SQUID GIANT AXONS

A MODEL FOR THE NEURON SOMA?

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ABSTRACT Insertion of electrically floating wires along the axis of a squid giant axon produces an apparent increase in diameter in the region where the wire surface has been treated to give it a low resistance. The shape of action potentials propagating into this region depend upon the surface resistance (and the length) of the wire. As this segment's internal resistance is lowered by reducing the wire's surface resistance, the following characteristic sequence of changes in the action potential is seen at the transition region: (*a*) the duration increases; (*b*) two peaks develop, the first one generated in the normal axon region and the second one generated later in the axial wire region, and; (*c*) blockage occurs (for a very low resistance wire). Action potentials recorded at the membrane region near the tip of the axial wire in (*b*) resemble those recorded at the initial segment of neurons upon antidromic invasions. Squid axon action potentials propagated from a normal region into that containing the low resistance wire also resemble antidromic invasions recorded in neuron somas. Hyperpolarizing current pulses applied through the wire act as if the wire surface resistance was momentarily reduced. For example, the two components of the action potential recorded at the axial wire membrane region noted in (*b*) can be sequentially blocked by the application of increasing hyperpolarizing current through the wire. Similar effects are seen when hyperpolarizing currents are injected into motoneuron somas. It is concluded that the geometrical properties of the junction of a neuron axon with its soma may be in themselves sufficient to determine the shape of the action potentials usually recorded by microelectrodes.

INTRODUCTION

The work of Eccles and his collaborators (Eccles, 1964 review) has shown that the motoneuron surface can be roughly divided into three regions which appear to exhibit different degrees of membrane excitability: (*a*) the axon; (*b*) the initial segment (axon hillock); and (*c*) the soma dendrite. The different excitabilities of these membrane regions have been interpreted from the observed differences in "voltage thresholds" for excitation of these regions. The voltage thresholds of the initial segment and soma-dendritic membrane were taken from the inflexion points observed during the rising phase of action potentials antidromically invading the cell soma (where the microelectrode was presumed to be inserted). The excitability of the initial segment was interpreted to be higher than that of the soma-dendritic membrane. This was in spite of the

fact that Coombs et al. (1955) noted that while the soma depolarization threshold for antidromic stimulation was almost 30 mV, the threshold for synaptic and direct electrical excitation was only about 10 mV. They concluded that the "actual threshold" is the lower value but were unable to resolve the discrepancy.

To quantitatively test the hypothesis of differential excitabilities, Dodge and Cooley (1973) developed a mathematical model of a motoneuron and ran computer simulations of a variety of experiments. Assignment of different thresholds and density of sodium channels to the different regions was justified in detail, and their results seem to fit the experimental data quite well.

However, without discounting the possibility that different membrane regions may indeed have different thresholds or densities of active channels, we want to present another possibility by describing experiments conducted on squid axons. Insertion of an axial wire simulates an increase in diameter and causes changes in action potential shapes at the transition region similar to those observed in motoneurons. Since squid giant axons are known to have uniform membrane characteristics, the observed changes in the shape of the action potential cannot be due to changes in membrane properties but must be produced by the lower internal resistance which is equivalent to an increase in the axon diameter. These experiments extend preliminary unpublished observations by del Castillo and Moore.

METHODS

Experiments were performed on giant axons dissected from squid (*Loligo pealii*) available at the Marine Biological Laboratory at Woods Hole, Mass. Fig. 1 taken from Ramón et al. (1975) is a schematic diagram of the experimental setup. The axons were dissected up to 2–3 cm beyond the main branching region and placed in an appropriate chamber with running natural sea water at a temperature of about 15°–18°C. A cut in the main trunk of the axon was made to introduce a platinum axial wire of 75 μm in diameter. Platinization of the axial wire was performed as described by Moore and Cole (1963). In some experiments this wire was allowed to float

