Huntington’s disease is a chronic progressive neurodegenerative disease inherited in an autosomal dominant manner. It is characterized pathologically by the loss of intrinsic medium-sized spiny neurons from the basal ganglia with subsequent neuronal loss in various output structures including the thalamus and cerebral cortex. These pathological changes manifest clinically in midlife as a triad of cognitive decline, psychiatric disturbance, and impairment of motor function. This devastating hereditary disease is currently incurable.

In the most widely used animal model of Huntington’s disease, excitotoxic amino acids, such as kainic, ibotenic, or quinolinic acids, are injected directly into the brain. Of these, quinolinic acid lesions provide the most reproducible representation of the striatal pathological condition of Huntington’s disease in terms of anatomical, biochemical, and functional criteria.

Structural damage has been recorded for concentrations of quinolinic acid as low as 12 nM, with biochemical changes, including a significant reduction in substance P, at doses of 75 nM or more. Thus structural and biochemical pathological characteristics have been observed at relatively low doses of quinolinic acid, but much higher doses appear to be necessary to produce functional deficits. Studies in which graft-mediated functional recovery has been demonstrated have involved the administration of higher doses of excitotoxin ranging from 120 to 240 nM. The extent of the damage caused with higher doses makes it difficult to determine any potential graft-mediated effect on structural changes associated with quinolinic acid–induced lesions. Low doses of quinolinic acid may cause damage that represents the earlier stages of the recognized pathological changes associated with Huntington’s disease in humans and provide an opportunity to investigate possible effects of striatal transplantation on both graft development and excitotoxically induced host structural damage.

Clinical trials of intrastriatal transplantation of human embryonic tissue have commenced in France and North America. They are based on an extensive body of scientific research involving graft models based on high-dose...
excitotoxic lesions in which the damage to the host neuropil is most likely to represent severe human disease. Grafts in these lesions have a characteristic heterogeneous appearance composed of striatum-like tissue (patch zones) and nonstriatum-like tissue (nonpatch zones) when visualized using acetylcholinesterase (AChE) and antibodies against the dopamine receptor–related phosphoprotein (DARPP-32). The patch zones contain morphologically distinctive striatal neurons, and dopamine receptors. It is currently unknown to what extent the severity of host striatal damage affects graft development and maturation. There is a lack of experimental scientific evidence of what is known of the human disease on which to base decisions concerning the timing of surgical intervention. Current patient selection criteria are thus inevitably based on clinical grounds alone. Previous and growing experimental evidence of neural transplantation in patients with Parkinson’s disease emphasizes the importance of accurate patient selection; however, it is not known whether grafts will survive any better in the mild or moderately damaged host striatum than in the more severely damaged neuropil.

The aim of this study was therefore to investigate, in a rodent model of Huntington’s disease, the morphological development of striatal transplants in environments in which there were relatively low levels of striatal damage and, also, to determine whether the presence of a graft had any effect on the evolution of quinolinic acid. Such a study may begin to provide an experimental basis that, used in conjunction with clinical criteria, may facilitate patient selection and the timing of surgical intervention in clinical trials of intrastriatal transplantation.

Materials and Methods

Experimental Design

Thirty female Sprague–Dawley rats, each weighing approximately 200 g at the start of the experiment, were housed in group cages in a natural light-dark cycle with free access to food and water. The animals were divided into seven groups: unlesioned plus graft (six rats), 20-nM lesion plus graft (six rats), 40-nM lesion plus graft (six rats), 90-nM lesion plus graft (six rats), 20-nM lesion only (two rats), 40-nM lesion only (two rats), and 90-nM lesion only (two rats). All striatal lesions were induced by administration of quinolinic acid. Seven days postlesioning, the animals received intrastriatal cell suspension grafts derived from the whole ganglionic eminence obtained in rat embryos of 16 days’ gestation. Eight weeks after grafting, the animals were killed and processed for morphological analysis.

Striatal Lesions

All surgical procedures were performed with the animals positioned in a small-animal stereotactic frame under halothane anesthesia and induced lesions were made by 4-minute infusion of a 0.5-μl solution containing 20 nM, 40 nM, or 90 nM of quinolinic acid in 0.1 M phosphate buffer (pH 7.4). Infusions were administered to two sites at the following coordinates (with anterior [A] measured from bregma, lateral [L] from the midline, vertical [V] from the dura, and the incisor bar set 2.5 mm below the interaural line): A: 1.2 mm, L 2.6 mm, V 4.5 mm; and A: 0.0 mm, L 3.4 mm, and V 4.5 mm.

