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Temperature Dependence of Accommodation and Excitation in Space-Clamped Axons

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ABSTRACT Accommodation and excitation in space-clamped squid axons were studied with the double sucrose gap technique, using linear current ramps, short (50 μsec) square wave pulses, and rheobasic square wave pulses as stimuli. The temperature was varied from 5° to 35°C. Experimental results showed a Q_{10} for accommodation which was 44% higher than that for excitation. Yet calculations on the basis of the Hodgkin-Huxley equations predict equal Q_{10}'s for excitation and accommodation. Although the Hodgkin-Huxley equations are spectacularly successful for so many nerve phenomena, the differences between calculations of accommodation and these experiments, which were designed to test the equations, show that the equations need modification in this area.

INTRODUCTION

Accommodation had been studied experimentally in whole nerve and muscle by Gildemeister, Lapique, Solandt, and others. In 1950 Tasaki, using isolated single toad fibers, was able to confirm the much earlier work of Lucas (1907) on whole sciatic nerve of the toad, viz. that a linearly increasing voltage is effective in evoking a response when it rises above the rheobasic voltage at a rate greater than the minimal gradient of the fiber.

Tasaki and Sakagushi (1950) excited isolated single nerve fibers of the toad with exponential voltage stimuli and showed that the relation between the final voltage, V, and the time constant, RC, of the voltage rise is expressed by a straight line, indicating that the rate of increase of the stimulating voltage, $dV(t)/dt$, has a constant value at the moment when the voltage, $V(t)$, crosses the rheobasic voltage.

Rashevsky (1933), Monnier (1934), and Hill (1936) independently proposed theories of excitation. However, they limited their discussions to situations in which there would not be oscillations, and this early, limited approach has been made more general by FitzHugh (1966).

Fundamental investigations into the nature of accommodation were car-

ried out in this decade by Frankenhaeuser in Sweden with Dodge and other coworkers.

Recently, Vallbo (1964) has used the term accommodation to describe the nerve's response to slowly rising currents, and he determined the rate of accommodation by measuring the critical slope; i.e., the minimum slope of a linearly rising current which gave rise to a propagated impulse or an action potential. It is in this sense that the term accommodation is used in the present paper.

Since the time course of accommodation shows variations between fibers from the same nerve, Vallbo (1964) measured accommodation and voltage-clamp currents in the same single myelinated nerve fibers of *Xenopus* in order to relate these variations to membrane permeability mechanisms. Linearly rising currents were applied, the critical slope for excitation was measured, and the Na permeability and rate constants were obtained from the voltage-clamp measurements. He found that accommodation was related to the inactivation of the Na permeability.

Frankenhaeuser and Vallbo (1965) computed the responses of the myelinated nerve equations to linearly rising currents while introducing a number of changes in the constants in order to find out whether the equations are capable of reproducing the experimental results. The effects on the critical slope of the various changes in the equation constants were computed and it was found that while changes in all constants could affect accommodation, the largest effects were obtained by varying the Na inactivation.

The temperature dependence of the excitation and accommodation processes is of considerable interest. The effect of temperature upon the change of excitability in a nerve subjected to a constant stimulus was studied long ago by Rosenberg (1920) and by Eichler (1933). Moore and Cole (Moore, 1958; Cole, 1968) investigated the temperature dependence of early peak and steady-state currents.

Since excitation and the alteration of excitability during accommodation are such fundamental and important aspects of nerve function, it was decided to investigate in detail the effect of temperature variation upon accommodation.

The situation in our present work is different enough from the problem as stated by Hill so that his extensive analysis is not appropriate, and we will therefore arbitrarily define accommodation time, λ, as the ratio of rheobase to minimum gradient of a linearly rising current that will just excite, or $I_o/(dI/dt)$, and excitation time, k, as Q_o/I_o, which we have called τ in previous work.

