

## THREE PARALLEL CONDUCTING SYSTEMS IN THE STALK OF A HYDROID

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### INTRODUCTION

The concept of a single conducting system in coelenterates, represented by 'the nerve net', is being replaced by the realization that there are often multiple conducting systems co-existing in the same tissue. Romanes, in 1877, demonstrated two kinds of conduction in the bell of the jellyfish *Aurelia* which were distinguished by having different thresholds, conduction velocities, and behavioural correlates. More recent work by Bozler (1926*a, b*) and Horridge (1955, 1956*a, b*) make it appear that multiple conducting systems in the subumbrellar epithelium is a general feature of coelenterate medusae. Multiple conducting systems are also known for coelenterate polyps. Anemones, the most studied coelenterate polyps, show rapidly conducted responses, which can be ascribed to activity in a nerve net, as well as very slowly conducted waves of muscle contraction (e.g. Batham & Pantin, 1954). Behavioural evidence indicates that there are two conducting systems in the column and oral disk of *Cerianthus* (Horridge, 1958), in the oral disk of the alcyonarian *Heteroxenia* (Horridge, 1956*c*), and in the coenosarc of the hydroid *Hydractinia echinata* (Josephson, 1961*a*). Mackie (1960) has clearly demonstrated two histologically distinct nerve plexuses in the same epithelium in the chondrophore *Velella* which should be regarded as a polyp (Mackie, 1959). Electrical recording indicates that there are at least two conducting systems in the column of *Hydra* (Passano & McCullough, 1962, 1963), even though histologically there appears to be but a single complete nerve plexus (Semal-Van Gansen, 1952). This paper will give evidence from electrical recording that there are three parallel but distinct conducting systems in the stalk of the hydroid *Tubularia*.

### MATERIALS AND METHODS

The animals used in this study were *Tubularia larynx* Ellis & Solander,\* collected from logs and pilings in the northern end of the Cape Cod Canal. *Tubularia* is a large hydroid; the hydranth is 2-3 mm. long and the stalk can be several cm. long. The hydranth and stalk are joined by a short, contractile neck region. The hydranth has two sets of tentacles, the large proximal tentacles and the smaller distal tentacles which surround the mouth. The distal tentacles and mouth are borne on a contractile proboscis.

*Tubularia* polyps were fastened to a cork platform on the bottom of a dish of sea water with staples made from small insect pins. Cold water was passed through a

\* I wish to thank Dr W. J. Rees of the British Museum for identifying this species from preserved material.

glass coil in the dish to maintain the sea water surrounding the polyp at 19° C. The stalk was stimulated electrically through silver wires, insulated to the tips and placed one on each side of the stalk. Unless otherwise stated, the stimuli were square pulses of 1 msec. duration. Electrical potentials were recorded between one or two electrodes in or on the polyp and an indifferent electrode in the sea water surrounding the polyp. The potentials were amplified with capacitor-coupled amplifiers having long time-constants, and the final display was on an oscilloscope or pen-writer.

The stalk of *Tubularia* is covered with a thick perisarc, so recording electrodes which penetrate the stalk must be strong and rigid. Micro-electrodes of platinum-iridium or stainless steel, fixed in a manipulator, were found to be satisfactory. The micro-electrodes were etched electrolytically (Green, 1958; Wolbarsht, MacNichol & Wagner, 1960) and insulated with lacquer. The indifferent electrode used with steel micro-electrodes was a coil of silver wire; the indifferent electrode used with platinum-iridium micro-electrodes was also of platinum-iridium. The platinum-iridium electrodes seemed to offer no advantages over the steel, and as the latter are easier to make they were used in most of the experiments. Freshly made metal micro-electrodes were often too noisy to record potentials of the small size found in the *Tubularia* stalk. Pushing the tip of the electrode into a piece of cork usually reduced this noise, presumably by removing some of the insulation from the tip and reducing the electrode resistance.

The hydranth is quite mobile and recording from it presents some special difficulties. Electrodes fixed in a manipulator are not satisfactory as the animal either pulls off the electrodes or they tear the hydranth tissue. Some successful recordings were made with electrodes etched from small insect pins (minuten nadeln) soldered to a loop of very thin copper wire. These flexibly mounted electrodes were inserted into the hydranth by hand. But the most satisfactory method found for recording from the hydranth was by means of suction electrodes, similar to the 'minute' suction electrodes used for recording from the toad heart by Kanno (1963). These electrodes were made by drawing plastic tubing (Tygon) over a small flame until it ended in a flexible, hollow filament. A silver wire was mounted in the tubing near the drawn end and the other end of the tubing was attached to a small syringe. Sea water was drawn into the tubing with the syringe until it reached the silver wire. The tip of the filament was then placed against a portion of the hydranth and the plunger of the syringe was drawn back until the suction was just great enough to hold the tip in place. The suction generally used was 40–50 mm. Hg, as calculated from the change in the gas volume of the syringe and tubing as the plunger was withdrawn. Potentials were recorded between the silver wire in the tubing and an indifferent electrode in the bath. The suction electrodes used had internal diameters at the tip of 30–90  $\mu$ . With such electrodes it is possible to record from any part of the hydranth, including bases, sides and tips of the proximal and distal tentacles. Because of their flexibility, the electrodes freely follow the hydranth as it contracts.

