MEMBRANE ACTION POTENTIALS FROM THE SQUID GIANT AXON [1]

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FIVE FIGURES

The electrical potential difference across the membrane of an axon and its variation during the passage of an impulse are outstanding characteristics of nerve and important guides to its structure and function. Resting and action potentials have most commonly been recorded between an electrode on normal nerve and another electrode on a portion rendered inactive by chemical or physical means. As is well recognized, these potential differences are less than those across the axon membrane by an amount depending upon the degree of short-circuiting by external electrolytes, and they may be calculated, by the integration from one electrode to the other, of the external field caused by local circuit current flow in the external electrolyte. The observed potentials are then clearly dependent upon the characteristics of every point of the nerve between the two electrodes, but for an ideal uniform nerve the potentials should be reduced images of the membrane potential under the active electrode.

An inactive end which produces a monophasic action potential is usually considered satisfactory although it may be difficult to produce and maintain. However the criterion itself is arbitrary because it is based on the assumption that there is a monophasic variation of the potential difference across the membrane, and because it ignores any effect of the procedure upon the cell as a whole. In an actual nerve, localized injuries in the active region and the boundary between the active and inactive portions may be expected to contribute to the observed action and resting potentials. Another method of recording the action potential is with both electrodes on an active portion of the nerve. When these electrodes are close together, the potential is

[1] Since the present experiments and those of Hodgkin and Huxley ('39) were made independently and practically simultaneously, we had hoped to have our work appear at the same time as their complete report. We are publishing this paper now only because the war will probably prevent a further publication by Hodgkin and Huxley in the near future.

approximately proportional to the first derivative of the membrane action potential (Hill, '34; Cole and Curtis, '38) which can then be obtained—except for proportionality factor—by mechanical or electrical (Schmitt, O. H., '39) integration of the "diphasic" potential. This procedure has the advantage that it includes only a short, and consequently probably more uniform, length of nerve and causes a minimum of damage to the tissue. It does, however, place more stringent requirements on the sensitivity and frequency response of the recording equipment and, as with the inactive end data, the calculation of the magnitude of the membrane action potential from diphasic records requires measurements of nerve characteristics which are not easily obtained. For longer interpolar distances the diphasic potential must be resolved by the solution of an integral equation (Hill, '34) unless the interpolar stretch is longer than the wave length of the action potential, as for example in Nitella.

The more direct approach is that of inserting and sealing an electrode into the cell interior and recording the potentials between the interior and exterior as has been done with several large plant cells (Osterhout et al., '27). This technique is impractical for ordinary nerve fibers but with the introduction of Young's squid giant axon preparation (Young, '36) it became an obvious possibility. This paper describes an experimental procedure for the insertion of a small electrode through the membrane and some distance into the axoplasm with a relatively small amount of injury to the axon as a whole. The potential measured between this inside and an outside electrode is shown to be approximately the potential difference across the membrane. Although both the resting and the action potentials of the membrane should be investigated by this method, only records of the membrane action potential will be given here because there are electrode and amplifier limitations to be overcome before measurements of the resting potential can be obtained (compare Hodgkin and Huxley, '39).

MATERIAL AND METHOD

The material used was the giant fiber from the hindmost stellar nerve of the common North Atlantic squid, Loligo pealii and the method of dissection and teasing was the same as previously described (Cole and Curtis, '39).

The measuring cell, shown in figure 1, is also similar to the one used previously. The axon was placed in the trough, 540 μ wide and 550 μ deep, cut in the top of a sheet of Victron. The tied ends of the fiber were in the enlarged portions of the trough at A and B. The entire

trough was filled with sea water and a cover slip placed over the portion containing the axon. The micro-needles were about 9 mm. long and ranged from 10 to 60 μ in diameter. These capillary ends were pulled from glass tubing of about 540 μ outside diameter which formed the shank of the needle and slid easily in the trough on both sides of C, figure 1.

