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Errant Nerve Cells Risk a Clockwork Death

Fetal nerve cells growing along the spinal cord literally race against the clock to create connections within the developing nervous system. Newly published studies by investigators at the Howard Hughes Medical Institute (HHMI) at the University of California, San Francisco (UCSF), show that growing neurons must reach a specific intermediate destination by a certain time or risk not receiving life-sustaining chemical signals from specialized spinal cord tissue.

According to Marc Tessier-Lavigne, an HHMI investigator at UCSF, the discovery likely reveals a new fail-safe mechanism that ensures proper wiring of the nervous system. This checkpoint allows the burgeoning nervous system to winnow wayward neurons whose meanderings could cause dangerous miswiring of neural circuitry.

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Tessier-Lavigne and former postdoctoral fellow Hao Wang, who is now a scientist at the Merck Research Laboratories, reported their discovery in the October 21, 1999, issue of *Nature*.

The new mechanism for intermediate control of neural wiring is different from, albeit complementary to, the well-known "final target-derived neurotrophic system," which governs the survival of neurons once they reach

their final destination in the nervous system and kills off neurons that inadvertently reach the wrong target. The newly described "intermediate control" system functions solely to ensure accuracy in neural connections.

"In the traditional, final target-derived neurotrophic system," Tessier-Lavigne explained, "the long cable-like axons of nerve cells must compete for limited life-giving chemical signals known as neurotrophins when they reach their final target." Competition for limited amounts of neurotrophins at a target cell kills off excess nerve cells, leaving only the proper population of neurons necessary for wiring a given neural circuit.

"We speculate that the nervous system might need this additional intermediate control system because if axons have gone astray, it would not be wise to wait until they have reached their final target to eliminate them," said Tessier-Lavigne. "In large organisms such as mammals, axons may grow for up to a week before reaching their target. If they make an error early on and are not programmed to die quickly, they might wander all over the place and eventually find some alternate target that really creates a messed up neural circuit."

The researchers named the new intermediate neural guidance mechanism "*en passant*," which in French means "in passing." In the *en passant* model of axon guidance, axons receive support from neurotrophins as they pass through the region of the intermediate target, rather than when they stop at the target, as occurs in the traditional neurotrophic mechanism.

The discovery of the *en passant* mechanism was made during experiments in which Tessier-Lavigne and Wang were studying growing fetal rat spinal cord neurons in culture dishes. The scientists chose to study commissural axons, which in the developing rat embryo extend themselves from one side of the spinal cord to the other.

Under normal conditions, commissural axons sprouting from one side of the spinal cord migrate toward a guiding structure at the base of the developing spinal cord known as the floor plate. They then are directed along the floor plate by chemical cues, covering a distance of nearly a centimeter, before departing for their final target on the other side of the spinal cord.

"We noticed that in cultures grown without adding floor plate tissue, the neurons would suddenly all die," said Tessier-Lavigne. "If we had floor plate tissue present, they would survive much longer."

The scientists measured the timing of cell death and found that the cultured nerve cells needed the presence of some chemical, or neurotrophin, produced by floor plate cells during their fourteenth day of growth about the same time that the axons normally reached the floor plate in developing rat embryos. After that period, when the axons would normally have left the floor plate, the axons developed an additional need for neurotrophins to continue to grow.

To learn whether the nerve cell axons needed to approach the floor plate cells to survive, Wang and Tessier-Lavigne grew nerve cells and floor plate cells in the same culture dish, but on opposite sides of a collagen gel. This gel physically separated the two types of cells, but allowed growth factors to pass readily from one cell type to the other.

The experiments revealed that the nerve cells survived only if their axons grew near the floor plate cells. Preliminary studies suggest that the life-sustaining substance is a small protein, said Tessier-Lavigne. His team also found evidence that the tip of the growing axon senses this survival protein and sends a signal back to the main nerve cell body to thwart the suicide program. Experiments are underway to isolate the survival signals.