NEUROSCIENCE

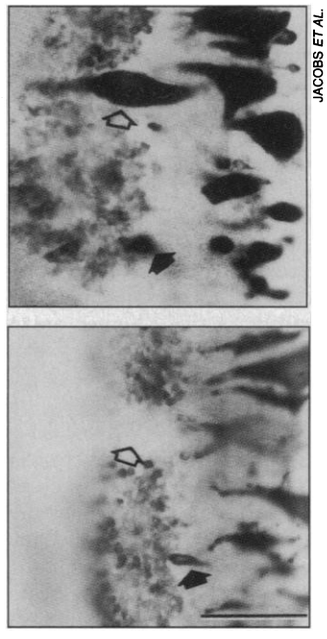
Are Pushy Axons a Key to Spinal Cord Repair?

When moving heavy loads at high speed, as any truck driver or railway engineer will tell you, it's wiser to pull than to push. Nature apparently learned this lesson long before humans did. In the developing nervous system, growing axons—the tendrils that transmit electrochemical signals from one neuron to the next and from the spinal cord to the body's muscles—are dragged to their destinations by oozing extremities called filopodia. These cellular locomotives can whisk their neural freight through the embryo at the breakneck pace of millimeters per day. But researchers studying one adult nervous system have now found a curious counterexample to this rule. In the primitive fish called the sea lamprey, some axons can push their way forward—a trait apparently responsible for the ability of the lamprey, unlike any higher vertebrate, to repair its spinal cord when it is severed.

In the 1 July issue of *The Journal of Neuroscience*, a group led by University of Pennsylvania neurologist Mickey Selzer reports that the lamprey axons seem to owe their mobility to neurofilaments, rods of protein previously thought to play a purely supporting role in axon growth. For unknown reasons, only some lamprey spinal cord neurons are adept at regenerating; the group found that in these cells neurofilament production bounces back after injury, while in those that stay put, production of the protein does not recover. They speculate that growing neurofilaments slowly push each axon forward, like poles gradually raising the canopy of a circus tent.

The finding could lead neuroscientists to revise their view of how neurons regrow after injury. The limited regrowth that does occur in higher vertebrates has been attributed mainly to actin and microtubules, the same components of the cell skeleton that drive filopodia during embryonic development. Now it appears that "we [may have] underestimated the dynamics of neurofilaments," says Itzhak Fischer, a cell biologist at Allegheny University of the Health Sciences in Philadelphia who studies the neuronal cytoskeleton. "It's intriguing to think about them as generating some kind of force."

The discovery may also put researchers one step closer to what New York University neurosurgeon Wise Young calls "the Holy Grail of neurobiology": a way to heal humans with injured spinal cords. "It's pure speculation at this point," explains Selzer, "but it may be that temporarily overexpressing neurofilament in people with central nervous system injuries would help the nerve fibers to grow, if we also can eliminate some of the extracellular barriers to regeneration." Many scientists remain skeptical of neurofilaments' healing potential, however, pointing to the large evolutionary and physiological gap between sea lampreys and humans. "It's certainly more complicated than saying 'If we could turn on this [neurofilament] molecule, Christopher Reeve would walk,' " says molecular biologist Nisson Schechter of the State University of New York (SUNY), Stony Brook.



JACOBS *ET AL.*

Bouncing back. A stain reveals neurofilament messenger RNA in lamprey neurons. Images taken before *(top)* and 10 weeks after their axons were cut show that neurofilament production recovers in only some cells (dark arrows).

Still, the finding opens a crack in the standard picture of how axons grow—and what restricts their growth. Over the decades, researchers have shown that during embryonic development these neuronal tendrils are led on their long journeys—which can stretch from the spinal cord to the big toe—by prickly, fanlike structures called growth cones. Filopodia on the growth cones, composed mainly of the structural protein actin, elongate and then contract like inchworms, tugging the axon forward. Just behind come microtubules, hollow rods built from the protein tubulin, which stiffen the trailing axon. Hours later, the axon is infiltrated by neurofilaments, which set up a permanent, rigid cytoskeleton.