In order to compare our experimental findings with the predictions of the Hodgkin-Huxley model for the squid axon, we asked Drs. J. W. Cooley and F. A. Dodge, Jr., of IBM Research to compute threshold conditions at

various temperatures for the theoretical axon. A brief description of their computational procedures is presented in an appendix. Computations were carried out for both the standard constants of the Hodgkin-Huxley equations ($A = 1$) and for the modification that all the ionic conductances were increased by a factor of four ($A = 4$), the modification that was introduced by FitzHugh (1966) to better represent the ionic current densities observed in voltage-clamp experiments at Woods Hole.

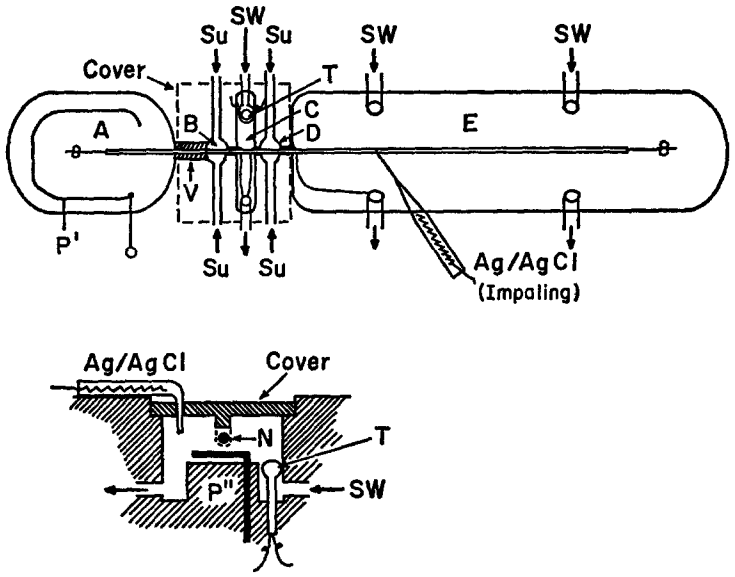

FIGURE 1. Experimental chamber used for studying temperature dependence of accommodation and excitation in space-clamped squid axons. Chamber is internally divided into five compartments, A, B, C, D, and E, by partitions provided with aligned clefts in which axon, *N*, rests. *P'* and *P''* are platinized platinum electrodes for application of current. The Ag-AgCl electrodes are used for potential measurement. *T*, thermistor. For further details, see text.

MATERIAL AND METHODS

The axon chamber used (Fig. 1) closely resembled that used in previous work (Guttman, 1966, 1968) except that the vaseline seals previously used between compartments were abandoned except between compartment A which contained KCl and compartment B, a sucrose gap. In most other respects the chamber could be considered a modification of the double sucrose gap chamber first developed by Julian, Moore, and Goldman (1962), but for the fact that the axon was laid down in grooves in partitions separating the compartments instead of being threaded through holes in Lucite partitions.

Compartment A contained 200 mM KCl, 20 times as concentrated as in seawater;

compartments B and D contained flowing isosmotic sucrose (0.75 M); compartment C, the experimental portion, contained flowing seawater at controlled temperatures, and compartment E also contained flowing seawater.

With the chamber dry and with the partition between compartments A and B vaseline-coated, the dissected and carefully cleaned axon was laid in place and the ligatures at its ends secured between double vertical stainless steel posts in the floor of compartments A and E. Additional vaseline was applied to complete the seal between compartments A and B. A Lucite cover with a central inferior projection positioned over the axon to help in establishing sharp shear lines between flowing seawater and sucrose solutions was placed over compartments B, C, and D. A small glass pipette containing an Ag/AgCl electrode was inserted in a hole in this cover so as to project into compartment C. Flow of solutions in compartments B, C, D, and E was started and an Ag/AgCl microelectrode was inserted into the axon in compartment E by means of a micromanipulator. The two Ag/AgCl electrodes were recording electrodes. Two platinized platinum electrodes for stimulation of the axon were permanently positioned in compartments A and C.

