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Longitudinal Conduction of Contraction Burst Pulses from Hypostomal Excitation Loci in *Hydra attenuata*

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Summary. 1. Contraction burst pulses are blocked more frequently by horizontal bridges than by vertical tissue bridges of the same width. S-shaped mazes blocked pulses more frequently than E-shaped mazes of the same width.

2. The conducting path for the contraction burst pulse was thought to be a longitudinally continuous pathway having discontinuous, randomly distributed horizontal transmission sites.

3. 'Contraction burst' pulses in isolated head preparations arise at distinct hypostomal loci immediately adjacent to the last tentacle of a sequence to contract.

4. Such a 'contraction burst' locus might be another hypostomal tentacle pulse initiation site whose activity had been facilitated to spread over the entire hypostome.

5. Cluster and crescendo firing of tentacles in more elliptically shaped heads are thought to represent incomplete 'contraction bursts' which can facilitate the occurrence of the 'contraction burst' itself.

6. Contraction burst pulses originating on one side of split-body preparations were conducted only down that side without being transmitted across the hypostome.

7. It is suggested that the longitudinal conducting paths are represented by the longitudinally running myofilaments of the epithelial cell muscle processes. Horizontal and vertical transmission sites between cells are represented by desmosomes and tight junctions. Pulse initiation is thought to occur at neuromuscular junctions on the conducting tracts of the column.

Introduction

The most easily accessible element of hydra's electrical repertoire is the large contraction burst pulse first described by Passano and McCullough (1963, 1964). Although much recent work has been directed towards an analysis of the contraction burst system (Rushforth, 1966, 1971; Rushforth and Burke,1971), its morphological basis and the nature of its conducting pathway remain largely speculative. Passano and McCullough (1964, 1965) initially believed the pulse to be generated and conducted in the ectodermal nerve net, and there have been later suggestions that it might be conducted in the epithelium alone (Joseph-

son and Macklin, 1967, 1969; Kass-Simon, 1970; Rushforth, 1971). Josephson and Macklin (1967, 1969) showed that during a body contraction, the entire body wall of the hydra becomes transiently depolarized and suggested, therefore, that the contraction burst pulse ("Contraction Pulse") itself was a result of a change in voltage across the body wall. Subsequently, however, Josephson and Macklin themselves demonstrated that the contraction burst pulse could represent only a small fraction of the total potential change which occurred during a body contraction (1969, Macklin and Josephson, 1971). In addition it was shown (Kass-Simon and Passano, 1969) that the contraction burst pulse could be conducted without the presence of endoderm. This suggested that the tissue conducting the contraction burst pulse might be limited to the outer ectodermal layer.

During the course of a series of experiments aimed at analyzing the Contraction Burst and Rhythmic Potential Pacemaker systems (Kass-Simon and Passano, in preparation), it was found that occasionally a contraction burst pulse was unable to be conducted across a horizontal strip of body wall. Experiments by Semal-Van Gansen (1952), however, had previously indicated that body contractions were transmitted across horizontal bridges when one side of an H shaped hydra was stimulated. This was interpreted to mean that transmission of contraction correlated electrical pulses was diffuse. Nonetheless, workers using other preparations have demonstrated preferential longitudinal conduction in coelenterate polyps (Parker, 1917; Robson, 1971).

The experiments described here were aimed at examining again the features of the contraction burst conducting path and some of the characteristics of the pulse initiation loci of the pacemaker system. Four groups of experiments were performed; the first two examine pulse conduction across horizontal and vertical tissue bridges, the third analyzes pulse initiation at the hypostome and the fourth attempts to show how pulses are conducted longitudinally down the body column from hypostomal initiation sites.

Recording and Evaluation Procedures

All experiments were performed on animals belonging to a single clone of the European hydra, *Hydra attenuata* Pall. The animals were maintained in glass baking dishes containing BVC solution (Loomis and Lenhoff, 1956) at 20° C ± 2° C. The cultures were fed an excess of *Daphnia pulex* every other day and water changed the day after feeding. The actual experimental animals were chosen at random from among the non-budding animals in the culture dishes. The experimental preparations were made by cutting the animals in a paraffin-wax lined petri dish with a fine scalpel. Recording took place at 20° C in a dimly lit room. Polyethylene suction electrodes containing a fine silver wire were used to lead pulses into the headstage of a cathode follower preamplifier. From the preamplifier

signals were fed simultaneously into an oscilloscope and a Grass Model 5 four channel ink writing polygraph. During recording the animal's behavior was constantly monitored through a stereo dissecting scope at 10 fold magnification; its behavior was concurrently noted on the polygraph record. In this way a given behavior could easily be correlated to a specific pulse. Each experiment lasted anywhere from 20 minutes to two hours.

Several experiments involved making tissue bridges and the width of these bridges was measured with an ocular micrometer before and after recording.

Where applicable, data was evaluated by a χ^2 test for 2×2 contingency tables, Fisher's direct probability or by a one way analysis of variance.

Experimental Procedures and Results

A. Conduction of Contraction Burst Pulses across Vertical and Horizontal Bridges of I and H Shaped Preparations

In this experiment the conduction of body contractions across H shaped preparations (Semal-Van Gansen, 1952) is reexamined; the conduction of the contraction burst pulse across the horizontal bridges of these preparations is compared with conduction across vertical bridges in I shaped preparations (Fig. 1). Several widths of bridges were made in order to examine the density and distribution of the connections in the conducting path.

Procedure

Vertical bridges were prepared by cutting the animal longitudinally in half on one side of the column and gently spreading the tissue out on the wax to obtain a more or less flat sheet of tissue. A single cut from each edge of the sheet towards the middle then created the desired bridge (Fig. 1). One recording electrode was attached just below the tentacle ring above the bridge, the other was attached in line with the first below the bridge on the lower third of the body column.

