

## COLONIAL CONDUCTION SYSTEMS IN THE ANTHOZOA: OCTOCORALLIA

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### SUMMARY

1. The octocorals *Alcyonium digitatum*, *Pennatula phosphorea* and *Virgularia mirabilis* each have a through-conducting nerve net. The nerve net demonstrated electrophysiologically may well be the same as that previously shown by the use of histological techniques.

2. It exhibits both facilitation and defacilitation in the rate of conduction of pulses.

3. The distance of spread of nerve net activity is not limited by the number of stimuli applied.

4. The nerve net controls fast muscle contractions; the frequency of pulses is important in determining which muscles contract and in which sequence.

5. The nerve net is 'spontaneously' active.

6. A previously undescribed slow system has been identified in *Pennatula*. It has many of the properties of slow systems in sea anemones and may well be ectodermal. It is suggested that multiple conduction systems are of common occurrence in the Anthozoa.

### INTRODUCTION

There is a preponderance of colonial forms in the Anthozoa and the six orders comprising the subclass Octocorallia all contain colonial species. A number of investigations have been undertaken into the control of behaviour in octocorals (Panceri, 1872; Parker, 1920, 1925; Nicol, 1955*a*, *b*, 1958; Buck, 1953, 1973; Buck & Hanson, 1967; Horridge, 1956*a*, *b*; Pavans de Ceccatty & Buisson, 1965; Buisson, Tricoche & Franc, 1967; Buisson, 1971). Much of our present knowledge of conduction systems and the control of behaviour in these animals comes from such work. Colonial activities may be classified into three main categories: (*a*) polyp retraction; (*b*) swelling, burrowing and related movements of the syndete; (*c*) propagated waves of phosphorescence, though not all of these are necessarily shown by every species.

Until now, the comparative lack of electrophysiological data has hampered progress in understanding the conduction systems involved in the control of such behaviour. The purely behavioural approach, moreover, has inherent shortcomings and it cannot be assumed that the presence or absence of a visible response at any point on the colony always indicates a corresponding presence or lack of nervous activity at that point (Shelton, in preparation). Furthermore, the discovery of two slow conduction systems in

addition to the nerve net in the sea anemone *Calliactis parasitica* (Couch) (McFarlane, 1969*a*), raises the possibility that similar systems may be involved in certain aspects of colonial co-ordination. A reappraisal of existing studies is required and it may be that activities previously ascribed to nerve net control are under the control of other systems.

This paper describes electrophysiological experiments conducted on octocorals, *Alcyonium digitatum* (Linnaeus), *Pennatula phosphorea* (Cuvier) and *Virgularia mirabilis* (Lamarck), in an attempt to pin-point the pathways of co-ordination present in members of the subclass Octocorallia. Some of the properties of these systems are described and suggestions made as to their anatomical basis.

#### MATERIALS AND METHODS

Specimens of *Alcyonium digitatum* growing on shells or stones were obtained locally as required. *Pennatula phosphorea*, collected by bottom-dredging, came from two sources – the Marine Biological Station, Millport, and the Scottish Marine Biological Association, Dunstaffnage. *Virgularia mirabilis* also came from Dunstaffnage and were individually gathered by a team of SCUBA divers. The rachis of each *Pennatula* and *Virgularia* was 'planted' in a substrate of fine sand and all the specimens were maintained in aquaria with running sea water at about 10–15 °C.

Electrical activity was recorded using extracellular, polythene suction electrodes similar to those described by Josephson (1965) with tip diameters of 100–200  $\mu\text{m}$ . Similar electrodes were used to administer square-wave electrical stimuli produced by a Tektronix Type 162 wave-form generator and a Tektronix Type 161 pulse generator. Recorded activity was amplified by differential pre-amplifiers and displayed on a Tektronix Type 564 storage oscilloscope.

It was found that the most convenient place from which to make recordings from *Pennatula* and *Alcyonium* was the oral disc of an autozoid. With care, the electrodes could be kept in contact even during polyp retraction. The polyps of *Virgularia* were too small for satisfactory electrode contact to be maintained in the oral disc region, however, and electrical recordings from this species were usually made from the column of an autozoid.

