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ELECTRICAL CORRELATES OF CILIARY
REVERSAL IN OIKOPLEURA

By C. P. GALT

Zoology Department, University of Washington, Seattle, U.S.A.

AND G. O. MACKIE

Biology Department, University of Victoria, Victoria, British Columbia, Canada

INTRODUCTION

'Almost all cilia and flagella seem to be under the control of the organism and such control may involve activation, inhibition and sometimes reversal' (Sleigh, 1962). Examples from among the protozoans, nemertines, ctenophores, sea anemones and corals, amphibians, brachiopods, bivalve and gastropod molluscs are mentioned in reviews by Sleigh (1962) and Kinosita & Murakami (1967). In addition, ciliary stoppages or reversals have been described in the polychaete *Chaetopterus* (work cited by Jørgensen, 1966), Amphioxus (Bone, 1958), echinoderm larvae (Mackie, Spencer & Strathmann, 1969) and, among the Tunicata, in ascidians (MacGinitie, 1939), doliolids (Fedele, 1923) and larvaceans (Lohmann, 1933).

There is strong evidence for nervous control in bivalve molluscs (Aiello, 1970), ctenophores (Horridge, 1965; Horridge & Mackay, 1964), doliolids (Fedele, 1923), and Amphioxus (Bone, 1961). Elsewhere among the Metazoa, evidence with regard to control is ambiguous, although it is likely that the nervous system is implicated in a number of cases.

In ciliate protozoans the cilia beat forwards when the cell membrane is hyperpolarized, reversing when the membrane depolarizes (Naitoh & Eckert, 1969*a*, *b*; Eckert & Naitoh, 1970). There is as yet no comparable evidence in the case of metazoan ciliated epithelia, but several authors have suggested that the excitation responsible for reversal or inhibition is propagated in a neuroid manner between the ciliated cells themselves (Bone, 1961; Baba, 1968; Mackie *et al.* 1969). The last-named authors recorded electrical events from ciliated epithelium which appeared to be correlated with the co-ordinated reversals of different groups of cilia, but the recordings were made with external electrodes and the source of the signals was not established.

In the present study we have investigated ciliary reversals in *Oikopleura*. This pelagic tunicate secretes a 'house' in which it lives and which serves as a filtration apparatus. Water is pumped through the house by undulatory movements of the tail and at the same time a ciliary current draws food particles into the mouth. The food is trapped in mucus and ingested while the water passes out through two ventro-lateral stigmata, as indicated by the arrow in Fig. 1. The current is set up by rings of ciliated cells located in the stigmata. Water can flow through the pharynx in either direction (Huxley, 1856). The normal flow (in through the mouth) is interrupted by

periods when the flow is reversed (Fol, 1872), and this was attributed by Lohman (1933) to a change in the direction of beating of the cilia in the ciliated rings. We have observed such events and have recorded the electrical signals accompanying them, using both internal and external electrodes.
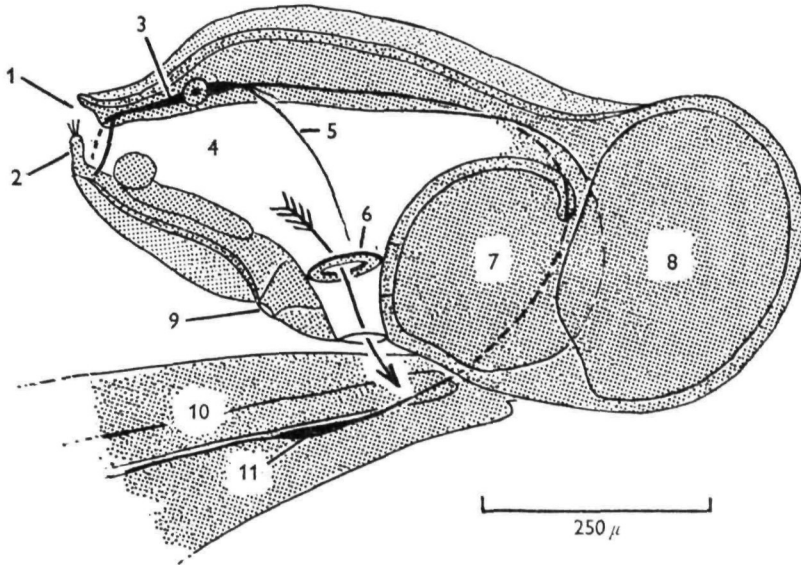

Fig. 1. Trunk and portion of tail of *Oikopleura* removed from its house, viewed from the left (schematic). 1, Mouth; 2, lower lip; 3. brain; 4, pharynx; 5, left lateral nerve; 6, ciliated ring of left stigmatal opening; 7, left lobe of stomach; 8, gonad; 9, anus; 10, tail; 11, caudal ganglion. Arrow shows direction of normal water flow.

