

NEUROMUSCULAR ACTIVITY IN THE SWIMMING SEA ANEMONE, *STOMPHIA COCCINEA* (MULLER)*

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INTRODUCTION

The spontaneous activity and responses to electric stimulation of actinians have been intensively studied (e.g. Pantin, 1935 *a, b, c, d*; Ross, 1952, 1957 *a, b*; Horridge, 1958) for the light which they might throw on the functioning of the 'elementary nervous system', i.e. a nervous network lacking the physical concentration of neurones found in ganglia and brains. It seems desirable to add to this knowledge data from the swimming sea anemone *Stomphia coccinea*. This animal, although not yet well known, is evidently widely distributed, having been recorded from Millport, in the Clyde, Scotland (Stephenson, 1935), and from President Channel, Puget Sound, Washington, U.S.A. (Yentsch & Pierce, 1955). It should be pointed out, however, that the species identity of animals from the two regions has yet to be established with certainty. Stephenson (1935) noted that *Stomphia* is capable of becoming curiously active (for a sea anemone) twitching its 'tail' and 'jumping'. Similar activities can be elicited from the Puget Sound specimens by two species of starfish from the same region (although not the same habitat), *Dermasterias imbricata* and *Hippasteria spirosa* (Yentsch & Pierce, 1955; Sund, 1958).

Details of the swimming activity have been described by Sund (1958). Since the species reacts to the starfish named, by giving a set of distinct reactions which are usually executed in the same sequence, it can be said to show a high level of integrated activity, possibly higher than any known in other actinians. The following distinct parts of the response can usually be observed, in the following sequence. The division and resulting emphasis is based on my own observations, but closely follows Sund's description:

- (1) Elongation, with uptake of water.
- (2) Increase in turgidity with expansion of oral disk.
- (3) Waving of whole oral disk, usually in a circular motion.
- (4) Release from substratum by extrusion of centre of basal disk and withdrawal of edges.
- (5) Alternate and rotating sequence of quick flexions causing 'swimming'.

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In addition to these complex reactions, there are almost certainly other, slower, forms of behaviour, which might be equally complex and meaningful. The reactions of the animals to starfish were analysed closely by Sund (1958) and by Ward (1958), who have shown that the elicitation of swimming depends upon a chemical substance exuded by the starfish. Sund found that swimming can also be elicited by electric shocks, and this has been further studied by Wilson (1959). The nature of responses to electric stimulation has not, otherwise, been investigated. Sund found that a single stimulus was not followed by a response, but that a contraction occurred in response to a second shock delivered 1 sec. after the first. He does not give sufficient details to decide exactly which muscles were responding. Wilson (1959) also found responses occurring only to the second shock and subsequent, closely spaced stimuli. He found that in the eighteen animals he examined, most of them gave swimming responses, comparable to those obtained by presenting *Dermasterias*, when *precisely* eight electric shocks were applied to the base of the body wall. This quite remarkable finding demands further investigation. The total number of shocks was much more important than other parameters such as stimulus strength (no effect), duration (no effect) and in particular the interval (frequency) which could be varied between 0.25 and 2 sec. without affecting the response. Sund (1958), who had also given eight shocks as the approximate number required to evoke swimming, still obtained the response at intervals of only 0.12 sec., with a total of eight shocks. Wilson states that at the higher frequencies (above four per sec.) more than eight shocks are required and that at intervals longer than 3 sec. the response does not occur.

Wilson also studied the effect of applying what he called the 'subthreshold' number of seven stimuli, which he repeated at intervals. He found that seven stimuli became adequate to evoke swimming after a single (unsuccessful) trial of seven. The initial stimulus he referred to as 'conditioning' and the subsequent ones as 'test' stimuli. The latter could be applied as long as 1 week after the first and still be effective. Wilson proposed to call this phenomenon 'long-term facilitation'. Subsequent to the 'conditioning' experiment, in which an animal came to respond to seven shocks, it could now be made to respond to six shocks by the same procedure, namely a single application of six shocks.

Some of the animals responded to a single contact with *Dermasterias*, others did not. The latter would, however, react to a smaller number of electric stimuli than usual (i.e. five, six or seven instead of the usual eight) by swimming if the shocks were applied soon after the starfish contact. Once the anemones had then reacted to this mixed stimulation they always subsequently responded to a single contact with the starfish. Wilson argued that there had been a long-lasting decrease in 'threshold' of the response. In looking for a site of the change he argued that since unlike stimuli (chemical, from the starfish, and electrical) effectively summed, the myoneural junction might be the site of a facilitation process giving the over-all appearance of a lowering of 'threshold' (= no. of shocks required to elicit) of the complex swimming response.

It is difficult to see the logic in this argument. Does it mean that the smaller

number of stimuli which cause swimming after 'conditioning' were eliciting the response from the nervous system all along but weakly so that the muscles did not respond? Why should an additional stimulus increase their intensity? Or does it fire a trigger mechanism? How could such a long-lasting 'facilitation' come about physiologically anyway? Although such questions cannot be answered, the major premiss, namely that there is a long-lasting facilitation at the level of the neuromuscular junction, can readily be tested provided an effective nerve-muscle preparation can be made of the responding muscles. The present work was undertaken principally with a view to finding out if the state of 'facilitation' in the excitation/response pathways using electric stimuli could be shown to undergo 'long-term facilitation'. At the same time a number of other observations were made on the animals, and these are reported here. It was not found possible to confirm the observations of Wilson, and many observations contrary to his views were made.

Animals were allowed to remain on their host shells (*Modiolus modiolus*, a mussel) or else they were induced to swim away by touching them with *Dermasterias* and then made to settle on paraffin wax in glass dishes. Once settled, they were pinned to the wax by a number of small entomological pins pushed through the margin of the basal disk. Animals prepared in this way would live quite well for up to 2 weeks, after which they were released. For recording, hooks made from entomological pins were passed through the body wall under the oral sphincter muscle and attached by linen threads to lightly weighted isotonic levers. They were kept in aerated, fresh sea water which was changed every few hours. Electric stimuli were applied through glass tubes with sea water applied to the surface of the animal, and containing thick chloride-coated silver wire. The stimuli were direct current pulses derived directly from Grass S 4 electronic stimulators and delivered at constant voltage. The stimulus duration was usually 0.5 msec., or from 0.1 msec. up to a maximum of 5 msec. The temperature of the water rose gradually from about 12° C. (slightly more on hot days) to 16° C., when it was changed. Animals of various sizes were tested, the smallest being 2 cm. in diameter at the basal disk. The largest one available had a disk diameter of about 8 cm. at the time of testing, and its length reached 10 cm.

