

Chapter 4

General Features of Neural Grafting

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General Features of Neural Grafting

Neural grafting has been hailed as.. one of the most promising approaches to have come from experimental neurobiology as a potential therapy for a variety of disorders involving damage to the central nervous system” (41). It has also triggered a debate among physicians and medical researchers about when a medical procedure should be advanced from a research tool in animals to a treatment in humans. The use of neural grafting to treat patients suffering from Parkinson’s disease has led to both dramatic claims of success and more cautious statements of results. A proposal for neural grafting research prompted the Executive Branch of the U.S. Government to forbid Federal support of transplantation procedures employing human fetal tissue from induced abortions. All of this controversy begs a clear answer to the question, What is neural grafting?

The term neural grafting, as used in this report, refers to the transplantation of tissue into the brain or spinal cord. Neural grafting differs from organ transplantation, wherein an entire diseased or injured organ, such as the heart or kidney, is replaced with a healthy one. Although neural grafting may entail replacing a diseased portion of the brain, animal experiments suggest that it may also serve as a drug delivery system, providing chemical substances to the central nervous system (CNS) of the graft recipient, or that it may be used to promote recovery of the host’s injured brain or spinal cord. In addition, a neural graft may be derived from various types of tissues, including fetal CNS tissue, peripheral nervous tissue, cells from other organs, or cell lines sustained in the laboratory. Thus, neural grafting is a generic term that embraces many different treatment goals and materials.

This chapter focuses on the salient features of neural grafting and issues related to its potential use to treat the diseased or injured CNS. Unless otherwise specified, data discussed in this chapter are derived from animal studies. Despite the publicity that has recently attended neural grafting attempts in patients suffering from Parkinson’s disease, the clinical usefulness of this approach is not certain. In the case of Parkinson’s disease, optimal methods for using neural grafting are still under investigation. In

general, the development of innovative techniques, including the use of genetically engineered cells, relies on extensive basic research. Furthermore, many questions concerning the functional effects of neural grafts, as well as the problems presented by their use, are unanswered. Neural grafting may, however, lead to promising treatments for neurological disorders, which often resist therapeutic intervention. Issues addressed in this chapter include:

- What therapeutic strategies are possible through neural grafting?
- What tissues and cells can be used for neural grafting?
- What factors influence the successful survival and function of neural grafts?
- What potential risks are presented by neural grafting?

THERAPEUTIC STRATEGIES

How a neural graft improves CNS function within the recipient is not completely understood. A neural graft may simply provide a depleted chemical substance to the brain or it may permit other therapeutic strategies as well (5,33,61). In fact, neural grafts display a wide range of capabilities. These diverse functions lead researchers to predict that neural grafts will be employed to accomplish different treatment goals in different neuropathological disorders. Continued research is necessary to determine precisely how neural grafts function and how those functions can benefit a graft recipient (37). In this section, potential therapeutic strategies are discussed. Neural grafts may:

- provide a source of depleted chemical substances,
- stimulate neuron growth and promote survival of neurons, and
- replace lost structures in the brain and spinal cord.

Source of Depleted Chemical Substances

Neural grafts may supply chemical substances that have been depleted in the CNS by injury or disease.

The loss of neurons within the brain or spinal cord can severely impair memory, the control of muscle

movement, and other functions performed by the nervous system. Impaired CNS function may result from a depletion of the chemical substances normally produced by the degenerating or missing neurons. Conventional drug therapy can be employed to replenish the supply of a depleted chemical in the brain. However, several factors can make drug therapy problematic. For example, some administered substances are prevented from entering the CNS by the blood-brain barrier, which excludes many cells and molecules in the blood from the CNS (11,74).

The current limited success in treating CNS disorders via drug therapy may be circumvented by the use of neural grafts. Cells that synthesize and secrete a neurotransmitter (a messenger molecule used by neurons in communication) or other chemical substance may be implanted in the CNS; it is thought such implants may provide a continuous supply of chemicals directly to the depleted region of the brain or spinal cord.

It is postulated that neural grafts supply chemical substances in one of two ways (5). If the graft is composed of cells that do not form connections (synapses) with the host neurons, it may simply synthesize and release a steady and diffuse supply of chemical substances to adjacent regions of the CNS, acting as a localized pump, or drug delivery system. If synapses are forged between graft and host neurons, integrating the graft into a neuron network, the release of chemicals may be more carefully regulated by the host neurons. In this case, rather than indiscriminately spewing chemicals near the graft site, the grafted neurons may discharge the chemicals in a more controlled fashion at synapses abutting host neurons.

Promotion of Neuron Growth and Survival

Neural grafts may introduce new substances or cells that promote and guide host neuron regrowth, prevent host neuron death, or both.

The complex network of nerve fibers within the mature brain and spinal cord attests to the tremendous neuron outgrowth and synapse formation that occurs during development. Developing neurons may form long fibers and establish contact with as many as 1,000 other neurons. While mature neurons do enjoy some regenerative potential, the ability to extend long fibers appears to be masked or inhibited in the mature CNS. Anything more than modest

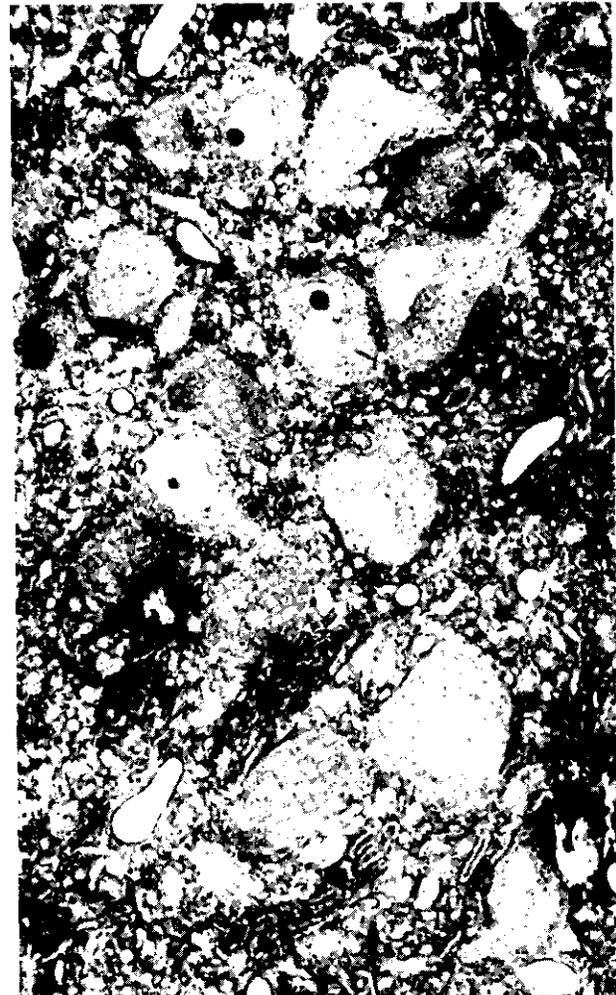


Photo credit: Laura Lee Hall

Injured nerve cells.

injury of mature neurons often proves fatal to them. In general, glial cells inhibit neuron regrowth following injury (20,76,84). Injured neurons may be permanently disconnected from their targets within the CNS, they may degenerate and die, or both. Furthermore, neurons that die are not replaced, since they lack the ability to reproduce themselves.

