An overview of central nervous system transplantation in human disease

Robert E. Breeze, M.D., and Marjorie C. Wang, M.D.

Department of Neurosurgery, University of Colorado Health Sciences Center, Denver, Colorado

Although its roots date back over a century, the field of neurotransplantation has been shaped mostly by advances over the past 30 years. Animal models of nigrostriatal disconnection in the 1970s allowed investigators to explore the feasibility of neural grafting. By the end of that decade, functional and behavioral effects had been demonstrated using fetal tissue grafts. In the 1980s, animal experimentation continued, as did clinical trials involving patients with idiopathic Parkinson's disease. Both autologous adrenal medullary tissue and fetal allografts were tested in the clinical setting, with the latter proving to yield superior results. Animal models of striatal cell loss provided the impetus for limited clinical trials in patients with Huntington's disease by the early 1990s, and work with both diseases continues today. Although much has been learned, neural grafting remains experimental. Broader applications are being explored even now, though, as transplant techniques are applied to animal models of dementia, spinal cord injury, cortical injury, and pain. Some very limited human trials have already begun in some of these areas. In this review some of the advances in the field are highlighted.

Key Words * neural transplantation * Parkinson's disease * Huntington's disease * review

For nearly a century, the specialty of neurosurgery has focused on the removal of abnormal tissue--neoplastic tissue, purulent or inflammatory tissue, extruded disc material, and clot, to name a few. In time, we became able to augment blood flow, divert cerebrospinal fluid, repair peripheral nerves, and reconstruct the protective structures of the brain and the spinal cord. Even so-called functional neurosurgery has focused on the destruction or removal of tissue believed to contribute to abnormal behavior (such as surgery for seizure control, psychosurgery, and thalamotomy). However, repair of the principal component of the nervous system--the neuron--remained unattainable. Certainly many of the operations performed by the neurosurgeon can improve the function or even ensure the survival of compromised neurons. Once a neuron has died, however, there has traditionally been no recourse. Over the last 20 years, advances in the field of neural transplantation have promised to change this. As the new millennium approaches, we are on the verge of being able to restore function to the victims of neurodegenerative diseases, stroke, and central nervous system (CNS) trauma by using neural transplants. In the following review we summarize the current status of neural transplantation, its history, and what problems remain to be solved.
Discussions of neural transplantation inevitably start with the work of W. G. Thompson dating from the late 19th century.[72] Thompson obtained a relatively large piece of adult cat cerebral cortex and placed it on the surface of a dog's brain. The recipient was killed after 7 weeks, and histological examination revealed that the transplanted tissue had retained "its identity as brain substance." Despite the fact the more modern reviewers have suggested that the grafted material probably contained no surviving neurons,[10] this work most likely represented the first serious attempt at CNS transplantation. Over the next several decades, numerous other investigators pursued questions related to the feasibility of neural grafts. In 1917, Dunn[21] stressed the importance of using immature donor tissue and the need for establishing a sufficiently vascularized bed in which to implant it. Although Dunn demonstrated some success by using neonatal tissue, in 1940 Le Gros Clarke[47] was able to demonstrate superior survival when implanting fetal tissue. Despite these and many other provocative studies in the first half of this century, rapid progress in the field of neurotransplantation would await later advances in neuroimaging and immunocytochemical techniques.

Experimental work more directly related to clinical applications began with the development of an animal model of Parkinson's disease (PD). Parkinson's disease is uniquely suited to transplantation experiments because the principal manifestations of the disease can be traced to the loss of a discreet population of cells with known projections. In 1970, the 6-hydroxydopamine (6-OHDA) model was introduced.[73] In this model, 6-OHDA is injected into either the substantia nigra or the medial forebrain bundle, destroying dopaminergic neurons on that side. The 6-OHDA-injected animal subsequently develops a stereotypic rotational behavior that can be elicited using a pharmacological challenge (apomorphine causes contralateral rotation due to receptor supersensitivity on the side of the lesion, whereas amphetamine causes ipsilateral rotation due to increased release of dopamine on the nonlesioned side). With a reproducible model of injury to the nigrostriatal pathway, it was then possible to explore different transplantation strategies. By the end of the 1970s, using this rodent model, two groups of investigators independently demonstrated functional and behavioral restoration after fetal cell implantation.[9,63] However, the complexity of PD is only very grossly approximated by rodent models. In the early 1980s, the discovery of the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) provided a nonhuman primate model of PD that would prove to be extremely useful.[45,46] The compound MPTP is a byproduct in the synthesis of 1-methyl-4-phenyl-4-propionoxy-piperidine, a meperidine analog marketed as a "synthetic heroin" in Northern California in the early 1980s. It selectively destroys dopaminergic neurons, either bilaterally with systemic administration or unilaterally with direct carotid artery injection. Its mechanism of action involves the formation of 1-methyl-4-phenylpyridinium, selective concentration within mitochondria, and subsequent formation of toxic free radicals through the inhibition of nicotinamide adenine diphosphate: ubquiquinone oxidoreductase.[16] Throughout the 1980s, numerous groups resolved many of the fundamental issues related to neurotransplantation by using MPTP primate models of PD.[3,4,8,22,25,66,68]

