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ELECTRICAL ACTIVITY IN THE HYDROID *CORDYLOPHORA*

BY G. O. MACKIE
Department of Zoology, University of Alberta, Edmonton, Canada

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INTRODUCTION

Much has been learned of the spontaneous electrical activity of *Hydra* (Passano & McCullough, 1964, 1965) and of *Tubularia* (Josephson & Mackie, 1965) but in the related gymnoblastic hydroid *Cordylophora* spontaneous electrical events have not been recorded. Josephson (1961) showed that stimulation of the stem in this hydroid elicits large electrical potentials, either singly or in bursts, the number evoked being related to the strength of stimulation. The potentials are propagated throughout the colony and can be recorded in the hydranths, which respond by contracting, as well as in the interconnecting stem which does not contract. This ability on the part of hydranths to contract in response to stimulation at remote points along the stem is best interpreted in terms of protective behaviour. The potentials associated with this response will here be termed 'Josephson pulses' (JPs). It may eventually prove possible to homologize pulse systems in various hydroids, in which event a common terminology could be adopted, but at the present time non-committal designations seem preferable.

The wire electrodes used in the original study did not lend themselves well to recording from the hydranths, and most of Josephson's data were obtained from the stem. With the small, flexible suction electrodes now available mobile regions such as the hydranth and tentacles can be recorded from without much difficulty. Their use in the present study has made it possible to record spontaneous electrical activity in the hydranths.

In both *Tubularia* and *Hydra* it has been found that the animals' state of nutrition is reflected in the long-term patterns of spontaneous electrical activity, quite apart from the short-term electrical effects associated with food capture and ingestion. In a well-fed *Tubularia* the bursts of neck potentials are longer and more regular than in unfed animals (Mackie, cited by Josephson, 1965). Passano & McCullough (1964) showed that *Hydra* exhibits a higher frequency of contraction pulse bursts when fed daily than when starved for one or more days. N. B. Rushforth (personal communication) finds that starved *Hydra canadensis* show rather few contraction pulses, these typically coming singly; some 45 min. after feeding to repletion there is a four- or five-fold increase in pulse frequency and the pattern is typically one of bursts. Preliminary recordings from *Cordylophora* were carried out on recently fed animals on the assumption that spontaneous activity would be most easily demonstrated in such animals. It soon became clear that while the unfed animal is electrically silent, feeding leads to a dramatic electrical awakening of the animal.

MATERIALS AND METHODS

Cordylophora lacustris Allman, obtained from the Supply Department of the Marine Biological Laboratory at Woods Hole, Mass., were grown in 50 ml. beakers in the solution known as '*Cordylophora*-versenated-distilled water' (CVD) developed by Fulton (1960). It was here used at double strength (2 CVD) or quadruple strength (4CVD) to facilitate electrical recording. The colonies were fed on recently hatched *Artemia* nauplii.

For stimulation, square-pulse shocks of 0·5–2·0 msec. duration were delivered through fine platinum wire electrodes insulated to near the tip.

The recording electrodes were made from polyethylene tubing drawn out to a fine flexible tip. An internal diameter at the tip of about 50 μ was suitable for recording on the body wall, 25 μ for recording from tentacles. A length of 0·006 in. diam. platinum wire, connected to the amplifier input leads, was inserted into the lumen of the electrode as far as it would go without blocking the lumen. Such an electrode filled with 2CVD has a resistance of 1·5 MΩ. Suction was applied through a syringe and the electrode was attached to the body wall or tentacles of the hydroid. In all the recordings shown here the electrodes were placed on the hydranth wall either in the area from which the tentacles arise or immediately proximal to this area, where there are no tentacles and where the ectoderm is not protected by the cuticular layer (perisarc) which extends a little way up the hydranth from the stem.

Differential input through conventional capacity-coupled pre-amplifiers (Tektronix 122) was employed, and signals were recorded on a Grass Model 7 polygraph, with simultaneous display on a dual-beam oscilloscope (Tektronix 502A). The full frequency range of the amplifiers was used in all the records shown here, but a 15 cyc./sec. low-pass filter proved useful where low level 'slow pulses' were being examined at high gain.

RESULTS

Pulse types recorded

Figure 1 shows the three major types of potentials which were recorded from the hydranth, shown here as they appeared under identical recording conditions.

(*a*) Josephson pulses (JPs). These large signals are fully described by Josephson (1961), who recorded them at amplitudes of 0·5–15 mV. with conduction velocities averaging 2·7 cm./sec. in the stem. Recorded with suction electrodes on the hydranth or, through amputated side branches, on the stem tissue the potentials often show an initial positive component (as in Fig. 1) or are biphasic with an intial negative component instead of being the essentially monophasic negative events described in the original study.

(*b*) Slow hydranth pulses (SPs). These are recorded from all parts of the hydranth not covered with cuticle (which includes the tentacles), but do not appear to spread into the stem or to adjacent hydranths. The slow pulse is recorded as a biphasic event consisting of an initial negative deflexion not exceeding 400 μV. followed by a similar (occasionally greater) positive deflexion. The durations of the negative and positive components are in the order of 800 and 1200 msec. respectively when seen at high amplification under the best conditions. The validity of this picture of SP form needs

to be checked using DC amplifiers because of the distortion possible with capacitative coupling. The pulses never occur singly, but always in a regular, rhythmic series. Recordings with two electrodes on different parts of the hydranth show that the SPs are rapidly propagated to (or arise nearly simultaneously in) all regions. There is no feature in the wave form sharp enough to serve as a reference point for accurate velocity measurements. In two-channel recordings from widely separated points no significant amplitude differences are seen.

