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# Patterns of electrical activity in comb plates of feeding *Pleurobrachia* (Ctenophora)

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## SUMMARY

The electromotor behaviour of ciliary comb plates was studied during prey-stimulated and electrically stimulated feeding by intact *Pleurobrachia pileus* (Müller). Comb plate electrical activity was recorded by extracellular electrodes attached directly to the cilia; comb plate motility was recorded by high-speed video microscopy. Comb plate electrical activity fell into two distinct classes, identified by waveform and amplitude: (i) excitatory postsynaptic potentials (EPSPs) in the comb plate (polster) cells and (ii) regenerative potentials in the cilia, as described previously (Moss & Tamm 1987). Slow phasic bursts of regenerative potentials (reversal volleys) were observed in comb plates of rows undergoing reversed beating during capture of prey or by rhythmic electrical stimulation of the tentacles. All plates of a given comb row exhibited virtually identical electrical activity. Timing and development of electrical activity in comb plates of the subtentacular (ST) rows were nearly identical even though separated by several centimetres; onset of the reversal volleys of plates of subsagittal (SS) rows were delayed on average by about 0.5 s relative to the ST rows, although individual EPSPs displayed very similar timing.

Microsurgery, combined with extracellular recording from comb plates and the tentacle and associated basal structures, revealed the presence of an integrative center in the tentacular bulb. This communicates with the comb plates by means of a diffuse pathway, presumably the nerve net, which itself is maximally sensitive to rhythmic input. The pathway underlying the reversal volley may innervate only the stimulated hemisphere. In addition to the rhythmic pathway, a through-conducting pathway runs from distal regions of the tentacle to the comb plate cells. Yet another excitatory pathway, possibly distinct from the tentacular through-conducting pathway, may mediate certain cases of global postsynaptic activity. The pathway that controls mouth movements during feeding is entirely independent of any comb plate pathway.

## 1. INTRODUCTION

Upon catching prey with one of two tentacles, the giant ciliary comb plates of the ctenophore *Pleurobrachia pileus* temporarily increase their rate of beating, driving the animal rapidly forward (Tamm & Moss 1985). Comb plates of rows on the hemisphere of the prey-catching tentacle then press against the body ('laydown'), followed by reversed beating at high frequency. This unilateral reversal sweeps the tentacle into the mouth, which plucks the prey off. Comb rows of the opposite hemisphere do not undergo reversal, although they may cease to beat. As a result the animal always spins toward the prey-bearing tentacle, wrapping it over the body.

We previously recorded intracellularly from comb plate cells (polster cells) of dissected, longitudinally split, comb row preparations during electrically evoked ciliary reversals (Moss & Tamm 1986*a*). Rhythmic stimulation leads to reversed beating which is preceded by, and correlated with, excitatory postsynaptic potentials (EPSPs) that give rise to regenerative potentials graded in amplitude to the degree of postsynaptic depolarization.

Extracellular suction electrode recording from the comb plate cilia ('extraciliary recording') of split-row preparations revealed that such regenerative potentials arise in the ciliary membrane, and are calcium dependent as in *Paramecium* (Dunlap 1977; Eckert & Brehm 1979; Machemer & Ogura 1979). Furthermore, because of their great length, comb plate cilia are not isopotential: regenerative activity propagates tipward along their length (Moss & Tamm 1987). Comb plate ciliary reversal, like ciliary reversal in *Paramecium* (Naitoh & Kaneko 1972, 1973), is calcium dependent (Nakamura & Tamm 1985).

*Pleurobrachia* possesses an ectodermal nerve net (Hernandez–Nicaise 1973*a*,*b*, 1974), similar to that of cnidarians (Spencer 1991; Satterlie & Spencer 1987). Polster cells are innervated (Horridge 1966; Horridge & Mackay 1964), suggesting that neurally mediated modifications of comb plate activity are responsible for much of the complex swimming behaviour of ctenophores (Horridge 1974; Tamm 1982).

In this paper we use extraciliary recording to

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investigate the spatial and temporal patterns of electrical signalling that regulate ciliary beating on different comb rows of intact animals. We describe underlying rhythmic electrical activity correlated with production of unilateral comb plate reversal and show that several conduction pathways must be present to account for the electrical and motor activities observed. A localized pacemaker drives oscillatory neuronal activity in the ectodermal pathway innervating polster cells. We also discuss how reversed waves of beating are generated and maintained during feeding. A preliminary account of some of this work appeared previously (Moss & Tamm 1986*b*).

## 2. MATERIALS AND METHODS

Large (greater than 2 cm diameter) *Pleurobrachia pileus* were collected locally during the winter, and maintained in flowing sea water at 4–8°C. Animals were fed copepods daily; those used for prey-elicited feeding were starved for three days before experiments. Unusually large animals collected in late Spring were used for pathway tracing.

### (*a*) *Body plan, ciliary system and feeding response*

The biradial body plan and ciliary system of the ctenophore *Pleurobrachia pileus* is reviewed in figure 1*a*. The eight meridional rows of giant ciliary comb plates run longitudinally from the aboral to oral end of the animal. Each comb plate consists of hundreds of thousands of ~1 mm long cilia which arise from a ridge of several thousand elongated epithelial cells (polster cells; Afzelius 1961). All of the cilia of a comb plate beat together as a unit due to mechanical coupling between the closely packed cilia (Tamm 1984). The power stroke of the comb plate is normally directed aborally, driving the animal mouth forward. Forward beating begins at the aboral end of the comb rows, and waves of beating travel to the oral ends of the rows. In *Pleurobrachia* this antiplectic metachrony (Knight–Jones 1954) is coordinated by hydromechanical interaction between successive comb plates (Tamm 1973). Two tentacles emerge from pouches on opposite sides of the body, defining the tentacular plane. The flattened pharynx and mouth lie in the sagittal plane, perpendicular to the tentacular plane. The two comb rows adjacent to each tentacle are termed subtentacular (ST) rows (total of four rows); while the four comb rows bordering the sagittal plane are subsagittal (SS) rows. The sagittal hemisphere of a tentacle thus contains two ST and two SS rows.

### (*b*) *Feeding experiments*

*Pleurobrachia* were pinned with the tentacular plane horizontal to a Sylgard (Dow Corning, Michigan) coated petri dish containing artificial sea water (Cavenaugh 1956). The preparation was maintained at 4–10°C for trials involving electrical stimulation. Since animals responded sluggishly to prey at such low temperatures, feeding experiments were conducted at ~15°C. Animals were oriented so that at least one ST comb row was imaged in profile (figure 1*a*,*b*).

Video and electrophysiological recording was performed as described previously (Moss & Tamm 1987), except that the whole animal was imaged at low magnification. A high-speed shuttered video camera (model PCSM 6500, Tritronics, Burbank, California), gave exposures between 0.4–1.0 ms and field rates of 60–180 Hz. Separate video images of oscilloscope records and comb plate activity were individually numbered (model QSI VFF6030 field counter, QSI Systems, Newton, Massachusetts), combined with a video mixer (Model SE-1, Echolab Ind., Burlington, Massachusetts) and recorded on a video cassette recorder allowing consecutive still-field playback (model 2051, GYYR, Odetics, Sunnyvale, California). Electrical activity was recorded at 100–1000 gain (high frequency 3 db rolloff at 10 KHz), with AC-coupled amplifiers (model P15, Grass Instruments, Quincy, Massachusetts), on an instrumentation recorder (model D, Vetter, Rebersburg, Pennsylvania), for subsequent playback on a chart recorder (model 220, Gould Recording Systems Div., Cleveland, Ohio) and correlation with the video record.

Comb plate electrical activity was recorded extracellularly by polished glass suction electrodes attached near the end of a fine sliver of a comb plate (Moss & Tamm 1987) (figure 1*b*). Relative positive polarity was represented by an upward deflection in the electrical record. Extracellular signals represented compound electrical events from a few hundred cilia (Moss & Tamm 1987), arising from an undetermined number of cells. Although the rest of the plate was free to beat and remained hydromechanically coupled to neighbouring plates, it was often obscured by the recording electrode. Neighbouring plates are nearly identical in timing and type of beating. Therefore, beating of a plate adjacent to the one from which we recorded electrical activity was usually used to determine beat rate and direction. Beat frequency was calculated from the inverse of the period between each mid-effective stroke position. The records thus represent the highest temporal resolution possible, since they show changes in timing for each beat cycle.

Feeding responses were evoked either by applying copepods or cladocera to one tentacle, or by electrical stimulation of a tentacle with a loosely attached suction electrode. Ten to twenty volt, 5 ms bipolar impulses repeated at 4 Hz for 10 s (model S44 stimulator and induction-coupled stimulus isolation unit; Grass Instruments, Quincy, Massachusetts) reliably elicited feeding (Moss & Tamm 1986*a*) and was routinely used for tentacular stimulation. Longer duration pulse trains were not more effective at producing feeding, while shorter trains were less likely to evoke a maximal response.