**STRATEGIES FOR TRANSPLANTATION IN PARKINSON'S DISEASE**

Parkinson's disease is the archetypal disease treated with neurotransplantation, and for this reason it is worth looking at the various issues related to a successful transplantation in some detail. The lessons learned in treating PD will most likely guide future therapies directed at other disease entities.
Although various abnormalities are noted in the brains of individuals with PD, the dominant pathological disturbance is the loss of a discreet group of some 500,000 dopaminergic neurons in the ventral mesencephalon--more specifically, in the pars compacta of the substantia nigra. These neurons project to numerous structures throughout the brain, but the majority of the efferent fibers go to the striatum. The loss of this input is presumably responsible for most of the clinical manifestations of PD. Although the architectonic structure of the striatum is relatively uniform, function is presumably not and, likewise, neither is the pattern of dopamine depletion. In a typical individual with idiopathic PD, the dopaminergic activity is relatively well preserved in anterior structures (head of caudate), whereas the greatest depletion is seen in the posterior putamen. It is generally assumed that the anterior striatum is more related to cognitive function and that the posterior striatum is more related to motor function. This could explain the fact that individuals with PD exhibit mainly motor dysfunction, with mild and variable cognitive involvement.

In terms of tissue grafting, the first issue to address is location. Although the goal of transplantation is to replace the lost dopaminergic neurons in the substantia nigra, it is unlikely that cells placed in the ventral mesencephalon of an adult human brain could send processes to the striatum.[64] Some evidence derived from studies in animals does suggest that tandem transplants (tissue placed both in the substantia nigra and directly into the striatum) may be superior to striatal grafts,[59] but all human trials to date have involved only placement of striatal grafts. The exact location within the striatum, however, has been somewhat controversial. The limited numbers of grafting procedures performed using open techniques have, obviously, been restricted to the head of the caudate, which is readily accessible via the frontal horn of the lateral ventricle. Stereotactic techniques allow for more widespread distribution of the grafted tissue, but even so, in many early trials the investigators targeted only more anterior structures. We have chosen to target the entire putamen, excluding the head of the caudate in our more recent work.[14] The wisdom of this strategy remains to be proven.

Having decided on a location, one must next choose the nature of the grafted tissue. The majority of early animal experiments directed at reconstructing lesioned nigrostriatal pathways relied on fetal ventral mesencephalon. Fetal tissue has much to recommend it. It is already genetically programmed to do the job at hand. Furthermore, the dopaminergic cells come with other supportive cells that presumably provide trophic factors. The optimal age of the tissue must be determined, but data obtained from both animal and human experiments have now established it to be approximately 6 to 8 weeks gestational age for human transplants.[29] This allows for terminal differentiation of the dopaminergic cells but is prior to the development of extensive neurite outgrowth that would be disrupted by harvesting.

Because fetal tissue grafts would almost always be allografts, immunological issues are raised. Fortunately, fetal tissue of the appropriate age has not yet begun to express Class I major histocompatibility antigens,[33] and the brain is a relatively immunologically privileged site. Nonetheless, it cannot be assumed that the graft will be accepted. Limited migration of mononuclear cells can be seen at graft sites,[5] although a humoral response has not been identified in humans.[1] Evidence derived from human trials does suggest that immunosuppression is not required,[27] but the issue remains controversial. The majority of investigators still use some form of immunosuppression in their human trials.

