple-time graphical analysis approach[37]. We used occipital counts from the same time-frames to generate the tissue reference input function. We used integrated images (time-frames 25.5–94.5) to identify the parameters required to transform the K_i images into standard stereotaxic Montreal Neurological Institute (MNI) space. The transformation matrix was then applied to the K_i images. We compared mean voxel values of the normalized K_i images throughout the midbrain and basal ganglia using a paired Student's *t*-test in SPM99. Any regional increases in [^{18}F]dopa uptake could subsequently be defined as a volume of interest and the mean K_i values for those volumes extracted using the appropriate SPM tool[18].

We then co-registered the integrated images to each patient's MRI scan for region-of-interest (ROI) analysis. We calculated the position of the catheter tip relative to the anterior–posterior line and centered an oval region of interest (6 mm × 12 mm) at the location of the tip. We then copied the ROI onto 2 planes on either side of the slice containing the calculated tip location, creating a 12 mm × 6 mm × 5 mm (0.36 cc) volume of interest centered on the catheter tip, which we subsequently used to sample ^{18}F activity. In the 4 patients on whom we operated bilaterally, the mean K_i values for the left and right regions of interest were averaged. The patient's K_i values were then subjected to a paired Student's two-tailed *t*-test.

Note: Supplementary information is available on the Nature Medicine *website.*

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Competing interests statement

The authors declare that they have no competing financial interests.

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ADDENDUM: Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease

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In response to a query on the limitations of presenting changes in drug treatment as percentage changes in levodopa equivalents, as shown in **Table 1** of our article, we would like to submit further supplementary information detailing the actual drug changes during the course of this phase 1 clinical study. This information would allow for a better clinical interpretation of the participants, who showed both significant off- and on-medication benefit. Accordingly, we have tabulated the drug and levodopa equivalent changes up to 24 months, beyond the 12-month clinical data and 18-month positron emission tomography data presented in our article.

	Baseline	3 months	6 months	12 months	18 months	24 months
P1	Madopar 250 mg TDS	Madopar 250 mg TDS	Madopar 250 mg TDS	Madopar 250 mg TDS	Madopar 250 mg TDS	Madopar 250 mg TDS
	Ropinirole 2 mg TDS	Ropinirole 2 mg TDS	Ropinirole 2 mg TDS	Ropinirole 4 mg TDS	Ropinirole 4 mg TDS	Ropinirole 4 mg TDS
*	666.7	666.7	666.7	733.3	733.3	733.3
P2	Sinemet CR QDS	Sinemet Plus x 5/day	Sinemet Plus x 5/day	Sinemet Plus x 5/day	Sinemet Plus x 5/day	Sinemet Plus x 5/day
	Entacapone QDS	Sinemet CR nocte	Sinemet CR nocte	Sinemet CR nocte	Sinemet CR nocte	Sinemet CR nocte
		Tolcapone 100 mg TDS	Tolcapone 100 mg TDS	Tolcapone 100 mg TDS	Tolcapone 100 mg TDS	Tolcapone 100 mg TDS
*	615.4	653.8	653.8	653.8	653.8	653.8
P3	Madopar 125 mg x 18–20/day	Cabergoline 6 mg/day	Cabergoline 6 mg/day	Cabergoline 6 mg/day	Cabergoline 6 mg/day	Cabergoline 6 mg/day
	Sinemet Half CR x 1–2/day	Sinemet Half CR x 5–6/day	Sinemet Half CR x 5–6/day	Sinemet Half CR x 4–5/day	Sinemet Half CR x 4–5/day	Sinemet Half CR x 5–6/day
	2,153.8	1,061.5	1,061.5	984.6	984.6	1,061.5
P4	Sinemet Plus x 6/day	Sinemet Plus x 5/day	Sinemet Plus x 2–3/day	Sinemet Plus x 5–6/day	Sinemet Plus x 6/day	Sinemet Plus x 6/day
	Lisuride 200 g QDS	Lisuride 200 g QDS	Lisuride 200 g QDS	Ropinirole 6 mg TDS	Lisuride 200 g x 6	Amantadine 100 mg BD
	Selegiline 5 mg BD	Selegiline 5 mg BD	Selegiline 5 mg BD			Pramipexole 0.7mg TDS
*	680	580	380	800	720	750
P5	Sinemet CR x 3/day	Cabergoline 4 mg/day	Cabergoline 2 mg/day	Cabergoline 2 mg/day	Cabergoline 3 mg/day	Cabergoline 2.5 mg/day
	Sinemet Plus x 1/day	Sinemet Plus x 3–4/day	Sinemet Plus x 2.5–3/day	Sinemet Plus x 2.5/day	Sinemet Plus x 2–2.5	Sinemet Plus x 1.75/day
	Cabergoline 2 mg/day	Zelapar 1.25 mg OD	Zelapar 1.25 mg OD	Zelapar 1.25 md OD		
	Zelapar 1.25 mg OD					
*	761.5	800	500	450	500	425

*Levodopa equivalents: 1 mg Pergolide = 1 mg lisuride = 10 mg Bromocriptine = 10 mg Apomorphine = 9 mg Ropinirole = 1 mg Cabergoline = 100 mg Levodopa + dopa decarboxylase inhibitor = 130 mg levodopa CR preparation.