GROW, NERVES, GROW

How can severed nerve cells be encouraged to reestablish functional connections?

By JULIE ANN MILLER

In an episode of Hugh Lofting's *The Story of Dr. Dolittle,* a resourceful band of monkeys spans a river by creating a "living bridge," each one's arms holding the legs of the one before it. Another kind of living bridge may be the key to promoting repair of damaged spinal cords and brains. In this case, the bridge is built of implanted tracts of cells derived from the more peripheral regions of the nervous system.

Injury to the brain or spinal cord – the central nervous system, or CNS – tends to produce permanent damage in people and other mammals. If nerve cells are killed, they cannot be replaced. Even when the cell bodies are spared, if the injury severs some of the millions of long fibers that carry signals, connections are lost and a person may suffer loss of sensory input or motor control. The cell body does not regenerate those thin cellular extensions.

In amphibia and fish, some parts of the brain and spinal cord *will* regenerate the long fibers, which are called axons. Even within the human body, the peripheral nervous system (PNS), which runs between the spinal cord and the muscles and sense organs, can regrow damaged processes. Medical researchers are making progress in encouraging this regrowth after PNS damage (SN: 9/21/85, p. 183). But the failure of nerve cells in the CNS to regenerate cut processes continues to frustrate physicians searching for treatment for the people – almost half a million each year in the United States alone – who suffer from head and spinal cord injuries.

Since the 1920s some scientists have suspected that the source of the problem lies not in the capabilities of the nerve cells but in an inhospitable environment. More recently, Albert Aguayo and his colleagues at McGill University in Montreal, Quebec, have demonstrated that, when placed in the environment of peripheral nerve cells, even CNS nerve cells can regrow axons (SN: 12/5/81, p. 363). Now Aguayo is taking advantage of this observation to direct the growth of rat CNS neurons, developing techniques that may be useful for direct repair of CNS injury and for transplanting brain tissue.

"Once you dress up like a peripheral nerve cell, you behave like one – within certain limits," Aguayo told a recent science writers' workshop at the Cold Spring Harbor (N.Y.) Laboratory.

The technique may be thought of as an environment transplant, Aguayo says. In his experiments, Aguayo transplants into an injured rat brain or spinal cord a segment of a peripheral nerve, the sciatic nerve of the leg. The nerve cell components rapidly degenerate, but the supporting cells, called the Schwann cells, divide and remain aligned in columns, which are surrounded by a continuous tube of material called basal lamina.

"The graft is not just a bridge," Aguayo says, "but a biologically active system." The Schwann cells secrete molecules that stimulate nerve growth. One of these substances is nerve growth factor, a molecule that causes axonal growth under a variety of conditions.

A search for other growth-stimulating substances is under way in several laboratories, including that of Eric Shooter at Stanford University. Shooter and his colleagues have identified several candidates. One is a protein that accumulates in peripheral nerves but not in central nerves. It appears to bind fat molecules spilling out of damaged nerves and provide material for new construction. Another candidate is a molecule found in the axon's growing tip, both in young animals and in regenerating nerve cells. The scientists expect that ultimately at least several proteins will be found to be involved in the natural switching on of nerve growth.

"We don't know yet what is crucial," Aguayo says. "But for the first time we are getting information about the molecular events that take place." The PNS graft may also provide a surface – the basal lamina – conducive to regeneration.

Absence of inhibitory factors may be another crucial factor in the PNS environment. Hans Thoenen and Martin Schwab of the Max Planck Institute for Psychiatry in Martinsried, West Germany, propose that the CNS contains substances that inhibit nerve outgrowth. These substances may be necessary in the uninjured adult nervous system to prevent axon growth and rearrangement of connections, processes that could be detrimental to brain function.

Whatever the underlying mechanism, Aguayo has clearly demonstrated that a peripheral nerve environment can trigger regrowth of CNS axons. In a typical experiment, he makes an incision into the rat's brain or spinal cord, thereby cutting many nerve cell axons. One end of a peripheral nerve segment, the PNS graft, is inserted into the incision. Some of the damaged nerve cells then regenerate axons that grow into the PNS graft (see cover and p. 195).

The scientists, thus far, have no control over which damaged nerve cells enter the graft. Aguayo says, "It's like ice fishing. We make bait and wait for something to take it."

