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ELECTRICAL STIMULATION OF THE INTERNODES OF SINGLE FIBERS OF NERVES WITH INTACT SHEATH*

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In a preceding communication proof was given that in response to stimulation by the outward flow of action currents all points of the internodes of myelinated nerve fibers produce in succession the EMF of the action potential. In this communication proof is given that applied cathodal currents can initiate nerve impulses at all points of the internodes.

Technique.—The experimental observations have been made with the long and slender peroneal branches of the Texan bullfrog (probably a variety of R. sphenocephala). In order to detect the response of a single fiber, a method originated by Adrian and Bronk is used. At the bifurcation of the peroneal trunk an opening is made in the external connective tissue sheath and all the nerve fibers are cut, except one large myelinated fiber.
The preparation is mounted in a chamber (Fig. 1a) which consists of a plate of Lucite in which three 2-mm deep compartments (pools) A, B, and C have been carved. The three pools are filled with Ringer's solution. The 600-μ-wide air gap between pools A and B is used for stimulation with 10-msec-long, rectangular pulses of current. At the axis of the gap the nerve is in contact with one edge of a 20-μ-wide chloridized silver plate (P), which is connected to the cathode of the stimulating device; the grounded anode is connected to both the A and B pools by means of long and thick chloridized silver wires. The 2-mm-wide, vaseline-filled gap between pools B and C is used to record, from the peroneal trunk, the action currents of the impulse carried by the single conducting fiber, by means of a 122 Tektronix differential amplifier. The long latency, up to 2 or 3 msec, of the threshold response leaves no doubt that the nerve impulse has been initiated at the stimulating cathode. The magnitude of the applied current is recorded by the second beam of the oscilloscope. The threshold current was taken to be that which initiated an impulse in two or three out of five applications.

At the start of the observations a 7- to 12-mm-long segment of nerve is located in pool A, and a longer segment, arranged in soft loops, in pool B. During the experiment the end of the nerve is displaced to the left, in steps of 0.5 mm, by means of a micromanipulator. After bringing the nerve back to its initial position, the observations are repeated. The nerve is always placed in position by means of soft brushes. If metallic instruments are used to handle the nerve, slight localized injuries to the nerve fiber are almost unavoidable.

The steady-state value of the electrotonic potential produced by an applied current has been determined by means of the model represented by diagram b (Fig. 1). An analysis of the steady-state electrotonus is sufficient when the stimulation threshold is measured with long rectangular pulses of current.

The model has a characteristic length \( \lambda = \sqrt{\rho/r_s + r_i} = 0.22 \text{ cm} \); \( \lambda \) varies from nerve to nerve, but the selected value has been often measured during a study of the fast electrotonus in frog nerves. Assuming \( r_s = r_i = 150 \text{ megohms/cm length of fiber} \), \( \rho = 15 \text{ megohms} \). In building the model 1000 times smaller resistances were used, but since thereby the ratio \( \rho/r_s + r_i \) was not altered, the distribution of the electrotonus in the model is the same as in an actual nerve fiber for which \( \lambda = 0.22 \text{ cm} \). On the assumption that the internal diameter of the nerve fiber is \( 14 \times 10^{-3} \text{ mm} \), the resistance of 1 cm² of myelinated membrane would be approximately \( 66 \times 10^4 \text{ ohm} \).

The model represents a nerve fiber extending from \( x = -40 \) to \( x = 41 \text{ mm} \). From \( x = 0 \) to \( x = 1 \text{ mm} \) the model is symmetrical. It has 10 sections from \( x = 0 \) to \( |x| = 100 \mu \), 20 sections from \( |x| = 100 \mu \) to \( |x| = 200 \mu \), and 100 sections from \( |x| = 200 \mu \) to \( |x| = 1 \text{ mm} \). The segment extending from \( x = 1 \text{ mm} \) to \( x = 5 \text{ mm} \) is divided into 100 sections, which are followed by one section 1 mm long, three sections 2 mm long, two sections 4 mm long, and two sections 10 mm long. The segment extending from \( x = -1 \text{ mm} \) to \( x = -2 \text{ mm} \) is divided into 200 sections followed by four sections 1 mm long and one section 34 mm long.

The conditions prevailing when the tripolar arrangement of electrodes (one narrow cathode between two diffuse anodes) was used were reproduced by making \( r_s = 0 \) in the sections of the model corresponding to \( |x| > 300 \mu \) and \( |x| = 10 \mu \) and connecting to the external conductor the cathode at \( x = 0 \) and the anode both at \( x = -300 \mu \) and \( x = 300 \mu \).

