THE NERVES AND MUSCLES OF MEDUSAE

I. CONDUCTION IN THE NERVOUS SYSTEM OF *AURELLIA AURITA* LAMARCK

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INTRODUCTION

In their classical works on *Aurellia*, both Eimer (1874) and Romanes (1876) demonstrated the propagation of the wave of contraction of the circular muscle in all directions across the bell. The rapid transmission of this contraction wave with a velocity of about 50 cm./sec. is responsible for the symmetry of the movement at each beat when the animal is swimming. Upon his discovery of 'varicose' fibres spreading out from each of the eight marginal ganglia, Eimer at once assumed that he had found nerves responsible for the propagation of the contraction wave. Romanes was more cautious; 'lest the fibres which Mr Shäfer (1878) describes as nervous should eventually prove to be the anatomical structures that are concerned in performing the physiological functions...I adopted...a neutral term' (he called the physiological pathways 'lines of discharge').

The passage of a wave of contraction along a strip of tissue cut from the bell is eventually prevented if the strip is made narrower by gradual removal of tissue. Romanes pointed out that the interruption suddenly appears at one point only, and persists at that point, in a strip that may be 1 cm. wide. Romanes was also able, in the following way 'to locate very precisely the line through which physiological continuity had been established'. While stimulation at any other part of a small area was always followed by only a small local contraction, stimulation of this 'line of discharge' produced a general contraction.

Bethe (1903) showed that a wave of contraction of the circular muscle passes muscle-free areas of the bell of *Rhizostoma*, but this tells us nothing about conduction in the regions of muscle. Mayer (1906) showed that the contraction wave passes damaged areas containing nerve fibres but is not transmitted across a previously damaged area where muscle fibres alone have regenerated. Mayer also showed that the wave passes an area where the muscle is anaesthetized with magnesium ions. These experiments leave open the question whether there is, in addition to the normal contraction wave, a slower conduction through normal muscle from fibre to fibre. Moreover, Mayer's identification of the separate elements is open to doubt. In a study of the behaviour of Scyphozoa, Bozler (1926) described reactions where decremental conduction is involved, but these are never concerned with the normal propagation of the beat. Bozler also pointed out that the contraction wave is

unlikely to be propagated through the muscle alone because the velocity of the contraction wave is the same along the line of the muscle fibres as across it. In the polyp *Calliactis*, Pantin (1935) measured the refractory period of the through-conducting system, and Bullock (1943) measured that of several species of medusae. These results show that the most rapid conducting system is distinct from the muscle and they are the best physiological evidence of nerves we have, but they do not disprove conduction by additional means, and none of the experiments definitely locate the pathway of the excitation.

Evidence will now be presented to show that the excitation associated with the contraction wave is conducted in either direction at least by the thicker nerve axons and that its passage is accompanied by an electrical impulse of the usual type.

THE FUNCTION OF THE OBSERVED AXONS

A piece of the bell was mounted on a microscope stage with the subumbrella epithelium uppermost. The larger fibres of the nerve net of such a preparation could be clearly seen by use of a phase-contrast microscope or oblique illumination (Horridge, 1953). Moreover, the fibres proved to be strong enough to tolerate a certain amount of manipulation under the microscope. This made it possible to show that some of the observed fibres in the epithelium are in fact essential for the propagation of the contraction wave.

With a little care it was possible to make a narrow bridge of tissue with a single observable axon going across in a bridge of epithelium from one part of a piece of *Aurellia* to another part. Supported on wax or cork, the bridge was held motionless under the microscope by several pins to prevent damage which might result from contraction of the preparation. To give the fibres of the circular muscle a chance to conduct the excitation across the bridge, some of the bridges were cut along the line of the muscle. The bridge was examined under a microscope and gradually made narrower and narrower until only one observable axon went across. During this process of whittling down, the propagation of the excitation across the bridge was tested from time to time (Fig. 1). In many experiments the propagation broke down while the bridge still contained several axons, but one could not tell whether these had no connexion with the through-conducting system or were merely damaged by the pulling and tearing. Running across the bridge were plenty of muscle fibres which did not appear to be damaged.
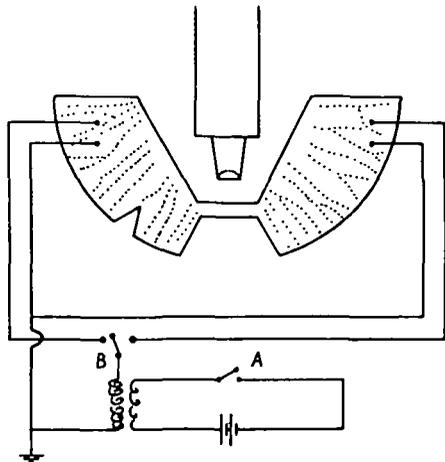

