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Nerve Membrane Excitation without Threshold*

Kenneth S. Cole, Rita Guttman,† and Francisco Bezanilla‡

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND, AND BROOKLYN COLLEGE, BROOKLYN, NEW YORK

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Abstract. Evidence is presented to show that for a squid axon membrane the potential response, V, is a smoothly continuous function of a stimulating current, I. This makes it unlikely that an all-or-none or sharp transition phenomenon is a major factor in the processes by which ions cross the normal squid axon membrane and, probably, other excitable membranes.

Spatially uniform V and I were first produced in the squid axon with internal and external electrode arrangements and later by isolating a short length of axon between external pools of sucrose. Under these simplified conditions, direct experiments and calculations based on the Hodgkin-Huxley empirical conductances agree in showing that the maximum response, R, is a continuous, single-valued function of the effect of the stimulus, S. The maximum value of $\Delta R/\Delta S$ decreased steadily as the temperatures were increased from 25° to 38°C. Uncontrolled fluctuations prevented direct observations of $\Delta R/\Delta S$ below 15°C where calculations showed that it rose rapidly as the temperature decreased. Since the conductances are experimental parameters and since $\Delta R/\Delta S$ as calculated from them remained finite and continuous even at 6.3°C, this is experimental evidence against an all-or-none threshold excitation.

However there is an all-or-none threshold for the initiation and propagation of an impulse along an axon where V and I are functions of both time and distance.

The all-or-none law may be considered as a cornerstone of classical electrophysiology. Its essential feature is that no trace of a stimulus below a critical value appears at a distance along an axon. Then all stimuli above this threshold soon result in identical impulses that move at constant speed along a normal, uniform axon. The interpretation and even the validity of the law have been questioned frequently—usually for abnormal situations. But in its simplest form this law has remained as one of biology's most intriguing challenges and uncounted explanations for it have been proposed.

The squid giant axon allowed an arrangement of internal and external electrodes which gave a uniform potential difference and current density for a defined area of the axon membrane.[1] This elimination of spatial differences made it easy to measure directly membrane characteristics that had been always difficult and usually uncertain. Under these simple conditions, the action potentials were very similar to those which were propagated along an axon. The spikes seemed to appear at various times or not at all in a random manner after constant stimuli.[2] This was attributed to spontaneous fluctuations of membrane potential and/or threshold. These observations were soon confirmed by better experiments.[3]

Next Hodgkin and Huxley[4] produced their empirical sodium and potassium conductance, g_{Na} and g_K, from voltage clamp data. With these they calculated near threshold membrane responses to short current pulse stimuli which were in excellent agreement with experiments at 6°C and gave the threshold within 1 and 2 mv. The first automatic computations[5] at 6.3°C then located the threshold to within 0.1 μv. There have been many subsequent calculations of many excitation phenomena for the membrane under uniform potential and current conditions. These have been compared[6] with experiments on axons under similar conditions with a short length of membrane exposed between pools of sucrose. In general there have been excellent agreements: significant differences may be attributed to minor inadequacies of the conductance formulations and/or to variations of experimental axons.

The ion conductances also allowed Hodgkin and Huxley[4] to make calculations for the shape and speed of an impulse conducted along an axon. These again agreed very well with experiments. In calculating the effect of temperature Huxley[7] not only showed that the ion conductances explained the wave form, speed, and high temperature cutoff but also discovered an unstable solution which gave an all-or-none threshold. Far more intricate computations again gave an all-or-none threshold for the initiation of a propagating impulse.[8]

The first hint that excitation might not be strictly all-or-none, with spatial uniformity of potential and current, came from the calculations of FitzHugh and Antosiewicz[9] at 6.3°C. At the limiting accuracy of the IBM 704 an increase of stimulus by one part in 10^8 increased the maximum response from 14 to 80 mv. Subsequent interpolations for stimulus changes of $1/10^{14}$ and $1/10^{19}$ showed that the responses were continuously graded: they also proved that there was no saddle point on the potential phase plane which would be required for strict all-or-none behavior. This was important as it cleared up controversial points of analysis. But it was easy to dismiss[10] this graded response as academic because in real work with real instruments and noisy real axons there was only a negligible chance that anything but all-or-none responses could ever be obtained during a real experiment.

