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Promoting Functional Plasticity in the Damaged Nervous System

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Not long ago it was commonly believed that the adult mammalian central nervous system (CNS) was structurally static—that severed neurites did not regrow and functionally significant structural adjustments did not occur. When recovery of function following brain lesions was observed it was attributed to the brain's capacity to circumvent the lesion by mechanisms such as denervation supersensitivity or the use of alternative pathways (*1*). In contrast, peripheral nerves were known to regenerate after being injured, sometimes with recovery of function (*2, 3*). The consequence of this pessimistic view was that scientific interest in neuronal plasticity was mainly directed at lower animals, immature systems, and peripheral nerves.

Terminal fields vacated as a consequence of CNS injury are commonly filled by the growth of axon collaterals, or collateral sprouting (*4*). Neuronal plasticity, and collateral sprouting in particular, now appears to be a ubiquitous phenomenon in the mature CNS that can have functional consequences (*5, 6*). A considerable body of information pertaining to the cellular biology of collateral sprouting and synaptic plasticity now exists (*7*). Here we discuss neuronal plasticity from the perspective of functional restitution; that is: Can neuronal plasticity be manipulated or exploited to obtain functional benefits?

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Axonal Plasticity in the Normal Nervous System

In the peripheral nervous system, severed neurites invariably regenerate. So vigorous is this regeneration that it persists in the stump even after removal of a limb, resulting in the formation of a neuroma (*2, 3*). As peripheral neurites grow, contact with Schwann cells stimulates myelination and some formation of new or additional basal lamina (*8–10*).

In the mature mammalian CNS, myelinated tracts, including those of the spinal cord, are unable to regenerate through the site of a lesion (*11, 12*). Certain categories of unmyelinated or thinly myelinated CNS neurites show substantial regeneration provided no physical barrier is produced when they are damaged (*13*).

Scarring at the site of a CNS lesion is greatly reduced in the immature animal; nonetheless, most studies agree that immature neurites cannot penetrate a CNS lesion to any greater degree than those of a mature animal (*14*). In most cases where regeneration is observed in either immature or adult animals, it occurs around, rather than through, the lesion site (*11, 15–17*). For example, function is spared after pyramidal and corticospinal tract lesions in infant animals (*15, 16*). The anatomical basis of this sparing is probably either a regrowth of late-devel-

oping fibers around but not through the lesion site or sprouting of long collaterals from uninjured pathways on the contralateral side (*15–17*). In both cases, immature neurons are likely to have an increased capacity to generate neurites, but only when the injured systems are still in the process of differentiation and development (*15*).

Submammalian species have several forms of axonal plasticity not observed in mammals. Partial regeneration of the transected spinal cord and optic nerve occurs in lampreys and other lower vertebrates (*18*). In amphibia, regeneration of the spinal cord is preceded and apparently guided by channels formed by ependymal cells (*19*). In crustacea and in the leech the distal segments of transected peripheral axons survive for extended periods and may re-fuse or synapse with the proximal portion (*20*).

Summary. Damage to the central and peripheral nervous system often produces lasting functional deficits. A major focus of neuroscience research has been to enhance functional restitution of the damaged nervous system and thereby produce recovery of behavioral or physiological processes. Promising procedures include surgical, physical, and chemical manipulations to reduce scar formation and minimize the disruption of support elements, administration of growth-stimulating substances, tissue grafts to bridge gaps in fiber pathways, and embryonic brain tissue grafts to provide new cells with the potential to generate fiber systems. Two elements are required for functional nervous system repair: (i) neurons with the capacity to extend processes must be present, and (ii) the regenerating neurites must find a continuous, unbroken pathway to appropriate targets through a supportive milieu.

Scarring and Disruption of the Normal Physical Environment

The differences between peripheral nerve and spinal cord are illuminated by their responses to being crushed. In peripheral nerve, crush injury destroys the neurites but is invariably followed by an almost complete restructuring and functional regeneration (*21*). In contrast, crushing the spinal cord completely destroys its structure, and the permanent outcome is the formation of a massive glial and connective tissue scar (*22*); the outcome is essentially indistinguishable from that of complete transection.

The most probable reasons for this difference are that the basal lamina survives crush injury of peripheral nerve (*23*) and that Schwann cells are able to migrate with the regrowing neurites and produce a new support structure (*9*). Even though the myelin is destroyed and Schwann cells are displaced by crushing (*23*), Schwann cells migrate with, and remyelinate the regrowing neurites (*24*). In addition, the basal lamina acts as a "guide tube" for newly growing sprouts and Schwann cells (*23, 24*). Thus peripheral nerve retains or reforms a columnar organization after being injured. In contrast, no basal lamina is present in the spinal cord, and the oligodendrocytes are not longitudinally organized (and thus have no basis for reorganization) (Fig. 1).

Peripheral nerve repair. Injuries resulting in transection of peripheral nerve trunks are usually accompanied by lasting motor and sensory deficits regardless of the method of repair (*2, 3*). Notwithstanding the poor functional recovery, severed peripheral nerves invariably regenerate and reach peripheral targets (*3*).

Mapping studies have shown that the connections between the leg muscles and spinal cord are inaccurate after epineural suturing of the severed rat sciatic nerve (*25*), whereas crush injury is followed by accurate targeting (*26*) and functional recovery. Thus the crucial elements of functional recovery after peripheral nerve injury probably involve the accuracy, not the vigor, of the regeneration (*27*). It has also been noted that wandering and deviation of regenerating peripheral neurites occurs primarily at the site of reunion; once the growing neurites reach the undisturbed tissue of the distal stump, their paths do not deviate further (*2*). It therefore seems reasonable to assume that disruption at the site of transection injury leads to misrouting of regenerating neurites and ultimately to inaccurate connections between the CNS and target tissues.

