VELOCITY VARIATIONS ON UNMYELINATED AXONS*

ALWYN C. SCOTT
Center for Nonlinear Studies, Los Alamos National Laboratory,
Los Alamos, New Mexico 87545, U.S.A.

and

UIA VOTA-PINARDI
Laboratorio di Cibernetica, Via Tolana 2,
Arco Felice (Napoli), Italy

(Received June 19, 1981)

Abstract

In a series of 'twin-pulse' experiments on squid giant axons, the ratio (R) of second pulse to first pulse velocity has been measured as a function of the average time interval between pulses (T). These measurements are compared with simple theoretical calculations of R(T) that depend on the shape of a solitary action potential and a parameter that is independent of axon diameter. Agreement between measurements and calculations of R(T) is within 5%.

1. Introduction

Following initial observations by Ramon et al. (1973), Donati and Kunov (DK) (1976) have described 'twin-pulse' studies on the giant axon of the squid in which the second pulse velocity was measured relative to that of the first pulse. These studies are particularly interesting because they suggest the possibility of a dispersive effect in nerve impulse propagation where the local speed of an impulse train depends upon the local value of repetition frequency. Their measurements show that for certain values of the pulse interval the second pulse may travel faster than the first, a 'supernormal' effect that has also emerged from recent numerical studies of the Hodgkin-Huxley (HH) (1952) equations by Rinzel (1979) and Miller and Rinzel (1981).

In this paper we report a series of squid axon experiments that confirm the observations of Donati and Kunov and extend the corresponding error analysis. Starting with the same assumptions as DK, we use a novel theoretical perspective to compute dispersive effects directly from the shape of an isolated waveform. Although the resulting dispersion formula is approximate, it is quite simple and gives a rather good representation of our data without adjusting parameters.

Observations of second pulse propagation at supernormal speeds differ on the squid axon from previously studied effects. On the sciatic of the green frog (Rana pipiens), for example, Graham (1934) found that the ability to exhibit supernormality developed slowly (4 to 14 hours) as isolated nerves rested in a moist testing chamber. Squid supernormality, on the other hand, is immediately observed on a freshly excised axon. Also, Graham found supernormal second pulse velocity for a time interval between the two pulses of several hundred milliseconds, an order of magnitude greater than the persistence of our effect. Similar comments can be made with respect to Bullock's (1951) study of the giant fiber of the earthworm (Lumbricus terrestris): Gardner-Medwin (1972) investigated twin pulse effects along the parallel fibers of cat cerebellar cortex and suggested that first pulse after effects persisting up to 100 milliseconds may be related to changes in the extracellular environment. Our observations, however, are compatible with a theoretical picture that assumes at most only slight changes in extracellular ionic concentrations.

2. Materials and methods

Our experimental parameters are almost identical to those described by Donati and Kunov (1976). Squids (Loligo vulgaris) were collected from the Bay of Naples and kept in an aquarium with filtered, constantly running sea water for no more than one week. We used the longest possible portion of the giant axon from the stellate ganglion, stimulated by platinum, extra-cellular electrodes. Action potentials were recorded intra-cellularly from KCl-filled glass microelectrodes connected to high input impedance preamplifiers with negative capacitance adjustments to cancel the positive capacitance of the microelectrode tips. Pulse waveforms were measured on a TEKTRONIX 565 dual-beam oscilloscope. The measurements reported here were made after several months of daily practice so we feel that the probability of unobserved surgical damage is rather small. The giant axons were usually left within a sheath of smaller fibers (i.e. 'uncleaned') and pinned to a microscope slide that was covered with Sylgard resin (a soft plastic).

