DELAYED INITIATION OF SS1 PULSES
IN THE SEA ANEMONE CALLIACTIS PARASITICA:
EVIDENCE FOR A FOURTH CONDUCTING SYSTEM

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SUMMARY

1. Single electrical shocks to the column sometimes elicit a series of
1-6 pulses in the SS1 (ectodermal slow system) but the first pulse does
not appear until 5–28 s after stimulation. These pulses occur in addition to
the early SS1 pulse which follows every shock and which has a conduction
delay of less than 1 s.

2. The threshold of the delayed SS1 response is different from the
thresholds of the three known conducting systems (through-conducting
nerve net, SS1, and SS2).

3. In the case of stimulation of the column, the delayed SS1 pulses do
not arise at the point of stimulation but probably originate in the tentacles
or upper column. The pulse origin can shift during a single burst.

4. The pathway from the point of stimulation to the site of origin of
delayed SS1 pulses is endodermal. We propose that this pathway represents
a fourth conducting system (Delayed Initiation System – DIS). The DIS
must connect, across the mesogloea, with the ectodermal SS1. The long
pulse delay and repetitive firing may derive from pacemaker activity in the
DIS. The DIS pacemakers closely resemble the pacemakers connected
to the through-conducting nerve net. The DIS may be neuronal.

5. Delayed SS1 pulse bursts from unattached anemones showed an earlier
onset, and more pulses/burst, than those from attached anemones.

6. Delayed SS1 pulses can also be evoked by electrical, and in some
cases mechanical, stimulation of the pedal disc, tentacles, and pharynx, but
there are regional differences in the number of pulses evoked, in their
delay, and in their site of origin.

INTRODUCTION

Behaviour in cnidarians is coordinated by multiple conducting systems. McFarlane
(1969a, b, 1974a, b) has described three systems in the sea anemone Calliactis
parasitica (Couch). The through-conducting nerve net excites fast and slow muscle
contractions. The SS1 (ectodermal slow system) inhibits ectodermal muscles and
also causes pedal-disc detachment. The SS2 (endodermal slow system) inhibits
endodermal muscles and nerve-net pacemakers. The SS1 and SS2 show some
properties of epithelial (neuroid) conducting systems and some properties of nerve
nets. They may involve linked neuronal/epithelial conduction of the type proposed in *Hydra* (Josephson & Macklin, 1969) or they may represent a separate category of conducting system (Shelton & McFarlane, 1976).

Some progress has been made in the analysis of control of behaviour in *Calliactis parasitica*. For example, the shell-climbing response (Ross & Sutton, 1961) is co-ordinated by the SS1 and SS2 (McFarlane, 1976). We are still, however, some way from being able to describe how the SS1 and SS2 interact to produce the complex of movements observed in shell-climbing behaviour. A major problem is that the three known conducting systems are through-conducting and symmetrical, whereas many components of behaviour are local movements. For example, the column bending seen during the shell-climbing behaviour is difficult to relate to conducting systems that show no restriction in spread of activity. Pantin (1935) was aware of the problem of control of local movements and he proposed that in addition to the through-conducting nerve net ('mesenteric system') there is a net in the column that shows interneural facilitation ('primary nerve net'). The facilitation requirement would provide the observed restriction in spread of activity. We here present evidence for a fourth conducting system and we suggest that it may be involved in the coordination of local movements.

**MATERIALS AND METHODS**

*Calliactis parasitica* were supplied by the Marine Laboratory, Plymouth, and were kept in aquaria at 10—14 °C. Most experiments used anemones gently detached from *Buccinum* shells. These unattached anemones were suspended from one or more suction electrodes attached to the column. This prevented the tentacles from touching the tank and was necessary because such contact often evokes a sequence of movements, apparently identical to shell-climbing behaviour, that may dislodge the recording electrodes (McFarlane, 1976). Some experiments used anemones attached to shells. Most recordings were from small specimens (oral disc diameter less than 4 cm) but similar results have been obtained with larger individuals. The recordings were from plastic suction electrodes, usually attached to tentacles. Suction electrodes were also used for stimulation. All stimuli were rectangular pulses 1 ms in duration.

