AXON DIAMETER AND FLUCTUATION IN EXCITABILITY

BY

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Introduction.

A nerve fiber responds with an action potential in a fraction of all trials, if stimulated with identical electrical stimuli at threshold intensity and at a frequency such that the interval between the stimuli is larger than the recovery period (BLAIR and ERLANGER, 1932, 1933; PECHER, 1936).

PECHER (1937, 1939) showed that this phenomenon is an inherent property of the nerve fiber. It is not due to variations in external circumstances, viz, stimulus intensity, hence the name, fluctuation in excitability. The sequence of these responses is a haphazard one; the probability of response has the same value each time and is independent of the preceding reactions (VERVEEN, 1960).

The probability of response depends on stimulus intensity. PECHER (1939) suggested already the existence of a Gaussian relationship between response probability and stimulus intensity. In previous studies on nerve fibers of frog and of crag-fish (VERVEEN, 1960, 1961) it was shown that these relations approximate the Gaussian type of distribution function. The `threshold' (the mean of the distribution function) depends on stimulus duration : the classic strength-duration relation. It was found that the `spread' (the standard deviation of the distribution function) also depends on stimulus duration. However, the quotient of spread and threshold, the `relative spread' is independent of stimulus duration. It is a measure of the width of the threshold range, relative to the value of the threshold.

The relative spread appeared smaller for the fastest conducting axons in the claw of crag-fish than for comparable fibers in the frog sciatic nerve. A difference in axon diameter exists between these two types of axons, apart from differences such as the myelin-sheath. This fact and the suggestion by FATT and KATZ (1952) that membrane noise might depend on axon diameter warranted a further investigation. Stellar nerves of the cuttle-fish *(Sepia officinalis)* were used for this purpose. These nerves contain axons of widely different diameters (one or two giant axons of about 250 u and a large number of fibers of a much smaller diameter) but all of the same structure (YOUNG, 1936). In this way differences in species and in structure are eliminated.

Methods.

Sepia officinalis (150-200 gr.) from the Bay of Naples were used in all experiments. After decapitation the stellar nerves were dissected in fresh seawater for lengths of about 40-60 mm. Nylon threads were tied to each end. The nerves were then mounted on the electrodes and covered with mineral oil. The complete

set was kept in a water-tight box in a bath of 20° C, regulated by an ultrathermostat. Only nerves containing one giant axon were used in the experiments.

The electrode system (VERVEEN, 1961) consisted of a tripolar symmetrical stimulating electrode and two recording electrodes. All were made of tungsten wire and firmly mounted in a plastic block. The distance between the central cathode and the pair of recording electrodes was fixed at 9 mm.

A constant current stimulator was used with the current initially fixed at 400 µA for stimulating the giant axons. The output was changed in steps of 0,28 µA, which amounted to a change in intensity of 0.7 ‰ per step. With a current output of 200 µA used for stimulating the smaller fibers each step caused a change in intensity of 1.4 =A=/ shunt was connected between the stimulating electrodes parallel to the nerve. This was necessary because of the fixed output of the stimulator.

A stimulus of 0.12 cosec /duration was given to the nerve every two seconds. The giant axon in each nerve was investigated as well as the fastest conducting axon from the group of the smaller fibers. In some cases it was possible to investigate another, still slower axon of this group in the preparation.

The conduction time was measured for each fiber. This is the time interval between the application of a suprathreshold stimulus and the first inflection of the baseline on the oscilloscope, indicating the arrival of the action potential at the recording electrodes.

The shunt was adjusted to such a value, that the chosen fiber reacted with a probability of about 50 %. The threshold region was scanned in 4 to 10 input levels. The resulting percentage of positive responses per step in current intensity was transformed into probits (FINNEY, 1952). The slope of the line indicating the relation between stimulus intensity and the transformed probability of response was estimated arithmetically. The product of the reciprocal value of this slope and the percentage intensity change per step is the estimate of the relative spread.

After each experiment the diameter of the giant axon at the point of stimulation was measured in vitro. The complete preparation was then preserved in 10 formalise in sea water for further histological procedures.

Results

l. Conduction time and relative spread.

Conduction time and relative spread were determined in a total of 15 giant axons and 25 smaller axons. The results are presented in fig. 1. This graph shows that a relation exists between the value of the relative spread and conduction time the shorter the conduction time, the smaller is the relative spread. Thus, the relative spread is smaller in those fibers with higher conduction rates.

PUMPHREY and YOUNG (1938) found, that conduction rate in *Sepia* varies approximately with the square root of the diameter of the nerve fibers. Therefore, the relative spread is the reciprocal value of some function of the diameter.