Experimental evidence suggests that the degenerative consequences of neuronal injury in the mature brain and spinal cord can be prevented, or at least ameliorated, if a growth-promoting environment, such as that found in the developing brain, is provided. In other words, recovery of injured neurons within the mature CNS may be enhanced by cells or chemical factors that promote neuron regrowth, neuron survival, or both (figure 4-1). It is

speculated that neural grafts may be used in this capacity, stimulating neuron functions such as nerve fiber and synapse growth or preventing neuron death (21).

A neural graft may lead to the recovery of injured neurons in several ways. Grafted cells may synthesize and release growth-promoting factors near the injured neurons, preventing neuron death and promoting neuron regrowth. Neural grafts may also be used to form a bridge between a group of neurons and their target within the CNS, bypassing the unfavorable environment of the mature CNS. These graft materials may enhance neuron outgrowth and provide a terrain over which it is directed. Neural grafts may also serve to reduce scar formation within the injured CNS or neutralize the growth-inhibiting effect of the mature CNS (85,90). Other graft activities, such as the removal of toxic substances, may also be possible (34).

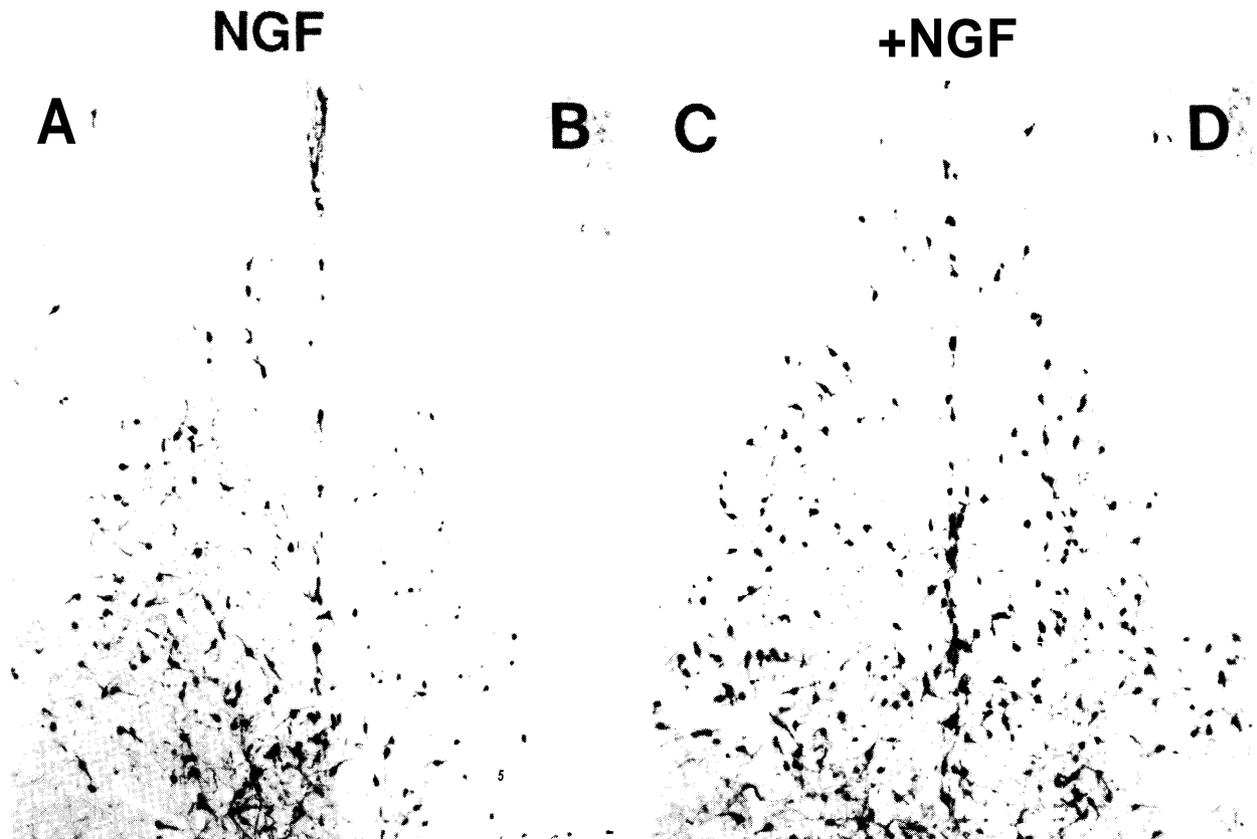
A neural graft used to promote regrowth and recovery of the host's own brain or spinal cord tissue may be required only temporarily, thus making long-term graft survival, which may be difficult to achieve, unnecessary.

Replacement of Lost Structures in the Brain and Spinal Cord

A neural graft may be used to replace nerve cells in the CNS that were lost to injury or disease.

Normal aging, injury, disease, or lack of oxygen can precipitate the death of nerve cells within the CNS. Since neurons generally are not replaced within the brain and spinal cord of adult mammals, their death leads to a permanent decrease in the number of neurons in the brain and creates a missing link in neuronal networks. This loss of neurons and disruption of neuronal networks may result in the

Figure 4-1—Injured Nerve Cells With and Without Growth Factor Treatment



Photograph of brain section; nerve cells are darkly stained. Injured nerve cells on side D received nerve growth factor (NGF) and therefore survived injury. Injured nerve cells on side B, in the absence of NGF, degenerated.

SOURCE: S. Varon, "Neuronal Growth Factors," *Neural Regeneration and Transplantation, Frontiers of Clinical Neuroscience*, vol. 6, F.J. Seil (ed.) (New York, NY: Alan R. Lies, 1989).

permanent impairment of a **CNS** function. Another therapeutic use for neural grafts may be the replacement of degenerated neurons.

Replacement of neurons and the reconstitution of neuronal networks within the adult CNS seem a remote possibility, considering the intricate interactions that occur between neurons within the nervous system. A single neuron may receive thousands of contacts from different regions of the brain. Despite this complexity, grafted fetal neurons demonstrate a remarkable ability to integrate into the mature CNS of a recipient. Grafts of fetal neurons can send nerve fibers into the host CNS and form synapses with host neurons, often in an appropriate and recognizable pattern. Grafted fetal neurons may also receive synaptic input from the host.

Complete and literal replacement of a group of neurons is probably impossible. For example, when neurons that normally project long distances within the CNS degenerate, replacement neural grafts are generally implanted near the target in the host CNS rather than in the original site of nerve cell degeneration. In this situation, juxtaposition of graft and host CNS target can be important for their interaction (41). Although the graft will probably not receive the full range of normal inputs from other CNS regions, it may be sufficiently integrated into host neuronal networks to restore a useful degree of function.

MATERIALS FOR NEURAL GRAFTING

Several types of biological materials may be used for neural grafting. The first and perhaps most important determinant of a particular material's usefulness is its ability to improve CNS function with minimal risk to the recipient. The availability and source of the graft material will also significantly influence its application in humans. Currently, several sources of tissue for neural grafting in humans seem possible, including human fetuses, cells maintained in cultures, or tissue from graft recipients themselves. Nonhuman species also represent a potential source of neural grafting material, although they may present serious functional and immunological problems (30,52,67,70). Each of these sources presents unique technical issues and questions about availability. In addition, the type and source of material used are central to the

ethical and legal questions surrounding neural grafting (see chs. 7 and 8). In this section, general features of the following potential neural grafting materials are discussed:

- tissue from the fetal central nervous system;
- tissue from the peripheral nervous system;
- peripheral autonomic neurons;
- tissue from outside the nervous system; and
- isolated, cultured, or genetically engineered cells.

