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TWO SLOW CONDUCTION SYSTEMS IN THE SEA ANEMONE *CALLIACTIS PARASITICA*

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INTRODUCTION

Studies of the behavioural physiology of coelenterates have produced a picture of multiple parallel conduction systems, often spontaneously active and sometimes interacting. The recorded electrical activity can sometimes be related to known nerve nets, but in other cases conduction seems to be by way of epithelial or musculo-epithelial cells. There are at least two conduction systems in *Hydra* (Passano & McCullough, 1964, 1965) and one is possibly the ectodermal musculo-epithelial cells (Josephson, 1967; Josephson & Macklin, 1967). Josephson (1965) recorded activity from two co-ordinating pathways in the stalk of the hydroid *Tubularia*. A third pathway was demonstrated but had no electrical correlate. Electrical activity has been recorded from the giant-fibre net and the diffuse net of medusae (e.g. Horridge, 1953; Passano & McCullough, 1960). Passano (1965) recorded activity in several conducting systems in hydromedusae. Non-nervous conduction, by way of epithelial cells, is found in siphonophores (Mackie, 1965) and hydromedusae (Mackie & Passano, 1968).

To date, the only clearly demonstrated conduction path in anemones is the nerve net and in particular the through-conduction system. This system was investigated by Pantin (1935*a*), who found that a single electrical stimulus to the column of *Calliactis parasitica* initiates a single propagated impulse which travels through a nerve net differentiated with respect to conduction velocity, the fastest part being the through-conduction path supplying the muscles involved in the fast withdrawal reflex. The main muscles giving this protective response are the ectodermal longitudinals in the tentacles and the endodermal sphincter lying in the mesogloea at the top of the column. Josephson (1966) recorded large pulses, probably muscle action potentials, preceding contraction in the sphincter and tentacles of the tropical anemone *C. polypus*. The protective response in *Metridium* results mainly from contraction of the retractor muscles of the mesenteries. Robson & Josephson (1969) recorded muscle action potentials preceding contraction in mesentery preparations; in addition they recorded small pulses which may represent activity in the fast nerve net innervating the retractor musculature. Pickens (1968) has recorded similar small pulses from the mesenteries of the burrowing anemone *Calamactis praelongus* and suggests that they originate in the nerve net supplying the retractors.

In view of the complexity of behaviour shown by many anemones it is clear that there must be other conduction systems in addition to the fast nerve net. Although the only recorded activity has been from the fast nerve net and its associated muscles,

other systems have been proposed. For example, Ross and Sutton (1964) have shown that the swimming response of *Stomphia coccinea* must be controlled by a pathway other than the through-conduction system.

The anemone *Calliactis parasitica* has been studied for many years and its behaviour and physiology are well known. The present study began as a re-investigation of the through-conduction system and associated muscles using modern recording techniques. In general the work of Josephson (1966) on *C. polypus* was confirmed but in addition two slow-conduction systems were found. This is the first clear demonstration of multiple conduction systems in a sea anemone. The co-ordination mechanisms of *C. parasitica* are thus shown to approach the same level of complexity as those found in other coelenterate groups.

MATERIALS AND METHODS

Specimens of *Calliactis parasitica* were supplied by the Plymouth Marine Laboratory and were kept in re-circulated sea water at 10° C. Experiments were carried out in the range 10°–15° C. The anemone is normally found attached to *Buccinum* shells inhabited by the hermit crab *Pagurus bernhardus*. Most recordings were from animals attached to empty *Buccinum* shells, but detached animals and isolated preparations were also used. The sizes of animals used ranged from 3–6 cm. longitudinally.

Suction electrodes were used for both recording and stimulation; contruction details are given by Josephson (1966). Signals were amplified by Tektronix 122 pre-amplifiers and displayed on a Tektronix 564 storage oscilloscope. The main technical difficulty lies in keeping the electrodes attached, as they are dislodged by strong contractions of the animal. As most experiments involved the use of single shocks the problem of contraction was reduced and electrodes would often remain attached for an hour or more.