(*c*) Fast hydranth pulses (FPs). These potentials are sharp, spike-like events, exhibiting a range of forms and amplitudes. In Fig. 1 they are predominantly negative, with amplitudes of 600–650 μV. and a slight positive overshoot. This is a typical appearance of FPs in an animal that has not been stimulated to contract strongly. Figure 2A shows a similar monophasic FP of nearly 1 μV., this being about the

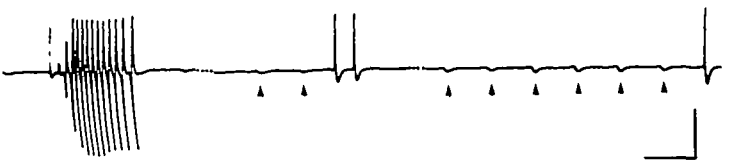

Fig. 1. Three pulse types recorded from a hydranth without change in the recording conditions, for comparison. Dots represent gaps of 50 sec. in each case. A JP burst is shown first following a shock to the stem. The three negative signals in the following parts are FPs, the small biphasic events shown by the arrow heads being SPs. Horizontal bar 4 sec.; vertical bar 600 μV negative

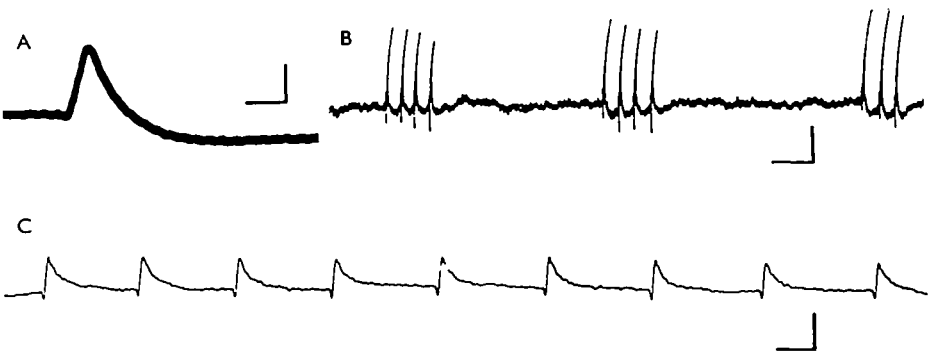

Fig. 2. Fast pulses. A. Monophasic FP photographed from the oscilloscope. The pulse is based on an SP whose presence is responsible for the lowered baseline following the FP. B. Typical interrupted burst pattern of biphasic FPs observed one minute after electrical stimulation of a hydranth. C. Typical continuous burst pattern seen during the first 10 sec. following stimulation. Small biphasic FPs are seen. Horizontal bars 50 msec., 2 sec. and 200 msec.; vertical bars 500, 100 and 100 μV.

maximum amplitude seen. A second type of FP, commonly seen in animals where the longitudinal muscle of the body wall is markedly contracted, is a smaller event, rarely above 250 μV. and usually having an initial positive component (Figs. 2B and C). Intermediate forms of FP exist and a burst of FP activity may demonstrate a transition from biphasic to monophasic sorts. Conversely, if the hydranth is made to contract during the performance of FP activity by stimulating the stem, the FPs can be seen to continue through the resulting JP burst, emerging on the other side with the

biphasic form where before they were monophasic (Fig. 3). This experiment also illustrates that the FP is an event quite distinct from the JP and one whose periodic occurrence can continue unaffected by JP activity or the contraction associated with it. There is a resemblance here to Passano & McCullough's demonstration (1964) of the essential independence of rhythmic potentials and contraction burst potentials in *Hydra*.

FPs may occur singly, in bursts of fairly regular frequencies or in patterns more or less rigorously based on the SP rhythm (see below p. 393). Like the SPs, FPs are recorded in all parts of the hydranth not covered with perisarc and are not recorded from the stem or from adjacent hydranths. Given the spiky character of the FPs it should be possible to obtain good figures for conduction velocities in the system transmitting them, but the shortness of the distances available for such measurements and the uncertainty about their point of origin make it hard in practice to do this. Measurements from base to tip of single tentacles give velocity values of 6·5–9·0 cm./sec. at 24–26 °C. Some FPs may originate in the tentacles, for series of pulses resembling small FPs and showing a characteristic FP frequency have been recorded from isolated tentacles following electrical stimulation.

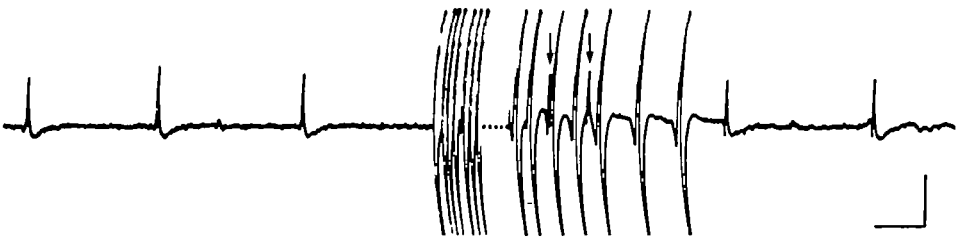

Fig. 3. Beginning and end of a long JP burst (the dots indicating a span of 42 pulses which have been excised) together with FPs induced by feeding some minutes earlier. The FPs continue through the JP burst and an FP pair can be seen within the burst (arrows). Horizontal bar 2 sec.; vertical bar 300 μV.