Only recently was it recalled that, in sucrose gap experiments of several years ago[6] at 30°C and, more so, at 35°C, the potential response had been graded over an observable range of stimulus currents. Threshold was arbitrarily taken to be the midpoint. This fortunately corresponded to the computer results which gave the stimulus for a 50-mv response.

It became interesting to know whether or not the spread of the computed responses emerged from the academic into the practical range as the temperature was raised and how well such calculations for spatial membrane uniformity might explain the experimental results in terms of the Hodgkin and Huxley ion conductances. It has also become particularly important to determine this membrane behavior as a guide and a criterion for theoretical models. It is obviously far simpler to construct such models with spatial uniformity of potential and current density than for the much more complicated extended axon in which both membrane potential and current density are functions of position as well as time. But usually the apparently reasonable assumption has been made that the goal for the membrane with spatial uniformity is an all-or-none threshold. Cooperative phenomena leading to phase transitions[11] are but the most recent of many proposed triggerlike models.

The present evidence leads to the conclusion that the uniform membrane potential response is a smoothly continuous function of the stimulating current. This suggests that a single abrupt threshold phenomenon does not have an important part in the processes by which ions pass through membranes.

Experiments. Membrane calculations: The Hodgkin-Huxley equations have been programmed for solutions of a wide variety of problems on the G.E. Mark II Time Sharing System from an NIH terminal. The time constants, τ_m, τ_h, and τ_n, were decreased by a factor of 3 for each 10° increase of temperature, $Q_{10} = 3.0$, or by 11.6% per °C. The membrane capacity, C_m, the maximum conductances, $\bar{g}_{Na}$, and $\bar{g}_K$, the effective electromotive forces, E_{Na} and E_K, and the axoplasm resistivity, r_2, were left unchanged by temperature.

Printouts were made of the maximum responses, R, to initial displacements as stimuli, S, at 5°C intervals from 10° to 45°C. Some of these are shown in Figure 1 plotted on log-log scales for comparison with axon data. Similar calculations were made for a hyperpolarization of 20 mv at several temperatures. In the upper temperature range these corresponded roughly to the resting potential results at a 5° lower temperature. A few calculations were made for the responses to step changes of stimulus current as for a rheobase threshold. These showed a similar, increasingly gradual response to the stimulus as the temperature was increased.

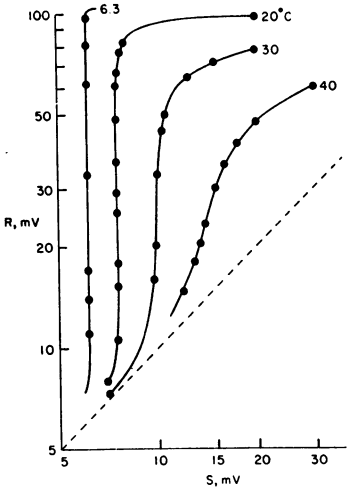

FIG. 1.—The peak membrane potential responses, R, calculated as functions of initial pulse stimulus displacements, S, at the indicated temperatures. The dashed line is the initial response for a passive membrane.

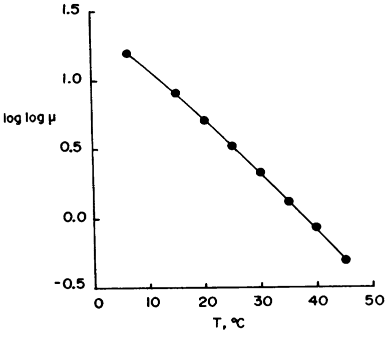

FIG. 2.—The dependence of the maximum rate of rise, μ, on temperature, T.

The slopes, $\Delta R/\Delta S$, may be considered as amplification factors. The maximum values, μ, are convenient measures for the sharpness of the S versus R relation:

T °C	6.3	15	20	25	30	35	40	45
μ	6×10^{15}	1.6×10^{8}	1.3×10^{5}	2.2×10^{3}	140	21	7.1	3.1

These results are plotted as T versus log log μ in Figure 2 to show their regularity. Although the decrease of μ with increasing temperature is striking, it is not until the region of 20°C that the calculations indicate a breadth of response which can be in the experimentally observable range.

