J. exp. Biol. **135**, 133–164 (1988)
Printed in Great Britain © *The Company of Biologists Limited 1988*

SYSTEMS THAT CONTROL THE BURROWING BEHAVIOUR OF A SEA ANEMONE

BY PETER E. PICKENS

Department of Molecular and Cellular Biology, The University of Arizona, Tucson, AZ 85721, USA

Accepted 2 September 1987

SUMMARY

The sand-dwelling sea anemone, *Phyllactis concinnata*, buries itself by attaching sand grains to the lower column as this is bent under the anemone by a travelling peristaltic wave. Some of the sand is then released as the column expands laterally. Once buried, the anemone increases in length over a period of several hours until the pedal disc finally attaches to a buried shell. Burrowing is controlled by bursts of pulses generated by the through-conducting nerve net. These pulses produce column shortening and peristalsis. Regular intervals between pulses and between bursts suggest that pacemakers are driving the nerve net. Pulse patterns are modified after sand has surrounded the column and when the pedal disc touches hard substratum. A second conducting system (the SS2) can, under specific experimental conditions, respond to mechanical and chemical stimuli and inhibit nerve net discharge, but its function during burrowing is not known, even though it produces a distinctive pattern of pulses. After the column has been buried, an anemone may contract spontaneously and rapidly. Two other types of behaviour, pharynx eversion and antiperistaltic behaviour (crawling), usually precede burrowing and each is associated with its own characteristic pattern of pulses.

INTRODUCTION

The behaviour of sea anemones ranges from simple, local contractions of the tentacles or column to complex activities such as swimming and shell-climbing (Shelton, 1982). In several anemones these activities are coordinated by one or more conducting systems. The properties of these systems have been determined by giving electric shocks to tentacles or column to evoke pulses which are recorded by suction electrodes attached to the tentacles. Such stimulation elicits nerve net pulses (NNPs) from the through-conducting nerve net (TCNN), larger pulses (SP1s) from an ectodermal, slow-conduction system (SS1), and smaller pulses (SP2s) from an endodermal, slow-conduction system (SS2) (McFarlane, 1969). In addition to conducting systems with recordable pulses there are other systems whose existence is inferred from the responses of the SS1 to certain kinds of stimulation (Jackson & McFarlane, 1976; Lawn, 1980). In general, the TCNN excites muscles to contract

Key words: sea anemone, burrowing, nerve net.

rapidly or slowly, the SS1 inhibits ectodermal muscles or causes the pedal disc to detach, and the SS2 inhibits endodermal muscles or a TCNN pacemaker (McFarlane, 1982).

Some anemones burrow into sand or mud. Burrowing is a rhythmic activity in which periodic contractions of muscles of the body wall change the shape of the column of fluid within the anemone so that the anemone is continuously driven into the sand until buried (Ansell & Trueman, 1968). The burrowing behaviour of *Phyllactis concinnata* has been described by Mangum (1970). The present study was undertaken to determine the role of the nervous and/or other conducting systems in controlling burrowing behaviour.

MATERIALS AND METHODS

The sea anemone, *Phyllactis concinnata*, has a flat pedal disc at the base of its column (Carlgren, 1951). It also has a ruff that projects from the upper edge of the column below the tentacles. The ruff is composed of hollow expansions of the body wall. Apart from the ruff, the anemone's anatomical features resemble those of well-known anemones. Animals used in these experiments were 15–40 mm across the oral disc and 20–70 mm tall, the average being about 35 mm. Specimens were obtained from the northern Gulf of California and maintained in a seawater system at the University of Arizona, in which artificial sea water (Instant Ocean) circulated through aquaria that were half-filled with sand. The aquarium room was on a 12 h : 12 h day : night cycle and was kept constantly at 19 °C.

To study burrowing, an anemone was placed in an experimental chamber with sand and sea water. The water trickled through the chamber during experiments which sometimes lasted as long as 8 h. The sea water in the chamber was maintained between 20 and 22 °C. In some experiments movements of the anemone were monitored by attaching two small, threaded hooks to the oral disc. Each thread was connected to a counterweighted lever that was part of a linear motion transducer. Output from the transducer went to a chart recorder. Biological potentials were picked up from the surface of the anemone by attaching two suction electrodes to the bases of two widely separated tentacles. These electrodes were connected to a.c. preamplifiers whose output went to the chart recorder and to an oscilloscope. The oscilloscope and an audiomonitor were used to confirm the identity of potentials recorded on paper. Electrical stimuli were given through two additional suction electrodes that were also attached, when needed, to the tentacles. The long and flexible suction electrodes seldom came loose during burrowing, even during rapid contractions. The sizes of all pulses increased several minutes after electrodes had been attached, possibly because the tentacles were less contracted. During these experiments reduction in pulse size seemed to be related more to tentacle contraction and other transient events than to the accumulation of mucus in the electrodes.

Magnesium chloride blocks contractions and all three conducting systems in anemones, with the SS1 being blocked sooner than the others (McFarlane, 1973*b*). The standard magnesium chloride solution used as an anaesthetic for anemones has

been a half-and-half mixture of sea water and a solution of magnesium chloride isotonic with sea water (final Mg^{2+} concentration $0{\cdot}18\,mol\,l^{-1}$) (Batham, Pantin & Robson, 1960). Mixtures of other percentages of magnesium chloride have been also used, so that a 10% solution had 10 ml of isotonic magnesium chloride mixed with 90 ml of sea water.

In *Calliactis* the threshold of the SS2 is lower than that of the TCNN when shocks of long duration are given, but when brief shocks are used the TCNN responds to a weaker stimulus (McFarlane, 1974*a*). This difference also exists in *Phyllactis*, so shocks of 1 ms duration were used to evoke NNPs and of 20 ms or more to evoke SP2s. However, when an anemone was exposed to higher concentrations of magnesium chloride it was not possible to elicit different pulses by this technique because the threshold of the SS2 had risen.

RESULTS

During this study of burrowing, four types of activity were observed: eversion of the pharynx, antiperistaltic behaviour, burrowing and the startle response. The first two usually occurred just before burrowing, the last one was a part of it. Each activity was characterized by its own pattern of pulses and comparisons between them contribute to an understanding of how burrowing is controlled. Three types of pulses, NNPs, SP1s and SP2s, occurred spontaneously during these activities, but could be evoked by electric shocks at any time (Fig. 1).

Most of the time during burrowing, tentacles moved very little. Once in a while they twitched briefly at the start of an NNP burst and they were all contracted during the startle response. Such contractions were usually accompanied by tentacle contraction pulses (TCPs). TCPs have been observed to accompany tentacle contractions in *Calliactis parasitica* (McFarlane, 1984).

Eversion of the pharynx

A burrowed anemone in the aquarium had its ruff and oral disc flush with the surface of the sand and its pedal disc attached to shell fragments several centimetres below the surface. After it had been transferred to the experimental chamber, SP1s occurred 7–20 s apart: the average interval between SP2s was 12 s. SP1 intervals were shorter when an anemone was taken out of the sand and hooks were attached to the edge of the oral disc. Mechanical stimulation of the anemone as it was dug up produced pharynx eversion, that is the pharynx ballooned above the oral disc after the mouth had opened (Fig. 2A). Sometimes an anemone that had just been dug up in the field also everted its pharynx so this was not just a laboratory phenomenon and might occur under natural conditions when strong currents or a browsing fish have exposed a buried anemone. The function of pharynx eversion is not known. The pharynx was usually withdrawn within 15 min, and thereafter SP1s occurred infrequently. During burrowing they were not consistently related to any particular phase of the burrowing cycle. If an anemone was bathed in a solution of 10% magnesium chloride in sea water, spontaneous SP1s disappeared, but burrowing

continued. It is concluded, therefore, that the SS1 is not involved in the control of burrowing.

Antiperistaltic behaviour

Once every few months an individual abandoned its burrow and crawled across the sand to another site. To reach it, the anemone propelled itself forward with tentacles leading and column on the sand. This activity, designated in this paper as

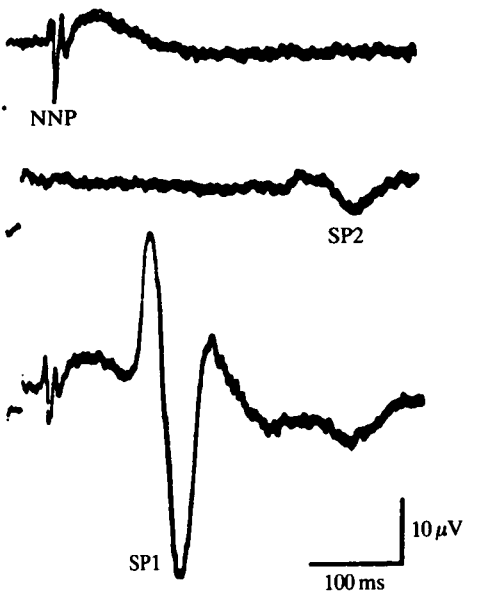

Fig. 1. Three types of pulses evoked by stimulating a tentacle of *Phyllactis* with electric shocks. In the top trace a nerve net pulse (NNP) and a broader, muscle action potential were recorded from a tentacle in response to a stimulus of 1 ms duration. The electrical events labelled NNPs in the following figures are dominated by muscle action potentials which follow the NNPs and are often much larger than the nerve impulses. In the middle trace a pulse (SP2) from the second slow-conducting system (SS2) was evoked by stimulating at a lower voltage with a shock of 20 ms duration. In the lowest trace a shock was used to evoke a pulse (SP1) from the first slow-conducting system (SS1) as well as an NNP, which precedes the SP1, and an SP2 which follows it.

Fig. 2. Four different types of pre-burrowing and burrowing behaviour, with traces showing typical pulse patterns recorded from tentacles. Each trace is based on one recording from each of four different anemones, but for the sake of clarity pulses actually recorded have been replaced by pulses of three standard sizes. The large pulses in A are SP1s. They are sometimes present during the types of activity shown in B, C and D, but are not shown here because they did not occur in these particular traces. Small pulses in all four traces are SP2s and the large pulses in B, C and D are NNPs except for the seventh pulse in D which is a compound potential composed of a burst of two or three NNPs and a large muscle action potential. Pulse patterns in C are similar to those that occur during the first phase of burrowing. Each drawing shows the shape of the anemone at the time pulses are occurring in the trace above.

