

Towards a Protocol for the Preparation and Delivery of Striatal Tissue for Clinical Trials of Transplantation in Huntington's Disease

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There is a growing body of scientific evidence contributing to the development of clinical transplantation programs in patients with Huntington's disease. Phase I clinical trials have already commenced in France and North America and are starting in the near future in Sweden and the UK. Protocols for patient selection, surgical implantation, and pre- and postoperative follow-up are well defined. However, considerable variability exists with respect to the harvesting, preparation, and timing of implantation of the donor material. In this article we review the scientific evidence on which a rational protocol for donor tissue preparation and delivery may be based. Strategies aimed at minimizing the variability of tissue preparation should reduce the variability of functional outcome of striatal transplantation observed in animal models of Huntington's disease.

Key words: Huntington's disease; Donor tissue preparation; Clinical trial protocol

INTRODUCTION

Huntington's disease (HD) is a chronic progressive neurodegenerative disease inherited in an autosomal dominant manner and expressed when a CAG trinucleotide repeat in the gene IT-15 is expanded beyond a critical number of repeats (>36) (86,88). It is characterized pathologically by loss of intrinsic medium spiny neurons from the basal ganglia with subsequent neuronal loss in various output structures, including the thalamus and cerebral cortex (34,35,75,76,90). 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 and the possible use of intrastriatal implants of fetal striatal tissue is being actively investigated as a possible therapeutic option (27,38,47,68,82).

The harvest and preparation of embryonic neural tissue constitutes the most critical phase of any neural transplant procedure. Two recent case reports of fatalities due to the implantation of inappropriate donor tissue in patients with Parkinson's disease (25,48) together with the general failure of adrenal medullary transplants [for review see (45)] emphasizes this fact. Another important factor is the need for standardized patient assessment before and after transplantation in both PD and HD (43,73). While the issue of patient assessment has been

addressed and is constantly being evaluated and modified, similar well-defined protocols for the preparation and stereotactic implantation of the neural tissue have not been so well developed.

FACTORS INFLUENCING THE DEVELOPMENT OF STRIATAL GRAFTS

A typical striatal graft is illustrated in Figure 1. Little is known about the survival and development of striatal neurons in grafts of embryonic ganglionic eminence. Approximately 60–70% of neurons surviving in ganglionic eminence grafts are of nonstriatal origin [for review see (103)]. The yield of dopamine and adenosine regulated phosphoprotein of 32 kDa (DARPP-32) cells in grafts of lateral ganglionic eminence (LGE) tissue has been estimated to be between 10% and 40% depending on the method of computation employed (57). One such method involved determining the proportion of striatal tissue within the graft. Knowing the number of cells implanted, an estimate of the number of striatal neurons was made by multiplying the proportion of striatum-like tissue within the graft and the number of cells implanted. Thus, implants of approximately 470×10^3 cells, which gave rise to 60% P-zones, were expected to contain around 280×10^3 striatal cells. The final number of DARPP-32 cells was 115×10^3 giving a survival of

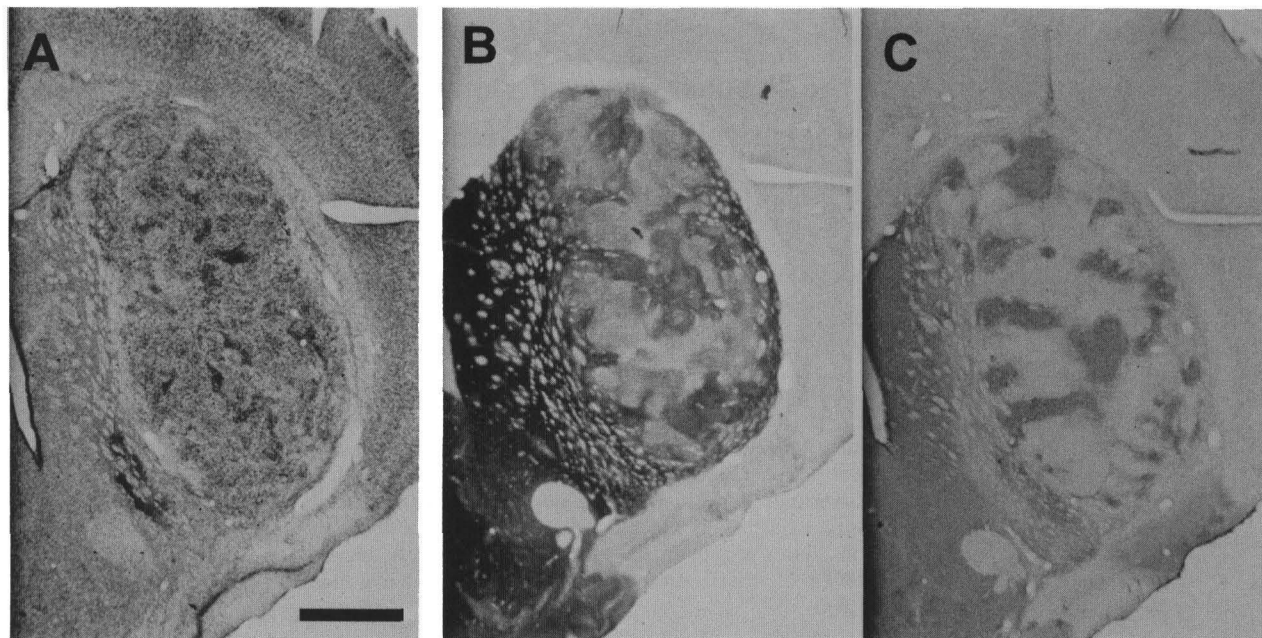


Figure 1. A typical striatal graft. (A) Nissl staining, (B) AChE staining, and (C) DARPP-32 staining. Striatum-like tissue is present as darker staining patches against a paler background of nonstriatal tissue. Scale bar: 1 mm.

