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Band-pass filtering by voltage-dependent membrane in an insect photoreceptor

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The membrane properties of short type blowfly photoreceptors (R1–6) were investigated in dark and light adaptation with single electrode current and voltage clamp techniques. The impedance of the cells was defined in frequency domain by using discontinuous current clamp and white-noise-modulated current injection. We found that the slow activation and relaxation of the voltage-dependent K^+ conductance transform the photoreceptor membrane effectively into a band-pass filter. This behaviour could be observed under current clamp as voltage-dependent outward and inward rectification of the membrane. The voltage-dependent band-pass filtering is likely to be present in all neurons with graded potentials and voltage-dependent membrane conductances.

The photoreceptor membrane is the final part of the complex machinery producing responses to light and regulating phototransduction gain. Photoreceptors code changes in illumination as graded potentials, continuous variation of their membrane potential. Light intensity increments elicit changes in membrane potential opposite to those caused by decrements [11, 12]. In insect photoreceptors a powerful voltage-dependent potassium conductance opposes the light-gated calcium and sodium conductances, compresses depolarising responses and regulates the membrane time-constant [9, 19].

The properties and light responses of insect photoreceptors have been studied in situ with intracellular recordings in numerous studies. The role of the photoreceptor membrane itself has only very recently begun to clarify with the combined use of intracellular and patch-clamp techniques [9, 19]. We have further investigated the modulatory role of the K^+ conductance in blowfly photoreceptors, where two types of potassium channels are activated in the operational voltage range of the photoreceptors [19].

The intracellular current and voltage recordings were performed in R1–6 photoreceptor somata of adult intact blowflies (*Calliphora vicina*) in room temperature. The recordings were performed after at least an hour of dark adaptation and when the photoreceptor voltage was changed by injecting continuous current or by adapting light background. The current and voltage clamps with a single intracellular electrode were done with the discontinuous (switched) clamp method [5, 22] with a switching frequency of up to 33 kHz (SEC-1L, NPI electronics, FRG). The resistance of the electrodes filled with 3 M KCl varied from 50 to 80 MΩ. The capacitance compensation of the electrodes was performed by monitoring the head stage output voltage. The voltage and current commands were produced with a laboratory computer and an ASYST language (Keithley ASYST, USA) based program. Data were sampled at a sampling frequency of 2 kHz, low-pass filtered at 500 Hz (Kemo VBF/23 0.01 Hz to 100 kHz low pass dual channel elliptic filter) and stored in hard disk. The light source, positioned at 50 mm from the surface of the eye, was a green light emitting diode (Stanley HBG5666X with a peak emission at 555 nm), which depolarised the photoreceptors about 20 mV above the dark resting potential with the maintained adapting light background of $2.1 \cdot 10^6$ effective photons/s. Both the current and the voltage clamp records were averaged up to 20 times. The input impedance of the cells in frequency domain was studied by pseudorandomly modulated current injections [21]. The current was injected via the recording electrode and the responses to the same pseudo-random sequence were averaged over 10 repetitions [6]. The input impedance of the cell was determined as the frequency response of a system (the injected current as the input and the recorded voltage as the output)

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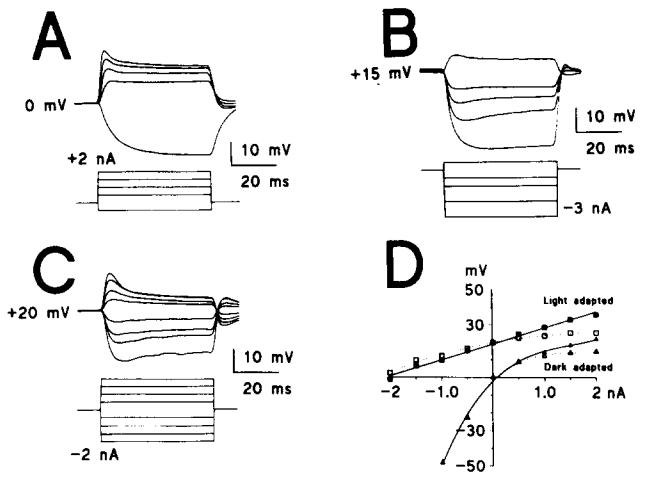

Fig. 1. Current-voltage relationship of fly photoreceptors demonstrating the outward and inward rectification of the membrane. A: voltage responses to injected current steps in dark. Resting potential (about −60 mV) is referred to as zero voltage. Depolarising current steps caused outward rectification of the membrane due to activation of delayed rectifier K^+ channels. B: the photoreceptor was depolarised 15 mV by current injection of about 2.2 nA whereon current steps were introduced. C: responses to current steps when the photoreceptor was light adapted with a background of 10^6 effective photons/s. Note both outward and inward rectification of the membrane. D: voltage–current relations of a photoreceptor determined in dark adapted state (lower continuous line) and in light adaptation (upper continuous line). Solid symbols: peak voltage induced by current steps; open symbols: steady-state voltages at the end of the 50 ms current step.

utilising Fast Fourier Transform [2]. The coherence function was then calculated from the squared cross-power spectrum and the input and output power spectra [2, 18].

Depolarising current steps applied in dark (Fig. 1A) revealed strong outward rectification which has been shown to be due to the activation of voltage-sensitive potassium channels [9, 19]. In response to hyperpolarising current steps the photoreceptor membrane demonstrated — as expected — passive charging properties like those of a simple filter consisting of a resistance and a capacitance (RC-circuit) with membrane time constant of 6–8 ms. When the photoreceptor membrane was depolarised either by increased adapting light background, or by constant depolarising current injection (Fig. 1B,C), the input resistance and the time constant of the photoreceptor membrane decreased, the latter to values near 1 ms. In addition to the outward rectifying membrane we found also an apparently inward rectifying property which is clearly seen in response to hyperpolarising current steps (Fig. 1B,C). The light adapted or otherwise depolarised photoreceptor membrane rectified approximately by the same amount to small depolarising and hyperpolarising current steps. The dark adapted membrane was strongly non-linear, but when light adapted (the membrane potential had depolarised about 20 mV above resting potential) the steady-state *I/V* relation was virtually linear (Fig. 1D).