Axons in the peripheral nervous systems of mammals retain some of this embryonic wanderlust throughout life, which is why surgery to reconnect severed fingers and other body parts often succeeds. But the central nervous system (CNS—the brain, eyes, and spinal cord) in adult mammals is soaked through with proteins that inhibit axonal growth.

Sea lamprey neurons flout these restrictions. As Selzer first reported in *Science* in 1985, many of the 2000 neurons in the lamprey brain that project axons to the spinal cord can regenerate after the cord is cut, forming functioning connections with the correct neurons on the opposite side of the gap. More recently, researchers in Selzer's lab found that the growth cones responsible for this feat have some unusual features: They lack filopodia, contain little actin, move extremely slowly compared to axons in embryos, and are packed with neurofilaments. "For all of those reasons, we realized that the actin-based, filopodia type of axon growth cannot be the mechanism of regeneration" in lampreys, says Selzer.

To dig further, Alan Jacobs, a former Selzer graduate student now at the University of California, San Francisco, decided to study whether regenerating lamprey neurons produce unusual levels of neurofilament protein. Jacobs cloned the gene encoding lamprey neurofilament protein and used its nucleotide sequence to construct complementary DNA probes that would bind to the gene's messenger RNA (mRNA) product. He then cut halfway through several lampreys' spinal cords, and while the primitive fish convalesced, he tracked neuronal mRNA levels to monitor the production of neurofilament protein.

In axons that were "bad regenerators," Jacobs and Selzer found, neurofilament mRNA production fell after the axons were cut and stayed low. In "good regenerators," however, neurofilament expression showed a smaller decrease and then—about 4 weeks later—climbed back up. The mRNA levels recovered even when Jacobs and Selzer made the spinal cord gap so broad that axons couldn't grow across it, suggesting that the revitalization isn't merely a consequence of axon regeneration but may help drive it.

The Penn researchers still can't explain why neurofilament production recovers in some neurons and doesn't in others. But the implication that evolution has given at least one vertebrate species a backup mechanism for neuronal growth in the CNS accords with similar hints emerging from a few other neuroscience labs. Ben Szaro at SUNY Albany, for example, has found that severed optic nerves of *Xenopus* frogs—another rare example of CNS neurons that usually regenerate—fail to regrow if they are exposed to antibodies that prevent the assembly of neurofilament protein subunits. Neurobiologist Dennis O'Leary at the Salk Institute for Biological Research in

La Jolla, California, has also found that the axons connecting the pontine nuclei—structures in the mammalian brain that link each hemisphere to the opposite half of the cerebellum—to the spinal cord grow very slowly and lack growth cones. "Something about the structure of neurofilaments allows for this [slow but sure] kind of growth," speculates SUNY Stony Brook's Schechter.

But other researchers caution that the hints of neurofilament-driven axon growth in other species don't mean that their axons can match the regenerative prowess seen in the lamprey. Lamprey nerves lack the myelin sheath that protects mammalian nerves, for example, and lampreys have only one type of neurofilament protein, while humans have at least three. Nor has Selzer figured out yet how to boost neurofilament production in lamprey nerve cells, let alone human paraplegics.

Thanks to the new finding, though, neurofilaments may not be the only things trading in their passive stability for dynamism and flux. The doctrines of many scientists studying neural growth and regeneration, says Allegheny's Fischer, "are less rigid now."

–Wade Roush

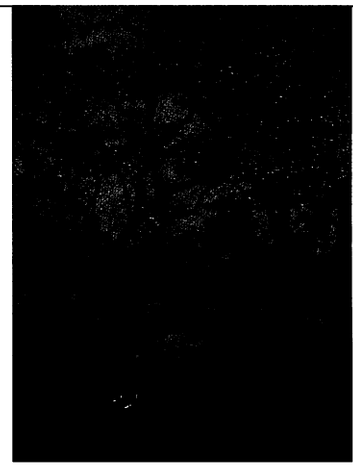
EVOLUTIONARY BIOLOGY

Longer Tusks Are Healthy Signs

NEW DELHI—The long tusks of some male Asian elephants may advertise the genetic vigor of their bearers, shows a new study by two Indian researchers. Unfortunately, long tusks are also a come-on for poachers, who take a heavy toll on the endangered elephant. Ivory hunters may thus be depleting the elephant populations of the individuals with the healthiest genes.