#### RESULTS

##### *Alcyonium digitatum*

This species proved extremely difficult to record from, due to the small size of the evoked potentials. Typically, a single 1 msec electrical stimulus of 10 V amplitude applied to a polyp, elicited a single pulse through-conducted over a wide surrounding area (Fig. 1*a*). In the best preparations, the pulses were up to 10  $\mu\text{V}$  in size and closely resembled a 'nerve net' pulse from *Calliactis parasitica* (McFarlane, 1969*a*). The system fatigued very readily in response to repetitive stimulation and very little 'facilitation' of the size of the pulse was observed following the second of a pair of stimuli.

##### *Pennatula phosphorea*

Two conduction systems were positively identified in this species. The first of these appeared to be a through-conducting nerve net (Fig. 1*b*). At 10 °C the mean conduc-

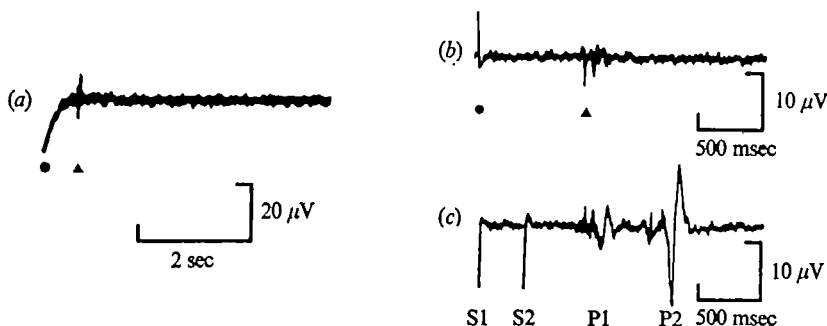


Fig. 1. (a) *Alcyonium digitatum*. Electrical activity (arrow) following a single stimulus (black spot). Suction electrode recordings from the oral disc of a polyp.

(b) *Pennatula phosphorea*. 'Nerve net pulse' (arrow) recorded from an autozoid in response to a single electrical stimulus (black spot) applied to the rachis. Note the complex shape of the pulse.

(c) *P. phosphorea*. Following two stimuli applied 320 msec apart (S1 and S2), two responses were recorded (P1 and P2). Note the facilitated muscle response P2.

tion velocity was  $7.5 \text{ cm sec}^{-1}$ . Both electrical and mechanical stimuli were effective in evoking activity in this system. Conduction occurred both up and down the rachis, peduncle and pinnae at equal speed; stimulation of any part of the outside of the colony elicited a pulse which spread to all parts of the colony. Due to the small size of the autozooids, it was not possible to measure the conduction rate within a single polyp. This may well be greater than the mean velocity of the net given above. The system was readily excited even by small stimuli and a stimulus-duration curve with a chronaxie of about 0.8 msec was obtained. This compares with a chronaxie of 2-4 msec for the nerve net in *Calliactis parasitica* (Pantin, 1935a).

The size of the response following the second of two stimuli close together in time was greatly increased over that following the first (Fig. 1c). A smaller 'facilitation' effect was also observed following the third and fourth stimuli. The increase in size of the second response depended on the interval between stimuli and reached a peak at an interval of about 250 msec with another smaller peak at about 600 msec. The complex shape of the 'nerve net' pulse could have been related to activity triggered in two sets of muscles (possibly the mesenteric retractors and the oral disc radials). These muscles would seem to be maximally activated at different stimulus frequencies. Using paired shocks, it was possible to determine the absolute refractory period of the nerve net. A value of 40-50 msec was obtained. Occasionally, following such double stimuli, three or four nerve-net pulses were recorded, indicating 'after-discharge' in the net. Spontaneous activity was also observed from time to time but no attempt was made to analyse this. It may have been in part stimulated by unknown outside factors. Repetitive stimulation led to complete retraction of all the autozooids in the colony.

The second conduction system revealed during these experiments produced characteristic pulses quite distinct from those of the nerve net. The pulses were through-conducted at a velocity of about  $6 \text{ cm sec}^{-1}$  at  $10^\circ \text{C}$ . A single supra-threshold electrical stimulation of any part of the outside of the colony evoked a single pulse in this system. Unlike nerve net activity, pulses could only be recorded from the oral disc region. The responses were extremely small and since the pulses were shown