Some caution must be used in evaluating potentials recorded from suction electrodes on *Tubularia*. Often brief, spontaneous potentials are seen which are recorded from only one of two adjacent electrodes and which are apparently caused by the presence of the electrode as their frequency increases if the suction is increased and declines

when the suction is reduced. Such potentials can be troublesome in long-term recordings, but were not a nuisance in the present study as attention was given only to evoked responses, coming in a short period after a stimulus.

## RESULTS

### *A. The distal opener system*

Stimuli of sufficient intensity to the stalk of *Tubularia* evoke a synchronous depression or opening of the distal tentacles of the hydranth. The conducting system in the stalk which mediates this response will be termed the distal opener system (DOS). Often potentials are recorded from an electrode in the stalk or hydranth which have precisely the same threshold as does the tentacle opening, even if the stimulus duration is varied from 0.01 to 50 msec. These potentials are an indication of activity in the DOS, and will be termed DOS pulses (DOSP's).

Distal tentacle opening and DOSP's are very stable responses. The threshold may change considerably during a long experiment but several threshold determinations made within a short interval, for example 1 min. apart, seldom vary from each other by more than 10%. Repetitive firing of the DOS has been seen only twice. In each case the animals were being stimulated at a low frequency, 1 per 5 and 1 per 10 sec., with shocks of not much above threshold intensity. After a number of stimuli had been given DOSP's began appearing in short bursts following each stimulus rather than the previous one pulse per stimulus. This repetitive firing lasted for only a few stimuli. Repetitive firing was seen at no other time, even when the stimulus intensity used was twice or three times threshold. When the stimulus frequency is greater than about 1 per 2 sec., the distal tentacles remain strongly depressed and responses to each stimulus are not seen. DOSP's follow each stimulus at frequencies up to 2 per sec. in most animals and in some animals the DOSP's will follow at least a few stimuli at 10 per sec., after which they appear after every other stimulus and still later erratically.

### *The DOS in the stalk*

DOSP's, recorded with a metal micro-electrode in the stalk, can be as large as 200  $\mu$ V., but are more generally less than 50  $\mu$ V. (Fig. 1 A, B). They are usually larger in the stalk just below the hydranth than more proximally. Successful recording of DOSP's often depends on critical electrode placement; a small change in the electrode position can greatly diminish or enhance the size of the recorded potential. The durations of the larger DOSP's recorded were between 5 and 15 msec. The durations of the usual small DOSP's can not be determined with any certainty, but appear to be in this range.

In about 20% of the animals from which successful DOSP recordings were made (8 out of 39), DOSP's appeared occasionally or regularly as double pulses rather than the usual single potentials (Fig. 1 C). Usually double DOSP's were seen from only one of two recording electrodes in the stalk, but in experiments on three animals they were recorded in both channels. In one of these three the occurrence of double DOSP's, and the intervals between the two peaks when double pulses were seen, varied irregularly in each channel. In the other two animals double DOSP's appeared regularly and the interval between the double peaks was greater in the recording

electrode more removed from the stimulating electrodes. These results suggest that the DOS in the stalk, rather than being a single conducting system, is a series of parallel conducting systems. The double peaks then would be due to activity in two adjacent subsystems which were slightly out of phase, and the changing intervals between peaks at different points in the stalk would be a result of different conduction velocities in the parallel subsystems.