**RESULTS**

*Electrical stimulation of the column evokes delayed SS1 pulses*

A single suprathreshold shock to any part of the column of an unattached anemone excited a single pulse in each of the three known conducting systems (through-conducting nerve net, SS1, and SS2). The SS1 pulse was conducted at 5—12 cm s−1 and so usually passed over the whole ectoderm in less than 1 s. This pulse, shortly following the stimulus, will be called the early SS1 pulse.

In addition to the early SS1 pulse, a single shock to any part of the column was often followed by a further single SS1 pulse or by a series of up to six SS1 pulses (Fig. 1). The delay between the stimulus and the first of these SS1 pulses was rarely less than 5 s and sometimes as long as 28 s. It will be seen that this delay greatly exceeds the conduction delay of the early SS1 pulse. We will show later that
Delayed SS1 pulses in Calliactis

Fig. 1. Three delayed SS1 pulses following a single shock to the mid-column region. Recording electrode on a tentacle 2 cm from the stimulating electrode. The early nerve-net and SS1 pulses are obscured by the large stimulus artifact from the high intensity (50 V) shock needed to evoke the delayed response. The early SS2 pulse (Δ) is visible just after the artifact. There is also a spontaneous SS2 pulse (▲) just before the third SS1 pulse. In this case the first delayed SS1 pulse was recorded just less than 10 s after the shock. This is a continuous record and each single sweep lasts 5 s. □ Delayed SS1 pulses. Vertical calibration, 10 μV.

this long delay is largely due to delayed pulse initiation. Consequently the observed pulses will be called delayed SS1 pulses. They are identified as being SS1 pulses because their shape and conduction velocity match those of the early SS1 pulse. Also, where the stimulus-response delay was less than 10 s the delayed SS1 pulse was clearly smaller than the early pulse, presumably because of the long-lasting process of fatigue known to follow a single pulse in the SS1 (McFarlane, 1969a). Delayed SS1 pulses sometimes occur against a background of spontaneous SS1 pulses but the frequency of spontaneous pulses is less than one a minute and it is usually possible confidently to relate delayed pulses to the applied stimulus. The cause of spontaneous SS1 pulses is not known; they may arise from mechanical stimulation (McFarlane, 1973) or from spontaneous activity of the fourth conducting system.

The voltage required to elicit delayed SS1 pulses did not match the threshold of any known conducting system. The threshold of the delayed response depended on the position of the stimulating electrode, and for electrodes with a tip diameter of 500 μm was lowest at the top of the column (10 V) and highest in the mid-column region (40–50 V in large specimens). The higher voltages were above the thresholds of the through-conducting nerve net, SS1, and SS2. Evidence presented later shows that, for stimulation of the column, delayed SS1 pulses often originate at a position several centimetres from the stimulating electrode. This means that excitation travels in a conducting system from the point of stimulation to the regions where the delayed SS1 pulses arise. This conducting system must connect with the SS1 but the system is not the SS1 itself nor either of the two other known systems. Here the term conducting system is defined, following Josephson (1974), simply as the elements responsible for communication over distances.
This newly described conducting system is at present characterized only by its function, not its structure, so we shall use the term Delayed Initiation System (DIS). The conduction velocity of the DIS may be extremely low but it appears that the long delay of the SSi pulses is due largely to a delayed initiation of pulses, either in the DIS itself, or at its connexions with the SSi. Delayed SSi pulses showed a wide range of delays at any stimulation point and this defied attempts to find the conduction velocity of the DIS. The results of a typical experiment will illustrate the difficulties involved. Three stimulating electrodes were attached to the column in a row, each electrode being 1 cm from its neighbour. Stimulating electrodes S1, S2, and S3 were on the base, mid-column, and upper column respectively. The recording electrode was on a tentacle less than 1 cm from S3. Stimulation at 50 V gave the following values for the delays of the first delayed SSi pulse: Si, 5·1–10·1 s (mean 7·0 s); S2, 5·3–10·7 s (mean 8·0 s); S3, 5·3–8·2 s (mean 6·5 s); n = 10 in each position. Results described later show that, for column stimulation, delayed SSi pulses arise in the upper column or tentacles. Clearly, even when the stimulating electrode was close to the point of SSi pulse origin there was still a considerable delay. A puzzling feature shown in this, and in most similar experiments, is that the mean delay for stimulation at the base of the column was shorter than the mean delay for mid-column stimulation, even though the base is further away from the probable region of delayed SSi pulse origin.

