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Coordination in Physonectid Siphonophores

G. O. MACKIE

Biology Department, University of Victoria, Victoria, British Columbia, Canada

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Intracellular and extracellular recordings from the stem, gastrozooids, palpons, tentacles and nectophores of physonectid siphonophores are presented. The stem organization previously described for *Nanomia* applies with only minor differences to *Forskalia* and *Agalma*. The endodermal epithelium of the stem is shown to be the pathway for slow potentials.

Pumping cycles and feeding activities are organized locally in gastrozooids and palpons. Protective retractions are coordinated, probably through a direct nervous link with the stem. This is also true of tentacles. The ectoderm of bracts is a conducting epithelium; excitation in it can induce nervous activity in the stem, but the mechanism is unknown.

Impulse traffic between stem and zooids is erratic and breaks down rapidly with repeated stimulation. The motor centres of the nectophores are connected to the stem by a labile nervous link, but an alternative epithelial pathway exists.

INTRODUCTION

Siphonophores present something of a paradox, being colonies in the phylogenetic sense but developing and behaving as individual organisms. For E. O. Wilson (1975) "the resolution of the paradox is that siphonophores are both organisms *and* colonies"; they originated as colonies but have evolved as individuals. They have progressed further in this direction than any other animal colonies, and have achieved an advanced grade of construction by making organs out of what were originally individual zooids (Mackie, 1963; Wilson, 1975).

Efforts to understand the neurophysiological basis and extent of coordinated behaviour in physonects (Mackie, 1964) failed to advance beyond a certain point because of lack of information about the fundamental components of behaviour, the conduction systems and the mechanisms of effector control. The stem, as the central coordinating region, was therefore studied in detail by electrophysiological methods (Mackie, 1973b, 1976b). Though incomplete,

this approach has now yielded enough basic information to justify reconsideration here of some of the problems of coordination.

At the same time, various other coelenterate colonies have been studied by electrophysiological methods. The earlier work on hydrozoans was reviewed by Mackie (1973a) but new studies have since appeared on the hydroids *Proboscidactyla* (Spencer, 1974), *Hydractinia* (Stokes, 1974) and *Millepora* (de Kruijf, 1976a, b; 1977) and the calycophoran siphonophore *Hippopodius* (Bassot *et al.*, 1978). All these forms have through-conducting systems in the connecting stolons which coordinate protective responses of individual zooids. Food capture and feeding are carried on locally.

The electrophysiological approach has likewise been applied to problems of coordination in scleractinian corals (Anderson, 1976a; Shelton, 1975a, c; Shelton and McFarlane, 1976) and alcyonarians (Anderson, 1976b; Anderson and Case, 1975; Satterlie, Anderson and Case, 1976; Shelton, 1975b). All these forms have colonial conduction systems, considered to be nerve nets, which coordinate polyp retraction. Luminescence and rachis contraction accompany polyp withdrawal in some pennatulids and are also spread by the nerve net. In addition to the nerve net, a second "slow" colonial conduction system is present in hard corals apparently serving to promote expansion of the tentacles and mucus production. A slow system also occurs in pennatulids but its behavioural role is obscure.

The general picture then for coelenterate colonies is of local action systems organizing most of the activities of individual zooids with coordinating impulse traffic passing across the colony by way of one or more, nervous or non-nervous, conducting systems. The function served by these coordinating systems is usually protective in some sense, which would include escape locomotion in the case of pelagic colonies, and luminescence in pennatulids and some siphonophores. The response spread by the colonial slow system in hard corals is exceptional in lacking obvious protective significance.

Except in the coral *Goniopora* (Anderson, 1976a) impulses spread through the colony by through-conduction. This does not mean that the activities of any one zooid automatically spread to others. Much activity is purely local. Nor, as Horridge (1957) showed, does it mean that spreading responses spread everywhere without decrement. In *Porites* (Shelton, 1975a, c) the retraction response spreads in decreasing increments with each through-conducted successive impulse, perhaps because conduction delays increase with successive impulses causing lengthened interpulse intervals and reduced temporal facilitation at remote points.

What has not yet emerged from any of these studies is the precise nature of the blocking or filtering mechanism at the interface between the individual zooid and the colonial net, which regulates the traffic between the two. As Anderson (1976c) points out this mechanism must be crucially important in

enabling the zooid to carry on autonomous local behaviour with the same action systems which at certain times come under colonial control.

MATERIAL AND METHODS

Nanomia cara were collected at Friday Harbor, Washington, U.S.A., while *Forskalia edwardsii* and *Agalma elegans* were studied at Villefranche-sur-Mer, France. The *Agalma* material consisted of incomplete specimens retrieved from plankton hauls. Intact specimens were available of *Nanomia* and *Forskalia*, which were obtained directly from the sea without the use of a net. *Forskalia* was used only for study of the stem action systems. The terminology used here follows Totton (1965).

Electrical correlates of behaviour were recorded with fine polyethylene suction electrodes. Amplified signals were displayed on a Grass pen-writing oscillograph, or were photographed from the oscilloscope screen using a Nihon Kohden recording camera and Kodak KIND 1732 film, or a Polaroid camera using Type 107C film. Intracellular recordings were made from *Forskalia* using 50 MΩ glass microelectrodes filled with 3M KCl, in conjunction with a field effect transistor DC amplifier.

For anaesthesia, isotonic magnesium was added to the sea water, usually in the proportion one part to five (1:5 Mg/SW). This concentration does not affect conduction in the stem conduction systems, but it greatly reduces the amplitude of muscle twitch contractions.

Light-microscope observations were carried out with a Zeiss microscope equipped with phase contrast optics. Methylene blue staining by the method of Unna (Pantin, 1958) was used.

RESULTS

1. The stem

The stem (Figure 1) develops as an elongated zone in the middle of the oozoid and although it becomes regionally specialized for the budding of secondary zooids it retains its essential unity as a coordinating pathway for activities throughout the colony. The float develops as an aboral invagination in the oozoid and possesses the same conduction systems as the stem.