Preparation of Cell Suspensions and Intrastriatal Transplantation

The cell suspension was derived from the whole ganglionic eminence obtained in embryos of 16 days’ gestation (E16, Sprague–Dawley; crown–rump length 15 mm). Time-mated rats were terminally anesthetized and the embryos were removed by cesarean section. The ganglionic eminence was dissected bitemporally from each embryo under aseptic conditions in sterile Hank’s balanced salt solution (HBSS) by using the classic method of dissection. Pieces of tissue from all embryos of a litter were pooled and washed in cold sterile HBSS after which they were incubated in 0.1% bovine trypsin at 37°C for 20 minutes. The trypsin was removed by washing in HBSS, and the tissue was incubated with 0.05% DNAse for another 5 minutes at 37°C. After additional washes in grafting medium 0.6% glucose and 0.05% DNAse in HBSS, the tissue was dissociated by trituration through fire-polished Pasteur pipettes of progressively smaller diameters. After the cells had been counted and viability determined by the trypan blue exclusion test, the cells were centrifuged at 1000 rpm for 5 minutes and resuspended in the final volume of grafting medium. The cells were resuspended to obtain a final concentration of 2.38 × 10^6 cells/ml with a viability of 95.5%.

Two deposits of 1 μl each were made into the right striatum in both lesioned and unlesioned animals at the following coordinates: A: 0.7 mm, L 2.9 mm, V 4 mm, and 4.5 mm.

Tissue Processing

After a fatal injection of sodium pentobarbitone (Euthatal), each rat was perfused transcardially with phosphate-buffered saline (PBS; pH 7.4) for 1 minute, followed by 10% formalin in 0.1 M PBS for 4 minutes. The brains were postfixed in 10% formalin for 24 hours at 4°C and placed in a 25% sucrose solution for 48 hours at 4°C. Sections were cut on a freezing microtome at 60 μm through the striatum and collected in Tris-buffered saline (TBS; pH 7.4).

One in six free-floating sections was processed for dopamine and adenosine regulated phosphoprotein of 32-kD (DARPP-32) immunohistochemical analysis, and a second series was processed for tyrosine hydroxylase (TH) immunohistochemical analysis. After quenching and washing in PBS, the sections were blocked with normal goat serum (30 μg/ml) dissolved in 0.2% Triton X-100 in TBS (TNTBS) for 1 hour at room temperature. The sections were transferred unwashed to the primary antibody solution (TH 1:3000; DARPP-32 1:20000) with 1% normal goat serum in TNTBS for 48 hours at 4°C. Bound antibodies were visualized using an avidin–biotin peroxidase complex system with diaminobenzidine as the chromogen.

Two additional series of mounted sections were also processed for AChE histochemical analysis (modified Koelle method) and for Nissl’s staining with cresyl fast violet.

Volume Determinations and Cell Counting

Morphological evaluation of the volume of the grafts, lesions, and striatum was performed on every sixth section by using a computerized image processing system. The margin of each structure was outlined manually on the video monitor and the surface area was expressed in square millimeters. The volumes were then calculated by summing the section areas and correcting for section thickness and frequency. The grafts were further subdivided into areas of dense AChE, TH, and DARPP-32 staining (patch zones) and areas in which staining was weak or absent (nonpatch zones). Total patch-zone volume was determined from digitized images obtained from camera lucida drawings of each section. Because the lesions and grafts were contained within the head of the neostriatum, all striatal volume measurements were truncated anatomically at the genu of the corpus callosum anteriorly and the decussation of the anterior commissure posteriorly. Striatal loss relative to the contralateral intact side was determined by the difference in striatal volumes between the lesioned and contralateral intact side.

Tyrosine hydroxylase fiber ingrowth to the graft was measured using optical density measurements. Maximal TH ingrowth was defined by the optical density of the dorsomedial striatum on the intact side (thereby avoiding internal capsule fibers), and a background level of staining was defined by the optical density of the corpus callosum.

The total numbers of DARPP-32 cells in the grafts and in both intact and lesioned striatum were counted stereologically.
**Statistical Analysis**

Results were compared by analysis of variance for which commercially available software was used. Neuman–Keuls tests were used to make unbiased post hoc comparisons between lesion groups and Sidak’s test was used to make independent pairwise comparisons between differences in striatal volume, ventricular enlargement, and DARPP-32 cell counts between lesion-only and grafted animals for each dose of excitotoxin.