ERRORS:

C o n d u c t i o n t i m e. According to PUMPHREY and YOUNG (1938) the time lapse between the application of the stimulus. and the arrival of the action potential

at the recording electrodes is a reasonably sound estimate for the conduction time. This is providing that the distance between the electrodes is not too small to decrease the error introduced by the latency. This influence is small because of the use of short-duration stimuli of suprathreshold intensity but nevertheless it is present.
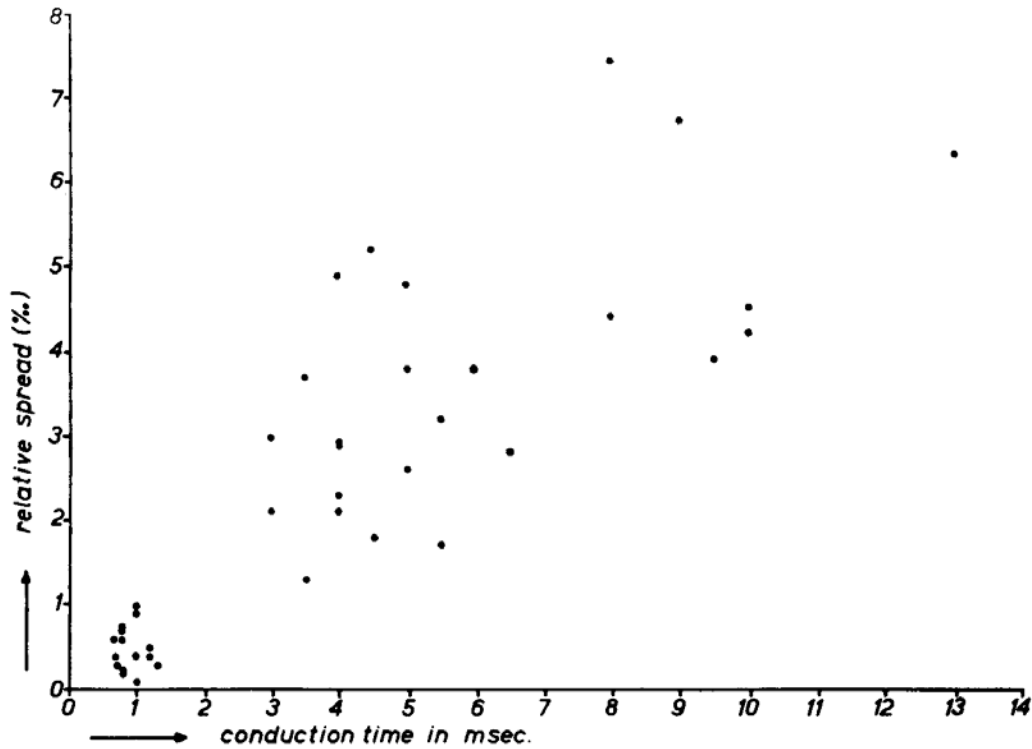

Fig. 1. The relation between relative spread and conduction time for nerve fibers in the stellar nerves of the cuttle-fish.

A second error is caused by the difficulty in estimating the moment at which the base-line begins to deflect indicating the arrival of the action potential.

A third error is introduced by the fact that the diameter of the giant fibers is not uniform over their whole length. This influences the conduction time, because conduction rate depends on the fiber diameter. Therefore, the action potential is conducted with different velocities over different stretches of the fiber. To reduce this effect the distance between the electrodes was kept small but that implies that the first error becomes a greater factor.

No attempts were made to obtain correction factors for these errors.

R e l a t i v e s p r e a d. In all these fibers the threshold region appeared to be very small. This implies, that variation of the stimulus will considerably influence the measurements. Direct measurement on the noise level of the stimulus was used to correct the values of the relative spreads of the fibers investigated.

In these experiments a second error arises from the fact that the threshold of the nerve fibers changes slowly, either increasing or decreasing steadily. The net

effect of this change is a compression or enlargement of the percentage value of the steps used for scanning the threshold region. A correction factor was calculated in each case by measuring rate and direction of the threshold change in time. Nevertheless, the existence of these errors greatly hampers accurate determinations of the relative spread.

Because of the existence of the errors no attempt was made to make a further analysis of the data presented in fig. 1, of relative spread and conduction time.

2. Fiber diameter and relative spread.

The fact that an inverse relationship exists between relative spread and axon diameter calls for the analysis of the data obtained in this study and those gathered in large series of experiments on frog and crag-fish axons mentioned before. Only the diameters of axons were studied. No attempt was made to include the myelin-sheath in the analysis. Whether or not this is of influence is difficult to determine at present.

