

EXCITATION OF NERVE FIBERS IN THE SQUID (*LOLIGO PEALII*)

By C. LADD PROSSER AND ALFRED H. CHAMBERS*

(From the Marine Biological Laboratory, Woods Hole)

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In the nerves of the squid axons with diameters up to nearly 1 mm. provide a unique opportunity for the study of the excitation of so called non-medullated nerves. Of special interest is the opportunity to find out whether the excitation is the same in an isolated nerve fiber as when that fiber is surrounded by other fibers in a nerve trunk.

Excitation in single nerve fibers has been studied in medullated nerve with the aid of pore electrodes (Grundfest, 1932; Sakamoto, 1933), by stimulating so as to excite only the most excitable fiber in a group (Blair and Erlanger, 1936), and by the isolation of one nerve fiber (Kato, 1934). These studies have shown that the time constants of excitation of single fibers in frog motor nerves are approximately the same as when a large group of fibers are stimulated. It has not previously been possible, however, to measure the excitation properties of a given nerve fiber both isolated and intact in a trunk.

The large fibers in the stellar nerves of the squid (Young, 1936 *a* and *b*; Bear, Schmitt, and Young, 1937), innervate the circular muscle fibers of the mantle. These nerve fibers range from 100 to 800 micra in diameter. A single condenser discharge, provided it is not too long or too strong, sets up a single impulse in the giant nerve fiber, this impulse then causing contraction of all the muscle fibers reached by the axon (Young, 1938). There are also smaller fibers (up to 50 μ in diameter) in the stellar nerves and some of these are motor axons to circular muscle fibers. It is possible to dissect away the giant fiber completely from all surrounding fibers and, since its

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threshold is nearly always lower than that of the smaller fibers, it is also possible to stimulate it independently without isolation. Since the tension produced through the smaller nerve fibers is at most only a very small fraction of that produced by the giant fiber, it is certain that when a large contraction of the muscle occurs the giant fiber has been stimulated. When the giant fiber is stimulated repetitively the muscle shows no increase in tension as the frequency of stimulation increases above fusion frequency (Prosser and Young, 1937).

For study of the excitability of smaller axons it is convenient to use the fin nerve, which contains fibers up to 50μ in diameter, the response to whose stimulation is a contraction of the muscles of the fin.

Preparations were made as described by Young (1938), the stellate ganglion and its nerves being exposed by a median ventral cut through the mantle. Of the ten or so stellar nerves the hindermost ("great stellar nerve") is much the longest, and contains the largest giant fiber. It is the most convenient to prepare and has been used in most of the following experiments, the smaller fibers in the more anterior nerves being studied occasionally for comparison. The nerve was tied close to the ganglion, freed from surrounding tissues, and placed on electrodes. The muscle and nerve were bathed with sea water. Most of the experiments were at 22–23°C. The muscle nerve preparation is not very viable and rarely remained in good condition for more than 20 minutes, as judged by constancy of threshold and size of response.

In the majority of the experiments the stimulating electrodes were calomel cells. These were made up with sea water and their ends dipped into paraffin tubes 6 cm. long leading to troughs in which were strips of wood 0.5 mm. in diameter. The tubes and troughs in the paraffin chamber were filled with sea water. The nerve lay across the edges of the sea water-soaked pieces of wood.

The D.C. resistance of the electrodes was 4000 ohms. The resistance of 10 mm. of isolated giant fiber was approximately 25,000 to 32,000, and of 2 mm. was approximately 5000–10,000 ohms. The resistance of the entire nerve was considerably less.

A few experiments were performed with chlorided silver wire electrodes, a few with chlorided silver plates, and a very few with agar-sea water pore electrodes into which dipped coils of chlorided silver wire.

Stimulation was by single condenser discharges. The circuit used was similar to that of Hill (1934), but a hand switch was used in place of the commutator. Either 50,000 or 100,000 ohms were in series with the tissue, and this resistance was shunted by 500 to 10,000 ohms. The time to discharge to $1/e$ of the charging potential was taken as the product of the condenser capacity and the shunt resistance (RC).¹

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Methods of Treating Experimental Data

The data from many experiments were first plotted in the conventional manner of voltage (V) against duration (RC). Chronaxie values ($0.37 RC$ for $V = 2 \times$ rheobase) were obtained directly from these strength-duration curves.

