

THE NERVES AND MUSCLES OF MEDUSAE

III. A DECREASE IN THE REFRACTORY PERIOD FOLLOWING REPEATED STIMULATION OF THE MUSCLE OF *RHIZOSTOMA PULMO*

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INTRODUCTION

In his paper on *Aurellia* and other medusae, Bullock (1943) introduces a method which makes possible the measurement of the refractory periods of the nerve and muscle in a strip cut from the bell of a medusa. During a repetition of this experiment, using the Mediterranean form *Rhizostoma pulmo*, it was soon noticed that the responses depended on the previous activity of the preparation, and this could not be explained by the known characteristics of coelenterate muscle. Bullock's method of stimulating a strip of the bell can be represented diagrammatically as in Fig. 1, where time is plotted downwards. A single stimulus at *A* is followed by a wave of contraction down the strip to *B*. If a second stimulus is applied at *B* after such an interval that it falls within the refractory period of the nerve at *B*, there will be no further response. However, if the stimulus falls when the nerve can again respond, but when the muscle is still refractory, a second wave of excitation will be propagated back along the strip. Although the muscle is refractory at *B* this second wave will bring about a second contraction at *A* if the strip is sufficiently long for the muscle at *A* to have emerged from its refractory period. Ideally then, two contractions are observed at the end *A* and one contraction at the end *B*. These contractions are represented at the sides of the diagram. The interval between the two responses at *A* depends on the length of the strip, the interval between the stimulus at *A* and that at *B*, and the velocity of propagation in each direction.

METHODS

Half of a bell of a large specimen of *Rhizostoma* was taken, and the regions round the mouth and along the margin were removed. This produced a curved strip about 25 cm. long and 5 cm. wide, which was pinned down in sea water in a way that allowed the contraction at each end to be recorded separately. In the absence of the tentaculocysts there were no spontaneous movements. The responses to stimulation were recorded on a smoked drum with light isotonic levers. Stimulation was by single break shocks from two induction coils, one for each end of the strip. The method of stimulating the two ends separately with a constant interval between stimuli was as follows. A heavy pendulum, of adjustable period, was fitted with a light contact which worked on a hinge, *H*, Fig. 2. Below the pendulum was clamped

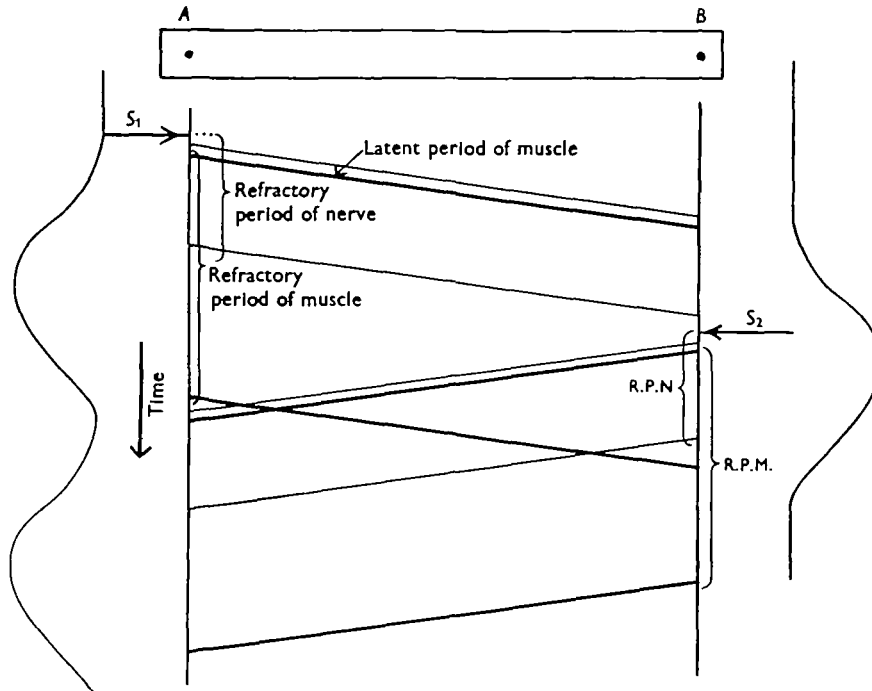


Fig. 1. A representation of the propagation of excitation following the first stimulus S_1 and the second S_2 with an indication of the response of the muscle. The time scale is not known accurately and is therefore not shown.

