Chapter 5

Applications of Neural Grafting Into the Brain and Spinal Cord

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Chapter 5

Applications of Neural Grafting Into the Brain and Spinal Cord

The technology of grafting into the brain and spinal cord to restore functions lost through disease or injury is still in the initial stages of development. Research in animals has indicated that neural grafting may provide beneficial therapeutic effects in some neurological disorders, notably Parkinson's disease. But in every case, including Parkinson's disease, there is still much information that needs to be collected before neural grafting can be adapted for general use in humans. The research currently being conducted in this field is aimed at learning more about basic mechanisms involved in grafting tissues into the central nervous system (CNS) and the actions and effects neural grafts can exert there. Information is being sought in three broad areas:

- the conditions necessary for graft survival and incorporation into the host,
- the functional role grafted material can play in alleviating deficits caused by disease or injury, and
- the mechanisms by which grafts produce any functional recovery that is observed..

Scientists use many different kinds of experiments with animals to obtain this information. Some experiments are designed to gain information about basic mechanisms related to brain development, the brain's response to injury, and the regenerative capabilities of nervous tissue. Others examine the ability of grafts to ameliorate or reverse experimentally induced deficits. Still others use animal models that either replicate or share features of human disorders in order to determine whether grafting could result in improved function. Thus, experimental work in the field of neural grafting provides information about basic brain mechanisms as well as the role grafts can play in neurological disorders.

The need for animal models that mimic a given neurological disorder in humans is as important in the field of neural grafting as it is in most other areas of clinical research. Animal models of a disease or injury can be either homologous (sharing a common origin with the human condition) or analogous (sharing similar organ damage, though not necessarily via the same disease mechanism). Currently, there are very few homologous models of human neurological disease. The closer an animal model is to the human condition under study (in terms of the neurological damage induced and the resulting behavioral effects), the easier it is to extend observations from the model to a human disorder. Virtually all scientists in the field of neural grafting believe it is essential to develop good animal models for use in grafting experiments.

Theoretically, the applications of neural grafting technologies into the brain and spinal cord could encompass many types of neurological disorders. The possible therapeutic applications of neural grafting range from the highly speculative to the actually implemented. This chapter provides an overview of some of the possible applications of neural grafting into the brain and spinal cord, the research that has been conducted so far, and the current status of each. In addition, the possible mechanism of therapeutic action of neural grafts for each condition is discussed. The neurological disorders are presented according to type, with an emphasis on those that have been studied most extensively.

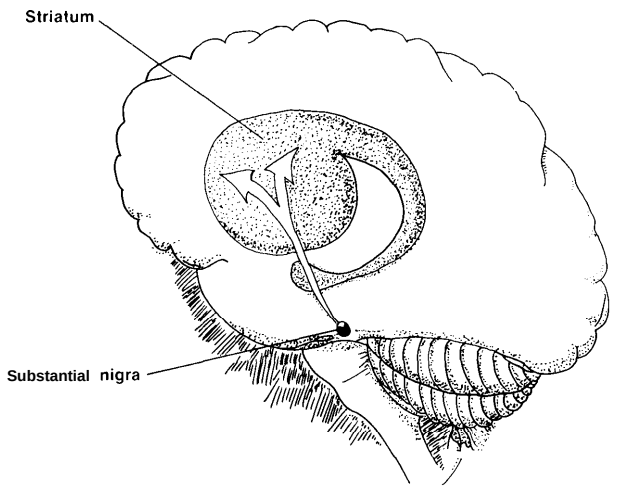
NEURODEGENERATIVE DISORDERS

Parkinson's Disease

The clinical use of neural grafting in the treatment of Parkinson's disease, begun in the early 1980s, has generated controversy in the scientific and medical communities. While more is known about neural grafting in Parkinson's disease than in any other neurological disorder, the efficacy of grafting in humans is unclear, and ethical concerns are unresolved. The concern about the discrepancy between the base level of knowledge and the actual clinical use of these procedures, even under experimental conditions, has increased public awareness of this controversy. This section provides a summary of the animal research and clinical use of neural grafting in persons with Parkinson's disease.

Parkinson's disease is well suited to the application of neural transplantation because the disease results from the degeneration of a discrete population of neurons located in an area of the brain called

Figure 5-l—The Substantial Nigra and the Striatum



The cells of the substantial nigra send their axons to the striatum. This pathway degenerates in Parkinson's disease.
SOURCE: R. Restak, *The Brain (New* York, NY: Bantarn Books, 1984).

the substantial nigra. These neurons produce dopamine, a neurotransmitter, and form synapses with neurons in another area of the brain, the striatum (figure 5-l). The neurons in the striatum are not destroyed in Parkinson's disease (67); however, the depletion of dopamine in the striatum, caused by the loss of cells in the substantial nigra, is associated with the symptoms of tremor, rigidity, difficulty in standing, and slowness of movement that are characteristic of Parkinson's disease. Neural grafting in Parkinson's disease has been aimed at correcting the loss of the dopamine-producing pathway between the substantial nigra and the striatum by either replacing the dying cells or supplying additional dopamine. To do this, neural grafting materials from two main sources have been used: 1) tissue from the adrenal gland and 2) tissue from the fetal CNS. A graft that would survive indefinitely could produce continuous relief from symptoms, but evidence suggests that the degeneration of the dopamine-producing neurons in the substantial nigra would continue. All research to date has been aimed at the ability of grafts to provide relief from symptoms and reduced disability, not cessation or reversal of the degenerative process.

The most obvious site for transplantation of dopamine-producing cells in Parkinson's disease would seem to be the locus of the degenerating cells, i.e., the substantial nigra. While cells placed in this site might receive some inputs from neurons in other areas of the brain that normally send fibers to the substantial nigra, axons from the grafted cells would have to grow several centimeters through the brain to connect with their targets in the striatum. Unfortunately, nerve fibers do not readily grow over such distances in the adult brain, and it has been shown that dopamine-producing grafts implanted into the experimentally damaged substantial nigra of rodents have no effect on the parkinsonian symptoms such damage causes (40).

Instead, dopamine-producing cells have been grafted (either as a cell suspension or as solid pieces) to sites close to their targets in the striatum, in order to facilitate formation of synapses. In fact, it is unclear whether the formation of synapses is actually necessary to produce an effect—it is possible that some of the effects of dopamine produced in the

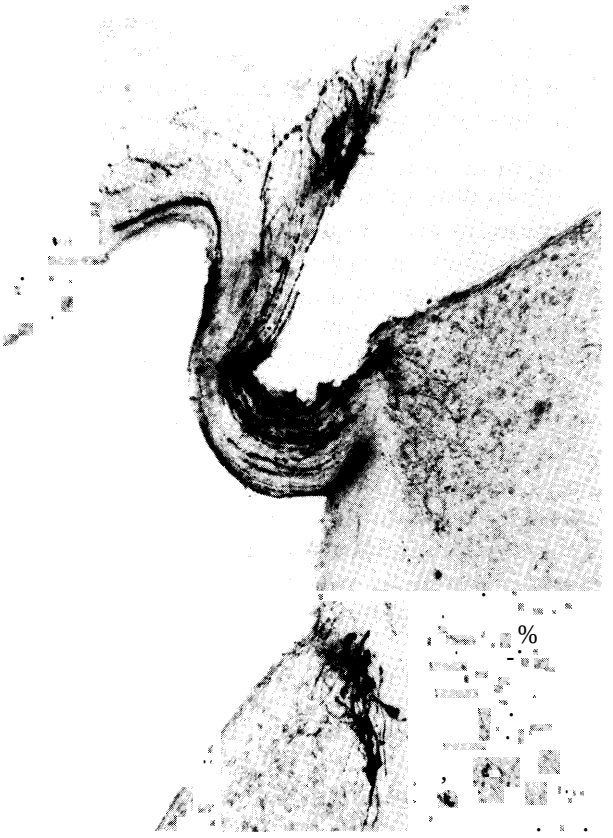


Photo credit: J.R. Sladek, Jr., University of Rochester Medical Center

Picture of a graft of monkey fetal tissue implanted into an adult monkey.

striatum by grafts may result simply from the diffuse release of chemicals in the area and thus do not require specific, point-to-point synaptic connections and precisely timed release.

Animal Research

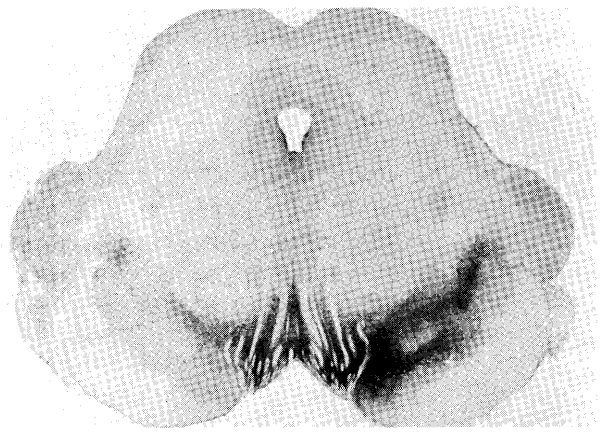
Fetal Tissue—The first indication that grafts might be effective in the treatment of Parkinson's disease was observed in experiments conducted in 1979 (19,1 19). These experiments used rats in which the pathway between the substantial nigra and the striatum had been destroyed by the neurotoxin 6-OHDA,[1] thus chemically affecting the same site that is thought to be central to the clinical signs of Parkinson's disease. Grafts of dopamine-producing neural tissue from rat fetuses, implanted into the striatum, were able to reduce some deficits caused by the wound (40,41); however, the extent and type of function that was restored depended on where in the striatum the graft was placed (40,41).

Since these initial experiments, many others using the same animal model have shown that implanted fetal tissue sends out fibers and makes contact with the host tissue, receives inputs from the host tissue, and performs typical neuronal functions within the host (3,20,54,55,103,131,139,141, 153).

Although some of the functional recovery produced by neural grafting may be due to the nonspecific release of dopamine into the host brain, studies have shown that much of the observed recovery is related to the extent to which the implanted tissue becomes incorporated within the host brain--i.e., the degree to which the graft sends connections to, and receives connections from, the host brain. This finding indicates that more than just a diffuse release of dopamine into the striatum is involved. In fact, when the graft is removed, the corrective effects disappear, suggesting that they are due to a direct action of the graft and not to an effect unrelated to it (17,42).

Dopamine-producing fetal tissue has also been studied in the MPTP model of parkinsonism (figure 5-2).[2] While it takes only days for MPTP to produce the symptoms of parkinsonism in laboratory animals, as compared to years for the disease itself in humans, most scientists still regard this as the best animal model of the disease (see box 5-A). Also, since MPTP is particularly effective in monkeys, it has provided scientists with an important primate model of parkinsonism. (Data derived from experiments using monkeys are more easily extrapolated to humans than are data from nonprimate animal models.) As with the 6-OHDA experiments, it was shown that grafted dopamine-producing fetal neural tissue can become incorporated into the striatum of treated monkeys and result in significant, long--lasting reductions in movement abnormalities, including tremor, rigidity, and slowness of movement (7,8,31,49,124,135). Recently, this effect of fetal tissue grafts in monkeys has been linked to the ability of the grafts to stimulate new growth from undamaged neurons (10).

Figure 5-2—Monkey Brain Exposed to MPTP



The brain of a monkey that was exposed to MPTP on one side. 'The substantial nigra on the unexposed side (right) is normal (black band). On the MPTP exposed side (left) the cells of the substantial nigra are destroyed.