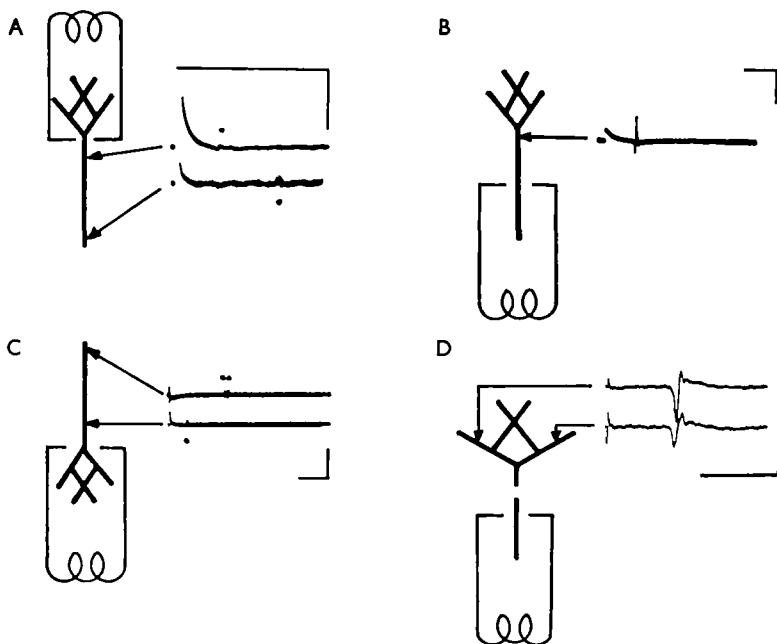


Fig. 1. DOSP's in the stalk and hydranth. (A) DOSP's as usually recorded in the stalk. (B) Unusually large DOSP recorded from the neck region. (C) Single DOSP's in one recording channel and double pulses in the other. (D) DOSP's from the proximal tentacles. A-C were recorded with metal micro-electrodes and each is several superimposed sweeps. D is a single sweep, recorded with suction electrodes. The dots (·) in this and following figures indicate the position of DOSP's where these are not obvious. Calibration: 200  $\mu$ V. and 100 msec.

The DOS conduction velocity in the stalk was determined for a number of animals from the difference in the time of DOSP appearance at two recording electrodes in the stalk. In one series five measurements were made on each of ten animals. Cases where the DOSP appeared as a double pulse were not considered. The stimulating electrodes were below the recording electrodes in the stalk, so the conduction was toward the hydranth. The average conduction velocity for this series was 15.4 cm./sec., and the standard deviation between the means of the individual animals was 2.0 cm./sec. In another series ten conduction-velocity measurements were made on each of four animals; first five with the stimulating electrodes below the recording electrodes and then five with the stimulating electrodes above the recording electrodes. Three of these animals showed single DOSP's, the fourth was one which gave double DOSP's at each recording site. For the three with single pulses, the average conduction velocity toward the hydranth was 15.9 cm./sec. and that away from the hydranth 16.0 cm./sec.

The absolute value of the difference in conduction velocities in the two directions averaged 0.4 cm./sec. It would seem, from this admittedly small sample, that conduction occurs equally well in either direction. In the animal showing double DOSP's, the conduction velocity was computed separately for the first and second pulse to appear at each recording site. The conduction velocities for the faster pulse were 16.8 and 17.5 cm./sec. in the directions toward and away from the hydranth respectively, and those for the slower pulse 15.1 and 14.9 cm./sec. The parallel subsystems postulated to account for the double pulses also appear to conduct essentially equally well in either direction.

#### *The DOS in the hydranth*

The DOS is found throughout the hydranth. DOSP's have been recorded with suction electrodes from the proboscis and from the sides and tips of both proximal and distal tentacles (Fig. 1 D). They have even been recorded from an electrode inserted through the mouth and ending somewhere on the inside of the proboscis. The DOSP's are usually between 50 and 100  $\mu$ V. when recorded with a suction electrode and, as in the stalk, are 5–15 msec. in duration.

When the recording electrode is on a distal tentacle or on the proboscis at the base of the distal tentacles, the DOSP is followed by a slow, usually positive potential, up to 800  $\mu$ V. in amplitude and 200 msec. in duration. This slow wave shows marked antifacilitation. If the stimulus frequency is greater than about 1 per 15 sec., successive potentials become progressively smaller (Fig. 2 A). It had been earlier noticed that distal tentacle opening was also an antifacilitating response. When using stimulus frequencies between 1 per 5 and 1 per 20 sec. the tentacle opening is most pronounced to the first stimulus and is less vigorous to subsequent stimuli. This similar behaviour of the slow wave and the distal tentacle opening was a first indication that the slow wave might be a result of activity in the tentacle-opening musculature.

Some experiments were carried out with excess  $Mg^{2+}$  to further establish the relation between the slow wave and the tentacle-opening response. A first effect of excess  $Mg^{2+}$  in coelenterates appears to be the blockage of neuromuscular transmission (Ross & Pantin, 1940; Bullock, 1943). A polyp was stimulated at 1 shock per 20 sec. After several minutes of stimulation isosmotic  $MgCl_2$  at 19° C. was added to the sea water surrounding the polyp until it made 25 % of the final solution. This was accomplished in about 10 sec. The stimulation was continued, and the DOSP, slow wave, and tentacle opening were all observed. This experiment was repeated three times, each time with essentially the same result. Tentacle opening and the slow wave began to decline immediately after the addition of  $MgCl_2$ ; after 60–80 sec. neither could be detected (Fig. 2 B). The DOSP's continued unchanged for 6–8 min. and then suddenly stopped appearing. Excess  $Mg^{2+}$  rather effectively separates the DOSP from the following slow wave. The most likely explanation for the sensitivity of the slow wave and the relative insensitivity of the DOSP to excess  $Mg^{2+}$  is that the DOSP is a result of nerve activity and the slow wave a muscle action potential, with excess magnesium blocking the link between the two.

