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REPETITIVE POTENTIALS FOLLOWING BRIEF ELECTRIC STIMULI IN A HYDROID

BY ROBERT K. JOSEPHSON
University of California, Los Angeles

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INTRODUCTION

Only recently have electrical correlates of conduction in coelenterates been recorded (Horridge, 1954; Yamashita, 1957; Passano, 1958; Passano & McCullough, 1960). But before that, such progress had been made with indirect methods that Pantin, the leading student of this group, was able to state: 'Though its electrical concomitant has not yet been detected, the existence of the "all-or-nothing" impulse is as clear in coelenterates as in vertebrates' (Pantin, 1952).

The properties of the coelenterate nerve net that had been supposed to indicate decremental conduction—that is, increased distance of excitation spread with stronger mechanical stimuli and greater responses near the stimulated area than at some distance—were convincingly explained by Pantin (1935*a*) on the basis of repetitive impulses engendered by the mechanical stimulus and of interneural facilitation in the nerve net. Pantin found no evidence for decremental spread following electric shocks in the sea anemone *Calliactis*. Using muscle response as an indicator, he demonstrated that an electric shock in this species initiates a single all-or-nothing event in the nerve net.

Recently, however, distance of spread of excitation varying with the stimulus strength and intensity of response varying with the distance from the point of stimulation have been found following single, brief, *electrical* stimuli in colonial corals (Horridge, 1957) and hydroids (Josephson, 1961). This paper will present evidence from electrical recordings that such responses, at least in hydroids, are due to repetitive impulses in the conducting system, even following brief electric shocks which are not much above threshold intensity.

MATERIALS AND METHODS

The species used in this study was the gymnoblastic hydroid *Cordylophora lacustris* Allman. Hydroid colonies were grown in sea water diluted to 10% by aged water from a freshwater aquarium. The temperature of the cultures was about 21° C. Several times a week the animals were fed on newly hatched brine shrimp. Under these conditions *Cordylophora* forms colonies consisting of a single stolon, firmly attached to the substrate, and, at 2–5 mm. intervals, short, upright stalks, each bearing a single hydranth. The stolons are 0·13 mm. in diameter; the stalks bearing the hydranths and the hydranths themselves are each about 1 mm. high. The older polyps and the coenosarc regress as new polyps form, and a complete colony grown under such conditions contains only three to six hydranths.

Electric potentials were recorded from the stolons of *Cordylophora* by stainless steel micro-electrodes etched and insulated as described by Green (1958). The electrodes used had tip diameters of from 1 to 3 μ. Potentials were recorded between one or two micro-electrodes and an indifferent silver electrode in the solution surrounding the polyp. Conventional capacitor-coupled amplifying and recording equipment was used.

Electric stimuli were delivered to the colonies through fine silver wires (0·2 mm. in diameter) insulated to the tip and placed one on each side of the stolon (Fig. 1). The stimuli were square pulses produced by a Grass S4 stimulator and were, unless otherwise stated, 0·5 msec. in duration.

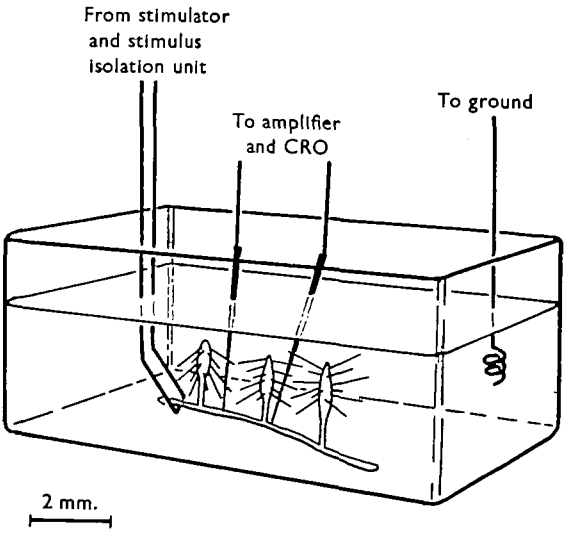

Fig. 1. The method of recording. The hydroid colony is shown as it might appear following a stimulus of not much above threshold intensity; the polyp nearest the stimulating electrodes is partially contracted, the next polyp is somewhat less contracted, and the most distal polyp is unaffected.

Five minutes were allowed between each stimulus or group of stimuli constituting a single test. Only with such long intervals between trials can repeatable results be obtained. The threshold continually rises and the characteristic tendency to repetitive firing declines with stimulus frequencies as low as one per minute. All experiments were done on colonies completely submerged in dilute sea water. If only a few stimuli are given at each trial, the colonies show no signs of deterioration during the course of an experiment, and measurements can be made for many hours.

