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Central circuitry in the jellyfish *Aglantha digitale*

IV. Pathways coordinating feeding behaviour

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Summary

The hydromedusan jellyfish *Aglantha digitale* feeds on small planktonic organisms carried to the margin by tentacle flexions. During feeding, the manubrium bends across ('points') and seizes the prey with flared lips. In immobilized preparations, pointing to a source of electrical stimulation was accurate, 70% of the time, to within 15°. Cutting experiments showed that the conduction pathways concerned with pointing and lip flaring are located in eight radial strands consisting of a radial canal, a giant nerve axon and a bundle of small axons with FMRFamide-like immunoreactivity.

Application of food juices to sites on the margin and tentacles evoked trains of impulses in the axon bundles (F events; conduction velocity 15.5±3.7 cm s^{-1}) and in the epithelium lining the radial canals (E events; conduction velocity 28.5±3.5 cm s^{-1}). Impulses were conducted circularly in the outer nerve ring (F events) or in the ring canal (E events).

Unilateral flexions of the manubrium during pointing arise from preferential excitation of one or more of eight longitudinal 'muscle bands' in the wall of the manubrium and peduncle. Lip flaring represents symmetrical contraction of all eight bands. Cutting experiments revealed that F events mediate pointing; E events mediate lip flaring. Thus the endodermal radial canals, which in other hydromedusae mediate protective 'crumpling', provide the conduction pathway for manubrial lip flaring. *Aglantha*'s alternative protective response – escape swimming – makes crumpling unnecessary, releasing the pathway for use in feeding.

Trains of E events, generated in the manubrium during ingestion, propagate to the margin and inhibit rhythmic (slow) swimming with a duration that depended on their number and frequency. Inhibition of swimming appeared to facilitate transfer of food from the margin to the mouth, but how it comes about is unclear.

Key words: *Aglantha digitale*, feeding, manubrium, swimming, Cnidaria, medusa, nervous system, FMRFamide, hydromedusa, nerve ring.

Introduction

Most neuroethological studies on hydromedusae, including our own previous papers in this series (Mackie and Meech, 1995a,b, 2000), deal with aspects of swimming behaviour. Relatively little attention has been paid to the mechanisms underlying feeding (Satterlie and Spencer, 1987). Earlier writers (e.g. Romanes, 1877; Nägel, 1894; Horridge, 1955a,b) showed how prey are captured by the tentacles and brought to the margin by tentacular contractions. The manubrium then bends laterally toward the site where the food is located ('pointing'), grabs the prey with its lips and ingests it. In some species, the margin flexes inward, bringing the food closer to the manubrium during the transfer process. These flexions of the margin that help to bring the margin and mouth into proximity are brought about by the contraction of radially aligned smooth muscles located in the ectoderm overlying the circular, striated muscles responsible for swimming. As these flexions are directional and involve the selective excitation of muscles on only one side of the animal, they are thought to be mediated by nerves.

The same radial muscles that function in pointing also function during the protective behaviour known as 'crumpling', but this is a generalized response involving contraction of muscles all the way around the bell and it is mediated, at least in part, by excitable epithelia (Mackie and Passano, 1968; Ohtsu and Yoshida, 1973; Mackie, 1975; Mackie and Singla, 1975; King and Spencer, 1981). Thus control of the radial muscles poses an interesting problem because the muscles take part in two quite different forms of behaviour, one of which is thought to be controlled entirely by nerves while the other involves a non-nervous conduction pathway. The excitable epithelia in question are those forming the canals in the subumbrellar endoderm, and the lamella that spans the area between them. These tissues conduct all-or-none impulses. It has never been suggested that impulse conduction

in the endoderm plays any part in feeding behaviour, but as the canals bridge the gap between the margin and manubrium the possibility that propagated events carried by them might play some role in coordinating feeding activity cannot be ignored.

A function such as feeding would be expected to involve chemoreceptors but there is little behavioural or physiological work dealing with this topic in hydrozoan jellyfish. Henschel (1935) found that the tentacles and manubrium of *Sarsia tubulosa*, after briefly contracting, elongated in solutions containing mussel juice. In bitter solutions (quinine, aloes) the bell contracted convulsively, and the tentacles and manubrium shortened maximally. *Stomotoca atra* are reported to stop swimming, contract and sink to the bottom in the presence of mesogloeal extracts of some other medusae (Lenhoff, 1964). In experiments by Arai (1991), *Aequorea victoria* were attracted to one end of a tank in which *Artemia* larvae were present in a screened compartment, indicating sensitivity to a prey-related chemical. *Mitrocoma cellularia* on the other hand, while showing clear behavioural responses to food chemicals, showed no tendency to move toward sources of food cues (Tamburri et al., 2000). Numerous workers have described sensory cells in hydrozoan medusae that might be chemoreceptors but the evidence appears to be entirely circumstantial.

The existing picture for Aglantha

Like many other hydromedusae (Child, 1918; Arkett, 1984), *Aglantha* is a ‘sink-fisher’. It shows regular cycles of upward swimming followed by ‘passive’ sinking, in which the tentacles are stretched out sideways and collect prey over a large area (Fig. 1). Sinking is not altogether passive, as the tentacles are heavily ciliated and strong ciliary currents propel water past the tentacles in the oral direction, driving the animal downwards with the apex leading (Mackie et al., 1989). The prey consists of a variety of small planktonic organisms including copepods, tintinnids, and the eggs and larvae of euphausiids (for references, see Arai et al., 1993). On contact, these objects are secured to the tentacles by discharged nematocyst threads and are brought by tentacle flexions to the margin where they can be transferred to the manubrium. Tentacular flexions seen during feeding are graded movements coordinated by a slowly conducting tentacle conduction system (Mackie and Meech, 1995a,b).

As to the histological basis of the conduction pathways involved in feeding, Singla (1978) described a bundle of some 20 small axons that runs radially up the subumbrella as part of each radial strand, lying close to the giant axons that mediate swimming behaviour, and overlying the radial canals (Fig. 2). The small axon bundle forms the only known neural connecting link between the margin and manubrium and is therefore an obvious candidate for a pathway mediating feeding behaviour. It contains axons with FMRFamide-like immunoreactivity (FaIR; Mackie et al., 1985). The FaIR axons in the bundle are thought to derive from sensory cells located in the margin and manubrium, but the role of the FaIR neuronal subset has remained uncertain.

Recordings with external electrodes over the radial strands

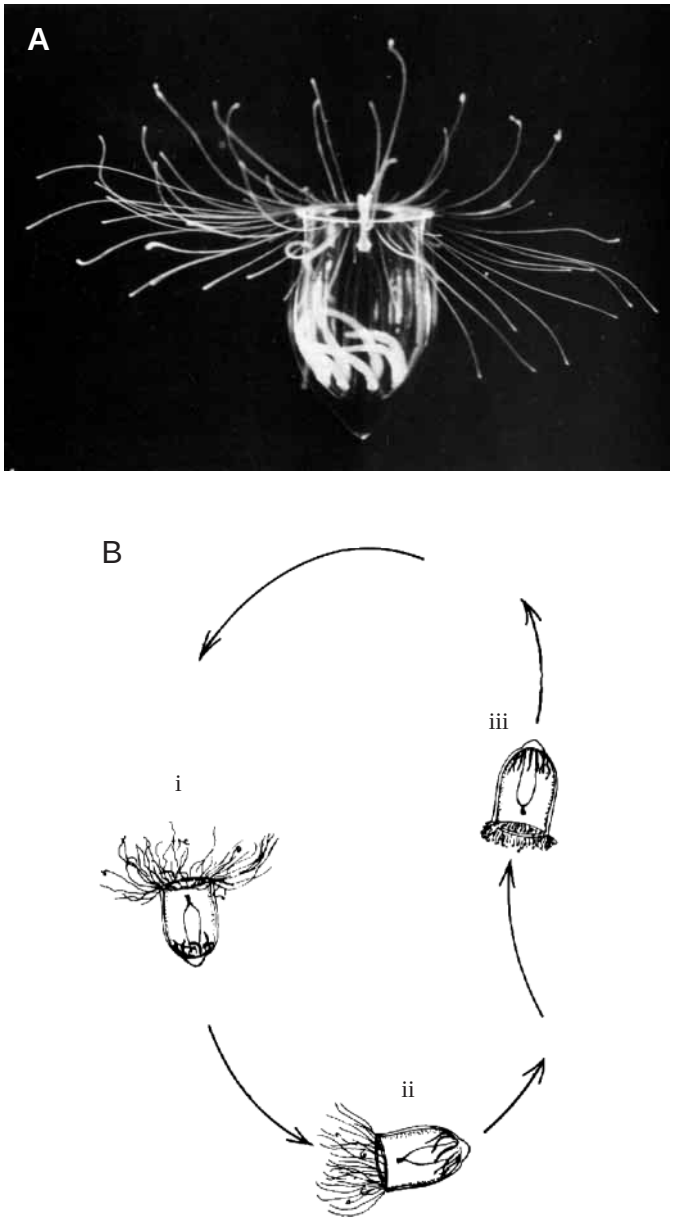

Fig. 1. Feeding in *Aglantha digitale*. (A) A specimen floating upside down in feeding posture, tentacles extended (photograph by Claus Nielsen). (B) Cyclical sink-fishing of *Aglantha*. The animal sinks passively, tentacles extended (i); at the bottom of the cycle swimming ensues (ii); the animal swims upwards with tentacles contracted (iii); then it stops swimming, turns over and starts sinking again (after Mackie, 1980).

show not only large electrical events associated with spike propagation in the motor giant axon but also much smaller propagated events referred to as manubrial (M) impulses by Mackie and Meech (2000), who assumed that they were involved in coordination between the margin and manubrium during feeding behaviour. While this now proves to be correct, our assumption that M events are carried by Singla’s small axon bundle turns out to be incorrect. To avoid possible confusion we have abandoned the terms ‘M impulse’ and ‘M

pathway' and will use new terms to describe radial conduction pathways involved in communication between the margin and the manubrium.

The primary purpose of this paper is to describe the conducting pathways and effectors responsible for transfer of food from the margin to the manubrium during feeding in *Aglantha*. We have found an unexpectedly complicated system in which both nervous and non-nervous pathways play a role.

Materials and methods

Behaviour

Specimens of *Aglantha digitale* Müller were caught off the dock at the Friday Harbor Laboratories, University of Washington, USA and were kept in glass containers at 7°C until used. Observations on the behaviour of whole animals were made in finger bowls under a dissecting microscope. Images were obtained through the microscope using a digital camera (Coolpix 995; Nikon Corp., Tokyo, Japan).

Physiological recordings

Specimens were dissected in seawater containing 115 mmol l^{-1} Mg^{2+} to immobilize them. For many purposes, parts could be studied in isolation, but to study coordination between margin and manubrium, the animal had to be left largely intact. It was found best to incise the subumbrella with inter-radial cuts as far as the base of the peduncle. Four such cuts were made so as to produce four equal flaps, each with two intact radial strands. This term refers to a tissue complex consisting of an endodermal radial canal plus a giant motor nerve axon and a bundle of small axons running radially in the overlying ectoderm (see Fig. 2B).

