GREGORY D. BAILEY and JOHN GRIFFIN

DESIGN AND USE OF A RADIATION MICROPROBE TO STUDY AXONAL TRANSPORT

Current standard methods of measuring axonal transport require sacrificing the animal and excising the nerve under study so that the distribution of a radiolabeled marker within the axon can be determined. Interest in a nondestructive method led to development of a small, movable radiation probe that is placed in direct contact with an exposed nerve and is moved stepwise along the nerve, mapping the distribution of the transported marker.

BACKGROUND

The nervous system of higher organisms is composed of the central nervous system (brain and spinal cord) and the peripheral nervous system, which provides the major interface between the central nervous system and the environment. Within the peripheral nervous system, afferent nerve fibers carry information in the form of electrical impulses from sensory nerve endings, such as thermal receptors in the skin, to the central nervous system. Motor fibers extend from the spinal cord and brain stem to the muscles. In the simplest reflex response, stretching a muscle tendon activates stretch receptors, resulting in a volley of firing in the sensory fibers that innervate that tendon. Some of the sensory fibers directly contact motor nerve cells in the spinal cord. An elongated extension of each motor nerve cell passes through a peripheral nerve to innervate a group of muscle fibers. These elongated cell processes are termed axons. Activation of the motor nerve cells by the incoming volley of impulses in the sensory fibers produces an outgoing response in the motor axons, resulting in muscle contraction. This is the reflex arc, most familiar as the basis for the "knee jerk" response.

The extremely long axons of nerve cells present special problems in maintenance. Figure 1 illustrates the relationship between the parts of a single motor nerve cell, consisting of the cell body in the spinal cord, the axon, and the nerve terminals. The axon and nerve terminals cannot synthesize the proteins and other essential materials necessary for their survival and functions. Rather, they depend on a transport system within each axon for the continuous delivery of materials from the cell body (anterograde transport). In addition, certain materials are returned from the nerve terminals to the cell body (retrograde transport).

Because abnormalities of axonal transport occur in a variety of neurological disorders, there is considerable interest in the relationship between these abnormalities and axonal disease. Study of the transport processes in an animal model can enhance the understanding of the same processes in humans, and it was for such animal studies that the radiation microprobe was developed.

Radioactive markers have been used extensively to study the nerve transport process in animals.[1-3] The following isotopic technique is in standard use for measuring anterograde axonal transport. A radiolabeled protein precursor (a substance used by the cell in the synthesis of protein) is injected near the appropriate nerve cell bodies (for example, motor nerve cells in the spinal cord), and sufficient time is allowed for incorporation of the precursor into protein by the nerve cell bodies and for transport of the protein along the axons away from the cell bodies. At an appropriate point in time, the distribution of radioactivity along the axons due to the transport process is determined by killing the animal, removing and cutting the nerve into segments (the size of the section determines the spatial resolution), and measuring the radioactivity in each of the nerve segments by liquid scintillation counting or by autoradiography.

Retrograde transport, in turn, is usually measured by injecting a radiolabeled protein near the nerve terminals, where the labeled protein is taken up by them and carried within the axons toward the cell bodies. The distribution of radioactivity is then determined in the same manner as described above. The rate of transport and the relative amount of radioactive material carried by the axons in either direction can be estimated by these methods; however, any single experiment gives only a "snapshot" of the transport process and requires sacrifice of the animal and excision of the nerve under study in order that the distribution of radioactivity in the nerve can be determined. Thus, these studies require large numbers of animals, excessive time, and care to compensate for variations among animals. In many experimental situations, the ability to follow axonal transport continuously for hours, days, or weeks in a single nerve would offer valuable advantages.