Fetal Central Nervous System Tissue

Animal experiments have shown that fetal tissue,¹ unlike mature CNS tissue, readily develops and integrates within a host organism following grafting. A majority of neural grafting research in animals has made use of tissue from the fetal brain and spinal cord. In pioneering experiments, fetal CNS tissue often displayed a considerable capacity for survival within the CNS of the graft recipient (for reviews see 7,35,89). During the 1960s and 1970s, technological advances heralded a new era of neural grafting with fetal CNS tissue. Reliable methods for distinguishing surviving graft tissue within the host were developed, as were improved surgical methods for inserting the graft material. When fetal CNS tissue was transplanted into the CNS of young animals, it matured and interacted with the host brain for a substantial period of time. Subsequent studies revealed that fetal CNS tissue could also be successfully grafted into the mature brain. In fact, the grafted fetal CNS tissue was shown not only to survive and develop in the host, but to integrate into the host brain in a predictable manner.

Research further established that grafted fetal nervous tissue often produced functional improvements in animals with neurological deficits. For example, in several studies grafted neurons were seen to increase brain hormone production in animals that demonstrated a deficiency of hormones (38,40,58). The absence of the hormones impaired the brain's regulation of kidney or reproductive organ function. When the appropriate fetal nerve cells from the same species were introduced into the CNS of the impaired animals, they produced the deficient hormones and restored control of kidney or reproductive function. More recent studies, directed toward the analysis of biological rhythms, have

¹In this chapter, when referring to humans, the fetal stage is considered the period from the end of the eighth week after fertilization until birth.

shown that fetal tissue from a region of the brain called the suprachiasmatic nucleus could be grafted and subsequently control daily cycles of behavior in hamsters (73).

Animal models for parkinsonism were employed to evaluate the effectiveness of grafted fetal nervous tissue (6,72). In rodents and nonhuman primates, chemicals were used to destroy the nerve cells that degenerate in humans suffering from Parkinson's disease. The chemically induced nerve cell death is manifested as abnormal rotatory movement in rodents and as abnormal body movements (similar to those seen in humans with Parkinson's disease) in nonhuman primates. When fetal neurons are grafted to replace the destroyed cells, the movement disorder is partially reversed. The success of this technique led to its adaptation for studies in humans suffering from Parkinson's disease. Ongoing clinical trials are examining the effectiveness of neural grafting with human fetal tissue for the treatment of persons with Parkinson's disease (see ch. 5).

Many different animal models of neurological deficits are being used to study the effects of grafted fetal CNS tissue. Results suggest that neural grafting with fetal neurons may one day be developed to treat several neurological disorders (see ch. 6).

The number of fetal nerve cells needed for neural grafting may be of critical importance, especially when the graft recipient's brain is relatively large, as is the adult human's. While too many grafted fetal neurons pose the threat of excessive growth and overenlargement within the CNS of the host, too few cells (a more common occurrence) may fail to improve CNS function significantly. For example, the amelioration of symptoms of Parkinson's disease reported in a single patient following neural grafting required CNS tissue from four human fetuses (62). Invariably, some neurons are lost while collecting the fetal tissue. In addition, identification of the region of the fetal brain required for grafting is difficult; the desired fetal brain region transplanted into humans with Parkinson's disease is approximately 1 millimeter long (less than 4/100 of an inch) (figure 4-2). Human fetal CNS tissue for neural grafting is derived from first-trimester elective abortions, which are commonly performed via vacuum aspiration; this results in fragmented fetal tissue, the transplantable components of which maybe difficult

to identify (18). Some researchers have estimated that human fetal tissue for neural grafting in Parkinson's disease is correctly identified in only 10 to 50 percent of aborted tissue analyzed (27,61). Furthermore, only 5 to 10 percent of the transplanted neurons may survive the grafting procedure. Improving graft retrieval and survival could diminish the amount of fetal tissue necessary for transplantation.

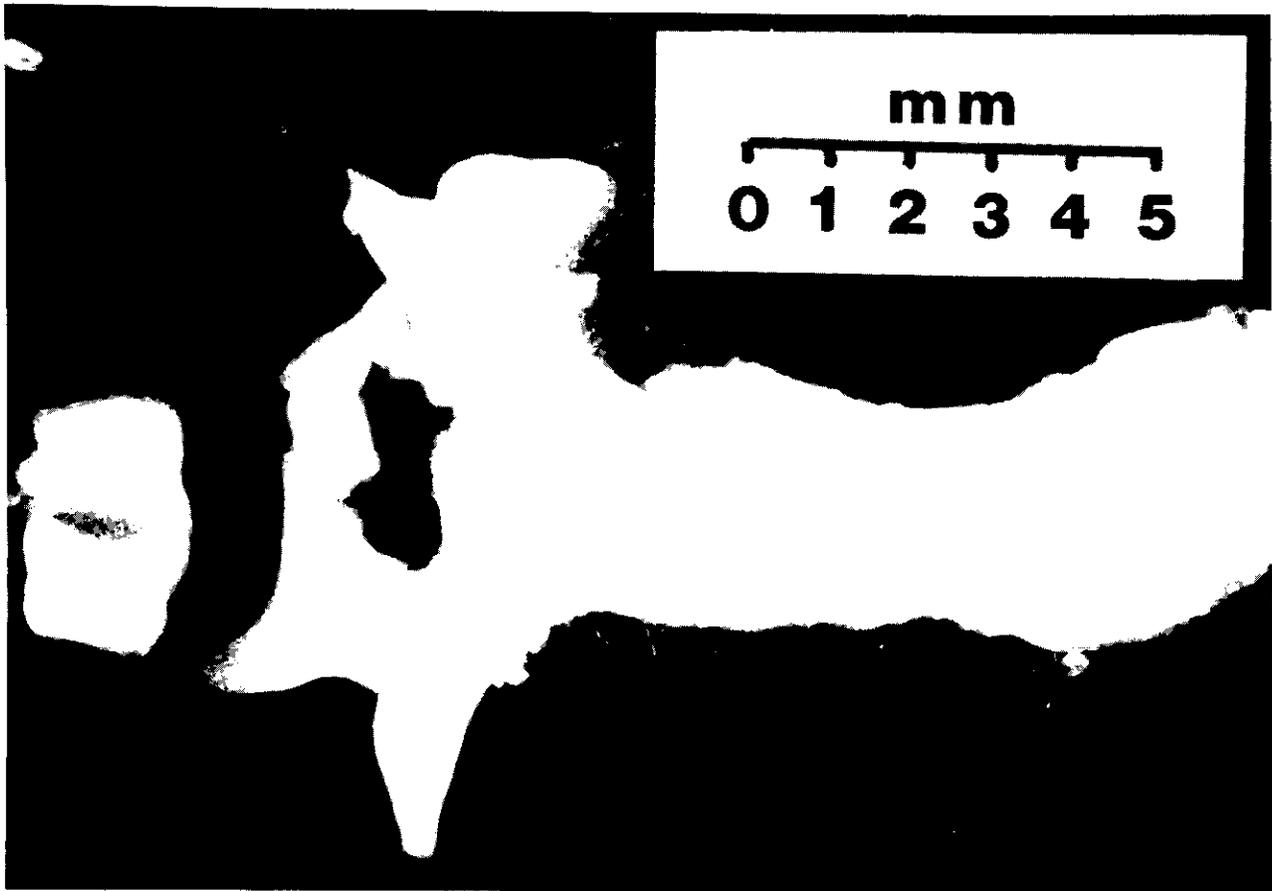
Like other tissue used for neural grafting, fetal CNS tissue presents some risks (see discussion of risks later in this chapter). However, many scientists consider fetal CNS tissue to be the most effective material currently available for neural grafting (46,93). Fetal CNS cells appear to be less vulnerable than adult cells to damage from, for example, lack of oxygen, which is encountered during the transplantation process. Also, cells within fetal CNS tissue can readily mature and integrate within the host; mature CNS tissue has lost these capabilities. Of all the graft materials available at the present time, fetal CNS tissue is most capable of reconstituting nerve cell structure and function within the host CNS. In addition, fetal CNS tissue may enjoy at least a temporary immunological advantage (see later discussion) and, like other potential graft materials, is amenable to long-term storage via cryopreservation (figure 4-3) (box 4-A). Despite the usefulness of fetal tissue for neural grafting, ethical, social, and political issues have created a barrier to its use in the United States and propel the search for alternative neural grafting materials.

