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Rita Guttman
CUNY Brooklyn College

Lon Hachmeister
CUNY Brooklyn College

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Effect of Calcium, Temperature, and Polarizing Currents upon Alternating Current Excitation of Space-Clamped Squid Axons

RITA GUTTMAN and LON HACHMEISTER

From the Department of Biology, Brooklyn College, Brooklyn, New York 11210, an d Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Mr. Hachmeister's present address is Department of Oceanography, University of Washington, Seattle, Washington 98115.

ABSTRACT   Alternating current threshold excitation of space-clamped squid giant axons was measured as a function of frequency, external calcium concentration, temperature (from 10° to 35°C), and hyper- and depolarizing steps. In normal axons there is usually an optimum frequency at about 120 Hz, at which the threshold is a minimum. The threshold rises at both lower and higher frequencies to give a resonance curve. Low calcium causes an increase in optimum frequency, a decrease in current threshold, and an increase in sharpness of tuning in both real axons and axons computed according to the Hodgkin-Huxley formulation; high calcium causes opposite effects. An increase in temperature causes an increase of optimum frequency, an increase in sharpness of tuning, and an increase in threshold current in both real and computed axons. The Q_{10} for the effect of temperature upon optimum frequency is 1.8 in real and computed axons at moderate temperatures. Hyperpolarization causes (a) a decrease in optimum frequency, (b) a decrease in sharpness of tuning, and (c) an increase in threshold. Depolarization causes opposite effects.

INTRODUCTION

In previous work (Guttman, 1969; Guttman and Barnhill, 1970) the natural frequency of subthreshold oscillations and repetitive firing was studied in space-clamped squid axons during variation of temperature and ionic environment, and the experimental results compared with computations based on the Hodgkin-Huxley (HH) formulation (1952). In the previous studies, the membrane was stimulated by threshold or just subthreshold steps of current. The present work investigates this subject further, using, however, sinusoidal stimulation and examining the resonance curves resulting from threshold stimulation at the various frequencies.

The topic is an old one. Classical experiments were carried out by Arvanitaki (1939), by Monnier and Coppée (1939), by Hill, Katz, and

Solandt (1936), by Tasaki and Sato (1951), and by still other earlier workers. These experiments were on the initiation of propagating impulses. When interpreted, these results were described in terms of the formal two-factor theory of excitation. An important conceptual simplification in the present work is the use of the sucrose gap space clamp on squid. Later the results are to be again compared with HH calculations, which it was not possible to do for the early work.

As was pointed out long ago by Katz (1939) the phenomenon of an optimum frequency in nerve is a natural consequence of the fact that a nerve fiber fails to respond to a given current if that current (*a*) does not last long enough or (*b*) does not rise fast enough. It is not therefore necessary to invoke some obscure "resonance" mechanism for nerve. Any model or equivalent circuit for the excitable membrane (cf. Cole, 1968), however, does have to take into consideration the resonance phenomenon and the natural frequency of subthreshold oscillation (such as is obtained in low calcium). Since calcium has such a marked effect upon excitability, it was decided to study the effect of variation of the concentration of this ion upon resonance curves.

Since temperature effects have long been felt to be an important aspect of any physical model that is developed for the excitable membrane, it was thought that it might be of interest to investigate the temperature dependence of resonance curves and optimum frequencies. Very little work has been carried out on the effect of temperature upon resonance curves of nerve. Conti (1970) did not study threshold directly but rather membrane impedance. Moreover, he did not study the effect of temperatures higher than about 16°C.

The present study includes an investigation of the effect of hyperpolarization and depolarization upon resonance curves, because, since we were dealing with axons in sucrose gaps and since the sucrose gap technique is known to result in hyperpolarization of the membrane (Blaustein and Goldman, 1966), we wished to establish that hyperpolarization did not in itself affect or obscure our data in any way.

Whenever calculated values are presented in the figures or discussed in the text, they were computed by Frederick A. Dodge, Jr., at our request.

MATERIAL AND METHODS

The dissection, the axon chamber, and the temperature control were identical with those used in previous work (cf. Guttman and Barnhill, 1970). The artificial seawater and the low and high calcium solutions were also made up as in the previous work. All axons were equilibrated in running natural seawater for at least a half hour before they were mounted in the chamber. In the calcium experiments, the axon was first bathed in a "holding" solution of 35 mM Ca (considered equivalent to seawater) and, when fairly well-equilibrated, the artificial seawater solutions containing the indicated amount of calcium were substituted. In all experiments involving alteration of the ionic environment, the temperature was maintained at 20°C.

Instrumentation   Stimulation of the axon consisted of a gated sinusoidal current provided by a Krohn-Hite 4100 signal generator (Krohn-Hite Corporation, Cambridge, Mass.) (Fig. 1). The gate was activated by pulse from a modified Tektronix pulse generator and shut on the completion of three cycles of the stimulus by counting the down transitions of the accompanying square wave output of the signal generator. These precautions were taken to prevent overstimulation of the axon, especially at high frequencies. The pulses were applied once every 2 sec to the platinum electrode in the central experimental compartment of the axon chamber through a 0.47 megohm isolating resistor. Stimulation current was measured by an operational amplifier and a 10 kilohm resistor serving as a current to voltage transresistor (Fig. 1).