The slow pulse rhythm

The periodicity of the SP rhythm has been measured at 2–4 sec. in various preparations at temperatures of 22–24 °C. In some preparations the SP pattern, uncomplicated by the occurrence of other electrical events, is seen to be a very regular one. A coefficient of variation of 3·1 % was calculated for a series of 50 consecutive pulses having a mean interval value of 2·3 sec. (The bottom line of Fig. 10 is taken from this series.) Over long periods the periodicity may alter slowly, and when FPs occur they usually upset the rhythm as will be seen.

The best sequences of slow pulses have been seen in animals that have been fed during the preceding 4 hr. Their hydranths are distended with food and peristaltic activity may be in evidence. Neither distention nor peristalsis is necessarily accompanied by SPs, however, and SPs can occasionally be observed in starved, inert animals. The SP rhythm is not definitively related to any observed form of behaviour.

The SP rhythm never starts or stops abruptly in the unstimulated animal. On its first appearance it may slowly emerge from the noise level of the recording building up steadily or in a series of 'surges' to higher amplitudes. In unfed animals (Fig. 4)

occasional, brief emergences of the SP rhythm, with or without some FP activity, may be the only activity seen, and usually no SP activity is seen. The periodicity of these brief emergences of the SP rhythm is about 1·5–3·0 min., much shorter than the periodicity of peristalsis which in starved animals is in the order of 15–30 min. (Fulton, 1963*a*) and was never less than 6·5 min. in fed animals studied in the present investigation.

Photic stimulation has no effect on an established SP rhythm and does not evoke SP activity in dormant animals. This is also true of FPs, and *Cordylophora*, like *Tubularia*, appears to be insensitive to light. Electrical stimulation has no clear effect on SP activity, although if it throws the animal into a general contraction the accompanying JP or FP activity suppresses or distorts the SP pattern.

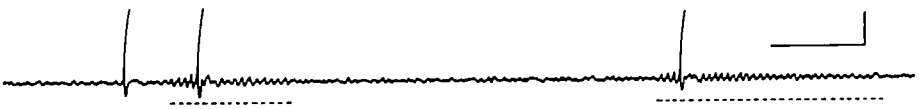

Fig. 4. Recording from a hydranth which had not received food for 18 hr. Occasional 'surges' of SP activity (indicated by the broken line drawn beneath the record) were showing briefly above the noise level at a periodicity of 2–3 min. in this preparation. Isolated FPs were also in evidence. Horizontal bar 30 sec.; vertical bar 250 μV.

Patterns of fast pulses

Unfed animals show no FP activity or only occasional, aperiodic pulses. Within at most 30 sec. of catching food, however, monophasic FPs ensue. They usually continue throughout the period when food is being held in the tentacles, ceasing or becoming infrequent when the last of the food has been ingested. The periodicity in single pulse sequences of this sort may be fairly regular, the pulses coming every 4–6 sec. in typical cases, but the pattern is not usually regular enough to suggest that it is based on an underlying SP rhythm. The sequence of FPs shown in Fig. 3 is typical of a hydranth that is holding food in its tentacles.

There is no clear correlation between individual pulses or pulse pairs and specific movements of the animal. While food is present in the area, the hydranth may sway from side to side, flex its hypostome and wave its tentacles, but these 'appetitive' movements can also be seen in the absence of FPs. For instance, if food juices or proline (to a final concentration of 70 μg./ml., Fulton, 1963*b*) are given instead of solid food, the movements are exhibited without accompanying FPs, or, if FPs do occur, they may only start after the movements have begun. FPs are evidently not an essential correlate of appetitive feeding behaviour. This behaviour would seem to be controlled by a coordinating system whose electrical concomitants are unknown, but which are not FPs.

Once food has been caught, 'consummatory' behaviour is exhibited, involving flexions of the tentacles toward the mouth, bending of the hypostome toward the food and, eventually, ingestion. With the exception of ingestion these movements have so far only been seen to occur with an accompaniment of FPs, although this coincidence may again be a fortuitous one.

Electrical stimulation of the tentacle tips was employed in an attempt to clarify these questions. If a tentacle tip is given a shock just above threshold strength the usual

response is for a few nematocysts to discharge, causing the tentacle to adhere to the electrode. The tentacle then shortens, pulling itself off the electrode. The hypostome may show a slight, unilateral flexion, which brings it over to the stimulated side, and there may be slight movements of other tentacles. At any time from about 5 to 25 sec. following a shock FPs may be recorded from the hydranth (Fig. 5A). However, they may not appear until after the muscular response has started or they may not appear at all. This supports the conclusion that FP initiation is essentially independent of the muscular movements seen in the hydranth. The long latency of the response might

