J. exp. Biol. (1980), **84**, 303–318
With 12 *figures*
Printed in Great Britain

THE GIANT AXON ESCAPE SYSTEM OF A HYDROZOAN MEDUSA, *AGLANTHA DIGITALE*

BY ALAN ROBERTS AND G. O. MACKIE

Department of Zoology, University of Bristol and Department of Biology, University of Victoria, Canada

(*Received* 26 *April* 1979)

SUMMARY

1. *Aglantha digitale* (Hydrozoa) has a ring giant axon, up to 35 μm in diameter, which runs all round the margin of the bell in the outer nerve-ring. Running from the margin, up the inside of the bell towards the apex are eight motor giant axons, up to 40 μm in diameter. These synapse with the subumbrellar myoepithelium and are therefore motoneurones. The myoepithelial cells line the inside of the bell and have striated muscular tails running circumferentially. Contraction of this circular musculature forces water out of the bell and propels the medusa through the water. A large axon (up to 7 μm in diameter) runs on the aboral side of each tentacle. The tentacles retract during swimming and contain longitudinally aligned striated muscle tails.

2. Intra- and extracellular recordings from the giant axons indicate that they are involved in the rapid escape swimming response of *Aglantha*. Stimuli which evoke escape swimming lead to a brief burst of two to six impulses (2–3 ms in duration) in the ring giant axon. These propagate round the margin (at up to 2·6 m s^{-1}). The large tentacle axons fire one-to-one with the ring giant axon and the tentacles contract. The motor giant axons are excited at chemical synapses (blocked by divalent cations and with a synaptic delay of 1·6 ± 1 ms), where large, facilitating epsps evoke an impulse which then propagates up the bell at up to 4 m s^{-1}. The overshooting motor giant axon impulses are Na^+ dependent, 2–3 ms in duration, and excite the myoepithelium at synapses with a 1·6 ± 1 ms synaptic delay. A regenerative muscle impulse is evoked. It is of long duration (15–70 ms), Ca^{2+} dependent, overshoots zero, and propagates through the myoepithelium at 0·22 to 0·29 m s^{-1}. A nearly synchronous contraction of the whole subumbrellar circular musculature is evoked. Isolated strips of muscle can reach peak tension in 40 ms. In the absence of sarcoplasmic reticulum, Ca^{2+} for excitation–contraction coupling probably enters the muscle during the impulse.

3. This preparation has allowed a clear understanding of how the two types of axon co-ordinate escape behaviour. It should also be valuable for the study of synaptic transmission, previously very inaccessible in Cnidarians.

INTRODUCTION

Extracellular recordings of potentials generated by various cnidarian tissues have thrown a great deal of light on the organization of their behaviour (Josephson, 1974). However, our knowledge of the cellular mechanisms and roles of neurones in cnidarian behaviour is still vague in most cases. This is because the neurones are usually too small to be seen in living preparations and this has made unambiguous single-unit recordings of their electrical activity very difficult (but see Horridge, 1954). We have studied a small hydrozoan medusa, *Aglantha digitale* (Fig. 1), which has two types of giant axons visible under a dissecting microscope. These giant axons appear to be involved in the rapid escape swimming which is brought about by a nearly synchronous contraction of the circular muscles lining the inner surface of the bell and velum. The escape swimming movement has been analysed by S. Donaldson (in preparation). We have used intracellular recording from the giant axons to unravel some of their properties and their precise role in this escape behaviour. *Aglantha* is unusual in having two modes of swimming: a rhythmic slow swimming similar to that shown by most medusae, and a very rapid escape swimming in which bell contraction is complete in about 100 ms. The subumbrellar muscle sheet is responsible for both fast escape swimming and the more usual slow swimming. We presume that smaller, conventional neurones co-ordinate slow swimming, but we understand these activities less well at the present and plan to investigate them later.

MATERIAL AND METHODS

Specimens of *Aglantha digitale* (Fig. 1) were dipped from surface water at the dock of the Friday Harbor Laboratories, University of Washington. They were kept in fresh, flowing sea water for up to three days. Detailed anatomical observations were made on living tissues using a Zeiss interference microscope. For electrical recording, whole animals or dissected pieces were placed in dishes with a Sylgard layer on the base. This allowed illumination from beneath and pinning with spines from the fruit of the cactus *Opuntia*. All observations were made at 15 ± 1 °C. Extracellular recordings were made with plastic suction electrodes. Intracellular recordings were made with 3 M-KCl-filled glass pipettes with resistances of 40 MΩ or more. For penetration of the ring giant axon and subumbrellar epithelial cells microelectrodes were advanced hydraulically and a piezo-electric prodder used for penetration (R. Weevers, personal communication). Electrical stimulation was with a bipolar stainless steel electrode. Experiments were done in sea water, sometimes with small amounts of divalent cations added (e.g. Mg^{2+}, Co^{2+}, Mn^{2+}). The sodium-free artificial sea water had the following composition. Tris Cl 440 mM; $CaCl_2$ 10 mM; KCl 10 mM; $MgCl_2$ 30 mM; $MgSO_4$ 20 mM – at a pH of 7·7. Most recordings were photographed directly from a storage oscilloscope; some were played back from a Tandberg FM tape recorder. Muscle tension was recorded with a Grass Instruments force transducer. Techniques for electron microscopy are described in Singla (1978).

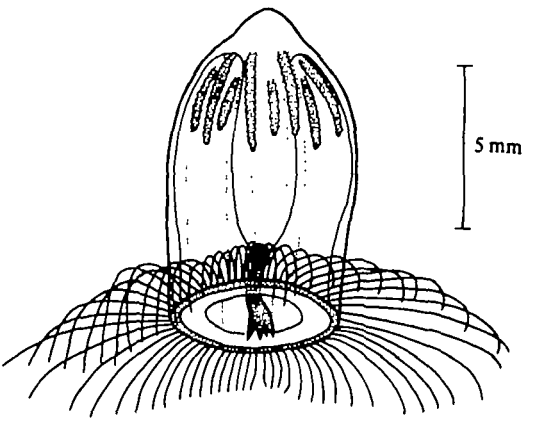

Fig. 1. Scale drawing from a photograph of *Aglantha digitale*, apex at top. In the relaxed state the tentacles stick out sideways from the margin as shown. The eight gonads (light stipple), peduncle and manubrium can be seen hanging from the apex through the transparent bell wall. The manubrium, with four feeding arms, protrudes through a circular opening in the velum.

ANATOMICAL RESULTS

Aglantha has a series of giant axons and certain other anatomical specializations which behavioural observations (Donaldson, in preparation) and the recordings to be described below suggest are related to its escape response. These axons have been observed in living animals using interference microscopy, which is helped by the transparency of the tissues. One giant axon lies in the outer nerve-ring near the velum and runs round the margin of the bell (Figs. 2, 3 and 4). This ring giant axon is of uneven diameter (from 5 to 24 μm in one 10 mm long specimen), being wide opposite tentacle bases and narrow between them. Its size (maximum diameter from 22 in small to 35 μm in larger animals), peculiar organization and cytoplasm, led previous workers not to think of it as a neurone (Hertwig & Hertwig, 1878; Singla, 1978). In life it is very clear and transparent. In fixed preparations (Fig. 4*b*), the bulk of the axon is occupied by a homogeneous flocculent material containing no organelles, and is surrounded by a membrane outside which lies a thin layer of normal cytoplasm with organelles and then the plasma membrane. The ring giant lies in parallel with the much smaller axons (up to 6 μm) of the outer nerve-ring and receives synaptic contacts from some of these. The ring giant axon is covered, on its outer aspect, by a single layer (4–5 μm thick) of epithelial cells (Figs. 3 and 4). Some of these are ciliated. Where the ring giant axon narrows between tentacles another series of specialized structures is found (Figs. 2, 3, and 4). These structures are bean-shaped, from 20 to 62 μm in length, and on their protruding long edge have a regular row of straight, immobile cilia (23–31 μm long). The number of cilia varies with the size of the pad. Like the ring giant axon these ciliated comb pads have a thin coat of epithelial cells.