Some complete time versus potential printouts were made at a few temperatures and plotted, as had been done[9] at 6.3°C. Those at 15° and 38°C are showni n Figure 3 for comparison with the axon records of Figure 4.

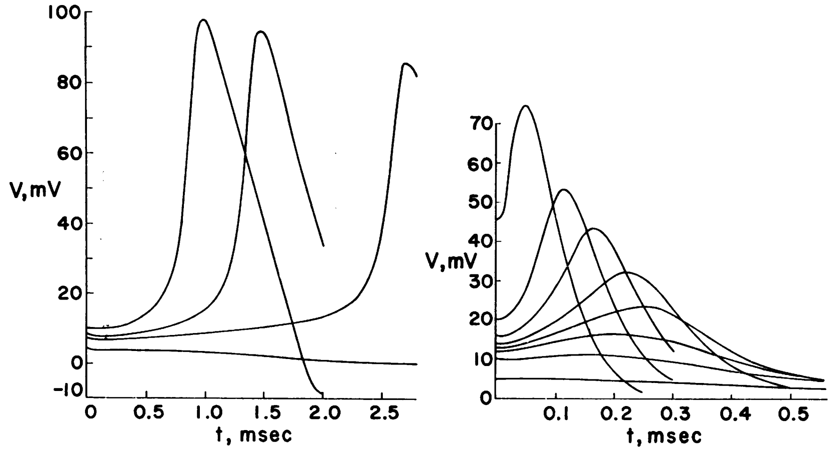

FIG. 3.—Calculated responses, V, at times, t, after initial pulse stimuli as shown at $t = 0$. *Left*, 15°C, *right*, 38°C.

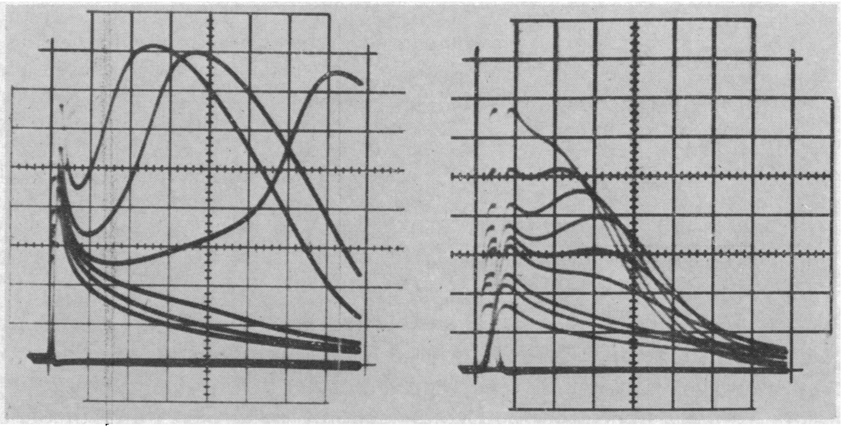

FIG. 4.—Oscilloscope records of membrane responses (ordinates 10 mv/div) vs. time (abscissae), at 15°C, left (0.2 msec/div) and 38°C, right (0.05 msec/div).

Membrane data: All axon measurements were made on a short length, about 1 mm, of squid giant axon in flowing artificial sea water between two streams of dextrose flowing over the axon. The stimulating current pulses of 20 μsec duration entered one end of the axon to flow through the central experimental area of membrane to the outside. The corresponding change of potential was measured between the opposite end of the axon and the outside. More details of axon preparations, chamber construction, flow, and temperature control systems and the electronic arrangements have been published.[6]

The data were obtained on axons from midsummer squid at Woods Hole. The resting potentials and action potentials averaged 10–15 mv lower than the best that can be obtained in a sucrose gap earlier in the season. Measurements made at the 15°C reference temperature before and after those at another temperature were usually in good agreement and this was a necessary condition for data acceptance. In particular the records of Figure 4 at 15°C were made immediately after the records were made at 38°C. Also this was the highest temperature at which satisfactory data were obtained.

