RESPONSE OF SINGLE RANVIER NODES TO ELECTRICAL STIMULI

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A VERTEBRATE motor nerve fiber is thickly covered with a layer of myelin sheath except at narrow gaps called nodes of Ranvier. As the myelin sheath is shown to be composed of an electric insulator, investigation of the physiological properties of these nodes seems to disclose the nature of the plasma membrane of an infinitesimal dimension and consequently to enable us better to understand the activity of the surface layer of a cell in general.

The present investigation is undertaken to secure a series of oscillograms which seemed to us to indicate the basic properties of the plasma membrane at the nodes. The temporal configuration of the electric responses and the latencies of the responses to rectangular current pulses are our main concern in this paper.

METHOD

The material and apparatus employed have been similar to those used previously by Tasaki and Takeuchi (8, 9). An artificially isolated motor nerve fiber of the toad was mounted on a platform made with two or three separate pieces of glass-plate and Ringer's fluid surrounding the fiber was divided into two or three independent pools. The gaps between the glass plates, or the bridge-insulators, served to insulate these pools from one another. In each pool of Ringer was immersed a non-polarizable electrode of Zn-$ZnSO_4$-Ringer type, which was connected with the stimulating circuit or with the input of the amplifier. Stimuli were as a rule rectangular current pulses generated by means of a Helmholtz pendulum and were delivered to the fiber at intervals not less than about 20 sec. The amplifier used was resistance-capacity coupled. As the RC products of the coupling circuits were between 0.5 and 1 sec., a rectangular current pulse not exceeding about 25 msec. suffered practically no recognizable deformation with this amplifier. The input resistance of the amplifier was about 0.1 megohm.

In all cases, it is shown experimentally that the size of the observed electric response of the nerve fiber varies proportionately as the input-resistance connected between the two lead-off electrodes. This obviously indicates that the strength of the current produced by the fiber remains constant no matter what the resistance of the current-detecting system is. The size of the electric responses of the fiber should therefore be presented, under these experimental conditions, not in terms of volts, but in *amperes*. Such strengths of current are readily obtained by dividing the observed potential differences between the two lead-off electrodes by the resistance connected between them.

RESULTS

Law of polar excitation. It is simple matter with the routine method of handling our preparations to demonstrate that an electric current flowing outwards through the plasma membrane at the node can throw the node into action. Figure 1 shows the set-up of such an experiment and an example of the results obtained.

A single nerve fiber, of which two nodes were exposed, was laid across a bridge-insulator. Of the two electrodes on both sides of the bridge-insulator,

one was led to the grid of the amplifier and the other constituted a terminal of the stimulating circuit. The other terminal of the stimulating circuit was grounded. In one of the pools, a concentrated narcotizing solution—in most cases a 0.2 per cent cocaine-Ringer solution—was introduced. With the narcotizing pool connected with the cathode of the stimulating circuit, it