RESULTS

Large negative potentials, 0·05–15 mV. in amplitude, can easily be recorded from an electrode impaling the stolon of *Cordylophora*. These potentials are not spontaneous; they are only seen following stimulation. The amplitude of the potentials depends as much upon the particular electrode used as on any other factor. Small changes in the position of the recording electrode cause little alteration of the measured response. There is considerable variation in the duration of the potentials; they can be as short as 20 msec. or as long as 120 msec. Similar potentials can also be recorded from an

lectrode inserted in the wall of the hydroid body. The flexible hydranth, however, is more difficult to impale than the rigid stolon, and the latter was the preferred location for measurement in this study. Since the potentials recorded are not yet known to be equivalent to nerve action potentials, the terms 'impulse' or 'spike' will not be applied. They will be called 'pulses' in this discussion.

A train of pulses can follow a single shock of less than $1\frac{1}{2}$ times threshold intensity (Fig. 2). As many as twelve pulses in a burst lasting several seconds can be produced

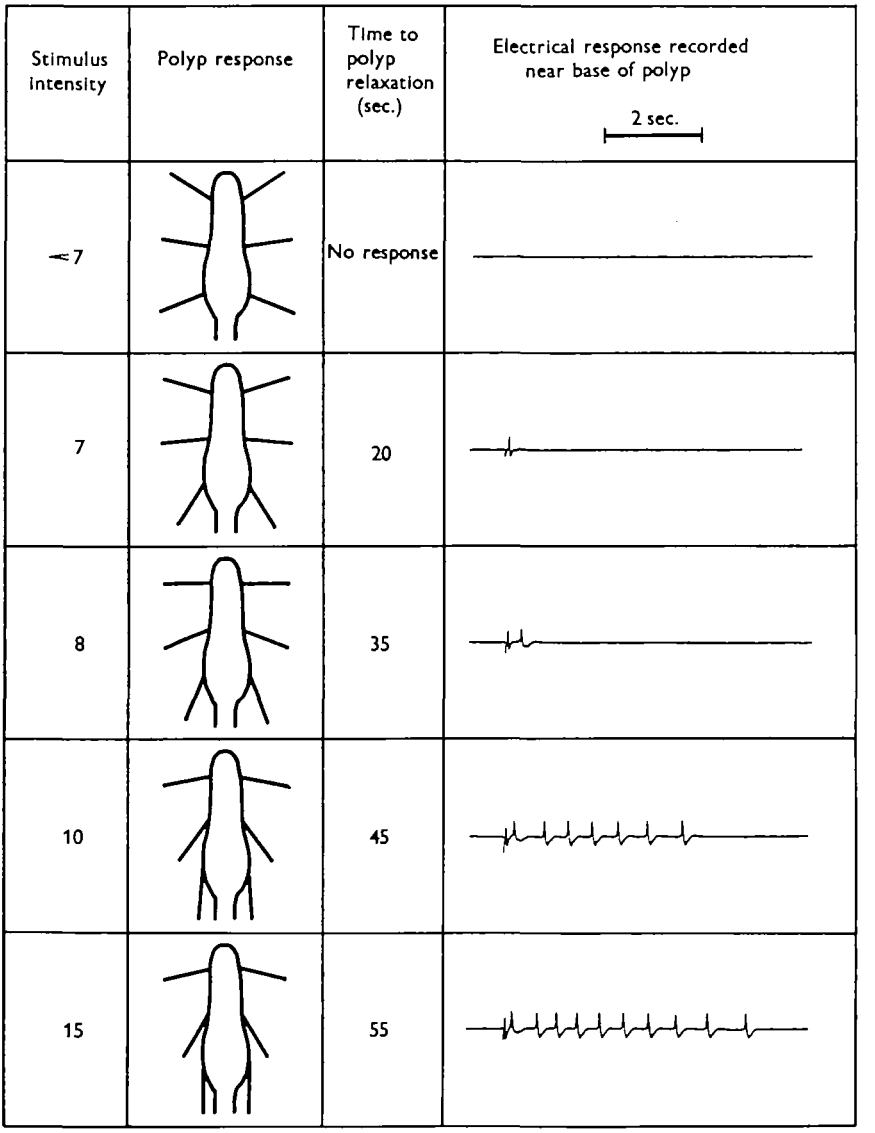

Fig. 2. Comparison of the response of a polyp and the electrical potentials recorded in the stolon near the polyp stalk. Only tentacle depression is shown in the diagrams of the responding polyp; hydranth shortening, which also occurs, is not shown. The times to relaxation are approximate since the end point (complete relaxation) is difficult to determine exactly. The deflection in the first electrical record is the stimulus artifact.

by a single 0·5 msec. stimulus. The shape of the pulses in such a series is quite characteristic. The first is short, 20–40 msec., and is usually the smallest in amplitude of the series. It tends to be more diphasic than any of the others, often being followed by a marked positive potential. The second pulse follows the first after an interval of about 200 msec. It is usually the longest pulse of the series. The remaining pulses are all similar in shape and height, usually having a form intermediate between that of the first two pulses, being higher and longer than the first and more diphasic than the second. They follow one another at increasing intervals. The interval between the second and third pulse is usually longer than that between any except the last few pulses of the train.

