Neural transplants in patients with Huntington’s disease undergo disease-like neuronal degeneration

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The optimal donor age for human striatal graft survival was 12 months (11). The clinical evaluation of neural transplantation as a potential treatment for Huntington’s disease (HD) was initiated in an attempt to replace lost neurons and improve patient outcomes. Two of 3 patients with HD reported here, who underwent neural transplantation containing striatal anlagen in the striatum a decade earlier, have demonstrated marginal and transient clinical benefits. Their brains were evaluated immunohistochemically and with electron microscopy for markers of projection neurons and interneurons, inflammatory cells, abnormal huntingtin protein, and host-derived connectivity. Surviving grafts were identified bilaterally in 2 of the subjects and displayed classic striatal projection neurons and interneurons. Genetic markers of HD were not expressed within the graft. Here we report in patients with HD that (i) graft survival is attenuated long-term; (ii) grafts undergo disease-like neuronal degeneration with a preferential loss of projection neurons in comparison to interneurons; (iii) immunologically unrelated cells degenerate more rapidly than the patient’s neurons, particularly the projection neuron subtype; (iv) graft survival is attenuated in the caudate in comparison to the putamen in HD; (v) glutamatergic cortical neurons project to transplanted striatal neurons; and (vi) microglial inflammatory changes in the grafts specifically target the neuronal components of the grafts. These results, when combined, raise uncertainty about this potential therapeutic approach for the treatment of HD. However, these observations provide new opportunities to investigate the underlying mechanisms involved in HD, as well as to explore additional therapeutic paradigms.

Huntington’s disease (HD) is a progressive, untreatable, and fatal neurodegenerative disorder caused by increased CAG repeats in the huntingtin gene (1). The clinical evaluation of neural transplantation as a potential treatment for HD was initiated in an attempt to replace lost neurons and improve patient outcomes (see refs. 2–4). Preclinical rodent and primate experiments demonstrated the feasibility of using embryonic striatal grafts for the treatment of HD. Fetal striatal grafts survive (5), induce behavioral benefits (35), although this afferent connectivity appropriate host target areas was postulated to mediate graft-derived behavioral benefits (35), although this afferent connectivity of the graft to the host has never been observed at human autopsy (25, 36). Additionally, recent reports of PD-like Lewy-body inclusions within grafts surviving long-term in patients with PD have raised concerns that genetically and immunologically unrelated grafts are susceptible to the disease processes as well (37–39). The brains of 3 patients with HD who received neuronal transplants a decade earlier were evaluated at autopsy. Patients were clinically monitored, as described previously (31, 32). Here we report in patients with HD that (i) graft survival is attenuated long-term; (ii) immunologically unrelated grafts degenerate more rapidly than the patient’s brain, particularly the projection neuron subtype; (iii) cortical neurons project to transplanted striatal neurons; and (iv) microglial inflammatory changes in the grafts specifically target the neuronal components of the grafts. We also provide evidence that excitotoxicity and inflammatory mechanisms play a significant role in transplant degeneration and HD as well.

Results

Clinical Outcomes. Autopsy results from patient 2 (W.G.) of our series (32) have been reported previously (25). The evaluations volume within grafts to greater than 50% (2, 3, 23, 25, 26), which was postulated to correlate with optimal behavioral improvement in rodent models of HD (4, 27–29). Others have used alternative methods of dissection, transplanting the entire ganglionic eminence (4, 30, 31), which includes at least 13 different cell types or nuclei that are not medium spiny neurons of the striatum (23).

Several programs initiated neural transplant trials to evaluate the safety, tolerability, and potential efficacy of human striatal transplantation for the treatment of HD (26, 30–32). Preliminary, open-label studies of neural transplants in patients with HD have demonstrated approximately 2 to 4 years of modest clinical benefits, followed by progressive clinical deterioration similar to the natural history of the disease (31, 32), although there is 1 anecdotal case report of more meaningful clinical benefit (33). The safety profile for neural transplantation in HD may differ significantly from neural transplantation in Parkinson’s disease (PD) (32, 34). Neuritic outgrowth of transplanted medium spiny-projection neurons to appropriate host target areas was postulated to mediate graft-derived behavioral benefits (35), although this afferent connectivity of the graft to the host has never been observed at human autopsy (25, 36). Additionally, recent reports of PD-like Lewy-body inclusions within grafts surviving long-term in patients with PD have raised concerns that genetically and immunologically unrelated grafts are susceptible to the disease processes as well (37–39).