Peripheral Nerve Tissue

The permanent deficit in function that frequently results from injury to the mature CNS reflects, in part, the stymied regrowth of neurons within the CNS. In contrast, axons in the peripheral nervous system (PNS), which lies outside the brain and spinal cord, can regrow following injury. Components of the PNS, including Schwann cells, a type of glial cell that produces the insulating myelin sheath around axons, promote axon growth (19).

Investigators have attempted to harness the growth-promoting capacity of peripheral nerves by grafting segments of peripheral nerve into the CNS. Early animal experiments showed that a piece of peripheral nerve placed into a lesion in the CNS would bridge the lesion, allowing host nerve fibers

Figure 4-2—Fetal Central Nervous System



A portion of the dissected human fetal central nervous system.

SOURCE: Curt Freed, Department of Medicine and Pharmacology, University of Colorado,

to penetrate and completely traverse the graft (22) (figure 4-4). Nerve fibers that regenerate through a grafted peripheral nerve penetrate only a short distance on reentry into the host CNS. However, even this shallow penetration back into the host brain permits some reconnection with target neurons. Thus, some recovery of function may be obtained by using this approach (55). This approach may be limited to certain regions of the CNS due to geometric constraints on nerve graft placement. For example, it may be impossible to interconnect deeply embedded regions of the brain with a nerve graft without damaging the surrounding brain tissue.

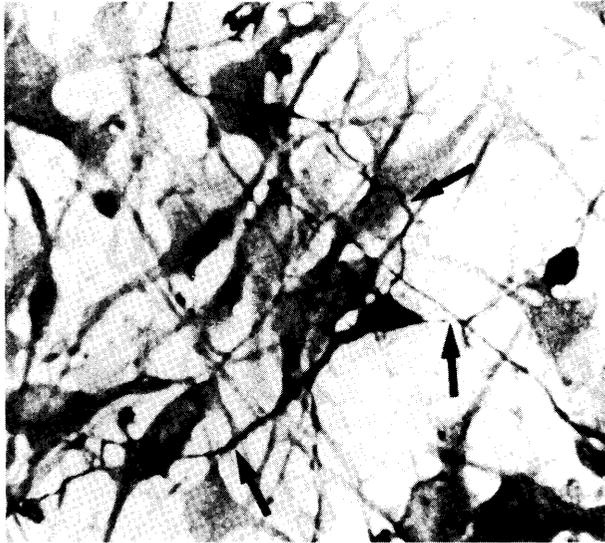
Most of these animal experiments have involved autografts; that is, the graft material has come from the animal itself. An autograft provides two advantages: rejection of the graft by the host immune

system is avoided, and the graft recipient serves as a readily available source of material.

Peripheral Autonomic Tissue

Some neurons are located outside the brain and spinal cord and interact directly with various organs, regulating body temperature, metabolism, and the body's response to stress. These peripheral neurons are part of the autonomic nervous system, and they synthesize neurotransmitters and other chemical substances that are similar to those found in the CNS. In addition, mature peripheral autonomic neurons can survive injury and redevelop nerve fibers. Because this class of neurons is easily accessible and exhibits a great potential for regrowth, several investigators have examined the usefulness of peripheral autonomic neurons for neural grafting. Autonomic neurons have been

Figure 4-3—Transplanted Fetal Nerve Cell



Photograph of a fetal nerve cell following cryopreservation and transplantation.

SOURCE: D.E. Redmond, Jr., F. Naftolin, T.J. Collier et al., "Culture and Transplantation of Human Fetal Mesencephalic Tissue Into Monkeys," *Science* 242:788-771, 1988.

grafted into the mature brain in a few animal experiments, and in some cases these grafted neurons survived and led to improvement in function within the injured CNS (54, 83).

Tissue From Outside the Nervous System

Some nonneuronal cells located outside the central and peripheral nervous systems share a common heritage with nervous tissue; i.e., they develop from the same type of embryonic precursor cell as nervous system cells. These cells (sometimes referred to as paraneurons) produce neurotransmitters and can be stimulated to extend nerve fibers. Included in this category are some cells in the adrenal gland, as well as other, smaller collections of cells in the body (e.g., carotid body cells, which monitor the concentration of oxygen in the blood). Because such cells resemble neurons, they have been considered potential candidates for neural grafting. Cells from the adrenal gland have been studied extensively.

The adrenal glands are located above each kidney, and they produce various hormones. The innermost region of the adrenal gland is the adrenal medulla. One type of adrenal medullary cell, the chromaffin cell, is derived from precursor cells that also generate neurons in the autonomic nervous system.

Figure 4-4—Peripheral Nerve Graft

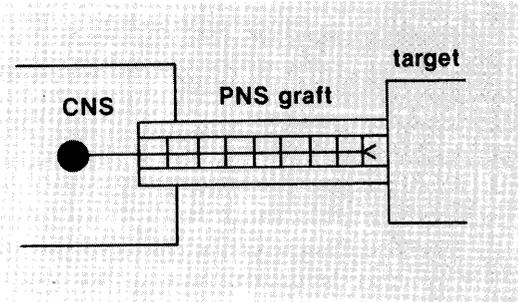


Diagram of graft from the PNS, permitting CNS nerve fiber regrowth to target.

SOURCE: A.J. Aguayo, "Regeneration of Axons From the Injured Central Nervous System of Adult Mammals," *Encyclopedia of Neuroscience*, vol. II, G. Adelman (ed.) (Boston, MA: Birkhäuser, 1987).

Chromaffin cells produce neurotransmitters chemically related to those made in the nervous system. One of the neurotransmitters produced by chromaffin cells is dopamine, the chemical that is deficient in the brain of persons with Parkinson's disease.

The finding that fetal neurons which produce dopamine could reverse parkinsonian symptoms in animals suggested the use of adrenal medulla cells for neural grafting (6,72). In the latter case, animals could provide their own chromaffin cells for grafting, thus eliminating concerns about a source of tissue and possible rejection of the graft. The adrenal medulla grafts were shown to reverse some of the abnormal body movements in animal models of parkinsonism (see ch. 5).

