TASAKI

NERVOUS TRANSMISSION

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By

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PREFACE

In the summer of 1933, when I started my research work on the nerve and muscle, as an undergraduate of the Medical Faculty, it was certainly a great horror for the assistants of the Physiological Institute, Keio University, to be given a suggestion by the director to work on isolated single nerve fibers. The technique of dissection was then rapidly improved by the effort of Dr. Z. Kaku, an ingenious Korean physiologist and surgeon, to whom I am deeply indebted for instruction in his technique. My interest at that time was concentrated mainly on the physico-mathematical aspects of the action of electric currents and narcotics upon the nerve fiber.

Much influenced by the work of Prof. G. Kato and Dr. Hallowell Davis, who with their collaborators advocated the existence of a non-decremental, uniform spread of impulse in a narcotized nerve, I approached the problem of nervous transmission by asking the question: "How is transmission along a single nerve fiber blocked by narcosis?" In 1935, I was convinced that narcotics act with extreme rapidity upon the nodes of Ranvier but not on the myelin-covered portion of the fiber. At the same time, I was very much puzzled by the fact that the rate of transmission could be reduced promptly down to 50 per cent of the normal value or still less by an application of a narcotizing solution of adequate concentration upon a single nerve fiber. Why can such a pronounced slowing of transmission velocity occur if the narcotic acts only at the nodes of Ranvier which occupy a length of about 0.02 per cent of the total length of the nerve fiber? This question remained unanswered until the end of 1938. Nevertheless, this series of work, done with an old, rusty Helmholtz pendulum combined with a pair of fine dissecting needles and published only in the Japanese language, has given me the title Doctor of Medicine.

Thus, all the experimental results described in this book have been obtained in Japan, an island next to the one where Robinson Crusoe had been secluded. The unfortunate warfare had made our cultural isolation from the rest of the world virtually com-

plete until the summer of 1948, when Dr. Davis sent me a set of the *Annual Review of Physiology* and his reprints, for which my friends in Japan and I are very grateful. The fact that the manuscript of this book was written in a place where no foreign physiological journals were yet available can probably be a partial excuse if I have omitted some of the important literature in this field of physiology.

The problem of "subthreshold responses" has become clear to me only after I have been given, by the generous Rockefeller Foundation and the kindness of Prof. A. von Muralt in Bern, a chance to discuss the matter with Mr. A. L. Hodgkin and Mr. A. F. Huxley in Cambridge. I am deeply indepted to these English researchers for their valuable advice and criticism. I am now fully convinced that the non-linear phenomenon which they discovered on the squid giant axon (Hodgkin, Huxley and Katz; *Arch. Sc. Physiol.*, 3:129, 1949) supplies us with a basis for a deeper insight into the problem before us. For the analysis of the latency and the rate of transmission at low temperature, the consideration of the non-linear ionic currents is undoubtedly essential.

Nevertheless, the content of this book is not more than a systematic presentation of a number of action current records taken under various experimental conditions. Although the preparations I used were taken mainly from Japanese toads, it would be no use doubting that, under similar experimental conditions, preparations from European and American animals behave in a similar manner. Nerve physiology never stops its steady progress, and old theories are very frequently replaced by new ones. But the experimental facts upon which the theories are built are certainly unalterable.

Publication of this book has been made possible by the kindness of Dr. R. W. Gerard and Mr. Charles C Thomas. It is a great pleasure to me to acknowledge my sincere gratitude to them.

ICHIJI TASAKI

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CHAPTER I

THE ACTION CURRENT

1. THE NERVE AND THE NERVE FIBER

When a brief pulse of an electric current is sent through one of the limb muscles of a vertebrate, for instance through the gastrocnemius of the frog, there generally ensues in the muscle a brisk contraction followed by an immediate relaxation. A similar contraction, or a twitch, of the muscle can also be evoked when the current is applied to the nerve entering the muscle, instead of applying it directly to the muscle (Fig. 1).

Although the nerve is a conductor of electricity, there is good reason to believe that the twitch brought about by application of an electric current to the nerve is not due to direct spread of electricity along the nerve to the muscle. If, for instance, the nerve is crushed with forceps or treated with a dilute cocaine solution beforehand, induction currents delivered to the nerve fail to evoke twitches in the muscle. As these procedures do not seem to utterly prevent the direct spread of electricity along the nerve, it is certain that some process which has not ordinarily been treated in the theory of electricity is brought into play in the nerve when the effect of an electric current reaches the muscle. This process is called the *nervous transmission,* without any implication as to its physico-chemical nature.