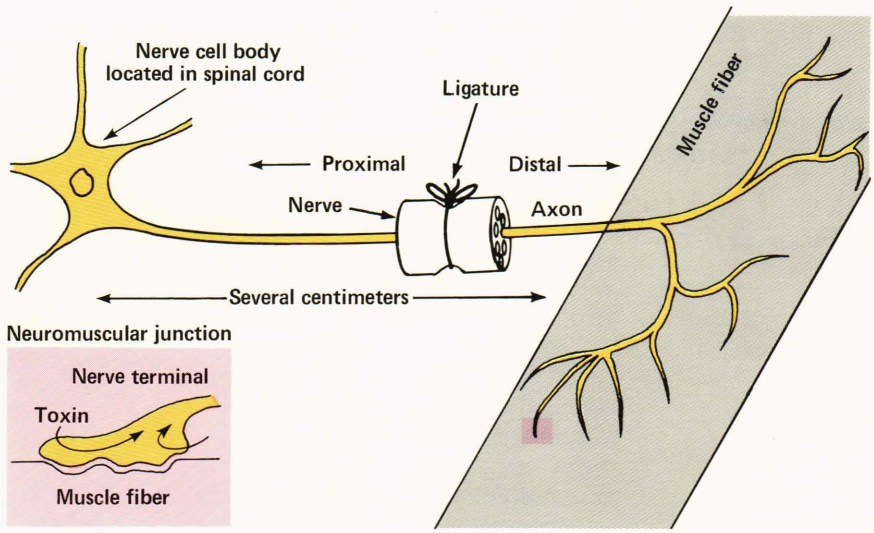

Figure 1 — Schematic representation of a motor nerve cell, consisting of the body, the axon, and the nerve terminals. The nerve is shown ligated, as used in some experiments. A peripheral nerve contains large numbers of individual axons extending from the central nervous system to targets in the periphery, such as muscle fibers.

If one could measure the distribution of these markers along the nerve *in vivo* (intact within the body) and without damage to the nerve, the transport processes could be studied in a single nerve by acquiring a number of "snapshots" of the distribution taken at different times, each of which would replace the sacrifice of an animal in the standard methodology.

RADIATION MICROPROBE

The sciatic nerve in the white rat lies approximately 0.5 centimeter below the skin surface of the hind leg. A radiation detector on the exterior of the leg is unsatisfactory because radiation passing from the nerve through the overlying tissue would be attenuated and scattered, severely limiting the spatial resolution of the source location. However, surgical exposure of the nerve during an experiment was considered acceptable, so the most straightforward way to achieve the desired capability appeared to be to construct a small, movable radiation probe that would be placed directly in contact with the exposed nerve and collimated with lead foil to provide simple spatial resolution. By measuring the activity of each nerve region directly under the probe, the distribution of a marker within the nerve could be determined without sacrificing the animal by moving the probe along the length of the nerve.

The feasibility of building such a probe for use on a small animal such as the rat depended on the selection of a suitable detection medium. The detector must be able to detect 28-KeV gamma rays emitted by the radioisotope ^{125}iodine (^{125}I) which is commonly used in retrograde transport studies. The amount of ^{125}I within the nerve is small, so the detector, in addition, must be very efficient.

The sciatic nerve in the laboratory white rat is only 1 millimeter in diameter, and only about 30 millimeters of the axon can be made readily accessible by surgery. The packaged detector, therefore, must be small and capable of being manipulated within the surgical cavity; this was probably the most stringent requirement.

Transport rates are affected by nerve temperature, so during an experiment the exposed nerve must be kept close to its normal temperature by illumination of the surgical cavity with a heat lamp. Therefore, the detector must operate satisfactorily at body temperature.

Crystalline cadmium telluride (CdTe) satisfies these requirements and has been used successfully in other medical experiments with ^{125}I.[4,5] The counting efficiency of cadmium telluride is high because of the high atomic numbers of cadmium (Z = 48) and tellurium (Z = 52); a 2-millimeter-thick crystal will stop and detect virtually all incident ^{125}I photons. A commercially available crystal of 3 × 3 × 2 millimeter dimensions is small enough after packaging to be moved about in the surgical cavity. Cadmium telluride also works satisfactorily at body temperature, experiencing only a slight increase in background noise level over room-temperature operation.

