Chapter 14

Recording Cilia Activity in Ctenophores

Tigran P. Norekian and Leonid L. Moroz

Abstract

Pelagic ctenophores swim in the water with the help of eight rows of long fused cilia. Their entire behavioral repertoire is dependent to a large degree on coordinated cilia activity. Therefore, recording cilia beating is paramount to understanding and registering the behavioral responses and investigating its neural and hormonal control. Here, we present a simple protocol to monitor and quantify cilia activity in semi-intact ctenophore preparations (using *Pleurobrachia* and *Bolinopsis* as models), which includes a standard electrophysiological setup for intracellular recording.

Key words Ctenophora, Electrophysiology, Behavior, *Pleurobrachia*, *Bolinopsis*, Cilia, Locomotion

1 Introduction

Ctenophores evolved unique locomotory and integrative systems [1–6], reaching a tissue and organ complexity comparable to bilaterian animals [7]. The evolutionary scenario includes independent origins of neurons [8, 9], synapses [10, 11], and alternative integrative systems [12], where ciliated structures (not muscles) diversified as the primary effectors.

The role of cilia in ctenophores is overwhelmingly rich and essential for virtually all functions and behaviors [6, 13, 14]. One primary example is the use of cilia for complex locomotion—ctenophores move in the water column with the help of eight rows of ctenes, which consist of the large mechanically fused swim cilia [14]. The entire coordination of multiple behaviors in ctenophores is also primarily controlled by variations in the activity of swim cilia, and these mechanisms were under intensive investigation [6, 13, 15–22].

Although cilia are the major effectors in ctenophores, with presumed neuronal control, little is known about synaptic regulation and neurotransmitters controlling cilia movement. Therefore,

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Leonid L. Moroz (ed.), *Ctenophores: Methods and Protocols*, Methods in Molecular Biology, vol. 2757,
https://doi.org/10.1007/978-1-0716-3642-8_14,

recording cilia activity in ctenophores is essential for their behavioral and functional analyses. Here, we describe a simple protocol that was successfully used to record and quantify cilia beating in ctenophores during our investigation of the physiological roles of different transmitters [23] and their evolution [24–26].

2 Materials

1. High Mg^{2+} seawater—333 mM magnesium chloride ($MgCl_2$) is added to filtered seawater at 1:1 ratio.
2. Sylgard-coated Petri dishes (World Precision Instruments, Sylgard Silicone Elastomer, SYLG184).
3. Small steel insect pins.
4. Glass microelectrodes—borosilicate glass micropipettes for intracellular recording (World Precision Instruments, standard glass capillaries, 2 mm OD with a thin filament, 1B200F-4).
5. Potassium acetate—3 M solution.

2.1 Equipment

1. Nikon stereoscopic microscope SMZ-10A.
2. Micromanipulators (Warner Instruments, Standard Manual Control Micromanipulators, MM-33).
3. Micropipette puller (Sutter Instruments, Flaming/Brown Micropipette Puller P-97).
4. Intracellular amplifiers (A-M Systems Neuroprobe 1600 Amplifiers).
5. Chart recorder (Gould WindoGraf 940 4 Channel Recorder).

3 Methods

1. Freshly collected animals were incubated in high Mg^{2+} seawater for about 1 h to prevent muscle contractions. We used large, 1–2 cm, *Pleurobrachia bachei* and medium-sized, 3–4 cm, *Bolinopsis microptera* for these experiments.
2. The animals were then tightly pinned to a Sylgard-coated Petri dish with small steel needles to prevent body movements other than cilia beating (*see* **Note 1**). Relatively small animals (1 cm *Pleurobrachia*) were used whole without dissection; larger animals (2 cm and above) were dissected, and parts of a body wall with 2–3 cilia rows were pinned the same way to the Petri dish.
3. The high Mg^{2+} solution was washed out by fresh seawater several times during 30-min intervals to restore the normal chemical balance.

4. The Petri dish was placed in a standard electrophysiological rig on a recording platform and connected to the Ag/AgCl reference electrode.
5. We used glass microelectrodes filled with 3 M potassium acetate to record cilia beating. The sharp microelectrodes were pulled using Sutter Electrode Puller.
6. The original resistance of sharp microelectrodes (made for intracellular recordings) was 20–40 MΩ. A narrow strip of thin paper was used to carefully touch the tip of the electrode to break off the fragile sharp end. The resulting electrode was more stable to further mechanical contact and had a resistance of about 10 MΩ. Electrodes with very low resistances below 2 MΩ were unsuitable.
7. The electrodes were then connected to the micromanipulators and the intracellular amplifiers (Neuroprobe 1600, A-M Systems).
8. With the help of micromanipulators and under visual control via a dissecting microscope, the tip of the electrode was carefully placed next to the cilia combs so that during cilia beating, cilia were touching the tip of the electrode (Fig. 1). This physical contact created a brief electrical signal picked up by amplifiers and recorded on paper and in digital form using Gould Recorder (WindoGraf 980). Thus, each cilia beat was translated into a fast electrical spike, which allowed a digital recording of cilia beat frequency, but not the amplitude and power of beating (*see* **Note 2**).
9. To understand whether the cilia activity pattern was modulated by polysynaptic inputs or inherent to cilia, chemical isolation was used by bathing the preparation in high Mg^{2+} saline for 5–15 min (333 mM $MgCl_2$ was added to filtered seawater at 1:1 ratio). High Mg^{2+} solution was applied into the recording dish using a graduated pipette attached to a long small-diameter tube. Elevated magnesium chloride solution suppresses synaptic chemical transmission and is widely used in comparative neurobiology [19, 20].