A histological exammation reveals that the nerve comprises a large number of small fibers, known as the *nerve fibers,* bound with a layer of connective tissue. A nerve fiber consists of a long thread of protoplasm, called the *axis-cylinder,* enclosed in a tubing of a complex fatty substance, i.e., in the *myelin sheath,* and further covered with a very thin layer of cells like connective tissue, namely with the sheath of Schwann. The axis-cylinder runs an uninterrupted course between the central nervous system and the periphery. It is also well known that the myelin sheath is not continuous but is broken in its course at an approximately regular interval of a few millimeters. At these breaks of the myelin

sheath, which are called the *nodes of Ranvier* (Fig. 2), the sheath of Schwann is known to cover the nerve fiber continuously, sticking closely to the surface of the axis-cylinder (de Rényi, 1929).

It is an established fact that the nerve fiber is the ultimate functional unit which performs the nervous transmission. It is easy to demonstrate that transmission does occur even after all the fibers

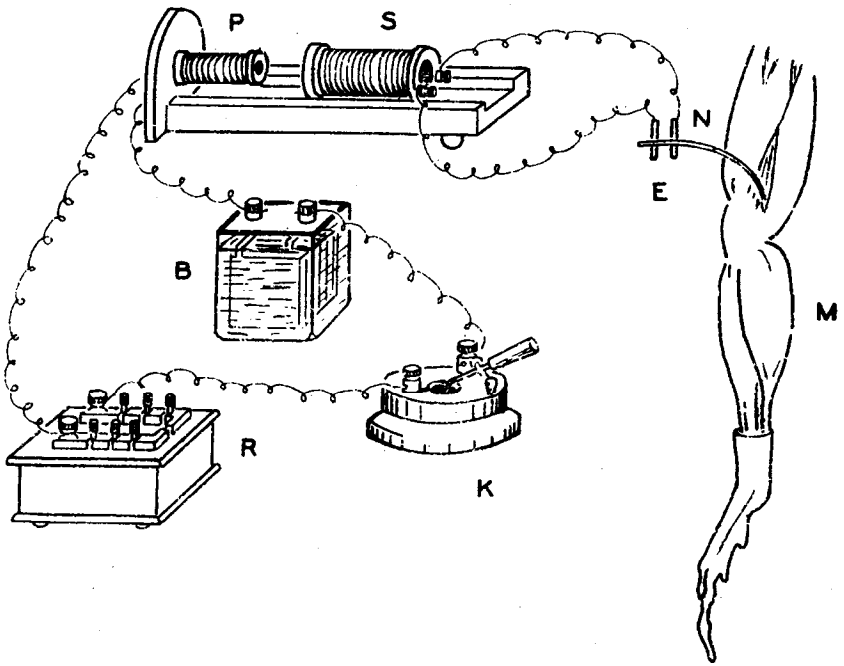

Fig. 1. Diagram of experimental set up to evoke a twitch in frog's gastrocnemius muscle by application of an induction shock to the sciatic nerve. *M*, gastrocnemius mucle; *N*, static nerve; *E*, a pan of platinum electrodes; *B*, battery; *K*, key for closing and opening the circuit, *R*, variable resistance. *P* & *S*, primary and secondary coil of an inductorium respectively.

except just one are cut across by a micro-operation, but never occurs after the remaining one fiber is also severed. The twitch of the muscle evoked through an intact nerve is in general much more powerful than that evoked through a single nerve fiber, but this is ascribed simply to the difference in the number of units acting in the transmission. It is therefore expected that, in the investigation of the process of nervous transmission, the use of

single nerve fibers, in place of whole nerve trunks, makes the experiments much simpler and more decisive.

The first successful attempt to reduce operatively the number of active nerve fibers in a nerve was made by Adrian and Bronk (1928). The technique was greatly improved by Shimizu, Kaku, Tasaki and others working under Kato (1934), and recently by von Muralt (1945) and Stämpfli (1946). The operation is now