Three sets of data are available for the analysis.

1. F r o g *(Rana esculenta)*. The relative spread was determined in a total of 80 axons (viz., nodes of RANVIER). Each axon was the most excitable fiber in a sciatic-phalangeal nerve preparation. With our experimental set up it was impossible to know which fiber was actually investigated. In histological preparations one of the largest axons of the phalangeal nerves was assumed to be the stimulated one because the most excitable fiber was stimulated. In this way the diameter of a total of 45 axons in 12 phalangeal preparations was determined histologically (BODIAN-ZIESMER). There was some shrinkage present. But the axon diameters are larger than the diameters of the nodes (HESS and YOUNG, 1952) actually active in the initiation of the action potentials. Therefore uncorrected diameter values are presented.

2. C r a y - f i s h. *(Astacus leptodactylus)*. The relative spread was determined in 15 axons, each being the most excitable fiber in the nerve studied. 17 Axons were prepared from comparable preparations which allowed measurements of their diameter in vitro. A sheath of connective tissue surrounds each fiber. A-thin layer of myelin is present as a bire-fringent zone surrounding the axon of which the inside diameter was determined.

TABLE I

Species	Relative Spread (R.S.)$^\circ/_{oo}$			Diameter (D) μm		
	Number of determinations	R.S.	S.D.	Number of determinations	D.	S.D.
Rana	80	11	5	45	4	0.8
Astacus	15	1.2	0.3	17	30	6
Sepia	15	0.5	0.2	14	243	64

3. C u t t l e- f i s h *(Sepia officinalis)*. Uneven shrinkage in the histological preparations prevented the estimation of the mean diameter of the largest axons in

the group of small fibers. Therefore, only the data on the giant fibers are used. The data on relative spread and axon diameter for each group of fibers were pooled and the three resulting pairs of mean values were compared. These data are presented in Table I. It is clear that relative spread and axon diameter are inversely related.

Discussion

Among the possible causes for the phenomenon discussed by PECHER (1939) the thermal agitation of particles within the axonal membrane might be an important factor. In their studies on spontaneous subthreshold activity at motor nerve endings, FATT and KATZ (1952) suggested that noise voltage across the axon membrane might be responsible for the occurrence of spontaneous excitation at nerve terminals. With the assumption that noise voltage effected the random fluctuation of the resting potential, they calculated the noise level for a model nerve based on the electrical constants of non-medullated axon. It followed from their calculations that the noise level for axons of these properties is related to its diameter. The numerical expression derived from their equation $\log E = 4.20 - 0.75 \log d$.

E: r.m.s. value of noise fluctuations in Volts.

d : diameter in μm.

The threshold potential is about 15 mV for frog nodal membrane (TASAKI, 1959) and 15-20 mV for *Loligo* axon (HODGKIN, HUXLEY and KATZ, 1949). Therefore, if we make the assumption that the threshold potential of peripheral axons is 15 mV, then the quotient (C) of effective noise voltage and threshold potential can be calculated. It follows that $\log C = -2.38 - 0.75 \log d$.
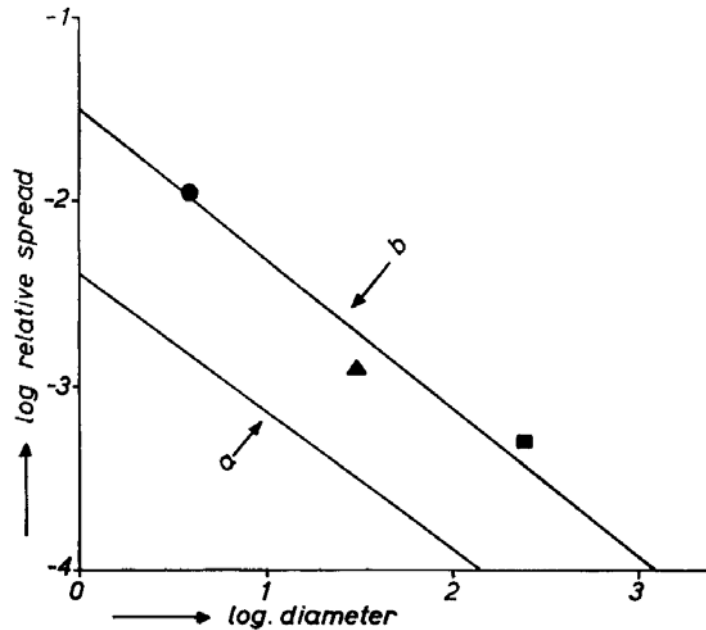

Fig. 2. Relation between relative spread and axon diameter for the fastest conducting nerve fibers in peripheral nerves of frog ● , cray-fish ▲, and cuttle-fish ■
a. theoretical relation, b. least square line.