The first type of needle used consisted of a soda glass tube filled with solder. These were made by the same method which is employed in making Taylor ('24) process wire. It consists in melting the metal in a glass tube and, after obtaining a clean surface, pulling a capillary

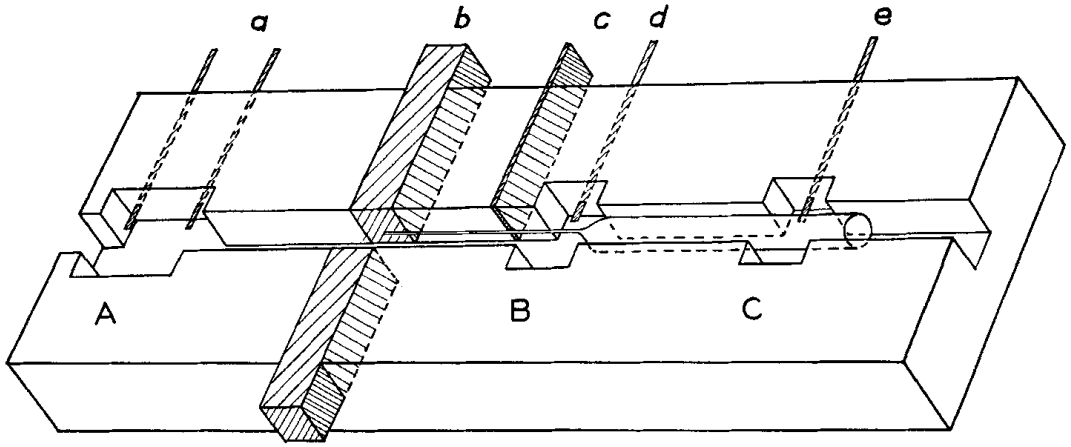


Fig. 1 Schematic drawing of chamber for measurement of the membrane action potentials in the squid giant axon. The ligated axon was laid in the central trough between A and B and the capillary electrode was moved in from the right to the position shown. b, are transverse empedance electrodes, a, c and d were used for stimulation, d and b were used for inactive end recording and e and b were used for capillary electrode recording of membrane action potentials.

in the usual way. In this case two pulls were necessary, the first to obtain a rod 540 μ in diameter, and the second to obtain the needle, which was pulled by the usual technique for making micro needles. It takes considerable patience to obtain a needle of the correct size and shape which does not have a break some place in the solder. The solder tip of the needle was coated electrolytically with platinum black to reduce electrode polarization. Figure 2 shows a portion of the cell and axon with one of these needles in place. The second type used was simply a Pyrex micro-pipette pulled to the proper dimensions in the usual way and filled with a KCl solution iso-osmotic with sea water (compare Bear and Schmitt, '39). Results obtained with the two different kinds of electrodes were identical and since the micro-pipettes were much easier to make, they were used for all the later experiments. The electrode

e in well C was electrically connected to the needle with mercury for a metal needle or with sea water for an electrolyte needle. A vaseline seal was placed between wells B and C to prevent current flow between electrode e and any of the other electrodes, except through the needle. To test the seal, the resistance of the needle was measured outside the cell and again in place in the cell without a fiber. The resistances were found to be the same when the seal was tight, and this test was made in every experiment. To verify the assumption that no current was flowing to electrode e from any place except the tip of the needle, the tip was sealed off in a flame and the resistance between electrode e and all other electrodes was found to be substantially infinite when this needle was in place.

