RAPID CONTRACTIONS AND ASSOCIATED POTENTIALS IN A SAND-DWELLING ANEMONE

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INTRODUCTION

The behaviour of sea anemones ranges from simple reflex activities which are complete within seconds to slow complex movements which last for several hours (Pantin, 1935a; Batham & Pantin, 1950; Robson, 1961b; Ross & Sutton, 1961). While the latter are difficult to analyse, the withdrawal or protective responses are very rapid, can be consistently evoked by appropriate electrical stimuli, and have been the most thoroughly studied of all the components of anemone behaviour. These responses are considered to result from the widespread activation of one or more groups of muscles by a through-conduction system, so that there is a sudden closing of the oral disk and symmetrical contraction of the column.

Until a short time ago analyses of the properties of the through-conduction system were dependent upon the nature of the evoked mechanical responses. Recently, however, Josephson (1966), using flexible suction electrodes, was able to record muscle action potentials for the first time from Calliactis. These potentials precede rapid contractions of the sphincter muscle by 12 msec. and their size is closely related to the size of the contraction.

In the present study the same technique has been employed in an analysis of neuromuscular transmission in a sand-dwelling anemone. This animal, Calamactis, differs from Calliactis in that it lacks a sphincter, and the major effectors are the well developed retractor muscles. The latter have the same general properties as the sphincter, but show distinct differences associated with their greater length and their longitudinal arrangement in this very elongate, burrowing anemone.

MATERIALS AND METHODS

Experimental procedure

Calamactis praelongus Carlgren, 1951, an anemone endemic to the Gulf of California, is found in sand flats. It acclimates well to aquaria with artificial sea water and will dig into a sand substratum within a few hours after it is brought into the laboratory. The annual range of monthly temperatures to which the anemone is subject in the field is 12–34°C. As a compromise between these two extremes, all experiments reported in this paper were carried out at 20–22°C.

Suction electrodes, similar to those described by Josephson (1966), were used to pick up muscle potentials which then were amplified and recorded with standard electronic
instruments. Movements of the anemone were recorded with a movement transducer consisting of an E & M type A Myograph with a long, counterweighted lever attached to the end of the myograph and passed through a pivot placed close to the spring. A thread from the free end of the lever was sewn through the oral disk or a retractor muscle usually as close as possible to the muscle from which electrical potentials were being recorded.

Unless otherwise indicated, electrical stimuli of 1 msec. duration and one and one-half times threshold strength were delivered through a suction electrode with a 1 mm. bore at the tip. Intervals between trials were variable and depended upon the time it took for the anemone to recover from an electrical or mechanical stimulus. In nearly all experiments movements were monitored on the physiograph, so that a baseline was established and the next trial was not started until the anemone had returned to its initial length.

Prior to recording, anemones were prepared as follows. In those instances when whole animals were used they were either pinned through the aboral end or permitted to move about as freely as the suction electrodes would allow. In most of the experiments, however, a longitudinal cut was made through one side of the stomodaeum, across the oral disk, down the column between two adjacent mesenteries and up to but not across the aboral end. The anemone was then pinned to the bottom of a shallow pan so that a considerable amount of longitudinal movement was possible.

Morphological features

*Calamactis* is a halcampoid anemone with no pedal disk. The anemone digs into sand with alternating contractions and expansions of the rounded physa at the aboral end. When the tide comes in, the oral disk is usually level with the surface of the sand and the anemone is extended maximally. At this time the length of a large animal may be 60 cm.

The strong retractor muscles, reniform in cross section (Carlgren, 1951) are the ones primarily responsible for rapid changes in length. They are widest (3 mm. in a large anemone) about one-third of the distance from the oral disk and, although they taper toward each end, are narrowest near the physa (Fig. 3), becoming flatter in cross-section at this end. The mesenteries themselves are thin, contractile membranes which suspend the retractors in the body cavity. The parietal muscles are also best developed in the upper third of the animal and may play an important part in slower movements. The circular muscles are moderately strong, but a sphincter is not present at the top of the column.