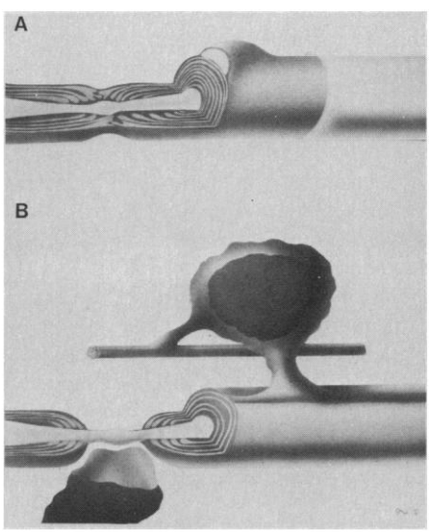

Fig. 1. Configuration of support cells in the peripheral and central nervous system [adapted from figure 4 in (*10*)]. (A) A peripheral myelinated axon. (B) A central myelinated axon. In the CNS the node of Ranvier is wide and not covered by myelin. The gap is covered by the "foot" of an astrocyte. In a peripheral nerve, the node of Ranvier is narrow and completely covered by interlocking loops of myelin; the entire structure is enclosed in basal lamina. The myelin is formed into more layers in the peripheral nervous system. Finally, in the peripheral nervous system, each Schwann cell myelinates one axon only. In the CNS, one oligodendrocyte may myelinate several axons. Thus, when a central axon is lost all traces of the former structure are lost.

This has long been recognized, and considerable effort has been devoted to surgical refinements of nerve repair methods (*2–4*). For example, perineural suturing may result in a greater accuracy of regeneration than epineural suturing. There has been no definitive demonstration, however, that this or any other refinement in surgical technique alone is sufficient to increase the rate or degree of recovery (*28*). For example, displacing the mechanical stress away from the actual site of injury by supporting the transected rat sciatic nerve with a rubber cuff (*29*) greatly reduces the histological disruption at the reunion site. No improvement in the functional outcome is observed, however (*30*).

It was therefore hypothesized that a more profound form of disruption takes place at the site of a nerve transection. To diminish this disruption, a combination of measures was used to decrease the width of the wound site. First, to obtain a clean cut of the nerve stumps after transection, the nerves were carefully and briefly frozen and trimmed with a vibrating razor blade. Without freezing, cutting the nerve inevitably further crushes and distorts the tissue.

Although the cut axonal membranes are ultimately sealed (*31*), immediately after being cut they are vulnerable to disruption of the ionic composition of the cytoplasm through osmosis and equilibration with the extracellular fluid. One means of counteracting this process would be to soak the nerve in a medium, such as Collins fluid (*32*), that mimicks the intracellular composition. This pro-

cedure would normally be destructive. Since the nerve was being cooled for cutting, however, metabolic processes were greatly slowed. The nerves could therefore be soaked in a modified Collins fluid, also containing either EGTA or chlorpromazine to chelate or counteract intracellular calcium. Calcium is thought to disrupt neurofilaments (*33*) and may trigger Wallerian degeneration (*34*). The normal extracellular fluid was restored as the nerve was returned to room temperature (Fig. 2).

These combined procedures functionally improved the recovery from peripheral nerve transection in rats (*30, 35*), whereas none of the individual manipulations alone altered the outcome (*30*). In terms of walking track performances, animals that received the combined treatment of support cuff, freezing, and modified Collins fluid recovered in each of 20 cases, whether the reconnection was performed immediately or 2 hours after transection. Although function returned to about two-thirds of baseline, the performance never equaled the original level, and in no case did the animals recover as rapidly as they did after crush injury.

Thus, refinements in the physical-chemical manipulation of regenerating neurites can improve functional outcome. Minimizing disturbances at the site of injury apparently allowed the neurites a relatively continuous, unbroken pathway from the start of regrowth, into the correct part of the distal stump, through a supportive matrix, and to appropriate peripheral targets. These manipulations have not specifically been shown to result in increased accuracy of regeneration. Nonetheless, these findings do suggest that more that mere stimulation of the rate or amount of regeneration may ultimately be required to produce a functionally significant restitution after injury to the spinal cord or brain.

Spinal cord repair. Beginning with the studies of Windle and colleagues (*36*), many methods of decreasing scar formation in the spinal cord have been studied in the hope of increasing spinal cord regeneration. The first of these, a bacterial pyrogen, pyromen, may have increased sprouting of new neurites; nevertheless, there was no permanent increase in the regenerative response of spinal cord neurites after lesions, and the ultimate outcome was unaffected (*37*). Subsequent studies have uncovered a variety of manipulations, including immunosuppression, "cuffing" the injured spinal cord with an encircling semipermeable membrane, the use of protein synthesis inhibitors, and administration of hormones, which decrease the density or the amount of scar tissue (*37, 38*). Although some of these manipulations may increase sprouting in the proximal stump, they do not result in regeneration of spinal cord fibers across the gap (*38*).

In some model systems, such as in hibernating ground squirrels or immature animals, in which scarring is minimal some regrowth of the severed neurites occurs. These neurites, however, reach the zone of injury and follow, but do not penetrate, the interface (*39*). In these cases the scar does not seem to form an impenetrable barrier, but regeneration is nonetheless thwarted at the scar boundary (*14, 39*). Thus spinal cord fibers seem to have the intrinsic potential for regenerative growth. Other proposed explanations for the failure of spinal cord regeneration include "contact inhibition," or the development of synaptic contacts within the proximal stump and a consequent termination of regeneration (*40*). Sealing of the injured spinal cord by basal laminae may impede regeneration (*41*). Nonetheless, the reasons these neurites do not penetrate the scar are unclear.

