THE CONTROL OF FAST AND SLOW MUSCLE CONTRACTIONS IN THE SIPHONOPHORE STEM

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The stem of the siphonophore Nanomia cara is the only coelenterate preparation so far found which allows intracellular recordings to be made both from muscles (Spencer, 1971) and nerves (Mackie, 1973); as such it offers unique possibilities for the analysis of neuro-muscular interactions at the cellular level. The stem muscle is also interesting as an example of an effector capable of responding by rapid, twitch-like contractions (both local and general) and also by slower, sustained contractions (Mackie, 1964). Such muscles are found in all three classes of the Cnidaria (reviewed by Josephson, 1974) but the ways in which the two sorts of response ('fast' and 'slow') are differentiated remains problematical. Only one histological type of muscle fibre seems to be involved; it is assumed that there are two excitation pathways. In sea anemones, the electrical correlates of fast contractions are known, but slow contractions can occur in the absence of recordable signals (McFarlane, 1973). In Nanomia, however, both fast and slow contractions have distinctive electrical correlates, the analysis of which is our present concern.

This work was done at the same time as a study on the giant axons mediating escape behaviour in Nanomia (Mackie, 1973) and is essentially Part II of the same account.

METHODS

In addition to the techniques noted in Mackie (1973) a method for stabilizing the stem for intracellular recordings was used here. This involved passing a glass rod up the lumen of the stem and stretching the stem along it by suction tubes attached at the two ends, these tubes doubling as stimulating or recording electrodes.

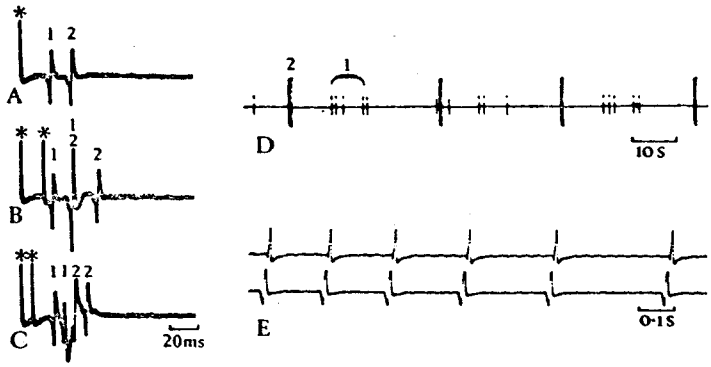

Fig. 1 Activity in the two nerve nets (1,2) recorded extracellularly in 1:10 Mg/SW. In A, a single shock (*) fires the two nets. N1 conducts faster than N2 so a second impulse in N1 can be made to catch up with (B) or overtake (C) a previously launched N2 impulse, demonstrating independence of the two pathways.

Both systems are spontaneously active, firing in bursts (D). The N2 burst shown expanded in E is from the same recording.

Pilloried stems show similar activity to normal stems. Magnesium ions, which elevate the muscle response threshold to nerve input were used in some experiments to subdue hyperactive stems. Even 1:15 Mg/SW (1 part of isotonic magnesium chloride plus 15 parts of sea water) has a perceptible damping effect on twitch activity. In the extracellular recordings, negative is up, positive down, except for Fig. 7 where the polarities were inadvertently reversed.

RESULTS

The Two Nervous Conducting Systems, N1 and N2

There are two independent, unpolarized through-conducting nerve nets in the stem ectoderm, each associated with a rapidly conducting giant fibre. Conduction velocity in each system is related to giant fibre diameter. Microelectrode recordings were obtained from these fibres and it was shown that impulses could pass around regions where the giant fibres were cut (presumably travelling in the two nerve nets) and reinvade the giant axons on the other side of the cut (Mackie, 1973). Fig. 1 documents the independence of the two nervous systems, the difference in their conduction velocities and their spontaneous activity. Both systems are photoexcitable. There are no nerves in the endoderm.

