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2D measurement of ion currents associated to the signal transduction of the phototactic alga *Chlamydomonas reinhardtii*

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ABSTRACT

Our objective was to develop a simple procedure for the detection of light-induced ion currents of photomotile cells in two dimensions. The novel technique was based on the light gradient method (LGM), and the model object was *Chlamydomonas reinhardtii*, a phototactic unicellular alga, ideal for such experiments. The conventional LGM cuvette was modified such that the electrode pair could be rotated around the sample and pick up the electric signals from arbitrary directions. The experiments were performed with and without the application of an auxiliary light beam preorienting the motile cells. The analysis of the detected traces revealed two main vectorial components of the signal by the help of singular value decomposition (SVD), in concert with previous experimental findings and theoretical considerations suggesting different origins of the "fast" and "slow" components of the photoelectric response of *Chlamydomonas* and Haematococcus cells. Using plausible assumptions, our method allowed a quantitative analysis of the signal, assigning size and direction to the two vectorial components. The method allows a rapid and accurate way to measure electric signals of photomotive cells in 2D, and particularly, to test the physiological activity and in vivo-kinetics of site-directed mutants of ChR1 or ChR2, providing novel photo-electrophysiological methods with important quantitative information.

1. Introduction

One of the most important goals in modern biophysics is to describe membrane-coupled signal and energy transduction processes on different levels of physiological organization. Such processes govern, e.g., nerve signalization, or the energy conversion in chloroplasts and mitochondria [1,2]. The phototactic unicellular alga, *Chlamydomonas reinhardtii* is a popular model system to investigate basic problems concerning both signal and energy transduction, and electric signals carry a lot of information about these processes. The light gradient method (LGM) [3,4] is ideally suited for the in vivo detection of electric signals because it has a fine time resolution and, unlike microelectrode techniques, it does not make any demands for the cell size or the consistency of the cell wall. Cells can be investigated by LGM under their natural living conditions, while the sensitivity and reproducibility of the measurements is competitive with other methods (patch-clamp, BLM, SSM, etc.) [5,6]. The results give us direct information about the kinetics and spatial properties of the underlying transport processes.

Chlamydomonas cells have a simple light-tracking system [7–9]. During their swim toward the light source, the algae are rotating counterclockwise around their longitudinal axes because of asymmetric strokes of their flagellae, so sustaining a helical pathway [7,10]. The two flagellae are radially oriented during the helical motion (with the dominating flagellum pointing to the outside). As a consequence of such a motion pattern, the photoreceptors of the eyespot receive sinusoidally modulated light intensity. This modulation decreases when the ideal orientation is approached and disappears when light- and tracking-directions coincide [11]. After reaching the ideal orientation, the algae should protect their photoreceptors from the absorption of further photons, so as to avoid disadvantageous direction changes. This is the reason why the eyespot normal points 45° outside of the beating plane of the flagellae. It should be noted that, under high intensity illumination, the algae move not toward the light source, but away from it.

Both types of phototactic motions are mediated by a cascade of transmembraneous ion currents, starting at the photoreceptor and ending at the flagella region. The cascade is initiated by two, rhodopsin-type ion channels called Channelrhodopsin 1 and 2 (ChR1 and ChR2) [5,12–16]. The channelrhodopsins belong to the G-protein-coupled receptor superfamily, consisting of two parts, a protein (opsin) and a chromophore (all-trans retinal). The opsin part of both ChRs is built up by about 700 amino acids, and have a core region of seven transmembrane alpha helices of the size of ca. 300 amino acids. In native algae, these ion channels are shown to be Ca^{2+}-dependent. ChR1 and 2 are light-gated ion channels, mediating a light-induced depolarization of the plasma membrane. If the depolarization reaches a threshold, voltage-gated Ca^{2+}

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channels are opened, which, in turn, leads to beating of the flagellae [11,13,14]. The electric signals detected by our method contain valuable information about this complex process.

2. Materials and methods

The electric signals of the cells were measured by a modified light-gradient method. LGM belongs to the bulk methods, where an overall electric asymmetry of the sample is a prerequisite for the measurements [3,4]. It is achieved via a gradient of the exciting light present across each cell body in the direction of the light propagation, due to the extinction of the cell. In LGM, usually a voltage signal is detected by the electrodes, that, under the time course of the measurement, is proportional to the sum of the individual currents flowing through the cell membranes [17,18]. *Chlamydomonas* cells are ideally suited for LGM because of an enhanced light gradient across the cells due to the strongly absorbent stigma underlying the photoreceptor membrane of the eye spot.

We developed a new layout of the light gradient method for the measurement of phototactic responses of the model cell *C. reinhardtii*. Similarly to the method of Sineshchekov et al. [19,20], the measurements were carried out in two different ways. The signals were detected while the cells were preoriented with a continuous dim light incident perpendicular to the exciting light, or, alternatively, without preorientation. The difference between our method and the one described in [19,20] is that in our system, the electrodes can be rotated around an axis perpendicular to the plane defined by the exciting and preorienting light. Hence, the electric signals induced by light flashes can be measured not only in the direction of the exciting or orienting light, but at arbitrary angles, as well. We carried out the experiments at eight different directions of the electrode pair, separated by 15° from each other.