Measurement of velocity ratio

Our measurement of velocity ratio as a function of the pulse separation can be described in the context of Fig. 1. The axon is stimulated by two consecutive pulses and the resulting action potentials are recorded at positions 1 and 2. The times of arrival of the first and second pulse on electrode 1 are called t1 and t2, while the corresponding times for electrode 2 are called t3 and t4. Thus the average pulse spacing for a particular measurement can be defined as

\[ T = \frac{1}{2}(t_4 + t_3 - t_2 - t_1) \]

and, at this pulse spacing, the ratio of second to first pulse velocity

\[ R = \frac{\dot{\theta}_2}{\dot{\theta}_1} \]
From a series of measurements of the four times \((t_1, \ldots, t_4)\), one readily obtains a plot of \(\bar{R}(T)\) which has the general form indicated in Fig. 2. This estimate, \(\bar{R}\), is approximated because it neglects the fact that the velocity of the second pulse changes as it travels between the two recording electrodes. It is shown in the Appendix that this error can be removed in the following way. (a) Choose a point [say \((\bar{R}(T_0), T_0)\) on Fig. 2] and construct the tangent; (b) find the value of \(T(T = T')\) at which this tangent intersects the horizontal \(R = 1\); and (c) compute

\[
R = \bar{R} = \frac{t_2 - t_1}{t_4 - t_3}
\]

\[
R = \frac{t_4 - t_3 + [t_4(t_4 + t_3 - t_2 - t_1) - T_0^2]}{t_2 - t_1}
\]

\[
\frac{R}{\bar{R}} = \frac{t_4 - t_3 + [t_4(t_4 + t_3 - t_2 - t_1) - T_0^2] + [1 - (t_3 - t_1)/T_0^2]/[1 - (t_4 - t_2)/T_0^2]}{t_2 - t_1}
\]
Since the correction implied by Eq. (4) is rather small (no greater than 2% throughout the course of our measurements), the measurement of the ratio \( R \) was not affected by absolute errors in time calibration of the oscilloscope. However, dial reading uncertainties on the vernier adjustment constitute an irreducible basic error. After some practice, we found the rms deviation in time difference measurements to be 0.003 milliseconds. Thus the rms error in measurement of \( R \) caused by uncertainties in the dial readings should be

\[
\Delta R = 0.003 \left( \frac{1}{t_2 - t_1} + \frac{1}{t_4 - t_3} \right).
\]

In the presentation below of our measurements, we plot \( R \pm \Delta R \) where \( R \) is obtained from Eqs (3) and (4) and \( \Delta R \) from Eq. (5). This error estimate does not include effects of pulse jitter nor, of course, accidental misreading and misrecording in the digits of \( t_1, \ldots, t_4 \).

3. A simple theory

In Donati and Kunov's (1976) analysis of velocity dispersion during a twin-pulse experiment, the aim was to find a simple analytical relation between the velocity ratio \( R \), defined in Eq. (2), and physical parameters describing the axon. Of course one can always return to the original HH equations, but \( R \) is then obtained only after a rather lengthy numerical computation. Thus DK turned to a 'piecewise-linear' model which assumes that membrane conductance switches instantly from a 'resting' value to an 'active' value as the leading edge of the pulses crosses a threshold voltage, \( V_r \). This model was introduced by Offner et al. (1940) and first solved correctly by Scott (1962). It was augmented to include a non-zero resting conductance independently by Kunov (1966) and Vorontsov et al. (1967). In this picture, determination of the active membrane conductance is a key step. For further details see Scott (1977).

Our aim is to relate the velocity ratio, \( R \), to information that can be obtained directly from measurement of the shape of a nerve impulse. To this end we find it convenient to find the relation between impulse velocity and the 'foot' (or exponential precursor) of an impulse. We consider the shape and velocity of the foot to be described by the linear diffusion equation

\[
v_{xx} - rccv_{t} = rgv
\]

where \( r \) is the series resistance per unit length of the axon, and \( c \) and \( g \) are respectively the membrane capacitance and conductance per unit length. (Subscripts imply partial differentiation; thus \( v_x = \partial v / \partial x \), etc.) This equation has a traveling wave solution with the exponential form \( v \propto \exp[(t - x(t))/\tau] \) where

\[
\tau = 2/[\rho c \theta^2 (1 + \sqrt{1 + 4g/[rc \theta^2]})].
\]

Setting \( g = 0 \) yields a reasonable approximation of the pulse velocity as \( \theta = 1/\sqrt{crt} \) (Scott, 1977), and more generally

\[
\theta = [\rho c t (1 + g/t)]^{-1/2}.
\]

Such a velocity formula was derived independently by Pickard (1966) and Markin and Chizmadzhev (1967) and was the one actually used by Zeeman (1972) to 'calculate' the speed of the Hodgkin-Huxley pulse at the close of his catastrophe theory analysis.