Eleven animals were each run through three separate classes of trials: (i) using copepods and/or cladocera as food, with concurrent electromotor recording from plates of either of the ST comb rows on the prey-catching side and sometimes of a ST row of the noncatching side; (ii) electrically stimulated on a tentacle while recording electromotor activity from ST




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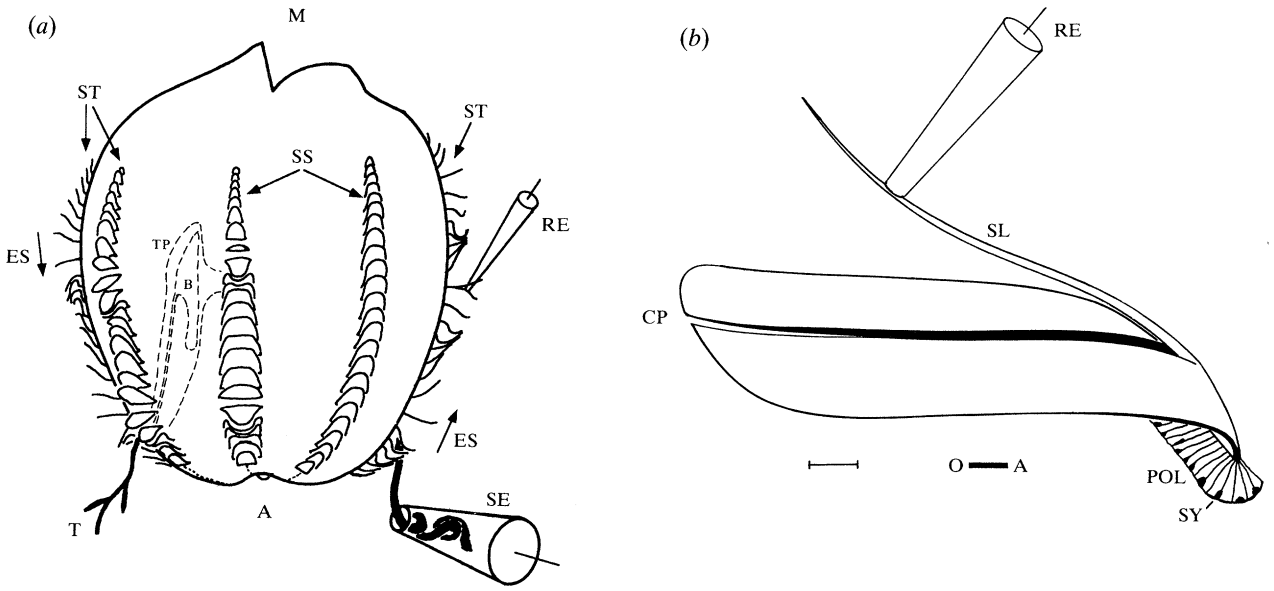


Figure 1. (*a*) Diagram of an immobilized adult *Pleurobrachia* with one extra-cellular recording electrode (RE) attached to a comb plate of a subtentacular (ST) row adjacent to the electrically stimulated tentacle. Plates of the ST row on the stimulated side are undergoing reversal (cf. effective stroke on the non-stimulated side). A tentacular pouch (TP) and tentacular bulb (B) are indicated by a dashed line for clarity. SE, stimulation electrode; M, mouth; A, aboral end of animal; SS, subsagittal comb row; T, non-stimulated tentacle; ES, effective stroke. (*b*) The recording electrode (RE) is attached by suction to a sliver (SL) of a comb plate (CP). Comb plate cilia arise from a ridge of polster cells (POL) which receive synapses (SY) from the ectodermal nerve net. For clarity, the number of poster cells is greatly reduced. Scale, 100 μm. O–A, oral–aboral axis.

comb rows on stimulated and nonstimulated sides; and (iii) electrically stimulated on a tentacle while recording from a ST and a SS row on the same side.

Five to seven days elapsed between trials for each animal. Trials appeared to have no deleterious effects; animals fed avidly between trials and typically increased in size.

### (*c*) *Neurociliary pathway and anatomical studies*

To determine the gross anatomical nature of the conduction pathway underlying reversal, different patterns of cuts were made with very fine iridectomy scissors in the ectoderm of *Pleurobrachia*. Incisions were shallow, to confine the cuts to the outermost surface. In experiments involving behavioural analyses, intact animals were used. Animals were stimulated either on the tentacle as described above or on the ectodermal surface with a large-bore polyethylene pipette attached midway between the ST rows. Typically, more intense stimuli (50–100 V) were needed to evoke unilateral reversal for such trials, probably because of increased shunting from a poor seal around the plastic stimulation pipette, and the low density of neurons in the nerve net relative to the bundle of axons in the tentacle (figure 12*b*). Comb plate responses were noted on both sides of the cut to determine whether the conduction pathway underlying reversal had been interrupted.

To determine the source of rhythmic bioelectrical activity underlying unilateral reversal, animals were immobilized with the sagittal plane horizontal to allow recording from structures in the tentacular region. For most experiments involving comb plate extraciliary recording and microsurgical lesioning, animals were split in half along the sagittal plane to provide more stable preparations for attachment of recording electrodes. Such preparations responded to stimulation much like the intact animal, and displayed mouth bending, reversal, and bioelectrical activity that closely mimicked the prey-catching side of an intact animal. Electrical recordings for all pathway studies were made from ST plates only, since previous studies indicated that these responded most reliably to stimulation (Tamm & Moss 1985).

Where access to the base of the tentacle and the tentacular bulb was required, a window was cut through the side of the animal into the pouch. Great care was taken to limit the size of the hole in the pouch wall, so that the conduction pathway to the outer surface remained intact, although the hole was enlarged sufficiently to admit a recording electrode when required. The tentacle was drawn through this hole for mapping studies, so that the entire length of the tentacle, and the base, could be mapped for bioelectrical activity. The response of a ST comb plate was always concurrently determined so that tentacle and tentacular bulb activity was examined in the context of the unilateral reversal of beating.

## 3. RESULTS

### (*a*) *Characteristics of extraciliary electrical recordings from comb plates*

Freshly pinned animals initially exhibited continuous




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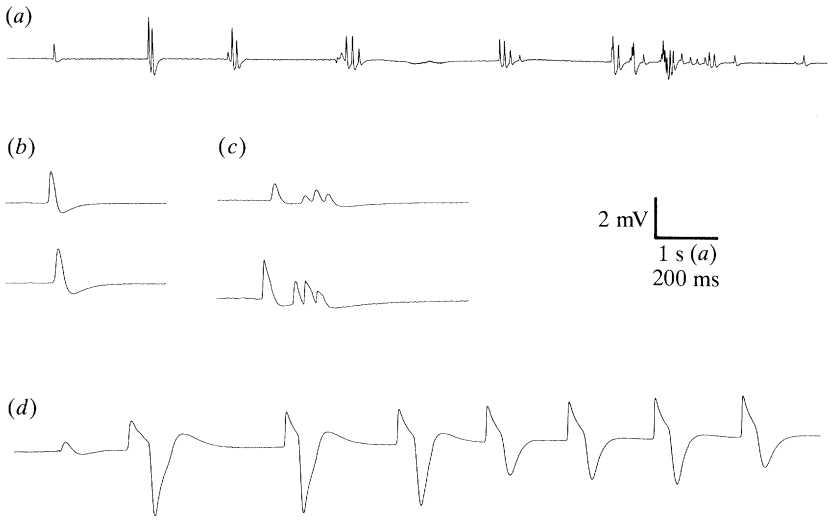


Figure 2. Range of comb plate electrical responses. Extraciliary recordings from intact animals. (*a–c*) Large EPSPs during fast forward beating in response to sucking a tentacle forcefully with the stimulating electrode. This motor response, but not the electrical response, has been described previously (Tamm 1982). (*a*) ST plate. (*b*) ST (above) and ss plates; note small lag (less than 20 ms) between events. (*c*) Oral (above) and aboral plates of an ST row (lag less than 40 ms). Note that distant comb plates (actually single or small clusters of cells of the plate) show virtually identical postsynaptic activity. (*d*) Volley potentials, ST plate, beginning with a small EPSP, and followed by several action potentials. Stimulus: 4 Hz, 5 ms, 10 V pulses applied to a tentacle for 10 s.

fast forward beating interspersed with tentacular contractions and reversal of beat direction of plates on all eight comb rows. Comb plate beating slowed a few minutes after pinning, and the tentacles relaxed. This 'resting' state, in which the comb plates beat slowly or not at all, was a prerequisite for eliciting the feeding response, whether by application of prey or by electrical stimulation.

Comb plate electrical activity fell into two categories. Rapid forward beating evoked by a noxious stimulus was often accompanied by comb plate electrical activity (figure 2*a–c*). These were positive monophasic waves, interpreted as arising from depolarization from EPSPs in the cell bodies that spread into the cilia (see figure 1*b* and Moss & Tamm (1987)). Such responses were also seen preceding regenerative activity and reversal during both prey-evoked and electrically evoked feeding. Regenerative potentials (figure 2*d*) were observed during unilateral reversal of beating that occurred during feeding episodes, and in freshly pinned animals during global reversal. Regenerative potentials had multiphasic waveforms, consisting first of a positive wave (that arises from the EPSP), a second positive wave which often merged and summed with the first, and then a pronounced, relatively large negative wave, sometimes followed by a minor positive wave (figure 2*d*). We interpret the second positive wave as action potential-based local circuit activity (Moss & Tamm 1987), and the negative potential as a net depletion of positive charge when the inward current of the action potential sweeps by. The action potential wave is reduced or absent in proximal regions of the comb plate (Moss & Tamm 1987). The shape of the recorded action potential wave thus varied with the site of electrode attachment along a ciliary sliver, and in some records is identified solely by its large amplitude (note vertical scale in recordings).

Regenerative potentials occurred in volleys (figures 2–11 and 13) during laydown and reversal. We term these rhythmic bursts 'reversal volleys'.

During electrical stimulation of the tentacle, but not during prey-evoked reversal, we additionally measured small positive monophasic potentials from comb plates which occurred one-for-one with each stimulus pulse. We interpret these potentials as arising from EPSPs in the cell bodies, triggered by the activity of a rapidly conducting nervous pathway that connects the tentacle with the comb plates. In accordance with previously established terminology (Josephson 1966) we refer to them as through-conducted potentials (TCPs). They are approximately the same amplitude in all plates of a row and in all four rows on the prey-catching or electrically stimulated hemisphere of the animal (see figures 5*b*, 6, 7*b* and 8).

### (*b*) *Properties of reversal volleys*

#### (i) *Feeding animals*

Reversal volleys were recorded from comb plates of ST rows on the prey-catching side before and during laydown and reversed beating (figures 3 and 4). ST plates typically exhibited a volley of 18–19 spikes lasting 4 s (table 1), which ended before cessation of reversal. The firing rate at volley onset was twice (factor of $2.1 \pm 0.1$; $n = 11$ animals) that at cessation. Volleys began as small EPSPs which grew quickly in size.