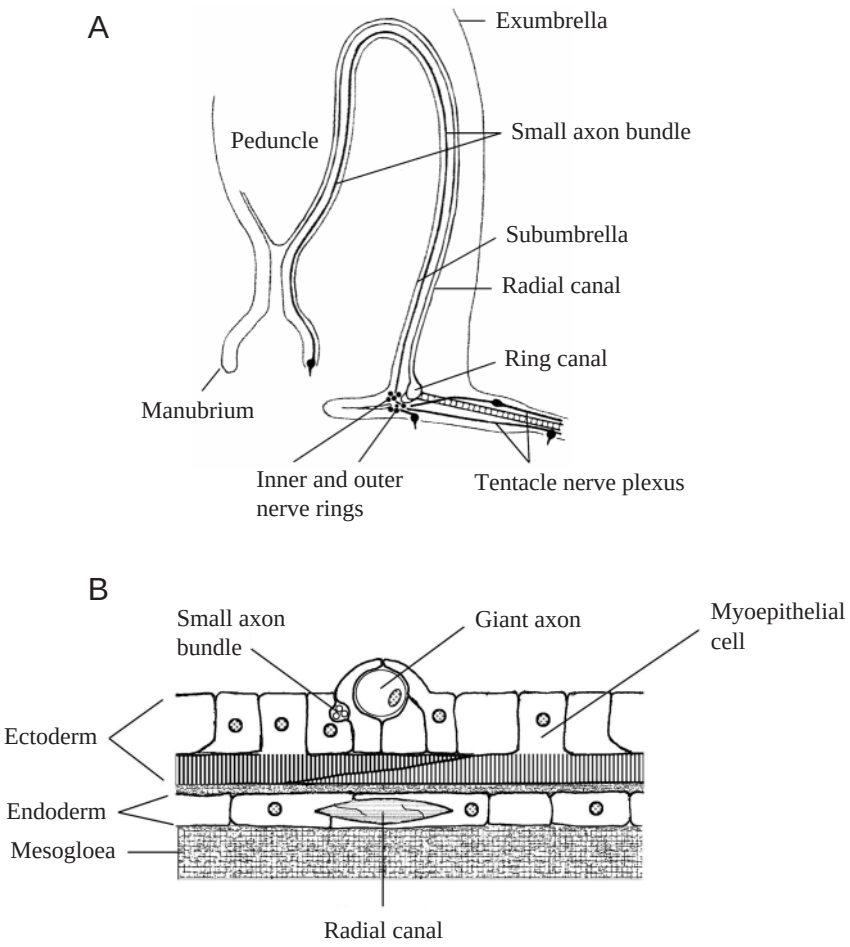

Fig. 2. Conduction pathways involved in feeding in *Aglantha digitale*. (A) Vertical section, apex of bell upwards, showing general structural relationships and distribution of neural elements having FMRFamide-like immunoreactivity (after Mackie et al., 1985). (B) Diagram of a transverse section through a subumbrellar radial strand showing the relationship between the radial canal, the small axon bundle and the giant axon.

The preparation was then pinned down with cactus spines, leaving the peduncle standing upright in the middle. The pinning of these quartered, splayed preparations had to be done carefully to avoid kinking the radial strands, as this could block conduction. In many experiments, the tentacles were cut off near the base as they tended to get in the way when suction electrodes were being attached.

Lesion experiments were carried out in order to locate the radial pathways mediating the pointing response. To prevent spontaneous swimming the specimens were placed in 0.3 mmol l^{-1} octanol or heptanol in seawater, both effective gap junction blockers (Johnston et al., 1980). Octanol (or heptanol) blocks conduction in the exumbrellar epithelium of *Aglantha* but has no effect on the production or propagation of sodium spikes in the motor giant axons (Mackie and Meech, 1995a).

Suction electrode recordings were carried out using polyethylene tubing pulled out to a fine tip (30–50 μm i.d.). For most recordings preparations were placed in 81–90 mmol l^{-1} Mg^{2+}, which prevented spontaneous swimming and reduced manubrial movements along with the complex electrical activity and movement artifacts that accompany them. Stimuli were applied externally through small, coaxial, bipolar, metal electrodes (SNE-100; Harvard Apparatus Ltd., Edenbridge, UK). Signals were amplified and displayed on an oscilloscope, a chart recorder, or a data acquisition system (PowerLab 8SP; AD Instruments, Castle Hill, NSW, Australia) with input to a laptop computer. Temperature was maintained at 10–14°C using a thermoelectric cooling stage (TS-4; Physitemp Instruments Inc., Clifton, NJ, USA).

Immunohistology

Pieces of tissue were pinned out in Sylgard-lined dishes with cactus spines and were fixed for 3–4 h in 4% paraformaldehyde

in 0.1 mol l^{-1} phosphate-buffered saline (PBS) at pH 7.3 followed by several rinses in 0.1 mol l^{-1} PBS containing 0.3% Triton X-100 and 0.03% sodium azide (PTA). For visualization of general nerve structure and layout, a mouse anti-tubulin antibody (N356; Amersham Biosciences, Cardiff, UK) was used, diluted 1:50 in PTA. Preparations were also treated with 1:200 rabbit anti-FMRFamide serum (IHC 8755; Peninsula Laboratories, San Carlos, CA, USA). After 8–12 h, preparations were rinsed in PTA and treated with the appropriate secondary antibodies for a further 8–12 h before washing and mounting in 50% glycerol containing 0.3% *n*-propyl gallate. Texas Red-coupled goat anti-mouse secondary antibody was used to display tubulin and FITC-coupled goat anti-rabbit secondary for FMRFamide. Laser scanning confocal microscopy, using a Bio-Rad MRC-600 (Bio-Rad Microscience Ltd, Hemel Hempsted, UK) at Friday Harbor and a Zeiss LSM 410 (Carl Zeiss Ltd, Toronto, Canada) at the University of Victoria was used to study the mounted preparations.

Electron microscopy

Pieces of tissue were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer at pH 7.4, rinsed in buffer and postfixed in 1% osmium for 1 h. They were then washed for 1 h and stained *en bloc* overnight in 2% aqueous uranyl acetate at 60°C, after which they were rinsed and dehydrated in a graded ethanol series followed by propylene oxide. They were infiltrated in Epon:propylene oxide overnight and then embedded in Epon. Sections were cut on a Reichert ultramicrotome (Hitachi Ltd, Tokyo, Japan), and examined in a Hitachi H 7000 electron microscope (Hitachi Ltd, Tokyo, Japan).

Results

Behavioural observations

Pointing

Depending on their size, individual *Aglantha* have 60–80 tentacles, separated from one another around the margin by about 5° of arc. Prey are caught by single tentacles or by local groups, depending on their size. Following capture of prey, the tentacles concerned flex orally, so as to bring the food object to the margin. Then the manubrium, which is normally vertical (Fig. 3A,C), bends across and seizes it with its flared lips (Fig. 3B,D). The histological evidence presented below shows that longitudinal muscle bands in the wall of the manubrium extend upwards to the peduncle running through the slender neck region, which acts like a hinge. Pointing is brought about by differential contraction within this set of muscle bands.

Lesion experiments were carried out in order to locate the radial pathways mediating the pointing response. To prevent spontaneous swimming the specimens were placed in 0.3 mmol l^{-1} octanol or heptanol in sea water. This treatment, which blocks gap junctions and inhibits epithelial conduction, had no effect on the pointing response and was fully reversible. Animals were operated on by cutting one or more of the eight radial strands (see Materials and methods). In the absence of stimulation, the manubrium has a vertical orientation (Fig. 4A). However, a single electric shock delivered to the outer nerve ring at the point where a radial strand joins the margin was enough to elicit an accurate manubrial flexion to that point (Fig. 4B). The pointing response was accurate 70% of the time to within 15°. Responses to shocks delivered inter-radially (Fig. 4C) were no less accurate. After cutting all eight radial strands, pointing was no longer obtainable (Fig. 4D). In preparations such as Fig. 4E, where all but two radial strands were cut, pointing was generally directed to the intact radius closest to the stimulus. In preparations such as Fig. 4F, the manubrium pointed to one or other of the two radii equidistant from the stimulus site.

These experiments show that coordination of the pointing response involves radial pathways restricted to the radial strands. Pointing is always towards the nearest intact strand, or strands. It is also clear that there are circular pathways at the margin that link the radial pathways and that the entire circuit is resistant to the gap junction blocker, octanol.

Lip flaring

The presence of food at the margin triggered not only manubrial pointing but also the sudden, symmetrical flaring of the lips, typically followed by irregular 'searching' movements of the whole oral region. The flaring response could be evoked by electrical stimulation of the radial strands or margin and evidently represents a more or less symmetrical contraction of the manubrial longitudinal muscles and their projections up the sides of the peduncle. The whole manubrium and neck region contracted longitudinally during lip flaring but these contractions were less obvious than the flaring itself. Lip flaring could still be obtained when radial stimulation was applied to the peduncle of animals that already had almost full stomachs (Fig. 3G).

Ingestion

Ingestion was observed by placing small pieces of fish or prawn muscle or whole small crustaceans directly against the mouth. The full sequence of events could be observed in isolated manubria attached to their peduncles, and appeared to be no different from the process as seen in whole animals.

If prey had not already been paralysed by nematocysts in the tentacles, they were immediately immobilized on contact with the manubrium, presumably by the nematocysts arranged around the edges of the mouth. On contact with food, the manubrial lips flared out widely and performed writhing, probing movements, which resulted in attachment of the mouth to the food. Ingestion then ensued, as the lips progressively engulfed the food. The four protruberant corners of the mouth led the advance of the lips around the prey. As in hydra (Beutler, 1924) the process evidently involves both ciliary and muscular effectors. The beating of the cilia lining the whole oral region was clearly responsible for the smooth, steady advance of the lips around the prey. Periodically, however, particularly in the case of large or unwieldy food objects, a

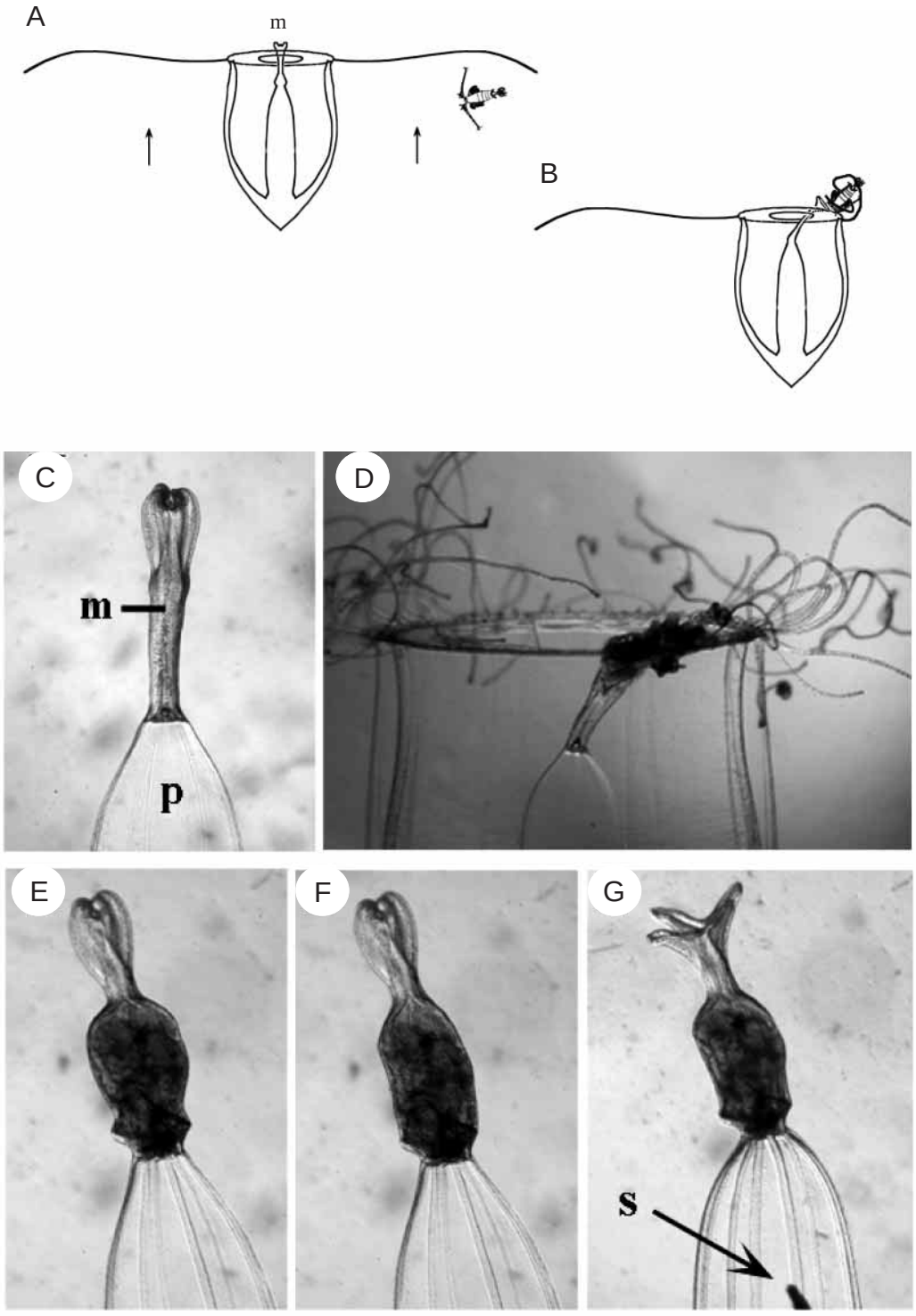

Fig. 3. Food capture, ingestion and digestion. (A) Ciliary beating drives water orally past the extended tentacles (arrows) and causes the animal to glide slowly through the water, bringing the tentacles into contact with a copepod. (B) Tentacular contraction and flexion brings the prey to the margin while the manubrium points across to it with flared lips. (C) Peduncle (p) and manubrium (m) of an unfed animal at rest. (D) Lateral flexion (pointing) to a site where food is held by the tentacles. (E,F) Changes in the diameter of the manubrium during peristalsis in a fed animal. (G) Lip flaring evoked by a shock from a stimulating electrode (s) on a peduncular radial canal.

sudden, muscular flaring of the lips, which caused them to detach briefly from the food, interrupted the process. The lips would then elongate again, lunge forward and reattach further down the prey, after which the ciliary traction process would be resumed. Lip flaring and lunging appeared to be particularly important in engulfing awkward, outlying parts of the prey such as the legs or antennae of crustaceans, as the muscular force exerted helped to fold these parts in. Lip flaring and lunging sequences continued until the lips met around the far side of the food. At this point the mouth closed, sealing the prey within.