A few recent experiments have employed another nonneuronal tissue, human amnion membrane matrix (HAMM), taken directly from the discarded human placenta, as a neural graft material (23,32). When positioned in an animal's brain, HAMM appears to serve as a bridge that supports neuron outgrowth. HAMM does not contain cells; rather, it contains a chemical substance that promotes and guides neuron regrowth. It does not seem to provoke rejection of the graft by the host immune system, and it is available in abundance. Although more research is necessary to evaluate the usefulness of HAMM in neural grafting, these experiments suggest that manmade materials, coated with a growth-promoting chemical, may ultimately be developed and used in neural grafting.

Box 4-A—The Deep Freeze: Cryopreservation of Fetal CNS Tissue

Neural grafting with fetal CNS tissue is constrained by the need for rapid implantation of freshly collected tissue into the graft recipient. The viability of fetal CNS tissue diminishes within hours of procurement. Extending the interval between tissue collection and implantation would greatly ease the logistics of neural grafting and allow assessment of tissue contamination, vitality, and genetic compatibility with the intended recipient. Cryopreservation, or freezing of cells and tissue at very low temperatures, can be used to extend the time between tissue collection and neural grafting. This technique is routinely used for the storage of continuous cell lines and other types of cells. In fact, cryopreservation of fetal CNS tissue has a long history.

Prior to cryopreservation, cells or tissue are typically treated with a cryoprotectant, a chemical that limits tissue damage during the freezing process. The cryoprotected material is then either abruptly or more gradually lowered to -196°C , the temperature of liquid nitrogen. Once at this temperature, cells or tissue can be stored for a long time; frozen cells can also be transported readily between clinical centers.

Fetal CNS tissue from several species, including humans, has been examined following cryopreservation. In general, cryopreserved fetal CNS tissue demonstrated a significant reduction in viability when thawed. Recently, however, improved cryopreservation of human fetal tissue has been reported, resulting in **95 percent** viability of thawed fetal neurons. Furthermore, cryopreserved human fetal tissue has been shown to survive following grafting into monkeys and humans.

SOURCES: T.J. Collier, D.E. Redmond, Jr., C.D. Sladek et al., "Intracerebral Grafting and Culture of Cryopreserved Primate Dopamine Neurons," *Brain Research* 436:363-366, 1987; T.J. Collier, C.D. Sladek, M. J. Gallagher et al., "Cryopreservation of Fetal Rat and Non-human Primate Mesencephalic Neurons: Viability in Culture and Neural Transplantation," *Progress in Brain Research*, vol. 78, *Transplantation Info the Mammalian CNS*, D.M. Gash and J.R. Sladek, Jr. (eds.) (Amsterdam: Elsevier Science Publishers, 1988); D.E. Redmond, Jr., "Fetal Tissue Transplantation: Animal and Human Studies," paper presented at the American Association for the Advancement of Science annual meeting, New Orleans, LA, 1990; D.E. Redmond, Jr., F. Naftolin, T.J. Collier et al., "Culture and Transplantation of Human Fetal Mesencephalic Tissue Into Monkeys," *Science* 242:768-771, 1988; D.E. Redmond, Jr., D. Spencer, F. Naftolin et al., "Cryopreserved Human Fetal Neural Tissue Remains Viable 4 Months After Transplantation Into Human Caudate Nucleus," paper presented at the Society for Neuroscience 19th annual meeting, Phoenix, Arizona, 1989; V. Silani, A. Pizzuti, O. Strada et al., "Human Neuronal Cell Viability Demonstrated in Culture After Cryopreservation," *Brain Research* 473:169-174, 1988; T. Sorensen, S. Jensen, A. Moller et al., "Intracerebral Transplants of Freeze-stored Rat Hippocampal Tissue," *Journal of Comparative Neurology* 252:468-482, 1986.

Isolated, Cultured, or Genetically Engineered Cells

The graft materials discussed thus far have been distinct types of tissue harvested directly from a donor organism. Technical developments that permit the separation of individual cells from a solid block of tissue, the maintenance of living cells in the laboratory, and the manipulation of genes within cells may expand the types and forms of materials available for neural grafting. The methods described in this section can be applied to many cell types. In this report, however, the cells derived from each tissue source via the methods described are treated as a separate neural grafting material because each gives rise to unique technological issues. The forms of neural graft materials presented in this section include:

- cell suspensions,
- cells in culture, and
- genetically engineered cells.

Cell Suspensions

Neural grafting can involve the placement of small, solid pieces of tissue into the brain (**8,61**); however, solid pieces of tissue cannot be implanted and sustained in all locations within the **CNS**. **In** order to overcome this limitation, procedures have been developed in which solid tissue is dispersed into individual cells or, more often, aggregates of cells prior to grafting (**8,16**). Dispersed cells in a supporting fluid are known as a cell suspension. Use of cell suspensions for grafting involves: 1) removal of the required tissue from a donor organism, 2) dissociation of the tissue into individual cells or small aggregates of cells, 3) suspension of the individual cells or aggregates in a supporting fluid, and 4) injection of the suspension into the host CNS.

Cell suspensions from several types of tissue have been used for neural grafting in animals (71). **Experiments have shown that fetal neurons grafted in the form of a cell suspension are capable of long-term survival and interaction**

with the host CNS and can restore function in a damaged region of the brain (8). In fact, neuron suspension grafts may provide more rapid and complete integration into the host brain than solid grafts. Another advantage of using cells in suspension rather than solid tissue for neural grafting is the ease with which suspensions can be manipulated. A solid piece of tissue contains a heterogeneous population of cells, the number and viability of which cannot be determined. In contrast, cell number and viability can be monitored more accurately in suspensions, and it may be possible to isolate a single type of cell for transplantation (16,63). Also, neural grafting with a cell suspension may provoke a less severe immune system response than solid tissue grafts.

Cells in Culture

Once solid tissue is dissociated into a suspension of cells, the living cells can be maintained in the laboratory for several weeks or months in culture. For primary culture, cells are taken directly from an organism and grown *in vitro* (literally, in glass). Culturing cells prior to grafting them increases the opportunity for manipulating the cells and thus may increase the versatility of neural grafting.

A vast amount of information concerning the *in vitro* culturing of different groups of nerve cells, each with its specific requirements, has emerged from basic neuroscience research (9), including a recent report of successful culturing of human neurons (78). Primary cultures of nerve cells, which are generally derived from embryonic or fetal tissue, have demonstrated the ability to survive implantation in the host CNS, to integrate into the host brain, and to promote recovery following an induced injury (15,39,51). In general, culturing fetal neurons diminishes their survival in the host following grafting.

The use of primary cultures of glial cells for neural grafting has also been studied. For example, neural grafts of astrocytes from the developing brain can reduce scar formation following CNS injury in animal experiments (90) and promote recovery of brain function (56). Schwann cells and oligodendrocytes, both myelin-producing glial cells, have also been grown in primary cultures and used for neural grafting. Cultured Schwann cells and oligodendrocytes have been implanted into the CNS of myelin-deficient rats, resulting in the formation of myelin within the host CNS (31,45).



Photo credit: National Institute of Neurological Disorders and Stroke, Bethesda, MD, 1990

Nerve cells in culture.