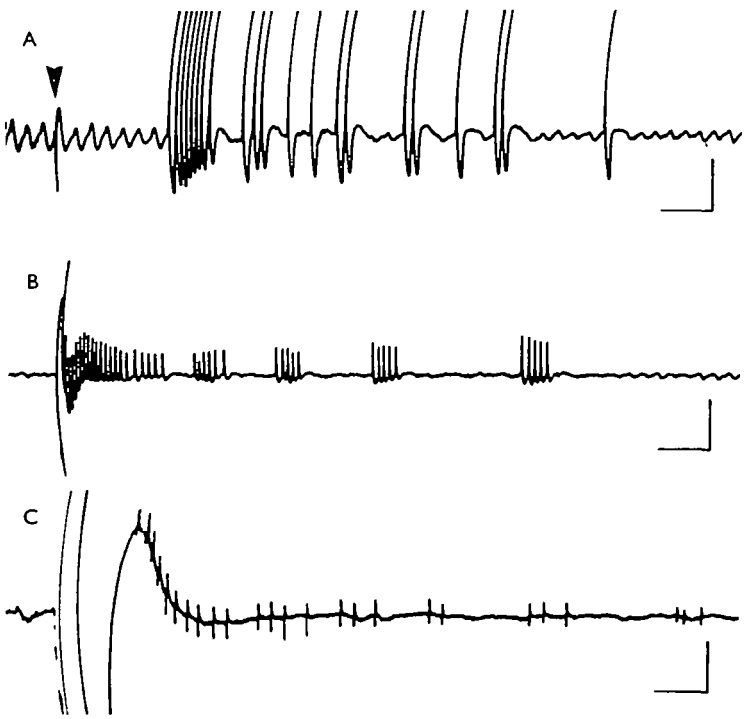

Fig. 5. FPs evoked by threshold stimulation of a tentacle (A) and of the hydranth wall (B, C). The three records are from the same hydranth and are recorded from the same electrode whose position was not changed. The shock artifact is indicated by the arrow head in A, and occurs at a corresponding level in B and C. In A the potentials begin 22 sec. after the stimulus and are of the large monophasic sort. No hydranth shortening occurred. In B potentials begin within 1 sec., are smaller but otherwise similar to those in A. Slight hydranth shortening occurred, lasting less than 1 min. In C the first potential shown comes after 5–6 sec. but some earlier ones may have been lost in the shock artifact. The potentials are of the small biphasic sort associated with a strongly contracted hydranth. Contraction here lasted 1–2 min. The pulse sequence eventually developed into the pattern shown in Fig. 10. Horizontal bars 10, 10 and 4 sec.; vertical bars 400, 600 and 400 μV.

suggest indirect activation of the FP system by some chemical mediator released at the site of stimulation and diffusing through the water. Burnett, Davidson & Wiernick (1963) give evidence that a feeding hormone is released when the nematocysts of *Hydra* discharge.

Stronger stimulation of the tentacles or direct stimulation of the hydranth in the tentacular region or hypostome will evoke a different response. Symmetrical shortening

of the whole hydranth and tentacles is exhibited in some degree, and FPs are elicited, immediately or within a few seconds, in the form of bursts of high frequency and of long duration (Fig. 5B and C). If the hydranth contracts markedly, the FPs are small and biphasic at first, becoming larger and monophasic as the response proceeds. Where we see an immediate response to stimulation as in Fig. 5B, it seems likely that the shock is directly activating an FP pacemaker.

Evidence for an FP pacemaker system is not hard to assemble. The series of pulses evoked by electrical stimulation characteristically shows a high initial periodicity, with the pulses separated by 0·5–0·6 sec. (Fig. 2C). The intervals between pulses gradually lengthen and the pattern breaks up into bursts of two to five pulses coming 0·8–1·5 sec. apart (Fig. 2B). The bursts become less frequent, with fewer pulses, further apart, and the pattern terminates in a few single pulses or pulse pairs. Pulse pairs or triplets may occur at any time (Figs. 1, 4, 10) and they typically show an inter-pulse interval of between 1·5 and 2·0 sec., this representing the 'pure' FP periodicity referred to below (p. 395).

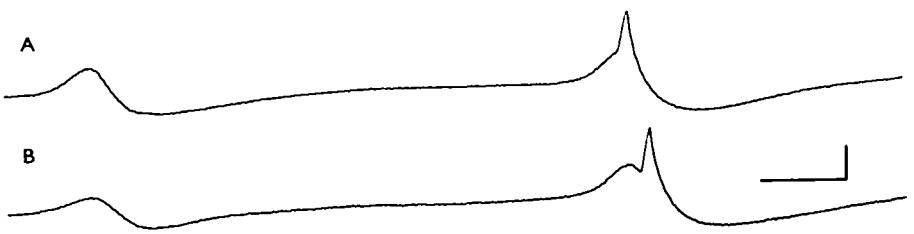

Fig. 6. Two recordings made a few seconds apart without change in the conditions. An SP appears alone on each line followed by an SP which 'spikes', producing an FP at its apex (A) or on its descending slope (B). Such a composite event is called an S/FP. Horizontal bar 500 msec; vertical bar 300 μV.

Josephson (1961) found that pinching a hydranth with forceps evoked a burst of JPs while gentler stimulation of the hydranth had no effect. This experiment has not been repeated. However, JPs were successfully evoked by strong shocks delivered to the wall of the hydranth. Stimulation of the tentacles and weak stimulation of the hydranth wall usually evoked FPs without evoking JPs. These results have not been quantified, but they seem to indicate a threshold difference for evocation of the two pulse types.

Interaction between SPs and FPs

During any period when both SPs and FPs are in evidence, the FPs tend to come at times specifically related to the SP rhythm; they arise from the negative peak of the SP or shortly thereafter (Fig. 6). The composite event is called a 'slow-based fast pulse', or S/FP. This occurs even when SP activity is exhibited at minimal visible amplitude (Fig. 4). Even when SPs are invisible in the record their presence can sometimes be inferred from the intervals between FPs.