Horizontal bridges were prepared in a similar manner. A scalpel was inserted into the mouth of a contracted hydra and a single longitudinal cut was made down one side of the column, again creating a nearly flat sheet of tissue. A narrow horizontal bridge was then cut by making a single incision from basal plate to the middle of the sheet which was then nearly met by another cut coming from the hypostome. Electrodes were placed beneath the tentacle ring of each half, about half-way towards the bridge. Bridges were cut at the following widths: less than 100 μ, 100 μ, 125 μ, 150 μ and greater than 150 μ. To make certain that narrow bridges had not actually severed the animals, rhythmic potentials (RP.'s) which are conducted on the endoderm (Kass-Simon and Passano, 1969, in preparation), were recorded from opposite sides of horizontal bridges of 100 μ in a sample of more than 10 animals; some of these represented the actual experimental animals.

In a few cases, among both H and I preparations, very much attenuated pulses appeared to be conducted across the tissue bridges without, however, producing any correlated column contractions on the other side of the bridge. (Conduction velocity in 4 cases was measured to be between 0.02 m/sec and 0.03 m/sec.) These animals are omitted from the data; since, although it is apparent that some conducting elements must have been present, they failed to activate the animal's contractile mechanism.

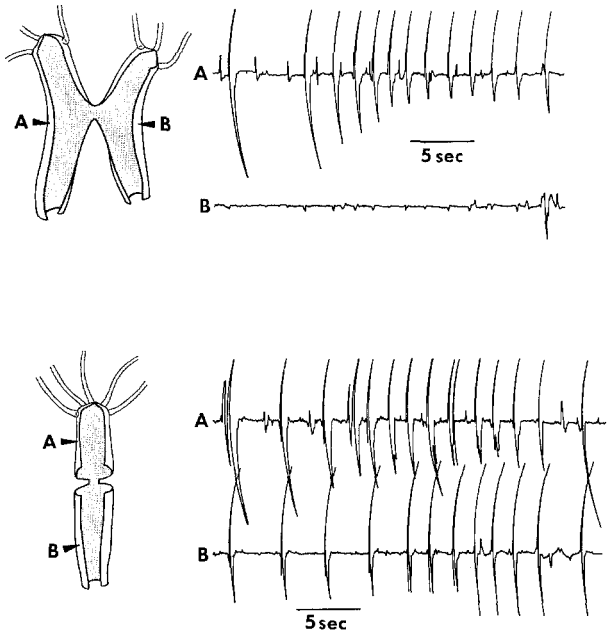

Fig. 1. Conduction of contraction burst pulses in horizontal and vertical bridge preparations. Contraction burst pulses are blocked by a horizontal bridge of 100 μ but are readily conducted across a vertical bridge of the same width. Arrow heads indicate electrode placement

Results

The data are summarized in Table 1. If both vertical and horizontal bridges are less than 100 μ wide, the contraction burst pulse and its concomitant behavior fail to be transmitted across the bridge. On the other hand both vertical and horizontal bridges greater than or equal to 150 μ readily conduct both pulse and behavior. Presumably, this means that the connections between conducting elements in the system are between 100 μ and 150 μ apart.

Between 100 μ and 150 μ there is a marked difference in the ability of horizontal and vertical bridges to conduct (see Fig. 1; Table 1). At 100 μ and 125 μ significantly fewer horizontal bridges conduct either the contraction burst pulse or its accompanying behavior than do vertical bridges. The difference is significant at $0.001 < p < 0.01$ [$\chi^2 = 12.46$ (at 100 μ) and $\chi^2 = 10.7$ (at 125 μ)]. Thus, there is apparently a longitudinal bias in the conducting network.

Table 1. Comparison of the ability of horizontal tissue bridges (H preparations) and vertical bridges (I preparations) to conduct contraction burst pulses

	Width of bridge:				
	less than 100 μ	100 μ	125 μ	150 μ	greater than 150 μ
	Ratio of the number of bridges conducting to the number of bridges blocking pulses:				
Horizontal bridge (H)	0:2	**3:15**	**6:9**	16:1	13:1
Vertical bridge (I)	0:19	**19:6**	**20:1**	15:2	2:0

Bold face indicates significant differences between the two types of bridges; see text.

B. Conduction of Contraction Burst Pulses in S Shaped and E Shaped Preparations

The previous experiment indicated that the conducting elements in the contraction burst system might be laid out in a pattern favoring longitudinal conduction; and it was originally argued that if that were the case, a tissue maze shaped like an S would be more likely to block the conduction of the contraction burst pulse than one shaped like an E. This however would only be the case if the horizontal path were comprised of separate, discontinuous elements.

Procedure

S shaped mazes were made by cutting an animal longitudinally in half down one side of the body column to obtain a flat sheet of tissue as in the previous experiment. Parallel horizontal incisions starting from each side of the sheet and stopping short of the opposite side were then made at the upper and lower thirds of the animal. E shaped mazes were prepared in the same way except that the two horizontal incisions led in the same direction (Fig. 2). Electrodes were placed at the upper, middle and lower thirds of the preparations. Bridges were made less than 100 μ, 100–125 μ, 150–200 μ and 225–350 μ wide.

Since the two bridges of any single preparation were not always the same width, the data for each type of preparation were arranged so that the conducting ability of first bridges of a given width of either S or E preparations could be compared with the conducting ability of second bridges of the same width. In addition the conducting ability of first or second bridges of any given width of S preparations could be compared with the conducting ability of first or second bridges of E preparations. Animals which failed to transmit pulses across the first bridge were necessarily excluded from this last statistic. Since pulses can originate at either end of the animal (Kass-Simon, 1970; Kass-Sinom and Passano, in preparation) and sometimes in the middle, a single animal can appear more than once in the compilation of results.