Histologically the stem shows a thick muscular ectoderm with the myofibrils arranged longitudinally on mesogloeal ridges (Figure 2). The endoderm is much thinner and, in *Nanomia*, possesses only a very rudimentary circular muscle component (not shown in Figure 2). In *Forskalia* this system is better

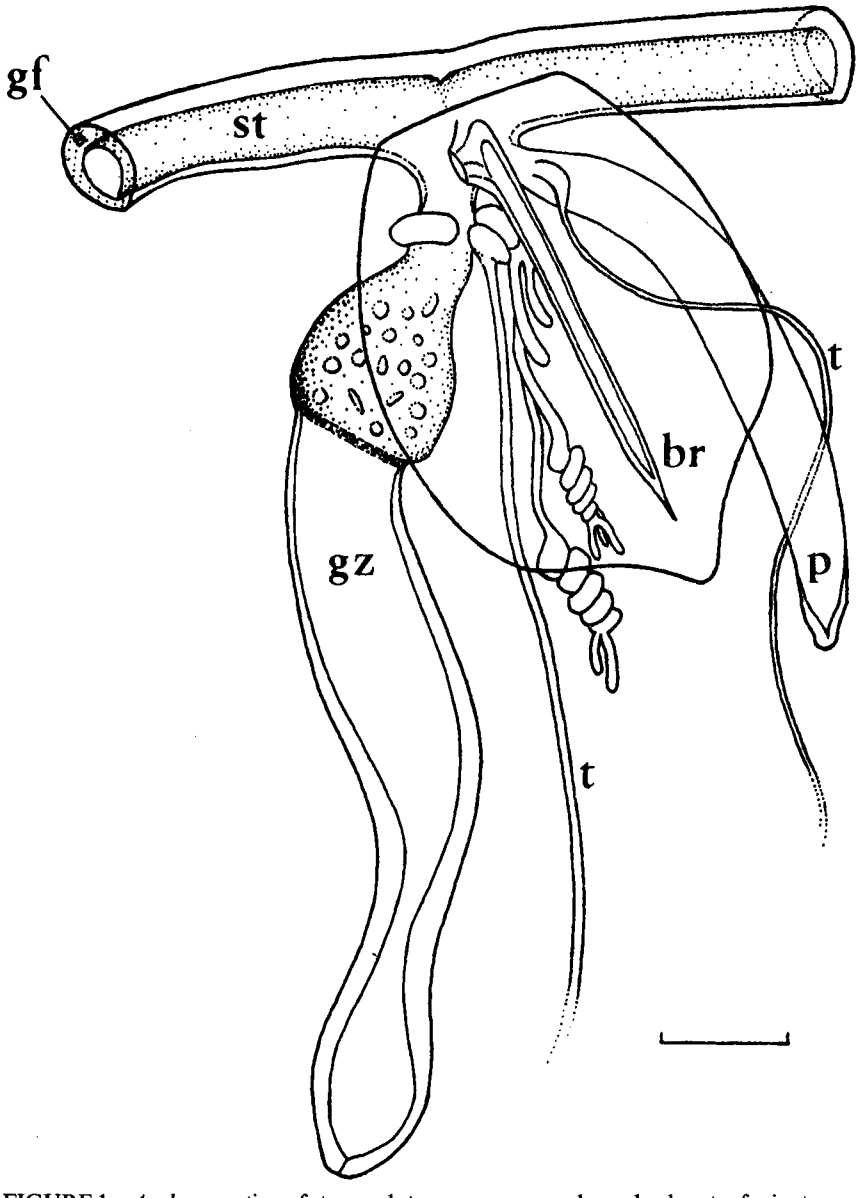

FIGURE 1 *Agalma*, portion of stem and stem-group app endages. br: bract; gf: giant nerve fibre; gz: gastrozooid; p: palpon; st: stem; t: tentacle. Scale: 1 mm.

developed. This circular system, first observed in *Physophora* by Korotneff (1884) may be responsible for the slow peristaltic activity of the stem. This

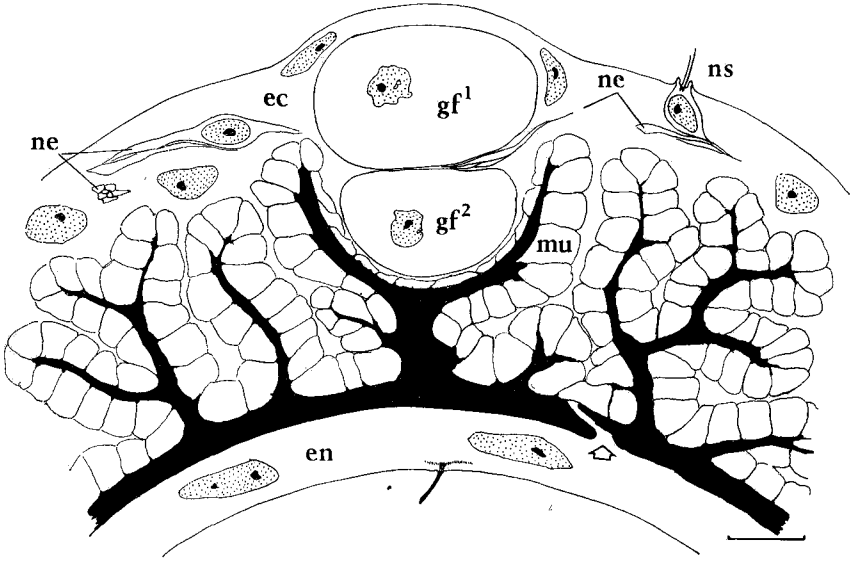

FIGURE 2 *Nanomia*, cross-section through the stem in dorsal midline. The boundaries between the epithelial cells are omitted. ec: ectoderm; en: endoderm; gf^1, gf^2: giant nerve fibres; mu: myofibril; ne: neurite of nerve plexus; the arrow shows an endodermal process crossing the mesogloea. Scale: 10 μ.

activity continues under deep magnesium anaesthesia. Cellular bridges connect the ectoderm with the endoderm across the mesogloea at numerous points.

The ectodermal nervous system consists of a diffuse plexus built up into two giant fibres (Korotneff, 1884; Schneider, 1892) along the dorsal midline (Figures 1, 2, 3a). Previous work (Mackie, 1973b, 1976b) has shown that the two giant fibres function independently as rapid conduction pathways and that each is associated with its own diffusely conducting peripheral component, presumably a portion of the diffuse nerve plexus. It has not been possible to distinguish these two sub-systems of the diffuse net clearly under the microscope, but phase contrast pictures (Figure 3c) indicate that many of the strands in the plexus are double. Possibly the neurites of the two sub-systems tend to associate in pairs, like the giant axons.

Methylene blue preparations (Figure 3d) show only those parts of the net close to cell bodies, and give no indication of the double strandedness seen with phase contrast. Some small neurons with fine neurites seen in methylene blue preparations may be neuro-sensory units. Korotneff (1884) described sensory elements in the stem, but their presence was denied by Schneider (1892) and Schaeppi (1898). Electron microscopy (C. L. Singla, unpublished) confirms their existence. Their presence accounts for the sensitivity of the stem to tactile stimulation.