RESULTS

SPONTANEOUS ACTIVITY

Time-lapse films of *Stomphia* made by Sund and Dr Dixie-Lee Ray show that the anemones are incessantly active, extending, contracting, bending, swaying, swirling, rippling and twisting in a great variety of motions, all of which are executed very slowly. Previous experience with other animals exhibiting 'spontaneous' activity (ascidians—Hoyle, 1952) had made me appreciate the need for taking into account the fluctuating states of an animal if one is to obtain consistent results when studying stimulus/response patterns in the whole animal. Accordingly, recordings were made of the spontaneous activity patterns of most of the animals which were tested electrically.

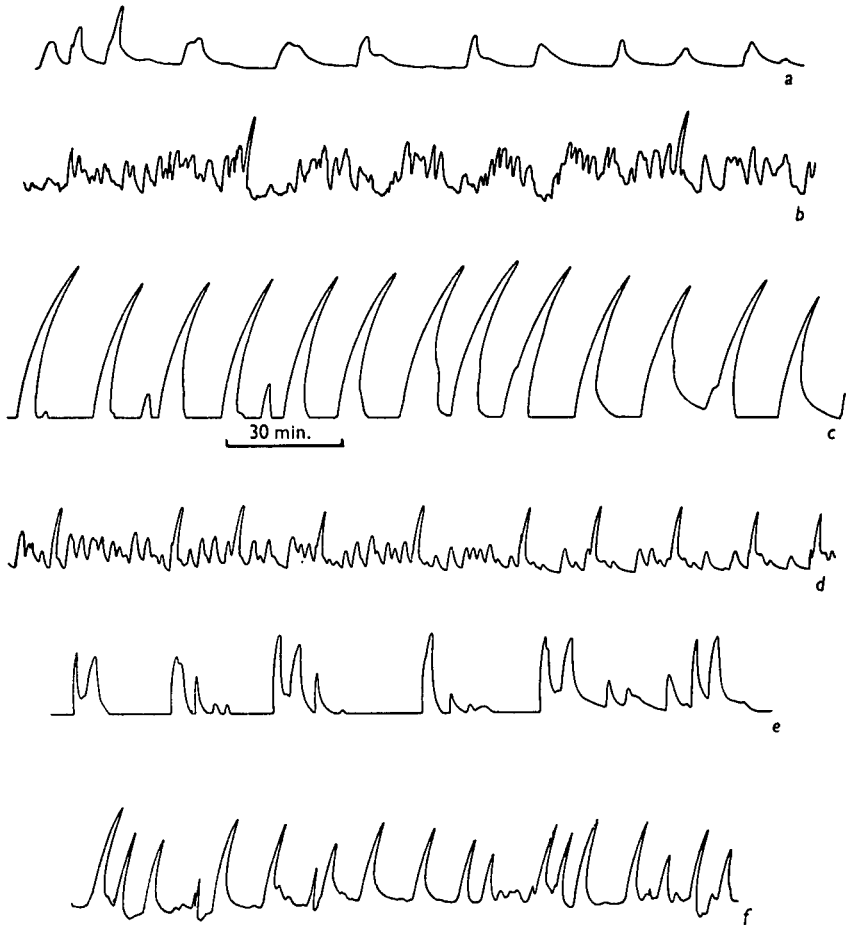


Fig. 1. Examples of spontaneous activity recorded from six different specimens of *Stomphia coccinea*.

In the tanks in which the *Stomphia* were kept, in running freshly-pumped sea water, it was evident that several of the specimens tended to be slightly shrunken during the day and expanded at night. There was, however, little evidence of a marked nocturnal/diurnal rhythm such as was recorded by Batham & Pantin (1950) in *Metridium* in the kymograph records of *Stomphia*. The specimens were equally active both during the day and at night, with only a slight extension occurring in the dark (i.e. at night). They were not given any food during the testing period. A rather bewildering variety of records was obtained, some examples of which are illustrated in Figs. 1 and 2. Some specimens gave an irregular pattern of slow movement, not involving any powerful contractions. Others gave, at fairly regular intervals, slow complete retractions always followed by a gradual extension to the fully expanded position. In the intervals between large contractions there were smaller movements due partly to bending, partly to contractions of the circular muscles.

One feature which was observed more than once, in different animals, was a full retraction followed by full extension and then a very gradual shortening caused by relaxation of the circular muscles, the whole process taking about half an hour. This movement would be repeated after about 2 hr. and again after a further 2-3 hr. (Fig. 2). An attempt was made to locate the origin of the spontaneous activity. Animals were anaesthetized in magnesium chloride solution and cut into rings; the anterior body wall including the oral sphincter and tentacles was prepared, and also rings from the basal region. These were allowed to relax in running sea water and then attached to kymograph levers. It was found that rings from both anterior and posterior regions showed marked periodic activity (Fig. 3). That from

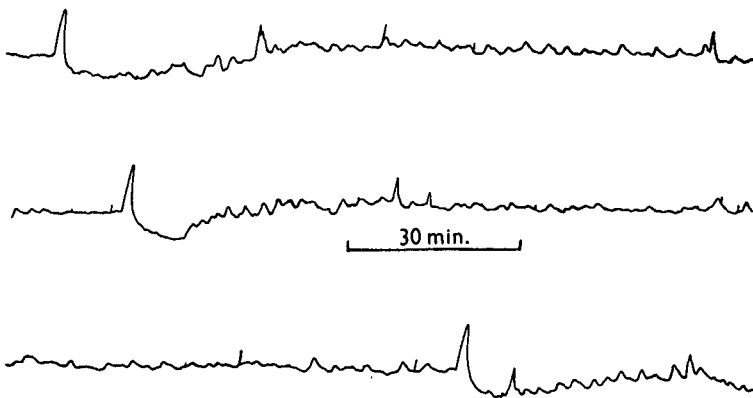


Fig. 2. Repeating pattern of slow activity of a whole animal not accompanied by strong periodic contractions.

the tentacles and sphincter region was never very regular, and contained a number of small movements as well as larger, very slow, contractions. By contrast, the activity of basal rings was sometimes extremely regular, tending to repeat itself at intervals of about half an hour (Fig. 3c). This activity, although probably due to circular muscles, is strikingly similar to that observed in one of the whole animals, in which it was due to the parietal muscles.