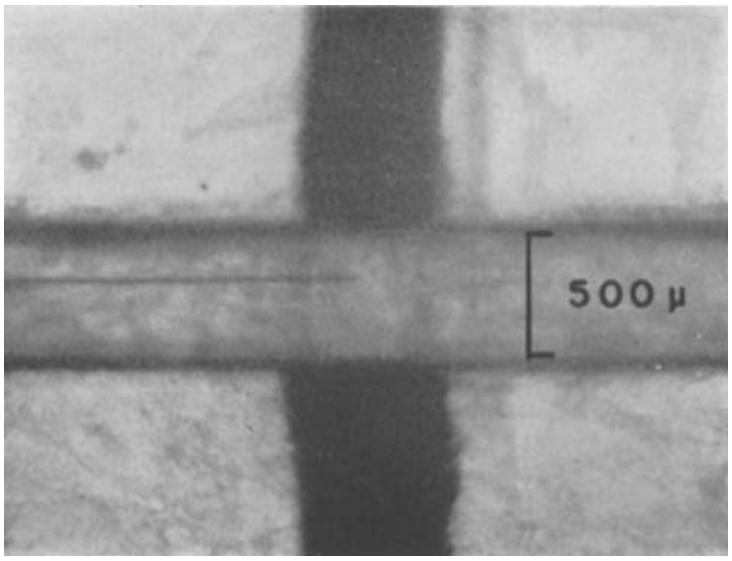


Fig. 2 Photograph of chamber with axon in trough between impedance electrode and metallic capillary electrode in axoplasm at the left.

All of the various electrodes in the cell were made of solder, electrolytically coated with platinum black. After the axon was in place and the needle inserted, electrodes b were connected in one arm of a Wheatstone bridge (Cole and Curtis, '37) to measure the change in impedance during activity (Cole and Curtis, '39) and electrodes a, c and d were used for stimulating or recording as desired. The stimulating circuit, amplifier and cathode ray oscillograph were the same as previously used except that larger coupling condensers were used in the amplifier. The high frequency response of this amplifier allowed a fairly accurate record of the action potential from outside electrodes. However, the needle electrode resistances, which were between 1 and 25

megohms, reduced the high frequency response sufficiently to cause considerable distortion in the recorded potentials. For the last experiment, an improvised "impedance changer" amplifier input stage was used and completely eliminated this error.

OBSERVATIONS AND RECORDS

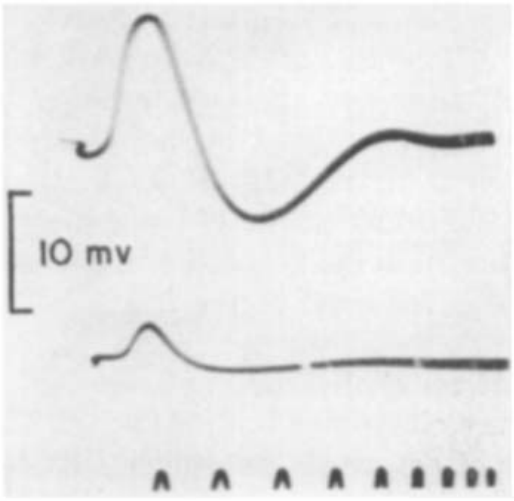
The survival of these impaled axons was quite good considering the adverse conditions under which they were kept. There was no provision for cooling and the sea water in the cell could not be circulated, but, even so, some axons remained excitable for as long as 4 hours after impalement. Usually the end which was punctured became inactive very soon and, starting from that point, the rest of the axon would slowly become inactive. The process of impalement did not change the height or form of the outside action potential, but the impedance change on excitation fell from about 4% for most of the fibers to 2% or less.

The axon was usually stimulated at an a electrode and the action potential recorded from any pair of the electrodes b, d, d and e. The most interesting of these potentials is of course the one taken between the needle, electrode e, and one of the b electrodes opposite its tip. One of these potentials, with the usual outside monophasic potential at the same amplification from electrodes b and d, is shown in figure 3. The membrane action potential spike is only about 12 millivolts, but in figure 4, it is about 44 millivolts. Potentials as large as 80 millivolts were observed, but 40 to 50 millivolts was more usual. The membrane potential is about five times the outside potential in figure 3 and about six times in figure 4. For other axons this ratio has varied between five and fifteen.

It is apparent in figure 3 that the potential recorded from the outside electrodes is somewhat faster than the needle potential. When the effect of the needle resistance and the amplifier input capacity was reduced by the impedance changer input, the spikes were practically identical as is seen in figure 4. The difference between the two potentials after the spike is probably due to the effect on the outside potential of some activity of the axon in the neighborhood of the "inactive" electrode d.