RESULTS

Electrical activity associated with fast contractions

Josephson (1966) recorded pulses associated with fast contractions in *Calliactis polypus*. Similar activity can be recorded from *C. parasitica*. An electrode on the

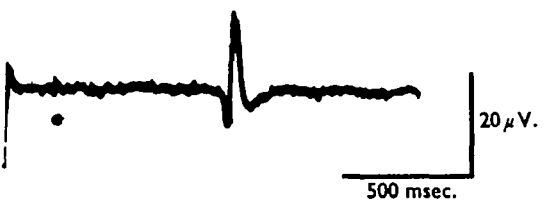

Fig. 1. Electrical activity recorded by a suction electrode on the sphincter region of the column in response to a single stimulus to the mid-column; inter-electrode distance 3 cm. The small pulse marked by the dot is associated with the through-conduction system; the larger pulse is from the slow system (SS1).

sphincter or tentacles records large pulses, up to 1 mV. in size, in response to electrical stimulation. They follow the second and subsequent stimuli in a series and are probably muscle action potentials.

Recordings from the tentacles of *Calliactis polypus* show small pulses following

single stimuli. These have the same response delay as the contraction pulses and Josephson suggests that they are seen because the tentacles are more excitable than the sphincter (as in *C. parasitica*, small twitches of the tentacles are often seen in response to a single shock). Similar small pulses are recorded from the tentacles of *C. parasitica*, but in addition single-shock responses are picked up from the sphincter; the dot in Fig. 1 marks the position of one such pulse. The delay of these pulses is close to that of the large muscle action potentials following the second and subsequent stimuli. They differ from the single-shock responses seen in the tentacles; they are much smaller and more constant in position and size. It is possible that the sphincter pulses represent activity in the fast nerve net supplying the sphincter muscle.

Slow-conduction systems

The slow-conduction systems will be termed SS1 and SS2. Activity recorded from them will be called slow pulses (SP1 and SP2). SS1 activity is recorded by electrodes on any part of the ectoderm (tentacles, oral disk, column and pedal disk) following electrical stimulation of any ectodermal region. The SP1 is largest when recorded

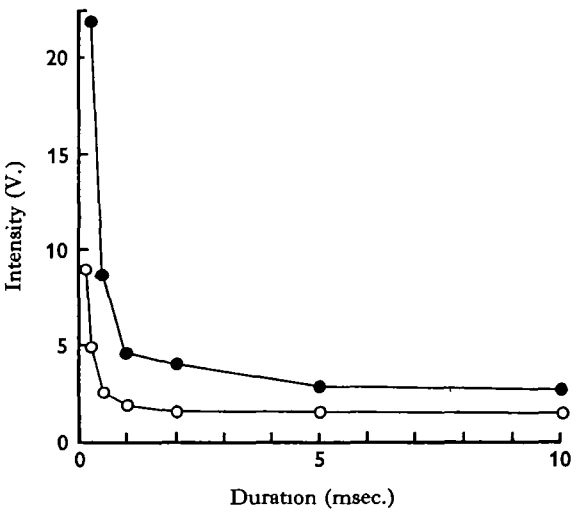

Fig. 2. Intensity/duration curves for the through-conduction system (○) and the SS1 (●). Recording from sphincter and stimulation of mid-column.

from the tentacles and upper column. Figure 1 shows an SP1 recorded from the sphincter region following stimulation of the mid-column. The first pulse is the single-shock response associated with the through-conduction system; the SP1 is considerably delayed in comparison. The amplitude of the SP1 may reach 30 μV. but is seldom greater than 10 μV. The pulse tends to be biphasic but is often predominantly negative when recorded from the tentacles. Pulse duration often exceeds 50 msec.

