

OSCILLATORY BEHAVIOR OF THE SQUID AXON MEMBRANE POTENTIAL*

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ABSTRACT

Squid axons impaled with a microelectrode have been treated with concentrations of xylene and benzene such that there is no change in threshold or resting potential at 20°C., while the spike height declines about 10 mv. A decrease in ambient temperature results in large, reversible, increases in threshold. While neither low temperature nor the added blocking agent induces repetitive firing from a single stimulus, the two treatments when combined do yield repetitive responses which commence at a sharply defined temperature. The alteration in the membrane responsible for the effects observed can be described by saying that there has been a large increase in the inductance of the equivalent electric circuit, and the temperature coefficient of the apparent membrane inductance has a $Q_{10} = 5$.

INTRODUCTION

In the course of some experiments to examine the temperature dependence of the action potential of the squid axon when it had been treated with a variety of blocking agents, it appeared that the combination of blocking agent and a decrease in the ambient temperature interacted in such a way as to produce both subthreshold oscillations of the membrane potential and repetitive spikes from a single stimulus. The further investigation of this effect forms the basis of this report.

The oscillatory behavior of the squid axon to be described does not appear to be qualitatively different from that observed by many previous workers, working with both squid and other invertebrate axons. Ellis *et al.* (1) found several drugs to induce repetitive firing in crayfish axons. Welsh and Gordon (2) also found several compounds which produced multiple responses in the crayfish axon, among which were paradichlorobenzene, naphthalene, DDT, and several DDT analogs. Burke *et al.* (3) observed tetraethylammonium to act similarly in crustacean nerve fibers. Wright and Coleman (4) have observed

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similar activity in crab nerve. The oscillatory activity observed in nerve subjected to low calcium solutions is well known and is very well illustrated in the work of Arvanitaki (5, 6) on *Sepia* axons. Hodgkin (7) pointed out that multiple spikes arise out of oscillatory subthreshold responses when they reach sufficient magnitude and rate of rise. Finally, potential oscillations have been observed in the giant axon of the squid. Cole (8) and Marmont (9) have observed the squid axon membrane potential response to outward currents to be oscillatory in nature. Lowered calcium and elevated potassium solutions were also found to induce oscillatory behavior. Shanes (10) observed the afterpotentials of the normal untreated squid axon to be oscillatory and highly damped. He also observed that veratrine, as well as low calcium, can undamp the oscillations.

Methods

Giant axons from the squid, *Loligo pealii*, were dissected out and a 5 to 7 mm. length carefully cleaned. Axons were mounted in a lucite trough filled with sea water and impaled with microelectrodes filled with 3 M KCl. The resistance of the microelectrodes used varied between 4 and 10 M ohm and these were selected for low (± 5 mv.) tip potentials (11). The microelectrode was inserted in the axon in the region of the cathode of the stimulating circuit and this was adjusted to coincide with the cleaned region. The potential was lead *via* a cathode follower to the input of the differential amplifier of the oscilloscope. Low temperatures were provided by the flow of precooled sea water from large Dewar flasks to the experimental chamber. As the flow was found to have some effect on the electrode system, it was interrupted while recording took place. Temperatures were measured with a 200 K ohm thermistor, maintained in contact with the axon, and connected in a bridge circuit. Temperatures could be read to 0.01°C. but in the absence of insulation around the chamber the accuracy was $\pm 0.1^\circ\text{C}$. Stimulation was effected with 1 msec. square wave pulses delivered from a stimulus isolation unit and the usual frequency of test pulses was 1/sec.

RESULTS

Previous studies with frog sciatic nerve (12) had demonstrated that 1,3,5-trimethylbenzene (mesitylene) was very effective in establishing a conduction block at 15°C. without appreciably affecting the properties of the nerve at 20°C. It seemed desirable, therefore, to test this compound on squid axon. The results of such trials showed that mesitylene affected most axons very slowly even in saturated solution; many axons could be exposed to a half-saturated solution in sea water for 30 minutes at 20°C. with no observable effect on either the threshold or action potential. Cooling such treated axons did show that their threshold *vs.* temperature characteristics had been slightly altered such that threshold rose somewhat more steeply with decreases in temperature than did that of control axons.