During ingestion, and subsequently during digestion, peristaltic waves of contraction were observed to travel from the oral region to the stomach. The waves constricted the gastrovascular cavity and its contents, propelling the food progressively further into the basal stomach region and freeing

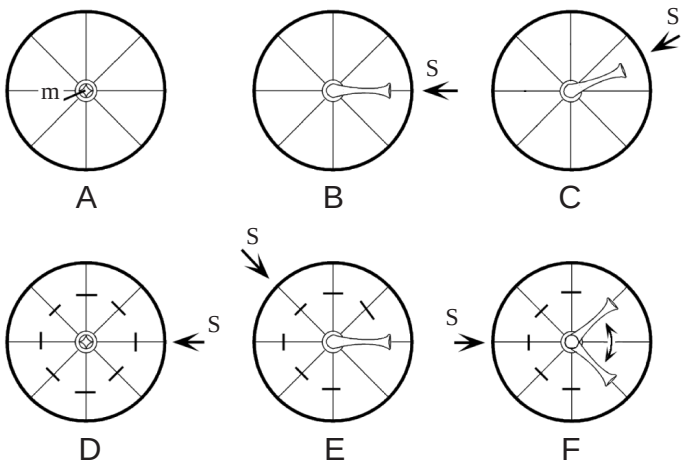

Fig. 4. Analysis of pointing pathways. (A) The manubrium (m) is at rest. (B) It points to a stimulus (s) located at the junction of a radial strand with the margin. (C) The stimulus was delivered interradially. (D) After cutting all radial strands, stimuli cease to evoke pointing. (E) After cutting six adjacent strands, stimuli cause pointing to the closest intact radius. (F) After cutting five adjacent strands, leaving two strands equidistant from the stimulus, pointing may be to either of the two equidistant radii.

up more of the oral region for spreading around the prey. Peristaltic waves tended to alternate with flaring–lunging movements of the oral region.

Once the prey was fully engulfed, flaring–lunging movements ceased and the manubrium settled down to a pattern of regular peristalsis with an approximately 20 s periodicity, churning the food within the stomach region (Fig. 3E,F). The food soon showed signs of enzymatic breakdown, becoming a rounded bolus surrounded by a thick fluid containing partially digested fragments. The bolus could be observed rotating slowly in the stomach as a result of coordinated ciliary beating.

Manubrial peristalsis not only churned the stomach contents but also was clearly instrumental in pumping digested nutrients out through the radial canals. With each peristaltic pumping cycle, the fluid was observed to move out along the canals, causing them to swell to two or three times the size observed in starved animals. The fluid contained many undigested particles. The cells lining the canals throughout the animal probably serve a secondary digestive function, endocytosing and digesting these fragments. Phagosomes were frequently seen in electron micrographs of the cells lining the canals.

Depending on the size and shape of the food object, ingestion might take a few seconds to several minutes. Digestion of small crustaceans usually took more than an hour and eventually undigested food matter was ejected by a combination of lip flaring and reverse peristalsis.

Ingestion appeared to be triggered primarily by contact with food rather than by chemosensory input, although both may be involved. The manubrium would attempt to engulf a wide variety of objects, including non-food objects. Once the process had started it usually went through to completion although rejection of food was seen sometimes where the food mass was very large or unwieldy. Rejection involved lip flaring and curling of the lips back upon themselves, which had the effect of disabling ciliary traction.

Inhibition of slow swimming during ingestion

It was repeatedly observed that swimming animals stopped swimming while ingesting food, and resumed it again once the food was safely in the stomach. This was seen under natural conditions and also when the food was presented directly to the lips without stimulating the tentacles or marginal structures in any way. Indeed, it still occurred when all the tentacles had been removed. Electrical stimulation of the manubrium confirmed that the response could be evoked by manubrial stimulation alone (Fig. 5A). The period of inhibition depended on the duration of the stimulus. A single stimulus produced little or no inhibition but two or three shocks at 3 Hz caused a perceptible increase in the swim interval. Longer trains of stimuli inhibited swimming for many seconds and the inhibition continued long after stimulation had ceased. In Fig. 5A (lower trace) inhibition was maintained for the duration of the 10.5 s stimulus and continued for at least 6 s. Fig. 5B shows a linear relationship between stimulus duration and the time interval between the end of the stimulus and the first post-stimulus swim. Each data value was normalized by dividing it by the average of the three pre-stimulus swim intervals and the line drawn through the points was fitted by linear regression. As expected, the intercept on the *y* axis was less than 1.0 because each stimulus period started midway between swims. The data in Fig. 5C (which was similarly normalized) shows the relationship between the stimulus duration and the interval between the first and second post-stimulus swims. The fitted linear regression line was forced to pass through the control value for the swim interval in the absence of stimulation and Student's *t*-test was used to test whether the slope was significantly greater than zero. The result (P=0.015) suggested that the effect of inhibition continued even though the animal had resumed swimming.

Evocation of escape swimming by strong manubrial stimulation

Pinching the manubrium with forceps or simply attaching a suction electrode to it was sometimes found to trigger escape swimming with an accompanying twitch contraction of all the tentacles, which was indistinguishable from the escape response obtained by stimulation of the tentacles and margin (Roberts and Mackie, 1980). In a few cases only the tentacular twitch component was evident. Escape swimming was sometimes seen under natural conditions when the manubrium was attempting to ingest violently struggling, bristly prey, such as small amphipods, but the response does not appear to be part of normal feeding behaviour and will not be further considered in the present study.

Chemosensitivity

Preliminary observations showed that pipetting food juices

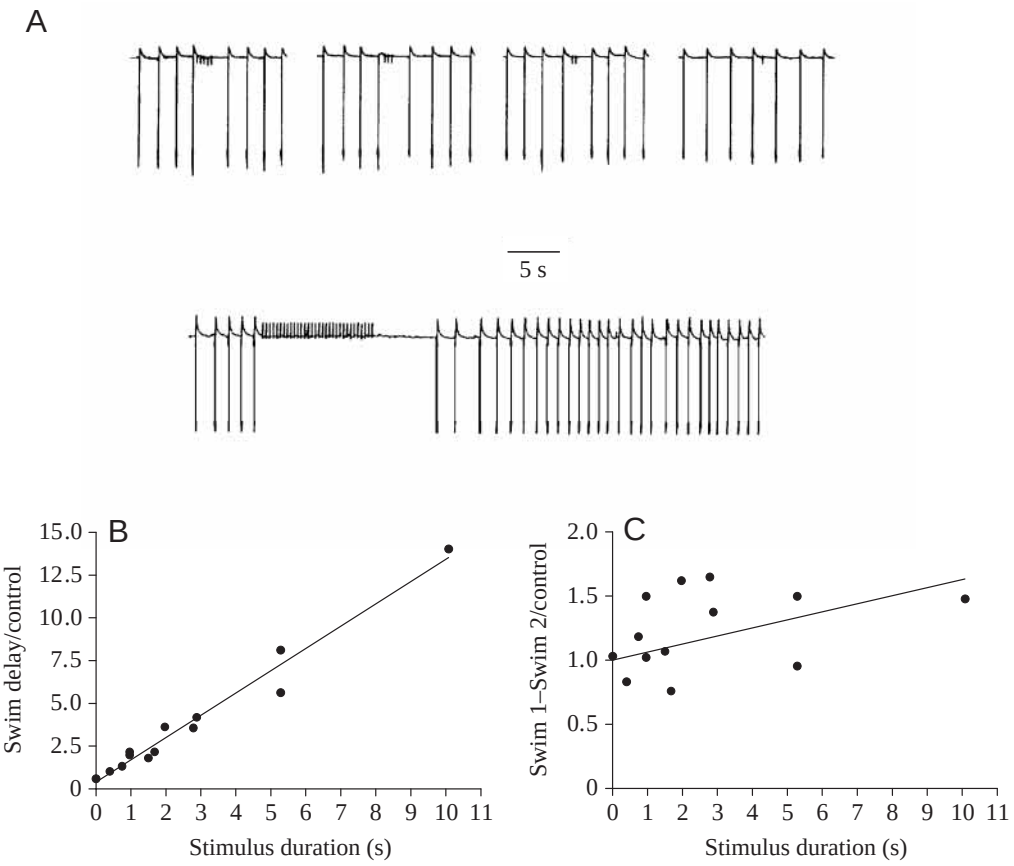

Fig. 5. Inhibition of rhythmic swimming by manubrial stimulation. (A) Portions of a chart recording from one animal in which swims were recorded electromyographically using a suction pipette attached to the velum. Each swim was registered as a large downward deflection; shocks to the manubrium appear as small stimulus artefacts (downward in top series of traces, upward in bottom trace). Stimulus pulse trains were delivered at 3 Hz. The top traces show that a stimulus train containing two or more pulses caused a perceptible increase in the interval between successive swims. In the bottom trace, inhibition outlasted the 10.5 s stimulus by at least 6 s. (The swim deflections have been enhanced to improve clarity). (B) Relationship between the stimulus duration and the time interval from the end of the stimulus to the first post-stimulus swim; each data point was normalized by dividing it by the average pre-stimulus swim interval (N=3). The line drawn through the points was fitted by linear regression (slope, 1.3 of control swim interval/second of stimulus; y-intercept, 0.40 of control swim interval). (C) Relationship between the stimulus duration and the time interval between the first and second post-stimulus swim; data points were normalized as above. The line drawn through the points was fitted by linear regression (slope, 0.06 of control swim interval/second of stimulus; y-intercept, taken as 1.0 of control swim interval).

from crushed amphipods or copepods onto the tentacles sometimes evoked manubrial pointing toward the general area where the juice was applied. After cutting off the tentacles the response could still be obtained when the juice was pipetted on the margin. The isolated manubrium also showed 'searching' movements in response to amphipod juice pipetted directly on to it, but waving a crushed amphipod near the mouth did not evoke a response until contact was made. Trials with reduced glutathione (GSH), a feeding activator in hydra (Loomis, 1955), were carried out. In some specimens, addition of GSH to the water to a final concentration of 25 μmol l^{-1} consistently evoked tentacle coiling and manubrial searching movements similar to those observed during normal feeding behaviour but other specimens failed to respond at all. These tests suggest that chemoreceptors located in the tentacles, margin and manubrium may play a part in feeding behaviour of animals, but responsiveness is labile and may depend on the animals' physiological state.

Electrophysiology

Radial conduction pathways

Stimuli applied to the margin or a radial strand evoked three well defined types of electrical signal when recorded at a site further along the radial strand, but still within the striated muscle field (see Fig. 6A). In the example shown in this figure the first event represents the stimulus artifact. It is followed by a spike in the giant axon (G) that could be eliminated by pricking the axon with a sharp point. G events could not be recorded beyond a point near the apex of the subumbrellar cavity where the giant axon terminates and the striated muscle field ends. The other two smaller events (E,F) propagated all the way to the manubrium and could be recorded at all points along the radial strands in the peduncle. Events recorded in the manubrium were often significantly larger than those recorded elsewhere.

