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CENTRAL CIRCUITRY IN THE JELLYFISH *AGLANTHA DIGITALE*

II. THE RING GIANT AND CARRIER SYSTEMS

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Summary

1. The ring giant axon in the outer nerve ring of the jellyfish *Aglantha digitale* is a multinucleate syncytium 85 % of which is occupied by an electron-dense fluid-filled vacuole apparently in a Gibbs–Donnan equilibrium with the surrounding band of cytoplasmic cortex. Micropipette recordings show small (−15 to −25 mV) and large (–62 to −66 mV) resting potentials. Low values, obtained with a high proportion of the micropipette penetrations, are assumed to be from the central vacuole; high values from the cytoplasmic cortex. Background electrical activity includes rhythmic oscillations and synaptic potentials representing hair cell input caused by vibration.

2. After the ring giant axon has been cut, propagating action potentials evoked by stimulation are conducted past the cut and re-enter the axon on the far side. The system responsible (the carrier system) through-conducts at a velocity approximately 25 % of that of the ring giant axon and is probably composed of small neurones running in parallel with it. Numerous small neurones are seen by electron microscopy, some making one-way and some two-way synapses with the ring giant.

3. Despite their different conduction velocities, the two systems normally appear to fire in synchrony and at the velocity of the ring giant axon. We suggest that, once initiated, ring giant spikes propagate rapidly around the margin, firing the carrier neurones through serial synapses and giving them, in effect, the same high conduction velocity. Initiation of ring giant spikes can, however, require input from the carrier system. The spikes are frequently seen to be mounted on slow positive potentials representing summed carrier postsynaptic potentials.

4. The carrier system fires one-for-one with the giant axons of the tentacles and may mediate impulse traffic between the latter and the ring giant axon. We suggest that the carrier system may also provide the pathways from the ring giant to the motor giant axons used in escape swimming.

5. The findings show that the ring giant axon functions in close collaboration with the carrier system, increasing the latter's effective conduction velocity, and that interactions with other neuronal sub-systems are probably mediated exclusively by the carrier system.

Key words: Cnidaria, jellyfish, *Aglantha digitale*, hydromedusan behaviour, escape swimming, tentacle contraction, nervous system, nerve ring, giant axon, intracellular recording, action potential, relay system, carrier system, pacemaker, central circuitry.

Introduction

This is the second of two papers describing the interactions of neurones running in the nerve rings of a rhopalonematid medusa *Aglantha digitale.* In the first paper (Mackie and Meech, 1995), the focus was on the relay system that mediates interactions between the pacemaker neurones that generate slow swimming and the ring giant axon that coordinates escape behaviour (see Mackie and Meech, 1985). It was noted that production of spikes in the ring giant axon requires more than relay system input: another system is involved, the carrier system. The carrier system is so closely associated with the ring giant that it has previously escaped detection. We show here that some of the functions previously assigned to the ring giant axon may more properly be assigned to the carrier system.

The ring giant is a unique axon structurally, being a multinucleate syncytium in the form of a perfect torus, 85 % of whose volume is occupied by a fluid-filled vacuole (Roberts and Mackie, 1980; Mackie, 1989). Electron microscopy of *A. digitale* larvae showed the ring giant axon as postsynaptic to some neurones but making symmetrical (presumably bi-directional) synapses with others (Mackie, 1989). We present similar evidence here for adult animals. The ring giant coordinates escape behaviour by receiving sensory excitation from all around the bell margin. It conducts impulses rapidly (at greater than 2.6 m s^{-1}) around the margin, causing twitch contractions in all the tentacles. At the same time, during escape behaviour, it excites the motor giants, probably through

a disynaptic pathway (Meech and Mackie, 1995). Its action potential shows the characteristics of a sodium spike (Roberts and Mackie, 1980). Spiking can be evoked by vibration of the tentacles or margin, and also by direct tactile and electrical stimulation. The ring giant axon is closely linked physiologically with the large axons that run down the tentacles on their aboral sides (tentacle giant axons). The two systems fire one-to-one during the escape response (Roberts and Mackie, 1980), but electron microscopy has failed to show any direct connections between the two. The ring giant receives input from hair cell mechanoreceptors located in the tentacle bases and velum, where the receptors cluster side by side in 'tactile combs'. The hair cells respond to vibrations and generate impulses in their basal neurites that cause summing excitatory postsynaptic potentials in the ring giant (Arkett *et al.* 1988). *A. digitale* is extremely sensitive to vibration and the escape response effectively removes it from the vicinity of the stimulus. A single escape swimming contraction with tentacles contracted can propel the animal a distance equivalent to seven body lengths (Donaldson *et al.* 1980).

Our purpose in this paper is to present new findings on the structure and physiology of the ring giant axon and to provide evidence for the existence of the carrier system, showing how it and the ring giant function together in the circuitry.

Materials and methods

Specimens of *Aglantha digitale* (Müller) were caught off the dock at the University of Washington Laboratories at Friday Harbor, USA. Methods of maintenance and dissection are described by Mackie and Meech (1995).