The results with mesitylene suggested that the molecule itself was too large to enter the membrane to the extent necessary to produce changes in threshold

and accordingly a new series of experiments was undertaken using 1,4-dimethylbenzene (*p*-xylene). When sea water $\frac{1}{4}$ saturated with *p*-xylene was introduced into the experimental chamber at 20°C., the spike amplitude of the axon declined from 103 to 93 mv. within 5 minutes while the threshold

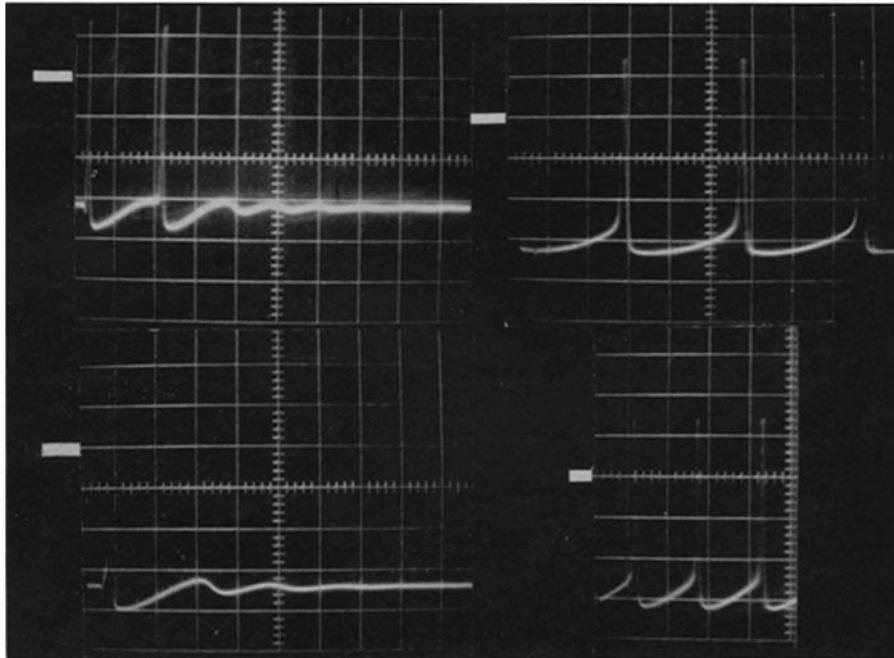


FIG. 1. *Upper left*, the two spikes shown occurred with a single stimulus and are followed by a damped oscillation of membrane potential. The axon was treated with sea water 0.2 saturated with *p*-xylene (temperature = 16.8°C.). *Lower left*, the single spike and following damped oscillation shown were obtained at 11°C. in 0.2 saturated *p*-xylene sea water. This axon had previously given a repetitive train of impulses at this temperature. Presumably fatigue or elevation of the threshold with sustained activity was responsible for the cessation of repetitive firing. *Upper right*, the train of spikes shown was obtained from a single stimulus in an axon treated with *p*-xylene (temperature = 6°C.). *Lower right*, this train of spikes was obtained from the above axon at 12°C. Grid scale, vertical, 1 unit = 20 mv., horizontal, 1 unit = 10 msec. The white markers denote zero potential.

and resting potential remained constant. As there was no change during the next 10 minutes, it appeared that the axonal membrane was in equilibrium with the applied concentration of xylene. A prominent feature of the action potential in the xylene-treated axon was a damped oscillation of considerable magnitude following the positive after-potential (see Fig. 1); this damped oscillation was also present after subthreshold stimulation and followed the

local response. When the temperature was reduced, the amplitude of the oscillatory response increased until at 16.5°C. repetitive responses to a single stimulus commenced. The duration of such a train of spikes was initially about 2 minutes. At all temperatures lower than 16.5°C. repetitive response could be obtained, although the frequency was much diminished at low temperatures. Upon warming beyond 16.5°C, a single stimulus would yield from 12 to 50 spikes, and this number would decrease, one spike at a time, until a temperature of 16.8°C. was reached; here repetitive activity ceased, and the axon responded normally to stimulation up to 22°C., the highest temperature used.