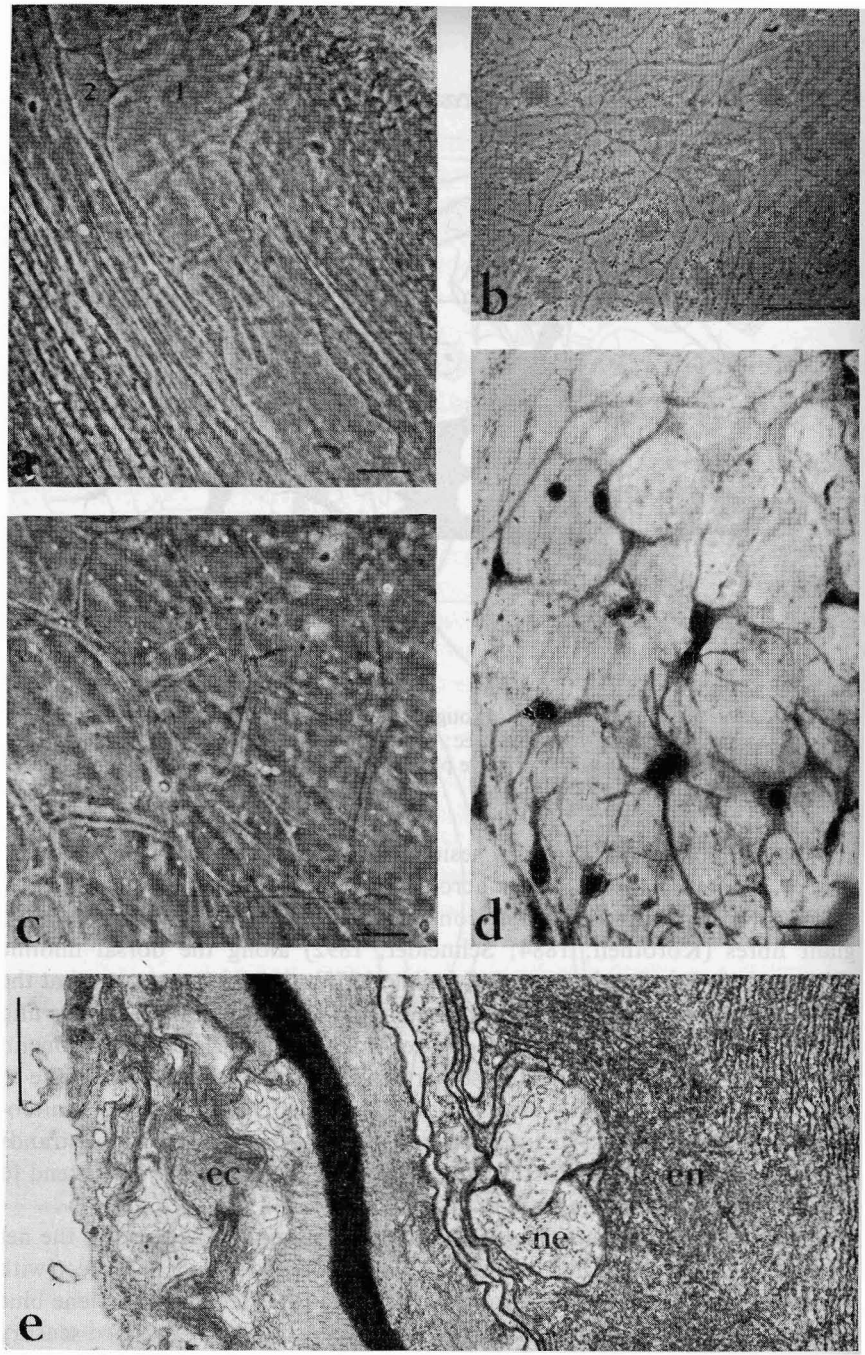

FIGURE 3 *Nanomia*, nerves and conducting epithelia: (a) whole mount of stem, phase contrast, showing the two giant nerve fibres. Compression of the tissue under the coverslip has deflected the superficial fibre (1) to one side, allowing the deeper one (2) to be seen; (b) bract ectoderm, an excitable epithelium. The small dots are mitochondria (phase contrast); (c) stem nerve plexus, phase contrast; plexus appears double stranded in places; (d) stem nerve plexus, methylene blue; (e) e.m. cross section through palpon wall. The dark line is the mesogloea. Ectoderm (ec) has longitudinal muscle, endoderm (en) circular. Neurites (ne) are shown only in the endoderm but occur in both layers. Scales: (a, c, d) 20 μ; (b) 50 μ; (e) 0.5 μ.

Physiologically, the stem exhibits three through-conduction systems. Two of these (n^1, n^2) are nervous, corresponding to the two giant fibres and their respective diffuse nets (Mackie, 1973b). The third system (Spencer, 1971; Mackie, 1976b) conducts slowly and is termed the *S* (slow) system. It had been considered on various indirect grounds to be the endodermal epithelium lining the stem cavity, but this was never proved by direct recording. *Forskalia* and *Agalma* have now been examined and prove to have the same three conducting systems. Conduction velocities for the three species are compared in Table I. Only short pieces of *Agalma* were available, and conduction values for the nervous systems were not obtained. The fastest value yet obtained for a siphophore, which is also the fastest coelenterate conduction velocity, is a value of 4.0 m/s (at 20°C) for n^1 in a large *Forskalia*.

TABLE I

Species	Vn^1	Vn^2	VS	temp. °C
Nanomia cara[a]	—	—	0.2	13
Nanomia cara	2.7	1.5	0.3	14
Forskalia edwardsii	3.7	2.0	0.4	20
Agalma elegans	—	—	0.3	20

Representative conduction velocities (*V*) in metres per second, nerves (*n*) and epithelial (*S*) stem systems.

[a] Spencer (1971).

Intracellular recordings from the n^1 giant fibre of *Forskalia* (Figure 4a) show a 95 mv spike and −60 mv resting potential. These values exceed the values previously reported for *Nanomia* (Mackie, 1973b) which were the highest of some rather low and variable values from fibres which may have been damaged. Action potentials recorded from neurons in the jellyfish *Polyorchis* (Anderson and Mackie, 1977) have amplitudes in the range 80–100 mv and resting potentials of about −60 mv, which again suggests that the *Nanomia* values were artificially low.

Attempts to record from the diffuse nerve plexus have been made in *Forskalia*. In one case, by probing at random, a neuron was penetrated, and it gave a few spikes closely resembling those obtained concurrently in a giant fibre.

Forskalia is much larger than *Nanomia* and it is possible to dissect the stem and to open it up so as to record from the endoderm. Extracellular recordings from the endodermal surface show *S* potentials as very large signals (8 mv in either polarity) compared with the same events recorded from the ectodermal surface, especially near the dorsal midline, where the ectoderm is thickest. Confirmatory evidence that the endoderm conducts *S* potentials was obtained by microelectrode recordings from a flap of stem laid out to the side with the endoderm exposed (Figures 4b, c, d). Electron microscopy (C. L. Singla,