The brains of 3 patients with HD who received neuronal transplants a decade earlier were evaluated at autopsy. Patients were clinically monitored, as described previously (31, 32). Here we report in patients with HD that (i) graft survival is attenuated long-term; (ii) immunologically unrelated grafts degenerate more rapidly than the patient’s brain, particularly the projection neuron subtype; (iii) cortical neurons project to transplanted striatal neurons; and (iv) microglial inflammatory changes in the grafts specifically target the neuronal components of the grafts. We also provide evidence that excitotoxicity and inflammatory mechanisms play a significant role in transplant degeneration and HD as well.


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reported here correspond to patients 1 (B.L.), 3 (M.C.), and 5 (M.S.) from the same series. Baseline data (age, CAG repeats, symptom duration, time from diagnosis, number of donors, and location of transplants), surgical and immunosuppression methods, clinical outcomes, and complications are described in Tables S1 and S2 and have also been previously described (32). More detailed clinical descriptions are contained in the SI Text.

Graft and Striatal Gross Morphology. Macroscopic examination of each patient's brain showed prominent ventricular enlargement and highly atrophied striatal structures. Disease severity (40) was Grade 3 for patients 1 and 3, and Grade 2 for patient 5.

Multiple grafts survived in 2 of the 3 patients (1 and 5). In patient 3, only 1 out of 16 transplants survived (Fig. S1). In patients 1 and 5, grafts were easily identifiable macroscopically and histologically in the host putamen. Complete loss of all caudate grafts was noted in all 3 patients, corresponding to the region where striatal atrophy was most severe. All histological analyses presented here therefore only pertain to patients 1 and 5. Volumetric analysis revealed that grafts replaced 2.9% and 3.8% of the corpus striatum volume of the right and left hemispheres, respectively, of patient 1, and 4.3% of the corpus striatum volume of the left hemisphere of patient 5 (see Fig. S2). The largest graft was identified in the left putamen of patient 1 and measured 39.7 mm³, similar in size to what was observed in an autopsy performed at 18 months after transplantation (3). Despite preservation of graft volume with time, brain atrophy was notable in all patients. Patient 1 exhibited a 56.5% putamenal shrinkage in comparison to control values and patient 3 demonstrated a 45% putamenal shrinkage, which is consistent with previous autopsy studies reporting a 5% yearly rate of striatal atrophy in HD (41) and a 43 to 47% putamenal atrophy in HD patients in comparison to age-matched controls (42).

Graft Survival and Cellular Composition. Immunohistochemical evaluation of surviving putaminal grafts demonstrated regions with striatal markers (P-zones) interspersed with areas of nonstriatal markers (non-P-zones), similar to what was observed in a previous case evaluated 18 months posttransplantation (25). The P-zones of grafts in patient 1 were selectively immunoreactive for various markers of interneuron populations, such as acetylcholinesterase (AChE) (Fig. 1A), NADPH-d (Fig. 2G and H), parvalbumin (PV) (data not shown), and calretinin (CR) (data not shown), as well as markers of projection neurons such as calbindin (CB) (Fig. 2C and D). P-zone areas represented an average of 58.4% of the grafted tissue in patient 1 (see Fig. 1). Graft P-zone volumes in patient 5 averaged 49% of total graft volume (data not shown).

Grafted P-zones had a decreased density of neurons in comparison to both similar P-zones evaluated 18 months posttransplantation (Fig. 2E and F) as well as host striatal P-zones (see Fig. 2C and D). Surviving grafts did not exhibit the morphology of healthy cells in comparison to grafts 18 months posttransplantation (25). Morphological changes typical of neuronal degeneration in HD were observed in nearly all medium spiny-projection neurons labeled with CB (see Fig. 2). These cells appeared rounded, vacuolated, and lacked structural integrity of the cytoplasm and dendritic arborization. On H&E staining, abnormal chromatolysis could be seen (see Fig. 1F). CB immunoreactivity within the graft was also diminished (despite nickel-intensified DAB enhancement used in this study) in comparison to CB immunoreactivity in control regions of this HD brain (see Fig. 2C,
bars: 40 μm.

(Inset) as well as in similar grafts at 18 months posttransplantation, where intensification was not necessary (see Fig. 2A and B). Caspase-3, a marker of apoptosis, was similarly present in both the graft (see Fig. 1J) and the host neurons (see Fig. 1K). In comparison, grafted interneurons had a healthier appearance with retained dendritic arborization, and maintained good immunohistological staining (see Fig. 2G).

