THE NERVES AND MUSCLES OF MEDUSAE

IV. INHIBITION IN AEOQUREA FORSKALEA

By G. A. HORRIDGE

Department of Zoology, University of Cambridge

(Received 2 February 1955)

INTRODUCTION
The large size of mature specimens of Aequorea forskalea, up to 25 cm. in diameter, makes this lepto-medusan a particularly favourable object for physiological work. This paper describes an investigation of the co-ordination of movement in Aequorea carried out along lines similar to an earlier study of Geryonia (Horridge, 1955).

MATERIAL AND METHODS
The anatomy of Aequorea is well known (Hertwig & Hertwig, 1878; Mayer, 1910) and its histology has been studied (Krasinska, 1914). The bell of the adult is saucer-shaped when relaxed and it will be convenient to distinguish three regions:

(a) The manubrium and stomach wall. In the centre of the bell hangs the manubrium, which is a thin transparent sheet of radial muscle fibres. When this is relaxed the mouth hangs down several centimetres and opens internally to the stomach, which is flat and disk-shaped, with a diameter about half that of the whole bell.

(b) The radial canals and gonads. From the stomach about seventy radial canals run to the periphery. Along the line of each canal runs a gonad, a flattened tube shaped like the blade of a sickle, hanging by one edge from the subumbrellar surface, and having a muscle strand running in the free edge.

(c) The margin. The gonads stop short of the margin, which contains two nerve rings and bears the velum and the tentacles, which are more numerous than the radial canals. These features can be seen in Figs. 1, 2.

Mechanical stimulation was usually applied with a glass rod or a piece of wire and electrical stimulation with a pair of platinum electrodes mounted in an insulating handle. Single short pulses of constant current ('square waves', with a duration of a few msec.) were most satisfactory, and the intensity of the stimulus was kept a little above threshold to minimize artifacts which might arise from stimulus escape. The contractions were recorded by writing levers attached with threads to hooks of bent wire embedded in the jelly. This was arranged so that both radial and circular muscle were recorded by the same lever; in the records (see Figs. 3, 4) the twitch-like contraction of the circular muscle is readily distinguished from the slow sustained contraction of the radial muscle.
THE CIRCULAR MUSCLE AND THE SWIMMING MOVEMENTS

The rhythmical contractions of the circular muscle, by which the animal swims, will be referred to as 'the beat'. As in all medusae the bell contracts symmetrically when beating normally, but in Aequorea during periods of comparative quiescence occasional local contractions can be seen, and the symmetrical action is not fully developed until the rhythm has been sustained for several minutes.

The beat is co-ordinated by a rapid through-conducting system which is confined to the margin. By recording from two points some 30 cm. apart, the velocity of conduction is found to be 70–90 cm./sec. at 20°C. Section of the ring nerve abolishes this rapid conduction, and in this respect Aequorea differs from Geryonia, in which the general conduction over the subumbrellar epithelium is as rapid as that in the nerve. It is likely that this rapid conducting system in Aequorea can be identified with the broad nerve fibres (Hertwig & Hertwig, 1878; plate VI, fig. 6, y) in the lower ring nerve, which sends axons over the subumbrellar epithelium and the velum.

After section of the ring nerve excitation to the circular muscle is still conducted, though more slowly, through the subumbrellar epithelium. By studying the responses of strips cut from the bell it can be shown that conduction takes place in all directions, but more readily in a radial than a circular direction. In one such preparation, the contraction wave traversed 8 cm. in a radial direction at the first stimulus, whereas several stimuli are usually necessary for the contraction wave to be propagated a similar distance in the circular direction. Variation of the strength of the stimulus above threshold has no effect upon either the amplitude of the contraction or the distance to which the contraction wave is propagated. At each successive stimulus the contraction wave spreads further but in any given region the amplitude of the contraction does not increase. In fact the observations indicate a similarity to the all-or-nothing contraction wave of the Scyphozoa.

When the whole bell is beating normally, excitation spreads from one rhythmical centre in the margin via the through-conducting system to all the circular muscles. Any isolated part of the margin beats at its own rate, so that in the normal beat the pace must be set by the centre with the highest natural frequency. A similar situation has been described in Aurelia by Pantin & Vianna Dias (1952).