Material for electron microscopy was fixed in 2.5 % glutaraldehyde in 0.2 mol l^{-1} phosphate buffer with sodium chloride added to raise the osmolality to 940 mosmol kg^{-1}, followed by postfixation in a 1:1 mixture of osmium tetroxide (40 g l^{-1}) and sodium bicarbonate buffer (25 g l^{-1}; pH 7.2). Pieces of tissue were embedded in Epon 812 for sectioning. Lanthanum was used to visualise gap junctions, using the method of Revel and Karnowsky (1967), as modified by Kerfoot *et al.* 1985)

For physiological recording, preparations were usually pinned out with the exumbrellar upwards so as to allow access to the ring giant axon. Extracellular recordings were made with polyethylene suction electrodes and intracellular recordings with glass micropipettes filled with 3 mol l^{-1} KCl, having resistances of 40–60 MΩ. Polarity in the extracellular recordings is negative upwards, except where otherwise noted. Conventional amplification and display equipment were used, details of which are given by Mackie and Meech (1995).

Results

Structure

A section through part of the outer nerve ring containing the ring giant axon is shown in Fig. 1A. The velum lies to the left and the inner nerve ring lies across a layer of mesogloea, out of the picture at the top. The bulk of the outer nerve ring lies out of the picture, to the right. A typical cross section through the margin shows about 100 axon profiles in the inner nerve ring and about 700 in the outer nerve ring. Roughly 10 % of the axons in the inner nerve ring have diameters exceeding 3 μm, compared with only 1 % in the outer nerve ring. Some or all of the large inner ring axons are probably pacemaker neurones, which are known to have a high conduction velocity. About 70 % of the inner ring axons have diameters in the 1.0–3.0 μm size range compared with 40 % in the outer ring. Very small axons, 0.25–1.0 μm in diameter, dominate the outer nerve ring, but most of them lie away from the velum, out of the picture to the right. The axons in the vicinity of the ring giant show a range of diameters resembling those of the inner nerve ring. As shown in the figure, part of the ring giant is ensheathed by epithelium, contacts with smaller neurones being concentrated in an unsheathed zone on the right-hand side. In this region, small neurones make one-way and two-way synapses with the ring giant (Fig 1B,C). Similar junctions were reported (Mackie, 1989) in very young (2.5 mm diameter) larvae of *A. digitale*, where the ring giant was in the process of formation. Gap junctions with epithelial cells are apparent in lanthanum-stained material (Fig. 1D). The chemical composition of the contents of the ring giant vacuole has not been investigated. Its high electron-density might be due to osmiophilic lipids or lipoproteins. In some blocks sectioned, the osmiophilic material appears to have leaked into the cytoplasmic cortex and even into adjacent epithelial cells, imparting high electron-density to these areas (as also noted by Mackie, 1989; their Fig. 6C). In other blocks, probably due to escape of the vacuolar contents near cuts, there is no electron-density and the vacuole appears clear. There is little evidence that the ring giant sends out lateral processes, although a few short processes are occasionally seen. Most of the outer surface is smooth, and it seems likely that all the functional contacts made with other cells are made at this interface. Thus, when looking for physiological pathways linking the ring giant with other systems, we have to think of connections involving separate, small neurites rather than processes of the ring giant itself.

Physiology

Intracellular recordings from the ring giant usually show a small resting potential, typically in the range −15 to −25 mV. On two occasions only we have recorded conventional resting potentials of −62 and −66 mV respectively. Given the structure of the axon, it seems probable that the latter were 'true' resting potentials from the cytoplasmic cortex, while the small resting potentials were from the central vacuole. Action potentials and synaptic events recorded in the two places showed the same polarity and similar amplitudes. It may be inferred that the inner (juxtavacuolar) membrane is inexcitable and freely permeable to current-carrying ions. The potential across it could arise from a Gibbs–Donnan equilibrium between the cytoplasmic cortex and the osmiophilic matrix that fills the vacuole.