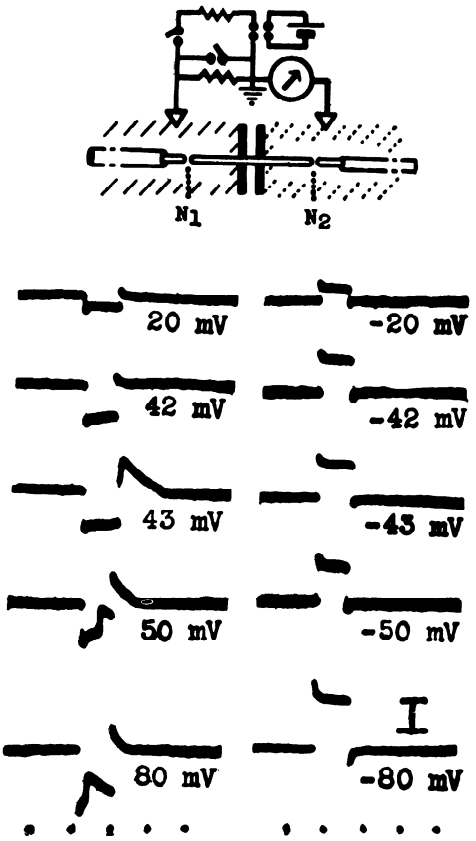

FIG. 1. Arrangements used for demonstration of the law of polar excitation in a single nerve fiber and an example of the records obtained. A battery, a current-reverser, two variable resistances and two break-contacts of a Helmholtz pendulum are diagrammatically shown on the top. Ranvier node N_1 is immersed in normal Ringer, while N_2 is in a 0.2 per cent cocaine-Ringer solution. Stimulating voltages given in the records are reckoned positive when the current tends to enter the narcotizing pool and leave the normal pool. Time msec. The bar subtends 2.5×10^{-9}A. 19°C.

was always observed that the cathode ray shows only a deflection of approximately rectangular shape representing the stimulating current itself. This deflection was naturally found to vary directly as the voltage applied to the fiber (see right column in Fig. 1). No break excitation was ever observed with this equipment for stimulating voltages not exceeding 10 times the rheobase.

With the normal pool connected with the cathode of the stimulating circuit, however, it was shown that action currents of all-or-none character were produced when the strength of the stimuli exceeded a certain critical value (left column in Fig. 1). The action currents thus evoked were of the configuration which we have designated as being "mononodal" (8). The

latency of the action current, as can clearly be seen in the figure, varied inversely as the applied voltage.

No action current from myelin-covered region of fiber. That no action current is produced in the myelin-covered region of the fiber can be demonstrated in various ways (9). The experiment, of which the arrangement and an example of the results are presented in Figure 2, furnishes another illustration for this statement. In this experiment, a single nerve fiber with two nodes exposed was laid across two sets of bridge-insulator. In the middle pool was introduced only the myelinated portion of the fiber, and in the two lateral pools the exposed nodes were immersed. The electrode dipped in the middle pool was connected both with the cathode of the stimulating circuit and

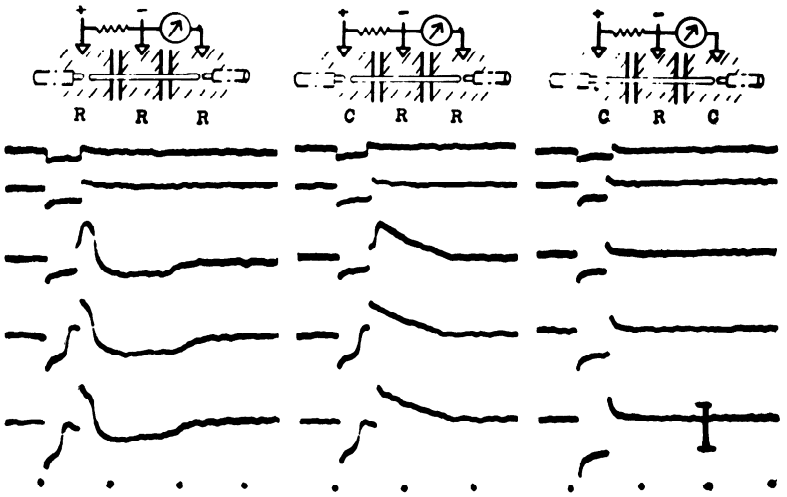

FIG. 2. Records showing the effect of rectangular current pulses applied to the myelinated region of a nerve fiber. Each column involves a series of records obtained under different experimental conditions. In the diagrams at the top, R refers to a normal Ringer and C to a 0.2 per cent cocaine-Ringer solution. Strengths of stimuli (in mV.); from the right top downwards, 25, 43, 50, 75, 105; middle, 25, 49, 75, 100; left, 25, 50, 75, 100, 150. The bar in the right corner subtends 2.5×10^{-9}A. Time msec. 19°C.

with the ground in the amplifier circuit. The two lateral electrodes were led to the anode of the stimulating apparatus and to the grid of the amplifier.