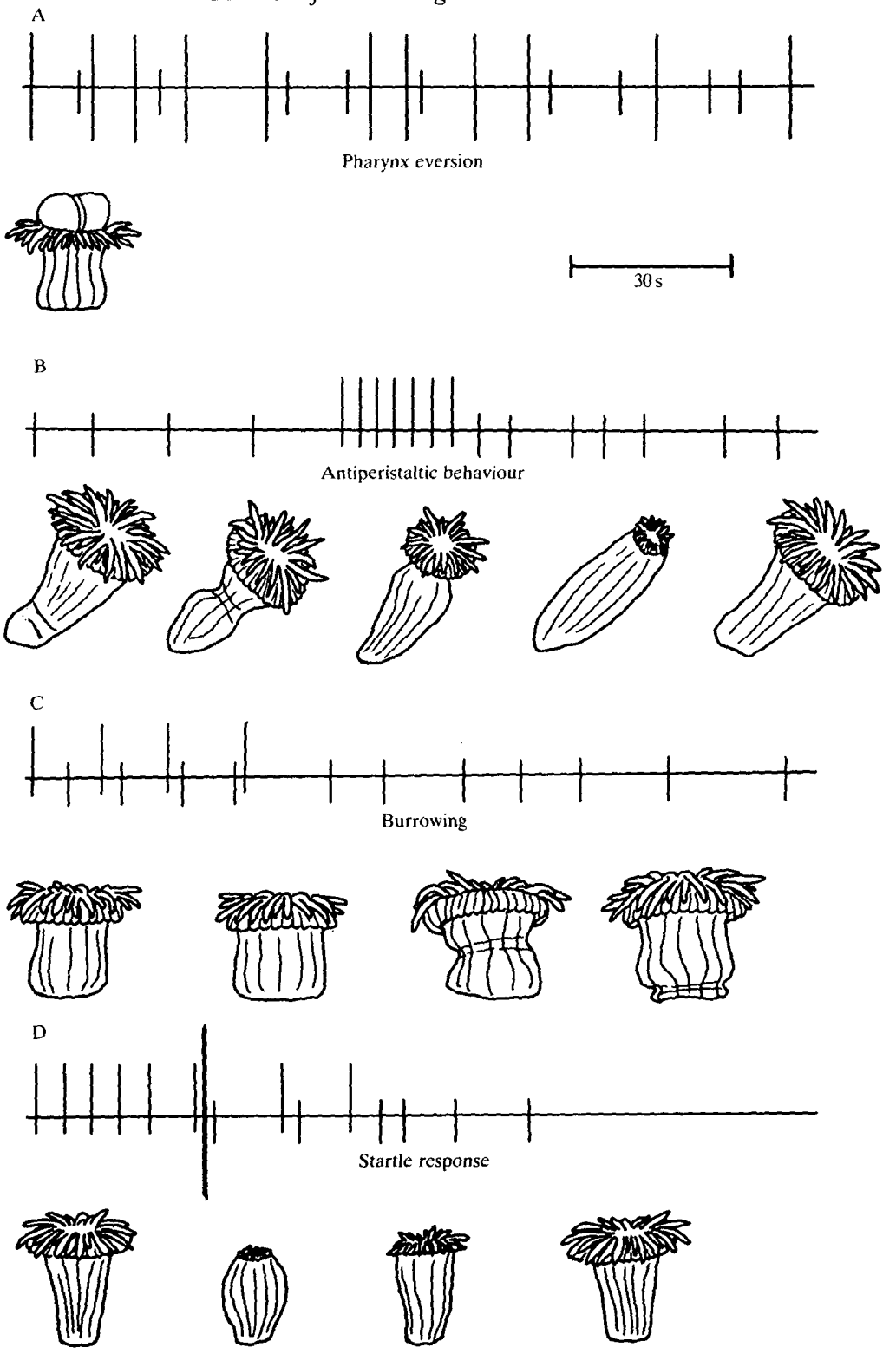
A
Pharynx eversion
30 s
B
Antiperistaltic behaviour
C
Burrowing
D
Startle response

Fig. 2

antiperistaltic behaviour, was also seen shortly after an anemone had been transferred to the experimental chamber. A major component of antiperistaltic behaviour was antiperistalsis (Batham & Pantin, 1954): the movement of a ring of contracted circular muscle up the column towards the oral disc. During burrowing peristalsis occurred, with the same type of ring travelling towards the pedal disc (Batham & Pantin, 1954).

Antiperistaltic behaviour (Fig. 2B) commenced with the formation of a ring at the base of the column. The ring then travelled upwards and, as it approached the top of the column, there was typically a burst of NNPs, the ruff (the fringe around the oral disc) contracted, the oral disc was lowered, and the top of the column narrowed. The ruff and oral disc then expanded, and the column lengthened, thus completing one antiperistaltic cycle. One cycle in a standard-sized anemone initially lasted about 120 s. The trigger for the next antiperistaltic ring is not known, but during the early cycles in a series a ring usually started up the column as the ruff was expanding again; that is, before the previous cycle was complete. As antiperistaltic activity continued, the interval between cycles increased and at later stages a ring could form as infrequently as once every 6 min, long after the completion of the previous cycle. An increase in column length was associated with continued antiperistaltic behaviour: after five or more cycles, an anemone would be nearly twice as long (65 mm for a standard-sized anemone) as it had been when it started.

The number of pulses in the NNP burst determined the degree of contraction of the ruff and upper column. A typical antiperistaltic burst consisted of 5–7 NNPs and lasted less than 20 s. The interpulse interval (IPI) was about 3 s. A burst of four or more pulses caused the ruff to withdraw completely. SP2s seldom occurred during the NNP burst; the first appeared from 5 to 25 s afterwards, as the ruff was expanding.

The column remained free of sand until the anemone started to burrow. At this point, sand adhered to the base of the column when the anemone was upright or to the side when it was horizontal. If the anemone was rolled around the test chamber after sand had adhered, other parts of the column did not pick up sand, so that when the rolling stopped the anemone came to rest upright or on the side weighted with sand. This, of course, is of advantage to an anemone subject to changing currents in its habitat.

Rhythmic burrowing

When the anemone finally began to burrow it was usually upright and had shortened so that it was about as tall as it was wide across the oral disc and ruff. If burrowing began before the anemone was upright, the column remained elongate while the anemone dug into the sand at an angle. As the anemone started to burrow the base of the column accumulated a ring of sand, and as burrowing proceeded the column slowly became coated with sand. Prior to burrowing there were no NNPs except during antiperistaltic behaviour and the intervals between SP2s were irregular. Burrowing behaviour was divided into three phases. In the first the column was buried, in the second the column was lengthened, and in the third the pedal disc

was attached to a buried shell. Each phase had its characteristic pattern of pulses. As burrowing started, bursts of NNPs occurred (Fig. 3A). The first NNP burst was a complete burst of 3–5 pulses; that is, bursts were not gradually built up from single pulses. An anemone could even switch abruptly from antiperistaltic behaviour to burrowing. After the third NNP in a burst the anemone began to shorten by a few millimetres and in 70–100 s shortening was complete. Then a ring of contracted circular muscle formed at mid-column and travelled towards the pedal disc at about $0{\cdot}5\ mm\ s^{-1}$ (Fig. 2C). If the ring did not form, burrowing did not occur. One burrowing cycle ended when the ring reached the base of the column. The next NNP burst began within 10 s after the ring had arrived. Bursts were repeated about every 150 s until the ruff touched the sand and the column was buried. In most anemones this first phase (Figs 3A, 4) was completed after 25–35 cycles, about one-sixth to one-quarter of the total number of cycles (100–130) that occurred during burrowing. After the column had been buried the interval between the start of one NNP burst and the start of another, the interburst interval (IBI), increased slightly. There was also a small increase in the number of NNPs per burst. The average IPI decreased to about 12 s and IPIs became less variable. During this second phase of burrowing, ruff and oral disc remained on the surface of the sand while the column lengthened as the pedal disc continued to penetrate the substratum.

The SS2 generated pulses during burrowing, but the pattern of SP2s changed as burrowing proceeded (Fig. 4). At the start of the first phase, the irregular SP2 pattern of the non-burrowing anemone continued. However, after a few cycles the SS2 did not begin firing until the nerve net had produced two or three pulses. At the start of the second phase, after the ruff had touched the sand, the pattern changed and SP2s tended to be grouped in and near the NNP burst. The first SP2 occurred after the second or third NNP and was followed by 2–5 more SP2s. The other major change was that the latency for the first SP2 after the first NNP in a burst decreased gradually over 5–7 cycles and then increased over one or two cycles. Startle responses were most likely to occur when the SP2 latency was short.

Startle responses

When the anemone was buried up to its ruff, there could be a sudden, rapid shortening of the column during an NNP burst (Fig. 2D). This startle response was identical to the protective withdrawal reflex of *Phyllactis* and other anemones (Hall & Pantin, 1937). During the second phase of burrowing it usually occurred about once every 5–7 cycles. When it happened, the tentacles contracted, the oral disc was pulled down, and the top of the column closed over the oral disc. In its extreme form the startle response produced an almost spherical anemone. The response was triggered only during an NNP burst, about 25–35 s after the first NNP. The burst in which a startle response was most likely to occur could be predicted: if the latency for the first SP2 was measured and fell below 20 s during successive bursts (e.g. bursts 73 and 88, Fig. 4), then a startle response was likely to occur one or two bursts later. The startle burst had more NNPs than a burrowing burst and IPIs were shorter. Furthermore, the pattern of pulses in a startle burst differed from that found in

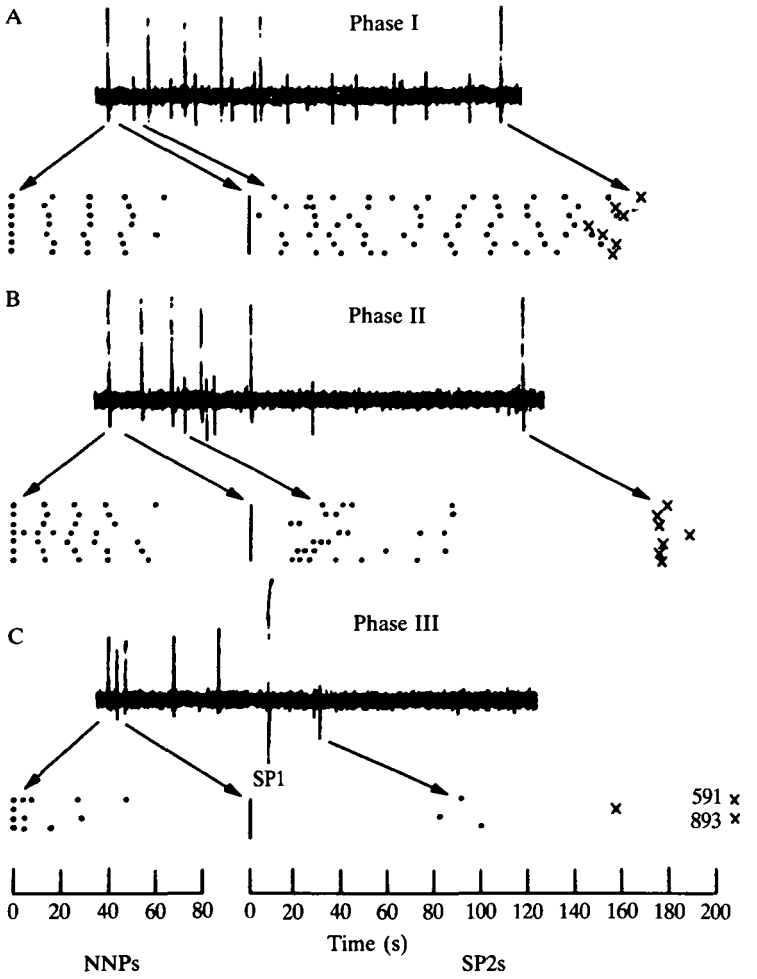

Fig. 3. Recordings of pulse patterns that are typical of each of the three phases of burrowing. Small pulses are SP2s and large pulses are NNPs except for the largest in the bottom trace which is an SP1. Below each trace the time of occurrence of each pulse is shown as a dot in a raster-like plot, with NNPs plotted separately to the left and SP2s to the right. Each row of dots represents a single burst and, for each of the first two phases, seven bursts are shown sequentially. The vertical line beneath each trace and the first dot on the left in every row indicate the time of occurrence of the first pulse in an NNP burst. Each × on the right marks the first pulse in the next NNP burst and is equivalent to the first dot on the next row. The time between the vertical line and the × is the interburst interval (IBI). Phase I is characterized by SP2s occurring throughout the IBI. In phase II most SP2s occur between the second or third NNP and the middle of the IBI. Phase III is characterized by NNP bursts with short intervals between NNPs at the start of a burst, few SP2s and more variable IBIs, some of which are very long; two lasted 591 and 893 s. These recordings were made with only a pair of recording electrodes attached to the tentacles.

either the burrowing or antiperistaltic bursts (Fig. 5A). The first group of NNPs in a startle burst consisted of four to six pulses that occurred, on the average, about 5 s apart. They were separated from a pulse that just preceded the startle response by a longer (8–9 s) interval; that is, there was a 'pause' in the burst. The single NNP that followed the pause was itself followed after 2 or 3 s by a group of two or three NNPs, 150 ms apart (range 80–200 ms) that triggered the startle contraction. After contraction had started the nerve net did not always continue firing. If it did, it produced up to four NNPs at the burrowing IPI of 12 s. Sometimes the two or three closely spaced NNPs and the startle response were absent, but the rest of the burst resembled the startle burst (Fig. 5B). In response to this 'non-startle' burst the anemone still shortened, but not as rapidly as it did during the startle response. Startle responses usually had little or no effect on the IBI, at most delaying the start of the next burst by a few seconds. After the startle response the level of the oral disc was 3 mm below that at the beginning of the startle burst. It was gradually raised again over several cycles. The transient shortening and lengthening that followed each NNP burrowing burst was superimposed on this gradual relaxation. Startle responses in most anemones are considered to be protective responses and can be triggered by poking or pinching the tentacles. However, the rapid contractions described here appeared to be triggered endogenously, occurring in the absence of strong mechanical stimuli. They were a normal part of burrowing, often being seen in an anemone without attachments after its column had been buried. Their function is unknown, but they might serve to widen the burrow. There were usually about seven startle bursts during burrowing, but numbers ranged from none to twelve.

