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Control of swimming in the hydrozoan jellyfish *Aequorea victoria*: subumbrellar organization and local inhibition

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SUMMARY

The subumbrella of the hydrozoan jellyfish *Aequorea victoria* (previously classified as *Aequorea aequorea*) is divided by numerous radial canals and attached gonads, so the subumbrellar musculature is partitioned into subumbrellar segments. The ectoderm of each segment includes two types of muscle: smooth muscle with a radial orientation, used for local (feeding and righting) and widespread (protective) radial responses, and striated muscle with a circular orientation which produces swim contractions. Two subumbrellar nerve nets were found, one of which stained with a commercial antibody produced against the bioactive peptide FMRFamide. Circular muscle cells produce a single, long-duration action potential with each swim, triggered by a single junctional potential. In addition, the circular cells are electrically coupled so full contractions require both electrotonic depolarization from adjacent cells and synaptic input from a subumbrellar nerve net. The radial cells, which form a layer superficial to the circular cells, are also activated by a subumbrellar nerve net, and produce short-duration action potentials. The radial muscle cells are electrically coupled to one another. No coupling exists between the two muscle layers. Spread of excitation between adjacent segments is decremental, and nerve net-activated junctional potentials disappear during local inhibition of swimming (such as with a radial response). Variable swim contractions are controlled by a combination of synaptic input from the motor network of the inner nerve ring, synaptic input from a subumbrellar nerve net, and electrotonic depolarization from adjacent, active muscle cells.

Key words: cnidaria, hydrozoa, jellyfish, locomotion, motor control, neurobiology.

INTRODUCTION

Medusae of the cnidarian class Hydrozoa show a dramatic variation in bell shape, ranging from prolate forms (thimble-shaped – height greater than bell diameter) to oblate forms (disc-shaped – bell height less than bell diameter). Many of the prolate jellyfish utilize an ambush-style feeding strategy, relaxing their tentacles during extended periods of non-swimming to produce a passive net for ensnaring prey (Colin and Costello, 2002). The tentacles are typically contracted prior to extended swimming bouts, or during escape swimming. The oblate forms, by contrast, tend to swim with the marginal tentacles extended and flowing in the contraction wakes. Colin and Costello (Colin and Costello, 2002) examined the biomechanics of swimming in several hydromedusan species and found the prolate forms primarily used a jet propulsive form of locomotion during a swim contraction. The oblate species derived only a small proportion of thrust from jet propulsion, rather using a drag-based rowing of the margin for thrust generation. This latter mechanism produced a series of vortex rings (toroids) that were well-suited for carrying food particles through the trailing tentacles, suggesting a more active form of feeding in the oblate medusae (Colin and Costello, 2002). Thus, the mechanics of bell contraction seem to be related to feeding strategy, at least for the species investigated.

Despite this apparent dichotomy in swim mechanics and feeding strategies, all hydromedusae, including those with both bell shapes, have what can be considered a common, basic organization for neuromuscular control (Satterlie and Spencer, 1983; Satterlie, 2002). This consists of an electrically coupled network of large neurons, found in the inner nerve ring, which acts as a distributed swim pacemaker system for swim contractions, and which communicates with overlying epithelial cells (post-synaptic cells) *via* chemical synapses (Anderson and Mackie, 1977; Anderson, 1979; Spencer and Satterlie, 1980; Satterlie and Spencer, 1983; Spencer, 1981; Spencer, 1982; Satterlie, 1985a; Satterlie, 1985b; Satterlie, 2002; Mackie and Meech, 2000; Lin et al., 2001; Mackie, 2004). As a second common feature found in all investigated hydromedusae, long duration action potentials in the post-synaptic epithelial cells are conducted to subumbrellar circular muscle cells *via* gap junctions, to produce (or help produce) swim contractions (Singla, 1978a; Spencer, 1978; Spencer, 1979; Spencer, 1981; Spencer and Satterlie, 1981; Satterlie, 1985b; Satterlie, 2002; Kerfoot et al., 1985; Satterlie and Spencer, 1983; Mackie, 2004; Mackie, 1975). Superimposed on these two basic features are species-specific neuronal and muscular organizations that allow unique behavioural repertoires for each species. For example, although the circular muscle sheets of the anthomedusa *Polyorchis penicillatus* are aneural, the motor network of coupled neurons extends from the inner nerve ring, up (orally) within each of the four radial nerves (which lie over the radial canals), and across the top of each quadrant, so excitation of the muscle sheet does not occur solely at the margin, but from all four sides of the quadrant (Lin et al., 2001). The combination of junctional coupling and action potential threshold is such that action potentials are conducted throughout the muscle sheet without significant decrement when generated from the motor network (Spencer, 1978; Spencer, 1982; Spencer and Satterlie, 1981).

In the trachymedusa *Aglantha digitale*, slow swimming is controlled by a similar network of inner nerve ring neurons,

although activation of the swim musculature is quite different (see Mackie, 2004). The marginal motoneuron network synaptically activates eight radially oriented motor giant neurons that overlie the radial canals (Weber et al., 1982; Kerfoot et al., 1985; Mackie and Meech, 2000; Mackie, 2004). The motor giants produce two types of action potentials, a calcium spike during slow swimming and a sodium-based spike during escape swimming (Mackie and Meech, 1985; Meech and Mackie, 1993). The motor giants, in turn, activate lateral neurons that help transmit excitation part of the way across the muscle sheets (Singla, 1978b, Weber et al., 1982; Mackie, 2004). Again, muscle electrical events are transmitted from muscle cell to muscle cell *via* gap junctions, however, the conduction is decremental since contractions in the slow swimming mode are relatively weak, and restricted to the margins of each subumbrellar octant.

Polyorchis is a prolate medusa, and produces symmetrical, relatively uniform contractions of the subumbrella. Superimposed on this swimming activity is another seemingly common type of behavioural response of hydromedusae that involves contraction of subumbrellar radial muscles, pulling the margin of the bell inward and upward toward the manubrium. These radial responses can be local, as in a feeding response in which a single tentacle or localized group of tentacles can be turned inward into the bell, ultimately to contact the manubrium (for prey transfer). A widespread radial response rolls the entire margin into the bell cavity, as a protective "crumple" response (Spencer, 1975; Spencer, 1978). Swimming is inhibited during a crumple, and sometimes during a feeding response. In either case, the radial responses are produced by contraction of smooth muscle that is either restricted to bands overlying with the radial canals (*Polyorchis* and several other species) or as more widespread subumbrellar sheets that overlie the striated swim muscle [as in the leptomedusae *Aequorea*, *Phialidium* and *Eutonina* (Satterlie and Spencer, 1983)]. The subject of this study, *Aequorea victoria* Murbach and Shearer (previously classified as *Aequorea aequorea*), which exhibits this latter organization of radial musculature, provides another excellent example of the superimposition of unique species-specific behavioural control on the basic hydromedusan neuromuscular plan.

As a relatively large oblate medusa, *Aequorea* spends a significant amount of time swimming, and does so with its tentacles extended. It shows both localized (feeding) and widespread (crumple) radial responses, but during the former, it can 'turn off' swimming in restricted sections of the bell while the rest of the subumbrella produces seemingly normal swim contractions (Satterlie, 1985a). This localized inhibition is unusual, and requires a mechanistic explanation since the 'basic' hydromedusan swim system includes the through-conducting, electrically coupled network of large motoneurons in the inner nerve ring (Satterlie and Spencer, 1983; Satterlie, 2002). This network has been studied in *Aequorea* (Satterlie, 1985a; Satterlie, 1985b), where it was found to function as both the pacemaker network for swimming activity and the primary motor network for synaptic activation of the overlying epithelial cells (Satterlie, 1985a). The neurons produce a rapid action potential burst that activates a single, broad action potential in the post-synaptic epithelial cells (Satterlie, 1985a; Satterlie, 1985b). The epithelial cells are in electrical contact with the subumbrellar swim muscle *via* gap junctions. The 'muscle' action potentials are variable in amplitude, and after a period of rest, they show a progressive increase in amplitude with each successive swim that is suggestive of a facilitatory mechanism (Satterlie, 1985b). Local inhibition caused by mechanical or electrical stimulation of the margin results in regional hyperpolarization in the swim motor network, and either a suppression of synaptic transmission to the epithelial cells, or a delayed burst in the motor network (Satterlie, 1985b).