The number of pulses in a burst, but not the shape of any single pulse, varies with the stimulus strength. The threshold for single pulses is sharp (Fig. 3). A just supra-threshold shock initiates a single pulse, a stimulus $1\frac{1}{3}$ times threshold frequently gives a short burst, and stronger stimuli can evoke longer bursts.

Similar potentials have also been recorded from the stolon of the hydroid *Tubularia crocea* during the conduction of excitation. Unlike *Cordylophora*, little tendency to repetitive firing was seen in the few experiments done on this species.

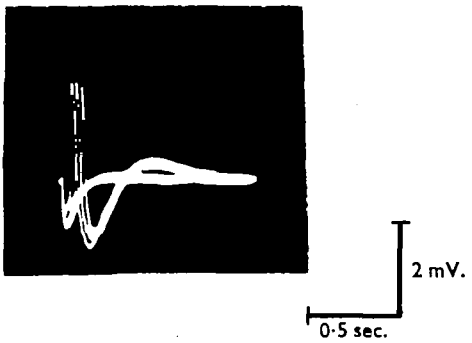

Fig. 3. Five superimposed sweeps taken 5 min. apart showing the sharp threshold and all-or-none nature of single pulses. The stimulus intensities were 8, 9, 10, 11 and 12.

Correlation between electrical activity and polyp response

When the stolon of a *Cordylophora* colony is stimulated, a wave of polyp contraction is initiated which involves some or all of the polyps of the colony. The tentacles are all depressed and the hydranth body shortened in a contracting polyp. The number of polyps responding, i.e. the distance of spread, is a function of the stimulus strength. With a just supra-threshold shock, one to three polyps near the electrodes contract. Increasing the stimulus strength causes more polyps to contract, and also leads to greater contraction in the polyps which previously responded. A second shock shortly following an effective one can increase the number of responding polyps and the degree of polyp contraction in the already active polyps.

The electrical activity measured in the stolon near the junction of the stolon and a stalk is exactly correlated with the behavioural response seen in the polyp on the stalk (Fig. 2). The larger the number of pulses recorded, the faster is the depression of the tentacles, the greater is the depression, and the longer is the period until relaxation is

complete. The polyp contraction is slow (taking several seconds) and smooth; it gives no hint of underlying repetitive activity.

Polyp responses are often graded with distance from the stimulating electrodes. The pulses, however, are not conducted with decrement. They are similar in height and shape wherever the colony is stimulated. When recording is made simultaneously from two electrodes separated by some distance in a long colony, the mechanism of graded response becomes apparent. The number of pulses seen at the two recording sites is often different; the electrode more removed from the point of stimulation records fewer pulses than does the electrode near the stimulated area. Polyps far from the stimulating electrodes, then, receive and respond to fewer pulses than do those near the point of stimulation. When the conduction velocity through the stolon is considered, it can be shown that it is the first pulses recorded in the electrodes nearer the point of stimulation which do not reach the distal electrode. The first pulses are not transmitted the length of the colony, but do pave the way for future pulses, a process suggestive of the interneural facilitation described by Pantin (1935*a*) from the oral disk of *Calliactis*.

Facilitation in the distance of excitation-spread can also be demonstrated with repetitive stimuli. In one experiment, for example, three shocks were given to the stolon at 1 sec. intervals. Two pulses were recorded from an electrode near the stimulated area following the first stimulus and one pulse was recorded following each of the next stimuli. A more distal electrode, however, recorded only one pulse, that following the third stimulus. The three pulses created by the first two stimuli did not reach the distal electrode, while the fourth pulse did.

Frequently two or three adjacent polyps form a behavioural unit; they all contract to the same degree following a stimulus and no one polyp of the group can be made to respond alone. If two electrodes record simultaneously from different places in a group of polyps, similar potentials are seen from each. Often all the polyps of a small colony form a single behavioural unit and potentials recorded anywhere in the colony are similar. It is only with electrodes widely separated in long colonies containing at least four polyps that facilitation of the distance of spread can be predictably demonstrated.

Characteristics of the pulses

(1) *Conduction velocity*

By measuring the latency between the appearance of pulses at two recording electrodes, the conduction velocity in the stolon of *Cordylophora* is shown to be 2·7 cm./sec. (average of ten measurements, range from 2·1 to 3·3 cm./sec., 22° C.). A similar figure is obtained by measuring the distance between the stimulating electrodes and a single recording electrode, and dividing by the latency between a just supra-threshold shock and the appearance of the pulse at the recording site. This indicates that the pulses originate in the stimulated area. The conduction velocity is constant for each pulse of the series, indicating a common conduction pathway.

