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CONTROL OF THE PACEMAKER SYSTEM OF THE NERVE-NET IN THE SEA ANEMONE *CALLIACTIS PARASITICA*

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SUMMARY

1. The rhythm of spontaneous nerve-net pulses is reset by intercalated evoked nerve-net pulses.

2. The origin of spontaneous nerve-net pulses can shift during a burst. There seem to be many potential pacemakers, widely distributed throughout the body, but apparently absent from the tentacles.

3. If a spontaneous or evoked pulse in the endodermal slow conduction system (SS 2) occurs during a burst, the nerve-net pulse intervals are increased during a 15–30 sec period following the SS 2 pulse. Additional SS 2 pulses cause a further increase in pulse intervals.

4. Nerve-net bursts are followed by a sequence of muscular contractions. The size of the contraction shown by any muscle group depends on nerve-net pulse number and frequency, the optimum frequency being different for different muscles. It is suggested that the SS 2 pulse action on nerve-net pulse frequency can significantly alter the behavioural output of nerve-net bursts. The SS 2 activity may represent sensory feedback on to the nervous pacemakers.

INTRODUCTION

In most higher invertebrates, flexibility of behaviour is possible because separate muscle groups are innervated by discrete single or multiple nervous pathways. A single muscle can operate in many different behavioural programmes because several independent central connexions can be made with each motor nerve. In sea anemones, however, all the muscles seem to be supplied by a single nerve-net, through which each conducted event spreads without decrement (Pantin, 1935*a*, *b*). These simple animals have no central nervous system to distribute signals to separate motor nerves; the various muscle groups nevertheless show a degree of independence because each has a different optimum frequency for activation (Pantin, 1935*b*). All muscles give slow contractions at low nerve-net pulse frequencies and some also give fast contractions at high pulse frequencies. Fast contractions may involve neuromuscular facilitation, so that a single pulse often does not directly excite the muscle but prepares the way for a subsequent pulse arriving before the facilitatory effect has decayed. In the sphincter muscle of *Calliactis parasitica* this effect lasts for up to 2 sec (Pantin, 1935*a*). All muscle groups and both types of contraction are excited by electrical stimulation at a single voltage threshold, that of the nerve-net (Batham & Pantin, 1954; Ross, 1957). There are in fact two other conducting systems in the sea anemones so far

studied, but these systems, the SS 1 and SS 2, both possibly non-nervous, inhibit inherent muscular activity and are not directly involved with muscle excitation (McFarlane, 1969, 1970, 1973*c*, 1974; McFarlane & Lawn, 1972). The SS 1 and SS 2 are slow systems, that is their pulses show a very low conduction velocity, and they appear to lie in the ectoderm and endoderm respectively.

A single nerve-net seems to co-ordinate excitation of the symmetrical reflexes and the slow spontaneous contraction sequences that together form a major part of the behaviour of sea anemones. In *Calliactis parasitica* strong mechanical stimulation elicits a high-frequency train of nervous impulses leading to fast contraction of the muscles involved in the protective withdrawal reflex – the sphincter, mesenteric retractors and tentacle longitudinals (Passano & Pantin, 1955). The nerve-net also shows low-frequency spontaneous activity. Bursts of nervous pulses have been recorded from tentacles of *Calliactis* and each burst elicits a sequence of slow muscle contractions (McFarlane, 1973*a*, *b*, 1974). Fast contractions are not evoked as the interval between pulses in bursts (3–10 sec) is greater than the time for decay of facilitation. Spontaneous contractions occur in unstimulated intact animals and clearly form a part of normal behaviour (Needler & Ross, 1958).

The present work describes the properties of the pacemaker system of the nerve-net and shows that behavioural flexibility is possible because the pacemaker firing frequency is not fixed but is reduced by accompanying SS 2 activity. Such changes in frequency give a significant alteration of behavioural output because of the different response characteristics of different muscles. The SS 2 activity may represent inhibitory feedback acting directly on the nervous pacemaker.

MATERIALS AND METHODS

Specimens of *Calliactis parasitica*, obtained from the Marine Biology Laboratory, Plymouth, were kept in running sea water at 10–14 °C. All recordings were from suction electrodes attached to tentacles. The recording and stimulating techniques are as previously described (McFarlane, 1969). Most experiments used half-animal preparations (McFarlane, 1973*a*); other preparations used will be described in the Results.

RESULTS

Pacemaker system of the nerve-net

Nerve cells are found in the endoderm of the column and the ectoderm of the tentacles and oral disc, but seem absent from column ectoderm and are sparse in oral disc and tentacle endoderm (Batham, Pantin & Robson, 1960; Robson, 1961). Each recorded nerve-net pulse is probably a small muscle action potential accompanying contraction of the ectodermal longitudinal musculature lying directly below the recording electrode (Josephson, 1966; McFarlane, 1973*a*), and although not a direct recording of nervous activity each pulse is taken to represent the passage of a single impulse in the nerve-net.

Bursts of nervous pulses can be recorded from half-animal preparations (McFarlane, 1973*a*). The interval between bursts (interburst interval) is usually 10–20 min. The interval between nerve-net pulses (pulse interval) normally gradually decreas