We wish to emphasize that there is nothing approximate about this calculation beyond the assumption of linear ionic currents ahead of the impulse. It is not particularly useful in developing the theory of nerve impulse propagation, because the result merely relates two dynamic properties: pulse speed, \( \theta \), and leading edge rate of rise, \( \tau \). However, for our purposes here, a relation between pulse speed and parameters describing the pulse shape is just what is desired.

In a twin-pulse experiment the velocity ratio defined in (2) becomes

\[
R = \left[ \frac{\tau_1 (1 + g_2 \tau_2 / c)}{\tau_2 (1 + g_1 \tau_1 / c)} \right]^{1/2}
\]

where \( \tau_1 \) is the exponential rate of rise on the foot of the first impulse and \( g_1 \) is the resulting membrane conductance, and \( \tau_2 \) and \( g_2 \) are corresponding parameters for the foot of the second impulse.

From the work of Donati and Kunov (1976) it is clear that the influence of the first impulse on the speed of the second is at least twofold. First, the membrane voltage in the tail of the first pulse has not returned to its resting value; and, second, the potassium conductance in the tail of the first pulse has not returned to its resting value. Thus the second pulse is propagating on an axon where both the membrane voltage and the potassium conductance are different from those seen by the first pulse. Let us now use Eq. (9) to determine the individual effects of 'after-voltage' and 'after-conductance' during the tail of the first pulse on the speed of the second pulse.

After-voltage.—Here we assume that after conductance, \( g_0 \), is constant but suppose the membrane voltage to differ from its resting value by an amount \( V \) at the leading edge of the second pulse. Below threshold, then, this leading edge has the form

\[
v = V + (V_r - V) \exp(t/\tau_2)
\]

as indicated in Fig. 3b. If the second pulse shape is assumed to be identical to that of the first pulse above threshold, then \( \theta(0) \) must be the same in Figs 3a and 3b which in turn implies

\[
\tau_2 = \left[ \frac{1}{V_r} \right] \tau_1.
\]

Taking Figs 3a and 3b to represent conditions at the leading edges of the first and second pulses respectively, the velocity ratio due to the effect alone is
After-conductance.—Here we wish to estimate the excess trans-membrane conductance in the tail of the first pulse which is seen by the leading edge of the second pulse. Following the analysis by Donati and Kunov (1976), we represent the undisturbed membrane by a resting potential battery, \( V_R \), in series with a resting conductance (per unit length), \( g_0 \). The after-potential is then the effect of a potassium battery, \( E_K \), in series with a conductance (per unit length), \( \Delta g_K \), representing the increase in potassium permeability. Then the absolute after-potential is \( V' = (g_0 V_R + \Delta g_K E_K)/(g_0 + \Delta g_K) \), so the after-conductance \( (g_0 + \Delta g_K) \) is related to after-potential, \( V' \), by

\[
g_0 + \Delta g_K = g_0 \frac{V_R - E_K}{V' - E_K}.
\]

(13)

(Note the typographical error in Eq. (14) of Donati and Kunov (1976).) An oscilloscope photograph of a typical after-potential is displayed in Fig. 4. From the Hodgkin and Huxley (1952) computation, \( \Delta g_K \gg g_0 \) at the maximum negative excursion of this potential; thus the (positive) voltage defined as \( V_+ \) on Fig. 4 is

\[
V_+ = V_R - E_K.
\]

(14)

If the after-potential as measured with respect to the resting potential is defined as \( V = V' - V_R \) (as in Fig. 3b), then Eq. (14) takes the form

\[
R' = \left[ 1 + \frac{g_0 \tau_1}{c} \left( 1 - \frac{V}{V_T} \right) \right]^{1/2} \left( 1 - \frac{V}{V_T} \right) \left[ \frac{1 + \frac{g_0 \tau_1}{c} \left( 1 - \frac{V}{V_T} \right)}{1 - \frac{V}{V_T}} \right].
\]

(12)
Velocity variations on unmyelinated axons

(13')

\[ g_0 + \Delta g_k = g_0 \frac{V_+}{V + V_+}. \]

Returning to Eq. (9), the effect of excess after-conductance indicated in Eq. (13') is to change the ratio of second to first pulse velocity by the factor

(15)

\[ R' = \left[ \frac{1 + \frac{g_0 \tau_1}{c}}{1 + \frac{g_0 \tau_1}{c} \frac{V_+}{V + V_+}} \right]^{1/2} \]

where \( \tau_1 \) is the time constant for the exponential segment below threshold on the leading edge of the first pulse.