The model has also been used to analyze the problem of stimulation of a single isolated fiber, placed in a practically unlimited volume conductor by a microelectrode which is displaced in small steps along the surface of the isolated fiber, the diffuse anode being placed in the medium at a large distance from the fiber. Under conditions such as these, the potential \( V_a \) at the surface of the fiber can easily be evaluated with sufficient accuracy in this manner.

At the points of the surface of the fiber in contact with the microelectrode \( V_a = \text{const.}/a, a \) being...
the radius of the microelectrode, and at all other points of the surface of the fiber \( V_r(x) = \text{const.}/|x| \), |x| being the distance from the center of the microelectrode. This potential distribution can be reproduced by making in the model \( r_s = 0 \) from \( x = -a \) to \( x = a \) and \( r_s = b[V_r(x) - V_r(x + \Delta x)] \) in all the other sections of the model, the constant \( b \) being chosen so that \( r_s \) is very small in relation to \( r_l \) and consequently the stimulated "fiber" cannot significantly alter the distribution of \( V_r \). If the stimulating device is connected to the external conductor, the cathode at \( x = 0 \) and the anode both to \( x = -40 \) mm and \( x = 41 \) mm, through adequate small resistances, the applied current brings all points of the surface of the "fiber" exactly to the potentials at which they would have been in the volume conductor.

Finally, the model with \( r_s = r_l \) throughout its length has been used to analyze the effect that low resistance nodes may have upon the electrotonic spread of current applied to the "fiber" by means of two electrodes connected to the external conductor at \( x = -40 \) mm and \( x = 0 \).

*Theoretical Argument.*—As is well known, impulses can be initiated only at those points of the nerve fiber at which the applied current is flowing outward through the membrane, i.e., only in a catelectrotonic zone. Since the nodes of Ranvier play no detectable role in the production of the action potential, the flow of current through the nodal membranes can play no direct role in the process of excitation. However, if the resistance \( \rho_s \) of the nodal membrane were small in relation to the resistance \( \rho \) of the myelinated membrane, the presence of the nodes would have an effect upon the electrotonic spread of the applied current, hence the need of determining the distribution of the electrotonus in core conductors having low resistance, transverse shunts (nodes) at distances, 2 mm, corresponding to internodal lengths.

The curves reproduced in Figure 2a give the distributions of the electrotonic potential in the segment of fiber extending from \( x = -300 \) to \( x = 300 \mu \) when one of the nodes was at the indicated distances from the cathode. The resistance of the nodal membrane was 6000 times smaller than that of the myelinated membrane.

In all cases the catelectrotonus had a maximum height at the cathode itself and decreased practically linearly with increasing distance from the cathode, to become zero in the neighborhood of the margins of the diffuse anodes. It is therefore

![Figure 2](image-url)

*Fig. 2.*——(a) Distribution of the electrotonus in the interpolar segment of a fiber having low resistance nodes, when currents are applied by means of a narrow cathode surrounded by two diffuse anodes. The dotted line joining the maxima of the catelectrotonus measured in arbitrary units the reciprocal of the apparent variations of threshold that would be measured along the internodes. (b) Distribution of the anelectrotonus in the extrapolar segments.
clear that, whether the nerve fiber has low resistance nodes or not, the nerve impulse can be initiated only within the short segment of fiber included between the margins of the anodes.

Curves $a$ in Figure 2 show that the maximum of the catelectrotonus had its minimal height when the cathode was at a node. In such a case the amount of current entering into the fiber through the diffuse anodes was largest, but the catelectrotonus had its minimal value because a large part of the current left the internal conductor through the low resistance node. The maximal height of the catelectrotonus rapidly increased when the cathode was moved away from the node, because the total amount of current leaving the internal conductor through the node rapidly decreased. The catelectrotonus reached its maximal value when an anode was at a node. But further displacement of the cathode toward the center of the internode...
caused only an insignificant decrease of the height of the catelectrotonus because the position of the nodes or even the presence of nodes in the anelectrotonic zones changes very little the total amount of current which enters into the fiber. Indeed, the curve of the electrotonic potential labeled 1000 \( \mu \) in Figure 2a is hardly distinguishable from the curve that was obtained with the cathode in the same position, in the absence of nodes in the model.

The curves in Figure 2a show that the presence of low resistance nodes must cause apparent changes in the threshold of stimulation of the internodes. The threshold, as measured by the magnitude of current needed to establish a given height of catelectrotonus, would be highest when the cathode is at a node and lowest when the cathode is at 300 \( \mu \) from the node. From this point on, the threshold should be found to be practically constant throughout a 1400-\( \mu \)-long segment of internode.