A broader response distribution was found for low resting potentials which was equivalent to the calculations at a higher temperature and vice versa. For these experiments it was concluded that the effective resting membrane potential corresponded most nearly to that of the original Hodgkin-Huxley formulations.

The net current flow across the entire membrane was zero after the stimulating pulse and this should also be true for small elements of area. The membrane potential and ion current density would then be spatially uniform unless modified by the negative resistance characteristic. An approximate steady-state analysis[2] showed that the critical length was close to 1 mm. However, a similar experiment was performed with internal current and potential electrodes. These space clamp results at 30°C fully confirmed those from the sucrose gap after correction for the differences of resting potential.

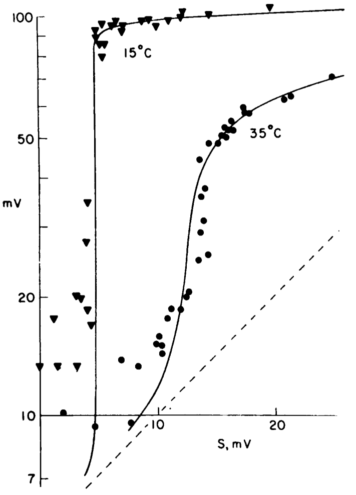

FIG. 5.—Comparisons of responses, *R*, vs. stimuli, *S*, for 15° and 35°C. Curves are calculated, points are by experiment.

Comparisons. The time versus potential relations as calculated and as recorded from an axon clearly show a close correspondence at 15° and 38°C in Figures 3 and 4. Further they agree in demonstrating that the responses at 38°C rise very much more slowly with stimulus than at 15°C. The results at the intermediate temperatures are similar and entirely consistent with these.

Of several possible methods for quantitative comparison of the axon records with the data from calculations, the maximum responses were plotted as functions of the stimulus on log-log scales and superposed. The normalizing factors were usually between 0.7 and 1.4 for each coordinate. The most extensive comparisons have been made at 15° and 35°C, as shown in Figure 5. Principal emphasis was placed on fitting the calculated curves at the higher values of response and stimulus. In spite of the deviations at both temperatures, the agreement is generally satisfactory.

Discussion. Time constants: One of the more uncertain features of the calculations is the temperature factor used for the time constants. The value of $Q_{10} = 3$ was chosen originally[3] as representative of the range from 2.7 to 3.5 found by a rough superposition of voltage clamp currents at different temperatures between 6° and 22°C. Since then there seem to have been no attempts to improve or even serious reasons to question this value, or to doubt that it was the same for all three time constants of the squid membrane or that it might apply at higher and lower temperatures. The most direct test in the upper temperature range may be the computations by Huxley[7] on propagating impulses. There is good general agreement with the experiments of Hodgkin and Katz:[12] the temperature at which block appears, for the propagating impulse only, are not importantly different—38°C experimental and 33°C calculated. The present experiments may be taken to give some support to the calculations.

Noise: Neither an obvious nor a simple way has been found to take into account spontaneous fluctuations of either the membrane potential or the threshold or a combination of them. No measurements of membrane noise or the effect of temperature on it have been found for squid. More extensive calculations and observations, particularly on latencies, may give more information on fluctuations and median response curves at lower temperatures. But at present it seems reasonable to assume that noise so dominates at temperatures below 15°C as to prevent direct observation of fractional responses.

Fractional responses: At this point it becomes appropriate to emphasize that the Hodgkin and Huxley conductances, g_{Na} and g_K, with their time and amplitude dependence on membrane potential are experimental facts for the squid membrane. To the extent that they are adequate they must agree with any other adequate experimental facts for the membrane. There have been so many comparisons[2] between calculations, based on the conductances, and direct observations and they have agreed so well that one cannot question the general validity and applicability of these conductances. Most of these tests have been performed in the temperature range from 5° to 25°C so there is the most reason to trust the conductances in this region. The most spectacular support for the conductances at higher temperatures is in the behavior of propagating impulses.