A graphical representation of threshold current intensity vs. log frequency of stimulation was displayed on a Tektronix 515A oscilloscope. The sinusoidal stimulus was first passed through a peak-to-peak AC-DC voltage converter and then to the vertical deflection plates of the oscilloscope. A frequency to voltage converter was used to translate the frequency of the stimulus into a DC voltage (cf. Philbrick Applications Manual for Computing Amplifiers, Philbrick Research Inc., 1966). The log of this voltage was taken and applied to the horizontal deflection plates of the oscilloscope. An "on effect" was occasionally noticed at high frequencies. In any case, responses to the first or second stimulus were ignored and only responses to the third sine wave were recorded. Thus the threshold criterion was a response to the third sine wave of the stimulus.

Differential recording was done between the two Ag/AgCl electrodes. The potential developed by each was buffered by an operational amplifier used as a unity gain follower, and the difference was taken by another operational amplifier acting as a subtractor with a gain of 10. This signal was monitored with a Keithley 610BR electrometer to give the resting potential and was also supplied to a base line stabilization circuit. This circuit consisted of synchronization and track-and-hold memory circuits, and an adder. The track-and-hold memory circuit was synchronized with the stimulus in such a way that the base line was sampled for 133 msec, ending about 16 msec prior to each stimulation. The negative of this potential was held for the remainder of the 2 sec repetition period and was added to the unaltered response signal. The sum produced the lower trace on the oscilloscope. The effect of this circuit was to set the oscilloscope base line to zero immediately before each stimulation, thus virtually eliminating the effect of changes in resting potential while introducing no distortion to the active response. In this way, large amplifications (up to 1 mv/cm) of interesting portions of the response became practical.

Computations   Computations were carried out on the basis of the Hodgkin-Huxley formulation in the manner of Huxley (1959). Voltage shifts corresponding to changes in calcium concentration were calculated from the formula:

$$\Delta V = -9.3 \ln \frac{[Ca]}{44}$$

where Ca concentration is expressed in millimoles.

In accordance with the above formula, the holding solution (35 mM CaCl₂) cor-

FIGURE 1. Block diagram of stimulating and recording circuits for and recording circuitry; B, current vs. log frequency CRO graphical study of AC excitation of space-clamped squid axons. A, stimulating display.

responded to a voltage shift of 1.87 mv. Low calcium (18.7 mm $CaCl_2$) for the computed axon involved a voltage shift of 8.0 mv, while high calcium (107 mm $CaCl_2$) corresponded to a voltage shift of -8.0 mv.

With regard to the computations involving the effect of temperature variation, it was assumed that only the rate constants were affected by temperature increasing with temperature with a Q_{10} of 3 (Huxley, 1959).

Deterioration In order to understand the effect of changing the ionic environment upon the resonance curve, it was first necessary to determine the effect of deterioration upon the curve. The resonance curve was monitored for 122 min in an axon at 10°C bathed in artificial seawater (Fig. 2, where the results are plotted
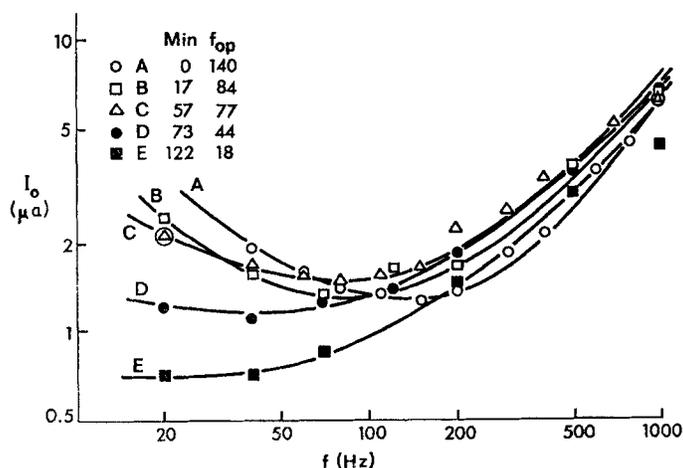

Figure 2. Effect of deterioration upon resonance curve of space-clamped squid axon stimulated by sinusoidal AC current. Threshold current, I_o, in microamperes vs. frequency (Hz) both on log scales. Curves recorded at times indicated. All points on same axon at 10°C in artificial seawater. See text for discussion.

logarithmically), when the excitability finally disappeared. (It must be confessed that after run C, the fiber was overstimulated out of sheer impatience to hasten its demise, and thus it would doubtless have survived longer under normal circumstances.) During this time interval a number of changes occurred.

1. The amount of current required at low frequencies for stimulation decreased. This might at first appear surprising; i.e., that the seeming "threshold" should decrease rather than increase. However, it should be kept in mind that we are dealing with a membrane hyperpolarized by sucrose gaps (cf. Blaustein and Goldman, 1966) and what we are probably actually observing is a gradual decrease in this hyperpolarization. The fall in resting potential with time, as monitored on an electrometer, favors this view.

2. With time, the optimum frequency (the frequency at which a minimum current intensity excites) decreased in this particular fiber from 140 to 18 Hz (Fig. 2). (Generally the optimum frequency of fibers in good condition varied from about 90 to 160 Hz at the start of experiments.)

