Nonlinear Cable Equations for Axons

II. Computations and Experiments with External Current Electrodes

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ABSTRACT We have investigated the steady-state potential and current distributions resulting from current injection into a close-fitting channel into which a squid axon is placed. Hybrid computer solutions of the cable equations, using the Hodgkin-Huxley equations to give the membrane current density, were in good agreement with experimental observations. A much better fit was obtained when the Hodgkin-Huxley leakage conductance was reduced fivefold.

INTRODUCTION

In the preceding paper (Arispe and Moore, 1979), we examined the steady-state spatial distribution of current and voltage in an axon into which current was injected. We demonstrated the validity and applicability of the Cole equation to extract the membrane current-voltage relation from the cable input characteristic. In this paper we take up the more frequently encountered but analytically difficult case of an axon bathed in a restricted medium through which current is passed between external electrodes. In 1965, Moore and Green made a preliminary report for solutions of this case, but we were unable to compare our solutions with the experimental results obtained by Cole and Curtis (1941) because the separation between their electrodes was not known. It was not given in the original paper, and Cole told us that the chamber had been disposed of so that it could not be measured. Furthermore they stated that "there were a number of unsatisfactory aspects to the potential measurement . . . and a number of compromises between theory and experiment which are not easily evaluated." In particular, their value of resting membrane resistance was 23 $\Omega \text{cm}^2$, some 50-fold lower than the generally accepted value of $\sim 1,000 \Omega \text{cm}^2$.

Therefore, the present authors decided to repeat and extend the experiments of Cole and Curtis. We also wanted to have data in the interpolar and extrapolar regions as well as at the electrodes for comparison with our calculated potential distributions. Furthermore, we wanted to see how much the well-known deterioration of axons after dissection would change the distributions of current and voltage along the cable.
CABLE EQUATIONS FOR EXTERNAL ELECTRODES

The experimental case to be simulated was that described by Cole and Curtis (1941), and consists of an axon lying in a narrow channel in which narrow current passing electrodes were placed in the wall of the channel as shown in Fig. 1. Because of the restricted outside volume of solution bathing the axon, the external resistance $r_e$ is finite and the potential drop outside the axon becomes very important.

Insight into the current and voltage distributions under these conditions comes from the physical principles used in cable equations augmented by running the problem on a hybrid computer which gave very rapid solutions.

Current flows through the axon as illustrated in Fig. 1. The current $I_0$ is applied between electrode $E_1$ and $E_2$. If there were no axon in the channel, all of the current flow would be intrapolar. Insertion of the axon causes some current to flow extrapolar to obtain access to the interior of the axon. All of the extracellular extrapolar current in the channel to the right of $E_2$ eventually crosses the membrane then flows towards the left inside the axon. To the left of $E_2$, current also enters the axon for part of the distance to $E_1$, bringing the axial current $i_a$ to a peak, and then $i_a$ declines as current leaves through the membrane. The axial current remaining at the position of $E_1$, continues to the left, is gradually lost through the membrane and returns to $E_1$ extracellularly. For an ohmic membrane, the peak of $i_a$ occurs at the midpoint between $E_1$ and $E_2$, but for a nonlinear axon membrane, it is shifted towards the depolarizing electrode.

In the interpolar region, the sum of the internal, $i_a$, and external currents, $i_e$, equal the injected current, $I_0$

$$I_0 = i_e + i_a.$$ (1)
Most of the current remains external to the axon, although a small part flows in or out of it depending upon the membrane potential at any particular point. At any extrapolar cross section, \( I_0 \) is zero and the current flowing external to the axon is exactly equal to and opposite in direction to the internal current.