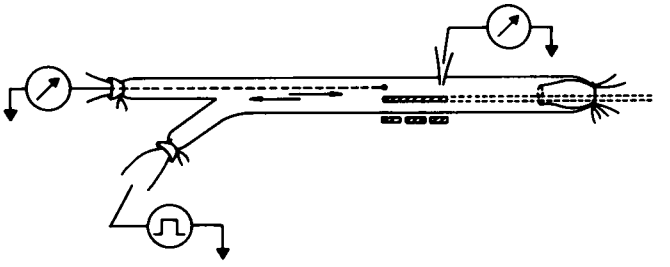


FIGURE 1 Schematic diagram of the experimental setup used to produce an axon region of apparently increased diameter. To the right of the figure is the cannula used to introduce the axial wire exposed only at a region (shaded area). The three small shaded areas facing the exposed region of the axial wire represent the platinum plates used in other experiments for voltage clamping. Through one of the axon branches, a platinum wire (exposed only at its tip) is introduced and used to record voltage changes. The propagated action potential is stimulated at the other axon branch. (Ramón et al., 1975; courtesy of *Fed. Proc.*)

electrically and in others, it was connected to a Grass stimulator (Grass Instruments Co., Quincy, Mass., model S88, output impedance of 100 KΩ) for delivering of current pulses.

The transmembrane potential was usually monitored via a platinum wire (about 25 μm in diameter) completely insulated except at its tip, which was melted to form a ball about 40 μm in diameter. This wire was inserted into the axon past a major branch and was connected to a Tektronix 565 CRO via an AC preamplifier (Grass model P511). For some measurements a microelectrode (with a tip resistance of about 15 mΩ) was introduced into the axon in the axial wire region and connected to the same CRO through a DC preamplifier (Grass model P16). Resting membrane potentials recorded by the microelectrode were about −60 mV. A large branch of the giant axon was stimulated to propagate an action potential, by applying a short pulse of electric current through an Ag/AgCl wire contained within a suction electrode filled with natural sea water.

To keep the experiments described here in perspective, we will limit ourselves to only qualitative comparisons when using the squid giant axon as a model of a neuron soma. For this purpose we will not give values for the resistance of the axial wire used in specific experiments. Suffice it to say that the platinized axial wire had a somewhat higher surface resistance (about 10–20 Ωcm^2) than that used for voltage clamp (2–3 Ωcm^2) by Moore and Cole (1963).

At first glance our system appears to be very much like a bipolar neuron (two axons attached on opposite sides of a central soma, one of which will be chosen for "antidromic" impulse invasion). However the dendriditic tree of the motoneuron is usually taken as a cylinder of passive membrane whose diameter is the same as that of the soma (e.g., Dodge and Cooley, 1973; Rall, 1969). As far as antidromic invasion is concerned, this additional passive load is indistinguishable from an additional active cylinder until enough of the active membrane is brought into the sodium conducting state to cause an action potential. Thus by making the simulated soma long enough, we should be able to approximate the soma plus dendritic tree. Indeed we observed that, for an axial wire of moderately low surface resistance, the double peaked antidromic action potential shape was rather insensitive to changes in the length (from 4 to 18 mm) of the exposed surface. Therefore the change of shape of the action potential at the transition appears to be more a function of the ratio of the diameters (see Ramón et al., 1975) than of the length of the large diameter region (above a reasonable minimum). This also means that the presence of an active axon on the far end of the soma has no appreciable effect on the events at the axon-hillock transition. From all this we can conclude that although our experimental model system is grossly oversimplified, it nevertheless shows the important and dominant phenomena associated with this region of transition.

THEORY

The neuron soma is usually a region of the cell having a larger diameter than the axon. A segment of increased diameter has a smaller internal resistance per unit length of axon (r_i), as compared to a region of smaller diameter and similar length. Therefore, experiments on an axon with a region of reduced internal resistance would provide a convenient way to analyze the effects that diameter changes may have on the shape of action potentials propagating antidromically towards the cell soma.

A region of apparently much larger diameter can be obtained in the squid giant axon by inserting an axial wire, insulated except for a length which is platinized to reduce its surface resistance. Such axial wires are similar to those used to deliver current for an axial wire voltage clamp of the squid giant axon (Moore and Cole, 1963). Because the internal resistance of the remaining axoplasm not displaced by the wire is now shunted by the low axial wire resistance, the equivalent internal resistance may be made

much smaller than normal. Therefore, the axon region containing an axial wire behaves like a region of increased diameter with a reduced axial resistance.