A more useful method of plotting, however, is that which has been extensively used by Hill, Rushton, and their associates, namely $\log V$ (or E) against $\log RC$. Hill has shown (1936) that many data when plotted in this way fall along a curve described by the equation

$$\frac{E}{E_0} = \left(\frac{RC}{K} \right)^{\frac{1}{K} - 1}$$

In the bi-log plots of Figs. 1 to 7, the line drawn in is the plot of this equation; the points were obtained experimentally. It will be seen from these figures that the fit is fairly good; in general it is better for the giant fibers intact in the great stellar nerve than when isolated (*e.g.* Fig. 2). Characteristic times (Hill's K values) were read directly from these log-log curves as the point where the slope of the tangent is $-\frac{1}{2}$. These points are indicated by arrows in the plots.

Blair has developed an equation which is similar to Hill's (Blair, 1935). It has the advantage of being tested by fit to a straight line by plotting $\log RC \frac{E}{E_0}$ against $\log RC + \log \frac{E}{E_0}$. A number of our experiments were tested by this method of plotting; *e.g.*, Figs. 1 and 7. The fit is fair in many of them for durations shorter than chronaxie. Blair's K is obtained from the slope and intercept.

Crozier (1937) has shown that probability considerations may apply to the strength-duration relation. He has applied the probability integral by plotting the excitability as a percentage of a saturation value; *i.e.*, $100 \times \frac{E_0}{E}$ against $\log RC$. When plotted on probability paper a straight line should result if the probability integral applies. Data from a number of these experiments have been plotted in this way; *e.g.*, Figs. 1, 2, and 7. The fit is as good as with the equations of Hill and Blair. The 50 per cent point, inflection point, or mode, and the slope give constants which are comparable to time constants derived by other means.

It is clear from the figures of this paper and from many others which have been constructed that each of these three expressions describes the strength-duration curves extremely well. It is not the purpose of this paper, nor is it possible from these data, to decide whether any one of these equations fits the data better than the others. In any case all of these formulations are based upon the same general type of assumptions about the process of excitation. The mere fit of a given equation does not tell much about the mechanism of excitation and the value of each of these equations will come only insofar as experimentally testable physical meaning is given to them.

A few measurements of "accommodation" in the giant fiber have been made by Miss M. H. Huntington according to the method of Solandt (1936). These show that accommodation is slow in this fiber and lambda values of 200-400 msec. were obtained.

Effect of Other Fibers in a Nerve Trunk upon the Excitation of One Nerve Fiber

Strength-duration curves were obtained from the excitation of the giant fiber intact in the great stellar nerve in nine preparations. Calomel electrodes making contact through pieces of wood 10 to 15 mm. apart were used. The characteristic times (Hill's K , or point where slope is $-\frac{1}{2}$ on bi-log plot) range from 0.33 to 0.46 and average 0.42 msec. In fifteen preparations the giant fibers were isolated for approximately 2 cm. The piece of wood at the cathode made contact with the proximal portion of this isolated fiber. In some experiments the anode also was on the giant fiber only, in others it made contact with the entire distal end of the cut nerve. One of these experiments with an isolated giant fiber is presented in Fig. 1. With electrode separations of 10 to 15 mm. the characteristic times of these isolated fibers ranged from 0.33 to 0.60 msec. and averaged 0.47. These experiments show that the excitation time constants—characteristic times, chronaxies, Blair's K , and other similar constants—are essentially the same whether the fibers are intact in the nerve trunk or are isolated.