SOURCE: R.A.E. Bakay, Section of Neurological Surgery, The Emory Clinic.

In Summary, a number of different studies have demonstrated that dopamine-producing fetal tissue can counteract some of the effects produced in the 6-OHDA and MPTP animal models of parkinsonism. In particular, some of the results seen following grafting in nonhuman primates treated with MPTP have been promising. However, in

[1] This analogous animal model is produced by the neurotoxin 6-hydroxydopamine (6-OHDA), which selectively destroys dopamine-producing neurons. In this model, the 6-OHDA is injected directly into the substantial nigra. While the lesion produced by this injection destroys the same neurons that are lost in Parkinson's disease, the action of the chemical and the temporal course of the neuron destruction do not mimic what occurs in the disease. Also, the functional deficits produced, while in some respects similar to those produced by Parkinson's disease, are not exactly the same.

[2] MPTP (1-Methyl-4-phenyl-1-2-3-6-tetrahydropyridine) is a chemical that, when administered, causes a syndrome similar to Parkinson's disease. Thus, the drug is said to result in parkinsonism rather than Parkinson's disease (see box 5-A).

Box 5-A—MPTP, a Key to Parkinson's Disease

On a July morning in 1982, a patient was brought into the emergency room of a hospital in Santa Cruz, California. He was stooped over and displayed the symptoms of rigidity and inability to initiate movement typically seen in persons in their sixties or seventies who have been suffering from Parkinson's disease for many years. The clinical picture was that of classic, late-stage Parkinson's disease. However, this patient was 24 years 014 and his symptoms had developed virtually overnight. With this startling patient began one of the most interesting and important chapters in the history of Parkinson's disease research and treatment.

A closer examination of this patient and five others who came to hospital emergency rooms in the northern California area at about the same time revealed that they were all heroin addicts who had injected some homemade narcotics that contained an impurity. The impurity was determined to be the chemical 1-methy1-4-phenyl-1,2,3,6-tetrahydropyridine, or MPTP. It was thought that the MPTP might have caused brain damage that mimicked Parkinson's disease, resulting in identical symptoms, This hypothesis was lent further credence by an autopsy report on another individual who had been exposed to MPTP. The report noted that the man's brain looked like that of someone who had suffered from Parkinson's disease for years: The dopamine-containing neurons in the substantial nigra, which are lost in Parkinson's disease, were destroyed. It was also found that when given L-dopa, the most common medicine for treating Parkinson's disease, the patients exposed to MPTP improved dramatically. However, the effectiveness of the L-dopa began to wear off after 2 to 3 years, just as it does in most Parkinson's patients after 5 to 10 years.

Immediately after the identification of MPTP as the agent responsible, scientists began to study the compound to find out how it causes this model of parkinsonism. They found that MPTP can enter the bloodstream through direct injection, inhalation, or contact with the skin. Inside the brain, an enzyme called monoamine oxidase B (MAO-B) breaks down MPTP into other chemicals, including an electrically charged molecule called methylpyridine (MPP+). The MPP+ molecules are taken up selectively by dopamine-producing neurons. It is thought that, once inside these dopamine neurons, the MPP+ may cause another chemical reaction to take place. This reaction produces toxic substances, such as peroxides, and other charged particles, called free radicals. The production of these substances destroys the cell. It was also found that if the breakdown of MPTP to MPP+ is prevented by blocking the activity of MAO-B, MPTP has no effect.

This understanding of the chemistry of MPTP has led to a new understanding of what might be occurring in persons with Parkinson's disease. It is possible that the same sorts of toxin-producing chemical reactions that MPTP causes in the brain occur in Parkinson's patients, What causes the chemical reactions to take place in the disease is unclear, but it may be some disruption in the normal biochemical activity of the dopamine-producing neurons. Indeed, it has been known for some time that MAO-B is also involved with the normal breakdown of dopamine in these cells. It is possible that this normal process gets disrupted somehow and that when the dopamine is broken down, peroxides and free radicals are produced. Based on these new MPTP data, a drug called deprenyl, which is known to block the action of MAO-B, was studied. It has been shown, at least in one study, that deprenyl appears to have a retarding effect on the progression of the disease. While this finding needs to be confirmed, it indicates how the information gained from the MPTP model of parkinsonism is giving scientists and doctors valuable new information about the disease,

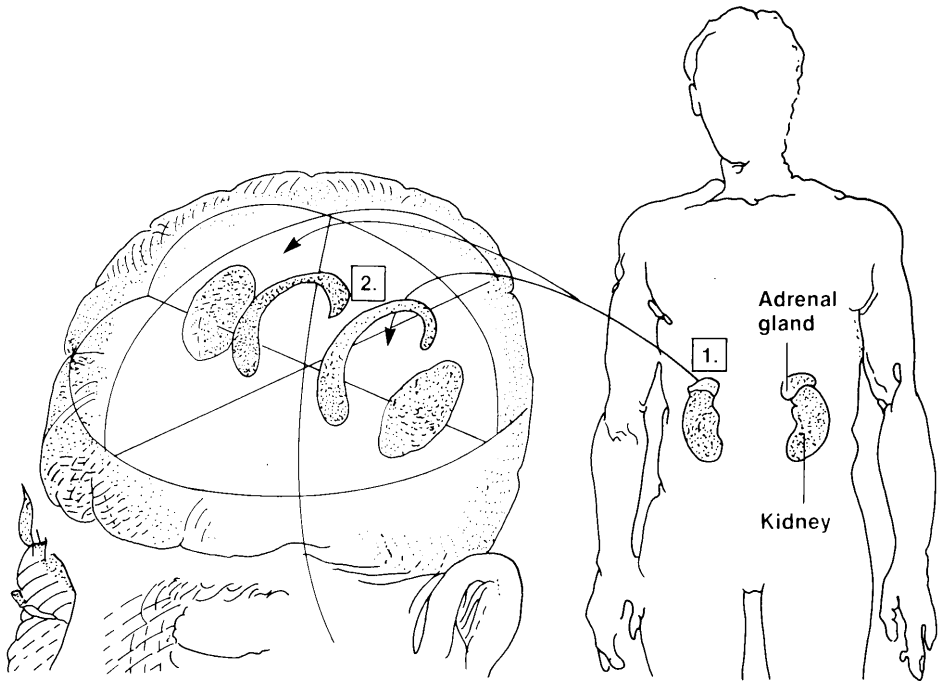
Another theory that has been proposed as a result of the discovery of MPTP is that Parkinson's disease might be caused by environmental exposure to MPTP or a chemical like it. While this is a possibility, no strong evidence of any link between the incidence of Parkinson's disease and exposure to MPTP in the environment has been found to date.

Interestingly, it has been found that MPTP does not affect all species. While it produces symptoms of parkinsonism in humans, nonhuman primates, and mice, it has little or no effect in rats, cats, rabbits, and a number of other species. The reason for this is not clear, but it is thought to be due to differences in the blood-brain barrier.

The discovery of MPTP has greatly advanced the understanding of Parkinson's disease. Not only has it provided important new insights into the possible mechanism that causes the disease, but it has given scientists an important and extremely useful new tool to study Parkinson's disease. The nonhuman primate MPTP model has been and will continue to be one of the best experimental tools for dete**rmining *the efficacy*** of the use of neural grafting to treat Parkinson's **disease.**

SOURCE: Office of Technology Assesstment, 1990.

Figure 5-3—Adrenal Medulla Neural Graft for Parkinson's Disease



Tissue from a patient's adrenal gland, which lies atop the kidney(1), is implanted into the striatum (2).
SOURCE: *Newsweek*, Apr. 14, 19S6, I.B. Ohlsson.

addition to the need to replicate and expand these findings, there is a need to answer the following questions by means of additional animal studies:

. Where in the striatum should material be grafted to achieve maximum effect?
• How long do the effects of the graft last?

Adrenal Medullary Tissue—There is considerable interest in finding alternative sources of dopamine-producing grafting material, for two reasons. First, there are ethical, legal, and technical obstacles to procurement of human fetal tissue for transplantation. Second, the use of autografts (tissue from the person's own body) eliminates possible immunological rejection of the graft. While other types of tissue for autografts have been studied [sympathetic ganglia, carotid body glomus cells, (15,83)], tissue from the medulla of the adrenal gland has attracted the greatest interest. In animal experiments, the adrenal tissue is collected from donor animals; in humans with Parkinson's disease, the patient's own adrenal tissue can be used for the graft (figure 5-3).

The efficacy of adrenal tissue is more uncertain than that of fetal CNS tissue. Questions have been raised about how effective adrenal tissue is in producing fictional recovery, what mechanism induces functional effects, and whether any observed functional effect is actually due to a direct effect of the graft or to some other factor.

Implantation of adrenal medullary tissue can ameliorate some of the deficits created in the 6-OHDA model in rodents; however, the range of functions recovered is narrower than when fetal tissue is used (52,96). The beneficial effects are thought to result from either a nonspecific, diffuse release of dopamine by the adrenal cells (53) or an increase in the number of new dopamine fibers near the graft site (112). The latter presumably reflects the degree to which the grafted adrenal medulla cells are converted into neuron-like cells that produce dopamine.

The number of adrenal cells that survive implantation is very low in both rodents (15) and monkeys (1 1,75,108). If the cells do not survive, the chance of functional recovery is small and any long-term

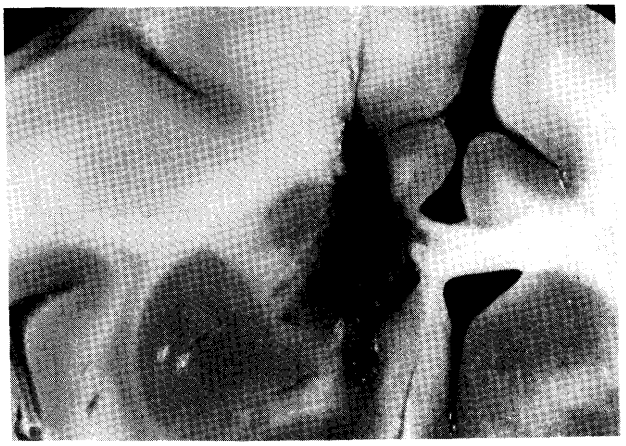


Photo credit: R.A.E.Bakay, The Emory Clinic

Section of a monkey brain showing a surviving graft of adrenal medulla tissue that was implanted along with other tissues that produce neurotrophic substances.

recovery that does occur is probably due to some effect unrelated to continued graft function. Adrenal cell survival is enhanced when neurotrophic factors, including nerve growth factor (NGF), are added to the graft site (16,88,140). In monkeys, increased fictional recovery is observed when adrenal cells are grafted along with cells that produce neurotrophic factors (88), although it has been observed in rats that the same level of functional recovery occurs when NGF is given in conjunction with either adrenal grafts or grafts of nondopamine-producing material (121). This suggests that the functional recovery observed in the latter case resulted, in part, from activation of the brain's injury response mechanisms due to surgical injury (including release of growth factors) and the nonspecific action of NGF to promote axon growth.

Another issue surrounding adrenal tissue grafts is the influence of donor age on graft efficacy. Grafts from younger rats are more effective than those from older rats in promoting functional recovery following destruction of dopamine-producing cells with 6-OHDA (51). Also, older tissue is less likely to undergo the conversion from adrenal medulla cells to dopamine-producing neurons (9). This may be due to a lack of growth-promoting factors, perhaps NGF, in the older tissue. When NGF is administered to rats in conjunction with grafts of older adrenal tissue, more conversion of cells to dopamine-producing neurons is seen and a greater improvement in movement occurs (140). Since many Parkinson's disease patients are elderly, donor age may be one reason for the limited effect of adrenal autografts used in these patients (see later discussion).