approximately 40%. However, this method is based on the assumptions that all grafted cells survive equally and no subpopulation of grafted cells undergoes expansion following transplantation.

Amount of Grafted Tissue

The effect of simply increasing the number of LGE cells implanted into the previously lesioned striatum has been investigated. Studies *in vitro* indicated that increasing plating density gave rise to a disproportionate rise in the number of DARPP-32 surviving for 7 days in culture (59). Previous reports of high proportions of striatal tissue within LGE-derived grafts had used between 100,000 and 200,000 viable cells per microliter (64,65). These results suggested that increasing the quantity of LGE tissue implanted could result in an improved survival of DARPP-32 cells contained within the grafts. Our results (94,95) showed that increasing the number of LGE cells implanted increased the total volume of the grafts (Fig. 2). However, the proportion of the striatal component, after an initial sharp increase, remained relatively constant at approximately 35% with single implants of 500,000 cells or more. Analysis of the number of neurons within the grafts showed a significant linear increase with increasing volume of LGE donor tissue. The increase in DARPP-32 cells was much slower and less significant. Clearly, increasing the quantity of donor tissue has only a limited effect on the striatal population

of these grafts. If the computational method of Nakao described above is employed with respect to this study, approximately 23% of implanted striatal neurons survive in implants of 200,000 cells, 40% survive in implants of 500,000 cells, 30% in implants of 1 million cells, and 34% in implants of 2 million cells.

The low proportion of striatum-like tissue within the grafts in different studies may be a result of variability in dissection of the LGE. It is known that LGE tissue from E14 donors contains a subpopulation of striatal interneurons that express somatostatin (63). Expansion of this or other populations of cells within the LGE tissue may explain the relatively small quantity of striatal tissue in these grafts. Interactions between LGE and medial ganglionic eminence (MGE)-derived cells have not been investigated to date. It is possible that cells derived from MGE tissue may regulate the development of the various cell populations contained within LGE tissue. Alternatively, expansion of cells in the donor tissue that do not express a mature neuronal phenotype may also be involved.

In further experiments to improve the yield of striatal neurons within striatal grafts we investigated the role of the carrier medium used in tissue implantation (96) and the effect of trituration by comparing grafts of cell suspensions with grafts of solid fragments of striatal anlage (91).

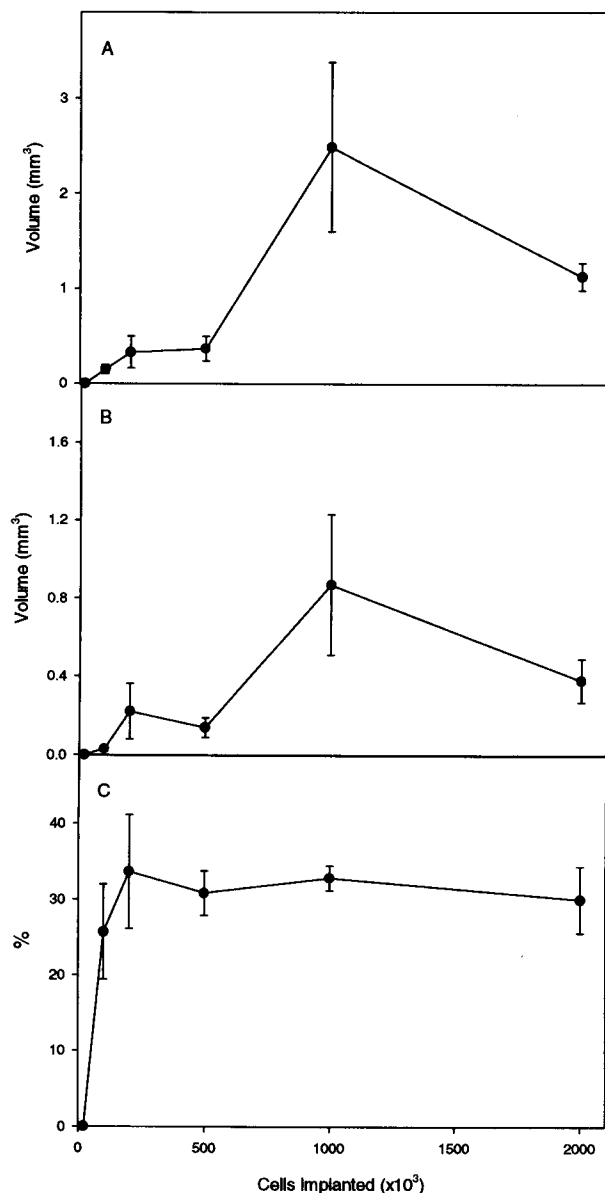


Figure 2. Graphic representation of the effect of increasing the amount of tissue implanted on the total volume of the resultant graft (A), the volume of striatum-like tissue within the graft (B), and the proportion of striatum-like tissue contained in the graft (C).

