Enhancement of the response to levodopa therapy after intrastriatal transplantation of autologous sympathetic neurons in patients with Parkinson disease

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Object. There is growing evidence to indicate that tissue transplantation can potentially be a restorative neurological treatment for patients with Parkinson disease (PD). In this study the authors investigated the clinical effect of unilateral intrastriatal grafting of autologous sympathetic neurons in patients with PD.

Methods. Four patients with PD who had been observed for 1 year after graft placement of autologous sympathetic neurons were selected for an analysis of the effect of that procedure. Sympathetic ganglionic tissue was endoscopically excised from the thoracic sympathetic trunk and grafted into the unilateral caudate head and putamen of the PD patients. No changes were made in the patients’ preoperative regimens of antiparkinsonian medications, and clinical evaluations were made principally according to those established by the Core Assessment Program for Intracerebral Transplantation Committee.

Whereas the sympathetic neuron grafts failed to affect clinical scores reflecting the patients’ motor performance, which was evaluated during either the “on” or “off” phases, the grafts significantly increased the duration of the levodopa-induced on period with consequent reduction in the percentage of time spent in the off phase. This beneficial effect may be explained by the results of the present in vitro experiment, which show that human sympathetic neurons have the ability to convert exogenous levodopa to dopamine and to store this synthesized dopamine.

Conclusions. Sympathetic neuron autografts were found to improve performance status in patients with PD by reducing the time spent in the off phase. This clearly indicates that sympathetic ganglion tissue, the use of which involves few ethical issues, can be an efficacious donor source in cell transplantation therapy for PD. Further studies are needed to determine whether the grafts may provide long-lasting clinical benefits.

Key Words • thoracic sympathetic ganglion • vesicular monoamine transporter–2 • aromatic L-amino acid decarboxylase • Parkinson disease • dopamine

PARKINSON disease is a neurodegenerative disorder characterized by a progressive loss of nigrostriatal dopamine neurons due to mechanisms that remain elusive. Tissue transplantation is a therapeutic modality designed to supplement the amount of dopamine in the brain of patients with PD. Over the last decade, authors representing several different centers have reported on results of a series of clinical trials in which human fetal nigral tissue has been transplanted into the brain of patients with PD. These results have shown that transplants placed in the dopamine-denervated striatum can survive and ameliorate parkinsonian symptoms. The positive results were substantiated by recent data obtained from the first double-blind, sham surgery–controlled study. The data provide direct evidence to indicate that the beneficial outcome seen after transplantation surgery is specifically derived from the grafted tissue and is not a placebo-induced effect. Despite these encouraging results, transplantation of fetal tissue raises a variety of religious, ethical, and legal issues that restrict the procurement of this tissue. Indeed, the clinical trials have been conducted in only a limited number of countries. Therefore, it is warranted to develop cell transplantation therapy that makes use of alternative donor sources that are universally acceptable in their ethical aspects.

Autologous tissue is a possible candidate as donor tissue, which involves few ethical issues. We have investigated the potential of autologous sympathetic neurons as a donor for implantation therapy of PD. It has been proven that autologous sympathetic ganglion tissue implanted in the striatum survives and improves motor deficits, including the drug-induced circling behavior and hypokinetik disorders seen in rodent and nonhuman primate models of PD. The results of animal studies prompted us to commence clinical trials in 1990 in which we autografted...
cervical sympathetic neurons in patients with PD. A long-term evaluation of the clinical course after grafting revealed that unilateral intranatural implantation of autologous sympathetic neurons leads to a significant improvement in parkinsonian symptoms, particularly akinesia and gait disturbance, with a consequent reduction in the patient’s daily intake of levodopa. We recently selected the thoracic sympathetic ganglia as a donor source for autotransplantation therapy. Following the development of video-guided endoscopic thoracic surgery, it is now feasible to excise three or more ganglia from the thoracic sympathetic trunk safely and in a less invasive manner. This option can augment the amount of donor tissue, thereby increasing the number of implantation sites in the dopamine-denervated striatum.