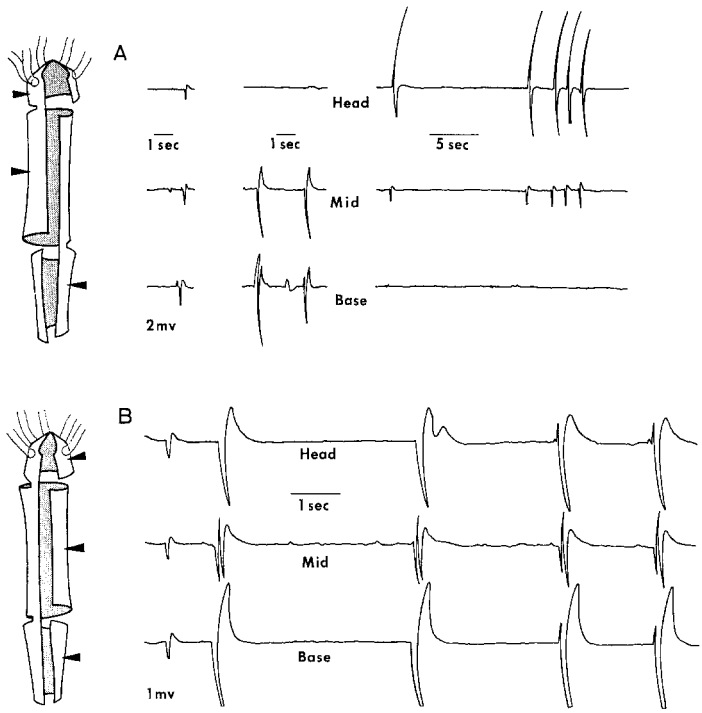

Fig. 2A and B. Conduction of contraction burst pulses in S and E preparations. In A contraction burst pulses originating at lower third of the animal is transmitted only as far as the middle section and are blocked by the upper bridge. Later, in the same preparation, contraction burst pulses coming from the upper third are also transmitted only as far as the middle section and are blocked at the lower bridge. In B the contraction burst pulse is transmitted across both bridges. Arrow heads indicate electrode placement

Results

Conduction across First and Second Bridges of S Preparations. The results are summarized in Table 2 and Fig. 2. Many more second bridges of S preparations blocked the conduction of contraction burst pulses and their concomitant behavior than did first bridges (Fig. 2), as would be expected if there were a discontinuity in the horizontal path. The differences in conduction ability are significant below the 1% level for widths of 150–200 μ and 225–350 μ ($\chi^2=6.09$, $p<0.01$ and $\chi^2=13.02$, $p<0.001$, one tailed, respectively). Although fewer first bridges 100 to 125 μ wide blocked contraction burst pulses than did second bridges, the difference here is not significant below the 5% level ($\chi^2=2.38$,

Table 2. Comparison of the ability of S shaped tissue mazes and E shaped tissue mazes to conduct contraction burst pulses

	Width of bridge:			
	less than 100 μ	100–125 μ	150–200 μ	225–350 μ
	Ratio of the number of preparations conducting to the number of preparations blocking pulses:			
S shaped tissue mazes				
1st bridge	2:0	**15:7**	**25:4**	40:2
2nd bridge	1:0	**7:11**	**7:13**	14:11
E shaped tissue mazes				
1st bridge	0:7	**22:16**	**24:2**	30:0
2nd bridge	0:1	**13:7**	**18:4**	26:4

Bold face indicates those widths at which the differences in the conducting ability of E and S shaped mazes are significant.

$0.1 > p > 0.05$, one tailed). The reason for this is not immediately clear, but it does appear that the axial level at which a bridge is made affects the conduction ability of the most narrow bridges: Significantly fewer first bridges of S preparations 100–125 μ wide conduct contraction burst pulses than do single vertical bridges made at the middle of the column ($\chi^2 = 5.07$, $0.025 > p > 0.01$, one tailed). Possibly there is a sparser distribution of conducting elements at either end of the animal, than in the middle.

Nonetheless, if the first and second bridges of an E preparation showed no difference in conducting ability, the results would support the notion of a discontinuous horizontal conducting path.

Comparison of First and Second Bridges of E Preparations. A comparison of the conducting ability of first and second bridges of E preparations (Table 2) shows that second bridges are as likely to conduct contraction burst pulses and their concomitant contractions as are first bridges. None of the differences are significant below the 5% level: [at 100–125 μ $\chi^2 = 0.069$, $p > 0.4$, one tailed; at 150–200 μ $p = 0.08$ (Fisher's exact probability); at 225–250 μ, $p = 0.06$ (Fisher's exact probability)]. Again, first bridges of E preparations 100–125 μ wide conduct significantly less often than do the same sized vertical bridges in the middle of the body column ($\chi^2 = 9.15$, $p < 0.001$, one tailed).

Comparison of First Bridges of E and S Preparations with Each Other and Comparison of Second Bridges of E and S Preparations. To be certain

that the differences in the conducting ability of the two preparations was not due to differences in the conducting ability of the first bridges or to some artifact caused by the shape of the preparation, comparisons were made between E and S preparations for both first and second bridges taken separately.

A comparison of second bridges shows that significantly fewer second bridges of S preparations conduct contraction bursts than do second bridges of E preparations [at 100–125 μ, $p = 0.012$ (Fisher's exact probability); at 150–200 μ, $\chi^2 = 6.86$, $p < 0.005$, one tailed; at 250–350 μ, $\chi^2 = 8.38$, $p < 0.005$, one tailed.] On the other hand, there is no significant difference in the conducting ability of first bridges of E and S preparations (at 100–125 μ, $\chi^2 = 0.87$, $p < 0.7$, two tailed).