The second type of giant axon runs from the margin up the inside of the bell in the myoepithelium. They parallel each of the eight radial digestive canals and we call them motor giant axons (Figs. 2 and 5*a*). They have conventional axonal ultrastructure and

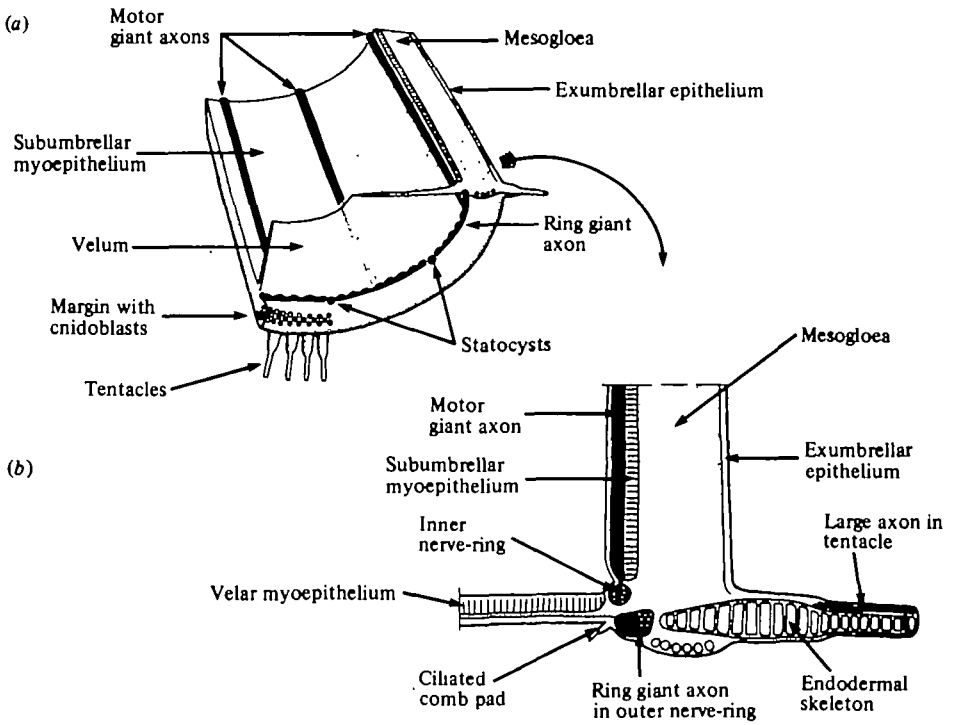

Fig. 2. Organization of giant axons and muscle layers. (*a*) Cut-away diagram (not to scale) of part of the bell to show locations of the giant axons. The ring giant axon runs all round the inner aspect of the margin next to the velum. The motor giant axons run up the bell in the subumbrellar myoepithelium. (*b*) Diagrammatic cross-section of the margin (not to scale) to show the nerve-rings, giant axons, a ciliated comb pad and the main epithelia. The ring canal and endodermal sheet are omitted.

synaptic junctions occur between them and the subumbrellar myoepithelium (Singla, 1978). The motor giant axons are therefore motor axons. They are up to 40 μm in diameter, generally of even diameter but becoming narrower towards the top of the bell (in one animal, maximum diameter 32 μm, minimum 7·5 μm), have some branches which extend circumferentially, and contain a few scattered nuclei. Each motor giant axon is accompanied by a number of smaller axons, some of which contribute to a nerve plexus in the subumbrellar myoepithelium. This myoepithelium lining the subumbrellar and inside surface of the velum provides the contractile force for the escape swimming response (S. Donaldson, in preparation; S. Donaldson, G. O. Mackie & A. Roberts, in preparation). It consists of a pavement epithelium. Roughly hexagonal cell bodies 5–10 μm across house the nucleus, mitochondria and other organelles. Below these the cells have narrow, helically striated muscle tails (5–8 μm deep and 1–2·8 μm wide) running circumferentially (Singla, 1978).

The final specializations related to the escape system are in the tentacles. A large axon (3·6–7·8 μm in diameter) with conventional axonal ultrastructure runs along the upper (aboral) side of each tentacle (Figs. 2 and 5). The tentacle also contains other smaller axons and on its oral side has a strip of longitudinal, ectodermal, striated, myoepithelial cells. Striated muscle has not previously been reported in hydromedusan tentacles.

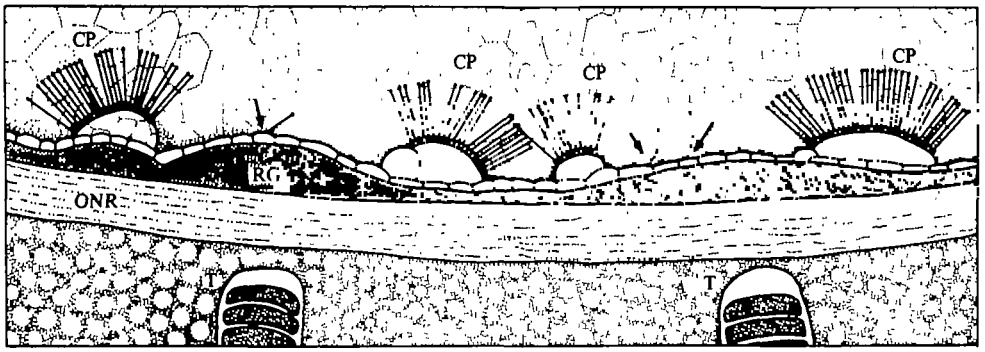

Fig. 3. Drawing from photomicrographs to show the inner aspect of the bell margin from the oral side. Comb pads (CP) are seen in view projecting over the velar epithelium (dotted cell and nuclear outlines). In optical section below are the ring giant axon (RG), its coating epithelial cells some of which are ciliated (arrowed), the outer nerve-ring (ONR), and the remaining margin tissue with the ends of the endodermal skeleton of two tentacles (T). The location of pairs of comb pads between tentacle bases, close to the narrow part of the ring giant axon, is typical.

