

NEUROPATHOLOGICAL EVIDENCE OF GRAFT SURVIVAL AND STRIATAL REINNERVATION AFTER THE TRANSPLANTATION OF FETAL MESENCEPHALIC TISSUE IN A PATIENT WITH PARKINSON'S DISEASE

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Abstract Background. Trials are under way to determine whether fetal nigral grafts can improve motor function in patients with Parkinson's disease. Some studies use fluorodopa uptake on positron-emission tomography (PET) as a marker of graft viability, but fluorodopa uptake does not distinguish between host and grafted neurons. There has been no direct evidence that grafts of fetal tissue can survive and innervate the striatum.

Methods. We studied a 59-year-old man with advanced Parkinson's disease who received bilateral grafts of fetal ventral mesencephalic tissue in the postcommisural putamen. The tissue came from seven embryos between 6½ and 9 weeks after conception. The patient died 18 months later from a massive pulmonary embolism. The brain was studied with the use of tyrosine hydroxylase immunohistochemical methods.

Results. After transplantation, the patient had sustained improvement in motor function and a progressive

increase in fluorodopa uptake in the putamen on PET scanning. On examination of the brain, each of the large grafts appeared to be viable. Each was integrated into the host striatum and contained dense clusters of dopaminergic neurons. Processes from these neurons had grown out of the grafts and provided extensive dopaminergic reinnervation to the striatum in a patch-matrix pattern. Ungrafted regions of the putamen showed sparse dopaminergic innervation. We could not identify any sprouting of host dopaminergic processes.

Conclusions. Grafts of fetal mesencephalic tissue can survive for a long period in the human brain and restore dopaminergic innervation to the striatum in patients with Parkinson's disease. In the patient we studied, clinical improvement and enhanced fluorodopa uptake on PET scanning were associated with the survival of the grafts and dopaminergic reinnervation of the striatum. (N Engl J Med 1995;332:1118-24.)

CLINICAL trials are testing the hypothesis that fetal nigral grafts are effective in the treatment of Parkinson's disease. In rodents and nonhuman primates, grafts of fetal nigral neurons consistently survive, produce dopamine, form synaptic connections, and ameliorate many behavioral deficits due to lesions of the nigrostriatal pathway.¹ However, the results of fetal-tissue transplantation in patients with Parkinson's disease have been variable. Some studies report a benefit after nigral grafting,²⁻⁵ whereas others report little if any improvement.^{6,7} This variation in the clinical response may be related to differences in the survival of implanted dopamine neurons, which has been shown to be critical for functional recovery in animal models.⁸ There have been only a few pathological studies after fetal nigral transplantation,^{9,10} and long-term survival of grafted nigral neurons has yet to be demonstrated in the human brain. Positron-emission tomography (PET) with fluorodopa has been used to determine graft viability.^{11,12} These studies, however, cannot distinguish between grafted neurons and the sprouting of host dopaminergic neurons, both of which have been proposed to account for functional improvement after nigral transplantation.^{13,14}

We studied the brain of a man with Parkinson's dis-

ease who had considerable clinical improvement and enhanced fluorodopa uptake on PET scanning after undergoing fetal nigral transplantation. Eighteen months later, he died from causes unrelated to the neurosurgery.

CASE REPORT

The patient was a 59-year-old man with an eight-year history of Parkinson's disease manifested by tremor, rigidity, bradykinesia, and gait disturbance. The findings on computed tomographic and magnetic resonance scans were within normal limits. Treatment with carbidopa-levodopa was initially associated with substantial improvement, but subsequently, there were motor fluctuations, dyskinesias, and dystonia during the "on" period (when the medication was working). Furthermore, there was progressive worsening of gait and bradykinesia with the development of a mild postural instability. Disability progressed to the point where the patient had to stop working. His symptoms could not be satisfactorily improved by increased or decreased doses of carbidopa-levodopa, the use of a continuous-release formulation of the drug combination, or the addition of selegiline or dopamine agonists. Before the surgery, he was receiving 50 and 200 mg, respectively, of the continuous-release formulation of carbidopa-levodopa six times per day and 25 and 100 mg, respectively, of the non-continuous-release formulation five times per day. Clinical evaluations with the protocol of the Core Assessment Program for Intracerebral Transplantation¹⁵ were performed at base line and 1, 3, 6, 9, and 15 months after surgery.