One remaining possibility is that it is not the scar per se that prevents regeneration. Rather, it seems possible that the absence of a normal neuronal support structure seems not to be conducive to sustained regenerative growth. If this is the case, no manipulation that decreases the formation of the scar will stimulate regeneration; regeneration will be possible only if normal conditions or conditions akin to those of normal spinal cord development, are restored (Fig. 1). Although it may also be necessary to apply exogenous forces to stimulate the regeneration of neurites, such efforts will probably be fruitless unless a medium that permits fiber growth can first be reconstructed.

Growth and Trophic Substances

Trophic, or "nourishing," and neurite-promoting agents are defined as chemical substances that cause a long-term increase in metabolic activity, neurite extension, survival, or differentiation of nerve cells (*42*). Nerve growth factor (NGF), the prototypic neuronal trophic substance, stimulates the outgrowth of neurites from sympathetic and embryonic sensory axons (*43*), promotes the neuronal phenotypic expression of the immature adrenal chromaffin cell (*44, 45*), prevents embryonic cell death, and guides growing neurites (*46–48*).

Nerve growth factor. Most of the well-documented effects of NGF occur in peripheral systems (*42, 43*). In fact, the most readily apparent effect of intracerebrally administered NGF is an ingrowth of peripheral sympathetic fibers (*43, 49*). Motor neurons, the primary targets in many regeneration studies, are unaffected by NGF (*43*). The effects of NGF in the CNS are controversial. There is some evidence that NGF is present in the CNS (*50*) and may affect cholinergic neurons (*51*). Catecholamines and substance P are probably unaffected (*52*). Facilitatory effects of intracerebral NGF in certain studies of CNS lesion recovery and behavior have been reported (*53*), but these effects may or may not be related to a growth of neuronal processes.

Cell surface components and substrates. Components of the outer cell membrane with hydrophilic properties, such as gangliosides and glycoproteins, are thought to be involved in cell-cell interactions (*54*). The extension of processes by a neuronlike cell increases cell surface area, with extension of processes into extracellular fluid (a hydrophilic event), and also leads to interactions with other cells, both other neurons and support cells. Therefore, cell surface components may play a role in the outgrowth of neuronal processes.

Gangliosides. Gangliosides, which are sialic acid–containing glycosphingolipids, have both lipophilic and hydrophilic properties. These molecules are found in many types of cells and are thought to be involved in cell-cell interactions (*55*). In heritable diseases characterized by excessive ganglioside accumulation, some neurons in the CNS develop large processes or extensions, termed "meganeurites" (*56*). Gangliosides also increase the formation of neuronal processes in tissue culture (*57*). These findings suggest a possible role of cell ganglioside content in the regulation of total cell surface area.

Several recent reports suggest that ganglioside G_{M1} may stimulate axonal plasticity after systemic injection. Ganglioside G_{M1} increases the numbers of neuromuscular junctions formed after crush injury to the sciatic nerve (*58*). After surgical disconnection, long-term administration of ganglioside G_{M1} promotes the survival and sprouting response of dopaminergic neurons (*59*). Gangliosides also promote behavioral recovery after certain brain lesion procedures (*60*). Although gangliosides seem to stimulate neuronal process formation in vitro and probably also increase axonal sprouting in some circumstances (*7*),

little evidence exists that gangliosides can increase neurite regeneration per se.

Glycoproteins. Glycoproteins are the primary proteinaceous components of the cell membrane that interact extracellularly. Blocking glycoprotein synthesis causes cultured neuroblastoma cells to retract their processes (*61*). Candidates for neuronal glycoproteins involved in process extension include fibronectin (*62, 63*), and NILE glycoprotein (*61*). Laminin, a component of the basal lamina, also stimulates neurite extension as a component of the substrate in tissue culture (*62, 64*). All of these substances may act in part by simply promoting adhesion of the cells to the substrate.

Heart-conditioned medium. One of the most extensively investigated neuronal growth-promoting substances is heart-conditioned medium, which contains substances extracted from culture medium in which heart tissue has been grown; it has trophic effects on cholinergic neurons (*65*). Heart-conditioned medium stimulates both cell survival and neurite extension; the property which stimulates cell survival is soluble, whereas the neurite-promoting property attaches to the tissue culture substrate (*42, 65*). One or both of these substances seems to be active on rat brain in vivo. To demonstrate this phenomenon, an iris was inserted into the hippocampus to interrupt the septohippocampal cholinergic pathway. Heart-conditioned medium was then injected daily into the septum. This procedure elevated choline acetyltransferase activity in the iris implants, suggesting an increased growth of cholinergic fibers into the implanted tissue (*66*).

Lesion-induced neuronotrophic factor. A chemically diffusable substance that promotes the survival of chick sensory neurons in tissue culture can be collected from the sites of brain lesions (*67*). The secretion of this substance is time-dependent and increases gradually to about 10 days after the lesion is made. Survival of brain grafts in wound cavities is optimal when the implantation of grafts is delayed until 7 to 10 days after the injury (*67*). Neuronotrophic substances have also been obtained from cerebrospinal fluid from patients with traumatic brain injury (*68*), from astroglial tissues (*69*), and from blood (*42*).

Peripheral nerve. The regeneration of peripheral nerves seems to be influenced by diffusable proteinaceous substances released from the distal stump of transected peripheral nerves (*42, 70*). Various substances including forskolin [an adenylate cyclase activator (*71*)], adrenocorticotrophic hormone (*72*), extracts of skeletal muscle from young rats (*73*), and ganglioside G_{M1} (*58*) also increase the rate of peripheral nerve regeneration or neuromuscular junction formation.