**Sources of Supplies and Equipment**

The Sprague–Dawley rats were obtained from Charles River UK (Margate, United Kingdom) and placed in a stereotactic frame provided by David Kopf Instruments (Tujunga, CA). Quinolinic acid, HBBS, and DNase were purchased from Sigma (Dorset, United Kingdom) and the bovine trypsin from Worthington (Freehold, NJ). We purchased the avidin–biotin peroxidase complex system (Dako streptavidin ABC kit) from Dako (High Wycombe, Bucks, United Kingdom) and the cell counter from Olympus, Denmark A/S (Albertslund, Denmark). The computerized image processing system was obtained from Seescan Cambridge Systems (Cambridge, United Kingdom). Statistical analysis was performed by using the Genstat Version 5.3 statistical package available from the Numerical Algorithms Group, AFRC (Harpenden, United Kingdom).

**Results**

**Morphology of Lesions and Grafts**

**Lesions.** Photomicrographs of specimens obtained in animals administered 20-nM, 40-nM, or 90-nM striatal lesions without grafts are shown in Fig. 1. The lesions manifested as a loss of neuronal cell bodies in the nissl and DARPP-32 stains and a decline in AChE staining in the neuropil.

In the 20-nM and 40-nM lesions, spared striatum with normal levels of AChE staining was present around the margins of the striatum, medially along the lateral ventricle, and dorsally and ventrolaterally along the external capsule. In the largest lesions there was also loss of the dorsal and medial margins of the striatum. The internal capsule fibers were spared in each lesion group.

**Grafts.** Striatal grafts were present in each lesioned grafted group and in the intact striatum of the unlesioned grafted group (Fig. 2). Grains in each lesion group were of a similar size on gross examination and were located in the striatum except for occasional extensions along the needle tract. Graft tissue outside the striatum was not analyzed further. The surrounding fiber bundles of the host internal capsule were compressed by grafts in each group but were not present in the grafts themselves (Fig. 2B).

Grafts in each group comprised two distinct compartments: the patch zones, which were densely staining for both AChE and DARPP-32, and the nonpatch zones, which lacked dense staining for these markers. There was no obvious difference in the number or size of the patch zones in grafts in each lesion group. Grafts in the lesioned group were visibly smaller with a smaller number of patch zones. Compression of surrounding internal capsule fiber bundles was less obvious (Fig. 2A).

**Volumetric Analysis of the Host Lesions**

**Striatal Volume.** There was no difference in striatal volumes on the intact side of animals that received a striatal implant into the contralateral striatum, regardless of whether they received lesions and/or grafts. However, there were differences between groups on the side of the lesion in both the animals receiving and not receiving grafts (Fig. 3A). Animals with lesions only showed a progressive decline in striatal volume with increasing concentration of excitotoxin compared with the contralateral unlesioned side (lesion × side F(3, 29) = 543.77; p < 0.001). The presence of a graft reduced the volume of striatal tissue loss (lesion × side × graft F(3, 29) = 32.47; p < 0.001). This effect was more pronounced at excitotoxic doses of 20 nM and 40 nM (Sidak’s post hoc analysis t = 4 and 4.1, respectively; both p < 0.01).

**The DARPP-32 Cell Count of the Host.** There was no reduction in the DARPP-32 cell count in the striatum on the intact side of unlesioned animals that received a striatal implant into the contralateral striatum. Grafting into the intact striatum resulted in a small, but still significant, loss of DARPP-32–positive neurons from the grafted side compared with the intact side. There was a progressive decline in the number of DARPP-32–positive neurons in
the striatum on the lesioned side with an increasing dose of excitotoxin (Fig. 3B; $F_{3,22} = 119.39; p < 0.001$). This effect was not influenced by the presence of a graft ($F_{1,22} = 2.98$; not significant).

**Volumetric Analysis of Striatal Transplants**

**Graft Volumes.** The largest grafts were seen in the 20-nM and 40-nM lesion groups and the smallest in the intact animals (Fig. 4A; $F_{3,23} = 45.46; p < 0.001$). The grafts in the unlesioned group were significantly smaller than those in any lesioned group (Neuman–Keuls post hoc analysis $t_{23} = 13.35; p < 0.01$). The grafts in the 90-nM lesion were significantly smaller than grafts in the other lesioned groups (Neuman–Keuls post hoc analysis $t_{23} = 5.84; p < 0.01$).