After obtaining informed consent, we grafted fetal nigral neurons bilaterally in staged procedures separated by four weeks, using fetal tissue from three donors on the right side and four donors on the left. Tissue was obtained according to methods described previously,¹⁶ with the approval of the local institutional review board and in accordance with federal, state, and local laws and the guidelines of the National Institutes of Health. Fetal tissue was stored for up to 48 hours at 8°C in a cool-storage ("hibernation") medium.¹⁷ Solid tissue derived from the ventral mesencephalon of fetuses 6½ to 9 weeks after conception was transplanted stereotactically into the postcommisural putamen. The stereotactic needle had a maximal outer diameter of 1.5 mm, which tapered to 0.9 mm at the tip. Multiple needle trajectories (six to eight per side) were used, and four tissue deposits were injected into each needle tract so that the deposits were separated by no more than 5 mm throughout the three-dimensional configuration of the target region. Immunosuppression with cyclosporine

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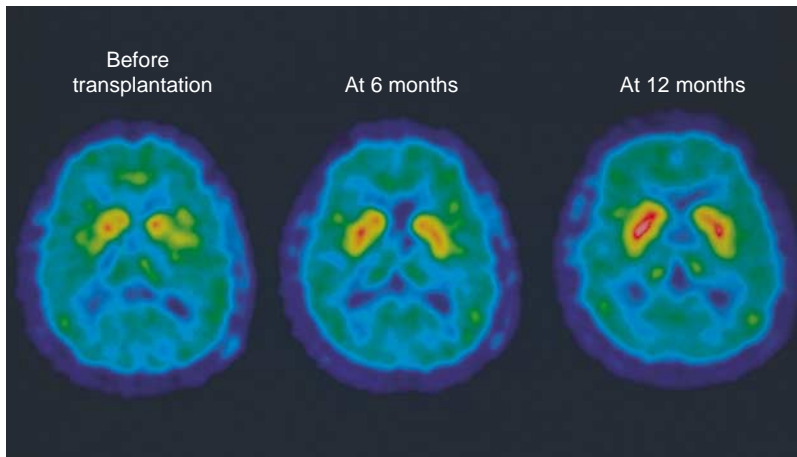


Figure 1. PET Scans Showing the Progressive Increase in Putaminal Fluorodopa Uptake before Transplantation and 6 and 12 Months after Transplantation. The caudate nucleus shows appreciable fluorodopa uptake even before transplantation.

(6 mg per kilogram of body weight per day) was initiated three weeks before the first transplantation procedure and maintained for eight weeks after the second procedure. The dose was subsequently lowered to 2 mg per kilogram per day, and the drug was discontinued six months after the second operation.

PET scanning with fluorodopa was performed before and 6 and 12 months after transplantation, with the use of an ECAT 953B/31 scanner according to a method described previously.¹⁸ The scanner has a reconstructed resolution of 5×5 mm, full width at half maximum (conventional index measure of PET resolution). Each scan was obtained with the patient in the same position. Regions to be scanned (8.8 mm in diameter) were placed along the axis of the striatum so that one covered the head of the caudate and three were distributed along the putamen. The same regions were used for each of the three imaging sessions and adjusted to account for any small differences in position. The constant for the rate of steady-state fluorodopa uptake was calculated by a graphic method with a metabolite-corrected, blood-derived input of function.¹⁹ The values for the three putaminal regions of interest were averaged for each side. The scan-to-scan variation with this method has been evaluated.¹⁹ In a patient with Parkinson's disease, a change of 0.002 in putaminal fluorodopa uptake is considered significant at the 0.05 level.