Subtentacular plates on the noncatching side of the animal usually remained electrically quiet regardless of whether they maintained forward beating or ceased to beat during reversal on the catching side. Some-




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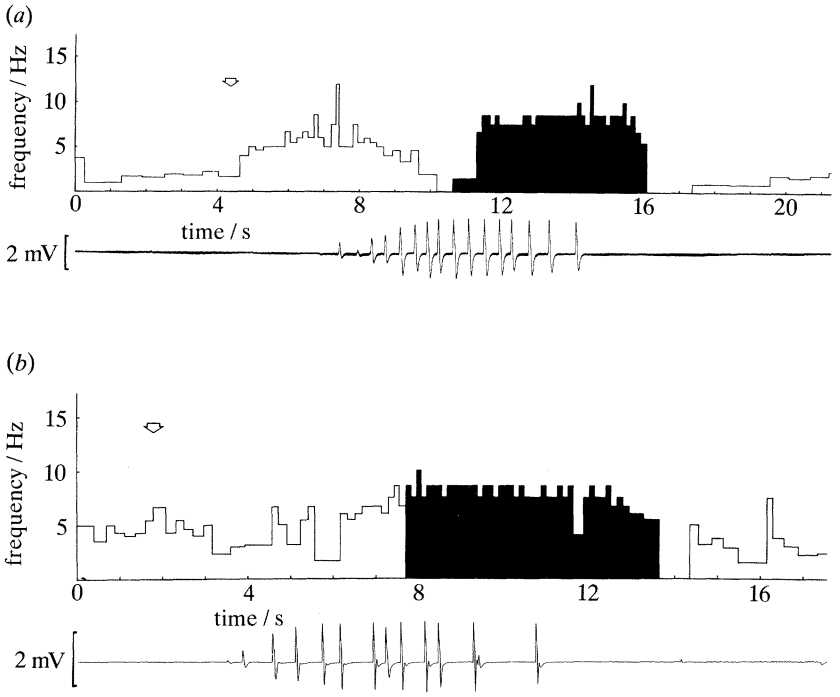


Figure 3. Electromotor responses of ST comb plates on the prey-catching side during copepod-induced feeding. (*a*,*b*) Two different animals. Upper graph, beat frequency; lower trace, extraciliary potentials. Open arrow, start of mouth bending toward the prey-laden tentacle. (*a*) Forward beat rate (open bars) increased from 1 to 8 Hz in the absence of electrical activity. Volley onset was associated with a decrease in the rate of forward beating (open bars) followed by a brief stoppage immediately prior to the onset of high frequency reversed beating (black bars). Reversed beating ceased 2 s after the last spike; eventually the plate returned to slow forward beating. (*b*) A ST plate of another animal exhibited a constant moderate forward beat rate (open bars) that dropped briefly after volley onset, then increased just before reversed beating began (black bars). Reversed beating persisted 2.9 s after the last spike, followed by a brief stoppage. Forward beating resumed after an additional 0.8 s.

times, however, they displayed brief bursts of synaptic and/or diminished action potentials which coincided with the onset of the reversal volley in ST plates on the catching side (figure 4). Each event of the truncated volley corresponded to an EPSP or spike on the prey-catching side of the animal. Truncated volleys may be due to abortive or unsuccessful attempts to capture prey by the tentacle on this side, but this seems unlikely in view of the precise timing of events on both sides. It seems more likely that truncated volleys arise from 'cross-talk' between weakly connected conduction systems on opposite sides or incomplete inhibition by the contralateral side (see below and Discussion). Note that cessation of beating on the noncatching side is not due to volley activity, since beating slowed and stopped prior to the onset of the first truncated volley (figure 4). In addition, the increase in forward beating before reversal on the stimulated side occurred well before the reversal volley.

(ii) *Electrically stimulated animals*

Unilateral reversals elicited by electrical stimulation of a tentacle were accompanied by volleys similar to those of feeding animals (table 1). Volley events were nearly identical in timing in ST and SS rows on the stimulated side. TCPs were typically observed from the onset of stimulation.

Reversal volleys began about 6 s after onset of stimulation, and continued 2–4 s beyond the end of the stimulus pulse train. Volleys thus did not require concurrent stimulus input. In the case indicated in figure 5, the stimulus train was prematurely terminated by breakage of the tentacle. After a single injury potential, TCPs ceased. Despite this, the accompanying reversal and its reversal volley still occurred. These results show that TCPs are not field effects of electrical stimulation, but are mediated by a biological conduction system. TCPs originate in and travel through the tentacles. Finally, some part of the conduction pathway must involve integrative activity, since the comb plate reversal volley is not temporally dependent upon concurrent bioelectrical input from the tentacle.

Reversal volley events ran free of the stimulus from inception, and could be distinguished from TCPs by this property. Figure 6 is a typical record in which volley potentials began 2 s before the end of the stimulus. The first event of the volley occurred out of phase with the stimulus and the TCPs, and the period between subsequent volley potentials increased. TCPs persisted out of phase with the rhythmic activity of the volley, and were evident by their consistent small size, monophasic shape, and 1:1 timing with the stimulus pulses (figure 6, asterisks and arrows). This indicates that the TCPs are conducted to the comb plate cells by a pathway independent of that which triggers the volley potential.

Stimulation at lower or higher frequencies did not




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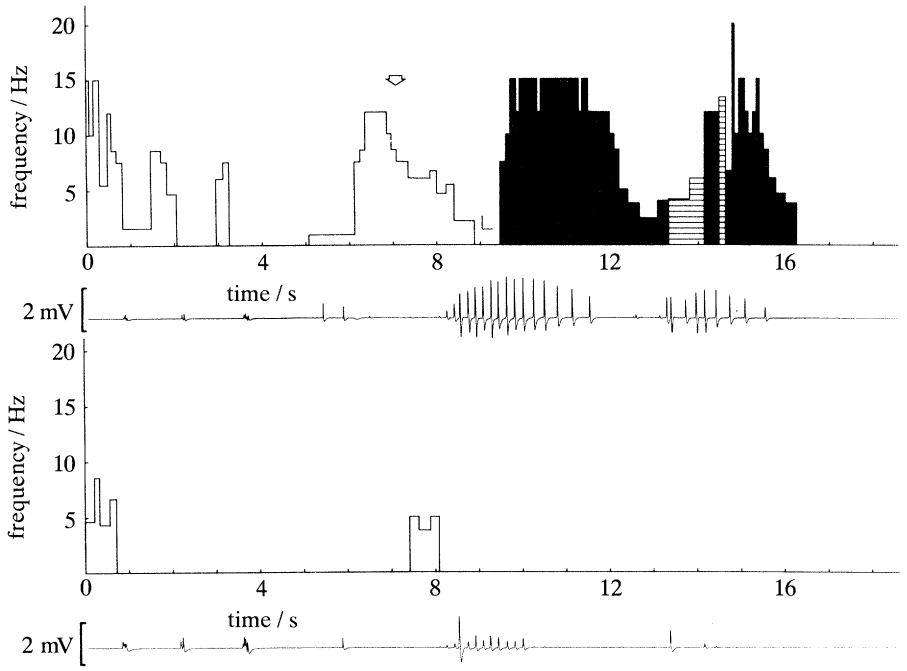


Figure 4. ST plate electromotor activity on prey-catching (upper records) and opposing non-catching (lower records) hemispheres during feeding upon copepods. An increase in forward beat rate occurs on the catching side prior to reversal, without detectable electrical activity in the comb plate. Laydown (L) and reversed beating are preceded and accompanied by a volley. On the non-catching side (lower records), decrease in beat rate and cessation of forward beat occur prior to a corresponding volley, which in each case is shorter and weaker (the second of the pair in fact shows almost no activity). These volleys initially have the same firing frequency as the respective volley on the catching side, but cease as the catching side volley slows. Note isolated events occuring simultaneously on both sides at the beginning of the record. Hatched bars, indeterminate direction of beating. Open arrowhead, mouth bending.

evoke reversal volleys and reversed beating (not shown). Instead only TCPS driven 1:1 at the stimulus rate were evident. At stimulus frequencies greater than 10 Hz, TCPS adapted and soon ceased to follow the stimulus pulse train.

### (*c*) *Intra-row coordination*

Reversed beating usually does not begin synchronously in all plates of a row, but is initiated at or very near the oral end (Tamm & Moss 1985). Following

Table 1. *Characteristics of reversal volleys in feeding and stimulated animals*

| category | no. animals | volley onset[a] ± s.e.m. s | events per volley[b] ± s.e.m. | length of volley ± s.e.m. s | frequency of volley s.e.m. Hz | range of lag between events ms |
|---|---|---|---|---|---|---|
| *feeding animals*: 44 sequences[c] | | | | | | |
| ST plates | 5 | n.a. | 18.5 ± 2.1 | 4.1 ± 0.6 | 5.0 ± 1.0 | n.a. |
| *stimulated animals*: recordings from stimulated side 224 sequences[d] | | | | | | |
| 1. ST oral | 11 | 6.0 ± 0.3 | 19.7 ± 1.7 | 7.0 ± 0.4 | 2.9 ± 0.2 | 10–60 |
| ST aboral | | 6.1 ± 0.4 | 19.0 ± 1.6 | 6.5 ± 0.3 | 3.1 ± 0.3 | |
| 2. adjacent | | 6.0 ± 0.4 | 19.6 ± 1.3 | 6.6 ± 0.3 | 3.1 ± 0.3 | 0–10 |
| ST plates | 10[e] | 6.0 ± 0.4 | 19.6 ± 1.2 | 6.7 ± 0.3 | 3.1 ± 0.3 | |
| 3. ss | 6 | 5.7 ± 0.5 | 19.7 ± 2.2 | 6.7 ± 0.7 | 2.8 ± 0.2 | 10–40 |
| ST | 6[f] | 5.2 ± 0.2 | 24.3 ± 0.9 | 8.1 ± 0.6 | 3.1 ± 0.3 | |

[a] After start of stimulus train. Applies only to electrically stimulated animals.
[b] Because identification of the bioelectrical response cannot be unequivocal (see Discussion, and Moss & Tamm (1987)), we identify the volley responses by the general term 'event'. Events include EPSPS and graded regenerative responses.
[c] Shorter volley length, and thus corresponding higher frequency, may result from the higher temperatures required to elicit feeding. Note that the number of events, however, remains constant across treatments.
[d] Data shown are for the stimulated (reversal) side only.
[e] One animal did not respond for this series of trials.
[f] Six of ten animals responded with reversal volleys in both ss and ST rows.

