RESEARCH ARTICLE

Electrical response hysteresis of crayfish photoreceptors to light intensity. Dependence on circadian time.

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Abstract

We studied the light-triggered current of crayfish photoreceptors. We found that when a train of light flashes of either increasing or decreasing intensity is applied, the current waveform presents the non-linear behavior known as hysteresis. Additionally, we observed that the extent of this response depends on the circadian time at which the pulses are applied. We hypothesize that positive feedback loops of biochemical networks underlying light energy transduction are responsible of the observed behavior. It has been demonstrated that a dynamical system hysteresis provides a mechanism that enhances its robustness against random perturbations. Taking into account this characteristic we hypothesize that the electrical-response hysteresis of crayfish photoreceptors: 1) makes the visual system more stable to environmental noise, and hence 2) adds stability to circadian clock oscillations.

Keywords: photoreceptor light-elicited current, light-intensity, hysteresis, circadian rhythm.

1 Introduction

Visual perception in invertebrate crayfish photoreceptors includes, as part of its initial events, a light-triggered, inwardly directed transient current. The light-transduction current activation occurs after a noticeably milliseconds latency, reaches a maximum (peak-amplitude) and then it desensitizes following a single-exponential time course. As photoreceptors are involved in both expression and synchronization of crayfish circadian rhythm, it is not surprising that the kinetics of recovery from light-induce desensitization depends on circadian time (CT).[1, 2, 3] The slow latency of current onset, as well as the slower CT-dependent recovery from desensitization phase clearly indicate that complex, not yet well determined, biochemical events regulate the light-triggered current, as it has been demonstrated to occur in mammals.[4, 5, 6, 7, 8]

It is known that biological process that involve complex biochemical cascades can show hysteresis.[9, 10, 11, 12] The latter means that system response to a significant input takes out different values, depending on whether inputs are presented in either an increasing or decreasing intensity manner. Both experimental and theoretical studies have demonstrated that hysteresis provides an enhancement of the robustness of the dynamical system against random perturbations.[12, 13] Additionally, it has been related with the presence of relaxation oscillations and period tunability rhythms, for example circadian rhythms.[14]

Herein we report that, interestingly, the light-triggered current of crayfish photoreceptors is endowed with a CT-dependent hysteric behavior. We hypothesize that this physical characteristic is the result of the underlying biochemical events that modulate the light-transduction current.

2 Materials and Methods

2.1 Biological material

Adult *Procambarus clarkii* crayfishes of indistinct sex in intermolt stage were used. The animals were collected in Chihuahua, México. Animals were kept in aerated aquariums at 16°C, and fed with fresh vegetables. For almost 1-month prior to the experiments, animals were maintained under 12 hours light (white light, 2.4kW/m^2) -12 hours darkness, to synchronize them to an adequate schedule. CT of the animal population was determined using the circadian rhythm of light sensitivity, as previously reported (Barriga-Montoya et al., 2017)[2]. CT 0 indicates the beginning of a subjective day, the time which an organism is normally active.

To excise eyestalks the animals were anesthetized by immersion in tap water at 0-4 °C, for 15 min. The structure was separated from the base using a fine scissor. Access to retinula was obtained through an opening (1mm^2) in the dorsal cornea effected with a razor blade. The eyestalk was bathed in crayfish physiological solution (see next section), in a continuous perfusing chamber (2-mL capacity, 0.5-mL/s flow rate) under constant darkness, with the access opening to the retina on the top of the chamber.

All experiments complied with the current laws of Mexico, the country in which they were conducted. Crayfishes were maintained under appropriate laboratory conditions to guarantee their welfare and responsiveness.

2.2 Solutions

Crayfish physiological solution composition was (in mM)[15]: NaCl 205; KCl 5; $MgSO_4$ 2; $CaCl_2$ 13, and Hepes 5 (pH 7.3–7.4). This is referred to as modified van Harreveld solution (VH).