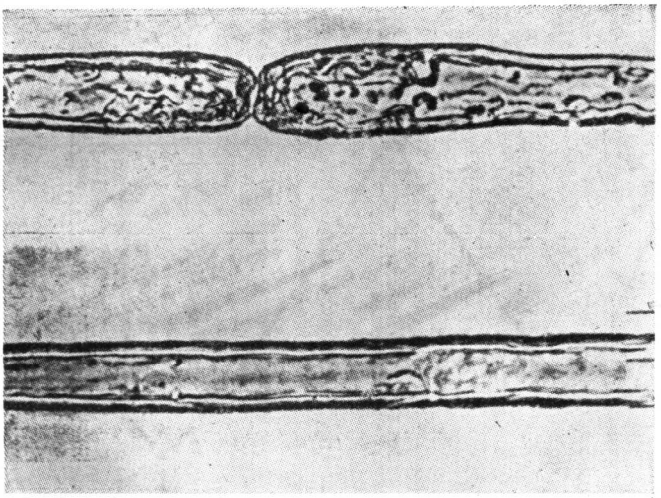

Fig. 2. Micro-photographs of a large motor nerve fiber of the cat. A node of Ranvier is shown in the upper picture. The scale on the right indicates the intervals of 10 microns. Dr. J. Nakai of the Department of Anatomy, Tokyo University, suggests that the pattern on the myelin sheath near the node in this picture might be due to the inadequacy of the ringer solution employed.

relatively easy to conduct and one can learn the technique of isolating exactly one fiber through two or three days' exercise.

Most of the experiments described in this book were carried out on single nerve fibers obtained by this technique.

2. ELECTRIC EXCITATION OF AN ISOLATED SINGLE NERVE FIBER

We shall proceed in this section to examine how or where an electric current acts upon the nerve fiber, if it is to initiate a transmission. For applying electric currents to an isolated single

nerve fiber floating in a shallow pool of ringer fluid on a glass plate, the use of a so-called micro-electrode seemed at outset indispensable. Let us now take an experiment which served as the first step in our investigation into the mechanism of nerve excitation and transmission.

In this experiment, as in most of the experiments which will be stated on the following pages, large motor nerve fibers entering into the gastrocnemius muscle of the toad were used. With such

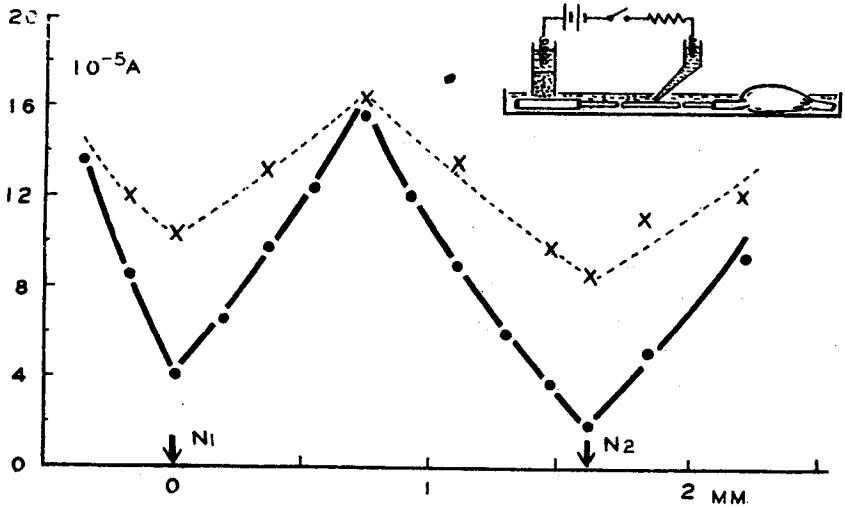

Fig. 3. Threshold strengths of long stimulating currents (in amperes) plotted against distances from a node of Ranvier N_1 of the fiber towards the neighboring node N_2 (in millimeters). Black circles show the results obtained with the cathode of the battery connected to the micro-electrode, and the crosses with currents flowing in the opposite direction. Toad's motor nerve fiber at 23°C.

preparations, one can readily observe by eye, on application of an electric current to the fiber, a distinct twitch in the muscle.

To apply electric currents to the nerve fiber, one small and one large electrode were used in this experiment (Fig. 3). The small electrode which was brought near the fiber was composed of a glass tubing of about 5 millimeters bore drawn to a point of about 0.1 millimeter in diameter. The other large electrode, which was kept far from the fiber, had an orifice of about 10 millimeters in diameter. The small electrode was kept in contact with

the fiber by means of a micro-manipulator. Both of these electrodes were non-polarizable, being of the Ag-AgCl- or Zn-$ZnSO_4$-ringer type.

If one starts, at a definite position of the electrode, with a very weak current pulse and increases its strength step by step, one finds that the muscle responds to the current pulse with a twitch only when the strength of the currens is above a certain critical value. One finds further that this critical strength of the current, or the *threshold strength*, varies remarkably as the small electrode is shifted along the fiber. The threshold depends also on the direction and the duration of the applied current pulse.