Polarized Conduction and Shifting Conduction Pathways. In three cases each of E preparations and S preparations there were instances of polarized conduction. That is, a pulse starting from either head or foot was transmitted to the opposite end of the animal, but a pulse starting at the opposite end of the same preparation was transmitted only as far as the middle and was blocked at the second bridge. This would indicate that the pulses traveled down different paths, either entirely or partially.

In two S preparations, there was an apparent shift in the conducting pathway. Thus a pulse from the head might be immediately blocked by the first bridge, while one coming from the base might get through as far as the middle section, passing its first bridge. In the next contraction burst, a pulse originating at the head would this time be transmitted across its first bridge and in a subsequent burst, a pulse starting at the base would be blocked at the first bridge and not be transmitted to the middle section as was previously the case. Bridges in one animal were 100 μ wide and in the other, one bridge was 100 μ and the other 150 μ.

C. Pulse Initiation at Several Loci in Isolated Head Preparations

Since contraction burst pulses appeared to travel down the column via distinct longitudinal paths, the question arose whether these pulses did not also originate at distinct loci on or at the conducting path. By severing the head of an animal just below the tentacle ring and allowing it to heal for different periods of time it is possible to create isolated head preparations which are elliptically shaped to varying degrees. Immediately after the head is severed, hypostome and tentacles form a circle. After one or two days, however, the head loses its circular appearance and takes on the elongated shape of an ellipse or oval. In these ellipses, the material is apparently redistributed so that the insertions of some of the tentacles fall somewhat below the plane of

the original tentacle ring, while the spaces between the tentacles are also altered so that they are no longer equal (Figs. 4 and 5). The initiation of contraction bursts from various focal points can then be determined by observing the preceding tentacle avtivity and monitoring the arrival times of pulses at electrodes placed around the ellipse.

Procedure

Heads were severed from body columns 0, 24 and 48 hours before recording. Four electrodes were placed around each isolated head: one was placed at each end of the ellipse between the tentacles and one was placed on each side of the tentacle ring near the middle of the oval. Recordings lasted from one to two hours for each head and a total of 20 heads were recorded from. Tentacles were numbered consecutively and their observed activity noted on the polygraph records. Since the column of the animal had been removed, a contraction burst (here referred to as 'contraction burst') is defined as those series of pulses which accompany the contraction of all of the tentacles. This definition seems justified since in normal animals it is the case that although some contraction bursts occur without the participation of all of the tentacles, the simultaneous contraction of the entire tentacle ring has never been observed without the concomitant contraction of the body column and the accompanying contraction burst pulses.

Results

About 60% of the total of 240 'contraction bursts' were immediately preceded by the contraction of one or more tentacles. Such bursts are termed tentacle initiated bursts (TIB) (Passano and Kass-Simon, 1969). (There was no statistical difference in the number of tentacle initiated bursts for 0 hr, 24 hr, and 48 hr ellipses.)

Sequential Tentacle Firing. Typically (Fig. 3), tentacle activity preceding a 'contraction burst' involved the firing of two, three or four tentacles near one side of the ellipse in *sequential* order. Sometimes the sequence would spread out from one tentacle in both directions. In all such cases the first pulse of the series of subsequent 'contraction burst' pulses would first arrive at that electrode which was closest to the tentacle which had last fired. The initiation locus of that particular 'contraction burst' was thus closest to that tentacle.

In a few cases, the first 'contraction burst' pulse arrived at more than one electrode at the same time. Such 'contraction bursts' were always preceded by tentacle activity which seemed not to be localized at one focal point on the ellipse. In general, however, tentacle initiated bursts were preceded by sequential tentacle activity culminating in a 'contraction burst' that originated near the last tentacle to fire.

Sequential firing of tentacles was also often seen between 'contraction bursts'. In all, somewhat more than half of all tentacle activity

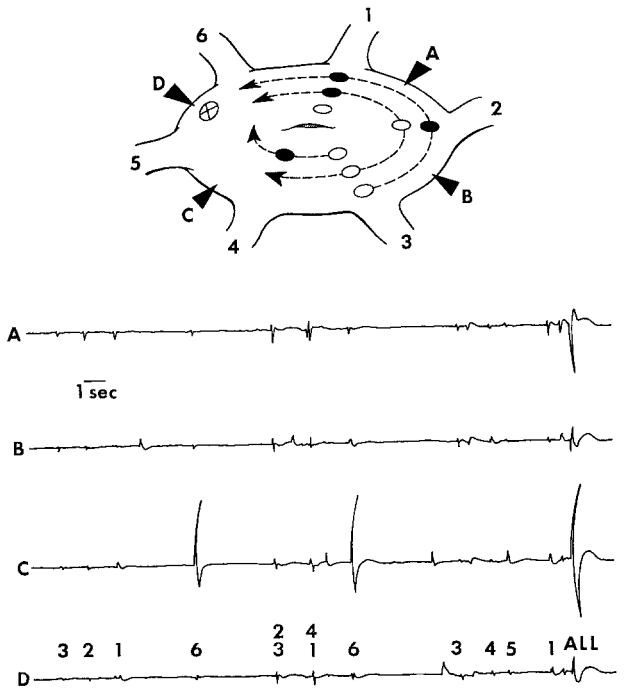

Fig. 3. Sequential tentacle firing in a day-old isolated head preparation. The open circles indicate the first tentacles of each sequence to contract. Subsequent contracting tentacles are indicated by closed circles. The last tentacle of a sequence is indicated by the arrow head at the end of the broken line. A new sequence begins with a contracting tentacle which is not immediately next to the preceding contracting tentacle. Each new sequence is indicated by the next inner circle. The 'contraction burst', indicated by the cross within the circle, is considered to begin near tentacle 6 since the 'contraction burst pulse' arrives at *D* first, followed by *C*, then by *B* and *A*. Electrode placement is indicated by arrow heads around circumference

involving three or more tentacles, either preceding a 'contraction burst' or during an inter-burst interval, was ordered in sequential fashion.