In the first series of observations (left column of Fig. 2), all the three pools were filled with fresh Ringer. It was observed in this case that, for current pulses above the threshold, action currents of the "dinodal" configuration (8) were brought out. Then a 0.2 per cent cocaine-Ringer solution was introduced into the lateral pool connected with the anode, and records were taken of the action currents evoked as before (middle column). The action currents observed in this case was "mononodal." The third series of observation (records in right column) was made after introduction of the narcotizing solution further into the remaining lateral pool, so that only the short myelin-covered portion of the fiber in the middle was left non-nar-

cotized. If the non-narcotized myelinated portion of the nerve fiber in the middle pool were capable of developing an action current, we should still be able to observe some deflection other than that having a rectangular configuration. But, within the limit of our observation, we were unable to recognize any component of the current which was not directly referable to the spread of the stimulating current along the fiber. Therefore, the distinct

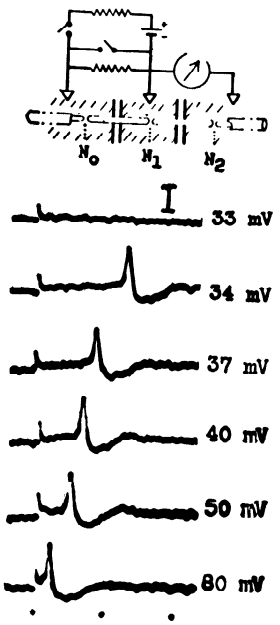

FIG. 3

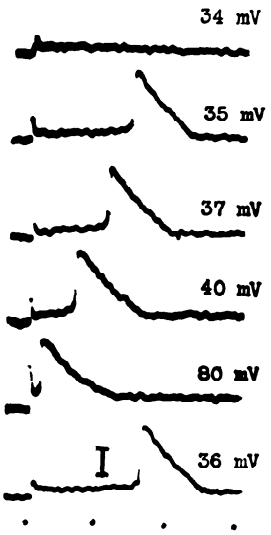

FIG. 4

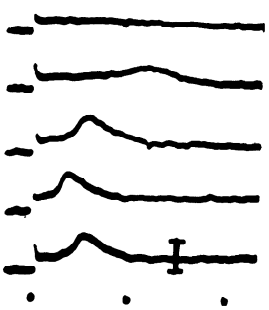

FIG. 5

FIG. 3. Tripolar arrangements used for recording the action current of a single nerve fiber and the records obtained. The bar subtends 10^{-9}A. Time msec. 22°C.

FIG. 4. Records of action currents developed by a single node of Ranvier. Experimental arrangements are the same as that shown in Fig. 3, top, except that the excitability of the nodes in the lateral pools (N_0, N_2 and others) are removed by narcosis. The bar subtends 10^{-9}A. Time msec. 21°C.

FIG. 5. Action currents developed by a single partially narcotized node. A 1.25 per cent urethane-Ringer solution was used as narcotic. Strengths of stimuli (mV.): from the top downwards, 75, 80, 85, 90 and 100. The bar subtends 2×10^{-9}A. Time msec. 22°C.

action currents observed before the narcotization of the lateral portions of the fiber must be regarded as being derived from the non-narcotized nodes of Ranvier.

Action current of a single node and strength-latency relation. When, in the experimental set-up described above, a node of Ranvier was introduced in the middle pool of Ringer, records which were entirely different from those in Figure 2 were obtained. Records presented in Figures 3 and 4 are examples of the results of such experiments obtained before and after introduction of the narcotic into the lateral pools.

In these experiments, the duration of the stimulating voltage was about

20 msec. and the threshold voltage for these rectangular current pulses could be regarded as the rheobasic. Attention should be called to the fact that the spread of the stimulating current to the amplifier is, under these experimental conditions, less marked than in the preceding experiments.