If, as we are assuming, the action potential difference recorded between the needle and an outside electrode opposite its tip is due entirely to the membrane action potential, it should be independent of the direction of propagation of the impulse. To test this, the axon was stimulated first at an a and then at the c electrode, and both action

potentials recorded on the same film. In several cases the potentials superimposed so completely as to be inseparable, but slight differences in the excitation and conduction times, as well as in the forms of the potentials, were sufficient to resolve those of figure 5. It is quite apparent that the amplitudes and forms of the action potentials are nearly identical for propagation in either direction.



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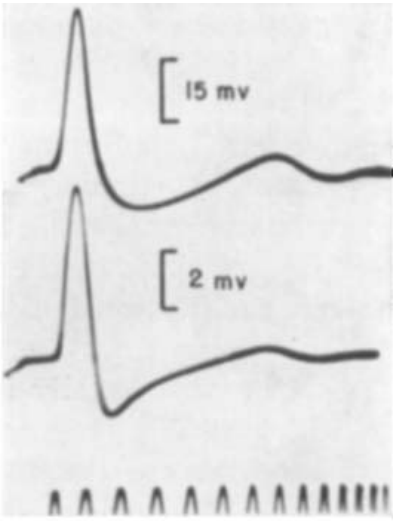

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Fig. 3 Action potentials of the squid axon. The upper record is the membrane action potential from the capillary electrode. The lower record is the action potential with an inactive end at the same amplification. The time intervals at the bottom are 0.5 msec.

Fig. 4 Action potentials of the squid axon. The upper record is the membrane action potential from the capillary electrode. The lower record is the action potential with a partially inactive end at a higher amplification. The time intervals at the bottom are 0.5 msec.

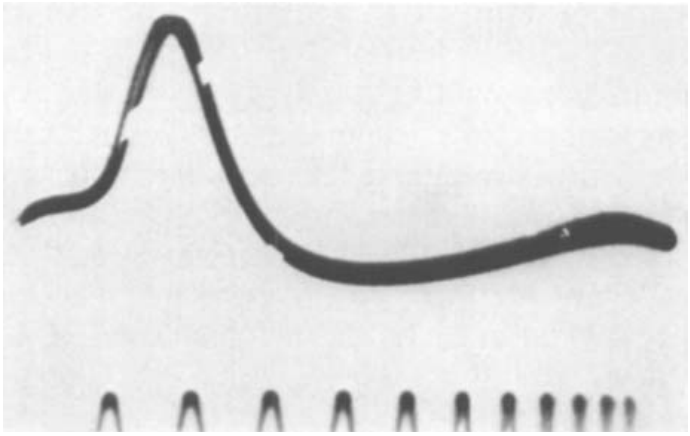

Fig. 5 Membrane action potentials of the squid axon from a capillary electrode. The continuous record was obtained with stimulation at the left hand end of the axon and the broken record with stimulation at the right hand end. The amplification was the same for both records. The time intervals at the bottom are 0.5 msec.

The records of figures 3, 4 and 5 show that the membrane action potential obtained with the needle electrode was far from monophasic. Both the metallic and the electrolytic needles gave the same form for this potential, and there was nothing in the amplifier characteristics, either with or without the impedance changer input, which would account for the effect. The impedance changer input did not alter it materially (fig. 4) nor was it dependent upon the direction of propagation (fig. 5).

Several attempts were made to use the impedance between the needle and an impedance electrode to obtain a direct measure of the resting membrane resistance and the decrease of impedance during the passage of an impulse. These were unsuccessful and the idea was subsequently shown to be impractical.