In the example of experiment furnished in Figure 3, the duration of the current pulse was about 0.5 second. The threshold strengths were determined at a number of points along the fiber. When the battery was so connected to the electrodes that the small electrode acted as the *sink* of current in the pool of ringer fluid, threshold determinations could be made with fair accuracy. It is seen that the threshold is lowest when the small electrode is placed on one of the nodes of Ranvier and is highest when it is attached to the fiber at a point half way between two neighboring nodes. At a given position of the electrode, the threshold is higher with the source of the current placed on the fiber than with the sink of the current kept in contact with the fiber.

These experimental facts, first demonstrated by Kubo and Ono (1934), undoubtedly bring to light the significance of the myelin sheath and the nodes of Ranvier in electric excitation of the nerve fiber. Analytical treatment of these data indicated very clearly that we can interpret all these results on the assumption that the myelin sheath is an electric insulator and consequently the electric current enters and leaves the fiber only through the nodes of Ranvier (Tasaki, cf. Kato's reviews published in 1934 and 1936).

A source and a sink of electricity placed in a pool of ringer fluid cause, according to the theory of electricity, a definite distribution of current in the fluid. This in turn means that a field of potential is generated in the fluid, and the equipotential surfaces are considered to be disturbed only slightly by the presence of the thin fiber in the fluid. The difference in the potential, to which two neighboring nodes of the nerve fiber are subjected,

should then generate an electric current, according to Ohm's law, which actually flows through the fiber. And, this current which enters and leaves the fiber, and not the current flowing through the surrounding medium, is actually found to be effective in initiating a process which precedes production of a twitch in the muscle.

Although this experiment illustrates very clearly the importance of the nodes of Ranvier in electric excitation of the nerve fiber, the field of potential generated by a micro-electrode in the fluid is of somewhat complicated pattern. To simplify the electric field acting on the fiber, the techniques which will be stated in the subsequent sections have been devised.

3. ELECTRIC CURRENTS DEVELOPED BY THE NERVE FIBER

Let us now introduce the operated region of a single nerve fiber preparation into a narrow groove filled with ringer fluid as shown in Figure 4, left top. If, with this arrangement, an electric current is sent through the fluid by means of a pair of electrodes dipped in the two pools on both sides, it is expected, in accordance with the theory of electricity, that the electric potential along the fiber under investigation varies appreciably only in the region of the groove. Since there is practically no potential difference along the nerve in the large pools, it is obvious from what has been stated in the preceding section that this procedure is effective in eliciting a response only from the fiber in the groove.

The strength of the current through the fluid in the groove increases, according to Ohm's law, in proportion to the voltage applied between the electrodes. If the applied voltage has a rectangular configuration, i.e., if a constant voltage is started suddenly at a definite moment and is withdrawn after a certain amount of time, the configuration of the current through the fluid is correspondingly rectangular. This can readily be demonstrated by means of cathode ray oscillograph used in conjunction with a vacuum tuble amplifier.

When the groove is made narrower, the resistance of the fluid in the groove, which is practically equal to the resistance between the two electrodes, becomes greater. This can very readily be shown by inserting the system, as shown by the diagram in Figure 4, in one of the arms of a Wheatstone bridge.

If the system composed of ringer fluid with a nerve fiber in it behaves in accordance with Ohm's law, it is expected that, after the bridge was once balanced very accurately, an increase in the voltage applied to the bridge would never result in bridge unbalance. And that this is actually the case can be demonstrated when the voltage between the electrodes is below about 20 millivolts.

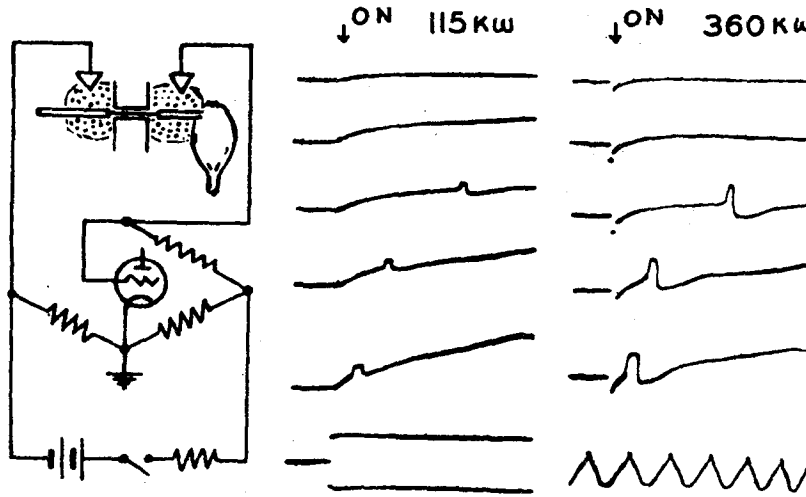

Fig. 4. Diagram of an arrangement to record electric currents developed by the nerve fiber (left) and an example of the results obtained by this method (right). The voltage between the two electrodes, generated by the bridge current starting at the moment marked by the arrows, were as follows: From right top downwards, 30, 47, 49, 60 and 80 mV; from middle top downwards, 20, 48, 50, 60 and 80 mV. Time marker shows the interval of 1 msec., and the record at the bottom in the middle column indicates the responses of the voltage recording system to rectangular pulses of ± 0.3 mV. The slight continuous bridge-unbalance during the passage of current is apparently due to polarization of the electrodes. Temperature 18°C.