Graft-Induced Inflammatory and Immune Responses. Cluster of differentiation 8 (natural killers and cytotoxic T cells, CD8) (Fig. S3a and b), cluster of differentiation 4 (T helper cells, CD4) (Fig. S3c and d), and human leukocyte antigen (HLA-DR) (data not shown), were positively identified within the graft, indicating an ongoing immune response at the time of death.

Although astrogliosis typical of HD was observed in the host striatum, the edge of the graft was demarcated by a strong astrocytic response (Fig. 3A), where GFAP-positive cells had a morphological appearance of reactive astrocytes. Within the graft itself, the more rarely encountered GFAP cells were recognizable by their nonactivated state (Fig. 3B). The pattern of astroglial response respected the boundaries of the solid transplants (Fig. 3C).

In contrast to the astrocytic response around the border of the graft, the microglial response differently recognized the P- and non-P-zones within the graft (Fig. 3D). In particular, there was activation of microglia within and surrounding those components of the grafts containing striatal markers. Grafted regions lacking striatal markers, which contained immunologically identical donor tissue, were relatively free of microglial activation (see Fig. 3D). Activated microglia were frequently intermingled with neuronal nuclei- (NeuN) positive cells and were periodically seen to engulf neuronal elements of the graft, more so on the edge of P-zones than in the host striatum suggestive of potential phagocytosis (Fig. 3E–G).

The absence of protein aggregates (ubiquitin) (see Fig. S3e) or of abnormal huntingtin protein (EM48) (Fig. S3g) within the graft suggest that the transplant was spared from the primary pathological effect of the abnormal huntingtin gene. However, ubiquitin (Fig. S3f) and EM48 (Fig. S3h) were expressed within the host striatum. Additionally, EM48 expression was also pronounced in layer 5 of the cortex (Fig. S3i and j).

Host Projections to the Graft. Synaptophysin immunoreactivity was demonstrated in patient 1 (Fig. 4A–D), suggesting establishment of synaptic contacts by transplanted neurons. Host-derived dopaminergic fibers grew into the transplant in both cases, as demonstrated by tyrosine hydroxylase (TH) immunohistochemistry (Fig. 4E and F). In the striatum, vesicular glutamate transporter 1 (vGlut1) axonal varicosities (terminals), found specifically in cortico-striatal axonal projections (43), were observed closely apposed to CB+ neurons in both the host striatum and the transplant (Fig. 4G). Electron microscopic examination of the graft P-zone in patient 1 revealed asymmetrical synaptic contacts established by vGlut1 immunolabeled axon varicosities (terminals) (Fig. 4H). Technical difficulties in achieving adequate preservation of the fine structure precluded a complete ultrastructural morphological analysis. Of note, the regions within the grafts receiving glutamatergic input colocalized exactly with the regions expressing microglial activation.

Discussion

We have demonstrated that graft survival in patients with HD is attenuated a decade following transplantation. Multiple surviving
Fig. 3.  Inflammatory responses to grafts. Both astrocytosis and microgliosis were evaluated and found to be consistent in all 3 hemispheres; representative photomicrographs of patient 1 are illustrated here. (A) The astrocytic response is significant in the host but minimal within the graft, as investigated by GFAP staining. The edge of the graft is demarcated by a particularly strong astrocytic response, characterized by activated astrocytes. A few nonactivated astrocytes are observable within P-zones (B). Abundant GFAP fiber staining is seen within the non-P-zone (C). These nonactivated astrocytes expressed fibers that extended throughout the non-P-zones but did not significantly invade P-zone. (D) Low-power photomicrograph of double immunostaining for the neuronal nuclear marker NeuN (brown; DAB chromogen) and the microglial marker Iba-1 (black; nickel-DAB chromogen) at the interface of a P-zone and non-P-zone and at the interface of the graft and the host. A pronounced microglial response is demonstrated cuffing the edge of the P-zone as well as within the P-zone. Microglial cuffing is seen predominantly at the interface between P-zones and non-P-zones, as opposed to the interface between the non-P-zone portion of the graft and the host. (E) Higher magnification of NeuN/Iba-1 staining at the interface graft/host. (F and G) Higher power photomicrographs depicting examples of grafted neurons interleaved with microglial cells. Of note, microglial cells were often observed engulfing neuronal elements, resembling an ongoing phagocytic event. (Scale bars: A, 25 μm; B, 20 μm; C, 100 μm; D, 300 μm; E and F, 25 μm; G, 12.5 μm.)

