RACING DOWN THE AXON

The field of neurobiology is filled with fascinating puzzles, but neurons also pose interesting questions in the study of cell biology. It is well known that neurons are extremely long cells stretching from the cell bodies in the central nervous system into all areas of the body. But how does the neuron transport neurotransmitters and other biologically important molecules to the ends of the axon? A piece to this puzzle was uncovered in the late 1960s when Raymond Lasek and Sidney Ochs independently described fast axonal transport.

Background

Neurons are highly specialized cells that have several interesting features. A neuron has four main components: the cell body, the dendrites, the axon, and the axon terminus. The vast majority of proteins and membranes are synthesized in the cell body. The axons extend from the cell body to the axon terminus where neurotransmission is carried out. Proteins, membranes, and organelles are needed at the axon terminus for neurotransmission to occur. Therefore, there must be a system to transport biomolecules along the axon. In the late 1960s, researchers took the first steps toward understanding this system of transport by trying to characterize the rate of transport. They found that radioactively labeled amino acids injected into ganglia could be taken up by the cell body of neurons and incorporated into proteins. This allowed the researchers to follow newly synthesized proteins as they were transported to the axon terminus. Using this technique, Lasek and Ochs discovered that not all proteins traveled along the axon at the same rate.

The Experiment

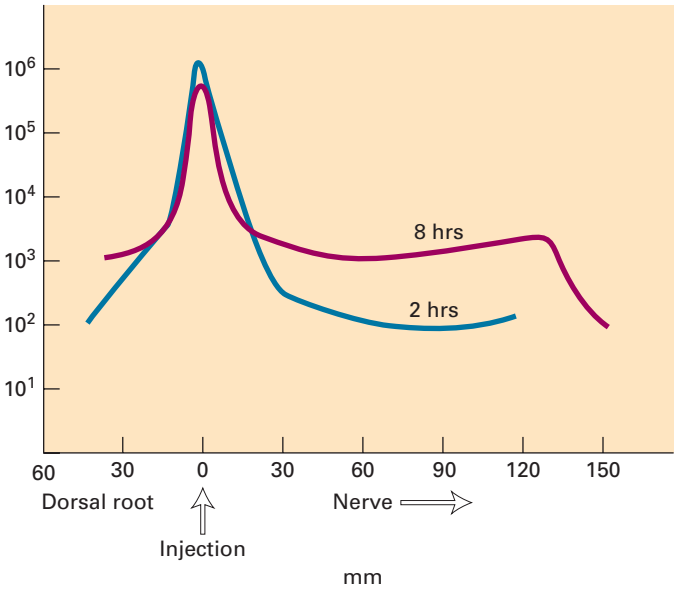
Lasek and Ochs set out independently to study axonal transport. To truly assess the rate of transport, each chose to study the sciatic nerve, which provides a long axon in which to study transport. Each of their experiments involved injecting radioactively labeled leucine ([^{3}H]leucine) into the L_7 dorsal root ganglia, the location of the cell bodies in the spinal cord. They analyzed transport along the axon by removing the nerve at various time points after injection, sectioning the axon into small pieces, and then measuring the amount of radioactivity by scintillation counting. By following this protocol, Lasek and Ochs were each able to determine the rate of transport in the axon.

Lasek devised a set of precautions to assure that the movement of radioactivity he observed was due to actual transport and not to passive diffusion. The choice of a cell with a long axon was one such precaution. He also performed a number of important controls. He used a combination of microscopy and autoradiography to demonstrate that [^{3}H]leucine did not diffuse more than 2 mm from the injection site, but rather it was specifically taken up by the neuronal cell bodies and other cells in the area. He then tested whether the axon itself could take up the radioactively labeled amino acids by injecting [^{3}H]leucine in the ventral root ganglia, an area devoid of cell bodies. If the axon took up radioactive amino acids, then Lasek would find radioactivity as far away from the ventral root injection site as he had found in the dorsal root injection site. One day after injections, he found little radioactivity

more than 15 mm away in the ventral root, whereas he found 40 times more in the dorsal root. These controls assured him that he was looking at the transport of radioactively labeled leucine in the axon.

To characterize transport of radioactively labeled amino acids and/or proteins, Lasek measured the distribution of radioactivity along the axon at time points ranging from 14 hours to 60 days after the initial injection of [^{3}H]leucine. By six days, a large amount of the radioactivity had been transported out of the ganglia and down into the axons. As expected, by 60 days the majority of the radioactivity had been transported down the axon. Interestingly, he could detect radioactivity up to 250 mm from the injection site only 14 hours after the injection, which corresponds to the product being transported through the axon at 500 mm/day. The majority of radioactivity traveled the previously observed rate of only 1.3 mm/day, suggesting that more than one mechanism transported some proteins.

Working with a similar system, Ochs further characterized this fast component of axonal transport. While he repeated the experiments Lasek had reported, he looked at transport using shorter time points after injection. Ochs sectioned nerves from 2 to 8 hours after injection (see Figure 19.1), which allowed a more complete characterization of fast axonal transport. As Lasek had observed, the majority of the radioactivity remained in the ganglia during these short time points. A small portion, however, traveled rapidly within the axon. After 2 hours, Ochs could detect radioactivity more than 90 mm away from the injection site; by 8 hours, he observed radioactivity up to 150 mm from the injection site. From these data, he estimated the rate of fast axonal transport to be 410 mm/day.



▲ FIGURE 20.1 Fast axonal transport was characterized by observing the movement of radioactively labeled proteins along the length of the axon. Researchers injected [^{3}H]leucine into dorsal root ganglia of animals. The radioactively labeled leucine is incorporated into proteins, which are subsequently transported within the axon. At various time points after injection, the nerves are removed and the axons sectioned and analyzed for the presence of radioactivity. The figure shows distribution of radioactivity throughout the axon at 2 hours (drawn in blue) and 8 hours (drawn in purple) after injection. [Adapted from S. Ochs et al., 1969, *Science* **163**:686.]

Discussion

Through a series of carefully controlled experiments, Lasek and Ochs were able to demonstrate that some proteins are transported within the neurons at much faster rates than others are. It is now known that biomolecules and organelle travel in the axon at three different rates. The rapidly transported proteins that Lasek and Ochs observed were likely part of vesicles or the smooth endoplasmic reticulum that are now known to move by fast axonal transport. These vesicles carry neurotransmitters to the axon terminus. It has been shown that these vesicles are carried along microtubules in a process that requires adenosine triphosphate (ATP). Subsequently, scientists have shown that families of molecular motor proteins, the dyneins and the kinesins, power the movement of vesicles by fast axonal transport. The study of movement along the axon—much of which is grounded in Lasek and Ochs's initial observations of fast axonal transport—remains an exciting field for researchers.