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Effect of Temperature on the Potential and Current Thresholds of Axon Membrane

Rita Guttman
CUNY Brooklyn College

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RITA GUTTMAN with the technical assistance of BEN SANDLER

From the Department of Biology, Brooklyn College, New York, and the Marine Biological Laboratory, Woods Hole, Massachusetts

ABSTRACT The effect of temperature on the potential and current thresholds of the squid giant axon membrane was measured with gross external electrodes. A central segment of the axon, 0.8 mm long and in sea water, was isolated by flowing low conductance, isoosmotic sucrose solution on each side; both ends were depolarized in isoosmotic KCl. Measured biphasic square wave currents at five cycles per second were applied between one end of the nerve and the membrane of the central segment. The membrane potential was recorded between the central sea water and the other depolarized end. The recorded potentials are developed only across the membrane impedance. Threshold current values ranged from 3.2 μa at 26°C to 1 μa at 7.5°C. Threshold potential values ranged from 50 mv at 26°C to 6 mv at 7.5°C. The mean Q_{10} of threshold current was 2.3 (SD = 0.2), while the Q_{10} for threshold potentials was 2.0 (SD = 0.1).

INTRODUCTION

The relations between nerve function and temperature have long been investigated on nerve trunks and more recently but less extensively on single fibers such as the giant axon of the squid. In order to prepare for work planned on the interrelations of the effects of narcotics and temperature on excitation, an investigation of the effect of temperature on the squid axon thresholds has been undertaken.

Hodgkin and Katz (1949) showed that the propagating spike of this axon increased in amplitude and duration with a decrease of temperature. In their analysis of voltage-clamp data, Hodgkin and Huxley (HH) (1952) found that the rate constants of the conductances used in their equations decreased by a factor of three for a ten degree decrease of temperature. The temperature coefficients of the conductances themselves were considered negligible and Moore (1958) has reported a linear decrease of 4 per cent per degree at 15°C.

Huxley (1959) has computed the effect of temperature on the propagating spike from the HH equations and found an excellent agreement with the earlier experiments. Sjodin and Mullins (1958) found that the potential threshold for a propagated impulse in normal as well as in slightly narcotized axons increased when the temperature was lowered. FitzHugh (1962) has made calculations on myelinated axon impulse initiation.

For the space-clamped or non-propagating action potential (Marmont, 1949), constant quantity thresholds were found for short stimuli (Cole, 1955; Bonhoeffer, 1953). Hagiwara and Oomura (1958) also used the method of space clamping by an internal electrode to give an ideally simple condition in which to study the relation between the absolute potential change across the fiber membrane of the squid and the current passed through it. They studied the change in membrane potential when the intensity and duration of the current pulse were altered and found that the critical depolarization was constant. They state that the threshold condition for constant current pulses is determined by the electrical constants of the resting membrane when the duration of the pulse is short (less than 2 msec. at 16°C).

Cole, Antosiewicz, and Rabinowitz (1955) computed current strength-duration curves from the HH equations, finding that the constant quantity at short times was little affected as the rheobase current increased two and a half times in going from 6.3 to 20°C. FitzHugh and Antosiewicz (1959) found that a mistake in the earlier computation made little difference in the threshold calculations for 6.3°C but they were not repeated for 20°C, so that the previous conclusions remain open to question. It thus seemed desirable to measure the effect of temperature on the membrane potential and current thresholds near rheobase in a space-clamped axon.

The space clamp as originally described by Marmont (1949) and subsequently developed and used by others with one or more internal electrodes was a difficult and drastic procedure. Huxley and Stämpfli (1951) and Dodge and Frankenhaeuser (1958) have employed electrical balance methods to eliminate outside leakage currents between adjacent nodes and allow measurement of node currents and potentials with external electrodes. Tasaki and Frank (1955) used air gaps for this purpose and Stämpfli (1954) has used isoosmotic sucrose. The sucrose insulation is directly applicable to non-myelinated axons as shown by Julian and Goldman (1960) for the lobster axon. It should also be useful for the squid axon provided the sea water gap between the two sucrose segments is kept small compared to the characteristic length of 4 to 6 mm for this axon at rest. This is the method that has been used in the present work.