Those axons that do enter the graft may grow to span even greater distances in the graft than they normally span in the brain. Severed axons in the retina, for example, can grow 2 to 3 centimeters, twice the length of the normal optic nerve. Spinal cord nerve cells have sent axons through a 7-centimeter graft.

The scientists find normal electrical activity in some of these axons, both spontaneous activity and response to stimulation. Axons in a graft to the retina have demonstrated, in response to light on the retina, electrical activity that is indistinguishable from that of axons in a normal optic nerve. And the axons that enter grafts to the part of the brainstem that receives signals from the rat's whiskers give an electrical response when the rat's whisker is tweaked.

"We assume that many cells don't respond, but now we know some cells do respond normally," Aguayo says. "We are

pleased by the fact that some of these cells communicate in appropriate language. But we don't know yet if there is communication with any target cells."

Although it is too early to conclude that all CNS cells can send fibers into PNS grafts, such growth has been demonstrated in dozens of areas of the brain and spinal cord. Aguayo has shown that the axon regrowth arises from cells of different sizes, geometry and chemistry. However, cell types differ in what fraction of injured cells will populate a graft. This variability "provides but a hint of the multiplicity of influences that shape neuronal responses to injury in the adult mammalian brain," Aguayo says.

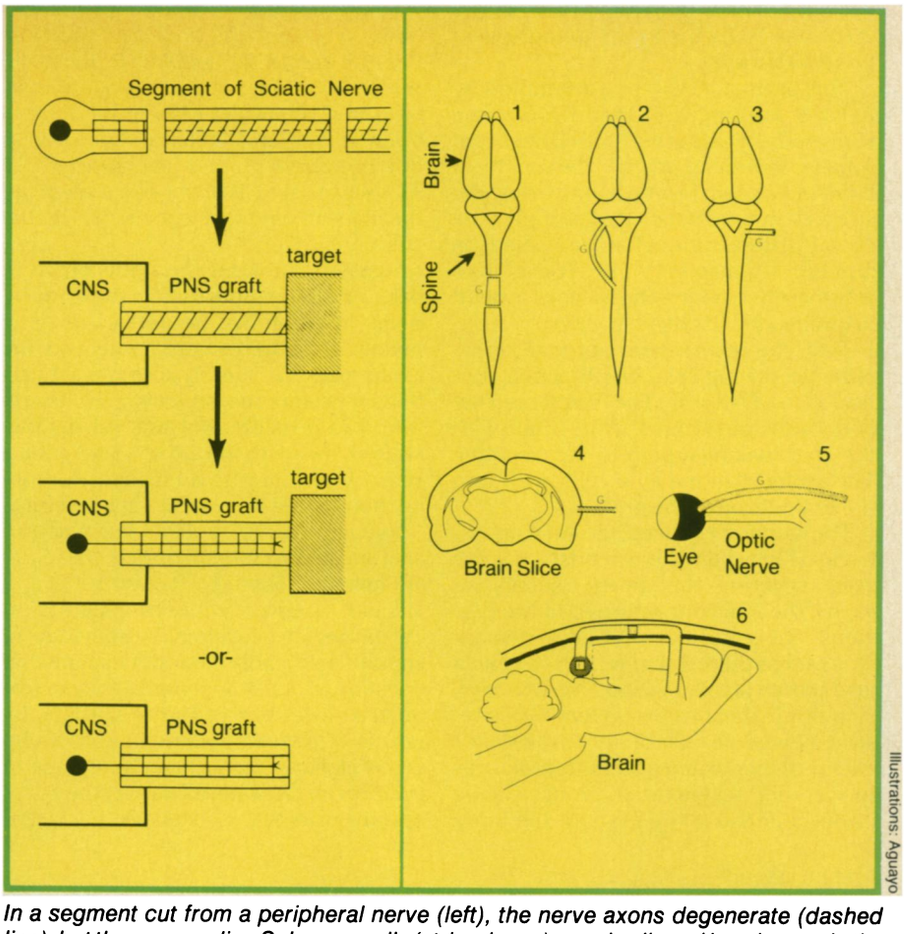
While Aguayo has demonstrated that axons can grow through a PNS graft, he does not yet know whether they can establish functional connections at the other end. Such connections might be necessary to the longevity of the axons as well as to restoration of brain function, Aguayo and his colleagues reported at the recent meeting in Dallas of the Society for Neuroscience.

