Researchers are suddenly confident they can make severed neurons grow, but can they translate laboratory finds into cures?

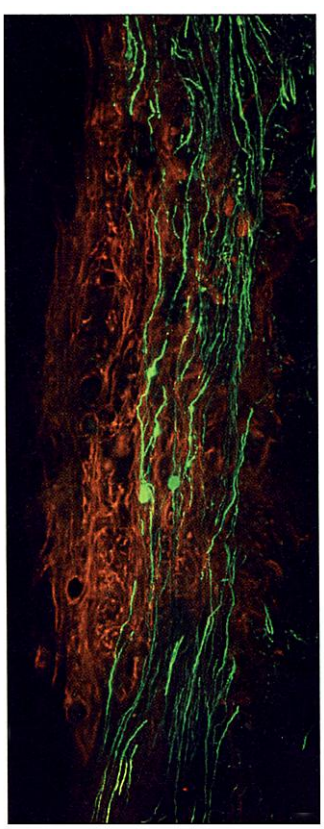
Animal Studies Raise Hopes For Spinal Cord Repair

In the summer of 1993, doctor-turned-entrepreneur Ron Cohen did something many of his colleagues considered a little nutty. He decided to set up one of the first companies—closely held Acorda Therapeutics in Hawthorne, New York—dedicated to developing treatments for spinal cord injury. At the time, it was not even clear that any such therapy was plausible, let alone ready for commercial development.

But Cohen was determined. His father had been a neurologist, so Cohen knew how desperate the world is for treatments for central nervous system disorders. Spinal cord injuries leave some 10,000—usually young—people permanently paralyzed every year in the United States alone; the total number of people with such damage is about 250,000.

Nearly 10 years later, Cohen's investment doesn't look so crazy. In the past few years, scientists have proved that they can regenerate spinal nerves, at least in rodents, enabling the animals to walk more normally and regain some primitive forms of sensation. Nearly a dozen research teams have recently reported in prominent journals ways of promoting such regeneration. And although much attention has focused on using stem cells to repair damaged spinal cords (*Science*, 3 December 1999, p. 1826), researchers are exploring many other options.

For example, some researchers have discovered specific growth inhibitors and blocked them, allowing neurons to grow and make new connections. Others have unveiled clever ways of building cellular scaffolds across the injury site that encourage neurons to traverse the damage, or chemicals that spur growth when injected into neurons.



Bridging the gap. Olfactory cells (red) transplanted into the damaged spinal cord of a rat encourage the regrowth of neurons (green).

"The explosion of findings has offered a sense of optimism I never expected to see," says Barbara Bregman, a spinal cord researcher at Georgetown University in Washington, D.C. Adds Geoffrey Raisman, a neuroscientist at the National Institute for Medical Research (NIMR) in London: "I've been in this field for 30 odd years, and for the first time, I'm beginning to think we're in reach of a treatment."

But despite the progress in the lab, researchers caution that, with possibly one or two exceptions, it will be at least a few years before these therapies are tested in human patients. Both the scientific and practical hurdles are immense, especially because many spinal cord therapies might require delicate surgery or the ability to successfully deliver protein molecules into the tortuous, largely unfamiliar terrain of the spinal cord. Developing such treatments also requires both financial resources and expertise that most basic researchers lack.

With a new facility for large-scale testing in animals and a drug just now entering advanced clinical trials, Cohen's firm is ready to fill this gap. Acorda sees potential profits in treatments for chronic spinal cord injuries, because most patients live 40 to 50 years after they are injured. This not only means that they might take a medication for decades but also that they—or their health insurance companies—might be willing to pay handsomely for a drug that helps offset individual, lifetime medical costs of $400,000 to $2.1 million.

But Acorda remains an exception. Most pharmaceutical companies are not interested in pursuing treatments for an ailment that affects so few compared to, say, cancer and heart disease. So it's still unclear who will develop most of these potential treatments, a problem the U.S. government and nonprofit organizations are trying to solve (see sidebar).

