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Dynamics of a sensory signaling network in a unicellular eukaryote

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The processing components and the dynamic signaling network that an individual cell uses to do signal integration and make decisions based on multiple sensory inputs are being identified in a well studied free-swimming unicellular green algal model organism, *Chlamydomonas.* It has many sensory photoreceptors and measurable behavior associated with its orienting and swimming with respect to light sources in its environment. Study of the dynamics of the beating of its two steering cilia reveals their complex specialization.

I. INTRODUCTION

Chlamydomonas reinhardtii is a single celled eukaryotic alga (10×7×7 μm) (Fig. 1), which swims in fresh water at a speed of about 120 μm/s with two beating cilia. It is a model organism with a mostly known genome [1] that is extensively studied using biochemistry, molecular genetics and classical genetics. We are interested in how a single cell integrates multiple signaling inputs and controls its phototactic behavior to orient relative to a source of light. Of particular interest to us is the role of dynamics of these processes, which take place on a wide range of time scales. With *Chlamydomonas* it is possible to measure the dynamic behavior of its ciliary beating which is altered on the time scale that the cell makes decisions based on these multiple sensory inputs. There are both biochemical and electrical networks that are responsible for intracellular signaling in this cell. Thus, it is much more complex than a bacterial system [2] which lacks electrical signaling, but much less complex than any multi-neuron behavioral system such as that of the nematode *C. elegans* [3].

Because of the inherent limitation that photons and chemicals have only positive concentrations within a cell, the signaling system would be expected to utilize various chemicals representing positive and negative signals and some integration of these. The electrical signaling and ion channel processing is also inherently nonlinear. However, the net result, apart from monotonic signal compression, is likely to look linear over the physiologically important bandwidth. It is hard otherwise to have stable feedback. To understand how the cell processes, interprets and makes decisions ultimately requires knowledge of the underlying nonlinear details. Nevertheless, it is valuable to trace the functional relationship of the cell to its environment, namely the mostly linear approximation.

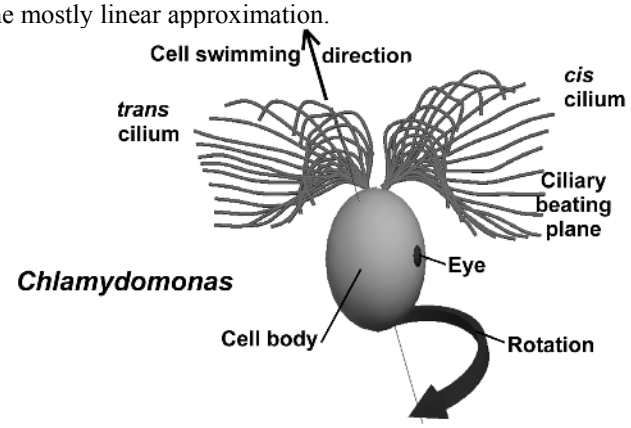

Fig. 1. *Chlamydomonas* cell showing its eye, the beating pattern of its *cis* and *trans* cilia and orientation to the axis of the helix it rotates around.

The ciliary control system, responsible for the steering with respect to light, is a particularly accessible system for studying signal processing dynamics within a single eukaryotic cell. *Chlamydomonas* uses its two cilia to steer with either a ciliary stroke (Fig. 1) (two oars in a breast stroke that pull the organism through the water) or a flagellar stroke (whip-like pushing of an organism as observed with sperm). *Chlamydomonas* swims consistently with left-handed rotation due to the two-fold rotation symmetry of the cilia and their slanting stroke plane. At the low-Reynolds-number regime in which it swims the net force and torque is proportional respectively to the translational and angular velocity. Its ciliary motion is an emergent property of the active forces within the cilium and their hydrodynamic interaction with the surrounding fluid.

There are three rhodopsins [4] photoreceptors that can under certain circumstances, can provide the cell with inputs of where the light sources are. They are all believed to be located in the eye (Fig. 1). There are also a number of other photoreceptors that influence phototaxis behavior. Principally, there are two red light absorbing receptors whose chromophore is a chlorophyll or a related pigment [5].