2.3 Electrical recordings

Intracellular electrical recordings were performed with an Axoclamp 2A amplifier (Axon Instruments). Electrodes were made of borosilicate glass (Kimax-51) pulled to 30-50 MΩ resistance when filled with 2.7 M KCl. Currents were measured with the discontinuous single electrode-switched voltage-clamp method. Switching rate was 500-1,000 Hz. Currents were digitized at a rate of 100 µsec/point with a Digidata 1200 Interface (Axon Instruments). Light stimuli (white light and 10 µsec duration) of different intensities were delivered from a Grass (PS33) photostimulator and applied in parallel to the eyestalk's longitudinal axis. Irradiance of light stimuli were (in kW/m^2) 1.7; 2.7; 7.2; 52.3, and 2738.4.

Currents were activated stimulating the eyestalk with the light pulse, with the membrane potential clamped at the photoreceptor's resting membrane potential (RMP). All experiments were performed in darkness, under a constant temperature (15°C), and were conducted at 0, 6, 12, and 18-h of the circadian cycle.

2.4 Data analysis

Currents were analyzed using Clampfit 9.2 (Axon Instruments, Inc.). To establish the kinetics of light-elicited current (*I*), we measured[1, 2, 3]: 1) activation latency (*L)*, the time elapsed upon the light flash delivery until the current achieves 10% of its peak value; 2) activation half-time ($t_{1/2}$), the time it takes to go from 10 to 50% of the current maximal value; 3) peak current (I_p), the maximal amplitude of the current; 4) peak current plateau, (I_{pl}), the length of time that current remains withing 10% of its maximal value. Curves were fitted using the Levenberg–Marquardt algorithm.

Results are expressed as the mean ± standard error (SEM) of 5 independent experiments. Means were compared with *F*-test, one-way analysis of variance (ANOVA). Significance level was set to 0.05. All computations were carried with QtiPlot 0.9.8.8.

3 Results

3.1 Kinetics of crayfish photoreceptor: light intensity dependence

Upon impinging crayfish photoreceptors light triggers an inward, transient, transduction-current. For a reference, Figure 1A compares light-elicited currents evoked by flashes of either 1.7 or 2738.4 kW/m^2 intensity and 10 µs duration, applied at 0-h CT (see Methods). Notice the transient character of the transduction currents, which reach a maximum, peak, amplitude in tens of milliseconds and thereafter decay slowly, following an exponential time course.[1,2] See that, as expected, the trace corresponding to the flash of higher intensity presents bigger amplitude (peak value) and faster activation kinetics, whereas the decay, inactivation, phase is not appreciably changed at these light intensities. Time between stimulus was 2 min to allow full recovery from inactivation.[1, 2, 3] These features are best seen in the Figure inset which shows the normalized, superposed, currents.

Interestingly, applying a series of light pulses of varying intensity we noticed that kinetics of the light transduction-current, activated by a light-pulse, depends not only on the activating pulse itself, but, unexpectedly, also depends on whether the prior pulse had a higher or a lower intensity than that of the activating light-pulse. The latter is illustrated in Figure 1B which compares two currents, both triggered by a flash of the same intensity (2.7 kW/m^2), although one of them (black trace) was elicited after the previous delivery of a flash of smaller intensity, whereas the other (red trace) was evoked after the prior delivery of a flash of higher intensity (see Figure legend). Notice how current kinetics depends on the intensity of the prior flash. That

is, crayfish photoreceptors response is determined by both the actual light intensity that triggers it, and by the stimulus history. The latter indicates that crayfish visual transduction-current presents hysteresis. It is important to point out that this behavior is not due to an incomplete recovery from current inactivation2 (see later).[2]