These observations show that pacemakers capable of initiating muscular contraction are probably present in all parts of the animal. A pacemaker with a period of discharge of about half an hour is located in the basal region of the body wall.

These results recall those of Batham & Pantin (1954) obtained by recording from rings of body-wall of *Metridium*. In their experiments they went further than in the present ones, recording at the same time from four rings cut in the sphincter, sub-sphincter, mid-column and pedal regions, having a strip of body wall remaining to connect them together. Spontaneous, recurring slow contractions were found in all rings, particularly the pedal. There was only a very limited co-ordination of the contractions, showing the presence of pacemakers in each ring.

If any generalization can be made from these observations it is that each animal behaves differently from all others in detail, but that there is a marked tendency in

all of them for certain movements to be repeated at fairly regular intervals, i.e. there is an inherent rhythmicity of action, comparable to and sometimes much more regular than that found in *Metridium*.

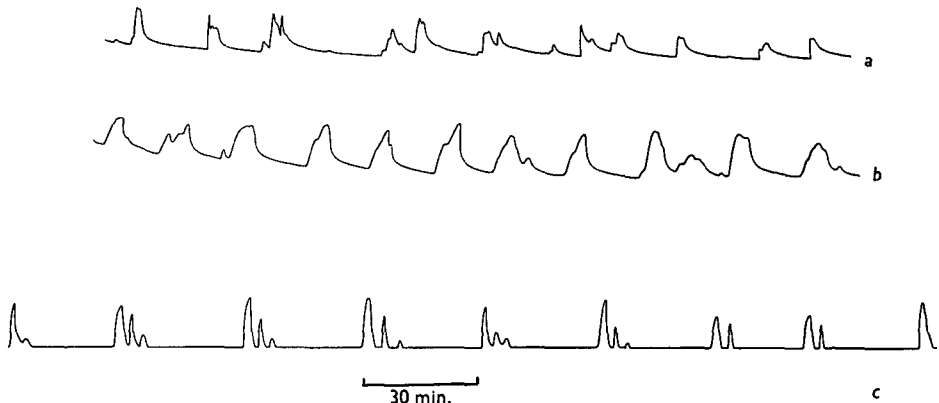


Fig 3. Spontaneous activity of isolated rings of body wall. *a*, Oral disk region; *b*, *c*, basal disk region. *a* and *b* were from the same animal. Note the very regular periodicity in *c*.

RESPONSES TO ELECTRIC SHOCKS

All the major muscle fields (Batham & Pantin's usage, 1951) found in sea anemones are well represented in *Stomphia*, and its parieto-basilar muscles are much more powerful than those of most others. They are the muscles responsible for the 'swimming' action. Since the animal is a fluid-filled sac, contractions in any of the muscles may be expected to produce a distortion which will be registered by the recording levers. Hence it is particularly desirable to confine the stimulation so that only one type of muscle responds.

The muscles present are: longitudinal retractors, parietals, parieto-basilar, circular muscles of the column, radial muscles and the oral sphincter. A diagram of their distribution is given in Fig. 4. The distinct oral sphincter can be isolated and studied independently, as was done by Ross (1957*a*) in *Calliactis*, but the responses of the other muscles (except the radial) can be studied only indirectly, in the whole animal, in which case it is impossible to be certain that only one type is contributing to the record. Nevertheless, a fairly complete effective separation is possible.

To obtain results of reasonable consistency from any muscle in the intact animal, the tests should be made only during the periods of relative quiescence which occur between major retractions. Even then, very considerable variations are encountered in threshold, magnitude of response and rate of relaxation.

(1). *Parieto-basilar muscles*. The reactions of these muscles cannot be distinguished with any degree of certainty from those of the parietals, but in other sea anemones in which the probable response of the parietal muscles is known it consists only of a slow contraction followed by a very slow relaxation. The magnitude is increased by increasing the number of stimuli applied, but the rate of rise

is not greatly affected. In *Stomphia* the parieto-basilar are well developed and there is a very quick, powerful contraction of the parietal group which is probably exclusively determined by the parieto-basilar. This response can therefore be separated from that which the two sets of muscles might show in common, which is a much slower contraction resembling that attributed to the parietal muscles in other sea anemones. There is at present no evidence to decide whether the slow response is the exclusive product of the parietal group, or whether it also involves the parieto-basilar, which would then be dually responsive.