In a previous series of patients with PD who received grafts of the autologous cervical sympathetic neurons, we changed their preoperative dosages of antiparkinsonian drugs after grafting, depending on the individual patient’s performance status. This could confound an assessment of the graft-mediated effect. To explore the mechanism responsible for the clinical effects of autografting sympathetic neurons, in the present study we chose to maintain the preoperative dosage of antiparkinsonian drugs after implantation and principally have followed the CAPIT protocol. Since the beginning of 1999, we have implanted endoscopically excised thoracic ganglion tissue into more extensive areas of the unilateral striatum in patients with PD, and have followed these patients’ clinical courses according to the aforementioned criteria. In the present paper we present the results of a 1-year follow-up review of four patients with PD who received grafts of thoracic ganglion tissue since 1999. Furthermore, using a tissue culture technique, we tested the ability of sympathetic neurons obtained in these patients to produce catecholamines, and we discuss possible mechanisms responsible for the clinical effects of the grafts.

Clinical Material and Methods

Patient Population

Since the beginning of 1999, we have transplanted autologous thoracic sympathetic ganglion tissue in a total of five patients with PD. Of these five patients, four who were followed according to the protocol described in this paper for more than 1 year postsurgery were selected for review (Table 1). One patient with PD who was unable to visit our clinic 1 month after surgery because of personal reasons was not included in the current study. The diagnosis of PD was based on the criteria recommended by the CAPIT Committee. Before graft placement, all patients had received levodopa and carbidopa along with dopamine receptor agonists such as pergolide and bromocriptine. The mean daily dose of levodopa was $700 \pm 115$ mg/day. All patients consented to undergo graft placement surgery after being informed of: 1) the potential risks of the surgical procedure; 2) the rationale for grafting sympathetic neurons in patients with PD, based on the experimental evidence; and 3) the possible lack of clinical benefit derived from the grafting. The entire process of the surgical procedure was approved by the ethics committee of Wakayama Medical University.

Clinical Evaluation

We conducted clinical evaluations of the four patients, which conformed in principle to the protocol established by the CAPIT Committee. To avoid the possibility that postoperative evaluations may be confounded by alterations in medication levels after grafting, the preoperative dosages of the antiparkinsonian drugs the patients had been using were held constant for at least 1 year during the present study, unless the individual patient’s condition required changes in the dosage of the drugs. The clinical evaluations were made according to UPDRS scores and Hoehn and Yahr stages assigned during both “on” and “off” states. Evaluation of a patient’s clinical condition during the off state was performed after the patient had not received antiparkinsonian medications for 12 hours (practically defined off phase). The on phase scores were determined at the time of the patient’s peak response to antiparkinsonian medications. The response of motor tasks to levodopa was examined before and after grafting by administering a single levodopa test. Thus, a single dose of levodopa (100 mg) with carbidopa was orally given during the practically defined off phase. Timed motor tests, such as supination–pronation, stand–walk–sit, finger dexterity, and hand–arm movements between two points, were conducted every 20 minutes until the time taken to complete each motor task reached the baseline values rated before drug intake. The patients recorded self-assessment diaries that illustrated the time courses of their conditions on an hourly basis. The self-assessment diary is composed of a line chart that exhibits changes in three levels of clinical conditions, such as on, off, and on accompanied by dyskinesia. The percentage of awake time spent during the off phase and the percentage of time spent during the on period with dyskinesia were calculated based on the hourly self-evaluation chart. Data were collected and averaged each month.

Grafting Technique

After general anesthesia had been induced in the patient and one-lung ventilation initiated, thoracic sympathetic ganglia on the right side were endoscopically excised as described elsewhere. Briefly, each patient was placed in the right-sided thoracotomy position with the upper limb abducted and raised. Three entry sites were made: one site was used for a 10-mm 0° endoscope, and the two other

### Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs)</th>
<th>Disease Duration (yrs)</th>
<th>Hoehn &amp; Yahr Stage (on/off)</th>
<th>UPDRS Score (on/off)</th>
<th>Time in Phase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50, M</td>
<td>6</td>
<td>2/4</td>
<td>16/69</td>
<td>Off 60 On 55</td>
</tr>
<tr>
<td>2</td>
<td>48, F</td>
<td>11</td>
<td>3/5</td>
<td>42/95</td>
<td>Off 95 On 0</td>
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<tr>
<td>3</td>
<td>45, M</td>
<td>3</td>
<td>1.5/3</td>
<td>20/31</td>
<td>Off 64 On 53</td>
</tr>
<tr>
<td>4</td>
<td>53, M</td>
<td>4</td>
<td>1.5/4</td>
<td>39/66</td>
<td>Off 66 On 0</td>
</tr>
</tbody>
</table>

* Dys = dyskinesia; off = off phase (period without antiparkinson medication); on = on phase (period during which antiparkinson medication is in effect).
sites for introduction of surgical instruments. After the parietal pleura was opened along the sympathetic trunk, ranging from the third to fifth ganglia on the right side, the chain including these ganglia was dissected en bloc by using scissors. Much effort was made to avoid the use of electrocautery to prevent diathermic injury to the ganglion tissue. After completing the excision of the sympathetic ganglia, a No. 16 French chest tube was placed in the thoracic cavity. On the following day the tube was removed after a chest x-ray study demonstrated no residual pneumothorax.

The sympathetic ganglion grafts were implanted unilaterally in the caudate nucleus and the putamen by using a computerized tomography–guided stereotactic technique. The implantations were performed on the side contralateral to extremities displaying more severe parkinsonian symptoms such as bradykinesia and rigidity. The excised sympathetic ganglia were prepared for implantation in a sterile Petri dish containing MEM. After the fibrous capsule of each ganglion was completely peeled off, the ganglion was cut into small tissue pieces measuring 1 to 2 mm each. A total of four graft deposits, each consisting of 80 to 100 tissue pieces, were implanted using a stainless steel cannula with a diameter of 2 mm. Three cannula tracks were planned to deposit graft tissue into three portions of the putamen, that is, the anterior, middle, and posterior thirds of this structure. One graft deposit was implanted centrally in the head of the caudate nucleus.

Several pieces of dissected ganglion tissue were subjected to in vitro measurements of catecholamines released from sympathetic neurons and to morphological analyses, described later.

**Sympathetic Neuron Culture and Measurement of Catecholamine Levels**

Tissue pieces of sympathetic ganglion were placed onto 12-well plastic dishes that previously had been coated with type I collagen. The explanted cell cultures were grown in serum-free, defined N2 medium supplemented with 2.5S nerve growth factor (100 ng/ml) at 37°C in a 95% air/5% CO2 humidified atmosphere. The culture medium was replenished every other day. After 7 days in vitro, sympathetic neuron cultures were washed with 0.1 M PBS three times and thereafter incubated in 15 mM HEPES-buffered MEM with Earle’s salts containing 20 mM glucose for 3 hours. The incubation medium was collected and examined to measure the amount of catecholamines released from cultured sympathetic neurons under basal conditions for a period of 3 hours. After being washed with PBS three times, the cultures were again incubated in HEPES-buffered, glucose-containing MEM in the presence of several test agents including levodopa for an additional 3 hours (Fig. 1). Catecholamine levels released from cultured sympathetic neurons exposed to the test drugs were measured and compared with those detected in the culture before addition of the test agents. The levels of catecholamines, such as noradrenaline, dopamine, and a primary metabolite of dopamine, DOPAC, were assessed using high-performance liquid chromatography with electrochemical detection as described in detail elsewhere.

**Morphological Analysis**

After sympathetic ganglion tissue was immersed in...
4% paraformaldehyde/0.1 M PBS, pH 7.4, overnight, sections were cut on a cryostat every 40 μm and collected in 0.1 M PBS. Free-floating sections were processed for TH, AADC, and VMAT-2 immunohistochemistry. Endogenous peroxidase activity was quenched for 7 minutes in 3% hydrogen peroxide/0.1 M PBS. After preincubation lasting 1 hour in 10% blocking serum/0.3% Triton X 100/0.1 M PBS, the sections were incubated overnight at 4˚C with polyclonal antibodies against TH (1:800), AADC (1:600), or human VMAT-2 (1:2000) in 0.1 M PBS containing 2% blocking serum. The sections were incubated for 1 hour at room temperature with a biotinylated secondary antibody (1:200; anti–rabbit immunoglobulin G). The bound antibodies were visualized using an avidin-biotin-peroxidase complex system.