This sequential patterning of tentacle activity seems to represent responses to excitation which is spreading around the hypostome. It also looks as though this excitation facilitates so that ultimately all the tentacles are involved; resulting in a 'contraction burst'.

Statistically, there was no difference in the number of sequential tentacle events among 0, 24 and 48 hour heads ($F = 1.1, df = 12, p > 0.05$) and the variability between animals was large.

Cluster Firing of Tentacles. In addition to sequential firing, there were many instances of cluster firing (Fig. 4). Cluster firing is defined

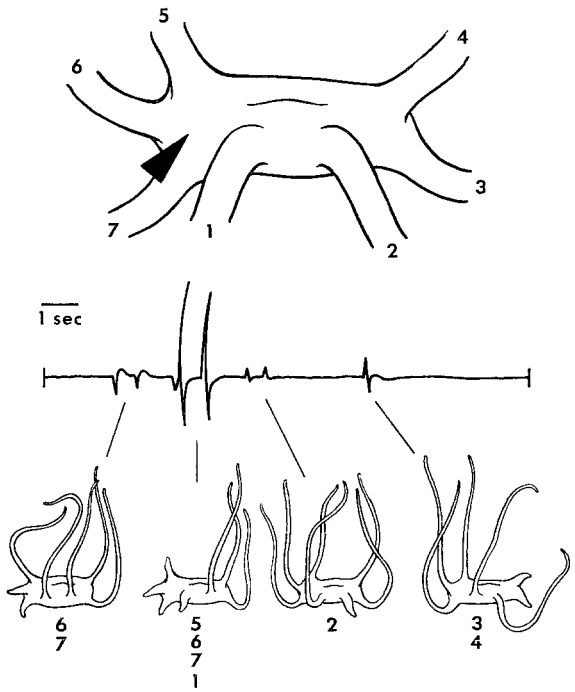

Fig. 4. Cluster firing in a two-day old isolated head preparation. The recording from only one electrode (arrow head) is shown. First the tentacles on the left side of the ellipse contract, then the ones on the right

as the *simultaneous* firing of two, three or four adjacent tentacles. Such cluster firing was especially evident among 48 hour ellipses and immediately preceded about half of all the 'contraction bursts' in this group. Among 24 hour animals only $^1/_7$ of all the bursts were preceded by cluster firing. And in 0 hour preparations cluster initiated bursts represented only $^1/_{12}$ of all the 'contraction bursts'. (The differences for the three groups are significant at $p < 0.001$, $\chi^2 = 39.5$, $df = 2$.) Thus, as the head becomes more elliptical, there seems to be less of a tendency to excite all of the conduction tissue at once, so that it looks as though the first event of 'contraction burst' is the firing of a cluster rather than the entire complement of tentacles.

Similarly, among 48 hour animals, there is an increase in the number of cluster firings which do not culminate in a 'contraction burst'. All of the animals in this group displayed cluster firing ($N = 5$) between 'contraction bursts'; among 24 hour heads five out of six animals displayed such instances of cluster firing and only one animal among the

seven 0 hour heads displayed cluster firing during an inter-burst interval. The total number of clusters fired per animal also varied significantly for the various aged heads: Compared to bursts of individual tentacles firing singly (but not necessarily in sequence) during inter-burst intervals, cluster firing represented $^1/_2$ of all inter-burst tentacle activity for 48 hour ellipses, whereas it only represented $^1/_5$ and $^1/_6$ of all inter-burst tentacle activity for 24 hour and 0 hour heads respectively. (If a cluster of tentacles fired several times in succession, it was counted here as a single instance.)

Among 48 hour animals about half of all inter-burst cluster events involved the repeated firing of the same cluster two or three times in succession. In one instance there were five successive firings of the same cluster of four tentacles at one end of the ellipse. This suggests that clusters represent partial 'contraction bursts' which were unable to spread around the entire elongated hypostome.

Crescendo Firing of Tentacles. In addition to sequential and cluster firing of tentacles there were many instances of what may be called crescendo firing (Fig. 5). Crescendo firing is defined as the firing in succession of an increasing number of tentacles which ultimately culminates in a 'contraction burst' or in the firing of a cluster at one end of the ellipse. Typically, such crescendos start with the firing of a single tentacle three or four times, followed by the firing of two or sometimes three tentacles simultaneously, followed by the 'contraction burst' itself or the repeated firing of a cluster. All of the 48 hour ellipses demonstrated several instances of crescendos, whereas only one animal in each of the 24 and 0 hour ellipses displayed crescendos.

Presumably this again means that as the hypostome becomes more elliptical and the distance between some of the tentacles increases, it becomes increasingly more difficult to excite all of the conducting tissue at once; so that each successive event, though insufficient in itself to cause a 'contraction burst', serves to facilitate conduction (or the spread of excitation) until a whole group or all of the tentacles fire simultaneously in either a cluster or a 'contraction burst'.