Targets. During development, neurons that do not develop appropriate connections with peripheral or central targets die (*46*). Greater than normal numbers of neurons can be induced to survive if additional target tissue is provided (*46*). Striatal target tissues also facilitate fiber growth from dopaminergic neurons grown in culture (*74*).

Hormones. The hormonal environment of developing animals differs from that of the adult. Possibly by duplicating some of the hormonal conditions present while the nervous system is developing, neuronal growth processes could be enhanced. Steroids are of particular interest. Adrenal corticosteroids suppress the formation of processes by adrenal chromaffin cells (*44, 45*). Adrenocorticotropic hormone stimulates peripheral nerve regeneration, an effect inhibited by corticosteroids (*72*).

Sprouting in the CNS after lesions is also influenced by steroid hormones. After removal of the entorhinal cortex, sprouting of the commissural-associational fibers is inhibited by dexamethasone and hydrocortisone (*75*). On the other hand, adrenalectomy suppresses sprouting of serotonergic fibers induced by lesions of the cingulum bundle (*76*). Estrogen also has some effects on neuronal process formation (*77*).

Electric fields. Cellular membranes generate electric currents that are important for normal cellular development and orientation. Unlike the short-lived currents that occur with neuronal firing, these currents last hours and days. A number of studies indicate that regeneration of limb, bone, soft, and nervous tissue may be stimulated by electrical

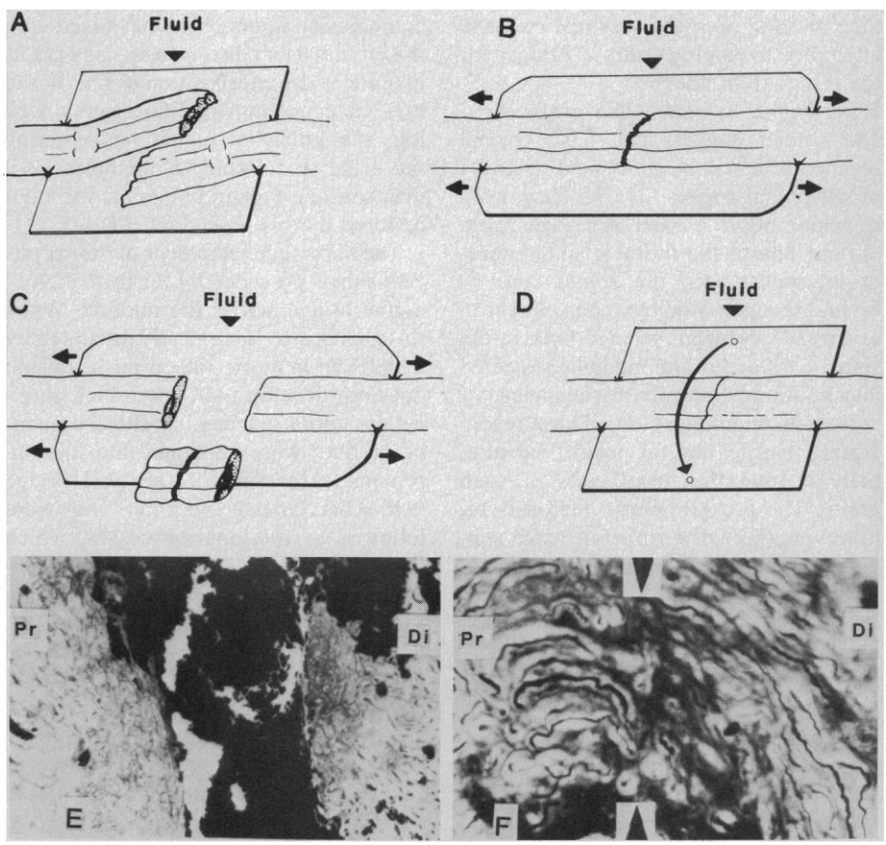

Fig. 2. Steps in a nerve reconnection procedure. (A) The transected stumps are sutured to a rubber support so that they overlap. The nerve is bathed in a modified Ringer solution containing chlorpromazine and polyvinyl alcohol at room temperature. (B) The nerve is cooled to below 12°C, soaked in Collins fluid containing chlorpromazine and polyvinyl alcohol, and the rubber support is stretched so that the nerve can be repositioned and aligned. (C) The nerve is slowly cooled to between −0.5° and −2.5°C, trimmed with a vibrating razor blade, warmed to 3°C, and the tension on the rubber band is released to reunite the stumps. (D) The temperature is raised to above 20°C, the nerve is rinsed with lactated Ringer solution, and the ends of the support are sutured together. Details are found in (*35*). (E) Histological appearance of a nerve repaired by conventional epineural microsuture 15 minutes after the operation (×200). The gap between proximal (Pr) and distal (Di) stumps is about 0.1 mm wide and is filled with blood. In other cases, the gap may contain fat, sheath fragments, or debris. (F) Appearance of a reconnected nerve (×600) 10 minutes after surgery. The reunion site (arrows) is narrow and free of extraneous material [figure 1 in (*35*)].

currents (*78*). Neurite outgrowth is accelerated and orients toward the negative pole or cathode of an electric field both in chick dorsal root ganglia and frog limb stump in vivo (*79*). In the lamprey, electrical currents applied to transected spinal cord stimulate axonal growth (*80*). Recent studies in animals indicate that a pulsed electromagnetic field may increase the rate of regeneration of severed peripheral nerves and spinal cords (*81*).