In TH-stained sections derived from each patient, the number of immunoreactive cells was counted in five randomly chosen areas by using a sampling grid placed in one ocular lens (250 μm × 250 μm for a magnification × 400). The TH-stained cell counts were averaged, and expressed as cell numbers per square millimeter in individual patients.

Statistical Analysis

Clinical assessment scores are each expressed as the mean ± SD of the four patients. One-factor analysis of variance was applied to test the presence of an overall time effect on the assessment scores. This test was followed by comparing mean values at a given time point with the mean preoperative baseline values by using a post hoc Scheffé test. Data obtained from the in vitro study were expressed as means ± SDs of four experiments, each using a piece of ganglion tissue derived from one patient. In each experiment, catecholamine measurements were repeated two or three times, and the data obtained were averaged. A two-tailed Student t-test was used to compare the level of catecholamines released from cultured sympathetic neurons after addition of test agents with the level measured before treatment.

Sources of Supplies and Equipment

The endoscope used to obtain grafts was purchased from Olympus (Osaka, Japan) and the 12-well, type I collagen–coated plastic dishes from Iwaki Glass Co. (Tokyo, Japan). The MEM and N2 medium supplemented with 2.5S nerve growth factor were obtained from Sigma Chemical Co. (St. Louis, MO). The HEPES-buffered MEM with Earle’s salts was acquired from Gibco (Grand Island, NY). Polyclonal antibodies against TH, AADC, and human VMAT-2 were purchased from Chemicon (Temecula, CA), Eugene Tech Inc. (Ridgefield Park, NJ), and Phoenix Pharmaceutical Inc. (Mountain View, CA), respectively. Anti–rabbit immunoglobulin G and the avidin-biotin-peroxidase complex system ( Vectastain ABC Elite Kit) were obtained from Vector Laboratories (Burlingame, CA).

Results

Intrastriatal Implantation of Autologous Sympathetic Ganglion Neurons

All the patients tolerated the entire grafting procedure well, including the endoscopic excision of thoracic sympathetic ganglia. Postoperative T1-weighted magnetic resonance images revealed graft deposits in the caudate head and in the putamen as slightly low-intensity areas, as well as some cannula tracks (Fig. 2). No serious complications associated with the grafting procedure were noted. The patient in Case 4 experienced recurrence of pneumothorax 2 weeks after surgery and required percutaneous chest drainage for 3 days. Because a bulla was found in the pulmonary apex during the surgery, it may be that surgical manipulations related to the endoscopic thoracic sympathectomy had some effect on the bulla to cause the pneumothorax.

Clinical Effect of Sympathetic Ganglion Grafts

In all patients, the preoperative dosages of antiparkinsonian drugs were held constant for 12 months postgrafting. There was no significant change in scores of clinical evaluation scales rated using UPDRS scores or Hoehn

<table>
<thead>
<tr>
<th>Timing</th>
<th>Pregrafting Score</th>
<th>Postgrafting Scores (mos postgrafting)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>on phase</td>
<td>29.3 ± 13.2</td>
<td>28.5 ± 13.0</td>
</tr>
<tr>
<td>off phase</td>
<td>65.3 ± 26.3</td>
<td>64.0 ± 28.2</td>
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</tbody>
</table>

* Evaluation of UPDRS scores in the off phase was made after patients had received no antiparkinson medications for 12 hours (practically defined off phase). The on phase score was determined at the time of peak response to antiparkinson medications.
and Yahr stages during the on phase for a period of 12 months after grafting (Table 2). No significant change in these evaluation scores was noted during the practically defined off phase (Table 2). Nonetheless, the grafts significantly decreased the time patients spent in the off phase, which had been calculated from data recorded in self-assessment diaries. Thus, the time spent in the off phase was attenuated over time postgrafting and was significantly decreased to approximately 60% of the pregrafting value from 3 to 12 months postgrafting (p < 0.01; Fig. 3A). Two patients (Cases 1 and 3) had suffered from levodopa-induced dyskinesias during the on phase. The duration of levodopa-induced dyskinesias tended to be decreased from 1 to 6 months postgrafting in these patients. Over the next 6 months, however, their dyskinesias worsened, and the durations increased. In one patient (Case 3), the extent of drug-induced dyskinesia returned to the pregrafting level 1 year after grafting.