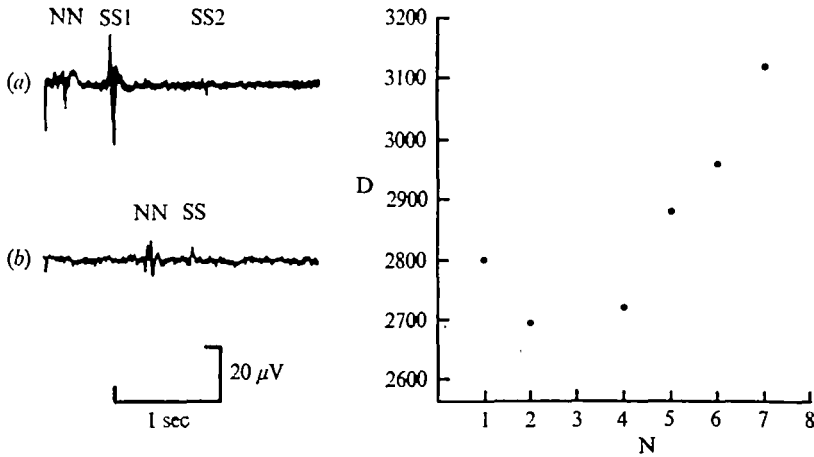


Fig. 2. (a) Nerve net pulse (NN), SS<sub>1</sub> pulse (SS<sub>1</sub>) and SS<sub>2</sub> pulse (SS<sub>2</sub>) following a single shock to the column of the sea anemone *Calliactis parasitica*. Recording from a tentacle. The same narrow-tipped electrodes were used as those employed to make the recordings from *Pennatula*.

(b) Comparison recording from *Pennatula* showing nerve net response (NN) and slow system response (SS).

(c) *P. phosphorea*. Fatigue in the slow system. Graph of response delay (D) measured in milliseconds against stimulus number (N) for a burst of stimuli administered to the peduncle 17 cm away at 1/5 s. Note that the response delay shows a small decrease before the effects of fatigue build up in the conducting system.

to conduct over the entire colony, it seems that the characteristics of the tissue may influence the success of the recording method. There are parallels here with the situation in sea anemones where 'slow system' activity can be recorded only from the tentacles and oral disc (McFarlane, 1969a). Fig. 2(a, b) shows a comparison between electrical activity following a single shock in *Calliactis parasitica* and the effect of a single shock to *Pennatula phosphorea*. On the basis of the similarities with sea anemone slow system activity (shape, size, low conduction velocity, recordings only possible from the oral disc region) it would seem reasonable to class this system as a slow system.

In response to repetitive stimulation, no facilitation in the size of the pulses in the slow system was observed. This was in marked contrast to the effect of repetitive stimulation of the nerve net. The slow system was very labile and readily fatigued. Fig. 2(c) shows a graph of the response delay following stimuli at 1/5 sec. Note the decrease in delay of the second and third pulses compared with the first, then the steadily increasing delays following subsequent stimuli. Continued stimulation ultimately caused total failure of the system. The rate of increase in response delay was greatest with high-frequency stimulation (e.g. 1/sec).

Unlike *Calliactis*, the slow system in *Pennatula* had a lower threshold than the nerve net (Fig. 3a) for stimuli of 1 msec duration. This adds further weight to the view that the nerve net and the slow system in *Pennatula* are distinct and separate.

#### *Virgularia mirabilis*

The nerve net in *Virgularia* seemed to be well developed, and recordings from the column of an autozoid revealed pulses of 10–15  $\mu\text{V}$  in size. As in *Pennatula*, the nerve net could be stimulated by both mechanical and electrical stimuli applied to any part

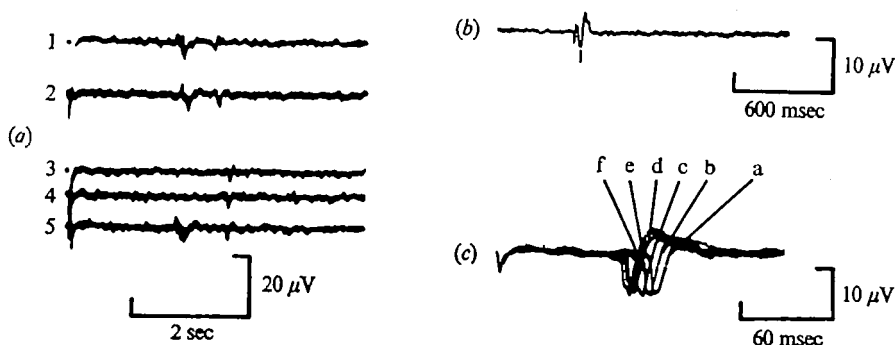


Fig. 3. (a) *Pennatula phosphorea*. Conduction system thresholds. Traces 1 and 2 show both pulses. Traces 3 and 4, the effect of reducing the stimulus voltage below 5 V (1 msec) – slow system activity only. Trace 5 shows the return of nerve-net activity when the stimulus voltage was raised again.