The goal of this paper is to provide an overview of the dynamics as presently understood for phototaxis in *Chlamydomonas*. Previously we have obtained some linear response functions which make it possible to predict

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approximately the behavior to any stimulus within the range of the original dynamic testing. Other goals are to characterize the multi-input multi-output (MIMO) relationships and to gain insight into the phototaxis control network, the specialization of the two cilia, and insight into the strategy for phototaxis.

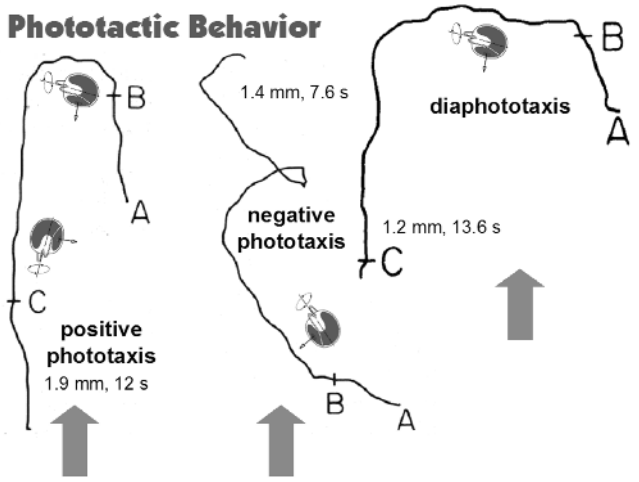

Fig. 2. Tracks of swimming cells showing the three phototaxis responses. The arrows depict light direction. A: start of trace, B: light on, C: light off.

The phototaxis system uses a single eye, the plasma membrane photoreceptor and the underlying visible eyespot or antenna [5], pointing approximately orthogonal to the net swimming path. The consistently left-handed rotation of the cell results in the eye systematically scanning the light environment. The signal from the eye is amplified and processed by electrical currents across the plasma membrane in the cell body and sent to the cilia by the net electric field induced in the plasma membrane. In turn the electric field signals are sensed by ion channels in the cilia [7]-[9] resulting in the unique individual responses [10] with each cilium contributing its own way to steering. For a free-swimming rotating cell its single lateral eye scans the light environment providing an orientation-error signal proportional to the needed angular correction that enables the cell (in the presence of a light source) to swim in an approximately helical path.

The cell steers the axis of this helical path toward, away or orthogonal to the direction of a light source, respectively positive, negative and dia-phototaxis (Fig. 2). Under some circumstances cells seem unable to decide between these three choices as seen in free swimming cells (diddling around) and held cell measurements show reproducible behavior that would not lead to orientation [10]. With sufficient stimulation the cells will back up with flagellar beating (swimming like a sperm). The choice among these and other options depends on a variety of external and internal factors. Unlike with chemical sensing [11] which results in the cell aligning with a chemical gradient, light sensing aligns a cell relative to the direction of a light source and not the gradient of light intensity [12, 6].

II. Methods

A. Ciliary stimulation and recording

The individual cell body is held stationary on a glass micropipette and the movements of each cilium are monitored with a microscope and detector system as previously described [10,13,14] (Fig. 3). The strain is 806, which is normally negatively phototactic (*agg*1 mutant). Modulated green light (543 nm) was a single input to the rhodopsin photoreceptors.