Fig. 1. Arrangement of the experiment with a single fibre in a bridge between the pieces of *Aurellia*. The switch *A* breaks the primary circuit, and the switch *B* directs the stimulus to one side or the other.

In six successful experiments a bridge was prepared with a single axon running across, and was found to conduct the contraction wave in either direction. In one experiment propagation was in only one direction and remained so after stimulation. Again it was impossible to decide whether this polarization, which other authors have found in long narrow bridges, was due to damage to the axons and their connexions. In each case section of the axon in the bridge stopped the propagation, and I have never succeeded in making a bridge which would conduct without an axon.

It is not likely that in each case failure to conduct after section of the nerve fibre was in fact due to damage of the muscle by the operation, and it is more likely that it was due to section of the nerve. A bridge containing muscle fibres but no nerve axon would not conduct and some bridges would not conduct although apparently undamaged axons were visible. In these cases narrow strips of muscle did not conduct the contraction wave and it seems unlikely that wider pieces of muscle would behave differently. This experiment showed that the contraction wave was propagated through the nerve fibres, not through the muscle, and that the excitation could be conducted in either direction along a single nerve fibre.

ELECTRICAL RECORDING FROM A NERVE FIBRE
Method

The diagram, Fig. 2, shows the arrangement of the apparatus for electrical recording. A microscope A with a 16 mm. objective had on its stage a flat dish made of a piece of glass 10 by 20 cm. with wax walls. A piece of *Aurellia*, lying in this dish with subumbrella epithelium uppermost, was kept moist with sea water from a hand pipette. In some cases it was necessary to flatten the piece of the bell by cutting off jelly from the exumbrella side of the thickest part of the wedge. It was found that the greater clarity obtained by the use of a phase-contrast microscope was not worth the time and trouble in making the continual adjustments as the preparation moved.

Attached to the same base as the microscope was a micromanipulator B which held the recording electrode in the field of view. With this a nerve fibre was picked up on a fine platinum wire and raised into the air above the surface of the epithelium.

The piece of *Aurellia* rested on an earthed plate arranged to avoid obstruction of the light passing through the microscope. All the apparatus rested upon a large sheet of galvanized iron, to which the whole was earthed. Fifty-cycle hum in the recording system could be avoided by keeping far from mains circuits, but extensive screening was necessary in a small room.

A screened lead from the electrode led to a condenser-coupled amplifier of usual design with a long time constant. The output of the amplifier was visible on a single-beam cathode ray tube S with a time-base, and also deflected one of the spots of the double beam tube Y.

The movements of a part of the *Aurellia* near the electrode were followed by the device shown at R. A potential difference from an h.t. battery was maintained between two electrodes in a bath of distilled water. Between these electrodes a light

wire lever was suspended. To this lever was attached a piece of cotton fastened at the other end by a hook to the *Aurellia*. Parts of this cotton were soaked in Bakelite varnish and then baked. This prevented electrical leakage from the movement recorder to the amplifier. The lever was connected to a terminal by a thin silver wire, which served as a light spring. The use of distilled water for the medium of the potential gradient prevented extensive electrolysis. For a shift control of the recording spot, some point in the middle of the h.t. battery *E* was connected to earth.
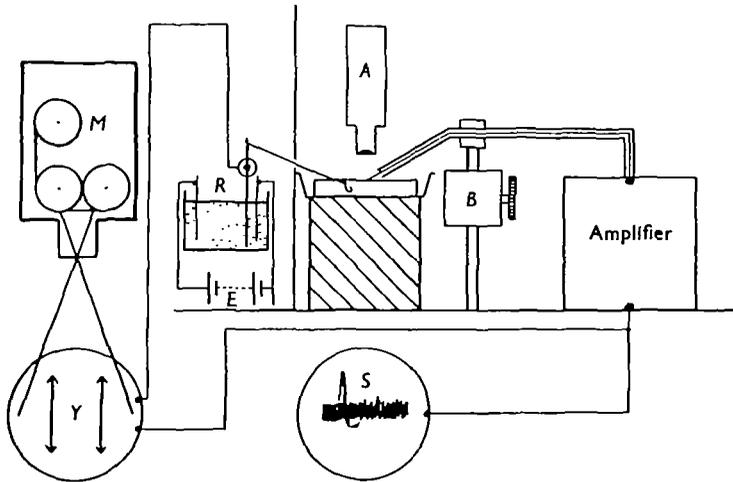