3. The resonance curve becomes less symmetrical as time elapses. Specifically, the low frequency portion becames flat, so that frequency variation has little effect upon excitability in that region; i.e., the membrane is very poorly tuned because of changes in threshold in the low frequency region. However, it should be noted that in this particular fiber and at this low temperature, this effect did not become marked until after about an hour had elapsed.

Of course, it would be interesting to know what changes in which membrane parameters are associated with this decrease in tuning. If deterioration alters the sodium conductance, one would expect the low frequency end of the resonance curve to be more affected than the high frequency end since the high frequency end is largely capacitative. This indeed seems to be the case (Fig. 2). The change at the low frequency end on deterioration might be caused by sodium inactivation or delayed rectification or a mixture of both.

It is a possibility, however, that sodium inactivation is not the cause of the change at the low frequencies since as will be described later, we were able to show (Fig. 9) that the resonance curve does not sharpen up when hyperpolarized.

It was suggested that the changes which occur on deterioration might be caused by increased leakage conductance. When, however, leakage conductance was increased for the calculated HH axon, the effect was opposite to what was found experimentally (*decreased* leakage conductance rather than increased leakage conductance mimicked the results found experimentally) so that this idea had to be abandoned.

The data shown in Fig. 2 suggest that only fresh fibers should be oscillatory because only fresh fibers are well tuned. In our experience with squid fibers, this has indeed been the case. We observed oscillation only in fibers from animals that were in excellent condition and then only when they were freshly dissected.

Now, with the effect of the lapse of time upon the membrane analyzed (whether this constitutes merely deterioration or other changes as well), it became possible to study the effect of varying the concentration of the calcium ion and the effect of variation of temperature upon the resonance curve.

RESULTS

When a strength-frequency curve, using threshold responses to AC sinusoidal stimulation, is plotted on log-log coordinates for a space-clamped squid axon, a nearly symmetrical curve is obtained, as was found earlier by Monnier (cf. Monnier and Coppée, 1939) for frog nerves. Such curves give information about the oscillatory nature of the membrane and some of the possible characteristics of the equivalent circuit.

One of the aspects of the curve that is of interest is the minimum, the frequency at which the threshold is the lowest, sometimes referred to as the optimum or natural frequency.

Another aspect of the curve which is of interest is where on the intensity parameter the entire curve or its minimum falls: the intensity threshold, I_o.

A third aspect which is significant is the sharpness of the curve; i.e., the degree of tuning (the reciprocal of the damping factor). Monnier (1952) has

suggested that the damping factor should be identified with Kennelly's bluntness of resonance, B (Kennelly, 1923). B can be readily calculated from the resonance curves. If f_1 and f_2 are respectively the frequencies at which the threshold current is $\sqrt{2}$ times the current, I_o, at the optimum frequency, f_{op}, then

$$B = \frac{f_1 - f_2}{f_{op}}.$$

Kennelly was the first to emphasize that resonance curves of electrical or mechanical systems are symmetrical in relation to the ordinate of the optimum frequency, if the frequencies are expressed logarithmically. This symmetry holds in most cases for resonance curves of nerves. Its consequences are the following:

1. The optimum frequency is the geometric mean of any couple, f_1 and f_2, of frequencies, above and below the optimum, measured at any multiple of the optimal current; i.e., $f_{op} = \sqrt{f_1 f_2}$ or $\log f_{op} = \frac{1}{2} \log f_1 - \frac{1}{2} \log f_2$. The symmetry of the curves, when it is evident, permits an accurate determination of the optimum frequency. The locus of the midpoint of the horizontal chords of the curve is a straight line. Its intersection with the curve indicates the optimum frequency.

2. The relative damping or bluntness of resonance, or width index of the curves (or their reciprocal, i.e., sharpness of resonance, acuity index, or degree of tuning) can be calculated from any couple of f_1 and f_2 frequencies, determined at any multiple of the optimal current, I_o.

Kennelly chose the value $\sqrt{2} I_o$ because in that case the damping factor B is simply related to the parameters of an R, C, L circuit:

$$B = \frac{R}{2} \sqrt{\frac{L}{C}}.$$

For practical reasons, i.e., a more precise determination of threshold, another multiple of the optimal intensity, I_o, may be used, for instance, $2I_o$. The couple of frequencies f_1 and f_2 then lies upon steeper factors of the curves, thus ensuring a better accuracy of their evaluation. In order to evaluate by simple inspection the relative bluntness of a set of resonance curves, these curves should be plotted on log-log coordinates. The length of the chords for any given increment of the ordinate above the optimum allows for a relative evaluation of the set of curves.

With regard to a comparison of our experimental squid membrane to an R, C, L circuit, it should be kept in mind that in our experimental setup the membrane is behaving very much like the excised Hodgkin-Huxley axon (1952) except that, as is well known, sucrose gaps tend to repolarize a mem-

brane, and this has an established effect on the apparent inductive reactance. According to Mauro, Conti, Dodge, and Schor (1970), if the membrane is hyperpolarized, the inductive reactance changes to a capacitative reactance with potassium turn off.

To recapitulate, then, we will consider three characteristics of the resonance curves in the following development: (a) optimum frequency, (b) threshold intensity, and (c) sharpness of tuning, and an attempt will be made to show how each is affected by variation of calcium ion concentration, by temperature variation, and by polarizing currents.

