J. exp. Biol. 150, 473–478 (1990)
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## SHORT COMMUNICATION

# DISTRIBUTION OF ION CHANNELS IN THE MEMBRANE OF THE DINOFLAGELLATE *NOCTILUCA MILIARIS*

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*Accepted 12 December 1989*

*Noctiluca miliaris*, a marine dinoflagellate, exhibits spontaneous flexion of its tentacle. The movement of the tentacle is always accompanied by perturbations of membrane potential, termed the 'tentacle regulating potentials' (TRPs) (Eckert and Sibaoka, 1967). Though the waveform of the TRPs is liable to variation, it consists of four successive basic components: (1) a $Na^+$-dependent depolarizing (positive) spike, (2) a depolarizing plateau potential, (3) a $Cl^-$-dependent hyperpolarizing (negative) spike, and (4) a long-lasting hyperpolarization (Eckert and Sibaoka, 1967; Oami *et al.* 1988). A slow flexion of the tentacle is seen during the plateau, and the flexion is suddenly accelerated when the negative spike occurs. The tentacle then extends during the long-lasting hyperpolarization. There is no correlation between the positive spike and the tentacular movement (Hisada, 1957; Oami *et al.* 1988).

To investigate the control mechanism of the tentacle movement by the TRPs, we have determined the distribution of the ion channels responsible for the TRPs over the cell surface by examining the membrane impedance and the membrane electric current in various regions of the cell surface. We found that the depolarization-sensitive $Na^+$ channels responsible for the positive spike were restricted to the cytostomal region, while the hyperpolarization-sensitive $Cl^-$ channels were present anywhere in the cell surface membrane, though they were most densely clustered around the cytostome region.

Specimens of *Noctiluca miliaris* were cultured in an artificial sea water (ASW) with their green algal food, *Dunaliella*, as previously described (Oami *et al.* 1988). A single specimen was held at the tip of a suction pipette, for either impedance or current recording, by lowering hydrostatic pressure inside the pipette. The TRPs were recorded using a conventional glass capillary microelectrode filled with $3\,mol\,l^{-1}$ KCl and inserted into the flotation vacuole of the specimen. For impedance recording, the suction pipette was 200 $\mu$m in inner diameter, and served

Key words: *Noctiluca miliaris*, $Na^+$ channels, $Cl^-$ channels, membrane impedance, bioelectric control.

as the unknown arm of a four-armed a.c. (2 kHz) bridge. Leakage resistance around the opening of the pipette was in the range 50–70 kΩ. The ratio of membrane area inside the pipette to that outside the pipette was about 1:20. The change in impedance of the unknown arm was therefore attributable mostly to a change in impedance of the membrane inside the pipette. The degree of imbalance of the bridge was proportional to the decrease in the membrane impedance. The a.c. output due to the imbalance was amplified and displayed on a cathode ray tube (CRT) together with the TRPs. For measurements of electric current the suction pipette was 30–100 μm in inner diameter. The current was amplified with a high-gain r.c.-coupled amplifier (time constant 0.1 s), and displayed on a CRT together with the TRPs. A positive deflection in the current trace corresponds to an outward current, and a negative deflection to an inward current. All the experiments were performed at room temperature (18–23 °C) in $Ca^{2+}$-free ASW, in which the specimen showed stable and conspicuous spike responses (Oami *et al.* 1988).

Changes in the impedance were measured at four different regions of the cell surface (inset of Fig. 1). A marked impedance decrease was always associated with each positive or negative spike, when the pipette was on the oral (cytostomal) region (Fig. 1A) or the tentacular region (Fig. 1B). However, when the pipette