The isosmotic sucrose solution (0.75 M) used in the double sucrose gaps was made up in glass-distilled water and deionized by passing it through a column of Crystalab Deeminite L-10 resin (Crystal Research Laboratories, Inc., Hartford, Conn.) while warm. The treated solution had a conductivity of 1 μMho/cm or less. A conductivity meter inserted into the system was used to monitor the flowing sucrose solution.

The solutions in the seawater and sucrose compartments were continuously circulated, flowing up from below, and being sucked off from above to maintain convenient flow rates.

The method of temperature control resembled that used in previous work (Guttman, 1966). The incoming seawater was used to control the temperature in the central compartment, C. It was first cooled nearly to freezing by flowing it through one tube of a stainless steel countercurrent heat exchanger as a 14% ethylene glycol solution (which has a freezing point of -4.8°C) was pumped continuously through the other tube from a tank containing frozen blocks of the same solution. The seawater was then heated to the desired temperature by a small heating coil as it passed into the axon chamber. The heating power was controlled by an error signal from a thermistor bridge (Cole, 1957) used to measure the seawater temperature.

The portion of the axon in the central compartment was close to a current electrode, P'', which filled the entire length of this compartment. An approximately uniform current density in the neighborhood of the electrode was expected because the length of axon between sucrose gaps (usually about 0.8 mm) was considerably less than the "characteristic length" of about 6 mm found for squid axon at rest (Cole and Hodgkin, 1939).

Short (50 μsec) and long (rheobasic) square wave pulses were provided by a Tektronix Model 161 or 163 pulse generator at one pulse per sec and applied through a 500 kΩ isolating resistance to the platinized platinum electrode in compartment A of the chamber. The platinized platinum electrode in compartment C was used as the reference electrode; the stimulating current was measured in this lead by an operational amplifier and 1 kΩ precision resistor operating as a current to voltage transresistor. This voltage was applied to the upper trace of a Tektronix Model 502

oscilloscope. Linear current ramps were applied through the same isolating resistance; they were generated by a gate-controlled constant current generator charging a fixed capacitor (Fig. 2). The capacitor voltage was buffered by an NPN transistor in a source-follower configuration and applied to the membrane through the 500 kΩ isolating resistor. The ramp slope was adjustable by varying the output of the constant current generator. A Tektronix 161 pulse generator was used to gate the ramp generator, and a switch allowed stimulation by square wave pulses from the 161 when desired.

Differential recording was made between a high impedance (about 10 MΩ) silver-silver chloride impaling microelectrode and a low impedance (about 2 kΩ) silver-silver chloride external electrode. The high impedance microelectrode fed a unity gain follower amplifier having a field effect input of 10^{13} ohms and provision for neutralizing input capacitance (Instrumentation Laboratory Model 181, Instrumentation Laboratory, Inc., Watertown, Mass.) The low impedance electrode fed an operational amplifier used as a source follower and having about 10 MΩ input impedance. No neutralization was needed with this electrode. The difference between these potentials was taken by another operational amplifier used as a subtractor; this was the membrane potential. The DC component of this signal (the resting potential) was measured by a Keithley Model 610 BR electrometer, and the action potentials were indicated on the lower beam of the oscilloscope.

The error in reading the photos was about 0.1 cm and in cases of high noise somewhat worse. With low total excursions (such as at low temperatures) this error could have been greater than 10%.

RESULTS AND DISCUSSION

Runs were made as follows. At each temperature three photographic records were obtained: superimposed subthreshold (local response) and threshold (action potential) records for (*a*) a very short square wave stimulus (50 μsec); for (*b*) a rheobasic square wave stimulus; and for (*c*) a linearly rising current ramp stimulus (Fig. 3). In every case a base line was also recorded.