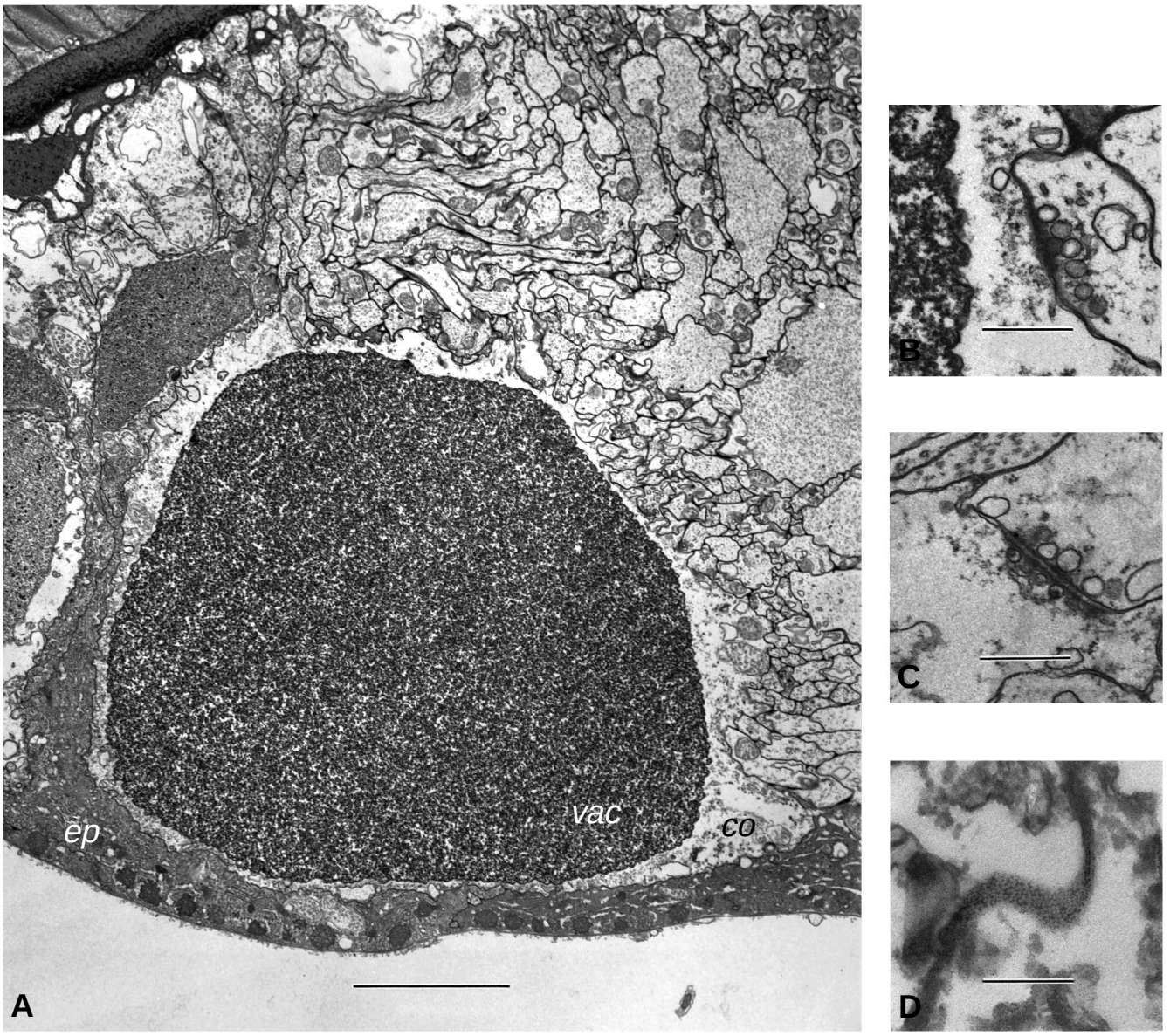

Fig. 1. Electron micrographs of the ring giant axon and its junctions with adjacent cells. (A) Low-magnification view of the ring giant showing its large, osmiophilic vacuole (*vac*) surrounded by a cytoplasmic cortex (*co*). The axon is partially enveloped by epithelial cell processes (*ep*) but, on the right, small neurones of the outer nerve ring contact the ring giant directly. (B) A small neurone (right) presynaptic to the ring giant axon. (C) Two-way synapse between a small neurone (right) and the ring giant axon. (D) Oblique section through a gap junction connecting the ring giant axon (left) and an epithelial cell. Connexon particles are outlined by lanthanum stain. Scale bars, A, 5 μm; B,C, 0.5 μm; D, 0.25 μm.

Intracellular recordings of spontaneous activity in the ring giant axon in sea water showed a considerable amount of synaptic input superimposed on an underlying oscillatory pattern (Fig. 2A). Most of the synaptic input is attributable to vibrations picked up by hair cells, which synapse directly with the ring giant (Arkett *et al.* 1988). As noted by these authors, hair cell spike bursts can be recorded extracellularly as small signals correlated with summing postsynaptic potentials (PSPs) in the ring giant (Fig. 2B). Treatment with 81 mmol l^{-1} Mg^{2+} reduced this input, allowing the underlying oscillations to be better seen (Fig. 2C), although tapping the bench top still evoked bursts of hair cell PSPs. The oscillatory rhythm showed a frequency of 0.5–1.0 Hz. The pattern was temporarily blocked by bursts of hair cell PSPs. High levels of magnesium (>105 mmol l^{-1}) virtually eliminated the oscillations.

