Kill the Messenger

Scientists are testing toxins that destroy the nerve cells that convey pain

By JOHN TRAVIS

It is easier to find men who will volunteer to die, than to find those who are willing to endure pain with patience.
—Julius Caesar

Military technology has made dramatic strides over the past few decades. Instead of indiscriminately raining shells on a city or torching a countryside with napalm, modern armies can now turn to precision-targeted cruise missiles and laser-guided bombs. The growing appeal of the latter two weapons stems from their ability to strictly limit the casualties or damage.

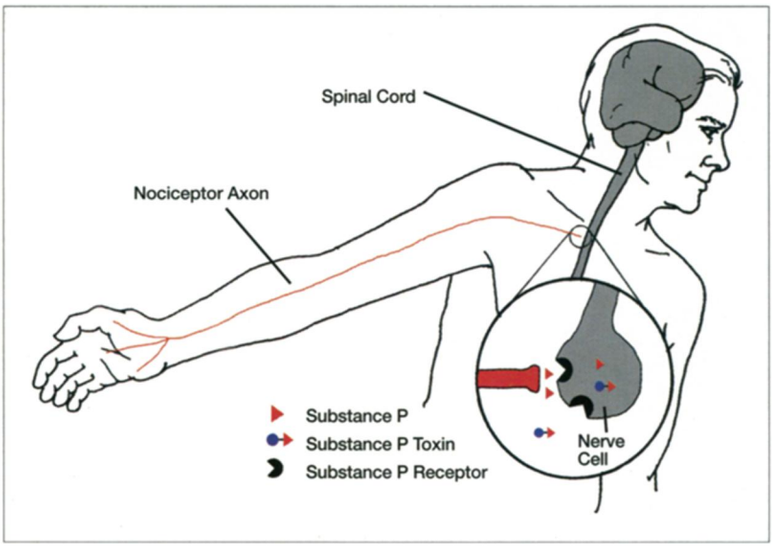
Scientists teasing out the nerve pathways that carry pain signals to the brain have begun to work with the molecular equivalent of these smart bombs. In the past, the researchers searched for these pathways by severing nerves in animals' spinal cords or applying substances that indiscriminately destroy the cells there. These tactics would occasionally make animals less sensitive to pain, but because they destroyed wide swaths of nerve cells, scientists couldn't distinguish which nerves carried the noxious signals.

Researchers today are instead using toxins that selectively kill the nerve cells that convey pain. In some cases, they're even using one of the body's own pain signals, a protein called substance P, to deliver the toxins to the correct cells.

"What we're trying to do is develop extraordinarily sharp scalpels. We want to take one class of neurons out of the neural network," says Ronald G. Wiley of Vanderbilt University and the Veterans Affairs Medical Center in Nashville.

Wiley and several other investigators reported on this cell-killing strategy, often called molecular neurosurgery, at the Society for Neuroscience meeting in Miami Beach in October. They've shown that the approach can relieve pain, but they've only tested small animals so far. Still, some scientists believe that these guided toxins may one day help people overcome pain that even morphine and other narcotics can't control. Moreover, the specificity of the toxins may allow them to bring about pain relief without the side effects, such as sedation and constipation, that can accompany heavy use of traditional painkillers.

"It's a very sophisticated technique. It represents a tremendous potential advance in how chronic pain is treated," says pain researcher Lorne M. Mendell of the State University of New York at Stony Brook.



Nociceptor cells sense painful stimuli in the body and convey pain signals along fibers, or axons, to the spinal cord. There, nociceptors release substance P, a chemical that triggers spinal cord nerve cells to relay pain signals along their axons to the brain. By attaching a toxin to substance P, scientists can kill these relay cells.

In need of a drink of water late one night, you walk in the dark to the kitchen and slam your bare right foot into a wall. In a split second, your brain receives pain signals from your stubbed big toe—and responds by making you hop around on the other foot while spewing profanities.