When effects of both after-potential and after-conductance are considered together, Eq. (8) implies a velocity ratio

(16)

\[ R(T) = \left[ \frac{1 + K}{1 - \frac{V(T)}{V_T}} \right]^{1/2} \left[ 1 + K \left( \frac{1 - V(T)/V_T}{1 + V(T)/V_T} \right) \right]^{-1/2} \]

where

(17)

\[ K = \frac{g_0 \tau}{c}. \]

In the following section we compare 'calculations' from Eq. (16) with 'measurements' as described in Eqs (3) and (4). To make this comparison definite, we describe how each number in Eq. (16) was determined.

**Determination of threshold voltage, \( V_T \).**—As is indicated in Fig. 3, \( V_T \) is the voltage level above which the rate of rise is determined by the nonlinear ionic dynamics. A precisely defined value for this level which was suggested by Donati and Kunov (1976) is the point of maximum slope. It was measured on both recording electrodes before each data run.

**Determination of relative potassium potential, \( V_+ \).**—As described above, this is a positive number that gives the maximum negative excursion of the tail (see Fig. 4). It was measured on both recording electrodes before and after each data run.

**Determination of the after-potential, \( V(T) \).**—The zero point for the time \( (T) \) scale is taken as the time at which the pulse goes through its threshold value. Measurement of the voltage is as indicated on Fig. 4. This function was measured before and after each data run on both recording electrodes.

\[ \text{Fig. 5. Comparison of calculations with measurement of the function } R(T). \text{ Calculations are from Eq (16). Measurements are as defined in Eqs (3) and (4).} \]
\[ K = \frac{4G_0 \tau^2 \theta^2}{\sigma d}. \]

In our calculations, we took \( \sigma \) to be the Hodgkin and Huxley (1952) value of 2.9 meters/mho and \( G_0 \) to be the average Donati and Kunov value (1976) of 10 mho/m². Thus

\[ \frac{4G_0}{\sigma} = 13.8 \text{ meters}^{-1}. \]

We measured \( \tau \) directly from the exponential rise of a single pulse on both recording electrodes. The axon diameter, \( d \), was measured under a microscope at ten randomly selected points between the recording electrodes to obtain an average value. Distance between recording electrodes (\( D \) on Fig. 1) was obtained by measuring under a microscope. Single pulse velocity, \( \theta \), was then computed as the delay of the first pulse divided into \( D \).

4. Comparison of experimental results with the simple theory

For thirteen consecutive axons we have measured and calculated velocity variations as described in the previous two sections. A typical result of these measurements and calculations is displayed in Fig. 5. There are four ‘calculated’ curves because the data \( V_+ \) and \( V(T) \) were taken from both recording electrodes before and after the velocity ratio measurements. Differences between these four curves provide some estimate of uncertainty in the calculations. Dial reading uncertainty in the measurement of velocity ratio as defined in Eq. (5) is indicated above the data.

One of the qualitative predictions from Eq. (16) is that \( R(T) = 1 \) for \( V(T) = 0 \). In other words, the velocity of the second pulse should equal that of the first when the pulse spacing is equal to the time between threshold and the first ‘crossover’ on the tail as is shown on Fig. 4. This prediction is independent of the precision with which the parameters \( V_T, V_+ \) and \( K \) have been determined. In Fig. 6 we compare such time intervals defined in the following way:

\[ T_1 = \text{pulse spacing at which } R \text{ [determined from (3)] is equal to unity}; \]

\[ T_0 = \text{time interval between threshold voltage on leading edge and first cross of tail (see Fig. 4).} \]

Fig. 6 indicates a general equivalence of these two times within the precision of which they are known. The way we have estimated the range of time within which \( T_0 \) lies is indicated on Fig. 5. This range, which we call \( \Delta T_0 \), is taken as the difference between the largest and smallest of the four values of \( T \) for which \( V(T) = 0 \) (i.e. (cross-over)). In a similar, although less precise manner, we have estimated \( \Delta T_1 \) by sketching outer limits for the measured point spread, again as is shown on Fig. 5. Evidently our measurements are somewhat more precise than our calculations.