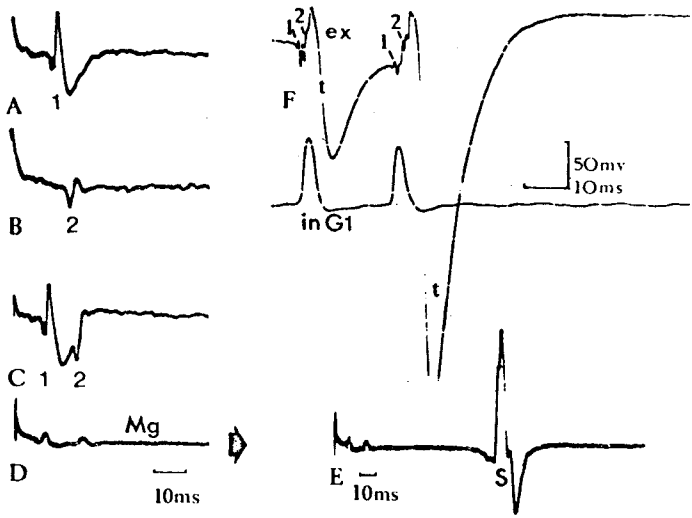

Fig. 2 Nerve spikes and associated twitch potentials recorded extracellularly. In sea water, nerve spikes are overshadowed by twitch potentials (A,B,C) but the latter are blocked in 1:5 Mg/SW (D). The slow system (S) continues unaffected (D,E). In (F), facilitation of the twitch (t) response to the second of the two N1-N2 pairs is recorded by a suction electrode on the stem surface (upper trace) while a microelectrode in the giant axon (G1) associated with the N1 records pure N1 action potentials simultaneously (lower trace, and amplitude calibration).

Twitch Response

As noted in the earlier report, events in N1 and N2 act synergistically upon the ectodermal muscle, producing graded, fast contractions (twitches). In extracellular recordings, the nerve spikes are augmented by after-events of variable magnitude representing locally induced myoepithelial potentials (Fig. 2A-C). In 1:5 Mg/SW the after-events are greatly reduced, although conduction of the nerve spikes is unaffected (Fig. 2D,E). The after potentials show facilitation during rapid firing of one or both nerve nets together (Fig. 2F,4) and the amplitude of the response judged visually varies accordingly. In nature, twitches would occur whenever the combined activity of the two nerve nets produced a barrage of several spikes in a tight frequency relationship and this might occur either spontaneously, in response to direct stimulation of the stem, or indirectly, to stimulation of one of the appendages. The effect would be a local or general withdrawal response. Strong responses are also accompanied by locomotion

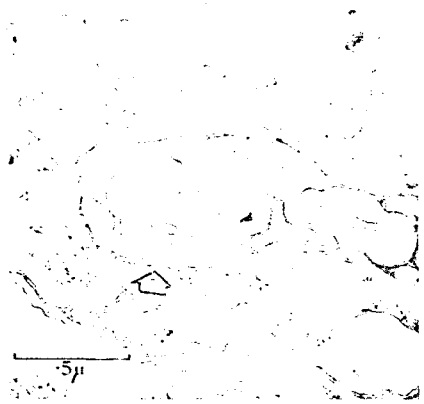

Fig. 3 Neuromuscular junction in the stem ectoderm. Such junctic occur in the superficial part of the myoepithelium.

(Mackie, 1964). For the most part, twitch amplitude is determined by the numbers and frequencies of nerve spikes, but twitches may be augmented or prolonged by input from another source, the slow system, as we note below.

Neuromuscular junctions (Fig. 3) are very numerous in the stem ectoderm, and doubtless represented chemical synapses mediati twitch responses. Twitches cannot propagate in the muscle itself, so unless there is limited local spread between the myoepithelial cells, we should assume that each cell is innervated, receiving input from either N1 or N2 or both. It has not been possible to differentiate the two nets or their junctions histologically.

Intracellular recordings from the muscle cells support this interpretation. When the extracellular electrode picks up a nerve spike, the intracellular electrode in the muscle records a small depolarization of about 4-6mv (Figs. 5,6) which is evidently an excitatory junctional potential (nEJP). Inputs from the two nets look similar, sum together, and facilitate one another. The amplitude of the muscle twitch potential evoked by a given input varies according to the physiological state and immediate past history of the preparation. This was shown in Fig. 2F and is also apparent in the intracellular records, e.g. Fig. 5B-D. None of the intracellular records used in this paper show a major twitch response, because such responses dislodge the electrode. Judging from the baseline deflection of the extracellular recordings, twitches commonly grade up to 50mv or more in preparations where both nerve nets have been made to fire at high frequencies along with the S system.