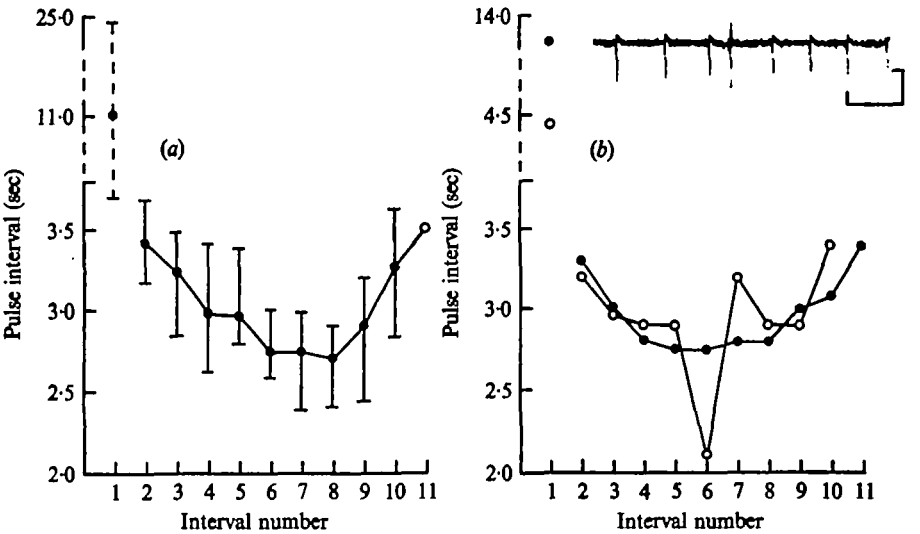

Fig. 1. Pulse intervals during spontaneous nerve-net bursts recorded from a half-animal preparation of *Calliactis parasitica*. (*a*) Mean pulse interval plotted against interval number for eight successive bursts. Bars show the range of intervals. The open circle shows the mean 11th interval in three bursts containing 12 pulses. (*b*) Action of an intercalated nerve-net pulse. Open circles: pulse intervals in a burst where the nerve-net was stimulated after the 6th spontaneous pulse. The 6th interval of the graph is that between the spontaneous pulse and the following evoked pulse. The 7th interval is that between the evoked pulse and the following spontaneous pulse. The evoked pulse caused a large increase in the interval between the 6th and 7th spontaneous pulses. The inset shows the electrical record obtained in such an experiment, the larger pulse being a muscle action potential associated with the evoked pulse. Closed circles: pulse intervals during the following spontaneous burst. Scale: 10 μV, 3 sec.

during the first part of the burst and increases towards the end of the burst. Fig. 1(*a*) plots the mean pulse intervals against the position of the pulse in the burst for eight successive bursts from a single preparation. Five bursts consisted of 11 pulses, three of 12 pulses. There is a marked similarity in pulse intervals for all the recorded bursts. As described later, this regularity in burst shape only occurs in the absence of accompanying SS 2 activity. Here the only irregular interval seems to be that between the first and second pulses (3·7–24·9 sec), suggesting that the mechanism operating to structure the burst and to maintain a regular pattern of changes in pulse interval is not operative for this first interval. The first pulse is, however, part of the burst even when the first interval is as long as 25 sec, for although single nerve-net pulses are recorded in the interburst interval, their frequency is extremely low.

A nerve-net pulse intercalated during a burst resets the pulse rhythm (Fig. 1*b*). A nerve-net pulse was elicited during a burst by electrical stimulation of the column; a 1-msec-duration shock at low voltage excited the nerve-net only. The tentacles give a fast contraction if the interval between the evoked pulse and the preceding spontaneous pulse is within the time for decay of facilitation. This is shown on the recording as a larger muscle action potential (see inset). An intercalated pulse, inserted at any stage of the burst, always causes an increased delay before the appearance of the following spontaneous pulse, regardless of whether or not a fast contraction is evoked. The effect is short-lived and the pulse intervals quickly return to the values shown in normal bursts (Fig. 1*b*). The evoked pulse can elicit a fast contraction that has been

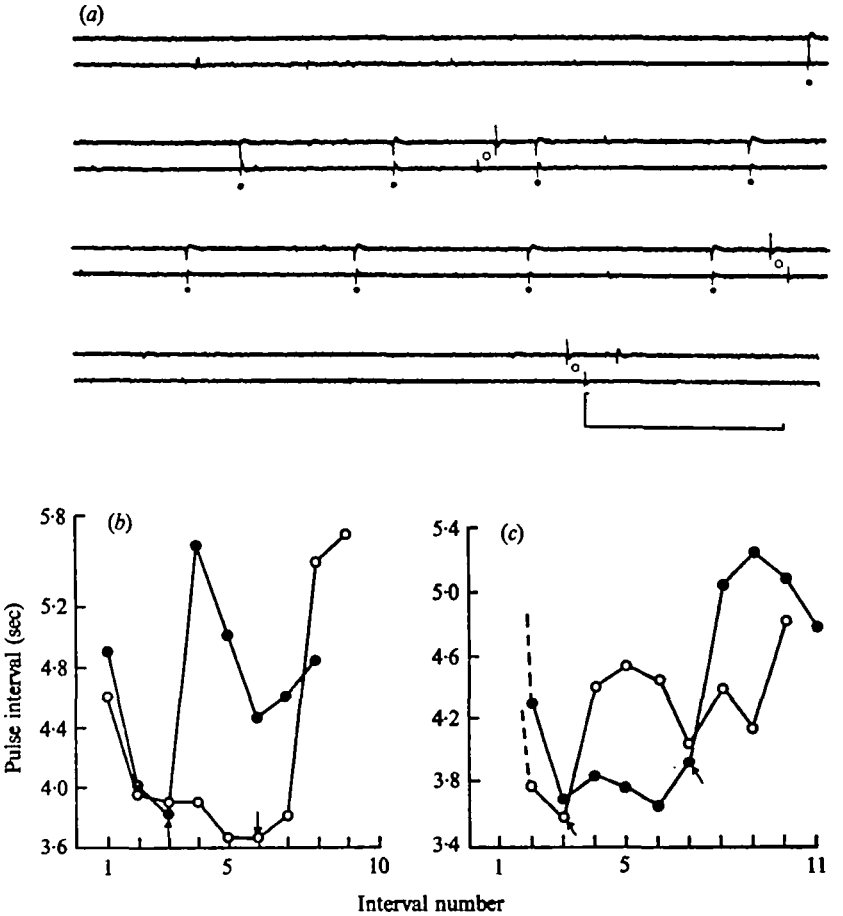

Fig. 2. The inhibitory effect of a single SS 2 pulse on the nervous pacemaker. (*a*) Complete record of a burst, showing nerve-net pulses (●) and accompanying SS 2 pulses (○). Two recording electrodes were used and the record is continuous from top left. (*b*) Pulse intervals during this burst (●). The arrows in this and subsequent graphs indicate the interval during which an SS 2 pulse appeared. Here there is an SS 2 pulse during the 3rd interval. The following pulse intervals are considerably extended, the effect persisting over several subsequent intervals. (○) Intervals in the following burst where an SS 2 pulse also occurred, but here during the 6th interval. (*c*) Two successive bursts, one with an SS 2 pulse in the 3rd interval (○), the other with an SS 2 pulse in the 7th interval (●). Scale: 10 μV, 5 sec.

facilitated by a preceding spontaneous pulse, but the next spontaneous pulse does not cause fast contraction as it always follows the evoked pulse by an interval longer than the time for decay of facilitation. This may explain Ross's (1952) observation for *Metridium senile* that only 3 % of fast contractions following single shocks were delayed, i.e. due to a spontaneous pulse appearing shortly after the evoked pulse.

