

A NEW MEASUREMENT OF ACTION CURRENTS DEVELOPED BY SINGLE NODES OF RANVIER

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INTRODUCTION

THE SALTATORY THEORY OF NERVOUS CONDUCTION in the myelinated nerve fiber is based on the following experimental evidence: 1) electric excitation takes place only at nodes of Ranvier, 2) action potential is produced only at nodes, and 3) the local current resulting from excitation of a node is strong enough to excite the neighboring node. The major portion of the evidence mentioned above was published before and during World War II (5, 8, 11; for review, ref. 9). When additional evidence along this line was reported from England in 1949 (4), the theory became widely accepted. At the annual meeting of the National Academy of Sciences in 1963, however Lorente de Nó and Honrubia (7) made an attempt to disprove the saltatory theory on the basis of rather indirect observations.

The present report describes a new experiment demonstrating that the inward-directed component of the action current of a single nerve fiber is produced at the node. By clamping the potential of the conducting medium by the use of an operational amplifier (3), it was possible to determine the absolute value of the action current of the node in a single fiber preparation immersed in a continuous fluid medium. This experiment adds a new piece of evidence to the previously published experimental support for the saltatory theory. An explanation is offered for the observation that under the experimental conditions adopted by Lorente de Nó, action potentials along the nerve did not show signs of saltatory conduction.

METHODS

Experiments were carried out on single nerve fibers isolated from a small branch of the sciatic nerve innervating the sartorius muscle of the toad, *Bufo marinus*. After isolation and cleaning, the single fiber preparation was transferred to a Lucite chamber (5 cm.x7 cm. in area and 2 cm. in depth) filled with Ringer's solution. The cleaned portion of the nerve fiber preparation was mounted on a small glass platform (6mm.x7mm.) placed in the chamber (see Fig. 1). Mineral oil was then poured into the chamber, and the Ringer's fluid in the chamber was reduced until a thin layer of fluid remained on the surface of the glass platform. A pair of metal electrodes (E_1 and E_2) was brought close to the fiber. The electrodes used consisted of two enameled nichrome-steel wires 25 μ . in diameter and fixed at a distance of about 20 μ . The enamel covering of the wires had been removed in about 50- μ . portions near the tips. One of the electrodes (E_2) was connected to a cathode follower (B) and the other electrode (E_1) to the output of an operational amplifier (Philbrick type U-2) in the manner shown in the diagram. The resistances in the d.-c. feedback loop (R_1 and R_2) were roughly 230 kilohms and r_1 and r_2 in the condenser-coupled feedback loop

were 50 and 10 kilohms, respectively. Each coupling condenser had a capacity of $0.1 \mu\text{F}$. Ringer's fluid in the chamber was grounded through a large silver electrode. Stimulating electric shocks from a Grass stimulus isolation unit (SIU-4B) were delivered to the preparation near its proximal end (S.E.) kept in mineral oil.

When the current electrode (E_1) was disconnected from the circuit by opening switch S , the action potential of the preparation could be recorded on the potential channel (V) of an oscilloscope. When the switch was closed, the potential of the medium at E_2 was maintained at a constant (zero) level by automatic control of the electric current supplied by the operational amplifier (A). The intensity of the current flowing into the medium through E_1 was determined by recording the potential difference across resistor r_1 with a differential amplifier (Tektronix type 122). A Grass kymograph camera and a Tektronix dual-beam oscilloscope (type 502) were used for simultaneous recording of the current (I) and potential (V). All the experiments were carried out at room temperature (23°C).

RESULTS

Measurement of the action current of a single node without partitions dividing the fluid medium. When the feedback circuit of the experimental arrangement of Fig. 1 is turned off, the setup is practically the same as that used previously for recording action potentials of a single nerve fiber in a continuous fluid medium (12). The amplitude of the observed action potential varied, under these experimental conditions, with the thickness (and uniformity) of the layer of Ringer's fluid on the glass platform. When the feedback system was in operation, the current supplied by the operational amplifier completely suppressed the potential variation resulting from the response of the single fiber preparation. The time course of the current (I) in the feedback system was therefore a direct measure of the current produced by the preparation.

An example of the records obtained with this experimental setup is furnished in Fig. 1. The records in the middle column were taken with the electrode set placed close to a node of Ranvier of the single fiber preparation. The potential variation observed under the condition $I=0$ (i.e., with switch S open) consisted of a relatively slow, positive phase (upward deflection) followed by a sharp negative phase. For stimulus intensity above threshold, the amplitude and the configuration of the potential variation was completely independent of the stimulus intensity, indicating that the observed potential variation was produced by the single nerve fiber under study. The electric current observed under the condition $V=0$ (i.e., with switch S closed) had a time course which resembled that of the action potential recorded under the condition $I=0$. When the recording electrode set was moved away from the node in either direction, proximal or distal, the negative component (i.e., downward deflection) of the single fiber response was markedly reduced. The slow, positive phase of the response was, on the contrary, decreased only slightly when the electrodes were moved away from the node.