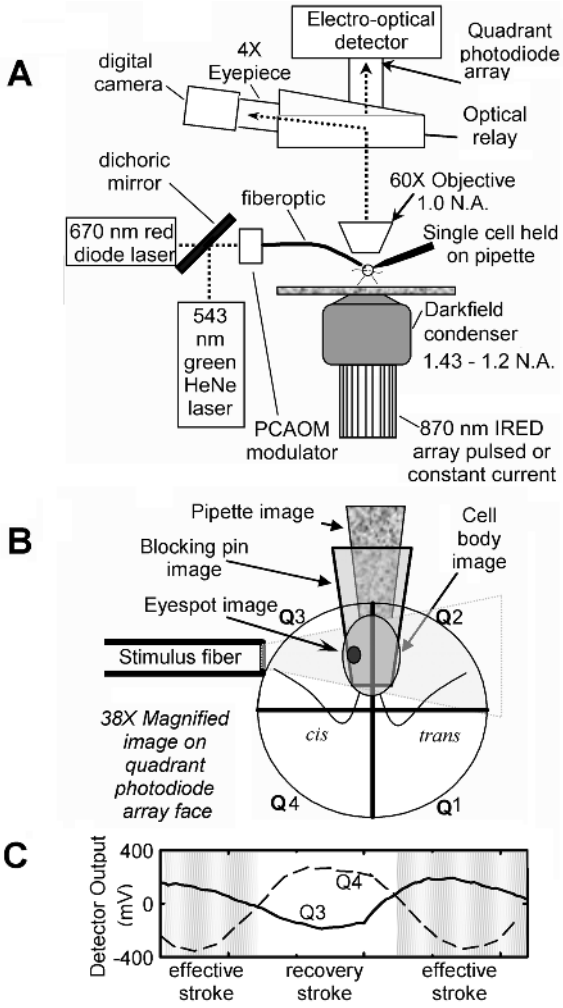

Fig. 3 **A.** Schematic drawing of the single-cell ciliary monitor with IR-diode illumination and digital camera. A digital camera synchronized with the pulsed IR (870 nm) diode array and the ciliary beat frequency enabled orientation of the cell as shown in **B**. Retractable prisms in the optical relay directed the image to the camera or a quadrant photodiode array of the electro-optical detector [13]. During data collection the IR diode array in constant current mode supplied uniform dark-field illumination of the cilia. An optical fiber with acousto-optical modulator (PCAOM) delivered amplitude-modulated green (543 nm) stimulation to the cell. The resulting signal is shown in **C**. Figure modified from [14].

Two measures of ciliary responses; beat frequency and stroke velocity, were assayed for each cilium. These responses were then used to interpret the steering of the cells. Beat frequency is defined as the number of complete beat cycles (effective plus recovery stroke) executed per second and is determined by time between successive positive/negative signal peaks and zero crossings (Fig. 3C) [10,13]. Ciliary stroke velocity, proportional to signal amplitude as a result of detector filtering, is defined as root-mean-square (RMS) detector output (Fig. 3C) [10,13]. Green light (543 nm) modulated by pseudorandom Gaussian white noise (GWN), applied to $\log_{10}$(light intensity), stimulated the cell with an intensity pattern changing at 12 ms intervals for 196.608 s (N = 16,384 = 2^{14} intervals). Many systems, including vision, have a response proportional to $\log_{10}$ (stimulus) [15]. This is also true for *Chlamydomonas* photoreceptors which respond to green light with approximately a $\log_{10}$ (intensity change)/(initial intensity) dependence [17]. Hence, the green light pseudorandom noise pattern was constructed to be Gaussian with respect to $\log_{10}$ (stimulus intensity). This also enabled stimulation over a large dynamic range (2.5 orders) [16]. Correlation of $\log_{10}$ (stimulus intensity) with beat frequency or stroke velocity yield their respective impulse responses.