Here, the method of transmission of muscle action potentials through the subumbrellar circular muscle sheet is investigated, as is the activity of the radial muscle during a radial response. These data are used to provide a physiological explanation of *Aequorea*'s ability to 'turn off' swimming in localized parts of the bell during restricted radial responses, and demonstrates yet another species-specific method of neuromuscular control of swimming that is superimposed on the 'basic' hydromedusan plan.

MATERIALS AND METHODS

Medusae were collected from the breakwater at Friday Harbor Laboratories (University of Washington, Friday Harbor, WA, USA) and held in tanks with flowing seawater. For electrophysiological recordings, strip preparations were created by cutting inward from the margin up to the level of the stomach, around the stomach margin, then back down to the margin to form the strip including three to seven intact subumbrellar segments (see Fig. 1). The strip was pinned in a Sylgard (Dow Corning, Midland, MI, USA)-coated dish with spines from the fruit of the prickly pear cactus (*Opuntia* sp.). Conventional intracellular recordings were used, with either $3\,mol\,l^{-1}$ KCl or $2\,mol\,l^{-1}$ potassium-acetate-filled microelectrodes. In both cases, the best electrodes had resistances of 15–25 MΩ. For dye injections, the electrode tips were filled with 4% Lucifer Yellow and back-filled with $1\,mol\,l^{-1}$ LiCl (Stewart, 1978). Dye was injected into a recorded cell by iontophoresis, with negative current pulses applied to the electrode using an amplifier bridge circuit. The currents were approximately 1–2 nA, and consisted of repetitive 500 ms pulses delivered at 1 Hz. The dye-fills were viewed and photographed live, in the recording dish, with a Nikon epifluorescence microscope and film camera. All experiments were completed in natural seawater. Swimming was blocked with a 1:1 solution of isotonic $MgCl_2$:seawater. This same solution was used to anesthetize the animals prior to initial dissection. Electrical stimuli were delivered by polyethylene suction electrodes (internal tip diameters approximately 50 μm) and a Grass S9 stimulator (Astro-Med, Inc., West Warwick, RI, USA).

Tissue for electron microscopy was anesthetized, dissected, and pieces were fixed for 2 h in 2% glutaraldehyde in either cacodylate or phosphate buffer (buffer and fixative calculated at 1000–1100 mOsm). After several washes in buffer, the tissue was post-fixed in 1% OsO_4 (1 h) in cacodylate or sodium bicarbonate buffer. The tissue pieces were washed and further dissected (if needed) and dehydrated in an ethanol series to propylene oxide, and embedded in Epon, an Epon substitute or in Spurr's resin. Thick and thin sections were cut on a Porter-Blum (Sorvall, New York, NY, USA) MT2-B ultramicrotome and mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and viewed in a Philips EM 201 electron microscope.

For immunohistochemical labelling, tissue pieces were fixed in 4% paraformaldehyde in phosphate buffer overnight, washed in phosphate buffer with either 0.05% Triton X-100 or 1% Tween 20, and incubated in 5% goat serum for 4 h. The tissue was then soaked in primary antibody (rabbit polyclonal anti-FMRFamide; Chemicon International Millipore Corp., Billerica, MA, USA; or mouse monoclonal α-tubulin; Developmental Studies Hybridoma Bank, University of Iowa, IA, USA) at 1:500 dilution in phosphate buffer for 24 to 48 h. In double labelling experiments, both primary antibodies were added together. After washing with buffer, the tissue was incubated in secondary antibody overnight (goat anti-mouse Alexa Fluor 488 for tubulin and goat anti-rabbit Alexa Fluor 594 for

FMRFamide; both from Molecular Probes, Invitrogen, Carlsbad, CA, USA). In double labelling experiments, both secondary antibodies were added together. The tissue was then washed in phosphate buffer and cleared and mounted in a 1:9 mixture of Tris buffer and glycerol. Specimens were mounted on glass slides using the same mounting medium, and examined with a Nikon epifluorescence microscope and photographed with a Spot Slider digital camera.

Staining in the subumbrellar neurons was not robust in either brightness and in resistance to fading, in particular, at the magnifications needed to view the neurons, fading was significant in the time for the camera exposure. In fact, if focusing the image took too long, most of the neurons in the visual field were too dim to show in the camera images. For this reason, confocal microscopy could not be used on these preparations. Also, in the higher magnification images (e.g. Fig. 7B,C), there is an under-representation of nerve net density.

The dual staining with the FMRFamide and tubulin antibodies seems to give consistent differential results in several species of hydromedusae, scyphomedusae and cubomedusae (R.A.S., personal observations and in preparation). Dual labelling experiments on *Aurelia* (scyphomedusae) and *Carybdea* (cubomedusae) indicate that the tubulin antibody does stain the FMRFamide-immunoreactive neurons, but the staining is extremely faint, with a low signal-to-noise ratio, so it does not show up in most observations except at high magnification (if the observer is quick). Because of these observations, there is a possibility that some neurons of cnidarians do not show up with either antibody, and are thus missed with our immunohistochemical techniques.

RESULTS

Subumbrellar organization

The subumbrella of *Aequorea* can be divided into four main regions, going radially from the margin up to the manubrium (Fig. 1). Outermost is the velum, an annular flap of muscular tissue that contracts with the subumbrella during each swim contraction and narrows the bell opening for more efficient swimming. The velum is attached to the marginal tissue, which includes the nerve rings and marginal canal, the latter a circumferential conduit of the digestive system. Occupying the summit of the subumbrellar cavity is a large sac-like stomach bearing a central manubrium. Between the stomach and the marginal tissue is a wide muscular sheet that includes circularly oriented swim muscle and radially oriented muscle that produces marginal curling during radial responses, feeding and righting behaviours. This muscular region is divided into numerous interradial segments by radial canals which, as branches of the digestive system, connect the apical stomach with the marginal canal. Each radial canal bears a single, raised ridge of gonadal tissue that incompletely interrupts the muscular layers of the interradial segments. The interradial segmental tissue is the subject of this investigation.

The subumbrella of a large medusa (approximately 8 cm bell diameter) is typically divided into over 50 interradial segments (Fig. 1). The segments are separated by 1 mm wide radial canals which are naked (no gonadal tissue) for their first 4 mm from the margin. The ridge of gonadal tissue extends from this point to the stomach as a 1.5 mm wide ridge that forms the roof of the radial canal. Each interradial muscular segment is thus bounded by two radial canals (with attached gonads), the marginal canal and the stomach. In an 8 cm diameter medusa, the interradial segments are nearly 30 mm tall, and up to 10 mm wide.

The ectoderm of the interradial segments includes two sheets of muscle, the outer one radial, and the inner one circular in orientation (Figs 2 and 3). The muscle sheets are totally interrupted in the gonadal regions of the radii, but are continuous with the musculature of the adjacent segment where the radial canals are naked. In the naked region, the radial canal is found beneath the muscular sheets. The subumbrellar side of the velum contains only circular epitheliomuscular cells, and is not divided by radii.

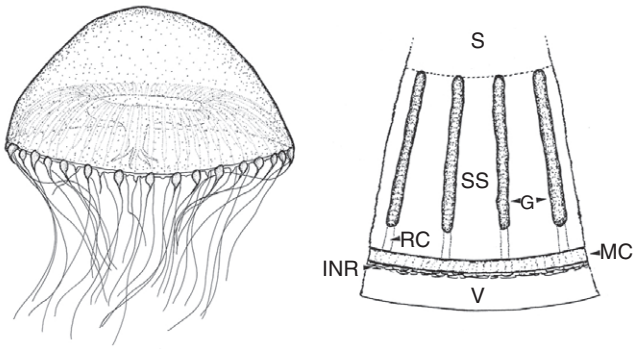

Fig. 1. Diagram of an intact medusa and a 'reduced preparation' consisting of three adjacent segments (viewed from the subumbrellar side). S, stomach; V, velum; G, gonad; SS, subumbrellar segment; RC, radial canal; MC, marginal canal; INR, inner nerve ring.

Subumbrellar ectoderm

Two types of muscle cells make up the ectoderm of the interradial segments. The outer layer is composed of surface epithelial cells with basal muscular processes oriented in a radial direction. The myofibrillar organization within the processes is of the smooth type (Fig. 2). Situated between the radial muscle cells and the mesoglea is a layer of circularly oriented, striated muscle cells (swim muscle). The radial muscle lies immediately over the striated muscle (Figs 2 and 3). Extensions of the circular muscle cells, to the free surface of the epithelium, have not been found. The ectoderm thus forms a pseudo-stratified epithelium.