Our interpretation of the above-mentioned property of the single fiber responses is as follows: the myelin sheath has a capacity of approximately $1.6 \mu\mu\text{F}/\text{mm}$. and a parallel resistance of 290 megohms mm. (10). When the potential of the exoplasm rises rapidly during the ascending phase of the

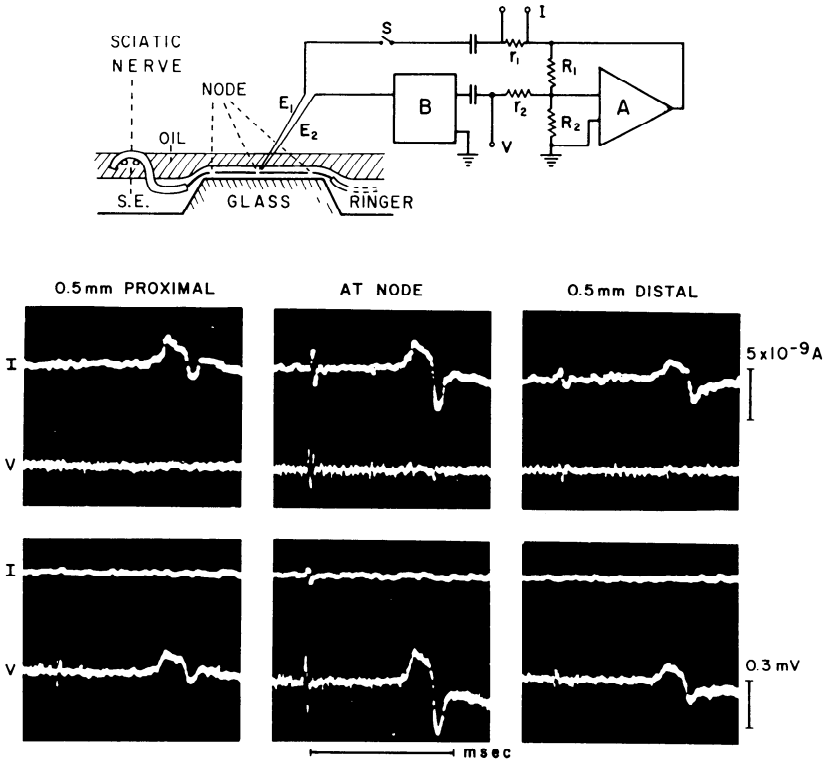


FIG. 1. *Top*: experimental setup used to measure potentials and currents developed by a node of Ranvier without partitions for dividing the surrounding fluid medium. "A" is an operational amplifier, and "B" a cathode follower. *Bottom*: oscillograph records obtained with switch S closed (top) and opened (bottom). Trace I represents the current in the feedback circuit and V the potential variation recorded with the cathode follower.

action potential, there is a relatively strong flow of capacitive current through the myelin sheath. This outward flow of current through the myelin sheath constitutes the component of the response which is insensitive to the distance from the node. The sharp, downward deflections in the records indicate the existence of an inward-directed membrane current at the node (for further details, cf. ref. 12). When the potential variation in the vicinity of the node was completely suppressed by the current from the feedback system, the current arising at the node should be equal in intensity with that deriving from the operational amplifier.

The argument mentioned above is strongly supported by the finding that the intensity of the inward current determined by the present method is practically independent of the thickness of the conducting fluid layer. The amplitude of the action potential varied directly with the thickness of the layer.

The intensity of the inward current at the node of Ranvier, determined by this method on six different fibers expressed in the unit of 10^{-9} A., was

4.5, 6.5, 4.0, 4.2, 3.8, and 3.9. The amplitude of the negative phase of the action potential, expressed in microvolts, in these preparations was 140, 700, 270, 330, 110, and 320, respectively. It is clearly seen in these data that the variation in the amplitude of the nodal current is far smaller than that in the action potential. The value of the inward-directed membrane current determined by the present method is in good agreement with the value reported previously, $(4-6) \times 10^{-9}$ A. (ref. 10, p. 649).