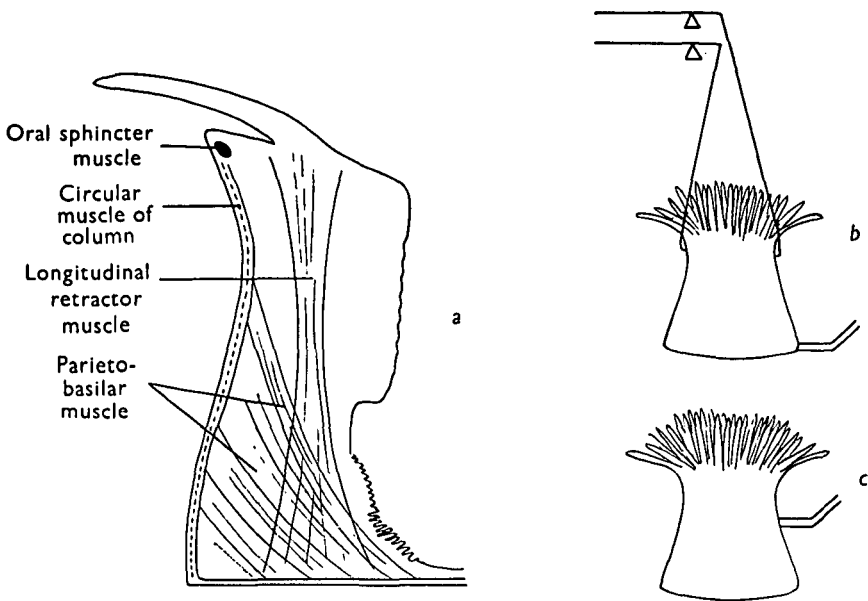


Fig. 4. *a*, Diagram of the principal musculature of *Stomphia* as seen in a perfect mesentery. The parieto-basilar muscles are strongly developed and attached to points high up on the column. *b*, Position of stimulating electrodes for eliciting responses of parieto-basilar muscles. *c*, Position of stimulating electrodes for eliciting responses of circular muscles of the column or of the longitudinal retractors.

A possible comparable quick response has been observed in the South American anemone *Bunodactis*. When this animal is touched lightly on the column a quick contraction occurs on the touched side only (Pantin & Vianna Dias, 1952) which could be due to the parieto-basilar muscles.

Selective fast responses of the parieto-basilar could be obtained by placing the electrodes at the very base of the column. The response obtained, in the fresh animal, is characterized by a contraction occurring locally, in the region under the stimulating electrodes, which will be referred to as ipsilateral (Fig. 5). The contralateral side, if it is affected at all, is either induced to relax slightly (see below) or else it gives a slight movement due to the passive elastic effect of the downwards movement on the other side. If the stimulation is continued, the responding region extends round the animal until the whole set of parieto-basilar muscles tends to contract, to roughly the same extent, with each stimulus (Fig. 13).

The magnitude of the response to a threshold or any constant-strength stimulus is very variable. It may vary in an irregular way, or it may wax and wane with a slow rhythm, if the stimulus is repeated at regular intervals of say 1 per 10 sec. (cf. Fig. 13). Responses may fail to occur for a time, only to return suddenly or gradually. The existence of this great variability makes it seem unlikely that the contractions are due to a direct excitation of the muscle fibres. Furthermore, there is no evidence of a direct excitation of the circular muscles, which lie between the electrodes and the parieto-basilar. Instead, it seems probable that the muscles are excited indirectly, via the local nerve net, the elicited excitation summing with the background activity occurring there.

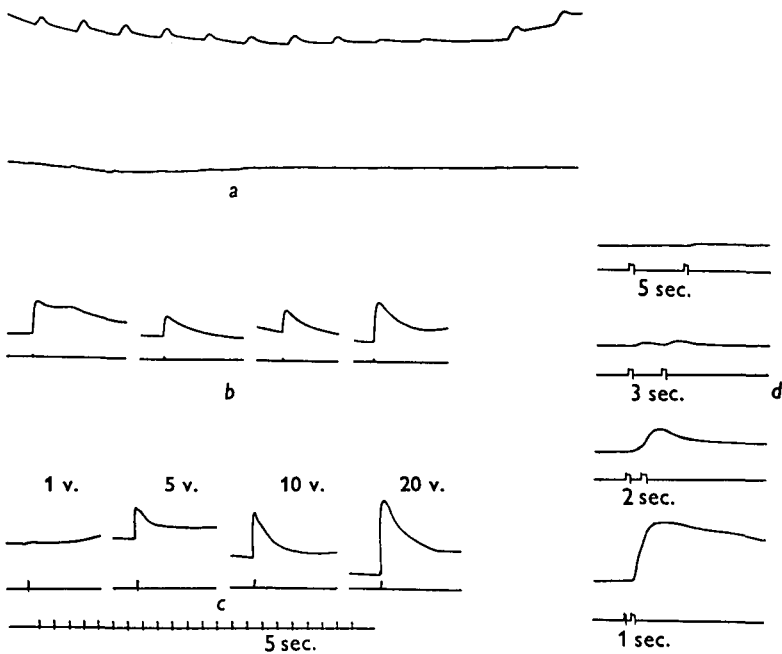


Fig. 5. Quick responses of parieto-basilar muscles. *a*, Constant-voltage stimuli delivered at one per 10 sec. Upper trace—responses of ipsilateral (stimulated) side. Lower trace—responses of contralateral (opposite) side. *b*, Four responses obtained at different times to single shocks at constant voltage all applied at the same site. *c*, Responses to increasing stimulus strength at the approximate voltages indicated. *d*, Facilitation is marked when the interval between a pair of weak shocks is reduced.

When a 'preparation' of the parieto-basilar is responding consistently, it can be shown that the magnitude of the contraction increases with increasing intensity of stimulation (Fig. 5*c*). This may in part be due to an increase in the area contracting, i.e. in the number of mesenteries brought into action. It appears visually also to involve a greater extent of local contraction, but this cannot be stated with certainty. Very strong stimuli appear to cause a small amount of active contraction also, on the contralateral side.

The fastest time required to reach peak contraction of a powerful parieto-basilar response in the intact animal is 0.2 sec. and relaxation takes 2-3 sec., although both times may be up to six times longer.