Razing roadblocks

Such a quandary exists thanks to rapid scientific advances in understanding the injured spinal cord. In the wake of an accident—say, a car crash, fall, or gunshot wound—inflammatory processes inflict additional damage on the cord, including severing nerve fibers that survived the original trauma. To curtail this spreading damage, in the early 1990s neurologists began treating spinal cord injuries with injections of the anti-inflammatory steroid methylprednisolone. But that drug must be administered within 8 hours of an accident, and it has only a modest effect at best.

So scientists searched for better therapies, in particular those that can help the many patients with preexisting injuries. They have discovered that inflammatory processes not only directly damage the cord but also inhibit its recovery by blocking nerve regeneration. In particular, neural supporting cells known as astrocytes deposit scar tissue that blocks neuronal outgrowth both chemically and mechanically.

In the early 1990s, Jerry Silver of Case Western Reserve University in Cleveland and his colleagues identified the main inhibitory component of the scar as glycoproteins called chondroitin sulfate proteoglycans. Silver's team showed that nerve cells growing in a dish stopped and turned away when they encountered a streak of proteoglycans. However, when the researchers added a bacterial enzyme called chondroitinase ABC that chops off the sugary branches of these glycoproteins, the nerve fibers grew where they wouldn't before.

Not until this year, however, did researchers find out how the enzyme would perform in an animal model of spinal cord injury. In work described in the 11 April issue of *Nature*, Elizabeth Bradbury of Kings College London and her colleagues infused chondroitinase ABC into rats immediately after they had partially clipped the animals'

SOURCE: Y. LI, P. M. FIELD, G. RAISMAN, *SCIENCE* 277, 2000 (1997)

spinal cords with forceps. The researchers found that the damaged nerve fibers regenerated and made functional connections across the injury site—connections that seemed to improve the rats' motor skills.

The treated rats took longer strides than untreated controls, which walked with a short, choppy gait, and regained finer sensorymotor skills such as the ability to traverse a grid and a narrow beam. However, the animals still failed to detect a piece of tape stuck to their paw—something normal rats would immediately strip off—because the axons governing conscious sensations did not grow far enough to reach their brain targets.

conduct neural impulses.

In healthy animals, Nogo might help cement the proper neuronal connections established during development by inhibiting further growth. But the downside is that Nogo also inhibits neuronal sprouting after injury. Schwab's team is now working to find ways of blocking Nogo's effects. In 1995, the Zürich researchers showed that a Nogo antibody helps spawn connections that enabled rats with damaged spinal cords to walk more gracefully, with better balance and longer strides.

More recently, in 2000, the Schwab team—as well as Stephen Strittmatter's group

SOME POTENTIAL NEW SPINAL CORD THERAPIES

Therapeutic substance	Mode of action	Stage of development
Chondroitinase ABC (enzyme)	Fosters nerve fiber growth by digesting scar tissue	Animal studies
Nogo antibody	Binds to the neuronal growth inhibitor Nogo, blocking its activity	Animal studies
Nogo peptide	Blocks Nogo action at the Nogo receptor	Animal studies
Cyclic AMP (intracellular messenger molecule)	Blocks action of growth inhibitors when injected into spinal neurons	Animal studies
Olfactory ensheathing cells	Forms a bridge to help neurons grow across the injury site	Animal studies
Bone marrow stromal cells	Bridges the wound	Animal studies
Fetal spinal cord tissue and protein growth factors	Bridges the wound and boosts neuronal growth	Animal studies
Fampridine (4-aminopyridine; potassium channel blocker)	Improves function of damaged neurons by preventing current leakage	Currently in large human trials
Methylprednisolone (steroid)	Inhibits inflammatory damage to cord	Completed large human trials. Standard of care.

Although chondroitinase alone is unlikely to get people in wheelchairs to walk, it "might be part of some overall strategy for treatments in humans," Silver says. Adds Bradbury: "Each time we find something like this that we know we can overcome, it's very exciting." However, the team has not yet explored whether the enzyme will aid recovery from long-standing spinal cord injuries. Also unknown is whether it might have any undesirable side effects.