The normalized experimental results are presented in Figs. 4 to 6. In Fig. 4 accommodation time, λ, which is defined here as the ratio of rheobase to minimum gradient of a linearly rising current that will just excite, or $I_o/(dI_o/dt)$, is plotted on a logarithmic scale against temperature in degrees centigrade on a linear scale for nine axons and the results for each axon slid vertically to give best fit. This is justified since accommodation time varies considerably from axon to axon at the same temperature. The composite plot was then positioned so that its average at 15°C coincided with the average value of the 15°C empirical data.

The variation with temperature in accommodation and excitation in experimental and computed axons is presented in Fig. 7.

In the experimental axon, accommodation time at 15°C varied from 2.6 to 4.7 msec and averaged 3.2 msec. The computed axon had an accommodation time of 3.4 msec at 15°C when $A = 1$ and 2.2 msec when $A = 4$.

In the experimental axon, excitation time at 15°C varied from 0.47 to 0.73 msec and averaged 0.62 msec. Excitation time in the computed axon at 15°C was 1.7 msec when $A = 1$ in FitzHugh's notation, and for a more powerful axon when $A = 4$, it equaled 1.0 msec at this temperature (Fig. 7).

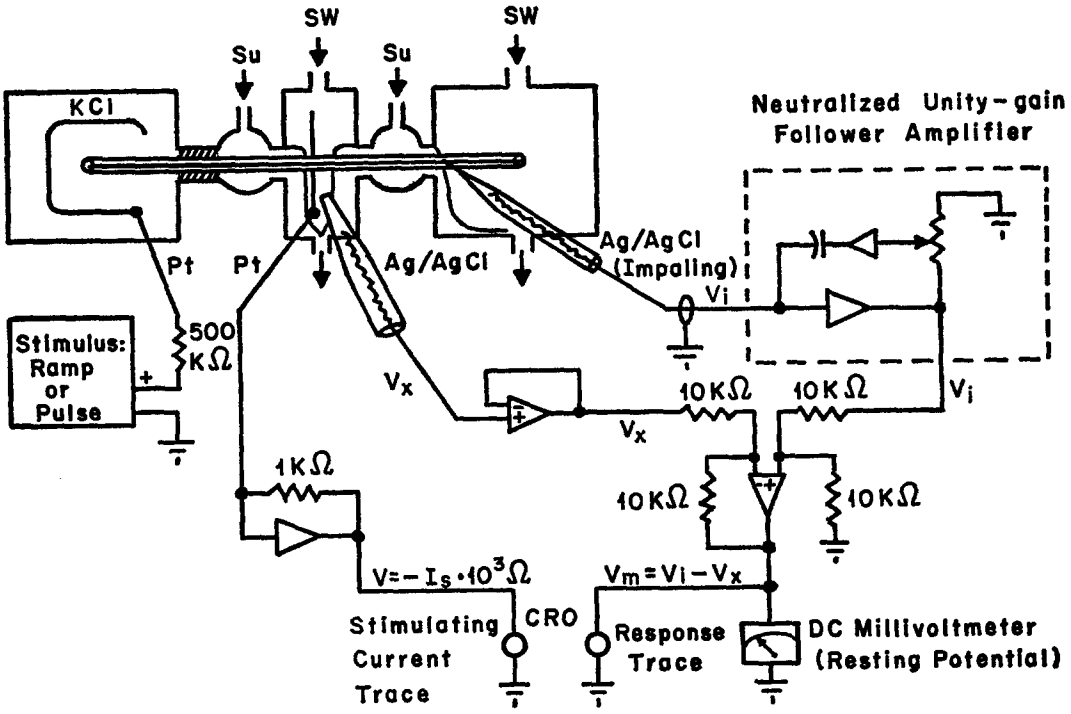

FIGURE 2. Circuits used in studying accommodation and excitation in space-clamped squid axons; chamber diagram not to scale. For details, see text.