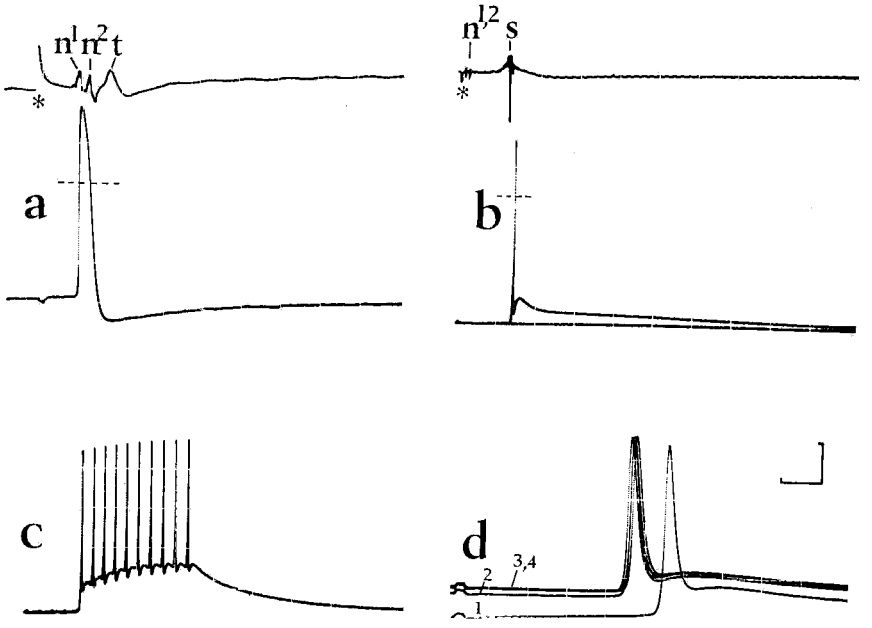

FIGURE 4 *Forskalia* stem in 1:5 Mg/SW to reduce twitch contraction amplitude: (a) a shock (asterisk) to the stem fires the two nerve nets (n^1, n^2) which causes a small muscle twitch potential (t), all three events monitored by a suction electrode on the surface of the ectoderm (upper channel). A microelectrode in the larger giant fibre records n^1 spike intracellularly (lower); (b) extracellular electrode (upper) records nerve potentials (n^1, n^2) and slow (S) potential following shock to the stem. Lower channel (intracellular) records S potential from an endodermal epithelial cell lining the stem canal; (c) intracellular record from an endoderm cell. A series of shocks at 30/s evokes a series of spikes whose after potentials sum progressively; (d) as in (c), four shocks at 4/s, sweeps superimposed, numbered in order, showing decreased conduction time of the later events in the series. Scales: (a) 0.2 mv (ex), 20 mv (in), 20 ms; (b) 0.5 mv (ex), 20 mv (in), 100 ms; (c) 20 mv, 100 ms; (d) 20 mv, 10 ms. Dotted line in (a) and (b) shows zero potential.

unpublished) fails to show any nerves in the stem endoderm (contrary to Schaeppi, 1898) so it can be concluded that the endoderm is a "pure" conducting epithelium, as earlier postulated.

The S potential recorded intracellularly has two main components, a fast, 85 mv, spikey, initial component and a 15 mv slow after depolarization. The resting potential is 60 mv. With repetitive stimulation late components sum (Figure 4c) and the conduction time of the whole event decreases (Figure 4d). This decrease is evidently due to reduction of initiation time at the stimulating site, the epithelium being residually depolarized from preceding events. It is interesting to note that conduction time also decreases with successive stimuli in the colonial slow conduction system of the pennatulid *Virgularia* (Shelton, 1975b), though not necessarily for the same reason.

These experiments were conducted with an external stimulating electrode. Attempts to fire the epithelium by intracellular current injection were unsuccessful, although this has recently been done in another conducting epithelium, that of the jellyfish *Euphysa* (W. Schwab, personal communication). The presence of two components in the active response recalls recordings from endoderm cells in the siphonophore *Hippopodius* where the late depolarization is associated with discharge of secretion (Mackie, 1976a). It is not known if secretion is involved in *Forskalia*, or if the late component is associated with another effector function. Another possibility is that the late depolarization represents a response of the ectoderm cells to which the endoderm is coupled.

It was shown in *Nanomia* that all three stem conduction systems can excite the ectodermal muscle. The evidence, though indirect, strongly indicates that the two nerve nets n^1 and n^2 excite the muscles through chemical synapses while the S system excites them through electrical junctions connecting the cells of the two layers; the same transmesogloeal couplings allow the endoderm to be reciprocally excited when the ectoderm is strongly depolarized (Mackie, 1976b). These interactions have not been investigated further in the present material, although the recordings from *Agalma* and *Forskalia* show very similar features to those which underlie the *Nanomia* model. In addition, neuromuscular junctions have been located throughout the ectoderm in *Forskalia* by electron microscopy (C. L. Singla, unpublished). Korotneff (1884) and Schaeppi (1898) talked of *bouton*-like nerve endings on the stem muscle fibres, but we find synapses to be of the *en passant* type; as in *Nanomia* (Mackie, 1976b) they are nearly always located in the superficial (non-muscular) layer of the ectoderm.

2. Gastrozooids

The gastrozooids and their basal tentacles (Figure 1) function in the capture, manipulation, ingestion and digestion of prey, and in the elimination of wastes. A review of the older literature on these activities, together with new data for *Nanomia* was given by Mackie and Boag (1963). Two or more gastrozooids may attach themselves to and partially ingest the same food object, but there is no indication that their activities are coordinated. Gastrozooids perform writhing, searching movements in the presence of food stimuli, and spread their mouths widely around food objects during ingestion.

Histologically, the gastrozooid shows the muscle arrangement typical of hydrozoan polyps, consisting of an outer, ectodermal layer of longitudinal muscle and an inner, circular layer lining the endoderm. Nerve plexuses are associated with both layers (C. L. Singla, unpublished), that of the ectoderm being equipped with sensory elements, as in the Portuguese Man-Of-War (Mackie, 1960).

Electrophysiological recordings from *Nanomia* (Figure 5) and *Agalma* show a pattern of signals associated with the rhythmic pumping movements which go on all the time in gastrozooids which are not engaged in feeding or egestion and are not protectively contracted. These movements consist of a peristaltic ripple which starts at the tip and runs down to the base, pumping fluids into the stem; this is followed by relaxation of the tip and contraction of the base. Fluid streams back in from the stem causing the tip to distend. After a short period of relaxation, a new peristaltic wave starts. At the very end of the emptying phase, just before filling starts, a burst of potentials is seen (Figures 5, 7). These events are associated with the basal contraction, and occur at the same time in the pumping cycle as do the "neck potentials" in tubularian

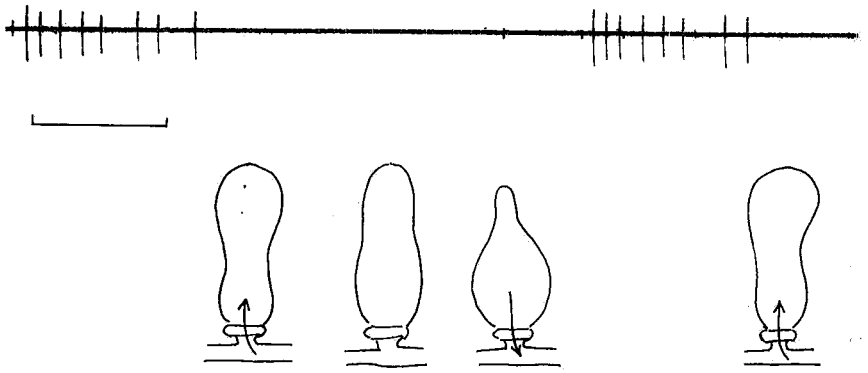

FIGURE 5 *Nanomia*, gastrozooid tidal cycle. Following a BP (basal contraction pulse) burst, the gastrozooid relaxes and fills with fluid from the stem. A peristaltic wave starting at the tip then drives fluid down into the stem again. The BP burst coincides with contraction of the base of the zooid during the final part of the expulsion phase. Scale: 15 s.