When the resistance of the nodes is increased, the changes in the height of the catelectrotonus are reduced, and already when the resistance \( r_n \) of the nodal membrane is made 300 times smaller than the resistance \( r \) of the myelinated membrane, the maximal change in the height of the catelectrotonus amounts only to 4 or 5 per cent, and consequently the change becomes undetectable during threshold determinations.

The distributions of the anelectrotonus when the cathode was at \( x = 0 \) and \( x = 700 \mu \) are given in Figure 2b. It is clear that the height of the anelectrotonus is too small to produce an anodal block of conduction, unless the applied current were made much larger than the threshold current.

The curves in Figure 3 present the distribution of potentials \( V_a, V, \) and \( V_i \) that appear when currents are applied to a nerve fiber placed in a conducting medium by means of a distant anode and a microcathode (radius 10 or 50 \( \mu \)) in contact with the surface of the fiber. The nodal membrane is supposed to have the same resistance as the myelinated membrane. That curves \( V_a \) completely agree with the theoretical curve shows that the model had been properly built.

The curves of the membrane potential \( V \), the ordinates of which are proportional to the density of membrane current, have wide catelectrotonic zones with sharp maxima at the microelectrodes, and two broad anelectrotonic zones having their minima at 1.5 and 2 mm from the microcathodes. The membrane potential reverses its sign in one case at about 500 \( \mu \) and in the other at about 800 \( \mu \) from the center of the microcathode. It is clear that since the catelectrotonic zone is about 1000 to 1600 \( \mu \) wide, microcathodes can effect localized stimulation only when near threshold currents are being used. It should be noted that in the neighborhood of the microcathode the \( V \) curves parallel almost exactly the \( V_a \) curves. It should also be noted that the curves obtained with the microelectrode of 50 \( \mu \) radius would have been duplicated with unessential changes if the microelectrode of 10 \( \mu \) radius should have been placed in the conducting medium with its center at 50 \( \mu \) from the surface of the fiber.

The distribution of \( V \) is modified by the placement of low resistance nodes in the model much in the manner shown in Figure 2a. The catelectrotonus has its minimal value when the microcathode is at a node, but the catelectrotonus rapidly increases in magnitude when the microelectrode is moved toward the center of an internode. When the distance of the microcathode to a node is 500 \( \mu \), the effect of
the nodes upon the catelectrotonus becomes insignificant, and hardly an effect is measured when the microelectrode is at the center of a 2-mm-long internode.

Under conditions such as these it is clear that when a nerve fiber is being stimulated with a microcathode, the applied current can initiate an impulse only within the catelectrotonic zone.

In experiments on stimulation of isolated nerve fibers with microcathodes reported in the literature, effective stimulation was obtained when the microcathode was displaced in small steps throughout the entire length of an internode. Consequently, unless stimulation was produced at a distance from the microcathode by escape of current, the experimental results had only one possible interpretation. The microcathode had initiated impulses at points of the internodes. Moreover, since in Figure 12 of reference 4 the variations of threshold along the internode amount to less than 6 per cent, it should have been concluded that the stimulation threshold is practically constant throughout the internodes of undissected single fibers.

Finally, Figure 4 presents the distribution of the electrotonus in the extrapolar segment when rectangular pulses of current are applied to the nerve fiber through two electrodes at its surface. The resistance of the nodes is given with each curve.

Since the density of membrane current through the nodes and through the internodes, are respectively, \( i_m = \frac{V}{\rho_n} \) and \( i_m = \frac{V}{\rho} \), it is clear that in the cases of curves II to IV the density of membrane current through the nodal membranes was much higher than the density of membrane current through the internodes. The important fact, however, is that the presence of low resistance nodes resulted only in unessential modifications of the distribution of the electrotonus in the internodes, which are the excitable parts of the nerve fiber. Indeed, in the case of curve II the modification is so slight as to be practically undetectable.