RESULTS

General behaviour

The behaviour of *Calamactis* is similar to that of other anemones, except that most of its activity is restricted to movements along the oral-aboral axis because it lives in a burrow. Three types of longitudinal movement, differing in the rate at which they occur, can be recorded under laboratory conditions. These are slow, sometimes rhythmic, contractions, slightly faster contractions which are associated with photic and low-frequency electrical stimuli, and rapid contractions in response to strong mechanical stimuli or electric shocks.
The responses have their counterparts in *Metridium* (Batham & Pantin, 1950, 1954) and one or more groups of muscles participates in each response. The retractor muscles of *Calamactis* are capable of fast and slow contraction as they are in *Metridium* (Pantin, 1965), but muscle potentials are recorded only when the rapid withdrawal responses occur. It is probable that the rest of the activity of an anemone results from smaller or slower changes in muscle membrane potential which could not be picked up with the recording equipment used in these experiments.

**Retractor responses to paired electrical stimuli**

The first of a pair of shocks applied to any part of *Calamactis* will not evoke a withdrawal response. If the second shock is given a short time after the first, a rapid contraction of the retractor muscles occurs. A recording electrode placed on a retractor muscle will pick up a large (up to 2.75 mV.) potential which precedes contraction by as much as 120 msec. The size of the potential is dependent upon the interval between shocks, but is independent of stimulus strength above threshold. The average duration of the potential is 30 msec. with a range of 15-150 msec. The direction of polarity in the Figures in this paper is such that negative is upward, and muscle potentials are primarily negative. With repetitive stimulation, there is a shift in the polarity of responses to later stimuli (Fig. 6).

Pantin (1935a) was the first to determine that two shocks were needed in order to evoke a withdrawal response. He postulated that the initial shock elicited a nerve impulse which was conducted in a through-conducting nerve net and facilitated transmission of excitation by the second nerve impulse at the neuromuscular junctions. Several recordings from one specimen of *Calamactis* show such an event; that is, two nerve spikes preceding a muscle potential (Fig. 1). The conclusion that these are nerve spikes is based on the fact that they are brief (1.5 msec.), all-or-none potentials from a region of the retractor muscle where large nerve fibres are known to occur. Each spike appears at a constant interval after a shock, but only the second is followed by a muscle potential.

A muscle potential and a contraction can be evoked by two shocks which are as much as 1.4 sec. apart. Contraction occasionally fails to appear after the application of paired shocks 1.2 sec. apart, as, for example, after the first muscle potential in
Fig. 4a, but a rapid withdrawal response is never seen when the interval between stimuli is 1-6 sec., even though small potentials are sometimes evoked by the second stimulus. As the interval between shocks is decreased the size of the muscle potential and the contraction increase (Fig. 2), so that up to a point there is the same correlation between the size of the potential and the size of the contraction in the retractor muscles of *Calamactis* as there is in the sphincter response of *Calliactis* (Josephson, 1966). The response of the retractor of *Calamactis* differs in that when electrical stimuli are closer together than 200 msec., there is no further increase in the average size of the potential or of the contraction, although the variability of the response increases. However, the minimum interval between paired shocks which will produce a response when using electrical stimuli of one and a half times threshold is about 30 msec. on
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the average (range from 10 to 90 msec. in 23 anemones). This must represent the relative refractory period. By increasing the strength of the stimulus the refractory period can be shortened. In one experiment, at ten times stimulus strength above threshold, the minimum interval was 2 msec.

Fig. 3. Regional differences in physiological properties of muscle and nerve net. Only two complete tentacles and one retractor muscle (black band) are shown in the sketch of a partly extended anemone. Sections at left, bounded by lines representing the edges of the retractor muscle, indicate the arrangement of nerve fibres on the face of the upper and lower thirds of the muscle (drawn from composite photographs of the nerve net of a retractor stained with methylene blue). The width of individual nerve fibres is greatly exaggerated and fibres of only two diameters are indicated although there is a gradation in size. Physiological properties are shown on the right with arrows indicating the parts of the anemone from which they were recorded. In determining threshold and refractory period, the recording electrode was kept on the upper part of a retractor muscle and the stimulating electrode was moved around to the points indicated. Threshold is expressed as milli-amps. of current required to evoke a muscle potential when paired stimuli 0.6 sec. apart were given to the retractor or body wall. Other details are in the text.
Regional differences in responses to paired stimuli

The results reported above were obtained with a stimulating electrode over a tentacle and a single recording electrode on the upper half of the retractor muscle. However, by using two recording electrodes and shifting the recording and stimulating electrodes to different points, it is possible to show regional differences in size of potential, conduction velocity, sensitivity, and refractory period. The values given in Fig. 3 are averages from three trials on one medium-sized anemone whose experimental resting length was 8 cm.