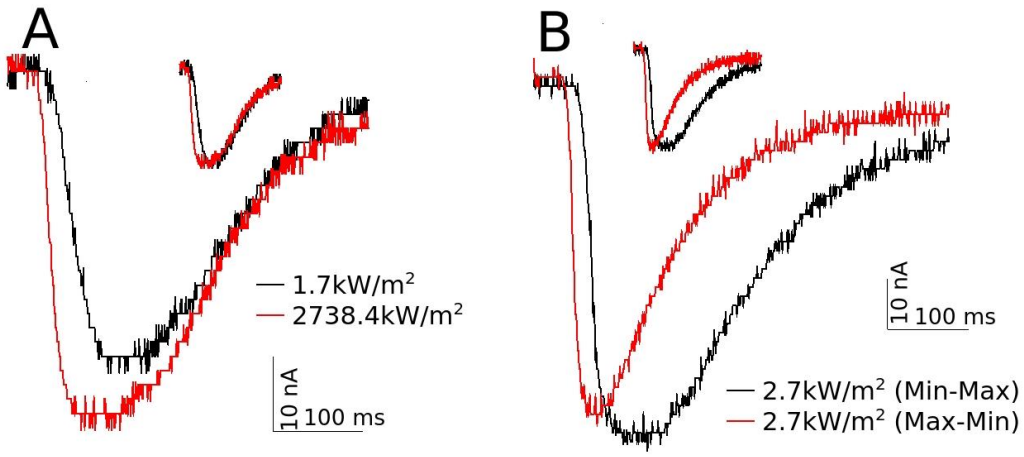

Figure 1: Light-triggered currents of crayfish photoreceptors. A) Currents evoked by a flash intesity of either 1.7kW/m^2 (black trace) or 2738.4 kW/m^2 (red trace). Note that current kinetics depends on light-intensity. To facilitate its comparison the inset shows the superposed traces, normalized to the peak current value. B) Currents elicited by a flash intensity of 2.7 kW/m^2 following the prior delivery of a flash of either a higher (7.2 kW/m^2, red trace) or smaller intensity (1.7 kW/m^2, black trace). Note that current waveform is determined by the stimulus history. Inset shows the superposed traces. Currents were recorded in control solution (VH) at 0-h CT. Membrane potential was clamped at RMP value.

Transduction-current hysteresis is best revealed when a train of stimulus is applied in either increasing or decreasing intensity order. The results of these experiments are quantified in Figure 2. Note that except the peak current amplitude ($I_p/I_{p,max}$, see Methods), all the kinetic parameters, namely: current activation rate ($t_{1/2}$), latency, and peak-current plateau-length take on different values, depending on whether stimuli are applied in an increasing or a decreasing intensity order. The lines are the least-squares fit of the points with an exponential equation of the form $y=y_0 \pm A*exp(-x/\tau)$ (see Methods), as corresponding (parameters are listed in Table 1). Note that, there are two exponential curves on each plot, one of them fits the data gathered in either the Min->Max (forward) direction of flash intensities, or in the backward direction, as indicated. Additionally, and importantly, notice that the fitted curves cross at both the lowest and highest intensity flash-values: this demonstrate that the observed behavior is not due to incomplete recovery from inactivation.[2]