Fig. 6. Comparison of \( T_1 \) with \( T_0 \) as defined in Fig. 5. Hodgkin–Huxley calculations by J. Rinzel are indicated as ‘O’.

Comparisons of measurements with calculations for the remaining twelve axons are displayed in Fig. 7. These twelve displays plus Fig. 5 present the main experimental results of this paper. Values of leading edge rise time (\( \tau \)), axon diameter (\( d \)), pulse velocity (\( \theta \)) and temperature at which the parameter \( K \) was calculated are recorded in Table 1 for each of the thirteen axons studied.

One of the basic assumptions in our derivation of Eq. (16) was that the velocity of the first pulse is independent of its nearness to the second pulse. To show that this assumption is valid, we plot (in Fig. 8) the time delay of the first pulse as a function of pulse interval recorded during the measurements made on axon 31–3–80B. During these measurements, which took about an hour, the total temperature variation was less than 0.1°C. Although there is a slight (3%) increase (decrease) in first pulse delay (velocity), any changes correlated to pulse spacing must be less than the dial reading uncertainty of 0.3%.
from an inspection of Fig. 8. Thus any ‘measured’ point has an equal probability of being recorded with decreasing or with increasing pulse spacing.

5. Discussion

The reader may have noticed that although measurements on thirteen axons are presented in Figs 5 and 7, only twelve boxes comparing $T_1$ with $T_0$ are plotted on Fig. 6. The explanation for this apparent oversight is that for axon 1–4-80A the tails did not ‘cross over’ as indicated in Fig. 4. Thus $T_1 = 9 \cdot 3 - 10 \cdot 8$ ms, while $T_0 = \infty$. The reason for this singular discrepancy is not clear: one can surmise that the axon properties at both recording electrodes were qualitatively different from the section of axon lying between them. In any case the overall shape of $R(T)$ is not badly represented by the calculated curves.

From (17) we expect the parameter $K$ to be proportional to the leading edge rise time $\tau$. In Fig. 9 we display corresponding values of $K$ and $\tau$ taken from Table 1 and also values calculated from data of Donati and Kunov (1976) on eight axons, and an approximate proportionality is observed. The slope is about $10^3$ seconds$^{-1}$ which, from (17), should be the value of $g_0/c$. Since we have assumed that $g_0 = 20n\cdot(10\text{ mho/m}^2)$, this implies a membrane capacitance of 0·01 farad/m$^2$ or 1 microfarad/cm$^2$ which is the standard HH value. Thus we feel fairly comfortable with the conclusion

\begin{equation}
K \propto \tau
\end{equation}

where the proportionality constant ($g_0/c$) is independent of the axon diameter for an HH axon. From Eq. (8) we can write

The pulse delay data in Fig. 8 does show a slight ‘aging’ of the axon during the measurement. In order to obviate systematic error from this effect, we always began with measurements at the largest value of $T$, decreased to the smallest value of $T$, and then increased again to the largest value of $T$. (This procedure is evident