Fig. 2 gives the results of one of the experiments in which a strength-duration curve was obtained before and after isolation of a single

giant fiber. This figure and the curves of other similar experiments show that the effect of the other nerve fibers and surrounding peri-

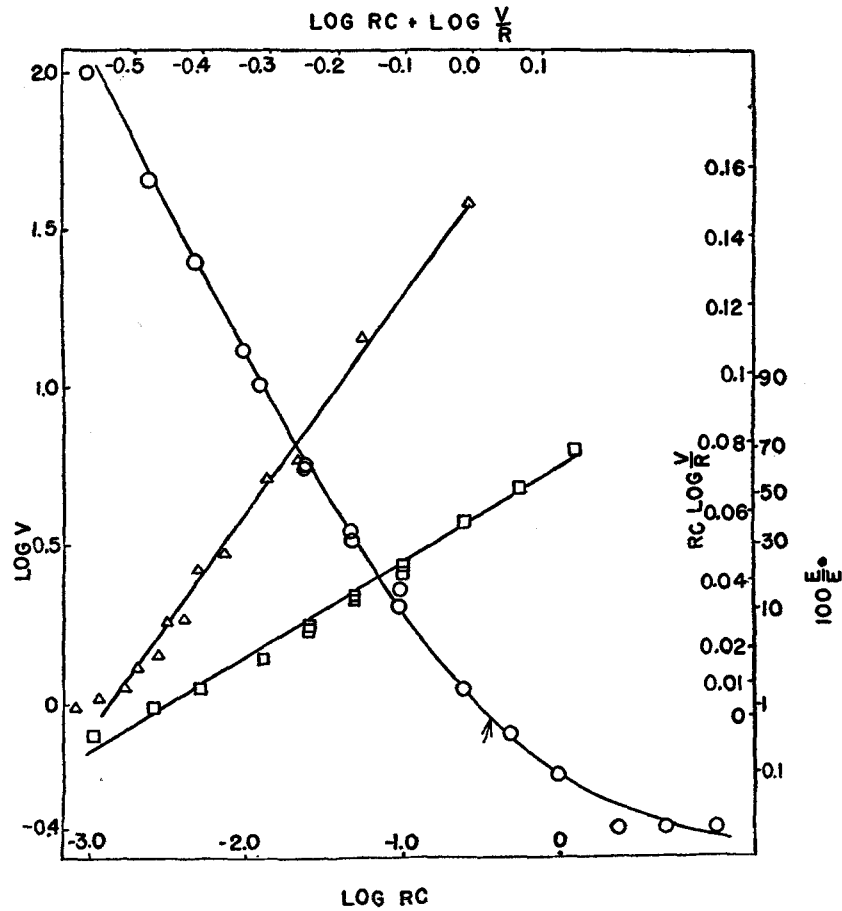


FIG. 1. Strength-duration curve (circles) of an isolated giant fiber. Calomel electrodes; electrode separation 13 mm. Solid curve—plot of Hill's theoretical equation; points obtained experimentally. Arrow gives Hill's K value. Lower straight line (squares)—probability plot of same data by Crozier's method, ordinates at extreme right. Upper straight line (triangles)—plot of same data according to Blair's equation, units on upper abscissae and inner right ordinates.

neurium upon the excitation of one fiber is to raise the entire strength-duration curve vertically. The time constants remain

essentially the same. The same elevation of the curve can be obtained by laying a thread soaked with sea water along the isolated nerve fiber. When plotted according to the probability relation of Crozier, the points from the data of the isolated fiber fall along the same line as those from the fiber intact in the nerve trunk (Fig. 2).