The observation that an intercalated pulse resets the rhythm suggests that the nerve-net pulses originate in a pacemaker, although this must remain a provisional interpretation of the results as the possibility that bursts are due to the patterned discharge of sense cells cannot be ruled out. The pacemaker must be diffuse as bursts can be recorded from isolated preparations. As shown later, there seem to be a number of potential pacemakers, normally linked so that the system acts as a single pacemak

Action of SS 2 pulses on the nerve-net pacemaker

Bursts do not always show the regular changes in pulse interval depicted in Fig. 1(*a*). Deviations from the normal rhythm seem associated with the occurrence of SS 2 pulses during the burst. No SS 2 pulse was recorded during the bursts shown in Fig. 1.

It has been previously shown (McFarlane, 1973*a*) that the activity of the nerve-net and the endodermal slow conduction system, the SS 2, are closely interrelated. During a burst few or no SS 2 pulses are recorded. Shortly after the burst the SS 2 activity rises to its maximum frequency (about 6 pulses/min) from which point the SS 2 pulse intervals gradually increase during the period leading up to the next burst.

An SS 2 pulse appearing during a nerve-net burst is followed by a marked increase in nerve-net pulse intervals. Fig. 2(*a*) shows a burst containing an SS 2 pulse after the third nervous pulse. Fig. 2(*b*) plots the pulse intervals and shows that the fourth interval, instead of being shorter than the third, was greatly extended. The normal curve was then resumed, with the intervals continuing to decrease, but they remained longer than in bursts without SS 2 activity. The other curve in Fig. 2(*b*) shows the following burst, containing an SS 2 pulse during the sixth interval. Here the intervals continued to decrease until the SS 2 pulse appeared. In both bursts there seems to be no extension of the interval containing the SS 2 pulse; in other cases this is also increased in duration. Two further SS 2 pulses appear in Fig. 2(*a*), both after the last nervous pulse. These show a similar delay in arrival at the two recording electrodes, suggesting that they originate from the same region, but this is clearly different from the origin of the first SS 2 pulse.

A spontaneous SS 2 pulse is effective at any stage of a burst. Fig. 2(*c*) shows two bursts from a half-animal preparation, one with an SS 2 pulse early in the burst, the other with an SS 2 pulse late in the burst. In both cases the subsequent nerve-net pulse intervals are increased compared with the same intervals in bursts without SS 2 activity.

Summarizing the results from a large number of recordings, the inhibitory action of a single SS 2 pulse has a slow onset and is long-lasting (15–30 sec), increasing the duration of several subsequent pulse intervals. A single SS 2 pulse is generally followed by a 25–70 % increase in the duration of the next nerve-net pulse interval. The action of an SS 2 pulse on the pacemaker rhythm differs from the action of an intercalated nervous pulse; the latter also increases the interval between spontaneous pulses but affects only the interval in which it appears. Stimulation of the other slow conduction system, the SS 1, had no obvious effect on pulse intervals.

Evoked SS 2 pulses also affect the nervous pacemaker. Fig. 3(*a*) compares nerve-net pulse intervals during three successive bursts, all apparently without accompanying spontaneous SS 2 activity. The SS 2 was electrically stimulated during the interval between the 3rd and 4th pulses of the second burst: a single SS 2 pulse is elicited by a low-voltage 200-msec-duration shock to a tentacle (McFarlane, 1974). This single pulse was followed by a marked increase in the duration of the subsequent nerve-net pulse invervals. Additional spontaneous or evoked SS 2 pulses are followed by a further increase in pulse intervals. In the burst shown in Fig. 3(*b*) the 2nd, 3rd and [redacted] intervals each contained one spontaneous SS 2 pulse. Here the nerve-net pulse

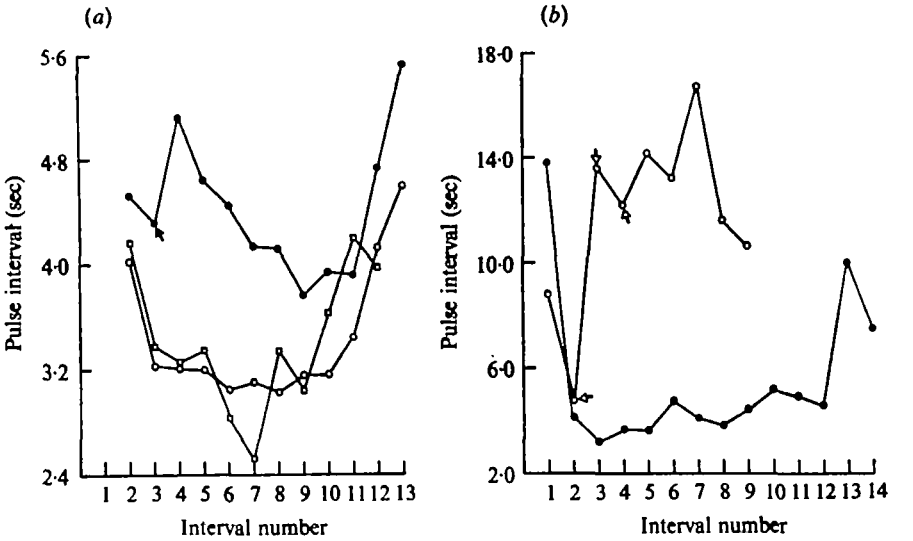

Fig. 3. (*a*) Effect of an evoked SS 2 pulse on nerve-net pulse intervals. (●) Intervals in a burst where SS 2 was stimulated during the 3rd interval; (○, □) intervals during preceding and following bursts. (*b*) Effect of several spontaneous SS 2 pulses on nerve-net pulse interval. (○) Burst containing 3 SS 2 pulses (note the extremely long pulse intervals); (●) following burst with no accompanying SS 2 activity.