The patient had had severe pain in his ankle, which was related to a fracture sustained before the diagnosis of Parkinson's disease. Eighteen months after the first transplantation procedure, he underwent an ankle fusion and died from a massive pulmonary embolism while recovering from the surgery. The brain was removed and placed in Zamboni's fixative²⁰ within four hours after his death. Every sixth section through the striatum bilaterally was stained for tyrosine hydroxylase (TH) immunoreactivity and Nissl's substance, as previously described.¹⁴

RESULTS

After the transplantation, the patient's motor function improved, and he could again perform all activities of daily living independently and engage in an active exercise program. Motor fluctuations, dyskinesias, and on-period dystonia virtually disappeared. From baseline to the evaluation performed 15 months after the transplantation, there was improvement in the total score on the United Parkinson's Disease Rating Scale in the "off" period (when the medication was not working), from 78 to 49.5. There was also improvement in the amount of off time (waking hours during which the medication was not working), which decreased from 48 to 0 percent; the amount of on time (waking hours during which the medication was working) with dyskinesias

(from 19.5 to 0 percent); and the amount of on time with dystonia (from 30 to 0 percent). These benefits of transplantation were observed between one and three months after the initial procedure and were sustained throughout the entire period of follow-up.

After the surgery, the patient was mildly confused. The dose of carbidopa-levodopa was reduced to 25 and 100 mg, respectively, four times per day, and the continuous-release formulation was reduced to 50 and 200 mg, respectively, five times per day. Drug treatment was subsequently unchanged. He had a single motor seizure one month after the second transplantation procedure, which was controlled by the addition of carbamazepine (250 mg three times a

day) to the drug regimen. No other adverse effects were noted.

Fluorodopa uptake (expressed as milliliters of fluorodopa per minute per cubic centimeter) within the right putamen increased from 0.0071 before surgery to

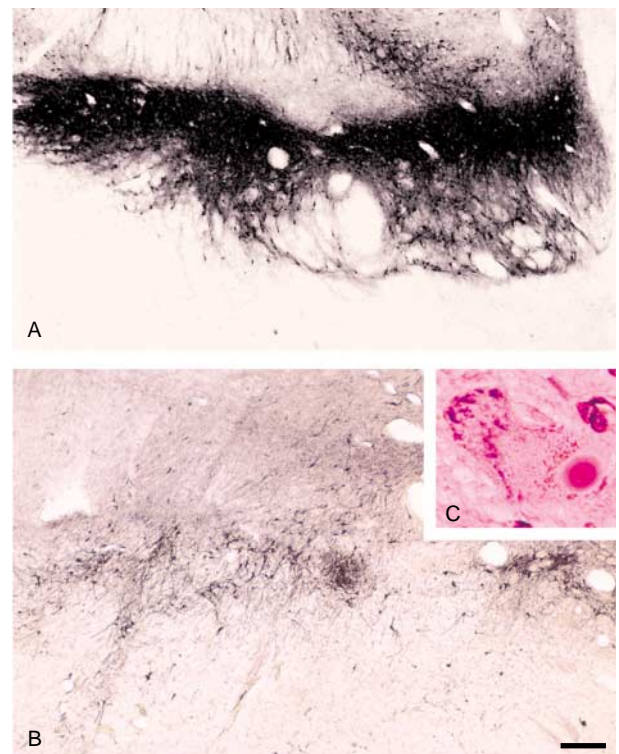


Figure 2. TH Immunoreactivity within the Ventral Midbrain. In a normal subject, TH immunoreactivity is densely expressed within neurons and processes in the ventral midbrain (Panel A). In contrast, the number of TH-immunoreactive neurons within the substantia nigra is dramatically reduced in a patient with Parkinson's disease who received fetal implants (Panel B). A neuron within the patient's substantia nigra pars compacta stained with hematoxylin contains a Lewy body (Panel C). The bar represents 120 μ m in Panels A and B and 20 μ m in Panel C.

0.0105 at 6 months and 0.0144 at 12 months ($P < 0.01$). Within the left putamen, fluorodopa uptake increased from 0.0087 before surgery to 0.0111 at 6 months and 0.0140 at 12 months ($P < 0.05$) (Fig. 1). The value of 0.0144 in the right putamen represents 72 percent of the value in normal subjects.^{18,19} Fluorodopa uptake also increased in the right caudate nucleus (from 0.0130 before surgery to 0.0143 and 0.0181 at 6 and 12 months, respectively; $P < 0.01$). No change in uptake was detected in the left caudate nucleus (0.0144 before surgery, 0.0143 at 6 months, and 0.0146 at 12 months).