(b) *Virgularia mirabilis*. 'Nerve net' activity following a single electrical stimulus. There seem to be at least two components to the pulse. The second phase was somewhat variable in shape and liable to fatigue. It is suggested that this represents electrical activity in muscles underlying the recording electrode.

(c) *V. mirabilis*. Six traces superimposed to show the increase in conduction velocity of six successive nerve net pulses (a-f) stimulated at 1 per 500 msec. Although the effect is produced at a higher stimulus frequency than the slow system would respond to in *Pennatula*, there is a similarity in the facilitation effect shown by the two systems.

of the outside of the colony. Most of the experiments on this species were carried out at about 16 °C. In response to a single supra-threshold stimulus, a single nerve-net pulse was produced which conducted to all parts of the colony at a velocity of 40–60 cm sec<sup>-1</sup>. There was the familiar relationship between the duration and the intensity of threshold electrical stimuli (Pantin, 1935a) with a chronaxie between 0.6 and 1 msec. There was no evidence of any interneural facilitation. The 'nerve net' pulse often had a compound nature (Fig. 3b) rather like that of *Pennatula*, but the second, slower component was very labile and was often lost after repetitive stimulation. This may have been due to the movement of a contracting muscle away from the recording electrode. Recorded pulses from the outside of the column of an autozooid did not show a marked facilitation in size following repetitive stimulation. Nevertheless, a facilitation in conduction velocity was observed (Fig. 3c). The extent to which this apparent 'speeding-up' of the pulses occurred was directly related to (a) the distance between stimulating and recording electrodes and (b) the frequency of stimulation (Fig. 4). The greatest decrease in conduction delay was thus produced over long distances with high frequencies of stimulation. Following this initial increase in conduction velocity, continued stimulation led to a progressive decrease until refractoriness in the nerve net caused total failure in the system. Multiple channel recordings proved most informative in pin-pointing the site of the block to conduction. In every case the nerve net was through-conducting, but with progressively increasing delays until the refractoriness reached a critical level; at which point no nerve net pulses were conducted from the site of stimulation to any part of the colony. If the stimulating electrode was moved to a fresh region, however, through-conduction was restored. This is to be expected since when the nerve net is fatiguing, pulses will always be closest together in time in the region surrounding the stimulating electrode, and thus the build-up of refractoriness will be greatest in that region. A

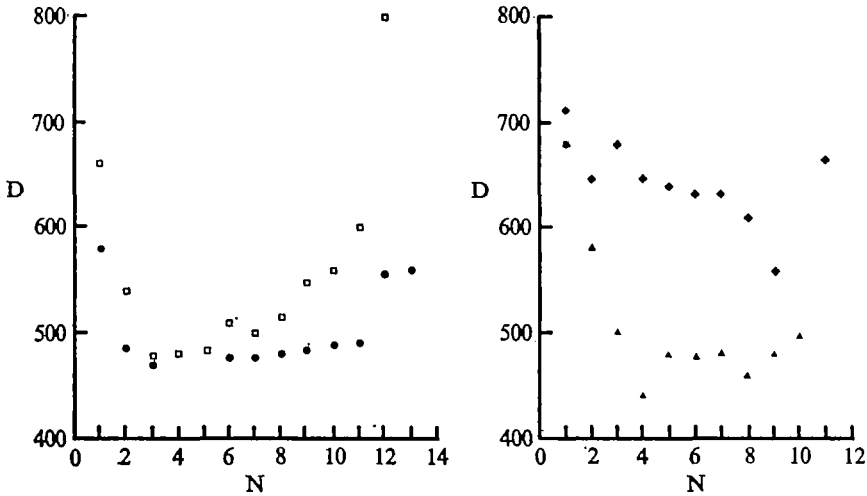


Fig. 4. *Virgularia mirabilis*. Graphs of response delay (D) and stimulus number (N) for different stimulus frequencies of the nerve net. Constant distance between stimulating and recording electrodes. □, Frequency of 1/100 msec; ●, 1/200 msec; ▲, 1/400 msec; ◆, 1/800 msec.

similar case has recently been demonstrated in the Madreporarian corals (Shelton, in preparation) and a full explanation of this effect will be published.