The data collected on adrenal grafts in the MPTP nonhuman primate model of parkinsonism agree with the data obtained in 6-OHDA experiments with rats. As noted previously, only limited survival of grafted tissue following implantation has been observed (11,75). This raises the question of what is responsible for the functional recovery that has been seen. New dopamine fibers growing in the host brain (47) and functional improvement (11) have been observed in both grafted animals and animals that had the surgery performed without tissue implantation. This suggests that the surgical procedure itself has some effect. As was true of the rats, the degree of functional recovery in nonhuman primates is less with adrenal medullary tissue than with fetal CNS tissue (100).

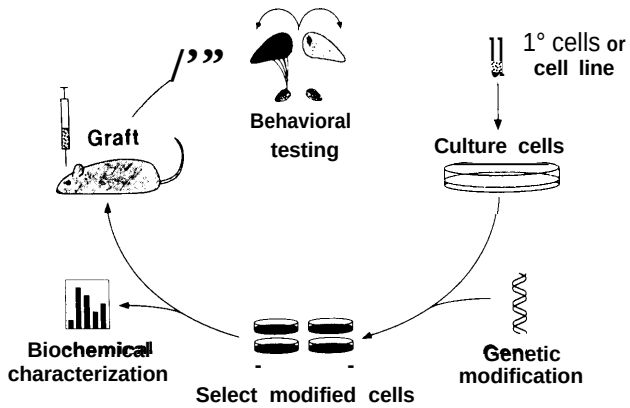
In summary, the ability of adrenal medullary tissue to ameliorate the deficits caused in animal models of parkinsonism is unclear. Questions that need to be answered include:

- Are the functional improvements that have been observed the result of the adrenal graft or the surgical procedure?
- If adrenal grafts do have a beneficial effect, what is the range of deficits that can be improved?
- What role can concomitant application of growth-promoting factors play in the survival and efficacy of adrenal grafts?

Genetically Engineered Cells-Genetically engineered cells have been shown to survive grafting into the CNS (58). The ability of genetically manipulated cells to decrease the abnormal behavior caused by administration of 6-OHDA to rats has recently been investigated (151). Certain skin cells, called fibroblasts, were taken from rats and genetically modified to produce the enzyme tyrosine hydroxylase (TH), which is required for the production of dopamine (figure 5-4). It was hoped that inserting these cells into the 6-OHDA rats would cause the animals to produce dopamine and thus reduce their abnormal behavior. The grafts of the genetically engineered cells did reduce abnormal behavior, by about 40 percent.

Although this important study indicates that genetically altered cells might be an effective grafting material, it was noted that the extent of

Figure 5-4-Grafting Genetically Modified Fibroblasts



The process of grafting genetically engineered fibroblasts consists of: 1) culturing the fibroblasts, 2) genetically modifying them, 3) selecting which cells to use, 4) testing them, 5) grafting into the brain, and 6) behavioral testing of the animal to determine the effect of the graft.

SOURCE: F. Gage, Department of Neuroscience, University of California, San Diego.

recovery was less than that which has been observed when fetal CNS tissue is used. One possible reason for this difference is that the manipulated cells do not produce as much dopamine as normal fetal brain cells. Nonetheless, since the use of genetically manipulated cells lines would circumvent the problems associated with fetal tissue and the immunological considerations connected with other sources, continued exploration of their use is an important area of research.

Human Application

Human trials of neural grafting in persons with Parkinson's disease have used both adrenal and fetal CNS tissue. In both cases, some observers have held that the trials were premature (137). In the case of adrenal grafting, many observers believe that there has been a rush to proceed with human trials without having first collected adequate data from animal experiments; in the case of fetal tissue grafts, while there is a larger base of animal data to draw on, there is still concern that widespread implementation of human fetal tissue grafting could proceed before adequate information has been derived from animal experiments. This question may place scientists who are doing the research at odds with patients and doctors, who must deal with the realities of devastating illness on a daily basis. The participants in this debate include scientists performing basic research, desperately ill patients who are willing to undergo virtually anything that might help them, and clinicians, who are motivated by the altruistic desire to help their patients and, perhaps, by the ambition to be on the cutting edge of a new and exciting field.

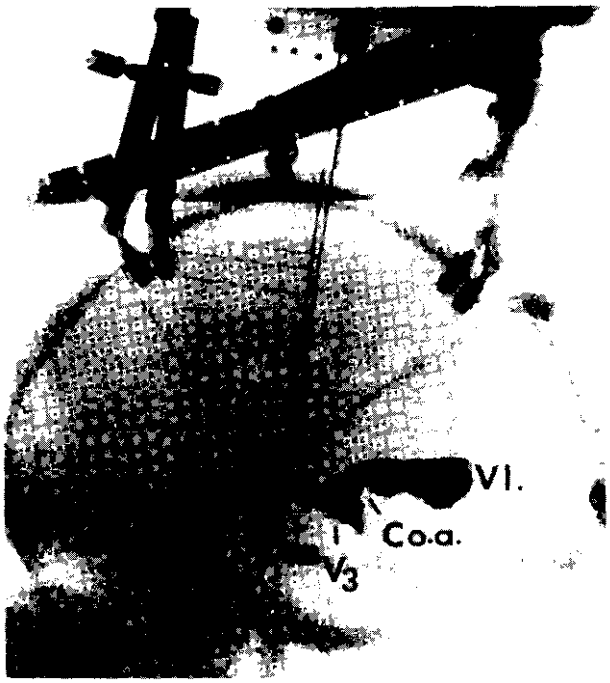
As of 1990, between 300 and 400 persons with Parkinson's disease had received neural grafts worldwide. About 100 of them have been implanted with fetal CNS tissue, the remainder with adrenal tissue. In the United States, approximately 130 patients have been treated with adrenal tissue, while fewer than 10 have had fetal tissue implants. The use of fetal tissue for implantation in this country is limited to privately funded ventures because of the moratorium on Federal funding for such research (see app. A).

Surgical Procedures--Implantation of adrenal or fetal tissue into the brain can be done using either a stereotactic or an open surgical approach. In a stereotactic procedure, the tissue is implanted by means of a long needle inserted into the brain through a small hole made in the skull; this procedure is guided by various computer-assisted imaging procedures and brain scans (figure 5-5). In an open surgical procedure, the area where the graft is to be placed is exposed so it can be seen by the neurosurgeon. This procedure, therefore, is more invasive than the stereotactic procedure. In addition, a patient undergoing an adrenal transplant must also undergo surgery to remove one of his or her adrenal glands for implantation.

Most stereotactic procedures are performed under local anesthesia, although general anesthesia is sometimes used. Implantation of tissue using a stereotactic procedure usually takes 1 to 1 1/2 hours. Following surgery, patients are often observed in a neurological intensive care unit overnight in case surgical complications develop. Provided there are no complications, the patient could recover from the operation within 24 to 48 hours and be fit for discharge a few days after that. In the case of experimental procedures, however, it is likely that the patient would be kept in the hospital longer, for observation and for comprehensive testing.

While an open approach is more invasive, it provides more flexibility in the way graft tissue can be implanted. A general anesthetic is given, and a portion of the skull is removed. For grafting in Parkinson's disease patients, the surface of the striatum is exposed by incising the overlying brain

Figure 5-5-Stereotactic Surgery



An X-ray of a patient in a streotactic apparatus.
SOURCE: E.A. Spiegel, *Encyclopedia of Neuroscience, vol.* II, G. Adelman (cd.) (New York, NY: Birkhauser Boston, 1987).

tissue; a cavity is made on the surface of the striatum, and the graft tissue is placed into that cavity. Sometimes the graft is held in place by surgical staples or by a covering of a specially treated material. The piece of bone that was removed from the skull is replaced, and the scalp wound is closed. This operation generally takes 3 to 4 hours. The patient is usually returned to the neurological intensive care unit for observation. In general, recovery from the operation takes several weeks.

When adrenal medullary tissue is used for grafting, one of the patients two adrenal glands has to be removed. This procedure is called an adrenalectomy. Since the adrenal gland lies atop the kidney, its removal requires abdominal surgery. Access to the adrenal gland is obtained through an incision in either the abdomen or the side of the patient. Normally, an adrenalectomy is performed by a surgeon working in tandem with a neurosurgeon, who is simultaneously preparing for the implantation. In the future, techniques such as cryopreservation may make it possible to retrieve the adrenal tissue, preserve it, and then wait until the patient has recovered from the abdominal surgery before performing the grafting procedure.

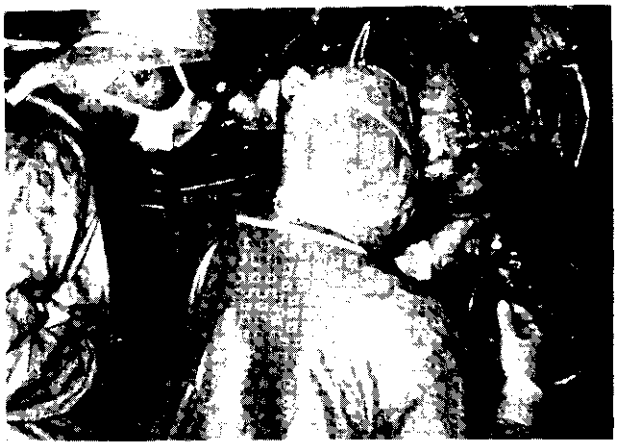


Photo *credit: R.A.E. Bakay, The Emory Clinic*

Adrenal medulla graft surgery.

Thus far, the majority of patients who have received adrenal grafts have had both the adrenalectomy and the brain surgery done at the same time. The procedure is quite debilitating, and recovery takes a long time, often weeks. Patients may also experience the surgical complications that can accompany abdominal surgery and neurosurgery. Based on the results presented by different groups in the United States that have done adrenal grafts, the mortality rate and the rate of complications have each been estimated at 5 to 10 percent (100).

Adrenal Medulla Grafts—Based on the early success obtained in rodent experiments using adrenal tissue, the first grafts of adrenal medullary tissue in humans were performed on two Parkinson's patients in Sweden in 1982; both patients had been experiencing severe fluctuations in the management of their disease by medications (the so-called on-off phenomenon[3](5). In a subsequent study, two additional patients were operated on (97). In all four patients, a stereotactic technique was used to transplant medullary tissue from the patient's own adrenal gland into the striatum on one side of the brain. These patients showed minor improvements in motor function that lasted for about 2 months. By

[3]The on-off phenomenon refers to sudden and unpredictable shifts between periods in which the symptoms of Parkinson's disease are waler control (on), and periods in which they are severe and uncontrolled (off). This occurs regardless of when and how much medication patients aregiven.It is thought that the phenomenon results from the continued progression of the disease. Most patients will eventually experience dose-related fluctuations in their symptoms, but the on-off phenomenon is more rare.

6 months, no positive effects remained. No adverse effects of the procedure were reported. Long-term followup of these patients has not shown any influence of the transplant on the course of the disease (96). In the face of these modest effects and the simultaneous development of the MPTP primate model of parkinsonism, it was widely considered that further animal research was needed before attempting additional grafts in patients.