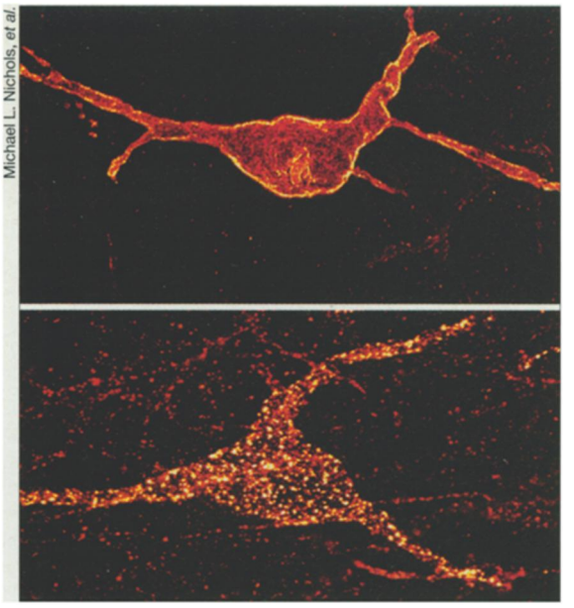
How does that pain signal get to the brain? The collision of wall and toe triggers specialized pain-sensing nerve cells, nociceptors, that reside in skin, muscle, and other tissues and keep the body aware of damage. Through thin fibers called axons, the nociceptors connect to the spinal cord, the pencil-thick bundle of nerves relaying information between the body's peripheral nervous system and the brain. In the cord, the ends of the nociceptor axons release neurotransmitters—chemicals such as glutamate and substance P—that trigger cord nerve cells to send a pain signal along their own axons that ascend into the brain.

The body's pain highway isn't a one-way street. The brain sends signals back down the spinal cord, through descending axons, to modulate the sensitivity of nociceptor cells. In fact, chronic pain often arises when nociceptors turn overly sensitive after an especially painful stimulus such as nerve damage. In these cases, previously harmless sensations, such as the feel of a light breeze on the skin, can become almost unbearably painful.

In the early 1980s, neuroscientists began to explore the role of various parts of the spinal cord in the transmission of

pain and other sensory information. Wiley and his colleagues used several toxins, including ricin, lectin, and volkensin, that axons take up and deliver to the main body of nerve cells. These toxins killed the cells and allowed the scientists to determine whether these cord cells had played a role in the animal's sensing of pain. Still, these toxins offered the neuroscientists little control over which nerve cells within the cord were destroyed.

Not content with such carpet bombing of the spinal cord, scientists achieved greater precision more recently by using antibodies to target specific cells, says Wiley. These Y-shaped proteins, made by the immune system, normally latch onto surface molecules of bacteria, viruses, and other infectious agents but rarely bind to anything else. Impressed by this talent for discrimination, scientists long ago learned to coax immune cells into generating antibodies specific for any chosen protein, including ones on the surface of nerve cells.



Pain-relaying nerve cells in the rat spinal cord (top) display the receptors (red and yellow) for substance P on their surface. When the rat feels pain, substance P binds to the receptors, which are quickly taken inside the nerve cells (bottom).

By fusing a cell-killing toxin to such an antibody, neuroscientists have created immunotoxins that seek out specific classes of nerve cells. For example, while at the University of California, San Francisco, William J. Martin started to work with immunotoxins that home in on a group of nerve cells, called noradrenergic neurons, that respond to the neurotransmitter dopamine. The antibody portion of the molecule binds to a protein on the surface of the neuron, enticing the cell to take in both the antibody and the toxin. Once inside, the toxin kills the cell. Martin, now at Merck & Co., in Rathway, N.J., compares the tactic to the infamous wooden horse that unleashed destruction once rolled inside the gates of Troy.

Some noradrenergic neurons in the brain send axons down the spinal cord that play a role in pain processing. For example, researchers believe that such neurons quickly dampen acute pain sensations such as those associated with a pinch.

The cells' exact role in pain processing remains murky, however, partly because attempts to kill the neurons in animal experiments have often resulted in the death of other nerve cells that have their own influence on pain. At the Miami Beach meeting and in the March PAIN, Martin described how his team used immunotoxins to kill only noradrenergic neurons and alter an animal's pain responses.