Longitudinal and radial resistance of the nerve trunk. When a nerve impulse travels along a single fiber within a nerve trunk the electric current associated with the impulse flows through the conducting medium in the

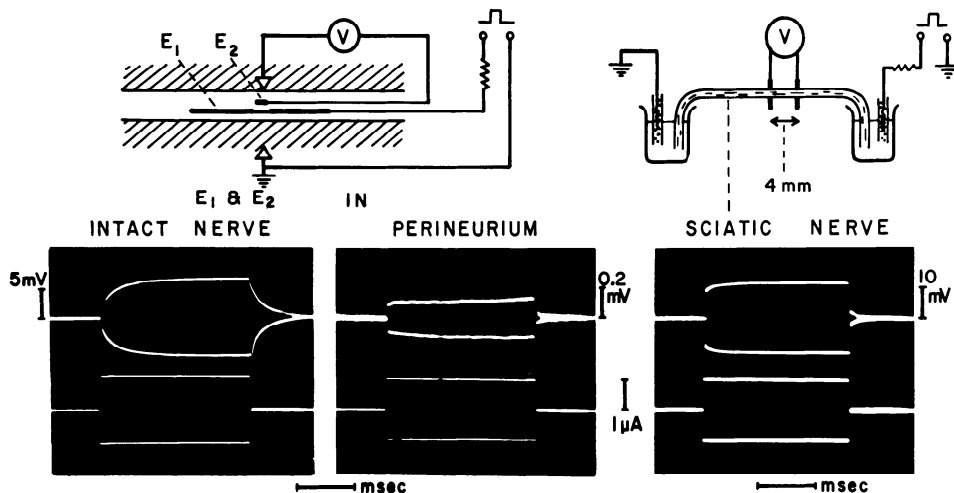


FIG. 2. *Top:* experimental setups (not to scale) used to measure the specific resistances of the sciatic nerve in the radial (left) and longitudinal (right) directions. *Bottom:* potential variations produced by rectangular current pulses ($+1$ and $-1 \mu\text{A}$. in intensity) observed with the axial wire electrodes inserted in an intact nerve (left), in a connective tissue sheath (middle), and with a pair of surface electrodes on an intact nerve (right).

trunk. If the nerve is immersed in a fluid medium the current spreads also into the surrounding fluid. The potential field around the nerve fiber is extremely complex and difficult to analyze under these circumstances. The major complicating factors are 1) anisotropy of the nerve trunk with respect to conduction of electric current, 2) the discontinuity in electric conductivity at the boundary between the nerve and the surrounding medium, and 3) the time dependence of the resistance of the nerve to radial current. The following observations were made to estimate the magnitudes of the factors mentioned above.

The experimental setup shown on the right-hand side of Fig. 2 was employed to evaluate the resistance of the sciatic nerve trunk to longitudinal current. Each end of the nerve trunk was immersed in a pool of Ringer's fluid in a glass beaker. Rectangular current pulses of 2.5 msec. in duration were applied to the nerve through large electrodes in the beakers. The

potential drop along the nerve was determined with a pair of fine electrodes of the Ag-AgCl type making contact with the surface of the nerve. In the example furnished in the figure, the current intensity employed was $1 \mu\text{A}$. The potential drop across a 4 mm. long portion of the nerve was 11.5 mV.; the longitudinal resistance of this nerve is then approximately 28,000 ohms/cm. By multiplying this value by the cross-sectional area of the nerve (1.06 mm. in diam.), the specific resistance in the longitudinal direction is found to be 245 ohms·cm., approximately three times the specific resistance of amphibian Ringer's solution (approx. 80 ohms·cm.).

The results of ten independent measurements of this type indicated that the longitudinal specific resistance is between two and three times that of Ringer's solution. Removal of Ringer's fluid on the surface of the nerve trunk by dipping it in an isotonic sucrose solution for 10 sec. did not alter the results appreciably.

The resistance of the nerve trunk to radial current was determined by introducing a set of wire electrodes into the whole nerve trunk along its axis. The electrode set employed was made with two enameled silver wires (50 μ . in diam.), one of which (current electrode) had a bare portion of 11 mm. and the other (potential electrode) a bare portion of 1 mm. These two wires were twisted together without making direct metallic contact. Rectangular current pulses were applied between the current electrode in the nerve trunk and the ground electrode in the surrounding medium. The potential drop between the center and the surface of the nerve trunk was measured with the internal and external potential electrodes.

The potential drop between the axis and the surface of the nerve showed an exponential time course under these experimental conditions. The time constant of potential changes was roughly 0.4 msec., which is, as expected,¹ in good agreement with the time constant of the myelin sheath (10). The steady-state resistance measured on five nerve trunks (approx. 1.0 mm. in diam.) was between 3,000 and 5,500 ohms·cm. The relatively large variation in different nerves can be ascribed to the difficulty of inserting the axial wires at the center of the nerve. The specific resistance to radial currents can be estimated by multiplying the value mentioned above by $2\pi/\ln(b/d)$, where b is the radius of the nerve and d the distance between the internal current electrodes and potential electrodes (e.g., see. ref. 2, p. 340). Since b is 500 μ . and d is between 25 and 50 μ ., the above-mentioned multiplier should be in the range between 2.1 and 2.7. Using the average value 2.4, it is found that the radial specific resistance of the nerve is between 7,000 and 13,000 ohms·cm., namely, between 90 and 150 times that of Ringer's fluid. Comparing these figures with the longitudinal specific resistance (i.e., 2-3 times that of Ringer's solution), estimation can be made of the degree of anisotropy that has to be dealt with in an analysis of the action potential of the nerve trunk.