Possible reciprocal relaxation

The quick contractions of the parieto-basilar, when used in swimming, recur at intervals of only a few seconds, on opposite sides of the body. There is no doubt that a mechanism of reciprocal relaxation of the parietal muscles on opposite sides would be of assistance in ensuring rapid motion. Sund (1958) mentions the possibility of relaxation of the circular muscles on the same side, which would facilitate the ipsilateral bending. In many of the records obtained, both during swimming evoked by starfish and during electrical stimulation of the parieto-basilar, there occur small but sharply defined relaxations of the contralateral side which are almost synchronous with the contractions (Fig. 6). They might have been due,

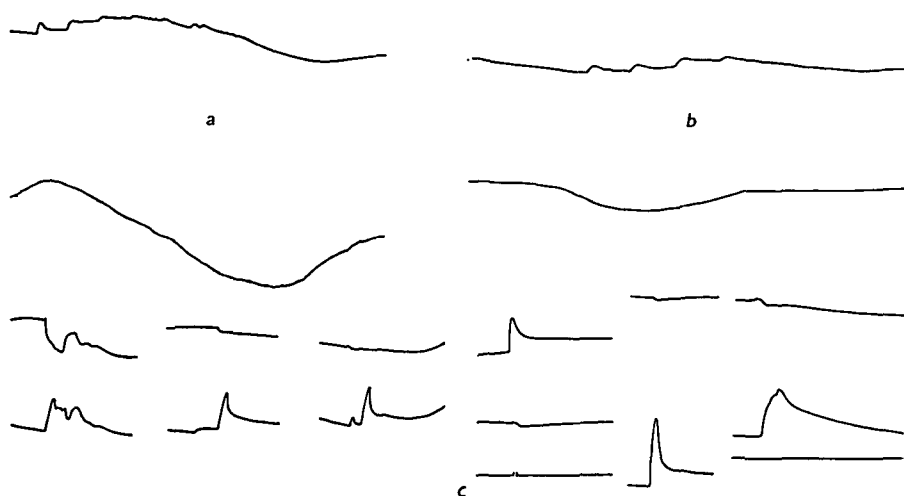


Fig. 6. Reciprocal relaxation in parieto-basilar muscle of non-stimulated side. Large contractions at side of stimulating electrodes. *a, b*, Short series of shocks applied at one per 10 sec; *c*, miscellaneous responses to single shocks.

in some cases, to simple mechanical stretch consequent upon the increased hydrostatic pressure, but they were also visible when the mouth was open and when a glass tube was inserted through the mouth into the gastric cavity. Although by no means conclusive, these results suggest that a mechanism exists whereby the 'tonic' nerve discharge to the 'contralateral' parieto-basilar muscles is inhibited when the 'ipsilateral' ones are excited strongly. The inhibitory process is not likely to be at the level of the neuromuscular junction, because the ipsilateral side can be electrically excited by locally applied electrodes during the relaxation.

Summation and facilitation

Contractions of the parieto-basilar in response to successive excitations summate completely. If the first response was a large contraction, the second and subsequent ones are all much smaller and there is no facilitation. But if the initial contraction was small in magnitude due to a weak shock the response to the second shock is always much larger, showing a facilitation effect. In the less vigorous animals there may even be no response to a single shock, but the second or third gives rise to one. When the interval between two shocks is progressively reduced, a marked growth in facilitation is apparent (Fig. 5*d*).

(2) *Parietal muscles*. Following the application of a train of six or more stimuli through electrodes placed to obtain the parieto-basilar response, there occurs, as the animal relaxes after the quick parieto-basilar contractions, a strong, delayed contraction (Fig. 7). It is similar to that obtained by Pantin (1935*d*) in *Calliactis* and can be attributed to the parietal muscles. It does not occur at frequencies above two per sec., or below about one per 5 sec., being optimal at one per 2 sec.

(3) *Circular muscles of the column*. The best site to place the electrodes in order to obtain responses of the ordinary circular muscles of the column is about one-third of the way down the column, i.e. near the upper attachment of the parieto-basilar. Below this point one is liable to obtain responses of the latter. In this position two or more shocks delivered at intervals of 5 sec. elicit a slow contraction (Fig. 8), starting under the electrode but appearing to affect the whole circumference synchronously. The local contraction may spread down the column causing an appreciable elongation of the animal. There are also occasional small quick responses to the single shocks, and also delayed parieto-basilar 'incipient swimming' responses may appear. Although there may be no direct responses of the parieto-basilar, the nerve supply to the latter is certainly affected by stimulation at the point for optimal response of circular muscles alone.

(4) *Longitudinal retractor muscles*. These muscles can be excited with the electrodes in the same position as for exciting the circular muscles (3) (Fig. 8) and also at most other places on the column. The advantage of using the former position is that it is relatively free from interference by contractions of the parieto-basilar muscles. Responses of the retractors can be recognized by the way in which they all contract in synchrony, causing a sharp withdrawal of the marginal disk. Responses occur occasionally to single shocks. Laws & Ward (1959) found such responses in 17% of the tests they made, although it is not certain that they distinguished the responses from possible parieto-basilar ones. However, there is no doubt that the frequency of occurrence of responses of the longitudinal retractors of *Stomphia* to single shocks is several times higher than the figure of 0.3% found by Ross (1952) in *Metridium*. As in the latter the characteristic quick withdrawal response occurs to the second shock in a closely spaced pair, the amplitude increasing with decreasing interval between shocks. The response is reproducible, but with less consistency in regard to amplitude, than is the case in *Metridium*.

(5) *Oral sphincter*. The oral sphincter was prepared as an isolated structure after