Preparation and Grafting Medium

The proportions of DARPP-32-positive striatal neurons in cultures of LGE tissue prepared in the different media were similar at approximately 1%. This result was consistent with earlier studies (56) and with our previous results (97). The proportion of striatum-like tissue in the grafts was approximately 60% in each group. However, significant differences in the number of surviving

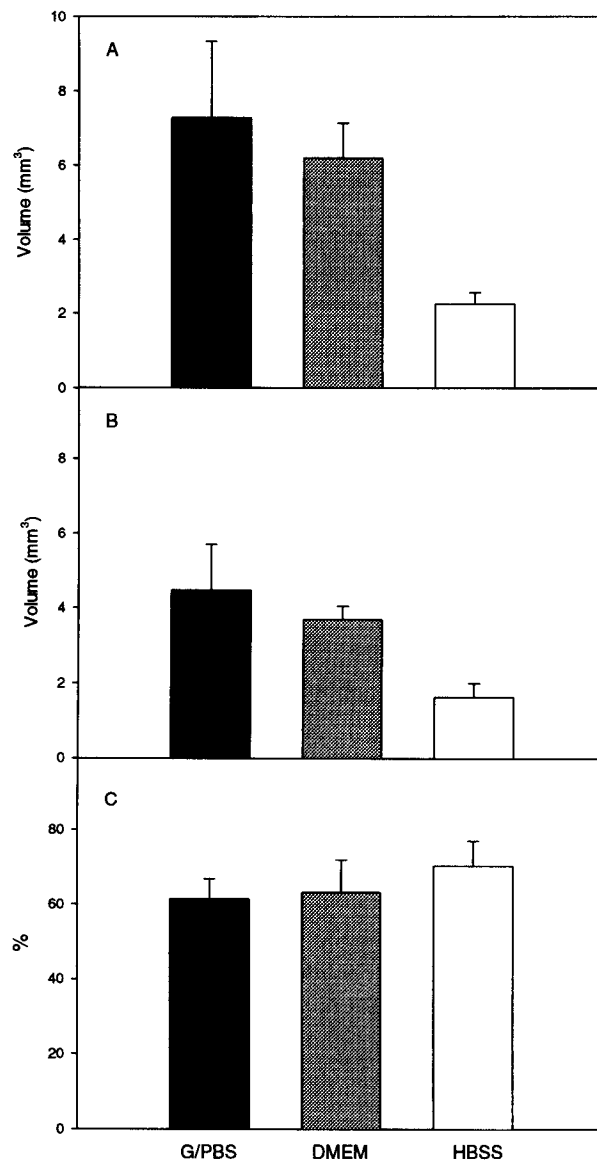


Figure 3. The effect of different types of media used for preparation and delivery of suspensions of embryonic striatal tissue. Media used: G/PBS (glucose/phosphate-buffered saline); DMEM (Dulbecco's modified eagle medium); HBSS (Hanks balanced salt solution). (A) Effect on overall graft volume, (B) effects on volume of striatum-like tissue within the graft, (C) effect on the proportion of striatum-like tissue within the graft.

DARPP-32 striatal cells were observed (Fig. 3). Glucose/phosphate-buffered saline (PBS) provided the highest yield of striatal neurons and Hanks balanced salt solution (HBSS), which is rich in potassium, the lowest where only 11% of the number of DARPP-32 cells present in the glucose/PBS grafts had survived. Applying the computational method described above (57), approximately 29% of implanted striatal neurons survived in the

glucose/PBS group compared to 5% in the HBSS group.

Tissue Dissociation

The comparison of striatal graft morphology between grafts derived from trypsinized cell suspensions and those from pieces of striatal tissue showed a minimal advantage of the former in terms of the number of striatal DARPP-32 cells present within the grafts (Fig. 4) (91). Again using the computational method of Nakao 33% and 30% of implanted striatal neurons survived in

the grafts of trypsinized cell suspensions and tissue pieces, respectively, assuming the same number of cells were implanted (quantified retrospectively).

These three studies suggest that little advantage is gained in the number of surviving striatal DARPP-32 neurons by implanting more than 500,000 cells (equivalent to approximately one LGE). Tissue pieces and properly prepared cell suspensions give rise to similar yields of surviving striatal neurons but the use of simple grafting media (e.g., glucose/PBS) appears to offer significant advantage in terms of the yield of surviving striatal neurons.

Taken together, these results have important clinical implications. Knowledge about the yield of grafted neurons in animal experiments is important for the development of clinical transplantation therapies, because it allows prediction of the graft size from the amount of donor tissue implanted.