In 1986, however, a group in Mexico conducted similar grafting experiments on patients with Parkinson's disease. These experiments differed from those done by the Swedish group in that the adrenal grafts were performed using an open surgical procedure and the tissue was placed into the brain in a slightly different fashion. Furthermore, the patients were generally younger and in a less advanced stage of the disease. In 1987, the Mexican group reported dramatic and persistent improvements in the first two patients (102). The surgeons placed several fragments of adrenal tissue into a single cavity on the surface of the striatum on one side of the brain and anchored them with miniature staples. Improvements in muscle movements were seen almost immediately after recovery from surgery, with continued improvement over the next 10 months. In a second report, in 1988, the outcomes for the Mexican group's first 11 grafting patients were described (38). All patients (ages 35 to 65) were said to have improved in gait and writing capability; tremor and rigidity were lessened and movement was improved in most cases; and medication could be reduced in all. The Mexican group has now performed the operation on more than 40 Parkinson's disease patients. Four of the patients have subsequently died (the relation of death to the surgery is not clear); no postmortem confirmation of surviving adrenal cells has been reported.

Despite the discrepancies between the Mexican and Swedish results, the 1987 Mexican report led to a torrent of adrenal medullary operations on Parkinson's patients around the world. Approximately 300 operations using adrenal medulla autografts have been carried out worldwide. In many of these operations, the neurosurgeons attempted to follow the procedure of the Mexican group, with only minor modifications. A detailed observation of 19 patients over a period of 6 months in Chicago, Tampa, and Kansas City has been published (69,1 18). A modest reduction in these patients' off periods (when symptoms are most severe) was found, but the patients were still disabled by the disease. Also, the grafting procedure did not permit dosages of their medications to be decreased. Frequent medical complications were associated with the procedure. A followup study of 7 of the 19 original patients reported that, 12 months after their operations, the overall amount of on time was increased and the severity of symptoms during off time was decreased (71).

Other studies, including one involving a group of 18 patients treated in Nashville (1) and several involving smaller groups of patients (2,94,95, 117,148), have been published or presented in abstract form at scientific meetings. They have reported variable results with the use of adrenal grafts. In an effort to establish a centralized repository for the data collected from these procedures, two registries have been established (see box 5-B). The results collected by each of these registries have been published (6,70), with the data from some centers included in both reports. One registry indicated that there was "a rather diffuse response with a general trend for improvement, but mostly modest improvement" (6) 1 year following surgery, and the other reported a statistically significant decrease in disability, as indicated by an increase in on time and less severe symptoms during off periods 1 year after surgery (70). Neither registry found that it was possible to decrease the medication patients received, and both reported a number of postoperative complications. The complications, which in some cases were transitory (lasting less than 3 months) but in others persisted for as long as a year, included respiratory problems, pneumonia, urinary tract infections, and a number of transitory psychiatric problems, including hallucinations or delusions, sleepiness, and confusion.

Data from both registries clearly show that there are fewer complications associated with removal of the adrenal gland through the patient's side as opposed to the abdominal cavity and by stereotactic placement of the tissue into the brain versus an open neurosurgical approach. Older, more severely affected patients were more likely to have adverse effects and less likely to benefit from the procedure, leading to the conclusion that, based on the data collected thus far, the ideal patient ". . . would be less than 55 years of age and only moderately disabled" (6). The mortality rate according to the data collected by one of the registries was 10 percent, though not all the deaths were thought to

Box 5-B—The Use of Registries To Collect Data

In 1987, in an attempt to document the activity occurring in the field of adrenal-neural grafting, the American Association of Neurological Surgeons (AANS), in collaboration with the National Institute of Neurological Disorders and Stroke (NINDS), established the General Registry of Adrenal-Fetal Transplantation (GRAFT'). Like the National Heart Transplant Registry established in the early 1980s to track the work being done in heart transplantation, the idea behind GRAFT is to provide a central clearinghouse where researchers in the field can send their data so that sufficient information can be collected to evaluate the various methodologies being used. Following the report from Mexico of successful adrenal grafting in persons with Parkinson's disease, a number of centers began trying to replicate the results. These studies usually involved small numbers of patients and used various modifications in the surgical technique. It was thought that, by pooling data from the various sources, an ongoing analysis could be performed . . of the evolving transplantation experience to document the potential risk factors and to guide future studies" (Bakay et al., in press).

At about the same time that GRAFT was established, the United Parkinson Foundation (UPF) announced formation of its own registry. In order to avoid duplication and competition between the two groups, it was decided that the focus of the two registries would be different. GRAFT would focus on safety, efficacy, and surgical issues by collecting data from as many centers as were willing to participate. The UPF registry would collect data from a smaller, more uniform group of investigators. Due to different methods of data acquisition, the UPF would be able to make a more quantitative evaluation of efficacy, as opposed to GRAFT, which would have to rely on qualitative measures of efficacy. As a result of this arrangement, some research teams have contributed data to both registries.

As of mid-1990, the GRAFT registry had collected data on 135 patients from 24 centers. Most are from the United States, with two centers each from Canada and South America and one from Europe and Mexico. The database includes preoperative information on demography, medical history, and prior treatment of parkinsonism, as well as qualitative evaluation of patients' responses to the procedure and complications at 3, 6, and 12 months after the operation. Followup information more than 1 year later is currently being collected.

The UPF registry has collected information on 61 patients from 13 centers in the United States and Canada. The same kind of demographic information was collected, but the pre- and post-operative data were collected in a more uniform manner: All the centers were asked to analyze the patients and their responses to the surgical procedure using the same rating scale. This made it possible to compare and evaluate the data from all the centers more easily. These data were collected 12 months tier the operation,

The l-year data from both registries have been published and both registries are continuing to collect information. Registries are an effective way of collecting and comparing information derived from a variety of sources in order to evaluate progress in the development of a surgical procedure. Their success depends on the willingness of investigators to supply their data and in some cases to comply with requests to collect data using standardized methods. When these criteria are met, data from registries are invaluable guides for future investigations.

SOURCES: R.A.E. Balmy, G.S. Allen, M.L.J. Apuzzo et al., "Preliminary Report on Adrenal Medullary Grafting From the American Association of Neurological Surgeons GRAFT Project," *Progress in Brain Research, in* press; C.G. Goetz, G.T. Stebbins, H.L. Klawans et al., "United Parkinson Foundation Neurotransplantation Registry Multicenter United States and Canada Database Presurgical and 12-Month Followup," *Proceedings of the Third International Congress of Neurotransplantaion: Cambridge (Amsterdam:* Elsevier Science Publishers, 1989).

have been due to the surgical procedure (70). The overall level of improvement and number of complications observed led to this summary of the data collected by both groups:

> This strongly suggests that there is an effect from the transplantation, although the effect inmost cases is not very dramatic. The mild-to-moderate improvement would be more enthusiastically received if the surgical morbidity were less (6).

The original Swedish results also inspired a series of adrenal medullary autografts in Beijing, China, beginning in 1986. The Chinese group used a stereotactic technique similar to that of the Swedish group, but it used a larger total mass of implanted tissue and administered antiparkinson drugs after the operation. Substantial improvements in movement and lessening of rigidity and tremor were observed in the first four patients for at least 6 months after surgery. To date, this group has operated on at least 10 patients, with claims of varying degrees of improvement in all cases (84). However, some scientists believe that it is difficult to interpret and compare the results of this study and others (60,143)

due to differences in the methods used for assessing benefit to patients and in the medical treatment they received pre- and post-operatively.

Adrenal medullary autografts have also been performed in the United States in a small number of patients with progressive supranuclear palsy (PSP), a neurodegenerative disorder marked by loss of neurons at various locations in the brain, including the substantial nigra. PSP, though much less common than Parkinson's disease, has many of the same symptoms, including difficulty in moving and standing up. The disease is invariably fatal and there are no satisfactory pharmacological therapies. One report (87) described three patients with PSP who had tissue from one of their adrenal glands grafted into the striatum. Only one of the three patients showed a small degree of improvement, mostly in his ability to stand. The authors conclude from these data that "adrenal medullary transplantation has only limited efficacy in progressive supranuclear palsy.'

To date, the success reported by the Mexican group in 1987 has been difficult to replicate. The reason for this is unclear. Questions about diagnostic and other criteria used for inclusion of patients, the methods used to measure the severity of the disease preoperatively, the handling of the patients' medications, and the methods used to quantify the improvements in movement that were observed have been raised about the Mexican study.

Based on the available data, the efficacy of adrenal medulla autografts is unclear. The relative effectiveness of the various open and stereotactic procedures used, their modes of action, and the existence of surviving, functional adrenal medullary cells have not yet been conclusively established. The efficacy of adrenal grafts has yet to be demonstrated in experiments using nonhuman primates, and the human clinical data are inconsistent, with several studies suggesting that there may be only minimal, perhaps transitory, improvement. Judging from animal experiments and the small amount of postmortem human data available, the ability of adrenal tissue to survive in the brain seems to be limited (80,120). Survival may be improved by concurrent administration of growth-promoting factors, but this needs to be explored further in the laboratory. If adrenal tissue does not survive, it is unclear whether any observed improvement is the result of the graft or of nonspecific effects. The observation made in many of the clinical trials that improvement was

Photo credit: R.A.E. Bakay, The Emory Clinic

Dissecting tissue to be used as a graft.

seen on both sides of the body when the graft was placed in only one side of the brain also suggests a general effect, perhaps circulation of graft-related factors in the cerebrospinal fluid, rather than a direct action of the graft. Additional animal experimentation can provide more information and help answer these questions. Regarding the use of the procedure in humans, the adrenalectomy and open neurosurgical procedures most widely used are associated with risk of severe complications in older, more profoundly afflicted patients. Also, the best age for donor tissue and a clarification of which Parkinson's patients are most likely to benefit fromthe procedure needs to be delineated.

Fetal Neural Tissue-Only about 100 persons worldwide have received grafts of fetal CNS tissue for the treatment of Parkinson's disease. Clinical trials with such tissue are going on in Sweden, England, Mexico, the People's Republic of China, Czechoslovakia, Spain, and Cuba. Approximately 10 patients have undergone the procedure in the United States. While the results of some of these procedures have been reported at various scientific and medical meetings, there are very few published

Some of the most thoroughly documented research has been conducted in Sweden, where two patients underwent this procedure in 1987 (100).

Dopamine-producing tissue from four human fetuses, 7 to 9 weeks (postconception) of age, was implanted as a cell suspension, stereotactically, on one side of the brain in each patient. Both patients received drugs to keep the graft from being rejected. Up to 6 months after the operation, although both patients showed some minor improvement in how quickly they could move and how well they responded to a single dose of antiparkinson drugs, the scientists reported that ". . . no major therapeutic effect from the operation was observed" (96). This same research group reported a greater degree of improvement in another patient, for whom they used different methods of handling and implanting the fetal tissue and thus may have increased the ability of the graft to survive (99). This patient exhibited a lessening of his symptoms and an increased response to his medications for the 6 months he was observed after the surgery.

Other positive results have been reported from groups in Mexico and England, but these reports have not been in the form of published papers in scientific journals providing information on how the procedure was performed, what criteria were used for assessing patients pre- and post-operatively, and giving all the details necessary to analyze the results.

In the United States, observations of a patient who was operated on in Colorado in November 1988 were reported 15 months after the operation (50). In this case, drugs to prevent graft rejection were not used. Some improvement in movement and motor coordination was observed in this patient, and the patient has reported increased ease in doing daily tasks (cutting food, brushing teeth, and so on). Also, the patient's medication could be decreased without any adverse effects. While the investigators involved state that the patient is not cured, they believe that the implants can reduce disability, smooth out medication-related fluctuations in symptoms, and increase mobility.