DISCUSSION AND CONCLUSIONS

The fact that a single fiber can remain alive and conduct an apparently normal and unaltered impulse when it has a glass rod one-tenth of its own diameter thrust through its axoplasm makes it seem rather unlikely that there is an internal structure in nerve which plays a significant role in the active mechanism of propagation. In fact, the only evidence which we have of more than a local injury due to the puncture is the reduction of the impedance change during activity. As previously pointed out, the impedance change is a more delicate test of the condition of the fiber than the height of the action potential, and as such it indicates a slight effect on the cell as a whole. On the other hand, the internal pressure of the axon may have forced axoplasm around both types of needles or into the tip of the electrolytic needle and the consequent reduction of axon volume would have reduced the measured impedance change without requiring an alteration of membrane properties. The data taken are not conclusive, but indicate that this effect was not appreciable and that there was a slight change of the entire membrane. In any case, although the axons were not normal, they were probably only slightly more abnormal after puncture than after dissection and teasing.

Although the puncture and the presence of the needle in the axoplasm did not seriously reduce the survival, the outside action potential or the impedance change on activity, they might have altered the membrane potential near the tip of the needle. The successful impalement of Valonia and Halicystis cells depended upon the formation of a seal around the needle at the point of puncture having a resistance which was considerably higher than that of the membrane as a whole. Other-

wise the membrane potential would be reduced by an amount depending upon the current flow through this shunting circuit. To take the worst possible case, we may assume that the membrane potential difference and resistance are reduced to zero at the point of puncture. At a distance x from this point, we find from cable theory that the membrane potential V is given by $V = V_0 (1 - e^{-x/\lambda})$, where V_0 is the normal membrane potential and λ is the characteristic length. The difference between V and V_0 is less than 5% when the distance from the point of injury is greater than 3λ. From previous work (Cole and Hodgkin, '39) an axon in the trough has a value for λ of 3 mm. or less, so we may expect a 5% decrease at 9 mm. from the point of puncture, indicating that a larger separation would have been desirable. Although the complete impulse is 3 or 4 cm. long, the distorting effect of an injury would still be confined to a distance of 3λ, where λ is now considerably less than for resting membrane in the parts of the impulse where the membrane resistance has fallen. This conclusion is borne out by the similarity of impulses propagated in opposite directions.

It has been shown (Hodgkin, '39) that the velocity of an impulse depends upon the effective resistance of the outside electrolyte, and should depend similarly on the internal resistance. The needle acts as a non-conductor but its effect on the internal resistance as a whole would be negligible because its cross section is only 1% of that of the axon. The impulses travelling in both directions may be cited again in this connection. As entertaining possibility is that of reversibly blocking an impulse by the injection of air or oil into the axoplasm from the needle.

The membrane potential difference can also be altered by current passing through the needle and measuring equipment. If the needle is perfectly insulated, its potential will be V_0 with respect to an outside electrode. Where Z_m is the impedance of the axon and Z_e of the needle and measuring equipment, both measured between the tip of the needle and the outside electrode, this potential will be reduced in the ratio $Z_m/(Z_m + Z_e)$. For direct current $Z_m = \sqrt{(r_1 + r_2)r_4}$ where r_1, r_2 and r_4 are the outside, inside and membrane resistances for a unit length of axon in the trough. Here we find $Z_m = 1.6 \cdot 10^4$ ohms, and since Z_e is $2 \cdot 10^6$ ohms, due to the needle alone, this error should be negligible.

We may now conclude that the needle injuries—due to puncture or other causes—and the measuring equipment probably have only a

small physical effect on the membrane resting potential at a distance of a centimeter and even less effect on the action potential.

We must now estimate the potential difference between the needle tip and inside surface of the membrane. If the needle tip is at the inactive end, corresponding to the conventional monophasic lead, the measured potential is less than the membrane potential by an amount equal to the potential drop due to current flow in the internal circuit (Cole and Curtis, '38). We are now able to move the needle away from the inactive end and along this internal circuit picking up potential as we go. When the inside and outside electrodes approach each other and the membrane, we should expect to lose only the potential drop in the quarter of a millimeter or less of axoplasm separating the needle tip from the membrane. In a resting axon with all injuries more than 3λ distant, the current flow through the axoplasm and membrane are negligible and the needle tip has the same potential as the inside surface of the membrane. During the passage of an impulse there is of course a radial component of current flow and a potential difference between the needle tip and the membrane. Although it seems obvious that this error should be small, a satisfactory approximation has not been found and the complete calculation has not been attempted. It is apparent that the impedance of the measuring equipment should be large compared to that of the needle and axon, i.e., considerably greater than $2 \cdot 10^6$ ohms. The vaseline seal around the needle between electrodes d and e eliminated short circuiting in the cell and the impedance changer amplifier input provided a high amplifier impedance.

