Regeneration in the Central Nervous System

Years of pessimism about the failure of mammalian central neurons to regenerate are giving way to new optimism about their regrowth potential

Injury to the brain and spinal cord is one of the most intractable of all medical problems. In the United States alone, more than 200,000 persons, many of them young and in what ought to be the prime of life, are paralyzed as a result of spinal cord damage. Loss of the ability to walk is only one of their problems. Spinal cord damage can also produce sexual impotence and inability to control the bladder and bowel.

Once damaged, the nerves of the mammalian central nervous system do not repair themselves. And currently there are no effective therapies for restoring abilities lost as a result of the damage. Clinical attempts to reestablish missing neural connections have generally met with failure and frustration.

For many years, this pessimistic outlook extended from the clinic to the basic neurobiology laboratory. As recently as 1970, says Lloyd Guth of the University of Maryland School of Medicine, "We agreed that there was no need for a conference on spinal cord regeneration because so little was known about it."

Thanks to the persistent urging of Alan Reich, who was then president of the National Paraplegia Foundation and who, among other things, raised the necessary funds, a conference was held anyway. The participants came away pleasantly surprised by the news that the potential for regeneration of neurons of the mammalian central nervous system is greater than they had thought. In the 10 years since the conference was held, several lines of evidence have shown that both brain and spinal cord neurons are often intrinsically capable of growing back, even though they normally do not, at least not in a productive way.

The emphasis now is on finding out why the neurons do not regenerate and on attempting to devise ways to circumvent the impediments to central nerve regrowth. The answers are clearly not in yet. But investigators are more optimistic than they have been in the past, because for the first time they have a number of tantalizing clues and promising avenues to explore.

Nerve cells in the spinal cord, like nerve cells in general, consist of a cell body, which contains the genetic material and synthesizes the proteins and other substances needed by the neuron, and at least one long projection called an axon. The axons lead out of the cord, often extending for long distances—1 meter or more in the case of nerves leading to the toe, for example—to their target cells.

What generally happens when the spinal cord is damaged is that the segments of the axon above the injury, which are still connected to the cell body, produce new sprouts, whereas the segments below the injury degenerate and die. The sprouting is short-lived, however, lasting only a week or two and failing to produce links to the neurons' original targets.

The damaged spinal cord, it seems, is a very hostile environment for nerve regrowth. Part of the hostility derives from the nature of the nonneuronal cells, principally glial cells, present in the cord. Albert Aguayo of McGill University Medical School says, "We need to consider very seriously the contribution of the glial cells to the failure of mammalian central neurons to regenerate."

The glial cells proliferate in the damaged region of the cord, forming a dense tangle that physically blocks the attempts of neurons to regenerate their axons. In contrast, the nonneuronal Schwann cells that are present in peripheral nerves, which do regenerate, serve to direct axon regrowth and may even foster it.

In experiments on laboratory animals, investigators have attempted to overcome the physical blockade to spinal nerve regeneration by removing the damaged portion of the cord and replacing it with a graft of peripheral nerve tissue. They hoped that the Schwann cells of the peripheral grafts would help to guide the regenerating spinal axons just as they do the peripheral nerves—and this is apparently what happens.

For example, a few months after Aguayo grafted pieces of peripheral sciatic nerve, which innervates the muscles of the leg, into the spinal cords of rats, he could detect many axons growing into the grafts. Some of these axons turned out to be from peripheral nerves that send projections into the cord, but others were spinal neurons that had crossed the junction between the end of the cut cord and the graft and traveled distances up to about 10 millimeters, the approximate length of the grafts. Aguayo says, however, that he was unable to tell whether the spinal axons reentered the cord on the other side of the graft and made new connections. He does know that none of the animals with the grafts showed any functional improvements.

In another series of experiments, Aguayo grafted pieces of the optic nerve, which is part of the central nervous system, into the sciatic nerve. Although sciatic nerve axons normally regenerate very well, in this case they grew a millimeter or two into the optic nerve grafts and then stopped. Aguayo explains, "the axons just had trouble getting into that dense jungle of astrocytic processes." (Astrocytes are one kind of glial cell.) Aguayo's findings confirm the importance of the nonneuronal cells, showing that the axons of damaged central neurons can regrow provided they are in an appropriate cellular environment.