Termination of burrowing

The second phase of burrowing usually ended when the pedal disc attached to hard substratum or, if none were present, when the column reached a length that was more than twice its starting length. The third and final phase could be characterized as a period of adjustment that lasted for 15–60 min (Fig. 4). There were fewer NNPs, NNP bursts were shorter, bursts began with short IPIs, later IPIs were more variable, and the IBI and SP2 patterns were irregular. Rhythmic activity eventually stopped. By this time the anemone's column was similar in shape to that of an anemone just extracted from its burrow in the field; it had increased in length from 30 to 70 mm and was tapered. The pedal disc was narrow and attached to shell fragments or to large sand grains if no shells were available. Buried anemones exhibited no short cycle rhythms, but longer cycle rhythms could be present as they are in *Metridium* (Batham & Pantin, 1950). In the dark, the column lengthened and the ruff was raised about 10 mm above the surface of the sand. In the light, a buried anemone produced few pulses of any kind. NNPs, when they occurred, were often in triplets with IPIs of 4 s. SP2s were not closely associated with NNPs and *vice versa*.

NNPs and muscle contraction

It has long been known that intervals between NNPs determine rates and types of muscle contraction in anemones (Pantin, 1935), so it was of interest to discover what

effect the different patterns of NNPs had on the muscles of *Phyllactis*. Different numbers of NNPs were evoked at the intervals recorded during antiperistaltic behaviour and burrowing. To record contractions, an anemone was hooked up to an isotonic myograph and either placed on the surface of the sand or suspended vertically or horizontally between myograph levers and a glass rod. The clearest results were obtained when anemones were suspended and these are shown in the figures.

During antiperistaltic behaviour circular muscles contracted to form a ring that moved up the column, while other muscles pulled in the ruff, pulled down the oral disc, and decreased the diameter of the upper column. No pattern of evoked NNPs was found that would trigger an antiperistaltic ring, but NNPs evoked at 3- to 4-s intervals caused the ruff to contract, the top of the column to become narrower, and the oral disc to be lowered. As the intervals were increased the size of these contractions decreased and, at intervals greater than 9 s, the ruff and top of the column contracted little if at all. Size of contraction was also dependent on the number of NNPs, but when NNPs were evoked at IPIs of 9 s even six or more produced little movement of the ruff. When NNPs were evoked at the 5- to 6-s intervals characteristic of startle bursts there were contractions of the ruff, oral disc and upper column that resembled those seen in antiperistaltic behaviour. However, the startle response itself could not be triggered by evoking NNPs 5 s apart. If a spontaneous startle response did not follow the initial group of 5–6 NNPs, i.e. when a non-startle burst occurred, the ruff usually stopped contracting because IPIs in the later part of the burst were longer than 9 s. Contractions of the ruff and oral disc were less consistent when NNPs occurred spontaneously than when they were evoked. Thus, during antiperistaltic behaviour, strong, weak or no contractions could occur in response to NNP bursts with identical numbers of pulses. Furthermore, the ruff would occasionally contract in the absence of NNPs, indicating that this, too, may be an inherent activity that is sometimes not controlled by the nerve net.

Shortening of the column occurred during a startle response and soon after the start of a burst of NNPs during burrowing. Shortening is produced by the contraction of one or both of two groups of longitudinally oriented muscles located in the mesenteries. The retractors are situated close to the centre of the anemone and can contract slowly but are typically associated with startle responses, during which their primary function is to lower the oral disc. The parietals, however, lie laterally near the inside wall of the column. They contract slowly and were responsible for most of the shortening that occurred when NNPs were evoked at intervals greater than 2 s. To study the effect of number of pulses and IPI on slower contractions, 3–7

Fig. 4. A complete record of the NNPs and SP2s produced by a single *Phyllactis* as it burrowed into the sand. The time of occurrence of each pulse is indicated by a dot as in Fig. 3. Each × on the right marks the first pulse in the next NNP burst and is equivalent to the first dot on the next row. The large arrows indicate the end of one phase and the beginning of another. Filled arrows on the left indicate bursts in which a startle response occurred; the open arrow points to a 'non-startle' burst (see Fig. 5). During most of phase II there are cyclical changes in SP2 latency.

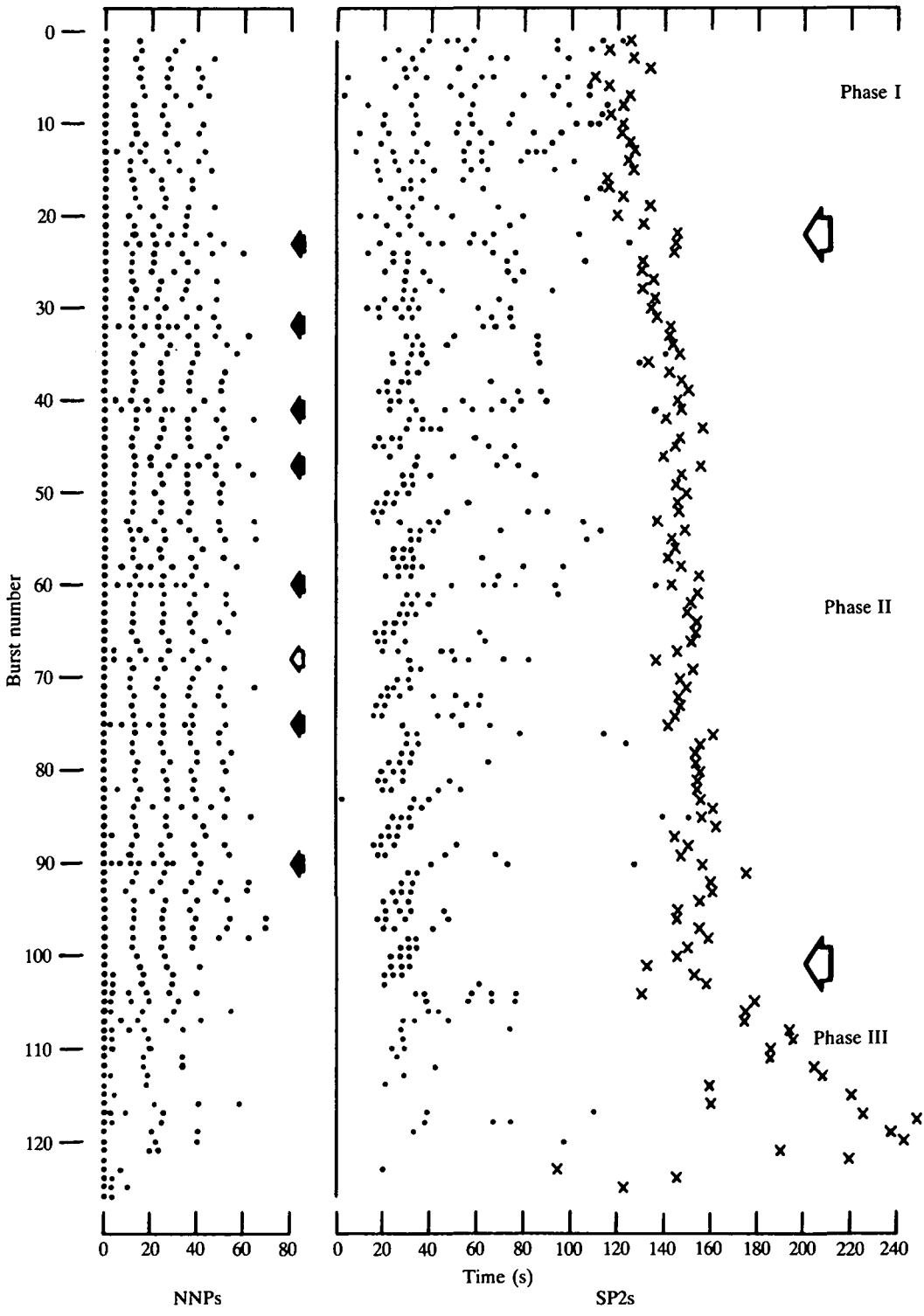
Burst number
0
10
20
30
40
50
60
70
80
90
100
110
120
Phase I
Phase II
Phase III
0 20 40 60 80
NNPs
0 20 40 60 80 100 120 140 160 180 200 220 240
Time (s)
SP2s

Fig. 4

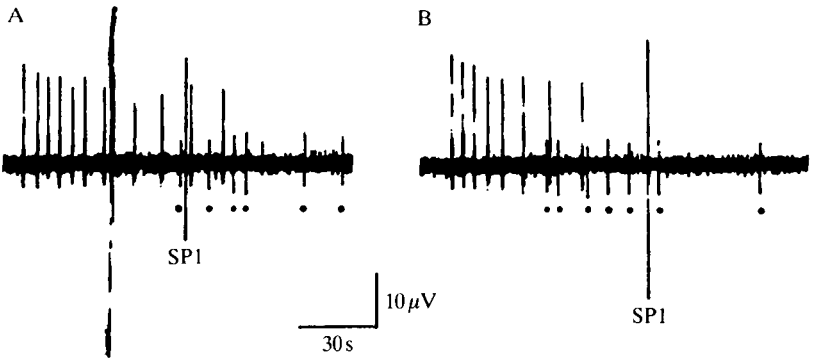

Fig. 5. NNP bursts during which a startle response did (A) and did not (B) occur. SP2s are marked with dots in this and all subsequent figures except Fig. 10. The large pulses are NNPs except for two SP1s and the large compound spike in A which includes three NNPs and a muscle potential. In A six NNPs 5 s apart are separated from the single NNP that precedes the compound spike by an 8 s 'pause'. In B the compound spike is missing.

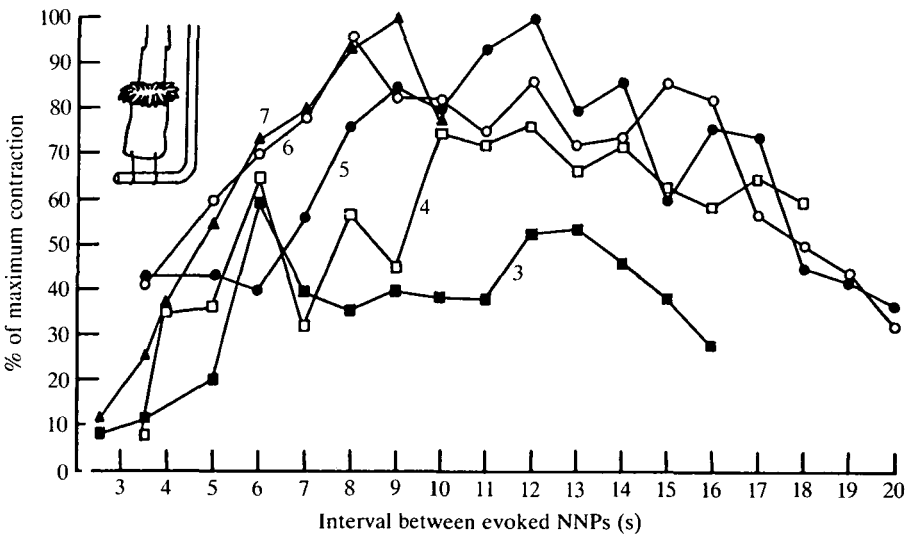

Fig. 6. Size of contraction in vertically suspended anemones as a function of interval between evoked NNPs and number of evoked NNPs (the number evoked is shown beside each curve). Each point is the average value at that interval for contractions in four anemones. A single NNP does not produce a contraction. Shortening begins after the second NNP, but amount of shortening in response to two evoked NNPs is usually less than 5 % of maximum, maximum contraction being the largest response seen when NNPs are evoked at intervals between 3 and 20 s.

