

XV. NEUROPHYSIOLOGY*

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A. MEMBRANE PROPERTIES

Resting squid nerve membrane actively extrudes Na^+ against a large concentration gradient, while another cation, K^+ , is concentrated passively within the cell by the Donnan equilibrium. During the passage of an impulse, large transient current flows across the membrane; some K^+ leaves, some Na^+ enters, but which ions carry the current at which instant, and how it is turned on and off cannot be told from simple external measurements. Thus Marmont, and later, exhaustively, Hodgkin and Huxley, applied a voltage clamp between the inside and outside of a squid giant axon, i. e., they applied step functions of voltage across the membrane and measured the current required to maintain the steps. By systematically varying the external concentration of different ions they were finally brought to a description of the nonlinear behavior of the membrane in terms of specific ion conductances, each conductance being dependent on the concentration potential of its species and certain rate constants. With this very careful job they accurately predicted the shape of traveling nerve spikes and after-potentials. However, that tour de force was meant to apply only to squid axon — the subject of their experiments.

Very soon other physiologists began using as laws interpretations which Hodgkin and Huxley had only diffidently suggested, and these laws were applied to nerve-nerve and nerve-muscle transmission with great ingenuity. While such guessing is fair in the absence of data, it is clear that one must in the event test, say, frog nerve, for what nonlinear conductances govern its behavior. Too many differences in chemical composition, speed of transmission, shape of spike, and so forth, exist between squid and frog nerve to allow the voltage-current relations for one to be used on the other. Some workers even suspect that certain quaternary amines rather than Na^+ are important in frog-nerve conduction.

The physical difficulty of examining other than giant fibers is very great. One can insert down a squid nerve of some 0.5 mm in diameter two reasonably large wires, one to measure voltage against some external probe, the other to supply current to some outer electrode. This technique is not possible with fibers that are only 10 μ in diameter. Cell bodies some 50 μ across could, in principle, admit two microelectrodes, one acting as a point source of current, but such a measure would be valid only if the cell were spherical. (Eccles' results with bipolar microelectrodes are not relevant

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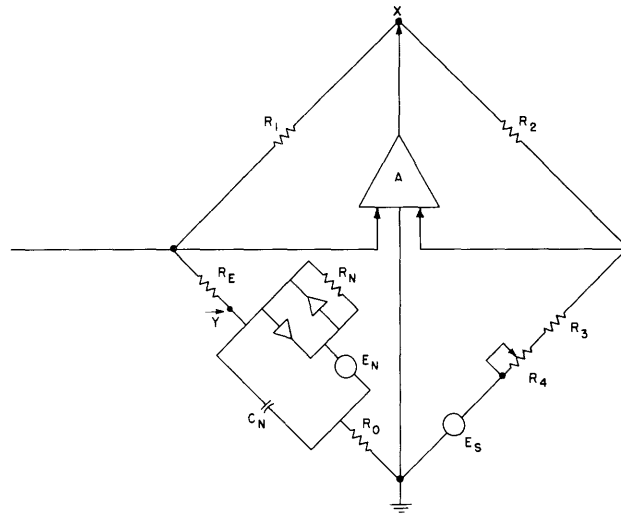


Fig. XV-1. Voltage clamp with one electrode.

here, since he impaled such things as motoneurons which have large irregular processes.)

The trouble in making a bipolar microelectrode (two compartments, fluid-filled, with a total outer diameter of less than 2μ) is great, the problem of filling it properly is worse, and the patience required in handling it in tissue must be staggering. Thus we honor Eccles but shall not imitate him except as driven to it. Instead, we have devised two methods whereby vertebrate nervous tissue can be submitted to a voltage clamp; both are also trying to the patience. The first method involves examining spherical ganglion cells such as those of the dorsal root. Here we use the circuit shown in Fig. XV-1. A is a differential amplifier (gain = 250) so arranged that the left-hand loop through R_1 is degenerative and the right-hand loop through R_2 is regenerative. R_N represents the variable membrane conductance, C_N the constant membrane capacitance, E_N the membrane potential which is interdependent with R_N , and R_0 the impedance to ground from the outside of the cell. Although this is not a true bridge it is operated as one in this fashion: Suppose that R_N remains constant for small perturbations of the voltage across C_N and that R_0 is zero. Then, with X disconnected from A and connected to some external generator of square waves, if the shunt capacitance to ground C at the junction of R_1 and R_E is compensated by $-C$, then $R_1/R_E = R_2/(R_3 + R_4)$ when the sharp front of the square wave is balanced out in the output of A (so that only the charging and discharging current of C is visible). If, with this configuration, X is reattached to A, the effect is that of a negative resistance, $-R_E$, in series between R_E and E_S , and Y follows E_S but not E_N . Thus, without access to Y except through R_E it is possible to keep Y at any constant voltage independent of wide changes in R_N and E_N .