The occurrence of an S/FP can modify the SP pattern both in amplitude and in rhythmic relationships. Following an S/FP, the next SP is usually of diminished amplitude or distorted form and, in extreme cases (Fig. 7), the SPs may disappear completely for short periods before re-emerging and building up to discernible

amplitudes again. In records where the SPs following an S/FP are clearly shown, even if small, it can usually be demonstrated that the SP rhythm has been 'reset' by the S/FP event; the first SP in the series following the S/FP occurs sooner than it would have done if the rhythm had continued unmodified. This is shown in Fig. 8, and the legend to this figure gives the details. The 'reset' value (time by which the rhythm is advanced) varies from 0 to 1·2 sec. in the records measured. This variation in reset value is hard to account for. If the FP comes late on its SP base (as in the third S/FP of Fig. 8) the reset value is usually low, but if this were consistently true the reset value should be lower after the first S/FP shown than after the second, whereas the reverse is true.

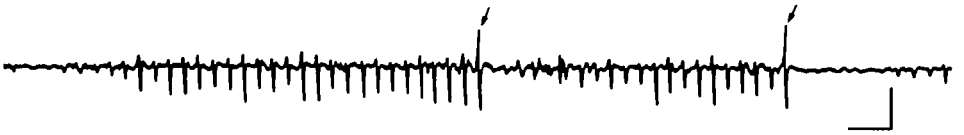

Fig. 7. SP activity seen in a recently fed hydranth. Each SP series is interrupted when there is an S/FP event (arrows), A new series of SPs commences at low amplitude after a short period. The SPs in this record are unusual in having a disproportionately large positive component. Horizontal bar 10 sec.; vertical bar 400 μV.

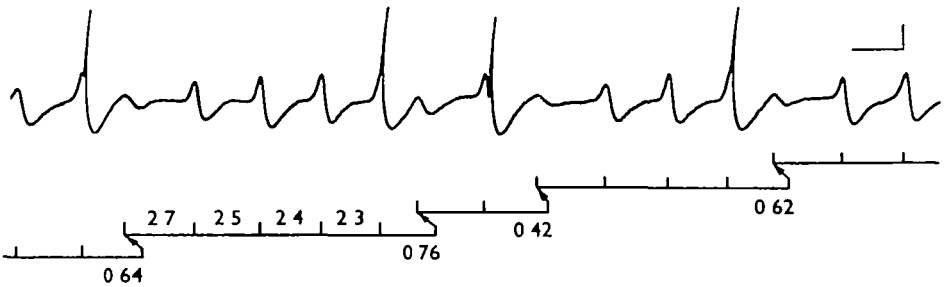

Fig. 8. Interaction between FPs and SPs. The FPs occur on the negative peaks of the SPs or soon thereafter, the composite event being an S/FP. Each time there is an S/FP the SP, rhythm is advanced by a fraction of a second ('reset value') indicated numerically by the lower set of figures and graphically by the arrow pointing obliquely up and back to a new point which represents the start of the new SP series. The shaft of the arrow starts from the point at which an SP would have occurred in the old series. At the same time, the SPs immediately following resetting are separated by intervals longer than the 2·3 sec. which is normal for the preparation. These intervals (in seconds) are given in the upper set of figures for one sequence. Horizontal bar 2 sec.; vertical bar 250 μV.

Figure 8 illustrates another feature of the interaction, namely the compensatory lengthening of the intervals between SPs following resetting of the rhythm. The SP system fires at abnormally long intervals for the first three or four SPs after resetting, and these lengthened intervals compensate for the displacement of the rhythm occasioned by resetting in the first instance. This is shown graphically in Fig. 9. It can be calculated that the average time gained by resetting (570 msec in these measurements) is progressively lost again over the next four SPs which occur after intervals longer than the normal, to a cumulative total of 620 msec. This represents, in fact, a slight overcompensation. Since this compensatory adjustment is achieved progressively over SPs 2–5, it follows that the occurrence of an S/FP in place of any of these four

pulses will reduce the possibility of full compensation being achieved. If SP 3 were an S/FP, for instance, only 350 msec. would have been made good toward the 570 msec. displacement, and the difference of 220 msec. would represent a permanent increment toward the displacement of the SP rhythm.

Analysis of records from several different hydranths reveals an interesting fact about the interval following an S/FP. This interval approximates quite closely to the normal interval between FP pairs, the 'pure' FP periodicity of 1·5–2·0 sec. referred to above (p. 393). Crudely expressed, it seems as if the FP system, having been excited to produce a pulse, will have a 'tendency' to fire again after 1·5–2·0 sec.; if no second FP

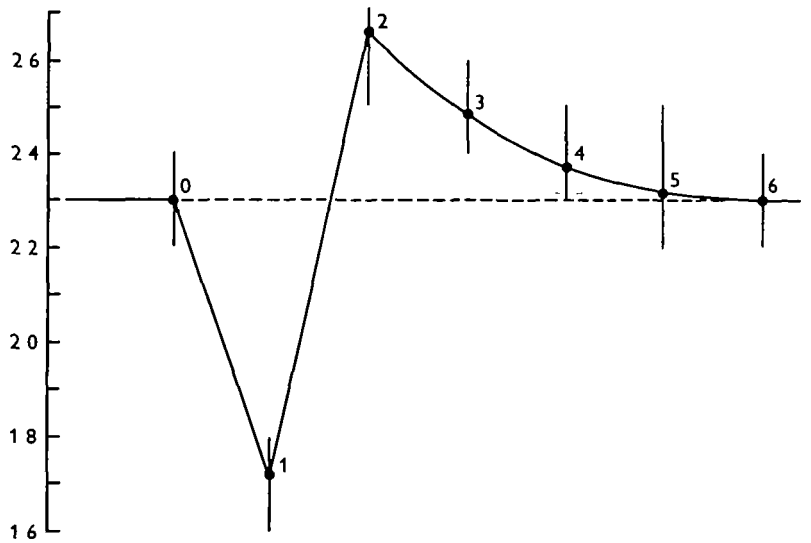

Fig. 9. Curve showing modification of the SP rhythm following an S/FP event (O). The numbers 1–6 represent intervals preceding SP 1–SP 6, the SPs following the S/FP. Scale at left shows intervals in seconds. Following the S/FP, the inter-pulse interval drops from 2·3 sec. (normal for the pure SP pattern in this preparation) to below 1·8, rising sharply to over 2·6, then declining slowly to 2 3 again. The curve passes through mean values obtained from ten pulse sequences selected from the record of which Fig. 8 forms apart.