The amount of tissue to be transplanted must also be considered. By definition, one fetus contains a full complement of dopaminergic cells. However, cell survival is far from complete. Available evidence suggests that most cell loss occurs between the time of harvesting and 1 week after transplantation.
estimate ultimate cell survival to be no better than 5 to 10% in our cases. It remains to be seen whether the use of various growth factors, either in culture or administered locally or systemically after transplantation, can improve this cell survival rate. The optimum number of cells needed for a successful transplant is not known at present. It is estimated that dopamine activity needs to drop to 20 to 40% of its normal level before a patient exhibits clinical signs and symptoms of PD.[44] In theory, these levels could be achieved using material from approximately four fetuses, even with our dismal survival statistics. This, of course, assumes that a one-to-one correspondence exists between the functional capacity of a native dopaminergic neuron in the substantia nigra and a transplanted dopaminergic neuron in the striatum. However, this is certainly not known. To date, in human trials material from between one and eight fetuses per patient has been used.[15,26,29,34,35,37,41,46,49-51,53,55,56,60,61,69,71,73,77]

The use of fetal tissue does have two significant drawbacks, however. One is purely logistical. Obtaining uncontaminated tissue of the appropriate age is a labor-intensive endeavor that requires highly trained and experienced personnel as well as access to a relatively large number of fetuses. With our current system, it is generally difficult to perform more than two fetal cell transplants per month at our institution. It is difficult to envision how this number could be radically increased if the procedure were to become a routine treatment for PD. The other problem with fetal tissue is that its use is inexorably tied to the issue of abortion, a divisive issue that throws all fetal cell research into an ethical controversy. Both issues have caused researchers to seek alternatives to fetal tissue.

One potential alternative to fetal tissue is autologous adrenal medullary tissue. The chromaffin cells within this tissue produce catecholamines, but unfortunately these cells do not survive long in the CNS without growth factors such as nerve growth factor (NGF).[70] Various strategies have been used in animal experiments to circumvent this by providing NGF, either via infusion mechanisms or by cografting natural sources of NGF such as sural nerve or Sertoli’s cells.[42,44] Although fetal adrenal tissue has also been used in animal and human trials, its use, inevitably, raises the issues attendant on fetal tissue in general. Other natural sources of catecholamines, such as sympathetic ganglion cells, have also been investigated.[40]

The use of genetically engineered cells has also been evaluated[13,44] and two distinct strategies can be used: 1) to provide a dopamine-producing cell by genetically altering a fibroblast, myoblast, or even an astrocyte, or 2) to alter cells genetically to produce NGF or other growth factors and then use these cells as cografts. Similarly, one could try to produce an immortalized cell line from germ cells or tumor cells and then genetically "force" the cells into neuronal differentiation.

Finally, one needs to consider xenografts. The use of fetal porcine cells, for example, avoids some of the ethical issues surrounding human fetal tissue, but it introduces new technological problems. Although the need for immunosuppression remains controversial in the setting of human fetal allografts, many experiments have proven that long-term immunosuppression is necessary with xenografts. Two strategies to circumvent this have been to coat the cells with a polymer capsule or to pretreat the cells with antibodies directed against species-specific Class I major histocompatibility antigens.[17,23,39,62] Both strategies are currently being tested in limited human trials.[17,18]

Up to this point, the discussions on the type of cell to transplant, the appropriate transplant site, and the optimum amount of tissue required have been predicated on the assumption that we need to provide a source of dopamine in the striatum to replace the lost cells in the substantia nigra. However, this may not be completely accurate. Several competing theories could explain the positive functional and behavioral
effects clearly demonstrated in animals and probably in humans with neurotransplantation experiments.[44] It is possible that the results are due solely to a lesion effect, analogous to those obtained in thalamotomy or pallidotomy. Alternatively, the act of transplantation may cause the release of trophic factors, from the transplanted tissue or host tissue, that results in the sprouting of native dopamine nerve terminals. The latter has been clearly demonstrated in some animal models.[11,12,20,24] The passive release of dopamine from the graft tissue may be the responsible mechanism. Perhaps, on the other hand, integration of the graft into the striatum, with the establishment of synaptic connections and complex feedback, is necessary for a truly successful transplant. Although there are data obtained from investigations in animals to support the last proposed mechanism (synaptic connections from graft to host can be demonstrated, and control grafts with fetal cortex or cerebellum do not show significant improvement), the issue remains controversial.[11,43,44,58] A combination of contributing factors may ultimately be proven to exist. Regardless, one can see that it is difficult to perfect a strategy if the path to the desired goal is unclear. Our group has proceeded on the assumption that widespread integration of the graft into the host striatum is necessary for a successful transplant. Other technical issues related to tissue storage, graft preparation, and surgical techniques will probably also prove to be important.[13]