FACTORS INFLUENCING ANATOMICAL AND FUNCTIONAL CONNECTIVITY OF INTRASTRIATAL STRIATAL GRAFTS

Striatal grafts receive afferent input from the ipsilateral frontoparietal neocortex, intralaminar thalamic nuclei, basolateral amygdala, substantia nigra and dorsal raphe (99,101). They also develop efferent projections to the host globus pallidus, where they develop appropriate synaptic connections (100,102).

Dopaminergic projections to striatal grafts are more extensive compared to those from the cortex, thalamus, or amygdala (101). These results together with studies of cortical, spinal cord, hippocampal, cerebellar, and thalamic grafts suggest a greater growth capacity for the more diffusely projecting aminergic systems compared to the more rigidly organized point-to-point projections (62,74,84,85,87).

Afferent and Efferent Projections

Our results indicate little difference between trypsinized cell suspension grafts and grafts of tissue fragments in terms of their dopaminergic innervation (91). Similarly, donor age did not substantially influence dopaminergic input to WGE grafts. Grafts of E16 LGE received more tyrosine hydroxylase (TH)-positive fibers consistent with the larger volume of striatal tissue in this group. Dopaminergic innervation of MGE grafts was limited, particularly with grafts derived from the younger donor group (92).

Efferent projections to the ipsilateral host globus pallidus were greater in trypsinized cell suspension grafts compared to tissue fragments. These results suggest that the looser association of cells within implants of cell suspensions may facilitate the elaboration of efferent outgrowth. Suspensions from younger donors had slightly

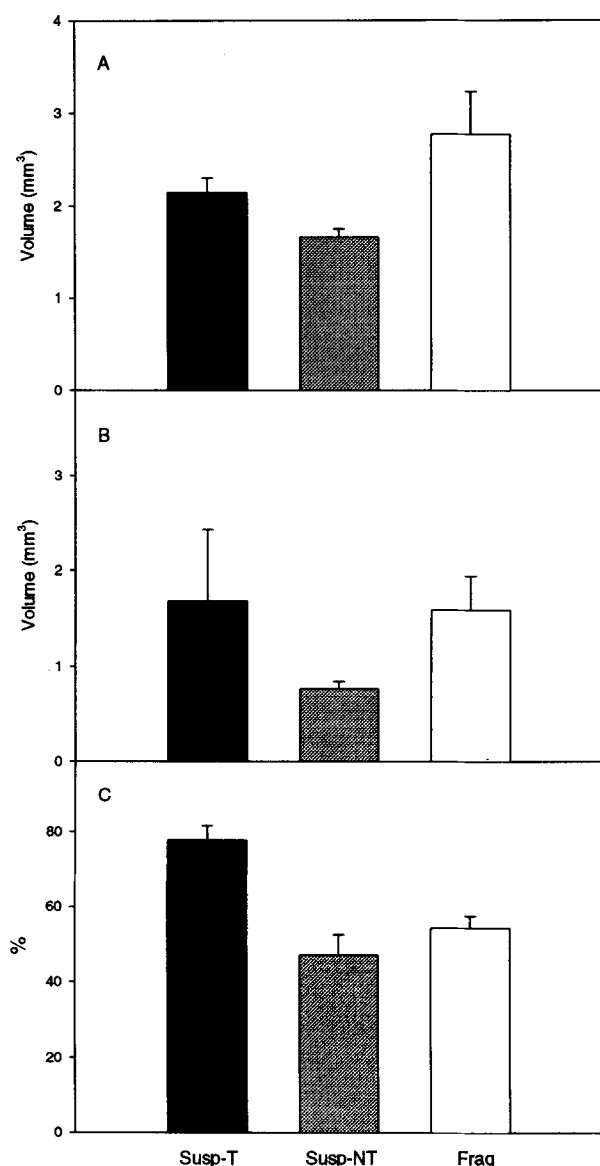


Figure 4. The effect of different methods of tissue dissociation on the graft volume (A), volume of striatum-like tissue in the graft (B), and proportion of striatum-like tissue within the graft (C). Susp-T: trypsinized cell suspension; Susp-NT: non-trypsinized cell suspension; Frag: tissue fragments.

more projections than their older counterparts. E14 LGE grafts had the most, reflecting perhaps the higher density of striatal projection neurons within these implants.

Functional Connectivity

The afferent and efferent connections of homotopic striatal transplants have been well characterized [for review see (103)]. However, the degree to which functional afferent connections are capable of modulating graft-derived striatal projection neurons remains unclear. Electrophysiological studies have shown responses in striatal grafts after stimulation of the frontoparietal cortex (77,104). More recently, electrical stimulation of the cortex has been shown to induce expression of the immediate early gene *c-fos* in striatal DARPP-32-positive neurons via both glutamatergic and dopaminergic receptors (40,46). Activation of *c-fos* indicates changes in neuronal activity and the expression of the fos nuclear protein is believed to be a link between short-term extracellular signaling events and longer term changes in genomic activity (54,78). In our study (92), the induction of fos expression by electrical stimulation of the frontoparietal cortex was used to identify corticostriatal postsynaptic responses within intra-striatal striatal grafts. Fos expression was identified bilaterally in the host striatum and in striatal grafts. Most of the immunopositive cells within the grafts were distributed in patches that corresponded to areas of AChE and DARPP-32 positivity. Many of the fos-positive cells were co-labeled with the retrograde tracer Fluorogold, which was used to identify graft-derived striatal projections to the globus pallidus. The relatively limited number of fos-positive cells observed in this study is consistent with the loss of large numbers of cortical projections to the grafts (99). However, fos expression has been demonstrated in grafts implanted into long-term lesioned striatum, indicating residual host corticostriatal fibers are capable of establishing functional synaptic contact (40). No fos expression was observed in our study in grafts derived from E16 donor tissue. Only grafts of WGE or LGE from E14 donors expressed *c-fos*.