Fig. 2. Diagram of the apparatus for recording the electrical effects and the movement. Explanation in the text.

A camera *M* with sensitive paper running between two drums followed the movements of both spots on the tube *Y*. In the records (Figs. 3, 4), the upper trace represents the output of the amplifier and the lower trace represents the corresponding change of the movement recorder.

It was unavoidable that the electrical and mechanical records were from points separated by a few centimetres. According to the direction in which the wave of contraction travelled as it passed these two points, the interval between the action potential and the record of the movement could vary a little. On account of this uncertainty, the accurate measurement of the latent period between the nerve impulse and the movement was not possible. This difficulty could have been overcome by the use of artificial stimulation, but the problem of stimulus escape made this impossible.

Results

At each spontaneous beat of the bell an action potential from the nerve was recorded, together with the corresponding movement of the muscle.

Of all the fibres tested only about one in thirty gave any result. An electrical impulse was obtained from eleven different specimens; in each case it was of the same characteristic appearance shown in Fig. 3. The nerve impulse appeared consistently once at each spontaneous beat of the bell, as shown in Fig. 4, and at no

other time was an electrical effect of this kind observed. In Fig. 3, the electrical impulse is shown on a background of noise and below is the corresponding response of the muscle. This figure also demonstrates the consistency of the response, which is in fact to be expected from a preparation of a single axon.

If the electrical change had its origin in the muscle fibres one would expect to find a larger proportion of active preparations and the same results would appear with the electrodes placed on the muscle. Neither of these expectations was realized. When the electrodes were placed on the surface of the muscle either
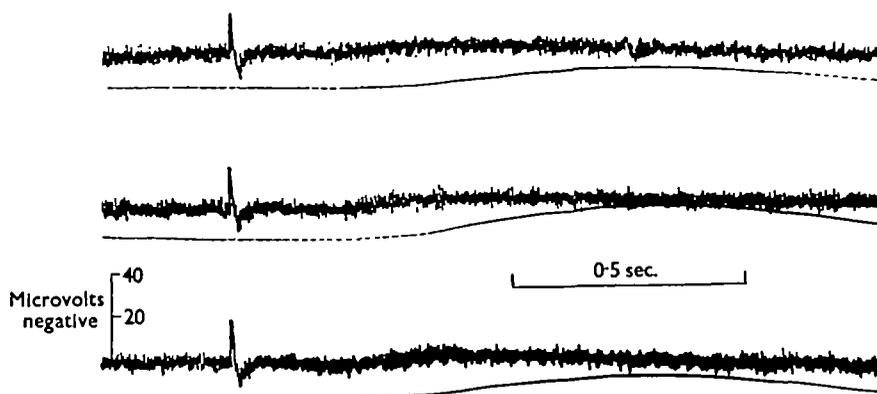

Microvolts negative

Fig. 3. Part of a typical trace showing the nerve impulse on a background of noise from the amplifier, and in the lower trace the accompanying movement.
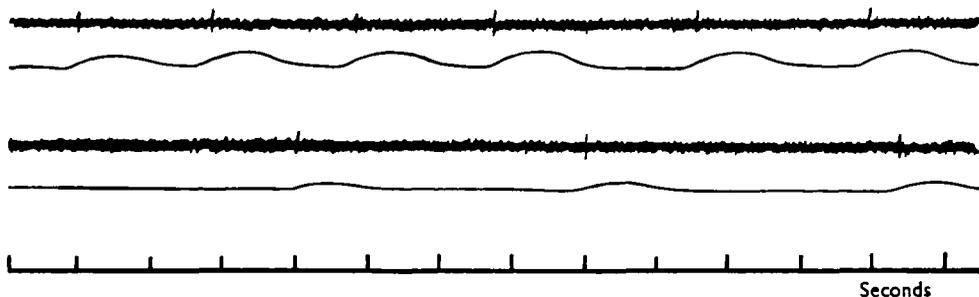