results, the 'tendency' is still manifest in the premature production of the next SP, which comes when the second FP 'should' have come. If we are correct in assuming that the SP and FP pacemaker systems are not merely the different expressions of a single pacemaker system, it must follow that the two systems are intimately coupled, each affecting the output of the other. The rhythmic modulations illustrated in Fig. 9 are to be viewed as a manifestation of competitive interaction between the two pacemakers, one of them tending to produce FPs every 1·5–2·0 sec. and the other to produce SPs every 2·3 sec. Where the latter system firing alone is capable of a sustained, metronomic regularity, the former normally exhibits a rather broad range of output frequencies. This may go some way toward explaining the variations in reset values commented on earlier (p. 394).

The conditions under which SP-S/FP patterns are shown are not exactly understood. Hydranths that have fed to repletion and have reduced the food to a semi-fluid consistency are most likely to show the pattern, but some such hydranths inexplicably fail

to show any electrical activity, show a simple SP rhythm or develop SP-S/FP activity only after they have been electrically stimulated. Figure 10 is an example in the latter category. Varying pulse patterns are shown from a record lasting over 30 min. from the time of stimulation. Where S/FP pairs are occurring around the 14 min. mark it can be seen that the following SP comes at the time a third S/FP might have occurred,

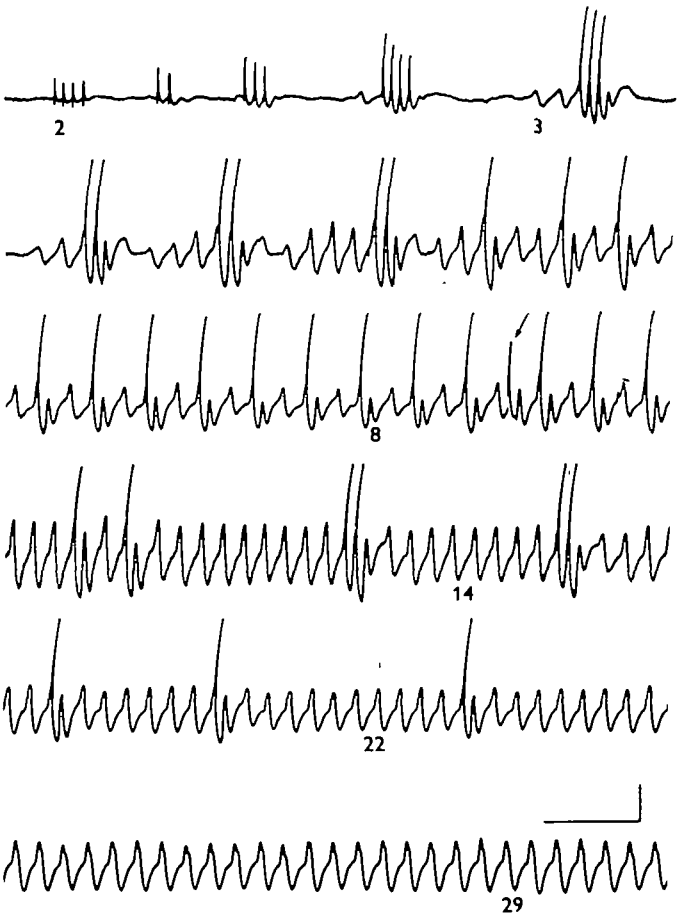

Fig. 10. Pulse pattern recorded following electrical stimulation of a hydranth fed 2 hr previously. The numbers represent minutes following the electrical stimulus. The second line follows directly on from the first but the others are separated by time gaps of varying durations. The initial, interrupted-burst pattern of small biphasic FPs, whose origin is shown in Fig. 5 C, develops into bursts of large, monophasic FPs which, after 3 min., start to become integrated with the emerging SP rhythm. S/FP activity is at its height around the 8 min. mark. The arrow here shows the only single FP in the entire sequence which was not based on an SP. S/FPs subsequently become less frequent and finally only the SP rhythm is left (bottom line). Horizontal bar 10 sec.; vertical bar 400 μV.

illustrating the point made in the preceding paragraph. The transition between biphasic and monophasic FP forms is also seen in the first line as well as their integration into, and progressive domination by, the emerging SP rhythm around the 3 min. mark and thereafter.

Pulse patterns associated with tidal exchange

Fulton (1963*a*) described the peristaltic activity of *Cordylophora*, a process which serves to mix and distribute the products of digestion throughout the colony. A regular tidal ebb and flow results, with a pumping of fluids down into the stem and their passage back again into the hydranth.