The limited data collected to date suggest that fetal CNS grafts may have a beneficial effect in controlling some of the symptoms of Parkinson's disease. However, more information must be derived before an ultimate determination of the efficacy of this procedure can be made. How long the grafted tissue survives, the optimal placement of tissue in the striatum, and the time course for seeing an improvement are unknown. Additional animal research can answer these questions. Then the details of how much tissue is needed to achieve a worthwhile therapeutic effect and the susceptibility of the grafted tissue to immunological rejection and to the underlying destructive mechanism of Parkinson's disease can be derived from human experimentation. As stated by the Swedish research group:

> Although our findings support the idea that neural grafting can be developed into an effective therapy in Parkinson's disease, further work is necessary to optimize the transplantation procedure. . . (99).

What Is the Next Step?

Whether clinical neural grafting experiments should continue before additional data are gathered from animal experiments is still a subject of scientific debate (93). In the case of adrenal tissue grafts, the lack of replication of successes and the lack of a solid basis in animal experimentation have made many persons in the medical and scientific communities retreat from the rush of enthusiasm that accompanied the initial reports.

The effectiveness of fetal tissue grafting in animal models and the initial reports of some success with its use in humans indicate that fetal tissue grafting may have a more solid scientific basis than adrenal grafting. The question that now arises is how to proceed from here. Many persons in the scientific and medical communities feel that the questions concerning fetal CNS tissue grafting can best be answered with additional basic animal research, coupled with limited human experimentation. Then expanded human trials could proceed to determine whether fetal CNS grafts area genuinely efficacious treatment for persons with Parkinson's disease. This evaluation has been expressed in the following statement by two prominent scientists:

> . . . too many questions remain unanswered about the use of embryonic nerve cells to propose anything more than limited fetal grafting in humans as a requisite next step. Although scientifically it seems logical to proceed, considerable information is needed before therapeutic success might be predicted . . . . We are still at a stage at which basic scientific studies are needed to elucidate important details. . . (60).

When clinical experiments do proceed, it will be necessary to standardize their designs, the criteria used for assessing pre- and post-operative levels of disability, and the methods of reporting results. For the most part, this has not been done in the clinical experiments carried out so far, making it difficult to interpret and compare studies.

Beyond the scientific issues, the legal and ethical controversies that surround the use of fetal tissue could impede the initiation of clinical trials. In the United States, the Federal ban on funding of transplants using human fetal tissue from induced abortions will limit clinical research (see app. A).

Huntington's Disease

Huntington's disease is a genetic disorder, the major pathological hallmark of which is a loss of neurons in the striatum. Symptoms of the disease include abnormal movements, especially of the face and extremities, and a progressive deterioration in mental ability. Unlike Parkinson's disease, where only one population of cells dies, Huntington's disease involves the destruction of several populations of neurons, generally located in the striatum, that contain different neurotransmitters. An animal model in which chemicals known as excitotoxins are used to destroy the cells in the striatum[4] has been employed to see whether implantation of fetal striatal tissue could reverse some of the deficits caused by the injury. This animal model exhibits some of the same characteristics as persons suffering from Huntington's disease (e.g., abnormal movements, inability to respond to sensory stimuli, and deficits in learning and memory). In most of these experiments, grafts have been inserted in hopes of replacing the lost striatal neurons rather than supplying a single neurotransmitter, as in the Parkinson's disease research.

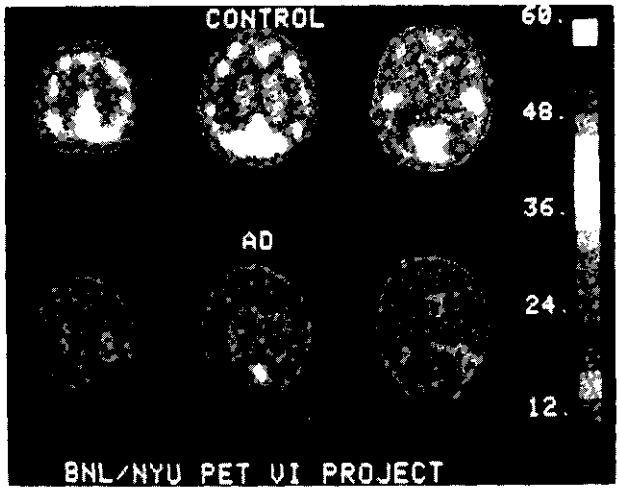
Studies have shown that rat fetal striatal tissue can survive following implantation into the striatum of adult rats that have been injected with excitotoxins (114). These grafts have also been shown to decrease some of the abnormal movement (36,66,81,129) and learning and memory deficits (35) produced in this model; however it has been reported that the animals' behavior is not returned to normal (114).

The mechanism by which the fetal tissue grafts act is not entirely clear. There are two possibilities. First, the grafted tissue may grow into the host brain, forming synapses and thus directly and actively play a role in the function of the striatum. Experimental evidence to support this includes the need for an intact, undamaged graft (66) at a specific region of the striatum in order to achieve a functional effect (82) (indicating the importance of graft integration into the host), coupled with the observation of an interaction between host and grafted tissues (128,147). The second possibility is that the grafted tissue could be exerting a growth-promoting effect that causes the undamaged parts of the host striatum to grow and assume some of the fictional activity of the damaged parts. Experimental evidence supporting this idea includes the conflicting observations that integration does not occur (101,146) but a functional effect may nevertheless take place (113) and that fetal grafts may be able to protect against excitotoxins (145) [although this has not been conclusively proven (11 l)]. Although the majority of the evidence indicates that grafts do integrate into the host, the data preclude an unequivocal statement. The possible role of fetal striatal tissue in the treatment of Huntington's disease must await further elucidation of both the graft's mechanism of action and the underlying mechanism of the disease itself.

There has been one report, not published in a research journal, that grafting of rat adrenal medulla tissue into the rat striatum can protect neurons against the destructive action of excitotoxins (132). Since one theory holds that excitotoxins may be involved in Huntington's disease, adrenal tissue could possibly play a role in treating this disorder. Despite the lack of conclusive evidence to support this hypothesis, a patient with Huntington's disease has received an adrenal medulla autograft (132). It was reported that the patient received no beneficial effect from the procedure. At this time, there is no evidence to support the idea that adrenal tissue can ameliorate any of the symptoms associated with Huntington's disease: the role of excitotoxins in the disorder has yet to be clarified, and the effect of adrenal tissue on the action of excitotoxins has yet to be proven.

[4]In this model, excitotoxins (kainic acid, ibotenic a@ quinolinic acid) are injected directly into the striatum. These toxins stimulate sites 011 neurons that are normally activated by certain neurotransmitters; however, these acids activate the cells so much (hence the name "excito") that they kill the cells. Whether these excitotoxins are actually involved in Huntington's disease in humans is unclear.

Figure 5-6-Brain Activity in an Alzheimer's Disease and a Normal Brain



Brain scans showing the activity (white areas) in a normal brain (control) and the brain of an Alzheimer's disease patient (AD). The scan of the Alzheimer's disease patient shows that many areas in the brain are not functioning properly.
SOURCE: R. Restak, *The Mind* (New York, NY: Bantam Books, 19S8).

Alzheimer's Disease

Unlike Parkinson's or Huntington's diseases, in which there is either a loss of a specific population of neurons or localization of cell destruction in one region of the brain, Alzheimer's disease involves a much more diffuse loss of neurons and affects a number of different groups of cells (figure 5-6). Cells in the front of the brain that contain the neurotransmitter acetylcholine and cells in the middle of the brain that contain norepinephrine are particularly affected in Alzheimer's disease. The acetylcholine-containing areas are involved with memory and learning, while the norepinephrine-containing regions are thought to be associated with controlling moods. Destruction of these areas results in the dementia and depression that are characteristic of Alzheimer's disease. Due to the diffuse nature of the damage in Alzheimer's disease, it is thought that if neural grafts have a role, it maybe to supply growth-promoting substances or to supply lost neurotransmitters rather than to replace lost structures.

At present there is no fully satisfactory animal model of the symptoms of Alzheimer's disease. The closest is aged monkeys, which can exhibit some of the memory and cognitive deficits or abnormal changes in brain cells, or both, seen in persons with Alzheimer's disease (122). To date no neural grafting experiments have been conducted on such monkeys; however, experimentation has been conducted on aged rats and rats that have had injuries made in the same areas of the brain that are destroyed in Alzheimer's disease. These animals exhibit some of the same types of memory and learning problems seen in humans with the disease, even though they do not truly mimic Alzheimer's disease.

To create animal models, wounds are made in some of the same areas of the front or middle part of the brain that are destroyed in Alzheimer's disease or in the pathways that connect these areas to other parts of the brain.[5] The result is that the animals have difficulty learning and remembering certain tasks, such as running through a maze. When rat fetal tissue is grafted into wounded adult rats near the areas to which the acetylcholine fibers project, the grafts take hold and there is a partial improvement in the rats' ability to learn and remember specific tasks (42,43,48,86,150). This occurs if the fetal tissue implanted is taken from areas in the fetal brain that correspond to the wounded areas in the adults. Similar results have been observed in adult rats when either grafts of fetal tissue from mice (32) or cultured cells that naturally produce acetylcholine (89) were implanted.

The ability of acetylcholine-producing grafts to reverse memory deficits caused by the ingestion of alcohol has also been examined(4). Large quantities of alcohol cause brain damage in rats, as in humans, especially in the regions of the cortex and hippocampus containing acetylcholine; this damage causes memory and learning deficits. When acetylcholine-producing tissue from the front part of the brain is implanted into either the cortex or the hippocampus of rats given alcohol, the deficits decreased. This does not occur when other tissue, not containing acetylcholine, is grafted. It is postulated that the positive effects are due to the increased supply of acetylcholine into the cortex and hippocampus.

[5]The acetylcholine-containing cells in the front part of the brain send their fibers to the hippocampus and specific parts of the cerebral cortex. Both the hippocampus and these cortical areas are very important in mediating learning and memory. The norepinephrine-containing neurons in the middle of the brain also connect to cortical areas. The wounds result in the loss of these acetylcholine or norepinephrine pathways and the cells giving rise to them.

Aged rats are also known to have more difficulty learning certain types of tasks than younger animals. Implantation of acetylcholine-producing fetal tissue into the hippocampus (56,57) or norepinephrine-containing fetal tissue into 'the middle of the brain (30) improves the performance of some aged rats on certain learning and memory tests.

It is thought that the grafts in these studies exerted their effect by releasing acetylcholine or norepinephrine into the brain. While the research conducted to date has provided invaluable information about the ability of neural grafts to decrease experimentally induced deficits in cognitive performance and deficits in learning and memory in aged rats, the extrapolation of that information to patients with Alzheimer's disease is problematic. These models share, at best, only a few characteristics of the disease. In addition, when injuries are produced in experiments, they are very specific to the area that is damaged, whereas in Alzheimer's disease the damage is not restricted to a single group of neurons.

Since acetylcholine-producing cells are one of the major populations of cells affected in Alzheimer's disease and nerve growth factor (NGF) is known to act on acetylcholine neurons, investigators have studied the ability of NGF to protect these cells. The implantation of cells that have been genetically engineered to produce NGF has been shown to prevent the acetylcholine neurons in the front part of the brain from dying following destruction of the pathway that connects them to the hippocampus (127) and other areas of the brain (45). In addition, the protected cells apparently started to grow new fibers in the direction of the implanted NGF-producing cells. Whether NGF can affect the progression of Alzheimer's disease is a question being investigated extensively.

A number of important points must be addressed before the potential value of grafting therapy in Alzheimer's disease can be determined. A better understanding of the mechanisms underlying the disease is needed in order to determine the types of graft tissue likely to have the broadest possible effect on the disease. Also, development of a valid animal model of the disease or its symptoms is necessary.