When we calculate the equation for the line relating the logarithm of the relative spread (RS) and the logarithm of axon diameter we find that

$$\log RS = -1.50 - 0.80 \log d.$$

The slope of both lines is nearly identical but the theoretical values are smaller (Fig. 2). This is in agreement with the suggestion of FATT and KATZ (1952) and of BULLOCK *et al.* (1953) and HAGIWARA (1954), that more factors may be involved than only the agitation of ions within the membrane.

In this study the assumption is made that for each species investigated the values of relative spread and diameter are determined from samples of the same population. Because of this assumption and of the errors signaled before we must be careful with the evaluation of the experimental relation derived from these three sets of data. The similarity with the theoretical equation is, however, very suggestive. The experiments support, therefore, the hypothesis that fluctuation in excitability might to a large part be due to thermal agitation of ions within the membrane.

Summary

The relationship between axon diameter and fluctuation in excitability was investigated. Fluctuation was characterized by relative spread, i.e. the measure of the threshold region width relative to the threshold value.

Experiments on intact stellar nerves of the cuttlefish revealed the existence of a negative relation between relative spread and fiber diameter. The larger the fiber is, the less the relative spread.

Consideration of the data obtained on three populations of nerve fibers (the most excitable fibers in the sciatic of the green frog, in the crag-fish claw and in the stellar nerve of the cuttle-fish) gave rise to the notion that the relative spread may vary with a negative power of the axon diameter.

Comparison of these data with the theoretically expected values of the membrane noise voltage supported the hypothesis that fluctuation in excitability might to a large part be due to thermal agitation of ions within the membrane.

Literature

1. BLAIR, E. A. and ERLANGER, J. Responses of axons to brief shocks. *Proc. Soc, exp. Biol.*, N.Y. **29**, (1932), 926-927.
2. BLAIR, E. A. and ERLANGER, J., A comparison of the characteristics of axons through their individual electrical responses. *Amer. J. Physiol.* **106** (1933), 524-564.
3. BULLER, A. J., NICHOLLS, J. G. and STROM, G., Spontaneous fluctuations of excitability in the muscle spindle of the frog. *J. Physiol.* **122** (1953), 409-418.
4. FATT, P. and KATZ, B., Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117** (1952), 109-128.
5. FINNEY, F. J., Probit Analysis, 2nd. ed. Cambridge University Press. (1952).
6. HAGIWARA, S., Analysis of interval fluctuation of the sensory nerve impulse. *Japan J. Physiol.* **4** (1954), 234-240.
7. HESS, A. and YOUNG, J. Z., The nodes of Ranvier. *Proc. Roy. Soc.* B. **140** (1952) 306.
8. HODGKIN, A. L., HUXLEY, A. F, and KATZ, B., Ionic currents underlying activity in the giant axon of the squid. *Arch. Sci. Physiol.* **3** (1949), 129-150.
9. PECHER, C., Etude statistique des variations spontanées de l'excitabilité d'une fibre nerveuse. *C.R. Soc. Biol.,* Paris. **122** (1936), 87-91.

10. PECHER, C., Fluctuations indépendantes de l'excitabilité de deux fibres d'une même nerf. *C.R. Soc. Biol.,* Paris. **124** (1937), 839-842.

11. PECHER, C., La fluctuation d'excitabilite de la fibre nerveuse. *Arch. int. Physiol.* **49** (1939), 128-152.

12. PUMPHREY, R. J, and YOUNG, J. Z., The rates of conduction of nerve fibres of various diameters in cephalopods. *J. Exp. Biol.* **15** (1938), 453-466.

13.TASAKI, I., Conduction of the nerve impulse. In: Handbook of Physiology. Section I. Neurophysiology. p. 95. ed. J. Field. Washington: Am. Physiol. Soc. (1959).

14. VERVEEN, A. A., On the fluctuation of the threshold of the nerve fibre. In : Structure and Function of the Cerebral Cortex. p. 282-288, ed. D. B. TOWER and J. P. SCHADÉ. Amsterdam; Elsevier. (1960).

15. VERVEEN, A. A., Fluctuation in Excitability. Research Report on Signal Transmission in Nerve Fibres. Ph. D. thesis, Amsterdam: Netherlands Central Institute for Brain Research. (1961).

16. YOUNG, J. Z., The structures of nerve fibres in Cephalopods and Crustacea. *Proc. Roy. Soc.* B, **121** (1936), 319-337.