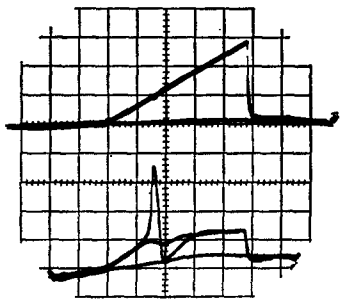

FIGURE 3. Typical record obtained with current ramp stimulus. Calibrations, 2 μa/cm, 20 mv/cm, 1 msec/cm. Temperature, 20°C.

In the case of λ, accommodation time, the experimental curve, though of a slightly different slope, was roughly bracketed by the two computed curves for $A = 1$ and $A = 4$, respectively. On the other hand, in the case of k, excitation time, the experimental curve fell below either theoretical prediction (Fig. 7).

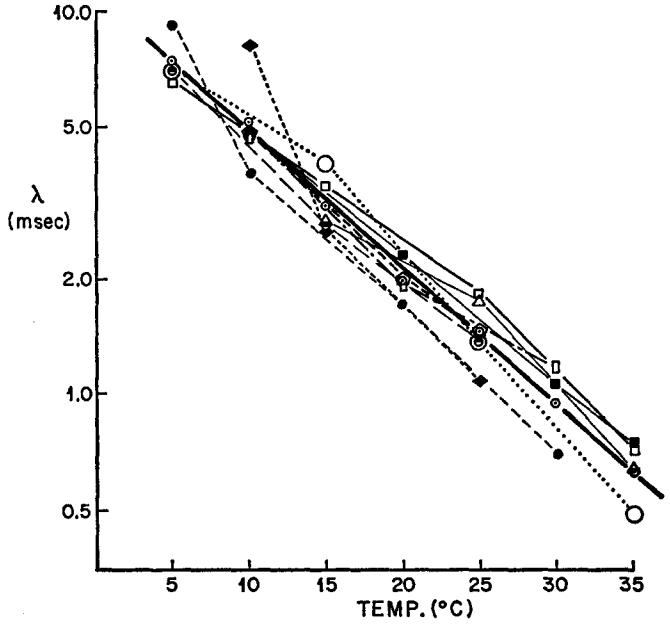

FIGURE 4. λ, which is accommodation time or $I_o/(dI_o/dt)$, rheobasic current divided by the minimum slope of the linearly rising current resulting in excitation, is plotted on a logarithmic scale against temperature in degrees centigrade. Data from nine axons have been slid vertically to give best fit and the composite plot positioned so that its average at 15°C coincides with the average value of the 15°C data. Heavy unbroken line represents the average of these plots.

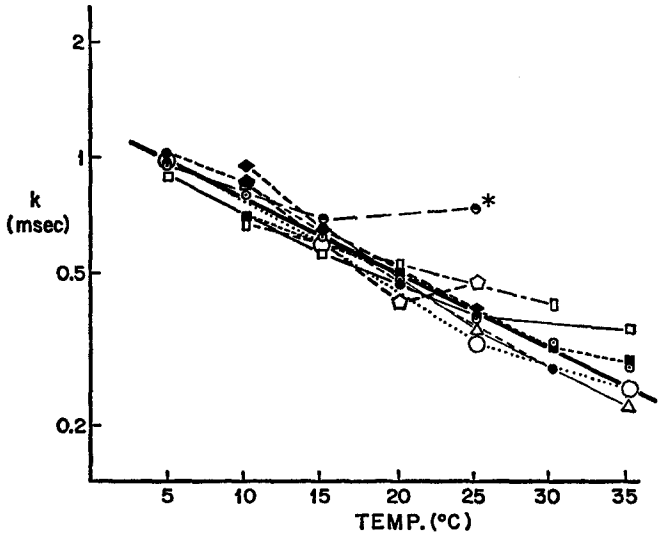

FIGURE 5. *k*, excitation time in milliseconds on a logarithmic scale, is plotted against temperature in degrees centigrade. Data from nine axons have been slid vertically to give best fit and the composite plot positioned so that its average at 15°C coincides with the average value of the 15°C data.