The difference in threshold between the SS1 and the through-conduction system is shown in Fig. 2, an intensity/duration plot. The points for the SS1 refer to single shocks. The nerve net threshold was found by giving two shocks 300 msec. apart and observing the resulting muscle action potential from the sphincter. This graph refers

to mid-column stimulation and in this position the threshold of the SS1 is 50–100% higher than that of the nerve net. The rheobase was determined for stimuli of 100 msec. duration and the chronaxies for the two systems in five animals ranged from 0·6 to 2 msec. for the nerve net and from 1 to 3 msec. for the SS1. Pantin (1935 *a*) found the chronaxie of the nerve net to be 2–4 msec.

SS2 activity has been recorded only from the tentacles. Three pulses follow stimulation of any other tentacle (Fig. 3A). The first may be a small muscle action potential associated with a small contraction of the ectodermal fast muscles, which are innervated by the through-conduction system. The second pulse is the SP1. This is shown by its threshold and by comparison of response delays in experiments with two recording electrodes, one on the sphincter and the other on a nearby tentacle. The third pulse is the SP2. Recorded SP2s are very small, occasionally reaching 10 μV. but usually less than 5 μV. Their shape is variable but they are often predominantly positive—the reverse of the polarity of the tentacle SP1. For tentacle stimulation, the threshold of the SS2 is slightly higher than those of the SS1 and the through-conduction system. The SS2 can also be excited by stimulation of the column or pedal disk but in these areas its threshold is at least 500% higher than that of the SS1.

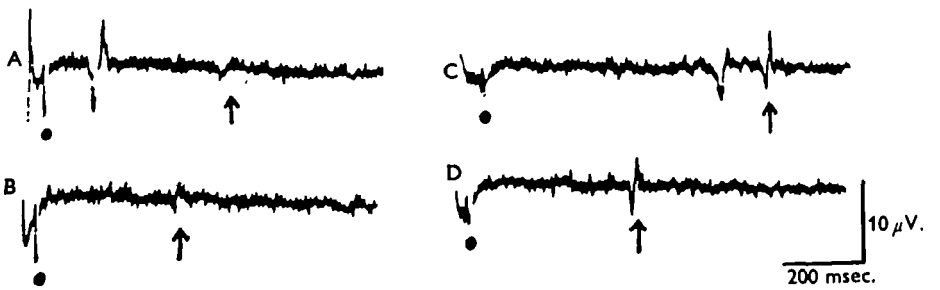

Fig. 3. Recordings from tentacles. A: Single shock to a tentacle 2·5 cm. away. The stimulus artifact is followed by three pulses; a pulse associated with the through-conduction system (dot), an SP1, and an SP2 (arrow). The SP2 is from the second slow-conduction system (SS2). B: Recording electrode in same position, stimulating electrode on the upper limit of the pharynx. Stimulation does not excite the SS1. C: Recording from a tentacle of a half-animal preparation. High intensity stimulation of column ectoderm excites all three conduction systems. Symbols as before. D: Stimulation of perfect mesentery in same preparation excites the through-conduction system and the SS2 but not the SS1.

It has not proved possible to link either slow system with known conducting elements, but the evidence suggests that the SS1 is ectodermal and the SS2 endodermal. Figure 3B is from the same tentacle as Fig. 3A but the stimulating electrode is on the mouth; the SS2 is excited but not the SS1. The SS1 boundary is marked by the junction of the oral disk and pharynx. The derivation of the lining of the pharynx is not clear, it may be endoderm or modified ectoderm (Stephenson, 1928). The pharynx wall consists of this lining layer separated by mesogloea from a layer of normal endoderm. Bearing in mind that the mesogloea cannot be positively excluded as a possible site for conduction, the SS2 seems to be located endodermally. The fact that the SS2 is excited by column stimulation only at high intensities suggests it is some way from the surface, perhaps in the endoderm. Figure 3C is a recording from a tentacle of a longitudinally bisected animal. All three conduction systems are excited by high-

intensity stimulation of the ectodermal surface of the column. In the same preparation, stimulation of a perfect mesentery excites only the through-conduction system and the SS2 (Fig. 3D). Mesenteries consist of two endodermal sheets separated by a layer of mesogloea. For stimulation of the retractor face of a perfect mesentery the SS2 threshold was about 75% higher than that of the through-conduction system. Large muscle action potentials have been recorded from the retractors preceding fast contraction; occasional delayed pulses of small size and long duration were seen in one preparation but this observation has not been confirmed. They may have been SP2s.