Radial muscle cells

Radial muscle cells have a surface epithelial component and one or more basal muscle processes (Fig. 2). Viewed from the surface, the cells are up to 50 μm in diameter (normally around 25 μm). The cells are drawn out basally into one or more muscle 'feet' that taper to less than 1 μm and interdigitate with similar processes of other radial muscle cells, and occasionally run between circular muscle cells to the mesoglea. Radial muscle cells are very thin (mean thickness, 1.39 μm; maximum 5 μm) with elongate nuclei and vacuolated apical cytoplasm. The myofibrillar region of the radial cells includes thick and thin filaments that are oriented in the long axis of the processes but otherwise randomly interspersed. Since no evidence of cross or oblique striations could be found, the radial muscle is considered to be smooth muscle (Fig. 2). In cross section, thick and thin filaments show no orderly or repetitive organization (Fig. 3A). Thick filaments are tubular in shape with a diameter of 20 nm, whereas the thin filaments appear solid with a diameter of 5–7 nm. Elongate mitochondria are scattered throughout the radial cells and are frequently found among the myofilaments.

Radial muscle cells are interconnected by gap junctions, found primarily near the apical surface of the cells, but also occasionally deeper in the muscle layer (Fig. 4). In the junctional area, the intercellular space is 3–6 nm as compared with 20–25 nm in non-junctional areas. Similar junctions between radial muscle cells and the underlying circular muscle cells were not found.

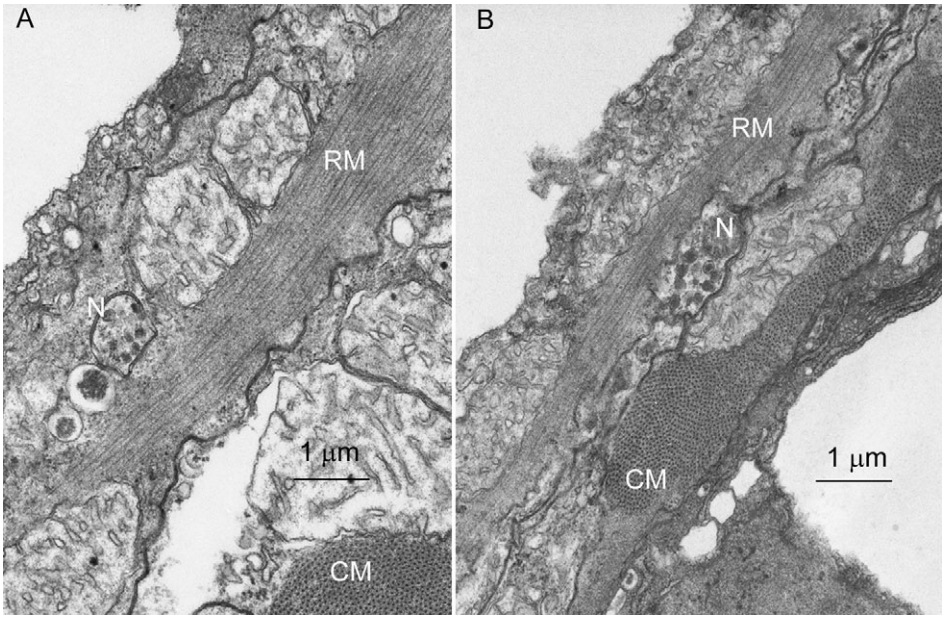

Fig. 2. Ultrastructure of the ectodermal muscle in a subumbrellar segment. Both A and B are radial sections, with the radial muscle (RM) cut longitudinally and the circular (swim) muscle (CM) in cross section. Note that the radial muscle cells are superficial to the circular muscle. In both A and B, neurites (N) are present, and contain dense core vesicles. The neurite in A is superficial to the radial muscle, whereas the neurite in B is between the radial and circular muscle processes. In both panels, the subumbrellar cavity is to the top left.

Circular muscle cells

Circular muscle cells lie between the radial muscle layer and a thin layer of mesoglea (Figs 2, 3). They are tubular or strap-like in shape with a mean cell thickness of 2.1 μm (maximum of 2.7 μm) and a mean width of 4.3 μm (maximum of 7.8 μm). Each muscle cell contains a single ovoid nucleus and numerous mitochondria located in the apical part of the cell. Contractile filaments are restricted to the basal region of the cells (the portion that abuts the mesoglea; Fig. 3A). The myofilaments are cross striated with a mean sarcomere length of 1.6 μm (range of 1.1 to 2.1 μm; N=75). Z-lines are not prominent, but M-lines are noticeable. In relaxed specimens, A-bands account for an average of 64% of the sarcomere length.

In cross section, thick and thin filaments are packed in an orderly array (Fig. 5). Thick filaments are hexagonally arranged with a centre-to-centre distance of 35–43 nm. The thick filaments are tubular with a diameter of 20 nm. Thin filaments are solid in appearance and are 5–7 nm in diameter (measurements derived from high magnification micrographs). The thick-to-thin filament ratio appears to be 1:8.

Circular muscle cells are interconnected by gap junctions that appear structurally similar to those found between pairs of radial cells (Fig. 3A, Fig. 4A). Where two muscle cells are joined ‘end-to-end’, a desmosome-like connection is usually found adjacent to one or more gap junctions. Circular muscle cells do not form a complete subumbrellar layer as radial cell processes occasionally extend to the mesoglea between the circular cells.

Subumbrellar neurites

Neurites and occasional neuron cell bodies are present throughout the subumbrellar segments. Neurites are up to 1.5 μm in diameter and are recognized by a relatively clear cytoplasm containing longitudinally oriented microtubules and either large dense-core or clear vesicles. Neurite swellings, including accumulations of dense-core vesicles (up to 140 nm in diameter) are frequently encountered (Fig. 2). Despite a common occurrence of such swellings, definitive neuromuscular junctions (including pre- and post-synaptic specializations) have not been found.

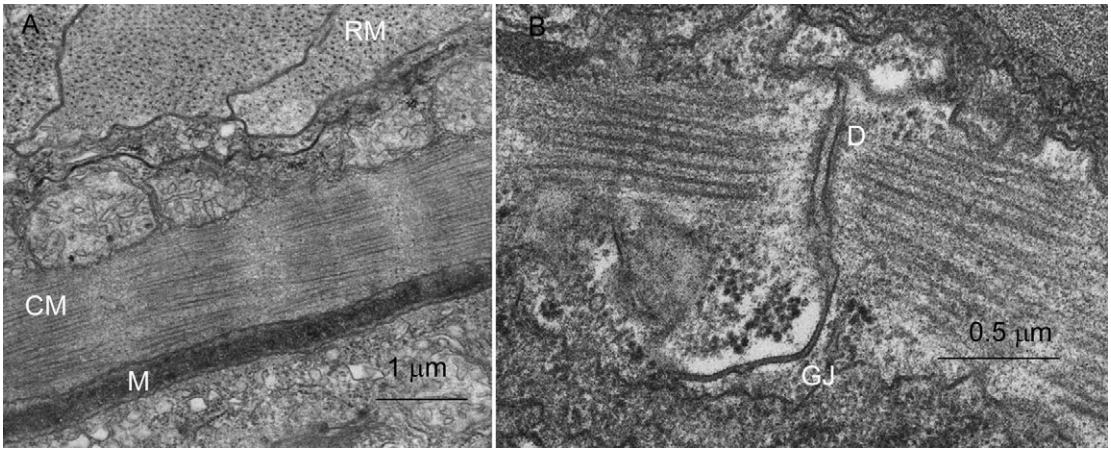

Fig. 3. Ultrastructure of the ectodermal muscle in a subumbrellar segment. In A, the circular muscle (CM) is cut longitudinally to show its striated nature. It also abuts the mesoglea (M). The radial muscle (RM) is cut in cross section, and shows a lack of regular organization of thick and thin filaments. In B, the end-to-end junction of two circular muscle cells show a desmosome (D) adjacent to a gap junction (GJ).