The radiation microprobe designed around the crystal detector is shown diagrammatically in Fig. 2. The crystal was mounted by the manufacturer at the inner face of a very thin-walled aluminum can 1 centimeter long by about 0.5 centimeter outer diameter, which was shielded by lead foil except for a 3 × 2 millimeter opening directly under the crystal. The hole is oriented along the axis of the axon to provide a degree of collimation that permits detection only of those photons originating in the 3-millimeter-long segment directly under the crystal, thus maintaining the desired spatial resolution. Polyethylene freezer wrap is used to isolate the nerve from the lead foil and to prevent body fluids from adhering to the probe. The detector can is held at the end of a 15-centimeter-long fiberglass tube, which is mounted to an X-Y positioner on a modified microscope stand so that the detector can be positioned three-dimensionally. The detector is connected by a 0.5-meter coaxial

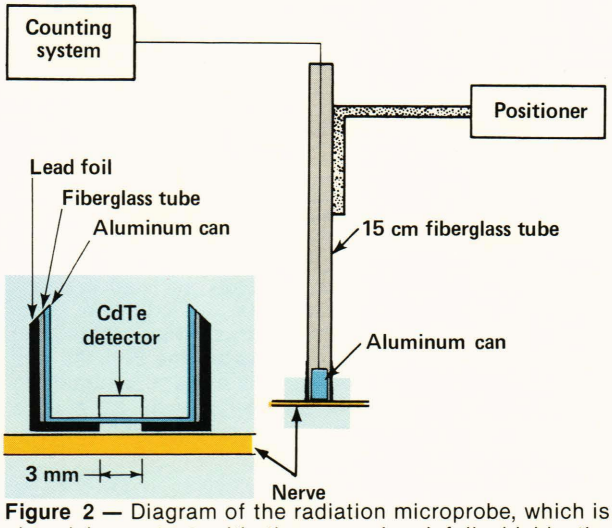

Figure 2 — Diagram of the radiation microprobe, which is placed in contact with the axon. Lead foil shields the crystal detector from all regions of the nerve except for the 3-millimeter segment of interest.

cable to the single-channel analyzer counting system diagrammed in Fig. 3.

In operation, 90 volts are applied to the crystal so that no free charges exist within it. As it is stopped within the crystal, an incident photon produces an electron-hole pair, each of which is swept out of the crystal by the applied electric field. These moving charges produce in the circuitry of the preamplifier a pulse of current the magnitude of which is directly proportional to the energy of the stopped photon. After amplification, the pulse is processed by pulse-height discrimination circuitry in the single-channel analyzer. If the photon energy corresponds to a ^{125}I source, the pulse is counted; the resulting count rate at the end of the counting period is a measure of the activity of the sample.

EXPERIMENTAL TECHNIQUE

During development of the radiation microprobe, ^{125}I-tagged tetanus toxin was used in the neuromuscular laboratory to demonstrate retrograde transport in the rat sciatic nerve.[6] The specific experimental technique was as follows. After the rat was anesthetized, the nerve was exposed and tied off (ligated) with a surgical suture at midlength to block transport mechanically within each axon. This blockage was necessary because the rate of retrograde transport is greater than the rate at which toxin is taken up by the nerve terminals and released into the retrograde transport system. No sharply defined front was seen in the distribution unless the nerve was ligated prior to injection to allow the labeled toxin to accumulate. The toxin was injected into the intrinsic muscle of the hind foot, where a small amount was taken up by the nerve terminals (see the inset of Fig. 1) and carried within each axon toward its cell body by normal retrograde transport.

Twenty-four hours after injection, the nerve was removed in preparation for the conventional liquid scintillation well counter. In the first test of the radiation microprobe on a nerve, the nerve was laid out on a flat surface and scanned by the probe. The probe was positioned at one end of the nerve by the researcher, who selected a 1-minute count time for each position and started the timer. At the end of 1 minute, the number of counts was displayed and recorded, at which time the probe was moved 3 millimeters farther along the axon. The procedure was repeated until the entire length of the removed nerve had been counted by the probe.