MATERIAL AND METHODS

The giant nerve fiber of the hindmost stellar nerve of the squid, *Loligo pealii*, has been used throughout. It was dissected out under running sea water, separated from neighboring smaller fibers under a binocular dissecting microscope, and mounted in

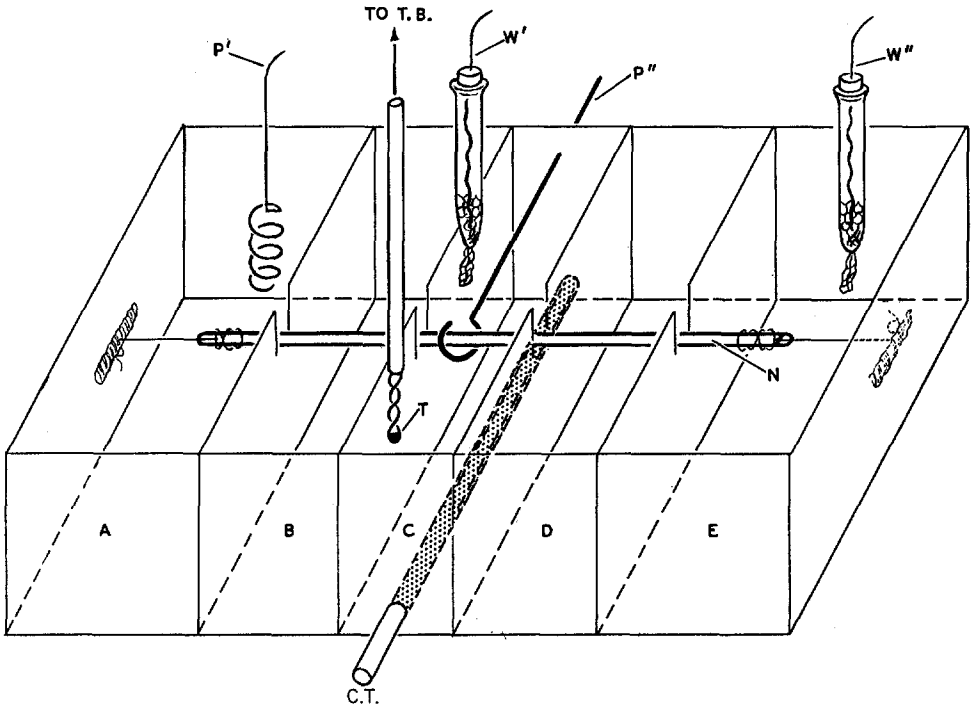

FIGURE 1. Measuring chamber used for studying threshold electrical characteristics for squid giant axon. Chamber is internally divided into five compartments, *A*, *B*, *C*, *D*, and *E* by partitions provided with aligned clefts in which axon, *N*, rests. *P'* and *P"* are platinized platinum electrodes for application of current. *W'* and *W"* are Ag/AgCl wick electrodes for potential measurement. *T*, thermistor. *T.B.*, thermistor bridge. *C.T.*, tube used for cooling. For further details, consult text.

a polystyrene chamber, divided internally into five compartments by four partitions (Fig. 1). The partitions were provided with aligned clefts for receiving the axon, which was sealed in each cleft by vaseline.

The length of the central sea water compartment (Fig. 1*C*) was 0.8 mm, and the two sucrose compartments (*B* and *D*) were each 6 mm long. (All partitions were 3 mm in thickness.) The extreme compartments (*A* and *E*) were filled with isoosmotic KCl, which served to inactivate the ends of the axon.

A number of methods were utilized for cooling the portion of the fiber in compartment *C*. In the early experiments when rapid cooling was desired, cooled sea water was flowed in under gravity or else injected manually by means of a syringe. Since the

axon was found to be extremely sensitive to pressure changes, the method finally selected was to pass cooled methyl alcohol through a stainless steel tube (*C.T.*, Fig. 1) running through the lower portion of compartment *C*. Temperature was recorded by means of a thermistor temperature bridge.