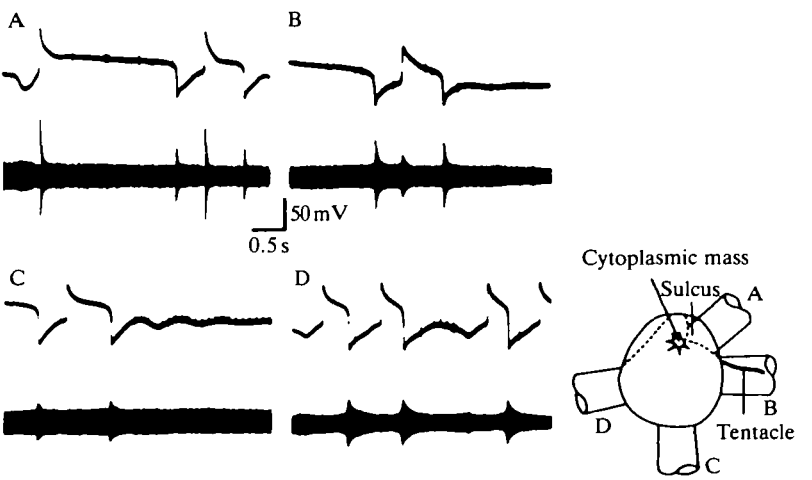


Fig. 1. Simultaneous recordings of the tentacle regulating potentials (TRPs; upper traces) and change in the membrane impedance (lower traces) in *Noctiluca miliaris*. The TRPs were recorded from the flotation vacuole. The impedance was measured in a region of the membrane inside a suction pipette. The suction pipette was placed just above the cytoplasmic mass (A), at the base of the tentacle (B) or at two aboral regions (C,D) of the membrane. The approximate size and location of the suction pipette are shown in the inset on the right of the figure. Each pair of traces was obtained in a different specimen. Because of the traumatic effect of the suction on the cell it was difficult to measure impedances at four different regions in a single specimen. See text for the details.

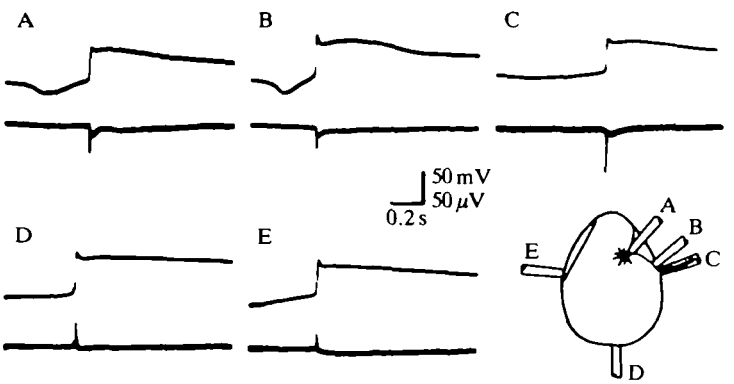


Fig. 2. Simultaneous recordings of the positive spike of the TRPs (upper traces) and local membrane currents (lower traces) in *Noctiluca miliaris*. The spike was recorded from the flotation vacuole. An electric current through a region of the membrane inside a suction pipette (30 μm in inner diameter) was measured for determining localized membrane current. The pipette was placed just above the cytoplasmic mass (A), at the base of the tentacle (B), to cover the tentacle (C) or at two aboral regions (D,E) on the membrane. The approximate size and location of the pipette are shown in the inset on the right of the figure. Positive deflection in the current traces corresponds to an outward current through the membrane and negative deflection to an inward current.

was on the aboral region, an impedance decrease was accompanied only by the negative spike (Fig. 1C,D).

The positive spike-associated membrane current was measured in five different regions of the membrane (inset of Fig. 2). The inward current was observed when the electrode was on the oral region (Fig. 2A,B). It was also observed when the tentacle was within the electrode (Fig. 1C). Since the ion species responsible for the positive spike is $Na^+$ (Oami *et al.* 1988), the inward current could be a $Na^+$ current. However, the current was always outward when the electrode was on the aboral region (Fig. 2D,E). The outward current could thus be a passive return current of the active $Na^+$ current. These results and the observation of the positive spike-associated impedance decrease only in the membrane region around the cytostome and the tentacle lead to the conclusion that the $Na^+$ channels responsible for the positive spike are present in that membrane region.

It should be noted that the positive spike-associated membrane current was always monophasic, whether it was inward or outward. This suggests that activation of the $Na^+$ channels occurs simultaneously in the membrane region around the cytostome and the tentacle.

The negative spike-associated membrane current was recorded in four different membrane regions (inset of Fig. 3). In contrast to the positive spike-associated current, the negative spike-associated current was always biphasic. It was first positive, then became negative when the suction electrode was around the oral or tentacular region (Fig. 3A,B); but was first negative then positive when the

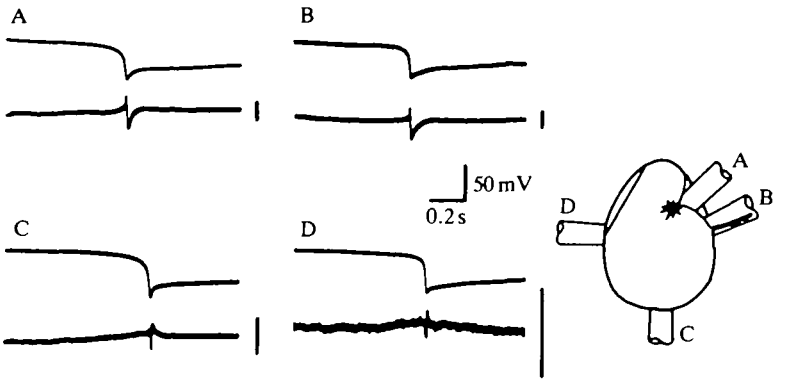


Fig. 3. Simultaneous recordings of the negative spike of the TRPs (upper traces) and local membrane currents (lower traces) in *Noctiluca miliaris*. The suction pipette (100 $\mu$m in inner diameter) for measuring the localized membrane current was placed just above the cytoplasmic mass (A), at the tentacle region (B) or at two aboral regions (C,D) of the membrane. The approximate size and location of the pipette are shown in the inset on the right of the figure. Vertical bars beside the current traces correspond to 50 $\mu$V. See the legend of Fig. 2 for more detail.