Effect of Calcium Ion Concentration   Depending upon our primary aim, we either (a) took runs as rapidly as possible, bracketing the run in the experimental solution of increased or decreased calcium between a previous and subsequent run in the holding solution: 35 mM $CaCl_2$ or (b) followed the effect of change in concentration of calcium with time by recording a number of resonance curves in abnormal calcium concentrations before returning to the control run in the holding solution.

Eight runs were carried out on seven axons treated with low calcium solutions and twelve runs were done on nine axons in high calcium.

Typical effects of varying the calcium ion concentration in the bathing medium of the axon are shown graphically for the experimental axon in Fig. 3
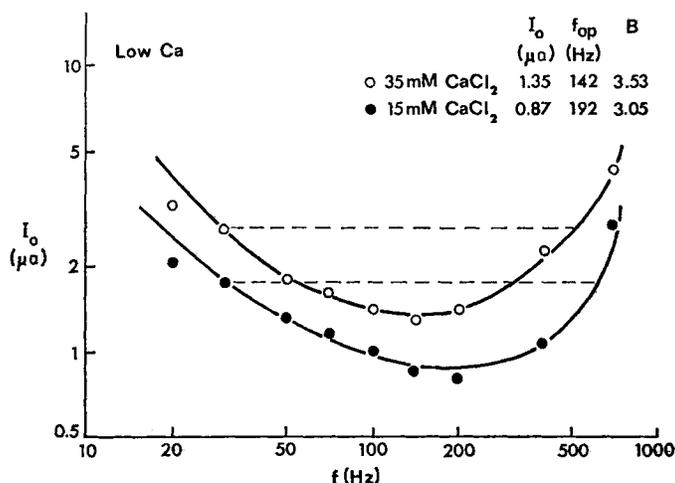

FIGURE 3.   Effect of low calcium (15 mM $CaCl_2$) on resonance curve of experimental axon. Threshold current, I_o, in microamperes vs. frequency (Hz), both on log scales. Note that lowering the calcium concentration causes a decrease in threshold current, an increase in optimum frequency, and a decrease in the width index, B. Dashed lines are drawn across the curves at a current intensity twice that of the threshold at the optimum frequency in order to compute the width index.

(low calcium) and Fig. 4 (high calcium) and for the computed axon, Fig. 5 (low and high calcium).

Effect of Calcium upon Optimum Frequency   It is clear that lowering the calcium concentration results in a raising of the optimum frequency in both experimental and computed axons. An increase in the calcium concentration, on the other hand, results in a decrease in optimum frequency of the resonance curve in both experimental and computed axons.

Effect of Calcium upon Threshold Intensity   Lowering calcium concentration decreases the current threshold in both the experimental and computed axon. Raising the calcium level increases the intensity of current necessary for excitation in both the experimental and computed axons.

Effect of Calcium upon Degree of Tuning   The sharpness of the resonance curve or the degree of tuning (which is related to the damping factor) is also affected by varying the calcium concentration. Rather than rely upon casual inspection, which can be misleading, the formula of Kennelly was invoked, as mentioned above. Lowering the calcium concentration was found to undamp the membrane (increase sharpness of tuning) in both the experimental and computed axons, which was to be expected since we know from experience that the membrane has a greater tendency to oscillate when deprived of calcium.

In the experimental fiber of Fig. 3, when the fiber was bathed in the holding solution of 35 mM $CaCl_2$ and then treated with a low calcium solution of 15 mM $CaCl_2$, the width index, B, dropped from 3.53 to 3.05.

Raising the calcium concentration in the experimental axon of Fig. 4 from the holding solution of 35 mM to 80 mM $CaCl_2$, caused increased damping, i.e. the width index rose from an initial 3.38 to 4.08, while in another fiber, raising the calcium concentration from the initial 35 mM to 112 mM, raised the width index from 2.80 to 5.87 (Fig. 4). Of course, the absolute values have little significance since they depend not only upon the concentrations of calcium used but also upon how long they were permitted to act before measurements were taken.

Effect of Calcium upon Computed Axon   The effect of varying the calcium concentration upon the resonance curves of the theoretical axon, calculated in accordance with the Hodgkin-Huxley formulation, is shown in Fig. 5, where threshold current in microamperes vs. frequency (Hz), both on log scales, is displayed. The results are similar to the experimental results: increasing the calcium concentration causes an increase in threshold current and the amount of damping, while it decreases optimum frequency. The sharpness of the curve in low calcium corroborates Cole's finding of a true resonance in low calcium and a pararesonance at higher concentrations (Cole and Marmont, 1942).
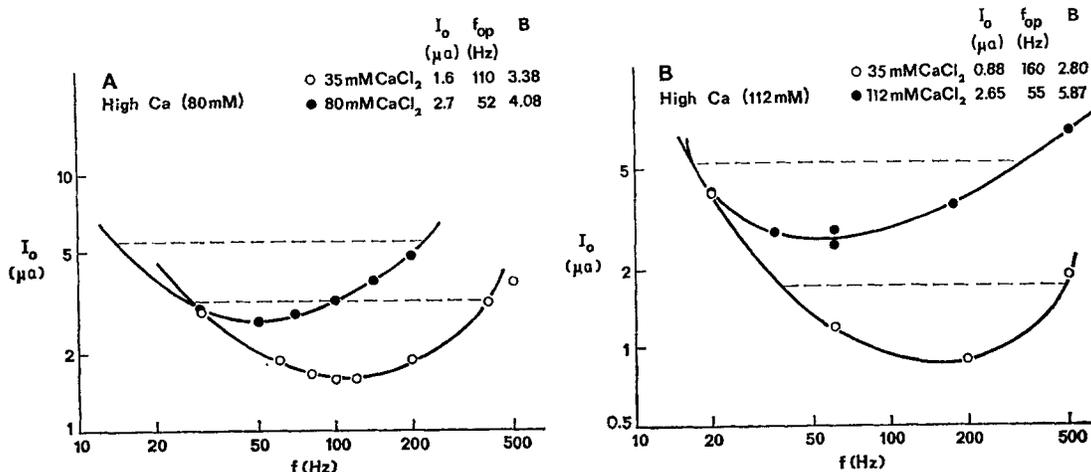
FIGURE 4. Effect of high calcium (A, 80 mM $CaCl_2$ and B, 112 mM $CaCl_2$) on resonance curve of experimental axon. Threshold current, I_o, in microamperes vs. frequency (Hz), both on log scales. Note that raising the calcium concentration has less effect at low frequencies than at higher frequencies. Increased calcium concentration causes an increase in threshold current, a decrease in optimum frequency, and an increase in the width index, B.