Grafts: As Bridges

During normal brain development, ependymal cells and later glial fibers provide a matrix for the growth of neurites (*82*). Beyond the loss of this supporting matrix with maturity, any form of CNS damage results in a further disruption of the support cell matrix. Thus CNS regeneration may be limited by the lack of an appropriate environment. For that reason, several investigators have attempted to employ grafts as bridges for the regrowth of fibers.

Peripheral nerve to CNS grafts. Several studies suggest that CNS axonal regeneration may be stimulated by grafts of peripheral nerves (*83, 84*). Segments of sciatic nerve grafted as bridges from the medulla to the thoracic spinal cord, or interposed into the spinal cord or brain, are innervated by axons of central origin (*84*). Neurons located both in the spinal cord and in the medulla projected into sciatic nerve grafts for distances of at least 30 millimeters (*84*). These regenerating neurites do not project substantially beyond the distal ends of such grafts. These experiments demonstrate, however, (i) that peripheral nerve support elements allow regeneration of central axons and (ii) that fully mature CNS axons have the capacity to generate new axonal processes.

Embryonic brain grafts. Some specific populations of unmyelinated or thinly myelinated fibers, particularly cholinergic and aminergic fibers, in the brain have considerable ability to regenerate when sectioned by techniques that do not create a gross disturbance (*14*). Neither these fibers nor any other CNS neurites have the ability to cross the gap created by electrolytic or knife-cut lesions or by other forms of mechanical disturbance (*12–14*).

When the fimbria-fornix is removed by aspiration, transecting the septohippocampal cholinergic pathway, the hippocampus is deprived of its cholinergic innervation. Grafting embyronic hippocampal tissue into the cavity that was created allows cholinergic fibers to reinnervate the hippocampus by growing through the implant (*85*). Several studies of embryonic brain grafts in the spinal cord have noted that the grafts fuse with the proximal and distal stumps to form a continuous uninterrupted structure with some properties of a bridge (*86–89*). These grafts might also conceivably act as bridges for spinal cord regeneration.

Artificial bridges. There have been numerous attempts to find artificial substances to support the regeneration of nerve fibers. For example, peripheral nerves will regenerate through silicone tubes for distances of 10 mm, becoming reorganized into fascicles with Schwann cells and regenerated perineurium (*42, 70*). Various polymers have been used to bridge gaps in transected peripheral nerves and spinal cord, with varying, but generally unsatisfactory, degrees of success (*90*).

Purified bovine collagen, injected into the site of a gap in the spinal cord after a compression injury, becomes fused with the proximal and distal stumps and greatly reduces the interposition of scar tissue (*91*). Catecholaminergic fibers grew well into the implants, sometimes entering the distal spinal cord. Nonetheless, somatosensory evoked potentials were not restored by this procedure (*91*).

The normal development of the corpus callosum is preceded by the formation of a glial bridge across the midline. When this glial bridge is surgically disrupted by a knife cut in utero, the corpus callosum does not develop (*92*). After such surgical disruption, pieces of cellulose membrane filter were implanted into the approximate location of the glial bridge (*92*). Glia coated the filter and were followed by neuronal processes, which formed a corpus callosum with an essentially normal appearance. Thus, when the appropriate substrate was provided, the remainder of the events in callosal development proceeded normally.

In sum, regeneration of intrinsic spinal cord axons was encouraged by peripheral nerve grafts into the spinal cord. These axons continued to regenerate only as long as they encountered the peripheral nerve milieu; when these axons again encountered a CNS environment (for example, the distal stump of the spinal cord), regeneration came to a halt. Apparently (i) these fibers are able to regenerate only when they encounter a peripheral nerve support structure; (ii) their regrowth is halted by properties of the distal graft-host interface; or (iii) both.

In contrast, regenerating central cholinergic neurites not only entered an embryonic brain graft, but also exited from the distal side and reentered the host brain, terminating in appropriate target regions (*85*). In this case, the severed neurites apparently had a greater inherent ability to regenerate, as they grew through not only embryonic brain tissue (the graft) but also mature brain tissue (the host brain distal to the graft). There were limitations in the regenerative capacity of even these neurites, however, as they could neither traverse the gap made by the wound cavity, nor could they circumvent the wound cavity by making a large detour through intact host brain tissue. Thus, although the growth of neurites may be enhanced by a supportive milieu, there are differences between CNS neurites in their capacity for growth.

Grafts: As a Source of New Cells

One of the most intriguing possibilities for promoting the reconstitution of damaged parts of the CNS is through the implantation of tissue grafts. There are two facets to this technique. First, in disorders characterized by the death of cells, transplantation may provide a means of replacing them. Second, and even more important, grafts are usually embryonic cells, which have the greatest potential to produce and extend new processes. Currently, transplantation of brain tissue has been applied to a number of neuronal systems, and in some cases brain grafts have been reported to partially reverse behavioral deficits consequent to CNS damage.

Nigrostriatal dopamine system. The most intensively studied clinical model of brain tissue transplantation is the dopaminergic innervation of the corpus striatum, which originates in the substantia nigra (SN) pars compacta. Damage to the SN dopaminergic neurons results in a variety of behavioral and motor dysfunctions in animals and humans in the form of Parkinson's disease (*93*). This system is attractive for the study of grafts largely because many of the consequences of SN lesions can be attenuated or reversed by the administration of dopamine agonistic drugs or L-dopa, the amino acid precursor of dopamine (*93*). This suggests that if grafts can be made to reinnervate the host brain there might be functional consequences even if the host brain does not in turn innervate or regulate the activity of the graft.