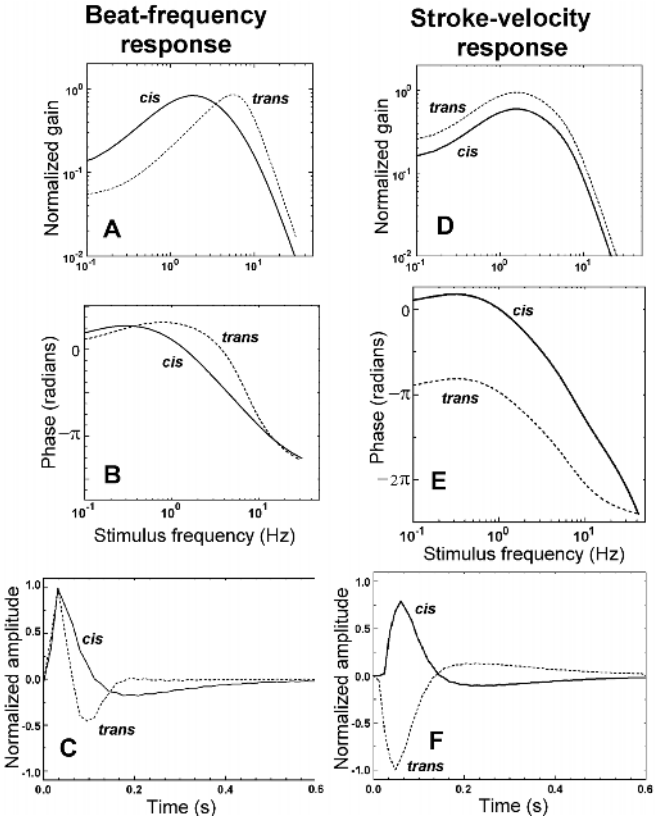

Fig. 4. Beat frequency and stroke velocity Bode plots (AB, DE) and equivalent impulse responses (C, F) of the negatively phototactic strain, 806 (drawn from data in [14]).

III. RESULTS AND DISCUSSION

The responses to millions of beating cycles has been measured [10,13,14]. Bode plots for the beat frequency and stroke-velocity responses and their equivalent impulse responses are shown in Fig. 4. Particularly noteworthy are the differences between the *cis* and *trans* cilia. The *trans* beat-frequency response peaks at 5-6 Hz significantly higher than does the *cis* cilium (Fig 4A).

The electric current, a measure of the electric field carrying the signal from the cell body to the cilium, has a peak response (~8 Hz) higher than the stroke-velocity (*cis* and *trans*) and the beat-frequency (*cis*) responses of the cilia, but similar to that of the beat-frequency response of the *trans* cilium [18]. While the *trans* and *cis* cilia have similar gain curves for the stroke velocity response (Fig 4D) the delay of the *cis* is markedly longer than for the *trans* (Fig 4F).

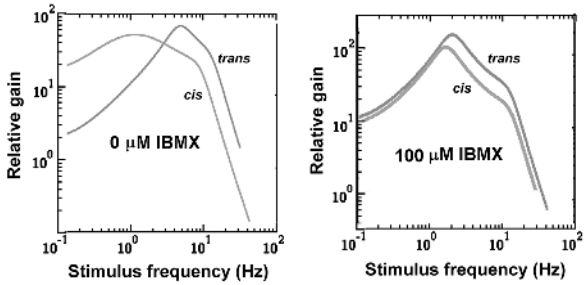

Fig. 5. Log frequency-gain plot of the *cis* and *trans* beat-frequency responses with and without IBMX.

Isobutyl-methyl xanthine (IBMX), a phosphodiesterase inhibitor, raises the level of cAMP in these *agg*1 mutants toward the level of wild-type cells and under sufficient green light intensity makes the cells positively phototactic like wild-type cells. The application of a background of red light (670 nm laser diode) in addition to the green stimulating light can also change the phototactic direction.

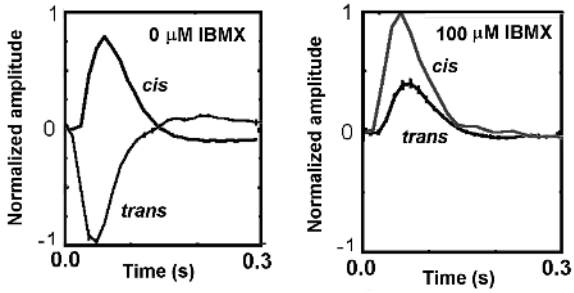

Fig. 6. The stroke-velocity impulse responses of the *cis* and *trans* cilium with and without IBMX.