PHYSIOLOGICAL RESULTS

(*a*) *The ring giant axon and co-ordination around the bell*

A search for electrical events related to escape swimming was begun in restrained animals pinned by their apex. Extracellular suction electrode recordings showed that strong swims were associated with a burst of spikes in the inner and outer nerve-rings. Such swims were evoked by crushing or tugging one or more tentacles, by prodding the margin area or velum, by water currents directed at the margin, by shock waves in the sea water, and by a brief electrical pulse to the margin or a tentacle. In other hydromedusae, swimming and tentacle shortening are co-ordinated in the marginal nerve-rings (Passano, 1976) so we analysed events in the nerve-rings more closely. To do this, the margin was separated from the main part of the bell and the velum removed. The tentacles and some myoepithelial tissue remained so that 'swimming' could be seen. However, movement of the margin was much reduced, so more controlled recordings could be made. Stimuli that normally evoke escape swimming evoked spike activity recorded at large amplitude in the outer nerve-ring over the ring giant axon (Fig. 6). Similar activity was recorded at much lower amplitude over the inner nerve-ring. The response to crushing a tentacle consisted of one to six similar spikes riding on a slow, positive potential. A closely similar response was evoked, at lowest threshold, by a 0·2 ms shock to the ring giant axon. During repetitive shocks the first spike was stable in occurrence and latency even with many stimuli at over 1 Hz. Shocks at about 1 Hz for a few seconds often led to a larger slow potential and more spikes. In 10 mM Mn^{2+}, 10 mM Co^{2+} or 81 mM Mg^{2+} sea water, muscular response and synaptic transmission were blocked. Crushing tentacles and water disturbances no longer evoked movements or a burst of margin spikes. However, direct mechanical jogging of the ring giant axon or electrical pulses still evoked a similar burst of spikes and slow wave (Fig. 6*b*). This indicates that at least some of the pathways evoking this response depend on chemical synaptic action whereas the burst of spikes and slow wave themselves do not. The

conduction velocities of the ring giant axon spikes ranged from 1 m s^{-1} to 2·6 m s^{-1} in larger animals. This would give a delay of about 4 ms from one side of the margin to a point diametrically opposite.

The origin of the extracellularly recorded spikes and slow waves was checked by intracellular recording from the ring giant axon under visual control. Recordings were made in preparations paralysed in 80 mM Mg^{2+} sea water and in one which was in normal sea water but contracted very weakly (Fig. 7). Resting potentials were low on penetration (from -10 to -20 mV) so hyperpolarizing current was injected to bring the resting potentials to about -50 mV. When brief electrical pulses were given to the margin the responses showed a close correspondence to the extracellular recordings. At threshold, a large potential was evoked consisting of one or more overshooting spikes riding on a slow depolarization. The spikes were up to 68 mV in amplitude, about 1·6 ms in duration and could repeat at intervals down to 3·5 ms. The underlying slow depolarization returned to the initial resting level in about 200 ms. Repeated stimulation for a few seconds at 1 Hz led to larger responses with up to six spikes. Usually the first one or two spikes were quite stable but later spikes came and went on repetition. In Mg^{2+} sea water the membrane potential was steady but in normal sea water irregular synaptic potentials were recorded (Fig. 7*c*). These occurred singly or in summating groups, and increased in amplitude when hyperpolarizing current was injected.

Our conclusion is that the extracellular and intracellular records are both from the largest neuronal element in the outer nerve-ring. This ring giant axon is excited by stimuli which normally evoke escape swimming or by electric shocks to the outer nerve-ring, and in response propagates a burst of impulses around the margin at up to 2·6 m s^{-1}.

(*b*) *The motor giant axons: motoneurones for the bell musculature*

If excitation travels rapidly round the margin in the ring giant axon it must next be carried up the inside of the bell to excite the subumbrellar myoepithelium which is seen to contract. Extracellular recordings were therefore made from the ring giant axon and a motor giant axon to see if the latter were involved. In preparations paralysed in 80 mM Mg^{2+} direct electrical stimulation of a motor giant axon evoked an all-or-none triphasic spike. However, stimulation evoking spikes in the ring giant axon did not excite motor giant axons. This suggested that chemical synapses between these two

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Fig. 4. Comb pads and the ring giant axon in the margin. (*a*) Photomicrograph of the inner aspect of margin. (See left half of Fig. 3 for identification of components.) Two comb pads illustrate arrangement of cilia, which are straight with enlarged basal regions and bulbous tips. The ring giant axon is seen in optical section. It is clear, with little or no sign of organelles. The outer nerve-ring lies immediately below the ring giant axon. Above the right-hand comb pad the faint striations of muscle-tails in the velum can be seen. (*b*) Electromicrograph of a transverse section through the ring giant axon (RG) and outer nerve-ring (ONR). The orientation is apex up, tentacles to the left, velum to the right. (This is a left-to-right reversal of Fig. 2*b*.) At the top, part of the inner nerve-ring (INR) lies above the grey strip of mesogloea (m), which would run into the velum off the section to the right. Below this lies the outer nerve-ring (ONR), where many small axons are seen in cross-section. The ring giant axon (RG) is on the external side of the outer nerve-ring. A single epithelial layer (ep) separates the giant axon from the exterior (*). A nucleus (n) of the ring giant axon is present lying in a bulge of cytoplasm. Elsewhere the cytoplasm forms a very thin layer (c) around an enormous central vesicle of homogeneous flocculent material (dark spots are stain contamination). Calibration lines (*a*) 20 μm, (*b*) 10 μm.