The glycoproteins targeted by chondroitinase are produced in response to injury, but researchers have found that the normal cord also makes compounds that inhibit neuronal growth. One of these is a protein called Nogo, discovered in the 1980s by Martin Schwab's group at the University of Zürich, Switzerland. The researchers found that Nogo is produced by the insulating sheath of myelin that surrounds all spinal nerve fibers and facilitates their ability to

at Yale and Frank Walsh and his colleagues at GlaxoSmithKline—cloned the *Nogo* gene, including its human version. This has enabled researchers to produce large amounts of the human protein, providing fodder for antibody production. It has also helped them identify the most active parts of Nogo, which are likely to be more precise antibody targets.

Despite the apparent lack of interest in spinal cord injury by most big pharma companies, Nogo has drawn some attention. A little over a year ago, Novartis licensed Schwab's Nogo antibody technology. The company is motivated, at least in part, by the idea that Nogo might prove useful in other neurological conditions such as multiple sclerosis, Parkinson's disease, and stroke, which affect far more individuals than spinal cord injuries.

Meanwhile, Strittmatter's group last year identified a receptor through which Nogo exerts its effects. In the 30 May issue of *Nature*, the Yale team showed that a small peptide portion of Nogo that gums up, rather than activates, the receptor could induce both neuronal regrowth and functional recovery in rats with spinal cord injuries. The results identify the receptor as a possible target for a small-drug Nogo inhibitor. Such an inhibitor would be attractive for pharmaceutical firms because it might be swallowed as a pill rather than needing to be pumped into the spinal cord, as proteins and antibodies do.

Indeed, recent data suggest that blocking the Nogo receptor might be even more effective than targeting Nogo itself. On 27 June, Strittmatter's team published evidence on *Science* online showing that a growth inhibitor called myelin-associated glycoprotein (MAG) binds to the Nogo receptor. In work published online by *Neuron* on 28 June, Marie Filbin and her colleagues at Hunter College of the City University of New York provide further support for the idea that MAG acts through the Nogo receptor. In addition, Harvard's Zehgang He and his colleagues reported in the 27 June issue of *Nature* that this receptor is also an attachment site for a third myelin-derived inhibitor, oligodendrocyte myelin glycoprotein. Thus, blocking the Nogo receptor might help thwart all three inhibitory influences.

Another more futuristic possibility for a small-molecule therapy also comes from Filbin's team. Previous work by that group showed that cyclic AMP, a molecule involved in the cell's internal signaling pathways, fosters nerve cell growth by overcoming growth inhibitors such as Nogo and MAG. The researchers are still working out exactly how this occurs. But in experiments described in the 13 June issue of *Neuron*, Filbin's team, and one led by Marc Tessier-Lavigne of Stanford University and Allan Basbaum of the University of California, San Francisco, showed that cyclic AMP injected into the cell bodies of certain rat spinal neurons before an injury induces regeneration of the branches of neurons that lead to the brain. Filbin predicts that the injections will also work when given after an injury.

Building a bridge

When the damage is severe, blocking inhibitors will probably not be enough, however. Regenerated neurons would still have to cross a difficult barrier: large, fluid-filled pockets that are produced by inflammatory processes in addition to dense, rubbery scar tissue. Work by NIMR's Raisman and his colleagues indicates that neurons can be coaxed across such forbidding territory with a bridge of tissue taken from the nose. Olfactory neurons spontaneously regenerate whenever they are damaged—say, by a cold virus or strong solvent that was inhaled—and grow into the brain to make connections that are

NINDS Delves Into Drug Development

The National Institute of Neurological Disorders and Stroke (NINDS) has traditionally left development of drugs for treating stroke, spinal cord injuries, and other neurological conditions largely up to the private sector. But NINDS will soon announce a dramatic departure from that policy: a new type of "translational" grant designed to bridge the gap between basic research and clinical trials.

"This is big stuff compared to the current situation," says Robert Baughman, NINDS associate director for technology development, who is heading the new effort. Given the explosion of promising findings related to neurological diseases (see main text) and a relative paucity of interest from the private sector, NINDS feels it is time to step in to encourage treatment development, Baughman says.