He and his colleagues injected an antibody-coupled toxin into the fluid around rats' spinal cords. After 2 weeks, the researchers confirmed the death of significant numbers of noradrenergic neurons in both the spinal cord and the brain. By observing the rodents' reaction to various painful stimuli, the researchers then determined whether the absence of this descending nerve pathway affected the ability to sense pain.

In one test thought to mimic the persistent pain people sometimes suffer after an operation, the researchers injected the pain-causing chemical formalin into one of the animals' paws. Compared with normal rats, those with destroyed noradrenergic neurons spent less time licking or raising the injected paw, indicating that they felt less pain. In contrast, these rodents seemed slightly more sensitive to acute sources of pain, such as a hot plate.

The death of the noradrenergic neurons is "associated with a reduction in persistent but not acute pain behavior," concludes Martin.

While immunotoxins kill particular classes of nerve cells, some of those cells may not be devoted to regulating and sensing pain. They might include neurons that control muscles or those that sense touch. The antibodies coupled to the toxins have other drawbacks as well. They're big, which hinders their ability to spread through the spinal cord, and they're difficult to make. Neuroscientists therefore wanted a better partner for their toxins, something small and specific to the nerve cells that convey pain.

Enter substance P. A short protein, or peptide, composed of just 11 amino acids, substance P is one of the many chemicals that relay signals between nerve cells. In particular, scientists believe that nociceptor cells release the peptide to trigger the cord nerve cells that pass pain signals on to the brain.

"There's a general agreement that these neurons that express the substance P receptors are a key component in the ascending conduction of pain," says Patrick W. Mantyh of the University of Minnesota and Veterans Affairs Medical Center in Minneapolis.

A decade ago, this realization set off a frenzy among pharmaceutical companies as they sought to develop drugs that would block substance P. Such drugs, however, proved disappointing because substance P isn't the only neurotransmitter that activates the spinal cord neurons relaying pain. In fact, glutamate seems to be the primary stimulus for these cells, notes Michael J. Iadarola of the National Institute of Dental and Craniofacial Research in Bethesda, Md.

What if the spinal cord's pain-relaying nerve cells themselves were destroyed? wondered scientists. Then, neither substance P nor glutamate would have anything to trigger, and pain messages would never reach the brain.

The obvious way to knock out these crucial relay cells was to turn substance P into the latest Trojan horse. Back in 1994, Wiley and his colleague Douglas A. Lappi built their initial version of such an insidious molecule. It joins substance P to saporin, a toxin produced by the soapwort plant, *Saponaria officinalis*. Saporin, the killer that scientists have employed in many immunotoxins as well, interferes with ribosomes, which are protein-synthesizing factories inside all cells.

"You only have to inactivate about 10 percent of the ribosomes and the cell dies," explains Wiley, who with Lappi has founded the firm Advanced Targeting Systems in San Diego to commercialize saporin-based toxins.

In Miami Beach, Wiley and other investigators described how injecting P-saporin into the spinal cord affects pain perception in rodents. For example, Mantyh and his colleagues, who also published their work in the Nov. 19 SCIENCE, reported using the toxin to destroy pain-relaying nerve cells in the spinal cord seems to block chronic pain felt by rats but leave intact their response to mildly painful stimuli.

In one experiment, the scientists damaged some of the rodent's peripheral nerves, a procedure that results in the animals' developing a lasting, heightened pain sensitivity. Such rats quickly withdraw from even the lightest touch. People who suffer nerve damage sometimes develop a similar chronic condition, which scientists call neuropathic hyperalgesia. Animals that received P-saporin injections in their spinal cords don't develop this persistent condition of abnormally high pain sensitivity, reports Mantyh.

Nor do they react as vigorously to a

chemical—capsaicin—that otherwise would cause a severe burning sensation on their skin. The rodents still feel other painful stimuli, however. They lick their paws if placed on a hot plate, for example.