In some animals the timing of the transplant may be important, according to Carl Kao of Georgetown University School of Medicine and the Washington Veterans Administration (VA) Medical Center. When Kao, who did much of his earlier work at the Madison (Wisconsin) VA Hospital, transplanted sciatic nerve tissue into the spinal cords of dogs immediately after injuring the cords, he failed to detect any significant growth of spinal neurons into the grafts. He found that over about a week, cystlike cavities developed at both cut ends of the cord, and they constituted still another block to axon regrowth.

Now Kao waits about a week after the injury and then cleans out both the damaged portion of the cord and the cysts before he inserts the transplants. If this is done without reinjuring the nerve ends, he says, the cysts do not reform and spinal nerves grow into the graft.

Kao's results differ from Aguayo's in an important aspect, however. The rats

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studied by Aguayo never recovered from their paralysis, even though their spinal neurons did show some regeneration. But, according to Kao, some of his treated dogs—about 10 percent—regained the ability to walk.

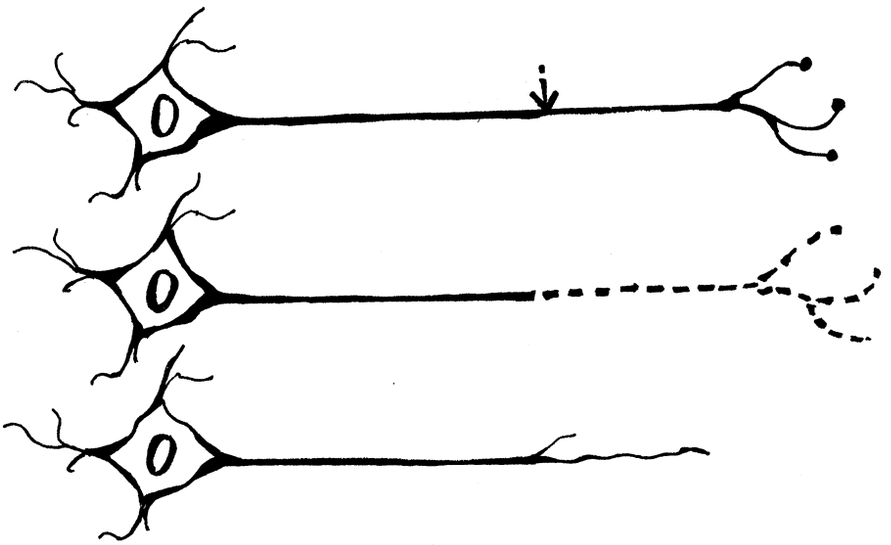
This claim is highly controversial. Some neurobiologists have suggested that the apparent ability of Kao's treated animals to walk may be explained by a simple spinal reflex, originating in the cord below the injury. True voluntary walking requires transmission of nerve impulses down the cord from the brain. As evidence against this possibility, Kao notes that the walking stops when the animals' spinal cords are injured a second time, above the original site. The second injury ought not to interfere with a spinal reflex, but would block the transmission of nerve signals from the brain.

Another criticism of Kao's work is that he did not demonstrate that regenerating spinal axons actually crossed the damaged region of the spinal cord, entered the lower cord, and formed new connections. This is a common omission in all the research on spinal regeneration done so far. Says Ray Lasek of Case Western Reserve School of Medicine, "The literature is filled with examples of partial functional recovery, which have not been subsequently documented." Within the past few years, however, methods for tracing the origins of axons have been developed and these may help to clarify the question of spinal axon regeneration.

A somewhat different transplant approach for repairing central nervous system damage has been tried by Richard Wyatt and his colleagues at the National Institute of Mental Health (NIMH). They first chemically destroyed a group of neurons, which are important for the control of movement, in the brains of adult rats. The degeneration of comparable neurons in humans causes Parkinson's disease, a severe neurological disorder marked by tremors and difficulty in initiating voluntary movements.

The NIMH workers then transplanted into the damaged rat brains grafts of the same neurons taken from fetal rats. The grafts survived, presumably because their location deep within the brain protected them from attack by the immune system, and sent out axons that made appropriate connections. As a result, says Wyatt, the abnormal movements seen in the rats were reduced.