Following stimulation at any point, nerve impulses are

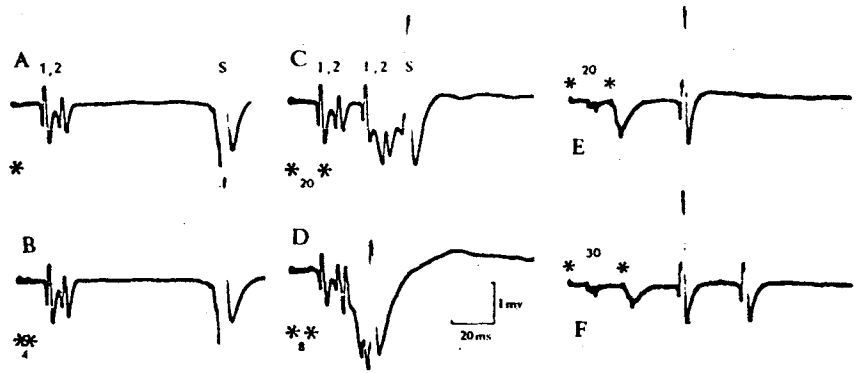

Fig. 4 Extracellular records of muscle twitches and slow (S) system responses. The response to two shocks 4 ms apart (B) is the same as to a single shock (A). A second shock >7 ms after the first is effective, producing a second N1-N2 pair, and a baseline deflection representing the evoked twitch depolarization of the myoepithelium (C,D). Twitch amplitude is a function of both number and frequency of N inputs. Reduction of S conduction time (see in text, 'piggy-back effect') is also seen.

E and F are from a similar preparation in which N events are recorded at lower amplitude. Each shock fires an N1-N2 pair as before. The twitch response to the second pair is markedly facilitated in E (shocks 20 ms apart), less so in F (30 ms). Note also the long refractory period of the S system.

initiated which propagate all the way along the stem in both directions, generating twitches as they go. If a section of the stem is placed in a magnesium bath, the twitch is abolished in this region, but appears again on the other side (Fig. 7). Thus, twitches are not propagated in the muscle itself, but are generated sequentially along the stem by the passage of nerve events, spreading at a velocity related to that of nervous transmission (<3 m/s).

Slow Conduction System (S) and Slow Muscle Response

Spencer (1971) described propagated events in the stem whose spontaneous production and conduction at 0.2 m/s was not blocked by magnesium. Extracellular recordings showed these events as large potentials (>1mv) while intracellular recordings (the first for any coelenterate tissue) from the ectoderm layer showed them as unexpectedly small depolarizations arising from a 50-80mv resting potential. In Spencer's Figs. 2A,D these events ('spon-

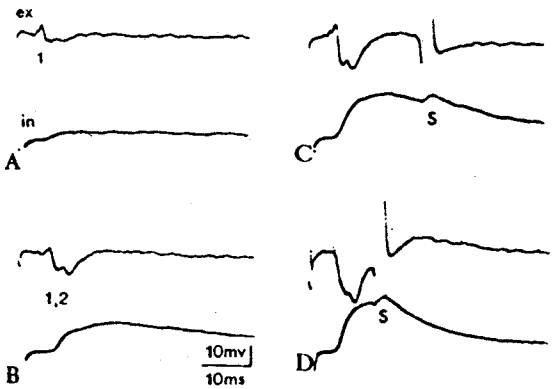

Fig. 5 Twitch depolarizations recorded intracellularly with a microelectrode in a muscle cell (lower trace and amplitude calibration) with simultaneous extracellular monitor (upper). In A, N1 fired alone, giving a single excitatory junction potential (nEJP) in the muscle. B, C and D are from a sequence in which similar N1-N2 pairs were evoked each time but where the twitch depolarization level was found to vary.

The S system fired each time but is off the record in A and B. With larger twitches, (C,D) reduction of S conduction time is apparent. The intramuscular correlate (sEJP) is seen mounted on the twitch depolarization, but distinct from it, indicating that the piggyback relationship had been lost earlier in the pathway.

taneous suprathreshold pulses') measure 10-12mv, but values up to 20mv are noted in the text. (The larger events shown in his Figs. 2B,C as responses to strong stimulation may include a twitch component. Spencer's 'subthreshold pulses' (Fig. 2B) would appear to correspond to my nEJPs). The propagated events seen in extracellular recordings are here called S (slow, or Spencerian) pulses. They are conducted at about 0.3 m/s in my recordings. They seem to occur spontaneously but not in consistent patterns. Their insensitivity to levels of magnesium which block twitch responses is shown in Figs. 2E,7. The correlated events seen in intracellular recordings from ectodermal muscle cells have amplitudes in the range 10-20mv, and arise from a 75-85mv resting potential. The small size of these events (ca. 25% of the resting potential) suggests that they are not being generated or propagated in the muscle itself. Moreover, much larger (twitch) potentials in the same cells clearly do not propagate. What we are recording is evidently a second type of junctional potential representing input from the slow conduction system, which may be referred to as an sEJP. The large size of the extracellular S pulse, its persistence at full amplitude in magnesium and its slow conduction suggest that it is neither a nerve