concert behaviour (Josephson and Mackie, 1965), but have a much smaller amplitude (50–200 μV). They originate at the base and are conducted to the tip at 7 cm/s (*Agalma*). They can be evoked by electrical stimulation in any part of the gastrozooid (Figure 6). The basal constriction appears to be due to contraction of the endodermal circular muscles and the potentials therefore probably represent activity in the endoderm, not, as in *Tubularia*, activity in both layers. They will be called basal contraction pulses (BPs). They are not blocked in 1:4 Mg/SW, a concentration which blocks neuromuscular transmission in the stem, and pumping rhythms continue unaffected in this solution. These observations suggest that BPs are endodermal epithelial potentials and that the rhythm is generated independently of the nervous system.

The peristaltic wave of the tidal cycle, and the peristaltic movements associated with ingestion and egestion are not accompanied by similar signals, although they too involve activity of the endodermal muscle system.

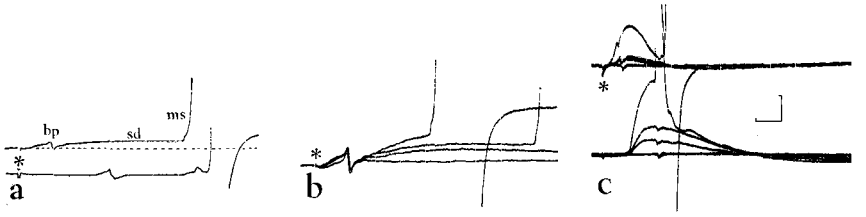

FIGURE 6 *Agalma* gastrozooid: (a) following a shock to the base of the zooid responses are recorded from an extracellular electrode near the base (upper) and from another near the tip (lower). BP: basal contraction pulse; MS: muscle spike; SP: slow depolarization; (b) same preparation, upper channel only, four shocks, 2, 3, 4 and 5 volts respectively, sweeps superimposed. With increasing shock strength a slow depolarization appears (3 v) and increases progressively giving rise to muscle spikes (4 v, 5 v) with decreasing latency. The BP appears unaffected by shock strength; (c) another preparation, stimulating the gastrozooid tip, recording from the tip (upper) and base (lower). Increasing shock strengths eventually give rise to a muscle spike at the base, which propagates back toward the tip. Scales: (a) 0.5 mv, 20 ms; (b) 0.2 mv, 20 ms; (c) 0.2 mv, 50 ms.

Stimulation of a gastrozooid causes a second type of electrical response in the form of depolarizations of long duration (slow depolarizations, SDs). In *Agalma*, these waves spread along the gastrozooid at 9.5 cm/s (Figure 6c). They are not accompanied by visible contractions. They can occur in the absence of BPs and are not related to the latter in any discernible way. Their amplitude varies directly with stimulus strength. Above a certain threshold the SD generates a large (8 mv) spike-like event (Figures 6b, c, 8c, d) and each event is correlated with a strong symmetrical contraction of the longitudinal, ectodermal muscle all around the zooid. This response will be termed protective retraction. The muscle spike (MS) appears to be an all-or-none event and it propagates in either direction at about 23 cm/s in *Agalma.* Spike threshold seems to be lowest near the base. Thus, a SD can propagate down the zooid to the base, where it generates a MS which spreads back up the zooid at its own, much faster velocity (Figure 6c). Above spike threshold, the stronger the shock, the larger the SD and the shorter the MS latency (Figure 6c). Both SD and MS are abolished in 1:5 Mg/SW.

It is apparent that the SD is a subthreshold response, presumably consisting of summed junction potentials, of the same cells which produce a propagated action potential (the MS), namely the cells of the ectodermal muscle layer. The fact that SDs are conducted more slowly than muscle spikes shows that another conduction system is responsible for spreading them. The "invisible" conducting system responsible for spreading SDs is doubtless the ectodermal nerve net. The amplitude and duration of the SD would depend on the number and frequency of the underlying nerve events. Although the nerve events themselves lie below recording threshold the SD, particularly in *Nanomia*, sometimes has an irregular form, consisting of a summating series of small

potentials, each of which probably represents junctional potentials at the neuromuscular junctions. Intracellular recordings will be needed to confirm this interpretation and to provide specific values for the spike and sub-threshold response parameters.

In the presence of food stimuli or reduced glutathione (Mackie and Boag, 1963) gastrozooids independently perform writhing movements, thrashing from side to side and abruptly changing in length. These movements must involve local or unilateral contractions in the same muscles responsible for protective retraction, but no specific electrical correlates have been associated with them.

None of the characteristic potentials associated with the gastrozooid are recorded in the stem, and none of the stem systems penetrate the gastrozooid. Stem events can be picked up in the gastrozooid pedicels, but these are functionally part of the stem, and, morphologically, are "miniature copies" of the stem, to use Korotneff's (1884) phrase. The true transitional region lies at the junction between the tentacle, pedicel and basigaster.

Pumping rhythms (BP patterns) are not coordinated between different zooids, and are independent of spontaneous stem events (Figure 7). Alternating tidal ebb and flow between a gastrozooid and neighbouring palpons has been reported (Mackie and Boag, 1963) but the electrical records show that these reciprocating rhythms arise fortuitously and are not maintained for long.

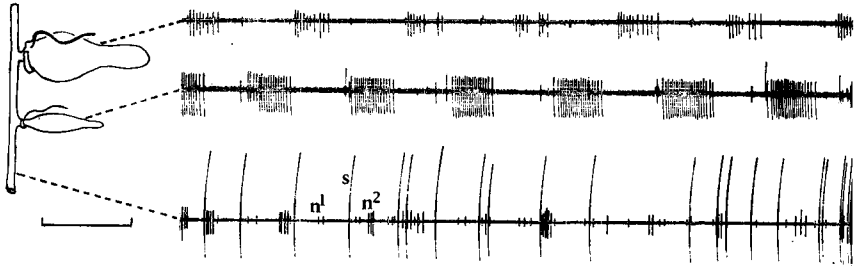

FIGURE 7 *Nanomia*, spontaneous rhythmic activities from gastrozooid (upper), palpon (middle) and stem (lower). The gastrozooid and palpon show independent BP bursts associated with their tidal cycles. The stem shows irregular activity in its two nerve nets (n^1, n^2) and slow (S) systems. Twitch potentials are suppressed by the anaesthetic. Scale: 1 min.