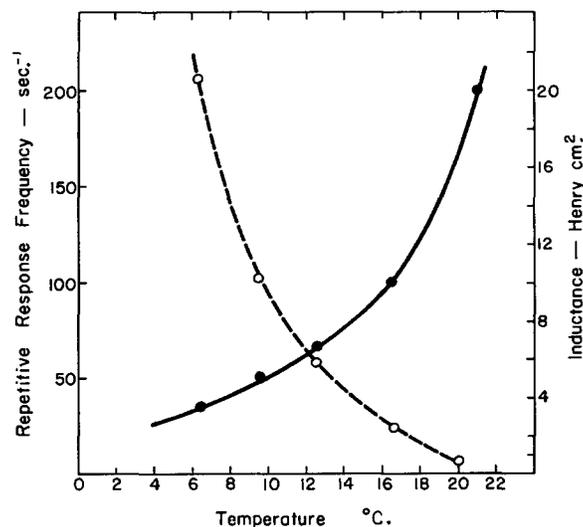


FIG. 2. The solid line shows the temperature dependence of the frequency of the repetitive responses found experimentally. The dotted line shows the corresponding calculated temperature dependence of L .

Repeated cooling and warming failed to alter the two transition temperatures by as much as 0.1°C. as long as the axon remained in good condition as indicated by a constant threshold at 20°C. When this experiment was repeated on other axons, the transition temperatures obtained were within $\pm 1.5^\circ\text{C}$. of each other.

A further point of interest is the dependence of the frequency of the repetitive response upon the temperature. It seems clear that after an impulse the refractory period of the membrane is fixed by both the time required for the recovery of the sodium-carrying mechanism, and the time required for the potassium permeability of the membrane to subside. These processes are not equally affected by a decrease in temperature and indeed it would appear that it is the slowness of the decrease of potassium conductance that is responsible

for the marked prolongation of the refractory period at low temperatures. It is, accordingly, somewhat surprising to find that the repetitive response has a characteristic frequency at a given temperature, as shown in Fig. 2, and there is no evidence of any asynchronous firing at any temperature used.

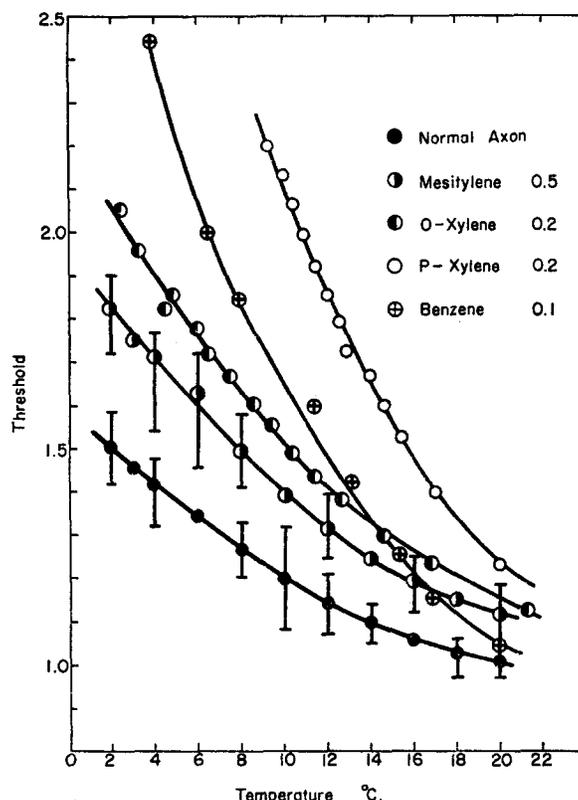


FIG. 3. Threshold (1.0 = normal) is plotted *versus* the temperature for axons subjected to different experimental treatments. The vertical bars denote the variability found with twelve different axons. Concentrations of compounds used are given as fraction of saturation in sea water.

The other two isomeric xylenes were tested and both were found to produce the repetitive response though their effectiveness, at the same concentration, differed as was evidenced by different transition temperatures. The transition temperature with 0.2 saturated *o*-xylene was 4.5°C. compared with 16.5°C. when *p*-xylene was used. The order of effectiveness noted was *p* > *m* > *o*. Benzene at 0.1 saturation was also found to produce similar results. These differences will be investigated in more detail in future work.