---

**Table 1**

<table>
<thead>
<tr>
<th>Axon</th>
<th>$\tau$ (ms)</th>
<th>$\theta$ (m/s)</th>
<th>$d$ (microns)</th>
<th>$K$</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–3-80A</td>
<td>0·093</td>
<td>19·90</td>
<td>423</td>
<td>0·110</td>
<td>17·6–18·0</td>
</tr>
<tr>
<td>7–3-80A</td>
<td>0·074</td>
<td>20·20</td>
<td>434</td>
<td>0·071</td>
<td>17·6–17·8</td>
</tr>
<tr>
<td>10–3-80A</td>
<td>0·131</td>
<td>14·32</td>
<td>320</td>
<td>0·152</td>
<td>15·0–15·8</td>
</tr>
<tr>
<td>11–3-80B</td>
<td>0·087</td>
<td>22·49</td>
<td>439</td>
<td>0·119</td>
<td>18·7–18·9</td>
</tr>
<tr>
<td>12–3-80C</td>
<td>0·117</td>
<td>17·18</td>
<td>492</td>
<td>0·113</td>
<td>17·6–18·2</td>
</tr>
<tr>
<td>13–3-80A</td>
<td>0·107</td>
<td>18·77</td>
<td>464</td>
<td>0·099</td>
<td>16·6–17·6</td>
</tr>
<tr>
<td>14–3-80C</td>
<td>0·100</td>
<td>17·10</td>
<td>406</td>
<td>0·098</td>
<td>14·9–15·1</td>
</tr>
<tr>
<td>15–3-80B</td>
<td>0·092</td>
<td>18·64</td>
<td>430</td>
<td>0·094</td>
<td>16·8–17·2</td>
</tr>
<tr>
<td>21–3-80A</td>
<td>0·091</td>
<td>19·40</td>
<td>433</td>
<td>0·099</td>
<td>17·3–17·4</td>
</tr>
<tr>
<td>24–3-80B</td>
<td>0·110</td>
<td>16·15</td>
<td>378</td>
<td>0·115</td>
<td>14·3–14·5</td>
</tr>
<tr>
<td>31–3-80B</td>
<td>0·108</td>
<td>16·05</td>
<td>393</td>
<td>0·105</td>
<td>14·4</td>
</tr>
<tr>
<td>1–4-80A</td>
<td>0·098</td>
<td>13·34</td>
<td>369</td>
<td>0·064</td>
<td>15·5–16·3</td>
</tr>
<tr>
<td>3–4-80A</td>
<td>0·079</td>
<td>19·97</td>
<td>349</td>
<td>0·098</td>
<td>20·1–20·7</td>
</tr>
</tbody>
</table>
Fig. 8. Time delay measured for the first impulse as a function of the pulse spacing.

\[ \tau \left[ 1 + \left( \frac{\theta_0}{c} \right) r \right] \theta^2 rc = 1. \]

Since \( \theta^2 rc \) is independent of fiber diameter for the HH axon, Eq. (19) implies that \( \tau \) is also independent of that quantity. Then from Eq. (18) we conclude that \( K \) should be independent of fiber diameter for an HH axon. (Note that although \( \theta^2 rc \) was observed by Pumphrey and Young (1938) to depend upon diameter as the \( 0.228 \pm 0.054 \) power, these authors suggest that this dependence may be related to systematic changes in axon loading by small branching fibers as the axon diameter increases.)

The temperature dependence of \( K \) should be that of \( \tau \) since \( g_0/c \) is independent of temperature for the HH axon. From (19) we see that \( \tau \propto \theta^{-2} \); and between 14°C and 22°C we find \( \theta = \theta_0[1 + 0.038(T_{\text{Temp}} - 18.5)] \) so we expect

\[ K = \frac{K_0}{[1 + 0.038(T_{\text{Temp}} - 18.5)]^2}. \]

From the data in Table 1 we calculate

\[ K_0 = 0.091 \pm 0.020 \]
where the ‘±’ indicates rms deviation. In Fig. 10 we plot values of \( K \) vs. temperature from Table 1 and also calculated from the data of Donati and Kunov (1976). Eq. (20) seems to give a fair representation of temperature dependence from 14°C to 24°C.

\[
K = \frac{0.091}{[1 + 0.038(T_{\text{Temp}} - 18.5)]^2}
\]

![Graph showing temperature variation of the parameter \( K \).](image)

**TEMPERATURE (°C)**

Fig. 10. Temperature variation of the parameter \( K \).

Examination of the comparisons between measured and calculated velocity ratios shown in Figs 5 and 7, reveals differences that appear to lie beyond the range of experimental uncertainty. Disagreements between measurements and calculations are notable for axons: 10–3–80A, 13–3–80A, 18–3–80A, 21–3–80A, 31–3–80B and 1–4–80A at smaller values of \( R \). In seeking an explanation for these differences, it should be emphasized that we have not adjusted any parameters in the theory. Every parameter has been individually measured with the exception of \( 4G_0/\sigma \) which was taken as 13.8 meters\(^{-1} \), the value assumed by Donati and Kunov (1976). There are at least three approximations made in the derivation of (16) which could lead to differences between calculation and measurement of \( R(T) \).