As the diameter of an axon is increased, the surface area per unit length increases, with an associated linear increase in both its membrane capacitance per unit length ($c_m = C_m \cdot 2\pi a$) and conductance per unit length ($g_m = G_m \cdot 2\pi a$).

Nevertheless these aspects of the increase in diameter do not affect propagation, as can be seen in two ways:

(*a*) The membrane time constant ($\tau_m = c_m/g_m$) is unchanged while the internal longitudinal resistance is reduced by the square of the diameter ratio ($r_i = R_i/\pi a^2$). Because both the passive and active properties of a membrane are uniquely related to its time constant, the effect of a region of increased diameter should be determined only by the decrease in internal resistance.

(*b*) Computations, using the unidimensional cable model of the axon, show that the same changes in the propagated action potential can be obtained either by increasing the cable diameter or decreasing the internal resistance, but not by increasing the membrane capacitance alone (Dodge and Cooley, 1971; Khodorov, 1974; Ramón et al., 1975). Also, a recent theoretical study by Goldstein and Rall (1974) describes the modifications in shape and speed of propagation of action potentials as they encounter regions of different diameter. They show that an abrupt change in the cable diameter produces modifications of the action potential shape (their Fig. 10) very similar to those we observed and describe here.

These reasons appear to be sufficient to justify our assumption that the reduction of internal resistance by insertion of an axial wire will give the same changes in action potential shapes as increasing the diameter of the axon and that the propagated action potentials will be similar to those seen at the initial segment and the soma of motoneurons.

RESULTS

The effects on propagated action potentials produced by the insertion of a low resistance axial wire can be controlled by the surface resistance and exposed length of the axial wire. Above a few millimeters of exposed low resistance surface, increases in length had no further effect and all of the changes observed were due to the apparent increase in diameter.

Much of our experiments were done under such a condition. When a wire with low surface resistance is made long enough it will approximate a voltage clamp (although actually floating electrically) and block the propagation of the action potential (see for example, Moore and Cole, 1963). This is due to the fact that the axial current available from action potentials propagating from a normal axon region into that containing the wire, would be insufficient to significantly depolarize the very large capacitance of the isopotential membrane surface area.

Fig. 2 shows changes in the action potential propagating into zones of transition to different simulated increased diameters (the direction for propagation is from top to bottom in this, as well as all other figures. The records were made via a wire intro-