Taken together, these studies confirm the ability of intra-striatal striatal grafts to establish appropriate functional connections with the host neuropil and suggest that this ability is donor age dependent.

EFFECT OF EMBRYONIC DONOR AGE AND DISSECTION: DONOR TISSUE COMPOSITION

The basal ganglia develop from the ganglionic eminence in the developing forebrain. The lateral part gives rise predominantly to striatal medium spiny neurons while the medial part gives rise to striatal interneurons, pallidal, amygdalar, and cortical structures (8,13,26,32).

Striatal neurogenesis occurs in two phases: at 12 days gestation (E12) proliferation begins in the ventricular zone of the forebrain vesicle followed by proliferation from a subventricular zone from 14 days gestation (E14) (2,3,14,83). The lateral part of the ganglionic eminence is the site of genesis of DARPP-32-positive medium spiny striatal GABAergic projection neurons (26). Neurogenesis occurs from 14 days gestation (E14) in the rat and the LGE constitutes a rich source of these striatal neurons (13,64). These are the principle neurons lost in Huntington's disease (90). Hence, the goal of striatal transplantation is to promote functional recovery by replacing these lost cells.

It is now widely accepted that the optimal donor stage for transplanting cells is during embryonic development when the cell type in question is in the differentiation and migration phase before axonal development has begun (7). Therefore, implants of LGE tissue from younger donors were tried and shown to increase the proportion of striatum-like tissue within the grafts, as defined by AChE and DARPP-32 staining, from approximately 30–50%, obtained with standard WGE grafts, to 70–90% (64,65). However, the resultant volume of the grafts and their striatal component was small and no comparison with grafts of whole ganglionic eminence was made in these studies.

We have investigated the role of donor age and embryonic dissection on the yield of striatal DARPP-32 cells in the donor tissue and tried to characterize the cellular composition of the donor tissue with respect to these parameters in vitro (97). The highest proportion of DARPP-32 neurons was obtained from the LGE of E14 and E15 donors. There was no difference between whole ganglionic eminence (WGE) and LGE for E16 embryos. DARPP-32 cell numbers were small, accounting for less than 1% of the cell population at day 2 in culture. This reflects the low plating density (25,000 in 30 μ l) that was used to facilitate quantification of the total cell population. These results were consistent with other reports of in vitro studies (58), and reveal a significant discrepancy with the mature striatum where medium spiny projection neurons constitute approximately 95% of the cell population (9). Genesis of DARPP-32-positive neurons continues from 14 days gestation to birth (26). Hence, the difference may be due to an unfavorable environment for the genesis and phenotypic expression of DARPP-32 cells in vitro. Alternatively, cell death may exceed the rate of neurogenesis or the lack of afferent and efferent connections may compromise the survival of DARPP-32-positive neurons. Little is currently known of the factors affecting the expression of DARPP-32. Co-cultures with nigral tissue have no significant effect (24). Increasing plating density has been shown to increase the number of surviving

DARPP-32 neurons *in vitro* in a disproportionate manner. For example, in one study a twofold increase in plating density produced a 16-fold increase in the number of DARPP-32-positive neurons (59).

The glial population of the donor tissue in our study was greatest from older E16 embryos but was still only a small fraction, less than 5%, of the total cell population. Approximately 60% of the cells were neuronal, expressing β -tubulin III, but were not characterized further in this study. Approximately 40% of the donor cell population did not express a phenotype that corresponded to mature neurons, astrocytes, or oligodendrocytes, and this major component of striatal cultures remains to be better characterized.

Thus, while LGE dissection in younger donors produces a higher yield of striatal projection neurons, they still constitute only a small fraction of the cells in the donor tissue.

EFFECT OF EMBRYONIC DONOR AGE AND DISSECTION: GRAFT MORPHOLOGY

The interaction between donor age and embryonic dissection on the yield of striatal DARPP-32-positive cells *in vitro* (97) suggested the potential for a similar relationship *in vivo*. Continuing controversy over the relationship between donor age, embryonic dissection, and the amount of striatal tissue within striatal grafts (11,69) emphasized the need for further studies. The use of LGE tissue alone rather than standard WGE tissue was found to increase the proportion of striatal tissue in the resulting grafts (13,64,65). The ability of striatal progenitors to develop phenotypic characteristics of striatal projection neurons appears to be confined to those derived from the LGE (63,64). It is independent of induction cues from the homotopic host environment and thus is an intrinsic property of the LGE striatal precursor population (64). The finding that striatal progenitors from E12.5 LGE were capable of elaborating striatal projection neuron phenotype (63) at a time when striatal neurogenesis has only just begun (2) suggests that these cells retain their fate specification through several cell divisions. Such phenotypic memory has also been described for the laminar fate of cortical progenitors (51). These studies further confirm the role of the LGE as the principle source of striatal projection neurons from a relatively early stage of striatal neurogenesis.