The concentrations of blocking agents used to treat axons at 20°C. were such that there was little or no change in threshold for excitation at this temperature.

A decrease in ambient temperature, however, markedly elevated the threshold for electrical excitation, irrespective of whether the axon produced single or multiple responses to the stimulus. Substances that were particularly effective in inducing multiple responses to a stimulus (*p*-xylene and benzene) also produced the greatest change in threshold with temperature, while with mesitylene, which does not give repetitive responses, axons showed only a slight change in threshold with lowering of temperature, when compared with untreated axons. The results are shown in Fig. 3. The fact that the introduction of blocking agent into the membrane and lowering the temperature both increase the threshold for electric stimulation and that these treatments interact is the reason for supposing that some of the changes in the membrane induced by the two treatments are similar.

DISCUSSION

The Linear Response of the Membrane to A.C.—When Cole and Baker (13) reported an inductive component of the membrane impedance, a theoretical basis for the occurrence of oscillatory behavior in nerve became available. An inductance in the equivalent electric circuit for the membrane, which we might take as a definition of the apparent membrane inductance L_m , together with the membrane capacitance and resistance, C_m and R_m , provides the requirements of oscillatory behavior. Cole (14) has discussed in some detail the implications of L_m and its possible relation to oscillatory processes. If we assume, for the moment, the validity of the equivalent circuit proposed by Cole, it is possible to make some calculations from the damping constant and frequency of oscillation of the membrane potential following spike as found in the present experiments. One can write the differential equation governing the voltage in the equivalent circuit as:

$$\frac{d^2V}{dt^2} + \frac{R}{L} \frac{dV}{dt} + \frac{1}{LC} V = 0 \quad (1)$$

The general solution of this equation is given by:

$$V = \frac{f_0}{f_1} V_0 e^{-(R/2L)t} \cos(2\pi f_1 t + \Phi) \quad (2)$$

in which

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{1}{LC}} \quad (3)$$

$$f_1 = \frac{1}{2\pi} \sqrt{\frac{1}{LC} - \frac{R^2}{4L^2}} \quad (4)$$

$$\Phi = \cos^{-1} f_1/f_0$$

f_0 is the frequency of the corresponding undamped oscillation, while f_1 is the damped frequency. Since we are assuming linearity, R is constant and f_0/f_1 is correspondingly a constant. The general solution can thus be rewritten as:

$$V = A V_0 e^{-kt} \cos(2\pi f_1 t + \Phi) \quad (5)$$

and A is a constant and

$$k = \frac{R}{2L} \quad (6)$$

The initial amplitude, V_0 , is seen to damp out with time by the factor Ae^{-kt} . If A is assumed to be very nearly unity, an assumption found to be justified, the damping is given by only the factor e^{-kt} . The oscillation is thus seen to damp out to $1/e$ of its initial amplitude in a time $1/k$. Since k and f_1 can easily be obtained from the record of the oscillations, R and L can be uniquely calculated from equations (4) and (6), assuming a value of $1.0 \mu\text{fd.}$ for C_m .

This procedure applied to the damped oscillations we have observed and also to those observed by Arvanitaki (5, 6) yields the values shown in Table I. R_d is the damping resistance of the equivalent circuit.

If there is any validity in the foregoing assumptions, one concludes that the membrane in these experiments has an inductive component of the im-

TABLE I

	Present data 18°	Arvanitaki (5) <i>Sepia</i>	Cole (14) A.C. impedance squid axon
$L_{\text{henry}} \cdot \text{cm}^2$	0.6	0.9	0.2
$R_d \text{ ohm} \cdot \text{cm}^2$	150	112	—

pedance of the order of that suggested for normal nerve and a resistance considerably below the normal value (1000 ohm cm^2).

The difficulties inherent in any analysis of this type are well realized (Cole (14); Schmitt (15)). Formally C and L imply energy storage mechanisms within the membrane while there is no reason to believe that the immediate energy for excitation is stored in any way other than in the ion concentration ratios. Schmitt points out, however, that any phenomenon that speeds up or delays a current process with respect to a voltage will cause the phase shift requisite for oscillatory behavior, even though the formally implied energy storage is absent.