On reaching the manubrium, the faster of the two small events (E) evoked symmetrical shortening of the manubrium and flaring of the manubrial lips. The slower event (F) was associated with unilateral manubrial flexions (pointing). These responses are described in more detail below. Velocities varied considerably depending on the size and physiological state of the animal and on the portion of the pathway under study, but where both systems were in evidence, the E system generally conducted roughly twice as fast as the F.

In order to identify the tissue substrates for these two

conduction pathways, specimens of *Aglantha* were selected in which the nerves running in the radial ectoderm deviated slightly to one side of the underlying endodermal canal, making it easier to cut either nerves or canal alone without damaging the other. Cutting the canal blocked the conduction of the faster (E) event while cutting the radial nerves abolished conduction of the slower (F) one. Cutting both ecto- and endodermal components of the radial strands blocked both conduction systems. The preparations used for these tests were examined by differential interference contrast microscopy with a 25× water immersion objective in order to verify that the pathways in question had been transected completely and that the remaining pathway was intact. Finally, tests such as those illustrated in Fig. 4 were repeated in preparations where only the radial canals had been cut, leaving a selected group of radial F pathways intact. The manubrium continued to flex toward the point where the intact radial strand closest to the stimulating site joined the margin, showing that pointing is mediated entirely by the F system.

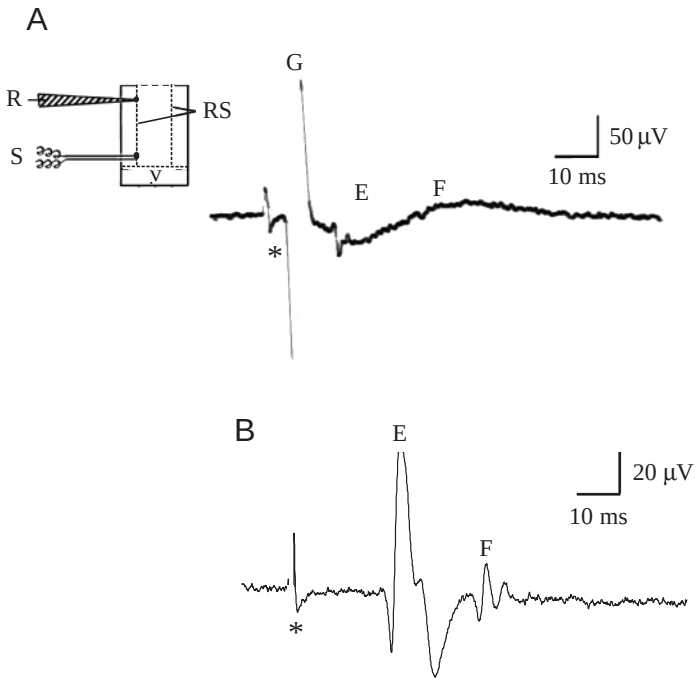

Fig. 6. External recording of propagated events evoked by radial strand stimulation. (A) A single shock (asterisk) evoked three propagated events: the first, G, (artificially enhanced for improved clarity) corresponds to the overshooting action potential in the motor giant axon; the second, E, corresponds to a propagating impulse in the endodermal epithelial conduction pathway; the third, F, corresponds to propagating impulses in the bundle of small-axons. Inset: experimental arrangement with stimulating (S) and recording (R) electrodes located on an intact radial strand (RS), subumbrella surface upwards. The stimulating electrode is near the velum (v). The dotted lines represent tissues containing one or more radial and circular conduction pathways as described in the text. (B) Recording showing E and F events in a peduncular radial strand close to its junction with the manubrium. Average of three responses evoked by single stimuli (asterisk) delivered to the same strand approximately 5 mm away.

We conclude from these experiments that the E events are conducted in the endoderm. The absence of organized nerve tracts in the endoderm (see below) implicates the endodermal epithelium itself as the excitable tissue. The F events, which were recorded solely in the radial ectoderm, are presumably conducted in Singla's small axon bundle (Singla, 1978). The only other nerves in the radial strands are the motor giant axons and the neurons running out laterally from them, which provide the motor innervation of the striated muscles (Roberts and Mackie, 1980; Kerfoot et al., 1985) but none of these extend beyond the apex of the subumbrellar cavity, whereas F events propagate all the way to the manubrium. Puncturing the motor giants did not affect passage of F impulses. This leaves the small axon bundle as the only plausible route.

The designation E stands for 'epithelial' as the events in question are conducted in an excitable epithelium. F stands for 'flexion', as these events trigger manubrial flexions in the pointing response.

The E system and lip flaring

The E potentials that propagated along the radial canals following stimulation at the margin were small events (<30 μV) with a mean conduction velocity of 28.5±3.5 cm s^{-1}. The endodermal radial canals lie within a flat, epithelial monolayer, the interradial endoderm lamella, which spans the gaps between all the canals. In many hydromedusae and siphonophores this lamella is also excitable (Anderson, 1980; Spencer and Schwab, 1982; Josephson, 1985) but in *Aglantha* excitability is restricted to the canals. Cutting the canals alone was found to be sufficient to block all E impulse conduction. In preparations where the ectoderm had been scraped away leaving the endodermal canals and lamella intact beneath a thin mesogloeal layer, E events could be recorded and evoked with electrodes placed directly on the canal, but not on either side of it.

Tests on preparations with several radial canals showed that E impulses propagated circularly at the margin, presumably in the ring canal (see Fig. 2A). E impulses were found to propagate down one radial canal, around the margin and up other canals. Similarly, impulses entering the manubrium *via* one canal exited *via* the others. Fig. 7A shows, in diagrammatic form, a splayed preparation with a stimulating electrode (S) on one of the radial strands (RS) visible in the peduncle (p). Recording electrodes were placed on another radial strand nearby (R1) and on the stimulated strand close to the margin (R2). This latter strand was cut between S and R2 at site *a*. A second cut at site *b* went through the ectoderm only, leaving the radial canal intact. As seen in Fig. 7B, a stimulus evoked both E and F events at R1 but only the E event arrived at R2. Passage across the manubrium (m) between canals on opposite sides took approximately 8 ms. The E system thus conducted through the entire canal system and manubrium on an all-or-none basis. E impulses were not recorded from the

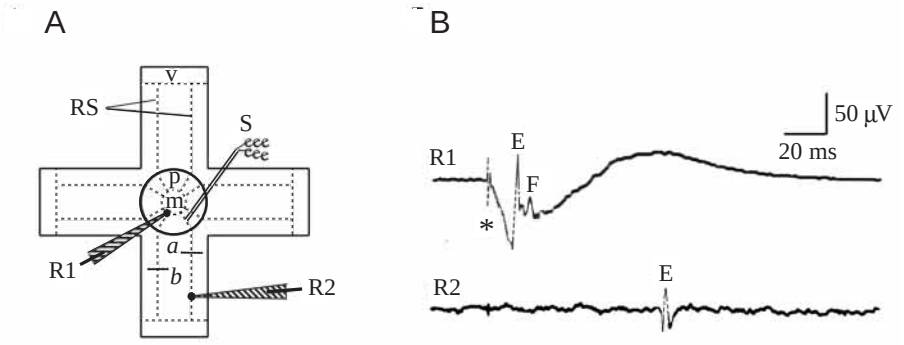

Fig. 7. Circular propagation of E impulses at the margin. (A) Representation of a quartered, splayed preparation with the stimulating electrode (S) located on a radial strand in the peduncle (p) and the recording electrode (R1) on another peduncular radial strand. A second recording electrode (R2) was located on the stimulated strand close to the margin. This strand was cut between S and R2 at site *a*. A second cut at site *b* went through the ectoderm only, leaving the radial canal intact. RS, radial strand; v, velum; m, manubrium. The dotted lines represent tissues containing one or more radial and circular conduction pathways as described in the text. (B) A stimulus (asterisk) evoked both E and F events at R1 although only the E event arrived at R2. Thus the E impulse must propagate down one radial canal, around the margin and up other canals.

tentacles, but could be evoked by stimulating the tentacles close to the margin, so the tentacle endoderm may be part of the same system.

The arrival of an E impulse in the manubrium was signaled by a sudden, symmetrical flaring of the lips (Fig. 3G), along with a less obvious but equally symmetrical shortening of the whole manubrium and adjacent wall of the peduncle. Flaring and shortening can both be attributed to longitudinal muscle contraction. Compared with E impulses recorded from the canals, those recorded from the manubrium were large events, frequently greater than 100 μV, as in Fig. 6B. Presumably the primary E event is augmented here by current arising from secondary muscle depolarizations.

In addition to causing lip flaring and manubrial shortening, incoming E impulses frequently triggered local activity in the manubium, in the form of writhing and searching movements lasting for several seconds and accompanied by much irregular electrical activity, some of it probably artefactual due to movement of the tissue sucked in to the electrode tip. It appears that the manubrium has its own local pacemakers, which generate these movements, because they continued in the absence of further E input. In some preparations, a series of small, sharp spikes was discernible within this mass of activity. These may represent the output of a local pacemaker driving the writhing movements but we have not explored this aspect further.

E activity in the presence of food

To confirm that the E system fires in response to the presence of food at the margin, recordings were made from radial canals on opposite sides of the peduncle close to their junction with the manubrium. The manubrium was amputated close to this neck region to reduce spontaneous activity and to eliminate the possibility of input from chemoreceptors in the manubrial lips. 10 μl of the fluid supernatant from an euphausiid crushed in seawater was dropped on the tentacles and margin of one quadrant of a splayed preparation with intact tentacles. Bursts of E impulses were recorded from the two electrodes, each impulse appearing first at the canal on the stimulated side. This experiment was repeated with similar results on other quadrants and with the electrodes repositioned accordingly. The E impulses always arrived first at the electrode on the stimulated side. These findings agree with visual observations (see above) that suggest the presence of food at the margin leads to manubrial lip flaring prior to contact being made with the food, and that chemoreceptors are present at the margin.

E activity during ingestion

Animals ingesting large or unwieldy prey showed frequent lip flarings that briefly caused the lips to detach from the surface. They were followed by lunging movements that had the effect of advancing the lips and helping them encompass projecting bits of the prey. Lip flaring was also evoked by food juices in the absence of solid prey and was accompanied by E impulses, which propagated through the entire E system and could be recorded at the margin after a delay representing conduction time along the radial pathway (Fig. 8). This activity

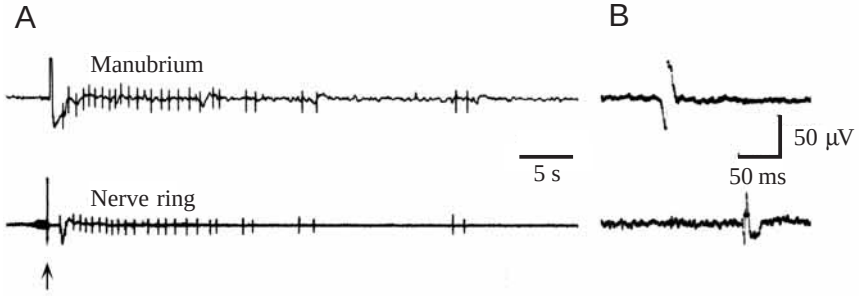

Fig. 8. E pulses evoked by crushed amphipod juice applied to the manubrium. (A) Amphipod juice was applied to the manubrium at the time indicated by the arrow. A train of E pulses was evoked, recorded with electrodes on the manubrium itself and on the marginal nerve ring. (B) A single E event on an expanded time scale. All E events in the train appeared in the manubrium first and were conducted to the margin.

all originated in the manubrium. Thus local excitation of the E system is a normal feature of the ingestion process. Also observed during ingestion were trains of smaller, sharper spikes that did not propagate down the canals. They showed high frequencies, being greater than 3 Hz during the early part of a burst. They were not studied in detail but may be regarded as the output of local pacemakers distinct from those generating E impulse bursts.