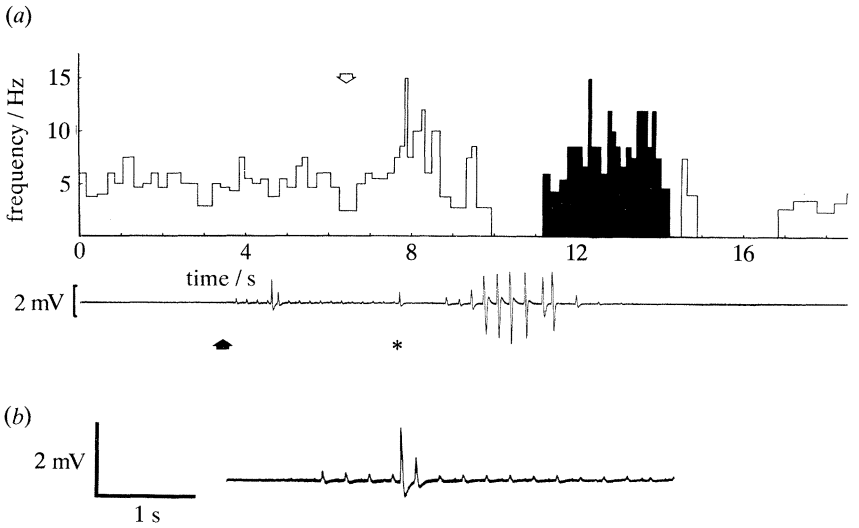


Figure 5. (*a*) ST plate responses evoked by electrical stimulation of the adjacent tentacle with 10 V, 5 ms biphasic pulses at 4 Hz for 10 s. Stimulus onset at heavy arrowhead. Small through-conducted potentials are evident after stimulus onset; the stimulus artifacts cannot be seen. A large monophasic event (injury potential?) occured when the tentacle broke (*) and retracted into its pouch, prematurely terminating stimulation. Yet, a volley and bout of reversed beating occurred. (*b*) Onset of stimulation and TCPs. Two larger potentials, out of phase with the stimulus, are seen.

the laydown, oral-most plates first perform a reversed recovery stroke, lifting up overlying plates successively in the aboral direction and mechanically stimulating them to perform reversed recovery strokes in turn, followed by reversed effective strokes (Moss & Tamm 1981). Waves of reversed beating thus propagate from the oral to the aboral end of the row, maintaining antiplectic metachrony (Knight–Jones 1954), but in the opposite direction to that during forward beating.

To examine the timing of electrical activity in comb plates along a given row, we recorded simultaneously from plates near the oral and aboral ends of the same ST row during electrically-stimulated initiation of reversed beating. Figure 7 shows that oral and aboral plates display virtually identical onset and timing of electrical events, yet the reversed beat occurred first orally and propagated aborally. Table 1 indicates that the oral plates, which are distal to the tentacular pouch opening (cf. figure 1*a*) received synaptic input at virtually the same time as the aboral plates. The size of the potentials recorded from oral versus aboral plates often varied, probably due to the tightness of electrode attachment, the number of cilia held by the recording pipet, or the absolute size of the bioelectrical response. Even so, oral and aboral volleys were very similar in both frequency and development; i.e. envelopes drawn around the volleys would yield very similar profiles. Therefore, initiation and propagation of reversed beating from the oral to the aboral end of the row is not correlated with the timing of volley potentials (either EPSPs or regenerative potentials) in successive plates along the row.

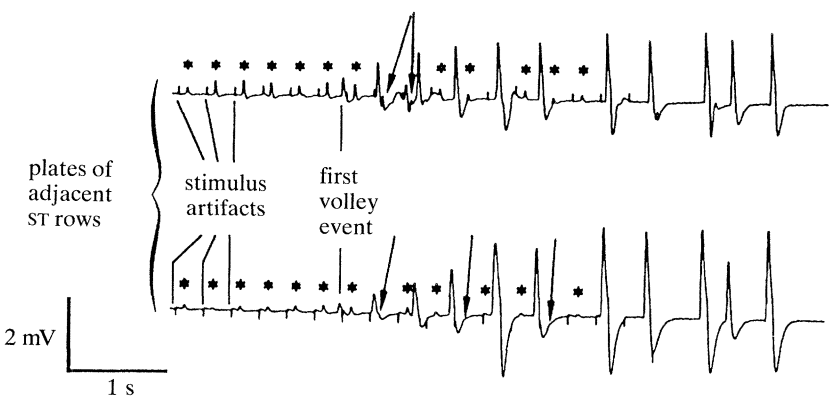


Figure 6. Temporal separation of through-conducted potentials (TCPs) and reversal volley potentials, as shown in responses of two plates of adjacent ST rows on the stimulated side. Stimulus artifacts are thin vertical lines of opposite polarity in the two traces. TCPs (*) were driven 1:1 with the stimulus. TCPs persisted within the volley as distinct potential changes as well as minor perturbations in the larger volley potentials (arrows); one TCP cannot be detected in the upper plate. The first volley event occurs between the sixth and seventh stimulus artifacts.




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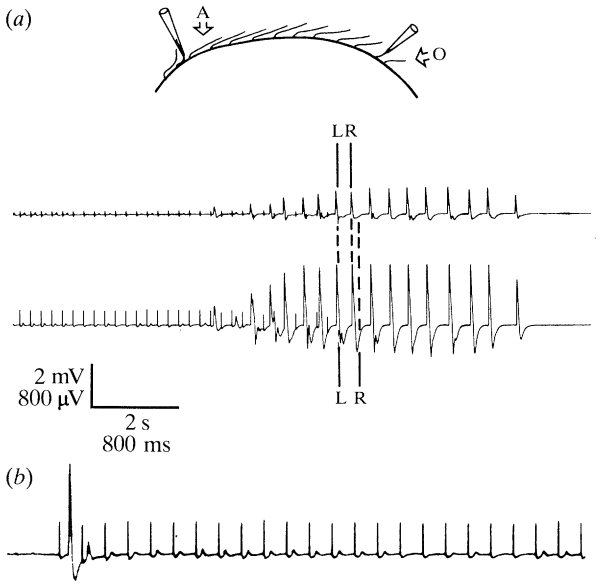


Figure 7. Concurrent recordings from plates at the oral and aboral ends of the same ST row during reversal evoked by electrical stimulation of a tentacle (arrow, onset of stimulus). (*a*) Animal traced from the video record. Aboral plate potentials were larger, perhaps due to tightness of electrode attachment and/or the number of cilia captured by the aboral electrode. Volleys were otherwise nearly identical in onset, frequency, timing and development. Laydown (L) occurred simultaneously along the row. Reverse beating (R) began at the oralmost end and traveled aborally as a single wave, passing first the oral, and then the aboral electrode. Open arrows in diagram indicate oral (O) and aboral (A) plates used for marking the passage of the first reversed metachronal wave. (*b*) Expanded record of the aboral record at onset of stimulation. The large first event occurred in both oral and aboral plates with identical timing. Stimulus: 10V, 4 Hz, 5 ms biphasic impulses.

### (*d*) *Subtentacular rows adjacent to the stimulated tentacle*

We previously showed that the onset of reversed beating occurs within 50 ms in the two ST rows adjacent to the prey-catching tentacle in feeding animals (Tamm & Moss 1985). The electrical activity of a plate in each of the ST rows adjacent to an electrically stimulated tentacle is compared in table 1, and an example shown in figure 8. As a rule we recorded from plates at about the same oral–aboral position along the two rows. On average, onset of reversed volleys occurred with identical timing in adjacent ST rows (table 1). All other characteristics of the adjacent ST row volley (events per volley, volley length, and volley frequency) were identical for each ST plate of a pair. Moreover, the lag between individual volley potentials was insignificant relative to the duration of the EPSP, the regenerative response, and certainly with respect to the motor response. However, while the electrical responses occurred with identical timing in plates of the two ST rows, and the volleys developed very similarly in both, the amplitude could be quite different (figure 8). In the case shown, reversal of beat direction was delayed by 0.5 s in the plate exhibiting a smaller amplitude volley, suggesting that the smaller amplitude potentials truly reflected the size of the electrical response, not technical differences in recording configuration. Such variation in the onset and duration of reversal in plates of adjacent ST rows is probably the result of the magnitude of synaptic depolarization. Because the regenerative response is graded to the magnitude of depolarization (Moss & Tamm 1986*a*), EPSP amplitude sets the resulting ciliary calcium influx.

### (*e*) *Subtentacular versus subsagittal rows*

Concurrent electrical activity in plates of adjacent ST and SS rows on the electrically stimulated side are compared in row 3, table 1, and examples shown in figures 9 and 10. In cases where reversed beating occurred only in ST rows, volley activity was confined to ST plates, and SS plates were electrically quiet (figure 10). When reversal occurred in both sets of rows, the respective volleys of ST and SS plates were somewhat different (table 1). In the example shown (figure 9), the volley and accompanying period of reversed beating occurred earlier and lasted longer in the ST plate than in the SS plate.

In figure 10, and in a number of other cases not shown, no electrical activity accompanied the initial increase in forward beating of a SS plate prior to reversal in ST rows, although several rather large EPSPs were sometimes observed during the corresponding frequency increase in the ST row. Low-to-moderate rates of forward beating therefore do not appear to depend on electrical input to the comb plates, consistent with previous results from dissected preparations using only longitudinally-split ST rows (Moss & Tamm 1986*a*). This is not to say, however, that membrane potential cannot play some role in regulating the beat rate (Moss & Tamm 1986*a*; below and Discussion).

We previously showed that initiation of reversed beating occurs within 50 ms on ST rows adjacent to the prey-catching tentacle (Tamm & Moss 1985). In 50% of the cases examined, reversal occurred on the SS rows. When reversal does occur in SS rows, it begins nearly simultaneously in the two SS rows, but about 0.5 s after onset of reversal in the ST rows. The current results on electrically stimulated reversal responses (six of ten animals displaying reversal on the ST rows alone; table 1) compares favourably with our findings on prey-elicited feeding responses. The volley activity of ST plates tended to precede that of the SS plates by ~0.5 s, although overlap in the data is large. What differences are seen (for example, the variation in onset of reversal in the two ST rows of figure 8) may reflect the different stimulation methods used (electrical versus food), orientation of the animals during stimulation (previously, animals were mounted with their oral-aboral axis vertical (Tamm & Moss 1985)), or to other seasonal and experimental differences between the two studies.