Not every shock gave delayed SSi pulses. If single shocks were given at 5 min intervals then each would invariably elicit delayed SSi pulses. Shocks given at 1 min intervals showed a rate of failure to evoke delayed SSi pulses that increased to 100% after about 12 shocks. This was not a failure of the SSi because the early SSi pulse was still present when the delayed response had failed. An increase in pulse delay and a decrease in the number of pulses evoked was usually seen before total failure occurred. The failure rate at any shock interval could be decreased by increasing the stimulus intensity. Another feature is shown in the results of one experiment where the anemone was stimulated every 2 min and the delay in seconds of the first delayed pulse for 10 successive shocks was 12·0, 13·6, 20·1, 20·9, failed, 11·9, 16·8, 17·6, failed, 11·4. These results show that delays increased with successive shocks but after a failure to evoke delayed pulses the next delay was reduced. Clearly there is a process of fatigue somewhere within the system, either in the DIS itself, or at its connexions with the SSi.

**The DIS connects with the SSi in the crown region**

SSi activity is recorded most readily from the tentacles, oral disc, and upper column (McFarlane, 1969a). Difficulties in recording activity from the rest of the column may arise because the corrugated nature of the epithelium prevents a good seal being made by the suction electrode. Recordings do, however, sometimes pick up very small SSi pulses from the base of the column. It is then possible, by having one recording electrode on a tentacle and another at the base of the column, to say something about the position of origin of the delayed SSi pulses.

In the first experiment the stimulating electrode was attached to the mid-column. The early SSi pulse after a single shock reached both recording electrodes more or
Fig. 2. Showing that the delayed SSi pulses caused by column stimulation (S) arise in the crown region. The cut (*) extended completely around the column and blocked SSi conduction only. A single shock evoked an early SSi pulse (1) which was recorded at the base of the column but not at the tentacles. The delayed SSi pulses (2, 3), however, were seen only at the tentacle. R1: electrode on base of column; R2: electrode on tentacle. There is a break of approximately 8 s between the two pairs of recordings. Calibration 1 s, 10 μV.

less simultaneously. The delayed pulses, however, reached the tentacle first, indicating that they arose above the mid-column region.

The point of origin of delayed SSi pulses could be located more accurately by cutting and scraping experiments. The ectoderm was cut through or scraped away in a complete ring around the column. This operation divided the SSi into two parts. SSi pulses stimulated in or arising in areas on one side of the cut were unable to pass the damaged region. In the case shown in Fig. 2 the cut was 3 mm from the top of the column. Stimulation of the mid-column gave an early SSi pulse recorded only at the electrode on the base of the column, whereas the delayed SSi pulses were recorded only at the tentacle. This showed that the delayed SSi pulses arose oral to the cut and also confirmed that the early SSi pulse is not involved in the formation of the delayed pulse burst. Two similar experiments were performed, both involving complete circular incisions around the oral disc. In one the cut was made around the mouth and in the other it was made through the disc at the oral side of the first cycle of tentacles. Column stimulation gave delayed SSi pulses in both cases. Thus DIS to SSi connexions exist in the upper column/tentacle region, but these experiments do not exclude the possibility of other connexions in the oral disc or pharynx.

Recordings from two widely spaced electrodes, both attached to tentacles, showed that the origin of delayed SSi pulses could shift during the course of a single burst (Fig. 3a). The alternative explanation, that the pulses arose at the same place but reached the electrodes by different paths, can probably be discounted because early SSi pulses never showed any evidence of this. Clearly the DIS to SSi connexions are scattered. It is not clear whether origin shifts are due to movements of the active pacemaker site or to additional DIS to SSi connexions becoming functional.