Ring giant spikes (Fig. 3A) were obtained by tactile or electrical stimulation of the margin and tentacles. They are 64–68 mV, non-overshooting events originally described as 'riding on a slow positive potential' (Roberts and Mackie, 1980) and could be recognised in extracellular recordings as biphasic (initially positive-going) deflections, typically with large, irregular after-potentials (Fig. 3B). The latter probably

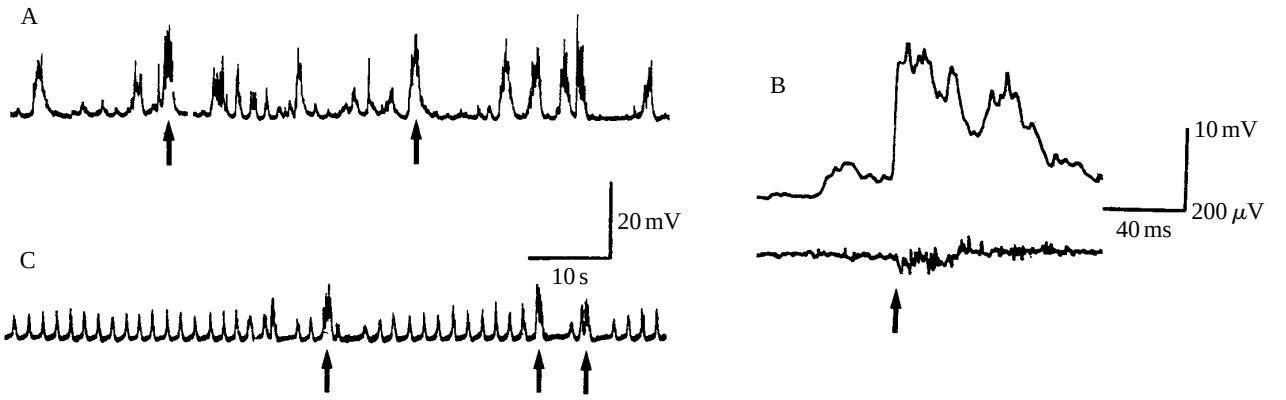

Fig. 2. Spontaneous electrical activity in the ring giant axon with superimposed mechanically stimulated events. Arrows show taps on the table top. (A) Preparation in sea water showing bursts of vibration-induced postsynaptic potentials (resting potential −25 mV). (B) Burst of postsynaptic potentials on an expanded time scale (upper trace; resting potential −20 mV), with an extracellular recording of hair cell impulse bursts (lower trace). (C) Preparation in 81 mmol l^{-1} Mg^{2+} showing the underlying oscillatory rhythm interrupted by vibration-induced PSPs (resting potential −18 mV).

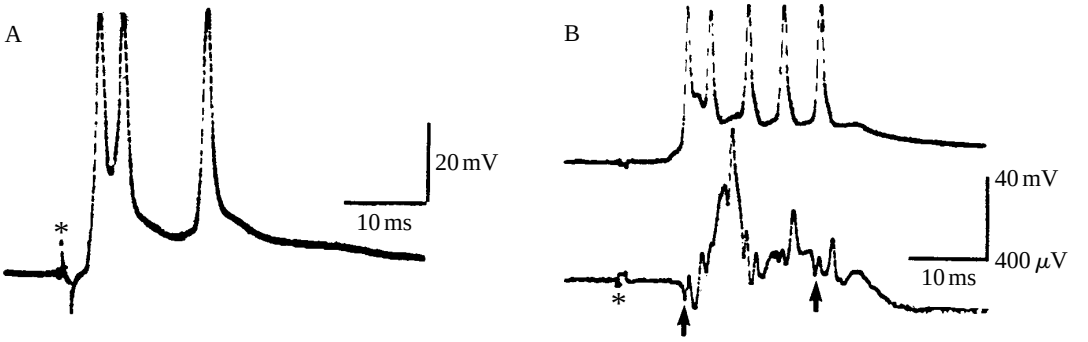

Fig. 3. Ring giant spikes. (A) A burst of three spikes evoked by a shock (asterisk) on the outer nerve ring. (B) Intra- (upper) and extracellular (lower) recordings compared. Asterisk shows shock artefact. Arrows below the extracellular trace show first and last spike correlates. Other larger events probably represent depolarisations from the covering epithelium because they could be reduced by treatment with octanol, a gap-junction blocker.

represent depolarisations spreading through the covering epithelium, whose cells are known to be interconnected by gap junctions (Mackie, 1989). The after-potentials could be reduced by treatment with 0.2–0.5 mmol l^{-1} octanol, a gap-junction blocker (see Fig. 3C in Mackie and Meech, 1995).

Recordings in sea water showed the first spike in a burst to rise and fall rapidly and smoothly without an obvious foot or inflection. Later spikes, however, frequently showed notches in their rising or falling slopes that betrayed the presence of an underlying synaptic potential (Fig. 4A). Such notches were often most pronounced at the ends of long bursts (Fig. 4B). Events interpreted as synaptic potentials that failed to cause spikes were seen occasionally within bursts also (Fig. 4C) and (more frequently) at the ends of long bursts (Fig. 4D), as if the ring giant axon was postsynaptic to a conducting system that normally fires in synchrony with it. This second system, which we call the carrier system, can continue to fire at the end of a burst, producing low-amplitude postsynaptic potentials that do not fire the ring giant. The absence of a notch on the early spikes in a ring giant burst suggests that these spikes were propagating on their own without a carrier input.