The Q_{10} for excitation, k, is 1.58 in the experimental axon. In the computed axon it is 1.89 when $A = 1$ and 1.92 when $A = 4$. The Q_{10} for accommodation, λ, is 2.27 for the experimental axon; it is 1.92 for the computed axon when $A = 1$ and 2.07 for the computed axon when $A = 4$.

It is clear that the values of Q_{10} for accommodation and excitation are practically identical in the computed Hodgkin-Huxley axon (see Table I). In the experimental axon, however, the Q_{10} of accommodation is 44% higher than the Q_{10} of the excitation process. This discrepancy is brought out in Fig. 6 where λ/k on a logarithmic scale is plotted against temperature on a linear scale for the experimental axon. The Q_{10} of λ/k for the computed Hodgkin-

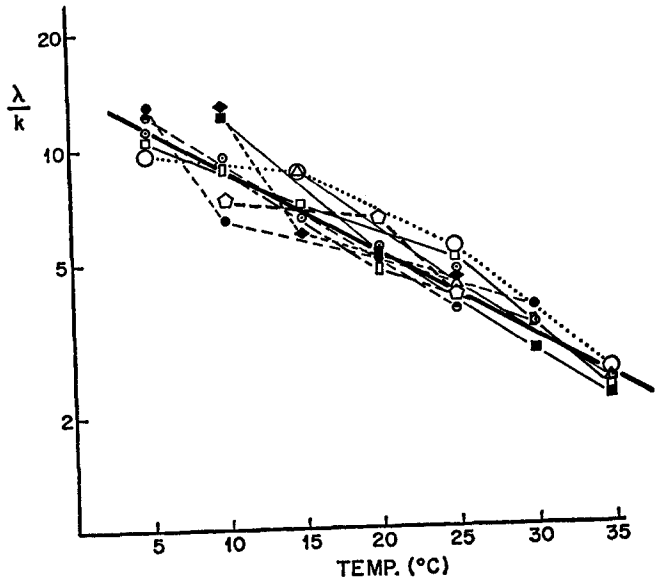

FIGURE 6. λ/k on a logarithmic scale is plotted against temperature, T, in degrees centigrade. λ is accommodation time, $I_o/(dI_o/dt)$, and k (which we have previously called τ), excitation time, equals Q_o/I_o. Data from nine axons have been slid vertically to give best fit. Heavy unbroken line represents average of these plots.

Huxley axon (Fig. 7) when $A = 1$ is 1.00 while that for the experimental axon is 1.62.

Although the Hodgkin-Huxley equations are spectacularly successful for so many phenomena, the difference between the calculations of the temperature dependence of the excitation and accommodation processes and these experiments designed to test the equations shows that the equations need modifications.

Both Hoyt (1965) and Stein[1] have indicated that temperature is likely to be an important variable in any attempt to deduce a physical model for

[1] Stein, R. Personal communication.