MATERIAL AND METHODS

Two species of *Oikopleura* were studied: *O. dioica* and *O. labradoriensis*. They were caught off the dock at the Friday Harbor Laboratories of the University of Washington and were studied within a few hours of capture, usually after leaving their houses. They were maintained at approximately 14 °C during feeding experiments and recordings.

A strobe light was used to facilitate observation of ciliary movements.

Electrical events were recorded extracellularly using suction electrodes (Josephson, 1965; Mackie & Passano, 1968) in conjunction with capacity-coupled amplifiers. Glass microelectrodes with input into a Medistor Model A–35 electrometer amplifier were used for d.c. recordings.

Specimens were fixed in Flemming's fluid (without acetic acid) diluted 1:3 with sea water and were subsequently dissected, pieces being stained in Heidenhain's haematoxylin and mounted in Canada balsam for study of nervous connexions.

RESULTS

Ciliary reversals and reversal of water flow in the pharynx

We can confirm that the direction of water flow is controlled by the ciliated rings. Preceding each change in the direction of flow a change is seen in the appearance of

e ciliated rings. It is not clear if the significant event is a change in the orientation of the cilia, a change in the direction of beating, or a combination of the two. Examination with the strobe light suggests that the observed change may be a synchronous shift of all the cilia which brings them into the orientation for continued metachronal reverse beating as observed in *Paramecium* (Párducz, 1956, cited by Eckert & Naitoh, 1970). For convenience we will simply speak of 'ciliary reversals'. The rate and direction of water flow through the pharynx appears to depend entirely on the activity of the ciliated rings. There is no muscle in the pharnyx and no squirting behaviour comparable to that shown by ascidians.

Ciliary reversals, if infrequent, cause only a slight interruption in the otherwise steady flow of water through the mouth. The only visible indication of a reversal may be the slight hesitation of a particle moving through the pharynx. Ciliary reversals occurring more frequently than about 1/s sum to create a reversal of the water flow. The higher the frequency, the smoother and more rapid the reverse flow, as the cilia are maintained in the reverse posture for only a short, finite time (less than 1 s) before starting to return to the forward posture. The cilia of both stigmata reverse in synchrony.

In animals observed under nearly natural conditions while still in their houses, ciliary reversals were observed when large particles of detritus struck or entered the mouth. Addition of carbon particles to the water evoked reversals, resulting in a sustained flow of water in the reverse direction if the material was not immediately dislodged by the initial reversal response. The flagellate *Tetraselmis*, when added to the water in low concentrations, evoked reversals in the same manner. A smaller flagellate, *Isochrysis*, was treated as food and ingested. Only when *Isochrysis* was present in concentration sufficient to cause congestion in the foregut were reversals observed. These examples suggest how the reversal mechanism may be of adaptive advantage to the animal in nature.

Electrical events recorded during reversals

Suction-electrode recordings from the trunk surface may pick up any of the following types of electrical events: (1) heart-beat pulses, (2) muscle potentials correlated with tail movements, (3) small potentials evoked by tactile stimulation of the outer epithelial covering layer of trunk (these events are also recorded by microelectrodes inserted through the epithelium, and may be 'skin impulses' comparable to those recorded in amphibian larvae; Roberts, 1969), (4) ciliary reversal potentials. We are here concerned only with the last of these.

Suction-electrode recordings

Suction electrodes placed on the outside of the trunk register sizeable electrical signals at each ciliary reversal. The pattern of these ciliary reversal potentials, or CRPs, accurately reflects the changing pattern of water flow through the pharynx (Fig. 2) as observed through the microscope.

CRPs are typically negative-going, simple or compound potentials having amplitudes up to 2 mV. They are picked up all over the trunk, including the specialized 'oikoplast' areas which secrete the house, and for a short distance down the tail.

The reversals recorded in Fig. 2 were evoked by collision of large particles of

detritus against the lips. The duration of the CRP burst and the frequency of t█
potentials within the burst seem to be directly related to the strength and duration
of impact, subjectively assessed. Fol (1872), who first described current reversals
following such impacts, stated specifically that the stimulating particles strike the
sense hairs which project from the lips. We find that tactile stimulation with a glass
needle or electrical stimulation of the lower lip both evoke ciliary reversals. In animals
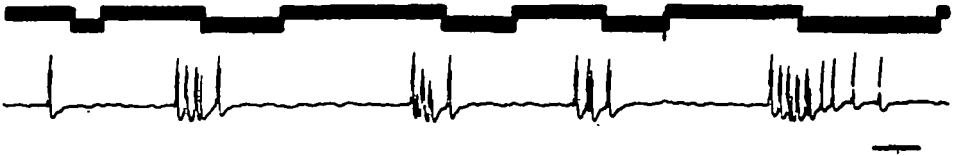

Fig. 2. Suction-electrode record from trunk surface, showing bursts of ciliary reversal potentials
presumed to be evoked by intermittent collision of carbon particles with the lips. Downward
deflexions of the solid bar above the record represent reversals of the water current as seen by
the observer. The time-lag represents the observer's reaction time plus delay due to inertia of
the water mass in the pharynx. Scale: 1 s.