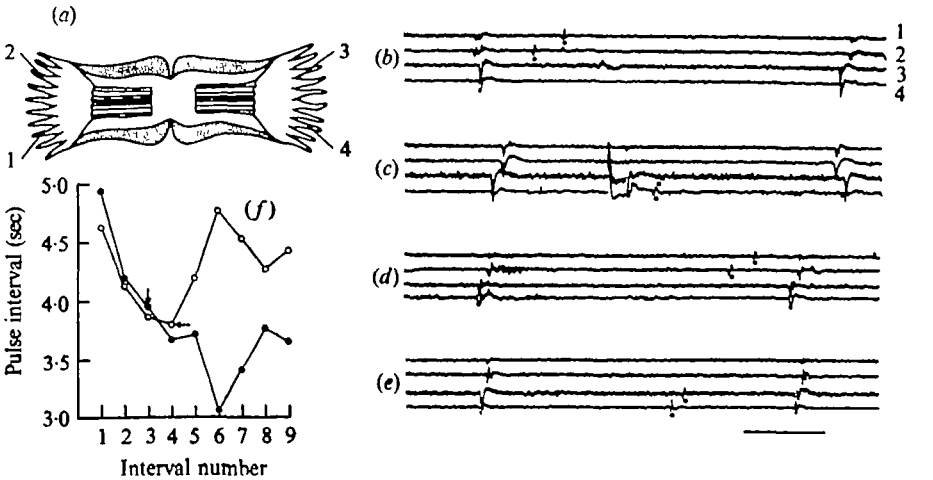

Fig. 4. (*a*) Split-animal preparation with four recording electrodes attached to tentacles as shown. (*b–e*) Spontaneous nerve-net pulses recorded from split-animal preparation. (*b*) Shift in nerve-net pulse origin following spontaneous SS 2 pulse. (*c*) Shift in nerve-net pulse origin following evoked SS 2 pulse. (*d*) No shift when SS 2 pulse is on opposite side to origin. (*e*) Occasionally origin does not shift after SS 2 pulse. (*f*) Pulse intervals from two bursts from a split-animal preparation, in one case (○) the SS 2 pulse was not followed by a shift in nerve-net pulse origin, in the other case (●) the SS 2 pulse was followed by a shift. Where the origin shifted the SS 2 pulse seems not to have altered the rhythm of the pacemaker. Time scale: 1 sec.

interval was increased to 14 sec from its normal value of 4 sec. The other curve shows the following burst that was without accompanying SS 2 activity.

One model for the observed results is that spontaneous or evoked SS 2 pulses spread throughout the endoderm and inhibit all potential pacemakers. The model can be tested by restricting the spread of SS 2 pulses. This is possible with split-animal preparations (Fig. 4*a*) where an anemone is divided vertically but a narrow pedal disc bridge is left intact. Each half is pinned down at the cut edge of the sphincter muscle. Nerve-net and SS 1 activity always pass freely from one half to the other, but in most preparations the bridge seems to act as a barrier to the SS 2. All experiments described below used preparations where the SS 2 pulse failed to cross the bridge; thus although the nervous activity of the two halves is linked, each half shows independent spontaneous SS 2 activity and the inhibitory action of an SS 2 pulse is restricted to only a part of the total population of nerve-net pacemakers.

Spontaneous nerve-net pulses are recorded from split-animal preparations and it is observed that the pulse origin often shifts from one side of the preparation to the other during a burst. The origin is taken to be on the side where the spontaneous nerve-net pulse is first recorded. This assumes that there is only a single origin for any observed pulse and there is no pulse collision. A major shift in nerve-net pulse origin usually follows the appearance of an SS 2 pulse during the burst. The halves of the preparation will be referred to by electrode numbers: 1/2 and 3/4 (Fig. 4*a*). In Fig. 4(*b*) the first nerve-net pulse shown originated in the 1/2 half and was shortly followed by a spontaneous SS 2 pulse recorded only in the 1/2 half. The origin of the next nervous pulse shifted to the 3/4 side. An origin shift may also follow an evoked SS 2 pulse (Fig. 4*c*); here stimulation of the SS 2 on the 3/4 side immediately after a spontaneous nerve-net pulse from the same half resulted in an origin shift. Shifting has not been seen when the spontaneous SS 2 pulse was in the other half of the preparation to the origin of the preceding nervous pulse (Fig. 4*d*). Occasionally, however, an SS 2 pulse on the same side as the preceding nerve-net pulse is not followed by origin shift in the same interval or in subsequent intervals (Fig. 4*e*).

It seems that there are many potential pacemakers and the actual origin at any time is presumably the most active of these. If an SS 2 pulse occurs in the same half as the preceding nerve-net pulse origin it will inhibit all the potential pacemakers on that side only, thus delaying the onset of the next spontaneous pulse and allowing a pacemaker in the other half of the preparation to assume leadership. If this is so, where a shift does occur (as in Fig. 4*b*) the rhythm of the nervous pacemaker should be more or less unaffected by the SS 2 pulse. On the other hand, where an SS 2 pulse is not followed by origin shift (as in Fig. 4*e*) there should be an increase in nerve-net pulse intervals just as in a half-animal preparation. Fig. 4(*f*) shows pulse intervals during two bursts, each containing a single SS 2 pulse. In one the origin shifted and there was no increase in pulse intervals, in the other the origin did not move and the subsequent intervals were extended.