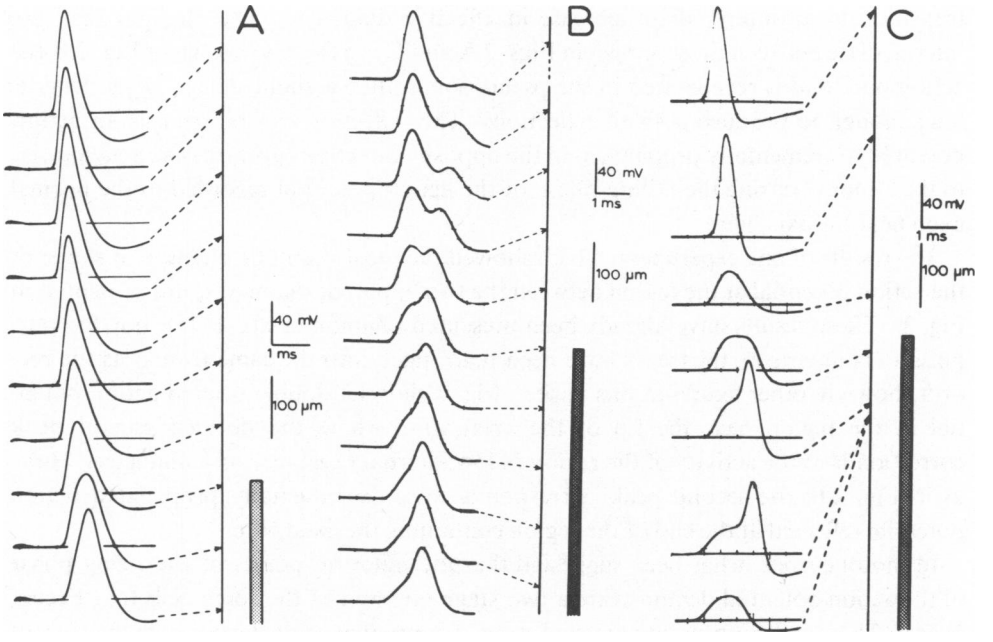


FIGURE 2 Propagation of action potentials in squid axon into regions containing axial wires of different surface resistances simulating regions of different diameter increases. In this, as well as in all other figures, the vertical line corresponds to the axon wall and the shaded area to the exposed and surface-treated part of the axial wire. The surface resistance of the axial wire decreases from records in part A through those in part C. In A, the action potential undergoes only small changes in shape as it travels into the axial wire membrane region. In B the propagated action potential is greatly affected, and in C its propagation is almost completely blocked. The bottom two records in C were obtained after repositioning the electrodes and the initiation of the spike does not exactly correspond with the other records (see text for further details).

duced through one of the axon branches and it was successively moved at points indicated by the arrows; the axial wire is represented by the shadowed area. In Fig. 2 A the action potential reaches the zone of transition between the homogeneous portion of the axon and the area containing the axial wire and continues on into the axial wire region with little shape change. There is a slight decrease of the maximum amplitude of the action potential and some broadening of the peak at the region near the tip of the wire (seen in the next to the last record).

In contrast to this situation the action potential is almost completely blocked when it enters the region of transition to a very large simulated increase in diameter as shown in Fig. 2 C. In this case the action potential is reduced to less than one-half its amplitude at the region proximal to the tip of the wire; however, it fully regenerates after entering the axial wire region. Marginal regeneration is shown in the last two records (taken on two successive sweeps) in the series in Fig. 2 C; the action potential seems almost completely blocked in the lowest record while there is a full regeneration of the action potential in the other.

Fig. 2 B shows changes in shape of the action potential as it traverses a region of

transition to an intermediate increase in effective diameter. The changes seen are intermediate between those shown in Figs. 2 A and C. In the case shown in Fig. 2 B the action potential is regenerated in the pseudosoma after a slight delay. This delay is long enough to produce a small reflection. The reflection can be seen as an action potential decrementally propagated in the opposite direction (upwards) and giving rise to the "hump" during the falling phase of the action potential recorded in the normal axon near the axial wire.

The results of one experiment, which showed the most dramatic changes in shape of the action potential at the region between the two apparent diameters, are presented in Fig. 3. These results have already been presented (Ramón et al., 1975); but for purposes of comparison, the traces have been rearranged into the same format as the records shown in other figures in this paper. Fig. 3 shows a double peaked action potential in the region near the tip of the axial wire, where the delayed second peak corresponds to the activity of the region of low internal resistance and diameter. Here, as in Fig. 2 B, the second peak corresponds to a decrementally propagated action potential reflected at the end of the region containing the axial wire.

In motoneurons it has been suggested that the inflexion points of the rising phase of the action potential demonstrate a two stage invasion of the soma cells (cf., Eccles, 1964). These inflexion points are said to be a consequence of delays in activation of different neuron regions and are due to the low safety factor for invasion of the action potential. This safety factor has been experimentally modified by either hyperpolarizing the cell soma (Coombs et al., 1955; Tauc, 1962) or by producing "fatigue" with repetitive stimulation (Tauc, 1962).

Similar experiments were conducted on a squid giant axon with an axial wire. The records shown in Fig. 4 were obtained at a single location (arrow), and preceding the arrival of the action potential at this point, depolarizing or hyperpolarizing current pulses were applied through the axial wire. Fig. 4 A shows the effect of hyperpolarizing pulses of different magnitude (increasing from top to bottom records) on the shape of the action potential. At the resting membrane potential (top record) or with weak hyperpolarizing pulses (upper records) the action potential shows a smooth contour. However, with strong hyperpolarizing pulses the propagated action potential shows two distinct peaks similar to those shown in the action potentials of Figs. 2 B and 3. Even stronger hyperpolarizing pulses (two bottom records) block the second peak of the action potential leaving only the early one which was generated in the axon region before the axial wire. In Fig. 4 B the arrival of the action potential is preceded by depolarizing current pulses. The top record shows the control action potential and the second and third records show the result of weak depolarizing pulses which do not affect the shape of the action potential. Stronger depolarizing pulses evoke an action potential at the membrane regions just beyond the end of the axial wire (last two records).

