# LIGHT-ACTIVATED CHANNELS IN *LIMULUS* VENTRAL PHOTORECEPTORS

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We have investigated the light-dependent conductance of the ventral photoreceptor of *Limulus* by using the patch-clamp technique. Our results show that ionic channels open in response to the sensory stimulus in these cells.

To prepare the photoreceptors for patch-clamp recording, the glial cells and connective tissue that surround the photoreceptor were removed by teasing them away using a suction pipette (20-$\mu$m diam), after treating the preparation with 2% pronase (Calbiochem, La Jolla, CA) for ~1 min (1). Although this procedure exposed the photoreceptor membrane (1), further treatment was necessary to obtain good patch recordings from the light-sensitive part of the photoreceptor because of the presence of microvilli on the membrane of this region of the cell (1). We found that mild sonication of the preparation allowed us to obtain patch-clamp recordings from this region, possibly because of alterations in the morphology of the cell surface. The sonication procedure did not, however, cause any obvious alteration in the physiology of the photoreceptor.

## RESULTS

Fig. 1 is a continuous recording of patch current at high temporal resolution and shows that single-channel currents are activated upon illumination of the cell. While the photoreceptor was in the dark, channel activity was extremely low. After the onset of a maintained light stimulus of moderate intensity, indicated by the arrow, channel activity was induced. The potential across the patch membrane during this recording was initially −40 mV (the resting potential of this cell, measured with an intracellular microelectrode). Light depolarized the patch by 5 mV during the steady-state phase of the receptor potential. The inward direction of the single-channel currents agrees with that of the macroscopic light-dependent current at the same membrane potential (2).

A current-voltage curve of the single-channel currents is shown in Fig. 2 *a*. The single-channel conductance, obtained from the slope of this curve, is 45 pS. (The average of five cells is 43 pS.) The reversal potential for the single-channel currents was 10 mV, in close agreement with that of the macroscopic current (2).

The open time of the channel has a distribution that can be fit well by a single exponential with a time constant, $\tau$, of 4.7 ms, the mean open time of the channel (Fig. 2 *b*. In other experiments, $\tau$ = 1.2–4.7 ms; average: 2.7 ms).

The ventral photoreceptor contains a variety of voltage-dependent channels (3). It is conceivable that the single-channel currents observed in Fig. 1 were activated by the light-induced depolarization rather than by a more direct effect of light. This problem is addressed in Fig. 3 *a*, which shows that the single-channel currents were activated by light and not by voltage. The top trace of Fig. 3 *a* shows the activation of channels during a stimulus of moderate intensity, while the patch was maintained close to the resting potential (−45 mV). Depolarization of the patch to 55 mV in the dark did not induce channel activity. However, channel activity in the depolarized patch is dramatically increased after the light stimulus was turned on, indicating that the channel activity was induced by

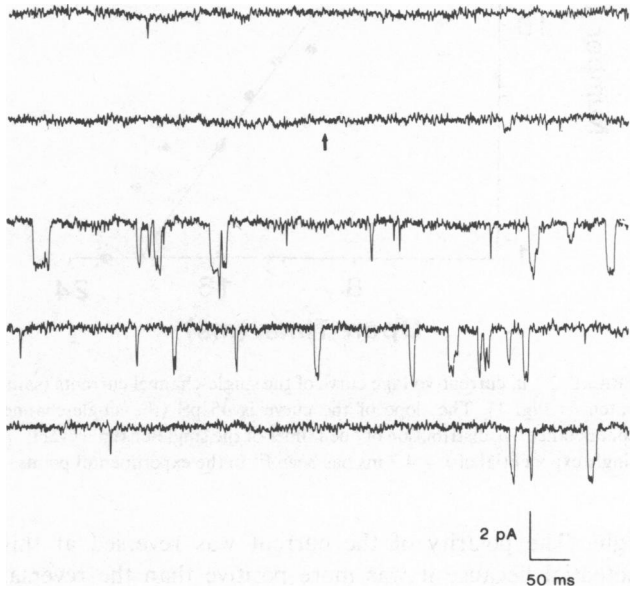


FIGURE 1 Continuous recording of patch current. The arrow indicates the moment the light stimulus was turned on. The illumination was maintained during the rest of the recording. Patch potential: −40 mV before light and −35 mV during illumination. $\tau$ of the recording system is 0.8 $\mu$s. Temperature: 20°C.