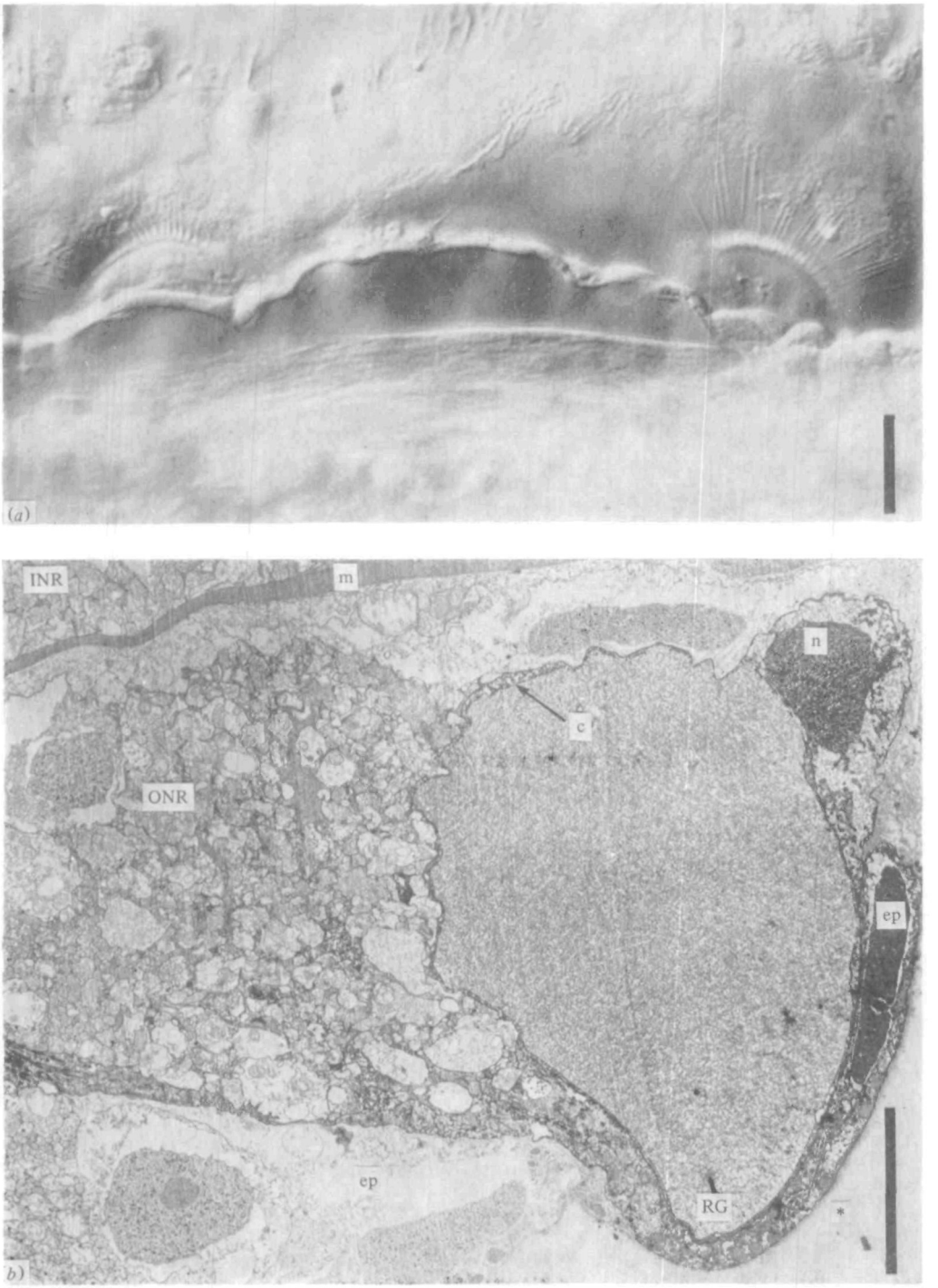
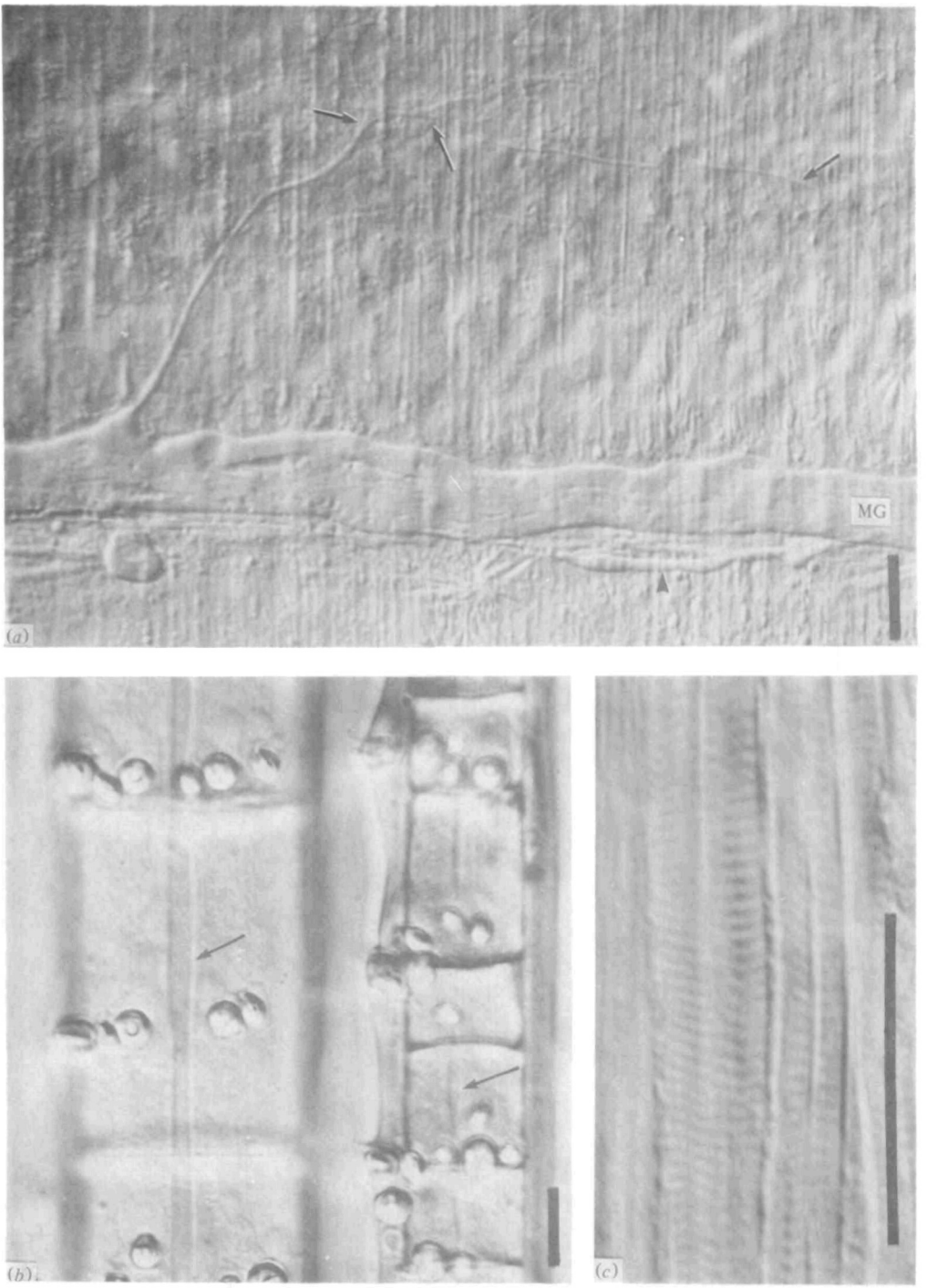


Fig. 4. For legend see opposite.



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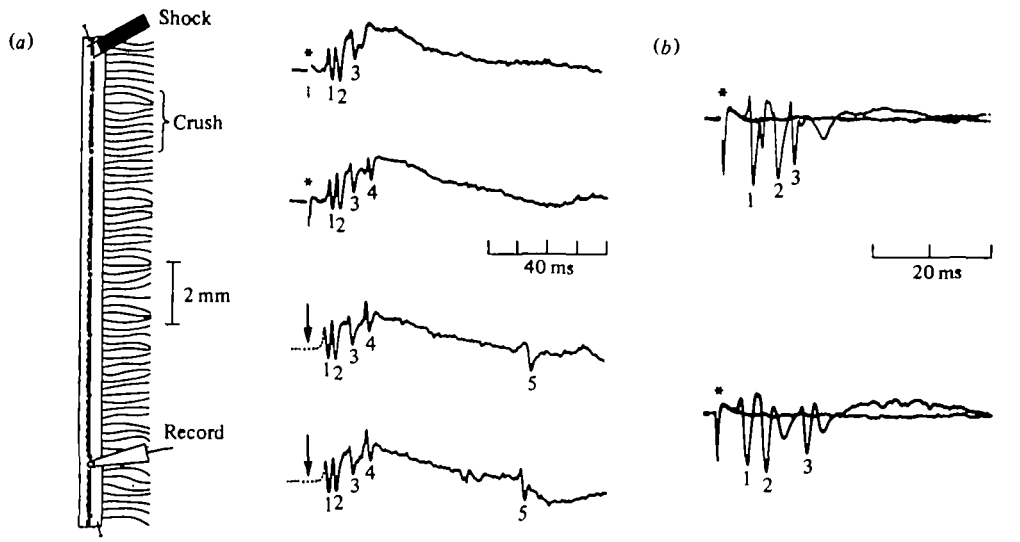

Fig. 6. Extracellular recordings from the ring giant axon. (*a*) The margin was separated from the bell and pinned out as in the diagram, where the location of stimulation and the recording suction electrode are shown. The recorded response to an electric shock (at asterisk) or crushing one tentacle (at arrow) was similar, consisting of a positive slow wave and from 3 to 5 spikes (numbered). (*b*) Responses to electric shock were similar in a straightened and cut margin in sea water (top) and in an intact circular margin in 10 mM Mn^{2+} where synaptic transmission was blocked (bottom).

types of giant axons were blocked by the Mg^{2+}. To avoid blocking these synapses while still recording motor giant axon responses clearly, muscle activity was locally abolished by raising the Mg^{2+} concentration in a small glass box sealed over a motor giant axon on the subumbrella with petroleum jelly. Electrical stimuli to the ring giant axon then evoked a single spike in the motor giant axon. In only one of 120 trials was there a later, second spike in the motor giant, about 60 ms after the first.