When the experiments of the type shown in Figs. 3 and 4 are performed together, records of the kind shown in Fig. 5 are obtained. This figure shows the action potential recorded at the regions marked with arrows from the normal resting axon (middle rec-

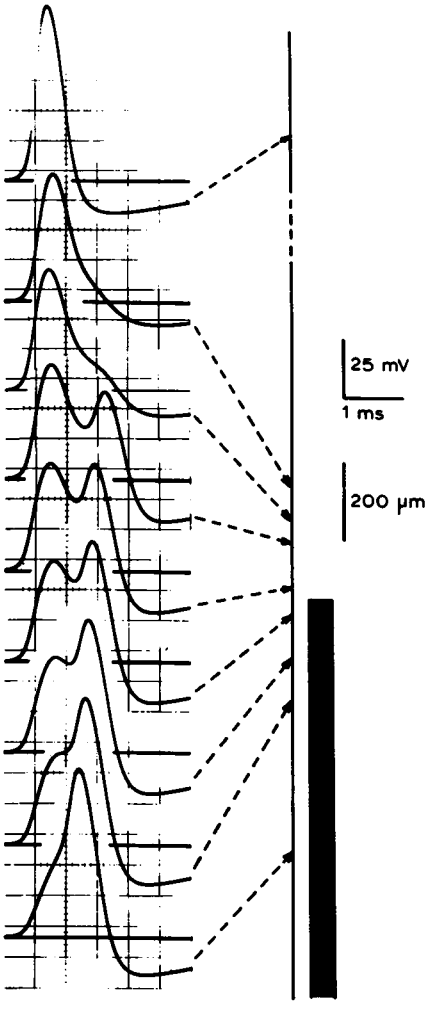


FIGURE 3

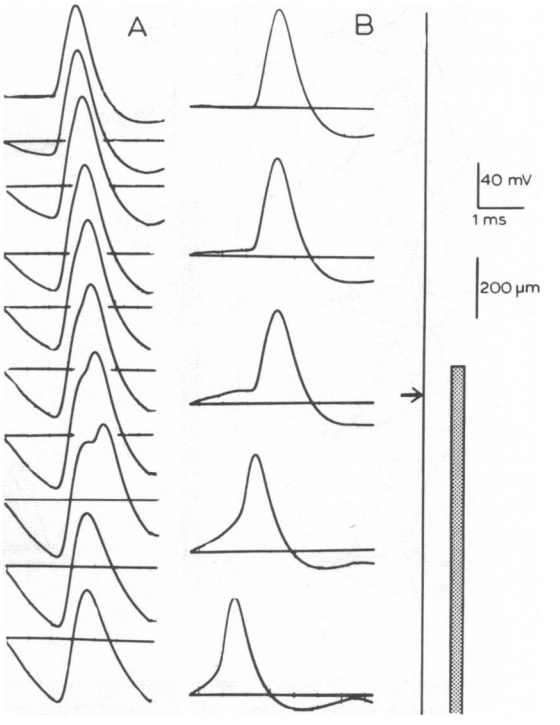
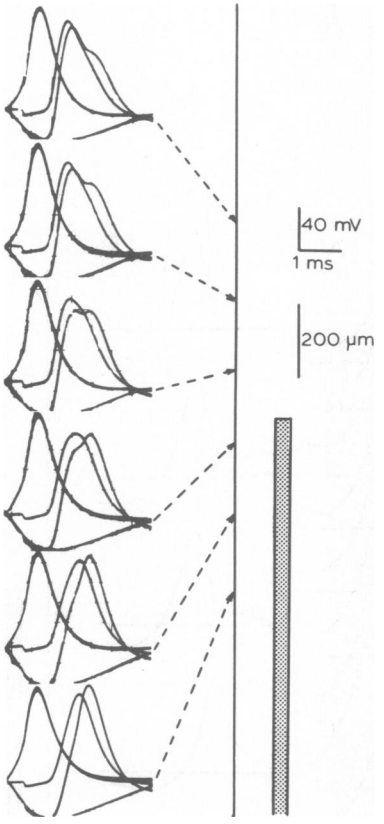


FIGURE 4

FIGURE 3 Changes in shape of an action potential propagating into a region of apparent diameter increase (axial wire surface has a moderately low resistance). Records are rearranged from those published elsewhere (Ramón et al., 1975; courtesy of *Fed. Proc.*) Note the shift of the "hump" from the falling to the rising phase of the propagated action potential as it enters the axial wire membrane region. At the region near the tip of the wire the action potential shows two distinct peaks similar to those shown in the action potentials recorded in neurons (Figs. 7 and 8).