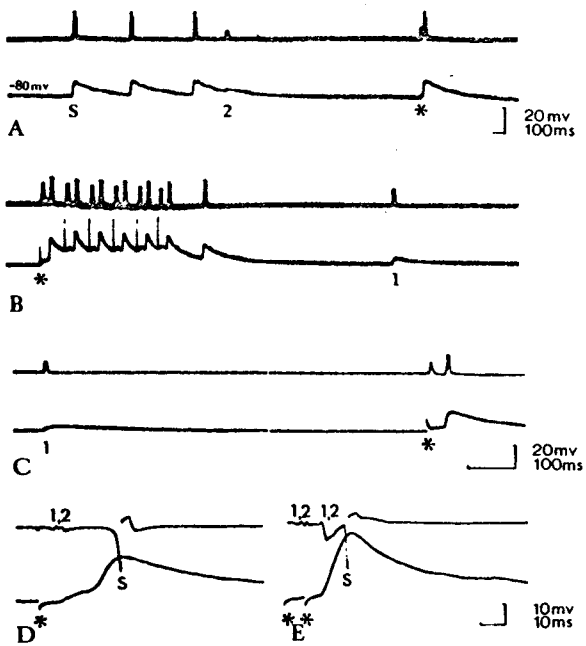

Fig. 6 Slow and twitch potentials recorded intra- (lower) and extra- (upper) cellularly, arrangement as in Fig. 5. In A,B and C the microelectrode registers nEJPs corresponding to spontaneous N1 and N2 events, and larger sEJPs corresponding to spontaneous S potentials. Shocks (*) evoke nEJPs followed by sEJPs. In B stimulation frequency is too low to permit twitch development and the mounting level of depolarization is due to summing of sEJPs, representing a fairly strong contraction of the 'slow' type.

D shows summation of nEJPs from N1 and N2 to which is added an sEJP. In E, four nEJPs and an sEJP have summed to produce a smooth 30mv twitch depolarization. (N.B.: the extracellular monitor which was slightly nearer the stimulating site, here records only the initial (positive) component of the S event due to amplifier overload. The event is really primarily negative-going, in contrast to twitches seen extracellularly, which allows it to be distinguished even where, as here, the two events coincide. In the intracellular record we see not the primary S event, but an sEJP which has the same polarity as the twitch and merges with it.)

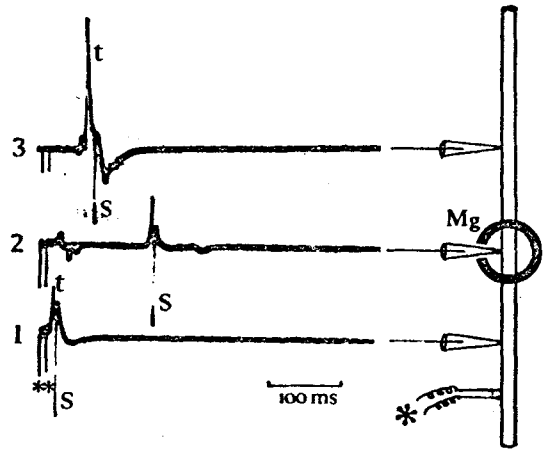

Fig. 7 Indirect production of twitches and slow potentials (polarities reversed). A shock pair produces two N1-N2 pairs which propagate up the stem through a region containir 1:10 Mg/SW. The twitch response (t) is blocked in the Mg bath (trace 2), but a twitch is again generated on the far side (trace 3) showing that twitches can be evoked by N combinations far from the site of stimulation.