(2) *Refractory period*

Attempts to measure the refractory period of the conducting system were made by giving a pair of shocks to the stolon separated by varying intervals. If the intensity was just supra-threshold, a single shock caused one pulse, and a pair of shocks, usually

only when separated by a sufficient interval, produced two pulses. The sufficient interval proved quite variable, and ranged from 12·5 to 700 msec. in different preparations. Since both stimuli were just above threshold, this would seem to be a measure of the relative refractory period. In one preparation, however, two shocks produced two pulses even with a vanishingly short interval between stimuli. This was verified in this preparation repeatedly. To investigate very short intervals, stimuli of 0·1 msec. duration were used. When the intensity was adjusted so that one shock gave one pulse, two shocks separated by any interval from 500 to 0·2 msec. consistently produced two pulses. Shorter intervals than 0·2 msec. could not be used with the equipment available. It would appear that the refractory period of the stolon of *Cordylophora* is exceedingly short or, more probable, non-existent, and that the minimum effective interval between two shocks seen in other preparations was a measure of something other than the refractory period. The lack of a refractory period and its significance for an understanding of the repetitive firing are discussed below.

If the interval between a pair of just supra-threshold shocks is greater than about 200 msec., the interval between the induced pulses is the same as the interval between the stimuli. If the interval between the stimuli is less than 200 msec., the pulses are always separated by about 200 msec. In the preparation mentioned above, even two stimuli 0·2 msec. apart evoked two pulses separated by 200–250 msec.

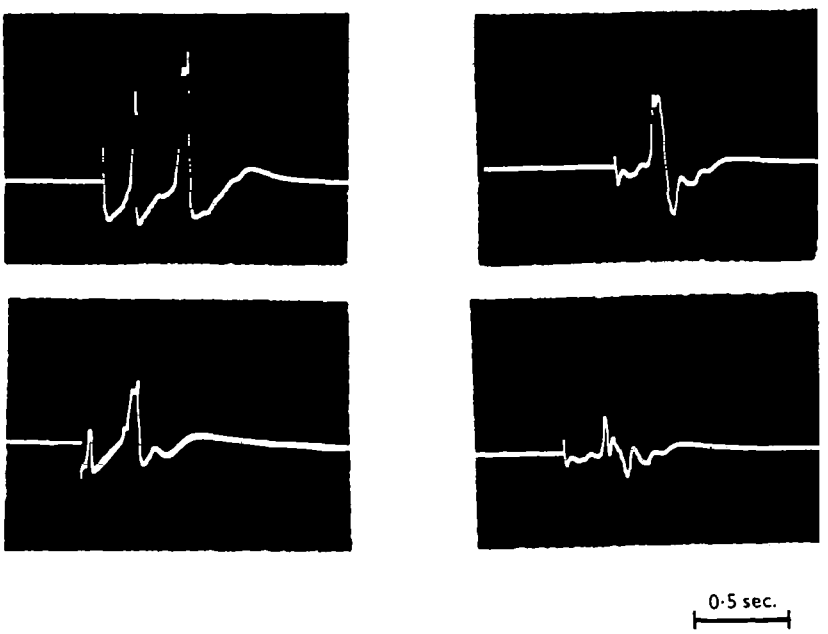

Fig. 4. Some atypical potentials which show the compound nature of the pulses.

(3) *The form of the pulse*

Although pulses are usually smooth in shape, potentials are occasionally recorded which reveal their compound nature (Fig. 4). On one occasion the first pulse of a train was a small asynchronous burst of potentials. These small potentials gradually coalesced so that the ninth and final pulse of the burst resembled those typically seen

Sometimes the single pulses following just supra-threshold shocks become more ragged with repetitive stimulation, but more commonly they retain their smooth form.

The shape changes seen in successive pulses in a burst are also seen in single pulses following repetitive stimulation. For example, if two just supra-threshold shocks are given to the stolon at a 1 sec. interval, the second pulse recorded is less diphasic than the first and is longer in duration—exactly the same changes as seen between the first two pulses of a burst following a single stronger stimulus. The change in the shape of the pulse following a second shock is not all-or-none, but appears gradually with decreasing intervals between the shocks, first being noticeable at an interval of about 5 sec. The different shapes of pulses in a burst, then, do not necessarily indicate different conducting systems, but more probably changes in the properties of a single conducting system.

(4) *Interaction between pulses*

Two pulses started from stimulating electrodes at opposite ends of a colony cancel one another where they meet and only the first pulse reaching the recording electrode is seen. If the pulses are timed so that they meet at the recording electrode, the amplitude of the recorded potential is not greater than that of a single pulse. On the contrary, it is somewhat smaller, although of a longer duration than the single pulses. This indicates that no new elements are contributing to the potentials during conduction in different directions. The lack of summation of two pulses where they meet and their mutual cancelling show that conduction in different directions probably involves the same conducting elements.

(5) *Facilitation of pulse height and number*

The amplitude of single pulses following just supra-threshold stimuli can usually be increased by repetitive stimulation. A change in the height of the second pulse is often seen with a pair of stimuli separated by as much as 10 sec. The facilitation of pulse amplitude reaches a maximum at stimulus intervals of 1 sec., and does not change with still smaller intervals between shocks. The increase in pulse amplitude following repetitive stimulation can be as much as 70%. Often, however, little or no facilitation can be demonstrated, and infrequently a second pulse is smaller than the first. The change in the amplitude of the first two pulses of a burst is probably due to the same facilitation that affects single pulses with repetitive stimuli. Similarly, in a burst the second pulse is usually higher than the first, although occasionally the two pulses are equal in height and atypically the second pulse is smaller than the first.