We may now turn from the physiological and physical complications involved in the technique to a consideration of the membrane potentials obtained with it. The wide variation in the observed values of the spike potential is probably an indication of the gradual mastery of the experimental difficulties and it is hoped that further work will give more consistent data or at least a correlation with the resting potential.

An outstanding characteristic of the squid membrane action potential is its diphasic form. Although this had been observed previously (Cole and Curtis, '39) in the outside potentials, it was so unorthodox that it was attributed to the technique rather than the axon. Furthermore the integration of the first derivative or "diphasic" action potential always gave a positive phase but this was discounted because of the uncertainty of the base line. However, Hodgkin and Huxley ('39) have also observed this form of action potential. From the present results alone, there is little reason to doubt the reality of the positive

phase. The type of needle was without effect and physical tests of the amplifier as well as the fact that this phase diminishes as the axon degenerates eliminated these possibilities. The identity of the impulses propagated in both directions, removes the possibility of an effect of injury due to puncture and other asymmetries. It seems that the positive phase is highly susceptible to injury but this may be due to the physical proximity of the injury, which can be determined by further experiment. It seems quite improbable that the diphasic wave could be caused by the outside metallic electrodes. Good monophasic potentials have been observed from other single fiber preparations (Hodgkin, '38, '39) so it may be that the positive phase is more apparent in large fibers or that the squid axon is exceptional.

The failure to obtain direct measures of the membrane resistance at rest and during the passage of an impulse is probably the result of the high resistance of the needle. The impedance between short electrodes inside and outside the fiber should be $Z = \sqrt{(r_1 + r_2)z_m/2}$ where z_m is the impedance of a centimeter length of membrane, $1/z_m = 1/r_4 + 1/z_3$ and z_3 is the dielectric impedance of the membrane, $z_3 = z_3 (j\omega)^{-a}$ For direct current, $\omega = 0$, $Z = 8 \cdot 10^4$ ohms. In series with a 2 megohm needle this amounts to 0.4%. This small difference requires more accurate temperature control and reproducibility of needle position than was available. Because of electrode polarization and the short duration of the effect, it was not convenient to use a bridge frequency of less than 10 kilocycles per second for detection of the impedance change during activity. At this frequency the capacity of the membrane introduces a large shunting effect, and Z varies during activity from $1.4 \cdot 10^3$ to $1.1 \cdot 10^3$ ohms or 0.015% of the total resistance. To make matters worse, for this resistance the unknown had to be shunted by a 10,000 ohm resistor and the difference then becomes less than 0.0001%.

SUMMARY

A technique has been developed for recording action potentials from the squid giant axon between one electrode inside and another electrode outside the axon. The membrane at one end of the axon is pierced by the capillary needle electrode, insulated except at its tip, which is then pushed along the axis of the fiber for a distance of about 9 mm. The needle produces only a local injury at the point of puncture which spreads slowly while the rest of the axon remains excitable for as long as 4 hours in some cases. The action potential recorded between the tip of the needle and an electrode directly opposite it on the outside of the fiber is approximately the action potential difference across the

membrane. This membrane potential had a usual spike height of about 50 millivolts although 80 millivolts was obtained. The potential of the membrane spike was about five to ten times that with one outside electrode on an inactive region. When the preparation was fresh the negative spike of the membrane potential was followed by a slow positive phase with about one-fifth amplitude which decreased as injury approached the recording region. The amplitude and form of the membrane action potential was shown to be the same for propagation of the impulse in either direction.

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