3.1 Illustrated Examples

The proposed protocol allows reliable registering and quantifying locomotory cilia activity in ctenophores. We experimented primarily with *Pleurobrachia bachei* and *Bolinopsis microptera*, although other ctenophore species, including larval and juvenile, can be used for that purpose.

Patterns of cilia beating in *Pleurobrachia* were always very variable, frequently with powerful bursts and inhibitory episodes (Fig. 2a; see also [23]). Such activity might represent intact behavior in a free-moving *Pleurobrachia* as an ambush predator [6]. Sometimes, however, the cilia beating in *Pleurobrachia* showed

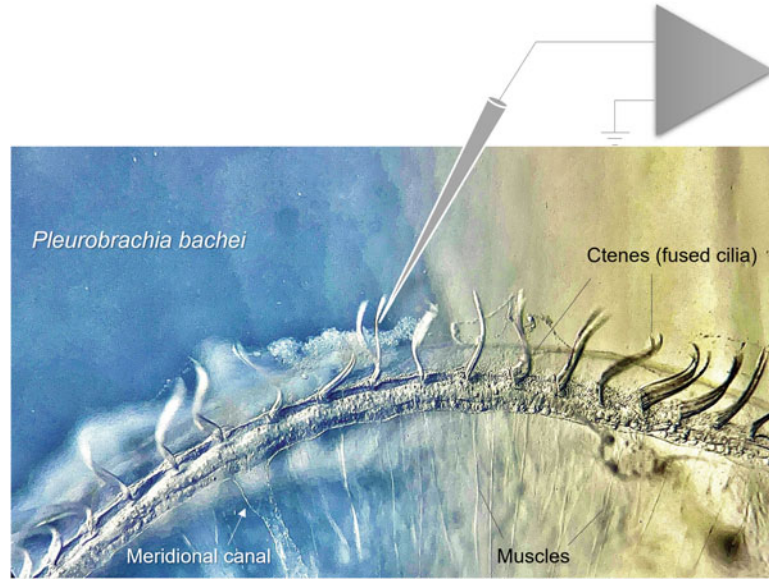

Fig. 1 Schematic diagram showing the position of the recording glass microelectrode next to the ctenes of swim cilia in *Pleurobrachia*

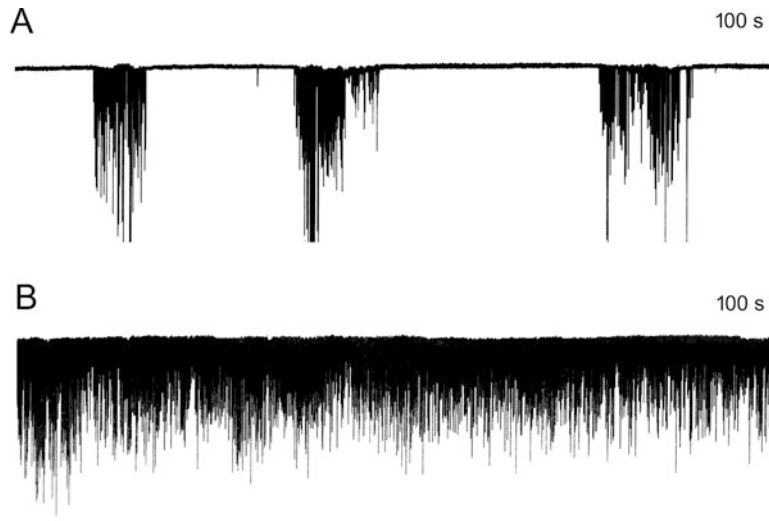

Fig. 2 Cilia beating in *Pleurobrachia bachei* is very variable and complex, similar to intact behaviors in free-moving animals. (**a**) Episodes of high-frequency bursting with periods of inhibition between them. (**b**) Tonic continuous cilia beating with possible brief episodes of acceleration. The numbers above the traces show the time of recordings

a constant active beating for a prolonged period (Fig. 2b). In contrast, *Bolinopsis* had more regular cilia beating with fewer activity patterns and lower frequency (Fig. 3a, b). Notably, the complex patterns of cilia activity were eliminated in the presence of a high