The number of pulses following a stimulus can be increased by repetitive stimulation. In three colonies in which this was examined with paired shocks, the facilitation as measured by the greater number of pulses produced by the second shock was maximal at stimulus intervals of 1 sec. The facilitation declined on either side of this value, and was not detected at stimulus intervals of 0·1 or 10 sec. It is interesting to note that facilitation of pulse number was not seen with just supra-threshold stimuli. As was stated above, a pair of such stimuli produce just two pulses.

Facilitation of pulse number is only seen with the first two or three stimuli of a series. After the first few shocks, the number of pulses created by each shock steadily declines (Fig. 5). Even single pulses cannot be obtained after seven to ten shocks when

using stimulus frequencies of 1/sec. or 5/sec. Occasionally the pulses of a very long burst evoked by strong repeated stimuli decline in amplitude toward the end of a train, but more often they retain the same height throughout the course of a burst.

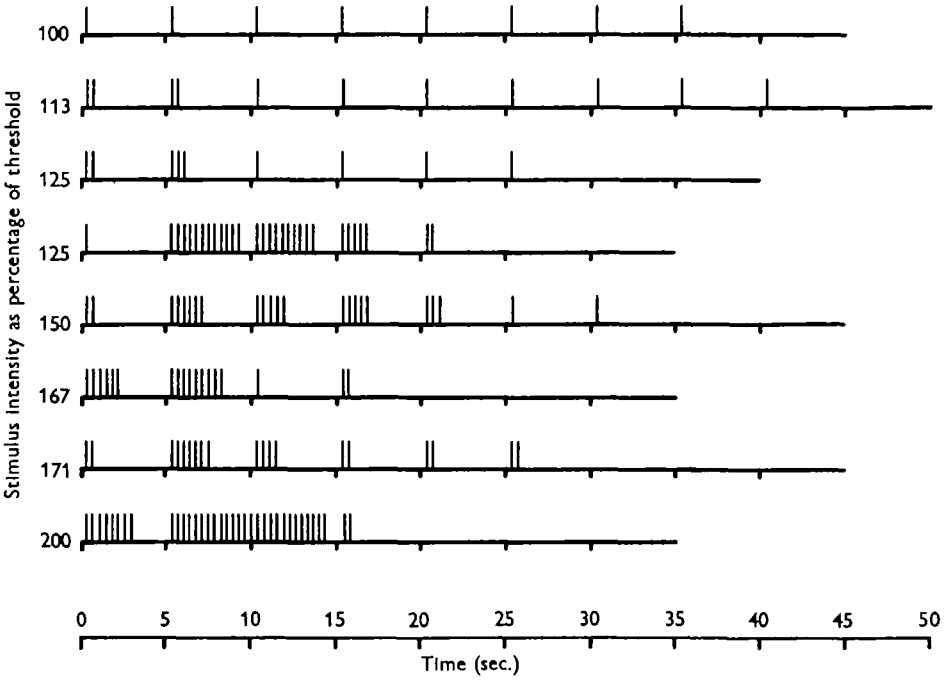

Fig. 5. The number of pulses initiated by each shock of a series at 5 sec. intervals. These are typical examples, and are taken from experiments done on several different colonies. The short bars below each line indicate shocks, the bars above each line indicate the pulses evoked.

Mechanical stimulation

Cordylophora colonies are quite sensitive to prodding of the stolon. Such stimulation usually leads to contraction of all the members of the colony. Pulses identical in form to those seen following electrical stimuli are recorded from the stolon following mechanical stimulation (Fig. 6). Gentle prodding of the stolon produces one or two pulses, more forceful prodding produces a burst of pulses. Prodding of the polyp usually initiates no electrical response in the stolon (nor does it cause contraction of neighbouring polyps) but pinching a polyp with forceps produces a burst of pulses and contraction of the polyps of the colony.

The site originating the bursts

One would expect the greatest concentration of nervous elements in a hydroid to be in the polyp body. The hydranth, therefore, falls under suspicion as the area which might initiate the repetitive activity recorded in *Cordylophora*. Removing all the hydranths from a colony, however, does not change the response seen in the stolon following stimulation. The stolon itself can give rise to a burst of potentials, and there seems to be no reason to believe it is not the stolon which normally initiates repetitive activity following stimuli applied to it.

In a few experiments involving simultaneous recording from two electrodes at increasing distances from the stimulating electrodes, evidence was found of pulses originating other than at the stimulated point. In these cases, following a pulse propagated in the usual direction, a second pulse appeared in the electrode more distal to the stimulating electrodes *before* it was seen at the recording electrode closer to the stimulated area. This pulse must have originated closer to the distal electrode than to the proximal one, and hence could not have arisen at the point of stimulation. Such multiple origin of pulses is not common; evidence for it was seen in only 3 out of over 100 simultaneous recordings from two electrodes.