Stimulation of a gastrozooid can nevertheless lead to stem activity and vice versa (Figure 8). Stimuli which evoke sizeable SDs in the gastrozooid cause twitch events in the stem. Twitches, as noted, are graded depolarizations of the ectodermal muscle brought about by synaptically mediated input from the two nervous sub-systems of the stem. Excitation arriving from a gastrozooid must therefore first enter the stem nervous system before causing a twitch. The stem muscle cannot propagate events by myoid conduction. It is not necessary

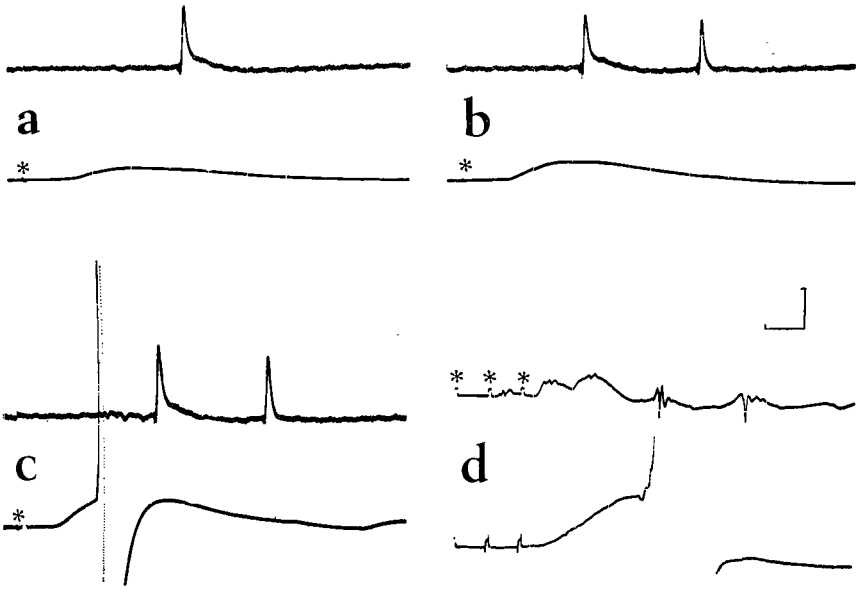

FIGURE 8 *Agalma*, gastrozooid–stem interactions: (a, b, c) stimulating on the tip of the gastrozooid, recording from its base (lower) and from the stem near the attachment point (upper). Events shown on the stem channel are *n*-induced muscle twitch potentials. Shock strength in (a) and (b) was 2 v, in (c) 3 v. Stimulation sufficient to produce a SD in the gastrozooid results in one or more stem twitch events, depending on SD amplitude, regardless of whether or not MS threshold is surpassed. Neither BPs in the gastrozooid nor *S* events in the stem were elicited in this case; (d) another preparation, three shocks at 50/s on the stem evoked a series of three facilitating twitches followed by two composite twitch-plus-*S* events. Activity spreads to the gastrozooid in the form of a large SD which reaches MS threshold. In the same preparation, two shocks at the same frequency led to a SD without an MS. Scales: (a, b, c) 0.2 mv (upper), 2 mv (lower), 50 ms; (d) 0.5 mv (upper, 0.2 mv (lower), 20 ms.

that an SD reach MS threshold for it to excite a twitch response in the stem (Figure 8a, b).

Either or both nervous sub-systems may be excited. There seems to be no consistent tendency for one to have a lower threshold than the other. The *S* system does not appear to be directly excited by input from the gastrozooid, although large twitches sometimes fire the *S* system secondarily as shown elsewhere. Since *S* potentials are conducted in the stem endoderm, and BPs (probably) in the gastrozooid endoderm, some functional connection between the two might be expected; but this seems not to be the case.

Flurries of nervous activity evoked by direct stimulation in the stem lead to depolarizations indistinguishable from SDs in the gastrozooid, and cause spiking if the SD reaches the necessary amplitude (Figure 8a). Communication between stem and gastrozooid is quickly lost with repetitive stimulation, and

a period of many seconds of rest must elapse before recovery. It is also blocked by magnesium.

3. Palpons

Palpons (Figure 1) are accessory digestive zooids which perform pumping cycles and protective withdrawals very similar to those seen in gastrozooids. They do not show feeding behaviour, and their muscle layers are relatively weakly developed. Both ecto- and endodermal (Figure 3e) nerve nets are present, and synapses have been observed between the units composing them (C. L. Singla, unpublished).

BP bursts accompany the tidal cycle as in gastrozooids and SD and MS events are elicited by stimulation. Communication with the stem works on the same two-way basis as in the gastrozooids.

4. Tentacles

Tentacles (Figure 1) grow from the bases of the gastrozooids and palpons and are best considered as parts of these zooids, not as separate zooids themselves, but like the tentacles of hydroid polyps, they have their own, local action systems. In *Nanomia* the palpon tentacles have two similar, fast conducting systems, and two chains of bipolar neurons which are assumed to be the two conduction pathways (Mackie, 1973b). These systems act synergistically upon the ectodermal muscle, causing graded twitch contractions as in the stem. The tentacle differs from the stem in lacking giant fibres and in having no equivalent to the *S* system, but in other respects it can be considered as a simplified model of the stem.

The tentacles do not take part in the rhythmic activity of the zooids to which they are attached; their conduction systems fire spontaneously, but no true rhythm has been observed.

Strong stimulation of the tentacles may cause firing of the n^1 and n^2 systems in the stem, which in turn may cause stem twitches. The *S* system may also fire. Conversely, tentacle contractions follow strong stem twitches. The n^1 and n^2 stem systems do not seem to be linked specifically with their counterparts in the tentacles. Conduction between the two regions fatigues easily, as in the case of gastrozooids and palpons.

5. Bracts

The bracts (Figure 1) are gelatinous structures lacking muscle and nervous tissue. The bract lamella, however, is muscular and can contract, causing autotomy. The bract endoderm is a narrow, blind ending tube, the ectoderm

a flat epithelial sheet (Figure 3b) enveloping the greatly expanded mesogloea. The ectoderm is an excitable epithelium which conducts at 0.25 m/sec at 20°C. Stimulation of this layer causes epithelial impulses to be transmitted to the stem, which responds by flurries of nerve spikes and evoked twitch potentials. This pathway is less sensitive to magnesium anaesthesia than are those between the stem and other appendages. While it is unclear how epithelial impulses enter the nervous system, it is clear that they do not pass via synapses, so magnesium would not be expected to block the pathway to the same extent as in synaptically mediated pathways. Transmission between bracts and stem does however show similar features to transmission from gastrozooids to stem, regardless of presumed differences in the mechanisms. The two nervous sub-systems are the primary stem units affected. One or both systems may fire, without apparent preference. The latencies vary erratically (Figure 9). This variability is unlikely to be due to initiation delay in the bract or to conduction velocity variations in the bract epithelium or nervous systems, so presumably it reflects lability at the interface between epithelial and nervous systems. Transmission only occurs predictably if an interval of several seconds is allowed between stimuli.

The conducting epithelium of the bract, like the excitable tail skin of a larvacean tunicate (Bone and Mackie, 1975), evidently serves as an extension of the sensory receptor field of the stem. There is no evidence that impulses travel in the reverse direction. The only effectors in the bract are nematocysts which are not fired by epithelial impulses, and the muscles of the bract lamella, connecting the bract to the stem. The latter are excited by stimulation of the bract, and probably not also by stem stimulation.