The long initiation delay and repetitive firing of SSi pulses suggest that pacemakers are found within the DIS. Note that the results do not show the pacemakers to be restricted to the upper column or tentacles, only that connexions between the DIS and the SSi occur in this region. Shifting of pulse origin during a burst is
Fig. 3. Nerve-net and SS1 bursts. (a) Simultaneous nerve-net and SS1 bursts after a single shock. The stimulating electrode was on a scraped area of the column, hence there is no early SS1 pulse. The early nerve-net pulse is partially obscured by the stimulus artifact. The shock was below SS2 threshold. Three delayed nerve-net pulses and three delayed SS1 pulses are visible. In this particular case the nerve-net burst started 6·7 s after the shock and 6 s before the beginning of the SS1 burst. The nerve-net burst continued after the displayed recording. Two recording electrodes (R1/R2) were used, attached to tentacles 2 cm apart. Comparison of the first and third SS1 pulses showed that the pulse origin appeared to shift. The first SS1 pulse reached R2 first; the third SS1 pulse reached R1 first. (b): Nerve-net burst evoked by a single shock below DIS threshold but above SS1 threshold. Single recording electrode on a tentacle; stimulating electrode at base of column. The early nerve-net and SS1 pulses are visible. The nerve-net burst started 13·2 s after the shock and contained six pulses. Each sweep in (a) and (b) lasts 5 s. □, SS1 pulses; ■, nerve-net pulses. Vertical calibration, 10 μV.

a feature also shown by the pacemaker system connected to the through-conducting nerve net (McFarlane, 1974b). Nerve-net bursts occur spontaneously but can also sometimes be elicited by single shocks, but in this case the shocks are effective at the threshold of the through-conducting nerve net. Nerve-net bursts evoked by shocks resemble the delayed SS1 pulse burst in that there is a long delay between the stimulus and the first pulse of the burst (Fig. 3b). It may also be significant that a long delay is seen between the first and second pulses of spontaneous nerve-net bursts. Nerve-net bursts normally contain 10–20 pulses and the pulse interval for most of the burst is usually 2·5–4 s. The interval between the first and second pulses, however, in eight successive bursts recorded from a single animal was 3·7–24·9 s (McFarlane, 1974b). Thus the interval between the first two nerve-net pulses in a spontaneous burst is very similar to the range of delays shown by delayed SS1 pulses following column stimulation. Whilst not showing that the same pacemakers connect with both the nerve net and the SS1 this does imply that the pacemakers for the two systems have very similar properties.

Several anemones gave simultaneous bursts of nerve-net and SS1 activity after a single shock (Fig. 3a). Sometimes just a nerve-net burst would be produced and sometimes only delayed SS1 activity. If, however, 5 min or longer was left between stimuli, it was very common to evoke both sets of activity. There were no apparent interactions between the two bursts.

The Delayed Initiation System is in the body wall endoderm

The threshold of the DIS is, for most stimulation positions, higher than that of the three known conducting systems. By itself, the high threshold reveals little
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about the location of the system because in the column the through-conducting nerve net and the SS2 have a low and a high threshold respectively, yet both appear to be endodermal. Regional differences in DIS threshold can, however, be best explained on the basis of an endodermal location for the DIS. Thus the threshold in any position may be related to the thickness of the overlying mesogloea; threshold is high in the mid-column region where the mesogloea is thick. Similarly, small specimens had a lower DIS threshold, and presumably thinner mesogloea, than large anemones. Two simple experiments confirmed the endodermal nature of the DIS.

A shallow flap was cut in the body wall. Such flaps consist of ectoderm and a thin layer of mesogloea. At any voltage, stimulation of the flap gave only a single, early SS1 pulse. Stimulation of the column under the flap, or of an area lightly scraped to destroy the SS1, gave delayed SS1 pulses but not an early SS1 pulse. This suggests that the DIS is endodermal and also confirms that the SS1 is not involved in the initiation of delayed SS1 pulses.

The second experiment showed that the DIS is present in the body wall but possibly not in the mesenteries. A deep cut was made around the mid-column region, penetrating the body wall completely but leaving intact parts of the perfect mesenteries. Nerve-net and SS2 activity could pass the cut because both systems are present in the mesenteries. Stimulation of the column oral to the cut elicited delayed SS1 pulses but stimulation pedal to the cut did not. We conclude that in the column the DIS lies in the body wall endoderm. The following results, however, show that the DIS is also present in other regions.

Delayed SS1 pulses can be elicited by electrical and mechanical stimulation of many parts of the body

The results have so far considered delayed SS1 pulses following electrical stimulation of the column. In fact, both electrical and mechanical stimulation of other regions can evoke delayed pulses but in some places the response differs from the column response.