### (*f*) *Activity of ST rows on opposite sides of Pleurobrachia*

ST plates on the side opposite to electrical stimu-




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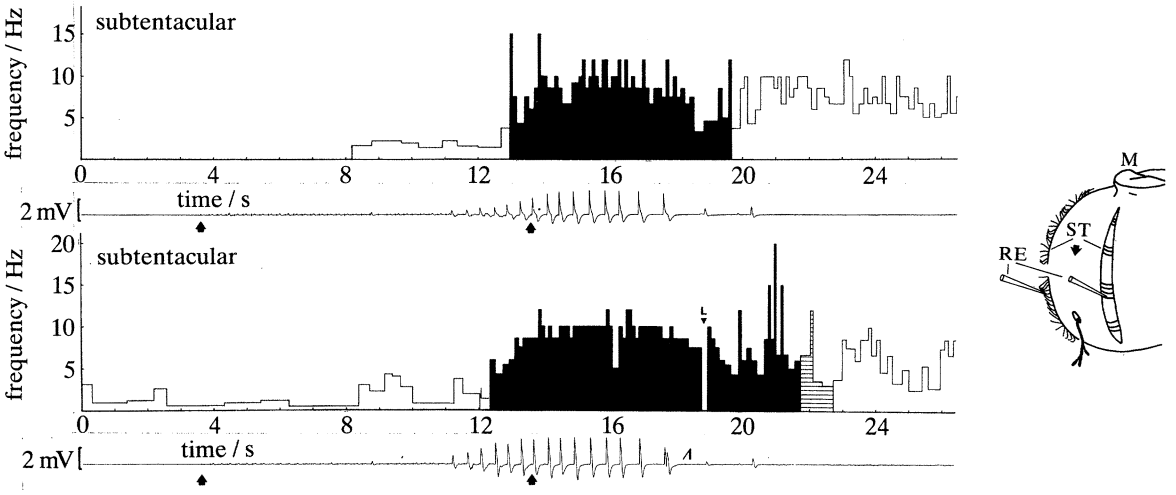



Figure 8. Motor activity in plates midway along adjacent ST rows bordering an electrically stimulated tentacle. Onset of volleys was identical in this pair of comb plates, although onset of reversed beating was somewhat different. In the lower trace, potential amplitude increased more rapidly, which may explain the earlier onset and longer period of reversal in this ST row. Spikes in the two plates occurred with identical timing. TCPs followed the stimulus at 4 Hz, while the average volley frequency was 2 Hz in both plates. Heavy arrowheads in electrical recording indicate onset and cessation of stimulus. Diagram is a tracing of the animal used for this trial: M, mouth; ST, ST rows; RE, recording electrodes; heavy arrowhead, direction of reversed metachronal wave. Stimulus as in figure 6. Hatched bars, indeterminate direction of effective stroke.

lation were typically electrically quiet, but did occasionally display truncated volleys similar to those in figure 4.

Global reversal was rarely observed in response to electrical stimulation. The few global reversal volleys observed were similar to unilateral reversal volleys, except that they occurred repeatedly in a given animal. Global reversals probably represent escape responses, not feeding behaviour, and are not considered further here.

### (*g*) *Isolated large electrical responses*

In many recordings from pairs of plates we observed simultaneous, isolated electrical events. These were monophasic (suggesting that they are EPSPs), and

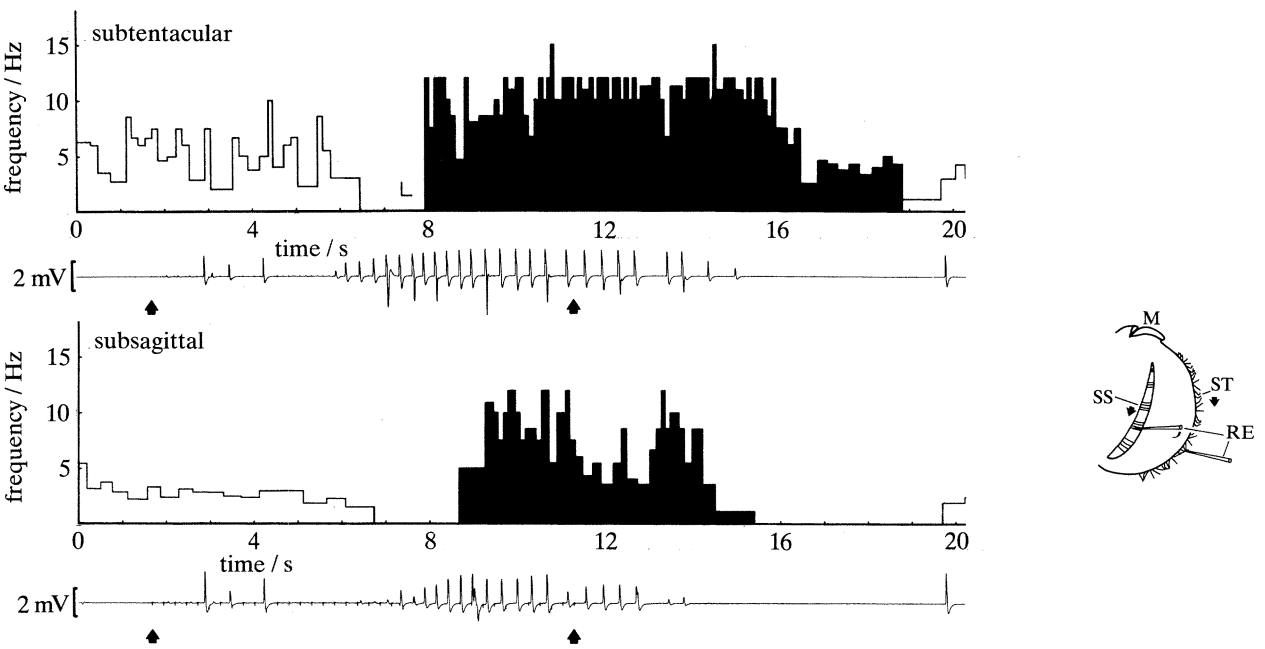
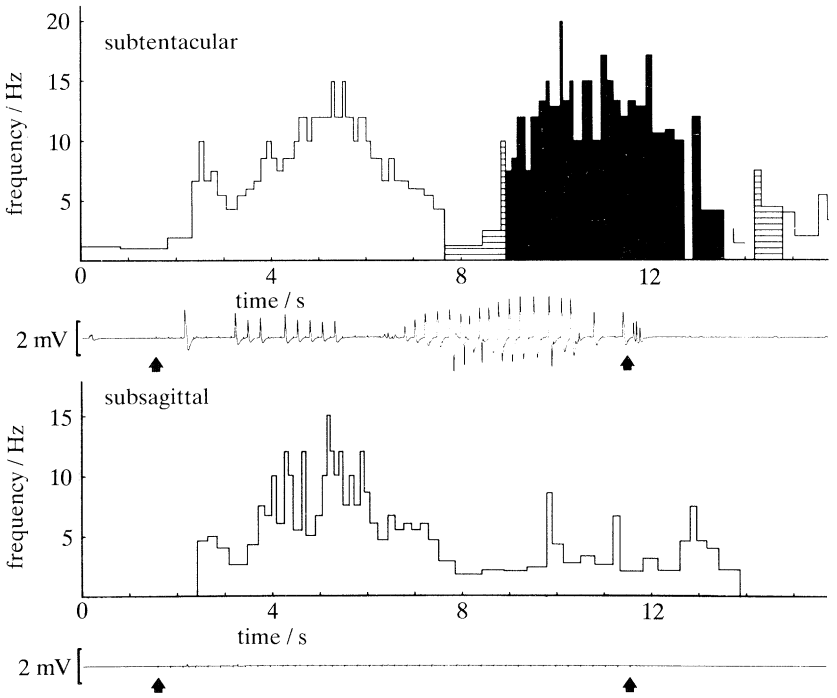



Figure 9. Electrical activity in laterally adjacent ST and SS plates that both exhibited reversed beating upon electrical stimulation of a tentacle. Three large spikes occurred concurrently in both plates prior to the volleys. Onset of the reversal volley in the ST plate preceded that in the SS plate by nearly 1 s, while ST row reversed beating also started approximately 0.7 s earlier. The ST volley was 2.5 s longer and reversed beating lasted 3.5 s longer than in the SS plate. Return to moderate forward beat rate was preceded by a single large event in each row. Stimulus as in figure 6.



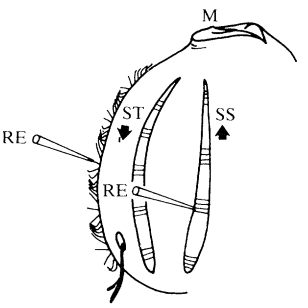




Figure 10. Concurrent, electrically stimulated ST and SS plate electromotor activity in a case in which reversed beating occurred only in the ST row. The SS row displayed no bioelectrical responses. The initial increase in frequency of forward beating in both rows was accompanied by large EPSPs in the ST plate only.

often quite large. They occurred prior to and/or after (figures 4 and 9) the reversal volleys, and were sometimes seen in nonstimulated animals. We recorded such activity in pairs of plates at various locations, both in the same hemisphere and in opposite hemispheres, suggesting that such responses can occur globally. These electrical events are not correlated with any consistent change in comb plate beating (cf. figure 4).

### (*h*) *Mouth bending*

Muscular bending of the mouth toward the prey-catching or electrically stimulated side (see Tamm & Moss 1985) typically begins before the onset of the reversal volley. Mouth bending occurred without any accompanying electrical activity in comb plates of any rows (figures 3, 4 and 11). Therefore, the neuromuscular conduction pathway responsible for mouth movements is entirely independent of the pathway innervating the comb plates.

### (*i*) *Determination of the unilateral reversal conduction pathway. Intact animals*

As with tentacular stimulation, electrical stimuli applied to the ectoderm of intact animals evoked unilateral reversal, as shown previously with vertically immobilized animals (cf. figure 3 in Moss & Tamm 1986*a*). However, the ST comb plate electrical responses to the two electrical stimulation methods were quite different, as shown in Figure 11. As expected, tentacular stimulation produced unilateral reversal. In the case shown (figure 11*a*,*b*), two reversal volleys occur with one tentacular stimulation train. The same preparation, when stimulated on the ectodermal surface (figure 11*c*–*e*), also displayed reversal, but with very different electrical activity. Reversal volleys were not seen in such ectodermally stimulated preparations. Instead, EPSPs appeared after some delay and grew in size while remaining 1:1 with the stimulus pulses. Action potentials appeared, again timed 1:1 with the stimulus, much as seen previously (Moss & Tamm 1986*a*; 1987). The regenerative potential waveshape shown in figure 11*c*–*e* shows that the electrode was attached to the basal region of the comb plate (see Moss & Tamm 1987), in order to prevent muscular contractions from pulling the comb plate free of the electrode. Occasionally, the bioelectrical response was not precisely timed with the stimulus (see figure 11*c* near beginning of the volley). As with electrical or prey stimulation of a tentacle, bending of the mouth occurred without associated electrical activity.