In summary then, tentacle activity tends not to be random but appears to be coordinated into spreading hypostomal excitation. 'Contraction bursts' originate at distinct loci which almost invariably can be localized to sites where the last tentacle of a sequence was active. This strongly suggests that 'contraction bursts' might be hypostomal excitation now facilitated to include all the tentacles but still emanating from a single locus near a tentacle. The farther such loci grow from each other, i.e., the older the ellipse, the more instances there are of simultaneous non-contraction burst tentacle activity: the more clusters and crescendos there are. And it looks as though these clusters and

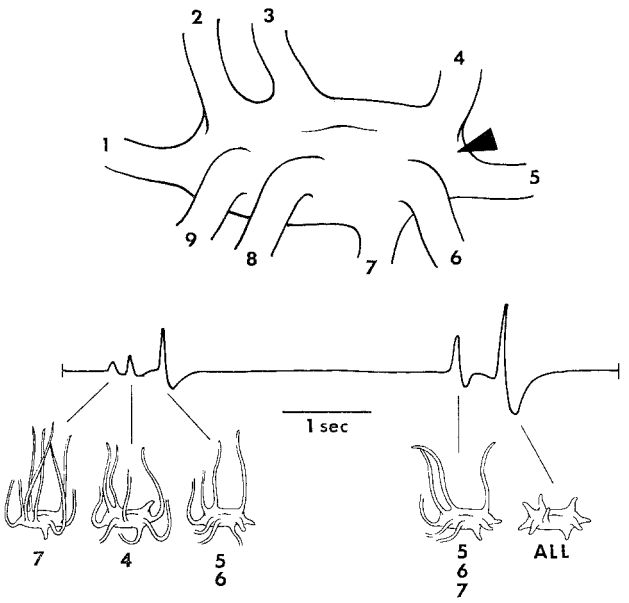

Fig. 5. Crescendo firing in a two-day old isolated head preparation. The recording from only one of the four electrodes (arrow head) positioned around the head is shown. In this preparation the 'contraction burst' began near tentacles 6, 7, and 8

crescendos may thus represent incompleted 'contraction bursts' which, because of increased distance, can only occur now after they have previously been facilitated by the cluster or crescendo.

D. Longitudinal Conduction of Contraction Burst Pulses Originating at Various Loci in the Hypostome of Split Body Preparations

If, as the results of the previous experiment suggest, 'contraction bursts' originate at distinct (but not fixed) hypostomal loci, then it ought to be possible to demonstrate the origin of body contractions from such focal points of activity. A split-body preparation can be made which manifests longitudinal column conduction of contraction burst pulses originating from distinct hypostomal loci.

Procedures

Twenty-four hours before recording, animals were cut in half from base to tentacle insertion. At the time of recording, the hypostome of 16 of the 24 animals used was split open on one side (Fig. 6), so that the two body columns which had formed were connected by a flat sheet of tissue. One electrode was placed at the lower third of each body column and one between the two tentacles above each body electrode.

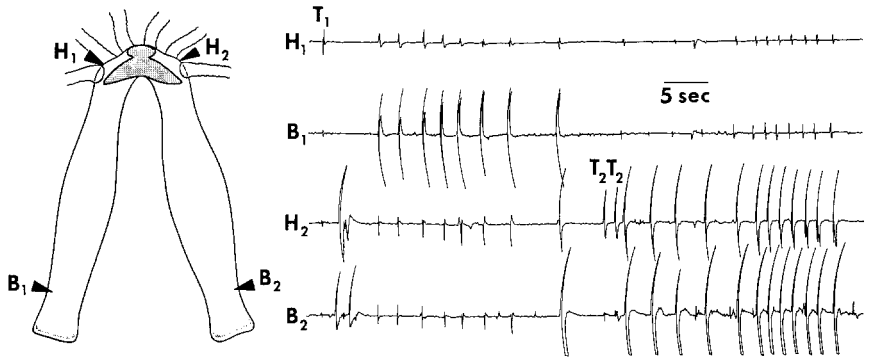

Fig. 6. Contraction burst pulse initiation and conduction in split body preparations. The sequence begins with a tentacle contraction on one side of the preparation (T_1) followed by a body contraction under that tentacle. The second body column begins to contract before the first column has ended its contraction burst. Arrow heads indicate electrode placement

Results

Both split and complete hypostomes gave identical results and the two types of preparations will be considered together. A total of 200 contraction burst events were observed. The following is typical of the type of activity recorded from this preparation: Tentacles at one end of the hypostome, i.e. near the cut edge, begin to contract one after another in typical sequential, cluster or crescendo fashion; such tentacle activity is then followed by a contraction of the body column beneath the active tentacles; these tentacles would mostly, but not always, continue to contract with the body column. The conduction velocity of the contraction burst pulse in the contracting column ranged between 0.028 m/sec to 0.048 m/sec for five animals. Contraction burst pulses arriving at the hypostomal and body electrodes of the inactive side were very much attenuated (Fig. 6) and seem to be transmitted electrotonically, since their arrival at both inactive electrodes coincides exactly with their arrival at the active hypostomal electrode. Since the hypostome thus effectively bars the conduction of tentacle and contraction burst pulses across it, contraction bursts originating at any point on the hypostome are limited to that side of the body directly beneath the active site of pulse initiation.

Nonetheless, either the electrically spreading excitation of tentacle and contraction burst pulses or some prior non-recorded conducted event appears to be able to stimulate other contraction burst excitation sites, since in 23 instances, during the course of a single contraction burst event, activity on one side of the animal was immediately followed by tentacle and subsequent contraction burst activity on the other side

of the animal. In many of these cases, after tentacle activity had begun on the second side of the animal, both the first and second body columns contracted together. In other cases, tentacle activity on the second side was initially followed only by the contraction of the second body column and then followed by the contraction of both columns together. In another 12 instances both body columns contracted after tentacle activity had occurred on only one side of the hypostome.

There are some cases where contraction burst occur without previous tentacle activity; 30 such contraction bursts were observed. In addition, there were two cases of column contractions preceded by tentacle activity on the opposite side of the hypostome: presumably a second site had become active before the first site had reached the contraction burst level.

In none of the 24 animals was either tentacle or contraction burst activity confined to one side of the animal.

