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Title of Study: THE NERVE IMPULSE

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Major Field: Natural Science

Scope of Study: This report has been undertaken as a comprehensive study of the physiology of the nerve impulse. However, to fully understand the physiology of the impulse, it is necessary to understand the physiochemical characteristics of the neuron in a resting state. Therefore, a chapter has been included dealing with the subject of a resting neuron. The physiological aspect of the nerve impulse is discussed with regard to three manifestations of an impulse: (1) electrical manifestations of an impulse; (2) chemical manifestations of an impulse; and (3) thermal manifestations of an impulse. A brief over-all discussion of the proposed theories of the mechanism of the nerve impulse is included in the final chapter of the report.

Findings of the Study: There is much literature available dealing with the neuron in books, research articles, and journals. Throughout the literature one finds rather close agreement on the discussions of the physiochemical properties of the resting neuron and the manifestations of an impulse. What has been written is generally based on sound experimental findings and has been substantiated by subsequent findings. However, when the subject of the nerve mechanism is reached, there is a wide divergence of opinions. This is a wide open field for experimentors. At the present time, the 'sodium hypothesis' is most widely accepted as the framework within which there are many proposed theories for the actual mechanism of operation. It will probably be several years before this question of the human body, i.e., by what mechanism does the neuron transmit an impulse, will be answered. Nevertheless, there is a great deal of research and experimentation taking place at the present trying to uncover the answer.

ADVISER'S APPROVAL L. Herbert Bruneau

THE NERVE IMPULSE

By

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THE NERVE IMPULSE

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CHAPTER I

THE NEURON AND ITS GENERAL PROPERTIES

Bernhard Katz states "The life of an organism, like that of an organized society, depends upon its system of communication." The healthy human body is a remarkable example of an integrated fascinating system which is extremely complex and about which much remains a mystery. In spite of the areas of uncertainty, scientists working together have acquired a good deal of knowledge about the mechanism of communication within an organism.

The structural unit of the nervous system is the nerve cell or the neuron. The functional role of the neuron is the nerve impulse. For the most part, this paper will be limited to the properties of a generalized neuron, the characteristics and mechanism of the nerve impulse, and a brief discussion of the theories which have been proposed to explain the excitable properties of the neuron membrane.

To understand the physiology of the nerve impulse one must first have an understanding of the physical structure of the resting neuron. When one uses the adjective 'resting' to describe a cell, it simply means that the cell is resting from its functional activity. In this case it implies that the neuron is not transmitting an impulse. Nevertheless, it is continually converting one form of energy to another and producing heat even when no signal is being picked up or transmitted.

Generalized Structure

The neuron is a very irregular cell in shape. Much of this irregularity is due to the extensions from the cell body or soma. There are two generalized forms of extensions. The axon arises from a cone termed the axon hillock on the main cell body and in some unknown way grows toward its proper peripheral station to make contact with muscle, skin, organ, or some other nerve fiber. In man, the adult axon may be several feet long, although it is less than .001 inch thick. Radiating from the soma are the second kind of extension , the dendrites. These extend about one millimeter and then break up into fine terminal branches.

In vertebrates, all axons except the smallest are surrounded by a sheath of fatty material known as myelin. At intervals of about a millimeter the sheath is interrupted by short gaps known as the nodes of Ranvier. This sheath improves the signaling efficiency of the peripheral nerve fibers. This is illustrated by the fact that in nonmylinated fibers the speed of nerve transmission usually is in the range of a few meters per second while in the thick myelinated fibers the speed is around 100 meters per second. It is believed that the electric signal is regenerated at the nodes of Ranvier. The increase in speed of conduction in myelinated fibers is due to saltatory conduction. This refers to the impulse hopping from one node of Ranvier to the next.

In lower animals, as well as a few axons in the vertebrates, the axon is unmyelinated. The fiber consists of a cylinder of protoplasm separated from the external medium by a membrane which is about 100 Å thick. A similar membrane is found at the nodes of Ranvier.

Within the soma the following structures are found: mitachondria, the Golgi apparatus, RNA granules, and the nucleus. The RNA particles found in the cytoplasm are observed as flakes which are termed Nissl substance. As the Nissl substance is found extending into the dendrites while it is absent from the axons, this substance provides a method for distinguishing between the extensions. Katz points out that the nucleus acts as a lifelong center of repair and brings its influence to bear upon the distant parts of the axon but the mechanism by which this is accomplished remains a mystery. Neurofibrils run from the axon into the soma.

The generalized motor neuron as depicted by Katz is drawn and labeled on the following page, portion a. Portion b of the illustration is an enlarged section of the axon showing the relationship of the myelin sheath to the axon. The Schwann cells are believed to form the myelin sheath by wrapping tightly around the axon during development.

Structurally one can have many kinds of neurons; hence there are many systems of classification. Some of these systems are based on such variables as 1) relative lengths of axons and dendrites, 2) the presence or absence of a myelin sheath, 3) the location of the cell body, and 4) the function of the neuron. Perhaps the most widely used system of classification is that method which is based on the function of the neuron. Using this method, there are three categories of neurons: (1) afferent or sensory nerve fibers which conduct signals toward the central nervous system, (2) efferent or motor nerve fibers which conduct signals away from the central nervous system, and (3) association fibers which are those nerve fibers located within the central nervous system.