Ectodermally stimulated animals behave very similarly to the greatly reduced 'split-row' preparations used previously for intracellular and extracellular recording (Moss & Tamm 1986*a*, 1987).

These results indicate that while the peripheral nervous system (presumably the anatomical nerve net previously described by Hernandez–Nicaise (1974)) can propagate rhythmic nervous activity elicited by stimulus pulses at 4–5 Hz, it is probably not the source of the rhythmic reversal volleys observed during unilateral reversal.

### (*j*) *Mapping a functional pathway in electrically stimulated intact animals*

We used a combination of microsurgery and beha-

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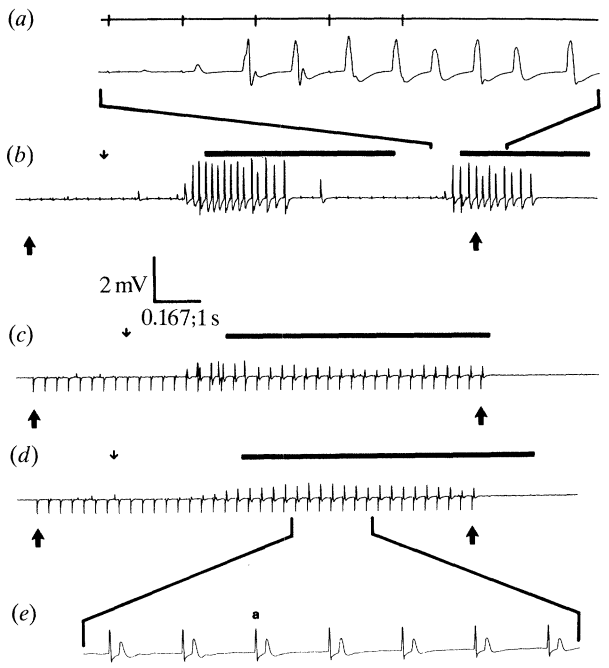
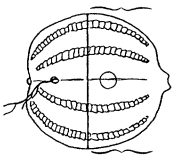
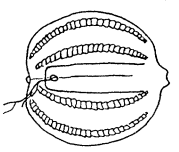
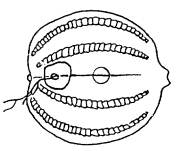
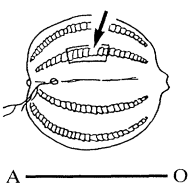



Figure 11. Comparison of direct electrical stimulation of the ectoderm versus tentacular stimulation. (*a*,*b*) ST comb plate ciliary electrical activity in response to tentacular stimulation. One 10 s stimulus train (10V, 5 ms duration, 4 Hz, indicated as starting and ending with the heavy arrows) resulted in two reversal volleys. Because the stimulus artifact is very small in these records, a stimulus marker line is shown above record (*a*). (*c*,*d*) Comb plate electrical activity in response to ectodermal pulse-train stimulation at 100 V on a different comb plate of the same ST row. Note that unlike in (*a*) and (*b*), electrical activity follows each large stimulus impulse in a 1:1 manner, and ends with cessation of the artifact. Positive-going stimulus artifacts in (*c*) and (*d*) (but not in the expanded scale record (*e*): 'a') are removed to allow clear view of the bioelectrical response at slow timescales. Small arrows, mouth bending.

Table 2. *Loss of unilateral reversal in microsurgically lesioned intact animals*

(Animals were electrically stimulated on the tentacle or midway between the ST rows (open circle) on the 'tentacular nerve' (Hernandez–Nicaise 1974) tract. The '+' and '−' refer to persistance or loss of unilateral reversal over a test region of the animal as follows, presented as number of trials meeting the criterion/total number of trials: (*a*) over the bracketed region; (*b*,*c*) over the whole hemisphere; (*d*) in the circumscribed 'island' denoted by the arrow.)

| | cut | stimulate | reversal + | reversal - |
|---|---|---|---|---|
| (*a*) |  | tentacle | | 8/8 |
| | | tract | 1/1 | |
| (*b*) |  | tentacle | 12/12 | |
| (*c*) |  | tentacle | | 9/9 |
| | | tract | 2/2 | |
| (*d*) |   | tentacle | | 2/2 |

vioural analysis to map the source of rhythmic nervous activity.

Lesioning demonstrated that the conduction pathway underlying feeding travels through the ectoderm in a diffuse manner (table 2). Annular cuts completely around the tentacular pouch opening always ablated all feeding responses and associated reversal volley activity recorded from the comb plate; leaving a small bridge permitted the feeding behaviour. Where the annulus around the pouch was complete, feeding could be restored by ectodermal stimulation as described above for intact animals. Equatorial cuts – which sliced across the stimulated hemisphere – had to be extended in both directions to the sagittal plane (i.e. halfway around the animal) in order to prevent mouth bending and reversal of comb plate beating in the region oral to the slice; reversal of beating, as expected, persisted in the aboral region. All attempts to modify or ablate the feeding response by cutting or removal (not shown) of the so-called 'tentacular nerve' (table 2; Hernandez–Nicaise 1974) were unsuccessful. Removal of all endodermal connections to the meridional canals, such as sectioning the radial canals, and cutting away all of the mesoglea had no effect on the feeding responses (not shown). Removal of the statocyst, always performed when making split-row preparations, also does not prevent comb plate reversal (cf. Moss & Tamm 1986*a*, 1987).

Taken together, these results show that the conduction pathway emerges from the tentacle pouch and travels in a diffuse manner to comb rows of that hemisphere through the ectoderm. An intact statocyst is not required. These characteristics are consistent with a nerve-net conduction pathway. The pathway appears to control one half of the animal, and is delimited by the sagittal plane. The nervous system thus appears to be partly bilateral, since a discrete conduction system associated with each tentacle triggers reversal of comb rows on that side. This conclusion is consistent with our observation above that electrical activity rarely passes from one side of the animal to the other. An alternate interpretation involves contralateral inhibition (see Discussion).

### (*k*) *A pattern generator in the tentacular base*

As with the ectoderm, cuts completely around the tentacle pouch (see figures 1 and 12*a*) prevented unilateral reversal upon tentacular stimulation. Con-

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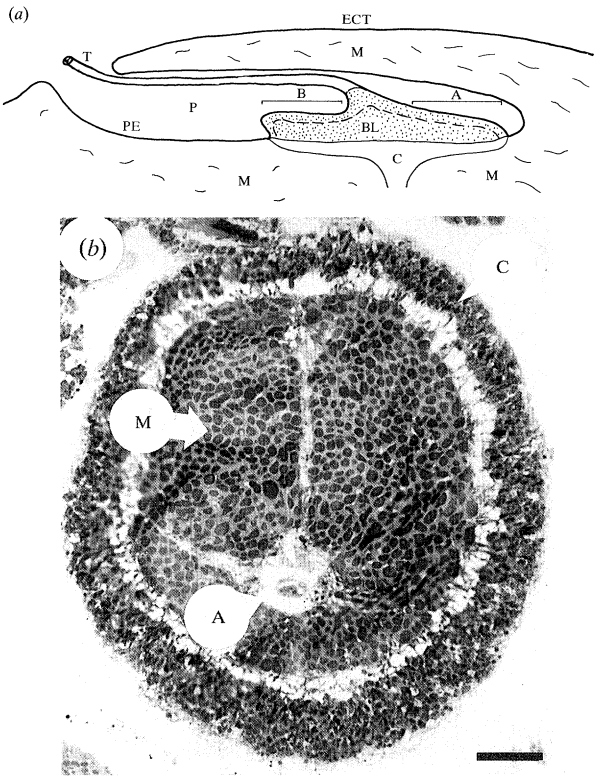


Figure 12. (*a*) Diagram of the tentacle, tentacular pouch, and tentacular bulb as viewed longitudinally within the pouch. ECT, ectoderm; T, tentacle; P, tentacular pouch; PE, pouch ectoderm; M, mesogloea; BL, tentacular bulb; stippled region, typical extent of the hyaline cortex; C, canal. Dashed line indicates the extent of the bulb internal canal, which is bifurcated (not evident in this view). A, transsectional cuts completely across the bulb in this region have no effect on comb plate unilateral reversal. B, transsection here entirely ablates the comb plate reversal volley. (*b*) Cross-section view of a tentacle well away from the hyaline cortex. Note two major muscle fiber bundles (M); A, axon fiber bundle; C, colloblast layer. Toluidine Blue O stained. Scale, 100 μm.

duction along the ectodermal lining of the pouch is also diffuse; leaving small bridges of intact ectoderm in any orientation allowed reversal volley activity in the comb plates. We were unable to record directly from the pouch ectoderm because it broke upon attachment of the suction electrode.

Electrical activity was therefore mapped by tentacular stimulation with *en passant* extracellular recording at various locations along the tentacle (figure 13). Considerable electrical activity was recorded, including very large amplitude complex waveforms which correlated closely with tentacular contraction (not shown). When such activity was observed, the tentacle invariably pulled free of the recording electrode. Fortunately, low intensity stimuli (5 V) were usually insufficient to excite muscle contraction, yet evoked individual action potentials that followed each stimulus one-for-one (figure 13*a*). Such tentacle spikes were uniformly 5 ms in duration (figure 13*b*) and had a large amplitude negative component that probably arose from the inward current of the action potential. Tentacle spikes were most easily recorded from the tentacular medial surface where the axonal bundle approaches the surface (figure 12*b*). Comb plate reversal and reversal volleys were invariably associated with tentacle spiking.

The base of the tentacle is called the tentacular bulb (Mayer 1912; Harbison & Madin 1982). Preliminary ultrastructural studies (A. G. Moss & S. Tamm, unpublished data) indicate that the bulb is hollow and surrounds a central bifurcated canal. The bulb itself consists of numerous small round cells embedded in a thick clear mound we term the hyaline cortex (see figure 12).

