TEMPERATURE DEPENDENCE OF OSCILLATION IN SQUID AXONS: COMPARISON OF EXPERIMENTS WITH COMPUTATIONS

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ABSTRACT The temperature dependence of the oscillatory behavior of experimental and computed axons was compared. In the experimental study of space-clamped giant axons of the squid, small oscillations after a single threshold spike were measured using the double glucose gap over the temperature range 10°–30°C during treatment with three concentrations of external $CaCl_2$ solutions. Calcium concentration had little effect on frequency, as was found also by Huxley in his computations at 18.5°C for a fiber at rest. The Q_{10} both for the experimental and for the computed axon of FitzHugh was 2.25. The experimental measurements of the frequency of oscillations near threshold agree extremely well with the Hodgkin-Huxley calculations.

INTRODUCTION

Repetitive spikes have long been a very important aspect of neuron function. Subthreshold oscillations were found later and recognized as a basic property of axons, underlying rhythmic function. Both repetitive spikes and subthreshold oscillations were described in a classic paper by Arvanitaki (1939).

Other early papers on the subject were published by Katz (1936), Brink et al. (1946), and Hodgkin (1948). Wright and Coleman (1954) studied oscillatory behavior in crustacean axons. Hagiwara and Oomura (1958) described trains of spikes in squid axons. Cole and Curtis (1941) showed records of subthreshold oscillatory potentials from squid axons during current flow below and above threshold. Cole and Baker (1941) found an inductive membrane reactance which permitted an analogous oscillatory equivalent membrane circuit (cf. Cole, 1941).

Optimal frequency of repetitive response to AC stimulation and the relationship of this to the Rashevsky-Monnier-Hill theory (cf. Hill, 1935), were investigated in frog nerve by Monnier and Coppée (1939). Le Fevre (1950) compared experimental observations of repetitive firing in the squid giant axon with excitation theory.

Bonhoeffer (1948, 1953) discussed repetitive spikes in the passive iron wire model for the excitation of nerve. He showed that chemical kinetics could lead to relaxation oscillations in this model.

Theoretical treatment of oscillatory membrane behavior was presented by Cole (1941), Hodgkin and Huxley (1952), Cole et al. (1955), Huxley (1959), FitzHugh (1966), Noble and Stein (1966), and Cooley and Dodge (1966).

It has long been known that a logarithmic relation exists between some variable—Agin has suggested membrane current (Agin, 1964)—and the frequency of repetitive discharge of nerve fibers in sensory systems. It is possible that current flow occurs between the receptor and the axon in the visual system, for example, and that the repetitive firing of the axon in this system depends on this. Thus, any study of repetitive firing has significance in connection with functional neurophysiology in general and sensory systems in particular.

In previous papers we studied the effect of temperature upon the time constants of excitation and of accomodation (Guttman, 1962, 1966, 1968 *a*, and 1968 *b*). The experimental work presented in those papers was compared to computations based on the Hodgkin-Huxley equations by FitzHugh (1966) and by Cooley and Dodge (cf. Appendix to Guttman, 1968 *a* and 1968 *b*).

The present study is an extension of the previous work and is concerned with the effect of temperature upon the oscillatory behavior of the space-clamped squid nerve fiber membrane, in an investigation of the quantitative predictions of Hodgkin and Huxley (1952).

The original plan for the present study had been to investigate experimentally the relationship between the type of response obtainable (viz. single spikes, repetitive spikes with decreasing or increasing amplitude, subthreshold damped and undamped oscillations) with various stimulus strengths at various calcium concentrations, following the plan shown by Huxley (1959, Fig. 15). This proved too complicated and involved too many variables, so it was decided to study first only the effect of temperature on subthreshold oscillations following one spike obtained by threshold stimulation, rather than true repetitive activity.

The time course and frequency of these oscillations have been computed from the Hodgkin-Huxley equations by Dr. Richard FitzHugh of the National Institutes of Health. The over-all agreement with the observations on the effect of temperature gives further support to the equations as expressions of the properties of the squid axon membrane. Appropriate calculations of the effects of external calcium have not been made but the few measurements suggest probable agreement for this variable.