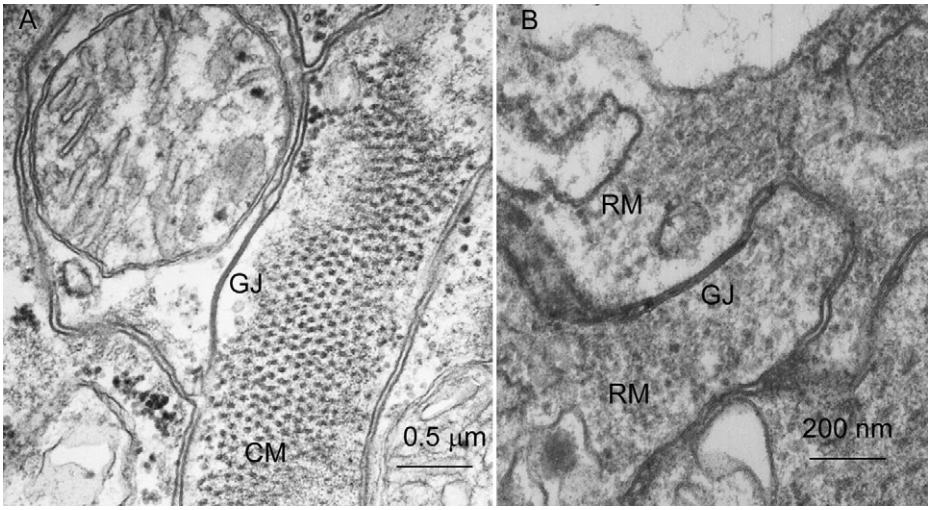

Fig. 4. Intercellular junctions between muscle cells. (A) Circular muscle cells (CM) have laterally positioned gap junctions (GJ) with other circular muscle cells. (B) Radial muscle (RM) cells have gap junctions (GJ) near the apical surface of the cells (they are also present deeper in the ectoderm – not shown).

Electrophysiology of circular muscle cells

Circular muscle cells were routinely penetrated with microelectrodes, and were recognized by a one-to-one correspondence of depolarizing electrical events and swim contractions. They were penetrated in all parts of the subumbrellar segments and in the gonad-less regions overlying radial canals. The mean resting potential for circular muscle cells was –62.3 mV (N=54).

With each swim contraction, the muscle cells exhibited one (or both) of two types of depolarizing potentials. During 'full swims' in the recorded segment, long duration action potentials (up to 125 mV above resting potential) were recorded (Figs 6 and 7). As with epithelial cells of the inner nerve ring (Satterlie, 1985b), muscle action potential duration was related to medusa size, and was up to 700 ms in some animals. The action potentials were variable in amplitude and duration, particularly when the area of recording produced a weak contraction or was inhibited during a radial response (Fig. 6). The second type of recorded potential was an apparent junctional potential of relatively large size (up to 56 mV amplitude) (Fig. 6). Junctional potential duration was 160–200 ms. Junctional potentials were recorded alone, or immediately preceding (giving rise to) action potentials in actively contracting segments (Fig. 6). In partially inhibited preparations, action potentials showed several types of variability. In addition to variation in amplitude and duration, they sometimes exhibited slow rise times with the reduced amplitudes, and either were not preceded by junctional potentials or exhibited junctional potentials that were smaller than those within the segments. Also, when junctional potentials were present with muscle action potentials, slight 'hitches' (delays) were sometimes observed between the peak of the junctional potential and the rise of the action potential (compare the action potential in Fig. 6A,B). In some cases in which swimming was suppressed in the recorded segment (*via* triggered radial responses), the recorded muscle cell still exhibited a junctional potential, but also showed a reduced-amplitude action potential followed by a second action potential that lacked a junctional potential and had a slower rise time (Fig. 6C). The second action potential in this 'saw-tooth' pattern is believed to result from electrotonic wash from an adjacent, active segment, or if close to the margin, from a velar action potential.

In all areas of a subumbrellar segment except immediately adjacent to the margin, a single junctional potential was recorded per swim, even when it gave rise to a full action potential (Fig. 6). In recordings from the vicinity of the inner nerve ring, multiple junctional potentials gave rise to action potentials, similar to those recorded from the post-synaptic epithelial cells that are coupled to the muscle cells in this region (Satterlie, 1985b). This 'bursty' junctional activity decreased with distance from the margin so within approximately 1 mm from the margin and beyond, only a single junctional potential was recorded per swim contraction.

Dual intracellular recordings from two circular muscle cells of the same segment showed identical activity in terms of the relative amplitudes of the action potentials (Fig. 7). Injection of hyperpolarizing currents into one of the recorded muscle cells showed time-locked hyperpolarizations in the other recorded cells, confirming electrical coupling between the cells (Fig. 7). Similar coupling was found between a circular muscle cell in a subumbrellar segment and another over the adjacent radial canal, as well as between cells of adjacent segments, although in the latter situation, very large currents had to be injected, and the associated hyperpolarization was extremely small. In dual recordings of a

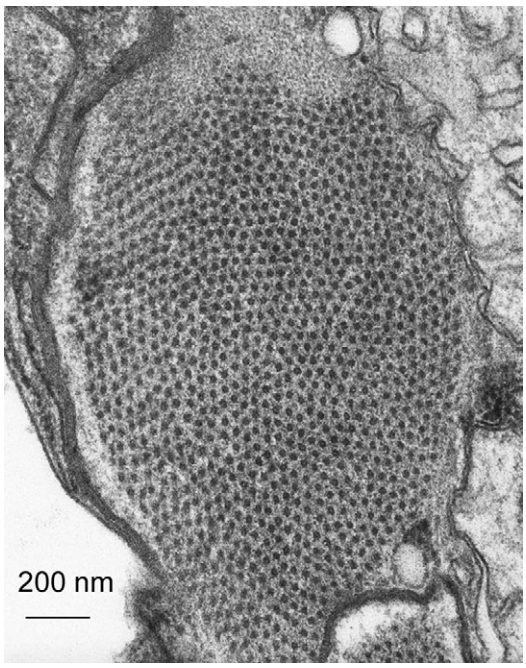

Fig. 5. In cross section, the striated circular muscle cells have an ordered arrangement of thick and thin filaments.

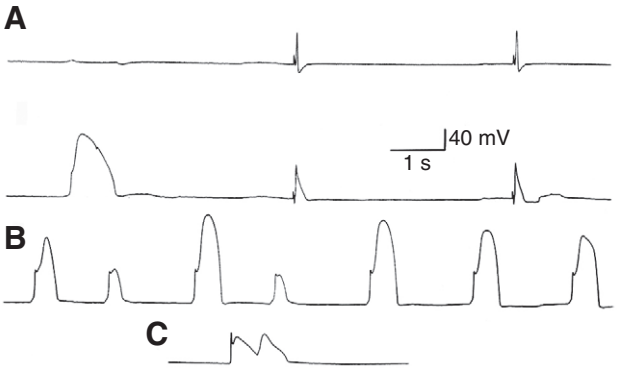

Fig. 6. Intracellular recordings from subumbrellar muscle cells approximately 2–2.5 mm from the nerve ring. (A) A double recording from a radial muscle cell (top trace) and a circular muscle cell (bottom trace). An initial spontaneous swim produced a full action potential (with initial junctional potential) in the circular muscle cell, but only a 'field effect' in the radial cell. When electrical stimuli were delivered to the segment with a suction electrode, both a junctional potential (circular muscle cell) and an action potential (radial muscle cell) were triggered. The small 'blip' before each event is the stimulus artifact. (B) Variability in the shape and amplitude of circular muscle action potentials. Each event is initiated by a single junctional potential. All were spontaneously generated in a reduced preparation that showed varying contractions. This recording was from a small medusa, 3 cm bell diameter, so the action potentials are of a shorter duration. (C) Complex electrical event from a circular muscle cell in a segment in which a radial response was triggered by a mechanical stimulus (light touch with a glass micropipette electrode) delivered to the velum in the region of that segment. The contraction in that segment was weaker than in the adjacent segments on each side.

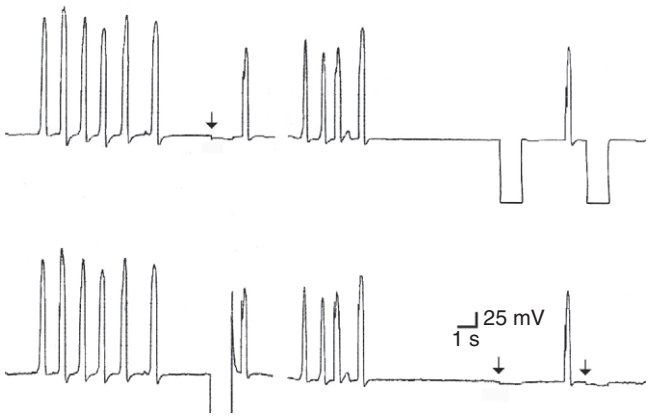

Fig. 7. Dual recording from a pair of circular muscle cells within 3 mm of the inner nerve ring. All muscle action potentials were spontaneous. The break in the traces represents a time break in the record. In the first segment, a single hyperpolarizing current (approximately 1 nA) was injected into the cell represented by the lower trace. The small hyperpolarization in the top trace (arrow) indicates the cells were electrically coupled. In the second segment, two current pulses were injected into the cell represented by the top trace. Again, small hyperpolarizations in the other cell (arrows) demonstrated similar coupling in the opposite direction.

circular muscle cell and a radial muscle cell, no electrical coupling could be demonstrated (data not shown).