In the experiment shown in Figure 3 all the pools were filled with a fresh normal Ringer throughout, while in that of Figure 4 a 0.2 per cent cocaine-Ringer solution was filled in the two lateral pools and only the portion of the fiber in the middle pool was kept in a normal Ringer. The difference in the forms of the action currents recorded in these two cases is naturally attributable to the component of the current developed by the nodes in the lateral pools.

It is clearly seen in the records that the latency is shortened as the stimulating voltage is increased. With barely supra-rheobasic voltages, the action current was found to appear about 3 msec. after the onset of the rectangular voltage pulse (at about 20°C.). The latency, *i.e.*, the interval between the onset of the stimulus and the beginning of the action current, was in all the experiments shown to be inversely proportional to the voltage above the rheobase of the fiber. Denoting the latency by t, the stimulating voltage by v and the rheobase by b, the relation between these quantities was found to be described satisfactorily by the formula $t=kb/(v-b)$, or $v=b(k/t-1)$, where the constant k, having a dimension of time, can be called the chronaxie for the strength-latency curve. As the applied voltage approaches the rheobase, the latency increases correspondingly; but there is a definite limit which is comparable to the "Hauptnutzzeit" in the case of ordinary strength-duration relation. The value of the chronaxie k was found to coincide practically with that obtained by the ordinary method (7) and was about 0.4 msec. at 12°C. For very strong rectangular current pulses, it was difficult to determine the latency accurately, as the beginning of the action current is obscured due to the continuous nature of the rising phase of the action current and to a prominent artefact caused by the make of the current.

Partial excitation. In the preceding experiment, we have seen that a normal node of Ranvier develops the action current in practically all-or-none manner; *i.e.*, the action current evoked by a barely supra-rheobasic stimulus was of practically the same strength and form as those elicited by much stronger stimuli. In the non-myelinated nerve fiber, however, it is known that the action current is of varying sizes according to the strength of stimulus (4, 5, and others). And we noticed that a similar phenomenon can be demonstrated in a narcotized single node of the toad's motor nerve fiber.

The arrangement and the procedure of the experiment done to show this was the same as those in the preceding experiment. After having examined the action current of a single normal node, a dilute narcotizing solution (a 1.25 per cent urethane-Ringer solution in the experiment of Fig. 5) was introduced into the middle pool. (The two lateral pools had been filled as before with a much stronger narcotizing solution sufficient to abolish the excitability of the nodes.)

Before the introduction of the weak narcotic into the middle pool, the rheobase of the node was 28 mV.; a 1.25 per cent urethane solution raised the rheobase up to about 80 mV. For rectangular voltage pulses exceeding 90 mV. in this case, the size of the electric response did not seem to vary with the strength of the stimulus. But, with 85 and 80 mV., we could obtain several pictures showing action current of variable size and of variable form. A similar variability of the size and form of the action current was observed in a normal node in an early stage during the relatively refractory phase.

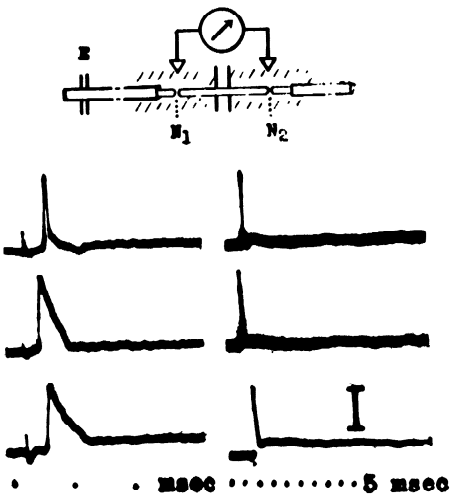

FIG. 6

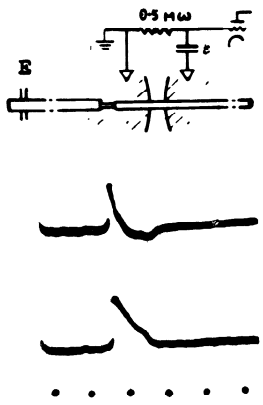

FIG. 7

FIG. 6. Records showing the effect of veratrine upon the action current of a single nerve fiber. Right and left column at different transit speeds. Top: both node N_1 and N_2 in a normal Ringer. Middle: N_1 normal, N_2 in a 0.2 per cent cocaine-Ringer solution. Bottom: N_1 in a 0.0001 per cent veratrine-Ringer solution, N_2 in the narcotic. The bar subtends 2×10^{-9}A. 21°C.