THE RADIAL MUSCLE AND THE FEEDING RESPONSE

The feeding response is made up of a series of movements brought about by groups of radial muscles and co-ordinated by physiological pathways that run largely radially. Food is caught on the tentacles, of which a few contract together; this region of the margin bends inwards towards the mouth; at the same time the manubrium is displaced towards the stimulated region and the mouth opens to receive the food. The whole process usually takes several minutes.

The inward bending of the margin is due to the contraction of the adjoining radial muscle in the region of the radial canals. This is shown by the direction of small folds which appear at right angles to the radial canals, and by the fact that the
Fig. 1. Part of the bell of *Aequorea* to show the arrangement of the velum, gonads, and muscles. C, circular muscle of the bell; R, radial muscle of the bell; R', radial muscle of the gonad edge; T, muscle of the tentacle; V, circular muscle of the velum.

Fig. 2. Following mechanical stimulation of any point on the subumbrellar surface the margin bends towards the mouth, which is displaced laterally to meet it. The response of the radial muscle does not spread all round the bell, though a wave of tentacle retraction may do so if the margin is entire.
The nerves and muscles of medusae

radial canals are not drawn closer together as they are during the contraction of the circular muscle. The inward bending is reinforced by contraction of the strand of muscle in the free edge of each gonad. The lateral movement of the mouth is brought about by the radial muscle of the stomach wall.

It can be shown by the familiar method of inter-digitating cuts that in the region of the radial canals excitation to the radial muscle is conducted predominantly in a radial direction, either centrifugally or centripetally. However on the sides of the lamellae of the gonads there is conduction at right angles to the radial line, as shown by the behaviour of the muscle strand along the free edge; if the gonad is divided in several places by transverse cuts, all parts of the muscle strand contract together with the neighbouring radial muscle. In the marginal region excitation to radial muscles spreads in a circular direction across the subumbrellar epithelium. Following stimulation of a tentacle, the radial muscle of the same radius contracts and brings about a local inward movement of the margin. Excitation then spreads circularly for about 10 cm. in both directions, as shown by inward bending of successive regions of the margin, immediately preceded by contraction of the corresponding tentacles (Fig. 2). The velocity of conduction appears to be 0.2-2.0 cm./sec. This circular spread of excitation is not prevented by section of both ring nerves but is prevented by a cut of 1.5 cm. or more into the margin. Co-ordination between tentacles, however, is abolished by a cut in the margin through both ring nerves, as in Geryonia.

A spread of excitation in the circular direction is also seen in the wall of the stomach. The lateral movement of the manubrium is due to the contraction of the radial muscles in the same radius as the stimulus, but after continuous stimulation the excitation spreads round the mouth, which in consequence is widely opened.

The contraction of the radial muscle is slow and sustained, in contrast to the twitch of the circular muscle (Figs. 3, 4). Unlike the latter it is evoked by gentle mechanical stimulation and if electrical stimuli are used, several may be required to produce a response.

In a piece of the radial canal region, deprived of marginal nerves, the radial muscles respond only so long as the stimulus continues to be applied, but with the adjoining ring nerves intact a contraction may sometimes be sustained for several minutes even after a single electrical stimulus.

THE INHIBITION OF CO-ORDINATED SWIMMING DURING THE FEEDING RESPONSE

During the feeding response, or when the radial system is electrically or mechanically stimulated, there are two effects of great interest. First, it can be shown that the excitation in the radial system inhibits the spontaneous rhythm of the beat. Fig. 4 shows a series of spontaneous beats recorded from opposite sides of the bell of an undamaged specimen. Electrical stimulation was applied to the margin, and as the contraction of the radial muscle develops the spontaneous beat comes to an end. Secondly, it can be shown that excitation in the radial system prevents the rapid marginal propagation of the beat, shown above to be distinct from the general
Fig. 3. Simultaneous records from two adjacent parts, A and B, of the bell. Contraction is registered upwards in the upper trace and downwards in the lower trace. Electrical stimuli on the margin at A (lower trace) produce a response of the muscle propagated to B (upper trace). But the radial muscle at A then contracts, as shown by the maintained contraction on which is superimposed the local response of circular muscle (at C). Now the propagation to B is inhibited and the top line (at D) shows nothing. The contractions at B are an example of temporal summation in Hydrozoa.