(i) Incorrect representation of potassium dynamics.—Our derivation of (13'), which gives the total after-voltage, followed the DK assumption that potassium conduction reaches stationary values appropriate for \( V(T) \) at each instant of time. That this assumption is not strictly true can be seen if we write (13') in the form

\[
G_L + G_K n^+ = (G_L + G_K n_0^+) \frac{V^+}{V + V^+}
\]

where the total (leakage plus potassium) conductance on the left-hand side has been expressed in the Hodgkin–Huxley form. Miller and Rinzel (1981) have used the HH equations to calculate \( n^+ \) with \( G_L = 3 \) mho/m², \( G_K = 360 \) mho/m² and, at a temperature of 18.5°C, \( n_0 = 0.3179 \), and \( V^+ = 10 \) mV (defined as on Fig. 4). Then (13') can be solved to obtain

\[
n = 0.3179 \left[ \frac{1 - 0.0815 V}{1 + 0.11 V} \right]^{1/4}
\]

as the relationship between \( n \) and \( V \) calculated under the assumptions made in the derivation of (16). Comparison of (21) with the numerical results shown in Fig. 6a of Miller and Rinzel (1981) indicates

Maximum error in \( n \sim 10\% \).

Then (13') implies

- Maximum error in \( G_K \sim 46\% \).
- Maximum error in \( G_K + G_L \sim 25\% \).

Since the constant \( K \) in (16) is about equal to 0.1, the corresponding error in \( R(T) \) is reduced by a factor of 1/20 to

Maximum error in \( R(T) \sim 1.3\% \).

(ii) Neglect of sodium turn-off dynamics.—In the derivation of (16), and also in the analysis of DK, effects of relaxation by the sodium turn-off parameter \( (h) \) to its resting value were ignored completely. From the original Hodgkin–Huxley work (see Fig. 19 of Hodgkin and Huxley, 1952), however, it is clear that the time
course of $h$-relaxation is roughly the same as that of $g_k$. For example at a temperature of 6°C and $T = 10$ ms. (which corresponds to $T/T_1 = 0.5$) the

Maximum error in $h \sim 10\%$.

To relate this error to that of $R(T)$, we turn to a little known work by Pastushenko et al. (1975) which discusses the effect of sodium turn-on dynamics upon conduction velocity. They show that for nerve parameters appropriate to squid axon the dependence of pulse velocity on initial value $(h_0)$ of sodium turn-on is

$$\theta \sim h_0^{1/3}. $$

Thus the corresponding

Maximum error in $R(T) \sim 1.3\%$.

(iii) **Error in estimating the resting conductance.**—From the Hodgkin–Huxley data (see Table 3 of Hodgkin and Huxley, 1952) membrane resting conductance can be expected to vary between

$$5 < G_0 < 20 \text{ mho/m}^2.$$

To evaluate the parameter $K$ in (16) we have assumed $G_0 = 10 \text{ mho/m}^2$ as did Donati and Kunov (1976). Thus the correct values of $K$ might be expected to vary by as much as one half to twice the values computed and displayed in our Table 1. The differential dependence of $R$ upon $K$ can be appreciated from inspection of the relative derivative

$$\frac{dR}{dK}R = \frac{KV}{2V_+} \left[ \frac{1 + V_+/V_T}{(1 + K)(1 + V_+/V_T - K V_+/V_T)} \right].$$

This gives the ratio of small per cent variations in $R$ to per cent variations in $K$. As previously noted, $R$ becomes independent of $K$ as $V$ goes to zero. For $V = 1/2 V_+$, we find

$$\frac{\% \text{ change in } R}{\% \text{ change in } K} \approx 60$$

so a 100% error in $G_0$ should lead to an error of about 1.7% in $R$.

In summary, each of the three sources of error in (16) could introduce errors of the order of 1.5%. It is evident from Figs 5 and 7 that these individual errors are not much larger than the ‘dial error’ and in many cases substantially smaller than the variation in the calculated curves.