S potentials appear on both channels 1 and 3 in the piggy-back time relationship. It is apparent that the S event in channel 3 must have been generated on the far side of the magnesium block along with the regenerated twitch. The S event which eventually arrives on channel 2 was carried piggyback as far as the Mg bath after which, with the collap of its carrier, it continued up the stem at its slow velocity. It never appeared on channel 3 having been blocked by the regenerated S event coming down the stem.

spike nor a combination of a nerve spike with locally induced myo-epithelial potential, like the twitch recorded extracellularly. The S pulse is almost certainly an epithelial potential conducted in the endoderm, although intracellular recordings from the layer are not available to prove this. The cells are connected by numerous gap junctions (C.L. Singla, personal communication). Endodermal epithelia are known to conduct in hydromedusae (Mackie and Passano, 1968) and impulses can propagate from ectoderm to endoderm, probably via the transmesogloeal tissue bridges which have been located histologically in appropriate regions. In Nanomia,

transmesogloeal bridges also occur between the two layers (Fig. 8). Although the histological evidence is still incomplete, it seems probable that the two layers are electrically coupled via these bridges, allowing slow pulses in the endoderm to be picked up as electrotonic junctional potentials (sEJPs) in the ectodermal muscle. In support of this interpretation, we may note that muscle twitches recorded extracellularly show the opposite polarity to S pulses, suggesting that the main components of the two events are not both being generated in the same cell layer. The sEJP however has the same polarity as the nEJP and is clearly recorded in the same cells.

Activity in the S system can be evoked without accompanying twitch activity (Fig. 6B). S bursts having interpulse intervals of 100-400 ms are accompanied by slowly developing contractions of the stem and of the bases of some of the zooids. Because of its long refractory period (25-30 ms) the S system cannot fire at more than about 30-40 Hz. When stimulated at these frequencies summation of sEJPs to a maximum of ca. 35mv has been observed, representing a strong, sustained contraction. N1 and N2 are fired concurrently but fail to generate twitches at these relatively low frequencies, so we are seeing an essentially pure slow muscle response. With more rapid stimulation a twitch component starts to develop and is added to the slow response. The ability of the S system to fire spontaneously in the absence of twitches probably accounts for the ability of the stem to maintain differing postural extensions in nature. Other possible roles for the S system remain unexplored. There is no true endodermal muscle, merely a few wisps of thin filaments in some regions.

When recorded concurrently with twitches, sEJPs may be seen mounted on twitch depolarizations in the 20-30mv range. In this position they show amplitudes similar to those of sEJPs recorded at resting potential from the same preparation, which again suggests electrical rather than chemical mediation.

Retroactivation of S System: Piggyback Effect

A striking feature of the stem preparation is the variable conduction time of S pulses. In the absence of nerve spikes, or where these are too few or far apart to generate twitches the S pulse propagate at about 0.3 m/sec, but where twitches are evoked concurrently with S pulses, the latter arrive earlier, this 'acceleration' being more marked the bigger the twitch, up to the point where the two events appear simultaneously all along the conduction route (Figs. 4,5). To explain this phenomenon, let us suppose that the S events are being carried 'piggyback' on the crest of a twitch depolarization for some or all of the distance over which they are recorded. When they fall off the carrier

Fig. 8 Transmesogloeal tissue bridge. The endodermal process splits into branches in the ectoderm, contacting several ectodermal cells.

event (or where the latter is wiped out by magnesium, Fig. 7), they continue to spread, but now their rate of spread is the (much slower) propagation velocity of the S system itself. In support of this interpretation we may invoke the same electrical coupling presumed to mediate sEJPs. Electrical junctions are typically non-rectifying, so retroactivation of the (endodermal) slow conduction system would be a reasonable expectation when the ectodermal muscle to which it is coupled fires in the twitch response. (Thus, in Fig. 9 two-way communication is indicated at the ecto-endodermal junctions.) We further assume that there is a critical level of twitch depolarization (piggyback threshold) above which coupling potentials invading the endoderm evoke S spikes. Fig. 10 shows this level as a dotted line on the muscle trace. S spikes would not be evoked with twitches below this level (probably about 25mv) although it is probable that the conduction velocity of an established S event would be increased by the depolarizing effect of the coupling potentials on the cells ahead of it in the conduction path. Thus a complete spectrum of conduction 'velocities' between 0.3 m/s and nearly 3.0 m/s would be expected, and is observed. It is not the velocity of S propagation which is variable, but the proportion of the conduction time spent on and off the piggyback.