Some hydranths exhibiting normal tidal exchange showed no electrical activity at all. Others showed slight amplitude changes in the SP rhythm. Those performing S/FP patterns, as described in the last section, showed marked changes in both amplitude and frequency of the pulses during the cycle. Figure 11 illustrates such a case. Where such changes were shown, the pattern was always the same: an abrupt decline in pulse amplitude at the onset of tidal ebb (shown by the inverted arrows), coinciding

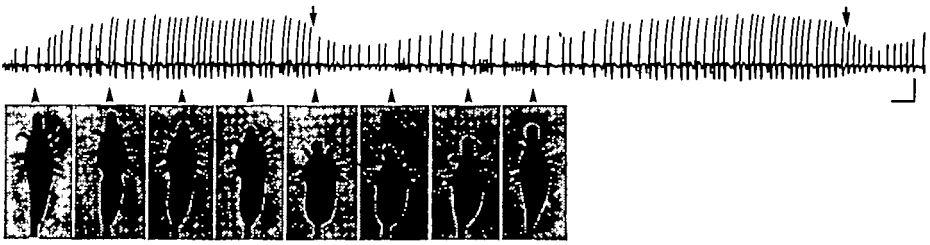

Fig. 11. Amplitude and frequency changes in S/FP activity of a hydranth showing tidal exchange with the rest of the colony. The photographs, taken one minute apart, show the changing silhouette of the hydranth over the cycle. The recording electrode can be seen projecting to the right of each picture. The inverted arrows above the electrical record show the points corresponding to the observed onset of tidal ebb (expulsion of fluids), the most clearly recognizable feature in the cycle. Horizontal bar 20 sec.; vertical bar 400 μV.

with the development of contraction in the endodermal circular muscles of the hydranth. As the hydranth fills (tidal flow), pulse amplitude rises again. The second photograph in the series shows a filling hydranth. The distal end swells up before the proximal, recalling the sequence in *Tubularia* (Josephson & Mackie, 1965). In the record shown, but not in all such cases, S/FP frequency was highest in the full hydranth, lowest in the empty. At the very start of the record and halfway through it, coinciding with 'low tide', the S/FPs are sufficiently infrequent for pure SP activity to emerge at conspicuous pulse amplitude in between. There are no marked changes in SP frequency during the tidal cycle.

Hydranths whose cavities are continuous via the stem show, as Fulton found, a rough synchrony in their tidal cycles. There is no synchrony in or evidence of interaction between the pulses themselves, but the onset of tidal ebb, signalled by observable fluid movement and by the dip in amplitude on the electrical record, tends to occur at about the same time in the whole interconnected group. Occasionally some hydranths are observed to be completely out of phase or to exhibit an independent rhythmicity. It is quite common for adjacent hydranths to be out of phase by about a minute over, say, a 7 min. cycle. It is likely that such synchrony as exists is achieved through the direct responses of individual hydranths to the flow of fluids and the accompanying pressure changes rather than through the transmission of impulses in the tissue of the stem. There is no reason to suspect the existence of a special 'triggering system' such as mediates communication between hydranth pacemakers in *Tubularia* colonies (Josephon, 1965).

Very fast pulses (VFPs, Fig. 12)

These pulses have only rarely been observed and there is not enough information on them yet to justify more than a brief description here.

So far they have been recorded only from hydranths distended with food in an advanced stage of digestion. The areas wherein they are propagated and their conduction velocity are unknown. VFPs are monophasic negative spikes (Fig. 12C) reaching amplitudes of 100 μV. with a rising phase lasting 1–2 msec. It has not proved possible to associate them with any observed movements, nor has it been possible to evoke them by tactile, electrical or photic stimulation.

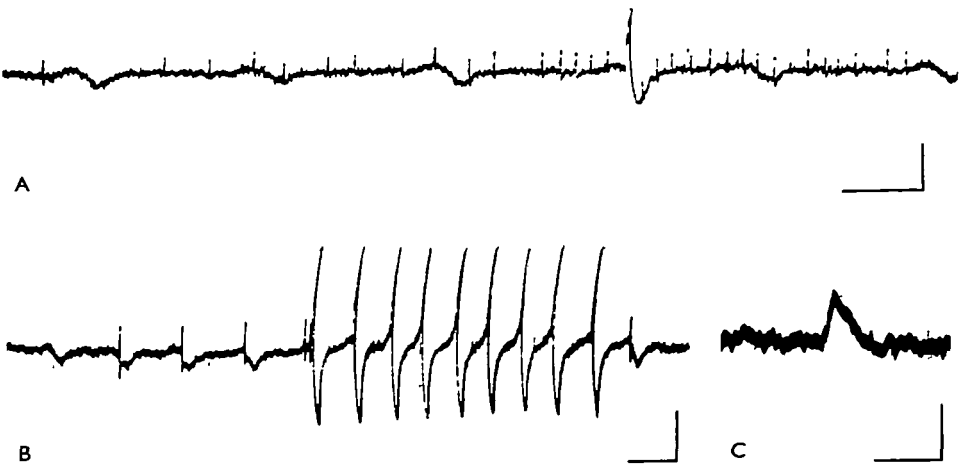

Fig. 12. Very fast pulses. In A a burst of VFPs is shown against a conventional SP-S/FP background. In B composite S/VFP events are shown, the series being interrupted by a pure FP burst (the large pulses). C shows VFP pulse form as seen on the oscilloscope. Horizontal bars 1 sec., 2 sec., 10 msec., vertical bars 200, 200 and 100 μV. respectively.

