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## Growing Neurons Use Silencing Mechanism to Avoid Disastrous Tug-of-War

Just as traffic signals switch off the red light when the green light comes on, the tips of growing nerve cells protect themselves from being led astray by switching off their sensitivity to an attractant protein when activating sensitivity to a repellent protein. This interlocking fail-safe mechanism might be a basic strategy used throughout the central nervous system to avoid the disastrous consequences of a tug-of-war between signals that attract and repel neurons. The discovery might also help explain why spinal cord neurons cannot be easily induced to regenerate after injury, said the researchers.

The discovery was reported by Howard Hughes Medical Institute investigator Marc Tessier-Lavigne and lead author Elke Stein in the March 9, 2001, issue of the journal *Science*.

Neurons wire themselves into networks by extending cable-like axons that grow toward specific targets in the nervous system. An axon's path towards a target neuron is steered by growth cones in the tip of the axon that receive cues about the best path to follow from chemical attractants and repellents secreted by cells in the nervous system.

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Tessier-Lavigne and his colleagues at the University of California, San Francisco, had previously identified a key attractant protein called netrin-1. Netrin-1 exerts its influence on growth cones by plugging into a receptor called DCC that nestles in the cell membrane. Conversely, a repellent protein

called Slit triggers axons to grow away from a site by plugging into a receptor called Robo. Thus, a growing axon is attracted by netrin to a target way station in the spinal cord and then repelled from it toward the next way station by Slit.

In their *Science* article, Stein and Tessier-Lavigne reported the startling finding that Slit's activation of the Robo receptor triggers it to bind directly to the DCC receptor to "silence" that receptor's sensitivity to netrin-1. The scientists saw the first evidence of this effect when they exposed growing cultured spinal axons from the frog *Xenopus* to both netrin and Slit.

"We knew they had opposite effects and that axons are exposed to both *in vivo*," said Tessier-Lavigne. "And we wanted to find out with this simple test more about what the rules are when axons encounter both at the same time. To our surprise, Slit completely silenced the attractive effect of netrin-1."

This silencing effect could stem from one of several mechanisms, said Tessier-Lavigne. The Slit protein might bind directly to netrin to silence it. Or, some Robo-related signaling pathway within the cell might indirectly affect the DCC pathway. Or finally, the Robo receptor might directly interact with the DCC receptor.

To help reveal the mechanism of netrin silencing, the researchers created a truncated version of the Robo receptor that lacked the domain that juts into the cell's cytoplasm. This truncated receptor failed to silence netrin's attraction, suggesting that the Robo receptor itself might be a key to the silencing effect.

Next, the scientists sought to rule out the possibility that Slit might be binding to netrin directly. They created "chimeric" Robo and DCC receptors that replaced the Slit or netrin attachment domains with a domain triggered by a different protein, called HGF. When either of these chimeric receptors was activated by HGF, they behaved just as if their natural proteins, Slit or netrin, had activated them. Thus, the natural proteins were not involved in the silencing effect.

Further studies revealed that the cytoplasmic domain of Robo did interact physically with the cytoplasmic domain of DCC to interfere with its action. The scientists also showed that blocking this physical interaction blocked the silencing effect.

"Thus, we've shown that if you have both of these receptors in a cell, the repulsive receptor dominates by taking hold of the attractive receptor and subverting it for its purposes, if you will," said Tessier-Lavigne. "And that also specifically involves the cytoplasmic domain." He speculates that an interlocking control mechanism might be present in other families of axon guidance receptors.

"This coordinated mechanism of switching off attraction to avoid errors of axon guidance makes so much sense that it will be surprising if it isn't found in other regions of the nervous system," he said. "It wouldn't necessarily have to involve direct receptor-receptor interaction, but might involve a cascade of events within the cell."

Interlocking mechanisms might complicate efforts to regenerate spinal cord nerves damaged by injury or disease, Tessier-Lavigne said. "It's known that one of the reasons axons don't regrow in the central nervous system is the presence of inhibitory factors," he said. "In the past, it has been thought that just flooding the system with attractant factors might overcome those inhibitory factors. But if the repellents can switch off the attractants, it might be necessary to try either to block the repellents or to affect the growth mechanism downstream within the growth cone, rather than affecting it from the outside. This poses new challenges as we attempt to develop a therapy for regeneration."