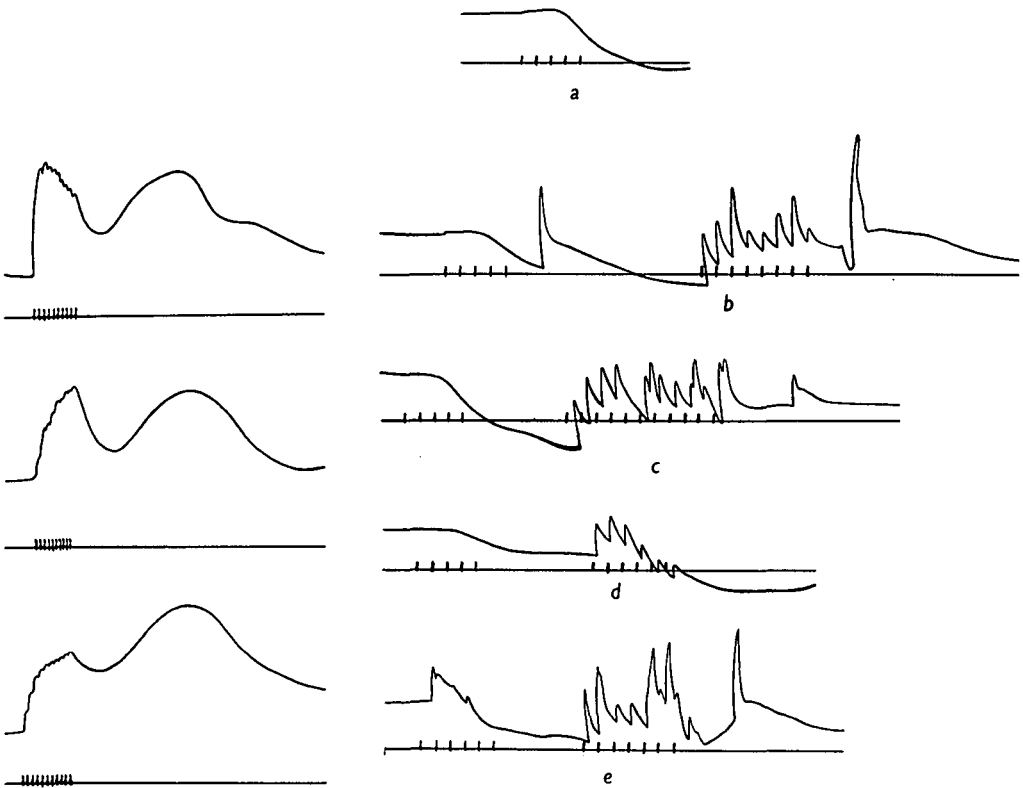


Fig. 7

Fig. 8

Fig. 7. Slow responses of parietal muscles. Ten shocks were delivered at one per sec. There are initially summing quick responses due to the parieto-basilar and these are followed by the (probable) slow response of the parietal muscles. Note that in no case was swimming evoked by the shocks.

Fig. 8. Responses of circular muscles of the column and the longitudinal retractor muscles. *a*, Elongation due to local contraction of a ring of circular muscle under the stimulating electrodes evoked by single shocks at one per 5 sec. *b-e*, Various examples showing: first, reaction of circular muscles to single shocks, then reactions to double shocks at the same frequency (two per 5 sec.). The evoked quick contractions are due to the longitudinal retractors. Additional (spontaneous) contractions are due to parieto-basilar muscles. Note the quick relaxation in *b* as the contralateral side contracted first in incipient swimming. Contraction of the circular muscles was inhibited when the longitudinal retractors were activated in *b*, *c* and *e* but not in *d*.

first anaesthetizing the animals in magnesium chloride solution. A ring of body wall containing the sphincter muscle was cut out of the column below the tentacles and a few mm. above the attachment zone of the parieto-basilar. These rings were kept in running sea water until the muscle relaxed to a diameter almost equal to that of the original animal at rest. Small pieces of nylon gauze were then sewn round the ring on opposite sides to provide tags for anchoring and recording from the ring. Electrodes were placed against the fixed-position tag. When the preparation was fresh, single stimuli evoked quick contractions of the sphincters, which were very variable in magnitude (Fig. 9). Later, these responses became very much

reduced in height and also slower, showing a summation on repetition at a slow rate. Following the delivery of eight to ten shocks at a rate of one per 5 sec. an additional response was elicited, appearing after a delay of a few seconds in some cases or roughly coinciding with the eighth or ninth shock (Fig. 10). This slow and smooth response resembles that obtained by Ross (1957*a*) from the isolated sphincter of *Calliactis*. The quick responses showed appreciable summation and facilitation effects (Fig. 11).

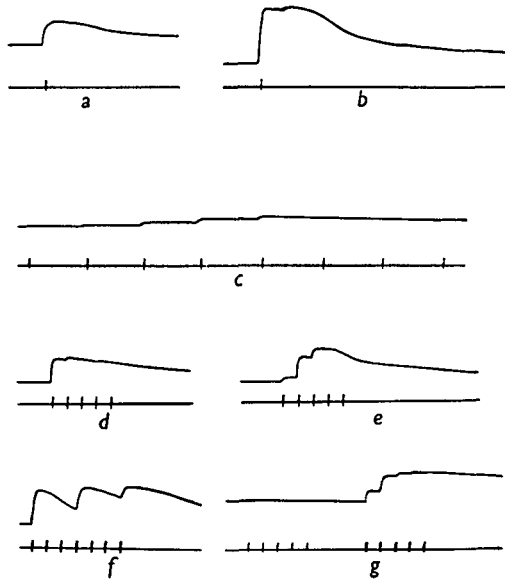


Fig. 9. Variable responsiveness of isolated sphincter muscle region of body wall. *a, b*, To single strong shocks; *c*, to weak shocks at one per 15 sec.; *d, e*, to single shocks at one per 5 sec.; *f* to paired shocks at two per 5 sec.; *g*, ageing preparation does not respond to single shocks but only to close pairs.

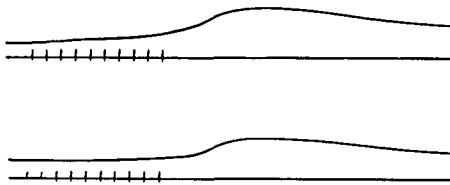


Fig. 10. Delayed slow response of isolated sphincter to weak shocks at one per 5 sec.

Elicitation of swimming responses. Preliminary tests using eight shocks delivered at one per sec. in order to test the Sund-Wilson finding were disappointing, giving swimming only occasionally and not consistently in the six animals tested. These animals showed no tendency to be more likely to respond after they had 'swum' once, or to respond subsequently to a smaller number of shocks. It was realized that to test the hypothesis adequately a large number of different animals of various sizes would have to be used. Wilson, in a private communication, tells me that the eighteen animals which were available to him were all small, with a disk diameter

of about 2 cm. This corresponds to the smallest specimens available for the present experiments; most were 2.5–3 cm. or more in diameter, being preferable for recording. It is probably worth while, nevertheless, to record some of the observations made. Although the majority of anemones probably remained stationary throughout the summer, two specimens at least changed their positions. Of course, they could have moved by 'walking', but one animal, which was in a small glass dish awaiting its turn for experiment, was actually observed to swim, probably following a spontaneous take-off. It had been handled, but not for an hour previously; the temperature of the water had not exceeded 16° C. and there was no immediate source of irritation. Stephenson, it will be recalled, had noted 'jumping' by an animal in a small dish (as the temperature rose) and Sund (1958) noted the preliminary movements of 'swimming' when an animal became heated up under the lights used for filming; this animal, nevertheless, walked, not swam, away. But these scanty observations show that swimming might well occur spontaneously, or in response to a variety of stimuli. The response may well be a general one to adverse conditions, rather than a specific one to a particular species of starfish.