The DOSP conduction velocity cannot be determined as accurately in the hydranth as in the stalk because the distance between the recording electrodes is necessarily small, 2.5 mm. or less. Further, the distance between an electrode at the base of the hydranth and one on the proboscis changes somewhat as the proboscis extends or

contracts. Conduction times between the hydranth base and the proboscis were measured in five animals. Five determinations were made on each animal. The distances between the recording electrodes were measured when the proboscis was fully extended. The average conduction velocity through the hydranth was 10 cm./sec. and the range in the means for individual animals was 8–13 cm./sec. The fact that the conduction velocity computed for the hydranth is lower than that for the stalk does not necessarily mean that conduction in the DOS is slower in the hydranth. It is not known where the DOS lies in the hydranth. If it is parallel to the hydranth surface, as is most likely, it must move laterally, away from the polyp midline, as it approaches the proximal tentacles and back toward the midline above the proximal tentacles. Thus the distance between the hydranth base and the tip of the proboscis is probably less than the conduction path length, and using this distance would result in a low value for conduction velocity.

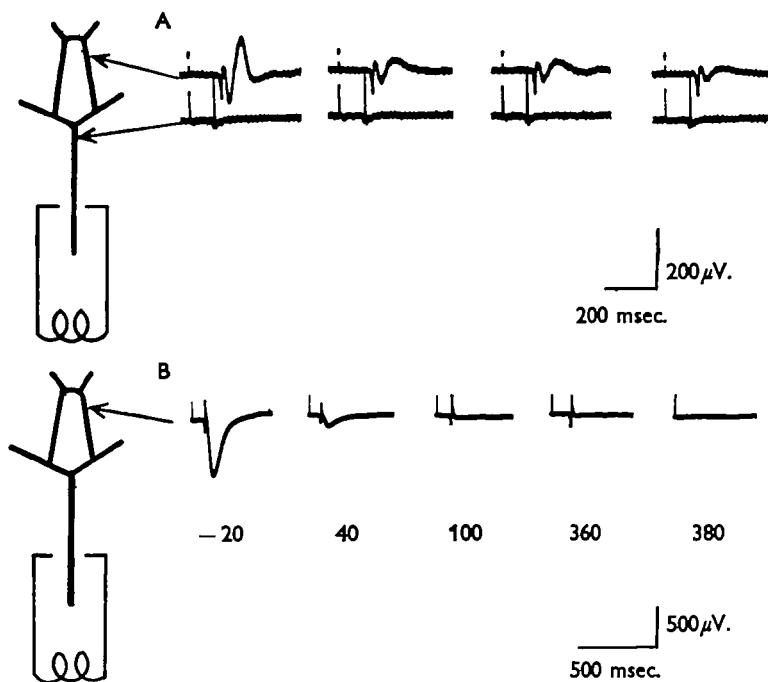


Fig. 2. DOSP's and the following slow potentials recorded near the base of the distal tentacles. (A) Successive potentials recorded with metal micro-electrodes during stimulation at 1 per 2.5 sec. Note the constancy of the DOSP and the antifacilitation of the slow potential. (B) Potentials recorded with a suction electrode 20 sec. before and 40, 100, 360, and 380 sec. after the addition of  $MgCl_2$  to the bathing solution. Note the rapid decline of the slow potential and the constancy of the DOSP until it suddenly stops appearing.

The latency between stalk stimulation and the beginning of the distal tentacle response is rather long. Some measurements of this latency were made using paired stimuli monitored with a loud speaker. The polyps were observed and the interval between the stimuli was adjusted until the second stimulus coincided with the beginning of the distal tentacle response. Three such latency determinations were made on each of five polyps, in each case with the stimulating electrodes 10 mm. down

the stalk from the base of the distal tentacles. The average latency determined in this manner was 280 msec., with a range in the individual means of 260–310 msec. DOSP's were not recorded in these experiments, but some estimate of the proportion of this latency which is due to conduction time can be made by using conduction velocities determined from other animals. If it is assumed that 2 mm. of the total length represents conduction through the hydranth and 8 mm. conduction through the stalk, the total conduction time should be about 75 msec. This leaves about 200 msec. for neuromuscular delay and the actual mechanical latency. It is clear, even from these rather crude figures, that the tentacle movement is just beginning at the time when the slow wave following the DOSP is subsiding or has completely subsided. It is presumably the relative long latent period which led to the low estimate for the DOS conduction velocity of 3.5 cm./sec., based on the stimulus to response time, in an earlier study (Josephson, 1961*a*).