Fig. 3. Suction-electrode record from a specimen showing a steady pattern of CRPs, accelera-
ted briefly by tactile stimulation of the lower lip with a glass needle (arrows). Scale: 1 s.

showing a steady pattern of CRPs in response to stimulation of the lip there is an
increase in the frequency of the potentials (Fig. 3). Stimulation of regions other than
the lips seems to have little, if any, effect on CRP frequency.

Microelectrode recordings

The following observations are based on some twenty microelectrode penetrations
through the body wall in various places, of which six reached stable recording positions
for CRPs in the vicinity of the ciliated rings.

On advancing the electrode through the body wall we generally record a potential
shift of about 50 mV negative as the electrode goes into or through the outer epithelial
layer of the body wall. CRPs are not picked up in this position. We tentatively regard
the 50 mV shift as representing the potential difference between the haemocoel and
the outside, as measured across the outer epithelium. On advancing the tip further in
the direction of the ciliated rings we record a second major shift of 50–70 mV negative
and CRPs are immediately recorded as 70–80 mV positive-going spikes. Although
some uncertainty remains regarding the exact position of the electrode tip, the resem-
blance of these recordings to conventional intracellular recordings, and the fact that
they were obtained only from the immediate vicinity of the ciliated rings, makes it
probable that they are intracellular records from the ciliated cells. On this assumption
we view the CRP as a depolarization of the ciliated cell membrane.

A typical microelectrode record of the type described is shown in Fig. 4. Such records were invariably monitored with an external electrode placed on the outer body surface. The CRP pattern recorded from the two places corresponds exactly.

Some, but not all, of the intracellular recordings show a pattern of low-amplitude fluctuations distinct from CRPs (Fig. 5A). Their frequency corresponds to the frequency of metachronal ciliary beating (10–15/s) measured stroboscopically, and we regard them as a record of the ciliary beat. We cannot determine whether these pulses represent fluctuations in membrane potential associated with propagation of the ciliary beat (cf. Horridge, 1965) or are merely an electrical artifact resulting from mechanical movement of the electrode tip due to the ciliary beat itself. In *Paramecium* ciliary beating is not accompanied by shifts in membrane potential (Eckert & Naitoh, 1970). Occurrence of CRPs causes consistent variations in the pattern of the ciliary beat record (Fig. 5B).
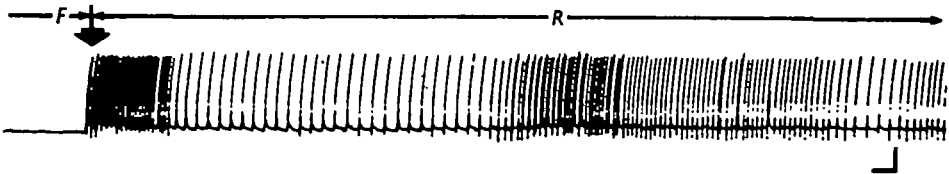

Fig. 4. Microelectrode record from ciliated ring. Current flow was observed to change from the forward direction (*F*) to reverse (*R*) at the point marked by the arrow, apparently in response to carbon particles lodging in the mouth. Horizontal scale, 1 s; vertical, 15 mV.
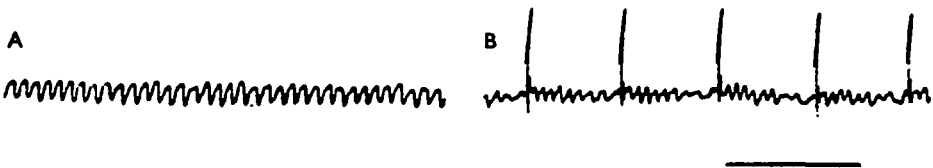

Fig. 5. Microelectrode records from ciliated ring. A, Low-amplitude deflexions at frequency of 11/s, representing ciliary beating. B, Ciliary reversal potentials are superimposed on the ciliary-beat record. Scale: 1 s.

Evidence of nervous control

Histological examination confirms the picture of innervation presented by classical authors, particularly Fol (1872). Nerves can be followed from the sensory processes in the lips to the brain (Fig. 1, (3)). The major posterior nerve trunk passes back along the dorsal midline and enters the caudal ganglion in the tail. A lateral nerve (5) arises from just behind the brain and runs down on either side toward the ciliated ring, continuing past it and disappearing under the gut. We have not followed out its posterior ramifications, and only the thicker, proximal part of the nerve is shown in the figure. However, we have discovered a dichotomizing side-branch coming from each of the lateral nerves, whose fine terminal processes (less than 0·5 μ in cross-section) can be traced to the cells of the ciliated rings. The two rings are separately innervated. Not all the cells in each ring are innervated.