Using intracellular recording, responses of motor giant axons to ring giant axon stimulation were examined. Movement was reduced to a minimum by removing the velum and cutting the subumbrellar epithelium along the margin and on either side of each motor giant axon. The resting potentials of motor giant axons were stable and usually close to -57 mV. Electrical stimulation of the ring giant axon above its threshold evoked an impulse in the motor giant axon. The subsequent contraction then dislodged the electrode. However, in less excitable preparations, impulses were not often evoked. Without motor giant impulses there was no subumbrellar contraction. Penetrations were

Fig. 5 (*a*). Motor giant axon (MG) in a photomicrograph of part of the subumbrellar myoepithelium (apex to the right). The circular muscle-tails of the epithelial cells can be seen as vertical striations. Their cross-striations are visible in the upper right part of the picture. The motor giant axon has a nucleus near where it gives off a branch which divides as it runs over the myoepithelium (arrows). Other axons running parallel to the giant axon lie just below it (arrowhead). (*b*) Photomicrograph of two tentacles to show the large, longitudinal axon in each (arrowed). The circular refractile cells are cnidoblasts. The large 'cells' of the endodermal skeleton produce a prominent cross banding of each tentacle (cf. Fig. 2*b*). (*c*) Photomicrograph of longitudinal muscle-tails on the oral side of a tentacle to show the clear cross-striations. Calibration lines are 20 μm.

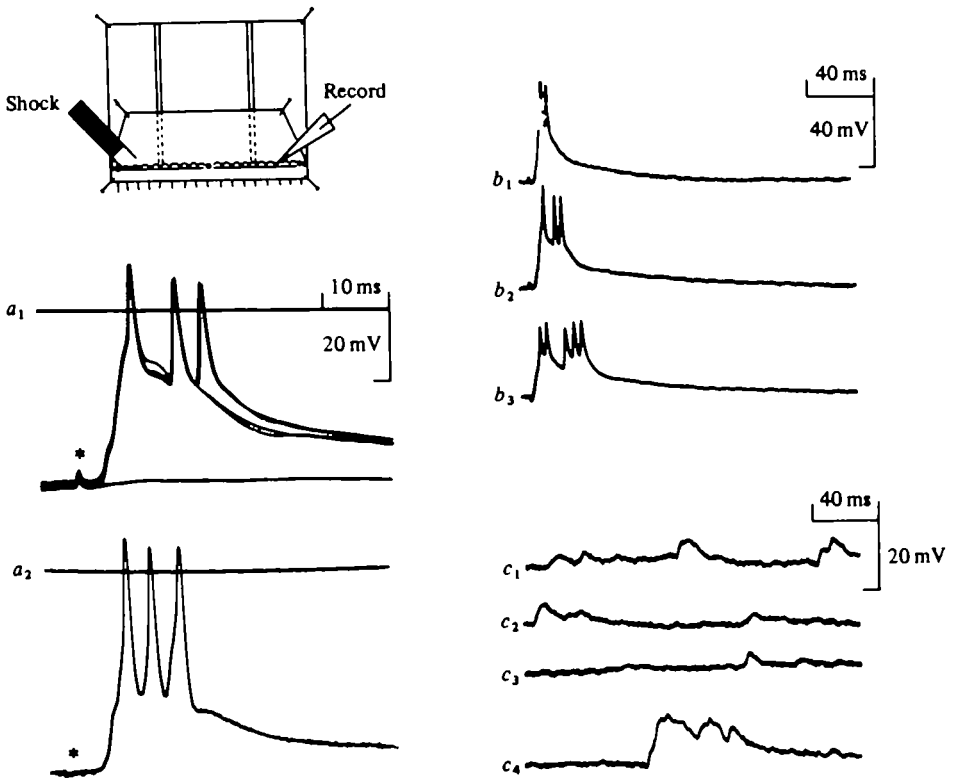

Fig. 7. Intracellular responses of the ring giant axon. The preparation (see diagram) consisted of one-quarter of a bell with the apex removed and tentacles cut short. This was pinned flat, and then the velum pinned down over the subumbrellar epithelium. The expanded parts of the ring giant axons on the velum side of the margin could then be seen clearly enough for insertion of a microelectrode. (*a*) and (*b*) Responses to shocks in 80 mM Mg^{2+}. (a_1) Through-threshold series of shocks (at asterisk) to show all-or-none response, in this case with 2 or 3 spikes overshooting zero potential line. (a_2) A single response from another preparation. (*b*) At lower gain and sweep speed these records illustrate the variety of response and long-lasting depolarization following the spikes. (*c*) Spontaneous postsynaptic potentials in normal sea water showing summation.

then maintained and the underlying postsynaptic potentials were clear (Fig. 8). With a microelectrode within 600 μm of the margin the synaptic delay between the presynaptic impulse in the ring giant axon (recorded with an extracellular electrode) and the psp in a motor giant axon was from 1·6 to 1·8 ms. The psps rose to a peak of up to 11 mV in 6–8 ms and fell to half amplitude in 40–50 ms. When later ring giant axon impulses evoked a second psp, these summated with the first and were facilitated.

The properties of motor giant axons were more easily studied by intracellular recording in preparations of the subumbrella paralysed in 10 mM Mn^{2+} (Fig. 9). Brief electrical pulses evoked a small, local potential and above threshold a regenerative impulse, up to 83 mV in amplitude but usually from 68 to 74 mV. At half amplitude the duration of the impulse was 1·2 to 1·4 ms. It had a long depolarizing after-potential, initially 5–13 mV in amplitude and taking about 40 ms to fall to half amplitude. Impulses evoked during this after-potential were of slightly reduced amplitude. Doubling the Mn^{2+} concentration had little effect on the impulse overshoot but increased the