Practical applications of this technique might someday include using a PNS graft as a bridge to span a damaged area of the spinal cord or brain. Aguayo has already begun studies in which a PNS graft, instead of an optic nerve, connects a rat's retina to its brain. The researchers drill a hole through the skull above the site that normally receives retinal input. They lay the graft on the top of the skull and thread it through the hole. "The axons are led by this curious course back to their target," Aguayo says.

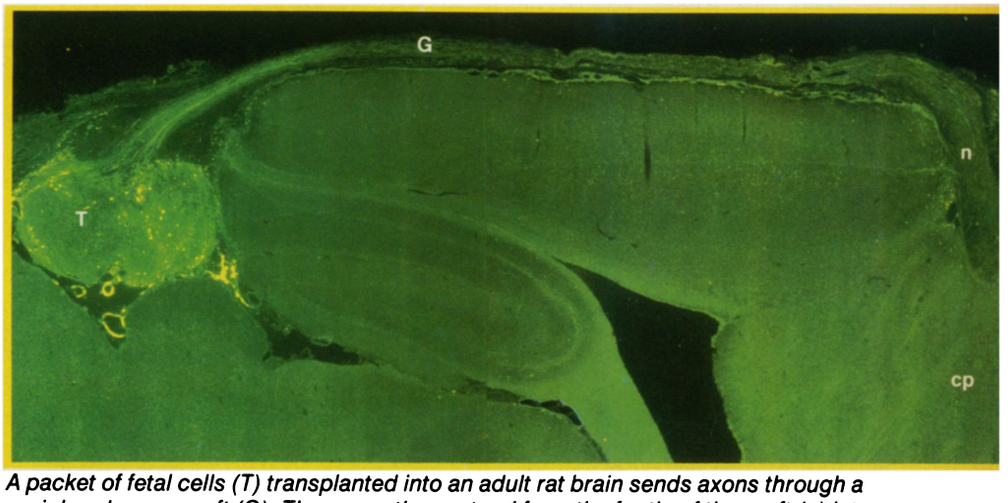
He finds that the axons from the retina spread radially as they leave the graft and grow about 1 millimeter to the appropriate brain area, the tectum. He has observed growth cones, the specialized axon tips characteristic of growing axons during embryonic development. "We are presently looking to see if the axons eventually culminate in differentiated synapses [the specialized junction across which nerve cells communicate with chemical or electrical signals]," Aguayo says.

This technique also may open up new possibilities for brain cell transplants. "We can use this strategy to establish new interactions between distant neurons," Aguayo says.

If fetal cells are implanted into an adult brain to replace damaged cells, the CNS environment discourages axonal growth. A PNS graft can encourage such growth as well as guide the axons across long distances. This procedure can provide not only a pathway but also a reservoir of fetal cells. Instead of implanting the cells into the brain, risking further brain damage, researchers can place them in a pouch constructed of peripheral nerve sheath and implant the pouch at a con-



In a segment cut from a peripheral nerve (left), the nerve axons degenerate (dashed line), but the surrounding Schwann cells (striped area) remain aligned in columns. In the transplantation experiments, researchers insert one end of the segment into the central nervous system (CNS). Axons from the CNS then grow through the graft, whether or not it is connected to a target tissue. At right, nerve axons grow through segments of peripheral nerve grafted into the central nervous system in many ways. The graft (G) may link cut ends of the spinal cord (1), bridge widely separated neural areas (2) or provide a conduit from the spinal cord (3), brain (4) or retina (5). The graft may also channel axons from a reservoir of transplanted fetal nerve cells into a chosen site in the adult brain (6).



A packet of fetal cells (T) transplanted into an adult rat brain sends axons through a peripheral nerve graft (G). The axons then extend from the far tip of the graft (n) into the selected region, the caudate putamen (CP) of the host brain. This micrograph was taken seven months after the grafting.

venient location outside the brain. The graft then leads the axons to the appropriate brain site.

This approach is being attempted by Aguayo, working with Fred Gage of the University of California at San Diego and Anders Björklund of the University of Lund in Sweden. In a rat's brain, they destroyed connections that supply the chemical dopamine to the area called the striatum. This surgery produces a characteristic behavior—the rat goes around in circles (SN: 11/20/82, p. 325).

