THE BIOPHYSICS OF THE NERVOUS SIGNALLING PROCESS

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For centuries the problem of nervous signalling has attracted the attention of biophysicists, physiologists, and psychologists. Whether the information is transmitted within the nervous and muscular systems only in the form of discreet nervous impulses at different frequencies or whether some other method is possible such as the migration of energy in colloidal cells, of electrical fields, or of electronic interaction etc. has not yet been decided.

N. E. Wedensky [1] proposed that there were two principal forms of excitation: the first was dynamic and spreading, and the second stationary, maintained, and confined to the region of action on the living system; it was held to be responsible for contrasting functional changes in a limited region around a parabiotic area.

At the end of his life, in 1920, N. E. Wedensky [2] expounded the concept of perielectrotonus, which contributed further evidence for the existence of a distinct continuous signalling process. One of us [4] using a single motor unit showed that the threshold for perielectrotonus is 2-4 times below that for normal excitation. Perielectrotonus is a subthreshold influence for the excitation of signals and has no refractory period; in other words it is not made up of discreet signals, but is a continuously variable quantity, and as such is not subject to the "all or none" law. It is susceptible to summation and reinforcement. Continuous signalling does not elicit a complete physiological response, but it resets the functional condition of the cells and organs comprising the system. Just like electrotonus it spreads more rapidly than do discreet nervous impulses. It is inseparably associated with the discreet signals, and prepares and adapts the living system to the optimal reception of certain patterns and forms of nervous information, or, on the other hand, it reduces the sensitivity of a system, making it indifferent to certain forms of nervous information. To explain its many aspects, quantitative biophysical investigations are required.

We have set out to determine functional changes in the parabiotic and periparabiotic region after inducing a condition of parabiosis by applying pressure graded in strength, gradient, and area to a phalangeal preparation.

METHOD

A phalangeal nerve-muscle preparation from a frog was placed in a moist chamber (Fig. 1); the nerve was placed on electrodes so that part of its upper region was supported by a perspex platform having a smooth surface, and it was then covered by a thin sheet of perspex or of glass. Through the latter, pressure was applied to the nerve from a lever loaded at one end by a flask filled with water from a syphon tube. By this means we were able to obtain forces which could be continuously increased up to 3.5 kg/cm^2 (pressure was measured on the cover plate, but not on the nerve itself).

The length of the compressed portion of the nerve was 3, 6, or 12 mm in the different experiments. We used rates of increase of pressure of 8.37, 4.19, 1.86, and 0.93 g/cm/second.

Above the compressed region there was a pair of electrodes from which stimuli were periodically applied to determine the condition of conduction in the compressed region. We recorded the time when conduction ceased and calculated the corresponding pressure. On the platform where the compressed part of the nerve was placed, level with its surface and below the pressure point, 3-6 pairs of electrodes were placed at a separation of 10-15 mm from each other, and were used to test the conduction in these regions, or to lead off potentials. All the electrodes were made of chlorided silver. In order to avoid the potentials being shunted through the fluid collected in the capillary space beneath the cover plate, in this region the nerve was dried with filter paper and covered with a drop of vaseline oil (a special test showed that this procedure did not appreciably influence the properties of the preparation).

To determine excitability and conduction we applied condensor discharges or squarewave stimuli of different amplitudes and durations; they were obtained from a ISÉ-1 pulse stimulator. The thresholds were determined either visually by observing the muscle contraction, or by recording the action potentials by means of an amplifier and cathode ray oscillograph.

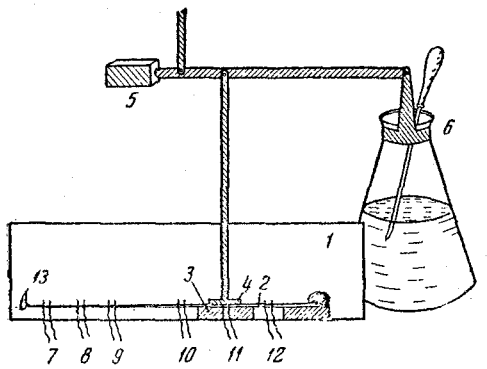

Fig. 1. Diagram of the device for applying a continuously graded pressure to induce a parabiotic condition mechanically. 1) Chamber; 2) preparation; 3) platform; 4) cover plate; 5) counter-plate; 6) load; 7-12) electrodes.