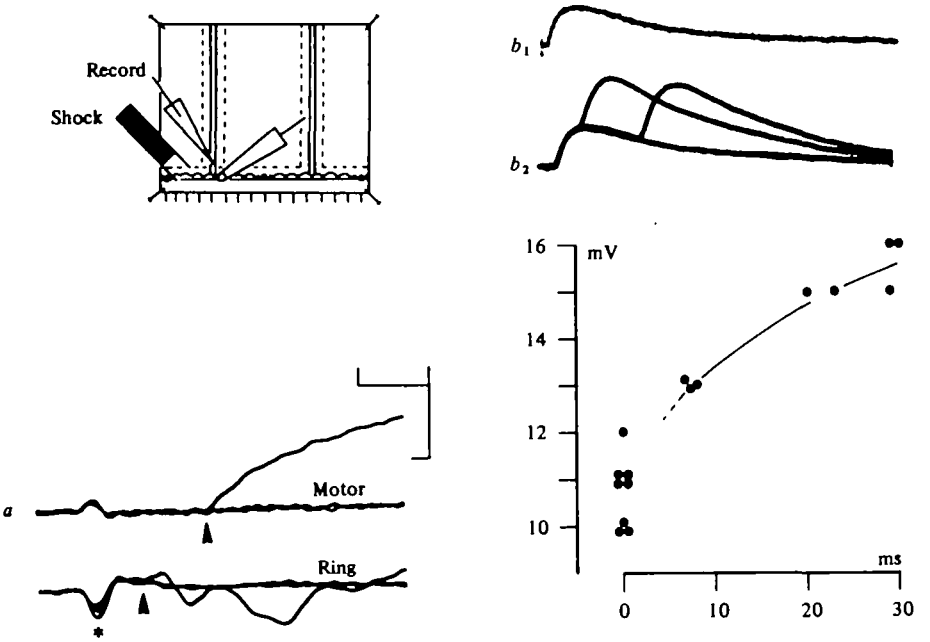

Fig. 8. Motor giant axon postsynaptic potentials in response to ring giant axon spikes. The preparation (see diagram) was similar to that of Fig. 7 but the velum was removed and the subumbrellar myoepithelium was cut on either side of each motor giant axon and along the margin (dashed lines). This reduced muscle contraction to a minimum. The stimulating and extracellular suction electrodes are on the ring giant axon. The recording microelectrode is in a motor giant axon. (*a*) Shows the delay between arrival of the presynaptic spike in the ring giant axon (arrowhead in lower trace) and the start of the postsynaptic potential in the motor giant axon (arrowhead in upper trace). Shocks to the ring giant axon (at asterisk) were just above and below threshold. (*b*) Shows the post-synaptic potentials in a motor giant axon (1) alone and in (2) summating and showing facilitation of the second psp in a pair (superimposed sweeps of 3 separate responses). This facilitation is illustrated in the graph where psp amplitude is plotted against the interval between psps. Calibration for (*a*) 2 ms, 10 mV and (*b*) 20 ms, 20 mV.

spike after-potential. However, replacing half the Na^+ in sea water with Tris reversibly blocked the impulse. The conduction velocity of motor giant axons, measured with extracellular electrodes, ranged from 1 m s^{-1} in small animals up to 4 m s^{-1} in large ones.

We conclude that the eight motor giant axons are excited by the ring giant axon via chemical synapses, which are blocked by Mg^{2+}, Mn^{2+} or Co^{2+}. The usual response of each motor giant axon to such excitation is a single impulse which is then propagated up the inside of the bell at from 1 to 4 m s^{-1}, exciting the circular muscles as it travels.

(*c*) *The subumbrellar myoepithelium: circular muscle layer for swimming*

It was clear from extracellular recordings in unparalysed animals that impulses in any motor giant axon led to contraction of the subumbrellar myoepithelium. Intracellular recordings from myoepithelial cells were made within 50 μm of a motor giant axon whose impulse was recorded extracellularly (Fig. 10). Contraction was reduced by cutting the myoepithelial cell layer on either side of each motor giant axon and by raising the Mg^{2+} concentration to 60·7 mM. Stable resting potentials were usually close

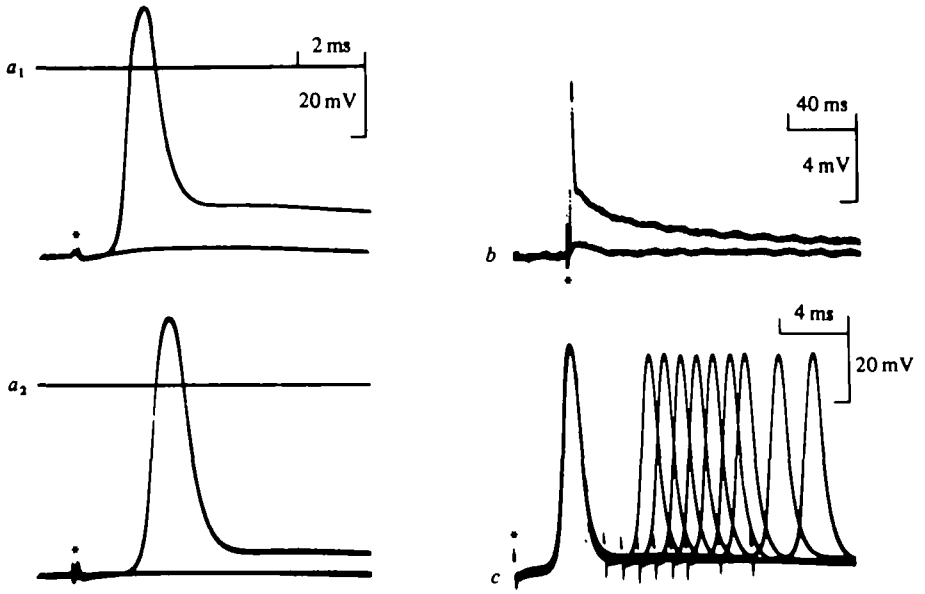

Fig. 9. Motor giant axon impulses in response to direct electrical stimulation (at asterisk). (*a*) Above and below threshold in (1) 20 mM Mn^{2+} and (2) 10 mM Mn^{2+}. Recorded in different axons. (*b*) At slower sweep speed and higher gain the depolarizing afterpotential is clear. Stimuli above and below threshold. (*c*) Paired shocks show that impulse amplitude is reduced during the afterpotential. (a_2), b and c were from the same axon in 10 mM Mn^{2+}. The preparation was as in Fig. 10 but without cuts in the myoepithelium.

to −55 mV. Postsynaptic depolarization started 1·7 ms after the beginning of the motor giant axon impulse. With the raised Mg^{2+} concentration, the myoepithelial impulse did not overshoot zero, but its whole shape could be seen. Its rise time was about 3 ms and it fell to half amplitude in about 18 ms. In normal sea water the rise time was usually just over 2 ms, the impulse overshot zero, and was 67 to 70 mV in amplitude. However, contraction invariably dislodged the electrode during the impulse. In 81 mM Mg^{2+} sea water a myoepithelial impulse could not be evoked by direct electrical stimulation, suggesting that the impulse is Ca^{2+} dependent. Responses of the myoepithelium to motor giant axon impulses were blocked by 81 mM Mg^{2+}, 10 mM Mn^{2+} or 10 mM Co^{2+}, presumably by a combined effect on neuromuscular synaptic transmission and the myoepithelial impulse itself.

Conduction of the impulse in the subumbrellar epithelium was studied using extracellular recording. In some preparations stimulation of a single motor giant axon would lead to contraction of the whole subumbrella, initiated by a myoepithelial impulse conducting circumferentially at 0·25–0·29 m s^{-1}. In other preparations the myoepithelial impulse was blocked at the next motor giant axon where the myoepithelial cells are much thinner. As a result, contraction only occurred in the segments of muscle on either side of the stimulated motor giant axon. The myoepithelial impulse propagates in all directions. By cutting a motor giant axon, the propagation velocity in the myoepithelium in a radial direction was measured as 0·22–0·25 m s^{-1} (Fig. 10*b*). The extracellular recordings from more intact preparations showed that the myoepithelial impulse could be up to 50 ms in duration.