All of these observations suggest that, although the carrier system is presynaptic to the ring giant axon and generates spikes in it, some ring giant spikes appear to be generated and propagated by the ring giant axon itself. However, further support for the role of the carrier system in generating ring giant spikes was found in the effects of divalent cations that compete with calcium at synapses. Treatment with 6 mmol l^{-1} Mn^{2+}, for instance, had little if any effect on the spike itself but increased the rise-time of the ‘slow positive potential’ on which the spike was based (Fig. 4E). Subsequent spikes in a burst were similarly delayed (Fig. 4F). This suggests that spike generation in the ring giant depends on synaptic input from the carrier system, that the ‘slow positive potential’ consists of summed postsynaptic potentials and that treatment with manganese ions, by diminishing the effectiveness of synaptic transmission between the two systems, delays depolarisation to spike threshold.

The existence of a system acting in close collaboration with the ring giant axon was confirmed in lesioning experiments. With the aid of a sharp micropipette held in a manipulator, it was possible to go along the ring giant cutting it and pulling large fragments aside without causing serious damage to the adjacent mass of small outer-ring neurones. Some shrunken fragments remained (and may account for the fact that the ring giant axon regenerated after 3–4 h), but for at least an hour after

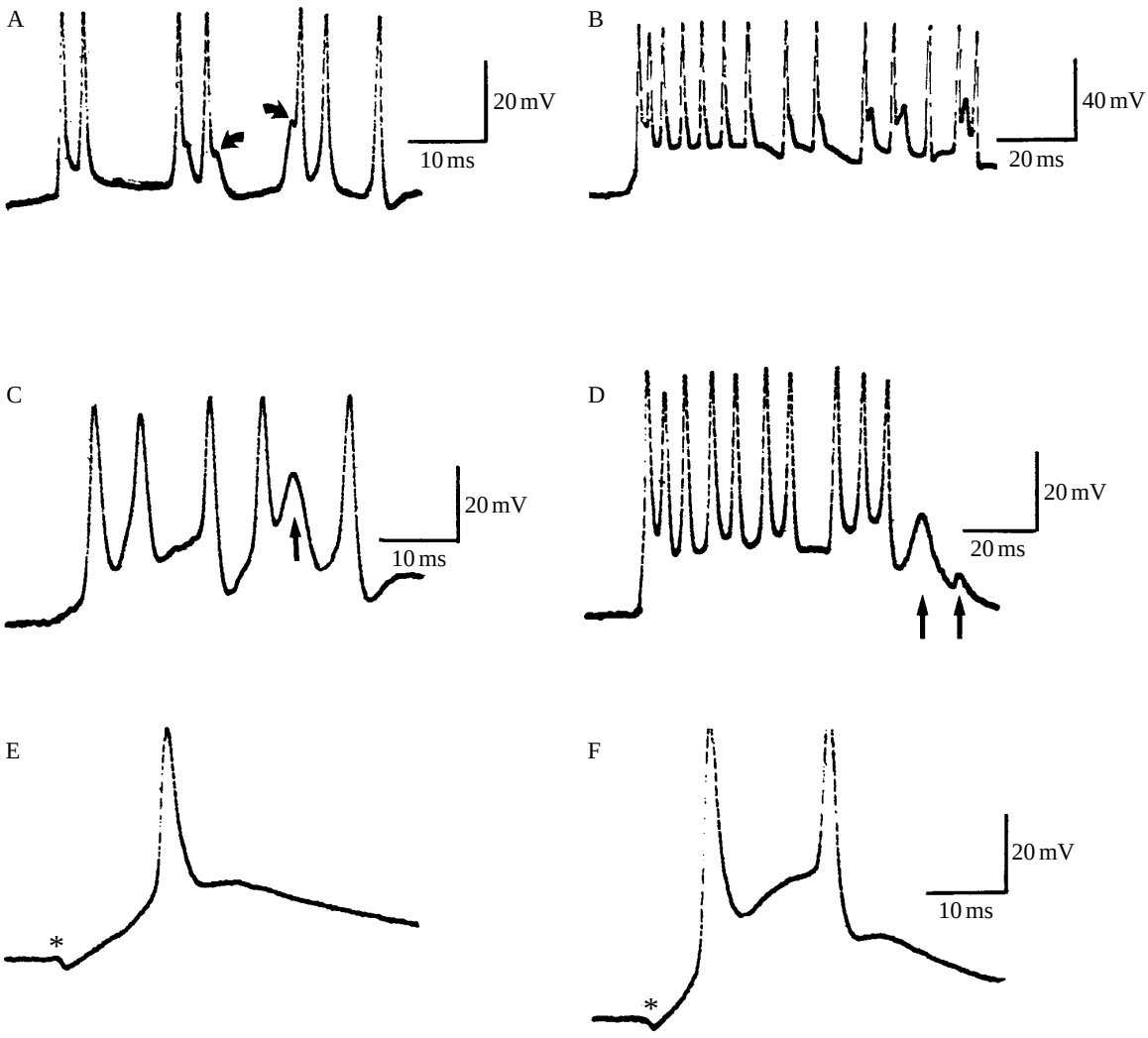

Fig. 4. Ring giant spikes evoked by shocks (asterisks) along the margin; shock artefacts are off the record to the left except in E and F. (A) Ring giant spike burst showing inflections (arrows) indicative of underlying postsynaptic potentials (PSPs). (B) Similar burst with inflections towards the end of the burst. (C) A PSP within a burst (arrow) fails to generate a spike. (D) A burst ends in subthreshold PSPs (arrows). (E) A single and (F) two ring giant spikes recorded from a preparation in 6 mmol l^{-1} Mn^{2+} showing the delayed rise-time of the carrier postsynaptic potentials.