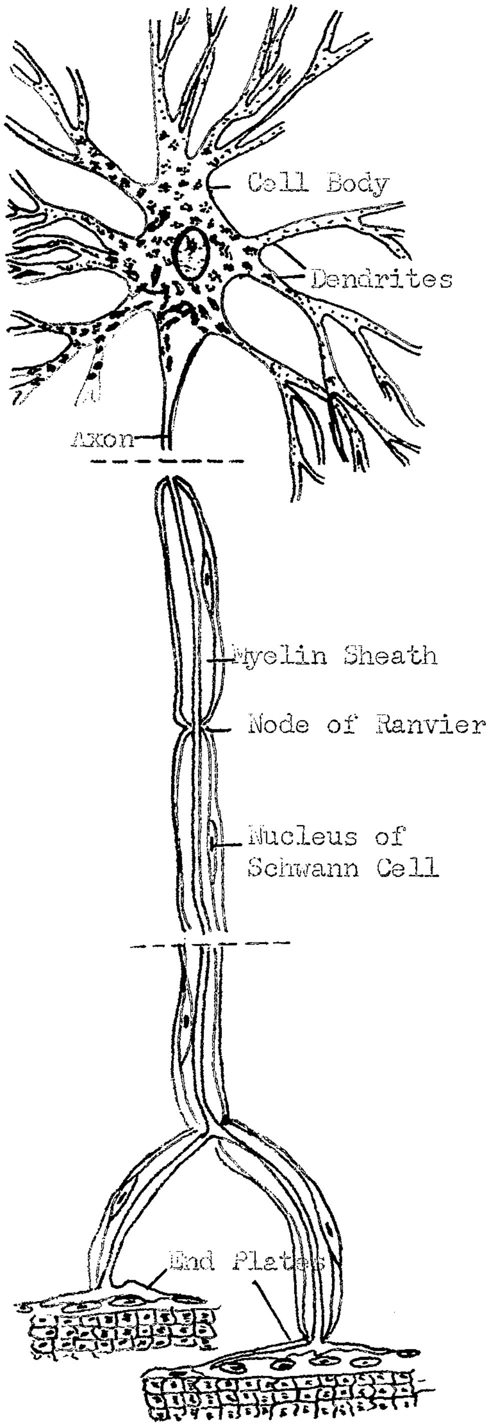

Fig. 1 The Generalized Motor Neuron.

One generally thinks of a dendrite conducting signals toward the soma while the axon conducts signals away from the soma. Bodian suggests because there are so many exceptions to this system it would be better to minimize the cell body, which may actually be located elsewhere, and say that dendrites conduct impulses toward the axon.

Electrochemical Properties of the Neuron

Dr. G. H. Bishop states "All cells are polarized as far as I know; I have not heard of any that are not and all cells have a polarizable membrane with the outside positive." This fact makes it necessary to examine the membrane of the resting neuron in order to attach any significance to changes which may occur during the active period. This steady potential is generally referred to as the resting potential to distinguish it from the potential which is observed during the transmission of an impulse.

The nerve membrane, as Katz aptly describes it, is a fascinating object; it is probably the most delicate and most important part of the nerve cell, and the one most intimately connected with the transmission of signals along the fiber. The reasons for this importance being attached to the nerve membrane can be illustrated in part by the following table. Table I, taken from The Conduction of a Nerve Impulse by A. L. Hodgkin, gives the concentrations of ions and other substances in freshly isolated axons of Loligo as compared with blood and sea water. While these figures do not hold exactly for all nerve fibers, the generalized picture remains the same.

TABLE I

COMPARISON OF ION CONCENTRATION

Substance	Concentration (mmole/kg H_2O)		
	Axoplasm	Blood	Sea Water
K	400	20	10
Na	50	440	460
Cl	40-150	560	540
Ca	0.4	10	10
Mg	10	54	53
Isethionate	250	-	-
Aspartate	75	-	-
Glutamate	12	-	-
Succinate + Fumarate	17	-	-
Orthophosphate	2.5-9	-	-
ATP	0.7-1.7	-	-
Arginine Phosphate	1.8-5.7	-	-
Water	865 g/kg	870 g/kg	966 g/kg

In this discussion we are mainly interested in the concentration differences of potassium ions, sodium ions, chloride ions and the organic anions. It will be noted that there is a high concentration of potassium ions and a relatively low concentration of sodium and chloride ions within the axon. The organic anions seem to be manufactured within the cell and are found only in the cell. This situation is found in many other cells as well. What is it about this situation which results in a steady membrane potential?

First one must examine some properties of the cell membrane. Within the axon are the fixed organic anions to which the membrane is relatively impermeable. This gives the inside a negative charge. Potassium ions are probably free, i.e., they are not bound to proteins or other large molecules. Therefore, they tend to be drawn into the axoplasm by an electrical gradient until the force of the electrical gradient is offset by the force of the diffusional gradient. The cell membrane is quite low in its permeability to sodium ions which results in the external medium carrying a positive charge. The passage of chloride ions into the axon is not prevented by impermeability of the cell membrane but there is another force keeping this ion out of the cell. In crossing the surface membrane inward the negatively charged chloride ions have to travel 'uphill' along an electrical gradient, i.e., from a positively charged to a negatively charged surface. It is the cell membrane which is responsible for separating and maintaining these two fields of electrical charges.

Ruch points out that by varying the external concentration of potassium ions, one can greatly alter the resting potential. In respect to these changes in the external potassium concentration the cell behaves much as though it were permeable only to potassium. This has given rise to an application of the Nerst equation for calculating the steady transmembrane potential. This equation is as follows: Equilibrium Potential $_{(K)} = 60 \times \log \frac{\text{K+inside the cell}}{\text{K+outside the cell}}$. To a first approximation, as pointed out by Galambos, this ratio gives a reliable picture of the resting transmembrane potential in nerve and muscle cells. However, deviations do occur because the Nerst equation can be written for every ion present in the system. It should be kept in mind that the actual situation, as is often the case, is much more complex than the difference in potassium ion concentration alone.

It has been pointed out that the permeability of the resting neuron membrane to sodium is very low; however, there is a small rate of leakage of sodium into the cell. To maintain the steady ionic concentration it is believed that there is a kind of pump located in the cell membrane which forces sodium ions 'uphill' and outward through the cell membrane as fast as they leak into the cell in the direction of the electrochemical gradient. While the details of this pump are generally unknown, it appears to trade sodium ions for potassium ions, i.e., for each sodium ion ejected through the membrane it accepts one potassium ion. When the potassium ion has been transported inside the axon it moves about as freely as the ions in any simple salt solution. While the cell is resting, the potassium ions tend to leak 'downhill' and outward through the membrane, but at a very slow rate. Eccles gives a diagrammatic representation of the 'sodium pump' in his book, <u>The Physiology of Nerve Cells</u>. This representation is drawn on the next page. The slopes in the flux channels across the membrane represent the respective electrochemical gradients.

The resting membrane potential lies around 70-90 millivolts with the inside negative to the outside. According to Eccles, this membrane potential is determined by the effectiveness with which the internal sodium-potassium pump operates and by the concentration of the fixed internal anions. The diffusional ionic exchange apparently plays no part in determining the membrane potential. However, this factor becomes of paramount importance in relation to the transient changes in this potential which occurs during transmission of the impulse as will be seen in the discussion of the neuron in its active state.