### B. *The slow system*

A strong stimulus to a *Tubularia* stalk often evokes both a DOSP and a second type of potential which is conducted at a considerably lower velocity than the DOSP (Figs. 3, 4). This second potential will be called a slow pulse (SP) and the system in which it is conducted will be termed the slow system (SS). SP's are typically bi-phasic, having a prominent negative phase followed by a usually smaller positive phase. SP's are much larger and longer than DOSP's; the initial negative phase is usually between 0.1 and 1 mV. in amplitude and 20–70 msec. in duration. The amplitude and form of recorded SP's are not altered by small changes in the position of the recording electrode. SS conduction must be non-decremental, for the size of SP's is not changed as the stimulating electrodes are moved toward or away from the recording electrode. Occasionally SP's show notches or double peaks (Fig. 4), indicating that they are of compound origin. Despite repeated attempts to find a function for the SS, neither behavioural responses of the polyp nor changes in the spontaneous electrical activity of the hydranth have been seen following SS activation.

The SS is very labile. SP's can be predictably demonstrated only in a fresh, unstimulated animal, and usually fail to appear after a few supra-threshold stimuli, even if the stimuli are separated by five minutes or more. The SP threshold in a fresh animal is two to three times that of the DOSP and increases with stimulation. The SS often fires repetitively if the stimulus intensity is increased above its threshold; as many as four SP's have been seen following a single stimulus (Fig. 3). The minimum interval between SP's in a burst is about 100 msec. Because of the lability of the SS it has not been possible to examine the relation between stimulus strength and the number of pulses evoked. Experiments with two recording electrodes in the stalk have shown that not all SP's are conducted throughout the stalk. Single SP's or the later SP's of a burst are sometimes recorded with an electrode near the stimulating electrodes but not at a more distal recording electrode.

The conduction velocity of the SS has been determined from the difference in the time of SP appearance at two recording sites in the stalk. In six animals it was possible to make five or more conduction velocity measurements before the SS stopped

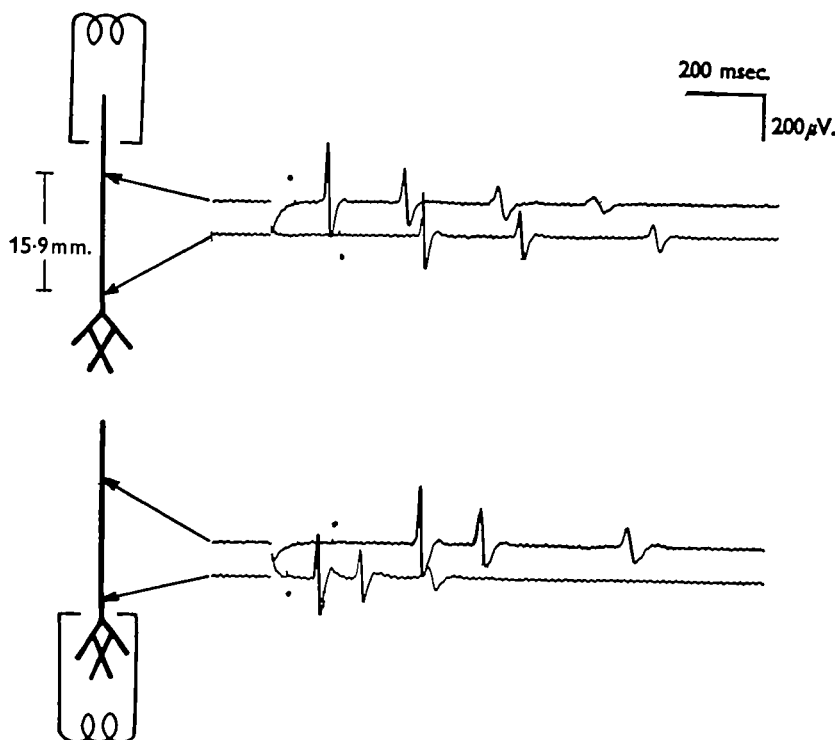


Fig. 3. Repetitive firing and non-polarized conduction in the slow system. The stimulus intensity in each case was about 25 % above threshold. The fourth SP in the upper trace of the first record never reached the second recording electrode. Note the decline in SP amplitude and the increasing conduction time between the recording electrodes through the bursts. Faster portions of the SP's have been retouched.