Injection of Lucifer Yellow into a single circular muscle cell produced widespread dye movement from the injected cell to other circular muscle cells (Fig. 8A). No dye spread was noted from a circular muscle cell to radial muscle cells.

Intracellular recordings from radial muscle cells were difficult to obtain, compared with those of circular muscle cells, however, stable recordings could be maintained for several minutes. The mean resting potential was –61 mV (N=21). The cells were silent unless stimulated. In particular, they were not active during swim contractions (Fig. 6A), although field effects from the long duration circular muscle action potentials were frequently recorded (not believed to be movement artifacts since contractions lagged slightly behind action potential generation). Relatively brief action potentials were recorded following electrical stimulation of the muscle sheet, and had a mean amplitude of 76 mV and a mean duration of 65 ms (Fig. 6A). Dual recordings from pairs of radial muscle cells were not obtained, although dye injections with Lucifer Yellow demonstrated spread of dye to surrounding radial (surface) cells, without spread to underlying circular muscle cells (Fig. 8B). When electrically stimulated *via* a distant suction electrode, radial muscle cell action potentials were always accompanied by junctional potentials in circular muscle cells (Fig. 6A). Likewise, electrical stimuli that triggered a circular muscle junctional potential always initiated an action potential in a recorded radial muscle cell. Initiation of a radial muscle action potential *via* current injection through the recording electrode (only two instances – data not shown) did not produce junctional potentials in circular muscle cells, however, suggesting that the coincident appearance of a radial muscle action potential and a circular muscle junctional potential is probably a result of their similar threshold to electrical stimuli and the close apposition of the two cell types or of the subumbrellar nerve nets.

Pattern of spread of circular muscle activity

The recording of junctional potentials in circular muscle cells from all parts of a subumbrellar segment following stimulation of the tissue within that segment, and their presence at the beginning of muscle cell action potentials, both electrically stimulated and spontaneous, suggest that a subumbrellar nerve net participates in spread of electrical activity throughout each segment. This is supported, in part, by the presence of neurites in these regions (Fig. 2). Yet, such a nerve net raises questions about how swimming can be 'turned off' in a portion of the subumbrella during localized radial responses. This problem required examination of nerve net conducting properties within the subumbrella.

As shown in Fig. 9, a recording electrode was placed in a circular muscle cell in segment A, and an electrical stimulus was delivered at a point represented by the letter A. In trace A (stimulating and recording electrode in same segment), junctional potentials were recorded in a 1:1 relationship with electrical stimuli, and the junctional potentials were roughly the same size as those triggering the three spontaneous action potentials in the same trace.

When the stimulating electrode was moved to an adjacent segment (segment B, stimulating position indicated by the letter B, and with the same recording position indicated by the letter R), junctional potentials were still recorded, but not with every stimulus. Trace B shows that only three of the four stimuli triggered a junctional potential, the potentials occurred with a much longer latency, suggesting a more circuitous conduction route. Furthermore, if repetitive stimuli were delivered at close to the normal swim frequency of the intact animal (about 1 Hz for the animals used), the faithfulness of junctional potential production in the adjacent segment became very labile (data not shown).

The delay between the stimulus artifact and junctional potential appearance was about 200 ms in trace B, which is several times greater than the expected delay of about 35 ms if the circular conduction velocity of swim contractions originating from the nerve

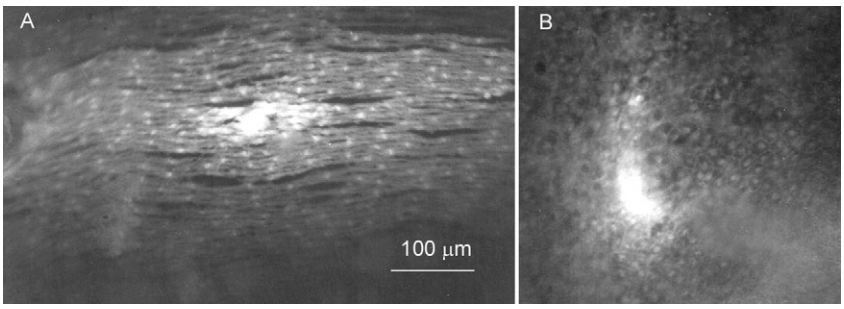

Fig. 8. (A) Lucifer Yellow fill of a circular muscle approximately one-third of the way from the margin in a subumbrellar segment, showing widespread dye coupling. The injected cell was in the middle of the bright spot. The small bright dots in the filled cells are nuclei. (B) Lucifer Yellow fill of a radial muscle cell roughly in the middle of a subumbrellar segment. The dye spread to the surface cells (radial muscle cells) throughout the epithelial tissue but not the underlying circular muscle cells. Again, the nuclei show up as bright dots. The scale bar applies to both A and B.

ring is considered (43 cm s^{-1} conduction velocity). The delay in trace B was calculated as a circular conduction velocity between adjacent segments of about 7.5 cm s^{-1}, a speed only two-thirds of the conduction speed in a radial direction in a single segment (12 cm s^{-1}).

When the stimulating electrode was moved over one more segment (Fig. 9, segment C), so that it was two segments away from the recording site, electrical stimuli strong enough to trigger contractions in the stimulated segment did not produce detectable junctional potentials or contractions in the recorded segment (Fig. 9C). This entire experimental series was repeated in three preparations from three different animals with identical results, suggesting that the conducting system of an individual segment, responsible for production of circular muscle cell junctional potentials, was not through-conducting in a circular direction around the bell, but was restricted between segments. By contrast, there seemed to be no such restriction in a radial direction, over similar distances, within each segment.

Evidence for participation of a subumbrellar nerve net in the spread of swim contractions

When stimulating a subumbrellar segment (with a suction electrode), junctional potentials in circular muscle cells appeared with a distinct threshold, and when the stimulus was above that threshold, the size of the junctional potential did not vary. Similarly sized junctional potentials gave rise to full action potentials in the muscle cells when a spontaneous action potential was triggered by normal inner nerve ring activity (Fig. 6; Fig. 9A). Furthermore, identical junctional potentials could be stimulated in non-swimming preparations when the stimulating electrode was placed well above (more apical to) the recording electrode. When the preparation was bathed in high-magnesium seawater, swimming was inhibited, but so was production of junctional potentials from direct electrical stimulation of subumbrellar segments (*via* a suction electrode). In these experiments, injection of current *via* the intracellular recording electrode showed the muscle cell was capable of electrogenesis. Finally, the presence of neurites detected in electron microscope preparations was confirmed by immunohistochemical staining using a commercial antibody to the neuroactive peptide, FMRFamide, which identified a diffuse nerve net within the subumbrellar segments (Figs 10 and 11). The neurites of this network of stained neurons were preferentially oriented in a radial direction. Staining was noted over the radial canals between segments as well as within the segments. It also extended to the inner nerve ring but could not be followed within the nerve ring because of high background staining in the region. Specifically, connections to immunoreactive neurons within the inner nerve ring could not be resolved.

A comparative investigation of similar FMRFamide-immunoreactive networks in a number of hydromedusae suggested they are associated with radial muscle, including the broad radial muscle sheets of leptomedusae, and not with the swimming system (Mackie et al., 1985). Mackie et al. noted that the stained neurons were a subset of the total neuron number found in electron microscopical investigations of these same leptomedusae, suggesting the presence of at least two independent nerve nets in the subumbrella of these jellyfish. To test this, double-label immunohistochemical staining was conducted using the polyclonal antibody to FMRFamide (rabbit primary, Alexa Fluor 594 secondary) and a monoclonal α-tubulin antibody (mouse primary, Alexa Fluor 488 secondary). The latter is a fairly non-specific neuronal marker in cnidarians (e.g. Satterlie, 2002), presumably staining neurotubules.