Long bursts of low-frequency 'spontaneous' nerve-net activity were observed (of the order of 1 pulse per 60 sec) but this activity has not yet been analysed in detail. As with *Pennatula* and *Alcyonium*, repetitive stimulation of the nerve net caused retraction of all the autozooids in the colony.

No electrophysiological evidence was found for the presence of a slow system, but since it proved impossible to make recordings from the oral disc region with the electrodes available this should not be taken as proof that no slow system exists. For reasons that are not immediately obvious, possibly related to the mechanical properties of the tissue, the recording position has a great effect on the size and kind of electrical activity recorded (McFarlane, 1969a).

#### DISCUSSION

The results reported here have strongly suggested that a through-conducting nerve net is present in each of the species tested. There seems little doubt that this is the system responsible for the control of polyp retraction and is the through-conducting system indirectly demonstrated in *Alcyonium digitatum* by Horridge (1956b). The morphological basis of the co-ordinating nerve net has in the past been disputed (e.g. Kassianow, 1908; Pavans de Ceccatty & Buisson, 1963), but more recently Titschack (1970a) has described a mesogloal 'nerve plexus' in *Alcyonium palmatum*. The identification of nervous tissue by the use of histological methods is notoriously difficult (Batham, Pantin & Robson, 1961), but Titschack's description of a sparse population of neurones with comparatively few interneural connexions fits in well with the electrophysiological evidence presented here, and may account for the relative inexcitability of *Alcyonium* and the difficulty of recording electrical activity from the nerve net. The musculature has been well described by Hickson (1895). The observation that the nerve net in each of the species studied is normally through-conducting

is interesting and there is every indication that this is a general property of Anthozoan nerve nets (Shelton, in preparation). Interneural facilitation, considered by Pantin (1935*a-d*) to be an important factor in the control of certain coelenterate behaviour, does not now seem to be a major influence in the colonial Anthozoa.

Titschack (1970*b*) has described a well-developed mesogloal nervous system in *Pennatula rubra* with neurones and interneural contacts similar to those found in *Veretillum cynomorium* (see Franc, 1968). The electrophysiological evidence certainly points to a much larger number of neurones per unit area in *Pennatula* than in *Alcyonium*. The nerve net is directly involved in the regulation of fast-muscle contractions, with the frequency of nerve-net pulses determining which muscles contract and in which sequence. The effects of changes in frequency of nerve-net pulses as they are conducted across the colonial nerve net will be considered in detail in a following paper.

The discovery of a slow system in addition to the nerve net adds a new facet to the possible modes of control of colonial behaviour. In the sea anemones, slow systems have been shown to be involved, during pre-feeding activities (McFarlane, 1970; McFarlane & Lawn, 1972), in the potentiation of certain secretory processes (McFarlane, 1969*b*); and to interact with the nerve net in the control of circular and parietal muscle contraction cycles (McFarlane, 1974). The possibility that a slow system could be involved in the control of similar activities on a colonial level has, as yet, hardly been considered by other authors. The system described in this work may well be ectodermal and homologous with the SS1 in sea anemones. It has a lower threshold than the nerve net (unlike the SS1) but the nerve net in *Pennatula* is buried deep in the mesogloea, which is filled with spicules (Marshall & Marshall, 1882). It would not be surprising to discover an endodermal slow system in these animals corresponding to the SS2; the failure to detect its presence so far almost certainly reflects the limitations of the recording method.

The selective advantage of the facilitation in the rate of conduction of high frequency nerve net pulses in *Virgularia* is open to debate. It should be borne in mind that a typical *Virgularia* may be 50–100 cm or more in length, compared with a typical *Pennatula* of about 10 cm. In certain Madreporaria, considerable delays between adjacent nerve-net pulses can build up with the distance conducted (Shelton, in preparation) and this can have the effect of decreasing the frequency to such an extent that the protective retraction response is not activated beyond a short distance from the point of stimulation. This cannot occur in *Virgularia*.

The colonial Anthozoa exhibit a wealth of interesting and poorly understood behaviour. The discovery of multiple conduction systems opens the way for a fresh approach to the study of the co-ordination of behaviour in these simple animals. It is to be expected that multiple conduction systems similar to those described will prove to be a common feature of most Anthozoa. It is clear already that even with the limited conduction pathways so far discovered, tremendous versatility is possible. Further investigations are now in progress.

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