Another way to evaluate the reliability of (16) is to compare its predictions with calculations on the Hodgkin–Huxley axon. A particularly important prediction is that $T_1 = T_0$; the pulse spacing for equal velocity is equal to the time of cross-over from hyperpolarizing to depolarizing phase on the tail. As Fig. 6 shows (24)

$$T_1 \approx T_0$$

for our experimental observations. Calculations by John Rinzel on the HH axon indicate values of $T_1$ and $T_0$ as in Table 2.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>$T_1$ (ms.)</th>
<th>$T_0$ (ms.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5</td>
<td>4.77</td>
<td>6.14</td>
</tr>
<tr>
<td>14.0</td>
<td>7.25</td>
<td>8.53</td>
</tr>
<tr>
<td>6.3</td>
<td>16.0</td>
<td>17.2</td>
</tr>
</tbody>
</table>

Thus

(25)  

$$T_1 < T_0$$

for the HH axon where the inequality in (25) seems to lie beyond the experimental error in (24). Since correction of the HH equations for potassium ion accumulation around the fiber (Adelman and FitzHugh, 1975) tends to depress the function $R(T)$ (George and Silberstein, 1977) and therefore increase $T_1$, failure to include this effect might explain the small difference between experimental observation and HH calculations.

Finally we note that in certain of the axons studied there was evidence of ‘triphasic’ tails which, from Eq. (16) implies that $R(T) = 0$ for one or more additional values of $T$. This is particularly evident for axon 12-3-80C (see Fig. 9) but also for axons 11-3-80B and 21-3-80A. The measured values of $R(T)$ for 12-3-80C show a rather clear triphasic behavior, but for 11-3-80B and 21-3-80A such a conclusion is obscured by the basic instrument error (‘dial error’).

6. **Conclusions**

(1) We confirm the observation by Donati and Kunov (1976) that there is a special time interval (we call it $T_1$) between action potentials (AP’s) on squid giant axons at which the speed of a following AP equals that of a leading AP. For time intervals shorter (longer) than $T_1$, the second AP goes more slowly (faster) than the first.

(2) We have derived an approximate expression, Eq. (16), for the ratio, $R$, of following to leading pulse speeds as a function of time interval, $T$. This expression depends only on the shape of an isolated AP and a dimensionless parameter $K$.

(3) $K$ is independent of fiber diameter for the Hodgkin-Huxley (HH) axon.

(4) Temperature dependence of $K$ is roughly as $K_0/[1 + 0.038(Temp - 18.5)]^2$ between 14°C and 24°C where $K_0 = 0.091 \pm 0.020$. 


Velocity variations on unmyelinated axons


APPENDIX

Calculation of $R/\bar{R}$

In this appendix we consider how the exact value of the velocity ratio, $R$, differs from the approximate value, $\bar{R}$, defined in Eq. (3). We assume that $R(T)$ is given exactly by the expression

$$R(T) = 1 + a \left( \frac{T}{T_1} - 1 \right) .$$

Then with $T(x)$ defined as on Fig. 1,

$$\frac{dT}{dx} = \frac{1}{\theta_1(x)} \frac{1}{\theta_1} = \bar{R} \left[ \frac{a(1 - T/T_1)}{1 - a(1 - T/T_1)} \right] ,$$

which can be integrated (with the boundary condition $T(0) = (t_3 - t_4)$) to obtain

$$\frac{x}{\bar{R}} = \frac{T_1}{a} \ln \left[ \frac{1 - t_3 - t_4}{T_1} \right] - \frac{T - t_3 + t_4}{T_1} .$$

From the boundary condition, $T(D) = t_4 - t_2$ (noting that $\bar{R} = D/(t_2 - t_1)$), we obtain

References


\[ a = \frac{T_4}{t_4 - t_3} \ln \left[ \frac{1 - t_3 - t_1}{T_1} \frac{t_4 - t_2}{1 - t_4 - t_2} \right] \]

from which \( R(T) \) in Eq. (A-1) is obtained as a function of \( T_1 \) and \( t_1, \ldots, t_4 \). The corresponding expression for the ratio \( R/R \) is presented in Eq. (4).

In general, of course, the exact function \( R(T) \) is not given by Eq. (A-1); it is a function with the character indicated in Fig. 2. However, if \( T_1 \) is replaced by \( T_1' \) as defined in Fig. 2, the exact \( R(T) \) can be approximated by a tangent with the form given in Eq. (A-1).