The study's finding—that male elephants with longer tusks have fewer parasites—supports a theory explaining such secondary sex characteristics put forward in 1982 by evolutionary biologist William Hamilton of the University of Oxford in the U.K. Hamilton proposed that males carrying genes for resistance to parasites will be healthier and, hence, in a better condition to develop expensive secondary sexual characteristics, which then enable females to choose mates carrying the best genes. Studies of invertebrates, fishes, reptiles, and birds have all supported the theory.

The elephant findings, which appear in a recent issue of India's *Current Science*, provide what co-author Raman Sukumar, an ecologist at the Indian Institute of Science in Bangalore, calls "the first demonstration" of its kind in mammals. For Raghavendra Gadagkar, a sociobiologist and chair of the Centre for Ecological Sciences at the Bangalore institute, it also suggests a pressing concern. "The importance of the present work lies not in its conceptual novelty, but in its implications for conservation of elephants," he says (see below), "for ivory hunters are most likely to cull the best males."



P. BAGLA

Size matters. Bigger tusks mean fewer parasites for Asian elephants.

The 3-year study was carried out at Mudumalai Wildlife Sanctuary in southern India. The researchers identified elephants from photographs and unique body markings and collected fresh dung samples from as many as 38 animals. They then tested the dung samples for intestinal helminth parasites, finding as many as 20 million parasite eggs per dropping. Although these densities "may not be life threatening," Sukumar says, in lean periods and in stressed conditions, the parasites could significantly weaken the elephants.

Sukumar and his colleague, microbiologist Milind Watve, also developed a standard growth curve of tusks using a series of field techniques—enlarged photographs, height measurements of live individuals and museum specimens, and postmortem examinations. They then plotted the amount by which each male elephant's tusk length exceeded the standard curve against the parasite densities found in its dung. The scientists found that the longer an elephant's tusks, the fewer parasites are found in the animal's dung.

Hamilton says he's "pleased to see these results from the 'king of mammals,' " adding that this study may convince skeptics, who offer other explanations for the correlation between exaggerated sex characteristics and parasites. In particular, the characteristics become exaggerated with age, while the number of parasites declines with age in most animals. Sukumar agrees that the findings are "compatible with, but not necessarily a substantial proof of," Hamilton's hypothesis in elephants.

Among other unanswered questions is whether longer tusks really do attract females, although Hamilton says females "contentedly mate with a dominant male" and that older and more dominant bulls usually display longer tusks. Nor is it clear yet that parasite resistance in elephants has a genetic basis.

But if tusk length is a sign of good genes, poaching may be weakening the elephant gene pool by removing parasite resistance genes from the population—something that could become a "serious health problem" for wild populations, says Sukumar. And Hamilton says that the solution, while obvious, isn't likely to be implemented. "Never cull the top bulls; cull old but small-tusked males," he says. "Of course, that is the opposite of what hunters do if they want to make a profit."

–Pallava Bagla

Pallava Bagla is a science writer in New Delhi.

Ivory Trade Seen as Threat

The decision last week by an international body to permit trade with Japan in ivory taken from elephants in three African nations is expected to put additional pressure on the dwindling number of Asian elephants, too. Although only stockpiled ivory from Botswana, Namibia, and Zimbabwe can be sold, the difficulty in identifying ivory's source could put all animals at greater risk, say environmentalists.

"A legal chink has been opened up in the international market," says Vinod Rishi, chief of a large conservation effort by the Indian government to protect its 27,000 elephants. "Now there is a chance of a large-scale massacre of elephants in India." Belinda Wright, of the Wildlife Protection Society of India, worries about a "dramatic and disastrous spate of poaching" and a further decline in the ratio of males to females, already as low as one to 400 in some parts of the country.

Ironically, the elephant's downlisting by the Convention on International Trade in Endangered Species comes at the same time the U.S. Congress is moving ahead with legislation to create an Asian Elephant Conservation Fund. The bill, sponsored by Representative Jim Saxton (R–NJ), would support research and conservation efforts to protect the animal and its environment. It is modeled after a program initiated in 1989 to help the African elephant. Although the bill would provide up to $5 million a year, a Saxton aide says that an annual budget of $1 million is more likely. **–P.B.**