There was some basis to expect that threshold phenomena might be unusually critical tests of the conductances and indeed some minor discrepancies have been found.[6] By and large the major threshold investigations and computations have been in good agreement in the 5° to 25°C range. And the few examples for mean responses at 30° and 35°C have been equally as good. We now have strong indications, both from conductance calculations and from experiments, for the dispersion of excitation at higher temperatures. There is thus even more reason to trust the computations based on the experimental conductances which predict this excitation dispersion at all temperatures from 5° to 40°C.

We may then conclude that the partial excitation, under conditions of uniform membrane potential or current density as derived from the Hodgkin-Huxley conductances, is a valid goal for theory to explain. Conversely any theoretical membrane model which aims to produce all-or-none threshold excitation under these conditions seems likely to be misguided and, to the extent that this aim is achieved, the element which is responsible for such behavior is probably wrong.

Perhaps this nonthreshold nature of excitation should have been expected from the ion conductances. These are continuous and have continuous derivatives as functions of membrane potential, time, and temperature. On the other hand, they have not yet provided a simple qualitative explanation for the increasing breadth of stimulus for the fractional responses as the temperature is raised. Another example of the difficulty of prediction is the failure of propagation at 38°C. There are no indications of critical behavior in experimental fractional responses at and below this temperature, although such a performance between 38°C and irreversible thermal injury at 40°C is not precluded by these experiments. It is at least as pertinent to note that the conductance calculations for uniform membrane excitation and the conductances themselves give no indication of irregularity in the neighborhood of 33°C. Yet it was at this temperature that calculations with the same conductances showed the propagation failure.

Analogs: One minor, but unhappy, consequence of this work is that it has made obsolete most of the interesting and, until now, useful analogs[2] of membrane behavior—mechanical, thermal, chemical, and electrical. Perhaps some of them can be retrieved by judicious choices of parallel components which will give only a single stable intersection with the N characteristics of these analogs. An outstanding exception is the BVP analog given by FitzHugh.[13] As he has well demonstrated to us, this model has the fractional response and the response broadens to populate his "No Man's Land" as the temperature parameter is increased. Further, with a series of Esaki tunnel diode BVP elements, Nagumo *et al.*[14] have shown how an initial response may rise or fall with time and distance to give a constant, uniformly propagating action potential or die as a local disturbance.

Other membranes: The observations, calculations, discussion, and conclusions have been limited to the squid giant axon membrane because more, and more complete, data are available than for any other excitable membrane or axon.

The characteristics of a lobster giant axon are very similar to those for squid. It seems safe to assume that the conclusions would be the same. Extensive data on the toad[15] and frog[16] nodes are available. Threshold calculations have not suggested fractional excitation at the standard temperatures but information on excitation at high temperatures has not been found. The behaviors at higher temperatures should be interesting because[17] τ_m has a Q_{10} of about 2 whereas for τ_h and τ_n it is near 3. Early calculations[18] indicated an all-or-none threshold for the initiation of a propagating impulse in a medullated axon and it seems unlikely that the conclusion would be changed with actual nodal membrane data. Enough data should be available[19] to compute threshold behavior for skeletal and cardiac muscle and direct experiments might be useful.

Although other excitable membranes differ significantly from the squid, the essential features are very similar so it seems probable that the conclusions will be much the same.

Conclusions. Measurements between 15° and 38°C show that the response of the squid axon membrane is a continuous graded function of the stimulus for the simple case of spatial uniformity of membrane potential and current density. These data and supporting calculations from the Hodgkin and Huxley conductances lead to the conclusion that although the all-or-none law applies to the initiation and propagation of an impulse along a normal squid axon, it is not applicable to an element of normal membrane under conditions of spatial uniformity.

It is recommended that theorists not attack the problem of all-or-none propagation of a nerve impulse directly: it is unnecessarily difficult and all of the important facts are to be explained by the ion conductances.

It is recommended that theorists not invoke mechanisms which will produce an all-or-none response in the far simpler situation of membrane uniformity: the conclusion is contrary to the best available evidence.

It is strongly recommended that theorists address themselves to the problem of explaining the Hodgkin-Huxley conductances: these are experimental facts of normal axon membranes upon which most of the important and interesting behaviors of excitable membranes and axons depend.

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‡ Present address: University of Rochester Medical Center, Rochester, New York.

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