NNPs were evoked at intervals from 2·5 to 20 s. Maximum contraction appeared to be relatively independent of IPI in the range 8–14 s when 5–6 NNPs were evoked in vertically suspended anemones (Fig. 6). At IPIs between 10 and 14 s four NNPs

were just about as effective as five or six. These two sets of results suggest that the size of the contraction during each burrowing cycle is affected minimally by small variations in IPI or in number of NNPs; each shorter interval or additional NNP produced only a small increase in contraction. The largest increase in size of contraction occurred in response to the third NNP; two NNPs usually produced shortening (about 5 % of maximum) that was barely noticeable, but the facilitatory effects of the second NNP caused a much greater response to the third NNP. Within the IPI range of 8–14 s, contraction in response to the third NNP was usually 40–50 % of maximum (Fig. 6). Compared to responses of longitudinal column strip preparations in *Calliactis* (McFarlane, 1974*b*), the onset of contraction and maximum contraction in *Phyllactis* occurred after fewer NNPs had been evoked and over a wider range of intervals. One other effect of evoked NNPs was expressed over several cycles. An anemone that had gone through antiperistalsis for a few minutes often had a column that was doubled in length. When NNPs were evoked in the burrowing pattern in this lengthened anemone, it shortened over several cycles until it reached the standard length of an upright anemone.

A ring could form without the column shortening and *vice versa*. However, during burrowing a ring usually formed at mid-column just after maximum shortening had occurred. As the ring travelled down the column there were sequential contractions of adjacent sections of circular muscle. Rings did not always travel, indicating that ring formation and ring movement are two separate events. This was best seen when *Phyllactis* was bathed in a solution of 20 % $MgCl_2$ in sea water. Before the anaesthetic blocked ring formation completely, it was possible to evoke NNPs that triggered a ring which travelled erratically before dying out. During recovery from the anaesthetic, after replacement of the $MgCl_2$ solution by sea water, a ring could form in response to each of the first 3–5 spontaneous bursts of NNPs, but several more bursts had to occur before a ring would move to the pedal disc as a smoothly travelling wave.

Facilitation of conduction is, of course, also required at the start of burrowing. However, even when the ring conducting system is primed, it is the intervals between evoked NNPs that determine whether a ring will form and travel. When NNPs were evoked at intervals of 6 s or less in upright anemones, rings formed at the top of the column, were narrow, and seldom travelled: those that did travel failed to reach the pedal disc. However, rings triggered by NNPs evoked at intervals greater than 7 s usually travelled to the base of the column. The point of origin of the ring also depended on intervals between evoked pulses. When IPIs were 7 s or less the rings originated at the top of the column, whereas when IPIs were 10 s or more rings began at mid-column. These observations are supported by data obtained when anemones, suspended horizontally, were stimulated (Fig. 7). When NNPs were evoked at short intervals, contractions occurred at the top of the column but not at the base. However, when NNPs were evoked at intervals of 10 s or more, only weak contractions were recorded at the top of the column because the ring now started at mid-column. Even though the point of origin of a clearly defined ring shifted, excitation still spread from the top and could be seen occasionally as a very weak

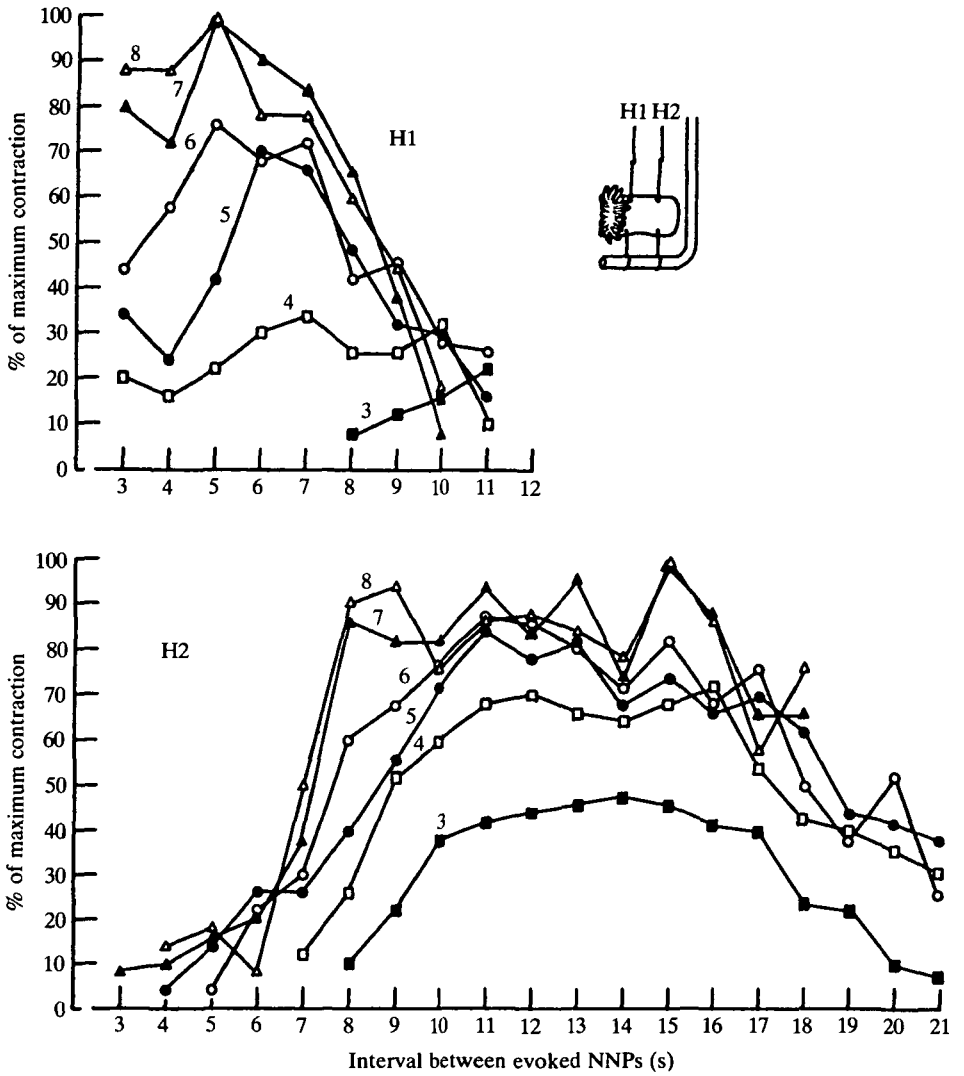

Fig. 7. Size of contraction in horizontally suspended anemones as a function of interval between evoked NNPs, number of evoked NNPs (number evoked is shown beside each curve) and recording site. Each point is the average value at that interval for contraction in four anemones. Short intervals between evoked NNPs produce local circular muscle or sphincter contraction in the upper column (recorded by H1), long intervals produce local circular muscle contractions that travel in the lower column (recorded by H2). Contractions recorded at H1 reached a peak 20–35 s after the third evoked NNP in a burst, those recorded at H2 85–105 s after the third evoked NNP (see Fig. 8).

contraction wave travelling down the column. It was impossible to distinguish between the rings that did not travel in the upper column and contractions of the sphincter. As in the responses of parietal muscles, the strength of contraction of circular muscle in forming rings was affected by the interval between evoked NNPs

and the number of NNPs. In the upper half of the column, contraction of the narrow, non-travelling rings formed under the ruff was greatest between IPIs of 4 and 6 s (Fig. 7, H1). At these IPIs the more NNPs there were, up to eight, the greater the size of the contraction. When NNPs were evoked at any interval shorter than 9 s, four or more produced a narrowing of the column below the ruff, but only when six or more NNPs were evoked did a well-formed ring appear. The maximum contraction occurred in the lower half of the column when intervals between evoked NNPs were between 10 and 16 s. Within this range, size of contraction was more or less independent of interval (Fig. 7, H2). Two NNPs rarely triggered ring formation at IPIs greater than 10 s, but they did facilitate neuromuscular junctions so that three evoked NNPs produced sizeable contractions. Again it appears that when pulses occurred at the IPIs and in the numbers that they did in burrowing bursts, small variations in IPI or NNP number had little effect on the size of contraction. Burst duration itself did not appear to be the significant parameter in ring formation and conduction, because when intervals between shocks were varied within a 40 s period of stimulation, rings formed under the ruff and did not travel, or rings formed at mid-column and did travel, or no rings formed at all, depending on the IPI (Fig. 8). McFarlane (1974*a*) has shown that the size of the ring contraction in *Calliactis* is related to SS2 activity: the longer the SS2 quiet period, the greater the contraction. This relationship was not studied in *Phyllactis*, but may be responsible for some of the variation recorded in these experiments.

Once a peristaltic ring started to travel, the conduction velocity was about $0{\cdot}5\,mm\,s^{-1}$ and appeared to be independent of NNP IPI between 7 and 16 s and NNP number between four and eight. Excitation that produced downward-moving peristaltic rings appeared to be conducted in the same pathway that was used when antiperistaltic rings moved upwards. Thus, if a peristaltic ring was evoked to travel towards a spontaneously produced antiperistaltic ring, collision occurred and the rings disappeared. As further proof, on rare occasions a ring travelling towards the base would reverse direction, head towards the top of the column, and trigger an antiperistaltic burst of NNPs (Fig. 9). However, this column conducting pathway was polarized in that maximum velocity for antiperistaltic rings was about $1\,mm\,s^{-1}$, a velocity that was about twice as high as that for peristaltic rings.

The function of the SS2

McFarlane (1974*a*) has proposed that one function of the SS2 in *Calliactis* is to inhibit endodermal muscles. If the SS2 has this function in *Phyllactis*, it would be expected that the SS2 would fire as the anemone shortens so that circular muscles would be inhibited, and then the SS2 would fire again when shortening ended so that longitudinal muscle would be inhibited as circular muscles contracted to form a ring. The pattern of SP2s during burrowing would appear to support this hypothesis. Towards the end of phase I, and in all of phase II, SP2s occurred at their highest frequency during that part of each cycle in which the anemone was shortening. In addition, at least one SP2 was recorded as the ring was forming, except during the later part of phase II. Unfortunately, proving that the SS2 inhibits muscles during

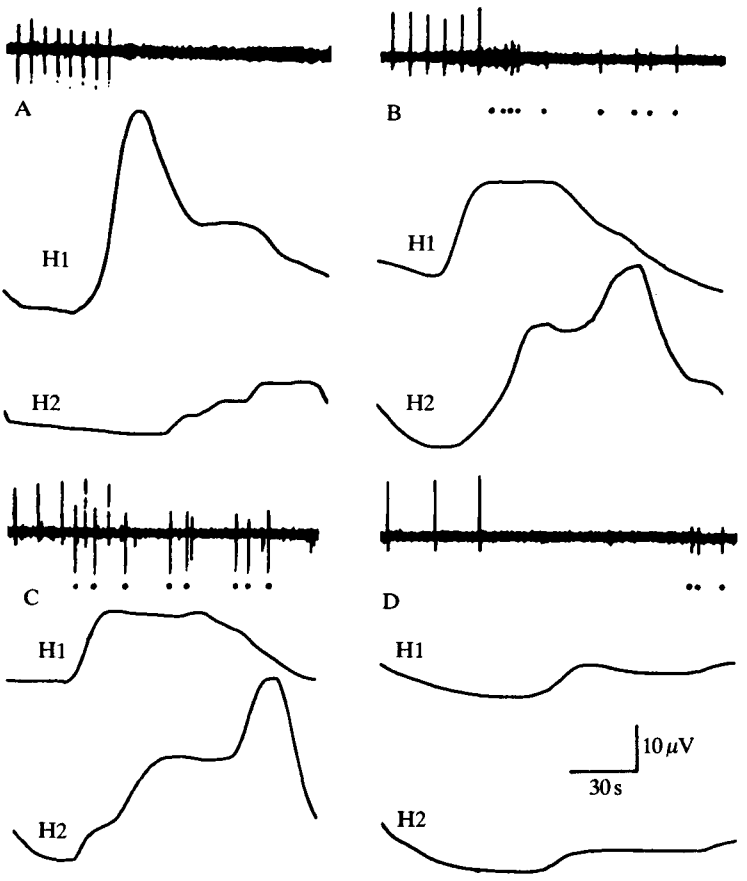

Fig. 8. Differences in ring formation as a function of the intervals between NNPs during a 40 s period of stimulation. Records are from four different anemones suspended horizontally. The top trace in each group is a record of pulses, the bottom two traces are records of contractions near the top of the column (H1) and near the bottom of the column (H2). H1 and H2 were about 25 mm apart. Intervals between evoked NNPs were 6, 8, 10 and 20 s in A, B, C and D, respectively. In A the ring formed at the top of the column but did not travel. In B the ring formed about one-third of the distance from the top of the column. In C the ring formed at mid-column. In D no ring was formed. The first upward deflections recorded in the H2 traces in this figure and in Fig. 13 are due either to symmetrical parietal or to local circular muscle contractions or to both. It is difficult to distinguish between the two, as McFarlane (1974*a*) has noted.

burrowing has been more difficult; when SP2s were evoked in a pattern similar to that which occurs during a burrowing cycle, the results were quite variable, usually providing little support for the hypothesis that one or the other group of muscles is inhibited. This is not to say, however, that evoked SP2s have no effect on muscles. On the contrary, if SP2s were evoked for several minutes at the average SP2 interval (15 s) characteristic of the anemone's preburrowing, quiescent period, any movements would stop. Thus, if SP2s were evoked during antiperistaltic behaviour,

antiperistalsis was inhibited and the burst of NNPs that typically followed did not occur. If similar trains of SP2s were evoked during burrowing, NNP bursts continued for several cycles but peristalsis and shortening stopped soon after stimulation began. This shows that the SS2 can have a tonic inhibitory effect, but whether it acts phasically during each cycle is yet to be determined.