The portion of the axon in the central compartment was surrounded by a current electrode (*P″*), which was more than semicircular. This electrode filled the entire length of the central compartment. An approximately uniform current density in the neighborhood of the electrode was expected because its length (0.8 mm) was considerably less than the "characteristic length" of about 6 mm found for this axon (Cole and Hodgkin, 1939).

An isoosmotic sucrose solution, deionized by passing through a Deeminac filter, model F4 (Crystal Research Laboratories), was flowed through compartments *B* and *D* in all experiments. When, as was usually the case, the sea water was not circulated, the amount of its penetration through the connective tissue under the vaseline was not certain (probably somewhat greater than the 0.8 mm between the ends of the vaseline). This may have an advantage in somewhat removing the junction area from the principal measuring area, but it also makes absolute values rather uncertain.

Square wave current was fed from a Hewlett Packard model 202A low frequency function generator at five cycles per second to platinized platinum electrodes located in compartments *A* and *C*. Potentials were picked up through two Ag/AgCl wick electrodes in compartments *C* and *E* and amplified by means of a differential DC amplifier. Current and potential readings were observed and recorded with a tektronix model 502 dual beam oscilloscope.

The square wave was biphasic and had a fixed half-time. The oscilloscope sweeps were synchronized with the beginning of the 100 msec. depolarizing current pulses.

The value of the threshold stimulating current was determined on the second beam of the oscilloscope by making it flow through a known resistance. At the instant of firing, the current was calculated from the value of the voltage drop across this known resistance. Current values were adjusted by varying the voltage output from the generator. Wherever values of the threshold potentials are given, they were read off from the trace at the instant of firing. Although no base line is shown, the approximate potential values were measured from the lowest point of the trace to the point of divergence of the largest local response and the action potential spike.

It is unfortunate that the use of biphasic square waves precludes the possibility of assigning an absolute base line against which the levels of the potential thresholds can be measured. This puts a qualification upon the interpretation of the results which is rather serious, and it is hoped to avoid this difficulty in future experiments with the use of monophasic pulses.

RESULTS AND DISCUSSION

The over-all effect of temperature on threshold was first shown by the membrane potential response for a current stimulus. The first response was fast, small, and subthreshold. As the temperature was decreased the responses

became slower and larger until the axon first fired at a certain low temperature and then again more promptly at a slightly lower temperature.

The point of separation of the subthreshold and threshold responses was taken from the records for the threshold potential change as shown in Fig. 2