The pathological findings were consistent with the clinical diagnosis of idiopathic Parkinson's disease. In comparison with the normal human substantia nigra (Fig. 2A), there was extensive neuronal loss in the mid-brain (Fig. 2B), with extracellular neuromelanin pigment and Lewy bodies in the remaining melanized neurons (Fig. 2C). Within the putamen, large viable transplants were observed bilaterally at all transplant sites. There were oval-shaped grafts as large as 1.5 by 14 by 1.5 mm in the mediolateral, dorsoventral, and rostrocaudal directions (Fig. 3). At autopsy, many of



Figure 3. TH Immunoreactivity at the Graft Sites.

A computer-inverted photograph of a coronal section through the right striatum shows substantial immunoreactivity within the caudate (Panel A), confirming the findings on all the PET scans. A large transplant can be seen within the dorsolateral putamen, which provides extensive TH-immunoreactive innervation to the dorsal half of the putamen. In contrast, the graft does not substantially innervate the ventral putamen, which contains few TH-immunoreactive fibers. Low-power photomicrographs show the dense collections of TH-immunoreactive perikarya and fibers at graft sites in both the right putamen (Panel B) and the left putamen (Panel C). The bar represents 8500 μm in Panel A and 1000 μm in Panels B and C.

the implants were larger than at the time of transplantation, reflecting the growth of the grafts *in vivo*. All graft sites contained dense clusters of TH-immunoreactive neurons (Fig. 3B, 3C, and 4A), and over 1000 surviving TH-immunoreactive neurons could often be observed in a single section. Cells were round or triangular and organized in clusters as seen in normal substantia nigra (Fig. 4A and 4B). Dopaminergic neurons were aggregated preferentially along the periphery of the transplant, with the center containing TH-immunoreactive fibers, nondopaminergic neurons, and glial cells (Fig. 3A and 3B). Elaborate neuritic arbors emanated from grafted nigral neurons within each graft site (Fig. 4C). All graft sites were highly vascularized. Substantial macrophage infiltration was noted at only one site, which still contained hundreds of viable dopaminergic cells. The grafted neurons did not contain discernible neuromelanin or Lewy bodies.

Each graft displayed seamless integration within the host striatum (Fig. 4D) and had extensive dopaminergic innervation to the putamen (Fig. 3A, 5A, and 5B). Dopaminergic innervation of host tissue extended 5 to 7 mm from the graft on the right side and 2 to 3 mm on the left. Thus, graft deposits separated by 5 mm usually provided confluent innervation of TH-immunoreactive fibers throughout the implanted regions of the striatum (Fig. 3A and 5A). Differences in the samples of fetal tissue, the order of grafting, or the longer survival of the grafts in the right putamen may have contributed to differences in outgrowth between the hemispheres. Differences in outgrowth did not correspond with the age of the donor or the storage time. The dense TH-immunoreactive staining of fibers and terminals within the putamen was associated with the location of the graft. Most grafts were placed in the dorsal putamen and gave rise to dense innervation, often encompassing the dorsal half to two thirds of the putamen (Fig. 3A and 5A). A few implants were located ventrally, and they gave rise to dense dopaminergic innervation only within the ventral putamen.

Although it is difficult to establish with certainty the source of particular fibers, these data, combined with the fact that ungrafted regions of the putamen had negligible TH-immunoreactive staining (Fig. 3A and 5C), strongly suggest that most of the dopaminergic fibers proximal to the implants were derived from the grafts. In both hemispheres, dopaminergic processes crossed the graft–host interface and innervated the parkinsonian striatum in a patch-matrix pattern²¹ (Fig. 5B). Dopaminergic fibers from the graft to the host ap-