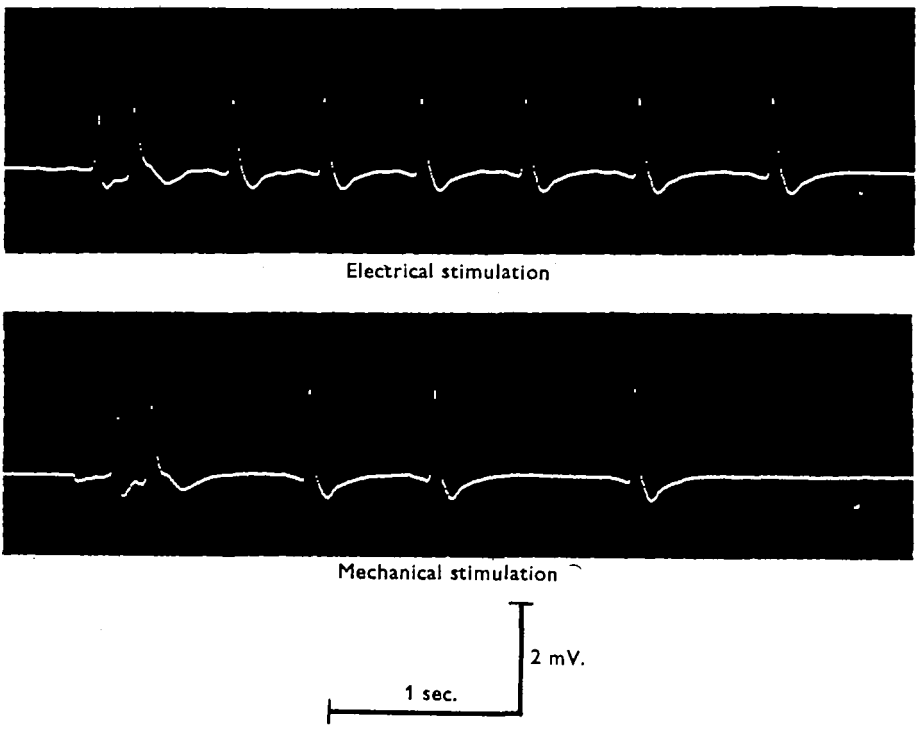

Fig. 6. Comparison of the recorded potentials following mechanical and electrical stimulation. The electrical stimulus was a single shock, 40% above threshold. The mechanical stimulus was a prod to the stolon. The positive deflexion of the base-line preceding the pulses in the lower record is probably a stimulus artifact.

Coenosarc movements during conduction

When the coenosarc of *Cordylophora* was observed with a magnification of 100 diameters, tissue movements were sometimes detected during the time a pulse was transmitted through the stolon. The movements usually consisted of a brief jerk, and involved displacement of certainly less than 15 μ. They were usually only seen following the first supra-threshold stimulus to a well rested colony. A second stimulus shortly after a first, although it often produced a longer train of pulses, usually did not cause discernible tissue movements. Although accurate measurements were not made, such tissue movements appear to be approximately temporally coincident with the passage of a pulse through the stolon.

Coenosarc movements could be due to active contraction of the observed area or to movements elsewhere in the colony mechanically transmitted through the stolon. They are not caused only by hydranth contraction, for they were also observed in colonies from which all the hydranths had been removed. Two observations on such hydranth-less colonies suggest that coenosarc movements may not be due to contraction of the area being observed. First, movements were often seen of only the fluid in the central space of the coenosarc and not of the tissue itself. Secondly, in one colony the movement seen was clearly an expansion of the coenosarc, which became more closely apposed to the outer perisarc. These observations indicate that contraction of the coenosarc of some area other than that being observed can cause a shift of fluid and an increase in the internal pressure of the stolon. Although this has not been investigated, the areas which come under suspicion are the terminal ends of the stolon, where the coenosarc is known to be contractile in some other hydroids (Berrill, 1949; Hale, 1960), or the cut ends of the polyp stalks.

Artifacts due to tissue movements do not appear to contribute to the recorded potentials. Pulses recorded when there was discernible movement near the electrode were identical to those recorded when no movement could be seen. When the wall of the stolon is displaced, there is produced a sudden, clearly observable shift of fluid and tissue in the stolon which is much greater than the movements described above. Such movements in the stolon can introduce artifacts in the electrical records (Fig. 6), but these are many times smaller than the pulses and do not interfere with measurements.

DISCUSSION

The significance of repetitive firing

The first point to be gained from this study is that while many conclusions can be drawn regarding conduction in coelenterates by the use of indirect methods, some properties of the conducting systems are elucidated only by electrical recordings. The muscular movements of *Cordylophora*, for example, are smooth and reveal no indication of underlying repetitive activity. Without direct measurements, one would suspect repetitive firing following single shocks only after eliminating other possibilities. Electrical measurements, however, make immediately evident the repetitive activity usually following stimulation. Analysis of conduction in *Cordylophora* by indirect methods is further complicated by the non-linear relation between stimulus strength and number of pulses initiated and by the changing effectiveness of shocks in a series, there being an early facilitation of pulse number followed by a decline in the number of pulses per shock (Fig. 5).