The patch zone volume varied between graft groups in parallel with differences in overall graft volume (Fig. 4B; $F_{3,23} = 29.01; p < 0.001$), but the proportion of the graft that comprised the AChE-positive patch zone did not differ between groups (overall mean $22.9 \pm 5.3\%$; $F_{3,23} = 0.42$; not significant).

**Tyrosine Hydroxylase Fiber Ingrowth.** A rich plexus of TH-positive fibers was seen growing into all grafts in both lesioned and intact striatum alike. The innervation was patchy and closely followed the distribution of patch zones seen in adjacent sections stained for AChE. The density of TH fiber growth into the patch zones of the grafts developing in each lesion group did not differ significantly ($F_{3,23} = 0.739$; not significant).

**The DARPP-32 Cell Content of Grafts.** The DARPP-32 cell counts of the grafts varied between groups (Fig. 4C; $F_{3,23} = 61.24; p < 0.001$). The grafts in the intact striatum contained significantly fewer DARPP-32 cells than grafts.
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in the other groups (Neuman–Keuls post hoc analysis $t_{3,23} = 13.55; p < 0.01$). The number of DARPP-32 cells in the grafts placed in the 20-nM lesions was significantly lower than the number of grafts in the other lesion groups, which did not differ (Neuman–Keuls post hoc analysis $t_{3,23} = 3.28; p < 0.05$).

The density of DARPP-32–positive cells within the grafts did not differ between the intact and 20-nM and 40-nM lesion groups, reflecting the progressive increase in the DARPP-32 content of the grafts. The density rose significantly in the smaller grafts in the 90-nM lesion group, which contained numbers of DARPP-32 cells similar to the larger grafts in the 20-nM and 40-nM lesion groups (Fig. 4D; $F_{3,23} = 8.52; p < 0.001$. Neuman–Keuls post hoc analysis $t_{3,23} = 3.66; p < 0.05$).

**Discussion**

The results indicate that striatal grafts survive and grow better when implanted into lesioned rather than intact striatum, as measured by both the volume and numbers of medium-sized spiny neurons within the graft. Only a modest lesion is necessary to produce this full survival-promoting effect; indeed, by some measures (such as graft volume), grafts survive less well when the lesion is more extensive. Furthermore, the presence of a graft reduced the extent of striatal atrophy induced by the lesions, but this effect is not due to differences in the numbers of surviving neurons per se.

**Striatal Lesions**

Loss of striatal neuropil in the anatomically defined area between the genu of the corpus callosum and the decussation of the anterior commissure occurred with all doses of excitotoxin. As expected, the lowest dose produced the smallest lesion with minimal loss of AChE staining. Increasing the concentration of the toxin increased monotonically the volume of the striatal lesion, which was consistent with previous work. This reflects a primary loss of the functionally important γ-aminobutyric acid (GABA)ergic medium-sized spiny neurons, which were identified in this study by DARPP-32 immunoreactivity. Even at the lowest dose, there was significant DARPP-32 cell loss (56.6%) at a point at which striatal collapse was relatively mild. The cell loss observed in these experiments has been reported at doses of quinolinic acid as low as 12 nM, with the GABAergic medium-sized spiny neurons being most vulnerable at these lower doses. The present loss of DARPP-32 cells substantiates this observation and further emphasizes the vulnerability of these striatal medium-sized spiny GABAergic cells. These observations suggest that each concentration of excitotoxin produced a lesion environment with characteristics of increasingly extensive damage within which...
to evaluate the impact of underlying striatal degeneration on the growth and maturation of intrastriatal grafts. The grafts were implanted 7 days after making the striatal lesions. In contrast with the slowly progressing nature of the human disease, quinolinic acid toxicity is acute. The relatively short half-life of the toxin of less than 1 hour and the subsequent death of striatal neurons within 1 to 5 days strongly suggests that any neuronal damage caused by the initial excitotoxic insult is likely to be complete by the time of implantation. This conclusion is supported by our own findings that good graft survival is obtained with implantation as few as 6 hours after stereotactic injection of excitotoxin.

**Lesion Effects on Graft Survival**

Grafts in the intact striatum were visibly smaller, with a smaller patch zone compartment and relatively larger non-patch-zone compartment. In contrast, grafts in the remaining three groups showed only minor differences. In particular, although the total graft volumes did not differ in the various lesioned groups, the patch-zone compartment was larger in the grafts in moderate lesions. These results suggest that the host environment has a significant effect on their maturation and morphology. Good graft survival in the presence of lesser degrees of striatal damage suggests that better graft survival is not simply a function of the available space.