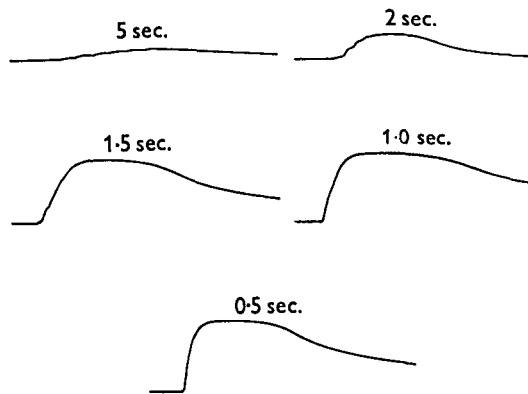


Fig. 11. Facilitation and summation in responses of isolated sphincter. Seven shocks applied at the intervals indicated.

Animals were stimulated electrically by applying the brief direct-coupled pulses through paired electrodes placed roughly mid-way up the column. The longitudinal retractor muscle response, which was probably that obtained by Sund and by Wilson as an initial response which preceded swimming, could be obtained in this position. The outstanding result of the experiments was simply the surprise engendered by their variability. One animal started to swim after only a single shock had been applied and went on to exhibit swimming movements without further stimulation (Fig. 11). The same animal could not be induced to swim by single electric shocks on later occasions. Other tests resulted in swimming in response to 5, 6, 7, 8, 9 or 10 shocks, or up to as many as 28 shocks, or no swimming at all. The 28-shock response (Fig. 12) occurred during a long series of shocks applied so as to evoke the parieto-basilar response and were delivered at the low rate of one per 10 sec.

More often than not there was an initial reaction, retraction followed by extension and waving, but without full swimming. As many of the animals used in these studies were tethered by pins they could not detach; a series of bendings following the initial reaction was taken to indicate that a full swimming reaction had occurred (Figs. 12, 13). The fact that these reactions occur at all, and continue for as long as normal free swimming, provides an important clue to the nature of the response. It cannot consist of a series of chain reflexes, for achievement of freedom from the attachment would surely constitute an important link in the chain of events.



Fig. 12. Swimming movements elicited by a single very strong electric shock. Note absence of preliminary activities. Traces from opposite sides of animal.

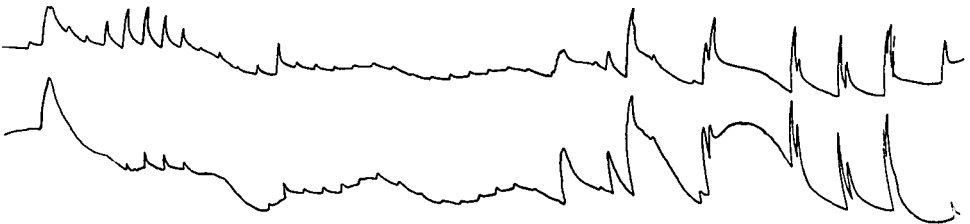


Fig. 13. Swimming movements elicited after twenty-eight shocks had been applied at one per 10 sec. Note initial part of swimming reaction after first shock. Swimming starts by a series of summing quick contractions; it is characteristic that these start not on the stimulated side (upper trace) but on the contralateral side. The great variability in the responses to the single shocks, due to the parieto-basilaris, should also be noted.

Animals came to be recognized as 'swimmers' or 'non-swimmers', although a non-swimmer' might surprise one on occasions. Wilson (1959) states that stimuli applied at intervals greater than one per 3 sec. do not cause swimming, but during the course of some of the present experiments (responses of the parieto-basilaris), full swimming movements occurred during repeated stimulation with single shocks at intervals as long as 10 sec., as in Fig. 13, though more than twenty stimuli were required.

Investigations on 'long-term facilitation'. The great variability encountered in the responses to stimulation aimed at evoking swimming made it impossible to use that approach extensively to test Wilson's finding of 'long-term facilitation'. The other methods suggested earlier were, however, extensively applied. They are:

first, testing the electrical threshold at intervals after first eliciting a motor response; secondly, testing the amplitude of the contraction to quantitatively similar stimulation applied at intervals after an initial response. These tests were applied to both the longitudinal retractor-muscle response and the parieto-basilar response. Wilson was probably arousing the longitudinal retractor response. But it is the parieto-basilar muscles, not the longitudinal retractors, which are used in swimming; this makes it difficult to see how the nerve-muscle junctions could be implicated. It would seem more probable that long-term facilitation would occur in the nerve net rather than at the nerve-muscle junctions as he suggested. Wilson was probably assuming that his electric shocks were exciting single impulse in a through-conducting nerve net, by analogy with Pantin's interpretation (1952) of the responses of the sphincter of *Calliactis*. Even if this is the case the excitation must in addition be affecting a great deal else, including sense organs and other parts of the nervous system, and these are likely to be affected in a much subtler manner.

Threshold changes

Examination of threshold changes would have no value as an experiment if the only reaction we expected was the simple one of eliciting a single all-or-nothing nerve impulse, but if there are also additional effects, on sense organs or nerve net, these might be affected differently from the observable motor response, and for them the shocks might well be suprathreshold. No effects were observed, however, as a result of giving shocks of a strength which just failed to evoke an observable motor response. There was no clearly detectable change in the subsequent response to suprathreshold stimuli, no increase in responsiveness to starfish; but the problem has not been tackled statistically, as it should be, owing to lack of time.