Although the connection between SS2 activity and muscle contraction during burrowing is either not understood or does not exist, SP2s were clearly seen to be associated with eversion of the pharynx; SP2s as well as SP1s occurred after the pharynx was everted (Fig. 2A). Attempts to record pulses as the pharynx was being everted in response to mechanical stimulation were unsuccessful. However, pharynx eversion could be triggered by chemical stimuli and it is probable that mechanical and chemical stimuli evoke the same pulse pattern. Proline added to sea water surrounding the anemone evoked a burst of SP2s in *Phyllactis* (Fig. 10), as

Fig. 9. This trace shows what happened when a peristaltic ring, triggered by the second burst of NNPs (large spikes), travelled a short distance towards the pedal disc and then reversed direction. When the ring reached the top of the column an antiperistaltic burst of NNPs (arrow) occurred between two burrowing bursts, just before another burrowing burst was expected. The latter may have been blocked because the nerve net was refractory, but the subsequent (last) NNP burst appeared at the time it was expected to occur, indicating that the IBI pacemaker was not reset by an antiperistaltic burst. This trace has two other features of note; an SP2 (marked by a dot) is present in the middle of an antiperistaltic burst, a rare occurrence, and the only SP1 present occurs shortly after an SP2. The latter event, an SP1 closely paired with an SP2, is not unusual and suggests that in certain situations, the SS1 may be triggered with or by the SS2.

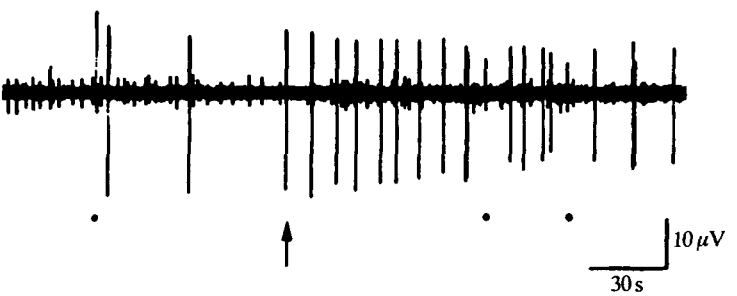

Fig. 10. Firing patterns of the SS2 and SS1 1 min after proline had been added to the bathing solution at a concentration (10^{-2} mol l^{-1}) sufficient to produce pharynx eversion in a buried anemone. A burst of SP2s (small pulses) is followed by a burst of SP1s (large pulses). Three spontaneous NNPs are marked with dots. The pharynx was completely everted by the time the third SP1 occurred (arrow).

McFarlane (1975) has shown in *Calliactis*. In *Calliactis* the pharynx is protruded, possibly because the SS2 inhibits the circular muscles of the pharynx and oral disc; in *Phyllactis* the protrusion was found to be exaggerated and to become an eversion. SP1s also occurred after the addition of proline, but not until eversion was nearly complete, so the SS1 did not respond directly to proline. The SS1 continued to fire while the pharynx was everted and stopped only when the pharynx was pulled back into the body cavity. Its function during eversion is not known. Pharynx eversion in *Phyllactis* could not be triggered by evoking SP1s with 10^{-2} mol l^{-1} betaine, just as pharynx protrusion is not elicited when betaine is applied to the column of *Urticina* (Boothby & McFarlane, 1986). It should be noted that SP2s evoked by proline occurred at frequencies similar to those recorded during phase II of burrowing, although during burrowing the mouth never opened. This shows that the effect of SP2s on circular muscles of the disc and pharynx is modified as a consequence of burrowing activity, but gives no indication of the function of SS2 during this behaviour.

The SS2 in *Calliactis* appears to affect the spontaneous discharge pattern of the nerve net (McFarlane, 1974*b*), so it could be doing the same in *Phyllactis* and thereby indirectly affecting muscle contraction. However, even though long trains of SP2s eventually inhibited the NNP bursts that occurred during burrowing, other examples of the SS2 inhibiting the nerve net pacemakers could be found only when *Phyllactis* was engaging in antiperistaltic behaviour. Inhibition of the entire antiperistaltic burst by the SS2 was recorded only once (Fig. 11, arrow), but in this instance SP2s occurred at short IPIs just prior to and during the period when an antiperistaltic ring was arriving at the top of the column. The result was that the expected burst of NNPs did not occur. Another more typical effect was seen when a single SP2 was evoked or occurred spontaneously during an antiperistaltic NNP burst; the next NNP in the burst was delayed. Exceptions to this rule were found, however, so that sometimes an SP2 in an antiperistaltic burst appeared to have no effect (Fig. 11, second and fourth bursts). This type of inhibition of the nerve net pacemakers of *Phyllactis* may be similar to, if not identical with, the type of inhibition that occurs in *Calliactis* (McFarlane, 1974*b*). However, in contrast to the effect of single SP2s on IPIs in a higher frequency NNP burst, single, pairs or even triplets of SP2s had no effect on IPIs during a burrowing burst (Figs 9, 12). Consequently, in *Phyllactis* there is no evidence of either a direct effect of the SS2 on the TCNN during burrowing or an indirect effect on muscle contraction.

Upon examination of a complete burrowing record, it might be concluded that there is interaction between the SS2 and the nerve net during the startle response. In phase II the latency for the first SP2 after the start of the NNP burst decreased over several cycles and then appeared to be reset to its initial value by a startle burst, producing a sawtooth pattern of rhythmic changes in SP2 latency (Fig. 4). Although startle responses were most likely to occur when the latency for the SP2s was short, they could occur at other times. Nevertheless, when there were no startle responses, the SP2 latency still changed periodically (Fig. 12). Thus, a startle burst did not reset the SS2 rhythm, but whether the state of the TCNN prior to a startle burst was

affected by the SS2 is still to be determined. What produces these periodic changes in SP2 latency is not known, but they appeared to occur when there were very small periodic changes in the length of a buried anemone. These changes of 0·2–1 mm were more noticeable when an anemone produced startle responses. After a startle response the oral disc was lowered and did not return immediately to its starting level, but was raised slowly over several cycles. Although SP2 latency decreased as the disc was raised, small changes in length could simply occur at the same time without being tied to the latter. It is of interest to note that NNP bursts occur in *Calliactis* when SS2 firing frequency is low (McFarlane, 1973*a*) and startle NNP bursts were found to be most likely to occur in *Phyllactis* when SP2 latencies were short. The cycles for both types of SS2 activity are about 15 min in length, which may be, of course, coincidental.

If length changes can produce an effect on the SS2, they might do so through stretch receptors, as suggested by McFarlane (1975) who noted an increase in SS2 activity when *Calliactis* was feeding. In *Phyllactis*, during phase II of burrowing, SP2s occurred when the anemone was shortening, suggesting that the SS2 could be responding to input from stretch receptors. A similar SP2 firing pattern could often be seen in a vertically suspended anemone (Fig. 13B), but even in this case it could be argued that the SS2 may be activated instead by the nerve net. In Fig. 13B, for example, SP2s begin before contraction begins. However, when an anemone is

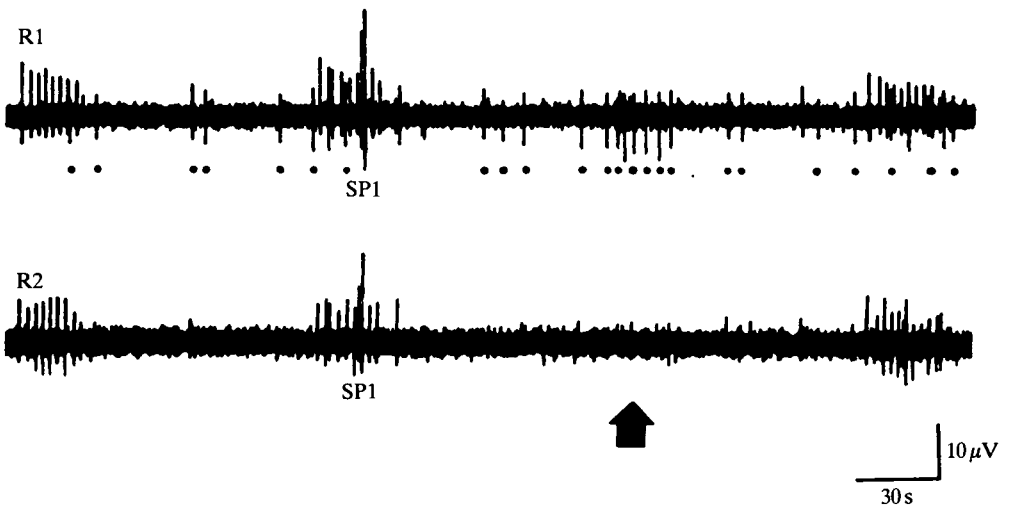

Fig. 11. Blockage of an antiperistaltic NNP burst by a burst of SP2s. Traces are from two electrodes (R1 and R2) recording simultaneously. SP2s are lost in the noise of the R2 trace, but are present in the upper trace as small pulses (marked by dots). All the larger pulses are NNPs except for a single SP1. The first two bursts of NNPs are a continuation of a series of bursts (not shown) that were occurring at regular intervals of 100–110 s whenever an antiperistaltic ring reached the top of the column. The third expected burst of NNPs is absent, clearly shown by the missing NNPs in the lower trace (arrow). In its place was a burst of SP2s (upper trace, third burst). SP2s are usually absent from NNP bursts following antiperistalsis, but in this record one or two occur in each NNP burst.

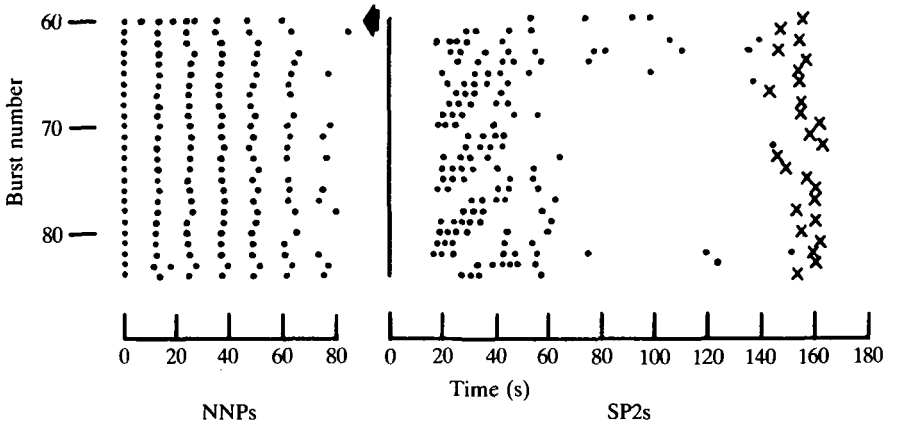

Fig. 12. The cyclical pattern of SP2s occurs even when startle bursts are absent. A sequence of bursts from one anemone plotted as in Figs 3 and 4. The only startle burst is marked with an arrow. Cyclical changes in SP2 latency, shown in the centre of the figure, are present in spite of the lack of notable change of NNP pattern within burrowing bursts. The average for all IPIs in bursts 62–82 is 12·44 ± 1·07 s (S.D.) (see Table 1). For the first IPI in all bursts the mean and S.D. are 12·82 ± 0·45 s. For the second, third, fourth, fifth and sixth IPIs the mean and S.D. are 11·84 ± 0·69, 11·87 ± 0·75, 12·24 ± 0·81, 13·43 ± 1·29 and 14·74 ± 2·69 s, respectively.

suspended horizontally (Fig. 13A) only one SP2 was recorded when no rings formed but several occurred when a ring formed and moved past a hook holding up the lower part of the column. Furthermore, stretching the column slightly by adding weight to the counterweight of the movement-transducing lever also increased the SP2 frequency for a brief period. Taken together, these observations suggest that proprioceptors may activate the SS2 when circular and, possibly, parietal muscles are contracting during burrowing.