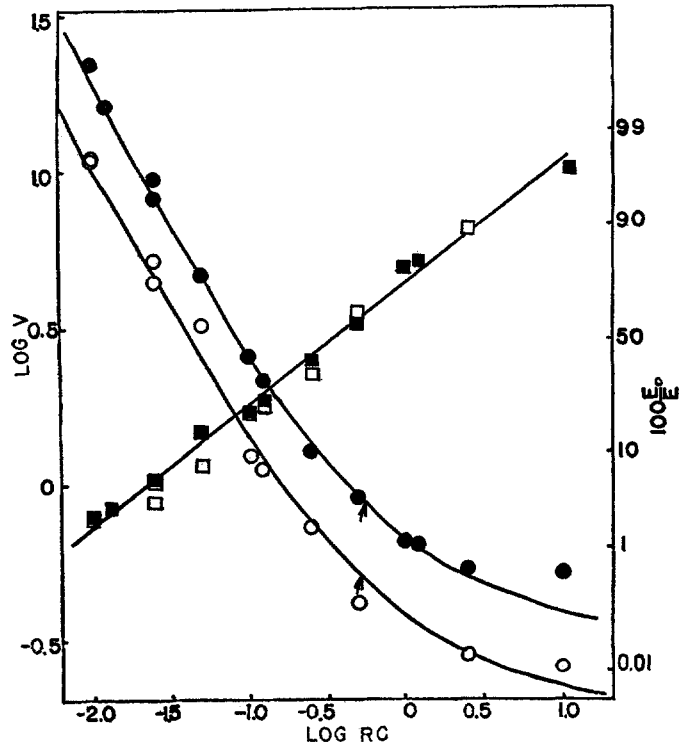


FIG. 2. Strength-duration curve of same giant fiber when isolated (open circles) and when intact in great stellar nerve (solid circles). Straight line—probability plot of same data; solid squares—fiber intact in nerve; open squares—isolated fiber. Electrode separation 13 mm. Calomel electrodes.

This is understandable if the excitability as plotted in the log probability relationship does not change. Furthermore, since the resistance of the isolated fiber is higher than that of the whole trunk, the effect of the other fibers must be that of a shunt rather than to keep the current flowing through the preparation constant.

We conclude that the other fibers in a nerve, when the most sen-

sitive fiber is being stimulated at threshold, tend to shunt the stimulating current and thus to raise the voltage necessary to excite at any given duration. It is reasonable to assume that this holds for all fibers whether large or small. These experiments prove conclusively, therefore, that the shape of the strength-duration curve and the time constants of a given fiber or group of fibers in a nerve trunk are the same as if that fiber or those fibers were isolated. The position of the strength-duration curve on the voltage axis is, however, higher for the nerve trunk.

Effect of Deterioration of the Preparation

The exposed giant fiber and mantle is very sensitive to fatigue and to external conditions. This makes the preparation a very poor one for extended experiments. In fact it is often difficult to obtain sufficient points for a satisfactory curve. It has been found from action current recording (Young and Hartline, private communication) that the nerve often lasts much longer than the muscle. The muscular response may gradually weaken before disappearing. More often it ceases very abruptly; in such cases we believe that the neuromuscular junctions have broken down. In some few experiments the nerve showed a decrease in its excitability during a few minutes preceding the cessation of the response. One such experiment is shown in Fig. 3. One curve is drawn through the points taken early in the experiment and the same curve is shifted to fit the points obtained later. The numbers give the order in which points were obtained. This experiment shows that with deterioration the entire curve is shifted diagonally upward and to the left with the result that the excitation time constants are shortened. In this experiment the K value in the fresh preparation is 0.58 msec., while when aged it is 0.47 msec. In the probability plots the slope is less steep as deterioration proceeds.

Effect of Interelectrode Distance

Fig. 4 shows strength-duration curves for an isolated giant fiber at electrode separations of 9.5 and 1.5 mm. Fig. 5 gives similar data for a fiber intact in the stellar nerve. The effect shown by these and other experiments is that as the interelectrode distance is decreased,

the entire strength-duration curve is shifted diagonally upward and to the left. The excitation constants (chronaxie, characteristic time, etc.) are shortened as a result. This effect has been observed by numerous investigators working with nerve trunks.