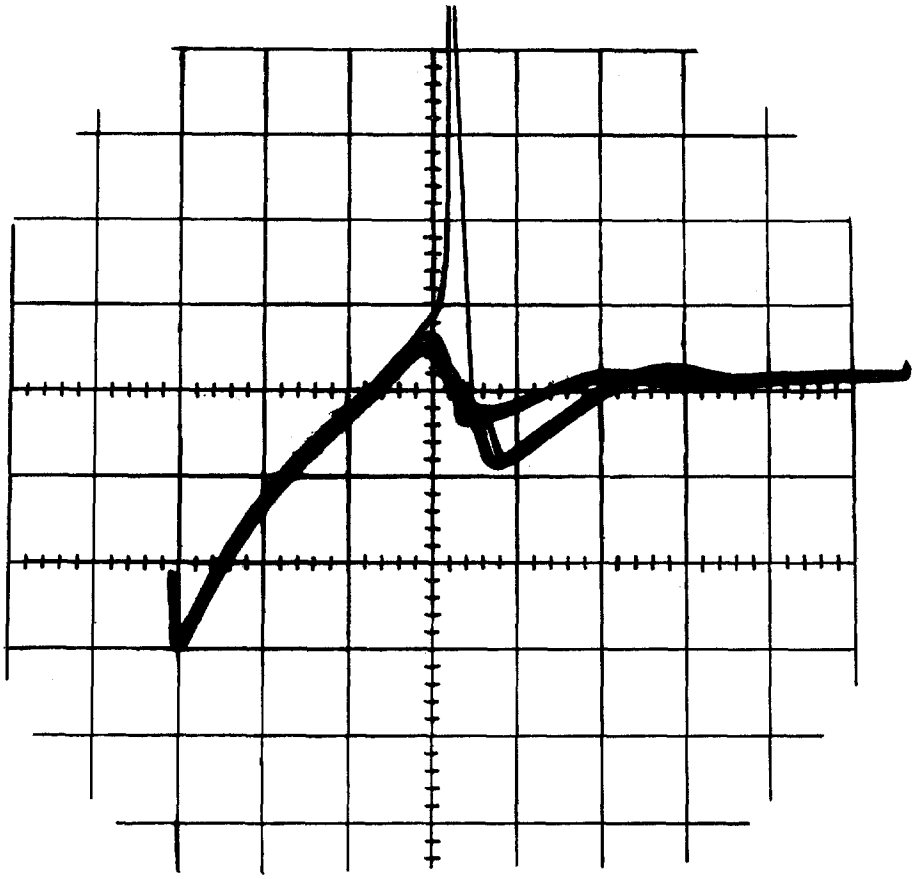

FIGURE 2. Record obtained with double sucrose gap method described in text. Five cycles per second square wave current (3.2 μa) sent in between central experimental section and one depolarized end of axon. Membrane potentials recorded between central section and other depolarized end. The recorded potentials are developed only across the membrane impedance and a small series resistance. The testing current, which results in a local response, is increased by a very small amount and this results in a spike superimposed on the local response trace. The point at which the two traces first deviate is a measure of threshold potential. Temperature is 25°C. Scale, 9.6 mv per division; 5 msec. per division.

and the corresponding current as the current threshold. No investigation was made of either the strength-duration relation or the effect of the repetitive stimulation. As quoted by Bonhoeffer (1953), Cole found a mean excitation time of $6 \cdot 10^{-4}$ sec. so that the stimulus duration of 100 msec. should give

the rheobasic values of current and potential. The time course of the rising phase of the potential changes is comparable to that found by Hodgkin (1948) in his study of repetitive activity in crab nerve.

Threshold Membrane Current

Cooling invariably and reversibly decreased the threshold current in all axons studied (Fig. 3); this result confirms the similar finding of Tasaki and Spyro-

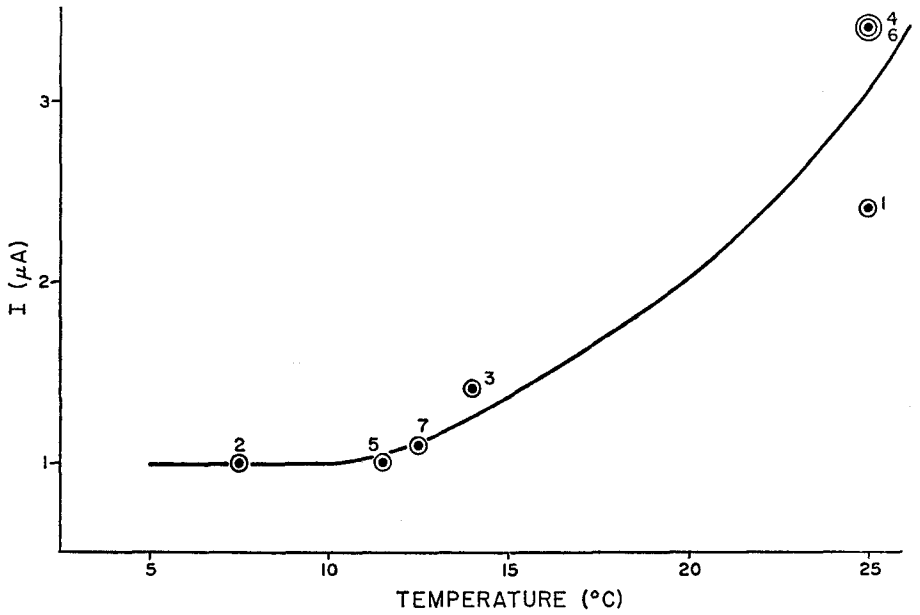

FIGURE 3. Temperature dependence of threshold current. Temperature in degrees C *vs.* current in microamperes. All points obtained on same axon. Numeral beside each point indicates order in which reading was taken.

poulos (1957). The threshold membrane current results are summarized in Table I, where the mean threshold current Q_{10} was 2.3 (SD = 0.2).