Nonsurgical therapies have also been tried to diminish scar formation and encourage spinal nerve regrowth. For example, in the 1950's, William Windle of Denison University found that treatment with steroid hormones or a fever-producing bacterial product called Piromen resulted in less dense glial scarring and enabled sprouting spinal axons to grow for months or even years instead of a week or two. Even so, Windle observed no improvement in the condition of the paralyzed animals.



When a nerve axon is cut or crushed (arrow), the segment of the axon below the injury degenerates. The cut axon may begin to regrow, although in the mammalian central nervous system the regrowth normally proceeds for only a short distance.

Use of enzymes such as trypsin and elastase to dissolve glial scars is another method that has been tested for improving spinal regeneration. In fact, the method is currently being used for human patients in the Soviet Union.

In 1973, L. A. Matinian and A. S. Andreasian of the Armenian Academy of Science reported that injection of the enzymes into the severed spinal cords of rats produced complete recovery from paralysis in 30 to 50 percent of the animals. This result, if true, would represent an unprecedented degree of recovery and a major breakthrough. But, says Guth, there were several "disquieting" aspects of the report, including Matinian and Andreasian's finding that Piromen, which Windle had studied for many years and always found to be ineffective in restoring movement, produced recovery in 25 percent of the rats.

Since then, an attempt to reproduce the Armenians' experiment has been made by investigators in this country, who found no recovery attributable to the enzyme therapy. Where recovery did appear to occur, it could be explained by incomplete severing of the cord in the first place, which may happen unless special precautions are taken to prevent it. Animals whose cords have not been completely cut often recover spontaneously, and this is what Guth thinks happened in the Armenian experiments.

Although some nerves, such as peripheral nerves, may regenerate well, they still do not necessarily make the appropriate connections. In a recent experiment, Thomas Brushart and M.-Marsel Mesulam of Beth Israel Hospital in Boston severed the sciatic nerves of rats and then attempted to repair them by stitching the ends together. The sciatic nerve consists of bundles of axons, each of which is encased in a connective tissue sheath. Brushart and Mesulam attempted to align the appropriate sheaths above and below the cut, in the hope that this would help to guide the regenerating axons to their appropriate targets. Nevertheless, the regenerating nerves did not form completely normal connections to the leg muscles. The connections were reduced in number and some were directed to the wrong muscles. The investigators suggest that the inaccurate regeneration may have resulted from improper alignment of the axon sheaths. They also suggest that, through microsurgery done at higher magnifications, it may be possible to line up the sheaths more accurately.

Even this may not be enough to ensure that regenerating neurons contact the right target cells, however. As important as the mechanical influences of nonneuronal cells are in fostering or hindering nerve regeneration, they are not the only factors affecting it. Chemical factors, acting to stimulate axon outgrowth or to help direct it to the appropriate targets, are also involved. In the 1950's, for example, Roger Sperry of the California Institute of Technology suggested that the regenerating axons of the optic nerve of lower vertebrates, including frogs and fish, found their way to the appropriate

regions of the brain by recognizing specific chemicals located on the target cells. Says Bernice Grafstein of Cornell University Medical College, "Sperry's ideas are still as good as any we have today." The problem has come in trying to identify the chemicals, none of which have yet been found.

One current candidate is nerve growth factor (NGF), a protein originally discovered by Rita Levi-Montalcini, of the Laboratory of Cell Biology in Rome and Washington University School of Medicine, and Viktor Hamburger, who is also at Washington University. This protein is needed for the development and maintenance of certain kinds of neurons, and may also be involved in guiding those neurons to their targets.

Because not all nerves are affected by NGF, most investigators view the agent as a prototype of what is probably a class of agents that enhance or direct neuronal growth. Silvio Varon and Ruben Adler of the University of California at San Diego are investigating several factors that act on neurons from the ciliary ganglion, a collection of nerve cells that do not respond to NGF. They found, among other things, that heart cells, which are normally innervated by neurons from the ciliary ganglion, produce at least two factors, one of which is required for neuronal survival while the other promotes the formation of axonlike projections by the cells. The latter factor apparently acts by making it possible for the elongating projections to adhere to the substratum of the culture where they are growing. (Nerve projections must adhere to their surroundings in order to grow.) Varon proposes that directional axon growth in the living animal may be aided by the production of one or another of these factors. Analogous agents for other types of neurons are expected to turn up as investigators begin looking for them.

