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


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 recovery (6, 7), and establish connectivity with the rodent brain (8, 9). Similar results have been reported with xenografts of porcine and human striatal tissue into rodents (2, 9–12). The human striatum is a small enough target to be approached surgically in HD with a realistic goal of achieving confluent graft integration into the host brain (2, 13, 14). Grafts can be functionally integrated (15–18), although such connectivity may not be necessary (19, 20). Similar behavioral results were reported in primate models of HD (21, 22).

The optimal donor age for human striatal graft survival was established in studies using animal models (4, 12, 23, 24). We chose to use embryonic striatal tissue derived from the far lateral portion of the lateral ventricular eminence to optimize striatal-like (P-zone) 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 graft 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.

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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 5.3% 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. 2 G 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. 2 C 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. 2 E and F) as well as host striatal P-zones (see Fig. 2 C 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 chromatin organization 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,

<table>
<thead>
<tr>
<th>Origin of Transplanted Tissue</th>
<th>NADPH</th>
<th>AChE</th>
<th>TH</th>
<th>PV</th>
<th>CB</th>
<th>Mean</th>
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<tr>
<td>P-zone (%)</td>
<td>73.4</td>
<td>55.8</td>
<td>59.4</td>
<td>59.1</td>
<td>45.1</td>
<td>58.4</td>
</tr>
<tr>
<td>Non P-zone (%)</td>
<td>25.5</td>
<td>44.2</td>
<td>40.5</td>
<td>41.9</td>
<td>54.9</td>
<td>41.5</td>
</tr>
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Fig. 1. Graft morphology. (A) Low-power magnification of a coronal section stained for AChE, which identifies graft placement within the putamen of the host, at the level of the anterior commissure within the striatum of patient 1. The graft is delineated by a dotted line. P-zone volume was calculated using tissue stained for the striatal markers AChE, NADPH-d, TH, PV, and CB (I). The mean percentage of P-zone volume per graft was evaluated to be 58% for patient 1 (see Results for patient 5). (B and G) Nissl staining showing the cell density discrepancy between P-zones and non-P-zones (B) as well as the notable difference in appearance between cells of the graft (black arrows in B and also C) and of the host (white arrows in B and Inset in C). (D and E) H&E staining also showing the cell-density discrepancies between P-zones and non-P-zones (D) as well as in the putamen of the host brain (E). Histograms of cell count (per square millimeter of tissue) in grafted P-zones versus host supports the observation of fewer cells within grafted P-zones than in the pathological host putamen (H). Note the morphological difference in appearance of cells in the P-zone vs. the non-P-zone (D). (F and G) Higher magnification photomicrographs of individual cells within the P-zone (F, and pointed by black arrows in D) and within the host putamen (G). In comparison to cells of the host (G), grafted cells appeared larger in diameter, balloononed, and vacuolated. Abnormal chromatin deposits exclusively within transplanted cells are discernible using the H&E staining (F). These characteristics suggest ongoing grafted neuronal degeneration. The immunostaining for the apoptotic marker caspase-3 (J and K) revealed equivalent neuronal degeneration in the host and graft. (Scale bars: A, 1 mm; B, 25 μm; C and inset, 12.5 μm; D and E, 50 μm; F and G, 6.25 μm; J and K, 25 μm.) CD, caudate; GP, globus pallidus; PUT, putamen.
Inset) as well as in similar grafts at 18 months posttransplantation, where intensification was not necessary (see Fig. 2 A and B). Caspase-3, a marker of apoptosis, was similarly present in both the graft (see Fig. 1 J) and the host neurons (see Fig. 1 K). In comparison, grafted interneurons had a healthier appearance with retained dendritic arborization, and maintained good immunohistological staining (see Fig. 2 G).

**Graft-Induced Inflammatory and Immune Responses.** Cluster of differentiation 8 (natural killers and cytotoxic T cells, CD8) (Fig. S3 a and b), cluster of differentiation 4 (T helper cells, CD4) (Fig. S3 c 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. 3 A), 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. 3 B). The pattern of astroglial response respected the boundaries of the solid transplants (Fig. 3 C).

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. 3 D). 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. 3 D).

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. 3 E–G).

The absence of protein aggregates (ubiquitin) (see Fig. S3 e) or of abnormal huntingtin protein (EM48) (Fig. S3 g) within the graft suggest that the transplant was spared from the primary pathological effect of the abnormal huntingtin gene. However, ubiquitin (Fig. S3 f) and EM48 (Fig. S3 h) were expressed within the host striatum. Additionally, EM48 expression was also pronounced in layer 5 of the cortex (Fig. S3 i and j).