Projects funded by the new initiative might include attempts to replicate promising experiments, toxicology studies, the development of test tube assays for large-scale screening of potential therapeutic compounds, or implementation of animal models of human diseases. NINDS also envisions supporting studies to determine the proper timing and dosages for drug administration.

At least some basic researchers think NINDS is on the right track. "In spinal cord injury, stroke, and head injury in general, there have been major discoveries in the last 10 years that point the way to treatments," says Oswald Steward, director of the Reeve-Irvine Research Center at the University of California, Irvine. "Somebody has to take that next step. We owe it to people like Christopher Reeve."

Some researchers might be concerned, however, that the institute has not set aside a specific amount of money to fund the new program and that the more applied projects will compete with basic science projects for the estimated $250 million NINDS is likely to budget for new research grants in fiscal year 2003. Indeed, because development work is pricey, and there are many good projects in the wings, Baughman predicts that translational research might eventually make up a significant fraction of the total pot.

Peer-review committees will evaluate the translational project applications. But because many scientists do not look favorably upon applied research, the institute is creating new study section guidelines for such applications that it hopes will provide a peer-review environment in which quality translational research projects can receive competitive scores.

In a policy more common to the private sector, investigators who are awarded the grants, which will be open to industrial as well as academic scientists, will be expected to meet specified milestones or risk losing their funding. In addition, the U.S. Food and Drug Administration has agreed to work closely with NINDS to help ensure that researchers take the steps necessary for eventual FDA approval.

NINDS is not alone in its quest to encourage translational research. The U.K.'s International Spinal Research Trust is planning a similar initiative. The project is not yet off the ground, but the plan is to pick at least two candidate therapies and hire a project management team, consisting of people with clinical, scientific, and industrial experience who would plot a critical path for each to the clinic.

And the Christopher Reeve Paralysis Foundation in Springfield, New Jersey, is announcing new support for scientists who wish to move beyond basic research. Reeve says the foundation is now "open for business" to fund large, multimillion-dollar grant proposals aimed at moving quickly toward, and through, clinical trials. "Why should we not challenge scientists to achieve the [medical] equivalent of the moon shot?" Reeve says. "I believe it will happen, and we have scientists who are ready to go."

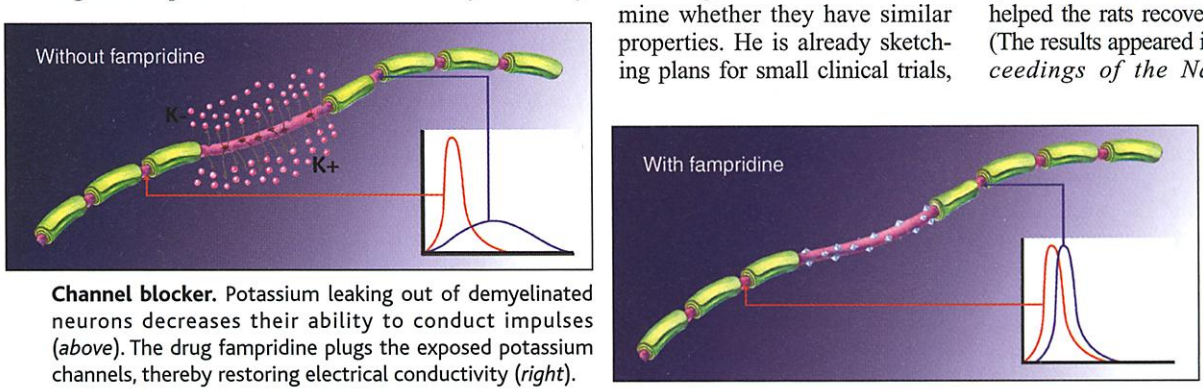
–I.W.

necessary for the sense of smell. Raisman discovered that these cells grow by extending their axons across a scaffold of supporting cells unique to the olfactory system.