The ectodermal nature of the SS1 is suggested by the fact that SP1s can be evoked by low-intensity stimulation of ectodermal surfaces only. Also, if a superficial flap is cut in the ectoderm of the column, stimulation of the flap excites the SS1 only. When the mesogloea under the flap is stimulated only the SS2 and through-conduction system are stimulated. As it proved impossible to cut flaps consisting solely of ectoderm, the superficial region of the mesogloea cannot be excluded as a possible site for the SS1.

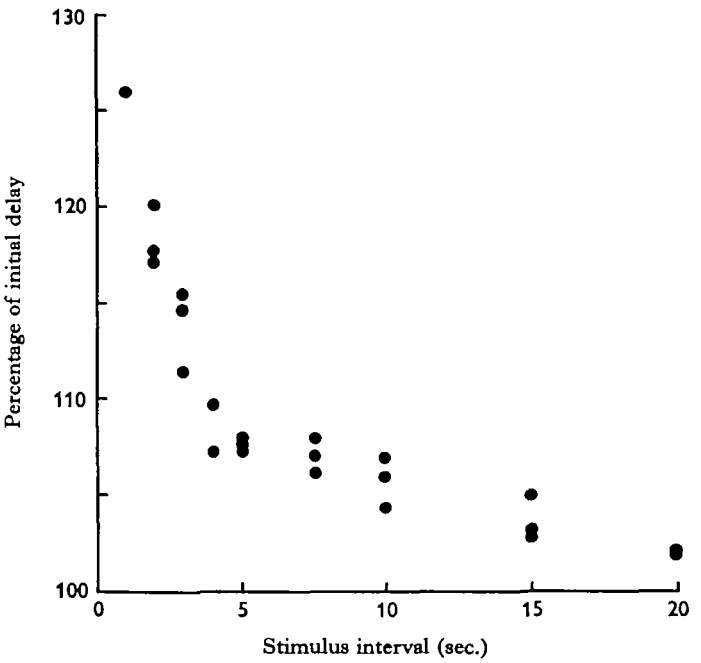

Fig. 4. The duration of the delaying effect of repetitive stimulation in the SS1. Recording electrode over the sphincter, stimulating electrode on mid-column region. Double shocks were given, with the interval between shocks varied from 2 to 20 sec. The delay of the SP1 following the second shock was expressed as a percentage of the delay of the SP1 following the first shock. This value was plotted against the interval between stimuli. Recovery of normal conduction after a single shock is incomplete even after 20 sec.

SS1 properties

Repetitive stimulation at frequencies between about one shock/3 sec. and one shock/20 sec. causes a decrease in the size and an increase in the delay of the response. Conduction rapidly fails at stimulus frequencies greater than one shock/3 sec. Figure 4 shows that there is still some detectable delaying effect when stimuli are 20 sec. apart. Paired stimuli at 1½ times threshold were given and the percentage change in delay of

the response to the second stimulus, compared with the delay to the first, was plotted against the interval between stimuli. The delay increases rapidly as the interval between the stimuli is decreased below 4 sec. The effect falls off slowly at intervals greater than 4 sec.

The relative refractory period of the SS1 is about 1400 msec. and the absolute refractory period about 300 msec. Stimuli of ten times threshold voltage failed to produce repetitive firing. Spontaneous SS1 activity was not seen. The system appears to be non-polarized. Excess of magnesium ions blocks conduction in the SS1 before the through-conduction system.