Fig. 4.  Graft connectivity. (A) Low-power photomicrograph of synaptophysin immunoreactivity inside the graft (dotted line). The density of synaptophysin reaction product is depicted in (B), in the form of an optical density plot taken along the line shown in (A). (C and D) High magnification of synaptophysin staining in (C) a P-zone of the graft and (D) in the host striatum. (E) Photomicrograph of TH immunostaining of the grafted P-zone (dotted line). (F) Numerous host-derived TH+ fibers are observed with seamless penetration of the graft border. (G) vGlut1 axon varicosities (black dots; arrows within the neuropile) were found closely apposed to cell bodies labeled for CB (brown, arrowheads) in both the host striatum and the transplant. (H) Example of a vGlut1-labeled axon varicosity (asterisk) within the transplant P-zone exhibiting an asymmetrical synaptic contact (between small arrows). (Scale bars: C and D, 25 μm; E, 100 μm; F, 25 μm; G, 20 μm; H, 500 nm.) av, axon varicosity; sp, spine.

Grafts were observed in only 2 of our 3 patients. Case 3 (see ref. 32) had only 1 of 16 surviving grafts, which is unusual for any PD or HD transplant recipient using similar techniques (3, 33, 14, 37, 38). Caudate grafts did not survive in any of these HD patients, similar to what we reported 18 months after transplantation (25). Because the caudate displays the greatest neuronal degeneration and astrogliosis in HD, the current findings suggest that this region is a poor transplant niche in this disease. Long-term putamenal grafts had decreased cellularity in comparison to those at 18 months posttransplantation. Grafted cells appeared unhealthy, particularly projection neurons within the grafts. The expression of intraneuronal caspase-3 suggests that striatal cell loss was induced, at least in part, by apoptosis. These results differ significantly from 18-month and 6-year posttransplantation cases where healthy graft survival was reported (25, 36). Neural degeneration at 10 years of neuronal transplant in HD patients is widespread and potentially incompatible with any long-term graft-derived benefit. These results differ from similar case reports in patients with PD (37–39). In those cases, 2 to 8% of transplanted cells demonstrated disease-specific markers (Lewy bodies) (37, 39) and 40 to 80% of dopaminergic neurons and cytoplasmic α-synuclein (38, 39). Despite this, cells were viable, metabolically active (38) and remained differentiated with neuritic reinnervation of the host striatum and with long-term clinical efficacy in individual anecdotal cases (37, 38).

There was diminished cellular viability in all grafted cells at 10 years (vs. 18 months) posttransplantation. Importantly, poor cellular viability was profound in the grafted striatal projection neurons in particular, as opposed to grafted interneurons that still showed distinct cellular cytoarchitecture and polarity but with fewer dendritic extensions than was observed at 18 months posttransplantation. The preferential degeneration of grafted medium-spiny neurons in comparison to interneurons recapitulates the pattern of
striatal cell loss seen in HD, suggesting that disease-like cell loss is also occurring within the genetically unrelated grafts.

Excitotoxicity may play a fundamental role in the degenerative processes seen within these transplants. Glutamatergic axon varicosities from the cerebral cortex were found closely apposed to striatal projection neurons in both the host striatum and the transplants. Within the graft, they were more abundant in the P-zones that contain the neurons that normally receive cortical glutamatergic innervation. Furthermore, microglial infiltration specifically cuffed the P-zones within the grafts, suggesting that microglial activation was responding to specific cues found within the striatal P-zone components of the graft. As the non-P-zone components of the allografts have the same immunological surface antigens as P-zone elements, the selective microglial response to P-zones in particular is compatible with the hypothesis that this response is mediated by neurotoxicity (44, 45) rather than a subacute immune rejection (14). Similar loss of striatal projection neurons is seen following excitotoxicity secondary to glutaric acid in the brains of patients with Glutaric Acidemia Type 1, which leads to an HD-like syndrome in young children (46, 47). In rats, no similar colocalization of glutamatergic input with P-zone-specific microglial infiltration has been described, suggesting that these pathological findings described in the study represent a neurotoxic event. However, it is also possible that the cortex is making appropriate synapses with the graft.

The lack of EM48 (a marker of the abnormal huntingtin gene) within grafts, in the presence of robust graft degeneration, indicates that the abnormal HD gene is not required within the striatal neurons to induce degeneration. Instead, the degeneration of striatal grafts may be a remote consequence of the abnormal gene expressed elsewhere in the brain. Among other regions, EM48 was located in layer 5 of the cortex, the origin of the vGlut1 innervation of both the striatum and our transplants. Normal huntingtin immunolabeling has similarly been identified in layer 5 cortico-striatal projection neurons of the normal rat (48). In addition to glutamatergic input to the striatum, cortico-striatal projections also provide trophic support to striatal neurons (49). These data suggest that grafts degenerate secondary to excitotoxic glutamatergic inputs from the cortex combined with microglial activation against striatal components of the grafts and to the loss of adequate host trophic support. The pattern of graft degeneration that so clearly recapitulates the pattern of striatal degeneration in HD suggests that findings in transplantation provide a possible model for the mechanisms of degeneration of the striatum of patients with HD as well.