Most cells survive for only a limited period in primary culture, either replicating a freed number of times or not at all. However, some cells can continue to replicate and thus can potentially survive indefinitely. These continually self-propagating cells may arise spontaneously, may be derived from tumors, or may be created via genetic engineering. Sustained, self-propagating cells in culture are known as continuous cell lines (CCLs). Because CCLs can produce a large number of cells and are in a sense immortal, they have been extremely useful in a number of areas of research.

Several CCLs have been studied extensively as model systems for neuron development and function, including PC12 and neuroblastoma cells. These neuronal CCLs, originally derived from tumors, can be induced to stop replicating and to develop features of adult neurons (e.g., formation of long nerve fibers and secretion of neurotransmitters). Experiments have shown that neuronal CCLs

can be successfully grafted into the CNS and may attenuate functional problems produced by lesions in the CNS (29,36,48,57).

Because CCLs are capable of continuous self-replication, they could provide an inexhaustible source of donor tissue for neural grafting; however, this very trait presents a critical obstacle to their use. As is true of all replicating cells, their potential for uncontrolled growth in the host CNS may lead to tumor formation. Tumor formation has been observed in some, but not all, animal experiments using CCLs for neural grafting (29,53,57). In order to eliminate the threat of tumor formation, strategies for chemically controlling the occurrence and arrest of cell replication are being evaluated (2,57). Although CCLs may offer an attractive option for neural grafting in the future, more research is necessary to characterize and control cell replication in tissue culture and in the graft recipient.

Genetically Engineered Cells

Research is demonstrating that cells may be designed to synthesize a specific chemical substance or to perform a specific function before being implanted into a recipient. This customized approach to neural grafting is made possible by the use of genetic engineering techniques. Genetic engineering permits the insertion of new genes into a cell. Genes code for proteins, which carry out many cell functions, and different genes direct the synthesis, or expression, of different sets of proteins. Cells derived from fetal CNS tissue, the prospective host, primary cell cultures, or a CCL can be genetically engineered.

Genetic engineering techniques have been applied to tissue from the CNS. In one approach, immature precursor cells from the developing CNS of rats, which are capable of developing into mature brain cells, were isolated and maintained in primary cell culture (42,66). There they replicated for a finite period and then matured. Genetic engineering methods were designed to permit the immature precursor cells to replicate indefinitely in the laboratory, but then subsequently to mature and to stop replicating when transplanted into a host brain or spinal cord. Using this approach, specific CCLs from the brain and spinal cord may be developed for use in neural grafting (10). The hope is that specific "immortal" precursors for each type of cell within the brain and spinal cord can be isolated and made available to

Figure 4-5-Genetic Engineering of Graft Tissue

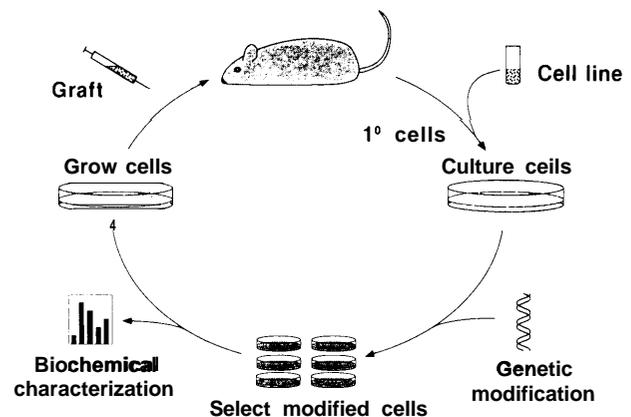


Diagram of methods used to graft genetically modified cells.

SOURCE: Fred H. Gage, Department of Neuroscience, School of Medicine, University of California, San Diego.

replace diseased or injured cells through neural grafting.

Two types of nonneuronal cells have been used for genetic engineering and neural grafting: astrocytes and fibroblasts (cells found in connective tissue) (figure 4-5). Early research demonstrated that genetically engineered cells can survive and function within the host CNS (34). In more recent experiments, fibroblasts have been genetically engineered to synthesize: 1) an enzyme important for the production of the brain chemical L-dopa, which is a precursor of the brain chemical dopamine; and 2) nerve growth factor (NGF) (25,79,97). When used for neural grafting, these genetically engineered cells enhanced survival and growth of neurons, improved CNS function, or both.

The use of genetically engineered fibroblasts, which are easily derived from the skin, as neural graft material has all the advantages of autografts: a ready source of tissue and no worry about immune system rejection of the graft. Although the use of nonneuronal cells precludes the graft from forming synapses with host neurons, genetically engineered nonneuronal cells may be able to function as a drug delivery system in the host. Fibroblasts, like other replicating cells, do present some risk of excessive replication within the host. However, since fibroblasts are primary cells, not derived from a CCL, their multiplication is inhibited by contact with other cells, minimizing the threat of tumor formation.

Many important questions remain concerning the grafting of genetically modified cells into the human brain. Perhaps most important, the genes controlling various cell functions within the CNS (e.g., neurotransmitter synthesis and growth factor production) must be identified. Once the genes controlling CNS function and disease are identified, they must be isolated and made available for genetic engineering. Providing multiple genes to cells for neural grafting and controlling their expression are long-term goals requiring extensive research. Nevertheless, current research represents an encouraging start toward many of these revolutionary therapeutic strategies.

DETERMINANTS OF SUCCESSFUL NEURAL GRAFTING

To survive grafting, cells must endure mechanical and metabolic disruption during their preparation for grafting, and they must incorporate into the foreign, and potentially hostile, environment of the host. The surgical technique and tissue used for neural grafting are important determinants of success. Several additional characteristics may figure prominently in the survival of a neural graft, including:

- . the developmental state of the graft material,
- the host's immune system response,
- . blood vessel formation into the graft, and
- . the age of the host.

Developmental State of the Graft Material

In general, immature tissue can survive grafting more readily than its mature counterpart. Several characteristics of immature tissue, especially fetal tissue, make it well-suited for grafting (1). In general, cells from fetal tissue replicate rapidly and can differentiate into functioning mature cells. Nutritional support provided by blood vessels from the host is easily accepted and probably promoted by fetal tissue. Fetal tissue is amenable to cell culture and storage techniques, thereby expanding its flexibility for use in grafting. These features, which make fetal tissue especially suitable for grafting, are diminished or lost with maturity.

Early experiments in neural grafting of tissue from the adult brain and spinal cord indicated that this tissue survives poorly in the host CNS (7,35,89). In contrast, tissue from the CNS of a fetus or

newborn exhibits great potential for survival and development following neural grafting. Neural grafting experiments in which cell suspensions are used demonstrate an even greater reliance on the use of fetal nervous tissue (8).

Although CNS tissue in later stages of development has been used with some success for neural grafting in rodents and nonhuman primates, survival is greatest when fetal CNS tissue is used (17,86). Apparently, immature neurons, which have not yet grown long and elaborate fiber-like extensions, are less vulnerable to the mechanical disruption associated with tissue collection. In addition, immature cells may be more resistant to other stresses' associated with the grafting procedure, such as a temporary reduction in oxygen supply. Because neurons throughout the CNS develop and mature asynchronously, different groups of neurons reach the optimal developmental stage for neural grafting at different fetal ages. The developmental stage of the CNS tissue used for grafting may present immunological considerations. Grafts of fetal CNS tissue fail to provoke an immediate immune response, probably because the cells and molecules that trigger graft rejection by the host immune system have not yet developed within fetal tissue. Fetal CNS tissue does possess the capability of expressing immunoreactive molecules on maturity, hence it could provoke a delayed immune system response.