Muscle potentials can be recorded from the tentacles and oral disk, but from nowhere else on the surface of an anemone. Within the animal, potentials may be picked up from the pharynx and retractor muscle. The size of the retractor muscle potential is greatest about one third of the distance from the oral disk and decreases proximally and distally on thinner parts of the muscle. The potentials shown in Fig. 3 are not the largest that can be recorded, but are responses to paired shocks 600 msec. apart. A shorter interval between stimuli was not used because of the long refractory period of the lower retractor (see p. 519). Potentials can also be recorded from the mesenteries, but these diminish markedly with increasing distance from each retractor muscle, suggesting that they are spread passively from the muscle and are not of sufficient size when they reach the body wall to be recorded externally.

Conduction velocity, as determined by the difference in time of appearance of a muscle potential at two different recording electrodes, is greatest along the upper face of the retractor and decreases toward the physa. In spite of differences in conduction velocity, conduction in any one section of the nerve net is about as fast in the oral as in the aboral direction. The conduction velocity is at least as rapid over the oral disk (0.86 m./sec.) as it is along the upper retractor muscle, but it is difficult to determine whether conduction is in a radial direction or around the circumference of the disk.

There appears to be a correlation between conduction velocity and the distribution of nerve fibres along the face of the retractor muscle. No detailed study of the histology of the nervous system has been made, but staining with methylene blue reveals that the nerve net of Calamactis resembles to a certain extent the nerve net of Mimetridium of New Zealand (Batham, 1965). There are large (up to 12 μ diameter) and long (up to 8 mm.) bipolar nerve fibres oriented in a longitudinal direction on the upper halves of the retractor muscles (Fig. 3). The lower halves are supplied with fewer, and generally thinner (5 μ or less), fibres oriented primarily diagonally or horizontally. Although rapid conduction appears to be associated with large, long fibres oriented longitudinally, there may be some correlation with the number of fibres per unit area as well.

Conduction velocity and the size of the potential at any one point on the retractor do not change when electrical stimuli are applied to different parts of the anemone. However, as the stimulating electrode is moved aborally, stronger stimuli are required and the minimum interval between an effective pair of shocks increases. There appears to be no difference between retractor muscle and adjacent body wall in the changes in sensitivity and refractory period as the stimulating electrode is moved toward the physa (Fig. 3). The increase in threshold might be explained as a decrease in density.
of excitable elements which, on the basis of the values obtained, would be about equally distributed along the surface of the retractor and over the body wall.

If shocks are given near the physa and potentials are recorded from the upper retractor, the refractory period is 500 msec. If shocks are given to the tentacles and potentials are recorded from the bottom of the retractor, the refractory period is also 500 msec. These two results indicate that it is not sensory elements or neuromuscular junctions or muscle fibres which are limited in their capacity to respond to shocks less than 0.5 sec. apart, but the through-conduction system in the lower part of the retractor, passing excitation in both directions. The refractory period of thin fibres is greater than that of comparable thick fibres (Bullock & Horridge, 1965), but even the finest fibres measured have refractory periods which are at least fifty times shorter than those recorded here.

The responsiveness of the tentacles to electrical stimuli is greater than that of any other part of an anemone (Pantin, 1935a). In fact, a single shock or the first of a pair will evoke a tentacle muscle potential and sometimes a twitch of the tentacles in Calliactis even though there is no response from the sphincter (Josephson, 1966). This is also true of the tentacles of Calamactis (Fig. 5), so that it is always possible to elicit a tentacle potential to the first shock if 5 min. are allowed to elapse between trials. A second tentacle potential evoked by a second shock is larger because of facilitation by the first only if the interval between stimuli is less than 1.4 sec., the same maximum interval between shocks which produces facilitation of the retractor potential.