Column. Electrical stimulation of any part of the column excited delayed SS1 pulses. These pulses appeared to arise in the upper column or tentacles. The delay was independent of stimulus intensity although stronger shocks did reduce the failure rate. Mechanical stimulation also evoked delayed SS1 pulses but was consistently successful only with strong touch to the base of the column in attached anemones. Failure to elicit delayed pulses by touch of other parts of the column may result from the thick mesogloea in the mid-column region and from the fact that the upper column is flexible and can readily move to absorb the impact. The base of the column of an attached anemone is the only area where the mesogloea is thin and the tissue is not free to move significantly. Mechanical stimulation evoked SS1 pulse bursts that contained the same number of pulses and showed a similar delay (4.0–17.0 s) as those evoked by electrical stimulation. Mechanical stimulation also elicits a single, early SS1 pulse (McFarlane, 1969b).

Pedal disc. Delayed SS1 pulses were evoked only by electrical stimulation. The parameters of the response were the same as for column stimulation.
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Table 1. The delay of the SS\textsubscript{1} pulse following a single shock to the pharynx is dependent on stimulus intensity

(Results from a half-animal preparation. The stimulating electrode was on the mid-pharynx and the recording electrode was on a tentacle 2 cm away. Stimuli were applied, in no set order of voltages, at 60 s intervals. Regression analysis shows that the means differ significantly (probability less than 1) with insignificant deviation from a linear regression line.)

<table>
<thead>
<tr>
<th>Stimulus voltage (V)</th>
<th>SS\textsubscript{1} pulse delay (s)</th>
<th>No. of failures</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 V</td>
<td>6.4 3.9-8.5</td>
<td>8 3</td>
</tr>
<tr>
<td>40 V</td>
<td>6.0 5.3-7.2</td>
<td>7 2</td>
</tr>
<tr>
<td>50 V</td>
<td>4.8 3.5-4.8</td>
<td>20 0</td>
</tr>
<tr>
<td>60 V</td>
<td>3.9 3.0-5.1</td>
<td>7 1</td>
</tr>
</tbody>
</table>

Table 2. Bursts of delayed SS\textsubscript{1} pulses show an earlier onset and more pulses in unattached anemones than in attached anemones

(The table shows results from a single anemone, first unattached then attached. Single shocks were given to the column at 2 min intervals and the delay of each delayed SS\textsubscript{1} pulse was noted.)

<table>
<thead>
<tr>
<th>Stimulus number</th>
<th>Delays of pulses in SS\textsubscript{1} burst (s)</th>
<th>Stimulus number</th>
<th>Delays of pulses in SS\textsubscript{1} burst (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unattached</td>
<td></td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.3 15.5 22.3 38.6</td>
<td>1</td>
<td>17.6</td>
</tr>
<tr>
<td>2</td>
<td>14.0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.2 15.7 20.5 34.2</td>
<td>3</td>
<td>16.6</td>
</tr>
<tr>
<td>4</td>
<td>12.7 17.0 23.8 30.2</td>
<td>4</td>
<td>16.4 24.8</td>
</tr>
<tr>
<td>5</td>
<td>13.0</td>
<td>5</td>
<td>17.0 22.9</td>
</tr>
<tr>
<td>6</td>
<td>10.2 14.0 24.2 31.6</td>
<td>6</td>
<td>19.0</td>
</tr>
<tr>
<td>7</td>
<td>10.3 14.0 16.2 24.2 26.8</td>
<td>7</td>
<td>20.8 26.8</td>
</tr>
<tr>
<td>8</td>
<td>10.3 18.4 28.6 35.2</td>
<td>8</td>
<td>20.4</td>
</tr>
<tr>
<td>9</td>
<td>10.2 16.3</td>
<td>9</td>
<td>19.7</td>
</tr>
<tr>
<td>10</td>
<td>9.0 12.2 16.4 21.0 26.4</td>
<td>10</td>
<td>20.6</td>
</tr>
</tbody>
</table>

Tentacles. Attempts to evoke delayed SS\textsubscript{1} pulses by electrical stimulation were rarely successful and then only immediately after the electrode was attached. Touching a single tentacle, however, regularly elicited delayed pulses but never more than three were seen, and the response delay (2.5-13.0 s) was somewhat shorter than for column stimulation. Note that this delay still greatly exceeds the delay of the early SS\textsubscript{1} pulse. The pulses always appeared to originate at, or close to, the point of touch. If touch did not make the tentacle contract then delayed SS\textsubscript{1} pulses were not seen. Early SS\textsubscript{1} pulses were rarely evoked by mechanical stimulation of tentacles.