The optimum donor age of non-CNS tissues for neural grafting has not been as extensively evaluated. Adrenal medulla transplants in rats demonstrate greater functional effects when derived from younger rather than older animals (28). Thus, optimal success in adrenal grafting maybe obtained by using young, and perhaps even fetal, adrenal medulla tissue. Grafted nerve cells from the autonomic nervous system do not survive well when derived from the fetus; however, mature autonomic nerve cells regenerate briskly when transplanted (82).

Immune System Response

Mammals have evolved an immune system to protect them from disease-causing agents encountered in the environment. This system is a finely tuned collection of tissues, organs, cells, and molecules that seek out, identify, destroy, and remember foreign cells and molecules. An individual's immune system vigilance against cells and mole-

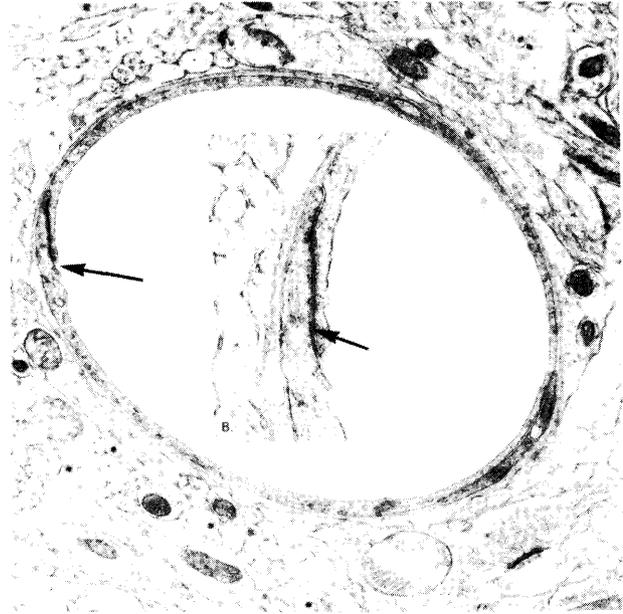
cules that do not originate within itself presents a major obstacle for organ or tissue transplantation. In order for a graft to survive for an extended period of time in the host, the host's immune system must be contravened or suppressed.

Rejection of a graft can be prevented by using tissue from the host's own body. As noted earlier, such a graft is called an autograft. Graft material from an identical twin—an isograft—should behave immunologically like an autograft. After these two types of grafts, compatibility is increasingly rare. Either an allograft—tissue transferred between different members of the same species—or a xenograft—tissue transferred between individuals of different species—can lead to graft rejection. Host rejection of grafted tissue may be prevented by reducing the genetic disparity between the donor and the host and by using drugs that suppress the action of the immune system.

It has long been thought that the CNS enjoys relative isolation from the immune system because it permits the entry of few cells from the immune system that recognize foreign cells or molecules (for review, see 69,94). This isolation has given rise to the concept of immunological privilege; i.e., the CNS is not subjected to the same degree of scrutiny by immune system components as the rest of the body. However, although allografts within the CNS seem to survive frequently without inducing rejection, xenografts in the CNS do provoke graft rejection (69). These data and others demonstrate that the immune system can penetrate the CNS and lead to graft rejection, thus casting doubt on the degree to which immunological privilege operates in the CNS.

Many immune system components are excluded from the CNS by the blood-brain barrier, an important agent of immunological privilege (figure 4-6). Injury, surgery, or infection can disturb the blood-brain barrier, allowing cells and molecules from the immune system to enter the CNS and cells from the CNS to enter the bloodstream. While there is debate about whether the blood-brain barrier is permanently disrupted by the implantation of tissue in the CNS (12,81), it is undoubtedly disrupted for at least a few days (13). The type of grafting material used also appears to affect the development of the blood-brain barrier within the graft (13,83).

Figure 4-6—Blood-Brain Barrier in Transplanted Tissue



Blood vessel surrounded by brain tissue shown in photograph. Inset B displays the source of the blood-brain barrier, i.e., the tight adhesion of endothelial cells, which line blood vessels.

SOURCE: Richard D. Broadwell, Division of Neurological Surgery, University of Maryland School of Medicine.

A second feature of the CNS which was believed to isolate grafts and hinder graft rejection is the lack of extensive lymphatic drainage. Lymphatic drainage returns fluids, molecules, foreign particles, and cells from various tissues in the body through the lymphatic system back to the immune and circulatory systems. It is through this drainage that grafted cells capable of triggering rejection reach the host's immune system. The CNS had been thought not to experience lymphatic drainage; however, some studies have suggested that molecules and cells can leave the CNS and enter the lymphatic system. Thus the CNS may indeed experience some lymphatic drainage and therefore be capable of provoking rejection (96).

The use of CNS tissue as a grafting material often fails to provoke rejection by the immune system, at least in the short term. Cells within the CNS normally lack, or express very few, immunoreactive molecules, i.e., molecules that trigger rejection by

the host's immune system (88,98).² Also, tissue from 'the CNS was thought not to contain cells that enter the immune system and trigger an immune response (47). Unfortunately, recent experiments suggest that the CNS can be provoked to produce immunoreactive molecules and that the CNS does possess cells which may reach the immune system and initiate graft rejection (3,50,92,98).

Another cell type that can provoke an immune response is endothelial cells (49,65), which form the inner walls of blood vessels and are commonly found in grafts of solid pieces of tissue. This cause of graft rejection can be eliminated by using neural grafts composed of a single type of cell, as in cell cultures or purified suspensions of cells, which do not contain blood vessels or endothelial cells.

The fact that many types of neural grafts survive in the host CNS suggests that the brain and spinal cord do enjoy some immunological privilege. However, research indicates that some grafts can be identified and destroyed by the host's immune system. Further experiments and analyses are required to delineate the precise relationship between the immune system and the CNS, as well as the immune system's response to neural grafts.

Blood Vessel Formation

The neural graft's ability to obtain ready access to nutritional support and a supply of oxygen from the host is critical for its survival. The failure of many early neural grafts may reflect inadequate incorporation of the grafted tissue into the host blood supply (8). Solid tissue grafts into the CNS may not receive an adequate blood supply for more than 1 week (13).

Several approaches to neural grafting have been developed to accelerate the provision of nutritional support to the graft. One entails placing the graft near a CNS surface that is naturally rich in blood vessels. The brain ventricles were a favored site for graft placement in animal experiments for this reason (82). Ventricles are cavities within the brain that contain cerebrospinal fluid (CSF). CSF may provide immediate nutritional support to the solid graft, and regions rich in blood vessels within the ventricles provide long-term support. Another ap-

Figure 4-7—Blood Vessel Growth in Transplant



Darkly stained blood vessels invading neural graft tissue in brain.
SOURCE: Richard D. Broadwell, Division of Neurological Surgery, University of Maryland School of Medicine.

preach is to create a rich vascular surface within the brain (91). Under normal conditions, new blood vessels are very rarely formed in the brain. However, when a cavity is produced surgically in the CNS, new blood vessel growth is stimulated and the cavity walls become heavily invested with blood vessels. Placing a graft within the cavity often results in a good supply of blood vessels to the grafted tissue. Greater blood vessel growth is produced by graft placement than by a wound alone (59). The choice of grafting material may also influence blood vessel formation: blood vessels can grow into suspensions of cells more readily than into solid pieces of tissue, and fetal tissue seems to stimulate blood vessel development better than adult tissue (figure 4-7) (77). Finally, certain drugs may be used to enhance blood vessel development within a graft (26).