Nerve-net pulse origin

The movements of the origin described above are the result of the restricted spread of SS 2 pulses and would not occur in intact animals. Nerve-net pulse origins can, however, move in the absence of SS 2 activity. They can shift not only from one side

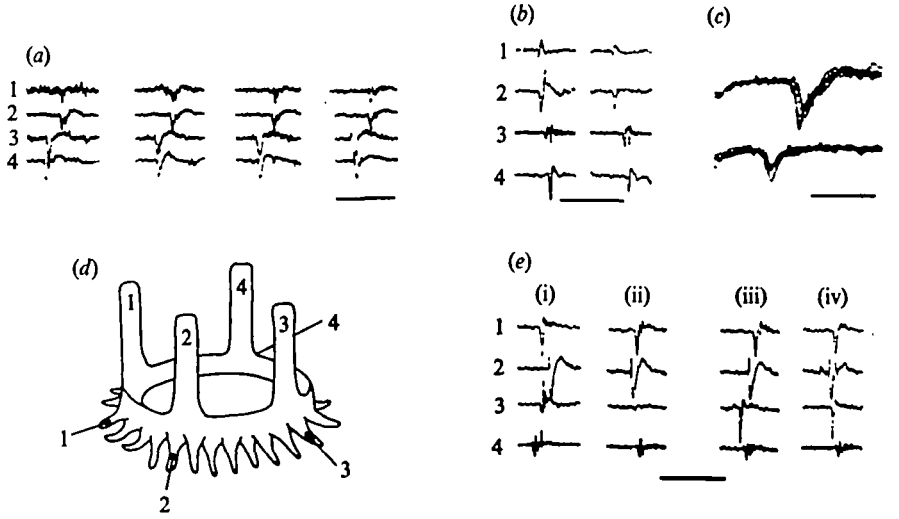

Fig. 5 (*a*) Four successive nerve-net pulses recorded from four electrodes on a split-animal preparation. Origin clearly on 3/4 electrode side but pulse arrives at electrode 4 first in the first case, and subsequently at electrode 3 first, suggesting that pulse origin can shift during a burst. (*b*) Two successive nervous pulses from split-animal preparation. The increased spread of the pulses in the second case again suggests that the origin has moved. This is not due to a change in conduction delay as (*c*) shows a record from two electrodes of superimposed nerve-net responses to 6 shocks at a stimulus frequency of 1 shock every 3 sec. There is very little change in conduction delay. (*d*) Preparation consisting of four connected longitudinal column strips with four electrodes on tentacles at bases of strips. (*e*) Nerve-net pulses recorded from such a preparation. Strip 4 was stretched gently, evoking a nerve-net pulse reaching electrode 4 first (i). This was shortly followed by a spontaneous burst, the first pulse of which is shown (ii), where all pulses originated close to electrode 2. Strip 3 was stretched (iii) and was again followed by a burst from region of electrode 2 (iv). Time scales: (*a*, *b*, *d*) 500 msec, (*c*) 100 msec.

of a split-animal preparation to the other but also to a different origin on the same side. Fig. 5(*a*) shows four successive nervous pulses from a split-animal preparation. If we consider the interval between pulse arrival at electrode pair 1/2 and at pair 3/4 as an indication of the position of the origin, then the origin appears to remain fixed. Closer inspection, however, reveals that the first pulse arrives at electrode 4 first, whereas the other pulses reach electrode 3 first. Another example of an origin shift is shown in Fig. 5(*b*) where the change in pulse spread in two successive pulses shows that the origin has moved, although remaining in the same half. This is not due to an increase in conduction delay as repetitive electrical stimulation results in only a slight change in delay of nerve-net pulses (Fig. 5*c*). The observed movements of pulse origin may indicate that there are a number of potential pacemakers.

Comparison of pulse arrival delays of spontaneous and evoked nerve-net pulses cannot accurately locate the pacemakers because they appear to be widespread throughout the endoderm. Fifty spontaneous nerve-net pulses were recorded from four electrodes attached to a split-animal preparation as shown in Fig. 4(*a*). Taking the time of pulse arrival at the first electrode as 0 and expressing the spread of delays at the other three electrodes as time after arrival at the first, the range of delays for the spontaneous pulses was 0–30, 105–195, 110–205 msec. Electrical stimulation of the nerve-net at the base of the column, mid-column, sphincter, pharynx and mesentery all gave arrival delays within the range shown by spontaneous pulses. Stimulation

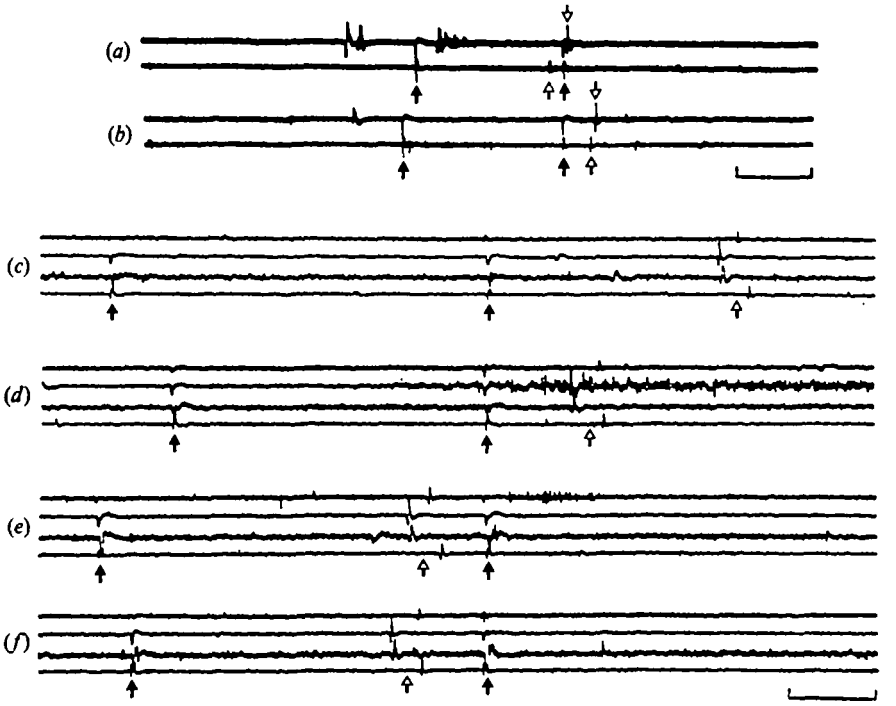

Fig. 6. (*a*, *b*) Two examples of short bursts containing just two nerve-net pulses. Recordings were from 2 electrodes attached to a half-animal preparation. Closed arrows show nerve-net pulses, open arrows show SS 2 pulses. (*c–f*) Records from 4 electrodes on a half-animal preparation. Each record shows the last two nerve-net pulses of four successive bursts. In all cases an SS 2 pulse occurred close to the end of the burst. Note that the SS 2 pulses all seem to have the same origin. Time scales: (*a*, *b*) 2 sec, (*c–f*) 1 sec.

tentacles, however, gave minimum values of 35, 220, 235 msec, outside the range shown by spontaneous pulses. It may be concluded that in this preparation at least, and during the period of monitoring, spontaneous nerve-net pulses did not arise in the tentacles.