electrode was on the aboral region (Fig. 3C,D). Since the positive current is a $Cl^-$ current (Oami *et al.* 1988), and the negative current is its passive return current, the positive–negative current sequence at the oral or the tentacular region corresponds to the generation of the negative spike and its subsequent propagation away to the aboral region. The negative–positive current sequence at the aboral region corresponds to the arrival of the negative spike from the oral region and subsequent generation of the spike in the aboral region. These results and the observation of the negative spike-associated impedance change all over the cell lead to the conclusion that, in contrast to $Na^+$ channels, $Cl^-$ channels are distributed all over the cell surface. However, it should be noted that the outward current was significantly larger in the oral region than in the aboral region (compare C or D with A or B in Fig. 3). This indicates that the $Cl^-$ channels cluster thickly in the oral region.

A small inward current was observed in association with a depolarizing plateau potential. The ionic mechanism and generation site of the depolarization remain to be investigated.

Motile activity of the tentacle and phagocytic activity in the cytostome membrane play a major role in the feeding activity of *Noctiluca*. We suggest that activation of the thick clusters of both $Na^+$ and $Cl^-$ channels around the cytostome and the tentacle is therefore involved in the bioelectric control of feeding activity. Feeding activity can be divided into two successive phases: (1) the food-gathering phase, in which flexion–extension of the tentacle is repeated for trapping algal food; and (2) the food-intake phase, which consists of an initial sustained strong flexion of the tentacle so that its tip with trapped algae touches the

cytostome, and subsequent phagocytic ingestion of the algae through the cytostome, accompanied by vigorous cytoplasmic streaming towards the cytostome region.

In the food-gathering phase, activation of the depolarization-sensitive $Na^+$ channels around the tentacle produces a regenerative positive spike, which, in turn, causes a depolarizing plateau potential, during which the membrane potential slowly shifts towards the hyperpolarizing direction. The hyperpolarization-sensitive inactivation of the $Cl^-$ channels is removed during the plateau, and the channels are activated to generate a regenerative negative spike when the membrane potential attains a certain activation threshold level (Oami *et al.* 1984). The subsequent long-lasting hyperpolarization is attributable, at least in part, to partial activation of the $Cl^-$ channels remaining after the negative spike. Inactivation of the $Cl^-$ channels gradually takes place during the hyperpolarization, and therefore the membrane potential tends to depolarize. The depolarization-sensitive inactivation of the $Na^+$ channels is removed during the hyperpolarization, and the channels are activated to produce the positive spike when the membrane potential attains the activation threshold (Oami *et al.* 1985). In this way *Noctiluca* repeatedly exhibits TRPs, and hence the flexion–extension of its tentacle. Therefore, thick clusters of both $Na^+$ and $Cl^-$ channels in the membrane near the tentacle are essential for the regulation of tentacle movement. The coupling mechanism between the TRPs and the flexion of the tentacle has been described (Oami and Naitoh, 1989*a*).

In the food-intake phase, cytoplasmic streaming takes place in association with a sustained hyperpolarization of the membrane (Nawata and Sibaoka, 1986). The hyperpolarization is $Cl^-$-dependent (Nawata and Sibaoka, 1986), as is the negative spike of the TRPs (Oami *et al.* 1988). Nawata and Sibaoka (1987) have suggested that the hyperpolarization-driven $Ca^{2+}$ influx induces cytoplasmic streaming. Therefore, overall distribution of the $Cl^-$ channels in the surface membrane is essential for the generation of the cytoplasmic streaming all over the cell that is seen prior to food ingestion.

Localized distribution of different kinds of ion channels in the cell membrane is particularly important for bioelectric control of effector activities in unicellular organisms (Naitoh, 1982, 1984; Oami and Naitoh, 1989*b*). In *Noctiluca*, the TRPs are generated across the membrane which faces the external solution (Oami *et al.* 1988), while a $H^+$-dependent flash-triggering action potential (FTP) is generated across the membrane which faces the flotation vacuole (Eckert and Sibaoka, 1968; Nawata and Sibaoka, 1979). *Noctiluca* provides a prominent example of differentiation of membrane function within a single cell.

We thank our colleague Professor R. Ridge for reading the manuscript. We also thank the following organizations for their financial support: Mitsubishi Foundation, Nippon Petrochemical Co. Ltd, Honda R & D Co. Ltd, and the Ministry of Science, Culture and Education of Japan (411802, 510902, 56105002).

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