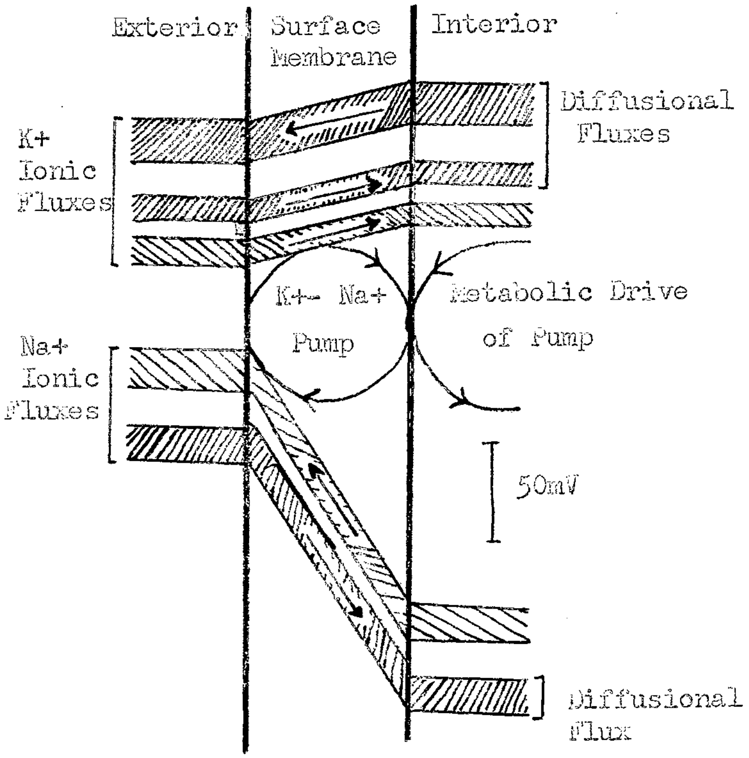

Fig. 2 The Theoretical 'Sodium Pump' of a Nerve Cell.

Before beginning the discussion of an active neuron, it might be well to summarize what is known about the resting neuron. According to Katz's summary, it is an electrically charged cell and the source of its electricity appears to be a twofold process which (1) keeps positive sodium ions outside by pumping them out, and (2) builds up and retains fairly large negative ions inside. This results in the inside being made negative to the outside. The potassium and chloride then distribute themselves according to this force: potassium is electrically attracted by the interior of the cell, chloride is rejected, and this goes on until the concentration gradients of these ions balance the electrical force. This means that as long as the cell is capable of pumping the sodium ion out and building up and retaining its organic anions, this state of affairs will continue. With this as a background, the exploration of the functional activity of the neuron, i.e., the initiation and transmission of the nerve impulse, can begin.

CHAPTER II

INITIATION AND MANIFESTATIONS OF THE NERVE IMPULSE

Any discussion of the 'resting' neuron merely serves to lay the groundwork for an understanding of the functional aspect of the neuron, i.e., the initiation and transmission of an impulse. It is recognized that man's understanding of the overall functioning of his nervous system, especially the functioning of his brain, is still at a primitive stage. But his knowledge is reasonably adequate to describe and partially explain how individual neurons generate and transmit the electrical impulses that form the basic code element of our internal communication system.

Initiation of the Impulse

The causal factor, or triggering device, of the impulse is the stimulus. Woodbury and Patton define stimulus as an environmental change. This change may occur anywhere on the soma or along the extensions of the neuron. Some types, and an example of each type, of stimuli are as follows: 1) mechanical, e.g., pressure, 2) thermal, e.g., application of heat, 3) chemical, e.g., applying chemical agents such as acteylcholine to a nerve, and 4) electrical, e.g., application of an electrical current.

Experimentally, electrical stimuli are used almost exclusively. The intensity of an electrical stimulus can be easily and quanitatively varied. Furthermore, mild electrical stimuli, even when repeated several times, do not damage the tissue. Therefore, in discussing the results of experimentally applied stimuli, the use of an electrical current is implied.

What does the stimulus actually do? It has been mentioned previously that all cells are polarized. However the application of a stimulus does not necessarily elict a response from any cell to which it is applied; to obtain a response, the cell membrane must possess the highly distinctive additional property of excitability. Bishop says that one must assume that irritable cells, i.e., cells capable of responding to a stimulus, have special mechanisms which make excitation possible. Woodbury and Patton explain the effects of a stimulus as follows: "In excitable cells, an environmental change may bring about a transient change in the ionic permeability of the membrane. This transient alteration of permeability brings about a transient change in the transmembrane potential." In other words, when the membrane's permeability is altered, something occurs which causes the depolarization of the cell membrane. The stimulus causes this depolarization; the mechanism by which this effect is brought about is still largely a mystery. Once initiated, the change in membrane permeability is propagated rapidly from the stimulus site to adjacent regions of the membrane. This property of the neuron is known as <u>conductivity</u> or <u>self-propagation</u>. The moving wave of depolarization followed by repolarization is known as an <u>impulse</u>; its electrical manifestation is called an <u>action potential</u>.

A stimulus must meet certain criteria in order to set off this self-propagating wave of excitation. (1) The strength of the stimulus may be varied but it must be of a certain magnitude. This means that the membrane potential must be lowered to some critical value called the threshold before an impulse will be generated. A current that just depolarizes the membrane to threshold is called a threshold stimulus. (2) The duration of the stimulus must be adequate. When a current is applied to the axon for a longer length of time, the membrane capacities become charged and the change in membrane potential will be maximal for that charge. Therefore, a prolonged stimulus has a threshold value which is lower than that value required of a stimulus which does not last long enough to charge membrane capacity completely. On the following page is drawn a standard Strength-Duration curve taken from the book, Medical Physiology and Biophysics, edited by Ruch and Fulton. It should be noted that this curve illustrates the relationship which exists between the strength and the duration of a threshold stimulus. Subthreshold values for stimuli have no bearing on this diagram. Woodbury and Patton point out that the shape of the strength-duration curve remains the same for all tissues although the time and current scales vary. (3) The rate of change must be sufficiently rapid. A prolonged subthreshold stimulus may either increase or decrease excitability of the neuron. In the giant squid axon the prolonged subthreshold stimulus decreases the excitability of the neuron. Therefore the threshold value of the stimulus is higher than it was before the prolonged application. When the neuron reacts to a prolonged subthreshold stimulus by a decrease in excitability it is said to have accomodated to the stimulus. Hodgkin points out that the rapid effect of depolarization is to increase sodium permeability; the slow effects are to deactivate the sodium-carrying system and increase the potassium permeability. This is to say that most nerves, when tested with a slowly rising current, pass into a refractory state without ever giving an action potential.