Initiation and termination of nerve-net bursts

Bursts can sometimes be initiated by mechanically or electrically excited nerve-net pulses (McFarlane, 1973*b*). Recordings from a preparation consisting of four partly isolated longitudinal column strips (Fig. 5*d*) show that the burst can originate in a different position from the applied pulse. In Fig. 5(*e*), a recording from such a preparation, strip 4 was stretched gently, eliciting a nervous pulse arriving first at electrode 4. This was followed after 10 sec by a burst with all pulse origins closest to electrode 2. Similarly when strip 3 was stretched the elicited pulse reached electrode 3 first but the burst that started 8 sec later had a constant origin closest to electrode 2. The same effect was seen with electrically excited nerve-net pulses. It is not known why any particular region should act as a leader.

The factors responsible for determining interburst interval and for initiating bursts are not known. Single nerve-net pulses are occasionally seen during the interburst interval and these do not initiate bursts. This may be because of the presence of some unknown excitatory input to the pacemakers that determines their readiness to fire.

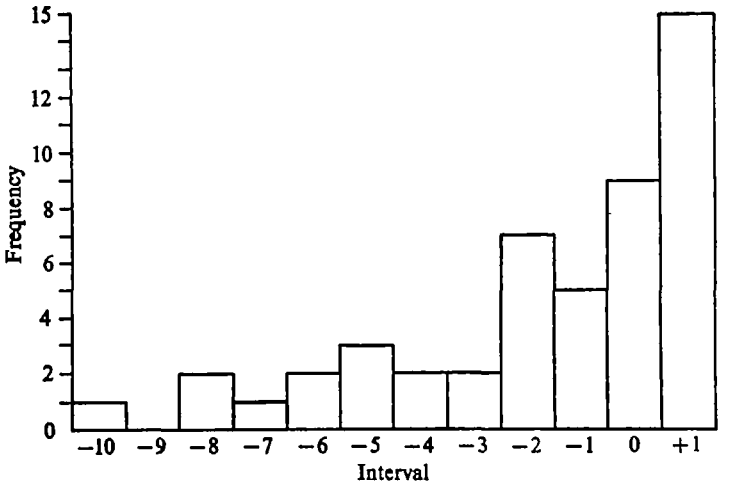

Fig. 7. Position of spontaneous SS 2 pulses in 29 bursts recorded from a single half-animal preparation. The final nerve-net pulse interval is shown as 0, and preceding intervals are counted backwards from −1 to −10. Any SS 2 pulses appearing after the last nerve-net pulse, but within a period equal to the last nerve-net pulse interval, are shown as +1. The frequency of occurrence of SS 2 pulses in each of these intervals is shown. There is a clear tendency for SS 2 pulses to appear towards the end of the burst, suggesting that they arise as a result of the effector output of the nerve-net pulses.

It may also depend on the level of SS 2 activity at the time the pulse appears. Eleven single nerve-net pulses were recorded from one half-animal preparation and all were followed by SS 2 pulses with a delay of 1·3–10·1 sec. The mean delay (4·5 sec) was less than the shortest interval between the first and second nerve-net pulses of bursts from the same preparation (5·4–12·1 sec, mean = 7·9 sec, $N = 11$), so it is possible that in some cases an SS 2 pulse occurring shortly after a single pulse can prevent triggering of the burst. This cannot, however, be a complete description of the factors controlling triggering, for single nerve-net pulses have been recorded that were not closely followed by SS 2 pulses and which did not initiate bursts. Short bursts consisting of just two nerve-net pulses are recorded very occasionally; these always seem to have closely associated SS 2 pulses (Fig. 6*a*, *b*). Again, whilst it is possible that the SS 2 pulses are preventing the appearance of further nerve-net pulses, a few full-length bursts also show early SS 2 pulses.

The number of pulses in a burst can vary considerably but is usually in the range 10–20 (McFarlane, 1973*b*). Burst termination appears to be related to the observed increase in pulse intervals towards the end of the burst. An SS 2 pulse might hasten burst termination by extending the pulse intervals, but bursts will continue even when intervals are increased well beyond those normally found at the end of a burst (as in Fig. 3*b*).

The occurrence of SS 2 pulses during a burst may be related to the effector output of the burst; the evidence for this is that SS 2 pulses show an increasing tendency to appear as the burst proceeds. Fig. 6(*c*–*f*) shows the last two nervous pulses from each of four bursts recorded from a half-animal preparation. In all cases there is an SS 2 pulse either in the last interval or shortly after the last nerve-net pulse. Note that here the SS 2 pulses all seem to have the same origin. The positions of the SS 2 pul

recorded during 29 bursts are shown in Fig. 7. The number of nerve-net pulses/burst ranged from 9 to 12 (mean = 10·6). The average number of SS 2 pulses/burst was 1·7, and only 5 bursts lacked an SS 2 pulse either in the last interval or closely following the last nerve-net pulse. The results indicate that SS 2 pulses tend to occur late in the burst, but it should be remembered that the SS 2 pulse may itself be partly responsible for burst termination.

Control of muscular contraction

Spontaneous nerve-net bursts are followed by a sequence of muscular contractions (McFarlane, 1973*b*, 1974). A small number of bursts have been recorded from intact animals and it is clear that the pulse frequency, and the type of contraction elicited, can vary considerably. As in preparations, reduction of burst frequency in intact animals seems to be associated with accompanying SS 2 activity. Some bursts are clearly followed by pulling-in of the oral disc, probably as a result of slow contraction of the mesenteric retractors followed by a slow sphincter-muscle contraction. Other bursts are followed only by column shortening, probably due to parietal muscle contraction. The type of contraction evoked seems to depend on both pulse number and pulse frequency. Pantin (1935*b*) showed that the frequency of nervous pulses was important in determining which muscle group contracts, but to date no quantitative study has been made of this phenomenon.