Amplitude of response

If the facilitation has occurred at sites between the excitable nerve elements and the muscles it should be detectable as an increased height of the response to a standard shock—single for the parieto-basilar, double for the longitudinal retractors. Once again it can be stated that if such effects occur at all they must be so small as to require a statistical test to detect them. Sometimes a facilitation was evident when the animals were tested soon after the initial stimulus, but this always died away after a few minutes. This effect could have been due to a relatively long-lasting 'arousal' of the nervous system, rather than to a persistent state of facilitation engendered at a particular set of junctions.

Reduced total number of shocks. Animals were also tested in the Wilson manner, by eliciting swimming and then applying a similar train of excitation but with one shock fewer. In none of these tests did a swimming response occur to the lower number of shocks, although there was usually some preliminary waving and occasional bending. Some animals could not be re-excited to swim at all for some time, and then only with a larger, not a smaller, number of stimuli than the original.

DISCUSSION

The failure, in all these experiments, to confirm the remarkably regular results obtained by Wilson is disappointing. Probably the difference must be attributed to differences in the animals. Wilson's specimens were all small and probably represented a very homogeneous group of young animals. If it should be confirmed that his results can be repeated with small, young animals, that will throw some interesting light on the changes which occur in the actinian nerve net as a result of maturation and experience, for the animals apparently become (untrainable) 'laws unto themselves' as they grow older, according to the indications of the present experiments. It is, nevertheless, hard to avoid the suspicion that the uniform way, for example, in which ten or so of Wilson's eighteen animals responded to precisely eight shocks was fortuitous. I find it difficult to imagine a mechanism by which a precise number of shocks could have such a well-defined meaning for a coelenterate nerve net, even an immature one, and be independent of frequency as a source of information to the net.

However, the fact that a non-biological stimulus in the form of a group of electric shocks can elicit the complex swimming reaction has been fully confirmed. The present interpretation, though, is that the swimming activity is a 'fixed-action' pattern, probably innate, which can occur spontaneously in response to certain extrinsic stimuli. I do not consider it to be a specific reaction to those starfish which elicit it and predict that other stimuli will be found which call it forth. It is indeed remarkable that so complex a reaction can occur in sea anemones at all. Where, and in what way, is the information required to activate the muscles sequentially stored in the nerve net? How is it called forth to elicit the phases of swimming, which take several minutes to complete? Since the sequence can be fully performed by tethered animals a chain-reflex interpretation cannot be applied to all stages. When the animal is cut in half whilst swimming (Sund, 1958; Robson, 1961) the halves of the animal continue to execute appropriate parieto-basilar contractions, so the integrity of the nerve networks of the whole animal is not essential for at least the later parts of the reaction. Robson (1961) has also shown that an isolated ring of body wall containing the tentacles and oral sphincter can react to a starfish by giving a prolonged contraction.

In considering the mechanism of initiation of swimming one is reminded of the way in which a variety of irritant substances increase the frequency of occurrence of responses to single shocks in *Metridium* (Ross, 1952). Rather randomly occurring impulses are evidently set up in the through-conducting nerve net by irritant chemicals, possibly by transmission across from another nerve net. It is visualized that in much the same way the secretions of certain starfish or electric shocks lead to impulses in a certain part of the nerve net which is caused, if the impulses persist long enough at a sufficiently high frequency, to evoke the sequence of nervous outflow which causes swimming. As the physiological state of the synapses between the nerve elements responsible is liable to be labile, it is not surprising that at different times different numbers of electric shocks or presentations of starfish

should be needed to evoke the response, or that there are times, for any animal, in which swimming cannot be elicited. Furthermore, like any innate behaviour pattern the 'strength' of the response may vary, appearing as weak swimming, brief swimming or a show of only the preliminary phases of the reaction.

The failure, in the present experiments, to demonstrate a 'long-term facilitation' could be due to the present animals being older, having unadaptable synapses and so forth. Only a full-scale investigation on animals of different ages, accompanied by a statistical analysis of the data, is likely to provide acceptable evidence on this point.

Regarding the responses of specific muscles the experiments have made it clear that a through-conducting nerve network, similar to that demonstrated by Pantin in association with the oral sphincter of *Calliactis* and the longitudinal retractors of *Metridium* and also present in *Stomphia* in connexion with the longitudinal retractor muscles, is not the only excitable element which reacts to the electric shocks. In particular, the sensory elements and other parts of the nervous network are likely to be reacting. The great variability observed in the responses of the parieto-basilar muscles suggests that they occur indirectly via the motor nerve elements, and that impulses elicited by electric shocks must summate with those due to normal 'background' activity. The responses of most of the muscles are not similar to the superficially simple ones of the longitudinal retractors. It is not possible to interpret them on the basis that single impulses are set up in the appropriate motor nerve elements by single electric shocks.

SUMMARY

1. The spontaneous activity and also the response mechanisms to externally applied electric shocks have been studied in the swimming sea anemone *Stomphia coccinea* obtained from Puget Sound, Washington.
2. Slow rhythmical activity with a repetition interval of the order of 30 min. is evident in kymograph records of whole-animal movements and also in rings of body wall isolated from the oral or basal regions.
3. By suitably placing the stimulating electrodes different parts of the musculature can be brought into contraction. Those studied were principally the parieto-basilar muscles and also the longitudinal retractors, the circular muscles of the column and the isolated oral sphincter.
4. The magnitude of the response of the parieto-basilar muscles to constant-strength stimuli was very variable. Responses occur to single shocks and these increase in magnitude with increasing strength of stimulus.
5. The parietal muscles give delayed, slow contractions in response to trains of about ten stimuli at one per sec.
6. The isolated sphincter muscle shows two kinds of contraction, a quick one which occurs to single shocks and a slow one which occurs following a brief train of fairly widely separated shocks.
7. The ordinary circular muscles respond with a slow contraction to a few widely spaced shocks. They do not give quick contractions.

8. Although in many cases animals responded to eight quick shocks by giving swimming reactions this number was not found to be a precise requirement, and it varied for the same animal at different times in an unpredictable manner as well as varying from one animal to another. On one occasion a swimming response occurred after a single shock and on another after twenty-eight shocks.

9. It was found that the state of 'facilitation' (occurring in the response to electric shocks) of the muscles used in swimming varied a great deal but did not show any marked long-term change following a single shock or the elicitation of a swimming response.

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