E activity and the inhibition of swimming

The inhibition of slow, rhythmic swimming during ingestion, described above, was abolished by cutting the radial canals but not by cutting the nerves in the radial strands, showing that it is mediated by the E system. Experiments similar to those shown in Fig. 5, but with the recording electrode placed over the ring canal, showed that a series of shocks that led to swimming inhibition all evoked E impulses at the margin. In *Polyorchis penicillatus*, epithelial impulses conducted in the exumbrellar ectoderm inhibit the neurons that generate rhythmic swimming by producing long-lasting hyperpolarizations in them (Spencer, 1981). It is likely that E impulses do the same in *Aglantha*, but we have not been able to record intracellularly from the equivalent neurons in our much smaller animal. On a few occasions, however, it was found that a series of shocks to the manubrium appeared to evoke rather to suppress slow swimming. The swim occurred approximately 600 ms after the shock that generated the last E event. Deducting 50 ms for conduction time along the radial pathway gives 550 ms for the latency between the arrival of the E event at the margin and the swimming event. Bearing in mind the long duration of the hyperpolarizations described in *P. penicillatus*, this long latency may well represent post-inhibitory rebound.

The F system and pointing

F impulses recorded along radial strands rarely had amplitudes exceeding 20 μV, and careful electrode positioning was required to see them at all. Their mean conduction velocity was 15.5±3.7 cm s^{-1} but it decreased with repeated stimulation. In one preparation, measured over a 15 mm stretch, the conduction velocity of the second of two shocks 250 ms apart was 73% that of the first. The small size of the F event is consistent with the small number of axons in the small axon bundle. The low conduction velocity is within the range expected for small-diameter axon systems in *Aglantha* (Mackie and Meech, 1995a). Direct recording with electrodes placed over the nerve rings confirmed that the events propagated circularly around the margin, as suggested by behavioural observations. F impulses continued to conduct circularly around the margin after the inner nerve ring had been cut. Cutting both nerve rings, however, blocked circular F conduction. Fig. 9 shows recordings from a quartered, splayed preparation with a stimulating electrode (S) placed on one radial strand and a recording electrode (R) on another near its junction with the manubrium. A cut through the radial strand at site *a* blocked that pathway to the manubrium. The upper trace (B1) shows propagation of both E and F events to the manubrium *via* circular pathways at the margin. The lower trace (B2) shows blockage of E events by cutting the circular canal at site *b*. This cut also went through the inner nerve ring, showing that F impulses can propagate circularly in the outer nerve ring.

F impulses entering the manubrium *via* one peduncular radial strand were found to emerge along all the other strands. Conduction across the manubrium between opposite octants was very slow, taking approximately 27 ms, probably because excitation has to travel *via* zigzag pathways through a dense, synaptic nerve net. This contrasts with the 8 ms latency found in the same preparations for E impulses which, as epithelial events, would encounter no synapses and travel by shorter routes. It is clear that, like the E system, the F system consists of a set of interconnected, through-conducting loops including tracts in the margin, radial strands and manubrium, and that impulses generated at any point in it will travel to all other points.

F events recorded from over the longitudinal muscles of the manubrium or from the extensions of these muscles that run up the wall of the peduncle were usually slightly larger than those recorded from radial strands. Sometimes, as in Fig. 6B, they showed after-potentials, presumably representing an evoked muscle response. There appears to be no local pacemaker system associated with pointing, however, as no continuing patterns of electrical activity were detected even

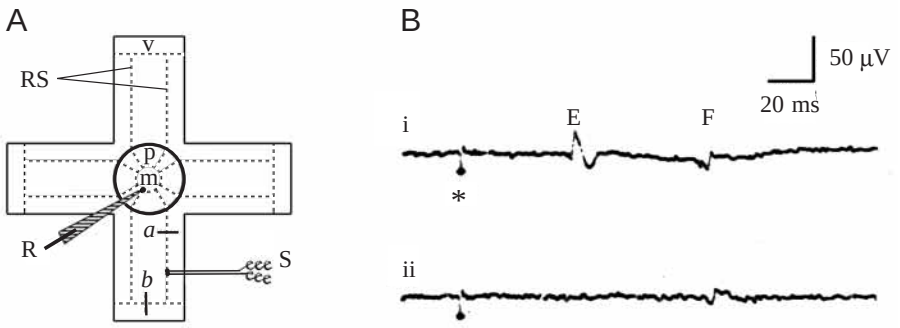

Fig. 9. Circular propagation of E and F events at the margin. (A) In a quartered, splayed preparation a stimulating electrode (S) was placed on one radial strand while a recording electrode (R) was placed on another near its junction with the manubrium (m). A cut through the radial strand (RS) at site *a* blocked that pathway to the manubrium. The dotted lines represent tissues containing one or more radial and circular conduction pathways as described in the text. p, peduncle; v, velum. (B) Upper trace (B1) shows propagation of both E and F events to the manubrium *via* a route that includes a circular component at the margin. Lower trace (B2) shows blockage of E events by cutting the circular (ring) canal at site *b*. This cut also went through the inner nerve ring, showing that F impulses can propagate circularly in the outer nerve ring.

when the electrode was placed directly over a visibly responding muscle band. The arrival of a single F event did not usually produce a perceptible amount of flexion, but two or three events arriving at 250 ms intervals produced visible, unilateral flexions and crinkling of the tissues around the junction of the peduncle with the manubrium. The flexions clearly represented contractions of specific bands within the longitudinal muscle field in contrast to E-evoked contractions, which were invariably symmetrical.

As described earlier, pointing could be triggered by a chemical stimulus applied to the tentacles and margin. If F impulses mediate pointing, they should be recordable as they travel along the radial strands to the manubrium after application of food stimuli at points around the margin. In a quartered, splayed preparation with intact tentacles (Fig. 10A) supernatant fluid from an euphausiid crushed in seawater was dropped on the margin of each quadrant in turn, with washes of clean seawater between each test. Two recording sites were selected on peduncular radial strands near the base of the manubrium. First, electrical stimulation was used to excite E and F systems and the recordings so obtained made it possible to differentiate E from F events by means of differences in their waveforms (Fig. 10B). Next, 10 μl of the fluid supernatant was applied to the tentacles of one quadrant. Trains of impulses, identifiable by their wave forms as F impulses, were found to propagate up to the manubrial base; in all tests they arrived first on the stimulated side (Fig. 10C). These tests confirmed that the margin and/or the tentacles contain chemoreceptors and showed that fluid extracts of appropriate prey can lead to trains of F impulses that propagate from the margin to the manubrium.

Histology

Radial pathways

The experimental evidence presented above shows that there are two conduction systems for electrical signals propagating between the margin and the manubrium and that one of these (E system) lies in the immediate vicinity of the endodermal radial canals and mediates lip flaring, while the other (F system) lies in the ectoderm overlying the radial canals and mediates pointing. We have examined the structure of these regions in particular detail.

Endodermal radial canals. The eight radial canals run from the margin up the subumbrella and down the peduncle, and open into the stomach compartment of the manubrium. The cells forming the wall of the radial canals (Fig. 11A, en) lack myofibrils and are interconnected by gap junctions (Fig. 11, inset). They bear flagella (not shown) that project into the canal lumen. Labeling with anti-tubulin and anti-FMRFamide (see below) failed to reveal nerves in the canals. Thin sections cut through canals in the subumbrella and peduncle and examined by transmission electron microscopy showed possible axon profiles in one or two places, but there were certainly no continuous nerve tracts or bundles running in the endoderm, and in most sections nothing resembling axons was seen at all. From these findings we conclude that the endodermal epithelium itself is an excitable tissue, as reported for several other hydromedusae, and conducts the E impulses. These events presumably propagate from cell to cell *via* gap junctions as in *P. penicillatus* (King and Spencer, 1979).

Ectodermal nerve bundles. We confirmed that in each octant a bundle of small axons runs up the subumbrella from the margin, lying close to the motor giant axon (Fig. 11A,B). While the giant axons terminate near the apex of the subumbrellar cavity, the small axon bundles continue around the apex, head down the peduncle and enter the manubrium (see Fig. 2A). We confirmed that axons in these bundles show FMRFamide-like immunoreactivity (FaIR), making the bundle as a whole easy to distinguish even where it runs very close to or beneath the motor giant axon, as the latter shows no FaIR (Fig. 12A). As the bundle travels down the peduncle, it maintains its coherence (Fig. 12B) until near the junction with the manubrium, where some of the

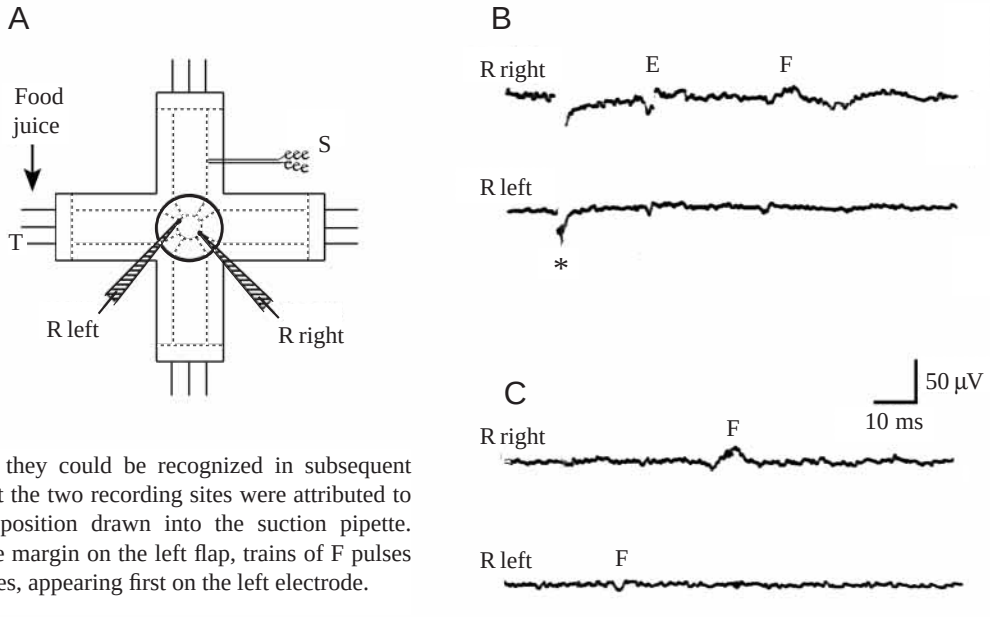

Fig. 10. Evocation of F events by food juices and their propagation to the manubrium. (A) In a quartered, splayed preparation recording electrodes (R) were placed on two radial strands within the peduncle equidistant from a stimulating electrode (S) on a third radial strand in a flap of body wall. Tentacles (T) were left intact. The dotted lines represent tissues containing one or more radial and circular conduction pathways as described in the text. (B) A control shock was used to establish the characteristic waveforms of E and F events so that they could be recognized in subsequent experiments. Differences in waveform at the two recording sites were attributed to differences in tissue volume and composition drawn into the suction pipette. (C) After application of food juice to the margin on the left flap, trains of F pulses were recorded at both recording electrodes, appearing first on the left electrode.