Functional recovery in rats with striatal grafts has been found to correlate best not with total graft volume but with the development of striatum-like patch zones within the grafts. Although all grafts in the present study showed differentiation of detectable patch zones, the volumes were smaller than has been reported in many studies. This observation is most likely a consequence of the interaction of donor age and striatal dissection.

The influence of the environment on graft volume and morphology raises the possibility that the striatal cell population in the donor tissue may expand differentially to compensate for the greater cell loss in the more severe lesions. In support of this hypothesis it was noted that the DARPP-32 cell content of the grafts developing in intact striata was lower than in all other graft groups. The cell content in grafts growing in mild lesions was lower than in grafts in moderate and severe lesions, which did not differ. This observation suggests that there may be increased expression of striatal phenotype or, possibly, striatal cell replication in response to cues in the host environment. Although we do not know what proportion of cells in the graft constitute the DARPP-32-positive subpopulation or their precursors, the mean DARPP-32 cell content of the grafts (13.5 ± 1 × 10⁶) constitutes only 0.28% of the donor cells originally implanted. Moreover, the number of surviving DARPP-32 cells represents less than 10% of the cell loss from the host (overall mean 22.3 ± 2 × 10⁶). Therefore, although striatal grafts can compensate for cell loss in the host, this compensation is small relative to the full extent of potential cell loss. The donor tissue used in these experiments was from the upper end of the range of effective donor ages, which may affect the ability of the DARPP-32 cell population to expand significantly in vivo.

**Graft Effects on Lesion Development**

Striatal atrophy was significantly reduced by the presence of a graft in the rats with smaller lesions compared with the rats with the largest lesions, in which this effect was less pronounced. This suggests that striatal grafts are able to reduce the extent of secondary neuronal loss, dependent on the severity of the primary injury. Previous studies have shown that intrastriatal striatal transplants can significantly attenuate excitotoxic damage caused by a subsequent quinolinic acid insult 7 days after transplantation. This protective effect has been shown to have a nonspecific host-mediated component in addition to the effect mediated by the transplant itself. However, the host-mediated protective effect had a relatively transient time course (7 days) compared with the graft effect, which persisted up to 4 weeks prior to excitotoxic injury.

However, these studies have not addressed the influence of a graft on the secondary consequences of striatal damage that has already occurred, as is the case in Huntington’s disease patients in clinical trials of striatal transplantation.

In the present study, striatal grafts reduced striatal atrophy. Although the grafts reduced the collapse in total striatal volume, they did not influence the actual loss of host striatal DARPP-32 cells. Because the cell loss is an acute, excitotoxicity-mediated event, striatal cell death would be expected to be complete at the time of transplantation. The subsequent atrophy may be attributable to secondary changes in intrinsic glia and retraction of afferent projections, as has been demonstrated in the cortex. There is no evidence of increased GFAP-positive reactive gliosis associated with striatal grafts. Rather, we have shown that the grafts receive afferent host TH fibers, and other studies suggest that there is additional ingrowth of cortical, thalamic, and raphe inputs. This indicates that grafts provide trophic and target-derived support of afferent fiber systems, which would otherwise retract, and form the basis for protection against atrophy of the neuropil. The observation that these effects were not present in the most severe lesion suggests that, once the damage has reached a critical level, any graft-mediated anatomical effect becomes attenuated.

**Clinical Implications**

These results have important implications for the timing of neurosurgical intervention. Our observations suggest that the ability of intrastriatal transplants to ameliorate structural damage in patients with Huntington’s disease may be critically affected by the extent of neural damage present at the time of implantation. Although striatal grafts do not survive well in a completely intact host environment, relatively mild striatal damage is sufficient to sustain good graft survival. The potential for improved graft development and amelioration of structural damage suggests that transplantation at an early stage of the disease progression is certainly not detrimental and may be beneficial to graft survival. Their effects on functional integration are still to be explored in humans. Nevertheless, pathological changes of the caudate and putamen (as determined by computerized tomography, magnetic resonance imaging, and positron emission tomography scan-
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ning) will provide important information to be considered in conjunction with clinical criteria in patient selection, as is advised in the current protocols for clinical trials.  

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References


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