**Host Projections to the Graft.** Synaptophysin immunoreactivity was demonstrated in patient 1 (Fig. 4 A–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. 4 E 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. 4 G). Electron microscopic examination of the graft P-zone in patient 1 revealed asymmetrical synaptic contacts established by vGlut1 immunolabeled axon varicosities (terminals) (Fig. 4 H). 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
microglial cells were often observed engulfing neuronal elements, resembling an ongoing phagocytic event. (Scale bars: 20 μm; 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 intermingled 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. 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 intermingled 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 within the graft 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 and non-P-zone. Numerous TH+ fibers are observed with seamless penetration of the graft border. (G) vGlut1-labeled axon varicosities (asterix) 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.
benefits after 10 years, vs. PD, where only 2 to 8% of grafted cells
Graft degenerative changes seen here are incompatible with clinical
grafted vs. host striatal neurons, as suggested in animal studies (51).
observed. It is possible that the immune responses to solid grafts in
axon varicosities and unlabeled dendrites within the graft were also
electron microscopy level, synaptic contacts between glutamatergic
istically unrelated to the patients with HD, and exposed to the disease
striatum, despite the fact that the grafts were young, immunolog-
strikingly more affected by pathological processes than the host
findings in transplantation provide a possible model for the mechanisms
recapitulates the pattern of striatal degeneration in HD suggests that
the origin of the vGlut1 innervation of both the striatum and our
abnormal HD gene is not required within the striatal neurons to induce
deregeneration. 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 activa-
tion against striatal components of the grafts and to the loss of adequate
host trophic support. The pattern of graft degeneration that so closely
recaptulates 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, immunolog-
ically 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
have α-synuclein inclusions (37–39), and clinical benefits lasted for
11 to 12 years in those patients (37, 38).
It has been postulated that if striatal transplants are to be
effective for the treatment of HD, they need to prevent local
neuronal degeneration within the striatum (26) as well as wide-
spread degeneration of cortical afferents projecting to the grafts
(2–4, 35). However, meaningful neuritic outgrowth was not dem-
onstrated in either the present study or previous studies of grafts
surviving 18 months (25) or 6 years posttransplantation (36). The
volume of striatal loss in our transplant recipients is similar to what
has been described in other patients with HD (42). Therefore, the
present study failed to demonstrate that embryonic striatal grafts
slowly degenerated the surrounding striatum via either neuritic
outgrowth or graft-derived neurotrophic expression (35). However,
the present study demonstrated that glutamatergic cortico-striatal
projections terminate onto transplanted striatal components of the
grafts. The functional significance of this interaction remains un-
known. Rather than the grafts positively influencing the cortex, the
pathology within the cortex appeared to induce neuronal degen-
eration within the grafts.
Risks associated with neuronal transplantation in this study are
not trivial. Two subjects developed a total of 3 subdural hematomas
that required surgical evacuation, out of a total of 14 hemispheric
transplants (32). This was likely because of cerebrospinal fluid loss
in the setting of brain atrophy leading to the formation of hygromas,
which frequently convert to hematomas. This also led to a targeting
error in the last needle tract in 1 patient. This safety profile differs
dramatically from that observed in transplant recipients with PD,
where the same surgeon (T.B.F.) had no surgically relevant sub-
dural hematomas in 66 consecutive transplant procedures (32).
Based on these results, it is our impression that any future surgical
therapy for HD should intervene in earlier stage patients with less
brain atrophy, and minimize cerebrospinal fluid loss to diminish the
risks of subdural hematomas and targeting errors.
Clinical efficacy has been postulated to require that greater than
50% of grafted volume is composed of P-zones (4, 27, 29) that
contain striatal projection neurons. This anatomical threshold was
achieved in both the present and our previous study (3). However,
clinical efficacy in our patients was mild in magnitude, short-lived,
subject to the limitations of small open-label trials (placebo effect,
as well as potential investigator and subject bias), and did not
prevent the unmitigating and fatal progression of HD. Similar
clinical outcomes have been reported by others (31), despite the fact
that their grafts were derived from a much more nonspecific
dissection of the striata in their first autopsy report (53). These
similar clinical results, using significantly different dissection meth-
ocds with a “subthreshold” quantity of P-zones within grafts, sug-
gests that clinical benefits, if any, are the result of nonspecific effects
of these grafts (19, 20). The combination of an unfavorable risk-
benefit profile, short-term and mild benefits at best, and significant
disease-like graft degeneration makes future trials of fetal-cell
transplantation using these techniques potentially unwarranted.
Therapeutic strategies (with or without neural grafts) aimed at
altering inflammatory or immune responses, host-derived neuro-
toxicity, and neurotrophic support in the brain may prove to be
more promising for the future treatment of HD.

Methods
Donor Tissue Preparation and Transplantation. Methods for tissue preparation,
neuronal transplantation, immunosuppression, location of the transplants, num-
ber of donors per patient, and clinical genetic as well as radiologic evaluation
have been described previously (3, 23, 25, 32). Solid-tissue transplants measuring
0.5 to 1 mm³ were derived from the far lateral portion of the lateral ventricular
eminen to optimize the percentage of tissue of striatal origin (23). All patients
received transplants into both the caudate and putamen derived from 5 to 8
striatal primordia per site. The 3 patients in this series represent patients 1, 3, and
5 from our clinical report (32).