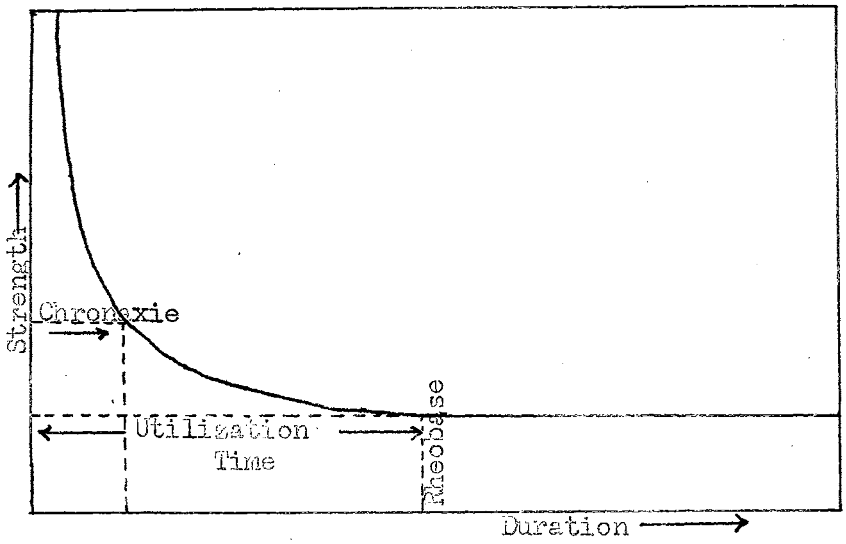

Fig. 3 Strength-Duration Curve. Rheobase - threshold value of the stimulus. Chronaxy or excitation time - the length of time a current twice theobase strength must flow in order to excite a cell.

Manifestation of the Nerve Impulse

Events which actually take place as the moving wave of depolarization followed by repolarization moves down the axon can be divided into three categories. These categories, in order of discussion, are as follows: (1) electrical manifestation of the impulse; (2) chemical manifestations of the impulse; and (3) thermal manifestations of the impulse. While this is a convenient organization for discussion, one must keep in mind that these events occur simultaneously and are interdependent on one another.

Electrical Manifestations of the Nerve Impulse

In Mary Brazier's article "The Electrical Activity of the Nervous System" which appeared in Science, December, 1964, she says "The single most important discovery in the exploration of nervous mechanisms was that the nerve impulse is identifiable with an electrical change." The discovery of this fact was due to a chance observation. Luigi Galvani, according to Katz, discovered accidentally that a frog's leg touching an iron railing propagated an electric current in 1786. The meaning of this observation became a matter of fierce dispute between Galvani and the physicist Alessandro Volta and not until some sixty years later was it clearly proved that nerve and muscle cells actually possess electrical charge and are capable of generating an electric current. Today the study of the functioning of the nervous system is centered around this electrical sign of activity for these signals are the neurophysiologist's clue to coding in the nervous system. What then makes up the electrical events which occur during activity?

While a subthreshold stimulus does not initiate an impulse, the axon shows the effect of the stimulus as a partial depolarization takes place. However, this is a local response, which, as the name implies, is local membrane activity and is nonpropagated. Katz diagrams the effect of subthreshold stimuli as follows:

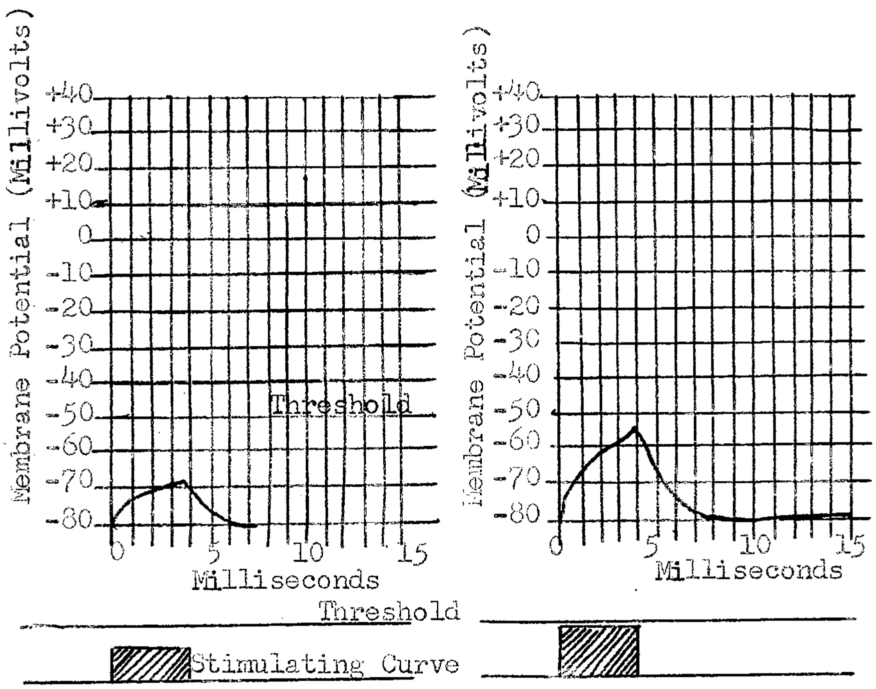

Fig. 4 The Effect of Subthreshold Stimuli

When the critical value of the stimulus is reached, i.e., threshold value, the action potential develops explosively and is entirely independent of the stimulus. Katz illustrates this self-propagation aspect of the impulse by comparing the axon to a communication cable. There is, in each, a relatively long cylindrical transmission line containing an electrically conducting core which is separated from the conducting outside fluid by an insulating sheath. But Katz quickly goes on to say that the resemblance is only superficial. While the communication cable depends on a thick insulating sheath of low capacity and high resistance to prevent leakage of the original signal, the neuron is in effect a chain of relay stations. This is to say that each point along the fiber receives an electric signal from the preceding point, boosts it to full strength and thereby enables it to travel a little further. In order to study this self-propagation concept, Hodgkin and R. Lorente de No proceeded to find out what it would take to block the signal. This was done by anesthetizing a stretch of nerve with cold or a drug. Anesthesia has the effect of making the nerve fiber inexcitable but does not interfer with its ability to conduct the signal in a cable fashion. The nerve fiber is a poor cable and the signal quickly loses strength as it travels. They found the block had to be long enough for the signal to lose ninety percent of its strength before it reached the next excitable point; otherwise, the signal would jump the block. In other words, just a little more than ten percent of the current generated by the nerve itself, independent of the source or strength of the stimulus, is sufficient to excite the normal nerve fiber.