MATERIAL AND METHODS

The giant nerve fiber of the hindmost stellar nerve of the squid, *Loligo pealei*, was used in this series of experiments. It was dissected out under running seawater, separated from neighboring smaller fibers under a binocular dissecting microscope, blotted on tissue, and then

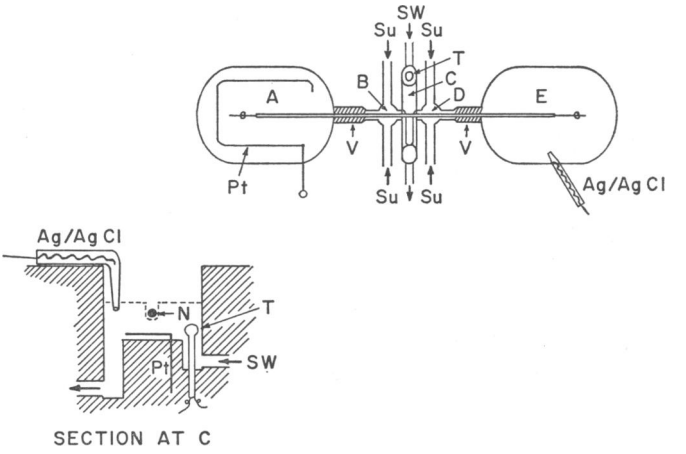

FIGURE 1 Mounting chamber used for studying temperature characteristics of oscillatory behavior in space-clamped squid axons. Chamber is internally divided into five compartments, *A*, *B*, *C*, *D* and *E* by partitions provided with aligned clefts in which axon, *N*, rests. Pt are platinized platinum electrodes for application of current. The Ag-AgCl electrodes are used for potential measurement. *T*, thermistor. For further details, see text.

mounted in a Lucite chamber. It was necessary to clean the axon of smaller fibers and connective tissue very carefully, if steady, clean seawater-glucose shear lines (described below) were to be obtained.

The axon chamber used (Fig. 1) was a modified and improved version of the one used in previous work (Guttman, 1966) in that no vaseline seals were interposed between the experimental and glucose compartments because the chamber design created smooth interfaces between laminarly flowing isosmotic glucose and experimental solutions.

The isosmotic glucose solutions (0.83 M) were made up in glass distilled water and passed slowly through ion-exchange resin (Crystalab Deeminite L-10 or Barnstead "Red Cap", Barnstead Still and Sterilizer Co., Boston, Mass.) while warm. The conductivity of the glucose solution was continuously monitored by a meter in the flowing system and was 1 μmho/cm or less.

The portion of axon in the experimental solution was between 0.5 and 1.0 mm long in various experiments. This short gap length contributed to the difficulties of measurement and from interference since with a small experimental area the percentage error was obviously greater. Nevertheless, measurements made through the microscope and those based on the electrical capacity (cf. Guttman, 1962), made when the apparatus was calibrated with known components, agreed within the experimental accuracy of a few per cent. The "visual" area measurement was used throughout.

However, with so short a gap length, less than three times an axon diameter, (constituting a space clamp) simple cable theory may not be adequate (Taylor, 1963). The approximation should not be serious between rest and rheobase where the characteristic length is between 3 and 6 mm and the membrane current density should be nearly uniform.

Long (40 msec) square wave pulses were provided by a Tektronix 161 pulse generator (Tektronix, Inc., Beaverton, Ore.) at the rate of one pulse per sec and applied through a 500 kohm isolating resistance to the platinum electrode in compartment *A* of the chamber. These pulses were considered as steps of current because all observations were completed within

their duration. Stimulating current to the platinum electrode in compartment *C* was measured by an operational amplifier and 10 kohm resistor serving as a current to voltage transresistor.

Differential recording was made 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 ten. The resting component of this signal was indicated by a Keithley 610BR electrometer (Keithley Instruments, Cleveland, Ohio).

The accuracy of reading periods of the oscillations from the photographic records varied greatly. In cases where there were many cycles of subthreshold oscillation following a spike (such as were obtained from very oscillatory fibers at high temperatures) the photographs could be read to within an estimated accuracy of 2%. In cases where only one period of small amplitude oscillation could be detected (such as frequently occurred at low temperatures) the error might have occasionally been as high as 30%. Even though the membrane was probably oscillatory at temperatures below 15°C, readings were not often made because it was not possible to observe or measure these low amplitude, long period oscillations in the presence of a small extraneous 60 Hz signal, which was always present. The amount of 60 Hz signal in the stimulus was approximately 20 NA and this was enough to produce a marked effect on the membrane at low temperatures, where the threshold stimulus itself was sometimes 100 NA or less.