The nerve was then divided into 3-millimeter segments, and the radioactivity in each segment was measured by the well counter. Figure 4 compares the results of the two counting methods: the net count rates (at 10% of their actual value) from each segment as measured by the well counter and the net count rates obtained by the microprobe as it scanned in 3-millimeter increments. Both distributions demonstrate a buildup on the distal side of the ligature and a sharp drop-off in activity across the ligature, indicating that labeled toxin has been carried from the nerve terminals as far as the ligature, where transport was halted. The count rate of the radiation microprobe is approximately 10% of the well counter rate in the region of the buildup, and the count rates by the two methods correlate very well in describing the distribution of the toxin. The count rates of the radiation microprobe are much smaller than the well counter rates because of the difference in geometry of the two methods: in the well counter, the sample is completely surrounded by detection material, whereas with the probe, only a fraction of the emitted radiation strikes the crystal.

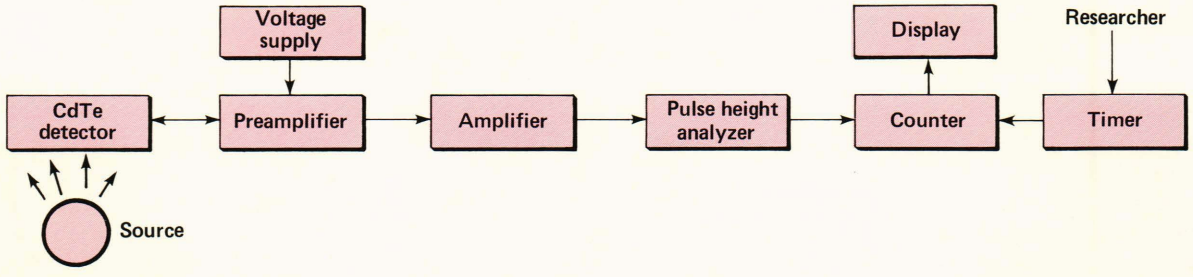

Figure 3 — Functional block diagram of the detector and counting system. The researcher manually starts a timer controlling the counter gate. A photon stopped by the CdTe detector creates a pulse whose magnitude is proportional to its energy, allowing the pulse-height analyzer to let only photons emitted by ^{125}I be counted.

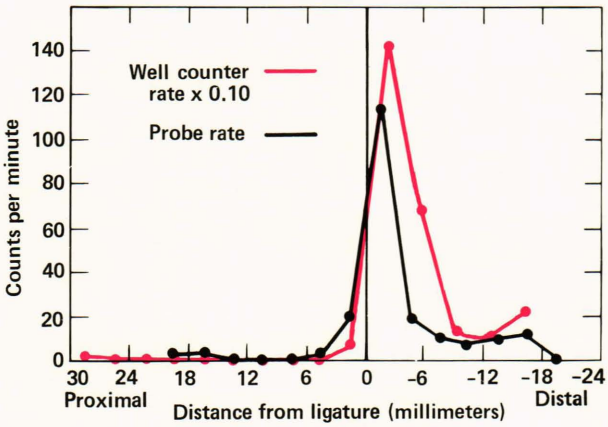

Figure 4 — An accumulation of tetanus toxin labeled with ^{125}I is produced by ligating the sciatic nerve. The nerve is removed and scanned with the probe. The distributions of toxin determined by probe and by well counter rates are compared. Note that the well counter rate is reduced by a factor of 10 for plotting.

This simple experiment demonstrated that the radiation microprobe is able to detect the very low levels of radiation that were used to monitor fast retrograde transport and that a buildup of labeled marker within the nerve can be located with spatial resolution that is comparable to using the well counter method.

Inherent limitations to the use of tetanus toxin as a marker of axonal transport include the facts that it measures fast retrograde transport only, and when tests were conducted *in vivo*, we found that the injected toxin was so widely distributed within the body of the rat that the resulting nontransported radioactive "background" counts completely overwhelmed the axonal counts. To extend the questions that can be studied using the microprobe, we have begun studies using an alternate method of labelling in which a radiolabeled marker capable of binding to nerve proteins is injected directly into the nerve. In principle, this approach should allow measurements of intraaxonal transport from the site of injection. Figure 5 illustrates changes in the amount and distribution of radioactivity in a single rat sciatic nerve following direct injection into the nerve of a protein-binding compound labeled with ^{125}I. Note the much higher levels of activity that result from use of the direct injection technique rather than from use of tetanus toxin that must be administered to muscle tissue. From the time of injection at day 0, two things are occurring, as seen by the probe: the total amount of radioactivity within the axon is decreasing, and the distribution of radioactivity is shifting, indicating the transport of labeled proteins toward the cell body. Such *in vivo* observations were unattainable before development of the microprobe. This labeling method has allowed extensive use of the microprobe *in vivo*, and a large body of transport data has been accumulated, demonstrating the value of the microprobe. In addition, by using different markers it