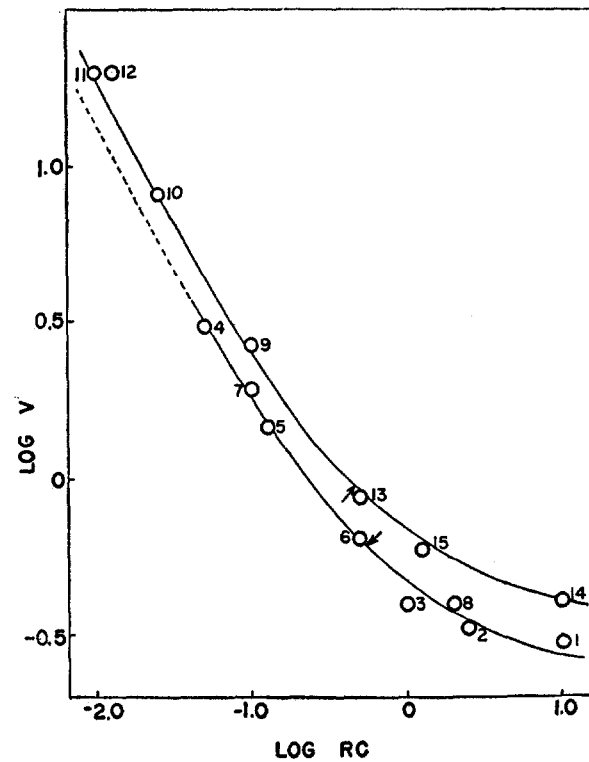


FIG. 3. Strength-duration curve of isolated giant fiber during deterioration. Numbers on points indicate order in which they were obtained. Same curve moved to fit early and late data. Note shift of K value. Calomel electrodes; interelectrode distance 13 mm.

The characteristic times in intact trunks with electrode separations of 12–15 mm. ranged from 0.40 to 0.46 and averaged 0.43 msec., while at 2–3.5 mm. in the same preparations they averaged 0.33 msec. (range 0.24–0.41). Similarly in four experiments on isolated giant fibers with interelectrode distances of 10–15 mm. the characteristic times averaged 0.51 msec. (range 0.46–0.58) while the same isolated

fibers with the same electrodes 3 mm. apart showed characteristic times averaging 0.38 msec. (range 0.16–0.42).

On the probability plots the curves with greater electrode separations have a lower slope and higher midpoint or modal value than

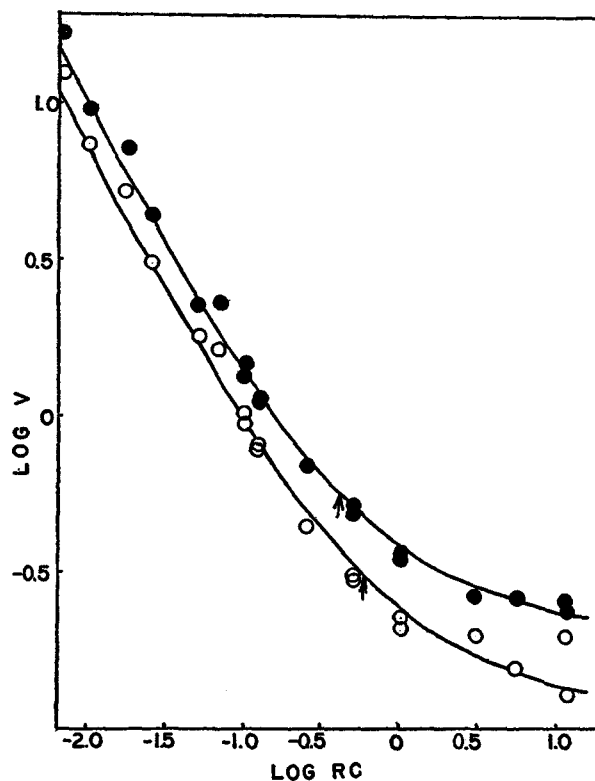


FIG. 4. Effect of electrode separation upon strength-duration curve of an isolated giant fiber. Lower curve (open circles) interelectrode distance 9.5 mm; upper curve (closed circles) electrode separation 1.5 mm. Curve of Hill's equation shifted to fit both sets of data.

those with electrodes close together. This higher value with greater electrode separations for the point where $\frac{E_0}{E} = 50$ per cent was predicted by Crozier (1937) on the basis of making available a larger population of excitable elements.

Effect of Electrode Size

The relation between electrode size and excitation time was investigated in the experiments in which silver-silver chloride electrodes were used. Data on the giant fibers obtained with silver wire electrodes 0.6 mm. in diameter and pore electrodes less than 0.1 mm. in diameter gave similar results, the fibers tested being 0.2 to 0.7 mm.