Pharynx. Delayed SS\textsubscript{1} pulses from the pharynx of half-animal preparations have been previously described (McFarlane, 1975). They have been evoked only by electrical stimulation and they differ from the column response in a number of ways. Single pulses only were elicited, the delay range was only 3-9 s, the delay was dependent on stimulus intensity (Table 1), and there is evidence that the pulses arise at, or close to, the point of stimulation.
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Comparison between attached and unattached anemones

Significant differences were found between the parameters of the delayed SS1 response shown by attached and unattached anemones. Table 2 shows the result of an experiment where the column of an unattached anemone was given 10 single shocks at 2 min intervals. The anemone was detached 1 h prior to testing. The anemone was then allowed to re-attach to a Buccinum shell and 24 h later it was re-tested. Similar results were obtained when the experiment was repeated with the anemone first attached and then unattached. Two features are obvious in Table 2. Firstly, when the anemone was attached there were fewer SS1 pulses in a burst. Secondly, the response delay was greater in the attached state. A further difference, not clearly shown in these particular results, was an increased rate of failure to evoke delayed responses in attached anemones. The delays for the unattached state in Table 2 are unusual in that they decrease as the stimulation series proceeds. This experiment was repeated, with essentially similar results, on four other individuals. In no case did an attached anemone give more than two SS1 pulses in a burst.

The differences between attached and unattached anemones might involve either an altered excitability of the DIS or a change in the likelihood of the DIS-SS1 connexions being functional. We found no satisfactory method to distinguish between these possibilities.

DISCUSSION

Distribution and cellular basis of the DIS

We assume that the common feature seen at all stimulation sites, namely production of delayed SS1 pulses, means that a single conducting system is involved in all the delayed responses and that the different response parameters at different sites simply reflect regional variations within this conducting system. If this is true, then the DIS has a widespread distribution and is present at least in the tentacles, column, pedal disc, and pharynx. The DIS is endodermal in the body wall of the column, but we do not know if it lies in the same layer everywhere. The DIS connects with the ectodermal SS1, presumably by links that cross the mesogloea. Connexions are present in the upper column/tentacle region and in the pharynx.

The regional differences in the response imply that DIS pulses do not show an unrestricted spread, in other words the DIS is not through-conducting but is a local system. It appears, for example, that the pharynx DIS is not linked with the body wall DIS (if it were we would expect occasionally to see multiple SS1 pulses after pharynx stimulation). This is supported by the observation that DIS information does not pass readily along the mesenteries, for these structures provide an obvious pathway between the pharynx and the body wall. This need not mean that the DIS is absent in the mesenteries, for here the system may require multiple firing to produce effective conduction. All we can say about the directions of conduction in the DIS is that there must be longitudinal routes in the column because stimulation at the base gave delayed SS1 pulses that arose in the crown region. The column and the pedal disc appear to be the only regions where the delayed pulses do not arise close to the point of stimulation. This may be due to a regional lack of either pacemakers or of DIS-SS1 connexions.
Speculation on the cellular basis of the DIS is difficult because we have no direct recordings of DIS electrical activity. It would be valuable to know the DIS conduction velocity and whether stimulation evokes single or multiple DIS pulses. Recordings from tentacles of *Calliactis parasitica* and *Stomphia coccinea* have shown pulses that are not in the through-conducting nerve net, SS1, or SS2, but apart from the observation that such pulses are local rather than through-conducted (McFarlane 1975, 1976; Lawn & McFarlane, 1976), there is no evidence to associate them with the DIS.

Three possible substrates for the DIS are a nerve net separate from the through-conducting nerve net, a neuroid system, and mechanical spread through the endodermal muscle layer. Mechanical spread can probably be eliminated because it is difficult to see how this could provide the observed pacemaker activity. If the DIS were a neuroid system this would presumably involve conduction in the endodermal musculo-epithelium; this, however, has already been suggested as the structural basis for the SS2 (McFarlane, 1969a). We consider that the DIS is nervous, although none of the following reasons firmly excludes the possibility of neuroid conduction. Firstly, connexions across the mesogloea could be made most readily by nervous elements. There is, however, a precedent for a neuroid system making a similar connexion in hydrozoans (Mackie & Passano, 1968). Secondly, although some suspected neuroid systems show repetitive firing (Josephson, 1974), the majority of cnidarian pacemaker systems appear to be nervous. Thirdly, there is no direct evidence for local conduction in neuroid systems. Theoretical considerations, however, suggest that this could happen (Shelton, 1975).