The experimental set-up allowed us to record over the full length of the tentacle and into the pouch. When inserted through an opening into the pouch (see Materials and Methods) and attached to the medial surface of the tentacle near its site of insertion in the bulb, volleys of monophasic waves were recorded in addition to the tentacle spikes (figure 13*c*). These 'cortical waves' increased in amplitude as the response developed. Cortical waves could be recorded anywhere on the surface of the hyaline cortex. Only if the electrode covered both the medial tentacle surface and the cortex were both cortical waves and the tentacle spikes recorded (figure 13*a*).

Like comb plate reversal volleys, cortical waves ran free of the stimulus and the stimulus-entrained tentacle spikes from inception. Onset of cortical waves preceded reversal volley onset in the comb plates, as revealed by concomitant recordings from ST plates and the hyaline cortex. Cortical waves appear to drive the ectodermal conduction pathway underlying comb plate reversal volleys, for the latter never occurred in the absence of cortical waves, and there was an approximate one-for-one correspondance between cortical waves and events in the reversal volley (figure 13*a*,*c*). Single large comb plate EPSPs, not associated with reversal volleys, also appeared to have a source in hyaline cortex activity, since we occasionally observed isolated cortical events (sometimes with a superimposed tentacle spike) which seemed to immediately precede isolated comb plate events (see beginning of record in figure 13*a*).

Cuts across the tentacular bulb itself had little effect on the comb plate responses unless the cut occurred between the site of insertion of the tentacle and the aboral end of the bulb (see figure 12). Such cuts clearly indicated an essential pathway within the bulb. Careful sectioning of the rearmost region of the bulb revealed that the pathway is very narrow there, and seems to be directly apposed to an apparent condensation of the nerve net, visible in differential interference contrast (DIC) light microscopy (not shown). This feature runs aborally from the end of the bulb to the entrance of the pouch. The ultrastructural basis for this pathway is under investigation.

## 4. DISCUSSION

### (*a*) *Neurociliary conduction pathways in ctenophores*

Neurociliary conduction pathways have long been




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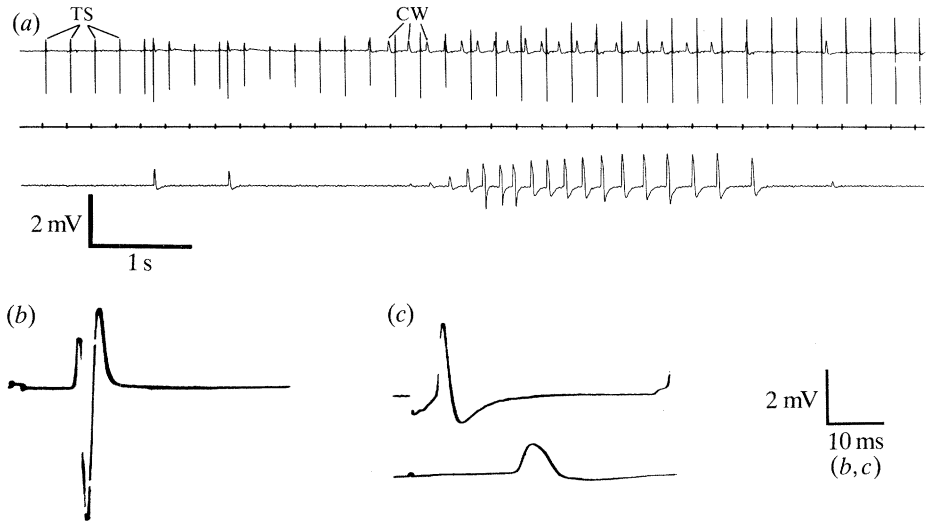


Figure 13. Cortical waves (CW), tentacular spikes (TS), and comb plate reversal volley potentials. (*a*) (Above) Record from an extracellular electrode attached over the medial axonal bundle where the tentacle enters the hyaline cortex, and (below) record from an extraciliary electrode attached to a subtentacular comb plate as described in the text. Twenty-one cortical waves preceded and accompanied 19 reversal volley events. Middle record marks the occurance of each stimulus of the pulse train. (*b*) Tentacular spike, recorded extracellularly from over the medial axonal bundle. (*c*) Extracellularly recorded cortical wave (above) and comb plate volley potential (below).

postulated in ctenophores, (Horridge 1965*a*, 1966, 1974). Horridge & Mackay (1964) described synapses onto the bases of comb plate cells by electron microscopy, and suggested that they were involved in the control of beating. Hernandez–Nicaise described a finely anastomosing nerve net in the ectodermal layer of *Pleurobrachia* by methylene blue staining (1973*a*,*b*, 1974), and described the detailed ultrastructure of ctenophore synapses (Hernandez–Nicaise 1974). Despite these efforts only one study has directly demonstrated that neural activity controls comb plate beating (Moss & Tamm 1986*a*).

This is the first report that characterizes the physiology of specific conduction systems in ctenophores. The most likely candidate is(are) the ectodermal nerve net(s), and we will assume here that the properties we describe are those of the nerve net(s). However, we cannot rule out that epithelial conduction or a combination of nerve net and epithelial conduction (see Shelton (1982) and Spencer (1991) for reviews) constitute the actual conducting pathway(s). Unequivocal confirmation that a nerve net is the anatomical pathway that controls comb plate beating awaits the development of an *in vitro* preparation such as those previously developed for scyphozoan (Anderson & Schwab 1983, 1984) and hydrozoan neurons (Przysiezniak & Spencer 1989). Our approach has the advantage of providing specific access to the comb plate neurociliary pathway. We are therefore able to use this method to trace the source of rhythmic activity and define the gross neuronal pathway of the feeding response.

### (*b*) *Conduction pathways in Pleurobrachia. Mouth bending*

Mouth bending preceded unilateral reversal in all of our trials. However, it was never observed in conjunction with electrical activity of the comb plates. Although ciliary reversal and mouth bending may both be mediated by nerve nets, it is clear that these two pathways are separate.

### (*c*) *Rhythmic behaviour of the ectodermal conduction system*

Our observations indicate that, in *Pleurobrachia*, the unilateral reversal conduction system innervating comb plates generates and conducts rhythmic electrical activity over a narrow frequency range. This latter characteristic is generally true throughout the conduction system, from the tentacle to the ectodermal nerve net. We show here that 4 Hz stimuli optimally activate unilateral reversal and trigger endogenous rhythmic behaviour in an integrative center that drives the ectodermal conduction pathway. Although it does not *generate* rhythmic activity, the ectodermal conduction pathway will conduct EPSPs at 4–5 Hz stimuli in a 1:1 manner if stimulated directly (figure 11*c*–*e*).

Four to five Hz biphasic stimuli produce a sustained 5–15 mV depolarization of the polster cells associated with increased frequency of forward beating. The sustained depolarization is also a prerequisite for the regenerative activity responsible for reversed beating (Moss & Tamm 1986*a*). Although higher stimulus frequencies (up to 15 Hz) depolarize polster cells further, regenerative activity is lost, presumably because the polster cell spike refractory period is of the order of 100–200 ms. Lower frequencies do not produce a summed DC potential. In contrast, the tentacular conduction pathway, probably the medial axonal bundle (figure 12*b*), follows each stimulus pulse in a 1:1 manner. As would be expected for an




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axon fiber bundle, low frequencies of stimuli (and so spiking) are faithfully carried by the tentacle; however, they produce only isolated cortical wave activity that never develops to ultimately produce a reversal volley. Thus, the rhythmic center, possibly the hyaline cortex of the tentacular bulb, is also maximally responsive to 4–5 Hz stimuli provided by tentacular spiking. Attempts to directly stimulate the hyaline cortex have so far been unsuccessful.

Thus, the entire reversal pathway is organised to provide a narrow range of rhythmic input to the polster cells. Trains of tentacular spikes evoke rhythmic activity in an integrative center in the tentacular bulb. The tentacular bulb produces an endogenous slow phasic discharge that occurs at an optimal driving frequency for the ectodermal nerve net. The nerve net in turn transmits rhythmic activity at a frequency that maximizes the electrical response of the polster cells. Moderate depolarization induces an increased rate of forward beating, while sufficient depolarization to produce ciliary spiking produces reversed beating.

### (*d*) *Separation of neuronal activity in the two halves of the animal*

A volley of ciliary regenerative activity on the prey-catching or electrically-stimulated side accompanies reversal of beat direction on that side, as expected. Rhythmic volleys rarely invade the opposite side of the animal. *Pleurobrachia* might achieve this by unilateral excitation of a neural pathway leading only to the side of prey capture. If so, *Pleurobrachia* has two independent, bilaterally arranged nervous systems which do not cross the sagittal plane. However, some connectivity must exist across the sagittal plane, because we occasionally observed weak non-stimulated-side volleys, as well as global volleys and reversals. Such observations suggest that reversal on the non-catching side might be actively prevented by inhibitory neural input at some point in the pathway to prevent the reversal volley. However, we have not observed a hyperpolarizing, or inhibitory, postsynaptic response in polster cells. We cannot rule out the possibility of an inhibitory shunting conductance in the polster cells that occurs near $V_m$ (for example elevated gK), such as has been seen in a hydrozoan (Chung & Spencer 1991).

### (*e*) *Stoppage of forward beating*

Unilateral reversals fall into several categories, with approximately 70% involving sustained forward beating on the non-stimulated side, while about 30% show complete stoppage on that side (Tamm & Moss 1985). The initiation and rate of forward beating in intact animals is regulated by the beating of pacemaker balancer cilia in the aboral statocyst (Tamm 1982). Coordination between each compound balancer cilium and the corresponding first plate of each comb row is strictly mechanical (Tamm 1973). Inhibitory input to the statocyst balancer cilia during unilateral reversal could therefore account for the 30% that undergo stoppage. To date, we have no direct evidence for such activity. However, support for this hypothesis comes from thin-section electron microscopical observations of numerous anatomical synapses at the bases of the balancer cells (Tamm 1982).

### (*f*) *At least one through-conducting pathway mediates global electrical activity*

A non-decrementing, through-conducting pathway is revealed by electrical stimulation of the tentacle. Each TCP clearly has its origin in a tentacular spike, which follows each stimulus precisely. In contrast, we never observed TCPs during prey-evoked feeding. We conclude that although electrical stimulation is a very convenient and simple method of evoking feeding responses, it activates at least two conduction systems. One is the reversal volley pathway, which may normally be activated by mechanical or chemical stimuli from captured prey. The other is the through-conducted pathway, of unknown function. There is also a third excitable element in the tentacle: higher intensity stimuli produce muscle contraction, either directly or by a third neural pathway. Attempts to record prey-induced tentacular spike activity have so far met with failure, because the tentacle contracts strongly during feeding. The large amplitude tentacular spike evoked by electrical stimulation might therefore mask a more subtle response actually responsible for unilateral reversal and feeding.