They appear singly or in bursts of up to 35 pulses. Pulse frequency is most commonly of the order of 1–3/sec., but in some cases the frequency increases during the burst reaching about 5/sec., as in Fig. 12A. This record also shows the remarkable and apparently typical lack of relationship between the VFP pattern and the SP and S/FP activity going on in the background. However, in one record (Fig. 12B) over a period of 8 min. VFPs appeared several times in association with the negative component of SPs the composite event (an S/VFP) being analogous to an S/FP event. The VFP burst pattern has not been found to show any correlation with pure FP patterns and appears to represent the output of an independent pacemaker system.

Effects of tetrodotoxin

JPs, SPs, FPs and VFPs have been observed to occur in *Cordylophora* more than 1 hr after addition of tetrodotoxin to 10^{-5} g./ml. The drug seems to produce no alteration in the normal electrical activity or in the behaviour of the animal. Two samples of the drug were used in separate experiments with the same effect. One sample was assayed by injection into the caudal vein of mice and was found to be immediately lethal at 10 μg./kg. body weight. This would be equivalent to a concentration of about $2{\cdot}5 \times 10^{-7}$ g./ml. of blood, and indicates full toxicity of the sample.

Tetrodotoxin blocks action potentials in squid axon by preventing Na^+ influx. Where transmission is not blocked, as in some crustacean cells, it appears that excitability depends upon Ca^{2+} rather than upon Na^+. In view of continuing uncertainty about the exact mode of action of the drug it would be premature to speculate about the significance of these findings in a coelenterate.

DISCUSSION

Attempts to assign the various recorded signals to specific cellular pacemakers and conducting elements have met with only limited success in the hydrozoans so far studied and *Cordylophora* is no exception. Epithelial conduction occurs in certain hydromedusae and siphonophores (Mackie, 1965; Mackie & Passano, in press); there is no evidence for epithelial pacemakers in these forms, and the pacemakers are probably nerve cells. Contraction pulses in *Hydra* are epithelial events (Josephson & Macklin, 1967) but they might be initiated by and even conducted in nerve cells, although this is still uncertain. In *Cordylophora* there is a diffuse, ectodermal plexus of nerve cells in the hydranth wall and tentacles but the most recent evidence indicates an absence of nervous tissue from the stem (Jha & Mackie, 1967). JPs, which can be evoked in the stem and are conducted in it, would therefore appear to be purely epithelial events. The other electrical events recorded from *Cordylophora* might involve nerves either in the initiation phase or in transmission, or both.

The relationship of the electrical patterns to behaviour is very puzzling in *Cordylophora*. SPs invariably, VFPs where they have been observed and FPs for much of the time are exhibited without detectable behavioural concomitants. Conversely, the animal can perform appetitive feeding behaviour, can respond to electrical stimulation of the tentacles by local tentacular and hydranth movements, can ingest food and can perform peristalsis without detectable electrical accompaniments. It is reasonable to infer that much if not all of the nervous activity in the animal is inaccessible to direct recording at the present time. The electrical correlates of muscular contraction also go undetected in the responses listed above. However, overall hydranth shortening and tentacle contraction are performed to the accompaniment either of JPs or of FPs depending either on the site of stimulation or, from the incomplete evidence now available, on its strength. Unlike the local movements listed above, these overall contractions have not been seen to occur without one or other type of pulse. If FPs and JPs are to be seen in this context as muscle-excitor pulses (and it is not strictly necessary to assume this) it would appear that they can excite the same muscle effector units in similar ways, for the overall response is fairly comparable in the two cases.

It is hard to treat the SPs as a component of the behavioural machine in the usual sense. They have no known motor correlates and are not evoked by electrical stimulation. They might have some metabolic significance remote from neuro-muscular activity, perhaps in relation to digestion or ionic regulation, for they reach their highest amplitudes a few hours after feeding, diminishing again after the digested food products have been dispersed. The SP rhythm itself is unlike anything seen in other hydroids although it bears a superficial resemblance to a certain pulse pattern observed in the velum of hydromedusae. This pattern is interpreted as consisting of a series of local depolarizations evoked in the myo-epithelial layer by pacemakers in the marginal

nerve rings (Mackie & Passano, in press). These local events alternate with propagated depolarizations of larger amplitude which are also driven by the marginal pacemakers. By analogy with this system the SPs might be regarded as pacemaker-induced local epithelial events with S/FPs as propagated events involving the same tissue elements. However, SPs can be recorded at remote points of a hydranth at the same amplitude, and are therefore no more 'local' than are the S/FPs. FPs show their own intrinsic rhythmicity which is readily distinguishable from that of the SP system, suggesting different pacemakers for the two systems. At the present time it seems best to assume that SPs and FPs are products of distinct systems although they are capable of interacting in a very obvious way.

SUMMARY

1. *Cordylophora* has two major hydranth pacemaker systems, one producing slow, biphasic pulses at 2·0–4·0 sec. intervals (slow pulse or SP system) and one exhibiting sharp, predominantly negative potentials singly or in bursts with a normal interval of 1·5–2·0 sec. between pulses (fast pulses, FPs).

2. Both SPs and FPs are recorded throughout the hydranth, but do not spread to and are not co-ordinated in adjacent hydranths.

3. SPs could not consistently be evoked artificially and showed no clear behavioural correlates. FPs occur during feeding and following electrical stimulation, where they may accompany a muscle response.

4. FPs appear superimposed on SPs when both systems are active. Such a composite event advances the SP rhythm by an amount close to the normal FP inter-pulse time, but compensatory adjustment of the SP rhythm then occurs.

5. Treatment with 10^{-5} g./ml. tetrodotoxin has no apparent effect on *Cordylophora*.

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