Responses to stimulus trains

When more than two shocks are given to the tentacles or retractor of Calamactis, evoked potentials increase in size with successive stimuli and then decline. If stimuli are far apart, the third or fourth retractor potential is the largest, but as the interval between stimuli decreases, the maximum size is reached earlier in a stimulus train (Fig. 4). Thus, facilitation of successive retractor potentials is similar to facilitation of successive sphincter potentials in Calliactis (Josephson, 1966). Regional differences in the size of the potential which is evoked in response to repetitive stimulation parallel the size differences seen when paired shocks are given. The greatest size of the retractor potential obtained with repeated shocks is smaller at the oral end of the anemone than at the physal end (Fig. 6c, d), which is to be expected in a thinner portion of the muscle with fewer muscle fibres. Furthermore, tentacle potentials reach a maximum size earlier in a series than do potentials from the upper retractor (Fig. 5), and potentials from the oral end of the retractor are maximal sooner than those from the physal end (Fig. 6c, d).

The mechanical responses to repetitive stimuli are similar to those reported for Calliactis and Metridium (Pantin, 1935 d; Hall & Pantin, 1937). Contractions increase in a step-like fashion, forming a 'staircase', and reach maximum size at the higher stimulus frequencies. When the frequency reaches approximately 16 stimuli per second, the 'staircase' appearance is lost and a single smooth contraction occurs (Fig. 4d). Contrary to the impression one gets from the gross morphological features of the retractor muscle, it does not act as a single unit. When a rapid withdrawal response is evoked, the upper half of the retractor muscle contracts, but the lower half relaxes or is passively stretched. However, it is not stretched except after the first
muscle potential (Fig. 6c); in other words, it is stretched only when the first contraction occurs in the upper half. This response is difficult to explain in view of the fact that potentials are recorded in the lower half, even though they occur after the contraction has started (see p. 521). A tentative hypothesis is that relaxation is a mechanical artifact and a function of the manner in which the anemone is restrained. Further studies are in progress.

![Diagram](image1)

**Fig. 4.** The relation between stimulus frequency and size of response. Mechanical (upper traces) and electrical (lower traces) responses to repetitive electrical (a–d) and strong mechanical (e) stimuli. Recording from a retractor muscle with the stimulating electrode over a tentacle. Intervals between shocks are (a) 1.2, (b) 0.8, (c) 0.2 and (d) 0.06 sec.

![Diagram](image2)

**Fig. 5.** A comparison of the tentacle potentials (upper traces) and retractor potentials (lower traces) evoked in response to repetitive electrical (a) and to strong mechanical (b) stimuli. Stimuli were given to an adjacent tentacle. Note in (a) the tentacle potential in response to the first stimulus.

The interval between the stimulus and the start of the contraction does not change with recording site. In contrast to this, the latency between the muscle potential and the muscle contraction decreases as excitation spreads from the oral disk to physa. Thus, potentials on the upper part of the retractor occur 120 msec. before the mechanical response, those from a point two-thirds of the way down the retractor occur
at the same time as the contraction, and potentials in the lowest part of the retractor appear as long as 50 msec after the contraction has started (Fig. 6g, h). The explanation for this decrease in electrical-mechanical latency is that the latency over the entire retractor is 120 msec, but that the latency measured in the middle of the retractor is only the time between the start of a potential in the middle and the start of contraction near the oral disk.

![Fig. 6. Mechanical (a, e, h) and electrical (b, c, d, f, g) responses from two different parts of the retractor muscle when multiple shocks are given to the tentacles. A thread from the transducer was tied to the retractor muscle close to the oral disk and a typical mechanical response is recorded in trace (a). A similar recording appears in (h), but is inverted, that is, contraction is downward. The middle of the retractor muscle was tied to the thread from the transducer in order to record trace (e). In (e) relaxation is upward and the amplitude of the mechanical response is four times that in traces (a) and (h). One recording electrode was placed on the upper part of the retractor muscle (b, d) and one on the lower part (c, f, g). Frequency of stimulation is 2/sec. Pairs of traces were recorded, in the following order (c, d), (a, b), (g, h), (e, f), with 7 min. intervals between trials.](image)