In preparations using a combination of filters, both green (tubulin) and red (FMRFamide) cell bodies and neurites were visible in the same focal plane of the subumbrellar segments (Fig. 11A). When separate filters were used, both types of neurons were apparent with the FITC filter, whereas with the TRITC filter, only the

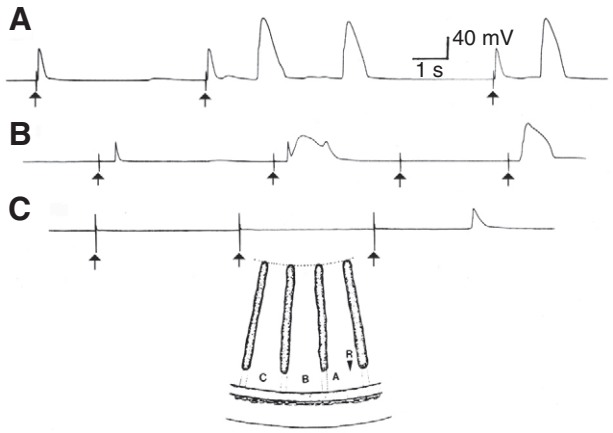

Fig. 9. Sequential experiment in which a single electrode was placed in a circular muscle cell (at the site marked R in the diagram), and a stimulating suction electrode was moved to the sites marked A, B and C. For trace A, the stimulus was delivered in the same segment as the recording site (stimulus site A), and a junctional potential was triggered with a short latency. Three spontaneous action potentials were also recorded, and each was initiated by a junctional potential of similar size to those electrically stimulated (note the slight inflection on the rising phase of the action potentials). When the stimulating electrode was moved over one segment (stimulus site B and trace B), junctional potentials were triggered by three of the four stimuli, but with a longer latency than in trace A. Following the second and fourth stimuli, weak contractions were initiated. The electrode was then moved over one more segment (stimulus site C and trace C). Electrical stimuli sufficient to produce weak contractions in the stimulated segment did not initiate junctional potentials in the recorded segment (two segments over). A few junctional potentials were recorded during these experiments (one is shown in this trace), however, they showed no regular relationship to imposed stimuli. It appears these are spontaneous events. Delivery of stimuli is marked by the stimulus artifacts (arrows). The recording site was 2.5 mm from the top of the nerve ring.

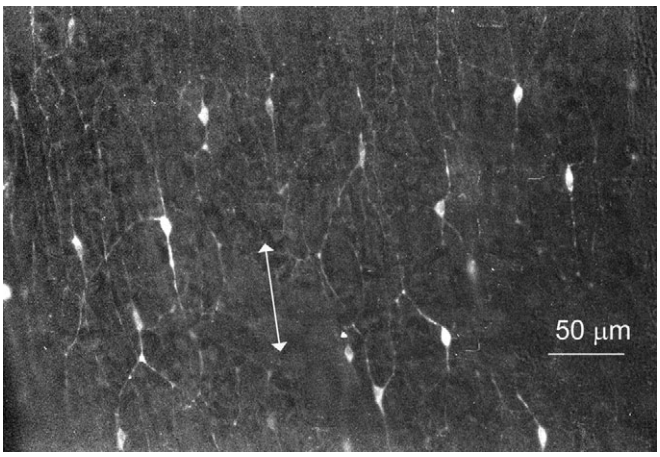

Fig. 10. FMRFamide immunoreactivity in a subumbrellar segment of *Aequorea* shows a diffuse nerve net with a definite oral–aboral orientation of neurites (direction indicated by arrow). The region was about one-third of the segment length up from the margin.

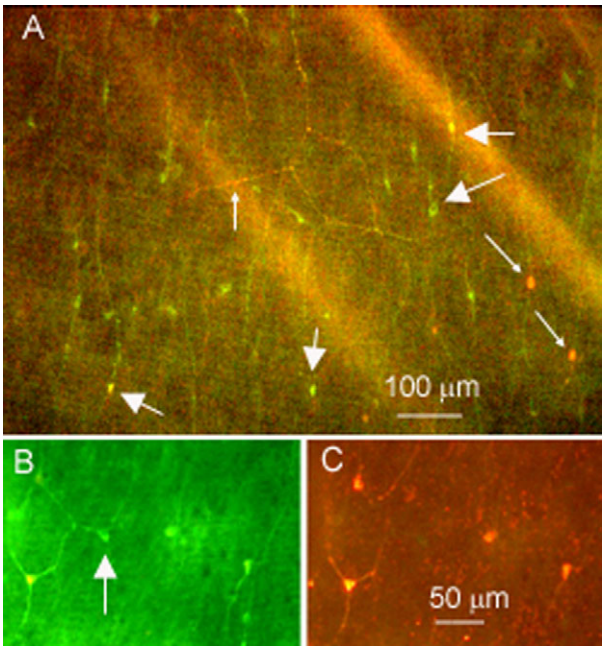

Fig. 11. Double-label immunohistochemistry using α-tubulin (green) and FMRFamide (red) antibodies. In A, a dual filter shows the two colours together. The small arrows indicate FMRFamide cell bodies and neurites, and the large arrows indicate some of the tubulin-stained cell bodies. B and C are from identical areas (no change in focus) with a change from a FITC filter (B) to a TRITC filter (C). Note the neuron indicated by the arrow in B is not present in C indicating it was immunoreactive to the tubulin antibody, but not to FMRFamide antibody. This suggests the presence of two distinct neuronal types in the subumbrellar segments of *Aequorea*.

FMRFamide-immunoreactive neurons were visible. For example, in Fig. 11B,C, one of the tubulin-immunoreactive neurons is clearly not stained with the FMRFamide antibody. Similar results were obtained in all parts of the subumbrellar segments in four different double-labelled preparations (four different animals), indicating the presence of two distinct nerve nets (at least based on immunohistochemical staining) in the subumbrellar of *Aequorea*.

An interesting aspect of junctional potential production is the relationship between action potentials in the swim generating network of the inner nerve ring, the putative nerve net that innervates the swim muscles, and the action potentials in the circular muscle cells themselves. In the inner nerve ring, each swim is triggered by a rapid burst of action potentials in the swim-generating neural network (Satterlie, 1985a). This is translated into a burst of junctional potentials and a resulting single action potential in the overlying epithelial cells (Satterlie, 1985b). These epithelial cells are in electrical contact with the circular muscle cells of the subumbrella and velum *via* gap junctions. Yet, away from the margin, only single junctional potentials were recorded in circular muscle cells with each action potential (Fig. 6), suggesting the action potential bursts observed in the motoneurons are converted to single action potentials in the putative subumbrellar motor nerve net.

How does a local radial response turn off swimming in a restricted portion of the bell?

Input from the motor network of the inner nerve ring, to the swim muscle, includes a direct synaptic activation of epithelial cells overlying the neurons and electrotonic spread of activity from the epithelial cells to the circular muscle cells. In addition, synaptic input from a putative subumbrellar nerve net supplements the excitation to the muscle cells. During a localized radial response, the burst production in the motor network was partially or totally inhibited (Fig. 12), blocking the direct synaptic input to the epithelial cells, and presumably the activation of the subumbrellar nerve net. However, if the region of inhibition was not too broad, muscle cell action potentials (and contractions) of the subumbrellar muscle cells could still be recorded, although with lower amplitudes, an absence of junctional potentials and slower rise times (Fig. 12). In preparations in which the area of inhibition involved several segments on either side of the recorded segment, muscle action potentials were not recorded, and swimming in that segment was totally blocked. This suggests that the electrotonic spread of action potentials through the subumbrellar gap junctions is decreasing. If so, the putative motor nerve net may play a crucial role in conduction of a full action potential (and contraction) within each segment. This would allow a decrease, or total inhibition, of contractility in the muscle sheet by a localized inhibition of activity in the segmental regions of the subumbrellar nerve net.