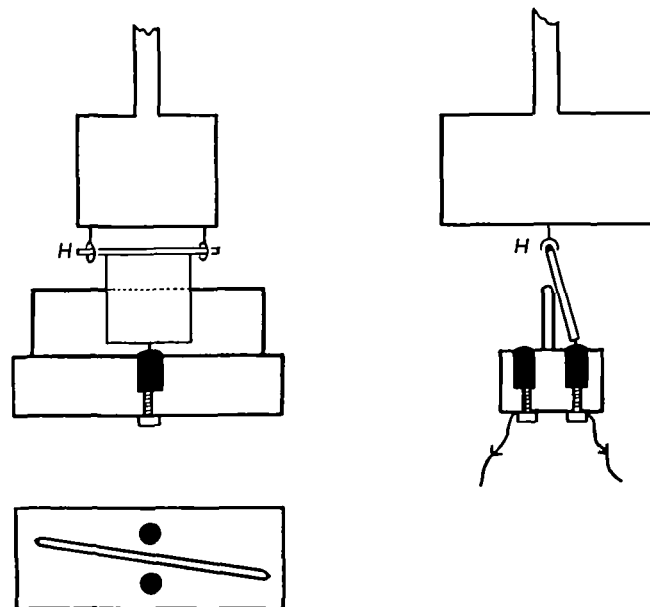


Fig. 2. The arrangement of the contact on the pendulum to give a constant interval between stimuli which are destined for opposite ends of the strip of tissue.

a block of insulating material in which two cups full of mercury were arranged as shown (black in the figure). Between these cups a partition was fixed in such a way that during the swing of the pendulum in one direction the contact would pass through the mercury in one of the cups, and during the swing back it would pass through the other. With the pendulum at rest the contact must be exactly between the mercury cups to ensure a constant interval between the two contacts irrespective of their order. The pendulum was always released from a fixed point to swing with the same amplitude on each occasion and was caught after each full swing. Each mercury cup was connected in the primary circuit of an induction coil, the secondary being adjusted so that only the 'break' shock exceeded threshold. To reverse the order of stimulation the pendulum was moved across to begin its swing from the other side. This movement caused a single stimulus, which is seen on the record Fig. 3 marked by a small arrow, α , β , γ .

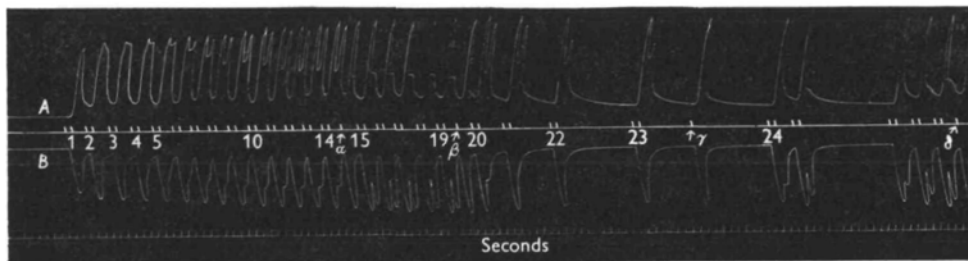


Fig. 3. Records from the two ends *A* and *B* of a strip 25 cm. long from the bell of *Rhizostoma*. Contraction is registered upwards at *A* and downwards at *B*. Between the *A* and *B* traces is the record of stimuli, two break shocks at a constant interval of 1 sec. Over the part of the record from the beginning to the point α and again between β and γ the first stimulus was applied at *A* and the second at *B*; between α and β and between γ and δ the order was reversed.

RESULTS

As can be seen from Fig. 3, the first pair of stimuli produce only a single contraction at each end, but at the end *A* the single contraction soon resolves itself into two parts until at stimulus pair no. 10 the response is characteristic of Bullock's method for measuring refractory periods, with two contractions at *A* and one at *B*. By stimulus pair no. 14 the single response at *B* shows signs of a second contraction. At the point α the order of stimulating the ends is reversed so that *B* receives the first stimulus. The double response now seen at *B* is similar to that formerly at *A*, and the response at *A* shows the small second response formerly at *B*. At β the order is again reversed and the symmetry is again evident. At this point the interval was increased before the next pair of stimuli, and it is seen that the second response at *B* disappears. The second response at *A* (no. 21) is reduced a little, further reduced at no. 22 and even further reduced at no. 23 after the still longer pause. The order of stimulation is again reversed at γ and after a pause of 10 sec. the end *B* receives the first of the pair of stimuli. There is now (no. 24) only a small second response at *B* and no second response at *A*, but a pair of stimuli immediately after give a response similar to those at nos. 15-19.

The progressive appearance of the second response is well shown by the first part of the trace, the decay of the phenomenon is shown by the later responses, and the reversal of ends at α shows that the effect is present at both ends of the strip of tissue. The progressive appearance of the second response at *B* is shown also in Fig. 4, but in this preparation it appears towards the end, rather than at the height, of the first response. In this record the responses at the end *B* for the contractions nos. 1 and 2 show marked facilitation, both in the greater height of the response and in the more rapid relaxation of the second contraction. Bullock called the latter effect 'facilitation of relaxation'. The appearance of the second response in nos. 7-15 (Fig. 4) is