Two different methods were used in these experiments. In axons 5 through 18, a testing current which was a fraction of the threshold current at the high temperature was sent through the axon throughout the course of the cooling process. The testing current results in larger and larger local responses. Finally the testing current becomes a threshold current for the low temperature and a spike appears. In axons 22 through 25, no current was flowing during the cooling process since Cole and Baker (1941) have shown that membrane impedance requires several seconds to return completely to the resting value after current flow and it was thought best to avoid this variable. In these axons, threshold current was determined immediately after the cooling process was completed.

An approximate calculation of the effective membrane area gives a very high value of the threshold membrane current density as compared to the calculated rheobase values at 6.3°C (Cole, personal communication). Until more experiments are available it is not possible to explain the discrepancy, but one possible factor is the spread of current into the nerve lying within the vaseline seals at the partitions, and another is the hyperpolarization which may occur with the use of the sucrose gaps. Julian, Moore, and Goldman

TABLE I

DECREASE OF THRESHOLD CURRENT ON COOLING

Axon	Run	Threshold at warm temperature		Threshold at cool temperature		Δ Temperature	Q_{10}
		μa	*°C*	*μa*	*°C*	*°C*	
5	a*	5.0	25	2.0	10	15	1.8
	b*	5.0	25	2.0	16	9	2.8
11	a	18	27	11	20	7	2.0
	c*	23	27	16	17	10	1.4
13	a	2.2	24.5	1.0	14.5	10	2.2
	b	2.2	24.5	1.0	16.5	8	2.7
	d	2.2	24.5	1.0	16.5	8	2.7
	e	2.2	24.5	1.0	14.5	10	2.2
16		3.0	30.5	1.0	18	12.5	2.5
18		2.0	28	0.5	15.5	12.5	3.0
22		3.2	26	1.5	8.0	18	1.5
23		2.4	25	1.0	7.5	17.5	1.7
		3.2	25	1.0	11.5	13.5	2.4
		3.2	25	1.1	12.5	12.5	2.3
25		2.5	26	1.6	12	14	1.4

* Axon slightly narcotized with 5 per cent ethanol in sea water.
In axons 22, 23, and 25, thresholds were determined after the cooling process was completed.

(1961), utilizing the double sucrose gap method, obtained action and resting potentials in the lobster axon that were higher than those reported by workers using other methods, as would be the case if the region close to the sucrose were hyperpolarized. Some evidence for a hyperpolarization effect with the use of sucrose gaps has been found in the squid axon in unpublished experiments by Adelman and Taylor (personal communication). This should be kept in mind in interpreting the results here presented since the possibility exists that there may be different degrees of hyperpolarization at different temperatures.

Threshold Membrane Potentials

The temperature dependence of the threshold potentials is illustrated in Fig. 4 where all points were obtained on the same axon. (The low value for the first point may be evidence that the axon had not yet reached a steady state