Why should twitch amplitude decline with distance, allowing S events to fall off their carrier potentials? A simple explanation is to be found in the fact that N1 and N2 conduct at different conduction velocities, so N-inputs to the muscle become spread out with distance, and twitch amplitude, which is frequency-related, will accordingly decline. In the deliberately oversimplified example represented in Fig. 10, N1 and N2 are initially close

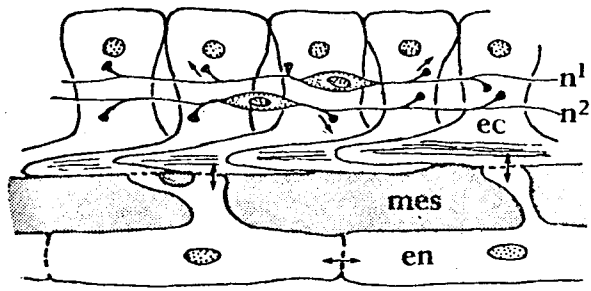

Fig. 9 Summary diagram of the histological relationships. The two nerve nets (N1, N2) synergistically fire the muscle through one-way synapses. The endoderm (en) is coupled to the ectoderm (ec) by transmesogloeal (mes) bridges, allowing two-way interactions to occur.

together, having been launched simultaneously. The further they travel, the more they spread out and finally N1 gets so far ahead of N2 that it barely facilitates the response to N2. Twitch amplitude now fails to attain the critical threshold needed for synchronous firing of the S system. S events however continue on their own.

The siphonophore has found an ingenious way of grading response intensity with distance from the site of stimulation. We can see how local twitch responses are feasible, even though the conduction systems are through-conducting.

It will be apparent that while S potentials can occur alone producing 'pure' slow muscle responses, any substantial twitch activity, whether local or general, will be accompanied by the production of propagated S events which will have the effect of reinforcing or prolonging the twitch contraction. Fast and slow contractions are therefore not sharply distinct in this animal - an untidy arrangement (nature is often untidy!) but one which probably makes the system more versatile and hence more efficient than would be the case if there were no way of mixing the two sorts of input.

DISCUSSION

Figures 9 and 10 summarize the relationships proposed in this paper to explain fast and slow muscle contractions. Other constructions may be possible but I can think of none which come at

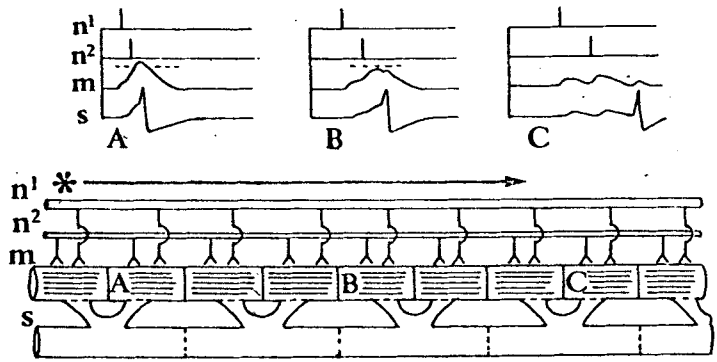

Fig. 10 Scenario to explain decline of muscle response intensity with distance (A - C) from stimulus (*). N1, N2 and S events are through conducted. N1 conducts about twice as fast as N2. The muscle (M) does not conduct. At point A, N1 and N2 are still close together; N1 facilitates N2 producing a large twitch in the muscle, which results in synchronous firing of the coupled S system. At B and C N1 goes progressively further ahead of N2, twitch level drops below the critical piggyback threshold (dotted line) leaving S to auto-propagate. M and S records are shown as intracellular recordings, but such records have not been made in the case of S, and are inferred.

all near to fitting the evidence. The critical experiments needed to substantiate the model are obvious: direct recordings from the endoderm to show that S pulses are conducted there; simultaneous recordings from the two cell layers to elucidate two-way coupling interactions, transducer recordings showing tension fall-off along the stem as a function of changing N-input relationships, etc. Nanomia is not an easy animal to work with and is not always easy to obtain, but it remains our best bet for this type of study.

My colleague, L.M. Passano, speaks of "the coelenterate way of doing things" and this work illustrate the point that while we may search for, and even find, neuromuscular mechanisms reminiscent of other phyla, giant axons, dual excitatory nerve inputs, etc. no facile comparisons can be made with animals whose muscles are arrange in discrete units. A single long, cylindrical muscle poses quite different problems from a series of short separate ones.

Siphonophores are colonies, the integration of whose component members has been explored at the behavioral level in other papers (e.g. [illegible]ie, 1964). The way in which stem activity is triggered by and in [illegible]n generates activity in the appendages needs to be re-

examined in the light of this analysis of the stem action system. Judging from my own (unpublished) observations, these input-output relationships are quite subtle but should yield many lessons of interest when critically examined. The morphological complexity of siphonophores may seem confusing, but the division of labour which accompanies polymorphism has resulted in certain striking simplifications of the functions of individual members which makes them useful for the investigation of the action systems one at a time, in isolation.

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