**CLINICAL TRIALS IN PARKINSON'S DISEASE**

In 1982 Backlund and associates [2] performed the world's first human neural transplant for PD at the Karolinska Institute in Stockholm. Despite the results of research in animals which supported the superiority of fetal mesencephalic tissue as the donor source, the authors chose to use autologous adrenal medullary tissue because of ethical considerations. The tissue was implanted into the head of the caudate unilaterally by using stereotactic techniques. A second case followed approximately 1 year later, and the series of two was published in 1985. The Swedish team reported modest results at best. In 1987 Madrazo and colleagues[54] reported significant clinical improvement in two patients in whom adrenal tissue was placed in the head of the caudate by using an open microsurgical technique. In subsequent studies conducted by multiple investigators (most of whom used stereotactic techniques) the same degree of clinical improvement was not achieved.[3,7,28,31,32] Some authors argued that the surgery-related trauma associated with the open technique was responsible for the greater degree of clinical improvement demonstrated in the series reported by Madrazo and colleagues. At approximately this same time, however, findings from additional animal studies showed the need for administering NGF to obtain prolonged survival with adrenal medullary grafts.[43,65] By the end of the 1980s, interest had mostly shifted away from adrenal transplants.

One of the earliest attempts at fetal cell implantation for PD was made by Madrazo in 1986.[56] In the first published report in the English-language literature, however, two cases from Sweden are described, the first having been performed in 1987.[49] It is probable that fetal cell transplants were performed in China even earlier than 1986. The issues of primacy notwithstanding, several groups around the world were poised to use fetal tissue in human trials at that time, and over the next 1 to 2 years, implantation of fetal tissue was performed at additional centers in Cuba, the United Kingdom, and the United States.[26,27,35,60,61] Numerous other centers throughout the world embarked on similar studies in the 1990s.[15,19,30,34,37,41,48,53,69,71,74] By the mid 1990s, a body of evidence had accrued to suggest that fetal cell implants were superior to adrenal medullary implants and could, in fact, produce lasting clinical effects in patients with PD. However, the lessons learned from the adrenal transplants were still quite fresh, and many people remained skeptical.

In 1994, our group proposed a prospective, blinded trial to evaluate the biological efficacy and safety of
fetal mesencephalic tissue implants for the treatment of PD. By design, the timing coincided with the change in the presidential administration and the subsequent lifting of the ban on the use of federal monies to support research that involved human fetal tissue. A National Institutes of Health grant was secured to support the project, and in the fall of 1994 patient recruitment began. The study design required the enrollment of 40 patients: 20 of whom would undergo tissue implantation, and 20 who would undergo a sham operation. Each treatment arm contained an equal number of patients older and younger that 60 years of age and an equal number of men and women. Tissue implantation was performed stereotactically, with two tracts in each putamen, oriented along the long axis of that structure. The sham operations included placement of a stereotactic frame and twist drill holes in the forehead but with no needle passes through the brain. The patients were recruited and evaluated by an independent team (the Movement Disorder Group at Columbia University). They underwent evaluation for at least 3 months preoperatively, and their medical management was optimized. Preoperative evaluations included standard clinical assessments such as the Unified Parkinson's Disease Rating Scale, videotapes, and diaries, in addition to 18-fluorodopa positron emission tomography scans. Once the patients were randomized to a treatment group, they traveled to Denver where they stayed for approximately 1 week and underwent either real or sham surgery. The two principal investigators in Denver were not blinded as to the nature of the procedure, but all other personnel in Denver were. The patients and all personnel in New York remained blinded for 1 year. Follow-up evaluations were performed at 3, 6, 9, and 12 months. A postoperative positron emission tomography scan was obtained at 12 months. Once "the blind" was broken, patients were given an opportunity to return to Denver to receive the actual implant if they had initially been randomized to the sham group. The 40 blinded procedures were performed between May 1995 and January 1998.