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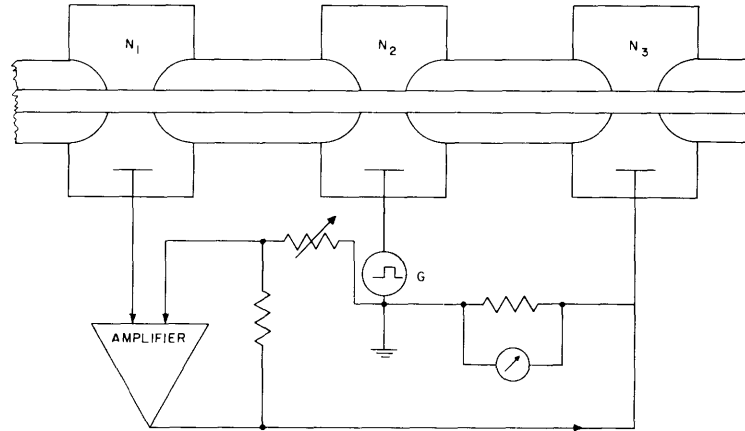


Fig. XV-2. Voltage clamp for node of Ranvier.

In this way it is possible to enter a cell with a resistive microelectrode, and through that electrode maintain the potential to ground constant across the cell membrane, measuring the necessary current at the output of A.

The second method involves applying a voltage clamp (Fig. XV-2) to a single node of Ranvier. Consider three sequential nodes, N_1 , N_2 , N_3 , lying in three troughs, the myelin between troughs dried off to isolate them, the two end nodes lying in KCl or K_2SO_4 , and the center node lying in Ringer's solution. The membrane potential of N_2 is given by $E_1 - E_2$, and the output of the amplifier is fed back degeneratively through E_3 . Then $E_1 - E_2$ will follow G independent of change in membrane potential and impedance at N_2 . With the visit of del Castillo we are using this method to measure the "quantal" nonlinear elements in the nodal membrane.

B. OTHER RECENT WORK

During the past quarter, we have completed work on two studies. First, on the question of the effects of a high-frequency burst of nerve impulses, we have examined the failure of the immediate appearance of the full potentiation. We found that, during the height of the hyperpolarization of the nerve fibers immediately after the end of the tetanus, there are signs of an interruption in the conduction of nerve impulses. Thus, while the individual nerve impulses are greatly increased in height, some are not conducted to the synaptic region. Next, we have examined the relation between the slow "synaptic potentials" and the excitability changes of the afferent fibers that end on the cells. There is no doubt that the changes seen in the skin sensory afferent fibers that end on internuncials are exactly correlated with the slow fifth-dorsal-root potential. In the case of the sensory fibers that originate in muscle and end on the motor cells, there

is a strong suspicion that the changes restricted to the active fibers are correlated with the slow ventral-root potential. However, a new series of experiments will be required to clarify this very important relation.

As a result of our experience in measuring the prolonged effect of a single nerve volley on internuncial cells, we can postulate that change in the speed of reaction of the nervous system in a very old animal might be caused by a change in the reaction of this type of cell. We have available a small number of ancient rats. We hope to establish the normal behavior of the internuncial cells in adult rats and then to compare their behavior in the senile rat.

Finally, we are starting a study of the behavior of spinal sensory internuncial cells in response to a touch on the skin. Our aim here is to extract the mechanism by which the localization of the peripheral stimulus is coded in the nervous system. Individual cells can be shown to respond to stimulation of huge areas of the skin. The pattern of response varies as the stimulus moves within the area. This is made particularly clear if two cells responding to the same area are examined simultaneously. We are making a catalogue of cells and it is already clear that they fall into a number of categories. In some the first response is at a very definite time interval after the stimulus, providing a time marker. Others fire off in a definite pattern which, however, is not exactly repeated at each stimulus. Others with a high spontaneous rate of firing do not respond by the addition of new impulses, but only by a temporary variation in the frequency of their spontaneous firing. We hope to extract various classes of cells from this plethora of types and to determine methods of coding, since it is clear that no one cell transfers all the information on the nature, intensity, and localization of a small touch on the skin.