such operations there can be no doubt that the structure was effectively destroyed as a conduction pathway. In a series of experiments, the ring giant was destroyed over distances between 1.0 and 2.3 mm. Conduction past the lesion was not blocked. On the contrary, typical bursts of spikes were recorded from the ring giant on the far side of the lesion following stimulation on the near side. Conduction velocity through the lesioned zone, however, fell dramatically from an initial 150–200 cm s^{-1} to values in the range 30–50 cm s^{-1}. These slow values presumably represent the conduction velocity of the carrier system conducting on its own.

Attempts to record intracellularly from small outer nerve ring neurones were not successful and, so long as the ring giant axon was intact, carrier system spikes could not be separately distinguished in extracellular recordings. Hence, the existence of the carrier system went unnoted in our previous work. After destruction of the ring giant axon, however, extracellular recordings in the regions from which it had been removed showed conducted events that met the specifications for the carrier system. The system in question fired singly or in bursts (bottom traces in Fig. 5A,B) at velocities within the range 30–50 cm s^{-1}. The bursts showed spike numbers and frequencies resembling those characterising ring giant bursts.

A close correspondence in the firing patterns of the ring giant and tentacle giant axons was noted by Roberts and Mackie (1980). Ring giant spikes are almost always matched one-for-one with spikes in the tentacle giant axon. Electron microscopy, however, has failed to show any direct contact between the two. The proximal processes of the neurones that fuse to form the tentacle giant dwindle in diameter and become lost within the mass of small neurites comprising the outer nerve ring (Bickell-Page and Mackie, 1991). It seems likely that, within this mass of neurites, components of the carrier system are arranged to form links between the tentacle and ring

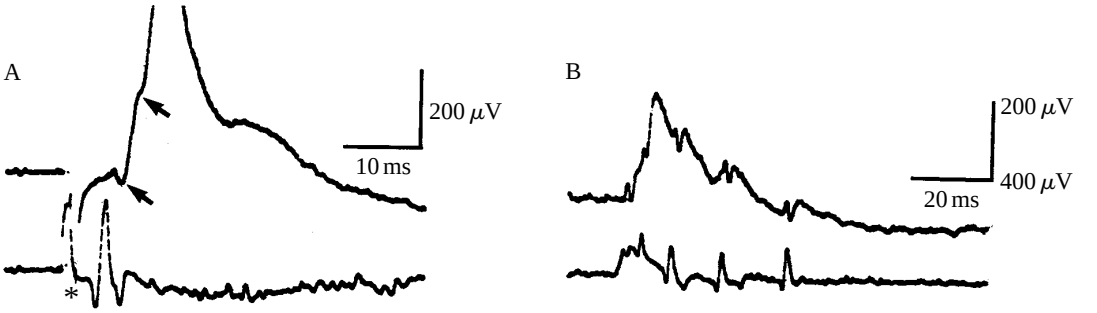

Fig. 5. Extracellular recordings from a tentacle (upper traces) and from the outer nerve ring in an area where the ring giant axon had been destroyed but the carrier system was intact (lower traces). In A, a carrier potential triggers a tentacle giant impulse (between arrows), which is followed by an after-potential representing a myoepithelial depolarisation. The asterisk marks the shock. In B, a burst of carrier potentials is matched one-for-one with tentacle giant potentials; summed epithelial depolarisations are also seen.

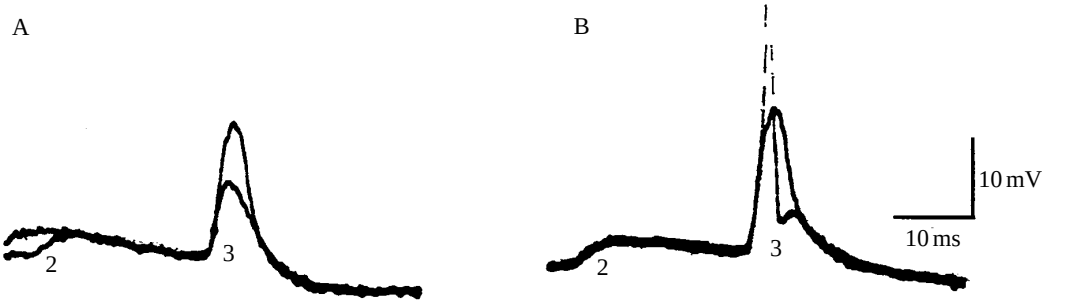

Fig. 6. Intracellular recordings from a ring giant axon during slow swimming showing steps 2 and 3 in the three-step depolarisation. Step 3 represents carrier postsynaptic potentials. (A) Two superimposed traces showing variability in carrier PSP amplitude. Both postsynaptic potentials were subthreshold. (B) Two superimposed traces in one of which the PSP reached spike threshold.

giants for, after ring giant destruction, impulses generated in the carrier system were still found to induce tentacle giant impulses on a one-to-one basis (Fig. 5A,B). Similarly, tentacle giant excitation passed to the carrier system. The carrier system may thus mediate two-way spike transfer between the ring giant and the tentacle giants.