The artificial seawater solutions with varying amounts of $CaCl_2$ were made up according to the method of Frankenhaeuser and Hodgkin (1957). Ca was varied in a Mg-free solution by mixing various proportions of 0 mM $CaCl_2$ solution and 112 mM $CaCl_2$ solution, made up as follows:

0 mM Ca solution (per liter)	112 mM Ca solution (per liter)
560 mM NaCl	112 mM $CaCl_2$
10 mM KCl	392 mM NaCl
5 mM Tris	10 mM KCl
	5 mM Tris

The pH was adjusted between 7.3 and 7.5 with HCl and NaOH. The fiber was first bathed in natural seawater and when a steady base line was obtained, the artificial seawater solutions containing various amounts of $CaCl_2$ and NaCl were substituted

The procedure was to probe for calcium concentrations which gave regular subthreshold oscillations following a single spike elicited by a threshold step of current. After this stimulus and response were photographed, a second record was obtained showing the response to a barely subthreshold stimulus as well. Using this method, a temperature run then was made. In most cases a second calcium concentration was then used and the procedure repeated. By this time the fibers had usually deteriorated so that they were no longer oscillatory, although good action potentials and resting potentials were still present.

RESULTS AND DISCUSSION

Experimental

Freshly dissected axons varied greatly in degree of oscillatory behavior shown. Fibers in apparently excellent condition from vigorous animals showed larger amplitudes

of oscillation and longer trains of impulses than poorer fibers. When axons deteriorated, oscillatory behavior dropped out first, followed by a decrease in spike height; the resting potential was affected last. Cole and Curtis (1941) found the impedance change also to be reduced very early in the deterioration process.

The effect of temperature upon frequency of subthreshold oscillation following threshold stimulation when the axon is bathed in solutions containing 18, 30, and 112 mM $CaCl_2$ artificial seawater solutions, respectively, is presented in Fig. 2. In this figure, temperature is plotted linearly and frequency is plotted on a logarithmic scale. Each run was first plotted separately and the results from sixteen threshold and three subthreshold runs on eight axons were then displaced vertically by amounts indicated on the figure for best fit to the calculated values in order to show the overall trend and range of variation from the trend. It may be noted that the data for 30.0 mM Ca gave a higher slope than that for 17.9 and 112 mM Ca. This apparently significant difference is not explained and is ignored in the composite plot shown in Fig. 2. It is reassuring that for the computed axon also Huxley (1959, Fig. 4) found that variation of calcium concentration had relatively little effect upon the period of

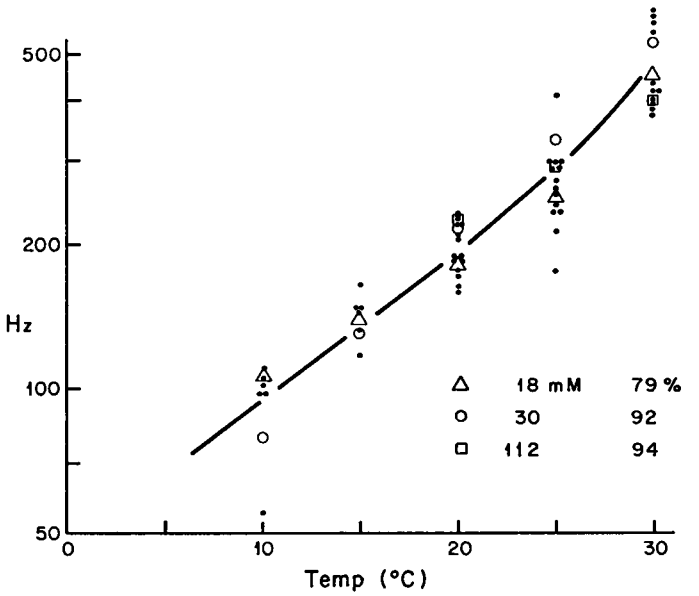

FIGURE 2 Effect of temperature upon frequency of subthreshold oscillation in space-clamped squid axons after near threshold current steps. Temperature in degrees centigrade on a linear scale vs. frequency (Hz) plotted on a logarithmic scale. Sixteen threshold and three subthreshold temperature runs on eight axons were first plotted separately and then displaced vertically for best fit to the continuous line, which represents computed values. Dots indicate individual experimental points. Averages for axon in 18, in 30, or in 112 mM $CaCl_2$ are plotted by symbols as shown above. Percentages indicate amount of vertical displacement necessary for best fit to calculated eigenfrequencies in the case of each calcium concentration.