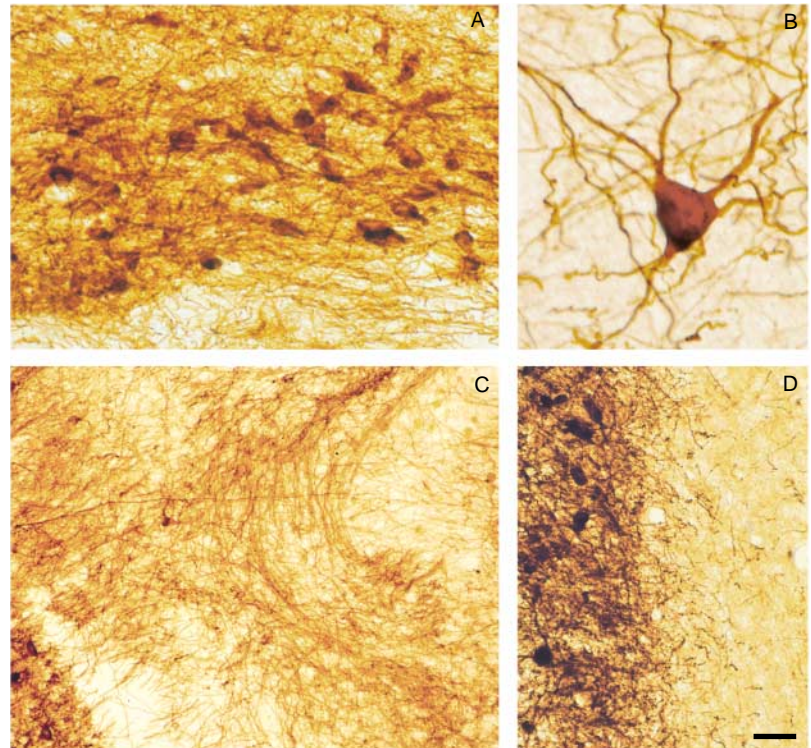


Figure 4. Morphologic Characteristics of the Grafted Neurons.

Medium- and high-power photomicrographs (Panels A and B, respectively) show the dense clustering and typical morphologic pattern of grafted TH-immunoreactive neurons. Within the graft, these neurons have given rise to an extensive network of fibers (Panel C). There is a seamless integration of graft and host tissue, with graft-derived fibers crossing the graft–host boundary (Panel D). The bar represents 80 μm in Panels A, C, and D and 30 μm in Panel B.

peared to be more vigorous in the mediolateral direction than in the dorsoventral direction. Graft-derived fibers were nonvaricose, unlike host fibers, which had numerous varicosities. Grafts placed in the medial portion of the right putamen gave rise to fibers that coursed between islands of the internal capsule to innervate the caudate nucleus, in keeping with the findings on PET scanning. Serial reconstructions of TH-immunoreactive stained sections failed to provide evidence of graft-mediated sprouting of host fibers within the putamen. As mentioned above, putaminal innervation was associated with the location of the graft. Furthermore, mapping of the host dopaminergic mesostriatal pathways failed to reveal aberrant sprouting of dopaminergic fibers to the perigraft region from potential sources of dopaminergic fibers, including the ventral tegmental area, substantia nigra, and nucleus accumbens.

DISCUSSION

The neuropathological findings we describe provide some fundamental information about the mechanism of action of fetal nigral transplantation and its potential usefulness as a treatment for Parkinson's disease. First, there was evidence of robust, long-term survival of transplanted nigral neurons 18 months after the initial procedure. Second, the grafted neurons displayed normal morphologic features and extended processes