In the present study, we also observed that the grafts were strikingly more affected by pathological processes than the host striatum, despite the fact that the grafts were young, immunologically unrelated to the patients with HD, and exposed to the disease process for only a decade. Cortical glutamatergic projections to both the graft and the host striatum were demonstrated. At the electron microscopy level, synaptic contacts between glutamatergic axon varicosities and unlabeled dendrites within the graft were also observed. It is possible that the immune responses to solid grafts in the absence of immunosuppression (14, 50), combined with the release of glutamate directly from microglia or glutamatergic cortico-striatal afferents, accelerated the degeneration process in grafted vs. host striatal neurons, as suggested in animal studies (51). Graft degenerative changes seen here are incompatible with clinical benefits after 10 years, vs. PD, where only 2 to 8% of grafted cells Graft degenerative changes seen here are incompatible with clinical benefits after 10 years, vs. PD, where only 2 to 8% of grafted cells.
ACHε (enzyme catalyzing hydrolysis of the neurotransmitter acetyl choline in cholinergic neurons), PV (Sigma; 1:1,000) or CR (Swant; 1:2,500). The immune/inflammatory response was investigated using GFAP (astrocytes; Dako Canada; 1:2,500), CD4 (T helper cells; Serotec; 1:2,500), CD8 (natural killers and cytotoxic T cells; Serotec; 1:2,000), HLA-DR (surface antigen of class II major histocompatibility complex; Serotec; 1:200), while other sections were processed for double immunohistochemistry to visualize the microglial response (Iba-1; Wako Chemicals; 1:1,000) to grafted neuronal elements (NeuN; Chemicon; 1:2,500). Genetic markers of huntingtin were evaluated using EM48 (abnormal huntingtin protein; 1:1,000) to grafted neuronal elements (NeuN; Chemicon; 1:2,500), and ubiquitin (Calbiochem; 1:200) to detect any neuronal elements. Finally, host-derived connectivity was assessed by TH (Pel-Freez; 1:1,000), synaptophysin (Calbiochem; 1:500), double immunostaining for CB (striatal projection neurons; Sigma; 1:2,500), and vGlut1 immunohistochemistry, but for single immunostaining were prepared using a slightly modified version of previously published protocol (92).

Three-Dimensional Reconstruction, Stratal P-Zone Evaluation and Cell Counts
Three-dimensional reconstruction was performed using the Serial Section Reconstruction method provided by the Neurolucida software, version 6.0 (Micro- brightfield) on Nissl-stained sections. Volumetric evaluation of graft size (Cavali- Heri method) as well as P-zone/non-P-zone areas for patients 1 and 5 were explicitly performed using the right hemisphere of both patients 1 and 5. Both grafts and P-zones were delineated using Nissl staining and the Tracing Contours option in the Stereo Investigator software, version 5.0 (Microbrightfield). Areas for either P-zones or non-P-zones were calculated using Contour Measurements option. Cell counts were performed using the Optical Fractionator probe (Stereo Investigator software) based on 3 different immunostaining markers for NADPH (interneurons), H&E (general cell marker), and CB (projection neuron). More detailed on the assessment of graft volume, extent of grafted striatum, estimation of strial P-zones, and 3-dimensional reconstruction are provided in SI Text.

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Significant cortical atrophy was noted on the preoperative MRI scan of patient 1 (B.L.). At the end of surgery, brain shrinkage because of cerebrospinal fluid loss was noted. The surface of the brain was ~8 mm from the inner cortex of the skull. No subdural hematoma was noted on the immediate postoperative MRI scan. However, cortical shrinkage led to targeting error in the last needle tract (in the right cuneate) (see Fig. S2). A 2-cm thick asymptomatic subacute subdural hematoma was noted on the localizing MRI scan done before the second operation and was drained uneventfully after completion of the contralateral transplant procedure. Follow-up MRI scans demonstrated complete resolution of the hematoma. The Unified Huntington’s Disease Rating Scale (UHDRS) score of bilateral hygromas postoperatively increased to 32 at 3 months postoperatively, but returned to baseline (1) by 18 months after surgery. Preoperative gait instability with falling improved for 2.5 years, whereupon a wheelchair was required and swallowing difficulties began to emerge. UHDRS scores stabilized until 3.5 years after surgery. Scores then progressively deteriorated to 52, 65, and 78 at 4, 6, and 7 years postoperatively. Cognitive function also progressively declined with Mini Mental State Evaluation (MMSE) scores worsening from 30 at both baseline and at 21 months postsurgery to 14 at 7 years postsurgery. She died at 67 years of age of an aspiration pneumonia complicated by a myocardial infarction 9 years postoperatively.