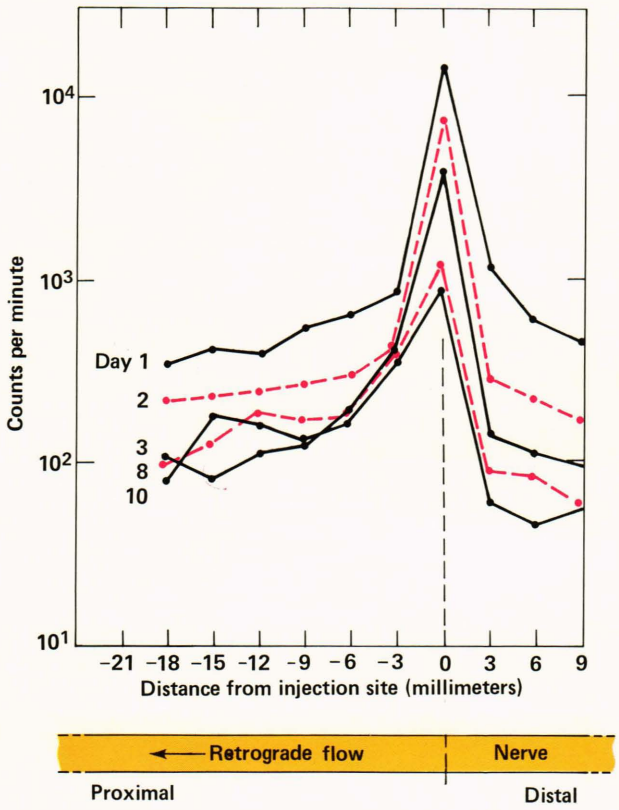

Figure 5 — *In vivo* distribution of radiolabeled ^{125}I within the rat sciatic nerve as measured with the radiation microprobe. Following direct injection into the nerve, some of the compound, having bound to proteins, is transported toward the cell body. Scans were made 1, 2, 3, 8, and 10 days following injection; by day 10, a front can be seen developing. Note the 100-fold increase in measured counts (compare Fig. 4) due to the use of ^{125}I instead of tetanus toxin.

should be possible to examine the migration of extraaxonal proteins moving in the endoneural fluid that lies between axons in a nerve. Little is yet known about the composition or kinetics of this "endoneural flow." Results of these studies will be published later.

CONCLUSION

The probe was designed to monitor, *in vivo*, the movement of radiolabeled markers within the axons so as to eliminate the need for sacrificing the animal and nerve, as in the standard methodology. The probe demonstrated on a nerve removed from the animal that it could detect the low levels of radioactivity in a typical experiment, that the distribution of a labeled marker within the nerve could be determined with a spatial resolution comparable to that of the standard well counter method, and that the probe can be used for a variety of studies of retrograde transport. In addition, use of ^{125}I-labeled protein bind ing agents to label nerve proteins will extend the range of questions that can be addressed with the probe methodology.

FUTURE PLANS

Future plans include development of a multidetector system employing six detectors like the one described above. By mounting each of them in an arm that allows precise positioning in a surgical cavity, they can be left in place during an experiment and the distribution of marker along the axon can be measured continuously at six different points. Control of the six counters and recording of the data will be handled by a personal computer. The technique can be applied to larger animals and, hopefully, human biopsies. Other isotopes are also being considered; for example ^{75}Se, which is higher in energy and therefore has greater range through tissue. This may lead to the use of ^{75}Se in situations where surgical exposure of the nerve is undesirable.

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