Motor Neuron Disease

Motor neuron disease (MND) is a family of disorders marked by degeneration of neurons located in the spinal cord and brain that are involved with regulating movement. Probably the best known MND is amyotrophic lateral sclerosis (Lou Gehrig's disease).

As in some other neurodegenerative disorders, such as Parkinson's and Huntington's diseases, the hallmark of MND is a selective loss of certain populations of cells. Since Parkinson's and Huntington's diseases are thought to be candidates for symptom amelioration by neural grafting, it can be speculated that persons with MND may also benefit from this procedure. The same strategy of replacing the lost cell populations with grafts may be applicable. The ability of fetal spinal motor neurons to become incorporated into the spinal cord of an adult has been demonstrated in rats (134) and mice (37). To date, no research on the ability of neural grafts to restore functional deficits caused by induced injuries to these cell populations has been conducted. Whether MND would be at all amenable to this therapeutic strategy is purely conjectural. The fact that the populations of cells lost in MND are distributed throughout the spinal cord and the brain may be a hindrance to the effective use of implants. It is also possible that the mechanisms involved in the development and progression of MND are such that graft technologies would not be suitable.

CENTRAL NERVOUS SYSTEM INJURY

Spinal Cord Injury

Injury **to the** spinal cord occurs most often when the bones of the spinal column (vertebrae) are pushed into the soft neural tissue of the spinal cord, bruising or tearing it. This ensues when a strong, rapid, mechanical force is applied to the back or neck, as can occur in automobile collisions or sporting injuries. Incidents such as these are the leading cause of spinal cord injury. Damage to the spinal cord can also be caused by a projectile or knife wound. In such cases the amount of tissue damaged is directly related to the strength of the force applied. A third cause of damage to the spinal cord is disruption of its blood supply or gradual compression (caused by various types of intruding bodies, such as tumors or blood clots).

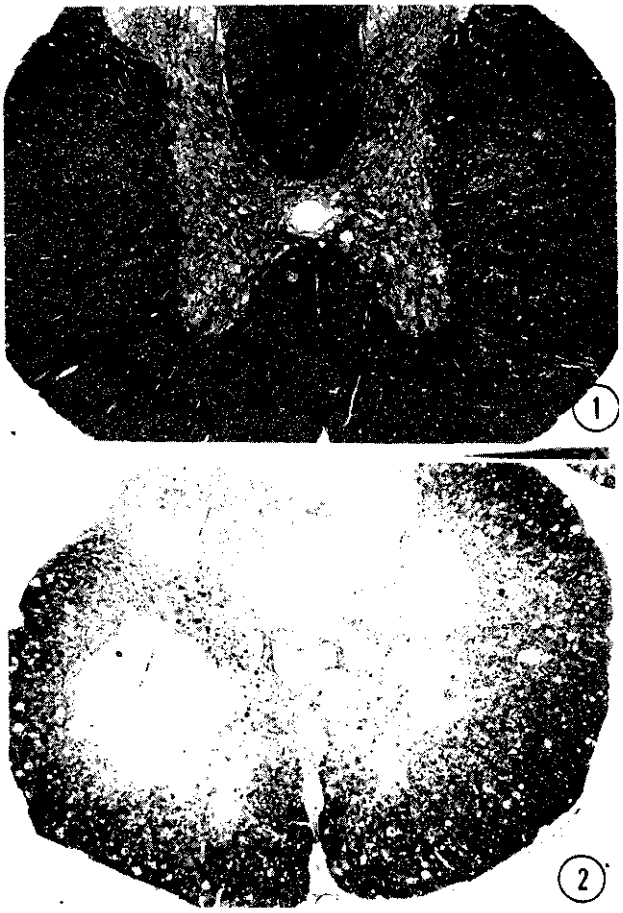


Photo credit: P.J.Reier, University of Florida

A cross-section through a normal(1) and an injured (2) rat spinal cord.

The spinal cord is made up of a central core of neuronal cell bodies surrounded by collections of axons. These axons make up the long pathways that connect the spinal cord and brain and the shorter pathways that connect areas within the spinal cord. Injury to the spinal cord, whatever the cause, can destroy both axons and neurons located in the damaged region. In addition, neurons that are located away from the injury site, whether higher up in the spinal cord or in the brain, and that contribute to the damaged pathways can die as a result of the injury (retrograde cell death). The functional deficits associated with spinal cord injury range from paralysis (loss of the power of voluntary movement in the extremities) and loss of sensation to the loss of reflex activities coordinated from within the spinal cord (motor reflexes and reflexes associated with sexual activity and bladder control). In most cases, these functional deficits are permanent.

Unlike the case in neurodegenerative diseases, in spinal cord injury the original neurons are mostly present, but damaged. Repair of the long ascending and descending nerve fibers that convey movement and sensory information between the brain and spinal cord would require regrowth of these fibers to reconnect with their original targets. Repair of the short pathways within the spinal cord would involve providing a means by which the intrinsic spinal cord connections could be reestablished.

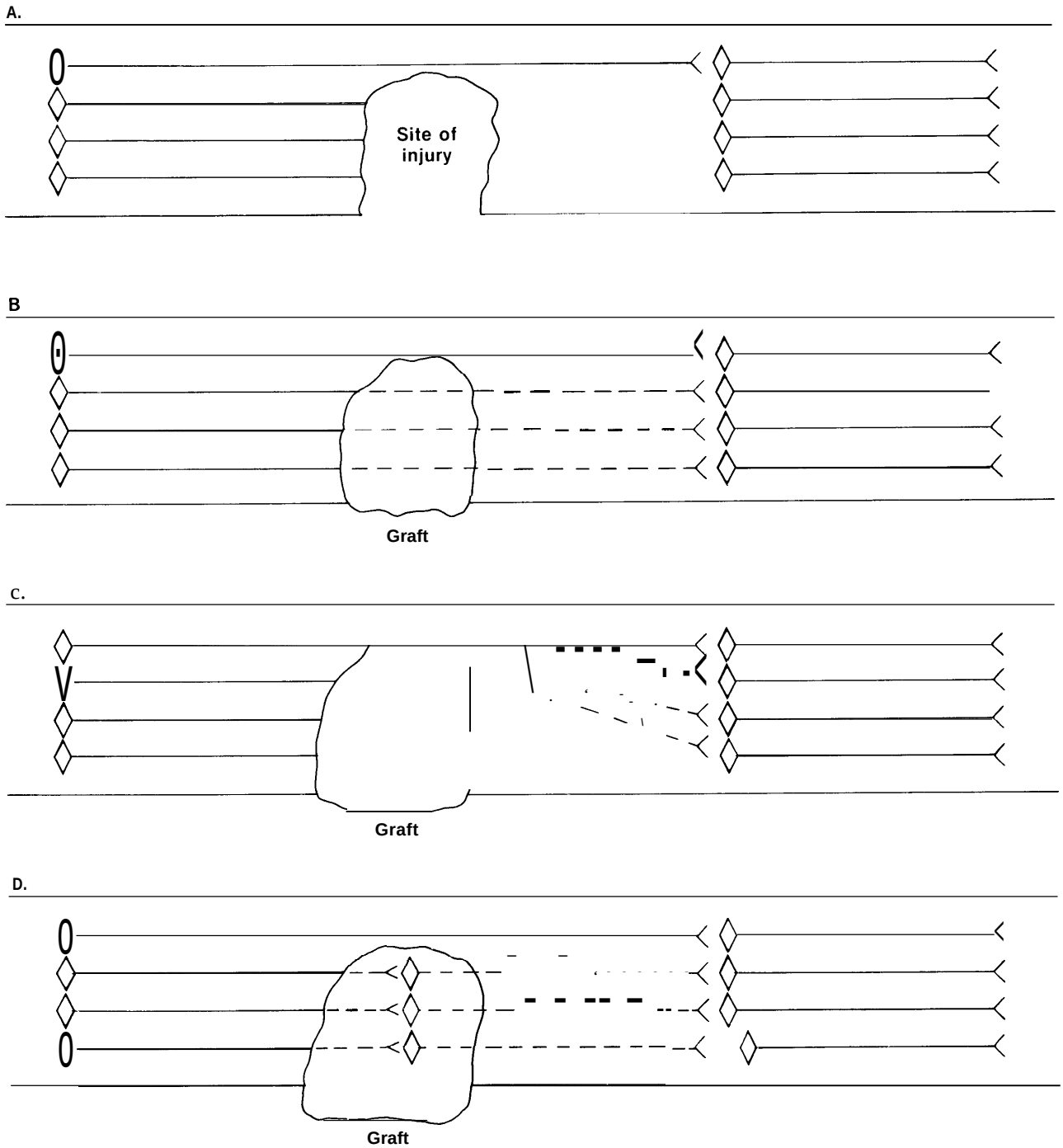
The goal of neural grafting in spinal cord injury is to repair or substitute for the damaged neuronal pathways to induce recovery of function. The aim is to achieve fictional recovery by reestablishing original synaptic contacts by the damaged axons; stimulating new outgrowth from uninjured neurons; or introducing new circuitries that would transmit functional information through the site of injury (figure 5-7). If growth of damaged neurons were to be stimulated, the grafts would serve as a substrate to induce the severed axons to reestablish their lost connections, thus regenerating the original anatomical structures. If undamaged neurons were to be stimulated, then the grafts would induce new growth from them to create alternative pathways, which would assume the role of the damaged pathways and restore functional activity without necessarily replicating the original anatomical connections (13). The third possibility is to construct a functional relay between separated regions of the spinal cord. ,

The questions to be addressed in determiningg the ultimate therapeutic role of neural grafts in spinal cord injury include:

- What grafting material will best integrate with the host spinal cord and create the environment most conducive for growth?
- Is there functional recovery following grafting?

To date, most research in this area has provided information regarding the first question; more basic research is needed to answer the second question. Scientists use a number of different types of experiments to study the mechanisms of regrowth in the damaged spinal cord. Some of the experiments closely approximate conditions that would be encountered in a human spinal cord injury. However,

Figure 5-7-Possible Actions of Neural Grafts in Spinal Cord Injury



Theoretically, neural grafts could repair the damage caused by an injury to the spinal cord (A) in three ways: B) stimulate regrowth from damaged axons; C) stimulate new growth from uninjured axons; D) provide a relay that would transmit information through the site of injury.
SOURCE: Office of Technology Assessment, 1990.

since different types of injuries can result in a wide range of insults to the spinal cord (the damage done in an automobile collision may be quite different from what occurs in a diving injury), no single model can be used to study spinal cord injury. The development of analogous animal models is a crucial step in exploring the usefulness of neural grafts in spinal cord injury.

What Makes a Good Graft?

Several sources of grafting materials have been examined in animal experiments. These include adult peripheral nerve and fetal CNS tissue, either in pieces or as cell suspensions. In general, autografts and allografts have been used in these experiments. The use of tissue from other species has not been widely examined; however, there is some evidence suggesting that such tissue is not as suitable as the others (142).

Peripheral Nerves—Nerves from the peripheral nervous system (PNS) are used to bridge a wound. They enable axons to cross the wound, reenter the far side of the spinal cord, and reinnervate their original targets. Over the past decade, some success has been achieved in implanting segments of peripheral nerves into the spinal cords of animals with experimentally induced injuries; these grafts have survived and become incorporated into the host spinal cord (126,130). The grafts support growth from the damaged tissue, but the extent to which the elongating axons can emerge from the graft and extend back into the host tissue is extremely limited (34,126).