¹ Note that in the limiting case, if the nerve fibers are very closely packed without deformation, a radial current will pass through the myelin sheath only.

The record in the middle of Fig. 2 shows that the connective tissue sheath on the surface of the nerve does not bring about any significant error in our evaluation of the radial resistance. In this experiment all the nerve fibers in the trunk were carefully removed and the empty sheath was filled with Ringer's fluid. The resistance between the interior and exterior of the sheath was determined by introducing the axial wire electrodes. It was shown that the observed resistance fell by a factor of 1/10 to 1/20 when the myelinated fibers were completely removed. When an incision was made in the connective tissue sheath there was another, significant fall in the observed resistance. This finding indicates that the potential field produced by nerve fibers near the surface of the nerve trunk could be distorted significantly by the resistivity of the connective tissue sheath. The role of the sheath as a diffusion barrier was vigorously discussed in the 1940's (6).

DISCUSSION

In an isotropic conducting medium the relationship between the current density i and the potential V is given by the well-known vectorial equation $i = \sigma \text{ grad } V$, where σ is the specific conductivity (i.e., the reciprocal of specific resistance) of the medium. Applying this equation to the two-dimensional fluid medium surrounding a single nerve fiber, an interpretation was given to the configuration of the action potentials recorded near and around a node of Ranvier (12). The observation described in this report proves the legitimacy of the previous interpretation.

It follows from the general relationship between the current and the potential that, when the conductivity varies discontinuously across a certain boundary in the medium, the direction of current flow should change abruptly at the boundary. The law of "refraction" of the current at the boundary is slightly different from that in optics; it is given by $\cot\theta_1 \cdot \cot\theta_2 = \sigma_2/\sigma_1$, where θ_1 and θ_2 are the angles between the normal to the boundary and the current vectors in the two media indicated by subscripts 1 and 2 (e.g., see ref. 2, p. 346). This type of discontinuous change in the current vector should take place at the interface between the nerve trunk and the surrounding fluid medium, thus complicating any attempt to interpret the potential field in the medium around the nerve.

In an anisotropic conducting medium, the conductance σ above has to be treated as a tensor (see e.g., ref. 1, p. 111); this means that the current does not in general flow in the direction of the potential gradient. We have seen that the conducting medium in the nerve trunk is highly anisotropic. A low radial and high longitudinal conductivity should tend to spread the current in the longitudinal direction of the nerve, even when the potential gradient is directed nearly radially. When a node of Ranvier within a nerve trunk produces an action current, the potential field in the vicinity of the node is determined by the anisotropic and time dependent conductivity of the nerve trunk.

Under the condition of the experiments reported by Lorente de N6 and

Honrubia (7), all the complicating factors mentioned above are present. It is difficult, therefore, to derive from their results any useful information concerning the mode of propagation of nerve impulses along individual nerve fibers.

It is interesting to note that the complicating factors described above do exist and have to be taken into consideration in analyzing the sources of electric activities of the central nervous system.

SUMMARY

1. A method is developed by which the intensity of the action current developed by a single node of Ranvier can be measured without using partitions for dividing the surrounding fluid medium.

2. The action current of the node determined by the new method is in good agreement with the values obtained previously by using the partition method.

3. The resistance of the nerve trunk to a current flowing in the radial direction is time dependent, due to the capacity of the myelin sheath of the nerve fibers. In the steady state, the specific resistance of the nerve trunk in the radial direction is nearly 50 times as large as that in the longitudinal direction.

4. Difficulties are discussed of interpreting the potential field produced by a whole nerve trunk immersed in a fluid medium.

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ADDENDUM

The full paper by R. Lorente de N6 and V. Honrubia appeared in *Proc. nat. Acad. Sci., Wash.*, 1964, 52: 305-312. They mention in this paper (p. 311): "Nerve fibers can be normal only if the external connective tissue of the nerve is normal." It should be noted, however, that isolated single nerve fibers carry impulses at a "normal" velocity and develop action currents of the "normal" intensity. It should also be pointed out that the current intensity necessary to excite a single motor fiber near the surface of the intact ventral root of the frog (under direct visual observation), by taking the action potentials of the leg muscle as an index, shows a sharp minimum at every node of Ranvier.

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