An elementary but essentially correct argument will serve to explain why nodes of relatively very low resistance can have only a negligible effect upon the electrotonic spread of currents in the internodes. Let it be assumed that 1 cm² of nodal membrane has a resistance as small as 660 ohms, i.e., that \( \rho_n = \rho / 100 \). Since the length of the nodes is 0.5 \( \mu \), the effect of the nodes upon the electrotonus can be duplicated almost exactly by introducing in the model (Fig. 1b) at 2-mm distances five 10-\( \mu \)-long segments having a transverse resistance \( \rho / \Delta x \) and longitudinal resistances \( r_s = r_f = 0 \). The density of the membrane current will be uniform throughout the 50-\( \mu \)-wide node, but the flow of membrane current also is very nearly uniform through the juxtanodal 50-\( \mu \)-long segments of the internodes, because in a homogeneous core conductor having a characteristic length \( \lambda = 0.2 \) cm., \( V(x + 50 \mu) = 0.97V(x) \). Consequently, the effect that each node has upon the elec-
trotonus amounts practically only to making a homogeneous core conductor 50 µ longer, and since a length of 50 µ is only a small fraction of the characteristic length λ, the effect of nodes having a resistance ρ_n = ρ/100 is smaller than the limits of error in measurements done in a well-controlled experiment on nerve.

Moreover, it will be shown in a following communication that there is no real reason to believe that the nodal membrane has a significantly lower resistance than the myelinated membrane.

Experimental Results.—The results presented in Figure 5 can be briefly described. The current applied to the single fiber by means of tripolar electrodes initiated impulses at all the numerous, tested points of the internodes. Consequently, in view of the curves in Figure 2, it is clear that all points of the internodes produced action potentials in response to applied currents.

Usually, but not always, immediately after the nerve had been mounted in the chamber, variations of threshold were measured when the nerve was displaced through the stimulating gap (Fig. 5, I, IV, VI). The variations were not periodic and were undoubtedly referable to slight injuries done to the single fiber during the dissection of the nerve. They rapidly became insignificant (Fig. 5, II, VII) even when the nerve was being kept in a hypotonic solution (50 parts Ringer's solution, 50 parts distilled water, Fig. 5, VII). The slight increase in threshold that appears in Figure 5, II, with increasing distance from the end of the nerve was undoubtedly referable to the effects of the flow of the demarcation current.

A mild anesthetic (1.5 mM xylocaine, pH 7.3) rapidly acts upon, i.e., raises the threshold of stimulation of, all points of the internodes. The effect of the anesthetic becomes detectable within less than 1 min, it increases progressively with advancing time, but it becomes practically maximal within 6–8 min. A remarkable peculiarity of the action of the anesthetic is that the previously observed variations of threshold along the fiber reappear in an exaggerated form (Fig. 5, III, VIII). The probable explanation of this phenomenon is this. Recovery from the effects of injury is affected by local flows of demarcation current from the uninjured into the injured segments of the fiber. The anesthetic interferes with those electrochemical reactions.
which underlie the restoration of nerve fibers by the anodal current (cf. ref. 6, chap. XIII).

A certain periodicity of the variations of threshold appears in curve V (Fig. 5), the period corresponding approximately to internodal lengths. The question, however, must be left open whether such a periodicity was coincidental or was due to the fact that with the nerve fiber in a certain state the anesthetic acts more strongly upon certain parts of the internodes than upon other parts.

Summary.—Evidence has been presented to show that all points of the internodes of peripheral myelinated fibers produce action potentials in response to applied cathodal currents, the stimulation threshold being practically uniform throughout each internode. Anesthetics act upon all points of the internodes. Model experiments have shown that the nodes of Ranvier can have only a negligible effect on the electrotonic flow of action currents, or applied currents, throughout the myelinated internodes.

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1 Lorente de Nó, R., and V. Honrubia, these PROCEEDINGS, 52, 305 (1964).
3 Working with isolated nerve fibers, evidence has been obtained, to be presented elsewhere, that the nodes of Ranvier either do not produce an action potential, or produce an action potential which is too small to be detectable with present-day techniques.
6 Lorente de Nó, R., A Study of Nerve Physiology (Studies from the Rockefeller Institute, 1947), vols. 131, 132.

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**DURATION OF THE QUATERNARY AND ITS SUBDIVISIONS**

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Estimates of the length of the Quaternary vary tremendously, as based on widely divergent lines of evidence such as astronomical calculations, rates of weathering, rates of sediment accumulation, and isotope abundance. The model presented here is based on changes of level of land and sea.

The essential reason for recognizing the Quaternary is climatic.1 The record of its history is stratigraphic.2 By the time the earliest accumulation of continental ice attained sufficient volume to lower sea level appreciably, rivers began to incise all land surfaces along routes leading to the oceans. Sediment deposition was accelerated around continental margins and shifted to progressively lowering levels during the waxing of the first glaciation. There is certainly no merit in postulating the start of the Ice Age during pre-Quaternary time on biological evidence. The essential criterion is the time when Quaternary sea level began to lower.

At the initiation of the Quaternary, sea level approximated the stand that would