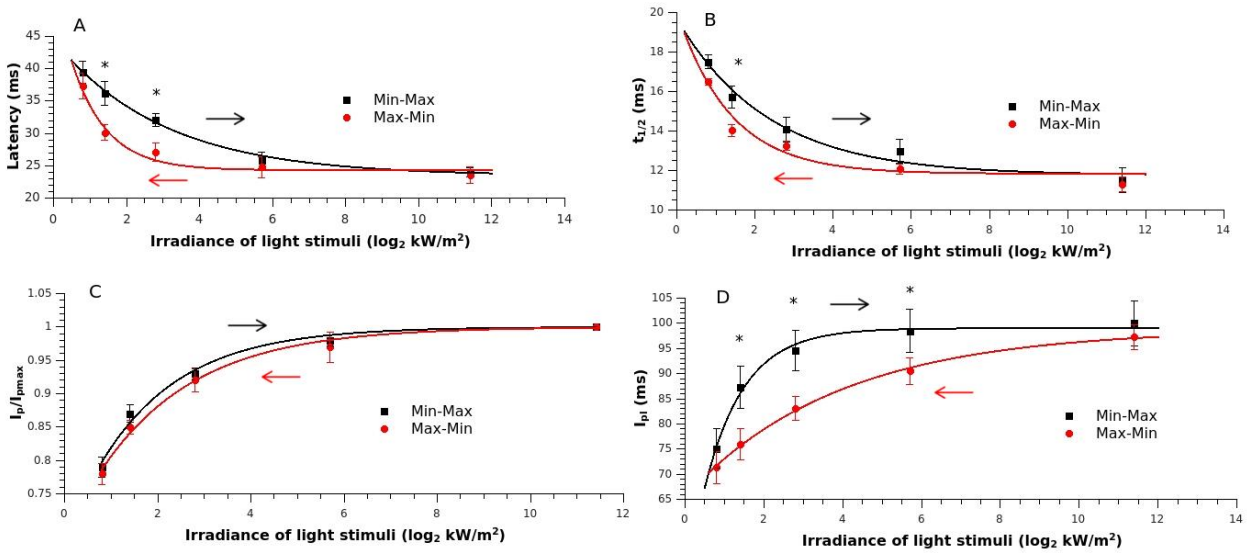

Figure 2: Kinetic parameters of light transduction-current vs. Light intensity. The plots show the values obtained when light flashes were applied in either an increasing (black) or a decreasing (red) order of intensity. A) Current Latency (L), B) Activation half time ($t_{1/2}$), C) Normalized peak current (I_p/I_{pmax}), and D) Peak-current plateau (I_{pl}) (see Materials and Methods). Statistically different points are marked with and asterisk. (n = 5). The lines are the least-squares fit of the points with the equation: $y=y_0 \pm A*\exp(-x/\tau)$, as corresponding. Notice the conspicuous hysteresis loop in panel A, B &D.

0-h CT	y_0	A	τ	R^2
$L_{Min\rightarrow Max}$	23.4±0.5	21.1±1.4*	3.0±0.4*	0.996
$L_{Max\rightarrow Min}$	24.3±0.9	28.3±1.5*	9.6±0.3*	0.929
$t_{1/2\ Min\rightarrow Max}$	11.8±0.6	8.2±1.8	0.9±0.3*	0.907
$t_{1/2\ Max\rightarrow Min}$	11.8±0.6	7.9±0.8	2.3±0.4*	0.941
$I_p/I_{pmax\ Min\rightarrow Max}$	1.0±0.1	0.32±0.02	1.7±0.2	0.989
$I_p/I_{pmax\ Max\rightarrow Min}$	1.0±0.2	0.32±0.02	2.0±0.2	0.992
$I_{pl\ Min\rightarrow Max}$	99.0±1.0	52.1±8.8*	1.0±0.2*	0.960
$I_{pl\ Max\rightarrow Min}$	98.9±1.2	33.3±1.1*	3.9±0.5*	0.998

Table 1: Parameters of the curve $y=y_0 \pm A*\exp(-x/\tau)$ that describes the light-intensity dependence of the transduction-current parameters, as in Figure 2 (see also Materials and Methods). The table gives the fitted parameters for flashes applied either in an increasing or a decreasing order of intensity, as indicated. The last column presents the goodness of fit.

In order to assess the magnitude of the hysteresis response shown in Figure 2, we measured the area between each pair of exponential curves that fit the flash-intensity dependence of the parameters that characterize the light-triggered current (see Materials and Methods). The latter is illustrated in Fig 3A which shows the area (A_L, shaded region) between the curves that describe the variation of current latency (L) as a function of light intensity. Clearly:

$$A_L = \int_{0.8}^{11.4} L_{\text{minmax}}\,\text{dx} - \int_{0.8}^{11.4} L_{\text{maxmin}}\,\text{dx}$$

The histogram in Fig 3B shows the result of these calculation applied to all current parameters. Notice that the latency of current activation, and, still more, the current plateau duration (i.e., the time interval during which average current remains at its maximal peak value), present the strongest hysteresis response, whereas peak current, a non-kinetic parameter, does not show hysteresis.