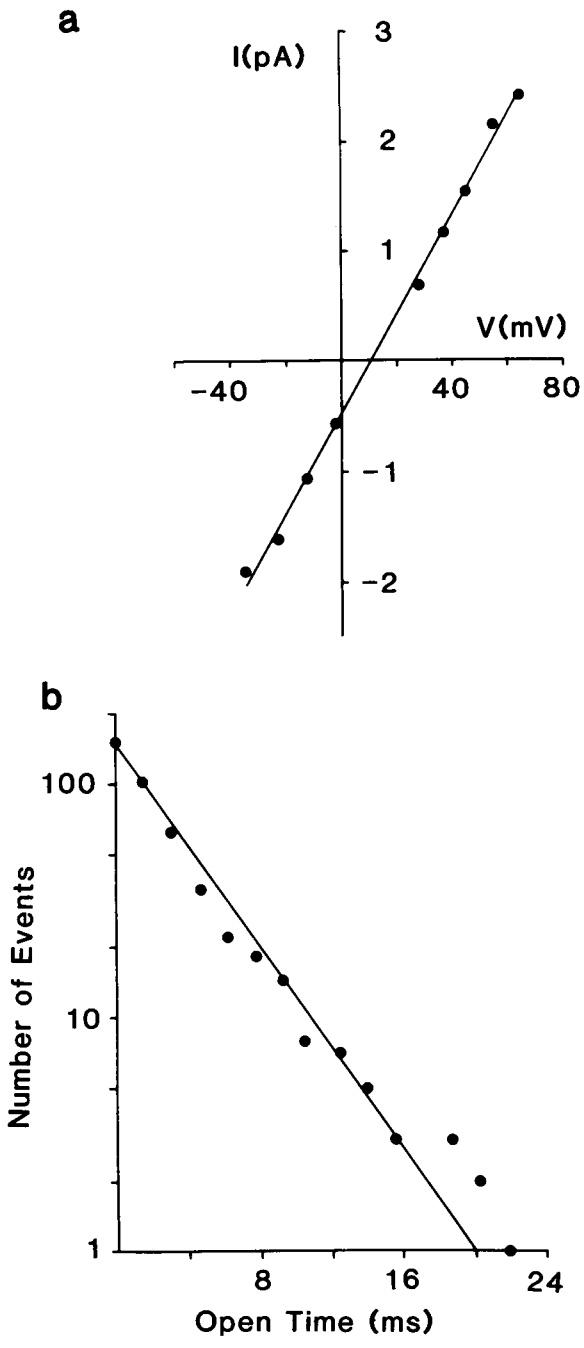


FIGURE 2 *a*, current-voltage curve of the single-channel currents (same patch as Fig. 1). The slope of the curve is 45 pS (the single-channel conductance). *b*, distribution of open times of the single-channel events. A single exponential of $\tau = 4.7$ ms has been fit to the experimental points.

light. The polarity of the current was reversed at this potential because it was more positive than the reversal potential (Fig. 2 *a*).

The outward-current glitch that occurs shortly after the onset of the light marks the initiation of the receptor potential and possibly reflects the charging of the patch-membrane capacitance during the rise of the receptor

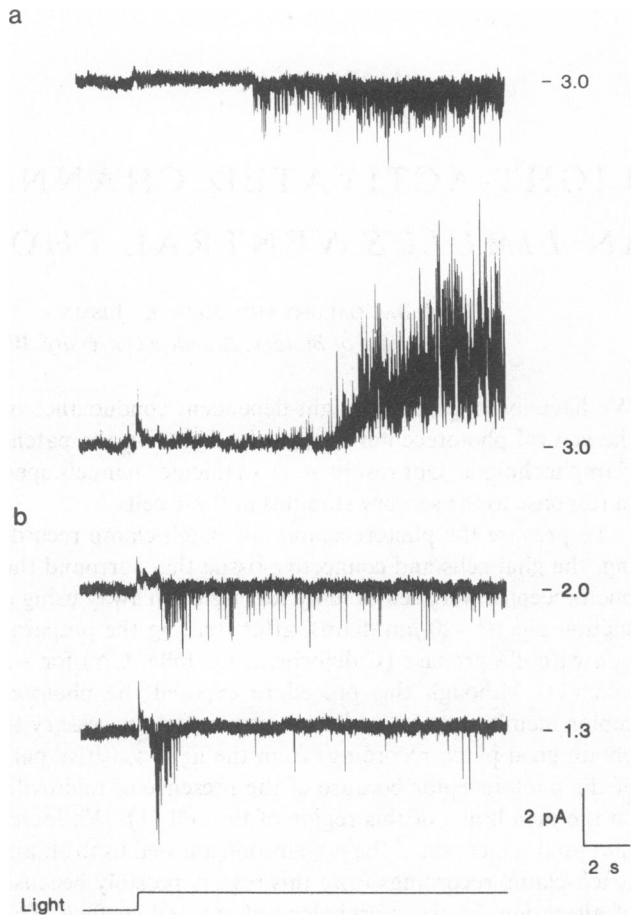


FIGURE 3 Single-channel recordings obtained at different patch membrane potentials and different light intensities. The numbers at the right indicate log intensity units relative to the unattenuated light ($1.4 \times 10^{14}$ photons $cm^{-2}$ $s^{-1}$; 520 nm). *a*, top trace: patch potential is about the resting value (−45 mV before light; −40 mV during light). Bottom trace: identical stimulus, but the patch potential is +55 mV. *b*, single-channel responses to two other light intensities; patch potential about the resting value.

potential. The delay in the activation of the channels was much longer than that of the macroscopic response to a comparable stimulus (~150 ms). This was consistently observed in our experiments. The reason for this abnormality remains unclear.