Patient 3 (M.C.) had an asymptomatic 1-cm cortical hemorrhage after her first operation, as well as a thin (3 mm) subdural hematoma without mass effect. She had postoperative confusion that resolved in 2 weeks. A 4-mm subdural hematoma was noted after the second operation, and the bilateral thin subdural hematomas both resolved on subsequent postoperative imaging without complications. Her preoperative UHDRS score of 33 remained unchanged until 10 to 12 months postsurgery, when scores improved to 28 and 21, respectively. Her preoperative balance difficulties and falling (once per month) improved postoperatively, and she did not fall for at least 2 years after surgery. Symptoms then worsened to a UHDRS score of 37 at 20 months after surgery and plateaued there until 3 years postoperatively. Cognitive function declined mildly with an MMSE score of 26 at baseline and 24 at 30 months postsurgery. Some depressive symptoms emerged at 2 years after surgery. The patient died at the age of 75 of a cardiorespiratory arrest as complication of end-stage HD, 9.5 years after surgery.

For ACH staining, sections were washed 3 times in distilled water and incubated in 0.2% acetylthiocholine iodide (Sigma) in a stock solution (copper sulfate, glycine, magnesium chloride, maleic acid in 4% NaOH, 40% sodium sulfate). They were then rinsed 3 times in 40% sodium sulfate, and incubated in 10% ammonium sulfide for 2 min. After washes in distilled water, sections were counterstained with Kernechtrot red (J.T. Baker) for 1 min and rinsed in tap water. Slices were mounted on gelatin-coated slides, air-dried overnight, dehydrated in ascending grades of ethanol, and cover-slipped with DPX mounting media (Electron Microscopy Science).

For H&E staining, sections were first mounted on gelatin-coated slides, air-dried overnight, and hydrated in 50% ethanol. They were then stained with hematoxylsin (Fisher Scientific) for 40 sec, washed in running water for 5 min, and placed in differentiator solution (0.5% pure glacial acetic acid in 95% ethanol) for 1 min. Sections were washed using distilled water and counterstained with Eosin Y (Sigma) for 40 sec, dehydrated in ethanol and xylene baths (3 × 90% ethanol, 2 × 100% ethanol, 2 × xylene) and cover-slipped with DPX mounting media.

Immunochemistry. Before immunostaining procedures, free-floating sections were washed 3 times in PBS 0.1M before histochemical staining for NADPH-d (marker for nitric oxide containing striatal interneurons), AChE (enzyme catalyzing hydrolysis of the neurotransmitter acetyl choline in cholinergic neurons), and H&E (brain cytoarchitecture). For NADPH-d staining, sections were washed in PBS 0.1M pH 7.4, preincubated in 0.25% Triton X-100 in PBS for 10 min and transferred in a fresh solution of 0.25% Triton X-100, 0.05% of the reduced form of nicotinamide adenine dinucleotide phosphate (b-NADPH; Calbiochem), 0.02% nitro blue tetrazolium (Sigma) in PBS for 5 min at room temperature and then at 37 °C for 8 h. Sections were rinsed in PBS, mounted on gelatin-coated slides, and kept at 37 °C overnight. They were subsequently dehydrated in ascending grades of ethanol and cover-slipped with DPX mounting media (Electron Microscopy Science).

Before single immunostaining, sections were subsequently washed in PBS and then preincubated for 30 min at room temperature in a blocking solution containing 0.1% Triton X-100 (Sigma) and 5% Normal Goat Serum (NGS, Wisent Inc.) diluted in PBS. Sections were incubated for 24 h at 4 °C in the same solution to which either anti-GFAP (Dako Canada; 1:2,500) or anti-TH (Pel-Freez; 1:1,000), or for 48 h at 4 °C with anti-PV (Sigma; 1:1,000). Sections were then washed in PBS and incubated for 1 h at room temperature in the blocking solution to which biotinylated goat anti-rabbit (for GFAP and TH) or biotinylated goat anti-mouse (for PV) (Vector Laboratories; 1:1,500) was added. Following 3 washes in PBS 0.1M, sections were placed in a solution of avidin-biotin peroxidase complex (ABC Elite kit, Vector Laboratories) for 1 h at room temperature. Antidodies were revealed by placing the sections in Tris buffer solution containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.1% of 3% hydrogen peroxide at room temperature. Reaction was stopped by washing in 0.05M Tris buffer and subsequent PBS washes. Slices were
mounted on gelatin-coated slides, air-dried overnight, dehydrated in ascending grades of ethanol, xylene, and cover-slipped with DPX mounting media.