Discussion

a) Conduction Path of the Contraction Burst System. As in *Corymorpha* (Parker, 1917) and *Gonactinia* (Robson, 1971), hydra's ectodermal conducting system demonstrates a distinct longitudinal bias. The nature of this bias is difficult to determine; histologically, all forms display a typical nerve net whose pattern does not account for the tendency toward longitudinal conduction. However, in *Gonactinia*, the muscle fibers underlying the nerve net are longitudinally laid out. And in hydra, electron micrographs show that the basal region of the epitheliomuscular cells is divided into numerous ridges coursing parallel to the long axis of the animal; these are muscle processes filled with myofilaments (Hess, Cohen and Robson, 1957; Lentz, 1966, p. 45). In addition, Lentz has found microtubules lying immediately above and running parallel to these muscular processes, and Slautterback (1963) has suggested that such microtubules are involved with potential changes. Lentz (1966) has further suggested that the muscle processes might therefore be capable of transmitting impulses. Since the muscle processes come in close contact with one another (Hess, Cohen and Robson, 1957), it is possible that they are made continuous by desmosomes and tight junctions between cells and that they represent or underlie the longitudinal conducting system of the contraction burst pulse. An analogy to the mammalian heart Purkinje system might be in order. In this regard Westfall, Yamataka and Enos (1971) describe gap junctions in association with desmosomes connecting the muscular bases of epitheliomuscular cells which resemble, according to them, the intercalated discs reported for canine cardiac Purkinje cells. The notion that the nerve net is not the actual site of contraction burst conduction has already

been suggested (Josephson and Macklin, 1967, 1969; Kass-Simon, 1970) and Mackie has described nerve free conducting epithelia in siphonophores (Mackie, 1965; Mackie and Passano, 1968).

If it were the case in hydra, that conduction of the contraction burst pulse occurs along the longitudinal muscle fibrils of the epitheliomuscle cells, the failure of some of the small through-conducted pulses to incite body contractions in E and S preparations might be explained in this way: If only one (set of) fibril(s) were conducting a contraction burst pulse and if the pulse failed to be transmitted to other neighboring longitudinal processes, the concomitant contraction would be very small and might indeed often be too small to be seen. In fact, is was the case that the body contractions accompanying small attenuated pulses were slower and less intense than body contractions accompanying large contraction burst pulses. Under this interpretation, a body contraction represents the activity of a number of simultaneously conducting and activated fibrils.

Among both E and S preparations, there were cases of polarized conduction; while two animals among S preparations demonstrated instances of shifts in the conducting path. These findings, together with an observation made by Josephson and Macklin (1969) that contraction burst pulses induced at the head are conducted at a different velocity than ones induced at the base, strongly support the idea that parts or all of the longitudinal conducting paths are different and distinct, although they may be very closely adjacent: Pulses travelling on one (set of) path(s) are evidently frequently prevented from spreading to another (set of) path(s).

S maze preparations consistently blocked contraction burst pulse conduction more frequently than E mazes. This fact can be accounted for by supposing that the horizontal elements of the conducting network have a scattered discontinuous distribution. Fig. 7 illustrates the sort of distribution cross-connectives might have. Again, it is difficult to assign anatomical entities to such postulated elements. But on the basis of current histological findings two possibilities present themselves: It is possible that the horizontal cross connectives are in fact the individual neurons of the nerve net. Alternatively, it is possible that between adjacent epihteliomuscle cells there are certain randomly distributed places which permit the transmission of contraction burst pulses. Such places might be actual chemical synapses but are again more likely to be the tight junctions and/or desmosomes connecting epitheliomuscle cells described by Wood (1959, 1961) and more recently by Westfall, Yamataka and Enos (1970, 1971). So far, it has not been possible to measure the conduction velocity of horizontally travelling contraction burst pulses in the body column. But, in the hypostome, it is the case that the horizontal transmission of contraction burst pulses is electro-

tonic; this would support the idea that the horizontal connectives between the longitudinal conducting paths are also best represented by tight junctions and desmosomes.

b) Pulse Initiation in the Contraction Burst System. Isolated head and split body preparations clearly demonstrate that contraction burst pulses arise at distinct hypostomal loci[1]. Thus 'contraction bursts' are easily pinpointed by the electrodes positioned around the hypostome. Tentacle sequences further show that excitation sweeps around the hypostome as one tentacle after another becomes active and that the 'contraction burst' which ultimately ends the sequence almost invariably emanates from a site close to the last active tentacle. This seems to suggest that a 'contraction burst' locus is simply the next pulse generating site in the sequence of activated sites; but now electrical activity from this site has been enabled to spread over the entire hypostome as the result of previous facilitation by activity associated with tentacle contractions (see Fig. 7). That such facilitation plays an important role in the formation of a 'contraction burst' is demonstrated by the appearance of cluster and crescendo firing in older, more elongated hypostomes. In older ellipses, the single or repeated firing of clusters frequently appeared as the final event of tentacle sequences, and suggests therefore that clusters represent incomplete contraction bursts that are unable to traverse the entire hypostome. This is given added support by the fact that cluster firing after a tentacle sequence is frequently followed by a 'contraction burst'. Again, this implies that 'contraction bursts' occur when excitation can spread over the entire hypostome and that this is made possible by the facilitory effect of previous activity associated with tentacle contractions. Similarly, the occurrence of crescendos indicates that each electrical event in a series of events associated with tentacle contractions facilitates subsequent events so that larger and larger areas of the hypostome are excitable.