Yet another functionally separate excitatory neural pathway may mediate global postsynaptic activity in all comb plates of all rows. Despite nearly complete separation of rhythmic electrical activity on the two sides of *Pleurobrachia*, isolated single postsynaptic events (indicating isolated spike activity in the pathway) can occur nearly simultaneously in well-separated comb plates, irrespective of the recording site. Whether such activity is carried by the above TCP system, or represents yet another anatomical pathway is unclear.

### (*g*) *Direction of metachrony during reversed beating*

Forward metachronal waves of comb plate beating start at the aboral end of a row, triggered mechanically by pacemaker balancer cilia in the aboral statocyst (Tamm 1973, 1980, 1982). In contrast, reversed metachronal waves travel from the oral to the aboral end of the comb row without obvious mechanical initiation of beating at the oral end.

What establishes and maintains the oral-aboral direction of metachrony during reversed beating? We show here that it is not related to the propagation of bioelectrical activity (Parker 1905; Horridge 1965*b*), since electrical activity at different sites along a row is nearly synchronous, with no suggestion of oral-aboral propagation. In addition, the cyclic rate of reversed beating far exceeds the firing rate of the comb plate cilia.

Our previous study using mechanical blocks to comb plate beating showed that reversed metachronal




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waves, like normal waves, are propagated by mechanical interaction (Moss & Tamm 1981). In addition, the shorter oral plates have an intrinsically higher beat rate than the longer aboral plates (Moss & Tamm 1981). Although this may establish a mechanical pacemaker activity at the oralmost end of the row, it is still unclear why the oralmost plates are the first to beat.

### (*h*) *Forward beating and electrical activity*

We previously showed a relationship between the synaptically driven, summed depolarization of polster cells and the forward beat rate of comb plates (Moss & Tamm 1986*a*). We could not test for this relationship here with intact animals, because our recording method detects only transient electrical activity. Our previous observation of summed depolarization in response to repetitive, excitatory postsynaptic activity (Moss & Tamm 1986*a*) should hold for intact animals. However, there were some cases in which moderately increased forward beat rate was not associated with EPSPs. Thus, there is no obligatory relationship between forward beat rate and membrane potential at low to moderate beat rates.

However, flurries of electrical activity are often observed when particularly fast beating occurs (figures 2 and 10 ST), such as during escape responses to noxious stimuli. All animals showed rapid, sustained EPSPs while displaying very rapid forward beating upon pinning to the Sylgard plate. This behaviour is probably an escape response to noxious stimulation. The polster cells of such animals were almost certainly depolarized, and this would indeed correlate with the observed fast forward beating. Nervous activity probably also increased the beat rate of the statocyst balancer cilia, so that they remained the mechanical pacemakers for the comb rows.

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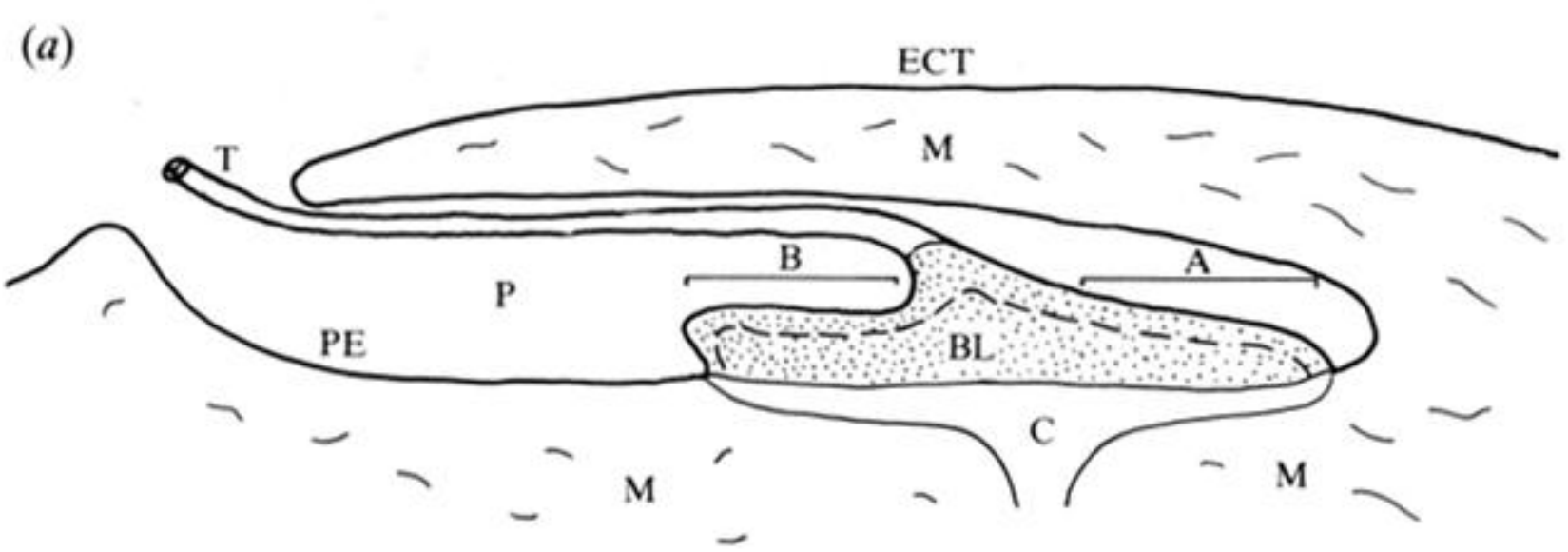


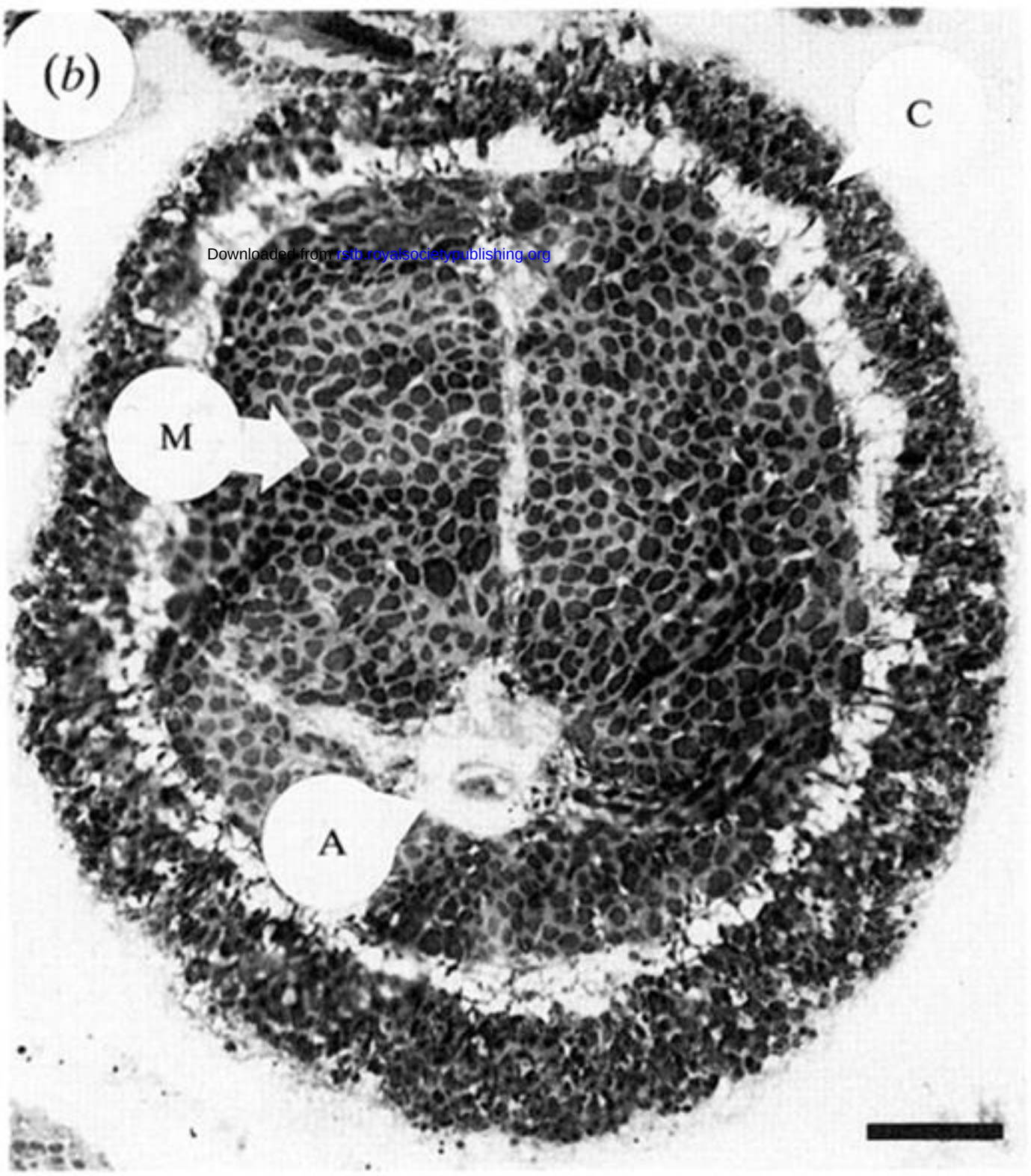




gure 12. (*a*) Diagram of the tentacle, tentacular pouch, d tentacular bulb as viewed longitudinally within the uch. ECT, ectoderm; T, tentacle; P, tentacular pouch; PE, uch ectoderm; M, mesogloea; BL, tentacular bulb; stippled gion, typical extent of the hyaline cortex; C, canal. Dashed e indicates the extent of the bulb internal canal, which is urcated (not evident in this view). A, transsectional cuts mpletely across the bulb in this region have no effect on mb plate unilateral reversal. B, transsection here entirely lates the comb plate reversal volley. (*b*) Cross-section view a tentacle well away from the hyaline cortex. Note two ajor muscle fiber bundles (M); A, axon fiber bundle; C, lloblast layer. Toluidine Blue O stained. Scale, 100 μm.