Other sections were immunohistochemically processed with nickel-intensification of DAB to enhance the chromogen signal. These sections were treated in a similar manner as described above except that the main buffer solution was composed of PBS 0.2M pH 7.4 and 1% BSA (Sigma) was added as a blocking agent in the primary and secondary antibodies, as well as in the ABC solutions. Sections were incubated 48 h at 4 °C with either anti-CB (Sigma; 1:2,500), CD4 (Sero; 1:250), CD8 (Sero; 1:200), HLA-DR (Sero; 1:200), ubiquitin (Calbiochem; 1:250), synaptophysin (Calbiochem; 1:500), EM48 (provided by X.J. Li, Emory University; 1:2,000) or anti-CR (Swant; 1:2,500) and in 1 h with biotinylated goat anti-mouse (for CB) or goat anti-rabbit (for CR) (Vector Laboratories; 1:1,500). After incubation with ABC, sections were washed twice in acetate imidazole 0.2M pH 7.2 followed by Ni-DAB solution (dH2O, sodium acetate 1M pH 7.2, imidazole 0.2M pH 9.2, nickel-sulfate 6.H2O, DAB, H2O2 30%). Immunohistochemical reaction was terminated by washes in acetate imidazole 0.2M (pH 7.2) followed by 0.2M PBS rinses. Slices were mounted on gelatin-coated slides, air-dried overnight, dehydrated in ascending grades of ethanol, and cover-slipped with DPX mounting media. In these experiments, immunohistochemical controls included omission of the primary or the secondary antibody, which completely abolished the immunostaining.

Other sections were processed for double immunohistochemistry to visualize neuronal nuclei (NeuN) and microglia (Iba-1) or calcium binding protein (CB) and vGlut1. After overnight incubation at 4 °C with an antibody against Iba-1 (Wako Chemicals; 1:1000) or 48 h at 4 °C with an antibody against vGlut1 (Sigma; 1:500), the sections were extensively washed in PBS and incubated for 1 h at room temperature in a PBS solution containing biotinylated goat anti-rabbit IgG (Vector Labs; dilution 1:1,500), Triton X-100 (0.1%), BSA (1%), and NGS (5%). After further washing in PBS, the sections were placed in a solution containing ABC (Elite kit; Vector Labs) for 1 h at room temperature. The bound peroxidase was revealed with nickel-intensified DAB as the chromogen. After immunostaining for Iba-1 or vGlut1, the sections were reincubated overnight at 4 °C with a NeuN antibody (Chemicon; 1:1,000) or 48h at 4 °C with an antibody against CB (Sigma; 1:2,500). The incubation procedures were the same as above, except that the incubation time in secondary antibody, goat anti-mouse (Vector Labs; 1:1,500), was 2 h and sections were revealed using DAB (Sigma) and 0.01% hydrogen peroxide in 0.05 M Tris-imidazole (pH 7.2) at room temperature. The reaction was stopped after 10 to 15 min by extensive washing in PBS. Controls included omission of either one of the primary antibodies to exclude cross-reactivity of the secondary antibodies.

Sections intended for electron microscopy were prepared as above for vGlut1 immunohistochemistry, but for single immunostaining only, without Triton X-100 in all solutions and using DAB as the chromogen. After revelation, these sections were osmicated, dehydrated in ethanol and propylene oxide, and flat-embedded in Durcupan (Fluka). Rectangular pieces within the grafted P-zone were removed from the flat-embedded vGlut1-immunostained sections, glued to the tip of resin blocks, and sectioned ultrathin (80 nm) with a Reichert Jung ultramicrotome. These sections were collected on bare 150 mesh copper grids, stained with lead citrate, and examined with a Phillips CM100 electron microscope (60 kV, Philips Electronique).