FIGURE 4 Propagated action potential recorded at a single point (arrow) as it travels into an axon region of apparently increased diameter. Synchronized with the stimulus for the action potential, hyperpolarizing (A) or depolarizing (B) current pulses were applied through the axial wire. In part A increasing (from top to bottom) hyperpolarizing currents produce, first no apparent effect, then a double-peaked action potential and finally, blockage of the second peak of the action potential. In part B, increasing (from top to bottom) depolarizing currents do not significantly affect the shape of the propagated action potential but strong currents elicit an action potential in the axial wire region.



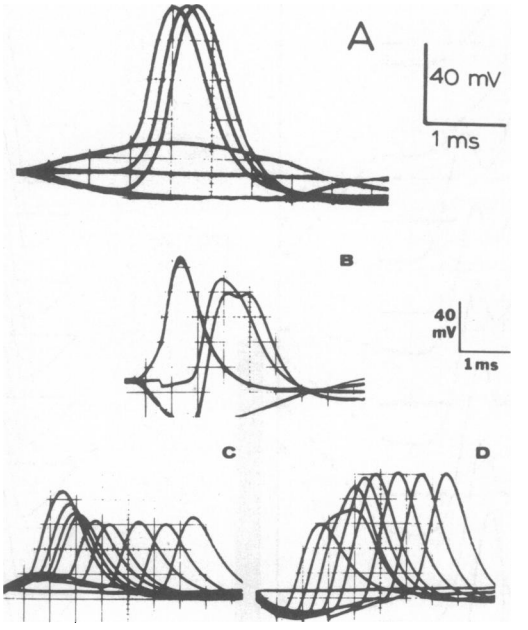


FIGURE 5

FIGURE 6

FIGURE 5 Propagated action potential, recorded at the points indicated by the arrows, as it enters a region of apparently increased diameter. In all records, the middle action potentials are controls and lower and upper action potentials correspond to those arriving synchronized with hyperpolarizing or depolarizing currents respectively. The action potential arriving to a normally polarized membrane region suffers small modifications, as also shown in Fig. 2 A, while that one arriving to a hyperpolarized membrane shows a "hump" and resembled that of Fig. 2 B.

FIGURE 6 Propagated action potentials recorded at the membrane region around the tip of the axial wire. The arrival of the action potential was synchronized with the application of hyperpolarizing and depolarizing currents through the axial wire. In part A, an axial wire of relatively high surface resistance produces only changes in latency of the propagated action potential when it encounters a region where the membrane has been hyperpolarized or depolarized. In part B a lower surface reistance axial wire produces a slight broadening of the action potential when the membrane is normally polarized, but two peaks are clearly shown when the membrane is hyperpolarized by the current pulse. In parts C and D, the time of arrival of action potentials was varied with respect to the initiation of depolarizing and hyperpolarizing currents. Depolarization produces very small effects on the duration of the propagated action potential, but hyperpolarization produces significant broadening.

ords) and when hyperpolarizing (lower records) or depolarizing (upper records) current pulses are applied through the axial wire in synchrony with the stimulus for the action potential. It is clear that the action potential arriving at the axial wire axon region with a normal resting membrane suffers only slight changes in amplitude and duration, which are similar to those seen in Fig. 2 A. However, when the axon membrane adjacent to the axial wire is hyperpolarized, the action potential undergoes changes in shape resembling those shown in Fig. 2 B. There is a "hump" on the falling phase of the action potential recorded in membrane regions above the axial wire and this hump shifts to the rising phase as the action potential enters the axial wire region.