After reaching threshold value, any increase in stimulus intensity has no effect on the amplitude of the action potential; thus its behavior is "all-or-nothing." This should not infer that all action potentials of an axon will have the same amplitude as there are other factors which can affect the axon response. Nevertheless, for any given set of conditions, the axon always responds maximally to a threshold stimulus.

Exactly what constitutes the action potential? Since the steady resting potential has been discussed, one can now examine the changes which occur in the membrane potential during nerve transmission, i.e., the action potential. The sequence of changes which occur is peculiar and unique to excitable cells during the excitation process. Drawn on the next page is a diagram of a generalized action potential which includes all fluctuations in the membrane potential that might occur. This diagram is taken from the article "Electrochemical Aspects in Excitable Cells II" by Abraham Shanes which appeared in Pharmacological Reviews in 1958. It should be remembered that this is an idealized membrane action potential.

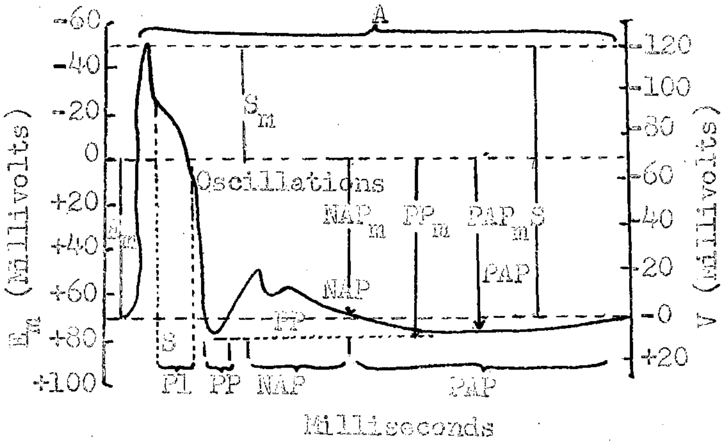

Fig. 5 An Idealized Action Potential
Amplitudes are given relative to cell interior which is taken as zero or relative to transmembrane potential just prior to the spike. The subscript "m" designates the transmembrane equivalent of the displacement potential.

That portion of the diagram labeled S represents the spike potential. The spike is composed of the collapse of the membrane potential, a reversal of the potential and the repolarization process. Some characteristics of the spike potential are as follows: 1) it is very short in duration, less than one millisecond; 2) there is a reverse polarization; 3) the spike follows the all-or-nothing property, i.e., the magnitude of the spike of a single neuron, under the same conditions, always remains the same regardless of stimulus intensity; 4) the spike does not stay in one place but travels very rapidly out from the point of origin; 5) the same spike is recorded after it has traveled for some distance, i.e., transmission occurs without decrement; and 6) recovery is rapid.

It has long been known that during most of the spike, another spike cannot be evoked regardless of the strength of the stimulus. This period is known as the absolute refractory period. Following this period for a short time a stimulus greater than that required for the first spike is necessary to initiate a second spike. This period is referred to as the relatively refractory period.

That portion of the generalized action potential labeled PP is the positive potential which may or may not be present depending on the nerve fiber in question. This portion represents a greater degree of polarization than normal following the spike. When present, the positive potential is very short in duration and small in magnitude.

The oscillations following the spike do not actually require the spike for their manifestations. Application of subthreshold stimuli can produce such oscillations which has been discussed previously as a local membrane response. However, since these oscillations generally follow the spike they will be discussed as a part of the generalized action potential.

The negative after-potential, that section of the drawing labeled NAP, is a decrease in the polarization of the cell. The excitability of the cell is increased during this period, i.e., the threshold value of the stimulus required to generate another spike is lowered. Lorente de Nó concludes that the presence of the NAP is due to the presence of the preceding positive potential rather than having a rising phase of its own. The negative after-potential may result from the accumulation of potassium. This observation is based on the fact that a rapid succession of spikes is accompanied by a progressive depolarization which attains a steady level governed by the rate of stimulation. (Shanes)

The positive after-potential, labeled as PAP on the diagram, is a slow, small hyperpolarization which frequently follows the negative after-potential. In the crab nerve this phase was shown to be related to the depletion of potassium from the extracellular space. However, this is the subject of much experimentation and discussion at the present.

For a lengthy discussion on the hypothetical considerations and experimentation methods and results concerning the generalized action potential the reader is referred to the article "Electrochemical Aspects in Excitable Cells II" which appeared in Pharmacological Reviews in 1958 and was written by Abraham Shanes.

This constitutes the electrical manifestations of a nerve impulse. To look for the cause of these electrical changes in membrane potential, one must turn to the chemical manifestations of an impulse. It is generally believed that the direct cause of the electrical changes observed is the movement of ions which occur during the transmission of an impulse.

Chemical Manifestations of an Impulse

As has been discussed, the voltage difference across the membrane is determined largely by the membrane's differential permeability to sodium and potassium ions. What makes the nerve membrane distinctive is that its permeability is in turn regulated by the voltage difference across the membrane. This mutual influence is the basis of the signaling process.

When the voltage difference across the membrane is artifically lowered, shown by Hodgkin and Huxley, the immediate effect is to increase the permeability of the cell membrane to sodium ions. The consequences of the alteration of the membrane in this manner is far-reaching. As the sodium ions leak into the cell, they cancel part of the negative charge at that portion. This in turn drops the potential further which allows more sodium ions into the cell. Therefore, the flow of some sodium ions into the cell makes it easier for others to follow. When the threshold of the stimulus is reached, sodium ions enter in such numbers that they change the internal potential of the membrane from negative to positive; the process flares up to create the action potential or nerve impulse. Hodgkin diagrams the relationship which exist between sodium permeability and membrane potential at the threshold level of stimulus as drawn below.