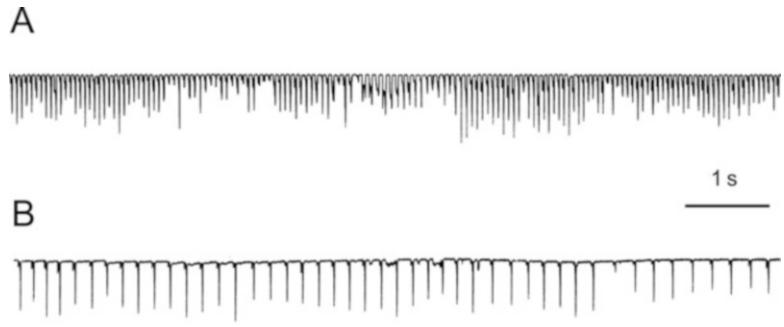

Fig. 3 Cilia beating in *Bolinopsis microptera* is less patterned and more regular than in *Pleurobrachia*. However, it demonstrates periods of higher-frequency beating (**a**) and slower activity (**b**)

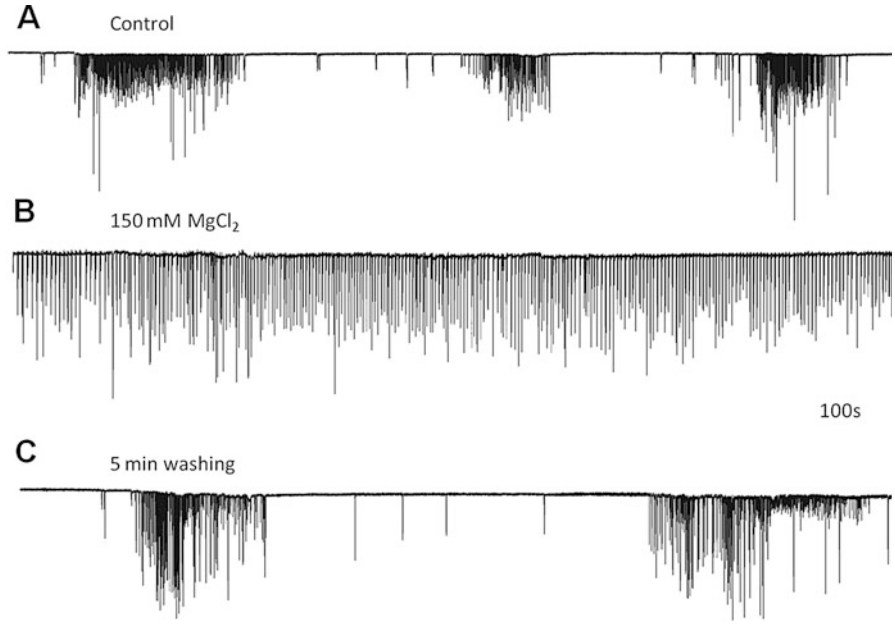

Fig. 4 High Mg^{2+} seawater blocked complex patterns of cilia beating (**a**) in *Pleurobrachia*, suggesting that synaptic inputs initiated episodes of high-frequency bursting and inhibition. (From [23]). The regular unvarying cilia beating was sustained during high Mg^{2+} solution exposure (**b**). The complete recovery was achieved within several minutes following washing in normal seawater, which fully restored episodes of bursting and inhibition (**c**). Numbers under the traces show the time of recordings

concentration of Mg^{2+} (Fig. 4; see [23]), known to suppress synaptic inputs [27, 28]. These findings indicate the presence of multifaceted chemical inputs (e.g., peptides [9, 29, 30] or nitric oxide [31]) and likely direct synaptic control of cilia, which is also confirmed by ultrastructural data with a remarkable diversity of synapses [5, 14, 32].

4 Notes

1. It was crucial for stable recording to have the ctenophore body wall tightly pinned to the Sylgard-coated Petri dish, with absolutely no movements in it, except cilia beating. If body wall muscles contracted and moved ctene row even a couple of mm away from the electrode, the electrode would stop picking up the signal, and recording continuity would be prevented.
2. It is important to note that this technique does not allow quantification of cilia beating amplitude and forces—only the frequency. The electrodes register mechanical contact with the cilia.

Acknowledgments

This work was supported in part by the Human Frontiers Science Program (RGP0060/2017) and National Science Foundation (IOS-1557923) grants to LLM. Research reported in this publication was also supported in part by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number R01NS114491 (to LLM). The content is solely the authors' responsibility and does not necessarily represent the official views of the National Institutes of Health.

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