Embryonic substantia nigra grafts. In animals with SN lesions, embryonic SN can be transplanted to cavities in the

cerebral cortex, into the lateral ventricles, or dissociated and injected directly into the striatum (*94–96*). These tissues survive, producing a new innervation of parts of the denervated host striatum. Under various circumstances, SN grafts will alleviate many of the consequences of SN lesions, including apomorphine- and amphetamine-induced turning behavior (*94–98*), sensorimotor neglect (*96*), and postsynaptic receptor supersensitivity in the corpus striatum (*97*). These effects seem to be related to production of a new dopaminergic innervation of the denervated striatum, although neither the reinnervation nor the behavioral effects are complete (Fig. 3). The grafted cells are spontaneously active (*99*), suggesting that dopamine is released from terminals of the grafted neurons either at synaptic contracts or nonspecifically in or near the denervated regions. Other explanations for the behavioral effects must also, of course, be entertained. For example it is possible that a reinnervation produced by other types of fibers, nonspecific release of dopamine or other trophic and chemical substances, or other unknown processes contribute to the behavioral effects.

Adrenal medulla grafts. The epinephrine-secreting chromaffin cells of the adrenal medulla have properties in common with neurons, such as the ability to store and release catecholamines. In response to changes in environmental conditions, chromaffin cells can change their biochemical and morphological properties (*44, 45, 100*). For example, decreased corticosteroids reduce the production of epinephrine in favor of a relative increase in norepinephrine and dopamine (*100*).

When adrenal chromaffin cells are transplanted to the lateral ventricle of the rat brain, they produce large amounts of dopamine (*101*) and develop some processes but do not innervate the host brain. Nonetheless, these grafts decrease the motor asymmetry produced by unilateral SN lesions (*102*). Apparently, dopamine secreted by the grafts influences the host brain through diffusion.

Although it is not necessary to use embryonic adrenal medulla for these experiments, tissue obtained from fully mature rats may be less effective (*45, 98, 103*) even though the grafts survive. The exact age of the donor for which behavioral efficacy decreases is still in doubt, however, and could differ from species to species. In rhesus monkeys, adrenal medulla autografts transplanted directly into the caudate nucleus survived but in amounts of no more than 300 cells per recipient animal (*104*). Thus these procedures are not yet ready for clinical application.

Spinal cord. The ultimate, and yet unaccomplished, goal of any studies of spinal cord transplantation is to find a means of restoring function below the lesion. There are essentially two possible avenues through which spinal cord function might eventually be reconstituted through tissue transplantation: (i) improving the milieu to permit regeneration of the endogenous fibers (bridge grafts) and (ii) providing new cells with an increased capacity to generate neuronal processes.

Peripheral nerve grafts into the spinal cord promote regeneration of axons inherent to the spinal cord; however, this regenerative growth ceases when the regenerating axons reach the distal border of the graft (*84*). Such grafts are likely to be successful, therefore, only if an unbroken pathway from the site of spinal cord injury directly to the muscle can be constructed (*84*). Moreover, functional effects might be extremely difficult to obtain given the complexities of simple peripheral nerve repair. The use of other tissues (such as embryonic CNS) as bridges would seem less likely to be successful, as CNS support cells and the surrounding environment tend to be less conducive to regenerative growth than that of peripheral nerve. This approach should not be abandoned entirely, however, for several reasons: First, embryonic CNS may provide a medium for axonal growth that is superior to mature CNS. Second, embryonic CNS might provide a bridging medium that would not result in generalized regeneration but would allow for a selective regrowth of certain specific CNS neurites. Third, embryonic tissue might be used as an interface between sciatic nerve grafts and the distal stump so that continued regeneration would be supported.

The use of embryonic CNS grafts presents additional possibilities, as embryonic neurons in early developmental stages would have the maximum potential for growth. Each CNS area has certain advantages. Cortical tissue grows well after transplantation (*86*). Spinal cord tissue from embryonic animals is another possibility (*89*). Finally, embryonic aminergic neurons have good inherent abilities for axonal regeneration (*87, 88*).

Retina. Embryonic retina (*105, 106*) or whole embryonic eyes (*98, 107*) survive transplantation into the brains of neonatal and adult animals. Grafts of intact or cultured retina to newborn hosts develop extensive projections to the host superi-