6. Nectophores

Physonectid nectophores are small, fragile and prone to undergo autotomy. They are inordinately difficult to record from in the intact animal, and despite several efforts to analyse their responses much remains unclear, including the intriguing mechanism whereby, as a group, the nectophores can be organized to swim either forwards or in reverse (Mackie, 1964). More progress has been made with calycophoran nectophores (Bassot *et al.*, 1978; Carré and Mackie, in preparation).

As reported previously (Mackie, 1964), a nerve tract runs across the exumbrella connecting the stem with the nerves in the nectophore margin. Sectioning this tract abolishes coordinated forward swimming, but the siphonophore can still swim backward in a coordinated manner, presumably because excitation reaches the motor centres via an epithelial pathway. In reverse swimming, the radial muscle bands (Claus' fibres) of the velum deflect the water jet forward. They are not active in forward swimming. The Claus fibres are directly linked to the epithelial conduction pathway through the nectophore and respond only

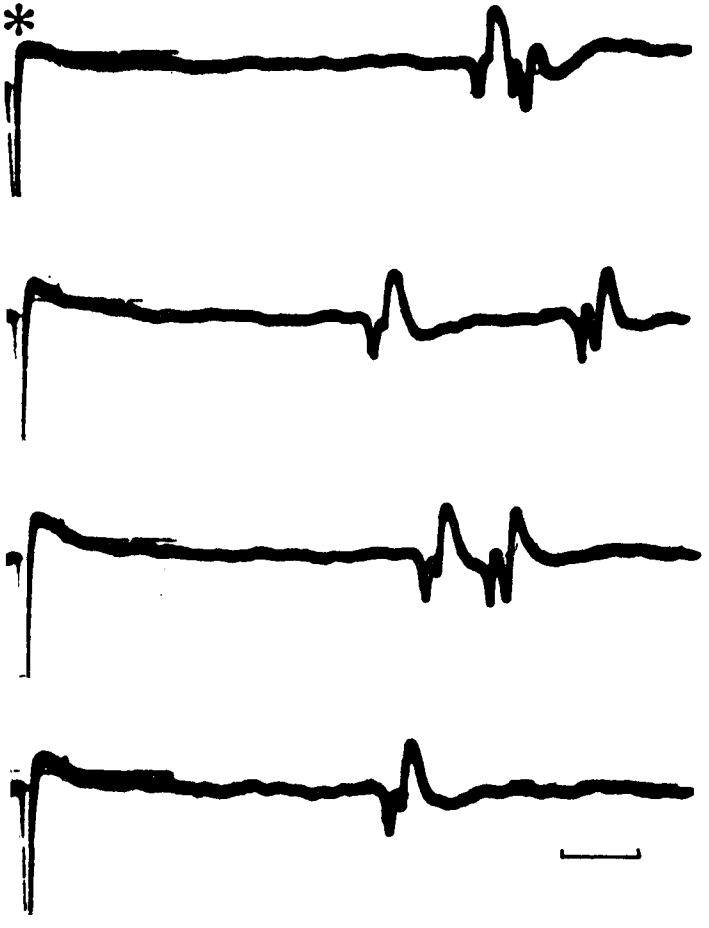

FIGURE 9 *Nanomia*, recording from the stem, stimulating on a bract in 1:7 Mg/SW to reduce muscle twitch component. The four responses were obtained successively without changing the electrode position or shock strength. n^1 and n^2, or n^1 alone were evoked with variable latencies. Scale: 10 ms.

when this system fires. The difference between forward and reverse swimming then resides in whether excitation arrives in the nectophore via an epithelial pathway or exclusively by way of nerves.

After eliminating the endoderm as a conduction route, excitation can still pass via the ectoderm (Mackie, 1964). However, the endoderm might still function as an alternative epithelial route in the normal animal; we now know this happens in the calycophores (Bassot *et al.*, 1978; Carré and Mackie, in preparation).

Bursts of nervous activity in the stem sufficient to cause sizeable muscle twitches, but not necessarily accompanied by *S* potentials, precede both forwards and reverse swimming in *Nanomia*. Two shocks to the stem 15–20 ms apart repeatedly evoked swimming in one responsive specimen. However, much isolated nervous activity can occur in the stem without any responses being picked up in the nectophore. It appears that there is a facilitation barrier at some point along the nervous route to the marginal motor centres. There is no indication of preferential involvement of one of the stem nervous systems. In *Nanomia* the nervous pathway across the nectophore consists of a single giant axon (Mackie, 1976b), but it is not clear if it is linked to n^1 or n^2 or to both. In *Forskalia* and *Halistemma*, the tract consists of several axons in parallel. The *S* system does not appear to penetrate the nectophore.

To resolve these uncertainties, a way must be found of preventing autotomy. Autotomy occurs because of intensive contraction of the ectodermal muscles of the nectophore lamella. These muscles are fired by epithelial impulses from the exumbrella epithelium so autotomy is easily provoked by attaching recording electrodes to the outside of the nectophore. Bursts of impulses in fact follow any contact with the exumbrella. They are conducted diffusely over the nectophore surface at about 30 cm/s at 20°C. Repetitive firing at a rate equivalent to 45/s has been observed during some of these bursts. Bursts of epithelial impulses spread to the stem and cause flurries of nervous potentials and associated muscle twitches.

DISCUSSION

The known or presumed locations of the conduction systems discussed in this paper are shown diagrammatically in Figure 10, along with an indication of the probable interaction pathways.

The results reported here support and in some respects confirm the model previously proposed for the stem. The demonstration that the endoderm is indeed the conduction path for the *S* system completes an important part of the picture. The way is now open for a more precise analysis of junctional events. It should be possible to explore the "piggyback effect" (Mackie, 1976b) by simultaneous intracellular recording from the ectoderm and endoderm. The *Nanomia* stem is the only coelenterate preparation so far described in which intracellular recordings can be made from all the tissues involved in behaviour, which in this case means two nervous sub-systems, one conducting epithelium, and one myoepithelium giving graded responses to mixed excitatory input from the other three.