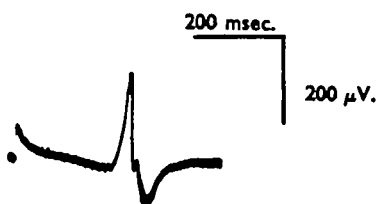


Fig. 4. SP with notch on the falling phase, indicating a compound origin for the pulse.

responding. The stimulating electrodes were below the recording electrodes, so conduction was toward the hydranth. The average of the first five conduction velocity measurements from each of these six animals was 5.7 cm./sec. and the individual means ranged from 4.5 to 6.9 cm./sec. SS conduction velocity in both directions in the stalk was measured in five animals by first stimulating below the recording electrodes and then above the recording electrodes. In three of these five it was possible to make only one pair of determinations, so only the first pair of measurements will be considered for each. The conduction velocity toward the hydranth averaged 6.0 cm./sec., that away from the hydranth 5.7 cm./sec. The SS, like the DOS, is essentially non-polarized; it conducts almost as well in either direction. When the



SS fires repetitively, the second pulse has a lower amplitude and is conducted more slowly than the first pulse (Fig. 3). During conduction velocity measurements, two or more SP's were recorded at both recording sites a total of eighteen times in experiments on five different animals. The conduction velocities for the first pulse in this group averaged 5.6 cm./sec. and those for the second pulse 4.4 cm./sec.

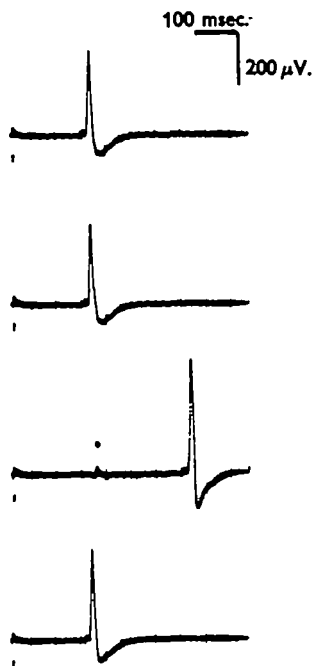


Fig. 5. Triggered NP's. Judging from the long latency, the NP in the third sweep is spontaneous and not triggered. Note that the triggered NP's in the other sweeps precede the DOSP seen in the third sweep.

### C. The triggering system

Behavioural observation and electrical recording show that there are a number of pacemakers in a *Tubularia* hydranth. One of the principal of these is located primarily in the neck region, between the stalk and the hydranth proper. This pacemaker produces a pattern of electrical activity consisting of a series of single pulses interrupted at intervals by short bursts of pulses (Josephson, 1962). These potentials are best recorded in the neck region, and are seen in an attenuated form in the upper hydranth and distal stalk, but can not be recorded in the stalk more than about 5 mm. from the hydranth. These potentials will therefore be called neck pulses (NP's). It was found that stimulation of the stalk could cause the appearance of pulses apparently identical to the normally spontaneous NP's (Fig. 5). There is considerable individual variation between polyps as to the effectiveness of stalk stimulation in triggering NP's. In some polyps almost every stimulus above a certain strength will trigger NP's, but more usually stalk stimulation is effective in triggering only if it comes within 3 sec. after a spontaneous or triggered NP, and even within this interval only 50-80% of the stimuli will trigger NP's. For some time it was thought that the ability to trigger NP's was one of the properties of the DOS, until it was found that the threshold for

triggering is 10 to 30% lower than that of the DOSP's. There must be a third conducting system in the stalk, one which triggers NP's. This system will be called the triggering system (TS). No electrical potentials have been recorded which can be definitely correlated with TS activity.

The system which produces the NP's is sensitive to triggering immediately following either a spontaneous NP or a triggered NP. This makes it possible to drive the NP system with a burst of stimuli if these are at a high enough frequency. For example, a burst of stimuli at 1 per sec. can trigger ten to twenty NP's before the NP system ceases to respond, while a burst at 1 per 5 sec. will usually not trigger more than a single NP. Not all bursts of stimuli of appropriate frequency will drive the NP system; the stimuli must begin immediately after a spontaneous NP. If the stimulation is started as late as 5-10 sec. after an NP, usually neither the first nor the following stimuli will trigger NP's. NP's can be driven at frequencies up to 5 per sec. with stalk stimulation, showing that the TS will follow to at least this frequency. The NP bursts produced by such stimulation closely resemble the NP bursts which appear spontaneously, except of course that the driven NP's are at a constant frequency while the NP frequency usually declines during the course of a spontaneous burst.

Often in a cluster of *Tubularia*, polyps can be found whose stalks appear to fuse proximally, giving a small Y-shaped colony of two hydranths, one on each of the arms of the Y. Such colonies might have arisen through branching during growth, or through the settling and subsequent development of an actinula larva upon an already formed stalk. In many but not all branched preparations there is tissue continuity through the intersection. Whether this continuity is primary, or formed secondarily through the fusion of tissues from two individuals is not known. When there is tissue continuity there is often continuity of the triggering systems of each polyp. A stimulus to one of the arms of the Y can trigger NP's in the hydranth on the other arm of the Y. This shows that conduction in the TS can occur in either direction, for the TS conduction is away from the hydranth on the stimulated arm and toward the second hydranth on the other arm. The occurrence of branched colonies is fortunate for the experimenter for otherwise, short of recording electrical activity from the TS, there would be no way to study TS conduction in both directions. The DOS can also be continuous between two hydranths on a branched preparation, but apparently DOS continuity is less common than TS continuity. In several cases stimulation of one arm of a Y preparation triggered NP's in the hydranth on the other arm even though strong stimulation did not give rise to DOSP's in the opposite arm. The converse, DOS continuity without TS continuity, has not been seen.