Impressed by the toxin's seeming ability to kill nerve cells that relay chronic pain without completely eliminating those involved in milder pain sensations, Mantyh's team then made two further observations that may bode well for a medical use of P-saporin.

First, the pain relief observed has lasted for more than 200 days, which is nearly half a rat's life. That's promising because even when physicians have resorted to the drastic step of cutting spinal cord nerves to eliminate chronic pain, the relief can be temporary. "Frequently, you would get the return of pain, and it could be worse than it was originally," says Mantyh.

Second, animals treated with P-saporin still respond to the ultimate painkiller, morphine. Investigators had been concerned that the pain-relaying nerve cells targeted by the substance P toxins were the same ones shut down by the narcotic. If they were, molecular neurosurgery could leave physicians without a backup way to relieve a patient's pain after a P-saporin treatment.

Morphine, however, seems to work on a separate nerve pathway in the spinal cord, says Mantyh. Researchers have recently shown that when a nerve cell sports substance-P receptors, it almost never has the receptors for morphine also, he explains.

"The animals [treated with P-saporin] are fully responsive to morphine," agrees Wiley, who has recently developed and is testing a saporin-based toxin that targets the morphine receptor.

Iadarola and his colleagues have joined substance P to different toxins, such as ones made by diphtheria and pseudomonas bacteria. These toxins interfere with a part of the protein-synthesis machinery other than ribosomes but produce the same result: cell death. In Miami Beach, Iadarola reported that his P-coupled toxins also reduced pain sensation in various rodent models, including chronic pain brought about by inflammation or nerve injury.

Scientists may do more than attach toxins to substance P. Someday, Mantyh speculates, they may affix compounds that shut down nerve cells rather than kill them, so the treatment could be reversible.

Several pharmaceutical companies, he adds, want to marry the peptide with a fluorescent tag to discover specific genes turned on or off during chronic pain. They expect this approach to provide leads for new pain therapies. The fluorescent construct would highlight pain-stimulated nerve cells in animals so that researchers could more easily compare the genetic activity of those cells with that in unstimulated nerve cells, says Mantyh.

Watching how many times a rat on a hot plate licks its paws isn't nearly as informative as asking a person whether a treatment reduces his or her pain. Still, it may be a few years before molecular neurosurgery reaches the clinic.

Investigators plan additional research in rodents and in nonhuman primates to confirm the technique's safety. The primary concern is whether the toxins will destroy nerve cells in the spinal cord that aren't related to pain transmission, such as motor neurons that control muscles involved in breathing or other activities. In some tests of P-saporin in rodents and primates, animals have developed muscle weakness and paralysis. Even if the motor neurons don't have substance P receptors—and most don't seem to, according to research so far—the cells can on occasion take up the toxins through other means.

Yet the muscle-related side effects arose at toxin doses far above what scientists believe will be medically useful, contends Wiley. At the expected doses, the toxins seem to kill only pain-conveying fibers, which reside in the outer layers of the spinal cord. In contrast, the motor neurons lie deep within the cord.

Consequently, Wiley and his colleagues remain confident that molecular neurosurgery may one day supplant the current treatment of last resort for chronic pain: severing nerve fibers in the spinal cord (SN: 2/13/99, p. 108). "For every neuron that you're cutting which is involved in pain, you've got to be cutting at least a 100 other ones whose function you don't know," says Mantyh.

The first patients to receive substance P–based toxins, predict researchers, will be people with terminal cancer who face intractable pain from tumors that have spread throughout their body. If the therapy eliminates their chronic pain without eliminating the milder pain sensations that they need to be aware of their surroundings, physicians may then consider the approach for a far wider range of chronic-pain conditions. Rheumatoid arthritis and neuropathic hyperalgesia, which afflict millions of people, are obvious targets.

Killing spinal cord nerve cells is admittedly a drastic step. "It's not for everyone, but it might offer an option when everything else fails," says David A. Thomas of the National Institute on Drug Abuse in Bethesda, Md., who organized the molecular neurosurgery symposium at the Society for Neuroscience meeting.

As a modern-day Caesar would say, some people may choose death, or at least cell death, rather than live with constant pain. □