Longitudinal column strip preparations (McFarlane, 1973*b*) were used to study how contraction size is related to number and frequency of evoked nervous pulses. The strips contain two main sets of muscles – the parietals and the mesenteric retractors. The parietals give slow contractions only, the retractors give both fast and slow contractions (Pantin, 1935*b*). Fig. 8(*a*) shows the effect of varying shock number at a constant stimulus interval of 10 sec. As described below, this frequency of stimulation elicits only parietal muscle contraction. Maximum contraction is reached after about 18 shocks. The curve rises steeply over the range 5–15 shocks, so the number of pulses is clearly important in determining the size of a contraction. Fig. 8(*b*) shows the effect of a constant number of shocks over a wide range of stimulus intervals. The peak at a pulse interval of 11 sec probably represents parietal muscle contraction. The part of the curve at intervals below 5 sec is due to retractor contractions. At intervals of less than 2 sec the retractors give fast contractions. The mesenteries were trimmed away from the strips to remove the retractor muscles; Fig. 8(*c*) shows that the contractions at high stimulus frequencies are lost in the response of a trimmed preparation. The small peak at 6 sec may be due to retractor remnants, but this peak was not obvious on the following day (Fig. 8*d*). Fig. 8(*c*) shows the response of the parietals to 12 shocks, Fig. 8(*d*) the response to 17 shocks. The peak seems to have shifted to a shorter interval in Fig. 8(*d*), but it is not clear if this is a result of ageing or is related to the number of shocks. If the latter is true, it may imply that the nerve-net has an inhibitory action, as well as an excitatory action, on the parietals. Such a dual effect of nerve-net pulses has been shown for the circular muscles (Ewer, 1960; McFarlane, 1974). The graphs for frequency show that nerve-net pulse frequency will be important in determining which muscles contract, so bursts that differ widely in frequency, for example those shown in Fig. 3(*b*), will produce significantly different behavioural outputs.

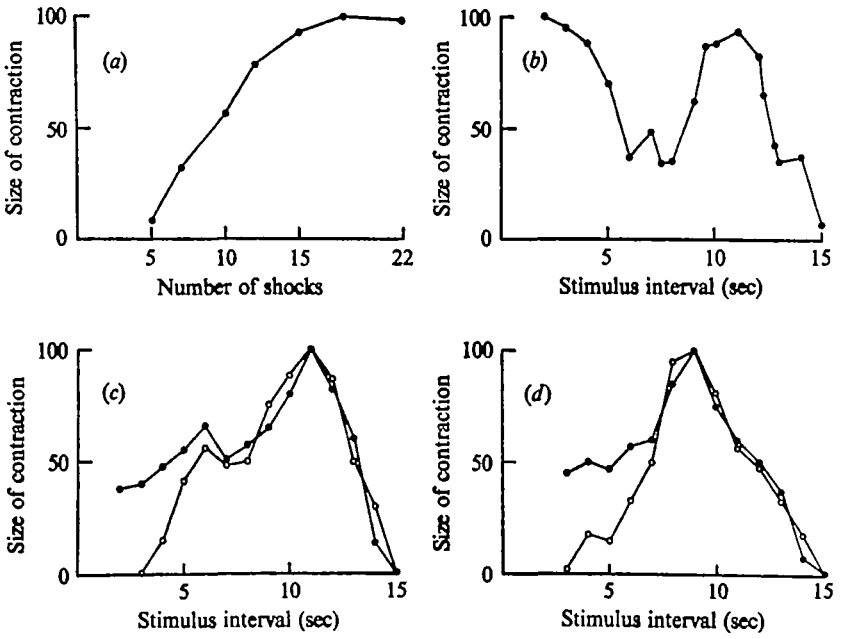

Fig. 8. Effect of stimulus number and frequency on size of evoked contractions of parietal muscles and retractor muscles. Contraction size is expressed as percentage of maximum contraction seen in each experiment. (*a*) Effect of number of shocks, at a constant frequency of 1 every 10 sec, on size of parietal muscle contractions. (*b–d*) Effect of stimulus frequency on contraction size. (*b*) Response of untrimmed preparation to 10 shocks at varying frequencies. The peak at 11 sec probably represents parietal muscle contraction; the peak below 5 sec is due to retractor muscle contraction. (*c*) Response to 12 shocks after mesenteries have been trimmed to remove retractors. The two curves show the results from two strips in a single preparation (1 day after operation). (*d*) Same preparation after 2 days; response shown to 17 shocks. Temperature: (*a*) 13 °C, (*b–d*) 14 °C.

DISCUSSION

Pacemaker system of the nerve-net

The results were interpreted as showing that there are several potential pacemaker sites and that the pulse origin may shift from one site to another during the course of a burst. An alternative explanation of the observed shift in pulse delays during some bursts is that the pulses originate in a single pacemaker but reach the electrodes via different routes on separate occasions. This is unlikely because evoked nerve-net pulses always show the same conduction delay from any one stimulation site and show no evidence for alternative pathways. Also the apparent shift in origin from one side of a split-animal preparation to the other is not easily explained in terms of altered conduction routes.

The nervous pacemakers seem to be widely distributed throughout the endodermal portion of the nerve-net, but may be absent from the ectodermal net of the tentacles. SS 2 pulses always slow the rhythm of the pacemakers and, as the SS 2 seems to lie only in the endoderm, this also argues for a strictly endodermal location of pacemakers. It may be possible to locate the pacemakers more accurately by recording from the nerve-net in the mesenteries.