Since TS potentials have not been recorded, it has not been possible to measure the TS conduction velocity with two-site recording as was done for the DOS and SS. The TS conduction velocity must be greater than that of the DOS, for when the stimulating electrodes are far down the stalk the triggered NP precedes the DOSP (Fig. 6). The TS conduction velocity has been determined in six polyps from the difference in triggered NP latency with changes in the distance between the recording electrode in the neck and the stimulating electrodes on the stalk. Two of these polyps were single, the other four were from two branched colonies. With a branched preparation the conduction velocities toward the hydranths of the two arms can be determined from triggered NP latencies with just three stimulating electrode positions;

one near the point of branching and one on each of the branches somewhat below the recording electrode in each neck region. Further, the conduction velocity away from each hydranth can be determined using the same three stimulating electrode positions by measuring the decrease in triggered NP latency in the opposite hydranth as the stimulating electrodes are moved from a branch to the junction. Single conduction-velocity determinations were made for each polyp, but these are based on the differences between averages of eight to thirty latency determinations at each stimulating electrode position. The conduction velocity toward the hydranth for the six polyps averaged 17 cm./sec. (range: 13–21 cm./sec.) and the conduction velocity away from the hydranth for the four polyps of the branched preparations also averaged 17 cm./sec. (range: 15–21 cm./sec.).

The triggered NP latency is usually rather variable. The coefficients of variation ( $= 100$  (standard deviation)/mean) for the twelve sets of latency measurements used in determining the conduction velocity toward the hydranth averaged 7.0. In several animals the latencies have not varied continuously, but have fallen into two discrete groups. For example, fifteen latency determinations were made with the stimulating electrodes 27.9 mm. below the recording electrode in one of the single polyps of the conduction-velocity experiments. Of these, seven were between 157 and 160 msec. and the other eight between 176 and 182 msec. The reasons for this discontinuous distribution are not known.

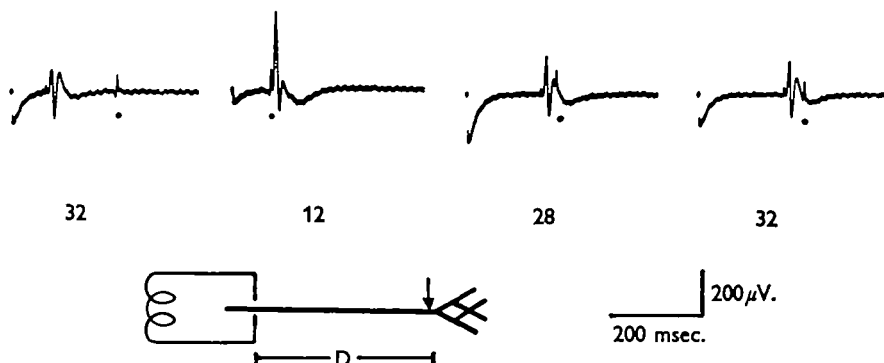


Fig. 6. Summation of DOSP's and triggered NP's. The distance (mm.) between the stimulating electrodes and the fixed recording electrode is given below each trace. Judging by the short latency, the NP in the first trace is not triggered but spontaneous. When the stimulating electrodes are 12 mm. from the recording electrode the DOSP just precedes the NP; at the greater distances the NP begins before the DOSP.

Although the TS conduction velocity, determined for the middle and lower stalk, is greater than that of the DOS, when the stimulating electrodes are near the hydranth the DOSP precedes the triggered NP (Fig. 6). This is because the triggered NP appears later than would be expected from its computed conduction velocity. This might mean that the TS conduction is much slower in the upper stalk, but more likely it indicates that there is some delay between the arrival of TS activity at the recording electrode and the first appearance of the triggered NP. This delay could be due to further TS conduction to the triggering site, a triggering latency at this site, and conduction of the NP back to the recording electrode. Some idea of the size of the total delay can be obtained by subtracting the expected time of NP appearance,

based on the TS conduction velocity and the distance between the stimulating electrodes and the recording electrode, from the actual onset of the NP. This has been done for the six polyps for which conduction velocities were obtained. The delay to the triggered NP, other than that due to conduction in the stalk, averaged 15 msec. (range: 7–33 msec.).