Differences between the empirical (Figs. 3 and 4) and computed axons (Fig. 5) are in these plots more apparent than real because of the distortion inherent in log-log plotting, but log-log plotting is convenient for showing the results compactly. Discrepancies between the empirical and computed axons are not believed to be significant.

Effect of Temperature The results of temperature variation upon resonance curves became clear only after improvement of instrumentation permitted a speeding up of readings so that many points could be taken before deterioration occurred. Only after the changes due to deterioration could be avoided, was it possible to analyze temperature effects with any degree of confidence.

Temperature has a marked effect upon resonance curves in a number of ways. Symmetrical resonance curves are obtained between 10° and 30°C. At 35°C the curve becomes irregular and at 40°C only a few readings can be obtained before excitability fails. As Figs. 6–8 clearly indicate, an increase in temperature caused an increase in optimum frequency, an increase in threshold current, and an increase in sharpness of tuning.

It should be noted (Fig. 6) that temperature affects the low frequency end of the resonance curve markedly, but not the high frequency end. This is not surprising, inasmuch as the high frequency end is largely due to capacitive effects.

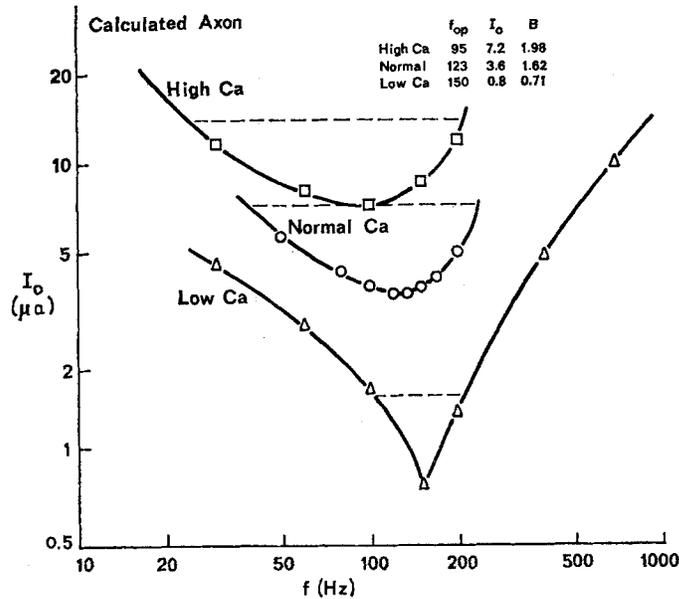The effect of temperature upon the threshold current at optimum fre-

FIGURE 5.   Effect of lowering and raising the calcium concentration on resonance curve of computed axon. Threshold current, I_o, in microamperes vs. frequency (Hz), both on log scales. See text for discussion.
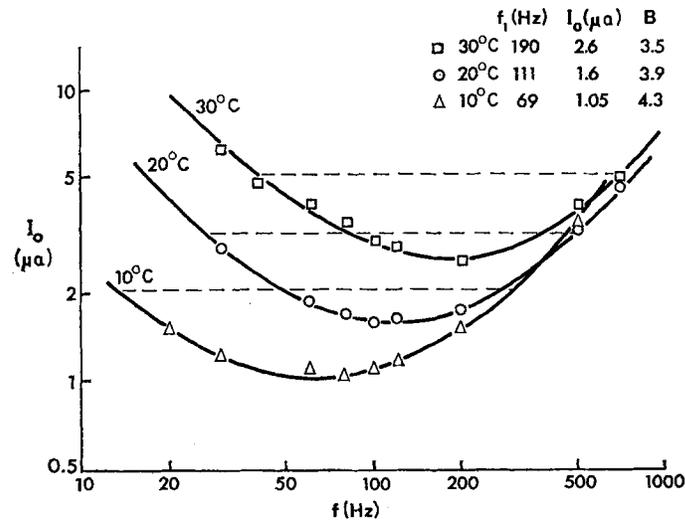