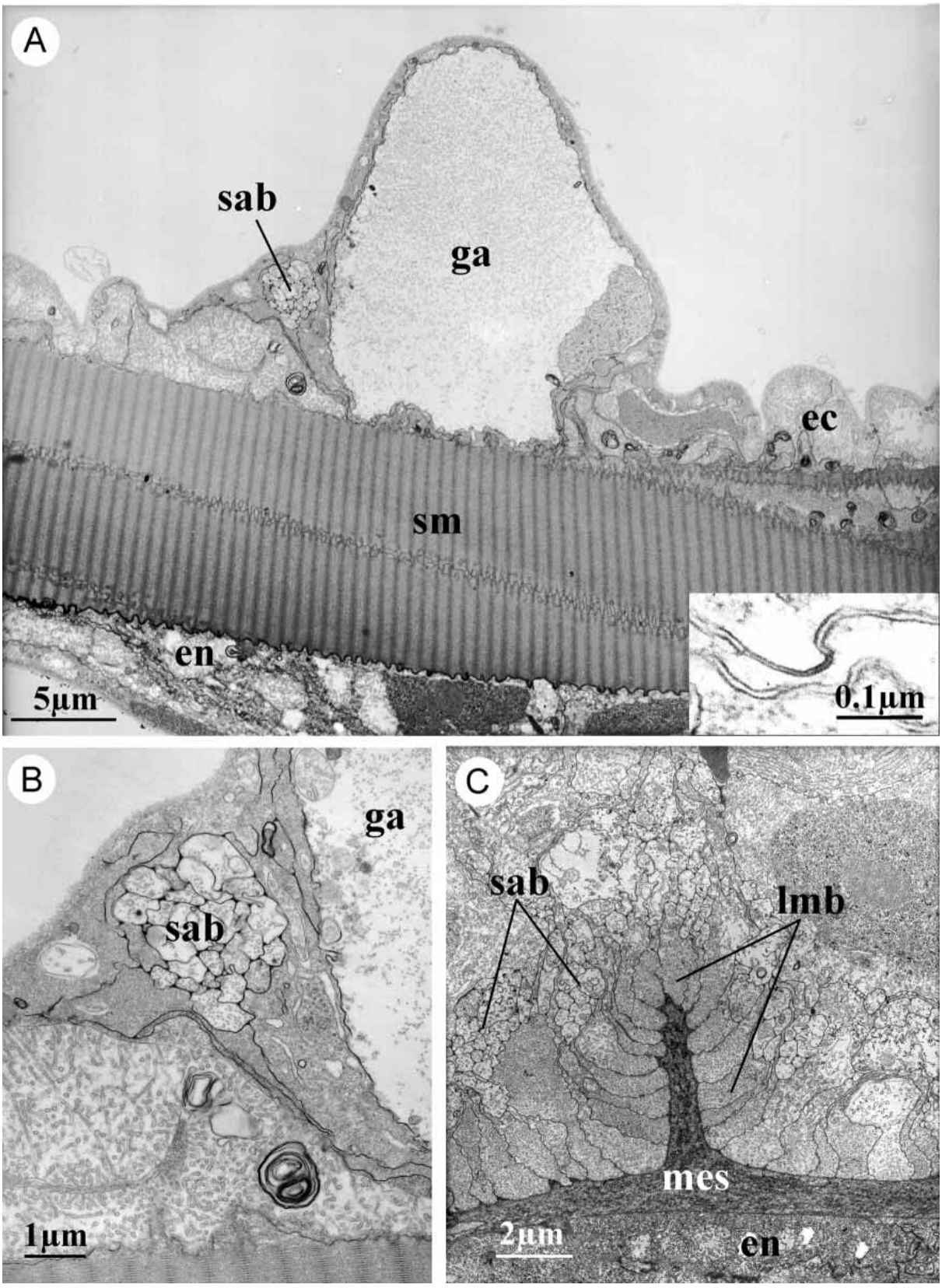

Fig. 11. Transmission electron micrographs of radial nerve pathways. (A) Transverse section across a radial strand as in Fig. 6. The scale bar lies within the lumen of the radial canal. The inset shows a gap junction in the endoderm of the radial canal. (B) Enlarged portion of A to show small axon bundle (sab). (C) Transverse section through a longitudinal muscle bundle in the ectoderm of the manubrium. ec, ectoderm; en, endoderm; ga, giant axon; lmb, longitudinal muscle bundle; mes, mesogloea; sab, small axon bundle; sm striated swimming muscle.

axons composing it fan out sideways. Even so all the divergent axons continue down into the manubrium along with the group of bundled axons (Fig. 12C).

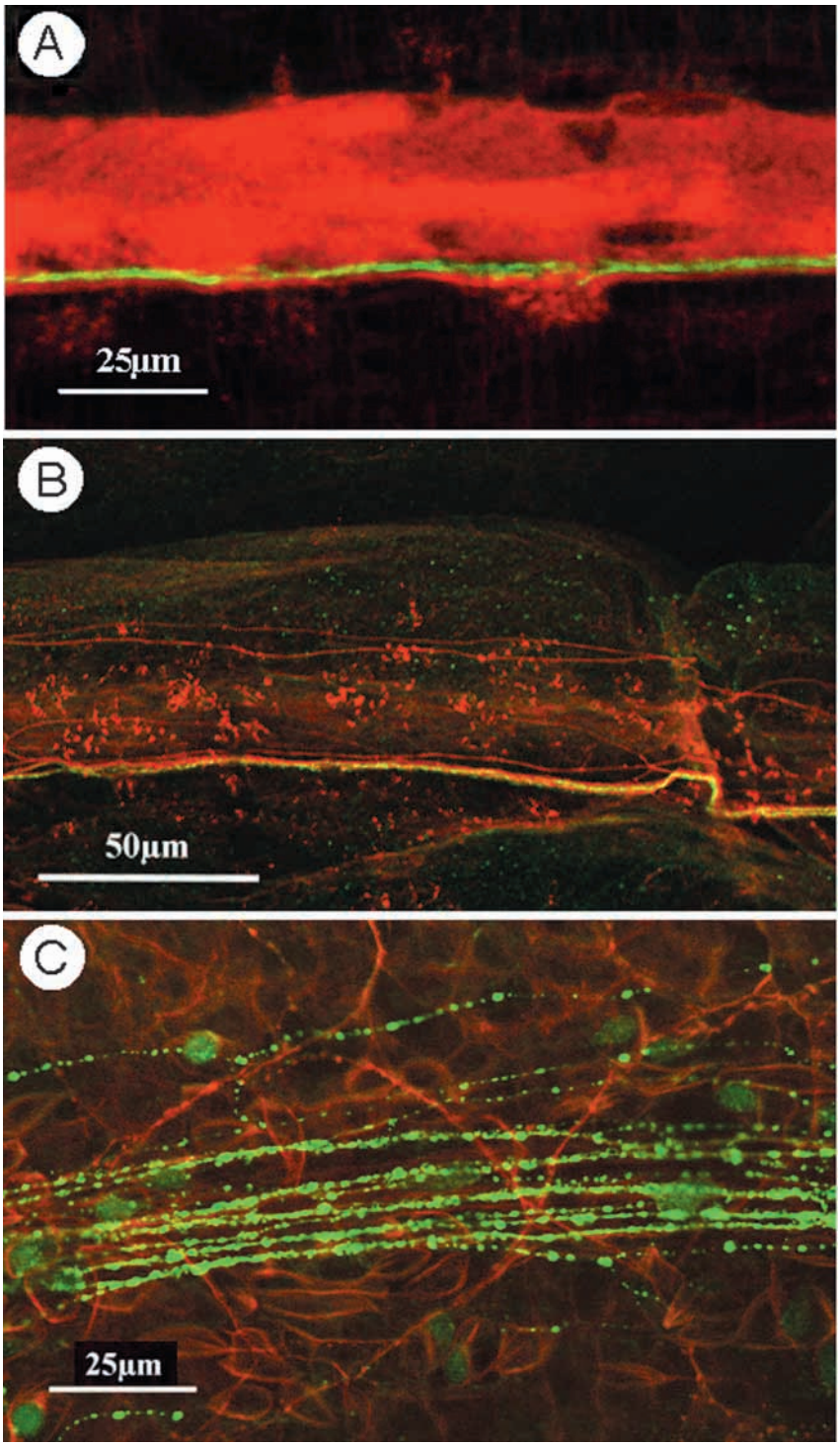

Fig. 12. Confocal overlays showing immunolabelled radial nerve pathways. Nerves showing FMRFamide-like immunoreactivity (FaIR) are green, other nerves red (anti-tubulin). (A) Subumbrellar radial strand showing the giant axon and the small axon bundle. (B) Peduncular radial strand showing small axon bundle and non-FaIR elements. (C) Manubrium, showing small axon bundle whose axons are now spread out, and underlying, non-FaIR plexus.

No connections were observed between adjacent FaIR nerve bundles in the radial strands even where they fan out in the peduncle before entering the manubrium. Cell bodies were never seen in the small axon bundle in its passage from margin to manubrium. Presumably the axons originate from cells in the manubrium or in the margin. Electron microscopy failed to show synapses between the axons in the small axon bundle or between them and other cells. Our transmission electron microscopy (TEM) sections showed 18–26 axons in the bundle close to the margin and a similar number higher up, near the apex of the subumbrellar cavity, but as the bundle entered the lower part of the peduncle and many of the axons diverged to either side, the number of axons in the bundle was reduced to 8–10. An early report by Roberts and Mackie (1980) that the small axon bundle '*contributes to a plexus in the subumbrellar myoepithelium*' is now known to be incorrect. Neurons certainly run out laterally across the muscle sheet, but they are connected directly to the motor giant axons not to the small axons (Weber et al., 1982; Kerfoot et al., 1985).

A second nervous component was observed in the peduncle using anti-tubulin immunolabelling. It was composed of neurites showing no FaIR. Like the FaIR component it was distributed in the ectoderm overlying the canals in the form of a plexus elongated in parallel with, but distinct from, the FaIR bundle and its divergent elements (Fig. 12B). No interconnections were seen between the plexuses of adjacent octants. The plexuses in question lacked cell bodies and appear to be aboral extensions of the non-FaIR plexus lying in the manubrial ectoderm.

Margin and tentacles

Ring canal. At the margin, the eight endodermal radial canals open into the structurally similar ring canal that runs around the margin. We have shown that all these canals conduct E impulses. Transmesogloeal epithelial bridges have been observed in sections of the margin, connecting the endoderm with ectodermal cells that lie close to, and sometimes envelop, bundles of axons in the nerve rings. These relationships are probably significant in the context of swimming inhibition, as discussed by Spencer (1981).

As E impulses can be generated by stimulation of the proximal region of the tentacles, the axial endoderm in this region is

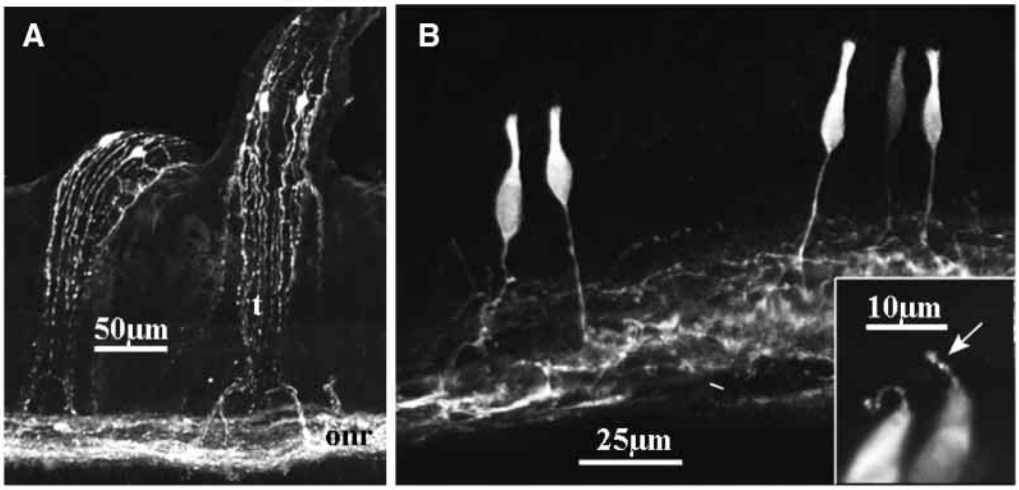

Fig. 13. Nerves associated with the outer nerve ring showing FMRFamide-like immunoreactivity (FaIR). (A) Outer nerve ring (onr) with tracts (t) leading to tentacles. (B) Monopolar sensory cells with their neurites running into the outer nerve ring. The inset shows sensory cilia (arrow) at the tips of these cells.