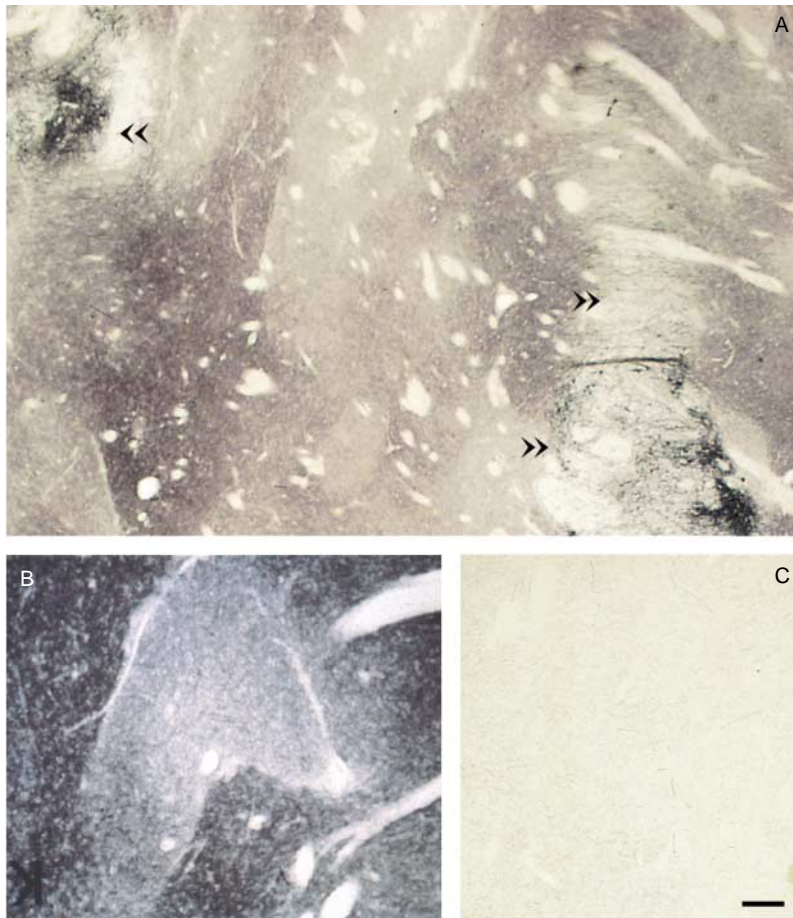


Figure 5. TH-Immunoreactive Innervation at the Graft Sites.

Grafted neurons have given rise to a dense pattern of TH-immunoreactive fibers and terminals that are confluent between two graft sites within the same coronal plane (arrows, Panel A). Dense graft-derived TH-immunoreactive innervation of the putamen is present in a patch-matrix pattern (lighter and darker areas, respectively; Panel B). There is a paucity of TH immunoreactivity 7 mm ventral to a transplant located within the right putamen (Panel C). Few dopaminergic fibers remain within the putamen distal to any graft site. The bar represents 350 μm in Panel A and 175 μm in Panels B and C.

in a pattern seen previously in studies in animals.²²⁻²⁴ Fiber outgrowth from the graft was sufficiently extensive to achieve confluent innervation of the putamen. Third, the robust survival of the grafted neurons, with the relative absence of macrophages, indicates that the neurons had not been rejected and continued to thrive for 18 months after the transplantation, even though the patient did not receive cyclosporine during the final 12 months of his life. Finally, the patient had a clinical benefit from the transplantation. That benefit was associated with the survival of the grafted nigral neurons and their innervation of the host striatum and was not associated with the sprouting of host dopaminergic systems.

The extensive survival of grafted nigral neurons in the brain of the patient we studied contrasts with survival in previous studies of transplantation in patients with Parkinson's disease. Redmond and coworkers⁹ reported negligible survival of transplanted cells from a 10-week-old fetus that had been cryopreserved before

grafting. Only a single dopaminergic neuron was identified adjacent to the graft site. Similarly, Hitchcock et al.¹⁰ reported marginal survival of grafted nigral neurons from fetuses over 12 weeks old. The few dopaminergic grafted neurons that were identified were atrophic, displayed minimal neuritic extension, and had massive accumulations of neuromelanin. The poor cell survival in both studies was associated with little, if any, clinical benefit.