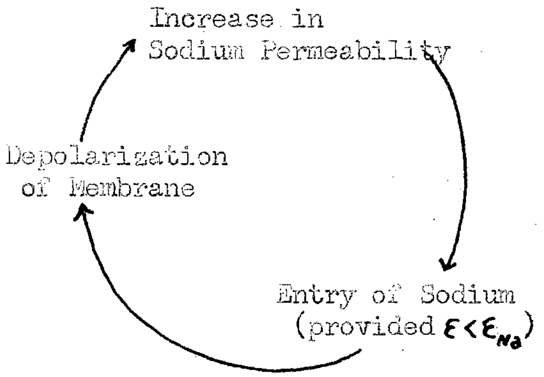

Fig. 6 Sodium Movement and Depolization

Since the permeability of the membrane to sodium ions increases smoothly as the membrane is depolarized, it seems natural to question why there is a threshold, or why the action potential should be all-or-nothing. The diagram above means that beyond a critical potential sodium ions enter the fiber at an accelerating rate and the potential moves rapidly toward the equilibrium potential of the sodium ion. On this basis, the threshold is that potential at which the inward sodium current just balances the outward potassium current. At the critical potential, the currents are equal and opposite and can turn upwards into an action potential or downwards to the resting level. Thus the threshold stimulus must be able to overcome the potassium current and once this is accomplished, through the force of the resulting sodium current, the action potential is generated maximally.

After the peak of the spike other chemical events take place. Permeability to sodium ions, which had been quite high during the rise of the spike, drops again and permeability to potassium ions increases. This causes an accelerated outflow of potassium ions which allows the membrane potential to return rapidly to the original resting level. The ionic permeabilities quickly return to their initial condition and the cell is ready to fire another impulse. This leads to the following generalization by Ruch et. al., the potassium ions are responsible for the resting potential while the sodium ions are responsible for the action potential.

The actual number of ions involved is very few and the duration of the impulse is so brief that the over-all internal composition is affected very slightly. The internal store of potassium is sufficient to fire thousands of impulses without replenishment. The sodium pump has no trouble keeping the normal nerve fiber in transmitting condition; therefore, our system of communication is quite efficient.

On the following page is a diagram taken from an article written by Richard D. Keynes entitled "The Nerve Impulse and the Squid." This article appeared in the December, 1958, issue of the Scientific American. The diagram illustrates the relationship of ionic movements to a simplified action potential.

Some of the theories which have been proposed to explain the movement of these ions are discussed in the final chapter of this paper. At this time it is sufficient to say that while the mechanism of switching off the sodium flow and switching back on the potassium flow may remain a mystery, this serves the extremely useful purpose of permitting the nerve to return to firing condition almost immediately.

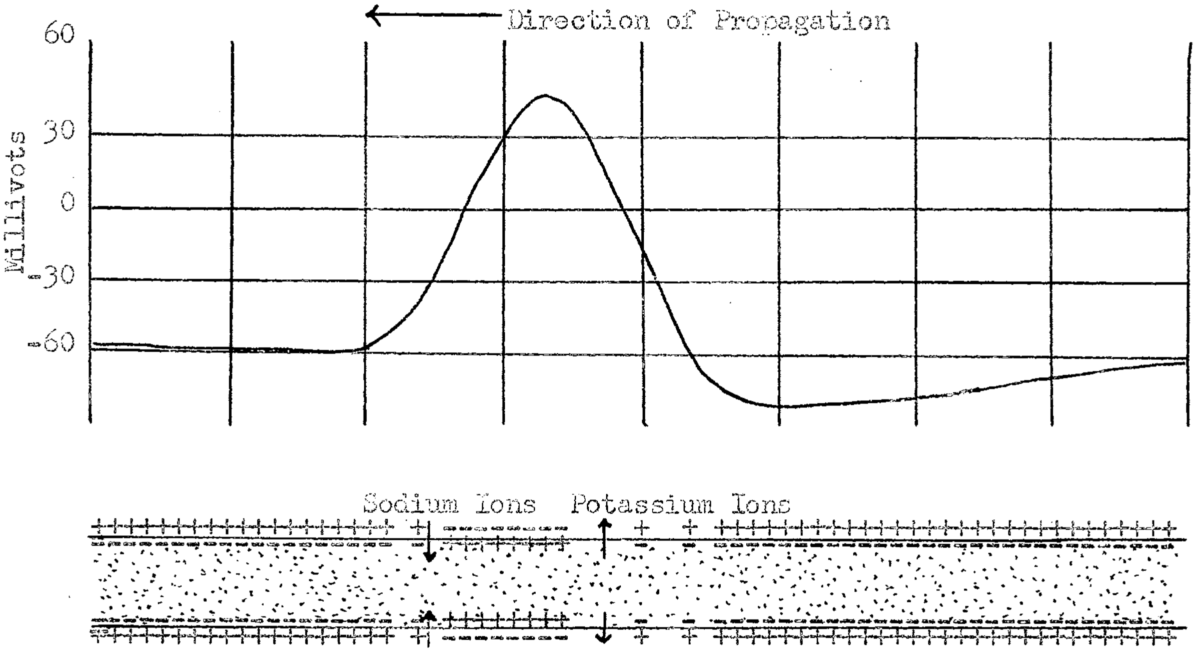

Fig. 7 Relationship of Ionic Movements to an Impulse

<u>Thermal Manifestations of an Impulse</u>

The very early studies of the nerve impulse indicated a complete lack of expenditure of energy. However, this concept began to lose favor rather quickly. In 1932, A. V. Hill considered the possibility of energy liberation in the membrane during activity. Nachmansohn quotes Abbott et. al., as follows: "It is difficult indeed to imagine an excitable membrane going through a complete cycle involving a several hundred fold increase of permeability to sodium ions followed by a similar increase of permeability to potassium ions, and yet behaving as a conservative system without a change of energy.... It is hard to believe that so drastic a cycle of physiochemical change could occur in material like that of the excitable membrane without the intervention of work or chemical reaction." Nevertheless, very little material can be found pertaining to the causes of the thermal aspects of an impulse.