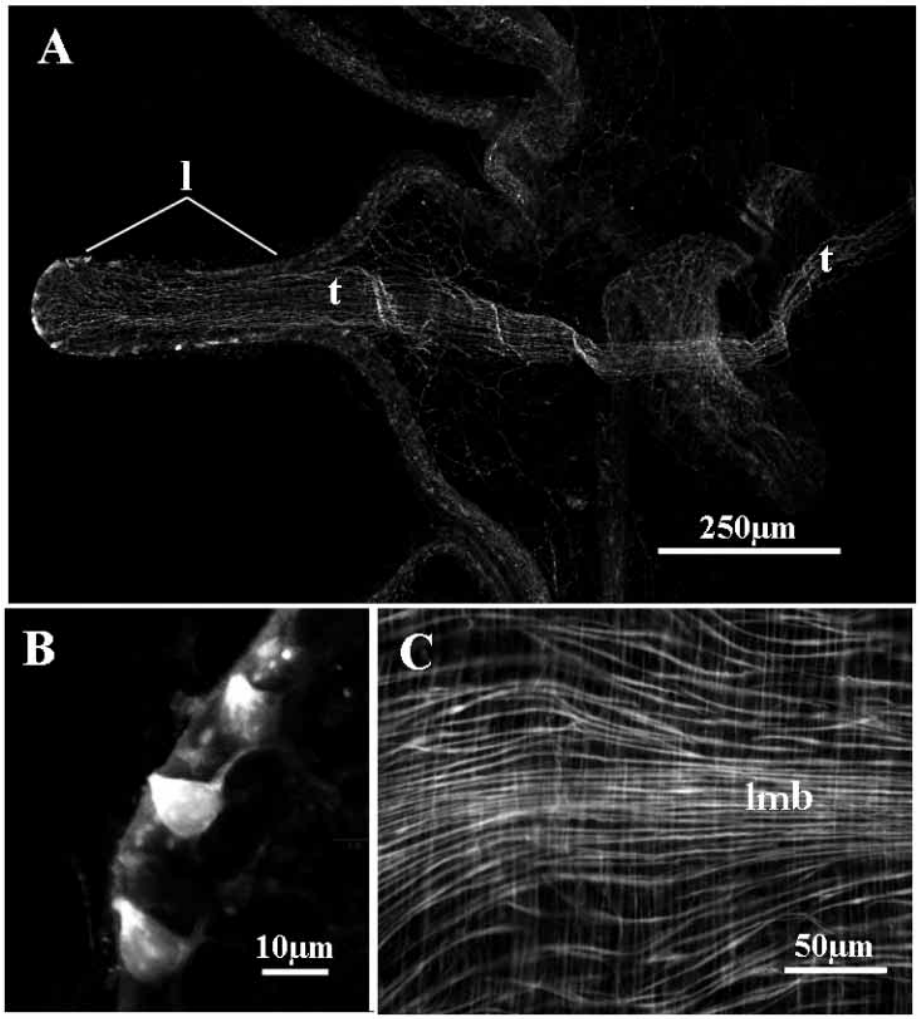

Fig. 14. Nerves and muscles in the manubrium. Nerves are labelled with anti-FMRFamide, muscles with phalloidin. (A) One of the four manubrial lips (l) with FMRFamide-like immunoreactivity (FaIR) in a nerve tract (t) running aborally. This tract becomes the small axon bundle that goes to the margin. (B) Sensory cells at the lip edge. (C) Longitudinal (horizontal) and circular myofibrils in the wall of the manubrium. The longitudinal muscles are concentrated into longitudinal muscle bands (lmb) that underlie the FaIR nerve tracts shown in A.

presumably part of the E pathway. The ring canal is in direct continuity with these cells.

Nerves. The radial nerve bundles fan out to left and right on entering the inner nerve ring. Some of the FaIR axons could be traced for short distances within the inner nerve ring but FaIR axons were most abundant in the outer nerve ring, where they were often traceable to sensory cell bodies (Fig. 13B). These correspond to the 'F cells' of Arkett et al. (1988). Neural connections between the two rings have frequently been seen in electron micrographs (e.g. Weber et al., 1982). Bearing in mind that the F impulses enter and spread circularly within the outer nerve ring, FaIR axons would be expected to cross between the two nerve rings, but this would be hard to demonstrate histologically without immunogold labeling. The only FaIR cell bodies observed in the nerve rings are those of the sensory cells in the outer ring. These therefore are probably the sources of axons entering the small axon bundles. We have confirmed that there is a rich FaIR innervation of the ectoderm of the tentacles consisting of sensory cells and their processes interconnected in the form of a nerve net with connections to the nerve rings (Fig. 13A). Some of these axons might also contribute to the radial, small axon bundle.

The observed distribution of FaIR sensory cells and axons in the margin, so far as it goes, is fully consistent

with our findings that chemosensory elements exist in the margin and tentacles and generate F impulses that propagate to the manubrium. The FaIR sensory cells seen in the outer nerve ring and tentacles would presumably be the sensors in question and their axons would carry F impulses circularly around the margin and up the small axon bundles.

Manubrium

The manubrium is a tubular structure whose proximal portion ('stomach') is where digestion takes place. The distal portion is formed into four lips around the mouth. By fixing manubria in the process of ingesting food, or when distended with food undergoing digestion, it was possible to make whole mounts in which the manubrial lips or stomach walls were stretched out as thin, flat sheets, allowing ready visualization of both nerves and muscles.

Phalloidin staining showed smooth muscle fibres running circularly in the endoderm and longitudinally in the ectoderm. The former were distributed evenly throughout the manubrium but the latter showed prominent local concentrations within a general distribution (Fig. 14C). Eight of these concentrations (longitudinal muscle bands) were observed, running from the oral margin to the narrow neck where the manubrium joins the peduncle and beyond the neck, up the sides of the peduncle and overlying the radial canals, for approximately 1 mm. The longitudinal muscle bands running up the sides of the peduncle were clearly continuous with those of the manubrium and can be regarded simply as extensions of the same system. Behavioural observations showed that pointing consists of a flexion at the neck region along with curvature of the walls of both the peduncle and the manubrium above and below the neck. Such unilateral flexions presumably represent contractions focused on the longitudinal muscle bands on one side.

In similar flat whole mounts it was possible to follow the eight FaIR nerve tracts that enter the manubrium from the peduncle (Fig. 14A). Four of the tracts ran all the way to the extended corners of the mouth. The other four fanned out into a more diffuse net-like array before reaching the edges of the mouth, but all eight nerve tracts were well-defined concentrations of axons for most of their length, each of them lying immediately over a longitudinal muscle bundle. The eight tracts were festooned with cell bodies and it was confirmed that the edges of the lips bear a well-defined row of FaIR sensory cells (Fig. 14B). The FaIR nerve tracts were interlinked in the manubrium by a sparse, diffuse plexus containing cell bodies, but the great majority of FaIR axons were precisely aligned along the muscle bands.

The non-FaIR nerves observed in the peduncular radial strands appeared to originate from scattered cells in the manubrial ectoderm. These were few in number and there was no evident concentration of non-FaIR axons associated with the muscle bands. A distinct network of fairly thick non-FaIR elements, probably axon bundles, was observed in the manubrial endoderm (Fig. 12C).

From these observations it seems fair to conclude that the FaIR nerves entering *via* the radial, small axon bundles are responsible for the selective motor innervation of individual longitudinal muscle bands, that underlies the pointing response.

Electron microscopy of the manubrium confirmed the presence of the circular and longitudinal muscle layers seen by light microscopy, the eight longitudinal muscle bundles and concentrations of axons associated with them (Fig. 11C). Neuromuscular synapses were not seen. Nerve processes were frequently seen in the manubrial endoderm, where they ran in close proximity to the contractile portions of the myoepithelial cells.

Discussion

Main components of feeding behaviour

We can summarize the main features of the sequence of events involved in feeding, as we now understand them, as follows:

Prey contact the tentacles. Contact evidently evokes nematocyst discharge because the prey are paralysed and held to the tentacles by nematocyst threads.

The tentacles shorten and curl round toward the margin. These flexions are almost certainly mediated by the slowly conducting tentacle nervous pathway described in earlier papers (Mackie and Meech, 1995a,b). Sensory cells are present in the tentacles, but it is not known what role they play or whether nematocyst discharge may itself be sufficient to trigger the movements.

Epithelial (E) impulses are triggered at the margin. They propagate *via* the radial endodermal canals to the manubrium, where they cause symmetrical flaring of the lips. This is followed by writhing, 'searching' movements of the whole oral area.

At the same time, F impulses are also generated at the margin. They propagate in the radial, ectodermal small axon bundles to the manubrium, where they cause directional pointing of the manubrium toward the site where food is held. Chemoreceptors in the tentacles and margin probably play a part in evoking pointing.

Pointing brings the mouth close to the margin, and lip flaring and searching movements bring the lips into contact with the food. The lips seize the food and start to spread around it, pulling it away from the tentacles. Nematocysts around the oral margin may assist attachment as well as stunning still struggling prey. When the food is secured, pointing ceases and the manubrium returns to its resting position.

Ingestion. Ciliary action causes the lips to advance over the prey and the lips continue to flare periodically, detaching from the surface and then reattaching further along. These movements are generated locally in the manubrium and are accompanied by E impulses, which propagate throughout the E system. Ingestion is further assisted by peristaltic contractions of the manubrial wall that propel food into the digestive chamber. Peristalsis is attributable to waves of contraction in the circular muscles of the manubrium. By

narrowing the manubrium, they also probably help push the lip region forward over the prey when the lips briefly become detached from the surface as a result of flaring.

Inhibition of swimming. E impulse trains generated in the manubrium during ingestion propagate to the margin and cause temporary inhibition of slow swimming.

Termination of ingestion. Once the food is fully engulfed, the mouth closes and the lips return to their resting position.

Digestion. Food is broken down enzymatically in the stomach, while the stomach performs peristaltic churning movements. Peristaltic waves originate just behind the mouth and travel aborally, constricting and elongating the manubrium. After passage of a wave of peristalsis, the manubrium shortens and swells. These movements, along with ciliary beating, drive partially digested food down the radial canals. Digestion continues intracellularly throughout the endoderm.

Egestion. Reverse peristalsis and lip flaring expel undigested material.

The E pathway and Aglantha*'s version of the 'crumpling' response*

This study of *Aglantha* provides the first evidence that endodermal epithelial conduction pathways (the radial and ring canals) may play a part in mediating feeding behaviour in hydromedusae. These canals are excitable in nearly all hydromedusae examined to date, but they have previously been implicated only in the protective 'crumpling' response. Adult *Aglantha* cannot crumple in the usual sense as they lack the subumbrellar radial muscles that are the main crumpling effectors, causing involution of the margin, in other hydromedusae. *Aglantha* retains an excitable endoderm, however, and the E impulses carried by this system excite the longitudinal muscles of the manubrium and peduncle, producing symmetrical shortening and lip flaring during feeding (summarized in Fig. 15).