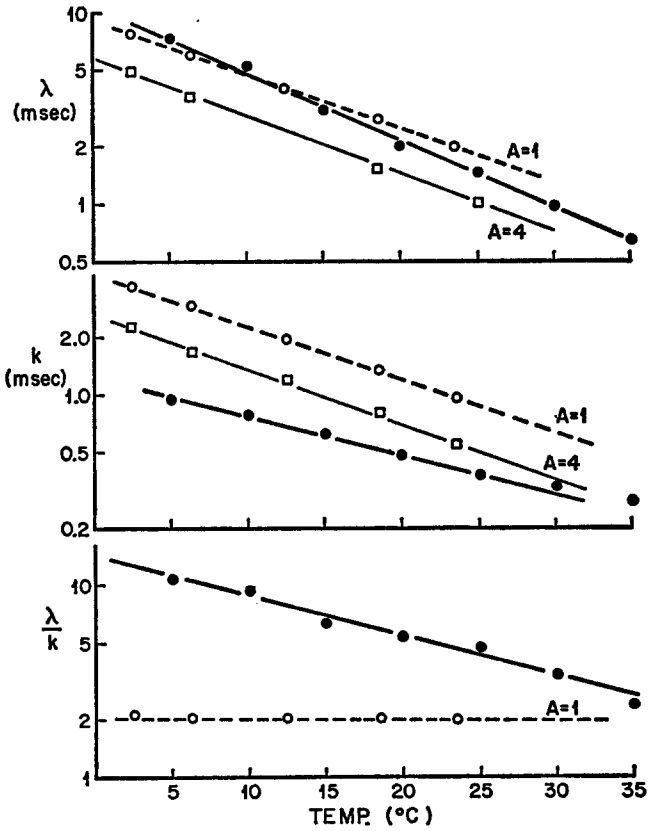

FIGURE 7. Comparison of experimental axon (heavy lines, solid circles, representing average values) with calculated axon (open circles for $A = 1$ condition; open squares for $A = 4$ condition) for λ, accommodation time; k, excitation time, and λ/k, all on logarithmic scale vs. temperature in °C on a linear scale. (For discussion of conditions $A = 1$, $A = 4$, see text.)

TABLE I

	Q_{10}		
		Theoretical	
	Experimental	$A = 1$	$A = 4$
k	1.58	1.89	1.92
λ	2.27	1.92	2.07
$\frac{\lambda}{k}$	1.62	1.00	1.08

membrane phenomena. The data presented here have been compared with computations on the Hodgkin-Huxley axon and it is hoped that such calculations will be carried out along with such variations of parameters as will give better agreement with experimental results.

The assistance of Dr. J. Y. Lettvin in the design of the linear current ramp generator, of Drs. John W. Moore and T. Narahashi with instrumentation in general, and Dr. Kenneth S. Cole's advice and suggestions throughout are all gratefully acknowledged.
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Appendix

Computation of Threshold for the Hodgkin-Huxley Model of the Squid Axon

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The system of four nonlinear ordinary differential equations for the "membrane action potential" (space-clamp constraint) was solved numerically using an iterative predictor-corrector method with a fixed, small value for the integration interval (Hodgkin and Huxley, 1952). Although such a procedure is not conservative of machine time, it was used to obtain sufficient temporal resolution in the computed wave form to determine the threshold. As one approaches very close to threshold, the peak response of the theoretical axon varies with the stimulus intensity; and the time to the peak is maximal for the threshold stimulus (FitzHugh and Antosiewicz, 1959). For the results reported, the threshold stimulus intensity was determined within a relative error of 0.2%.

Thresholds were computed for three stimulus wave forms; an instantaneous displacement of the membrane potential to determine the threshold to a very brief pulse, a step of current to determine rheobase, and a linearly increasing current to determine the critical slope. As pointed out by FitzHugh and Antosiewicz, a critical slope does not exist in principle for the Hodgkin-Huxley model; because the singular point of the equations goes unstable over a range of currents, a ramp stimulus will ultimately excite a spike (or train of spikes) no matter how small its slope. In the computations, the response to a very slow ramp shows at least one subthreshold oscillation preceding the spike; hence for the purposes of this paper we can operationally define the critical slope as the minimum slope required to excite a spike within the time to the first maximum of the membrane potential.

To simulate the various experimental conditions, we have modified the parameters of the Hodgkin-Huxley model in the manner described by FitzHugh (1966). In his notation, the subset of parameters which we have calculated is, A equals 1 and 4, B equals 0. Briefly, this corresponds to assuming that temperature affects only the rate constants of the empirical parameters m, h, and n with a Q_{10} of 3.0. Whereas A equals 1 corresponds to the standard values of the maximum ionic conductances, A

equals 4 corresponds to multiplying these by a factor of four to better represent the fact that voltage-clamp measurements on axons in Woods Hole typically show higher current densities than those reported by Hodgkin and Huxley (1952).

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