oscillation, although it did have a marked effect upon the decrement of oscillation. To repeat, these data give moderately good support to the suggestion that calcium concentration has little effect on the form of the response, but merely affects the degree of vertical displacement necessary to obtain a good fit to the computed values.

The best linear approximation for the calculations and experimental data on Fig. 2 gives $Q_{10} = 2.25$. The corresponding approximation on an Arrhenius, log f vs. 1/T, plot gives the critical thermal increment of 13.7 kcal/mol.

The relatively small effect of changes of external calcium upon the frequency measured at threshold over the temperature range is very similar to that at the resting potential at 18.5°C as computed by Huxley, 1959 (cf. Fig. 5). In our Fig. 3, this comparison of our experimental values at threshold over the temperature range: 10° to 30°C (circles) and Huxley's calculated values at resting potential and at 18.5°C (crosses) is presented. In this figure, external calcium concentration is indicated on the abscissa on a logarithmic scale and the ratio f_0/f, where f_0 is the calculated frequency at 44 mM $CaCl_2$, (equivalent to calcium concentration in Plymouth seawater), is indicated on the ordinate.

The maximum absolute variation of frequency at threshold over the temperature range 10° to 30°C is about 20% for external calcium concentrations from 18 to 112 mM. These frequency changes are nearly the same as calculated by Huxley at rest and 18.5°C. The comparable Hodgkin-Huxley calculations have not been carried through for the threshold case, but it seems entirely reasonable to expect that they will not be in serious conflict with the present experimental data.

In discussing variability of frequency and of degree of damping in experimenta

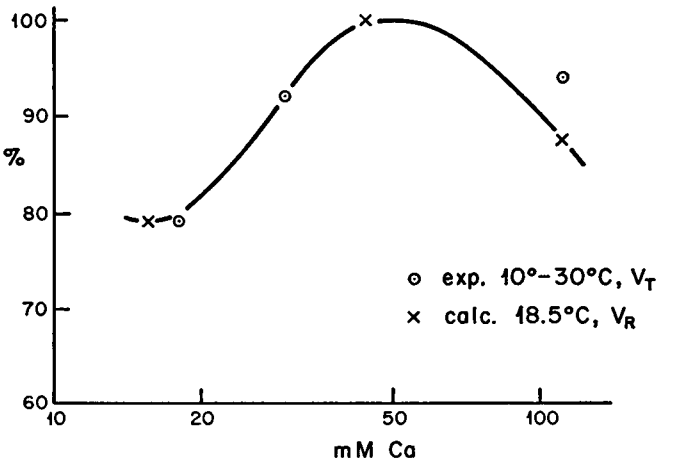

FIGURE 3 Effect of external calcium concentration on frequency of oscillation. Calcium chloride concentration in mM on logarithmic scale on abscissa; ratio of f_0/f, where f_0 is calculated frequency at 44 mM $CaCl_2$, on ordinate. Circles indicate experimental values over temperature range, 10° to 30°C, at threshold. Crosses are calculated values obtained by Huxley at 18.5°C at resting potential.

axons, Hodgkin and Huxley (1952) mention that a fair degree of variability is to be expected since both frequency and damping depend on the values of the resting conductance. They suggest that of these, g_{Na} and g_K depend critically on the resting potential, while $\bar{g}_l$ is very variable from one fiber to another.

They also believe that, following the suggestion of Cole (1941), the process underlying oscillations in membrane potential is closely associated with the inductive reactance observed with alternating currents and that the inductance is due partly to the inactivation process and partly to the change in potassium conductance, the latter being somewhat more important. They mention that the calculated inductance increases 3-fold for a 10°C fall in temperature and decreases rapidly as the membrane potential is increased. It disappears at the potassium potential and is replaced by a capacity for $E > E_K$.