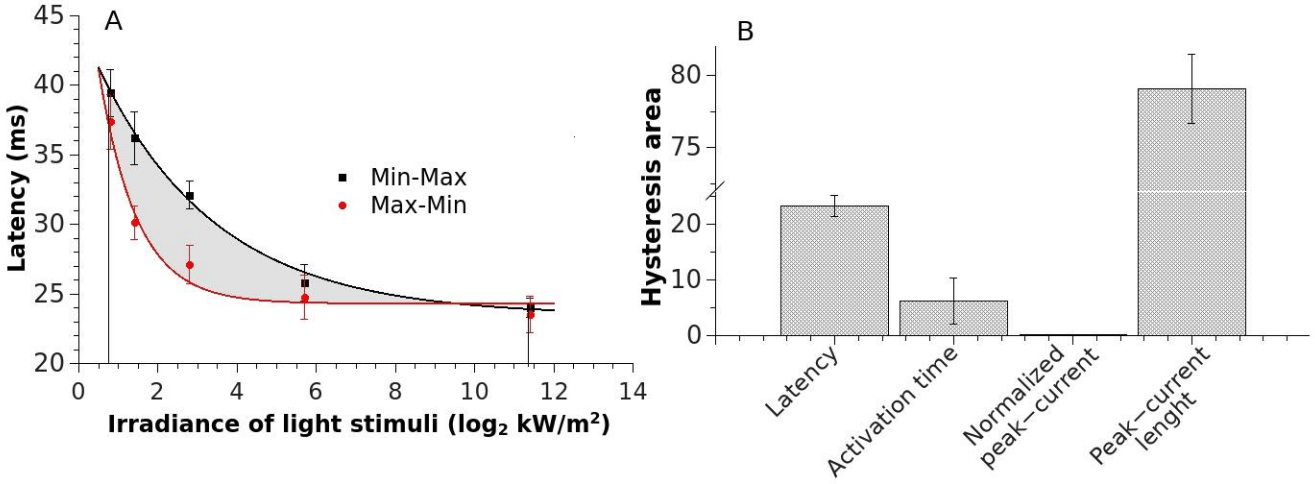

Figure 3: Hysteresis magnitude assessment. A) Current latency vs. Light intensity. The area between the curves obtained with flashes of either increasing or decreasing intensity (shaded region) is used as a measure of hysteresis magnitude of this parameter. B) Magnitude of hysteresis of the indicated parameters, assessed as in A. Units of hysteresis magnitude for latency, activation half-time and peak current plateau are: ms $\log_2 kW/m^2$; for normalized peak current is (% nA/nA $\log_2 kW/m^2$).

3.2 Circadian time dependence

Our next goal was to determine whether current hysteresis to light intensity depends on circadian time. Hence, experiments as in Fig 2 were carried out at 6, 12, and 18-h CT. Fig. 4 shows the CT-dependence of the hysteresis response of light-triggered current, evaluated as the area between forward and backward curves on each case. Notice that, interestingly and puzzling: (a) in contrasts to its lack of hysteresis at 0 CT, the non-kinetic parameter peak-current shows clear hysteresis at other CT; (b) there is either a minimum (latency & activation half time) or a maximum (peak current & plateau duration) hysteresis at 6CT.