In the mid-1990s, Raisman and his colleagues transplanted these so-called olfactory ensheathing cells into rats with spinal cord injuries. They found that the transplants not only coaxed nerve fibers to extend across the lesion, but they also restored the rats' ability to use their paws to reach out and grasp a piece of food. And in as-yet-unpublished work, the group has discovered that the transplants can induce severed neurons to create connections needed for climbing, a very complex movement involving the entire rodent body. What's more, the scaffold works even if it is built 6 months after the injury.

Now Raisman is implanting human olfactory ensheathing cells into rats to determine whether they have similar properties. He is already sketching plans for small clinical trials, to be conducted in collaboration with neurosurgeons in London.

Other types of cells, including stem cells, might also form effective scaffolds. Lars Olson of the Karolinska Institute in Stockholm, Sweden, and his colleagues have achieved promising results with bone marrow stromal cells, a kind of stem cell that can turn into cartilage and bone. His team transplanted the cells into rats with spinal cord injuries and showed that the cells coalesce into bundles that extended across the scar, creating an environment that encouraged neuronal growth. If grafted 1 week after the injury, the cells also helped the rats recover crude walking skills. (The results appeared in the 19 February *Proceedings of the National Academy of Sciences*.)



Channel blocker. Potassium leaking out of demyelinated neurons decreases their ability to conduct impulses (*above*). The drug fampridine plugs the exposed potassium channels, thereby restoring electrical conductivity (*right*).

CREDIT: ACORDA THERAPEUTICS

Plugging holes

Despite the focus on regeneration, rebuilding cut nerves might not always be necessary for promoting significant recovery of the injured spinal cord. More than half of human spinal cord injuries are incomplete, severing some but not all of the cord's fibers. In these cases, improving the function of the remaining fibers might be an equally promising route. That's the tack being taken at Acorda. The company's chief of research, Andrew Blight, discovered in the 1980s that the myelin sheaths of the spared fibers often

sustain damage that impairs their ability to conduct neural impulses.

One of myelin's jobs is to cover channels that would otherwise allow potassium ions to flow out of neurons. This prevents current from leaking from the cells and impeding conduction of the neural impulse. Acorda has developed a drug called fampridine (chemically, 4-aminopyridine, or 4-AP) that helps compensate for gaps in the myelin by directly blocking the potassium channels at those gaps. Blight and his team, then at New York University, showed that giving 4-AP to cats with spinal cord injuries restored the ability of surviving neurons to conduct electrical impulses and stimulate a normal pattern of electrical activity in the cats' muscles. In 1991, they showed that the treatment could improve standing and walking ability as well as bladder and sensory function in pet dogs that had become paralyzed in car accidents or after ruptures of their spinal discs.

Acorda began trials in humans about 5 years ago and has since treated more than 200 patients. Fampridine improved the patients' sensory and motor functions only modestly. But the drug significantly decreased spasticity, or stiffness and involuntary jerking of limbs, in some patients. It also improved bladder, bowel, and sexual function in treated individuals compared to controls. So far, the only significant side effect is a small risk of seizure, presumably because the compound also increases the excitability of healthy neurons. Large-scale human trials of the drug began in early June.

In the future, Cohen hopes his company will help develop new medications that provide more than partial benefits. Acorda has now constructed what he says is the biggest animal facility in the world for testing treatments for spinal cord injury. There, researchers can test compounds on several hundred rats at a time and get clear, statistically significant answers about whether to pursue a possible remedy.

The right combination

Most researchers in the field believe that no single therapy will be up to the job of treating spinal cord injuries. Instead, they propose that it will take a combination of different remedies to overcome the multiple barriers to neural regeneration that scientists believe exist in the injured spinal cord. "Clearly, we're not looking for one best treatment but a combination of treatments," Olson says. For example, a clinician might build a cellular bridge across the damaged area, administer protein growth factors to boost a neuron's intrinsic capacity for growth, and deliver enzymes that digest scar tissue. Antibodies or small molecules to neutralize the effects of inhibitory factors such as Nogo might also be added to the mix.