FIG. 7. The time course of the potential difference between two points on the nerve trunk induced by the action current from a single nerve fiber. C: condensor of 0.5 μF. E: platinum electrodes. The lower record was obtained after application of a 0.3 per cent cocaine-Ringer solution to the portion of the nerve in the distal pool. Time msec. 20°C.

Refractoriness, as well as narcosis, decreases the tenacity of the node with which production of the action current takes place.

Retardation of recovery process by veratrine. In the experiments described above, attention has been paid to the first predominant portion of the action current, namely, to the spike. We notice, however, that in the action current of a single node there is, following the spike, a phase during which a weak but long-lasting current is observed. Veratrine, which is known to augment the after-potential in the nerve trunk (2, 3), was found to be extremely effective to increase (or to induce) a long-lasting, inward-directed current through the plasma membrane of a recovering node. The records furnished in Figure 6 are an example of the results showing the effect of veratrine upon the action current of a single nerve fiber. In this experiment, a nerve fiber was laid

across a bridge-insulator, and the fiber was brought into action by means of an induction coil connected to the portion of the fiber existing within the nerve trunk. While at first the two pools were filled with fresh, normal Ringer, records were taken of the normal "dinodal" action current at two different transit speeds. Then the fluid in the distal pool (right pool in Fig. 6) was replaced with a 0.2 per cent cocaine-Ringer solution and, by this procedure, the action current was made "mononodal."

Finally the fluid in the proximal pool was replaced with a 0.0001 per cent veratrine-Ringer solution, and the action current records were taken from time to time at intervals of about 5 minutes until at last the size of the action current was reduced suddenly to about 0.25 the original value. The lowest records in Figure 6 were taken about 40 minutes after application of the drug. When examined 5 minutes later the spike was found to be very low; this indicates that the proximal veratrine-poisoned node in the immediate neighborhood of the bridge-insulator had lost the ability to respond during this interval. The strength of the current observed after the end of the spike can reach, under these experimental conditions, about 6×10^{-10} ampere (about one-quarter the normal spike-height) or more. We have seen previously that the action current developed by a node is strong enough to excite the neighboring node even when its spike-height is reduced by the action of narcosis to about one-sixth normal (8). As the normal spike-height is generally $2\text{–}2.5 \times 10^{-9}$ ampere at 20°C., it is evident that the above stated "after-current" acts upon the neighboring node as a stimulus of supra-rheobasic strength when the latter node is kept in a normal Ringer. In a personal communication Sugi has stated that, in a veratrine-poisoned nerve fiber, a single induction shock may cause repetitive responses. In fact, we also have encountered several cases in which a repetition had actually taken place in isolated single nerve fibers. We may conclude that such a repetition is effected through stimulation by the after-current.

Action potential of the nerve trunk. The time course of the action current of a single nerve fiber presented in the preceding pages is apparently different to a considerable extent from those recorded by previous investigators (*e.g.*, 1). The question now arises as to how this difference is to be explained. When a nerve fiber existing within a nerve trunk is thrown into action by some means, the current produced by the fiber traverses through the surrounding inactive fibers and through the conducting medium in the nerve trunk. This flow of current naturally causes, according to Ohm's law, differences in potential on the surface of the nerve trunk, and the ordinary method of recording nerve action potentials consists in leading off these potential differences by means of a potentiometer with an infinite internal resistance. It should therefore be possible, on the basis of our knowledge of action current of each constituent nerve fiber, to map out the temporal configuration of the action potential of the nerve trunk (6, 9).