To record the slow potential changes we used a mirror galvanometer with a compensating circuit. A unipolar lead was used, and the indifferent electrode was placed on a vertebra or on one end of a nerve killed by heat.

In some experiments pressure was applied to the distal end of the nerve, and the conductivity of this region and the excitability of the several parts of the nerve were determined from the action potentials led off on stimulation from the proximal end.

The results obtained were treated statistically.

RESULTS

Failure to Conduct with Pressure Applied to the Nerve

In a nerve exposed to a slow even pressure, no spread of excitation occurred. The pressure caused conductivity to cease, and the effect was at first reversible. If the load was removed or reduced, then conductivity was usually restored within 1-3 min. If however the pressure was maintained for 5-15 min after the block, the changes in the nerve were irreversible.

TABLE 1. Results of Experiments Made at Various Seasons

Season	Pressure at which conduction failed (g/cm^2)	Percentage cases of irreversible block
April-June	1062 ± 66	56.6 ± 6.7
September-October	1430 ± 91	25.0 ± 10.8
December-February	1432 ± 65	29.8 ± 5.7

Cessation of conduction due to pressure on the proximal portion of the nerve developed in the different experiments at pressures between 100 and 3,000 g/cm^2, the average value being 1,333 ± 39 g/cm^2; these figures were obtained from 230 measurements. When pressure was applied to the distal end of the phalangeal preparation where there are only a few nerve fibers, conduction ceased on average at a pressure of 533 ± 181 g/cm^2 (results of 18 measurements). After removal of the block, conductivity recovered in only two preparations out of the sixteen.

We found that there were seasonal differences; as can be seen from Table 1, preparations investigated in the spring and summer were considerably less resistant to pressure than were those studied in autumn and winter.

In spring and summer conductivity was blocked by a smaller pressure than was required in winter, and it more readily became irreversible. The differences were highly significant ($P = 0.01$).

When the area of nerve compressed was increased, the conductivity tended to fail earlier (Table 2).

However, the differences were not statistically significant ($P > 0.01$), so it is not legitimate to infer that the block occurs more rapidly the greater the extent of the region compressed.

At a rate of application of pressure of 8.37 g/cm^2/ sec, which was the maximum rate used, 25-30% less time was required to establish the block than in the case when the pressure increased more slowly ($P = 0.01$). There were no appreciable differences related to the other three rates of pressure change.

Change in the Polarization of the Nerve with Pressure

In the compressed region a negative potential of 15-55 mv develops. When the proximal part of the nerve was compressed, in 33% of the experiments the electrical response commenced before the block began, the two processes began together in 28% of the experiments, and the electrical response followed the block in the remaining 39% of the tests. After the load had been removed, conductivity was restored, but the potential alteration in the affected area remained unchanged. With pressure applied to the distal region of the phalangeal preparation, the electrical change was observed to occur in every case $\frac{1}{2} - 1\frac{1}{2}$ min after complete block had been established.

Removal of the load did not alter the electrical potential, and did not restore conductivity. In two cases however when the load was removed immediately after blockage had occurred but before the electrical response had taken place, the conductivity was re-established.

No electrical potential was recorded from the compressed area itself.

The results afford evidence for the view that mechanoparabiosis (and here we refer to the reversible stage of the process) may take place without changes of electrical potential. When investigations are made of a whole nerve trunk, the results are greatly complicated through the uneven development of the parabiotic process in the different fibers. Death of the least robust fibers causes them to become depolarized, and the electrical potentials which develop as a consequence may exert an electrical influence on the remaining functional fibers.