Fig. 6 shows the effect of the surface resistance of the axial wire on a propagated action potential recorded near the tip of the axial wire. Fig. 6 A shows the effect of a high surface resistance wire. In this case, when the action potential arrived simultaneously with the delivery of hyperpolarizing or depolarizing currents, the latency is affected but its shape does not change. In contrast, Fig. 6 B shows the action potential arriving at an axon region containing an axial wire of low surface resistance. The action potential appears to be elicited by the depolarizing current and is smooth but of shorter duration than with no current applied. Hyperpolarization again brings out the two characteristic peaks. Figs. 6 C and D show the effects of hyperpolarizing or depolarizing currents on an action potential arriving at different times with respect to the onset of the current pulse. The action potential becomes smaller but with minor duration changes when arriving at a depolarized membrane (Fig. 6 C); however it becomes larger and very broad when the membrane has been hyperpolarized (Fig. 6 D).

DISCUSSION

The demonstration that the insertion of an axial wire into a squid giant axon produces a system similar to a neuron requires comparison of experimental data from it with that from neurons. Fortunately many experiments such as ours have been performed on neurons and reported in the literature. For example, Fig. 7, taken from a paper by

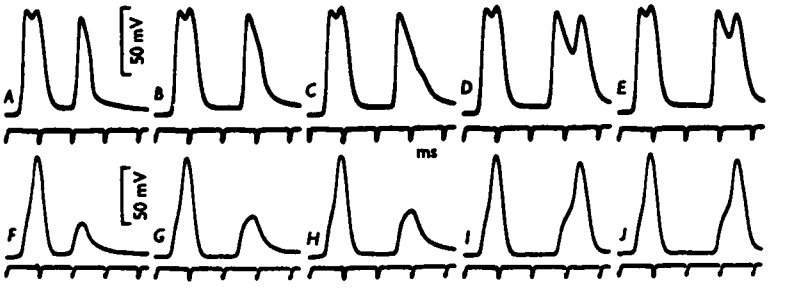


FIGURE 7 Antidromically propagated action potentials recorded by means of a microelectrode in the initial segment of a cat spinal motoneuron (top row) and the motoneuron soma (bottom row). (Coombs et al., 1957; courtesy of the authors and the *J. Physiol. (Lond.)*). Note the similarity of these records with those obtained from the squid giant axon when the recording electrode is at the region near the tip of a moderately low surface resistance axial wire, as in Fig. 3.

Coombs et al. (1957) is one case in point. For the upper row of records of action potentials, the microelectrode was presumably placed in the initial segment of a spinal motoneuron and two stimuli at various intervals were delivered to the motor nerve to elicit antidromically propagated action potentials. The second row of action potentials were obtained after the microelectrode was presumably moved into the cell soma. It is clear that the action potentials shown in Figs. 2 B and 3 of this paper are qualitatively similar to those recorded in motoneurons. Furthermore, the results shown in our Fig. 4 A are also similar to those shown by Coombs et al. (1955, Fig. 7) wherein the motoneuron soma was progressively hyperpolarized.

Similar observations have been made in other neurons and is demonstrated in Fig. 8, taken from a paper by Tauc (1962), where the records were made from an *Aplysia* giant neuron with microelectrodes positioned as shown in the diagram of the figure. The effect of progressively decreasing the hyperpolarizing current, applied to the soma via electrode P, is shown in records 1 through 5. Our records in Fig. 3 closely resemble those obtained by Tauc.

The double peak action potential recorded in the transition membrane region near the tip of the axial wire in giant axons (or in the initial segment of the motoneuron) clearly shows the difficulties encountered by an action potential as it sweeps into a region of larger diameter. This region may be considered as one of low safety factor for propagation whether in squid giant axons or in motoneurons (Eccles, 1964). Effects due to a region of increased diameter can be enhanced by further decreasing the already low safety factor for invasion; i.e. hyperpolarization of the enlarged diameter region (or the cell soma).