**Polarity and after-discharge in the through-conduction system**

In his studies of *Calliactis* Pantin (1935c) found anatomical and functional polarity in the tentacles, but conduction in the column was not polarized. This lack of polarity in the column is found in *Calamactis* under most conditions, for when two recording electrodes are used there is a one-to-one relationship between the muscle potentials recorded from the upper retractor and those recorded from some point lower down, no matter which end of the anemone is stimulated (Fig. 7a). However, in a few instances, usually after prolonged stimulation, an extra potential will be recorded from the lower part of the retractor and not from the upper. Figure 7b (lower trace) shows such a potential, which could be either an after-discharge or the first of the two components of a compound muscle potential that failed to arise synchronously with the second. In either case, the impulse which preceded this potential did not travel toward the oral disk. Two or more potentials triggered by a single stimulus are not unusual events in anemones (Pantin, 1935c; Josephson, 1966), but what is of interest is that this appears to be a local event in a through-conduction system. The behavioural correlate of this locally conducted potential is not known.
After-discharges are rare in *Calamactis*, but when they occur they usually originate close to the point of stimulation and are conducted in both directions along the through-conduction system. However, occasionally, they arise at some distant part of the conducting system and travel back toward the site of stimulation. This ‘reflexion’ of excitation is often seen when the physa is given a strong mechanical stimulus, but appears less frequently when the tentacles are stimulated (Fig. 7c, d). ‘Reflexion’ of nerve impulses takes place within the axons of single giant fibres of annelids and crustaceans according to Bullock & Turner (1950), who also report after-discharge and failure of conduction. These properties are undoubtedly associated with parts of the conduction pathway where nerve spikes have a low safety factor (Bullock & Horridge, 1965). It is possible that the through-conduction system in anemones behaves more like one of the septate giant fibres of the earthworm than like a chain of interneurons in one of the higher metazoa, so that some of the integrative properties of these single axons can be equated with activity in the through-conduction system and vice versa.
Calamactis will contract locally at the point of contact when it is touched lightly with a glass rod, but a sharper prod is required before it will exhibit a protective response. This is the typical anemone reaction to mechanical stimuli (Passano & Pantin, 1955). When a prod that is just strong enough to evoke a withdrawal response is applied with a handheld probe to the tentacles or oral disk of Calamactis, a single muscle potential appears and is followed by a single contraction. If an anemone has not been disturbed for 5 min., the potential and the mechanical response are of maximum size when compared to responses evoked by paired electrical stimuli. For example, the first potential in Fig. 4e is the same size as the first in 4d, which suggests that two impulses less than 200 msec. apart are present in the through-conduction system.

When a stronger or more rapidly applied mechanical stimulus is given, several muscle potentials occur (Figs. 4e, 7a). In general, the stronger the stimulus, the more potentials there are, the closer together are the potentials, and the greater is the contraction. The minimal interval between successive potentials evoked by mechanical stimulation was found in these experiments to be 60 msec. This is also close to the interval between repeated electrical stimuli at the highest following frequency for the first five muscle potentials (Fig. 4d). However, the nerve net appears to be capable of firing at higher frequencies; that is, in response to paired shocks the refractory period is 30 msec. or less.

There are regional differences in response to mechanical stimuli which parallel those to electric shocks. First, local conduction velocities and potential sizes are comparable to those listed in Fig. 3. Secondly, the sensitivity to mechanical stimuli decreases from tentacles to physa. Thirdly, when severe mechanical stimuli are given to different points along the body wall, the interval between evoked potentials increases as the prodder is moved from tentacles to physa. Thus, impulses originating from the physal
part of the through-conduction system are farther apart and this is probably related to the long refractory periods in the lower retractor. One consequence of the difference in spacing between nerve impulses is that a prod to the tentacles invariably evokes much larger potentials in the upper retractor muscle as a result of the facilitation of successive potentials than a similar prod to the phylla. Of course, some of this difference in potential size is due to the difference in diameter of the two parts of the muscle. There are two other cases in which responses to mechanical stimuli resemble responses to electrical stimuli. First, the lower half of the retractor muscle relaxes when mechanical stimuli are applied to the tentacles. Secondly, the first tentacle potential in a burst evoked by a sharp prod to the tentacles is recorded when there is no response from the retractor (Fig. 5b). It can be concluded from this that the neuromuscular junctions in the tentacles of the anemone are permanently facilitated.