When the technique of selective dissection was applied in transplanting human striatal tissue into rats the maturation and development of the grafted tissue was found to be protracted (5,55,72). It was unclear if this was a reflection of donor–host discordance, the heterogeneity of the age of the donor material, or an intrinsic problem with transplants of human striatal tissue *per se* (27,31,55). Furthermore, one study (31) identified better

survival in grafts of combined LGE and MGE tissue. This raised the possibility that the MGE may have a positive effect on the survival and development of striatal grafts.

Striatal cholinergic interneurons are the earliest striatal neurons to develop from 12 days gestation (E12) onwards (70). Cholinergic neurons have been demonstrated in grafts of MGE but not LGE tissue, suggesting that striatal cholinergic neurons arise predominantly from MGE (63). We have also identified AChE expression within MGE grafts that do not express DARPP-32 or TH positivity (92). In our study the distribution of the cholinergic neurons differed between donor ages. In E14 grafts there was a more even spread throughout the graft compared to a more patchy, peripheral organization in the E16 MGE grafts. High levels of AChE were also expressed in both LGE and WGE grafts of all donor ages. This was expected because the enzyme is expressed by cholinergic and somatostatin-containing striatal interneurons, as well as dopaminergic axons and terminals from the substantia nigra (6,12,44). Thus, AChE expression alone in LGE tissue may not be a reliable and specific marker of cholinergic neurons.

The volume of striatum-like tissue in our study (92) was significantly greater in WGE grafts compared to LGE grafts using tissue derived from E14 donors (Fig. 5B). In contrast, the proportion of striatal tissue in the grafts was greatest in the LGE grafts (Fig. 5C). Thus, more striatal tissue is obtained when using WGE compared to LGE from younger donors. This result suggests that the presence of MGE potentiates the maturation of striatal projection neuron progenitors even though it is probably not a source of these cells. These data are consistent with the results of our earlier *in vitro* comparison of WGE- and LGE-derived DARPP-32-positive cells (97).

When grafts derived from older (E16) donors were compared there was no difference in the volume or proportion of striatum-like tissue within the grafts (Fig. 5). This result is also consistent with the *in vitro* data and suggests that the beneficial effect of the presence of MGE tissue on the maturation of striatal progenitors is donor age dependent.

It remains to be resolved whether the initiation of LGE progenitor specification results from cell intrinsic mechanisms or early inductive signals specified by developmental control genes and what, if any, role is played by MGE-derived tissue. Evidence from other studies suggests that MGE is the origin of striatal cholinergic neurons, which migrate into the developing LGE-derived striatum (63). The ontogeny of these cells between E12.5 and E15.5 is consistent with our results (92). It is not known whether striatal cholinergic interneurons have an inductive effect on striatal projection

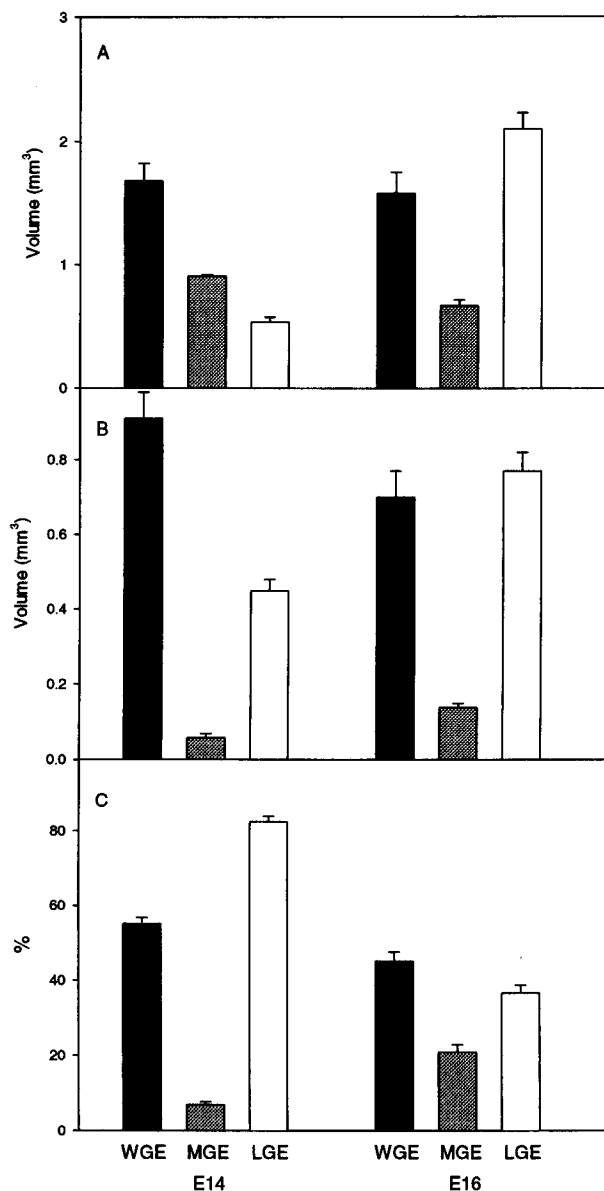


Figure 5. Effect of embryonic donor age and dissection on graft volume (A), volume of striatum-like tissue in the graft (B), and proportion of striatum-like tissue within the graft (C). WGE: whole ganglionic eminence; MGE: medial ganglionic eminence; LGE: lateral ganglionic eminence. E: embryonic day.

neuron progenitors but their neurogenesis and migration coincides with the periods of neurogenesis within the striatal anlage (2).