Multipolar nerve cells may provide the cellular basis for the DIS. Bipolar, multipolar, and sensory cells are present in the endoderm. Areas of the net, in particular the bipolar cells in the mesenteries and in the sphincter region, are clearly associated with the through-conducting nerve net (Batham, Pantin & Robson, 1960; Robson, 1961, 1965). The multipolar cells are found in the same region as the DIS, the presence of several neurites allows for complex interconnexions with other conducting systems, and being nervous these cells could provide the observed pacemaker function. Multipolar cells are also thought to be the pacemakers that connect with the through-conducting system in *Calliactis parasitica* (McFarlane, 1974b) and to be the pacemakers of the swimming contractions of *Stomphia coccinea* (Robson, 1963). Two interacting nerve nets may also be present in the pennatulid *Reniula* (Anderson & Case, 1975).

**The DIS pacemakers**

The long initiation delay and repetitive firing of delayed SS1 pulses suggest that the DIS has pacemaker properties. The multipolar nerve cells have been proposed as being the pacemaker cells of both the DIS and the through-conducting nerve net. The simplest explanation of the results is that DIS pacemakers connect with both the SS1 and the through-conducting nerve net. Certainly there are similarities between nerve-net and SS1 bursts. The inter-pulse interval is similar in both and even more striking is the similarity in delay following initiation by single shocks. In both cases the pulse origin can shift during the burst. There are, however, three important differences between the bursts. Firstly, nerve-net bursts occur spontaneously
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(McFarlane, 1974b) whereas SS1 bursts have only been seen following shocks. Secondly, although nerve-net bursts can also be initiated by single shocks, these are at the threshold of the through-conducting nerve net. Thirdly, nerve-net bursts usually contain many more pulses than SS1 bursts.

If DIS cells connect with both the through-conducting nerve net and the SS1 then there are two possible arrangements. Either all cells connect with both systems or there are two separate populations of cells, each making only one type of connexion. The second arrangement may be considered more reasonable because spontaneous nerve-net pulses are not always accompanied by SS1 pulses. On the other hand, special conditions may have to exist for the DIS–SS1 connexion to be functional. We have already noted that delayed SS1 pulses are more readily elicited in unattached anemones than in those attached to shells. Careful study is required to see if unattached anemones are also more likely to show spontaneous or evoked nerve-net bursts. The observation that SS1 bursts are evoked at a higher stimulus intensity than nerve-net bursts does not deny that the DIS pacemakers are connected to both systems. Perhaps the intense stimuli evoke multiple DIS pulses, and such repetitive firing may be needed to activate the connexion with the SS1. The differences in burst length may be explained by proposing that nerve-net pulses, but not SS1 pulses, feed back onto the pacemakers in an excitatory fashion.