A possible function has been found for the SS1 and this will be described in the following paper.

SS2 properties

Small pulse size and general lability of this system have made investigation difficult. The SS2 follows low-frequency stimulation in a fresh animal but fatigues rapidly. On repetitive stimulation at intervals shorter than 20 sec. the SS2 shows a more rapid increase in delay than the SS1 and fails more readily. The delay change before failure may be as much as 100%

In one half-animal preparation SS2 activity was very clear and the system was found to be spontaneously active, reaching a maximum firing frequency of one pulse/3 sec., but with long inactive periods. The parietal and circular muscles of the column are both endodermal and spontaneously active but it is not yet known if the spontaneity of the muscles and of the SS2 show any correlation. No clear behavioural correlate has been found for the SS2.

Conduction velocities

Most determinations of conduction velocity were made by recording the changes in response delay when the stimulating electrode was moved. True resting length of the animal is difficult to measure owing to long-term and short-term size variations; this

Table 1. *Slow system conduction velocities* (10–13° C.)

System	Location	Conduction velocity (cm./sec) A	B	C	D	E
SS1	Column longitudinal (adoral)	4·4	6·5	5·5	5·8	5·5
SS1	Column longitudinal (aboral)	3·6	—	—	—	—
SS1	Column circular (sphincter)	4·5	6·5	5·6	6·0	—
SS1	Column circular (base)	3·5	—	—	4·9	—
SS1	Oral disk	11·9	10·0	12·0	14·0	14·6
SS2	Oral disk	4·5	4·4	4·0	—	5·0

problem is less, however, with *Calliactis* than with other anemones. All measurements of velocity were made between 11° and 14° C. Response delay was plotted against conduction path length; the slope is the conduction velocity. Table 1 shows the results of velocity determination for the SS1 and SS2 in different regions of five animals.

The small size of the SP1 recorded from the lower parts of the column makes it difficult to measure the velocity of conduction towards the foot. In all cases where it has been found it is lower than the longitudinal column velocity in the opposite

direction. The circular conduction velocity of the SS1 is the same as the longitudinal at the top of the column but is lower near the base. The SS1 reaches maximum velocity in the oral disk. For eight animals the column velocity orally was 4·4–6·5 cm./sec. (mean = 5·2 cm./sec.) and the oral disk values were 10·0–14·6 cm./sec. (mean = 12·3 cm./sec.). The SS2 conducts at 3·0–5·3 cm./sec. in the oral disk but deviations away from a straight line delay/distance relationship suggest that the conduction path is not direct. The conduction velocity of the SS2 in the mesenteries seems close to that of the SS1 in the column. The conduction velocities of the slow systems and the through-conduction system are compared in Fig. 5.

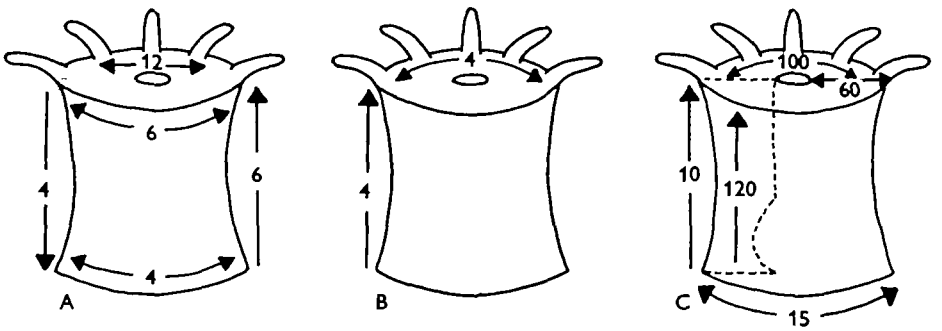

Fig. 5. Comparison of conduction velocities in various regions of the SS1 (A), SS2 (B), and through-conduction system (C). Values in C taken from Pantin (1935*b*) and refer to measurements at 18° C. Values in A and B obtained at 14° C. Velocities in cm./sec.