FIGURE 6.   Strength-frequency (resonance) curve of space-clamped squid axon, stimulated by AC sinusoidal current at 10°, 20°, and 30°C, respectively. Threshold current in microamperes vs. frequency (Hz), both on log scales. Note that the effect of temperature is much greater at low frequencies than in the high frequency (capacitative) region. Note also that both optimum frequency and threshold current increase with increasing temperature, while the width index, B, decreases. A dashed line is drawn across each curve at a current intensity twice that of the threshold current at the optimum frequency for calculation of B, the width index.

quency for both experimental and computed axons is shown in greater detail in Fig. 7. Experimental runs on eight axons are displaced vertically up or down by an average of 17.5% for best fit by eye and give a Q_{10} of 1.6. The units on the ordinate apply to the theoretical curve (solid circles, heavy broken line) only, which has not been displaced to fit the experimental curves. It is clear that not only does the theoretical curve have a higher threshold, but also more importantly that the theoretical axon shows more temperature dependence than most of the real axons.
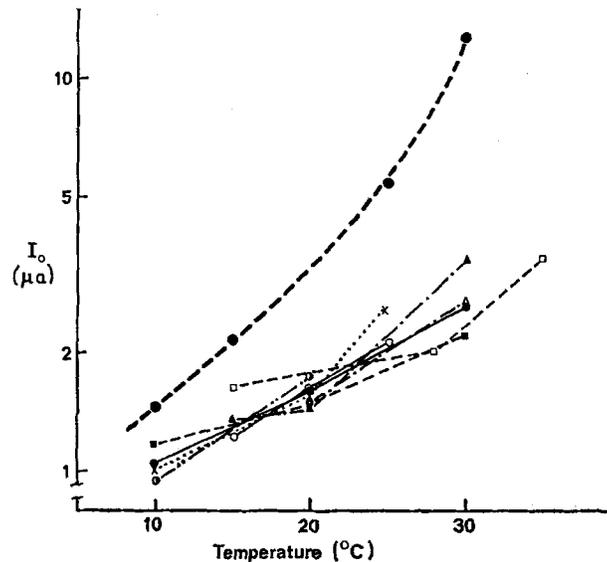

FIGURE 7. Effect of temperature on threshold current, I_o, required at optimum frequency for both real and computed axons. Current in microamperes on log scale vs. temperature in °C on linear scale. Composite of runs on eight real axons displaced up or down by an average of 17.5% for best fit. Theoretical run not displaced. Units on ordinate refer to computed axon only. Note that the computed axon is more temperature-dependent than the real axons. See text for further discussion.

The effect of temperature upon the optimum frequency of real and calculated axons is shown in Fig. 8. In this figure, the runs for the real axons have not been displaced vertically for best fit. Also, in this figure the optimum frequency values for the computed axon are based upon calculations of AC admittance. AC admittance gives the same shape of curve as AC stimulation would have (cf. Chandler, FitzHugh, and Cole, 1962) but is much easier to calculate. Both the linear portion of the curve for the theoretical axon and the curves for real axons give the same Q_{10} of 1.8 for the temperature dependence of the optimum frequency. Using entirely different methods, Conti (1970) found a Q_{10} variable from 3.7, around 4°C (contrary to our result) to 1.9, around 15°C (similar to our result) for the temperature dependence of the
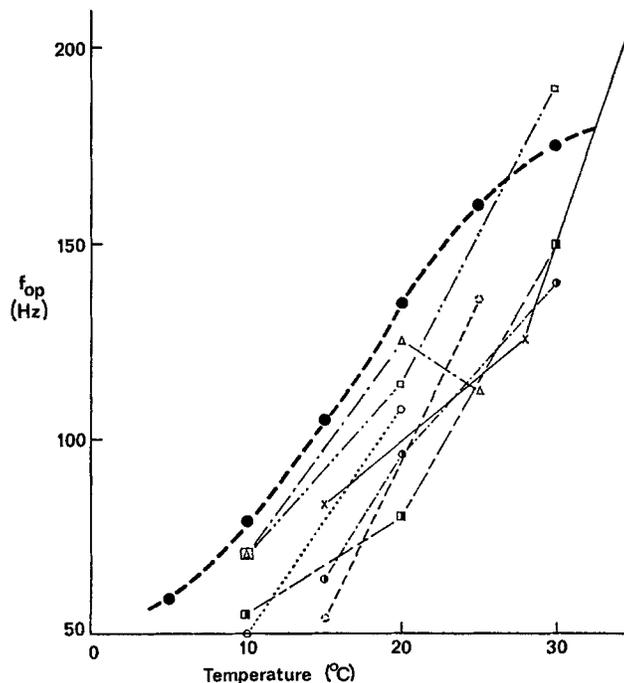
FIGURE 8. Effect of temperature upon optimum frequency of real and calculated axons. Optimum frequency in Hz vs. temperature in °C both on linear scales. Heavy broken line and filled circles indicate results for computed axon. See text for discussion.

optimum frequency. He did not, however, investigate temperatures above 16°C.

Effect of Polarizing Currents   Since it was known that the double sucrose gap method, which we were using, results in hyperpolarization of the membrane (Blaustein and Goldman, 1966) and also since deterioration, which is always a factor to be reckoned with, was shown to cause a loss of this hyperpolarization with time, it was decided that it would not be amiss to study the effect of depolarizing and hyperpolarizing currents per se upon the resonance curves and upon optimum frequency in particular (Figs. 9 and 10), for axons held at various temperatures.