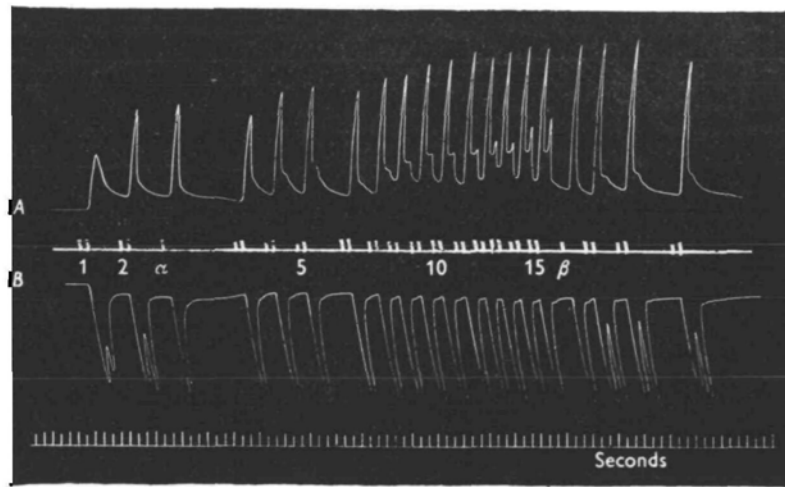


Fig. 4. See legend to Fig. 3. In this preparation the interval between the stimulus pairs was 0.88 sec.

accompanied by both of these long-term effects, but this does not mean necessarily that they are causally related to it. It may be objected that the progressive appearance of the second response is due to the facilitation of relaxation, i.e. that the second response has been present from the beginning but cannot show on the record until the muscle has relaxed after the first response. However, an examination of the lower trace of Fig. 3 shows that apart from the first response the rate of relaxation is the same for all; again, in Fig. 4, the second response at the end *A* grows from the end of the relaxation.

DISCUSSION

Let us first consider the effect at the end *B* (Fig. 3). Here the first contraction of the experiment is a single peak, and after several repetitions a smaller second contraction appears immediately following the first. The first contraction at *B* is due to the first stimulus, which is applied at *A*. Initially there is no second response at *B* because the muscle fibres are all refractory to the second stimulus. The progressive

appearance of a second contraction at *B* may be due to a progressive *increase* in the velocity of the excitation wave from *A* to *B*, so that the stimulus at *B* comes later relative to the excitation arriving from *A*. Alternatively, it may be due to a progressive shortening of the refractory period of muscle fibres at *B*. There are no other obvious factors which might undergo a progressive change to give this effect, namely that more and more of the muscle at *B* is able to give a second contraction.

Now consider the effect at *A*. At first there is only a single contraction due to the first stimulus, which is applied at *A*. Progressively there appears a larger and larger second contraction which is due to excitation spreading from the second stimulus which is at *B*. Initially stimulation at *B* produces no second contraction at *A*. This could be interpreted by saying that the stimulus at *B* comes within the refractory period of the nerve at *B*, so producing no second excitation. However, a second contraction at *A* appears progressively, not all at once, as it would do if the effect were due to a shortening of the refractory period of the nerve; for the nerve acts in an all-or-nothing manner and with constant time relations cannot produce a progressively increasing second contraction at *A*. In fact there must be a second excitation of the nerve at *A* on every occasion to which the muscle is able to respond with successively greater contractions.

The events at *A* can be explained by a *decrease* in the velocity from *B* to *A*, so that the muscle at *A* has more time to come out of its refractory period before the nerve impulse arrives from *B*. But, as shown above, an *increase* in velocity from *A* to *B* is required to account for the events at *B*. When the order of stimulation is reversed the second contraction still persists at both ends. This shows that whatever the effect responsible for the progressive increase of the second contraction, it is maintained irrespective of the direction in which the excitation travels. In fact the velocity of propagation cannot decrease in one direction and increase in the other.

The satisfactory explanation of all the observations is the progressive shortening of the refractory period of more and more muscle fibres. The first stimulus is applied at *A*, and after an interval a second excitation arrives from *B*, at first during the refractory period of most of the muscle fibres. Progressively more and more of the muscle fibres have come out of the refractory state when the second excitation arrives, and a larger and larger second contraction appears.

The phrases used in the last paragraph suggest that this sheet of muscle is envisaged as a population of individual fibres, each of which acts in an all-or-nothing manner, in which the strength of the contraction will then depend upon the number of fibres responding to a stimulus. But whether the unit is the individual fibre, part of a fibre or a group of fibres the same explanation would apply. The shortening of the refractory period at the neuromuscular junction has similar time relations to the facilitation of the strength of the response, but it is only a speculation to consider it a part of one general phenomenon.

SUMMARY

Evidence is presented to show the shortening of the refractory period of some of the elements in a population of coelenterate muscle fibres following frequent stimulation of the bell of *Rhizostoma pulmo*.

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REFERENCE

- BULLOCK, T. H. (1943). Facilitation in Medusae. *J. Cell. Comp. Physiol.* **22**, 251.