On the basis of the results reported above it appears that the same conduction pathways are used when the anemone responds to mechanical stimuli and when it responds to electrical stimuli. Passano & Pantin (1955) were able to show facilitation of sphincter contraction in Calliactis when a shock was given just prior to a mechanical stimulus. The same interaction between stimuli occurs in Calamactis. If, for example, a light prod is given during a series of shocks, it will evoke a single muscle potential and produce a larger than expected potential in response to the next electrical stimulus (Fig. 8a, b), presumably because two impulses, one evoked by mechanical and the other by electrical stimulation, appear close together at the neuromuscular junction.

A strong mechanical stimulus, on the other hand, if given during a similar series of electrical stimuli, will evoke several muscle potentials, but responses to subsequent interspersed electric shocks fail to appear (Fig. 8c, d). The simplest explanation for this is that the threshold for excitation of the through-conduction system rises as a result of the increased frequency or number of impulses in the system. Judging from the sizes of the potentials to the last effective electrical stimulus in Fig. 8c, d, the neurons under the stimulating electrode were not refractory at that moment, but became so shortly afterward as a result of the passage of two more impulses. Consequently, an electrical stimulus of one and a half times the initial threshold was no longer strong enough to initiate a response. This might also explain why Calamactis will always respond to a strong prod even though it no longer reacts to repeated shocks.

**DISCUSSION**

An analysis of the rapid withdrawal response of Calamactis has turned up little that does not fit in with the many conclusions drawn from studies of the nervous system of anemones since Pantin’s pioneering work in 1935. However, while there is nothing that is surprisingly different about Calamactis, there are some interesting modifications of the basic anemone neuromuscular organization.

The recording from an anemone of what appear to be genuine nerve spikes gives dramatic support to the concepts developed by Pantin 30 years ago; namely, that two impulses are required in order to evoke a rapid contraction. The spikes which precede the larger potentials are all-or-none and of the same duration as impulses recorded from higher animals. However, they are shorter in duration than nerve
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Spikes found in jellyfish (Horridge, 1953; Passano, 1965) and hydroids (Josephson, 1965).

In agreement with Josephson (1966), the large, graded potentials are considered to be muscle potentials. Those recorded from the retractor of *Calamactis* differ in polarity from those recorded from the sphincter of *Calliactis* (Josephson, 1966), but the tentacle potentials in both species are the same. This difference in polarity is probably due to slight differences in the geometric relationship between recording electrode and tissue. Apart from this difference in sign, the sphincter and retractor potentials have almost the same duration and size, although in *Calamactis* potentials recorded from the tapering ends of each retractor are smaller than those recorded from the middle. Reduction in size of potential toward both ends of the muscle might be indicative of passive spread of excitation from the middle of the muscle. However, potentials appear sequentially from the oral disk to the physa. On the other hand, muscle conduction may occur along the lower half of the retractor. While this cannot be discounted, it should be noted that large, single muscle potentials, evoked by paired electrical stimuli 200 msec. apart and appearing in the middle of the retractor, are not conducted all the way to the physal end of the muscle. It is hard to believe that the muscle is refractory or that muscle conduction would fail to occur under these conditions. Consequently, the evidence seems to indicate that muscle potentials are evoked by neuronal activity.

The through-conduction system runs along the retractor from oral disk to physa. There is a good correlation between velocity of conduction in this system and the distribution and size of nerve fibres. What is surprising, however, is that the velocity in the upper half is not higher, considering how well *Calamactis* appears to be adapted to rapid longitudinal movement in a burrow. The maximum value recorded so far, 0.9 m./sec., is lower than the conduction velocities along the mesenteries of *Metridium* and *Calliactis*, 1.2 and 1.0 m./sec., respectively (Robson, 1961a; Pantin, 1935b). Perhaps individual muscle fibres shorten more rapidly in *Calamactis*.

Although some of the differences between the responses of the subtropical anemone *Calamactis* and the tropical anemone *Calliactis polypus* are associated with the organization of the nerve net, some of the dissimilarities in response can be attributed to differences in habitat and experimental temperatures. Pantin & Vianna Dias (1952) and Josephson (1966) discuss temperature effects and conclude that among anemones there is no compensation for temperature in two physiological properties, namely, refractory period and the decay of facilitation. At 20–22°C, the temperature used in these experiments, the refractory period of 30 msec. and the maximum interval between effective stimuli of 1.4 sec. are close to those of *Calliactis parasitica* (Pantin, 1935a) and *Metridium* (Hall & Pantin, 1937) at about the same temperature. *Calamactis* might be expected to react at 32°C as rapidly as the tropical *Calliactis polypus* (Josephson, 1966). However, reaction time, or the latency between the start of the muscle potential and the start of the contraction, is about ten times longer in *Calamactis* than in *Calliactis polypus*. How much of this difference is due to temperature and how much to some other factor is not known.