The gastrozooid (or palpon) is organized completely differently from the stem, more closely resembling a solitary gymnoblastic hydroid, with its

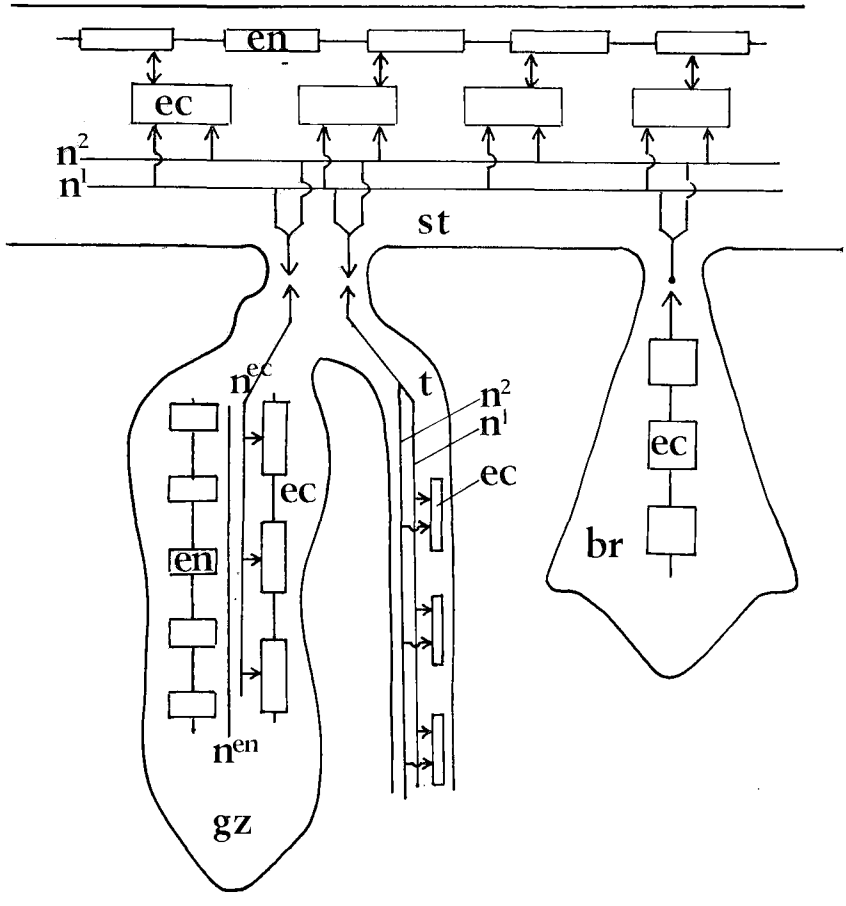

FIGURE 10 Interactions between conducting systems of stem (st), gastrozooid (gz), tentacle (t) and bract (br). Palpons are organized like gastrozooids. Nectophores are omitted owing to inadequacy of data.

Two ectodermal nervous sub-systems (n^1, n^2) run in both stem and tentacles, producing twitch contractions in the ectodermal epithelio-muscular cells (ec) which are not tightly coupled. The stem endoderm (en) is the conducting system for slow potentials. Its cells are shown connected by tight-coupling lines. Interactions occur between the endoderm and ectoderm (two-headed arrows).

In gastrozooids (and palpons) the ectodermal epithelio-muscular cells (ec) are gradedly excited, producing slow depolarizations, by input from ectodermal nerves (n^{ec}), but they can also propagate muscle spikes, and are shown as coupled. The endoderm (en) is tentatively regarded as the conduction route for basal contraction pulses. Nerves (n^{en}) run in the endoderm but their function is unknown.

The endoderm of tentacles and bracts does not conduct and does not contain nerves.

The ectoderm (ec) of bracts is a non-muscular excitable epithelium somehow wired into the stem nervous system.

spiking ectoderm and rhythmic contraction cycle. It has nerves in both layers, not just in the ectoderm. Since the nerve signals lie below recording threshold it is impossible to say for sure if there is a single ectodermal net, or whether it is double, as in the stem and tentacles. The ability of the ectoderm to show graded slow depolarizations as well as spikes is novel, and deserves to be explored further using intracellular recording techniques. Depolarizations which fail to reach threshold were not seen to produce any movement, but further research might reveal a contractile response; it would be hard to explain the utility of the graded electrical responsivity if there were no corresponding gradation of the contractile response.

The nervous events underlying these graded slow depolarizations (SDs) are not visible, but it is safe to assume that they exist, and that SDs are spread by nerves. Variations in SD amplitude with shock strength presumably reflect the number of neurons activated and their firing frequency. The SD then is the outer, visible sign of underlying, invisible nervous activity. SDs can be evoked directly by stimulating the gastrozooid or indirectly from the stem. If stem stimulation is repeated at a suitable frequency, producing a substantial barrage of nerve input, the SD may reach spike threshold, which results in a strong symmetrical contraction as the spike propagates rapidly through the epithelium. Nerve impulses from the gastrozooid can likewise invade the stem and trigger n^1 and n^2 events, which cause muscle twitches and, by a "translation" step, fire the *S* system.

The two-way link between the gastrozooid and the stem is easily fatigued, and probably requires repetitive nerve input to transmit even when rested. Here, if anywhere in coelenterates, we would expect to find interneural facilitation (Pantin, 1935), but this is a mechanism which has never been conclusively demonstrated, even in colonial anthozoans where it has often been invoked to explain incremental spread.

Whatever their *modus operandi* these labile junctions are probably useful to the organism as they interpose a barrier between zooids and stem which only substantial or prolonged stimulation can overcome. It is clearly not advantageous to have activities throughout the colony disrupted by minor disturbances in restricted localities.

The endodermal action system organizing rhythmical pumping movements in gastrozooids and palpons is independent in each zooid. No transmission paths seem to exist whereby such activity could spread to the stem. The organization of this system and the role of the endodermal nerves remain to be elucidated.

The tentacle seems to be a simplified version of the stem (Mackie, 1973). There are two fast conduction systems, probably represented by two nervous sub-systems, for which microscopical evidence is available in the case of palpon tentacles, and the ectoderm shows graded twitch contractions. There

is no counterpart to the endodermal *S* or *BP* systems. Communication with the stem works in both directions, by labile junctions, as in gastrozooids.

The bract presents an intriguing example of one-way epithelio-neural transmission. Impulses propagated in the ectodermal epithelium become "translated" into nervous activity in the n^1 and n^2 systems in the stem. The mechanism is obscure. The epithelial cells might envelop stem neurons in the attachment region of the bract, and excite them by external currents, as proposed for epithelio-neural interactions in *Stomotoca* (Mackie, 1975), and *Calliactis* (McFarlane and Jackson, 1976), or epithelial impulses might enter the nervous system through superficial sensory receptors, as in a tunicate example (Bone and Mackie, 1975).

It is interesting that although the linking device between bract and stem must be quite different from that between gastrozooid and stem, similar properties of lability are in evidence. Presumably, the functional requirement for some kind of barrier or filter at these interfaces has applied in both cases and has led to evolutionary convergence.

Coordination and control of nectophores, in some ways the most interesting problem, is also the most intractable, and the information available is not complete enough to justify including a nectophore in the model shown in Figure 10. Data on calycophoran nectophores will be presented elsewhere (Carré and Mackie, in preparation).

While this article has dealt primarily with coordination of zooids in a colony, it seems likely that the sorts of mechanism by which the action systems of the zooids are linked within the colony are similar in principle to those linking semi-autonomous parts of individual zooids, so we are really looking at a problem which applies broadly to all coelenterates, both colonial and solitary. The advantage of colonies is that the organism is spread out in space with a clear pattern and clear lines of demarcation between the action centres, which makes investigation more straightforward.

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