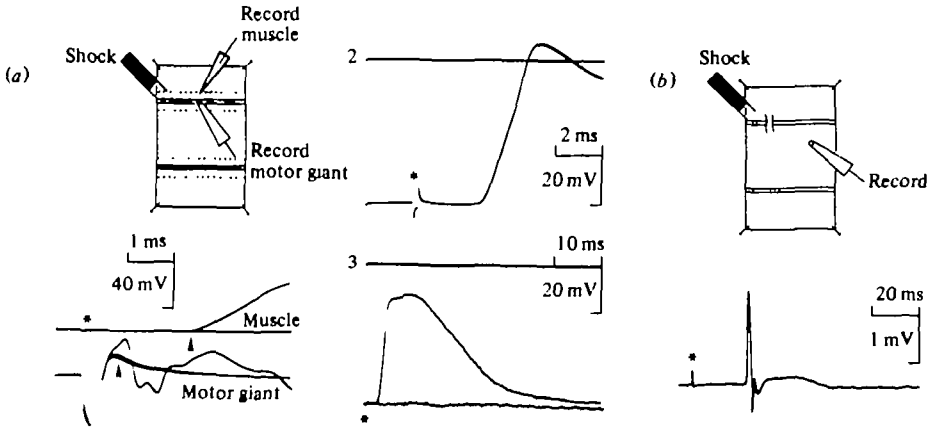

Fig. 10. Responses in the subumbrellar myoepithelium to motor giant axon stimulation (at asterisk). (*a*) The preparation (see diagram) was a pinned-out quarter bell with apex and margin removed. The myoepithelium was cut on either side of each motor giant axon (dashed lines). (a_1) Shows the synaptic delay between the arrival of the extracellularly recorded presynaptic motor giant axon impulse (arrowhead in lower trace) and the start of the intracellularly recorded myoepithelial cell depolarization (arrowhead in upper trace). (a_2) Shows the rising phase and normal overshoots of the myoepithelial impulse in sea water. Movement dislodged the electrode later during this response. (a_3) In 60·7 mM Mg^{2+} the myoepithelial impulse is reduced and does not overshoot zero, but its full form can be seen. Stimulation just above and below threshold for motor giant axon impulse. (*b*) When the motor giant axon is cut (as in diagram) an extracellularly recorded impulse will propagate through the myoepithelium. Its form is shown in the record. Stimulation on either side of the cut in the motor giant axon evokes a myoepithelial impulse.

Contractions of the subumbrellar myoepithelium in response to stimulation of a motor giant axon were recorded in one preparation where the myoepithelial impulse did not propagate across the giant axons (Fig. 11). Contractions had times to peak and half relaxation down to 40·5 and 94 ms respectively. Peak tension ranged from 440 to 780 mg.

We conclude that the eight motor giant axons excite the subumbrellar myoepithelium directly via a series of chemical neuromuscular synapses distributed along the length of the giant axons. Myoepithelial impulses are initiated that propagate from cell to cell away from the motor giant axons through the myoepithelium and trigger rapid contraction of the subumbrellar circular muscle.

(*d*) *The velum response to ring giant axon activity*

When the ring giant axon fires impulses, extracellular electrodes on the inner myoepithelium of the velum record an impulse similar to that in the subumbrellar epithelium. It propagates over the velum but its coupling to the ring giant axon has not been studied.

(*e*) *The tentacle response to ring giant axon activity*

During escape swimming the tentacles usually shorten to form tight coils. Ring giant axon impulses lead to a similar shortening. In animals paralysed in 81 mM Mg^{2+} or 10 mM Mn^{2+} a fine suction electrode was applied over the large axon on the outer side of a tentacle. A second extracellular electrode on the margin at the base of the tentacle

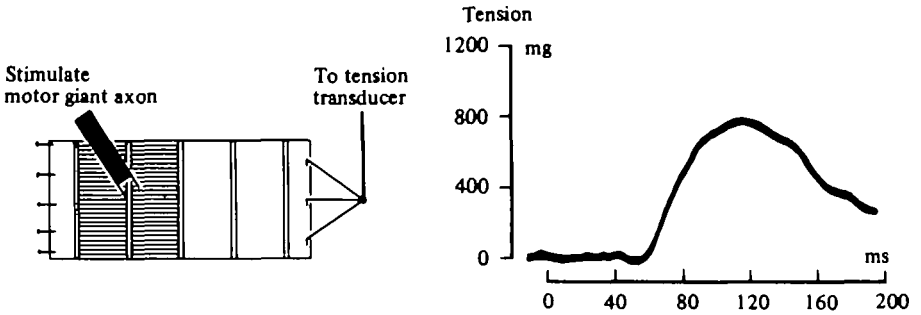

Fig. 11. Contraction of the subumbrellar myoepithelium in response to motor giant stimulation. The apex and margin were removed from a piece of bell wall which was pinned at one end as in the diagram. Stimulation of one motor giant axon led to contraction in the muscle sections on either side. This contraction was recorded with a transducer attached to the free end by a three-pronged hook. A typical contraction is shown to the right.

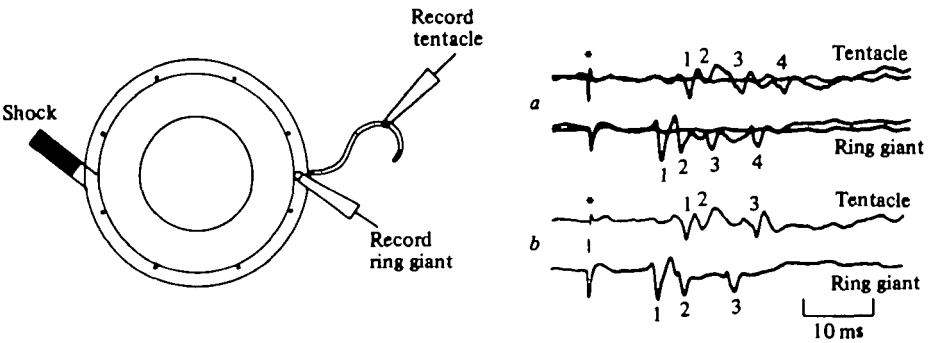

Fig. 12. Responses in the tentacles to ring giant axon impulses in 10 mM Mn^{2+}. The placement of electrodes on a paralysed whole animal viewed from the oral surface is shown in the diagram. (Dots show the positions of the statocysts.) (*a*) Two sweeps just above and below threshold for ring giant. This shows that threshold for appearance of spikes in ring giant axon and tentacle was the same. The number of spikes in both sites was also the same, in (*a*) 4 spikes and in (*b*) 3. Shocks were given at asterisks.

was used to record ring giant axon activity. When the margin was stimulated electrically, the threshold for impulses at the two sites was the same, as was the number and pattern of impulses (Fig. 12). Conduction velocities in these large tentacle axons ranged from 0·4 to 0·8 m s^{-1}.

The tentacle axons therefore appear to be in electrical continuity with the ring giant axon. It could be that the tentacle axons are branches from the ring giant axons. Alternatively, the two could be coupled by electrical synapses unaffected by Mg^{2+} or Mn^{2+}. It seems likely that the large tentacle axons excite the striated longitudinal muscle in the tentacles and thus initiate shortening, but recordings were not made from these muscles.

DISCUSSION

Organization of the escape system

The escape swim of *Aglantha* results from a rapid, nearly synchronous contraction of the circular muscles lining the inside of the bell (Donaldson, in preparation). Our results show that two types of giant axon and a propagated myoepithelial impulse are involved in achieving this synchrony. The first giant axon, the ring giant, forms a closed ring in the outer nerve-ring. When it is directly stimulated it can evoke a complete escape swim. More usually it would be excited indirectly via receptors in the margin, velum or tentacles. The comb pads may provide some of this sensory input. The ring giant axon response to indirect excitation is blocked by raised concentrations of Mg^{2+}, Mn^{2+} or Co^{2+}, so is probably via chemical synapses. Such synapses on to the ring giant axon have been seen in electron micrographs (Singla, personal communication) and our recordings of spontaneous, depolarizing, postsynaptic potentials in unparalysed preparations provide physiological confirmation of chemical synapses. The ring giant axon appears to act as a co-ordinating interneurone, receiving sensory inflow and in turn exciting motoneurones (see below). Its role is similar to that of many other giant axons controlling rapid escape behaviours (e.g. earthworm medial and lateral giant axons (Bullock, 1945); squid first-order giant axons (Young, 1938); crayfish medial and lateral giant axons (Wine & Krasne, 1972); teleost Mauthner cells (Furukawa, 1966)). Similar giant axons are shown in the outer nerve-rings of another trachyline medusa (*Rhopalonema*) and of a narcomedusa (*Aeginopsis*) by Hertwig & Hertwig (1878) but are labelled as empty spaces! We expect that the presence of ring giant axons in these and other medusae will correlate with rapid swimming. This is because the ring giant axon can help synchronize contractions around the bell in two ways. In the first place synchrony can be helped by their rapid conduction velocity. When sensory excitation is local (e.g. from one tentacle), impulses in the ring giant will propagate quickly from their site of initiation to the opposite side of the bell. In the second place, their ring structure and large diameter will allow subthreshold potentials to spread and sum. Thus, when excitation is more diffuse (e.g. a shock wave in the surrounding water which excites receptors all round the margin), subthreshold depolarizations originating at many sites around the margin could spread passively within the ring giant axon and lead to near-synchronous impulse initiation all around the ring.