The frequency of the oscillating local potential was observed to depend greatly upon the temperature. In the formal analysis, this would mean a dependence of L_m upon temperature. Fig. 2 shows the calculated temperature dependence of L_m ; temperature coefficient is seen to be negative and rather large ($Q_{10} = 5$).

The Linear Response of the Membrane to D.C.—The Hodgkin-Huxley equations (16) for the squid axon can be reduced to linear form for small (1 to 2 mv.) displacements of the membrane potential. As these authors point out, the equation for the K^+ current yields an oscillatory transient with the L_m of about 0.4 henry cm^2 , a value quite similar to that obtained from A.C. imped-

ance studies. The temperature coefficient of this L_m is necessarily the same as that for the rise of potassium conductance, $Q_{10} = 3$, while we find that with xylene-treated axons a Q_{10} of 5 is obtained. A difficulty with applying directly the Hodgkin-Huxley equations in order to discover which parameters have been affected by our treatment is that the membrane threshold is insufficiently defined analytically. While it is obvious that, to produce repetitive spikes, the damping constant k must have diminished greatly, the nature of the change in the equivalent circuit of the membrane is not apparent.

The damping coefficient k depends upon the ratio R/L , and we suppose that it is the increase in L_m observed with decrease in temperature that diminishes the damping.

Non-Linear Membrane Responses.—As soon as the displacements of the membrane potential exceed 1 to 2 mv., the use of any analog such as an equivalent electric circuit becomes exceedingly complex, because such an analog must in effect represent the full Hodgkin-Huxley equations. It is of interest, however, to contrast two types of experimental treatment that yield repetitive responses. Axons in sea water containing a low $[Ca^{++}]$ have low thresholds (17), low R_m , a potassium conductance corresponding to about 25 per cent of the maximum value, and a sodium-carrying system that is greatly inactivated (18). The membrane potential oscillates spontaneously and damping may be negative so that spontaneous action potentials are generated with a frequency of 350/sec., a value rather close to the calculated undamped frequency (19). Axons treated with xylene are indistinguishable from normal at 20°C., never exhibit spontaneous oscillations of the membrane potential, develop repetitive responses at a critical temperature where the threshold has been raised appreciably, and have a lower frequency of repetitive response corresponding to a damped oscillation. The difference in the subthreshold situation between low Ca^{++} and the narcotized axons can conveniently be represented as the difference between a low or negative R_d and a normal positive one. During an action potential, the rise of Na^+ conductance is capacitative, while its decline is inductive. The time course of the K^+ conductance change is inductive and makes the larger contribution to the inductive reactance.

The threshold of squid axon shows a marked minimum at a frequency of stimulation of 150 to 200/sec. (20) which is precisely the frequency range of the damped repetitive responses we have been considering. The variation of threshold with frequency of stimulation is a general phenomenon and has been discussed in detail by Monnier (21). The threshold for stimulation is made lower and the frequency *vs.* threshold minimum is sharpened as Ca^{++} is removed from sea water (20). In our experiments, the threshold at a frequency of 1/sec. rises considerably but from this observation we cannot infer that the threshold is also increased immediately following an impulse. Indeed, it appears

necessary to suppose that the threshold in our treated axons is greatly lowered immediately following the positive phase of the action potential and that the decline of the membrane potential from about +12 to -4 mv. suffices to give an excitation which is analogous to that of an anode break excitation. In the Hodgkin-Huxley notation, the conditions necessary for such an effect are that the potassium conductance is more thoroughly turned off at the peak of the positive phase, or that Na^+ inactivation is more thoroughly removed, so that h rises above its normal value at the level of the resting membrane potential, or that both of these effects act in concert. The nature of the interaction of temperature and narcotic in the axon remains obscure at present. Since cooling alone never gave rise to repetitive responses, it seems clear that the temperature coefficients of the underlying processes are very similar, actually 3 in the Hodgkin-Huxley formalism. The action of the narcotic might be to impose a higher temperature dependence on some process so that subsequent cooling suffices to separate it on a time axis from the others. This would be consistent with the elevated Q_{10} of 5 found in these experiments.

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