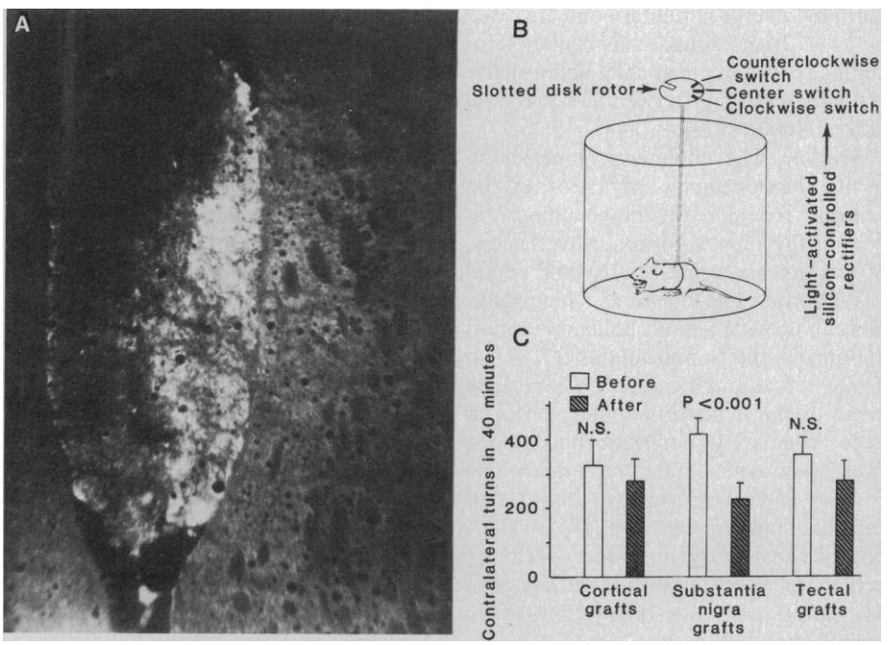

Fig. 3. The use of embryonic substantia nigra (SN) grafts to decrease rotational behavior consequent to unilateral SN lesions. (A) Appearance of an embryonic SN graft in the lateral ventricle of the rat processed for catecholamine histochemical fluorescence 3 weeks after implantation. The light areas in the graft indicate the presence of catecholamine cells and fibers, and the light areas in the host brain indicate reinnervation of the host brain by catecholaminergic fibers from the graft. The reinnervation of the host brain is always limited in extent, rarely penetrating more than 1.5 mm into the host brain even after 2 years. (B) Diagram of the device used to measure rotational behavior. A complete counterclockwise-clockwise rotation is recorded for each time the switches are activated in the sequence center, counterclockwise, and clockwise. [Adapted from figure 1 in (*98*)]. (C) Effect of SN grafts and control tissues on apomorphine-induced rotation (*97*).

or colliculus (*105*), but in adult hosts these connections are much less extensive (*106*). Electrical potentials can be evoked from embryonic eye grafts adjacent to the superior colliculus (*107*). There is as yet insufficient evidence that intracerebral eye grafts are functional in terms of bestowing a behavioral response to light in blinded animals (*98*).

Cortical grafts. Cerebral cortex is readily transplanted to the intact cerebral cortex of newborn animals (*108–110*). Many of the efferent and most of the afferent connections of cortical grafts in the cortex are uncharacteristic of normal animals (*109*). Cortical grafts grow very large in comparison with other embryonic brain tissue grafts (*98, 110*).

Aspiration lesions of the medial frontal cortex causes deficits in the performance of certain spatiomotor tasks. Grafts of late embryonic frontal cortex into the site of these aspirations improve behavioral performance (*111*). The specific graft efferents responsible for this effect are unknown. In general the excellent growth properties and extensive connections formed by cerebral cortex grafts make them good candidates for studies of the functional effects of embryonic brain grafts.

Neuroendocrine systems. Grafts of hypothalamic tissues into the third ventricle or directly into the hypothalamus partially reverse hereditary diabetes insipidus (*112*) and gonadal insufficiency resulting from a hypothalamic abnormality (*113*) and can also alter sexual behavioral differentiation (*114*).

Septum. The cholinergic innervation of the hippocampus originates in the septum, reaching the hippocampus by way of the fimbria-fornix. After lesions of the fimbria-fornix, embryonic septal tissue grafts adjacent to the hippocampus can provide a new cholinergic innervation to the hippocampus (*7, 115*). These septal grafts can partially reverse some of the deficits in spatial performance caused by fimbria-fornix lesions (*116*). Both solid septal grafts in cortical cavities and suspensions of dissociated cells are similarly effective (*116*). This is one of the few systems for which the behavioral effect of brain grafts has been associated with the restoration of an identified axonal pathway.

Artificial "Grafts" and Devices

Attempts to use electromechanical and electronic devices to aid in the functioning of damaged or destroyed nervous tissue have been numerous. Cardiac pacemakers can partially or totally replace extrinsic and intrinsic rhythm generators of the heart. Some coordination of movements in paralyzed limbs is possible with appropriately placed sensors and computer-driven feedback (*117*). "Artificial ears" capable of converting human words into electrical signals, which in turn stimulate a microelectrode array implanted in the cochlea give some, if limited, speech discrimination (*118*). Electromechanical and electroelectronic visual processing systems have shown promise by either transducing images into tactile stimuli or directly stimulating the visual cortex (*119*). Several groups are developing microchips with implanted electrode arrays capable of monitoring single neuronal units as well as stimulating the same neuron (*120*). Since very complex circuitry can be built into these devices, the microchip may eventually form the basis for very sophisticated electroprosthetic devices.

Conclusions

Functional neurite regeneration requires (i) neurons having the inherent capacity to regenerate new axonal, and possibly dendritic processes, and (ii) a continuous, unbroken pathway to the neurites' appropriate targets through an undisturbed perineuronal environment.

To provide the first requirement, there are at least three avenues. (i) The neurons may already have this potential, but it may not be expressed because of disturbances in the milieu. (ii) Through trophic influences, this potential might be brought out in mature neurons. (iii) Embryonic neurons, with greater powers to generate axonal tracts, might be substituted, through transplantation, for the mature cells.

To provide an environment permissive to regeneration, it is probably not sufficient to suppress scarring. Rather, an active intervention to provide a surrounding comparable to that experienced by developing neurites is probably necessary. It is unclear exactly how this might be accomplished, but there are some hints. The use of artificial bridges is one possibility. The peripheral nerve environs, consisting of basal lamina and Schwann cells, are highly conducive to regenerative growth. If reconnected with minimal disturbance, peripheral nerve consistently and functionally regenerates. When transplanted to the CNS, peripheral nerve segments promote axonal regeneration, even through the proximal junction of graft and host (*84*). When the growing axons reach the distal graft-host junction, however, regrowth is curtailed (*84*), perhaps because they again encounter disturbed surroundings. A less ideal, but still favorable, milieu may exist in the normal undisturbed brain and spinal cord. When these structures are damaged, however, the favorable environment is not spontaneously reconstructed and cannot be restored simply by suppressing the formation of scar tissue. Several methods of delaying or decreasing the formation of scar tissue after spinal cord injury have been described (*37, 38*). That none of these manipulations has caused the spinal cord to regenerate strongly suggests that diminishing scar formation alone is insufficient.