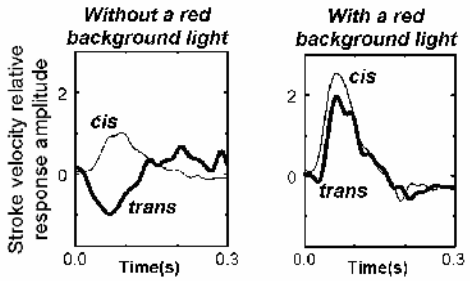

Fig. 7. The *cis* and *trans* stroke-velocity responses with and without a 25 W/m^2 constant red light background (10 minutes conditioning with red and green, then 20 minutes

data collection).

Interestingly, one of the effects of IBMX is to alter the beat-frequency responses of both the *cis* and *trans* cilia (Fig. 5). An effect of both IBMX (Fig. 6) and red (670nm) light (Fig. 7) is to change the sign of the *trans* cilium impulse response. This correlates with a change in phototaxis direction. Note that the delay in the *trans* cilium is now as long as for the *cis* cilium implying that a different process comes into play when the cell makes a positive response.

Red light also plays a direct role. Two separate red-light triggered signals control beat frequency and stroke velocity with an approximately 80 times slower time course than the parallel green light control [17]. They are delayed for a few hundred milliseconds implying a diffusive signaling pathway either to trigger a slow electric field change in the plasma membrane which envelops the cell body and cilia or by molecular diffusion to sites in the very narrow cilia.

IV. MODEL

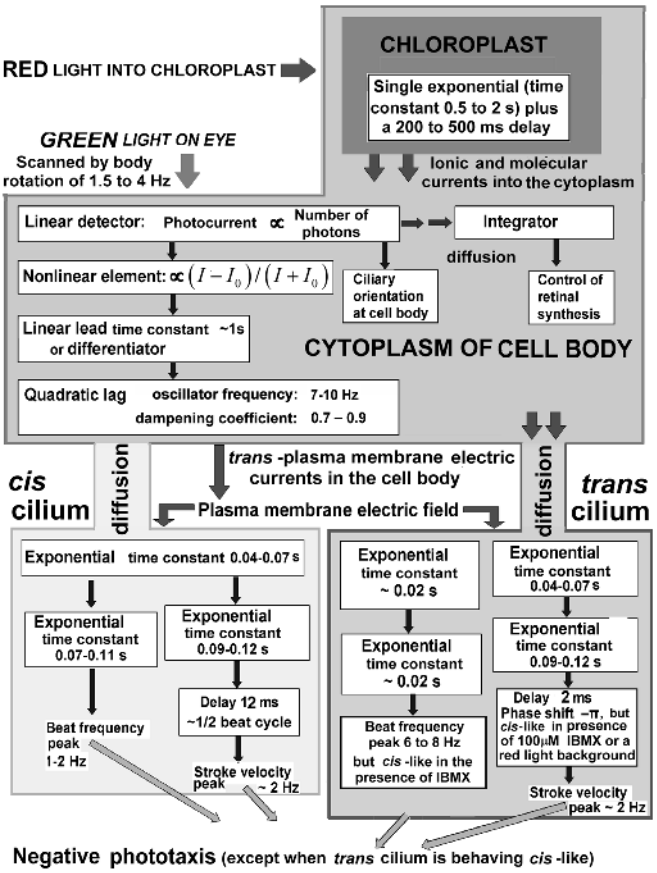

Fig. 8. Current mostly-linear working model with suggested processing elements involved in negative phototaxis.

V. CONCLUSIONS

Using the available evidence a tentative model (Fig. 8) has been developed that incorporates what is known with respect to the negative phototaxis response. These cells likely move away from light because, as seen in Fig. 4F, when the cell body rotates and the light beam then hits the eye, the stroke of the cilium directed toward the source (*cis*) increases its amplitude and velocity while the stroke of the cilium on the other side (*trans*) decreases its amplitude and velocity.