The cable equations of the first paper are modified to include \( V_a \) and \( V_e \), the internal and external potentials, respectively. The membrane potential difference \( V_m \) equals \( V_a - V_e \) so that

\[
\frac{dV_m}{dx} = \frac{dV_a}{dx} - \frac{dV_e}{dx} = -r_a i_a + r_e i_e, \tag{2}
\]

where \( r_a \) and \( r_e \) are the axial and external resistances per unit length. Again, from conservation of charge, the exit of current per unit length of membrane must equal the loss in the axial current and the gain in the external current,

\[
i_m = \frac{d}{dx}(i_a - i_e). \tag{3}
\]

**Computer Solutions for the Nonlinear Membrane**

Because we must consider two current sources in this case, the integration of these equations again presents the problem of instability associated with integration proceeding away from current sources. It is not possible to generate the full solution by reflection of the current and voltage distributions about the point of current injection, as was done in case a of the preceding paper, because the intrapolar patterns must be different from the extrapolar distributions.

For speed and convenience in solving this problem, we used a hybrid computer system, in which the 580 analog computer (Electronic Associates, Inc., Westland Branch, N.J.) solved the cable equations and a PDP-15 (Digital Equipment Corp., Marlboro, Mass.) computed the membrane current density. Although integration on an analog computer is carried out with respect to time, we followed conventional practice and substituted the space variable \( x \) for time and thus simulated integration over distance.

A digital program was written in FOCAL language for a PDP-15 computer to operate and control the analog computer and provide the membrane characteristics from the Hodgkin and Huxley (1952) equations. A table of the nonlinear current density-membrane voltage characteristic was calculated for increments in voltage of 0.1 mV and stored in the buffer memory of the computer.

Simultaneous computation of Eqs. 2 and 3 were carried out on a hybrid system shown in Fig. 2. The digital computer samples the membrane potential from the output of amplifier 18, looks in the stored table for the corresponding value for the membrane current density in mA/cm², and feeds back this value to the analog computer. Potentiometer 29 is set to convert the digital output of membrane current density \( I_m \) to membrane current per unit length \( i_m \). This is integrated to give a voltage proportional to the axial current \( i_a \) (Eq. 3). The values of the potential inside \( (V_a) \) and outside \( (V_e) \) the axon were found as a function of distance by integration of \( +i_a r_a \) and \( -i_e r_e \). The membrane potential was obtained by summation of \( -V_a \) and \( V_e \) sign inversion. Initial conditions were established as follows:
(a) The value of the extrapolar $V_a$ was chosen as 1 mV or less (as in Arispe and Moore, 1979). Here $V_e/V_a = r_e/r_a$ because $i_e = -i_a$.

(b) The initial value for $V_a$ was calculated from the relation

$$V_a = V_m/(1 + r_e/r_a).$$

This initial value also equals $i_{a0}$ times the input resistance (set by potentiometer 27) for a linear cable. Potentiometer 01 is adjusted to give the calculated value for $V_a$, also setting the appropriate initial value of $i_a$ in the process. This initial value of $i_a$ is less than the value chosen (by

\[ \text{potentiometer 04) for the axial current between the electrodes. Therefore, the comparator opens the electronic switch. Thus, the initial value of } i_e \text{ (output of amplifier 08) is the negative of the initial value of } i_a. \]

(c) Potentiometer 10 was set to the ratio $r_e/r_a$ to establish the initial value for $V_e$.

(d) The initial value of $V_m$ (output of amplifier 18) is automatically established as the difference, $V_a - V_e$.

Integration is initiated in the extrapolar region to the left of $E_1$ (see Fig. 1) and proceeds towards this electrode. When $i_a$ has reached the level selected by potentiometer 04, the electronic comparator closes the electronic switch (SW), and the electrode current $I_0$ is added to the external current. The integration proceeds in this condition while $i_a$ first increases and then returns to the chosen switching value. Thus, the comparator senses when electrode $E_2$ is reached and opens the switch to remove $I_0$. As the integration proceeds, $V_m$ and $i_m$ both return to zero beyond the second electrode.
EXPERIMENTAL METHODS

All experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., in a chamber similar to that used by Cole and Curtis (1941). A cleaned squid giant axon was placed in a narrow slot (600 μm wide and 680 μm deep) between two side pools cut in the top of a lucite block. External stimulating current was applied between a pair of thin (50 μm wide) electrodes embedded in the chamber on opposite sides of the channel and a large indifferent electrode in an end pool of the chamber. Such a chamber allows a reduction in the length of axon required because the polarity of the thin electrodes can be reversed, providing two data points for each location of measuring electrodes. Axial potentials were recorded with a low impedance micropipette which had a long stem (several centimeters) of uniform outer diameter making it possible to slide the micropipette axially back and forth into the extrapolar and the intrapolar regions without touching the inner side of the axon membrane. The external potentials were recorded with low impedance micropipettes placed just outside the axon. Simultaneous recording of the potential inside and outside the membrane as well as their difference ($V_m$) were made at several locations on both sides of the stimulating electrode pair by moving the tips of both recording electrodes together.

FIGURE 3. Computed spatial steady-state distribution of currents and voltages resulting from external application of current to an axon in a narrow channel.
Figure 4. (A). Computed family of membrane voltage distributions for a range of currents. (B) Family of experimental observations for same conditions.
RESULTS

The computed spatial steady-state distribution of the currents and voltages associated with a Hodgkin-Huxley axon in a narrow channel is shown for external current injection in Fig. 3. The external resistivity of sea water was taken as 20 Ωcm² and that of the axoplasm as 35 Ωcm². Note the relatively small ratio of axial to external current.

A family of computed distributions of transmembrane potential is shown in the upper part of Fig. 4 and a family of experimental observations below. Although these figures appear satisfactorily similar, a more direct comparison of the model and experiment can be made by plotting the input current-voltage relationship.

![Graph showing the input current-voltage relationship for an axon of diameter 432 μm.](image)

**Figure 5.** Experimental steady-state $I_0 - V_m$ relations showing changes with the duration of the experiment. The points on curve 1 were determined as quickly as possible after placing the axon in the chamber. The axon quickly settled into a stable condition (curves 2 and 3) up to nearly 1 h. Then the resting potential began to drop, and curve 4 was taken.

The experimental $I_0 - V_m$ input relation varied somewhat with time and an example is shown in Fig 5. The first curve (curve 1) was taken immediately after mounting the axon in the chamber. A few minutes later it had changed to that labeled curve 2 (without a resting potential change) and was rather stable for nearly 1 h (curve 3). Later the resting potential declined and the $I_0 - V$ relation changed to (curve 4). The stable $I_0 - V$ relation (points in Fig. 6) could not be fitted by the cable model with Hodgkin-Huxley equations shown as a heavy line. However, the cable model fit can be improved greatly for hyperpolarizations by reducing the leakage conductance in the Hodgkin-Huxley equations fivefold,
corresponding to a better physiological condition. It is possible that the poorer fit this gives for depolarizations could be partly overcome by also changing the leakage equilibrium potential but the meaning of such an additional change is not clear.

**DISCUSSION**

We found, as did Cole and Curtis (1941), that the input current voltage relation of squid axons changed with time. However, it was usually in a stable condition for many minutes, long enough for at least a few full families of data.

Our experimental observations of the current-voltage relation for axons in a narrow channel with external electrodes were in reasonable agreement with calculations for a cable with a Hodgkin-Huxley membrane. A better fit was obtained when the leakage was reduced fivefold. This means that our axons were in as good or better condition as those of Hodgkin and Huxley. They were in much better condition (−60-mV resting potential, 100-mV spike) than those of Cole and Curtis who reported an average resting potential of −50 mV. If we estimate a −60- to −65-mV resting potential for the Hodgkin-Huxley axon and calculate the membrane conductance at −50 mV we find ~250 Ωcm². This is still 10 times larger than the 23 Ωcm² which they report.

Because of the stated problems with the Cole and Curtis (1941) experiments, we still are not able to determine the reason for their extraordinarily low membrane resistance. However, we see no reason for continued concern because our new observations are in such good agreement with the Hodgkin-Huxley equations.

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