Neurociliary synapses have been described in ctenophores, using electron microscopy (Horridge & Mackay, 1964). Bone (1961) attributes a cilio-effector function to at

least some of the fibres running in the gill bars of Amphioxus. We assume that we dealing with a similar type of innervation in *Oikopleura*.

To determine whether the CRP pattern represents the activity of a nervous pace-maker in the brain we removed the anterior part of the animal. This operation, carried out several times, results in the removal of the brain and the isolation of the two lateral nerves. The CRP pattern was shown consistently in animals following this operation, from which we conclude that if a nervous pacemaker is involved, it must lie in the nerves associated with the ciliated rings themselves. Surprisingly, in these preparations, the two rings remain synchronized.

DISCUSSION

The existence of nervous pathways connecting the sensory processes in the lips to the ciliated rings, taken in conjunction with the observed excitatory effect of lip stimu-lation on the CRP pattern, can be confidently interpreted as evidence that the ciliated rings are under some form of nervous control. However, the fact that CRPs continue after removal of the brain and anterior sense organs shows that excitation from the anterior nerve centres is not the only way in which ciliary reversals can be evoked.

We favour the concept of a ciliary reversal pacemaker associated with each ciliated ring, the control of whose output would be a property of the nerves coming from the brain. Control might take the form of excitation of the pacemaker, inhibition of it, or both. Nothing is known of the internal 'wiring' of the larvacean central nervous system.

To explain the continued co-ordination of the ciliated rings after severance of all known nervous connexions between them requires the existence of a conducting path-way between the two rings or their pacemakers. Neuroid conduction in the inner epithelium of the body wall between the two rings is a possibility. Propagated action potentials are known in certain epithelia of hydrozoan coelenterates (Mackie, 1965; Mackie & Passano, 1968) and amphibian tadpoles (Roberts, 1969).

The fact that CRPs can be recorded as large signals on the *outside* of the body suggests that a relatively large number of cells are active simultaneously, creating a major bioelectric disturbance which is picked up electrotonically like an electrocardio-gram from the body surface. The alternative possibility, that CRPs are propagated to the outside epithelium, appears unlikely, since microelectrodes inserted across the outer epithelium fail to pick them up while picking up the 'skin impulses' mentioned on page 207.

The evidence from microelectrode recordings from the ciliated rings can be most conveniently interpreted in terms of the protozoan model, namely that the ciliary reversal potentials are depolarizations of the membranes of the cells of the ciliated rings. The steady negative potential from which they arise would thus be a transmembrane resting potential. However, it is conceivable that recordings from an extracellular fluid space enclosed within active, neuroid epithelia might simulate intracellular recordings, and until we have resolved the question of where the electrode tip is located, this pos-sibility must be kept in mind.

As Kinosita & Murakami (1967) point out, it has always seemed likely that ciliated metazoan cells and ciliate protozoans operate on the same basis in view of the similarity

the action of drugs such as KCl on the two systems. In the case of metazoan cells, specifically those like *Oikopleura* where there is evidence of nervous control, we propose that the depolarizations responsible for reversals originate at the neurociliary synapses. Since not all the ciliated cells in *Oikopleura* are innervated, the depolarizations may be supposed capable of neuroid spread within groups of ciliated cells, and possibly, as mentioned earlier, across the epithelium connecting the two ciliated rings.

Ciliary reversals occur in the pharynx of ascidians (MacGinitie, 1939). We have observed them in *Diplosoma macdonaldi* and have recorded the correlated electrical events. The picture here is complicated by the existence of musculature in the pharynx wall, and of spontaneous squirting activity (Hoyle, 1953). It is of interest to note that the squirting behaviour appears to involve a local pacemaker in the pharynx, like the CRP system in *Oikopleura*, since in both cases the activity continues after removal of the brain.

SUMMARY

1. Reversal of the water current through the pharynx of *Oikopleura* is brought about by a change in the action of the cilia of the two stigmatal ciliated rings. These 'ciliary reversals' occur synchronously in the two ciliated rings and can be evoked by the addition of particulate material to the incoming water or, by tactile or electrical stimulation of the lips.

2. Nerves run from the lips via the brain to individual ciliated cells, and it is therefore likely that the ciliated cells are under nervous control.

3. At each ciliary reversal an electrical potential can be picked up on the body surface. The same events are recorded by microelectrodes inserted into the ciliated rings. The microelectrode recordings resemble intracellular recordings, and the reversal potentials are considered to represent depolarizations of the membranes of the ciliated cells.

4. Ciliary reversals continue after removal of the brain, suggesting the existence of a peripheral pacemaker.

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