Seconds

Fig. 4. The electrical and mechanical effects. The electrical response appears delayed relative to the mechanical response because recorded some distance from it.

nothing was observed at each contraction or there were slow electrical changes that lasted for more than a second. Occasionally a fast spike, as in Fig. 3, could be picked up from a piece of raised epithelium which contained both nerve cells and muscle fibres. Sometimes the nerve axon on the electrode showed both the spike and the slow potential change which accompanied the movement. Typical action potentials were only found with a living nerve fibre on the electrode. There seems no doubt that the single action potential at each beat came from the axons of the nerve net, and the slow changes can be attributed to summation of the effects of numerous muscle fibres.

DISCUSSION

The experiment with a nerve fibre in a bridge of tissue has shown that the fibres formerly thought to be nerves do in fact conduct the excitation which leads to a contraction of the muscle. The nerves are clearly visible with suitable illumination, and these observations suggested the possibility of recording an electrical concomitant of the nerve impulse. Such an experiment is interesting on several accounts. Pantin (1935) showed that the excitation in the through-conducting part of the nervous system of *Calliactis* has essentially the same properties as the nerve impulse of higher animals. It is of interest to know whether the expected electrical concomitant of the nerve impulse can be recorded from the nerves of a Coelenterate. In fact, an action potential has been recorded, and this provides a confirmation of earlier conclusions and shows a more complete resemblance to the nerves of higher animals.

The propagation of the contraction wave across the bell is only one of several examples of transmission of excitation across the bell of Scyphozoa. Romanes (1876) demonstrated transmission of a wave of tentacle retraction in *Aurellia* and also the initiation of a beat from a tentaculocyst following stimulation at a distant point. He distinguished this 'excitational continuity' from the 'contractional continuity' of the wave of contraction. Bozler (1926) has demonstrated in *Rhizostoma*, and also in *Pelagia*, many examples of transmission of excitation other than the contraction wave.

The observations presented here show that the large nerve fibres carry the excitation which leads to a contraction wave. This is shown by the experiment where a single nerve fibre is sufficient and necessary for conduction across a narrow bridge between two pieces of tissue. In both Actinozoa (Pantin, 1935) and Scyphozoa (Bullock, 1943) this wave of contraction is followed by a refractory period. This refractory period indicates that a single impulse has gone from axon to axon throughout the effective system, which has now been demonstrated by direct recording from a single axon at each spontaneous contraction wave. A single impulse produces a single contraction, if the muscle is in a condition to respond. A contraction is therefore produced by the leading impulse of any train of impulses, so that any excitation in the form of impulses which is propagated along the system must be accompanied by a movement of the muscle. There are the following observations:

(1) A single impulse produces a contraction.

(2) The whole system (effectively) is active at each contraction wave.

(3) The direction that the excitation takes across the bell is of no importance.

(4) More than one kind of excitation may be transmitted.

On safe grounds it is assumed that only one kind of nerve impulse can travel through each axon, and that the effect of this impulse does not vary from occasion to occasion, so that it cannot produce different effects at different times. With these limitations it does not seem possible that each of the observed nerve axons can transmit excitation of two kinds, only one of which is accompanied by a contraction

wave. To use Romanes's terminology, it has now been shown that 'excitational continuity' cannot be maintained in the same conducting system as 'contractional continuity'. There must be a second conducting system, independent of the 'contractional' network, for the transmission of excitation which is not accompanied by a contraction wave. This second system is diffuse; there is evidence to suggest that it is a nerve net; altogether it seems safe to conclude that there are at least two nerve nets. The characters of the two nerve nets will be discussed more fully in a later publication.

SUMMARY

1. It has been shown that a single large bipolar nerve cell in the subumbrella epithelium of *Aurellia* is necessary and sufficient to conduct the contraction wave across a narrow bridge from one part of the bell to another.

2. This nerve impulse is accompanied by an electrical impulse of the kind usually recorded from the nerves of higher animals.

3. These nerve cells are concerned only with the excitation leading to a contraction wave. There must be other pathways for other transmitted excitation.

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