The histology of the nerve-net is well known but the pacemaker action cannot

[redacted]be ascribed to a specific cell type. The following description is from Batham, Pantin & Robson (1960) and Robson (1961, 1965). The endodermal nerve-net comprises sense cells and bipolar and multipolar nerve cells. The bipolar cells are widespread but are especially abundant in the region of the retractor muscles. The neurites of bipolar cells are often several millimetres long and they seem specialized for rapid conduction of nervous impulses to the muscles so as to give the fast protective contraction. The sense cells are mainly concentrated in the region of the longitudinal muscles, where the mesenteries join the body-wall. Each sense cell has a distal flagellum and 1, 2 or 3 basal neurites. The rather sparse multipolar cells lie mainly over the circular muscle of the body wall. In *Calliactis parasitica* the cell bodies are 10–15 μm in diameter and have 3, 4 or 5 neurites, which are 500 μm or more in length. They occur at a density of 50–100 cells/mm^2. The neurites of a multipolar cell may contact other multipolar cells, sense cells or bipolar cells. These three cell types will now be considered as possible sites for the pacemaker activity of the nerve-net.

Multipolar cells. Robson (1963) proposed that multipolar cells in the column of *Stomphia coccinea* are the pacemakers of the swimming contractions. She points out, however (Robson, 1965) that the multipolar cells in *Calliactis* are much smaller than those in *Stomphia* and may not be strictly comparable. Also, although multipolar cells are found in the column endoderm adjacent to the sphincter muscle (Robson, 1965), isolated sphincter rings do not show spontaneous contractions (Ross, 1957). Isolated circular muscle rings, cut from any level of the column, give regular contractions (Ewer, 1960). Nerve-net pulses have, however, not yet been recorded from these preparations and caution must be exercised in regarding such contractions as evidence for pacemaker activity, as it is known that circular muscles can contract in the absence of recorded nervous activity (McFarlane, 1974). Perhaps the pacemaker is inactive in any isolated ring preparation, possibly because the population of multipolar cells in these preparations is too small to give rise to pacemaker activity. Spontaneous nervous pulses have been recorded from *Metridium senile* (McFarlane, 1973*c*), but as yet multipolar cells have not been found in this species although there are occasional tripolar cells in the mesenteries (Pantin, 1952).

Sensory cells. As mentioned earlier, the results do not completely rule out burst origin by patterned discharge of sensory cells. Alternatively it is possible that these cells function as both receptors and pacemaker cells influenced by sensory input. There is in fact no direct evidence that these cells are sensory: Passano & Pantin (1955) suggest that these cells are responsive to mechanical stimulation but consider that the sensitivity may lie in the neurites rather than in the cell body, and that the flagella may be chemosensitive.

Bipolar cells. As described below, the refractory period of the bipolar cells appears to be too low for them to produce the observed low-frequency pacemaker discharge. It remains possible, however, that part of the population of bipolar cells has different characteristics.

The shape of the burst, an early decrease in pulse intervals followed by a later increase, is typical of the pacemaker output of nerve cells in many other invertebrates (e.g. tentacle contraction pulses in *Hydra*, Rushforth & Burke, 1971; lobster swim[redacted]et motoneurones, Davis & Murphey, 1969). A computer simulation describing

burst structure in swimmeret motoneurones was based on values for threshold, absolute and relative refractory period, and a sinusoidal change in excitatory input (Davis & Murphey, 1969). The pacemaker cells in *Calliactis parasitica* must have a long refractory period to produce low-frequency bursts by a mechanism of this sort. We have little information about the refractory period of different parts of the nerve-net; the mesenteric nerve-net has an absolute refractory period of only 45–60 msec (Pantin, 1935*a*), but the nerve cells in the pharynx have a relative refractory period of 800 msec (McFarlane, 1973*b*).

Action of the SS 2 on nerve-net pacemakers

Rhythmic behaviour patterns controlled by pacemakers are common throughout the animal kingdom. The rhythm is usually endogenous but is often modified by external stimulation and peripheral feedback (Huber, 1967). There are usually both excitatory and inhibitory influences on the pacemaker. In Scyphozoa the pacemakers of the swimming contractions lie in the marginal ganglia and their output is fed into the giant fibre nerve-net. A second, diffuse-fibre nerve-net can act on the ganglia, increasing pacemaker output (Horridge, 1956). Mechanical stimulation can inhibit pacemaker output but the conducting system involved has not been identified. In the hydroid *Tubularia* the distal opener system (DOS) inhibits both single pulses and bursts in the polyp neck (NP) pacemaker system (Josephson & Uhrich, 1969), but here the cellular basis of the conducting systems is not known.

The nervous pacemakers in *Calliactis parasitica* seem to be inhibited by SS 2 activity. The properties of the SS 2 are very different from those of known nerve-nets (McFarlane, 1973*b*) and the SS 2 may be a non-nervous conducting system. Neuroid conduction in coelenterates has been identified clearly only in certain hydromedusae and siphonophores (Mackie, 1965) but is suspected in many other cases. A possible location for the SS 2 is the musculo-epithelial cell layer of the endoderm. The nerve-net lies just above the muscle tails of this layer but specialized junctions between nerve cells and musculo-epithelial cells have not been described, although neuromuscular junctions presumably exist to convey impulses from the nerve-net to the contractile elements. Some other type of junction might be expected if the SS 2 action on the pacemakers is direct. The SS 2 action might, however, be indirect as it is already known that SS 2 activity inhibits inherent contractions of certain endodermal muscles (McFarlane, 1974) and such an action may remove some excitatory input to the pacemakers. The SS 2 action on the muscles requires several SS 2 pulses, however, and has a very slow onset, whereas the action on the pacemaker requires only one pulse and appears within a few seconds.

The origin of the spontaneous SS 2 pulses is not known. They clearly have many different sites of origin and it has been suggested (McFarlane, 1974) that they arise as a result of stress between two opposing muscle groups, the circulars and the parietals. Their tendency to occur late in nerve-net bursts suggests that they result from the effector action of the nerve-net pulses, and they may provisionally be regarded as being a form of inhibitory peripheral feedback on the nervous pacemakers. This ability to modify the frequency of spontaneous nerve-net pulses and hence to control the extent of contraction of different muscle groups is obviously of great importance in an animal where, although body shape is dependent upon

interaction between many muscle groups, there are no functionally separate nervous pathways to provide for independent control of individual muscles.

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