Next, the researchers put fetal dopamine-producing cells into a pouch connected to a PNS graft. They implanted the pouch outside the back of the brain, with the graft making a path to the striatum. The axons from the fetal cells grew into the brain through the graft.

The implant reduced the turning behavior. But when the scientists cut the graft, severing the newly established axons, the behavior returned to the previous abnormal level. "This demonstrates that there is a new pathway and a new source of innervation," Aguayo says.

In this instance, normal function is restored by nerve cells simply providing a chemical. Important questions remain as to whether regenerated axons will be useful in those many parts of the brain where the specific pattern of connections between cells underlies function. Can the axons, all jumbled in the graft, recognize their appropriate connections? Do the target cells in the mature brain still show the characteristics that in the embryo guided incoming axons? As Aguayo asks, "Will the axons smell out the determinants of specificity in the adult organ?"

Aguayo's work on the optic nerve fibers of rats is addressing these questions. In amphibia, he notes, cut optic nerves are able to regenerate and the axons make sufficiently accurate connections to restore the animal's sight. Therefore it is possible, but not yet demonstrated, that with the aid of a PNS graft, a sensory system with all its specific connections could be restored in a mammal.

Aguayo likes to quote an early neuroanatomist who foreshadowed these developments. Santiago Ramon y Cajal, a Spanish scientist, wrote in 1928, "... if experimental neurology is someday to supply artificially the deficiencies in question, it must accomplish these two objects: It must give to the sprouts, by means of adequate alimentation, a vigorous capacity for growth; and place in front of the disoriented nerve cones ..., specific orienting substances." □

Letters continued from p. 195

brothers), but it would seem to be a first step toward a sterile dead-end. It may be a clever piece of programming, but it can hardly be called "scientific." As an artist and professional computer programmer myself, it strikes me that the use in this context of computers and so-called "scientific discourse and scientific criticism" will lead to little other than spurious and unwieldly metaphors.

Cecil Bloch
Los Angeles, Calif.

I would be surprised if the "rules and steps needed to recreate the basic structure in a typical Diebenkorn painting" could really be stored in "about 8 bytes of data." If, however, this is true, it would only strengthen my prejudice that there is little substance or content in modern art.

Jeff Grothaus
Cincinnati, Ohio

If there is, indeed, a demonstrable grammar for shape in art, and if this grammar is not an idiosyncratic construction unique to the artist (or at least if this grammar represents some part of an underlying universal set of rules applicable across "school" or culture), then perhaps those linguists who posit some gene for language or grammar would do well to take the step up from specific to generic and begin searching for a basic grammar of representation (or composition) itself, some inherent scaffold on which might be hung words, music, graphic arts, sign language, symbol and even dance, as needed.

It seems more likely that a single basic grammar is applied to each newly evolved form of human communication as it appears, than that a new grammar is evolved for each new form of communication.

W. Gregory Stewart
Los Angeles, Calif.

David vs. Goliath?

The real story in "Millions of Digits of Pi" (SN: 2/8/86, p. 91) was in the last paragraph. That Bill Gosper can compute millions of digits of pi using a Symbolics Lisp machine, when others use Cray-2 supercomputers to do the same, tells us something: There is scope in this world for both brute force and genius, fortunately.

Berthold K.P. Horn
Visiting Professor
Dept. of Electrical Engineering
University of Hawaii at Manoa
Honolulu, Hawaii

Elephantine rumblings

I was interested to read of the discovery by Cornell University researchers of infrasonic communications amongst elephants ("Elephant calls that humans can't hear," SN: 2/22/86, p. 122), but imagine my surprise when a few days later I read the following in *Death in the Dark Continent* by Peter Hathaway Capstick (1983, St. Martin's Press):

From about eighty yards ahead, a low rumble sounding like a very distant mutter of thunder would be discerned intermittently. Once thought to be the noise of moonshine-vat stomachs doing what came naturally with hundreds of pounds of fodder, this weird sound is now accepted by most hunters and scientists to be a proximity signal, a way of locating each other in very thick bush while (possibly) the elephants' hearing is a bit dampened by the sound of their own chewing. That it can apparently be stopped instantly when suspicion of danger pops up seems to bear this out. Ever try to squelch a stomach rumble in the middle?

Perhaps Cornell and the World Wildlife Fund could save some research money by reading Capstick's books.

Harvey Wysong
Atlanta, Ga.