While the ability of peripheral nerve grafts to induce growth in the injured spinal cord of experimental animals has been confined, the ability of these grafts to restore function has yet to be demonstrated.

Fetal Spinal Cord Tissue—Another major source of grafting material in many animal experiments has been fetal spinal cord tissue, usually from the same species. Given its ability to support growth, fetal tissue could act as a bridge across a wound or as a relay station between the severed axons and their target sites on the other side of the wound by providing a system of intervening neurons. Research into the use of this tissue does not have as long a history as that into peripheral nerve grafts. Experiments have shown that fetal tissue can create an environment that supports limited growth in the damaged adult spinal cord. Also fetal tissue can sometimes limit the scarring that occurs following an injury (and that can act as a barrier to growth) (77) and prevent retrograde cell death (26).

One interesting model has demonstrated the effectiveness of fetal spinal cord grafts in promoting growth at the boundary of the central and peripheral nervous systems. The fibers conveying sensory information from the body (e.g., sensing when the bladder is full) into the spinal cord are called dorsal roots. These fibers travel in both the peripheral and central nervous systems and traverse the boundary between the CNS and PNS when they enter the spinal cord (see figure 3-5). In spinal cord injuries the dorsal roots are sometimes torn. When damaged, these fibers will regenerate in the periphery until they reach the PNS-CNS boundary at the spinal cord and then stop growing. This phenomenon clearly demonstrates the ability of fibers to regrow in the peripheral but not in the central nervous system. Animal experiments have shown that if fetal spinal cord tissue is placed where the dorsal root enters the spinal cord, the severed root will continue to grow into the implant (133,144) and in some cases, to a limited extent, through it and into the host spinal cord (78). Fetal grafts, then, can support regeneration of these specialized peripheral-central fibers within the CNS and, to a certain degree, overcome the barriers present at the PNS-CNS boundary.

Fetal spinal cord implants can also support growth of CNS axons in an animal that is still growing and developing (24,25). In the newborn rat, axons from the brainstem are still growing into the spinal cord. If the spinal cord of a newborn rat is cut, the growing axons cannot reach their targets, and in many cases the neurons in the brain that send fibers into the spinal cord die. When fetal spinal cord tissue is placed into wounds made in the spinal cords of newborn rats, the tissue supports the continued development of the growing spinal cord and prevents neuron death in the brain. Although these studies clearly show that fetal spinal cord grafts can facilitate survival and regrowth of CNS axons in the immature, growing spinal cord, they do not indicate whether fetal spinal cord grafts would have any effect in the fully formed, adult spinal cord.

The ability of fetal spinal cord grafts to become integrated into the adult spinal cord in rats has been demonstrated (77,125). Fibers from the host spinal cord can grow into the grafts; however, the outgrowth from fetal grafts, whether by spinal axons

Photo credit: B. Bregman, Georgetown University

A cross-section through a rat spinal cord with a neural graft of rat fetal tissue.

transversing the graft or by neurons from within the graft sending their axons into the host spinal cord, is very limited. This again demonstrates the inability of axons to grow in the environment provided by the mature CNS.

Neurotrophic and Neurotropic Factors—The mechanism by which peripheral nerves or fetal tissue induces growth of damaged axons in the spinal cord is thought to involve certain chemical factors (see box 3-A). Neural grafting into the spinal cord may owe its success thus far to the insertion of tissues that contain the correct mix of these chemicals at the injury site, establishing the proper biochemical environment to support regrowth of the damaged axons and to allow reestablishment of lost synaptic contacts. Providing the proper chemical messengers to turn the various components of the regeneration system on and off may be a crucial element in the repair process. As more is learned about neurotrophic and neurotropic factors and the processes by which these substances regulate growth in the nervous system, abetter understanding of exactly what is necessary to produce healing can be determined.

Fetal Brain Tissue—Another grafting strategy that is being investigated involves the use of tissue from areas in the fetal brain that are known to regulate activity in the spinal cord. When the spinal cord is injured, descending fibers (originating in the brain and containing a specific class of neurotransmitters called catecholamines) are damaged. It is known that these fibers modulate the activity of local centers in the spinal cord which control certain

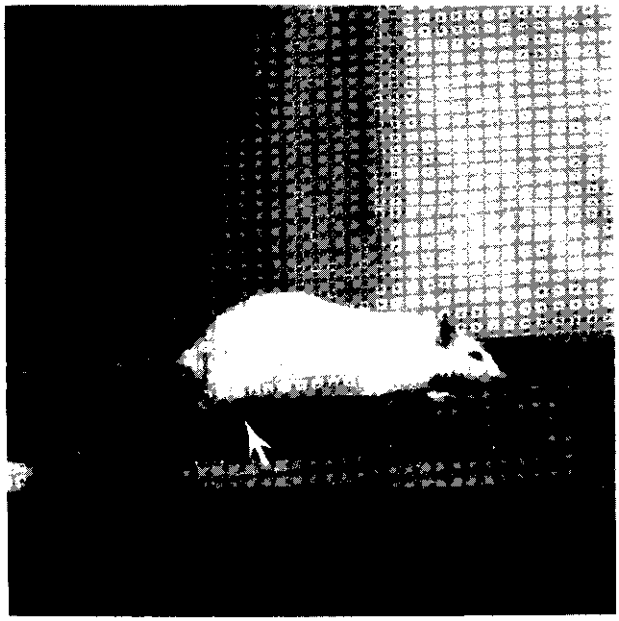


Photo credit: B. Bregman, Georgetown University

A rat being tested for the effects of a spinal cord injury. The rat has difficulty walking across the grid (arrow).

patterns of movement. There has been some investigation of whether these grafts could replace the lost catecholamines and regulate activity in the affected regions of the spinal cord. Grafts of fetal tissue from the relevant areas of the brain, implanted either as solid pieces (115,1 16) or in a cell suspension (18,123), become integrated into the host spinal cord and send fibers containing the catecholamines into the surrounding tissue.

Is There Functional Recovery?

The most important question regarding these techniques as they relate to possible clinical applications is whether there is any functional recovery from an injury after grafting. None of the studies previously discussed examined whether the grafts restored functions that were lost as a result of the injury. Most research to date has been aimed at determining and establishing the conditions most conducive to a successful implant and the degree to which the graft can integrate with surrounding host tissue. As a result, information regarding functional recovery following grafting is meager.

One study showed that grafting fetal rat spinal cord tissue into a small wound made in the spinal cords of adult rats decreased the difficulty in walking that the animals normally would have shown following such an injury (14). Significantly,

when the spinal cords of the animals that had received grafts were examined, it was seen that there had been very little regrowth of the severed fibers. The authors of the study concluded that the beneficial effects were probably" due to the growth-promoting factors in the grafts stimulating new growth from surviving neurons. They speculate that the surviving neurons assumed control of the lost functions by establishing new pathways that bypassed the injured area.

Injection of a cell suspension of rat fetal brain stem cells into the spinal cord can enhance leg reflexes in rats in which catecholamine-containing neurons have been destroyed (27). The catecholamine-containing cells in the suspension integrate into the spinal cord and establish new synaptic contacts with the spinal cord cells. It was hypothesized that some of the restoration of the leg reflexes was due to the development of new catecholamine synapses, not regeneration of damaged cells.

In neither of these studies was the functional recovery observed thought to be due to regrowth of the damaged neuronal pathways at the site of the wound. In the first study, neurotrophic factors were thought to be involved, while in the second, the enhancement of reflexes was due partly to an increase in catecholamine synapses and partly to other, unknown factors.

Some investigators have found evidence of recovery of function related to reconstruction of the neuronal pathways at the siteofawound(91). Grafts of fetal spinal cord tissue placed into the growing spinal cords of newborn rats lessen the deficits in walking that occur when the spinal cords of these animals are injured. Since neural grafts in newborns allow the growing spinal cord to assume a nearly normal circuitry, it has been proposed that the recovery of function is due to this rewiring of the spinal cord at the site of the injury by the still-growing spinal cord.

Questions That Need To Be Answered

Neural grafts may be able to ameliorate deficits caused by spinal cord injury; however, it is difficult to predict if and when these technologies will enter the clinical research arena. Animal experimentation in this area is yielding a wealth of information concerning basic biological mechanisms involved in growth and development of the nervous system, how it responds to injury, and the feasibility of grafting tissues into the site of a spinal cord injury. A number of questions remain to be answered before it can be known what clinical application, if any, this research will have.

Can Grafted Biologic Material Exert a Healing Effect on the Damaged Spinal Cord?—Implicit in this question is the notion that the spinal cord has no capacity to heal-once it is damaged, it is damaged forever. Evidence to date shows that this is false. The normal response of the spinal cord to injury indicates that it has some regenerative capacity. If this capacity, coupled with the mechanisms controlling growth in the immature spinal cord, can be harnessed and manipulated, then some form of healing can be achieved.

Can the Healing Effects of a Graft Restore Function?--The ability of grafted material to become incorporated into the damaged spinal cord serves no clinical purpose in itself. The ultimate clinical goal of grafting is to restore function. The requirements for restoration may differ from function to function. For simple functions, such as awareness of when the bladder is full, it may be possible to restore the necessary sensory input by reconnecting the dorsal root sensory nerves that convey this information to an intact area of the spinal cord. The graft would enable the sensory fibers to cross the PNS-CNS boundary and would allow the message that the bladder is full to enter the nervous system. While this may not restore complete motor control, the patient would be able to tell when it was time to empty the bladder.

In the case of more complex functions under the control of the spinal cord circuitry (blood pressure and temperature regulation, sexual reflex activity, bladder control, motor reflexes), reestablishment of function may require bridging the wound to reconnect the spinal cord control centers back into the reflex pathways or to reinstate the higher level control of these centers. The small degree of outgrowth from grafts that has been observed in many experiments may be sufficient for bridging spinal cord wounds. If the control centers themselves are damaged, then grafts would have to provide new cells to reform them. This would require a more complex interaction than just bridging an injury site. Restoration of the motor and sensory deficits that result in paralysis would require an even more comprehensive mending process, one that would initiate regeneration or generation of

pathways that could mediate the activity necessary for these functions.

The more complex and coordinated a function is, the more complex the type of repair needed to reinstate it. Exactly how much repair is necessary to restore a given function is not known. It is known from animal models that, even if only a small percentage of fibers is spared at the time of injury, there can be marked recovery of function. It may be that reestablishment of only a small proportion of connections, either by direct reconstruction or by stimulation of new pathways, may be sufficient for significant fictional recovery. In order to realize this recovery, it may be necessary to follow the reconstructive surgery with extensive rehabilitative therapy.

What Characteristics of the Graft Are Necessary for Functional Recovery?—The processes set in motion by implantation of neural tissue must be understood before this technology can be used as a therapeutic tool. At the present time, grafting into the spinal cord is analogous to inserting a "black box.' Very little information is available as to what causes the observed morphological changes and functional effects. Is an integrated, solid piece of tissue necessary to supply the proper mixture of neurotrophic factors to the right location and in the right form to induce growth? Or is it sufficient just to supply the growth-promoting factors alone? Is a mixture of the two needed? Do these needs differ depending on what type of functional recovery is desired? The answers to these questions are still being sought.

What Are the Possible Unwanted Effects of Grafting Into the Spinal Cord?—It is possible that inserting tissue into the spinal cord or stimulating growth from the damaged host tissue could cause abnormal pathways and connections to be formed. This could result in unwanted effects, notably the development of abnormal motor functions, such as muscle spasticity and increased reflex actions, or pain or other discomforting sensations (e.g., burning, tingling).