Postmortem Tissue Preparation and Histological Evaluation. Postmortem
samples were 5, 4, and 5 h for patients 1, 3, and 5 respectively. The brains were
bisected, cut serially into ~1 cm slabs in a coronal plane and immersed in Zamboni’s
fixative (25) for 8 days at 4 °C. The brain of patient 5 was collected in the same
fashion as patients 1 and 3, but only one-half of the brain was immersed in
Zamboni’s solution. The other half was immersion-fixed in 4% glutaraldehyde
prepared in cacodylate buffer for electron microscopic examination. Following the
8-day fixation period, brain slabs were then placed in a 20% sucrose in 0.1 M
PB cryoprotectant solution until they sank and were cut frozen into 40-μm thick
sections. Series of adjacent sections were processed for histochemical and immunohis-
tochemical (see Supplemental Methods in SI Text for protocol details) analyses for
the visualization of H&E (brain cytoarchitecture) and various striatal subtypes of
interneurons; NADPH-d (marker for nitric oxide containing striatal interneurons),
ACHE (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; 1:250), CD8 (natural killers and cytotoxic T cells; Serotec; 1:200), 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; 1:2,000) and ubiquitin (Calbiochem; 1:250). Finally, host-derived connectivity was assayed by TH (Pel-Freeze; 1:1,000), synaptophysin (Calbiochem; 1:500), double immunostaining for CB (striatal projection markers of fetal grafts provided by X.J. Li, Emory University; 1:2,000) and vGlut1 (cortico-striatal projections; Sigma; 1:500), and electron microscopy (see Supplemental Methods in SI Text for protocol details). Sections intended for electron microscopy were prepared as above for vGlut1, but for singlet-prepared only using a slightly modified version of previously published protocol (52).

Three-Dimensional Reconstruction, Striatal P-Zone Evaluation and Cell Counts

Three-dimensional reconstruction was performed using the Serial Recon-struction method provided by the Neurolucida software, version 6.0 (Micro- brightness) on Nissl-stained sections. Volumetric evaluation of graft size (Cavali- lier 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 (neurons), H&E (general cell marker), and CB (projection neurons). More details on the assessment of graft volume, estimation of striatal P-zones, and 3-dimensional reconstruction are provided in SI Text.

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**Supplemental Clinical Outcome.** 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 caudate) (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 decreased from 37 postoperatively to 33 and 31 at 10 and 14 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 a complication of end-stage HD, 10.5 years after transplantation.

Patient 5 (M.S.) was noted to have significant cortical and striatal atrophy on the preoperative MRI scan. She had 7-mm thick hematomas postoperatively which increased to 10 cm after the second operation. She tripped at home, hitting her head 2 weeks after her second operation, with a 1- to 2-min loss of consciousness. A CT evaluation demonstrated conversion of her hygromas into subdural hematomas that required surgical drainage bilaterally. The hematomas were 2.5-cm thick on the left side and 1.0-cm thick on the right side at the time of surgery. The CT scan 1 month later demonstrated complete resolution of the subdural hematomas. She never improved back to baseline. Her UHDRS score was 27 before surgery and 31 after drainage of her subdural hematomas. She deteriorated to a score of 39 by 9 months postoperation that remained stable until 2.5 years after surgery, when she began a more rapid deterioration to a score of 53. By 5 years postoperatively, her UHDRS score was 64. MMSE scores declined from 27 at baseline to 16 at 5 years postsurgery. She died at the age of 68 of a cardiorespiratory arrest, secondary to end-stage HD, 9.5 years after surgery.

**SI Methods**

**Histochemistry.** 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 coverslipped with DPX mounting media (Electron Microscopy Science).

For AChE 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 Kernichrot 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.

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 hematoxylin (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.

**Immunohistochemistry.** Before immunostaining procedures, free-floating sections were washed 3 times in PBS 0.1M pH 7.4 and placed in 3% peroxide in 0.1M PB for 30 min at room temperature. For 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. Antibodies were revealed by placing the sections in Tris buffer solution containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.1% of 30% 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 (Serope; 1:250), CD8 (Serope; 1:200), HLA-DR (Serope; 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 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 6H2O, 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%). 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.

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 Durcapan (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 Neurolucida 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 Neurolucida 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.
Fig. S3. 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.)
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Data from ref. 1 and subsequent clinical evaluation.

Table S2. Clinical outcomes

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MMSE, Mini Mental State Evaluation; UHDRS, Unified Huntington’s Disease Rating Scale.
Data from ref. 1 and subsequent clinical evaluation.