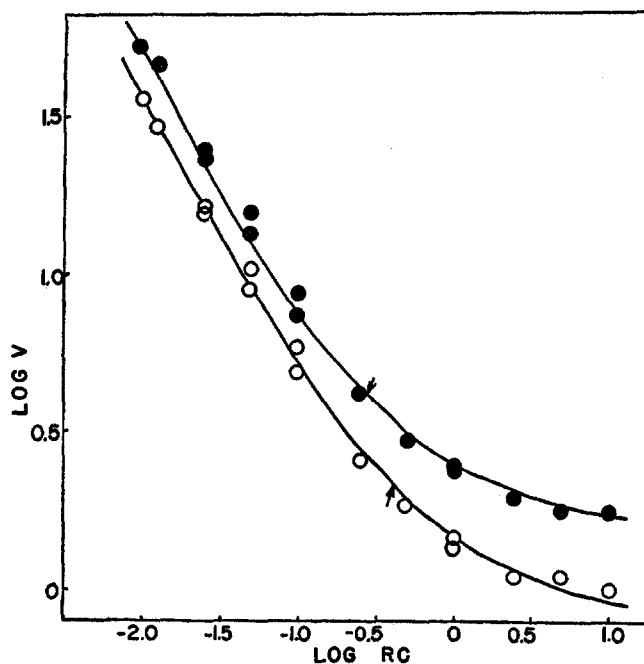


FIG. 5. Effect of electrode separation upon strength-duration curve of a giant fiber intact in great stellar nerve. Lower curve (open circles) electrode separation 15 mm; upper curve (closed circles) electrode separation 3 mm. Note shift of Hill's K value as indicated by arrows.

in diameter. The characteristic times with wires are 0.13 to 0.70 and with pore electrodes 0.34 to 0.66 msec. This shows that the wire electrodes acted as small electrodes for these huge fibers. Only when larger chlorided silver plate electrodes 7 mm. in diameter were used did longer excitation times appear. With the plates characteristic times ranged from 0.45 to 1.2 msec. The ranges of characteristic times with the large and small electrodes overlap considerably

and the results are less dependable than in the experiments in which calomel electrodes were used.

Effect of Fiber Diameter

The fibers of the fin nerves are very much smaller in diameter than the giant fibers, and range from 1 to 50 micra in diameter. They

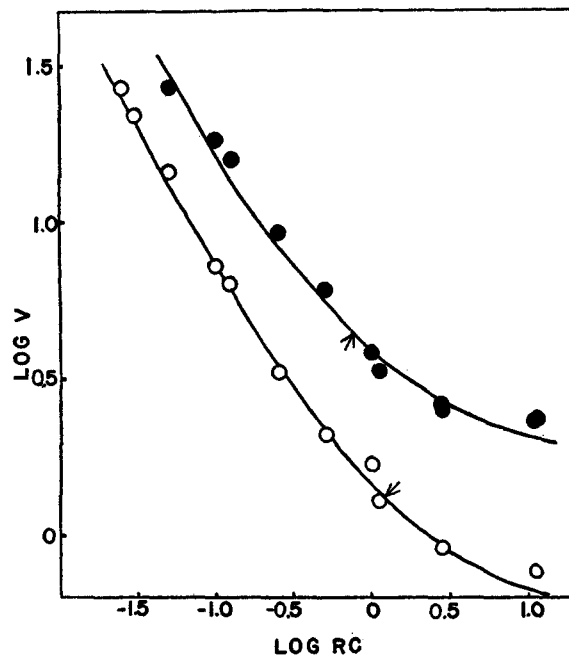


FIG. 6. Strength-duration curves of one fin nerve. Calomel electrodes. Lower curve (open circles)—interelectrode distance 12.5 mm. Upper curve (closed circles) electrode separation 3 mm. Solid lines—plots of Hill's equation. K values indicated by arrows.

yield consistently longer time constants than do the giant fibers. Fig. 6 shows strength-duration curves for low threshold fibers of one fin nerve with calomel electrodes at 12.5 and 3.0 mm. separation. The characteristic times are 1.2 and 0.77 msec. respectively. Fig. 7 is a curve obtained with chlorided silver wire electrodes 3 mm. apart. In a series of experiments with silver-silver chloride electrodes the characteristic times ranged between 0.92 and 2.4 msec. These values

are two to five times longer than with the giant fibers. The variability of the data from the fin nerves is greater than with the giant fibers because the end-point of the contraction is not so sharp, but all of the excitation curves lie well to the right of those for the giant fibers. Rosenberg (1935) obtained similar values (1.4 msec.) for the characteristic time in the fin nerve of *Sepia* which contains fibers up