Assessment of Graft Volume and Location. Nissl staining, as described previously (2), was used to perform the 3-dimensional reconstruction of transplantation sites using NeuronLucida modeling software (Microbrightfield) attached to a E800 Nikon microscope (Nikon Instruments) (see below). Two distinct sets of calculations were performed in relation to graft volume. The first calculations, which are found in Table 1 of Fig. 1, depict the volumes of the entire corpus striatum (putamen, caudate and the globus pallidus). Representative serial sections (1 in 10) were compared to equivalent sections from the corpus striatum of a control brain. The second set of volumetric measurements was used to evaluate the degree of brain shrinkage of patients 1 and 5. For this measurement, serial sections of the putamen of the transplant recipient were compared to equivalent serial sections of the putamen in an age-matched control brain.

Estimation of the Striatal Zones (P-Zones) Within the Grafts. Volumetric evaluation of graft size (Cavaleri method) as well as P-zone and non-P-zone areas for patients 1 and 5 were explicitly performed using the right hemisphere of both patients 1 and 5. Both grafts and P-zones were delineated using Nissl staining and the Tracing Contours option in the Stereo Investigator software, version 5.0 (Microbrightfield). Areas for either P-zones or non-P-zones were calculated using Contour Measurements option.

Three-dimensional reconstruction. Three-dimensional reconstruction was performed using the Serial Section Reconstruction method provided by the Neuroulucida software, version 6.0 (Microbrightfield). Briefly, the caudate, putamen, and transplant sites were traced using the Tracing Contours function for each section. Subsequently, each tracing was imported into the NeuroExplorer software, where the drawings were aligned to complete the 3-dimensional reconstruction. This procedure allowed calculation of structure and graft volumes, which took into account section thickness (40 µm).

Fig. S1. Necrotic grafts. Examples of necrotic grafts found in the putamen of patient 3 stained with (a) AChE (arrows) and (b) Nissl staining. (Scale bars: a, 250 μm; b, 50 μm.)
**Fig. S2.** Graft locations. (a) Axial view of an MRI scan performed 24 h after the first surgery of patient 1 showing localization of graft sites (red arrows) in both hemispheres. (b–f) Computer-generated 3-dimensional reconstruction of transplantation sites, based on a virtual representation of the Nissl staining of a series of 1 in 10 coronal sections of 2 of the 3 postmortem hemispheres analyzed in the present study (patients 1 and 5). Data derived from patient 1 is illustrated in (b) through (d) and has been colored in green. Patient 5 is illustrated in (e) and (f) and is colored in blue. Transplant sites in both cases are depicted in red. All putamenal grafts but not all caudate grafts were located. Total graft volume in patients 1 and 5 was 3 to 4% and 4.3% of the total host striatal volumes, respectively. (Scale bars in c and f, 1 mm.) A, anterior; CD, caudate; D, dorsal; GP, globus pallidus; P, posterior; PUT, putamen; STR, striatum; V, ventral.
Immune responses to grafts. (a) Photomicrographs of CD8 immunostaining (cytotoxic suppressor T-cell subset) demonstrated a greater CD8 immune response within the transplant (arrows) compared to the host (b). A similar difference was found for CD4 (subpopulation of T lymphocytes) immunostaining, where CD4-positive staining was seen within the graft (c, arrows) but not within the host (d). Neither ubiquitin staining, showing protein aggregates (e and f, arrows), nor EM48 (g and h), identifying the abnormal huntingtin mutant protein (arrows) within Nissl-stained cells (arrowheads), showed expression in the graft (e and g) as compared to the host putamen (f and h), where the abnormal huntingtin gene is expressed. These photomicrographs were taken from patient 1 but similar observations were made for patient 5. (i and j) EM48 expression was also pronounced in layer 5 of the cortex (arrows in j) (pictures taken from patient 3). Photomicrograph in (j) represents a higher magnification of (i) (see Inset). Depicted in (j) is also a higher magnification of the layer 5 distribution of nuclear inclusions (Bottom Inset) and higher magnification of a doubly stained neuronal nuclear inclusion (Nissl/EM48) (Top Inset). (Scale bars: a and b, 100 μm; c and d, 50 μm; e and f, 50 μm; g, 25 μm; h, 14 μm; i, 300 μm; Insets j: Top, 6.25 μm; Bottom, 50 μm.)
<table>
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<tr>
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<td><strong>Number and location of transplants</strong></td>
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<td><strong>Surgical complications</strong></td>
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Data from ref. 1 and subsequent clinical evaluation.

Table S2. Clinical outcomes

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<tbody>
<tr>
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<td>27 (31 after subdural hematoma drainage)</td>
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<td>24 (30 mo)</td>
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<td>14 (7 yrs)</td>
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MMSE, Mini Mental State Evaluation; UHDRS, Unified Huntington’s Disease Rating Scale.

Data from ref. 1 and subsequent clinical evaluation.