The effects of sensory input

Reafference may produce changes in the firing rate of the SS2 in a horizontally suspended anemone, but it is not known what effect this has on the nervous system or on muscle. However, two other types of transient mechanical stimuli do influence nerve net output during burrowing. The first is the weak, tactile stimulus which, in the environment, might occur when a floating object touches the tentacles of a burrowing anemone. This weak stimulus is probably comparable to the one that occurs in the laboratory when a suction electrode is reattached. Reattaching an electrode (as was done, for example, just before burst 13, Fig. 4) usually reduced the interval between NNPs and added one or two more pulses to a burrowing burst. The additional pulses were generated by the pacemaker and were not the result of the tactile stimulus evoking NNPs within milliseconds, as occurs just prior to a startle response. The anemone appeared to habituate rapidly to these brief, weak mechanical stimuli, so that the effect they had on nerve net output seldom lasted for more than two cycles. The second type of stimulus is more intense and occurs if an

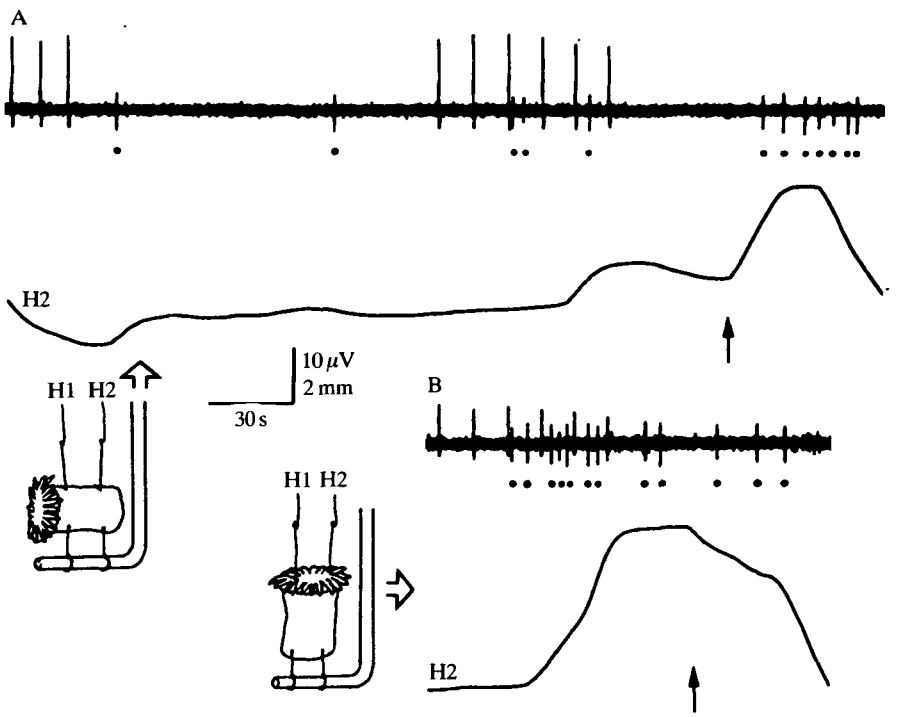

Fig. 13. Records from two different anemones showing the correlation between contraction and firing of the SS2. Upper traces in A and B are records of pulses recorded from tentacles, lower traces show contractions. Large pulses are evoked NNPs, small ones are spontaneous SP2s. In A a ring does not form in response to the first burst of evoked NNPs but does in response to the second. A filled arrow indicates the time at which the ring was forming at mid-column. Seven SP2s occurred as the ring passed H2. In B SP2s occurred at a high frequency as the anemone was shortening and they continued throughout the cycle. The arrow marks the time of formation of the ring at mid-column. There are a few more SP2s in B than there are in any record from an anemone burrowing without hooks attached (e.g. Fig. 3A,B) suggesting that even though SP2s may be generated in response to internal proprioceptive stimuli, a few additional SP2s may be produced in response to recording devices.

anemone's tentacles are clamped onto or bitten by another animal. A stimulus of this intensity was given in these experiments by pinching a tentacle with forceps. This evoked two or more NNPs within a few milliseconds and triggered a startle response. The response might have been expected to affect subsequent NNP bursts or to alter burrowing behaviour, but the effects of startle responses, like those of weak, mechanical stimuli, did not last long even when an anemone had hooks attached to its oral disc. An evoked or spontaneous startle response could delay the start of the next burst for several seconds, but it had no effect on the number of NNPs or the interval between them (Fig. 4, burst 47).

Tonic mechanosensory input appears to be responsible for maintaining the pulse pattern characteristic of each phase of burrowing. During phase I the number of and

interval between pulses seemed to be related to the absence of sand around the column, so that if a buried anemone, producing pulses in the phase II pattern during an experiment, was raised up and set on top of the sand, it continued to burrow, but the phase II pattern was replaced by that of phase I. The phase II pattern, however, depended on the column being covered. This could be shown in experiments with vertically suspended anemones which often exhibited spontaneous burrowing movements. These movements could continue for hours with an anemone producing the same pulse pattern as during phase I of burrowing. If the column of the suspended anemone was covered with sand, there was a change to phase II activity (Fig. 14A). This change included not only an increase in the number of NNPs per

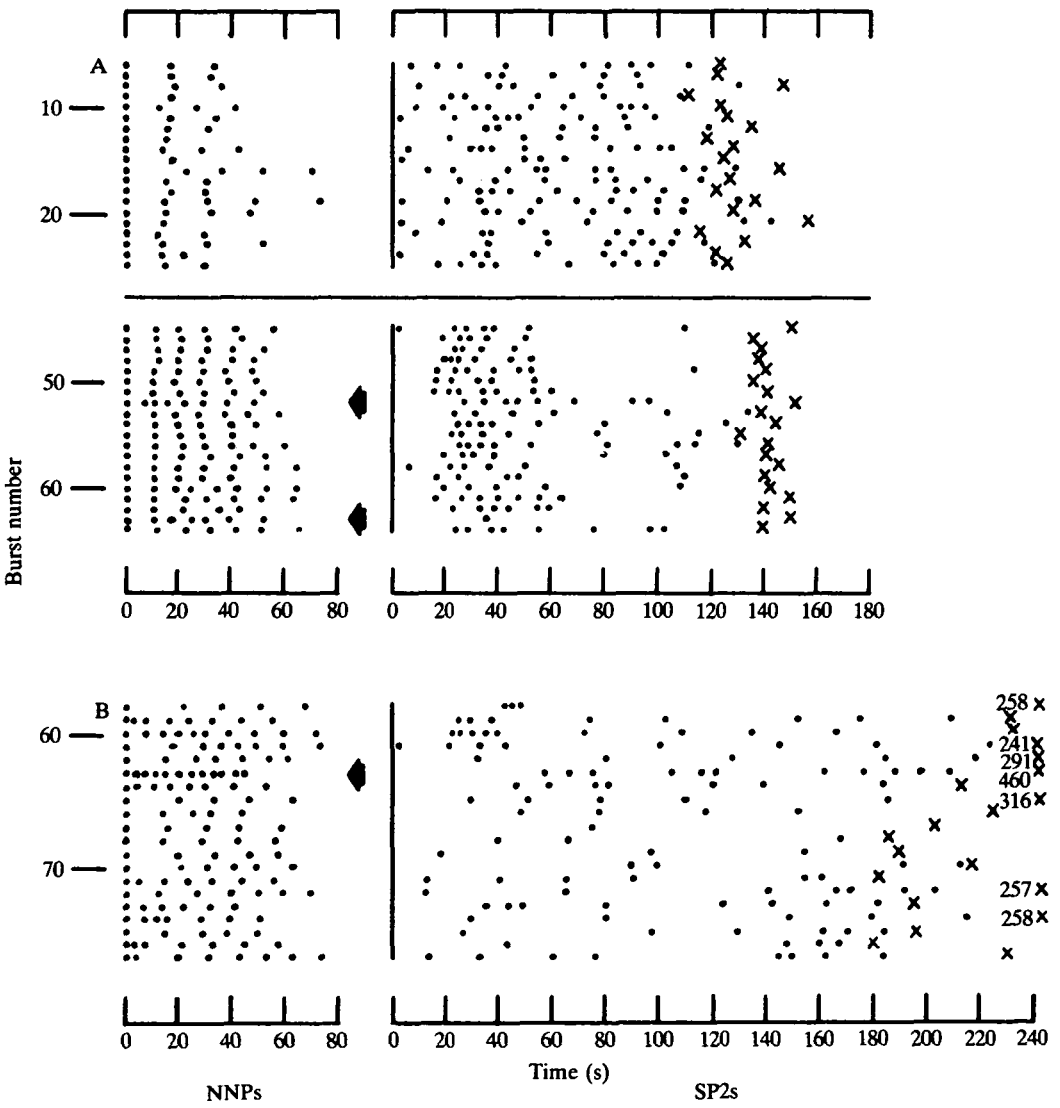

Fig. 14

burst and a decrease in IPI, but also an increase in IBI, a decrease in the variation in IPI and IBI, the introduction of cyclical changes in SP2 latency, and an increase in the probability of the occurrence of spontaneous startle responses. The sensory input that initiates phase III must come from receptors in the pedal disc when the latter touches solid substratum. However, phase III activity also began in the absence of shell fragments or rock if the column had been extended to about twice its starting length, suggesting that the anemone was sensing column length through proprioceptors. This type of input could affect the initiation of phase III in another way, illustrated by an experiment in which an anemone was allowed to burrow into sand that was no deeper than the initial length of the column. The pedal disc touched hard substratum before the anemone's column had lengthened and as a consequence the anemone did not attach or produce the phase III pattern of pulses (Fig. 14B). Instead the anemone went through several cycles of antiperistalsis (not shown in Fig. 14), then moved laterally ('walked') on the Plexiglas beneath the sand, and much later attached to it. Prior to that it had produced pulse patterns that were a combination of parts of patterns from all three phases (Fig. 14, legend). This type of walking also occurs when *Phyllactis* is placed on a glass plate on top of the sand (Mangum, 1970). Whether this modified behaviour is related to a short column or to the absence of deep sand is not known. Input from the pedal disc not only affected behaviour, but also affected the length of the column. If there was no sand or hard substratum beneath the pedal disc when an anemone was suspended vertically, for example, the anemone tended to lengthen while going through spontaneous burrowing movements. However, if sand was placed under its pedal disc at the start of the experiment, its length did not change when burrowing movements occurred. If more sand was added to cover the entire column, the column then lengthened and activity changed from phase I to phase II.