Excitability Changes in the Nerve at Different Distances from the Compressed Area

During the application of a slowly increasing pressure to the proximal end of the nerve, the electrical threshold for stimulation was measured in different regions of the distal portion at intervals of $\frac{1}{2} - 1\frac{1}{2}$ min. The measurements were made: a) during the application of the pressure and before block, and sometimes after block also; b) while the pressure remained constant, and after block had developed; c) during gradual release of pressure until conductivity was restored, until the whole of the load had been removed; d) for 10 min after gradual and complete removal of the pressure, or after the pressure had been removed rapidly. Because the time required for conduction to fail varied greatly from one preparation to another, we thought it best in each case to determine the period T from the time the pressure was applied until the onset of block, and all the measurements of thresholds during the pressure application referred to the first, second, third, or fourth quartiles of the period T.

TABLE 2. Effect Due to the Length of Nerve Compressed

Length of nerve compressed (in mm)	Pressure at which block occurred (in g/cm^2)
3	1280 ± 96
6	1219 ± 69
12	1180 ± 76

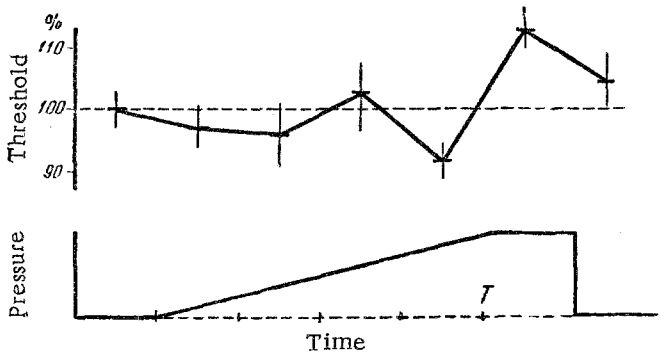

Fig. 2. Changes in the excitability of a nerve 5-15 mm below the compressed region. The lines indicate the extent of the variation.

We took into account the possible influences of the following factors: 1) the distance of the point tested from the compressed region (5-15 mm and 25-52 mm); 2) linear extent of the compressed region (3, 6, 12 mm); 3) rate of change of pressure (9.93, 1.86, 4.19, and 8.37 g/ cm^2/ sec); 4) characteristics of the test electrical stimuli (from short stimuli of 0.05 m sec to long ones of 25 m sec).

In order to be able to compare the results of the different experiments, in all cases the thresholds were expressed as a percentage of the original value (the mean value of the threshold at the test point of the particular preparation before the application of pressure was taken as 100%).

Figure 2 shows the mean percentage changes of excitability in a region 5-15 mm from the compressed part at various periods during the compression, and the range of variation.

After complete block had occurred in the compressed region, it was found that the electrical excitability in a part 5-15 mm below it had fallen, so that the threshold had risen on average by 12.5% (changes significant, $P < 0.01$). In this region, shortly after the occurrence of block (in the last quartile of T) it was most probable that there was a small increase of excitability; the threshold fell on average by 8.3% ($0.01 < P < 0.05$%). In the region 25-52 mm from the compressed part, no statistically significant changes of excitability were observed. We could find no difference between results obtained with different lengths of compressed area, with different rates of pressure application, or different kinds of electrical test stimuli.

The phasic changes of excitability in the periparabiotic region demonstrate that an influence may be exerted by one part of a living system on another either by means of discreet nervous impulses or by a continuous signalling process.

SUMMARY

Part of a nerve containing a small number of nerve fibers was compressed by different amounts, different rates, and over different areas; it formed part of a nerve-muscle frog phalangeal preparation. Excitability, conduction, and electrical properties were studied in the compressed area where parabiosis had been induced, and in the peri-parabiotic area. Gradual compression of the nerve caused no spread of excitation, but blocked conduction, at first reversibly.

In the part of the nerve distal to the compressed area, where the number of nerve fibers was small, polarization developed only after conductivity had failed, and then the blockage was irreversible. When the proximal end containing a greater number of fibers was compressed depolarization occurred before and after conduction failed, and was evidently due to nerve fibers being destroyed at different times.

Changes of electrical excitability were detected 5 to 15 mm from the compressed area. These phasic changes of excitability in the peripara biotic area indicate that one area of a living system may influence another by means of continuous signalization, a process quite distinct from the passage of discreet nervous impulses.

LITERATURE CITED

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4. P. O. Makarov, Problems of the Microphysiology of the Nervous System. [in Russian], Medgiz (1947).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