Because the results just described can be taken as a graft-derived increase in the duration of the on phase, we further analyzed the effect of the grafts on the response to levodopa by using a single-dose levodopa test. In all timed motor tasks, the duration of the on phase induced by a single dose of levodopa was significantly increased after implantation. Nonetheless, the scores on any motor tasks evaluated during the practically defined off phase were not improved after grafting. We chose a pronation–supination test as an index representing general motor conditions to analyze the graft-mediated change in the response to levodopa: 1) the latency of the levodopa response; 2) the duration of the response; and 3) the time needed to perform the motor task at the time point of maximum response. The levodopa response was defined as a reduction by more than 33% in the time taken to perform the motor task.30 The latency of the levodopa response varied and no significant difference was seen from the corresponding value before grafting. A significant increase in the duration of the on phase induced by a single dose of levodopa was noted in both hands after grafting (Fig. 3B). The time needed to perform the motor task was not significantly reduced after grafting, even at the time point of peak response to levodopa. This agrees with the results that neither UPDRS scores nor Hoehn and Yahr stages evaluated during the on phase were improved by the grafts.

**Morphological Analysis of the Sympathetic Ganglion Tissue**

Sympathetic ganglion tissue was found to contain numerous TH-immunoreactive cells. The cell density was estimated to be 276 ± 87 cells/mm² (Fig. 4A). There was no appreciable difference in cell density among the ganglion tissue obtained from the four patients. Cells containing VMAT-2 (Fig. 4B) or AADC (Fig. 4C) were also present in the ganglion tissue, and the densities of these cells were comparable to that of TH-positive cells. Sections stained with hematoxylin and eosin revealed no histological evidence for the presence of Lewy bodies.

**Catecholamine Production in Human Sympathetic Neurons In Vitro**

Short neurites were found to emanate from sympathetic ganglion tissue at 2 to 4 days in vitro. The neurites grew over time and a number of long neurites were noted at 7 days in vitro (Fig. 5A). Under basal conditions, noradrenaline and DOPAC were detected in culture media collected after a 3-hour incubation of sympathetic ganglion tissue. Although levels of DOPAC, a primary metabolite of dopamine, were measurable, dopamine levels were below the detection limits provided by the present method (Fig. 5B). When tissue cultures were exposed to levodopa (1 μM) for 3 hours, the dopamine concentrations in culture media reached detectable levels (Fig. 5C). The addition of levodopa also elevated the levels of nonadrenaline and DOPAC. An incubation with culture medium that was identical, except for the absence of levodopa, failed to in-

![Fig. 3. Bar graphs showing the effect of sympathetic ganglion autografts on the response of patients with PD to levodopa. A: The effect of grafts on the duration of the off phase. Time spent in the off phase was decreased to approximately 60% of the pregrafting value from 3 to 12 months postgrafting. *p < 0.01, significant difference from pregrafting value. B: Effect of the graft on the duration of the on phase induced by a single dose of levodopa (100 mg) with carbidopa. *p < 0.01, significant difference from pregrafting value. Values are each expressed as the mean ± SD of four patients.](image-url)
crease catecholamine levels (Fig. 5C). Neuronal depolarization in response to the incubation of a high level of potassium (40 mM) enhanced the levodopa-induced increase in the release of catecholamines including dopamine and noradrenaline (Fig. 5D). The addition of the AADC inhibitor, benserazide (1 μM) significantly attenuated catecholamine production in response to exogenous levodopa (Fig. 5E). Cotreatment with levodopa and the VMAT inhibitor, reserpine, also reduced levels of dopamine and noradrenaline that were released into culture media, whereas cotreatment led to a significant increase in DOPAC levels (Fig. 5F). The findings indicate that the levodopa-induced increase in dopamine levels is attributable to both the metabolism of exogenous levodopa and the storage of synthesized dopamine with the aid of AADC and VMAT-2, respectively, in human sympathetic neurons.