It is of interest that in both *Sarsia tubulosa* and *Stomotoca atra*, longitudinal muscles in the peduncle and manubrium contract symmetrically during crumpling (Mackie and Passano, 1968; Mackie and Singla, 1975) though lip flaring has not been reported. It appears then that the E-mediated responses seen in *Aglantha*'s feeding behaviour may have evolved as a modification of the ancestral, crumpling response. The same epithelial conduction pathways and some of the same effectors are common to both. Except perhaps in the early post-larval stage (Mackie and Singla, 1997), *Aglantha* does not respond to potentially damaging stimulation by protective involution and withdrawal of vulnerable parts into the bell interior like a typical medusa, but it has developed an

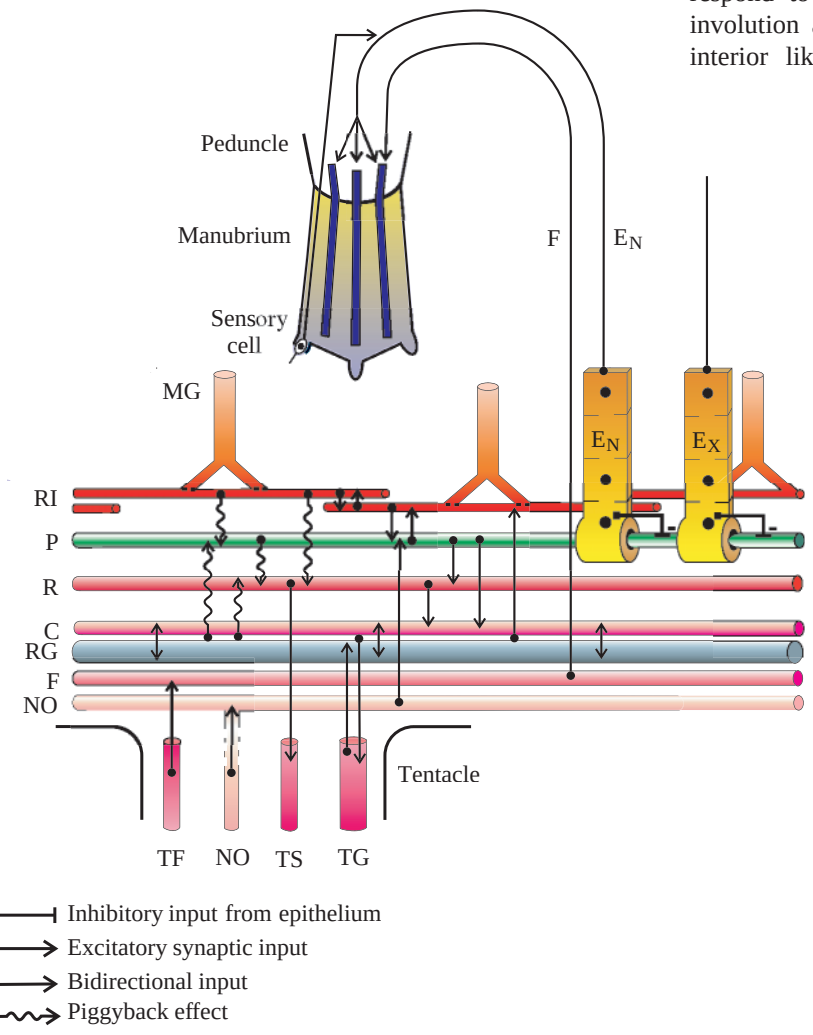

Fig. 15. The principal pathways involved in locomotion, the control of tentacle contractions and food manipulation. Three of the eight longitudinal muscle bands lying in the wall of the peduncle and manubrium are shown. The diagram is based on Mackie and Meech, (2000), but includes two new pathways, the flexion system (F) that mediates the pointing responses seen during feeding, and the endodermal, epithelial system (E_N) that mediates lip flaring and swimming inhibition (*via* the swimming pacemakers system, P). The exumbrellar epithelial conduction system (E_X) also inhibits swimming, but impulses do not pass between it and E_N, so the two are shown as functionally separate systems. The cells in these epithelial pathways are coupled by gap junctions, shown as incomplete membrane partitions, and conduction in them is unpolarized. Impulses spread in all directions in E_X while in E_N they spread laterally along the ring canal and up or down all eight radial canals (not shown). E_X is not the subject of this paper and in the text 'E' simply refers to E_N. MG, motor giant axon; RG, ring giant axon; TF, tentacle nerves that feed into the F system. The experimental basis for other components of the nervous system, the carrier system (C), the nitric oxide pathway (NO), the relay system (R), the rootlet interneurone system (RI) and the tentacle systems (TG, TS), are given in Mackie and Meech (1995a,b, 2000).

alternative protective response – escape swimming – that makes crumpling unnecessary. This has allowed it to retain and modify certain crumpling components for use in feeding behaviour. Lip flaring and the ensuing (locally generated) searching movements are set off by E impulses transmitted from the margin and increase the probability of contact being made between the lips and the prey. Lip flaring continues to be exhibited periodically during ingestion and assists advancement of the lips around the food. Lip flaring is also seen during rejection and egestion of food materials.

The inhibition of swimming associated with E impulses also finds a clear counterpart in other hydromedusae. *Aglantha* itself has an excitable exumbrellar epithelium, impulses in which inhibit swimming (Mackie and Singla, 1997), and the same is true in many other medusae and siphonophore nectophores (Spencer and Schwab, 1982). Such responses are typically evoked by collision or contact with external objects and, like crumpling, are considered to be protective. In *P. penicillatus*, the epithelial impulses hyperpolarize the swimming pacemaker neurons, inhibiting swimming for up to several seconds. It is not clear exactly how these hyperpolarizations are brought about. Epithelial processes envelop the swimming neurons but do not synapse upon them. An electrical field effect may be responsible. Whatever the mechanism, it is likely that the same process occurs in *Aglantha* and applies both to excitation arriving *via* the exumbrellar ectoderm (E_X in Fig. 15) and *via* the subumbrellar endoderm (E_N in Fig. 15). In the latter case, excitation must cross from the endoderm to the ectoderm before it can hyperpolarize the swimming pacemaker neurons, and presumably does so *via* transmesogloeal epithelial bridges, which are known to exist at the margin.

The inhibition of swimming that accompanies feeding in *Aglantha* is probably not protective in the usual sense, but seems to be an adaptation serving to facilitate transfer of food from the margin and its subsequent ingestion. The jerky movements of a swimming animal would make it harder for the manubrium to attach to prey successfully. Here too then, *Aglantha* has adapted a basic medusan response for a novel purpose.

We have seen that E pulses propagate in both directions along the radial canals during different phases of normal feeding behaviour. They travel from the margin to the manubrium, communicating information that the tentacles have caught prey, and setting off lip flaring and searching movements. Then they travel in the reverse direction at a slightly later stage in feeding, ‘telling’ the swimming pacemakers to cease firing while the manubrium is trying to ingest food. Of course the first set of E-impulses should also affect the animal’s swimming rhythm, but it is difficult to test this because it is rare to get an animal that swims with such metronomic regularity as to permit us to measure changes associated with the first lip-flaring.

Questions remain as to how E pulses, which are endodermal epithelial events, bring about contractions in the ectodermal muscles of the manubrium that cause lip flaring. Transmesogleal epithelial bridges have been seen in the manubrium, and so excitation could cross between the layers and excite the longitudinal muscles directly. Work on the *P. penicillatus* crumpling response, however, strongly suggests that in this case epithelial impulses, having crossed transmesogloeal epithelial bridges to the ectoderm, then excite nerves that innervate the muscles (King and Spencer, 1981). Some such process probably occurs in the manubrium of *Aglantha*, given the apparent involvement of local neural pacemakers in generating searching movements following the arrival of E events. Likewise, the E impulse trains that travel from the manubrium to the margin during swimming inhibition may originate in neurosensory cells in the manubrial ectoderm (as shown in Fig. 15) and pass from there *via* transmesogloeal epithelial bridges to the endoderm. Interactions of a very similar sort are believed to take place in the stems of physonectid siphonophores (Mackie, 1976; Fig. 9).

The F system and pointing

Pointing is mediated by the F system, a set of neural pathways that excite the manubrial longitudinal muscle bands selectively, in contrast to the E system that mediates symmetrical responses involving all the muscle bands (Fig. 15). Whereas E-mediated responses in the manubrium probably involve local neural pacemakers whose activity can be triggered by the arrival of E events, the F pathway appears to excite the muscles directly without local pacemaker involvement. The symmetry and generality of the E-mediated responses is typical of responses spread by excitable epithelia. The F system, by contrast, is an example of a ‘labeled line’ system, as found in all higher nervous systems, and considered to be one of the main reasons why nerves evolved (Horridge, 1968).

The pointing response shows a high degree of precision. When food is captured at a point on the margin where a radial strand joins the margin, pointing to that site must result from F impulses traveling straight up that strand to the muscle band located at its end. Where the marginal site is equidistant between two radial strands, two lateral muscles would have to contract equally. Where the site is closer to one radial strand than the other, the two muscles would both contract but one would contract sooner or more strongly than the other. Clearly, the distances traveled by the F impulses are all-important. The muscle band that is closest to the food site will be first to receive F excitation and it will contract most strongly.

The only trouble with this model is that F impulses travel not only by the most direct routes but also spread around the entire periphery and enter the manubrium by all eight radial strands. Why therefore do all eight muscle bands not contract equally in rapid succession, defeating any tendency to point directionally?

This question cannot be answered conclusively at present but, in an animal in which the distance from the margin to the manubrium along a radial strand is 18 mm, and in which impulses travel at 15 cm s^{-1}, an F impulse generated at the margin will take 120 ms to reach the manubrium by the most

direct route. To travel *via* an adjacent strand, it will first have to go around the margin for a distance of approximately 2.2 mm. This will add 15 ms to the conduction time. To reach the radial strand opposite the one providing the most direct route will require an additional 60 ms, giving a total conduction time of 180 ms in this case. These differences in arrival time of impulses will ensure that contraction will start first in muscle bands closest to the food site, and this alone may be enough to initiate flexion toward that point.

In addition, however, pointing seems to require repetitive firing of the F system, and we have found that the second of two impulses evoked 250 ms apart travels more slowly than the first: the velocity of the second was found to be 73% of the first. A second impulse initiated 250 ms following the first will therefore travel at only 11 cm s^{-1}, and take 160 ms to arrive at the manubrium by the most direct route and 243 ms *via* the least direct route. The two impulses will in fact be 370 ms apart after traveling by the most direct route but 493 ms apart going by the least direct route. If successive F impulses arriving in the manubrium cause contractions that sum on a frequency-dependent basis, the summing effect in the muscle band closest to the food site will be enhanced relative to summation at other muscle bands, and this will tend to reinforce the directionality of pointing.

The small axon bundle

It is hard to find a counterpart to this structure in other cnidarians. Far from being a conventional nerve net, or even a condensed nerve net, we can speak of it as 'a nerve' in the same way that we speak of the sciatic nerve or the vagus nerve. It is a compact bundle of axons that do not appear to synapse with one another and whose cell bodies lie at the two ends. The bundles are not interconnected, so each bundle selectively excites a particular muscle. In that sense it is a motor nerve. However, the axons appear to originate as the basal processes of primary sensory neurons, so it is also a sensory nerve. Counterparts may exist in the marginal nerve rings where six physiologically defined neural pathways run in parallel (Mackie and Meech, 2000), but these are central systems that interact synaptically along their courses, whereas the small axon bundle is a peripheral nerve innervating effectors some 2 cm away in a large *Aglantha*, and making no synapses with other systems *en route*.

The bundle is reactive with antisera raised against FMRFamide, making it easy to follow in immunolabelled whole mounts. Where the bundle fans out on entering the inner nerve ring and again where it enters the manubrium, all the divergent axons show FaIR, so there is no reason to suppose that non-FaIR elements are included in the bundle. Members of the RFamide family of neuropeptides are believed to function as neurotransmitters or neuromodulators in cnidarians (Grimmelikhuijzen et al., 2002), but it is not known which particular members of the family are present in *Aglantha*, nor have their physiological roles been investigated. In our present, brief, TEM survey of the longitudinal muscles in the manubrium we were unable to see any neuromuscular junctions there and so we would have to consider some form of non-synaptic release.

While present evidence is too incomplete to justify much speculation, we hypothesize that the bundle consists of axons that function both as sensory and as motor neurons. Neurons combining the diagnostic ultrastructural characteristics of both occur in hydra (Westfall, 1973; Westfall and Kinnamon, 1978). We propose that FaIR sensory neurons in the margin and tentacles supply axons that travel *via* the small axon bundles to the manubrium, where they provide the selective motor innervation of the longitudinal muscle bands required for pointing. It is likely that both mechano- and chemosensory receptors provide this input. The effectors (longitudinal muscle bands) in the manubrium would respond to a neuropeptide released from the axons in their vicinity. The fact that the FaIR neurites form tracts immediately overlying individual muscle bands is in itself suggestive of a functional relationship. Non-FaIR neurons in the manubrium showed no such concentrations.

There remains a question regarding the role of the FaIR sensory cells in the manubrial lips and elsewhere in the manubrium. Their basal axons mingle with those entering the manubrium from the margin, so they too might contribute to the selective innervation of specific muscle bands, helping to maintain or fine-tune pointing flexions during the stage when the manubrial lips first contact prey at the margin. It also seems likely that they trigger the trains of E impulses seen during ingestion and evoked by food juices applied to the manubrium. Here, as noted earlier, a neuro-epithelial transition step is required, as the E impulses are propagated to the margin in the radial canals.

Similar questions regarding the passage of excitation between nerves and conducting epithelia, and *vice versa*, exist wherever we find excitable epithelia, not only in hydrozoans but in groups like salps, where two-way interactions of this sort have been nicely demonstrated by other workers (reviewed by Bone, 1997). This remains one of the most interesting problem areas for future research.

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