Bregman and her colleagues at Georgetown University have done some of the most promising studies combining a scaffold, in this case made of fetal spinal cord tissue, with infusions of growth factors. Together, they found that this combination produced more complete neural regeneration than either growth factors or fetal tissue transplants do alone. Furthermore, last December in *The Journal of Neuroscience*, Bregman's team reported that delaying this combination treatment 2 to 4 weeks after an injury in rats produced even better recovery than administering it immediately. The delayed treatment enabled the rats to walk on treadmills and climb stairs, whereas immediate treatment did not.

Such combination therapies are likely to reach the clinic later, after each individual treatment has been carefully tested alone. Indeed, given the potential dangers of such approaches, some researchers recommend extreme caution before trying any of the current experimental strategies in humans. They worry that some of the more invasive strategies might end up doing more harm than good. "It's very exciting stuff we're doing," says W. Dalton Dietrich, scientific director of the Miami Project to Cure Paralysis at the University of Miami, "but we need to spend a couple more years [doing research] before we're ready to push something into people."

However, many researchers and patients do not want to wait for the perfect treatment if there is a more immediate possibility of a beneficial one. And many argue that without human tests, one will never really know whether something that improves function in rats will be of any use whatsoever in the clinic. After all, humans and rats are dramatically different—from the way they walk to the size of their spinal cords.

For his part, Cohen is betting that his new facilities for escorting early research to human trials will attract many of the creative minds in this rapidly ripening scientific field. "It's like *Field of Dreams*," Cohen says. "If you build it, they will come."

–INGRID WICKELGREN

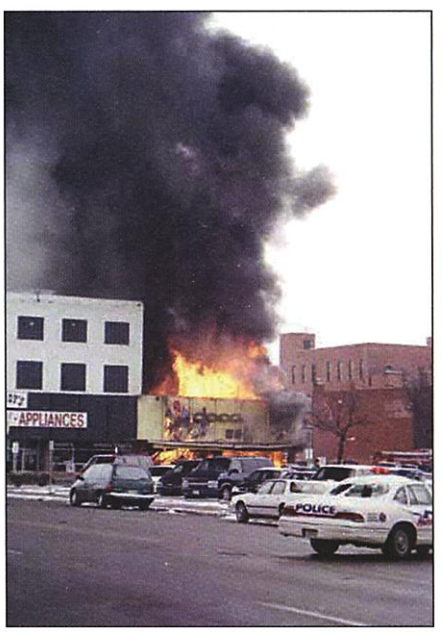
GEOSCIENCE

Data Dilemma: Stow It, Or Kiss It Goodbye

As storehouses burst with bulky samples, an NRC committee proposes a temporary cure for geology's down-and-dirty case of information overload

When Woody's Appliance Store in Hutchinson, Kansas, blew up 17 January last year, the 20-meter-high flames immediately got it pegged as a natural gas explosion. Firefighters shut off the city supply, yet the gas and flames still roared. That night, geysers of natural gas and water began to erupt a few kilometers east of downtown. One exploded under a mobile home, killing two people.

Suspecting that the gas had leaked from underground storage caverns, the Kansas Geological Survey (KGS) went in to figure out how the gas was moving. Within hours, survey scientists had created maps of the local geology from digitized records of thousands of wells drilled over past decades for energy exploration. Once they had fingered a particular layer of rock, the geologists went back to the survey's warehouse and dug out a continuous core of rock drilled some 40 years earlier. With this and other information, they quickly advised the gas company where to drill holes to vent the leaked gas.



To the rescue. A warehoused rock sample helped geologists solve a mysterious fire.

CREDIT: HUTCHINSON, KANSAS, FIRE DEPARTMENT

It was a dramatic step into the limelight for a dusty cylinder of rock. To geoscientists, such archived cores—bored out of rock and sediment by hollow drill bits—are standard reference tools for assessing hazards, searching for oil and other resources, and gathering an array of basic geologic information. Yet across the United States, many collections of cores and other samples are threatened by improper storage or simply being sent to the dump. The vast store-