Brain Injury and Stroke

Since grafts may serve to replace lost or damaged tissue or to stimulate growth from damaged areas, it is possible that neural grafts could restore functional losses caused by injury to the brain. Animal experiments have demonstrated that fetal tissue can survive and become incorporated into a wound made in the brain of an adult (e.g.,33,72,110). This includes tissue damage caused by inducing a stroke in the experimental animal (usually rats) (104,109). The stroke is caused by clamping one of the vessels that supplies blood to the brain, thus mimicking what happens in humans suffering a stroke. The area of the brain that loses its blood supply dies. When rat fetal tissue corresponding to the damaged area is placed in the wound, the graft integrates into the host brain and becomes incorporated into the host blood supply. The cells in the graft send signals like normal neurons, indicating that the graft is active. The grafts survive best when they are implanted at least 1 week after the stroke, a phenomenon that is observed when wounds are made in either the brain or spinal cord. Neither the stroke studies nor the others previously mentioned examined whether the grafts reversed any functional deficits caused by the wounds.

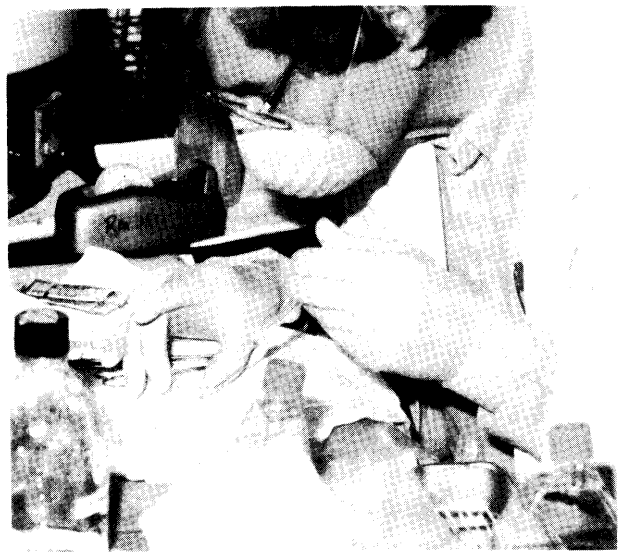


Photo credit: R.A.E. Bakay, The Emory Clinic

Preparing a cell suspension for grafting.

Studies of other kinds of brain injury in rats have shown that implantation of rat fetal tissue into wounds made in the cerebral cortex can help alleviate the deficits caused by the wounds (46,92,152). Grafting of fetal tissue into these models results in functional improvement, but only if the tissue implanted is from regions of the fetal brain that correspond to the areas destroyed in the adult.

The mechanism by which grafts exert their effects in these animal models of brain injury is unclear. Recovery may be due either to integration of the grafted material into the host brain or to effects not related to integration or even graft survival. It has been observed that the beneficial effect occurs for only a short time after implantation of the tissue (44) and that the same degree of functional recovery is seen whether a fetal graft, a suspension of glial cells, or a piece of sponge that has been sitting in the wound of another animal is placed into the wound (85). This indicates that part of the remedial effect may result from the presence of neurotrophic factors produced when the wound was made or secreted by cells other than neurons contained in the graft. These factors could stimulate growth from the injured host brain. Thus, both incorporation of the graft into the host and the effects of growth-promoting factors could play a role in recovery of function. Some investigators have stated:

> It appears. . . that transplants can facilitate functional recovery by more than one mechanism, including promotion of survival and reactive synaptogenesis [synapse formation] of host neurons, stabilization of the damaged environment and replacement of neurons (85).

The ability of grafts to reverse functional deficits caused by brain injury is still unclear. If a functional effect can be produced, the underlying mechanism of that effect is uncertain and the requirements for the grafted material are unknown. Additional basic research is needed to answer these questions and to define the ultimate role of grafts in brain injury.

EPILEPSY

Some success has been achieved in using neural grafts to block the occurrence of epileptic seizures in a very specific animal model of epilepsy called the "kindled" model. It is thought that the kindled model of epilepsy is analogous to temporal lobe epilepsy (68), which is the most frequent form of the disease in adult humans. In this model, an area of the brain called the locus ceruleus (LC), which inhibits the ability to induce seizures, is destroyed in rats. Investigators can therefore induce seizures in the hippocampus of these animals more easily than in normal, intact rats.[6] A graft of fetal rat LC tissue placed in the hippocampus, however, can form connections with the cells in the hippocampus and inhibit the induction of seizures (12,98). Thus, the LC grafts mimic, to a certain degree, the normal action of the intact LC in this model. LC tissue grafts have also been shown to inhibit seizures in another rat model of epilepsy that uses a combination of surgery and drugs to induce seizures (28).

While these studies show that LC grafts can inhibit the *induction* of seizures in these models, the relationship to *naturally occurring seizures in* persons with epilepsy is unclear. There is no evidence to date that LC grafts can inhibitor otherwise affect naturally occurring seizures.

The ability of grafts of other types of fetal brain tissue to suppress naturally occurring seizures has been examined in rats that have a genetic predisposition to seizures. The severity of seizures in these rats can be decreased if either of the neurotransmitters noradrenaline or gamma-aminobutyric acid (GABA) is injected into certain areas of the brain; however, grafts of tissue rich in these neurotransmitters, implanted into the proper areas of the brain, did not appreciably reduce the intensity of the rats' seizures (138).

Experiments conducted to date indicate that grafts can affect the induction of seizures. However, additional animal research is needed to determine what role grafts could play in reducing naturally occurring epileptic seizures.

NEUROENDOCRINE DEFICITS

The ability of grafts to supply chemicals makes them well suited to reduce deficits caused by a loss or imbalance of normal hormone levels. Neurons in an area of the brain called the hypothalamus regulate the release of hormones from the pituitary gland (which lies under the hypothalamus) into the bloodstream. In addition, some hormones are made by neurons in the hypothalamus itself and are released directly into blood vessels. Once in the blood vessels, the hormones travel throughout the body.

[6] Seizures are induced by electrically stimulating a portion of the brain called the limbic system, which includes the hippocampus. The electrical stimulation is given in small, progressive steps which make the limbic system so sensitive that even a mild electrical shock will induce a generalized seizure. Thus, the seizure is said to have been kindled. The locus ceruleus is thought to inhibit the kindling phenomenon by sending noradrenaline fibers to the hippocampus. Kindling takes place more quickly and easily when these fibers are destroyed.

If the area of the hypothalamus that secretes the hormone vasopressin (VP) directly into the bloodstream is destroyed in rats, the resulting low levels of VP cause water loss in the urine and produce dehydration. The same phenomenon is observed in a mutant strain of rat that has a congenital absence of VP-producing neurons. If VP-producing neurons are grafted into either of these animal models, the grafts become incorporated into the host and secrete VP into the circulatory system (59,105,136). Grafts of VP-producing cells can also ameliorate some of the deficits in fluid regulation seen in these animals (61,106). Finally, these grafts can prevent the death of hypothalamic neurons that normally occurs when a wound is made, perhaps by the release of growth-promoting factors (107).

Another model that has been used in this line of research is a special strain of mice with a genetic abnormality that keeps them from producing the hormone GnRH (gonadotrophin-releasing hormone). GnRH, which is released from cells in the hypothalamus, causes cells in the pituitary gland to release hormones that control sexual maturation. Mice of either sex with this genetic abnormality have immature reproductive organs and are sterile. The implantation of fetal grafts of hypothalamic tissue from normal, GnRH-producing mice into the hypothalamus of adult mice with this dysfunction can correct many of the reproductive deficiencies that occur in these animals (22,29,62-65,90). The grafts result in increased levels of pituitary hormones, maturation of sexual organs, and initiation of sexual behavior.

Neural grafts have been shown to restore sexual potency and sexual behavior in aged, impotent male rats (79). The sexual behavior of such rats before and after fetal hypothalamic tissue was implanted into the hypothalamus was observed. Before the implants, the animals did not engage in sexual behavior when exposed to receptive females. After the grafts, the sexual behavior of most of the rats was restored, resulting in increased sexual activity and impregnation of the females.

The ability of neural grafts to reverse deficits and imbalances in hormone levels due to neurological damage or abnormalities has been repeatedly shown in a number of animal models. Since analogous clinical conditions in humans are rare, and when they occur are treated with drugs, the ultimate role of neural grafts in human clinical neuroendocrine conditions is yet to be elucidated.

Photo credit: C.S. Raine, Neurocellular Anatomy in Basic Neuroohemistry, *4th ed., G. Siegel, B. Agranoff, R. Albers, and P. Molinoff (eds.) (New York, NY: Raven Press, 1989) p. 29.*

A photograph through a microscope showing a Schwann cell (N) and the myelin it produces (black ring) surrounding an axon (A).

DEMYELINATING DISORDERS: MULTIPLE SCLEROSIS

Demyelinating disorders disrupt nervous system activity by causing a breakdown in the insulating sheath that surrounds many axons. Loss of the myelin sheath interferes with the normal transmission of signals between neurons, causing messages to travel more slowly than normal. Specialized glial cells (oligodendrocytes in the CNS, Schwann cells in the PNS) produce the myelin covering of neurons. Within the CNS, demyelinating diseases attack either the oligodendrocytes or the myelin they produce.

A number of disorders can affect myelin in the CNS. While most are rare, one, multiple sclerosis (MS), is more common. MS destroys patches of

myelin in an erratic and random fashion throughout the CNS. The cause of MS is unknown.

Demyelination can also occur in certain types of spinal cord injuries. Injuries caused by a compressive force on the spinal cord often result in an area of demyelinated axons surrounding a core of dead tissue (149). The ability of grafts to restore the myelin covering of the appropriate axons could aid in the recovery of lost functions.

The therapeutic strategy underlying the use of neural grafts in patients with MS or other demyelinating conditions would be to provide a source of myelin that could become incorporated into the affected area and restore the lost myelin sheath. Any graft tied at replenishing lost myelin would have to include either oligodendrocytes or Schwann cells. Since these cells, when implanted into the CNS, can migrate some distance from the site of insertion, it is thought that they may be usable in treating disorders that result in patchy areas of demyelinated axons.

Over the past decade, animal experiments have shown that it is possible to replace lost myelin by injecting the appropriate cells into regions in which experimentally induced demyelinating lesions have been produced. This has been demonstrated using suspensions of Schwann cells from peripheral nerves (21,22,39,76), cell suspensions of CNS tissue (23), and fragments of CNS tissue (73,74). Additional animal experimentation is needed to determine whether these grafting procedures can restore functional deficits caused by demyelination. Also, it is unknown whether implanted myelin-producing cells would be susceptible to the underlying disease process that caused the original demyelination.

SUMMARY AND CONCLUSIONS

The possible therapeutic uses of neural grafting into the brain and spinal cord are varied and diverse, encompassing a wide range of neurological deficits. Of these, the application of neural grafting in persons with Parkinson's disease is the most advanced. But in Parkinson's disease, as well as all other applications, no definitive statement about the actual usefulness of neural grafting as a therapeutic procedure can be made at this time. The data from the basic research that has been conducted thus far provide tantalizing hints of the potential usefulness of neural grafting procedures, but additional animal experimentation needs to be conducted to clarify that potential. To increase the applicability of animal data to human disorders, animal models of the various neurological disorders under study need to be developed. Neural grafting holds the promise of new therapeutic interventions for neurological disorders, but a final determination of its usefulness must await the accumulation of more information about the mechanisms underlying neurological disease and injury, graft functions, and how those functions relate to various neurological disorders.

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