We have shown in the companion paper (Mackie and Meech, 1995) that ring giant spikes often occur during slow swimming and that this is due to a summed, three-step depolarisation of the ring giant, representing input from the pacemaker, relay and carrier systems acting sequentially and in that order. As with ring giant spikes evoked by direct stimulation, so in the case of these indirectly evoked spikes there was clear evidence that carrier postsynaptic potentials lie concealed or partially hidden within most if not all of the spikes. Occasionally the carrier PSP failed to produce a spike and could be seen on its own. Such events showed variable amplitudes (Fig. 6A). Those of sufficiently large amplitude to cause spikes rose fast enough for their ascending slopes to merge with the rising phases of the spikes they generated, allowing the spike to take off without any perceptible break (Fig. 6B). With smaller, more slowly rising, postsynaptic potentials a break or shoulder was apparent at or around spike threshold (Mackie and Meech, 1995, their Fig. 4D). A notch was frequently seen in the depolarisation phase, where the spike dipped briefly below the more slowly declining PSP (Fig. 6B). Three-step depolarisations summing to 20 mV elicited spikes in the ring giant; assuming a resting potential of −66 mV (see above), spike threshold would appear to lie at about −46 mV.

Discussion

The findings reported here reveal the presence of a ‘carrier’ system closely associated with the ring giant axon that fires when it fires, that can carry impulses through regions where the ring giant has been lesioned (albeit at a reduced conduction velocity), that can re-excite the ring giant on the far side of the lesion, that probably serves as the link between the ring giant and the tentacle giants and that is responsible for the third step in three-step depolarisations of the ring giant that generate ring giant spikes during slow swimming. These interactions are summarised diagrammatically in an ‘end-on’ view of the central nervous system (Fig. 7), where it must be imagined that the margin has been cut radially and that the observer is looking into the cut end.

It seems very likely that the carrier system also connects the ring giant axon with the motor giant axons during escape swimming. The delay of 1.6–1.8 ms between the ring giant spike and the fast postsynaptic potential in the motor giant axon suggests a disynaptic pathway (Meech and Mackie, 1995). If so, the first synapse would probably be between the ring giant and the carrier system and the second between the latter and the motor giant. This concept is incorporated in Fig. 7, which shows the carrier system as presynaptic to the motor giant axons.

If the carrier system is responsible for these input–output functions, what is left as a function for the ring giant? We see it as serving primarily to increase conduction velocity around the margin during escape behaviour. We know that it is excited by carrier postsynaptic potentials and that it conducts four or five times as fast as the carrier system. If we assume that

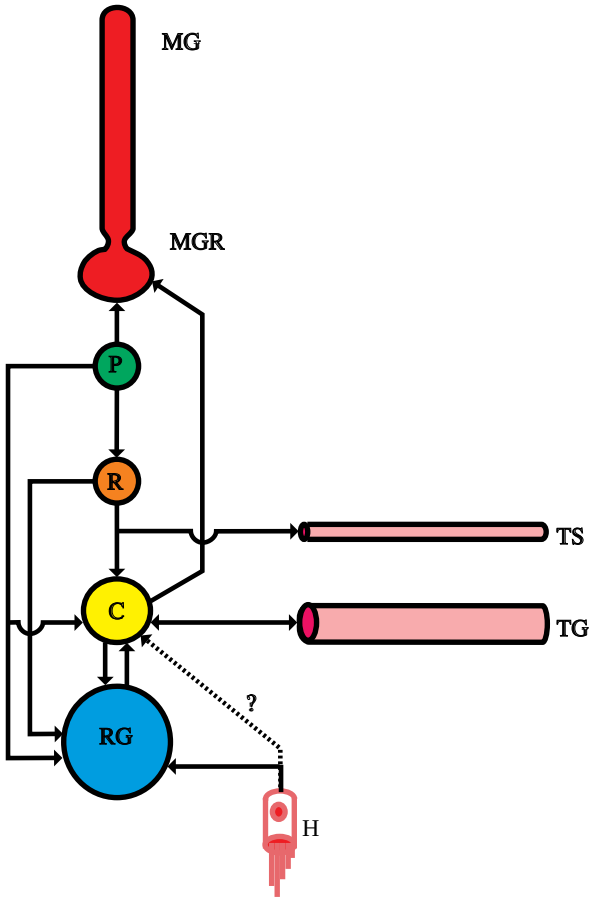

Fig. 7. Diagrammatic ‘end-on’ view of the central nervous system summarising the interactions between the motor giant axon (MG), the motor giant rootlets (MGR), the pacemaker system (P), the relay system (R), the carrier system (C) and the ring giant axon (RG). The ring giant/carrier system receives an input from numerous hair cells (H). Outputs to the tentacle, the giant axon (TG) and the slower conducting small nerves (TS) are also shown.