All the runs displayed in Fig. 9, were taken on the same experimental axon at 10°, 20°, and 30°C, and these are typical results. At each temperature, a "normal" curve was compared with a curve obtained during the passage of a 15 mv depolarizing[1] or a 15 mv hyperpolarizing pulse and then another normal curve was obtained, showing almost perfect reversibility. The effects of

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[1] In accordance with the accepted sign convention, "depolarizing currents" will be used to indicate a departure from the resting potential in the direction of a decrease in the absolute potential. "Hyperpolarizing current" has the opposite connotation.
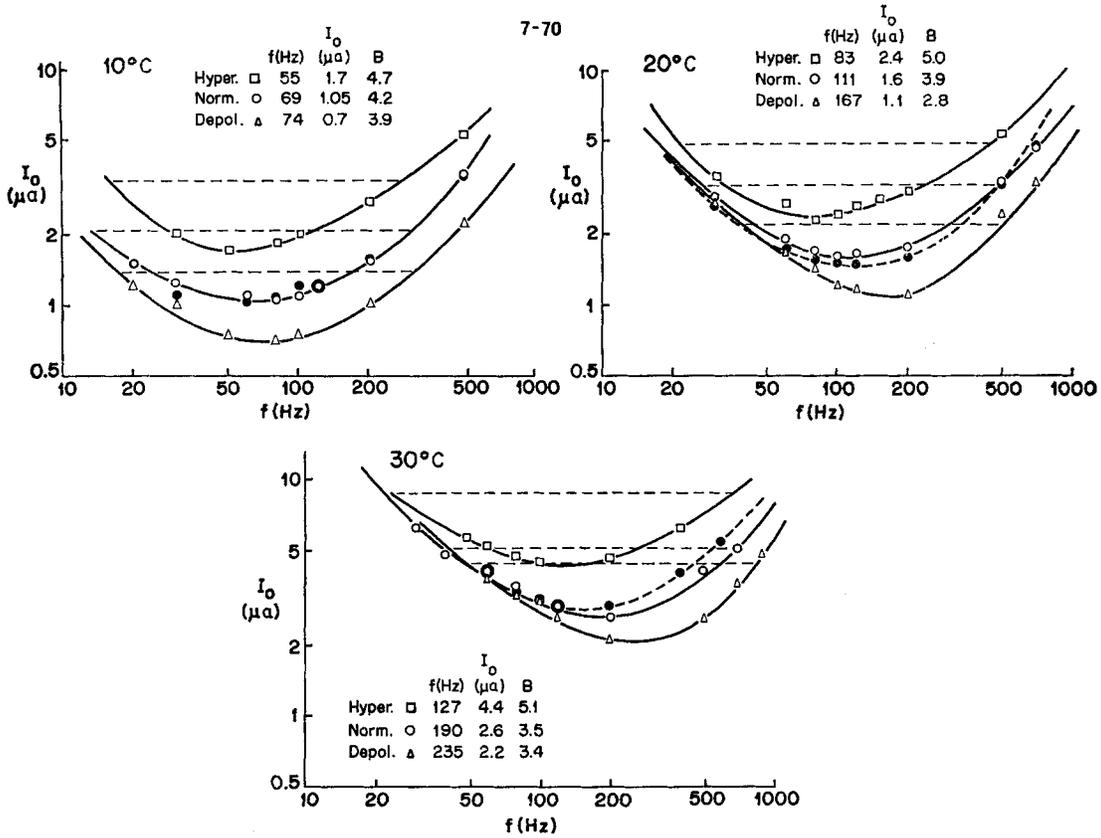
FIGURE 9. Effect of 15 mv depolarizing and hyperpolarizing currents on resonance curves of real space-clamped squid axons at 10°, 20°, and 30°C. Threshold current in microamperes vs. frequency (Hz) both on log scales. All points taken on same axon. Note excellent recovery (solid circles) in each case. Typical results. See text for discussion of results.

hyperpolarizing pulses were: (a) an increase in threshold current, (b) a decrease in sharpness of tuning, and (c) a decrease in optimum frequency. The effects of a depolarizing current pulse were (a) a decrease in threshold current, (b) an increase in sharpness of tuning, and (c) an increase in optimum frequency.

Fig. 10, where optimum frequency is plotted against temperature for the same hyperpolarized, normal, and depolarized axon presented in Fig. 9, indicates that experimental hyperpolarization does not markedly alter the temperature dependence of the optimum frequency. Thus, although our normal axons were hyperpolarized by the sucrose gaps, we have established that the temperature dependence of the optimum frequency is not affected by this hyperpolarization of the axons.

As mentioned above, we investigated (cf. Fig. 2) the effect of deterioration

upon resonance curves and demonstrated a decrease in sharpness of tuning developed primarily by changes in threshold in the low frequency range. It was suggested that the change at the low frequency end on deterioration might be caused by sodium inactivation or delayed rectification or a mixture of both. Since hyperpolarization does not result in increased sharpness of tuning (Fig. 9), there is a possibility that it is not sodium inactivation that is causing this change at the low frequencies on deterioration.