Hodgkin-Huxley Computations

In order to find the oscillatory nature of the current-space clamped Hodgkin-Huxley axon the two complex and two real eigenvalues of time for the linearized equations were computed for currents from 0.1 × to 0.5 × rheobase and for temperatures from 6.3° to 30°C. Although the frequency will approximate these values for the linearized equations at small amplitudes of oscillation, there was no indication of the differences after either a spike or a near threshold potential. The variation of frequency with temperature agreed surprisingly well at rheobase with the squid membrane data. However most of the experimental frequency measurements were made after a just threshold spike. These agreed well with the just subthreshold frequencies when both were recorded.

FitzHugh calculated the time courses of potential after small perturbations of current near rheobase (after $I_0 + 0.5\%$ and after $I_0 - 0.5\%$) at each temperature (Fig. 4) and these showed the Hodgkin-Huxley equations to be good representations of the surviving axon. The times of maxima and minima were interpolated on a computer, courtesy of Dr. H. M. Mel (Fig. 4). The calculated intervals are more consistent when measured between successive maxima t_1, t_3, t_5, and between successive minima t_2, t_4, rather than from maximum to minimum, minimum to maximum, etc. The intervals between maxima and between minima—except the first maximum (t_1) and the first minimum (t_2)—are within 5% of t_∞, which they both approach as the amplitudes become smaller.

At lower temperatures, the differences between sub- and superthreshold frequencies are larger than at higher temperatures for the first two intervals. In this range, the frequency for superthreshold is always higher than that for subthreshold in the computed axon. However, in the few experimental observations of just subthreshold oscillations, mostly at 18 mM $CaCl_2$, the frequencies were as often above as below those of the oscillations after a near threshold spike.

The agreement between the experimental and the Hodgkin-Huxley calculations of

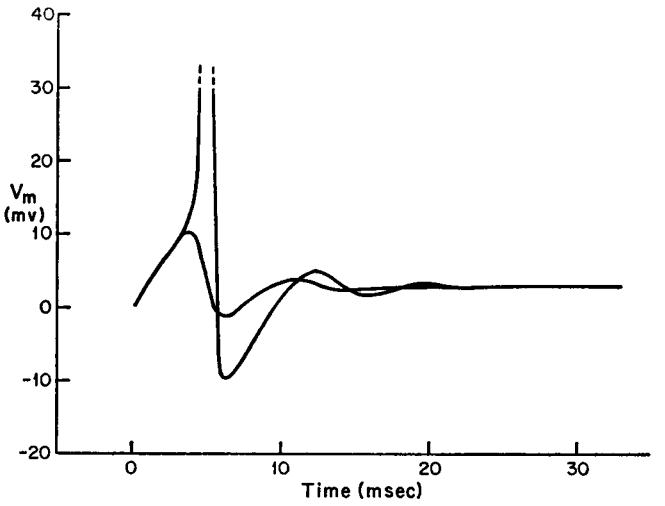

FIGURE 4 Calculated time course of potential following small perturbations of current: after I_0 + 0.5% (4.15 μa), and I_0–0.5% (4.11 μa), at 15°C. Potential in mv vs. time in msec. See text for discussion.

frequency is very good when measured near the threshold, I_0. The spread is much larger and the agreement not as good if frequencies are considered as functions of the membrane current, as Agin has done for his calculated axon (Agin, 1964). This is not surprising in view of the significant difference between the experimental and the Hodgkin-Huxley calculated rheobase thresholds which have been reported (Fitz-Hugh, 1966; Guttman, 1966).

A qualitative conclusion to be drawn is that the uncertain and uncontrolled factors which cause the differences between axons operate in much the same manner on the parameters which determine the subthreshold frequencies and the rheobase threshold.

In summary, calcium concentration has little effect on frequency in both experimental axons near threshold over the temperature range 10°–30°C and in the computed axon of Huxley at 18.5° at rest. The Q_{10} for both the experimental axon and for the computed axon of FitzHugh is 2.25, with a corresponding Arrhenius thermal increment of 13.7 kcal/mol. The experimental measurement of frequency of oscillation near threshold agrees extremely well with the Hodgkin-Huxley calculations.

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