There are different phases of heat liberation and absorption observed during neuron activity. One difficulty encountered when trying to measure this heat is the very small quantity involved. At the present time, the heat of ionic change can be derived only from very indirect methods as the quantities involved are much too small for direct measurements in a calorimeter.

It is believed that there are three successive phases of heat associated with the discharge of an impulse: (1) the first phase was always a positive heat, produced most likely simultaneously with the discharge; (2) the positive heat is followed closely by a negative phase in which heat is absorbed; and (3) a prolonged third phase which again produces heat and sometimes masks the second phase. Earlier studies had combined the first two phases of heat and termed the phase <u>initial heat</u>.

One explanation which was offered to explain the cycle of positive and negative heat phases was the "condenser theory." According to this theory, as proposed by Hill and explained by Nachmansohn, it is possible that the positive heat is derived from the energy released during the rising phase of the action potential in the discharge of the condenser which exists all over the excitable membrane. The negative heat would then be due to the absorption of energy in recharging the condenser during the falling phase. However, the time relations seem to be in error in application of this theory to the actual events taking place.

It should be pointed out that the great number of messages which the nervous fibers continually carries throughout the nervous system makes it necessary to perform this function in an economic way, i.e., with a minimum expenditure of energy. As has been seen, the Creator did indeed develop a mechanism which requires very little energy, as indicated by the small amounts of heat production. It is now up to man to discover this mechanism and with improved technique and equipment, it is quite likely he will do so in the near future.

CHAPTER III

MECHANISM OF THE NERVE IMPULSE

The study of the events which take place during neuron activity is not nearly as complicated as is the study of why these events take place. When one comes to this area, he finds all kinds of theories which have been proposed. There is an overwhelming amount of experimentation going on which sheds light on the already proposed theories and leads to the proposal of new theories. The purpose of this chapter is to give a brief over-view of some of these theories.

Membrane Theory

This theory was put forth at the beginning of this century by the German physiologist Julius Bernstein and for some forty years was accepted by most physiologists as a very satisfactory explanation of the electrical phenomena observed in nerve fibers.

Bernstein proposed that the nerve membrane might be relatively permeable to potassium ions but completely impermeable to sodium, chloride or other ions present in and around the cell. This situation would explain the maintenance of large differences in concentration of ions across the cell membrane and thus account for the electric potential which exists across its surface boundary. In the neuron at rest, potassium ions, which are concentrated inside the cell, would tend to diffuse out of the cell but are held in by the electrostatic attraction of the organic anion which cannot pass through the membrane. Since the sodium ions cannot enter the cell, the interior of the cell is negative to the surrounding fluid. At rest the pressure of the potassium ions to diffuse out of the cell is balanced by the electrical pressure keeping it in. Bernstein believed this balance of pressure was delicately poised.

Bernstein explained the activity during impulse transmission in the following manner. He suggested that the excitation is a breakdown of stability of the membrane which results in a transient change in the permeability of the cell membrane. Momentarily the membrane would be permeable to other ions besides potassium and the electric field would collapse. Electric currents generated by the movement of the ions would then spread the loss of selectivity to the next section of the membrane. Thus the impulse was made to travel by self-regeneration down the length of the fiber.

This theory stood as such until around 1940 even though there was little direct evidence to support it. The high speed of the reaction, the minute quantities of material involved, and the tiny dimensions of most nerve fibers defied the experimental ingenuity of the investigators. Then in 1933, J. Z. Young undertook a study of the nervous system of squids. He pointed out that the giant axon of the squid would provide excellent research material for the propagation of the impulse. Before long investigations were underway and new techniques of study were being perfected. By the late 1930's, the foundation of the membrane theory was becoming quite shaky.

As experimentation results became available it became clear that certain modifications of this theory were necessary. The most important modifications are as follows: (1) E. J. Conway, professor of biochemistry in Dublin, showed that the surface membrane was permeable to chloride ions as well as potassium ions. Soon other investigators discovered, by means of radioactive tracers, that sodium ions could indeed cross the membrane though with more difficulty than potassium and chloride ions encounter. Therefore, the observation that the ion concentrations remain essentially the same on either side of the membrane does not necessarily indicate a rigid impermeability of the membrane to either chloride or sodium ions. (2) While the first evidence concerning the passage of an electrical current increasing the permeability of the membrane seemed to support Bernstein's theory, two sets of investigators, Cole and Curtis at Woods Hole and Hodgkin and Huxley at Plymouth, discovered simultaneously and independently that the membrane potential did not simply drop to zero as would be the case if the membrane became equally permeable to all ions. Instead, the potential is reversed with the inside becoming positive to the outside. This then ruled out the theory that the membrane simply 'breaks down' and becomes permeable to all ions.

Nevertheless, as Brazier points out, most of the theories proposed today are derived from Bernstein's bold and ingenious theory because two essentials of this theory still stand. These essentials are: (1) the membrane of the nerve when inactive is polarized, the inside of the neuron being negative to the outside; and (2) the action potential is a self-propagating depolarization of this membrane.

Sodium Hypothesis

This hypothesis, proposed by Hodgkin and Bernhard Katz, is the most satisfactory explanation for the reversal of the membrane potential at the present time. Many writers simply refer to this hypothesis as the modified membrane theory but Katz refers to it in his writings as the modern 'sodium theory'.

The 'sodium hypothesis' holds that the nerve membrane does not merely lose it's selectivity during the rising phase of the spike, as Bernstein supposed, but that it becomes highly and specifically permeable to sodium ions. Since the sodium concentration outside the membrane is about ten times greater than that inside, this could account, in an idealized case, for a reversal of potential of nearly sixty millivolts. It is further proposed that the original internal negativity of the resting nerve is restored by the subsequent exit of potassium ions from the intracellular fluid.

Investigators began testing the validity of this hypothesis. Using the giant axon of the squid they found that the exchange of sodium and potassium ions is large enough to be detected after reasonably short period of stimulation. Keynes, working with cuttlefish axons, used radioactive isotopes of sodium and potassium to make measurements on the inflow of sodium and the outflow of potassium. The result of his study, supported by the results obtained by other investigators, is that the measured movements of sodium and potassium ions are large enough to alter the potential across the membrane to the extent observed. The close fit of the electrical and chemical observations lends strong support to the sodium theory.