In the experiment of Figure 4, the contact K in the battery circuit was closed by means of a thyratron tube at the moment marked by the arrows. The series of records on the right column was obtained when the groove filled with ringer fluid was approximately 1 millimeter long and 0.1 millimeter wide. Its depth was slightly less than 0.1 millimeter and a glass cover was placed on the groove. The bridge was accurately balanced at a low

voltage and the resistance between the electrodes was found to be about 3.6×10^5 ohms. The applied voltage was increased step by step until it reached about 80 millivolts.

When the voltage between the two electrodes rose to 48 millivolts in this case, the bridge went off balance at approximately 3 milliseconds after the onset of the voltage. The time interval from the onset of the voltage to the bridge unbalance is further shown to decrease with increasing voltage. It is of great significance to note that when, and only when, there occurs bridge unbalance there is a twitch in the muscle into which the motor nerve fiber under investigation enters.

The manner in which the bridge unbalance takes place in this experiment would easily be accounted for either if the nerve fiber is assumed to produce an electric current of a definite temporal configuration in response to the applied voltage or if the electric resistance of the fiber is assumed to change in that case. Judging from the sign of the potential difference which constitutes the bridge unbalance, the change in the resistance in the second assumption should be such that the resistance would increase during the first, predominant phase of the bridge unbalance. Since, however, the presence of the very fine nerve fiber (about 10 microns in diameter) in the fluid in the groove has practically no measurable effect upon the resistance between the electrodes, the second possibility stated above is excluded and we are left with the first explanation that the nerve fiber generates an electric current in response to an applied voltage pulse.

The electric current produced by the nerve fiber, which we call the *action current*, is of a strength approximately independent of the voltage applied to the fiber to induce it. This can be seen very clearly in the oscillograph records in the figure.

We will now turn to the effect of the electric resistance of the fluid in the groove upon the strength of the action current. The oscillograph records in the middle column of the figure were obtained after the width of the groove was broadened slightly. The resistance between the two electrodes was decreased by this procedure to approximately one third of the previous value. Action currents were found to be evoked again with voltages above a definite critical, or threshold, value. And, this threshold voltage was practically equal to that observed before. But, the magni-

tude of the observed potential variation resulting from the action current had now decreased to one third of the value found before. This observation indicates very clearly that the observed potential variation, or the *action potential*, varies within a certain limit directly as the resistance of the fluid medium in which the nerve fiber is immersed.

According to the principle of superposition in the theory of electricity, the potential difference recorded by the amplifier system in the figure should be given by the algebraic sum of the effect of the action current and that of the current arising from the battery in the figure. The experimental fact, therefore, that the observed action potential varies directly as the resistance of the fluid medium indicates that the current produced by the nerve fiber in action is practically independent of the resistance of the medium. And, this property of the action current can easily be accounted for under the assumption that the action current is produced by an electro-motive force existing within the nerve fiber and it has to flow through a very high resistance before it reaches the fluid medium.

If the ringer fluid in the groove in Figure 4 is replaced with vaseline, or with air, it is found that the resistance between the two electrodes increases readily up to several tens of megohms. Under such circumstances, the observed action potential can be 10 millivolts or more; but in that case the observed potential does not increase directly as the resistance of the electrode system. Furthermore, the observed action potential suffers a considerable deformation under these conditions. Our potential recording system does not follow a rapid variation in the electro-motive force to which such a high resistance is connected.*

4. POLAR EXCITATION

It has been shown possible to imbed a short myelinated region of a nerve fiber in liquid paraffin, or even to expose it to air, with-

* It is practically impossible to reduce the capacity of the short lead wire connected with the pool of fluid on the glass plate to a value below 10^{-11} farad. If the electromotive force within the nerve fiber is to charge up this system through a series resistance of the order of 5×10^{7} ohms, a time lag of the order of 5×10^{-4} second is unavoidable in the record of potential taken through the lead wire. This difficulty can partly be overcome by using an amplifier of the cathode-follower type in a proper way.