Although the effects of temperature and polarization upon damping are not as clear-cut as the effects of temperature and polarization upon threshold and optimum frequency, certain trends are nevertheless apparent. At normal polarization, the bluntness, B, of the resonance curve (related to the damping factor) decreases with temperature increase (Table I). Also, at a given temperature, hyperpolarization results in an increase in B, while depolarization causes a decrease in B (Table I).
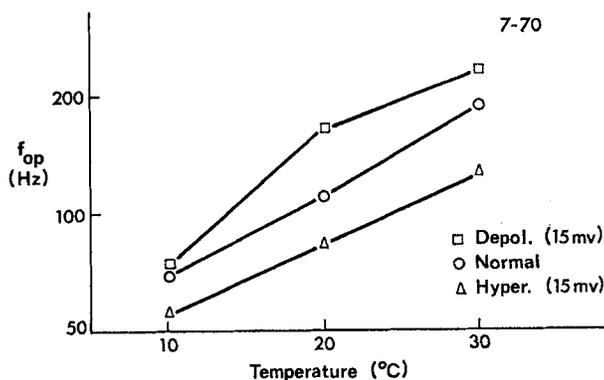

FIGURE 10.   Effect of temperature on optimum frequency of space-clamped squid axon hyperpolarized by 15 mv (squares), normal (circles), and depolarized by 15 mv (triangles). Optimum frequency (Hz) on log scale vs. temperature in °C on linear scale. Same data as in Fig. 9.

TABLE I

WIDTH INDEX, B, VS. POLARIZATION* AND TEMPERATURE

Experiment	Temperature °C	Hyperpolarization	Normal	Depolarization
7	10	4.7	4.3	3.9
	20	5.0	3.9	2.8
	30	5.1	3.5	3.4
8	10	4.6	3.9	3.2
	20	5.0	3.7	3.0
	30	3.8	3.4	2.5
9	15	5.8	4.5	3.5
	20	4.9	4.5	2.2
	30		3.9	2.7

* By 15 mv.

DISCUSSION

Hill et al. (1936) studied the effect of temperature upon the optimum frequency of frog nerves stimulated by alternating currents in normal Ringer solution and in high calcium. They were not able to extend these studies to nerves treated with citrate, since the results were very complicated. They felt this might have been caused by the fact that with the long-lasting waves of alternating current at low frequencies, the nerve may have responded repetitively to each half-wave, for Katz (1939) had shown that a series of action currents may occur when a constant current is passed through a medullated nerve when accommodation is very slow.

Working with axons deprived of calcium ions, both Arvanitaki (1939) and Brink, Bronk, and Larrabee (1946) felt that the frequency of conducted impulses along the fiber is determined by the frequency of the local excitatory process. On the other hand, we were able to show that when spikes and subthreshold responses appeared in squid axons intermixed in a train, the spike frequency was significantly lower than the frequency of subthreshold oscillations (Guttman and Barnhill, 1970).

Monnier and Coppée used the term, "pararesonance" rather than "resonance" in describing their results since the curves they obtained were considerably less sharp than true resonance curves. Cole and Marmont (1942) demonstrated that in the absence of calcium, the squid axon membrane showed increased inductive and capacitive reactances and decreased zero-frequency resistance, properties of a well-tuned resonant circuit. In the absence of calcium, then, true resonance rather than pararesonance was demonstrated. Cole 1941) then proposed for the normal membrane in seawater an equivalent circuit containing an inductive element. He postulated an equivalent inductance of 0.1 henry cm^2 and suggested that it is the structural characteristics of the membrane which govern the periodic activity and natural frequency of nerve. Later measurements (Cole, 1949) gave two capacitive elements. This was accentuated in high calcium.

Using impedance as the dependent variable, Conti very recently (1970) found that the optimum frequency remained the same when the concentration of calcium (and other ions) was varied. This is contrary to what we found when threshold sinusoidal current intensity was used as the dependent variable, for in that case low calcium increased and high calcium decreased optimum frequency reversibly.[2] (Phenomenological impedance of squid giant axons and natural frequency of oscillation were studied by Mauro et al. [1970].)

The membrane is acting as a rectifier and the reactance is associated with

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[2] It should be kept in mind, however, that the amount Conti altered the calcium concentration from the normal was considerably less than in our experiments.

its nonlinearity. One would expect that varying the calcium content of the environment would somehow affect this aspect of the membrane. Our results confirm this view.

In conclusion, this work has investigated the characteristics of living cell membranes by studying the effect of variation of the ionic environment upon alternating current excitation. Specifically, changes in calcium concentration strongly affect the natural frequency and damping of squid axon membranes.

A resonance curve used for studying AC excitation is a second-order phenomenon and not a primary one. Thus, it cannot directly give new insights into membrane mechanisms. However, it is a way of showing where the equations are not entirely adequate and so should help in elucidating the mechanisms involved.

The agreement obtained here in the investigation of AC excitability of real and computed axons suggests that (a) the manipulation of the concentration of a divalent ion in a solution bathing a real axon is adequately explained by a shift in the voltage of all parameters of the HH model according to the method of Huxley (1959); (b) the effect of temperature is to change the time scale of all conductances with a Q_{10} of 3 (Hodgkin, Huxley, and Katz, 1952); and (c) polarization involves setting the balance of resting Na and K conductances by adjusting them with the level of the resting potential (Mauro et al., 1970).

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