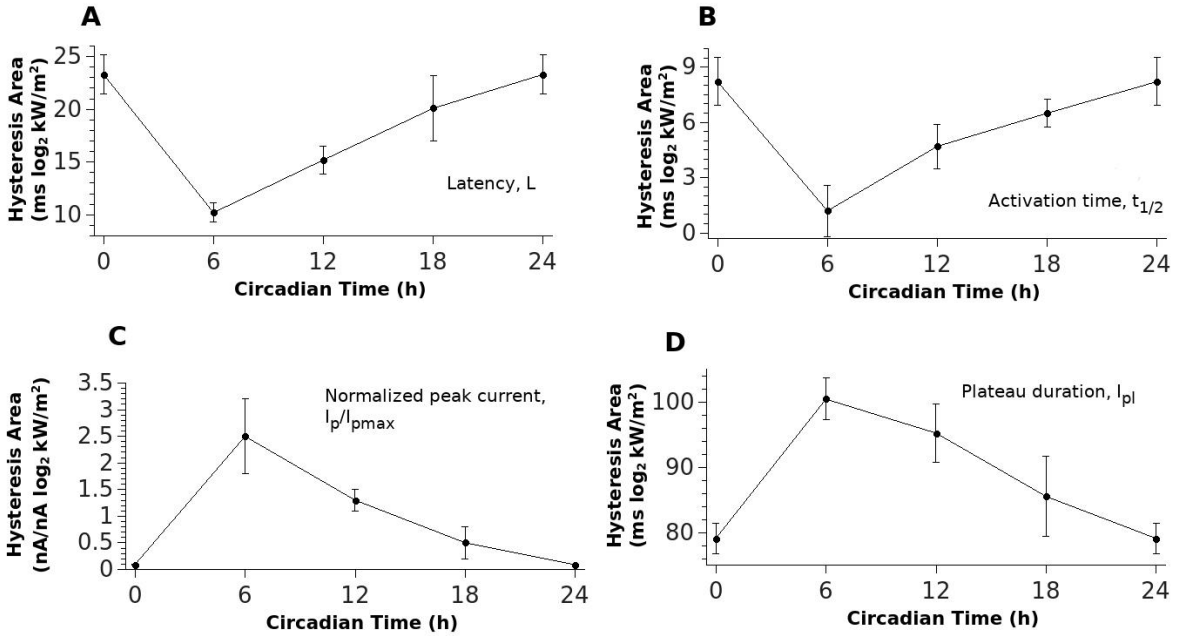

Figure 4 Transduction-current hysteresis vs. Circadian time. The points are the area of the hystereis loop, assessed at the indicated CTs, as in Figure 3.A) latency, B) activation half time, C) normalized peak current, and D) Peak current plateau duration.

4 Discussion

Herein we reported that: 1) upon impinging crayfish photoreceptor cells light trigger an inward transduction-current that is a nonlinear function of light intensity that presents hysteresis; and 2) the extent of this non-linear behavior depends on the circadian time. We hypothesize that this dynamic characteristic is the result of the non-linearity of the biochemical networks that modulate the visual transduction-current; the presence of these networks is clearly implicit in, for example, the conspicuous latency of current activation.

Regarding the above, it has been shown that linking between the light absorption by rhodopsin and the photoreceptor inward transduction-current there is a signaling pathway involving the G protein (G_q)-phospholipase C cascade. The subsequent PIP_2 hydrolysis and release of phosphatidylinositol biphosphate, and diacylglycerol should cause Ca^{2+} release from cells endoplasmic reticulum, hence raising the cytoplasmic concentration of this second-messenger ion. [4, 5, 6, 7, 8, 16, 17] Moreover, the transduction current itself is carried by a mixture of Na^+ and, the second-messenger Ca^{2+} ions.[1] Additionally (Miller and Glantz, 2000)[18] have demonstrated that crayfish photoreceptors have a voltage-dependent potassium conductance, who likely participates in membrane repolarization.

In order to get further insight regarding the mechanism of the transduction-current hysteresis herein reported, further work is first necessary to determine the nature of the ion channels that produce the inward transduction current and how are they linked to second-messenger pathways.

Whatever the underlying mechanism is, our observations support the proposal that the eyestalk contains a clockwork which permits the flow of environmental information to be modulated. Taking into account noisy light fluctuations and spectral composition over the 24-h cycle of light intensity and spectral

composition, the presence of hysteresis is expected to provide stability to the system (visual system) output.

It is known that, at least for the mammalian circadian clock, hysteresis induces a robust (stable) oscillation. That it is: 1) hysteresis in the crayfish visual system might suppress interference, making the visual system more resistant to noisy signals, and hence 2) hysteresis might enhance circadian clock stability.[10, 19, 20, 21] Hysteresis responses are found in many physical (mechanical, electrical) and biological (biochemical) systems. A hysteric switch can be created with just a single positive feedback loop, as often done in engineering systems. Thus, we hypothesize that underlying the mechanisms of photoreceptors hysteresis are the presence of positive feedback loops of second-messenger pathways that participate in the light transduction process. [10, 12]

To conclude, even though phototransduction is a complex process, it should be possible to find the minimal set of nonlinear biochemical cascades that may account for the mechanisms underlying the hysteric response. Future work is needed to test associations between cascade components and ion channels kinetics through pharmacological manipulation and/or mutant proteins. The observations herein reported may provide a useful basis for developing a more complete descriptions of the biochemical and biophysical process underlying the electrical response of crayfish photoreceptors.

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