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Crossing the Line

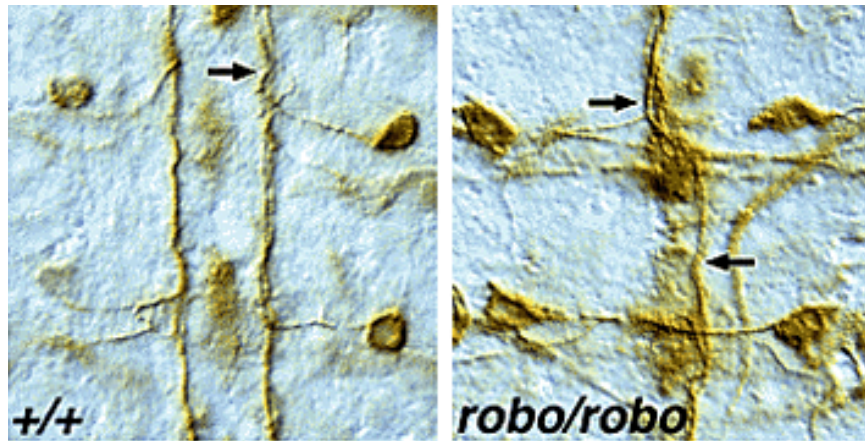


Image Title: (Left panel) In wild type *Drosophila* embryos, neurons (arrowhead) extend growth cones toward the midline but do not cross it. (Right panel) In *robo* mutant *Drosophila* embryos, neurons (arrowhead) cross the midline and then recross (lower arrowhead). - Courtesy of Neuron, Vol. 20, 25-33, January, 1988.

The long journey of nerve cells that wire the developing brain and spinal cord has been a longstanding secret, driven as if by mysterious molecular forces. In January, however, three teams of HHMI researchers identified and demonstrated how specific molecules influence an axon's trajectory by responding to repulsive cues.

The findings help explain how axons, the long

projections of nerve cells, grow toward and across an organism's midline whether in the mammalian spinal cord or its equivalent structure in flies and worms to wire up both sides of the body. These researchers found that these same "guidance" molecules shepherd the growth of axons in nematode worms and fruit flies, and also apparently in rats. It also appears that these factors direct construction of the basic nerve network infrastructure in all beings, including humans.

"The system for wiring the brain seems to be so ancient, so highly conserved that we can go back and forth between organisms to help us understand how events are coordinated," said Corey Goodman, a Hughes investigator at the University of California, Berkeley.

Goodman and Hughes investigators Marc Tessier-Lavigne and Cornelia Bargmann, both at the University of California, San Francisco (UCSF), are establishing how both growth promoters and growth inhibitors attract or repel axons as they search for targets in the central nervous system. The three scientists led research teams that recently discovered a family of receptor proteins on the surface of nerve cells that recognizes signals from outside the axon. These signals guide the path of the axon once it reaches the midline.

One of the key players at the midline is a receptor nicknamed Robo, short for "roundabout," because a faulty form of the protein causes nerves to circle back and forth around the midline of *Drosophila*. Goodman's team reported cloning the *robo* gene in the January 23, 1998 issue of the journal *Cell*.

Robo influences an axon's ability to cross the midline because it detects a chemical signal that deflects the axon away from the midline. During development, axons display low levels of Robo protein until they cross the midline. After the midline is crossed, a large amount of Robo is produced to ensure that the axons pick up the signal not to cross back over the midline.

Goodman's team also learned that *commissureless*, another key gene, encodes a protein, Comm, that interacts with the Robo protein and helps regulate it. They report in the January 1998 issue of *Neuron* that Comm may turn down Robo concentration so that axons can cross the midline.

At UCSF, Bargmann and her team independently cloned a gene in the nematode worm *C. elegans* that mirrors the action of Robo. Sax-3 guides motor and sensory nerves as they grow toward the midline of the worm. Once the axons reach the midline, Sax-3 prevents them from crossing over. This Sax-3 function is reminiscent of the Robo midline function, and remarkably *sax-3* is highly similar to the *Drosophila robo* gene. Thus both worms and flies control midline crossing with similar receptor proteins, Bargmann said. Her team isolated and sequenced the protein encoded by *sax-3* and published their results in the January 23 issue of *Cell*.

Next door to Bargmann, but working independently, Tessier-Lavigne's group, in collaboration with Goodman's team, cloned human and rat genes that encode proteins similar to Robo. They found that these genes were also expressed by axons that grew toward the midline of the spinal cord, suggesting that similar mechanisms establish the pattern of the vertebrate nervous system.

Several years ago, Tessier-Lavigne and colleagues discovered a class of molecules called netrins that guide axonal growth in vertebrates. Netrins help form intricate connections between neurons. Homologues of several netrins have been found in *Drosophila* by Goodman and in *C. elegans* by others in

the field.

The challenge facing the researchers is to determine how axons use the various chemical cues to wire and build the nervous system. In December, an important clue came as a team of researchers that included Tessier-Lavigne reported in *Neuron* that individual axons displaying netrin-1 can find the same chemical cue either attractive or repulsive.

Nerves "clearly change preferences and responsiveness to a given signal," Tessier-Lavigne said. They make the journey in segments, he explains, and might in some cases do this by first being attracted to "a", then repulsed by "a," and newly attracted to "b," then repulsed by "b."

Uncovering some of the attractive and repulsive cues that guide an axon's long journey constitutes a major step forward in identifying the molecular mechanisms that shape all nervous systems, whether they be in *C. elegans* or in humans. "We now have an opportunity to ask how groups of molecules are acting together to form a pattern," said Bargmann. "It may be that a few very conserved signaling systems are used over and over again to generate a complicated system."

"You can make an analogy to cars," she explains. "In *C. elegans*, we are trying to understand how a '64 VW Beetle operates so that eventually we can fathom the workings of a '98 Ferrari."

The difference between the brain of a fruit fly brain and a human is, in essence, small and subtle, "which is both fascinating and humbling," said Goodman. "Somehow differing cognitive abilities come out of these brains in ways that we don't understand."