Although it is possible that the mechanism for the development of a body contraction burst pulse might differ from that for the formation of a tentacle 'contraction burst' pulse, split body preparations clearly show that body contraction burst pulses can arise from the same distinct but unfixed hypostomal loci which form the initiation sites of 'contraction burst pulses'. Thus the contraction of one or the other of the two body columns is almost always accompanied by the firing of a cluster of tentacles on the given side of the hypostome and is usually preceded by a tentacle sequence which ends on that side. Again, this seems to imply that some kind of priming or facilitation is often necessary for the establishment of a body contraction burst locus. This is given added support by the fact that tentacle and body contraction burst

1 This statement refers only to those contraction bursts actually originating at the hypostome.

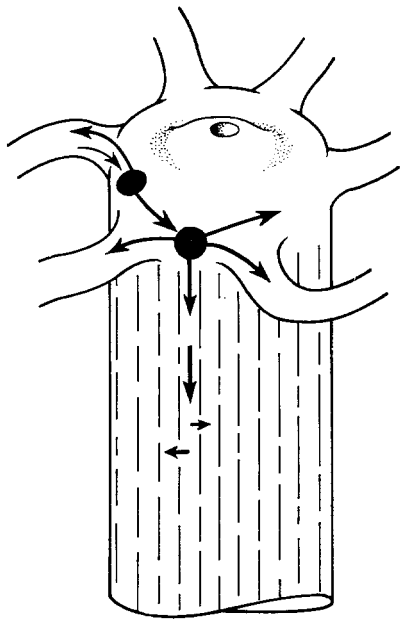

Fig. 7. A conceptualization of the pulse initiation sites and conduction pathways in *Hydra*. Closed circles represent initiation sites. Arrows indicate transmission of electrical activity. The conducting system in the column is represented as a series of continuous longitudinal paths with discontinuous, randomly spaced horizontal transmission sites. See text for further explanation

activity on one side of the preparation frequently induced tentacle and body contraction pulse activity on the other side, i.e. created a second contraction burst locus on the opposite side of the preparation.

The inference to be made that body and tentacle contraction burst systems share a common pulse initiation mechanism is given strong support by Rushforth's recent findings (1971). Tentacle and body contraction burst patterns were found to be closely similar and Rushforth has also suggested that the mechanism of pulse initiation is the same for the pacemakers of both systems.

If the contraction burst conduction system can be considered to consist solely of epitheliomuscle cells, the question now arises as to the anatomical basis of pulse initiation. According to Burnett and Diehl (1964), the nerve net is especially dense near the base of the tentacles and above the basal plate. In both E and S preparations, by far the vast majority of contraction burst pulses originated at the upper and lower thirds of the preparations. Further, there is some evidence that both tentacle contraction pulses and body contraction burst pulses are initiated as cholinergic neuromuscular events (Kass-Simon and Passano,

in preparation). And recent electron microscope studies by Westfall, Yamataka and Enos (1970, 1971) demonstrate the presence of chemical synapses, i.e., synapses with vesicles, both between neurones and between neurones and epitheliomuscular cells.

Taken together with the evidence for longitudinal rather than diffuse conduction in the contraction burst system and the evidence for the identity or at least close proximity of tentacle and body contraction burst pulse initiation sites, these findings seem to suggest that the nerve net, rather than representing the conduction path for the contraction burst pulse might function as a part of the 'pacemakers' of the tentacle and body contraction burst systems. In this regard, Westfall, Yamataka, and Enos (1971) describe neuromuscular synapse near the gap or tight junction between the myofilament-containing basal regions of two epitheliomuscular cells. The nerve net acting on the epitheliomuscular cells could then be considered to initiate tentacle and body contraction burst pulses as neuro-epitheliomuscular events. A pacemaker function for the nerve net alone has long been postulated by Passano (1963). Josephson and Macklin (1967, 1969) on the other hand, have subsequently suggested that column contraction burst pulses are generated by the membranes of the epitheliomuscle cells; but point out that the continued propagation of these pulses might occur as a result of electrical spikes being triggered (either electrotonically or chemically) by a wave of activity conducted in the nerve net of the column.

The suggestion that is being made here, however, is that the contraction burst conduction system is comprised entirely of epitheliomuscle cells and that pulse initiation occurs at neuromuscular sites close to or on the conducting tracts. Under this interpretation, it is possible to view the results of the foregoing experiments in this way (see Fig. 7).

Spontaneous activity in the hypostomal nerve net at any neuromuscular site close to or at the base of a tentacle[2] triggers a pulse in the epithelium surrounding the tentacle which is instantaneously transmitted down the epithelium of the tentacle causing the tentacle to contract. At the same time, the activity producing this pulse and facilitated by it (indicated by the arrow returning from the tentacle in Fig. 7) also spreads or is conducted via the hypostomal epithelium or nerve net to an adjacent hypostomal site near another tentacle, triggering it to fire. If the pulse initiation site lies on the conducting epithelium of the column, or, if, as a result of previous facilitation,

2 It is possible that the pulse initiation sites are in the tentacles themselves and are represented by the "tentacle pacemakers" described by Rushforth (1967, 1971), especially those considered to be at the bases of the tentacles, but the simultaneous firing of two or more adjacent tentacles is so common among all the preparations examined that it appears likely that the primary active sites are formed by the hypostomal epithelium and nerve net surrounding the bases of the tentacle.

a distant locus depolarizes part of the body conducting epithelium, a column contraction burst pulse occurs which is propagated through the conducting tracts of the column. These tracts are thought to be longitudinal, continuous paths as shown in Fig. 7, with dispersed, discontinuous horizontal transmission sites. Analogous to the mammalian heart Purkinje system, pulses are thought to be transmitted at finite rates possibly via the microtubules running closely parallel to the myofilaments of the longitudinally laid muscle processes of the epitheliomuscular cells. Horizontal and longitudinal transmission sites between cells are probably best represented by the tight junctions and desmosomes existing between them.

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