In addition to all of the above similarities, the records obtained from several types of neurons (Coombs et al., 1955, 1957; Fuortes et al., 1957; Tauc, 1962; Mellon and

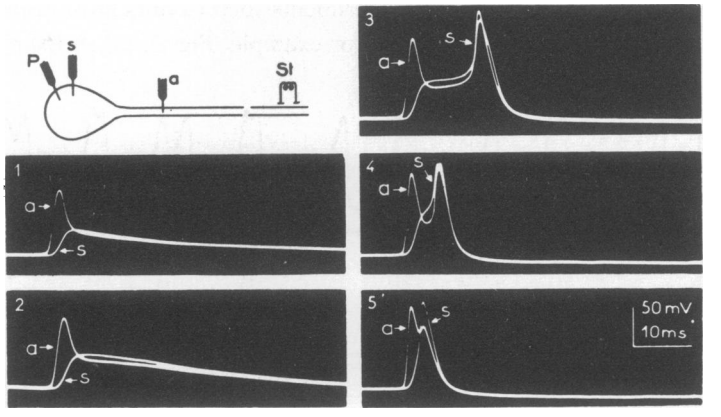


FIGURE 8 Antidromically propagated action potential recorded as shown in the diagram, from an *Aplysia* giant neuron. (Tauc, 1962; courtesy of the author and the *J. Gen. Physiol.*). The membrane is hyperpolarized by current pulses applied through the electrode labeled P and the hyperpolarization is decreased from records 1 through 5. Compare with our Figs. 4 and 5, where hyperpolarizing currents were injected into squid axons.

Kaars, 1974) further reassure us that squid giant axons, modified as to have a region of apparent increased diameter, represent a useful preparation with which to study and understand some of the behavior of neurons. The qualitative similarity of our results and those obtained in neurons makes very clear the profound importance that the geometry of a neuron has in determining the shape of the action potential recorded with microelectrodes in the cell soma.

In spite of the similarity between our records and those from neurons, we are aware that the resemblence which the modified squid giant axons bears to neurons in aspects other than action potential shapes has yet to be proved. Nor do they allow us to reach a conclusion as to whether or not motoneurons have different channel density at different regions, as suggested by Dodge and Cooley (1973). However our experiments do allow us to conclude that geometrical changes alone are in themselves sufficient to cause bizarre changes in shape of action potentials similar to those observed in neurons. Further investigation should be undertaken to resolve whether there are also membrane channel density changes.

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REFERENCES

COOMBS, J. S., D. R. CURTIS, and J. C. ECCLES. 1957. The interpretation of spike potentials of motoneurons. *J. Physiol. (Lond.).* **139**:198.

COOMBS, J. S., J. C. ECCLES, and P. FATT. 1955. The electrical properties of the motoneuron membrane. *J. Physiol. (Lond.).* **130**:291.

DODGE, F. A., and J. W. COOLEY. 1971. Excitation and propagation of impulses in various non-uniform axons. *Biophys. J.* **11**:51*a*. (Abstr.)

DODGE, F. A., and J. W. COOLEY. 1973. Action potential of the motorneuron. *IBM J. Res. Develop.* **17**:219.

ECCLES, J. C. 1964. The Physiology of Synapses. Academic Press, Inc. New York.

FUORTES, M. G. F., K. FRANK, and M. C. BECKER. 1957. Steps in the production of motoneuron spikes. *J. Gen. Physiol.* **40**:735.

GOLDSTEIN, S. S., and W. RALL. 1974. Changes of action potential shape and velocity for changing core conductor geometry. *Biophys. J.* **14**:731.

KHODOROV, B. I. 1974. The Problem of Excitability. B. Haigh, translator and F. A. Dodge, editor. Plenum Publishing Corp., New York.

MELLON, deF., and C. KAARS. 1974. Role of cellular geometry in conduction of excitation along a sensory neuron. *J. Neurophysiol.* **37**:1228.

MOORE, J. W., and K. S. COLE. 1963. Voltage clamp techniques. *In* Physical Techniques in Biological Research. W. L. Nastuk, editor. Academic Press, Inc., New York.

RALL, W. 1969. Time constants and electrotonic length of membrane cylinders and neurons. *Biophys. J.* **9**:1483.

RAMÓN, F., R. W. JOYNER, and J. W. MOORE. 1975. Propagation of action potentials in inhomogeneous axon regions. *Fed. Proc.* **34**:1357.

TAUC, L. 1962. Site of origin and propagation of spike in the giant neuron of Aplysia. *J. Gen. Physiol.* **45**:1077.