The startle responses observed during burrowing have been described as spontaneous after many failed attempts to find even subtle kinds of sensory input that might trigger these responses. There must be specific, as yet unknown, conditions

Fig. 14. The effects on pulse patterns of sand around the column and hard substratum under the pedal disc. (A) Bursts of pulses (plotted as in Fig. 3) recorded from a vertically suspended anemone are shown before (top) and 40 min after (bottom) enough sand had been added to the experimental chamber to cover the column (bursts 26–44 are not shown). After the addition of sand the following changes were evident. There were more NNPs per burst, IPIs were shorter, IBIs were longer, SP2s were grouped near the start of each cycle and there were startle responses (arrows). (B) Part of the record of another anemone, 35 mm long, that burrowed into sand 35 mm deep. By this time in a typical record of burrowing, NNPs and SP2s would be produced in the phase II pattern (e.g. bursts 60–80, Fig. 4). However, because hard substratum was encountered by the pedal disc shortly after the ruff had touched the sand (burst 51), a combination of pulse patterns occurred. Features characteristic of phase III are the long and irregular IBIs, the absence of cyclical changes in SP2 latencies, and the presence of short IPIs at the beginning of some NNP bursts. However, most of the NNP bursts are similar to those recorded during phase II, and one is a startle burst (arrow). The one feature characteristic of the phase I pattern is the presence of SP2s throughout each cycle.

that have to be present before a spontaneous startle response will occur. For example, although startle responses could be triggered by evoking two or more NNPs at intervals of less than 1 s, they could not be triggered by evoking NNPs with shocks in the pattern typically recorded during burrowing just prior to the startle response, that is evoking five NNPs at 5 s intervals and then a single NNP after an 8 s pause. Furthermore, a startle response was never triggered by an antiperistaltic burst even though some IPIs in the burst could be as short as 2·5 s. Finally, when hooks attached to the oral disc were touched or suction electrodes were reattached during the experiment, IPIs in the burrowing burst decreased and could be close to those seen during a startle burst; at such times a startle response did not occur. The triggering of this response requires further study.

DISCUSSION

Burrowing is controlled by the through-conducting nerve net. A burst of NNPs produces peristalsis and it is this activity, as well as the mechanisms that cause sand to adhere to the column, that appears to be responsible for driving the anemone into the sand. NNPs occur at regular intervals within a burst and there are also regular intervals between bursts, suggesting that pacemakers are present.

The first pacemaker is one that appears to control the TCNN during an antiperistaltic burst, during the third phase of burrowing and during experiments in which an anemone is exposed to magnesium chloride. The average IPI during an antiperistaltic burst is a little more than 3 s (Table 1, line 1). Similar but slightly longer IPIs are recorded at the beginning of NNP bursts during phase III or when an anemone is bathed in 5–15 % solutions of magnesium chloride in sea water (Table 1, lines 2, 3). All intervals in the antiperistaltic burst are about 3 s apart, but during phase III and when the anemone is in $MgCl_2$ there are only a few short IPIs at the start of a burst and two or three NNPs, after which the IPIs are longer (e.g. Fig. 4, bursts 102–126). Nevertheless, it is believed that the same pacemaker controls output of the TCNN in all three situations. This pacemaker in *Phyllactis* has a cycle length that is close to that of the 4 s pacemaker in *Calliactis* (McFarlane, 1974*b*) and shares two additional features with it. First, in a burst of NNPs the IPIs are slightly longer at the beginning and at the end than in the middle of the burst (Fig. 2B) and, second, a single, evoked or spontaneous SP2 during an NNP burst can delay the appearance of the next NNP. Activation of the 3 s pacemaker during phase III may be associated with attachment to a buried shell. A similar NNP firing pattern has been recorded when a detached *Calliactis* resettles on a shell (McFarlane, 1983).

A different pacemaker may be operating during a startle burst. The first few NNPs before the pause in the burst occur, on average, about 5 s apart (Table 1, line 4). Although the lower range of IPIs in startle bursts and the upper range in antiperistaltic bursts overlap, the first part of a startle burst is often followed by a closely spaced pair or triplet of NNPs that will trigger a startle contraction, whereas

Table 1. *TCNN pulse and burst intervals during different types of activity*

Intervals	Means ± S.D. (s)	S.E.M.	Number of intervals sampled	Number of anemones sampled
(1) IPI in antiperistaltic bursts	3·15 ± 0·65	0·04	331	10
(2) Short IPI at the start of burrowing bursts in phase III	3·65 ± 0·76	0·06	153	10
(3) Short IPI at the start of burrowing bursts when the anemone is in $MgCl_2$	3·65 ± 0·66	0·04	219	5
(4) IPI in startle bursts before the 'pause'	5·20 ± 1·17	0·06	332	10
(5) IPI in non-startle bursts before the 'pause'	5·28 ± 1·00	0·17	36	5
(6) IPI in burrowing bursts, phase I	12·95 ± 3·12	0·11	791	10
(7) IPI in burrowing bursts, phase II	12·22 ± 2·59	0·09	864	10
(8) IPI in burrowing bursts 62–82 shown in Fig. 12	12·44 ± 1·07	0·10	109	1
(9) IBI during burrowing. Last 25 IBIs of phase I	146·18 ± 17·03	1·08	250	10
(10) IBI during burrowing. First 25 IBIs of phase II	157·65 ± 17·38	1·10	250	10
(11) IBI during burrowing, phase II. Sample with the smallest variation	151·04 ± 4·61	0·92	25	1

The data seem to indicate that four pacemakers control the intervals.

the latter never occurs during or following an antiperistaltic burst. Whether startle responses are due to the influence of a specific pacemaker, to an SP2 pattern, or to some other factor has not yet been determined.

A third type of pacemaker regulates intervals in NNP bursts during phases I and II of burrowing. Even though IPIs in phase I bursts are more variable than those in phase II bursts, and the IPIs are slightly longer in the former, the average IPI in each phase is between 12 and 13 s (Table 1, lines 6, 7). The greater variability in phase I is assumed to be due to intervals being modified by input from transient sensory stimuli and this input appears to be reduced once the column is buried. It is also assumed that under conditions in which changes in sensory input are reduced, a high degree of uniformity in NNP intervals during a series of bursts might indicate that pure pacemaker output was being recorded. Such conditions appear to have occurred when one anemone was in phase II of burrowing (Fig. 12). The average IPI for 21 bursts was 12·44 s and the variation in interval (S.D. ± 1·07 s) the smallest for any

burrowing anemone during these experiments (Table 1, line 8). [Two other characteristics of this particular series of bursts were that the first interval was the least variable, and that the shortest IPIs occurred in the middle of the burst (Fig. 12, legend). Larger variations in the second and later intervals could be due to the nature of the burst generator itself or be related to sensory feedback that occurred after muscle contraction had begun. Although SS2 activity started at about the same time as contraction, the cyclical changes in SP2 latency did not appear to affect the interval between NNPs.]

The fourth pacemaker, with a 150 s cycle, controls intervals between bursts. During the first phase of burrowing the interburst interval (IBI) is about 145 s, but it increases to about 155 s during the second phase (Table 1, lines 9–11). This increase is probably due to the change in tonic sensory input to the column during phase II. It is concluded that the 150 s interval is controlled by a pacemaker rather than being a product of reflex activity, even though a reasonable alternative hypothesis would be that IBI is determined by the time required for a ring or its underlying wave of excitation to travel the length of the column, so that when the ring arrives at the base it triggers the next burst of NNPs. However, this is not likely, even though the next burst usually occurs within 10 s of the arrival of the ring, for the following reasons. First, in most cases, at least three bursts of NNPs must occur at the start of burrowing before a ring will form and travel. Second, when antiperistalsis precedes burrowing, the interval between the last antiperistaltic burst and the first burrowing burst is sometimes about 145 s, suggesting that the IBI pacemaker is activated prior to the first burrowing burst. Third, when an anemone is bathed in 15 % magnesium chloride in sea water, IBIs increase after a while, but rings travel towards the pedal disc at their normal rate and so reach the base many seconds before the next burst begins. Then, after the anemone has been in 15 % magnesium chloride for 2 h, rings no longer form, yet NNP bursts continue in their absence. Fourth, an anemone often generates spontaneous rhythmic bursts of NNPs that continue for several hours while the anemone is suspended between a pair of hooks and a glass rod in a seawater bath, yet rings do not always form under these conditions. Fifth, if three or four NNPs are evoked by electric shocks after a spontaneous burst of NNPs has occurred, the earlier, spontaneous ring will die out and be replaced by the second, evoked ring that reaches the pedal disc after the next spontaneous burst of NNPs. Parenthetically, this result also shows that the TCNN can be inhibitory as it is in *Calliactis*, although in *Calliactis* it is an antiperistaltic ring that is inhibited (McFarlane, 1974*a*). Finally, an anemone with half a column produces bursts of NNPs at standard IBIs. These were recorded in an anemone that was cut in half transversely to study the distribution of pacemakers. After recovery, the top half produced rings that travelled and reached the cut edge of the column in 120 s, but the next NNP burst did not begin until 60 s later.

Pacemaker cells have not been identified in this or any anemone, but results of experiments with bisected anemones suggest that they are not localized and, in *Phyllactis* at least, can be easily regenerated if lost. When *Phyllactis* is cut in half, either transversely or longitudinally, and halves are allowed to recover from the

Table 2. *The earliest time after anemones were divided in half that different types of behaviour and pulses were observed or recorded*

(1) Travelling peristaltic rings observed	3 days	(All halves)
(2) Anemone burrowed into sand	3 days	(Bottom half of anemone divided transversely)
(3) Travelling antiperistaltic rings observed	1 week	(Bottom half of anemone divided transversely)
(4) Pharynx everted	2 weeks	(Top half of anemone divided transversely)
(5) Antiperistaltic bursts recorded	2 weeks	(Top half of anemone divided transversely)
(6) Burrowing bursts recorded	2 weeks	(Top half of anemone divided transversely)
(7) Startle responses recorded	2 weeks	(Top half of anemone divided transversely)
(8) Length of phase I similar to that in whole anemone	6 weeks	(Half of anemone split through discs)
(9) IBI similar to that in whole anemone	6 weeks	(Half of anemone split through discs)
(10) IPIs in burrowing bursts similar to those in whole anemone	6 weeks	(Half of anemone split through discs)
(11) Cyclical changes in SP2 latency observed	6 weeks	(Half of anemone split through discs)
(12) Complete regeneration of discs	12 weeks	(Anemones divided transversely)

Anemones were divided transversely or through oral and pedal discs and allowed to recover in the seawater system.

operation, each half will produce peristaltic rings and will burrow within 2 weeks, even though complete regeneration may take up to 12 weeks (Table 2). At first, burrowing is slow, but after 6 weeks each half generates NNP bursts at the same intervals as it did when it was part of the whole anemone.

Pacemakers can generate bursts of NNPs in the absence of SP2s, so it does not appear that an interaction between the SS2 and the TCNN produces rhythmicity. This can be shown by bathing an anemone that is burrowing in a solution of 15–20 % $MgCl_2$ in sea water. The anemone stops burrowing after 2–3 h but, before it does, SP2s disappear while the NNP bursts continue. Pulse patterns in an anemone bathed in $MgCl_2$ resemble those of anemones in the third phase of burrowing. IBIs are longer and much more variable, there are short IPIs at the start of each NNP burst, and the number of startle responses is reduced. Unfortunately, after 2 h, $MgCl_2$ also affects muscles or neuromuscular junctions so shortening and ring formation no longer occur and it is impossible to determine the effects of pacemakers and the TCNN on burrowing behaviour in the absence of SP2s.

Pacemaker cycles are affected by column burial, by the pedal disc touching a shell and when there are transient, weak mechanical stimuli present. Transient, weak mechanical stimuli appear to have only minor effects on burrowing because the strength of circular muscle contraction is changed very little by small changes in NNP number or IPI when these occur within the range of numbers or intervals recorded during burrowing bursts. Another type of transient sensory stimulus is provided by the passage of a peristaltic ring down the column. When this happens the SS2 is excited, but what effect SP2s have on behaviour is not known. The sustained changes in sensory input that occur after the column is buried will shorten IPIs and lengthen IBIs, but how these are related to elongation of the column and attachment is not known. Another function of sensory input is to trigger different types of behaviour. When a strong mechanical stimulus is given to an anemone, it evokes NNPs and a startle response. However, the startle responses recorded during burrowing are not triggered by strong mechanical stimuli and it appears that most of the mechanical stimuli to which *Phyllactis* was exposed during these experiments were weak. The lack of overt stimuli, whether mechanical, chemical or otherwise, has made it difficult to determine what triggers the startle response, antiperistaltic behaviour or burrowing. A series of shocks can trigger burrowing behaviour, that is the production of peristaltic rings, but burrowing itself is initiated by an anemone if conditions are right and it has not been possible to determine what these conditions are.