DISCUSSION

In a comparative sense, the architecture of the hydromedusan nervous system has a basal organization upon which specializations are superimposed to account for differences in behavioural ecology of the individual subgroups and specifically of individual species. One basic structural and functional commonality seems to be the organization of the motor network for swimming in the inner nerve ring (Satterlie and Spencer, 1983; Satterlie, 2002). The electrically coupled network of large neurons provides a means for rapid circular distribution of motor activation around the bell, and also a means of receiving symmetrical and asymmetric inputs from sensory structures that are distributed around the margin of the animal (Spencer and Arkett, 1984; Arkett and Spencer, 1986a; Arkett and Spencer, 1986b; Arkett et al., 1988). This is ultimately translated into locomotory activity that is dependent upon coordinated contraction of circular musculature that lines the inside of the bell – another common feature that produces a forceful ejection of water from the bell cavity (Singla, 1978a; Spencer, 1978; Spencer, 1979; Spencer, 1981; Spencer and

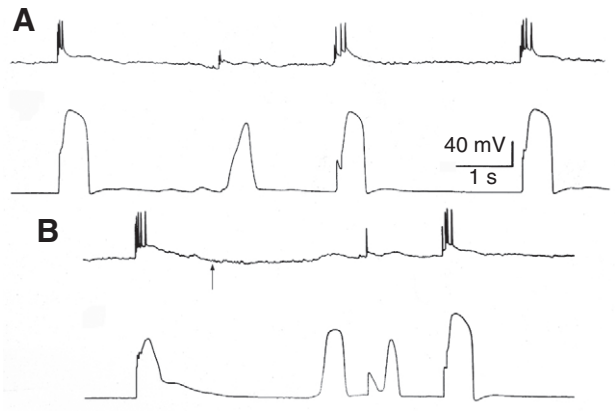

Fig. 12. Double recordings from a swim motor neuron in the inner nerve ring (top traces of each pair) and a circular muscle cell approximately 0.5 mm from the neuron recording site (bottom traces). In A, a spontaneous radial response occurred between the first and second swim. Note the inhibition of neuronal action potential burst and the smaller muscle event with a slow rise time. In the third swim, the preparation had partially recovered from the inhibition, and it was fully recovered by the fourth swim. (B) Another radial response from the same preparation produced total inhibition of the neuronal burst in the second swim (which occurs after a long delay), a single neuronal spike in the third swim, and full recovery on the fourth. Note the changes in the muscle action potentials during the inhibition.

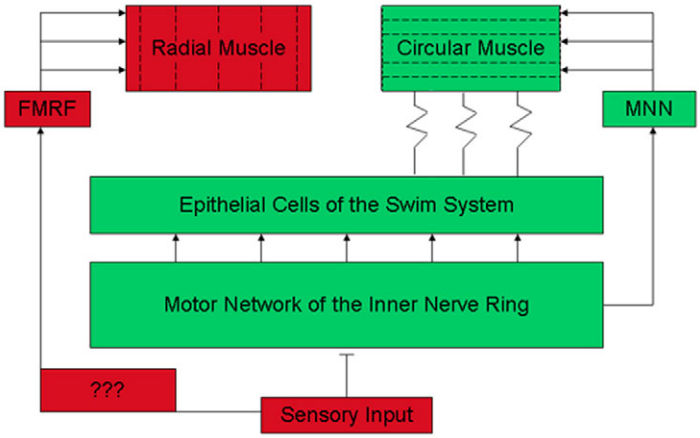

Fig. 13. Schematic of the neuromuscular organization of the subumbrella of *Aequorea*. The arrows represent excitatory chemical connections and the bars, inhibitory connections. The resistor symbols represent electrical coupling, as do the dotted lines within the radial and circular muscle boxes. FMRF, FMRFamide-immunoreactive nerve net of the subumbrella; MNN, motor nerve net of the subumbrella; ???, unknown pathway responsible for activation of the FMRFamide-immunoreactive nerve net following mechanical stimulation of the margin. The circular and radial muscle sheets refer to the musculature from a single subumbrellar segment.

Satterlie, 1981; Satterlie, 1985b; Satterlie, 2002; Kerfoot et al., 1985; Satterlie and Spencer, 1983; Mackie, 2004).

In the several medusae studied, the swim motor network of the inner nerve ring is composed of a compressed network of oversized neurons that activate overlying epithelial cells *via* chemical synapses (Anderson and Mackie, 1977; Anderson, 1979; Spencer and Satterlie, 1980; Satterlie and Spencer, 1983; Spencer, 1981; Spencer, 1982; Satterlie, 1985a; Satterlie, 1985b; Satterlie, 2002; Mackie and Meech, 2000; Lin et al., 2001; Mackie, 2004). Either local or distributed nerve nets of smaller neurons may further conduct excitation from the motor network to the muscles, as seen in the extreme in *Aequorea* where a subumbrellar nerve net is found throughout the muscle sheets (Fig. 13). Other specializations are superimposed on the basic organization, as in *Aglantha*, where motor giant neurons distribute impulses between the motor network and the swim musculature (Weber et al., 1982; Kerfoot et al., 1985; Mackie and Meech, 2000; Mackie, 2004). Also, use of the word 'oversized' to refer to the motor network of *Aglantha* loses some meaning since the neurons are dwarfed by the ring giant (in the outer nerve ring) that is involved in escape swimming (see Mackie, 2004). Again, however, this illustrates how species-specific neuronal peculiarities are superimposed on the basic system.

In *Aequorea*, the pacemaker/motor network of the inner nerve ring is somewhat unusual in that it produces a burst of action potentials to trigger each individual swim contraction (Satterlie, 1985a). Other medusae examined produce a single action potential per swim (Anderson and Mackie, 1977; Anderson, 1979; Spencer and Satterlie, 1980; Satterlie and Spencer, 1983; Spencer, 1981). The action potential burst in *Aequorea* induces a burst of junctional potentials in the postsynaptic epithelial cells that overlie the inner nerve ring, and in the immediately adjacent circular muscle cells, which are electrically coupled to the epithelial cells (Fig. 13) (Satterlie, 1985b). Electrical coupling is found throughout the circular muscle sheet; this represents another basic feature of hydromedusan locomotory systems that aids in the spread of excitation between muscle cells. The degree of 'coupling' varies so aneural conduction is sufficient to transmit a muscle action potential throughout the muscle sheet without decrement in *Polyorchis* (Spencer and Satterlie, 1981; Spencer, 1982), whereas the same conduction is apparently decremental in *Aglantha* [in slow swimming (Kerfoot et al., 1985; Mackie, 2004)] and in *Aequorea* (this study). This variation in muscle cell 'coupling' may be due to differences in gap junctional conductance, to threshold differences for full action potential production, differences in the regenerative properties of the action potentials, or a combination of these and other properties. As an example, in *Polyorchis*, the muscle action potential is conducted through the aneural circular muscle sheet without decrement when it is generated *via* normal inner nerve ring activity. However, the epithelial cells in the region of the inner nerve ring (the neuromuscular synaptic region) form an incrementally conducting region that requires the simultaneous input of junctional potentials from multiple sites to produce the conducted action potentials in the muscle sheet. A compressed network of small neurons is found in this area (Spencer, 1982) that presumably serves as a distributor network for additional neuromuscular input. In this way, this restricted region may act like the entire subumbrella of *Aequorea*, and the distributor network may serve the same function as *Aequorea*'s subumbrellar motor nerve net. In this case, it is interesting to suggest that the motor system of *Aequorea* represents a more diffuse organization of this non-regenerative band of *Polyorchis*, or that the latter is a compressed version of the former.

Regardless, the nature of subumbrellar conduction holds important consequences for the control of swimming, and in particular, the effect of other behaviours on swimming, as seen here in *Aequorea*. In other words, when the two basic properties of hydromedusan swimming systems are considered (electrically coupled motor network that synapses onto postsynaptic epithelial cells, and widespread electrical coupling throughout the circular

muscle sheets), the organization and physiology of these two basic features is where we see significant variation that is related to the structure of the medusa as well as its lifestyle.

In terms of neuromuscular control, perhaps the simplest case is that of the anthomedusa, *Polyorchis*. The electrically coupled motor network of the inner nerve ring extends up the four radii and across the top of each quadrant (Lin et al., 2001). This provides synaptic activation of the four circular muscle sheets along the entire periphery of each quadrant. As mentioned above, transmission in the muscle sheet is strictly *via* gap junctions. Radial muscle is not present in the quadrants, instead it is restricted to smooth muscle strips overlying the radii, so that radial responses in these prolate medusae, including crumpling, are triggered by radial contraction at the four radii (Singla, 1978a; Spencer, 1978; Spencer, 1979).

In what is possibly the most complex elaboration, *Aglantha* (a trachyline medusa) has the 'basic' organization for slow swimming, however, giant neurons are involved in this and in a faster escape response, with two distinct kinds of action potentials in the motor giants serving as the muscle input that determines the type of subumbrellar contraction [slow or escape (Mackie and Meech, 1985; Meech and Mackie, 1993; Mackie, 2004)].