The second category of giant axons are the 8 motor giants. Singla (1978) has shown that these make synaptic junctions with the subumbrellar myoepithelial cells. They are therefore motoneurones whose rapid impulse conduction velocity will help synchronize muscle contraction at different levels in the bell. They are excited by the ring giant axons, but the anatomy of this pathway is unclear. It is blocked by divalent cations, which interfere with chemical synaptic transmission, and the delay between pre- and postsynaptic depolarizations is in the same range as that at the junction between a motor giant axon and the myoepithelium which is monosynaptic (Singla, 1978). These results suggest that the ring giant axon makes direct excitatory, chemical synaptic junctions with each of the motor giant axons. The motor giant axons synapse directly with the myoepithelial cells which wrap them (Singla, 1978). The giant axon can excite the myoepithelium along its whole length. Thus neuromuscular synaptic structures must occur along the whole length of each motor giant axon. In effect, the whole giant axon

is like a long, presynaptic, neuromuscular terminal. This unusual situation may be useful for the study of the properties of presynaptic membrane. Occasional branches run from the motor giant axons. We presume that these also make neuromuscular synapses with the subumbrellar myoepithelium.

In other giant axon pathways, both chemical synapses (squid second- to third-order giant synapses) and electrical synapses (crayfish central to motor giant synapses) are found. In *Aglantha* it is possible that the large axons in the tentacles make electrical synapses with the ring giant axon. However, at present we cannot exclude the possibility that these tentacle axons are simply branches of the ring giant axon. The tentacle axons fire one-to-one with the burst of impulses in the ring giant, which is significant, as a single impulse is insufficient to cause full tentacle retraction.

An impulse in the motor giant axons is sufficient to initiate a myoepithelial impulse. This can propagate in any direction, but is presumably most important in carrying excitation circumferentially into the areas between motor giant axons. (Motor giant axon branches may also contribute to circumferential spread.) We assume that propagation in the myoepithelium is by intracellular current flow. Similar propagated myoepithelial impulses associated with swimming contractions are present in other hydromedusae (e.g. *Stomotoca* (Mackie, 1975); *Spirocodon* (Ohtsu & Yoshida, 1973); *Polyorchis* (Spencer, 1978)). As a result of the myoepithelial impulse the muscle tails contract very rapidly (down to 40 ms to peak tension). The rapidity of contraction and presence of giant axons are *Aglantha*'s specializations which together contribute to the effectiveness of its escape swimming.

Whether the ciliary comb pads distributed round the margin in close association with the ring giant axon are mechanoreceptors concerned with the escape response remains to be established (cf. Tardent & Schmidt, 1972). Similar structures are shown by the Hertwigs (1878) in *Rhopalonema* and *Aglaura* (Hydrozoa, Rhopalonematidae) and labelled 'Tastkämme' (touch-sensitive combs). In *Aglantha* rubbing the margin, in the area of the ciliary comb pads is not particularly effective in evoking escape swims. The pads could, however, be sensitive to disturbances in the surrounding water. In this case many pads could be excited together and spatial summation could allow them to fire the ring giant axon. The ring structure of the ring giant axon would increase its potential for spatial summation of inputs distributed around the margin.

Properties of the giant axons, myoepithelial cells and synapses

The motor giant axons of *Aglantha*, with diameters of up to 40 μm and conduction velocities of up to 4 m s^{-1}, are similar to some giant axons in siphonophores (Mackie, 1978) and appear to be larger and faster than other cnidarian axons. Though exceptional within the cnidaria, their structure and membrane properties seem fairly routine in a broader context. Their axons are simple and unsheathed. Resting potentials in the motor giant axons and myoepithelial cells were similar to each other (-55 to -60 mV) and to those of large axons in another hydromedusan, *Polyorchis* (Anderson & Mackie, 1977). However, the impulses of motor and ring giant axons in *Aglantha* are briefer than those in *Polyorchis*, *Forskalia* and *Nanomia*, which have the only other axons in the cnidaria where intracellular recording has been possible (Mackie, 1973, 1978). The motor giant axon impulse overshoots zero, is blocked by lowering the external Na^+ concentration,

and is little affected by 10 mM Mn^{2+} or Co^{2+}. This indicates a mainly Na^{+}-dependent rising phase (cf. Anderson, 1979). Both motor and ring giant axons have slow, depolarizing after-potentials during which peak impulse amplitudes are slightly reduced. In contrast to these neuronal impulses, the myoepithelial impulse is slower, longer, and blocked by 10 mM Mn^{2+} or Co^{2+} and by 80 mM Mg^{2+}. It would appear therefore to be Ca^{2+} dependent. As these cells contain virtually no indication of any sarcoplasmic reticulum (Singla, 1978), it seems likely that contraction is initiated by Ca^{2+} entering during the impulse from the extracellular space. It seems probable that this situation is widespread in the cnidaria. Consequently, divalent cation paralysis could result from direct action on the muscle and cannot therefore be used unambiguously as a method for blocking chemical synapses.

Our results provide directly physiological evidence of synaptic transmission in the cnidaria, extending previous results from intracellular recordings in neurones of *Polyorchis* (Anderson & Mackie, 1977) and in myoepithelial cells of *Nanomia* (Mackie, 1976). We have the following results indicative of conventional chemical synaptic transmission: (1) clear synaptic delay at interneuronal and neuromuscular synapses, (2) interneuronal synaptic transmission blocked by Mn^{2+}, Co^{2+} and Mg^{2+} ions, (3) long depolarizing postsynaptic potentials present which can summate and show facilitation. The blockage of synaptic transmission from the ring giant to the motor giant axons by the divalent cations and the abolition of spontaneous postsynaptic potentials in the ring giant also show that these ions are blocking chemical synapses as they do elsewhere.

When synapses are blocked by divalent cations, it is clear that the multiple-impulse response of the ring giant axon is still present, apparently unchanged. This response to a single brief current pulse (0·5 ms) is unusual, as is the ultrastructure of this giant axon. The multiple impulses could depend on the membrane properties of the ring giant axon itself. Alternatively, the giant axon might be electrically coupled to smaller neurones in the nerve-rings and the multiple impulses depend in some way on interactions with these other cells. The uneven diameter of the ring giant axon is presumably an adaptation to avoid damage due to buckling during swimming contractions when the margin circumference is dramatically reduced.

We would like to thank the National Research Council of Canada for support, the Royal Society for a travel grant to A. R., the University of Washington for the provision of facilities at its Friday Harbor Laboratories, and Drs S. Donaldson and C. L. Singla who helped with ideas and by providing Figs. 5*c* and 4*b* respectively.

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