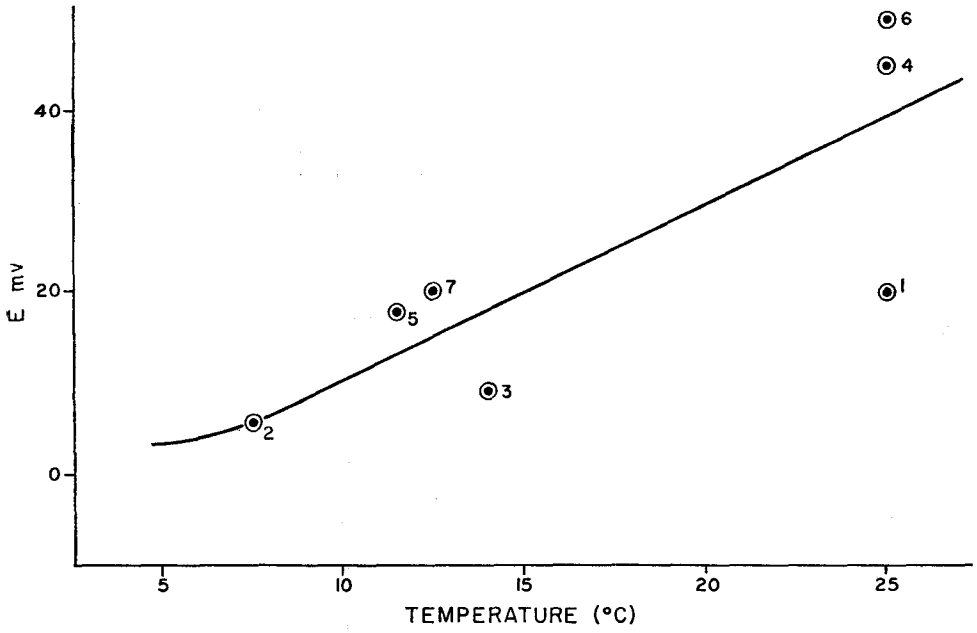

FIGURE 4. Temperature dependence of threshold potential. Temperature in degrees C. *vs.* potentials in millivolts. All points obtained on same axon. Numeral beside each point indicates order in which reading was taken. Same axon as in Fig. 3.

in the chamber.) This figure shows a marked and reversible decrease in threshold potential as the axon was cooled. Similar decreases in threshold potentials on cooling occurred in every axon studied (Table II). Again it should be stressed that the lack of a reliable base for measuring threshold potential must be kept in mind in evaluating these results. These potential measurements do include the effect of current flow through some axoplasm and sea water, which should be small. The mean threshold potential Q_{10} was 2.0 (SD = 0.1).

TABLE II

DECREASE OF THRESHOLD POTENTIAL ON COOLING

Axon	Threshold at warm temperature		Threshold at cool temperature		Δ Temperature	Q_{10}
	mv	°C	*mv*	°C	°C	
22	50	26	17	8.0	18	1.8
23	20	25	6.0	7.5	17.5	2.0
	9.0	14	6.0	7.5	6.5	1.9
	45	25	18	11.5	13.5	2.0
	50	25	20	12.5	12.5	2.1
25	5.0	26	2.0	12	14	1.9

Thresholds were determined after the cooling process was completed.

FitzHugh (personal communication) called attention to the possibility that the difference in stimulus duration may account for the fact that Sjodin and Mullins (1958) found an increase in the membrane potential threshold when the temperature was lowered while a decrease is found here. They used 1 msec. pulses instead of the present 100 msec. pulses, and so the experiments may not be comparable. Further, a comparison with the Sjodin and Mullins results may also not be justified in view of the lack of a reliable base line for measuring threshold potentials in our experiments. Tasaki and Spyropoulos (1957) found a decrease in threshold membrane depolarization as the temperature was lowered.

FitzHugh also mentioned that his computations on the effect of temperature on the rheobase current from the HH equations gave a Q_{10} of approximately 2. The experimental finding is thus another support for the effectiveness of these equations in representing the squid giant axon.

Preliminary experiments, utilizing the measurement of the amplitude of the output wave as an indication of membrane resistance (*cf.* Teorell, 1946) suggest that the change in membrane resistance on cooling depends upon the magnitude of the current used to measure it (the rectification effect, Guttman and Cole, 1941; Cole, 1941). Cooling resulted in a decrease in apparent resistance (chord resistance) if the threshold current for the low temperature was used for measurement, and usually resulted in a slight increase in apparent resistance if the threshold current at the high temperature was utilized for this purpose.

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