In many of the disc-shaped leptomedusae, including *Aequorea*, radial muscle is not restricted to discrete bands, but rather forms sheets that overlie the circular swim muscle (Satterlie and Spencer, 1983). Radial responses thus rely on more diffuse radial musculature (Fig. 13), possibly because of the challenge of the inward curling of the wide, oblate bell. A unique behavioural peculiarity of *Aequorea*, with its large number of subumbrellar segments, centres on its ability to invert a variable number of segments, and to inhibit swimming in those segments only (Satterlie, 1985a). Although the behavioural significance of this is obvious, the mechanisms required to turn off swimming in a variable portion of the subumbrella, with the basic swim system organization mentioned above, is not so simple.

The excitability of the subumbrellar circular muscle sheet of *Aequorea* appears to be less than that of 'normally' triggered contractions in *Polyorchis*. In the former species, muscle activity that spreads from one subumbrellar segment to another shows a decrease in amplitude and rise time characteristic of decremental transmission through gap junctions. For normal swimming, this electrotonic current spread could require 'supplemental' synaptic input from a peripheral nerve net to bring muscle cell electrogenesis to that of full action potentials. A full contraction in a subumbrellar circular muscle cell would thus require a combination of electrotonic current spread and synaptic input.

Evidence for a subumbrellar motor nerve net (in addition to the electron microscopical data) includes both electrophysiological and immunohistochemical data. Although the motor network in the inner nerve ring produces a burst of action potentials per swim, and this is reflected in the junctional potentials seen in the postsynaptic epithelial cells, the synaptic inputs to circular muscle cells throughout each subumbrellar segment consist of a single junctional potential. This holds for normally initiated swims and for contractions initiated by direct electrical stimulation of the subumbrellar tissue. In the latter case, stimulation of any point of a subumbrellar segment produces a junctional potential and contraction in the muscle cells of that segment, indicating a non-directional transmission in the putative nerve net.

Immunohistochemical experiments with a commercial FMRFamide antibody show the presence of a subumbrellar nerve net. Neuronal RFamides appear to be ubiquitous within the Cnidaria (Grimmelikhuijzen, 1983; Grimmelikhuijzen et al., 1996), and they are neuroactive (Spencer, 1988). The presence of an FMRFamide-immunoreactive nerve net in *Aequorea* has to be viewed with caution, however, since data from a number of other hydromedusae suggest that RFamide-immunoreactive subumbrellar networks are associated with radial, smooth muscle rather than the striated swim musculature (Grimmelikhuijzen and Spencer, 1984; Mackie et al., 1985). Perhaps the best data, in this regard, come from the anthomedusa, *Podocoryne*, where double labelling with an antibody specific for cnidarian smooth muscle was coupled with a FMRFamide antibody to directly show the structural correlation (Weber, 1989). However, in their comparative study of several hydromedusae, Mackie et al. (Mackie et al., 1985) found the density of subumbrellar neurites exceeded the density of FMRFamide-immunoreactive neurons, suggesting the possibility of multiple, separate nerve nets in the subumbrellar ectoderm. In *Aequorea*, this possibility is backed up by data from double labelling experiments, which clearly show the presence of two separate nerve nets in the subumbrellar segments, only one of which stains with the FMRFamide antibody. This is currently under further investigation in *Aequorea* using electron microscopical examination of immunohistochemical preparations stained for FMRFamide.

The extreme variability in muscle cell action potential size and shape in *Aequorea* is not always completely 'predictable' in either a normal swimming preparation or in one that is undergoing a spontaneous or triggered radial response. Current evidence suggests several neuromuscular properties of the subumbrellar motor nerve net may contribute to this level of electrophysiological (and contractile) variability. First, synchrony of inputs to the subumbrellar muscle cells may be important in production of full muscle electrogenesis. If an isolated muscle cell is depolarized, the surrounding, coupled muscle cells may represent a current sink that could decrease the amplitude and slow the rise time of the junctional potentials, thus decreasing their efficacy. Simultaneous depolarization of all neighbouring muscle cells would significantly decrease the current sink effect. Synchrony of activity within the inner nerve ring motor network of *Polyorchis* was found to influence action potential shape (Spencer, 1981), and the same could be true for the subumbrellar muscle cells of *Aequorea*. Any inhibitory input that decreases the synchrony of synaptic inputs to the muscle sheet could alter the amplitude and rise time of muscle action potentials. This was found in the postsynaptic epithelial cells of *Aequorea* during radial responses, where a delayed production of motor network spike bursts produced smaller postsynaptic action potentials with slow rise times compared to swims with unaltered synchrony (Satterlie, 1985b). It may also explain the slight delay between the peak of the junctional potentials and the rise of the action potentials seen in Fig. 6B (compared to the full action potential in Fig. 6A), and more clearly in the three full action potentials in Fig. 12A.

Second, the subumbrellar nerve nets of *Aequorea* show a distinct preferential orientation (radially; Fig. 10). This could also reflect a preferred physiological orientation as well, particularly if the overall pathway in one direction is restricted, as seen in the inter-segmental regions because of the radial canal gonadal tissue. Restriction of conduction is suggested by the data in Fig. 9. As an extreme case, a nerve net could be through-conducting in the preferred direction (radial in this case) and incrementally conducting in the perpendicular (circular) direction. This could show up as a labile conduction of repetitive events in the nerve net, including failures and re-appearances. An incrementally conducting nerve net has been described in an anthozoan (Anderson, 1976). Also possible, however, are alterations in conduction patterns in a through-conducting nerve net that merely alter the synchrony of activity within the nerve net with consequences as previously mentioned.

Third, the action potentials of swim muscles of *Aequorea* are variable in amplitude even during normal swimming bouts (see Fig. 7). In a rested preparation, a swimming bout is initiated by epithelial (and muscle) action potentials that increase in amplitude with the first several swimming contractions, reminiscent of neuromuscular facilitation (Satterlie, 1985b). Despite this, no significant correlation could be demonstrated between action potential amplitude and the preceding inter-spike interval, suggesting that a strong frequency-dependent facilitation is not in operation (unpublished observations). Yet it appears that muscle cell electrogenesis is influenced by the electrophysiological history of the cell, and this could contribute to some of the observed variability.

Finally, as an unexplored possibility, the swim muscle cells may be directly sensitive to the neuroactive substance (presumed an RFamide) released from the FMRFamide-immunoreactive neurons during a radial response. In this scenario, the nerve net associated with the radial muscle would have opposing effects on the two muscle types. Thus two levels of chemical inhibition could be acting on the swim system during a radial response: direct inhibition of the inner nerve ring motor network (Satterlie, 1985a; Satterlie, 1985b) and a peripheral modulatory action on the muscle cells. Thus far, permeability difficulties posed by the tight epithelium of the subumbrella have prevented direct testing of this possibility.

A combination of these nerve net and neuromuscular properties, in conjunction with the electrical coupling parameters of the swim muscle sheet, may be required to explain the full range of variability seen in the swim muscle cell recordings during normal swimming and during radial responses. In any event, the 'turning off' of restricted subumbrellar segments appears to be based on both central (nerve ring) and peripheral (subumbrellar) mechanisms. Add to this the ability to totally inhibit activation of the subumbrellar nerve net (as shown in Fig. 12), sometimes with action potentials of apparently electronic origin, and it is apparent that a flexible range of muscle force suppression/inhibition can be achieved in this system.

As a final word on the swimming system of hydromedusae, the organization of the motor network of the inner nerve ring is influenced by parallel sensory networks of the inner and outer nerve rings (Spencer and Arkett, 1984; Arkett and Spencer, 1986a; Arkett and Spencer, 1986b; Arkett et al., 1988; Mackie, 2004). Furthermore, fourteen distinct conducting systems have been described in *Aglantha* (reviewed by Mackie, 2004). In *Aequorea*, multiple statocysts are found throughout the bell margin (Singla, 1975), and righting responses to tilt involve asymmetrical swimming contractions of the subumbrella, possibly including the local inhibitory mechanisms discussed here. These observations suggest that parallel and point-source pathways provide excitatory or inhibitory input to the swim motor network in the inner nerve ring of hydromedusae, pointing to an integrative organization that forms what can be argued to be a true central nervous system in these radially symmetrical organisms. In these animals, the 'primitive' condition of diffuse, non-directional nerve nets has been modified in some conducting systems into a series of compressed, function-specific nerve networks that interact in the complex neural structures of the inner and outer nerve rings. As an example of the richness of function of the hydromedusan nervous system, one has to look no further than the organization of *Aglantha* (Mackie, 2004) to be impressed with the neural complexity of this 'simple' animal group.

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