It has been debated whether blood vessels within a neural graft are derived from the donor or the host. The relative contribution of each may depend on the type of tissue transplanted, whether it is transplanted as a solid piece or in a cell suspension, and the amount of tissue damage sustained by the recipient during placement of the graft (13,24,60,69,83). Blood vessels within the graft may or may not fully develop a blood-brain barrier, depending on the type of tissue utilized (13,14,68,80,95). Both of these observations, the presence of donor cells in the graft blood vessels and the formation of the blood-brain barrier, have important implications for graft rejection.

²The presence of specific molecules, including the major histocompatibility complex, or MHC antigens, is an important determinant of a tissue's immunogenicity. Detection and measurement of these molecules can be difficult (71).

tion. (See the earlier discussion of immune system response.)

The Age of the Host

In general, the younger the graft recipient, the more likely the graft is to survive and integrate within the host brain. A younger host animal, especially a newborn, maybe better able to support grafted material because it can more vigorously form blood vessels (8). Furthermore, an adult recipient rejects neural grafts more rapidly than an immature recipient (64). The age of the host, however, is less critical to graft survival than the age of the donor, immunological response, and extent of blood vessel formation.

POTENTIAL RISKS

A major goal of neural grafting is to improve CNS function following disease or trauma. As with any surgical intervention, however, neural grafting presents risks to the recipient. Problems may result from:

- immune system reaction or suppression,
 - . unwanted psychological effects,
 - . the surgical procedure itself,
- excessive growth of graft material, or
- infection or other effects on the host CNS.

Unfortunately, many of the risks attributed to neural grafting are either poorly understood or simply speculative. Before any routine application of neural grafting in humans, the risks must be carefully delineated, minimized, and measured against expected benefits.

Immune System Reaction or Suppression

The transplantation of tissue from one individual to another presents the risk of graft rejection. The foreign tissue triggers a cascade of events in the graft recipient, culminating in destruction of the graft. In general, allografts implanted in the CNS do not appear to suffer immediate rejection by the host's immune system, although rejection in the long term may be possible.

When tissue transplantation is performed outside the CNS, drugs that suppress the immune system are employed to prevent rejection and promote survival of the graft. Immunosuppression poses serious risks to the graft recipient, including increased susceptibility to infection and the development of some

forms of cancer (87). Uncertainty about immune system reactions in the CNS complicates attempts to balance the risks of immunosuppression against those of neural graft rejection. Studies of fetal CNS tissue grafting in humans with Parkinson's disease have both applied and abstained from applying immunosuppression therapy (27,62,75).

Unwanted Psychological Effects

Implantation of tissue into the human brain raises the possibility of unwanted psychological effects. Assessment of adrenal medulla grafts in humans with Parkinson's disease indicates that some psychological changes consistently accompany this procedure, including hallucinations, confusion, and somnolence (43,44). These psychological responses proved, in general, to be transient. How such changes are produced is unknown, but they may reflect either trauma to the brain from the surgical procedure itself or the effects of chemical substances released by the grafted adrenal tissue. Similar effects have not been reported in the few Parkinson's patients who have received fetal CNS tissue grafts.

Effects of the Surgical Procedure

Aside from its suspected role in producing temporary psychological changes in the recipient, the surgical procedure used to insert a neural graft presents other serious risks. Graft placement in the brain, especially the more invasive surgical procedures (see ch. 5), can cause serious damage, such as excessive bleeding or injury to brain tissue. Injury may result in the loss of CNS function or exacerbate the recipient's immune response to the grafted tissue (41). In addition, surgery disrupts the blood-brain barrier for at least a week; even the less invasive method of graft insertion probably disrupts the blood-brain barrier for 1 to 3 days (14,94). The CNS's protected environment may thus be lost temporarily near the graft site, posing a risk to the graft recipient.

Excessive Growth of Graft Material

Fetal CNS tissue, some nonneuronal tissues, and continuous cell lines can continue to replicate in the CNS of the graft recipient, presenting the risk of excessive graft enlargement. Brain tissue can be compressed and permanently damaged by an expanding mass of tissue. In addition, an enlarging neural graft placed in the brain ventricles can

obstruct the flow of CSF, which can dangerously increase pressure in the brain. The number of cells used for neural grafting is thus an important consideration; the number chosen must reflect a balance between the risk of overenlargement and the more common problem of inadequate cell survival. As discussed earlier, some replicating cells pose the added threat of tumor formation.

Infection and Other Effects on the Host CNS

Transplanted cells can transmit bacterial and viral infections, placing the graft recipient at risk for such diseases as hepatitis, AIDS, or herpes simplex encephalitis. Several actions can reduce this risk. The likelihood of a potential donor carrying an infectious agent can be assessed. The potential donor or graft material can be screened for infectious agents. In addition, graft material can be treated with drugs, such as antibiotics, to destroy susceptible infectious agents prior to implantation.

Materials used for neural grafting may disrupt or alter CNS function in the recipient. For example, non-CNS tissue, such as cells from the adrenal medulla or fibroblasts, may prevent the reestablishment of the blood-brain barrier near the graft (13,59,83). The implantation of certain fetal CNS tissue has been shown to produce seizures in some experimental animals (33). In addition, injection of brain tissue into the abdomen of animals has led to experimental allergic encephalomyelitis (EAE) (4), a potentially fatal inflammatory disease of the CNS in which immune cells attack components of nerves. Although EAE has not been reported in neural grafting experiments in animals, it represents a serious risk, especially in the case of xenografts. Finally, the graft may be susceptible to the pathological processes that underlie the neurological disorder being treated. Since many neurological disorders are of unknown etiology, it is difficult to assess the likelihood of this risk factor.

SUMMARY AND CONCLUSIONS

Neural grafting involves many therapeutic goals, materials, and procedures. Tissue or cells may be transplanted to the brain and spinal cord in order to deliver chemical substances, to promote neuronal growth and survival following injury, or to replace lost nerve cells. Numerous materials have been used for neural grafts, including fetal CNS tissue, peripheral nerve tissue, and tissue from outside the nervous system. In addition, several types of tissue have been

manipulated, using cell culture and molecular biological techniques, in preparation for neural grafting. While genetically engineered cells present an exciting possibility for the future of neural grafting, at present fetal CNS tissue is demonstrably the most effective graft material available. The developmental stage of the grafted material, the host's immune response, vascular support within the host, and the age of the host also influence graft survival and function. Depending on the material chosen and the surgical procedures employed, neural grafting technology does present some risk to the host.

The potential use of neural grafting for the routine treatment of the diseased or injured CNS requires much more research, even for Parkinson's disease, where the technology is most highly developed at present. Research is necessary to evaluate and optimize transplant procedures. Other neural grafting approaches, including the use of genetically engineered cells, will require even more extensive basic research. Factors that determine the long-term survival and function of a neural graft, especially the immune system reactions, must be probed more deeply. The influence of the disease process on the grafted material must also be addressed. Perhaps most important, a more complete understanding of the basis of disease and malfunction within the CNS is required for the development of treatments such as neural grafting.

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