impulses propagating in it can, in turn, excite the carrier system through serial synapses so that carrier spikes are piggybacked along at the same high velocity, then we have the makings of an effective partnership where the carrier system acquires the high conduction velocity of its giant *Doppelgänger*. The fact that early spikes (and some later ones) in ring giant bursts show no trace of a postsynaptic potential is to be expected, given that the ring giant conducts much faster than the carrier system, so even if a ring giant spike were generated by a spike in the carrier system, it might overtake the latter and arrive at the recording electrode before it. Elsewhere, the two systems are presumably firing slightly out of synchrony so that carrier postsynaptic potentials or their tail-ends become visible again.

While carrier input may be the main source of ring giant excitation, it should not be forgotten that the pacemaker and relay systems also produce postsynaptic potentials in it and that hair cell input can certainly affect its excitability. As indicated in Fig. 7, we do not know whether the hair cells synapse with carrier neurones or only with the ring giant axon. Electron micrographs show that the ring giant is postsynaptic to numerous small neurites running along beside it in the outer nerve ring. Some of the small units are probably hair cell processes, while others might be carrier, relay or pacemaker processes. At present we have no way of distinguishing between them. In addition, there are symmetrical synapses between the ring giant axon and adjacent small neurites. The latter may well be carrier units, and the symmetrical synapses could mediate the bi-directional transmission called for by the physiological findings. Symmetrical synapses occur in *Cyanea capillata* (Scyphomedusae), and recordings from the two sides show that transmission can occur in either direction (Anderson, 1985). The *Cyanea capillata* recordings show synaptic ‘backfiring’ where a spike on one side generates a spike on the other that generates a postsynaptic potential on the first side. Some of our recordings suggest that similar interactions may occur between the ring and carrier neurones of *A. digitale*, but this must remain conjectural until some way is found of recording intracellularly from both sides simultaneously.

The peculiar structure of the ring giant axon merits comment. Mackie (1989) suggested that the vacuole was a hydromechanical device for preventing the axon from buckling or kinking when the margin constricts during swimming. Its high conduction velocity suggests that the axon has a relatively low core resistance. If current flow were restricted to the narrow cytoplasmic cortex of the cell, it is difficult to see how this could be achieved. If, however, as the evidence suggests, the juxtavacuolar membrane is readily permeable to ionic currents, it would effectively lower the core resistance, allowing forward flow of current within the vacuole as well as within the cytoplasm during impulse propagation. We assume that the difference in membrane potential between vacuole and cytoplasm arises from a Gibbs–Donnan equilibrium. Extensive speculation seems unnecessary in the absence of detailed information about the contents of the vacuole, but we suppose that it consists of a non-permeant, polyvalent matrix which draws fluid and permeant ions from the cytoplasm. The fluid entry, which gives the the vacuole its rigidity, is opposed by the hydrostatic pressure supplied by the surrounding cytoplasm, but it nevertheless dilutes the permeant ions present and hence produces the membrane potential difference observed between vacuole and cytoplasm. Ion pumps in the vacuolar membrane compensate for the steady leak of permeant ions down their concentration gradient by ensuring their return to the cytoplasm.

Having an enormous surface area, the ring giant will have a high membrane capacitance, and if it also has a low core resistance it must be difficult to excite. The carrier system, or any small neurone system, that excites the ring giant would have to make numerous serial synapses with it for the synaptic currents to sum to a level required to fire the giant. The electron microscope evidence is consistent with this picture, as numerous synaptic inputs are observed. Evidence of the difficulty of raising the ring giant to spike threshold is seen in the observation that the production of a spike in it during slow swimming requires a cascading series of summing inputs from no less than three different presynaptic systems (Mackie and

Meech, 1995). These points clearly need to be substantiated by measurements of the axon's cable properties.

A final question concerns the possible homologies of the ring giant and carrier systems with conduction systems in other hydromedusae. Outside the Rhopalonematidae, there is no sign of anything resembling a ring giant axon. We regard it as a neomorph evolved by a group of holoplanktonic medusae living in a densely populated, midwater zone of the sea where frequent, potentially damaging, contacts with fast-swimming crustaceans such as amphipods and euphausiids have made escape behaviour selectively advantageous. The carrier system also appears to have evolved as part of the escape circuitry, and we can find no obvious counterpart to it in other medusae. The only hint of a possible homology uncovered in the present work concerns the background pattern of oscillations recorded in the ring giant. The 'O' system of *Polyorchis pencillatus* , a non-spiking coupled neuronal network, also shows very regular membrane potential oscillations at frequencies around 1 Hz (Spencer and Arkett, 1984) and the 'ring' system of *Stomotoca atra* (Mackie, 1975) may also belong in the same category. The membrane potential oscillations of the ring giant might represent input from an O-type system but there is no other evidence for the existence of such a system, and the oscillations could be intrinsic rather than extrinsic. Further research will be needed to determine their source.

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