It seems reasonable to suppose that natural selection would have favoured contractions in the presence of noxious stimuli that resulted in tighter closure of the sphincter or greater retraction of the oral disk and tentacles. An increase in the size of a con-
traction will occur if the interval between impulses is reduced, and it might be expected that size of contraction would continue to increase until the system was refractory. This does occur in Calliactis polypus (Josephson, 1966) but not in Calliactis parasitica (Pantin, 1935a) or in Calamactis (Fig. 2). In these last two anemones, the maximum contraction occurs when impulses are about 200 msec. apart, but the relative refractory period is 30–40 msec. The significance of this difference between species is still to be determined.

The duration of the refractory period in Calamactis varies from one part of the through-conduction system to another, so that a limit is placed on the amount of information which passes from oral disk to physa. For example, with the stimulating electrode over a tentacle, a pair of electrical stimuli 200 msec. apart will evoke a muscle potential in the middle but not at the physal end of the retractor, while in response to multiple stimuli applied at the same frequency, potentials can be recorded near the physa after every second or third stimulus. From an analogous experiment, it is inferred that weak mechanical stimuli evoke only two nerve impulses while stronger stimuli produce a burst of impulses, so that the latter rather than the former elicit potentials at the physal end of the anemone. Thus, impulses travelling toward the physa are filtered out if they occur during the refractory period of the lower nerve net. Only severe and prolonged mechanical stimulation of the oral end of the anemone will cause the physal region to respond, possibly with a slow contraction.

This phenomenon is another example of increasing spread of excitation with repetitive stimulation. It differs in three ways from the spread of excitation across a coral colony (Horridge, 1957) or around the oral disk of Calliactis which Pantin (1935a) has suggested is due to interneural facilitation. First, each impulse travels the length of the through-conduction system of Calamactis and blocks for a brief period of time the passage of the next impulse in the lower nerve net. In interneural facilitation each impulse travels only a short distance to a synapse where it facilitates the passage of the next impulse. The second difference is that the nerve net is through-conducting to the first impulse and this distinguishes it from the nerve net around the edge of the oral disk in Calliactis which is not through-conducting until several impulses have been evoked. Finally, there is no polarity in the direction of spread of excitation by interneural facilitation, but in Calamactis incremental spread proceeds from oral disk to physa and not in the opposite direction, because stimuli given to the physa evoke responses anywhere along the retractor muscle if they are 0.5 sec. apart and no response at all if the interval is shorter.

**Summary**

1. Two kinds of electrical potentials can be recorded from the surface of the retractor muscle of the anemone, Calamactis, during rapid contraction. These are large muscle action potentials and smaller pulses which are thought to be nerve spikes. The latter resemble nerve impulses of higher organisms in that they are all-or-none and of short duration.

2. A nerve spike follows each of a pair of electrical stimuli, but the muscle potential and contraction occur only after the second shock, indicating that facilitation is required at the neuromuscular junction.
3. The size of the muscle potential and of the contraction are correlated with the interval between paired electrical stimuli. Maximum size is reached when stimuli are 200 msec apart even though the minimum effective interval is 30 msec.

4. A muscle potential precedes contraction only along the upper part of the retractor muscle and this is the part that contracts rapidly during the withdrawal response. The lower retractor does not contract.

5. Conduction velocity along the upper retractor is higher than along the lower. The histological correlate of rapid conduction is a nerve net with large, long, longitudinally oriented fibres.

6. The refractory period of the conducting system of the upper retractor is shorter than that of the lower retractor. Consequently, spread of excitation toward the aboral end is limited if paired stimuli are further apart than 250–300 msec.

7. A mechanical stimulus which is just strong enough to elicit a withdrawal response evokes a single muscle potential of maximum size, suggesting that two nerve impulses closer together than 200 msec precede the muscle potential. Stronger mechanical stimuli evoke a burst of muscle potentials.

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