Many writers feel that the sodium theory does nothing except explain what is taking place. Woodbury and Patton write "The properties of the excitable membrane are described in terms of changes in membrane permeability, or conductance, because no information is yet available regarding the mechanisms which give rise to the large and specific changes in the ionic permeability of the membrane." However, the sodium theory is used as a foundation for many investigators on which to base their proposals regarding the operation of the controlling mechanism.

There have been many attempts to explain the mechanism by which these permeability changes occur. Some of the proposals are discussed briefly in the following pages. It should be remembered that these are proposed explanations of how the permeability of the cell's membrane is controlled.

Nachmansohn's Chemical Theory

Nachmansohn has long believed that the ionic movements during activity stop far short of being the full story of the chemical manifestations of an impulse. While in no way disagreeing with the accuracy of the sodium theory, he and Quastel question the cause of the sudden breakdown of resistance in the membrane and the increased permeability to sodium. He believes the most likely assumption to be a change in the protein or lipoprotein in the active membrane such as the folding or unfolding of some protein by a rapid chemical reaction. He proposes that acetylcholine is bound to a protein or lipoprotein. By flow of current, i.e., by accelerated ion movements, the ester is released from the complex and acts upon some receptor protein. The effect of the ester on the receptor protein is a polymerization which is responsible for the change in permeability. The acetylcholine is rapidly hydrolyzed by cholinesterase and the receptor protein returns to its original form. This accounts for the quickness of the transient changes.

Keynes, as he discusses the chemical aspects of the controlling mechanism, recognizes that since a very small change in potential brings a relatively huge change in permeability this suggests some rearrangement of charged groups in the structure of the membrane. However, he feels that nothing is known about the actual chemical nature of these groups and goes on to point out that no chemical compound yet described can discriminate between sodium and potassium so effectively as a nerve membrane.

Carrier Theory

It has been observed that the permeability of the membrane to sodium varies with the electric field. On this basis, Katz postulates the carrier theory which he admits is still only speculative. It is quite probable, says Katz, that sodium moves across the membrane in several steps, not as free ions but perhaps attached to molecules within the fatty material of the membrane. In other words, a sodium ion may first combine with a negatively-charged "carrier molecule" which acts as a guide through the membrane. The ion then may jump off as a free ion into the water of the inner surface. These negative carrier molecules may be held immobilized to the positive outer surface when the electric field is large, and may become mobile when the electric field is reduced.

Pore Structure of the Membrane

L. J. Mullins of the Biophysical Laboratory at Purdue University presented a paper at the American Institute of Biological Sciences in Washington D. C. in 1956, dealing with the structure of the nerve cell membrane. He proposed that perhaps the evidence favored a membrane structure perforated with pores. He further suggested that it is an oriented macromolecular liquid membrane in which pores are formed by the inability of the macromolecules, in their most efficient packing, to fill all the space in the liquid. Below is a diagrammatic drawing illustrating Mullin's suggested arrangement for the membrane structure.

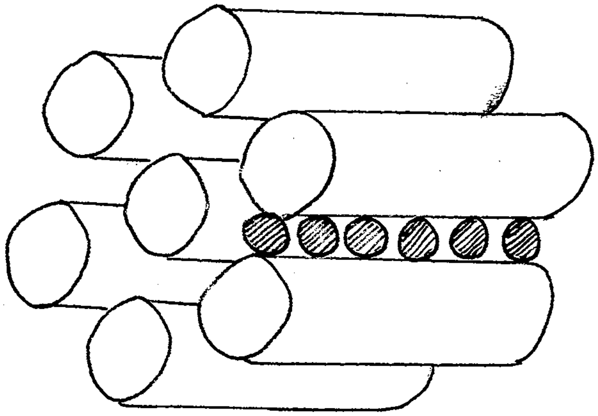

Fig. 8 Theoretical Structure of the Cell Membrane
Macromolecular cylinder, representing structural elements of the cell membrane, are shown in hexagonal packing. The spacing between the molecules has been greatly expanded to show the membrane pore that is formed between any three cylinders, and molecules are shown passing through one pore. The length of the cylinders in the membrane thickness, and the diameter is a function that sets the pore size.

With such a membrane, we have a mechanism for discriminating between sodium and potassium that resides solely in the size of the ion. Sodium ions are usually considered to be larger than potassium ions and about the same size as calcium ions. A change in the electric field could allow the macromolecules to assume new positions. This could account for the change in the permeability of the cell membrane. However, this brings up the question of why the membrane appears to be selectively permeable to sodium during the rising phase of the spike. It also does not explain why the macromolecules assume a new position with a change in electric field. Perhaps this structure could be working in combination with some chemical feature also.

Mullins readily admits that there are many difficulties to contend with in assuming that this is an accurate picture of the cell membrane. However, as he points out, the membrane is an extremely complicated structure and one must assume some things in order to have a foundation for study. Cell membrane structure is a subject of great length and about which much speculation is taking place. Nevertheless, Mullins gives his proposal of pore structure in light of events which are known to take place during cell excitation.

Speculative Ideas

In addition to those proposals which have been studied to some degree, there are many ideas tossed about attempting to explain the mechanism of the nerve impulse. Some of these ideas are as follows: (1) Sodium and potassium act merely as "lubricants," rather than direct carriers, of the transfer of charge across the fiber membrane; (2) it may be possible that the charge is transported across the membrane by hydrogen ions, or even by electrons, rather than by sodium and potassium ions; and (3) it may be that the membrane mechanism exploits some physiochemical difference between sodium and potassium ions, the subtlety of which at present eludes us. As Katz points out, these issues will remain controversial for years to come.

Summary

When one studies the events which take place during initiation and propagation of the nerve impulse, there is a temptation to feel that this field of study has been pretty well covered. However, after reading the material dealing with the theories and proposals regarding the mechanism of the nerve impulse one realizes the vast amount of research and experimentation that remains to be done. Proceeding from the foundation that nerve signals are propagated step by step by some electrochemical relay mechanism, the path toward solving the mystery of that mechanism is largely untraveled. Sifting through volumes of experiments and research data, investigators study and ponder this question daily. Hattie Orr put into words the summation of a formidable task in education and her summation very aptly applies to the study of the mechanism of the nerve impulse. "There are thousands of problems, and for every problem there are a thousand blind alleys before you find something that seems to work. And when you find it, if you do, you have other problems with other blind alleys pressing in upon you." But the explanation will be found and when it is, investigators will turn with anticipation to other fields of mystery.

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