The simplest way of testing the validity of our argument would probably lie in leading off the action potential of a single nerve fiber from two points

on the nerve's surface distant from each other not more than the internodal distance. If there were no node of Ranvier lying in the region between the two lead-off electrodes, the potential difference between these two points should be of the same temporal configuration as that of the "dinodal" action current and its magnitude should be equal to the product of the action current of the single nerve fiber and the resistance between these two lead-off electrodes. The records of Figure 7 are those obtained in an experiment done to test our inference in this respect.

Single motor nerve fibers innervating the sartorius or semitendinosus muscle of the toad were selected for the experiment. The slender intact portion of the nerve fiber preparation was laid across a bridge-insulator. The portion of the nerve suspended in the air gap between the two pools of Ringer was about 0.5 mm. in length and between 0.1 and 0.2 mm. in diameter. The fiber was brought in action by induction shocks applied to the fiber near its proximal stump. The electrode immersed in the proximal pool was grounded and the distal electrode was led directly (or at times through a condenser) to the grid of the amplifier. Between these two electrodes a known resistance (0.5 megohm or infinite) was connected. Prior to recording the action potential of the nerve trunk, the ohmic resistance of the stretch of the nerve trunk between the two pools was determined by inserting this system in a Wheatstone bridge, the amplifier and the cathode-ray tube serving as a current detector. As the nerve shows a slightly capacitive character, it was not feasible to determine the resistance very accurately, and an ambiguity of about 10 per cent seemed to be unavoidable.

As can clearly be seen in the figure, action potentials recorded under these experimental conditions bear in most cases a striking resemblance to the "binodal" and "mononodal" action currents of a single nerve fiber. The magnitude of the observed action potential was actually found to be proportional to the resistance of the interpolar stretch of the nerve trunk; it was regularly of the magnitude given by the product of 2×10^{-9} ampere and the resistance between the two lead-off electrodes.

Discussion

The methods of "bridge-insulator" consists in desiccating the internodal myelinated region of the nerve fiber by suspending it in the air. It is indispensable for us to rule out the possibility that the desiccation might bring about some electrical or physiological changes in the nerve fiber.

The fact that no progressive change occurs in the threshold and action current during the course of the desiccation seems to attest that there is no such possibility. Furthermore, as the production of the action potential in an intact nerve trunk can fully be accounted for on the basis of the results secured by the use of the bridge-insulator method, we are convinced that the desiccation of only the myelinated region brings about no serious change in the nerve fiber.

SUMMARY

With a view to visualizing the physiological properties of the myelinated nerve fiber, oscillograms were taken of action currents of toad's single nerve fibers under varying experimental conditions.

1. Records were taken showing the law of polar excitation in the isolated region of a single nerve fiber (Fig. 1).

2. It was shown that the action current of a nerve fiber derives from the Ranvier nodes and not from the myelin-covered region of the fiber (Fig. 2).

3. Using tripolar arrangements, action currents resulting from the responses of a single Ranvier node were recorded (Fig. 4). The relation between the latency and the strength of the rectangular current pulse was examined on a single Ranvier node (Figs. 3 and 4).

4. It was observed that a weakly narcotized node develops action currents of variable form and size when it is stimulated by current pulses of rheobasic strength (Fig. 5).

5. Veratrine prolongs the descending phase of the action current and makes a node produce a long-lasting current which corresponds to the negative after-potential in the ordinary recording (Fig. 6). Repetition caused by application of this drug to a nerve fiber is interpreted as being due to restimulation by this long-lasting current.

6. Records were taken of the difference of potential at two points on a nerve trunk when a single nerve fiber in it was thrown into action (Fig. 7). It was shown that, if the inter-lead distance is less than the internodal distance, the recorded action potential resembles in its configuration the action current of a single nerve fiber as recorded by the method of bridge-insulator. The magnitude of the action potential was given by the product of the interpolar resistance and the action current of a single nerve fiber.

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