At the time of this writing, the data accrued over 1 year of blinded follow up are being prepared for publication. In addition, data on the crossover patients and longer-term data on the now unblinded transplant patients are being collected. Several conclusions can be drawn from the available data, but the reader is advised that the following material has neither been subjected to peer-review nor replicated by other groups. With that disclaimer in mind, it appears that we now have class I evidence that fetal cell tissue implanted in the putamen can improve the signs and symptoms of PD. This can be achieved with a high degree of safety and without immunosuppression. The effect appears to be related to dopamine production; it continues to increase beyond 1 year, and it is more dramatic in younger patients. Unfortunately, some undesirable physiological effects may be associated with the grafts, and the beneficial effects are highly variable from patient to patient. In summary, the study appears to provide the most solid evidence to date for the efficacy of neurotransplantation in PD, and at the same time, the results underscore the need for further research.

**NEUROTRANSPLANTATION FOR HUNTINGTON'S DISEASE**

Huntington's disease (HD) is a neurodegenerative condition that is characterized by choreoathetosis, psychiatric disturbances, and a relentless deterioration of higher brain function. It is inherited in an autosomal dominant fashion, with onset of clinical manifestations usually in the fourth or fifth decade of life. Death usually ensues within 10 to 20 years of onset. The gene responsible for HD has been identified; it codes for a protein called "huntingtin" and contains CAG repeats in affected individuals.[36] Although multiple regions of the brain show pathological changes in HD, the most dramatic change is striatal atrophy. Some authors have argued that many of the other observed abnormalities are, in fact, secondary changes related to the loss of striatal neurons. The atrophy demonstrated in the caudate and
putamen is due to the loss of Golgi Type II neurons that comprise approximately 95% of the striatal neurons.[65] The large cholinergic neurons present in the striatum are relatively preserved. This loss of a discreet population of cells (similar to the situation in patients with PD) coupled with a complete lack of effective therapies, has led investigators to pursue transplant strategies in the treatment of HD. Their efforts have been aided by the use of animal models in which they use intrastratial injections of excitotoxic amino acids (such as ibotenic, kainic, and quinolinic acid) to mimic the neuronal loss observed in cases of the human disease.[38,44,52] The results of experiments in animals have demonstrated that a remarkable degree of striatal reconstruction can be achieved with fetal transplants, with relatively normal graft-to-host and host-to-graft synaptic connections being formed.[75,76] Given the complex interconnections of the striatum with other regions of the brain, one might hypothesize that to be successful, a striatal graft in patients with HD would have to reconstruct, at least partially, these connections, as opposed to simply releasing a neurotransmitter as the case may be in PD (although some of us believe that such connections are important to a truly successful graft in PD as well).

In 1991, Madrazo and coworkers[57] reported the first neural transplant for HD. Since then, several groups have embarked on clinical trials. Many of the issues raised in the treatment of PD will also need to be addressed with HD, keeping in mind that the transplanted tissue is now fetal striatum, not fetal mesencephalon. As in PD, alternative tissue sources are being explored.

**NEUROTRANSPLANTATION IN OTHER DISORDERS**

Following the pioneering work on the nigrostriatal pathway from the 1970s, neuroscientists have used transplantation techniques to explore a number of different problems related to stroke, Alzheimer's disease, and spinal cord injury. Although most work in these areas has not reached the stage of clinical trials, a team at the University of Pittsburgh has begun a phase I trial in which they are assessing a commercially available neuronal cell line in the setting of stroke (D Kondziolka, personal communication, 1998).

Another area of clinical interest is pain management. Adrenal medullary transplants have been placed in the lumbar theca to treat cancer pain,[67] not to replace lost cells but to act as a "drug pump," presumably releasing endorphins.

**CONCLUSIONS**

Although its roots date back over a century, neural transplantation has been shaped most by advancements over the past 30 years. Although possibly premature, the field was thrust into the realm of human experimentation in 1982 with the pioneering work of Backlund and associates.[2] Since then, clinical research has proceeded simultaneously with basic research. Today, much is known about neurotransplantation as it relates to PD, although more must be learned before it becomes a routine treatment. Other applications in neurodegenerative diseases, stroke, and trauma are just beginning to be explored clinically.

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Address reprint requests to: Robert Breeze, M.D., University of Colorado Health Sciences Center, 4200 East Ninth Avenue C-307, Denver, Colorado 80262.