Gametes of *C.* reinhardtii UTCC 100 strain were prepared according to the procedure described in detail in [19], with minor modifications. Briefly, mature cells were transferred to a nitrate-free nutrient solution, and kept shaked under constant illumination for 4 days. The exciting light was provided by an excimer laser-pumped dye laser system (Lambda-Physik XeCl excimer laser, exciting a home-made coumarin 307 dye laser). The frequency of the excitation was 0.5 Hz in the absence of orientation, and the energy of the light pulses was 1.2 mJ. Alternatively, in the presence of orienting light, preorientation of the cells was carried out by a Spectra Physics laser (Stabilite 2016, 488 nm, providing a flux of 5×10^{16} photons/m^2). After 8 s of preorientation and 2 s of darkness, a single exciting flash was applied, and then the cycle started again. The signals were amplified by a home-made voltage amplifier (100 kΩ load resistance, 1 kHz low-pass filter).

The amplified signals were recorded by a fast transient recorder (LeCroy), and the raw traces were processed by the MATLAB software package, with the singular value decomposition (SVD) tool. Briefly, the $n \times m$ data matrix, $\mathbf{D}$ was decomposed as $\mathbf{D} = \mathbf{U} \times \mathbf{S} \times \mathbf{V}^{\mathbf{T}}$, where n is the number of time points, and m is the number of electrode angle values the measurements were performed with. $\mathbf{U}$ ($n \times m$) contains the orthonormal abstract kinetic vectors, the $\mathbf{S}$ ($m \times m$) diagonal matrix consists of the singular values, while $\mathbf{V}$ ($m \times m$) contains the orthonormal vectors describing the angular dependence.

3. Results

3.1. Measurements

The results of the raw measurements are depicted in Figs. 1 and 2. Some general statements can be made based on the qualitative features of the signals. They all show a bipolar decay under the time resolution of the experiments: a few ms-long fast phase is followed by a slower period in the 10 ms range, with amplitudes of opposite sign. The fast phase is called photoreceptor current (PC) in the literature [8,12,19,20], and usually assigned to the opening of light-gated ion channels in the eye spot, while the slow phase is called the "regenerative response" (RR) which is assumed to be derived from the flagella region [19,20]. In the random case (Fig. 1), the maximal amplitude of the signal is observed when the electrodes are in the same direction as the exciting light (blue[1] trace).

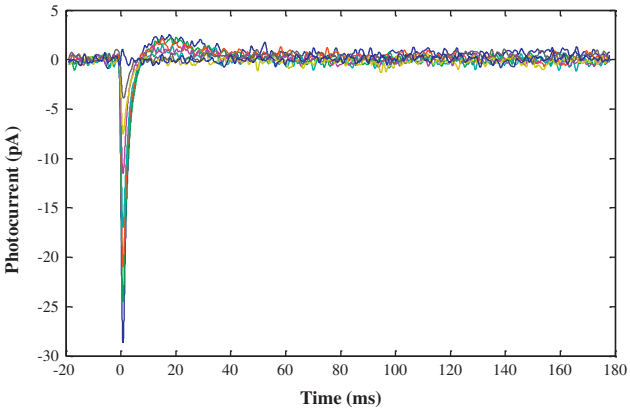

Fig. 1. Series of LGM signals recorded on a random sample at different angles between the directions of the electrode pair and the exciting light. Each trace is the average of of 400 flashes.

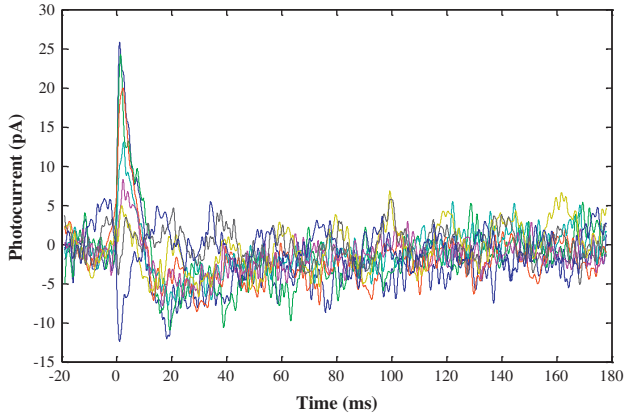

Fig. 2. Series of LGM signals recorded on an oriented sample at different angles between the directions of the electrode pair and the exciting light. Because of the prolonged measuring protocol as compared to the random case (see Section 2), each trace was the average of 32 flashes.

In case of the oriented sample, the maximum signal is observed when the electrodes are perpendicular to the exciting light (and parallel with the orienting light).

The directly detected traces are processed by the SVD technique. Since only one component was found to be significant both in the random and oriented case, the SVD components were used for noise filtering, by reconstructing the data matrix ($\mathbf{D}$) from the significant component vectors of $\mathbf{U}$, $\mathbf{S}$ and $\mathbf{V}$. Fig. 3 shows the significant $\mathbf{U}$ components for the random and oriented case, their weight as a function of the angle of observation ($\mathbf{V}$), as well as the noise-filtered traces, regenerated from the SVD analysis, leaving out the insignificant SVD components (rightmost inserts).

[1] For interpretation of color in Figs. 1–7, the reader is referred to the web version of this article.