Some properties of excitation-spread following electrical stimulation in some colonial corals and hydroids are difficult to explain on the basis of Pantin's now classical scheme of nerve nets. These properties are: (1) the greater distance of spread with more intense single shocks; and (2) the greater response in polyps near the point of stimulation than in those more removed from the stimulated area. Horridge (1957), in discussing his observations on corals, suggested that these properties could be explained on the basis of a nerve net in which only a portion of the available elements in any area carry a nerve impulse when a wave of excitation passes. He postulated that the greater distance of spread with stronger stimulation was due to a larger number o

units being initially activated and hence a greater probability of the excitation finding long pathways in the net. The decrease in the magnitude of the polyp responses with greater distance was suggested to be correlated with a decrease in the density of active units with greater distance from the stimulated area.

It seems more probable on the basis of this and previous studies (Josephson, 1961) that the anomalous excitation-spread in some colonial coelenterates is due to repetitive activity, even following single shocks. This is certainly the case in *Cordylophora*. The increased distance of spread with stronger shocks finds its explanation in more pulses being initiated, and hence more barriers crossed by interneural facilitation (or its counterpart if spread in hydroids turns out to be by means of some other system than the nerve net, a proposition which now seems unlikely). The polyps near the stimulating electrodes respond more than those at some distance because they receive more pulses. Thus the explanation advanced by Pantin (1935*a*) for responses following mechanical stimulation also applies to responses following electrical stimulation in some species.

Repetitive firing following prolonged or very strong electrical stimuli is frequently encountered in animals. Even repetitive firing following brief electric shocks is not uncommon, and has been reported especially for crustacean nerves and giant-fibre systems in annelids (see, for example, Barnes, 1934; Bullock & Turner, 1950; Kao & Grundfest, 1956, 1957). Repetitive activity following prolonged stimulation is seen for luminescence in sea pens (Buck, 1953; Nicol, 1955, 1958; Davenport & Nicol, 1956) and for polyp contraction in some colonial hydroids (Josephson, 1961). Pantin (1935*b*) reported supernumerary contractions in some specimens of the sea anemone *Calliactis* following a battery of stimuli, indicating after-discharge in the nerve net, and Pantin & Vianna Dias (1952) reported a similar phenomenon in the jellyfish *Aurellia*. This after-discharge, at least in *Calliactis*, is unlike repetitive firing in *Cordylophora* in that it bears only a very indirect relation to the stimulus, both in time of appearance and in number of pulses. Despite this wide occurrence of repetitive firing in the Animal Kingdom, it was still surprising to find patterned bursts of output following stimulation, obviously an integrating mechanism, to be so well developed in such phylogenetically primitive and seemingly undifferentiated tissue as that of a hydroid stolon.

Conduction in hydroids

The potentials recorded from the stolon of *Cordylophora* are associated with the conduction of excitation. They may be created by the conducting system itself or only a reflexion of activity in the conducting system. They could, for example, be muscle potentials associated with conduction in a nerve net. There would seem to be two important problems related to the electrical correlates of conduction in a hydroid stolon: (1) the nature of the conducting system; and (2) the origin of the potentials.

(1) *The conducting system*

Some of the properties of conduction are best explained on the basis of a nerve net. The compound nature of the potentials indicates activity in a number of parallel channels. The failure of such potentials to become temporally dispersed with increasing distance from the point of stimulation, a phenomenon characteristic of nerve

trunks, is to be expected from a system like a nerve net with many lateral connexions. In such a system, the fastest conducting elements could continually excite neighbouring parallel fibres by cross-fibres joining them. The conduction velocity of the whole system, then, would be determined by the conduction velocity of its fastest elements. In the best case seen in this study demonstrating the compound nature of these potentials (described above) it was the early pulses of a burst which were dispersed. The coalescing of the small potentials into the more usually formed pulses at the end of the burst is interpreted as due to an increase in the effectiveness of the lateral connexions because of facilitation, with the result that for the later pulses the system fired as a nearly synchronous whole. An increase in the effectiveness of lateral connexions and perhaps recruitment of more conducting elements, both due to interneural facilitation, may be the basis of the changes in the shapes of pulses during a burst. And, in further support of nervous conduction, Mackie (1961) has recently found neurons histologically in the coenosarc of *Cordylophora*.

Muscle is the only tissue other than nerve generally credited with the ability to conduct excitation over some distance in metazoans. There is conflicting histological evidence for muscular tissue in hydroid stolons. Allman (1853), Schulze (1871, 1873), Citron (1902), Berrill (1949), and Hale (1960) failed to find muscle fibres in hydroid stolons; Hamann (1882) reported epithelial muscle cells in this region. The coenosarc of hydroids is capable of movement. Evidence for contractility has been seen in *Syncoryne* (Josephson, 1961), and a limited region of the coenosarc is contractile in *Obelia* (Berrill, 1949) and *Clytia* (Hale, 1960). The coenosarc of *Cordylophora* shows exceedingly slow shape changes, and small twitches are occasionally—but importantly not always—seen during the conduction of pulses. Muscular conduction through the stolon cannot be ruled out, but, because there is often conduction without any visible movement of coenosarc tissue, seems improbable.