Is human application possible? The ultimate purpose of experiments aimed at manipulation of CNS plasticity is an application to human disorders. With a few exceptions, such as certain devices and physical training (*121*), these procedures are not generally thought ready for human application. It is appropriate to ask what criteria should be met before a procedure can be applied to humans and the circumstances under which an application should be made. We suggest several general criteria.

1) Any procedure should be effective in animal models of specific disorders, according both to functional measures (behavioral or physiological) and to anatomical or biochemical mechanisms that underlie the effects.

2) The effect shown for animals must not be marginal or demonstrable only in statistical terms. It should be robust and statistically significant for small groups of animals. If a procedure is to be applied to humans, a high rate of success should be anticipated.

3) Some basis for scaling the procedure up to humans should be obtained. This probably means investigating the procedure in species of various brain or body sizes.

4) The procedure should be functionally effective in a larger and higher species, such as dogs, cats, or subhuman primates. Although it may not always be possible to demonstrate an effect behaviorally or functionally in primates, the procedure should at least be shown to be practicable in anatomical terms.

5) Every effort to avoid adverse reactions or side effects should be made. These include tissue rejection, induced autoimmune reactions, other immune phenomena, and undesired side effects from the procedure or from surgery.

These criteria involve only the scientific aspects of such research. In addition, any procedure considered for human application should adhere to ethical

standards for medical and surgical practices. The most important criterion for the ethicality of any procedure, however, is that it should be studied thoroughly in animals so that when applied to humans it might be expected to succeed.

Many procedures such as surgical manipulations, application of trophic chemical substances, grafting of tissues as bridges for regenerating fibers, grafting to supply new cells with regenerative potential, and artificial stimulation can be conceived of as ultimately applicable to human disorder. In addition, there are others that have not been discussed (*121*, *122*). Although none of these procedures are ready for human application, avenues for human application can in some cases be visualized. This can be said only for certain well-understood disorders where the problems of restoration are very simple. For example, regeneration occurs in peripheral nerve. It needs only to be better organized to succeed functionally. In another relatively simple case, Parkinson's disease, little specific cell-to-cell organization is apparently required, as chemical agents mimicking the natural neurotransmitter can alleviate symptoms of the disease. All that is necessary is to provide a new catecholaminergic input. These two disorders thus represent opposite ends of a spectrum. In peripheral nerve, regeneration occurs without intervention, but organization must be aided artificially. In the nigrostriatal system, new cells which have the ability to regenerate axonal processes must be supplied. Very little specific cell-to-cell organization seems to be required.

Such disorders are probably the exception rather than the rule. Most CNS disorders, such as spinal cord injury or general trauma, are far more complex. Such regeneration as does occur is abortive or insufficient to restore normal amounts of interneuronal connectivity. Even if cells with regenerative potential can be supplied, the degree of organization supplied by specific chemical affinities (*123*) might be insufficient for functional restitution. Interneuronal connections involving dendritic processes and complex relations between various nuclei that may also be disturbed would be difficult to restore by any simple manipulation. Multiple techniques—many of which probably have not yet been developed—might be required. For many CNS disorders, it may be premature to investigate therapeutic approaches directly. It is therefore essential that basic research in neuronal plasticity continue to be vigorously pursued so that new approaches can be developed.

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RESEARCH ARTICLE

Three-Dimensional Flow in the Upper Ocean

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Regularly spaced, long, narrow surface slicks or rows of flotsam are commonly observed on the surface of windswept lakes and seas. In 1927, while on an Atlantic crossing to England, Irving Langmuir (*1*) observed such parallel lines of floating seaweed. After he returned home to New York, Langmuir conducted a series of ingenious flow visualization experiments in Lake George, showing that the slicks are formed in regions of convergent surface flow which are associated with counter-rotating helical vortices near the surface of the lake. The rotational axes of the vortices were horizontal and nearly parallel to the direction of the wind. These helical flow patterns are now called Langmuir circulation or, individually, Langmuir cells. Today we know that surface slicks are caused by other processes as well as by Langmuir circulation. However, in the open ocean, under moderate to heavy winds, surface slicks or rows of floating seaweed that are aligned nearly parallel to the wind are taken as evidence that helical Langmuir circulation is present within the mixed layer.

Meteorologists and physical, chemical, and biological oceanographers are interested in Langmuir circulation and in any other organized three-dimensional flows in the upper ocean because such flows should be effective mechanisms for transporting horizontal momentum, heat, nutrients, and organisms vertically through the wind-stirred upper boundary or mixed layer of the ocean. The field measurements collected to date, however, are insufficient to describe the persistence, depth of penetration, and amplitude of the helical flow. Thus, only limited evidence for such vertical transport exists.

Woodcock (*2*), for example, observed that buoyant *Sargassum* was carried under the surface in the regions of convergent surface flow. Accordingly, phytoplankton, with small terminal velocities (*3*), should be carried along with the helical flow and experience large changes in irradiance (*4*), affecting their photosynthesis (*5–7*); yet that conclusion is presently based almost entirely on laboratory experiment (*5, 8*) and theoretical inference (*9–11*). Recently, in Loch Ness, Thorpe and Hall (*12*) observed tongues of warm water extending downward toward the base of the mixed layer from beneath surface slicks. Such evidence from field studies (*13, 14*), recent laboratory experiments (*15*), and numerical models (*16*) suggest, but do not prove, that three-dimensional flow such as Langmuir circulation plays an important role in upper ocean processes.

Recently we began a study of the physics and biology of the mixed layer, including an investigation of the role of organized, three-dimensional flows. Our first goal was to develop the capability to make accurate measurements in the upper ocean of the vertical and horizontal components of velocity, the temperature, the conductivity, and the concentrations of chlorophyll a (a good indica-

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