The number of striatal projection neurons expressing DARPP-32 was also significantly greater in E14 WGE-derived grafts in our study compared to E14 LGE grafts (92). This effect was not observed with E16-derived grafts where slightly more striatal projection neurons were found in the LGE grafts. These results further em-

phasize an as yet unidentified role for the MGE in the development of the striatal component of striatal transplants from younger donors.

Applying a method of calculating the yield of striatal neurons described previously (57), we found that approximately 29% of implanted striatal precursor cells survived in E14 LGE grafts compared to 63% in E14 WGE grafts, 43% in E16 WGE, and 104% in E16 LGE grafts. While the actual values must be interpreted with caution for the reasons outlined earlier in this article, they strongly suggest that isolating LGE tissue at a critical phase of striatal neurogenesis might actually compromise the yield of striatal projection neurons within the striatal grafts.

EFFECT OF EMBRYONIC DONOR AGE AND DISSECTION: BEHAVIORAL RECOVERY

Grafts of embryonic neurons are capable of producing behavioral effects via several putative mechanisms including diffuse release of neurochemicals, neurotrophic support, and the reestablishment of neural circuits (21). If neural transplantation is to be a rational rather than simply an empirical surgical therapeutic option it is important to determine whether a graft-derived functional effect is influenced by the amount of surviving striatal neurons within the graft.

Striatal grafts can ameliorate several behavioral abnormalities induced by striatal excitotoxic lesions. Drug-induced motor asymmetry and spontaneous locomotor activity are examples of simple motor disturbances, and deficits of skilled forelimb use are examples of more complex motor disturbances. More complex cognitive deficits in choice reaction time tasks and delayed alternation have also been attenuated [for review see (8)]. Despite the extensive number of reports demonstrating behavioral recovery mediated by striatal grafts, relatively few studies have explored the relationship between these effects and graft morphology. High neuronal density in striatal grafts has been associated with improvement in spontaneous hyperactivity (15). Small grafts have been shown to reduce lesion-induced spontaneous locomotor hyperactivity but not drug-induced motor asymmetry, which required larger grafts from multiple deposits (15,16,80). However, the grafts producing these effects contained relatively small amounts of striatal tissue as defined by AChE and D₁ and D₂ receptor labeling (15,61,80). More recently, grafts derived from single deposits of WGE cell suspension have ameliorated apomorphine-induced rotational bias (29). These grafts contained approximately 30–40% P-zones.

While amelioration of simple motor deficits may be mediated by local graft–host interactions, the recovery of more complex motor deficits has been hypothesized

to require at least partial restitution of appropriate striatal circuits (20). Indeed, skilled paw use depends on the integrity of intrinsic striatal neurons, the sensorimotor cortex, and dopaminergic innervation of the striatum (98). Striatal grafts have been shown to be capable of ameliorating retrieval deficits in several studies (22,30,53,57) but not in others (29). Correlation with the amount of striatal tissue within the grafts has been described in two reports (30,57). We have also experienced variable effects of striatal grafts on simple motoric tests. Thus, little difference was observed between grafts of cell suspensions and solid grafts on amphetamine-induced rotational bias where little functional recovery was observed in one study (91). However, modest recovery was observed in contralateral paw reaching deficits in grafts of tissue pieces but not cell suspensions 3 months after grafting. This effect was lost on retesting at 6 months postgraft. It is possible that an element of relearning is necessary in motoric aspects of graft-mediated functional recovery similar to those described for graft-mediated improvements of more complex cognitive behavioral parameters (50). Attenuation of drug-induced motor asymmetry and improved paw reaching in grafts was observed in another study (92). Here graft-derived functional improvement was observed from younger (E14) rather than older (E16) donors, a result that is consistent with previous reports (30). Drug-induced rotational deficits were reduced by grafts of E14 LGE tissue. These grafts had the highest proportion but lowest actual volume of striatum-like tissue of all WGE and LGE graft groups in this study. This suggests that graft-mediated functional effects are not solely a function of the amount of striatal tissue implanted. This hypothesis is further supported by the observation in one study that recovery of contralateral paw reaching deficits, while improved in all graft groups relative to lesion-only controls, achieved significance only in animals grafted with tissue derived from medial ganglionic eminence (92). This effect, present 3 months after grafting, is sustained in trials 6 months postgrafting. These data strongly suggest that MGE-derived tissue makes an unrecognized but potentially significant contribution to graft-mediated functional effects. The relatively poor connectivity of MGE grafts indicates that the effects are mediated by either local connections with the host neuropil or via more nonspecific mechanisms that are poorly understood.