The conduction velocity in *Cordylophora* is slower than one expects from nervous conduction. Conduction in a diffuse nerve net would involve many synaptic delays, so a slow conduction velocity is not totally unexpected.

(2) *The origin of the potentials*

The short duration of the potentials makes it seem likely that they are due to activity of nervous tissue. The separate potentials making up the compound pulse are probably shorter or, at a maximum, the same duration as the pulse. That they are shorter is indicated in some of the records showing the compound nature of the pulses, where evidence for potentials of quite short duration is occasionally seen (notice the short potential superimposed on the longer pulse in the first record of Fig. 4). Although it is unwise to speculate on the membrane potential *v.* muscular contraction relations in a phylum in which they have not yet been studied, a 20 msec. action potential seems quite fast for an animal whose contractions, as seen in the polyp, usually take several seconds to complete. The only clear recordings from single nerve fibres in coelenterates which have been published are those of Horridge (1954). As closely as I can measure from his figures, the action potentials from the large fibre system of *Aurellia* are about 8 msec. in duration, rather long when compared to those of other animal phyla and certainly not too short to be considered as components of the compound potentials described in this study.

The size of the potentials (up to 15 mV.) and the ease with which they can be recorded are not what one would expect from a diffuse nerve net. The stolons of hydroids may be close to ideal for electrical recording. Shunting would be minimized because of their small dimensions and by the surrounding cover of thick perisarc which may be electrically insulating. Recording from hydroid stolons might be equivalent to recording from a fine nerve completely immersed in oil. Potentials can also be easily recorded from the hydranths of *Cordylophora*, however, where these conditions do not prevail. The explanation for the large size of the pulses probably lies in the summing of simultaneous potentials from a number of elements in a system held in synchrony because of the activity of lateral connexions.

Although the evidence is far from incontrovertible, it seems to indicate that both conduction in *Cordylophora* stolons and the potentials associated with this conduction result from activity in a nerve net.

The initiation of repetitive firing

For a system to fire repeatedly following a single brief shock, it must have a memory. It must 'know' after it has fired once that it has yet to fire again. An axon with its all-or-none action potentials has no such memory. In firing and the subsequent restoration of the membrane it destroys evidence of the local responses which initiated the firing. Some cell bodies, synaptic areas, and sensory terminations have a memory. These areas often do not fire in an all-or-none fashion and can maintain a local depolarization capable of initiating spikes in some other part of the cell. Such areas are integrative in that they can sum activity, often both excitatory and inhibitory and from many inputs, over some time period (see Bullock, 1957, for a discussion of the role in integration of membranes which normally do not show propagated spikes). Since *Cordylophora* stolons show repetitive activity following brief shocks, it may be concluded that the area initiating the pulses (it need not be part of the conducting system itself) is like such integrative areas in that it has a memory, and therefore probably does not show all-or-none action potentials. One would not expect such an area to have a refractory period, and the inability in one case to demonstrate a refractory period in *Cordylophora* is in confirmation of the concept of a locus initiating the pulses which responds in a graded manner. The minimal interval between a pair of effective just supra-threshold stimuli more usually seen is probably due to decay with time of the excitation at the initiating area coupled with the refractory period of an all-or-none conducting system.

A minimal interval between successive potentials following progressively more closely spaced shocks has been reported several times for vertebrate nerves. It has been explained as due to: (1) the second potential travelling in an area left refractory by the activity of the first potential and having an initially slower conduction velocity (Gasser & Erlanger, 1925); or (2) a longer latency for the second potential because of a prolonged shock-response interval (Rosenblueth, Alanis & Mandoki, 1949). The similar phenomenon in *Cordylophora* may be due to yet another mechanism; a slow return in the sensitivity of the conducting system following firing, either directly or secondarily induced by the stimulus, until its threshold matches the intensity of a prolonged excitatory state maintained by an impulse-initiating area.

SUMMARY

1. Electrical pulses (amplitude −0·05 to −15 mV.; duration 20–120 msec.) have been recorded from the stolon of *Cordylophora lacustris* following stimulation. These pulses are propagated with an average velocity of 2·7 cm./sec. at 22° C.

2. Brief electric shocks of little more than threshold intensity can evoke bursts of pulses. The number of pulses in a burst increases with stimulus intensity, but the shape and size of individual pulses do not.

3. Repetitive stimulation causes facilitation of both size of single pulses and number of pulses in a burst. Refractory period, if present, is variable. The minimum interval between two pulses is about 200 msec.

4. Mechanical stimulation evokes pulses identical to those evoked by electrical stimulation.

5. The greater the number of pulses recorded in the stolon near a polyp, the greater and faster is the contraction of that polyp.

6. The number of pulses, but not their individual sizes, decreases with increasing distance from the point of stimulation.

7. It is concluded that conduction in the stolon and the electrical pulses are due to nervous activity and that the conducting system is a network having interneural junctions which sometimes require to be facilitated.

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