The survival of dopaminergic neurons may be related to specific features of the transplantation, such as fetal age and storage of cells. Graft survival has been shown to be related to the ontogeny of the human nigrostriatal system.²⁵ Embryonic dopaminergic nigral neurons are first detected in the ventral mesencephalon 5½ to 6½ weeks after conception and begin to extend neuritic processes by 8 weeks.^{26,27} In a study of rodents with 6-hydroxydopamine-induced lesions, robust survival was routinely observed with cell-suspension grafts obtained from human fetuses five to eight weeks after conception and with solid grafts obtained six to nine weeks after conception.²⁵ Viability was not observed with grafts from human fetuses older than nine weeks. Storage of graft material is essential to screen for infectious agents and to allow for the acquisition of grafts from multiple fetuses. Cryopreservation is associated with the diminished viability of implanted cells.²⁸ In contrast, stor-

age of cells in cold-hibernation medium for up to 48 hours is not associated with a loss of viability.¹⁷ In our study, the exclusive use of embryonic donors that were 6½ to 9 weeks old and the storage of tissue for no longer than 48 hours in cold-hibernation medium may have contributed to the extensive cell survival, confluent striatal reinnervation, and clinical benefit we observed.

Serial PET scans showed a significant and progressive increase in putaminal fluorodopa uptake bilaterally 6 and 12 months after grafting. Vingerhoets and coworkers have recently established the base-line values for fluorodopa uptake within the striatum in normal subjects and patients with Parkinson's disease.^{18,19} Using the same scanner and procedure, we found that fluorodopa uptake bilaterally within the putamen was at least 70 percent of the normal value one year after transplantation. Other groups have observed increases in fluorodopa uptake and have interpreted their findings to indicate graft viability.^{11,12} However, the sprouting of host dopaminergic fibers could theoretic-

cally account for these findings and the reported clinical benefits. In our study, the increased fluorodopa uptake was clearly associated with graft-derived fiber outgrowth, since no sprouting of host fibers was detected. Fluorodopa uptake was also increased in the right caudate nucleus, even though all grafts were targeted to the putamen. Morphologically, it appeared that grafts placed within the right medial putamen sent dopaminergic processes through the internal capsule to innervate the caudate nucleus, and this innervation was associated with the observed increase in fluorodopa uptake. Similar graft-derived innervation was not observed in the left caudate nucleus, where there was no enhanced fluorodopa uptake on PET scanning. Taken together, these findings support the concept that increased striatal fluorodopa uptake after fetal nigral transplantation is an index of graft survival.

The need for immunosuppressive therapy after transplantation remains unclear. Although fetal allografts in animals usually survive for long periods without immunosuppression, this finding has yet to be demonstrated in humans. Furthermore, isolated examples of allograft rejection have been reported in rodents.²⁹ In our patient, allogeneic grafts from seven immunologically unrelated fetuses were transplanted sequentially in two surgical procedures separated by four weeks, thereby increasing the risk of rejection. The apparent absence of rejection after the second procedure is similar to the findings in an animal model.³⁰ We used a low-dose regimen of cyclosporine to increase the likelihood that the grafted cells would survive beyond the period when the blood-brain barrier was most likely to be disrupted.^{31,32} Cyclosporine was initiated three weeks before transplantation and was discontinued six months after the second procedure. Viability was excellent at all graft sites, even though immunosuppressive therapy was not administered during the patient's final 12 months of life.

Although fixation prevented the direct examination of immune markers, a number of observations indicate that no rejection process was under way at the time of the patient's death. The grafted cells displayed morphologic features of healthy neurons, few macrophages were observed within the perigraft region, fluorodopa uptake continued to increase on repeated PET scans, and functional improvement continued after the cessation of cyclosporine treatment. Although the possibility of a slow rejection of the graft cannot be ruled out, our data suggest that long-term immunosuppressive therapy is not required after the grafting of fetal tissue.

In conclusion, neuropathological studies demonstrated robust, long-term survival of implanted dopaminergic neurons and extensive graft-derived neuritic outgrowth with confluent reinnervation of the putamen in a patient with advanced Parkinson's disease who had sustained clinical improvement and progressive enhancement of striatal fluorodopa uptake on PET scanning after fetal nigral transplantation. These results suggest that transplant-induced clinical improvement

in patients with Parkinson's disease depends on the reinnervation of the putamen by viable grafts of embryonic dopaminergic neurons. Our findings support fetal nigral transplantation as an effective treatment for Parkinson's disease.

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