Behavioural significance of the DIS and the delayed SS1 pulses

In the relationship between Calliactis parasitica and the hermit crab Pagurus bernhardus, the anemone is solely responsible for achieving attachment to the shell, which it does by a complex shell-climbing behaviour pattern evoked by tentacular contact with an unknown chemical on the shell surface (Ross & Sutton, 1961). SS1 and SS2 pulses are recorded from tentacles during this behaviour (McFarlane, 1976) and one of the most important functions of the SS1 pulses is to coordinate pedal-disc detachment (McFarlane, 1969b). In order for detachment to occur there must be at least 30 SS1 pulses, with a mean pulse frequency greater than 1 every 10 s. Most of the SS1 and SS2 pulses seen during climbing appear to originate from tentacles in contact with the shell, and they have thus been interpreted as representing a chemosensory response. We now know, however, that mechanical stimulation of tentacles can evoke delayed SS1 pulses. Tentacles that respond to the shell appear to adhere and then contract longitudinally. This contraction may excite the DIS (or may even be a result of DIS activity). Possibly, then, some of the observed SS1 pulses arise via activation of the DIS. This cannot explain all the pulses, because the DIS pacemakers fatigue rapidly and would appear incapable of producing the large number of SS1 pulses actually seen during climbing. Nevertheless, any additional pulses that arise via the DIS may function to counteract any tendency towards adaptation by the SS1 chemoreceptors and thus ensure that the SS1 pulse frequency remains high enough for pedal-disc detachment to be speedily executed. This may also play a role in other Pagurid/Calliactis relationships where the crab plays a more active part in the transfer by poking the column of the anemone until it detaches (Ross & Sutton, 1970). It is likely that the crab's behaviour evokes SS1 pulses that cause the observed detachment of the pedal disc: mechanical stimulation of the base of the column in C. parasitica evokes single early SS1 pulses. The present
work shows that touch in this region can also evoke delayed SS1 pulses. Again these pulses may function to maintain a high SS1 frequency. In C. polypus detachment in response to electrical stimulation requires a stimulus frequency greater than 1 shock every second (Ross & Sutton, 1970).

The shell-climbing response of Calliactis parasitica is shown only by unattached anemones or by those on inorganic substrates, not by anemones already attached to shells (Davenport, Ross & Sutton, 1961). It is thought that this is an example of modification of nematocyst or spirocyst threshold, triggered here by the nature of the substrate to which the anemone is attached. In the light of the recent model for the involvement of the SS1 in the control of spirocyst threshold (McFarlane & Shelton, 1975) it would be interesting to see if the DIS–SS1 system is more sensitive in anemones attached to glass plates than in those on shells. We also require comparative information from species which do not show a shell response to see if the DIS–SS1 link is of common occurrence or if it may just be a specialization appropriate to species with complex behaviour patterns involving SS1 activity.

The DIS lies in the endoderm in the column and it is likely that the system has endodermal actions. One possible function would be the control of local muscle movements. The DIS may be the primary nerve net postulated by Pantin (1935). Restricted spread of pulses in this net was explained by a need for interneural facilitation. We have no direct evidence for such a process in the DIS although the high stimulus threshold may reflect a necessity to evoke multiple DIS pulses in order to get activity to spread to the connexions with the SS1.

This work has shown that an endodermal conducting system connects with the ectodermal SS1. Such a connexion (but working in the opposite direction) has been previously postulated (McFarlane, 1973) because SS1 stimulation causes column extension, presumably by exciting contraction of endodermal circular muscles. Studies of the swimming anemone Stomphia coccinea (Lawn, in preparation) have provided elegant evidence that SS1-to-endoderm connexions are present in this species also. The majority of links were shown to occur in the upper column. Thus two studies, the present work and the earlier work of Lawn, show SS1-to-endoderm connexions in much the same region but working in opposite directions. It remains to be established if there is a single type of non-polarized link or whether Lawn’s SS1-to-endoderm connexion is with a conducting system other than the DIS. A demonstration that the DIS elicits symmetrical contraction of circular muscles would not disprove the suggested local nature of the system because multiple inputs from the SS1 could convert an actual local system into an apparent through-conducting system.

Organization of conducting systems

Fig. 4 summarizes possible interconnexions between conducting systems and connexions between conducting systems and effectors. This is based on an earlier diagram (McFarlane, 1974 a) with additions derived from the present work, Lawn (1976 a, b) and McFarlane & Jackson (1976). The DIS is shown at right angles to the three ‘through-conduction’ systems to emphasize that conduction in this system may be local. The DIS may be a population of linked pacemakers. The pacemakers that connect with the through-conducting nerve net may be within that population.
**Delayed SS₁ pulses in Calliactis**

Fig. 4. Summary of the actions and interactions of the four known conducting systems in *Calliactis parasitica*. M, mechanoreceptive input; C, chemoreceptive input; P, pacemaker.

The diagram does not include all the known muscle groups. The horizontal inputs to the muscle groups (excitatory and inhibitory inputs from the nerve net, SS₁, and SS₂) may affect the entire muscle group. The vertical inputs (possibly from the DIS) show that, with the possible exception of the sphincter muscle, there are local excitatory inputs giving rise to local contractions. The major assumption made in this summary is that demonstrated interactions are direct. In no case, however, has this been positively established.

**REFERENCES**


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