The next steps are to measure the plasma membrane electric field, an intermediate signal that carries the cell body signal to the cilia, and to tease out the subtle nonlinearities that indicate the underlying processes to be identified with the biological components.

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REFERENCES

[1] G. J. Pazour, N. Agrin, J. Leszyk, G. B. Witman. "Proteomic analysis of a eukaryotic cilium." *J Cell Biol.* vol. 170, pp. 103-113, 2005.

[2] H. C. Berg, *E. coli in motion.* . New York: Springer-Verlag, 2004, pp. 133.

[3] W. R. Schafer, "Deciphering the neural and molecular mechanisms of *C. elegans* behavior," *Current Biology*, vol 15, pp. R723–R729, 2005.

[4] J. Saranak, "The three rhodopsins of *Chlamydomonas*," unpublished.

[5] T. Takahashi and M. Watanabe, "Photosynthesis modulates the sign of phototaxis of wild-type *Chlamydomonas reinhardtii:* Effects of red background illumination and 3-(3',4'-dichlorophenyl)-1,1-dimethylurea," *FEBS Let,* vol. 336, pp. 516-20, 1993.

[6] K. W. Foster and R D. Smyth, "Light antennas in phototactic algae," *Microbiol. Rev*., vol. 44, pp. 572- 630, 1980.

[7] O. A. Sineshchekov, E. G. Govorunova, A. Der, L. Keszthelyi, ad W. Nultsch, "Photoelectric responses in phototactic flagellated algae measured in cell suspension," *J. Photochem.Photobiol., B: Biol.,* vol. 13, pp. 119-134, 1992.

[8] C. Beck and R. Uhl, "On the localization of voltage-sensitive calcium channels in the flagella of *Chlamydomonas reinhardtii*," *Biophys. J.* vol. 125, 1119-1125, 1994

[9] E.-M.Holland, F.-J. Baun, C. Nonnengässer, H. Harz, and P. Hegemann, "The nature of rhodopsin triggered phototcurrents in *Chlamydomonas*. I. Kinetics and influence of divalent ions. ," Biophys. J. vol. 70, pp. 924-931, 1996.

[10] K. Josef, J. Saranak, and K. W. Foster, "Ciliary behavior of a negatively phototactic *Chlamydomonas reinhardtii, ". Cell Motil & Cytoskeleton, vol.* 61, pp. 97-111, 2005.

[11] H.C. Crenshaw, "A new look at locomotion in microorganisms: Rotating and translating," *Am. Zool.* vol. 36, pp. 608-618, 1996.

[12] J. Buder, Zur Kenntnis der phototaktischen Richtungsbewegungen. *Jahrb. Wiss. Bot,*vol. 58, pp. 105-220, 1917.

[13] K. Josef, J. Saranak, and K. W. Foster, "An electro-optic monitor of the behavior of *Chlamydomonas reinhardtii* cilia,"*Cell Motil & Cytoskeleton,* vol. 61, pp. 93-96, 2005.

[14] K. Josef, J. Saranak, and K. W. Foster, "Linear systems analysis of the ciliary steering behavior associated with negative-phototaxis in *Chlamydomonas reinhardtii," Cell Motil & Cytoskeleton,* submitted for publication.

[15] G.T. Fechner, Elements of Psychophysics, D.H. Howse and E.L. Boring, Eds. New York: Holt, Reinhardt, and Winston, 1966. Published originally in 1860.

[16] E. D. Lipson, "White noise analysis of *Phycomyces* light growth response system II," Biophysical J., vol. 15, pp.1013-1031, 1975.

[17] K. Josef, "The nonlinear phototaxis signaling network of *Chlamydomonas* investigated by observing ciliary responses of individual cells to green and red light," Ph.D. dissertation, Physics Dept., Syracuse University, Syracuse, NY, 2005.

[18] C. Capano, "Electric current responses in *Chlamydomonas*," unpublished.