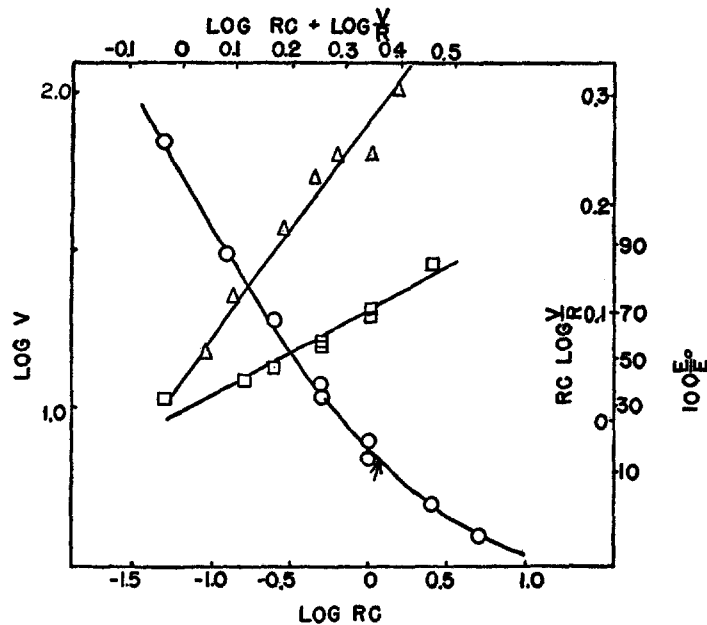


FIG. 7. Strength-duration curve of low threshold fibers in fin nerve. Chlorided silver wire electrodes. Curve is plot of Hill's theoretical equation. Lower straight line (squares)—probability plot, ordinates at extreme right. Upper straight line (triangles)—plot of Blair's equation; abscissae at top, ordinates inside at right.

to 35μ in diameter (Young, 1936 *c*), and Bugnard and Hill (1935), using the same nerve, obtained values of 2.5 msec. when electrodes were separated by 1.5 mm. and of 4 msec. when they were separated by 20 mm.

"Subordination"

There is no indication of any "subordination" effect. In a series of fourteen experiments in which chlorided silver wire electrodes were

used, the chronaxies for the uncut giant fibers of the stellar nerves were between 0.05 and 0.37 and for the cut ones between 0.05 and 0.12 msec. Chronaxies for uncut fin nerves fell between 0.28 and 0.59 msec. and for cut ones between 0.43 and 0.56 msec. (except 1). However, the ganglia of the squid are highly sensitive to oxygen lack and it cannot be assumed that under the conditions of these experiments the cell bodies were in a normal condition. It is not clear what factors are responsible for the discrepancy between the results here obtained and those of Lopicque (1929) who found chronaxies of 2-3 msec. in the great stellar nerve of *Loligo*.

SUMMARY AND CONCLUSIONS

1. Strength-duration data for the giant fiber of the great stellar nerve of the squid (*Loligo pealii*) can be approximately described by several mathematical formulations.

2. Excitation time constants for isolated giant fibers are essentially the same as constants of the giant fibers in the intact nerve.

3. The strength-duration curves of the fibers in the intact nerve lie higher on the voltage axis than those of the isolated fibers. It is concluded that the principal effect of other fibers upon the excitation of one fiber in a nerve trunk is that of shunting the stimulating current.

4. Deterioration of the nerve shifts the curve upward and to the left, resulting in shorter time constants.

5. Decreasing interelectrode distance also shifts the curve upward and to the left.

6. Excitation time constants of the giant fibers are larger with plate electrodes than with wire or pore electrodes.

7. The strength-duration curves of the smaller fin nerve fibers lie consistently to the right of, and the time constants are longer than those of the giant fibers.

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