The lack of clear correspondence between graft anatomy, connectivity, and functional motor effects in this study parallels other reports in rats (15,16,29) and in primates (37). Behavioral abnormalities in these models of Huntington's disease provide an incomplete representation of the functional deficits seen in the clinical condition. For reasons that are unclear no choreic or

dyskinetic movements are seen following excitotoxic striatal injury in rodent models of HD. In contrast, these movement deficits have been observed in primates following the administration of dopamine agonists to previously lesioned animals (4,10,33,81). However, increased spontaneous and drug-induced hyperactivity can be seen in rats and is more readily observed following bilateral striatal lesions (17,18,36,49,79,89). These limitations emphasize the importance of graft-mediated recovery of motor and cognitive function in primate models of HD (37,66). These results provide clear support for clinical trials of striatal transplantation but should be replicated in further studies. The similarities between primate and human anatomy provide an ideal model for evaluating the details of the neurosurgical techniques appropriate for delivering biologically active tissue to specific intracerebral targets (67). These studies should run in parallel with ongoing clinical programs so that questions arising from clinical application of intracerebral transplantation can be more readily answered in a rigorous scientific manner.

In addition to limitations in the animal models the heterogeneity of striatal function based on regional differences in the function of afferent cortical connections and underpins the idea of functionally disparate cortico-basal ganglia-thalamocortical loops (1,52) may also be a factor in the inconsistent correlation between graft survival and functional improvement. Hence, large doses of excitotoxin that destroy most of the striatal neuropil may result in functional deficits that currently available functional tests are too crude to characterize.

The degree of impairment of drug-induced rotation and skilled paw reaching appears to be dependent on the location and size of the lesion, but reports are conflicting. Thus, Dunnett and Iversen (23) reported no rotational effect of small striatal lesions at six different striatal loci whereas Norman et al. (60) reported apomorphine-induced contralateral rotation following anterior lesions and ipsilateral rotation after posterior lesions. Fricker et al. (28) obtained no drug-induced rotation after anterior lesions but marked ipsilateral rotation after posterior lesions. Similarly, impairment of skilled paw function has been shown to be influenced by the location (28,71) and size (57) of the preceding lesion. Inevitably, such heterogeneous and conflicting lesion-induced deficits will result in subsequent variability in any graft-mediated functional effect.

RELATIONSHIP BETWEEN GRAFT DEVELOPMENT AND HOST STRIATAL DAMAGE: IMPLICATIONS FOR THE TIMING OF SURGICAL INTERVENTION

Despite the ongoing clinical trials of striatal transplantation in patients with HD, it is currently unknown

to what extent the severity of host striatal damage affects graft development. 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. This issue has been addressed in a study in which it was shown that the presence of a striatal graft was able to attenuate striatal atrophy in mild and moderate, but not severe, lesions (93). Grafts grew to a larger size in the lesioned compared to nonlesioned striatum and in the mild and moderate lesions compared to severe lesions.

These results are consistent with previous reports (19,42) in which grafts implanted in the nonlesioned striatum were initially similar in size to those in the neuron-depleted striatum but did not grow further at longer survival times (42). Moreover, grafts growing in the lesioned striatum showed larger DARPP-32-positive P-zones than those placed in the intact host. Grafts implanted into severely atrophic striata (i.e., long-term lesions) attain 70–80% of the size of grafts implanted into more recent lesions (41).

Taken together, these results suggest that grafts implanted at an early stage of the disease process are more likely to survive and attenuate host damage. Because significant neuronal degeneration occurs before the clinical phenotype becomes manifest, clinical information (38,39) used in conjunction with the scientific evidence supports the hypothesis that striatal transplantation may be more effective at an earlier rather than an advanced stage of the disease process.

CONCLUDING REMARKS

Morphological and behavioral studies of striatal transplants in animal models of HD suggest that the stereotactic implantation of embryonic striatal tissue constitutes a potential strategy for amelioration of the neurological deficits found in patients with Huntington's disease. There is now sufficient scientific evidence to begin to formulate a protocol for the preparation and delivery of fetal striatal tissue.

The balance of evidence to date suggests that the optimal grafts for functional efficacy will be obtained using whole ganglionic eminence from younger donors, in rats E14–15, CRL 10–14 mm. This corresponds to Carnegie stage 18–21, which is equivalent to approximately 44–52 days gestation (postconception) in humans. The tissue can be prepared as either a trypsinized cell suspension or as solid pieces of tissue. The carrier vehicle should preferably be a simple media such as glucose/PBS. Surgery should be considered earlier rather than later in the disease process, and the volume of tissue implanted should be approximately one ganglionic eminence per implantation site. The heterogeneity of functional outcome and the relatively poor correspon-

dence between anatomy and function in these and other studies means that striatal transplantation remains an essentially empirical therapy. While early clinical reports are encouraging, further results in terms of clinical outcome in well-controlled experimental trials are required before concluding that neural transplantation can provide an effective therapy for HD.

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