Single-channel activity was time- and intensity-dependent, as illustrated by Fig. 3. A moderately dim light stimulus produced a maintained channel activity, but at brighter lights the channel activity was initially higher and then dropped to a lower maintained level. The macroscopic light-dependent current presents qualitatively similar characteristics: it consists of a maintained inward current at low light intensities and of a relatively large transient followed by a lower steady-state phase at brighter lights (2). The latency of the single-channel response was also intensity-dependent, as seen in Fig. 3, in a way similar to that of the macroscopic light-dependent current.

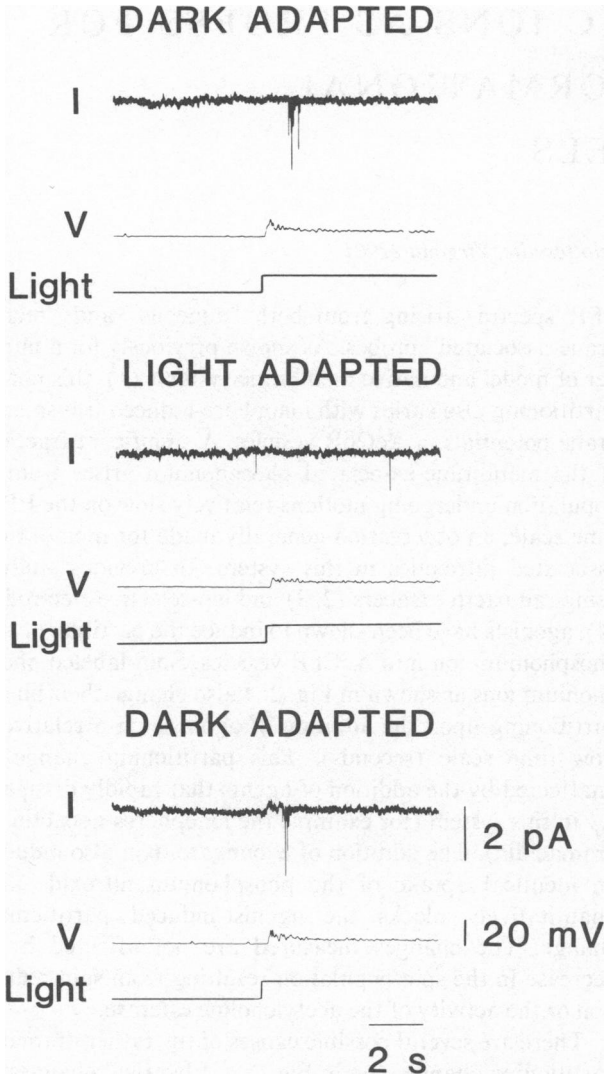


FIGURE 4 Effect of an adapting light on the response. Between the first (top set of traces) and second (middle set of traces) stimuli the cell was exposed to an adapting light. Between the second and third (bottom set of traces) stimuli, the cell was allowed to dark-adapt. The small bumps ("quantum bumps") seen in the voltage recordings of the dark-adapted cell (top and bottom *V*-traces) before the light is turned on are responses to single quanta of light and serve to indicate the state of dark-adaptation of the photoreceptor. They are absent in the light-adapted state (middle *V*-trace). *I*-traces: single-channel currents. *V*-traces: membrane potential recorded with an intracellular microelectrode. At the bottom of each set of traces are indicated the light stimuli, of identical intensity (log intensity −3.0).

Photoreceptors can be light adapted. The response to a test stimulus decreases when the cell has been previously exposed to a bright (adapting) light, as shown by the membrane potential recordings in Fig. 4. The current recordings in this figure show that the single-channel response also adapts to light in a qualitatively similar manner. The single-channel activation evoked in the dark-adapted state was depressed when the test stimulus was presented to the light-adapted photoreceptor (middle *I*-trace). If the cell was allowed to dark-adapt again, the ability of the stimulus to activate channels was recovered (bottom *I*-trace).

The excitation of a single rhodopsin molecule by a photon produces a quantum bump. $6 \times 10^8$ electronic charges are mobilized during a typical bump (2 nA, 100 ms.; reference 4). From the ratio of the charge mobilized during a bump and the charge mobilized during an average channel opening ($3 \times 10^4$), we calculate that the isomerization of an individual molecule of rhodopsin produces $\sim 10^4$ channel openings. The considerable gain of this process suggests that an internal transmitter mediates the activation of the channels (5).

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