Discussion

After unilateral intrastral transplantation of autologous sympathetic neurons, the time patients spent in the off phase was significantly reduced, although the grafts failed to affect clinical evaluation scores, such as UPDRS scores and Hoehn and Yahr stages, rated in either the “best on” or the “practically defined off” phases. Our findings indicate that the grafts enhance the duration of the effects of antiparkinsonian drugs including levodopa. This is further supported by observations during a single-dose levodopa test. Thus, the duration of the effect of a single dose of levodopa given in the practically defined off phase was significantly increased after grafting. Neither the speed of movements for motor tasks at the time of the maximum drug effect nor the latency of the drug response were significantly affected by grafting.

The mechanism for the graft-mediated increase in the duration of the levodopa effect remains to be explained (Fig. 6). In relatively early stages of PD, presynaptic dopamine nerve terminals remaining in the striatum can convert administered levodopa into dopamine. The dopamine terminals can also play a role in the storage and release of dopamine synthesized from levodopa for several hours, even after plasma levodopa concentrations have returned to baseline levels. As the degeneration of nigrostriatal dopamine terminals progresses, the synthesis and the storage capacity become insufficient to maintain the duration of the levodopa effect. Therefore, one possible explanation for the clinical effect of the transplantation is that grafted sympathetic neurons may compensate for the metabolic function of dopamine terminals that have been lost. Thus, sympathetic neurons implanted into the dopamine-denervated striatum could convert administered levodopa into dopamine and could also store dopamine synthesized from exogenous levodopa (Fig. 6). Human sympathetic ganglion tissue grafts were indeed found to contain numerous cells that were immunopositive for AADC and VMAT-2, which are important proteins for the synthesis and the storage of dopamine, respectively. Although the results of animal experiments have suggested that in the brain there is endogenous AADC activity in nondopaminergic neurons as well as in nonneuronal cells such as astrocytes, it is possible that an added activity of AADC achieved by grafting sympathetic neurons can augment the decarboxylation of levodopa to synthesize dopamine, particularly in PD-affected brains, in which the capacity to convert levodopa to dopamine is limited. Furthermore, the graft-mediated introduction of VMAT-2, which plays a pivotal role in sequestering monoamines, including dopamine, into synaptic vesicles, can decrease the rate of dopamine metabolism. Therefore, when a given dose of levodopa is administered, a more sustained production and release of dopamine could possibly be achieved in PD patients with sympathetic ganglion...
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Grafts expressing both molecules than in those patients who have not received grafts. This hypothesis is further supported by the present tissue culture experiment, which indicated that cultured human sympathetic neurons can convert exogenous levodopa into significant levels of dopamine and can store the synthesized dopamine. Moreover, the in vitro finding that cultured sympathetic neurons failed to produce detectable levels of dopamine in the absence of levodopa is consistent with the lack of clinical effects of the grafts during the practically defined off phase.

The observed clinical benefit might also be attributable to a mechanism by which tissue grafting may induce sprouting of the remaining dopamine nerve terminals by neurotrophic effects derived from the host brain in response to surgically induced tissue trauma or from the grafted sympathetic ganglion cells themselves. The sprouting of host dopamine nerve fibers has been noted in response to adrenal medulla and carotid body grafts in animal models of PD. Although sympathetic ganglion tissue contains numerous Schwann cells, which have been shown to produce neurotrophic molecules, to date there is no experimental evidence to indicate that implantation of sympathetic ganglion tissue produces trophic effects to induce regeneration of nerve fibers in the host brain.