Although the SS2 produces a well-defined pattern of pulses during phase II, its role in the burrowing behaviour of *Phyllactis* is not understood. IPIs between SP2s are short, about 3–4 s, but the SS2 also fires at this frequency in response to proline or when an antiperistaltic NNP burst is inhibited (Figs 10–12) and the latter two are associated with two entirely different types of behaviour. The behaviour and SS2 firing frequency associated with the addition of proline to the bath is of interest because it shows that the SS2 of *Phyllactis* is not different from that of other anemones, but responds in the same way under the same set of conditions. Proline causes the mouth to open and the pharynx to evert. Similar behaviour occurs in *Stomphia* and *Calliactis* when there are spontaneous or evoked SP2s (McFarlane, 1975), although mouth opening and protrusion of the pharynx are not as exaggerated as they are in *Phyllactis*. Another effect of the SS2 that also suggests inhibition of endodermal muscles occurs when SP2s are evoked at 5 s intervals in an unattached *Calliactis*: some of the tentacles collapse (McFarlane, 1976). In *Protanthea simplex* an increase in SS2 activity is associated with a sudden loss of muscle tone and collapse of the entire anemone (McFarlane, 1985). However, when *Phyllactis* is burrowing and SS2 activity is high, the mouth remains closed and the tentacles remain extended. It is obvious that when *Phyllactis* is not attached to a shell the SP2 activity associated with a settled anemone is modified. This appears to be true also for unattached *Stomphia*: it will not feed even though there is an increase in SS2 activity (Lawn & McFarlane, 1976). Rejection of food is also characteristic of a burrowing *Phyllactis* (Mangum, 1970). One other type of behaviour associated with increased

SS2 activity is that which occurs when *Stomphia* settles on a shell (Lawn & McFarlane, 1976). Among other things there is constriction of the mid-column and inflation of the pedal disc, both of which also occur after SP2s are evoked. In contrast, when *Phyllactis* is attaching to the substratum at the end of burrowing, the SS2 frequency decreases. However, *Phyllactis* does respond if SP2s are evoked with shocks every 10–15 s for several minutes. At this SP2 frequency, which is characteristic of the preburrowing period, any activity ceases, including burrowing. Under these conditions the SS2 appears not only to inhibit muscle contraction but also to block NNP bursts. Interaction between the SS2 and the TCNN in *Calliactis* and in *Protanthea* has been described by McFarlane (1974*b*, 1985). In these anemones when TCNN activity increases SS2 activity decreases and *vice versa*. Furthermore, in *Calliactis* a single SP2 can increase the interval between NNPs. Interaction between the two systems appears to occur in *Phyllactis* in that SP2s are seldom seen in antiperistaltic and startle bursts and, in one instance, NNPs were not present in a burst of SP2s (Fig. 11). However, in *Phyllactis* single SP2s were sometimes effective in altering the interval between NNPs in antiperistaltic bursts but never in burrowing bursts. Further studies are being planned to determine the role of the SS2 in burrowing behaviour.

In this paper the terms 'nerve net' and 'through-conducting nerve net' (TCNN) have been used interchangeably, but it is possible that anemones may have at least one other nerve net (McFarlane, 1982). In *Phyllactis* the column conducting system could be one. Batham & Pantin (1954) showed that the peristaltic ring of *Metridium* moves too slowly to be conducted by the TCNN, so they suggested that conduction might be from muscle cell to muscle cell. However, it is difficult to see how excitation originating at the top of the column of *Phyllactis* could be conducted downwards as a weak contraction and give rise to a clearly defined ring at mid-column. Furthermore, excitation that passes along the column not only conducts the rings but also causes vesicles on the column to release or pick up sand. Finally, the wave of excitation that starts at the top of the column produces a ring either at the top or at mid-column, depending on the interval between NNPs. This suggests that synapses or neuromuscular junctions at different levels in the column have different facilitation requirements. Other physiological or anatomical differences at the level of the synapse might explain why maximum velocity is about twice as fast when a ring is moving towards the top of the column than when it is moving in the opposite direction. Unfortunately, it has not yet been possible to show the existence of an anatomically distinct nerve net in the column of *Phyllactis*. If the column conducting system is another nerve net, it might be similar to the one on the inside of the column of anemones such as *Stomphia*, where there are networks of small bipolar cells (Robson, 1963).

Antiperistalsis has been observed in other anemones. In *Metridium*, for example, antiperistalsis accompanies defaecation (Batham & Pantin, 1950). However, in *Phyllactis* it is present primarily when the anemone moves out of its burrow to a new location in the aquarium. Antiperistalsis and movement of the ruff are used by the anemone to crawl out of and along the sand. Mangum (1970) reported that *Phyllactis*

could also move by pedal locomotion, the behaviour in which an anemone remains upright and glides across a hard surface with its pedal disc. This type of locomotion has been described in a number of anemones and is reviewed by Shelton (1982). When *Phyllactis* crawls it is a much more vigorous activity than pedal locomotion. The antiperistalsis that it involves is not initiated by TCNN activity in contrast to what happens in isolated preparations of *Calliactis* in which antiperistalsis can be triggered by evoking NNPs (McFarlane, 1974*a*).

Peristalsis, in contrast, has never been observed to occur in *Phyllactis* in the absence of NNPs. It has to be triggered by the nerve net and usually facilitation is required before a ring will form. The interval between NNPs must be at least 7 s if the ring is to form and travel. This requirement for a long interval ensures that during antiperistaltic behaviour the antiperistaltic burst with its NNPs occurring at short IPIs will not trigger a peristaltic ring that might collide with the next antiperistaltic ring and erase it. Ring formation in other anemones is apparently different. In *Metridium* rings will form and travel when the anemone is given 10 shocks at 3 s intervals (Batham & Pantin, 1954), and in *Calliactis* peristalsis occurs only when the SP1s and SP2s are evoked together (McFarlane, 1976).

The function of startle responses during burrowing is unknown. Batham & Pantin (1954) suggested that random, spontaneous NNPs in *Metridium* might occur just before or just after a single shock given during an experiment and the pairing of a spontaneous with an evoked NNP might trigger an unexpected startle response. In the case of *Phyllactis* it could be argued that even though no shocks were given during an experiment, the anemone was irritated by the attached electrodes and contracted rapidly. However, even when the same anemone was free to burrow in a large aquarium it still produced startle responses, sometimes at regular intervals. Under experimental conditions the time of occurrence of a startle response is predictable. It will always occur during an NNP burst which has a certain pattern of pulses and this pattern is most likely to be present when the latency of the first SS2 pulse after the start of a burrowing burst is shorter than 20 s. Although it has been suggested that a startle response widens the burrow, serving a purpose during burrowing, this rapid shortening occurs during a period when the column is gradually lengthening and would seem to be counterproductive.

Phyllactis does not penetrate the sand by using the fluid within its central cavity as a hydraulic system to force the column into the sand as *Peachia* does (Ansell & Trueman, 1968). Indeed, an intact hydrostatic skeleton is not required, because anemones cut in half will burrow without it. Instead *Phyllactis* burrows by attaching sand grains to the lower column as it is bent under the anemone by a travelling peristaltic wave. Sand is carried peripherally when the column straightens and a few grains are then released. A few more grains beneath the anemone are moved peripherally by the scraping action of attached sand as the column expands. This method of burrowing is inefficient: it takes *Phyllactis concinnata* about an hour to bury its column.

Because of the regular firing pattern of the TCNN during burrowing it might appear that *Phyllactis* could be made to burrow simply by evoking NNPs at the

intervals at which they occur during burrowing, but all attempts to do this have failed: this anemone cannot be driven into the sand. Evoked NNPs will trigger peristalsis, but the decision to burrow is made by the anemone alone and appears to involve primarily a decision about whether or not to attach sand to the lower part of the column. Whether this information is supplied by the TCNN or the SS2 or by some inherent mechanism remains to be determined.

REFERENCES

ANSELL, A. D. & TRUEMAN, E. R. (1968). The mechanism of burrowing in the anemone *Peachia hastata* Gosse. *J. exp. mar. Biol. Ecol.* **2**, 124–134.

BATHAM, E. J. & PANTIN, C. F. A. (1950). Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. exp. Biol.* **27**, 377–399.

BATHAM, E. J. & PANTIN, C. F. A. (1954). Slow contraction and its relation to spontaneous activity in the sea-anemone *Metridium senile* (L.). *J. exp. Biol.* **31**, 84–103.

BATHAM, E. J., PANTIN, C. F. A. & ROBSON, E. A. (1960). The nerve-net of the sea anemone, *Metridium senile* (L): the mesenteries and the column. *Q. Jl microsc. Sci.* **101**, 487–510.

BOOTHBY, K. M. & MCFARLANE, I. D. (1986). Chemoreception in sea anemones: betaine stimulates the pre-feeding response in *Urticina eques* and *U. felina*. *J. exp. Biol.* **125**, 385–389.

CARLGREN, O. (1951). The actinian fauna of the Gulf of California. *Proc. U.S. nat. Mus.* **101**, 415–449.

HALL, D. M. & PANTIN, C. F. A. (1937). The nerve net of the Actinozoa. V. Temperature and facilitation in *Metridium senile*. *J. exp. Biol.* **14**, 71–78.

JACKSON, A. J. & MCFARLANE, I. D. (1976). Delayed initiation of SS1 pulses in the sea anemone *Calliactis parasitica*: evidence for a fourth conducting system. *J. exp. Biol.* **65**, 539–552.

LAWN, I. D. (1980). A transmesogloeal conduction system in the swimming sea anemone *Stomphia*. *J. exp. Biol.* **83**, 45–52.

LAWN, I. D. & MCFARLANE, I. D. (1976). Control of shell settling in the swimming sea anemone *Stomphia coccinea*. *J. exp. Biol.* **64**, 419–429.

MCFARLANE, I. D. (1969). Two slow conduction systems in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **51**, 377–385.

MCFARLANE, I. D. (1973*a*). Spontaneous electrical activity in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **58**, 77–90.

MCFARLANE, I. D. (1973*b*). Spontaneous contractions and nerve net activity in the sea anemone *Calliactis parasitica*. *Mar. Behav. Physiol.* **2**, 97–113.

MCFARLANE, I. D. (1974*a*). Excitatory and inhibitory control of inherent contractions in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **60**, 397–422.

MCFARLANE, I. D. (1974*b*). Control of the pacemaker system of the nerve-net in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **61**, 129–143.

MCFARLANE, I. D. (1975). Control of mouth opening and pharynx protrusion during feeding in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **63**, 615–626.

MCFARLANE, I. D. (1976). Two slow conduction systems co-ordinate shell-climbing behaviour in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **64**, 431–445.

MCFARLANE, I. D. (1982). *Calliactis parasitica*. In *Electrical Conduction and Behaviour in 'Simple' Invertebrates* (ed. G. A. B. Shelton), pp. 243–265. Oxford: Oxford University Press.

MCFARLANE, I. D. (1983). Nerve net pacemakers and phases of behaviour in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **104**, 231–246.

MCFARLANE, I. D. (1984). Nerve net and conducting systems in sea anemones: two pathways excite tentacle contractions in *Calliactis parasitica*. *J. exp. Biol.* **108**, 137–149.

MCFARLANE, I. D. (1985). Collapse behaviour in the primitive sea anemone *Protanthea simplex*. *Mar. Behav. Physiol.* **11**, 259–269.

MANGUM, D. C. (1970). Burrowing behavior of the sea anemone *Phyllactis*. *Biol. Bull. mar. biol. Lab., Woods Hole* **138**, 316–325.

PANTIN, C. F. A. (1935). The nerve net of the Actinozoa. II. Plan of the nerve net. *J. exp. Biol.* **12**, 139–155.

ROBSON, E. A. (1963). The nerve-net of a swimming anemone, *Stomphia coccinea*. *Q. Jl microsc. Sci.* **104**, 535–549.

SHELTON, G. A. B. (1982). Anthozoa. In *Electrical Conduction and Behaviour in 'Simple' Invertebrates* (ed. G. A. B. Shelton), pp. 203–242. Oxford: Oxford University Press.