#### DISCUSSION

It is quite clear that there are at least three conducting systems in the stalk of *Tubularia*. But what are the cellular elements of these conducting systems? Apparently there is no information available on the types and distribution of nerve cells in the stalk of *Tubularia*. Leghissa (1950) reports that there is a single, widely distributed nerve plexus in the hydranth of *Tubularia*, based on results with staining by silver impregnation. Mackie (1963, personal communication) also finds a single nerve plexus in *Tubularia* hydranths with reduced methylene blue staining. The effects of excess  $Mg^{2+}$  on the DOSP indicate that it is of nervous origin, and the distribution of DOSP's throughout the hydranth is in agreement with the wide distribution of the epidermal nerve net. It therefore seems quite likely that the system which conducts DOSP's in the hydranth, and by extension in the stalk as well, is nervous. I can only speculate on the substrates of the other two conducting systems. The TS is similar to the DOS in its conduction velocity, threshold and ability to follow repetitive stimuli. These similarities suggest that the TS is also nervous. The SS, on the other hand, is rather different from the other two systems. Its threshold is much higher, its conduction velocity is much lower, and it is far more labile. SP's differ from DOSP's in their size, long duration, and insensitivity to small changes in the position of the recording electrode. If the DOS is nervous, it seems likely that the SS is not. Mackie (1960, 1964) gives several examples of tissues in coelenterates in which conduction can be demonstrated but in which nerve fibres have not been found. He suggests that conduction in these cases is by way of cells of the epithelial layer or the muscular bases of epithelial cells. The possibility of epithelial conduction should be considered for the SS of *Tubularia*, but this problem cannot be solved until the histology of the stalk is better known.

Electrical potentials have been recorded from two other hydroid genera, *Hydra* (Passano & McCullough, 1962, 1963) and *Cordylophora* (Josephson, 1961*b*). Two conducting systems have been found in the column of *Hydra*. One, termed the RP system, conducts small amplitude potentials which appear to be very similar in form to the DOSP and following slow wave as these are recorded from an electrode near the distal tentacles of *Tubularia*. In *Hydra* the fast initial component has a duration of 50 msec. or less while the following slow component lasts about 500 msec. It is tempting to regard the RP system of *Hydra* and the DOS of *Tubularia* as homologous. It would be most interesting to know if the slow component of the *Hydra* potentials could be selectively reduced by anaesthetics. The second conducting system of *Hydra* produces potentials of large amplitude which immediately precede contraction of the longitudinal muscles. It is difficult to compare the second *Hydra* system with those of the *Tubularia* stalk, as the *Tubularia* stalk apparently lacks muscle elements and it is likely that part or all of the potential associated with activity in the second *Hydra* system is a result of muscular activity. Only one conducting system is known

from the stalk of *Cordylophora* although others may be present, for example one which maintains the synchrony of peristaltic waves in the individual hydranths of a colony as found by Fulton (1963). The conducting system of *Cordylophora* is quite like the slow system of *Tubularia*, for it conducts slowly (2.7 cm./sec.), produces large potentials with a long duration, fires repetitively to stimuli whose intensity is not much above its threshold, and is very labile. These similarities put some doubt on the proposed nervous nature of the *Cordylophora* conducting system.

Perhaps the best parallel to the conducting systems in the *Tubularia* stalk is found in the subumbrellar epithelium of the scyphozoan *Cyanea* (Horridge, 1956*b*). Here there are two nerve nets; one, the giant net, controls the symmetrical contraction of the bell and the other, the diffuse net, can increase the frequency of the swimming beat by acting on the marginal ganglia. But there are slow contraction waves as well, which sweep over the muscles with a velocity of about 2 cm./sec. These should be considered as representing a conducting system distinct from the diffuse net for, according to Horridge, activation of the diffuse net is not always accompanied by a slow wave of contraction. Thus there appear to be three conducting systems in the bell of *Cyanea*, two nervous and one presumably not nervous; just the situation which is here postulated for the stalk of *Tubularia*.

#### SUMMARY

1. There are three, non-polarized conducting systems in the stalk of *Tubularia*. These are termed the distal opener system (DOS), the triggering system (TS), and the slow system (SS).

2. The DOS controls opening of the distal tentacles of the hydranth. Activation of the DOS produces a small electric pulse in the stalk which is conducted at about 15 cm./sec. At the base of the distal tentacles this pulse is followed by a slow potential. The slow potential but not the pulse shows antifacilitation and is quickly blocked by excess  $Mg^{2+}$ , as are distal tentacle movements. This is taken to indicate that the pulse and slow potential are a result of nerve and muscle activity respectively.

3. The TS can trigger the potentials which normally appear spontaneously in the neck region of the hydranth. The TS conducts at about 17 cm./sec and has a lower threshold than the DOS. No electrical correlate of TS activity has been found.

4. Activation of the SS produces large (up to 1 mV.), slowly propagated (about 6 cm./sec.) potentials in the stalk. The SS is very labile. In a fresh animal the SS threshold is two to three times that of the DOS and the SS often fires repetitively to stimuli above its threshold. SS activity has apparently no effect on polyp behaviour or on spontaneous electrical activity in the hydranth.

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