In nonhuman primate models of PD, surgically induced

![Image](image_url)
tissue trauma per se has been shown to induce recovery of motor behaviors.\textsuperscript{4,46} Behavioral recovery is believed to be mediated by axonal sprouting of the remaining dopamine neurons and/or their functional enhancements, both of which can be induced by the trophic effects afforded by surgically induced trauma.\textsuperscript{32,38} The resultant increase in the amount and function of the remaining nigrostriatal dopamine neurons can restore dopamine levels in the striatum, attenuating parkinsonian motor symptoms without supplementation of exogenous dopaminergic drugs. Therefore, if the trauma-induced sprouting of striatal dopamine nerve terminals had played a role in clinical outcome in the present study, one could also have noted some improvements in motor performance during the off phase.

Based on the present findings that the significant effect was detected only in the duration of levodopa-induced on periods, it seems more likely that the clinical effect depends on the function of grafted sympathetic neurons. The graft function could be derived from the ability of sympathetic neurons to convert exogenous levodopa into dopamine, as demonstrated in the tissue culture experiments.

It has been shown that intrastriatal transplants of fetal mesencephalic tissue increase the duration of the on phase as well as improve performance status in PD patients during the off phase.\textsuperscript{7,18,33,45,52,53} The lack of beneficial effects of sympathetic ganglion grafts on motor symptoms during the off phase may be due to the fact that the majority of neurons present in the sympathetic ganglion are noradrenergic and that dopaminergic neurons constitute a small proportion of ganglionic neurons.\textsuperscript{10,21,47} The present in vitro study indeed showed that sympathetic ganglion tissue obtained from patients with PD can release no detectable levels of dopamine under basal conditions. It is plausible that, without the supply of exogenous levodopa, the level of dopamine that is released from grafted sympathetic neurons is very low, if any, and not sufficient to produce clinical effects.

Although sympathetic neuron grafts were placed into the unilateral striatum, the mediated clinical effects were noted bilaterally. Bilateral beneficial effects have also been demonstrated in unilateral intrastriatal implantation of adrenal medulla tissue\textsuperscript{20,34,36} and fetal mesencephalic dopamine cells.\textsuperscript{7,18,33,45,52,53} A possible mechanism for the bilateral effects is that dopamine and/or trophic factors released from grafts may disperse into the contralateral striatum via cerebrospinal fluid. A more likely explanation can be made, based on several lines of evidence, to suggest the presence of anatomical neural connections that allow for functional interactions between bilateral brain structures related to motor functions. On either side the supplementary motor area, which receives afferent fibers from the ipsilateral striatum, regulates motor functions of the bilateral extremities.\textsuperscript{7} Experimental modifications of activities in the unilateral substantia nigra have been shown to induce bilateral alterations in basal ganglia glucose consumption.\textsuperscript{7,46-50}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig6}
\caption{A hypothetical mechanism for the increase in the levodopa effect in patients with PD who received grafts of autologous sympathetic neurons. In relatively early stages of PD, the residual nigrostriatal dopamine nerve terminals in the striatum can metabolize administered levodopa into dopamine. As the degeneration of nigrostriatal dopamine terminals progresses, the synthesis and the storage capacity may become insufficient to maintain the duration of the levodopa effect. Sympathetic neurons grafted into the dopamine-denervated striatum could convert administered levodopa into dopamine, and could also store dopamine synthesized from exogenous levodopa, thereby compensating for the metabolic function of dopamine terminals that have been lost.}
\end{figure}

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Conclusions
A 1-year-long clinical observation of four patients with PD who received autologous sympathetic ganglion grafts clearly indicates that the transplantation can improve the performance status of patients with PD by reducing time spent in the off phase. The increase in the duration of levodopa effects may be mediated by a possible mechanism by which grafted sympathetic neurons could provide a site for the conversion of exogenous levodopa to dopamine and for storage of the synthesized dopamine. Although the neurodegenerative process of PD has been proposed to involve peripheral autonomic ganglion tissue as well as central monoaminergic neurons, the results of the present study show that autologous sympathetic ganglion cells, the use of which involves few ethical issues, can constitute an efficacious donor tissue in cell transplantation therapy for PD. Further studies are warranted to determine whether the autologous sympathetic ganglion grafts may provide long-lasting clinical benefits.

References

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