Fig. 4. A series of spontaneous beats recorded from opposite sides of the undamaged bell. Contraction is registered upwards in the upper trace and downwards in the lower trace. Twenty electrical stimuli applied at the place from which the upper record was taken produce a raising of the base-line as the radial muscle contracts, and the rhythm presently comes to an end.
conduction to the circular muscle over the subumbrellar epithelium. Fig. 3 is a record from two adjacent regions of the bell about 10 cm. apart. Electrical stimuli applied to the margin at the region from which the lower trace was recorded result in twitch-like contractions of the circular muscle in this region (A) which are also propagated to the adjacent region (B). After the tenth stimulus the radial muscle of the stimulated region develops a maintained contraction, on which the twitches of the circular muscle are superimposed (C). However these twitches are no longer propagated to the adjacent region (D). The significance of these observations is discussed below.

DISCUSSION

It has been shown that during the feeding reaction, groups of radial muscle are co-ordinated by a conducting system that runs predominantly radially, and that this system is physiologically distinguished from that co-ordinating the beat. A similar sharp distinction was found in Geryonia in which it was shown (Horridge, 1955) that the pointing reaction of the manubrium was unimpaired while the contraction wave was propagated continuously round the bell. However, in the normal Aequorea, or parts with the marginal nerves complete, contractions of the radial and circular muscles do not occur simultaneously. This does not arise from any property of the epithelial conducting elements or of the muscles themselves, for simultaneous contractions of both sets of muscles follow electrical stimuli (Fig. 4, C).

The inhibition of both rapid conduction and spontaneous rhythm when the radial system is active depends on some process in the margin, most likely in the ring nerves, which have ganglion cells all along their course. In inhibited sectors propagation of excitation to the circular muscle resembles that in sectors where the marginal nerves have been removed; an excitation is then transmitted only by slow pathways in the subumbrellar epithelium. The result is that during the feeding reaction the bell stops beating, at least in part and sometimes entirely. This observation is of some interest since it is to date the clearest example of inhibition in a coelenterate nervous system.

Aequorea is not the only medusa which shows an inhibition of the beat when the radial muscle contracts. The same observation has been made in the following genera: Bougainvillea, Cunina, Melicertum, Mitrocoma, Oceana, Phialidium, Podocoryne, Rathkea, Rhapolonema, Sarsia, Solmaris, Steenstrupia and Neoturris, but cannot be conveniently recorded from small species.

In N. pileata a spontaneous beat was never observed in a region where radial muscles contracted locally, but a spontaneous beat in another region affected all the circular muscle, which also would respond to a single electrical stimulus. In this case spontaneous activity is inhibited but not rapid conduction. In other medusae, e.g. Geryonia, there is no inhibition of either kind. These differences between genera seem to be related to the type of movement involved in the feeding response, e.g. in Aequorea the margin bends inwards and the manubrium is displaced laterally towards it whereas in Geryonia there is no local bending of the margin, which can easily be reached by the long pseudomanubrium of this genus.
G. A. Horridge

A further point of interest arises out of the consideration of the inhibition. In normal swimming the circular muscles contract regularly with no accompanying contraction of the radials. The pathways to the radial muscles are not inhibited, for mechanical stimulation will arouse a feeding response that in its turn inhibits the beat. It seems that the physiological pathways from the radial system to the rhythmical can act in one direction only. The same unidirectional path at this point of interaction between radial and rhythmical systems was noted in Geryonia, and a similar state of affairs is found in Scyphozoa. For example, in Aurelia, the marginal tentacles do not contract at each beat but stimulation of one of them may initiate a contraction wave in a preparation containing a tentaculocyst (Romanes, 1877).

SUMMARY

1. The co-ordination of the swimming movement (beat) and feeding response in Aeurorea forskala has been studied with particular attention to the pathways taken by the excitation.
2. The rapid through-conducting system which co-ordinates the beat is sharply distinguished physiologically from the radial system which co-ordinates feeding.
3. The spontaneous origin of the beat and its rapid marginal conduction are both inhibited while the radial muscle is contracting.
4. Inhibition of this type has been observed in other genera of Hydromedusae,

For help with the apparatus and provision of material, I am very pleased to express my gratitude to Dr R. Dohrn and his staff at the Stazione Zoologica, Naples, where this work was carried out during tenure of a grant from the Department of Scientific and Industrial Research.

REFERENCES
Romanes, G. J. (1877). Further observations on the locomotor system of medusae. Phil. Trans. 167, 659.