While some investigators have been concentrating on the external factors, such as glial cells and chemical agents that might affect nerve regeneration, others have focused on the intrinsic neural activities on which axon regrowth depends. Because most—if not all—axon constituents are made in the cell body, which may be some distance from the growing axon tip, transport of materials from the cell body down the axon is one of these essential activities. In particular, investigators are looking at the transport of the proteins comprising the cell skeleton, a framework of tubules and filaments described by John Griffin of Johns Hopkins University Medical School as "the scaffold on which the axon hangs as it grows out."

Axonal transport occurs at different rates, being as fast as 400 millimeters per day for some proteins and as slow as 0.2 millimeter per day for others. The building blocks of the cell skeleton are transported exclusively in the slower components.

Almost 40 years ago, the noted neurobiologist Paul Weiss suggested that the axon contents move as a continuous column from the cell body to the axon terminal. Although the discovery of different transport rates for different components required a modification of Weiss's ideas, their general thrust remains correct. The various transport components move continuously, whether or not the axons are elongating. "In a sense," says Lasek, "nerve axons are regenerating all the time."

When an axon is cut, the cell body undergoes a number of biochemical changes, among them a step-up in the rate of synthesis of cytoskeletal proteins. But Lasek points out that axonal regrowth begins well before the wave of newly synthesized proteins, which move just as leisurely in regenerating axons as they do in nongrowing ones, reach the elongating ends. Apparently, the normal rates of cytoskeleton synthesis and transport are sufficient to support regeneration, even though the synthesis may be augmented in order to enhance regrowth after an injury.

Because the cytoskeleton is constantly being made and sent down the axon, there must be some means of disassembling it at the ends of axons that are not growing. Lasek has proposed that protein-digesting enzymes (proteases), which are activated only in the nongrowing tips of axons, might accomplish this goal. One possible protease activator is calcium ions, which move into the axon terminals when the nerves fire.

According to Lasek, the transport of one component of the cytoskeleton, the microfilament network found mainly in the growing ends of the axon, may be rate-limiting for regeneration. The rate of transport of the microfilaments, which is 4 millimeters per day, is about the same as the rate of axon growth. Moreover, when the rate of this slow transport is decreased, as in the aging animal, the rate of regeneration is also decreased.

Other investigators have also observed that nerve regeneration is diminished with age. For example, Carl Cotman of the University of California at Irvine reports that, when neurons in certain areas of the brain are injured, undamaged neurons may respond by sending out new sprouts to replace those that were lost. This response is less pronounced in aging animals than in younger ones.

According to Cotman, glucocorticoids, steroid hormones secreted by the adrenal cortex, slow down the sprouting in younger animals. Other investigators have found that glucocorticoid production is higher in older animals than in younger ones, and Cotman suggests that this might be one factor contributing to the slowing down of regeneration with advancing age. How the hormones might work—whether by affecting axonal transport or some other factor regulating axon growth—is unknown.

Cotman's observation may be significant for the therapy of central nervous system injuries, because glucocorticoids are often administered to prevent swelling of the brain or spinal cord. If the hormones also inhibit sprouting, they may actually be retarding recovery from an injury. Cotman emphasizes, however, that this is by no means certain. Sprouting is not necessarily beneficial and may be harmful if it interferes with reestablishment of the correct neural connections. Wrong connections may leave the injured animal—or human patient—worse off than with no reformed connections at all.

Whereas glucocorticoids may slow down nerve regeneration, other treatments may speed it up. Grafstein, with Irvine McQuarrie, also of Cornell University Medical College, found that if a nerve is injured a second time, about 2 weeks after the first injury, it regrows faster than it originally did. Cotman observed a similar effect in the brain.

According to McQuarrie, who is currently on sabbatical at Case Western Reserve, the faster regrowth may reflect the availability of an increased supply of cytoskeletal and other structural axonal proteins. Because of the slow rate of transport of these proteins, they would not arrive at the growing axon tip until a week or two after the first injury, where they would be immediately available for growth if the axon were to be reinjured.

Research on nerve regeneration is proceeding in several directions, with some investigators emphasizing the external factors involved and others the internal factors. All concede, however, that both types of factors probably participate, although perhaps not equally. "In certain situations," says Lasek, "local environmental factors may dominate; in others, intrinsic neuronal factors may be more important. The question now is, can we identify individual factors that are so influential that their modification is sufficient to permit regeneration in the central nervous system?"—JEAN L. MARX