DISCUSSION

The through-conduction system in *Calliactis parasitica* is well-defined anatomically (Robson, 1961, 1965). The constituent neurites are bipolar cells, on the faces of the retractors, in the oral disk and in the sphincter region. In some places the bipolar cells make contact with a multipolar nerve net.

There is as yet no clear evidence for the anatomical basis of either slow system. There is an ectodermal nerve net in the column of anemones that are regarded as primitive (e.g. *Protanthea*) and the SS1 may represent the remnants of such a system. It is possible that the smooth shape and long duration of the SP1 recorded from the sphincter region result from activity in a number of parallel neurites, but such an accumulation of cells has never been described. The long refractory period and the lability of the SS1 contrast strongly with the properties of the through-conduction system but do not deny a nervous basis for the SS1. The susceptibility to magnesium anaesthetization may favour the view that the SS1 is nervous. However, the conduction velocity of the SS1 seems extremely low for any form of nerve net. The possibility that the SP1 is a muscle action potential can be eliminated as there is no ectodermal musculature in the column and the ectodermal muscles of the tentacles are innervated by the through-conduction system. The large SP1 in the sphincter region cannot represent a slow contraction of the sphincter muscle, as fast and slow contraction are elicited at the same threshold (Ross, 1957).

The SS1 may be an epithelial conduction system, in other words the conducting

elements may be the ectodermal cells themselves. This method of co-ordination has been proposed in other coelenterates where recorded activity is not directly attributable to a nerve net or muscle sheet. The only clearly demonstrated non-nervous systems are in siphonophores (Mackie, 1965) and hydromedusae (Mackie & Passano, 1968). In the siphonophore *Hippopodius* the exumbrellar epithelium conducts at 20–50 cm./sec. and the refractory period is comparable to that of nerve. In comparison the SS1 conducts at 5–15 cm./sec. The nerve-like properties of the siphonophore system are suited to its function as a through-conduction system controlling protective responses, and is in this way equivalent to the fast nerve net in *Calliactis*. The slow systems in *Cordylophora* and *Tubularia* conduct slowly (3–5 cm./sec.) but differ from the SS1 in that they tend to fire repetitively (Josephson, 1961, 1965). The slow systems in these hydroids may be non-nervous but there is no histological evidence that nervous conduction can be positively excluded. The *Tubularia* slow system is very labile and shows antifacilitation of pulse size and conduction velocity.

The SS2 seems to be endodermal but again it is impossible to correlate with any tissue component. Endodermal muscle is a musculo-epithelium so the SS2 may be related to slow muscle contraction. However, these muscles are thought to be excited at the threshold of the through-conduction system. It is possible that the musculo-epithelium can both propagate the observed SP2s and contract in response to excitation from the nerve net. There is a multipolar nerve net in the column endoderm (Robson, 1961).

Further evidence for the location of the slow systems may come from knowledge of their functions. No function has yet been found for the SS2 but the SS1 seems to have the unusual action of controlling detachment of the pedal disk from the substratum. This is fully described in the following paper (McFarlane, 1969). A histological study of the pedal disk may reveal the mode of action and site of the SS1.

SUMMARY

1. Suction electrodes record electrical activity associated with three conduction systems in the sea anemone *Calliactis parasitica*. The two slow systems (SS1 and SS2) are previously undescribed. The third system is the through-conduction system.

2. Evidence is given that the SS1 and SS2 are located in the ectoderm and endoderm respectively. The conductile elements have not been identified.

3. The conduction velocity of the SS1 is 4·4–14·6 cm./sec. at 11° C. and is highest in the oral disk. The SS2 velocity is 3·0–5·3 cm./sec.

4. Both slow systems show a marked increase in response delay on repetitive stimulation and fail at stimulation frequencies higher than one shock/3 sec.

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