How can the described membrane properties be interpreted? Two alternative explanations exist for this inward rectifying quality of the light adapted membrane. Either there were a distinct inward rectifier conductance or the kinetical properties of the earlier discovered outward rectifying K^+ channels could be responsible. To distinguish between these possibilities we studied in situ the conductances activated at different membrane potentials using single electrode voltage clamp [5, 19, 22]. In addition to the delayed rectifier potassium channels we found no evidence for any other type of conductance activated by voltage in the studied photoreceptor somata (n=57) when depolarised either by injected current or by light (although this does not hold for the photoreceptor axons [20]). Instead, as illustrated in Fig. 2, both the outward and inward rectification can be well explained by the relaxation of the very same type of conductance, i.e. the voltage-dependent K^+ channels. It must be stressed that the inward rectification is in principle a quality of the

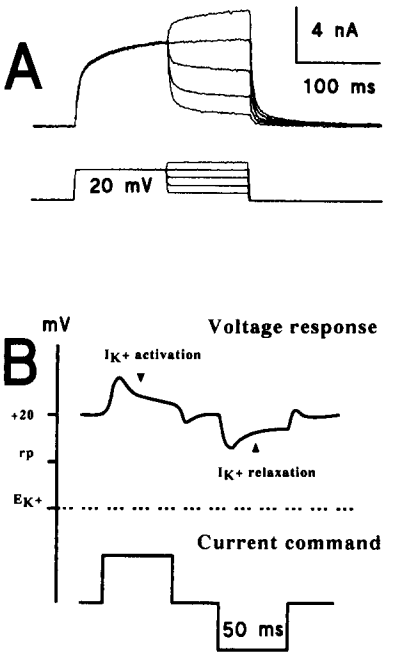

Fig. 2. Voltage clamp currents show that the relatively slow activation and relaxation of the K^+ current was the basis of both the outward and inward rectification. A: the activation and relaxation of the current elicited by a depolarising voltage command of 20 mV from resting potential. B: a schematic representation of a current clamp experiment illustrating how — in light-adapted photoreceptors — the voltage-dependent K^+ conductance creates the outwardly and inwardly rectifying membrane. During the response to a step of depolarising current injection the increasing activation of the K^+ conductance shifts gradually the membrane potential towards the E_K, whereas the increasing deactivation of the conductance shifts the membrane away from the E_K because of the light-activated depolarising conductance.

membrane under current clamp and — in this case — it cannot per se be seen under voltage clamp. When the outward rectifying conductance is being activated by depolarisation, the membrane potential begins to shift — after a transient — towards E_{K+} that is, it hyperpolarises. When the outward rectifying potassium conductance is relaxing due to the reduction of the depolarising influence, the membrane potential is, again after a brief transient, shifting *away* from E_{K+}, producing an apparent slow inward rectification (Fig. 2B).

How are the outward and inward rectifying properties, i.e. activation and relaxation, of this K^+ conductance matched with the functional voltage range and the speed of response of the photoreceptors? To resolve this question we looked more closely at the currents activated (and deactivated) by voltage steps from different holding potentials, the potential thus mimicking the (depolarised) membrane potential in light adapted photoreceptors. Fig. 3A shows that when the holding potential was increased, the activated conductance began to saturate. During the voltage steps the activated currents did not show significant inactivation, but partial decrease of the conductance was seen only in the holding current, where about 50% of the originally step-activated current was sustained in the holding current at 35 mV above the resting potential (Fig. 3B). This long-term inactivation may reflect the voltage-dependent shift from the fast channel type to the slow K^+ channels.

The voltage dependence of the K^+ conductance could be well fitted with either a Boltzmann equation (with V_{50} between −57 and −47 mV, and steepness factor between 6.0 and 12) or with an equation describing first order kinetics of channel opening, supposing a simple two-state model that is likely to be valid for the slow type channel [19] (Fig. 3B). The two components of the K^+ conductance in these cells were differentially activated from different holding potentials, as can be expected on the basis of the previous patch clamp study [19]. The difference in the activation range of the fast and slow channels results in varying activation and relaxation of the total voltage-activated conductance (Fig. 3B,C). As the holding potential was depolarised, the activation and deactivation become faster (Fig. 3C). This fits well with the speeding up of the rest of the phototransduction in light adaptation [3, 7, 8, 11, 17]. The increasing speed, with which the K^+ conductance was being modulated by voltage, lead to significant changes in the membrane's filtering properties. These were determined using the new method of impedance measurement with white noise modulated current injection [21] (Fig. 4A). At 20 mV below resting potential, the photoreceptor behaved like a passive spherical cell that is possible to model with a single low-pass RC-filter (Fig. 4B). At increasingly depolarised holding potentials, the activation of the K^+ conductance and the decrease of its activation and deactivation time constants transform the photoreceptor membrane to a band-pass filter. The relaxation time constants of the conductance in voltage clamp were very close to the time constants derived with the impedance measurement in frequency domain (compare Figs. 2A, 3A, and 4B). For example, with the impedance method the fitted relaxation time constant at 15 mV above the resting potential was 26 ms, whereas in voltage clamp measurements it varied between 21 ms and 25 ms, depending on the initial holding potential.

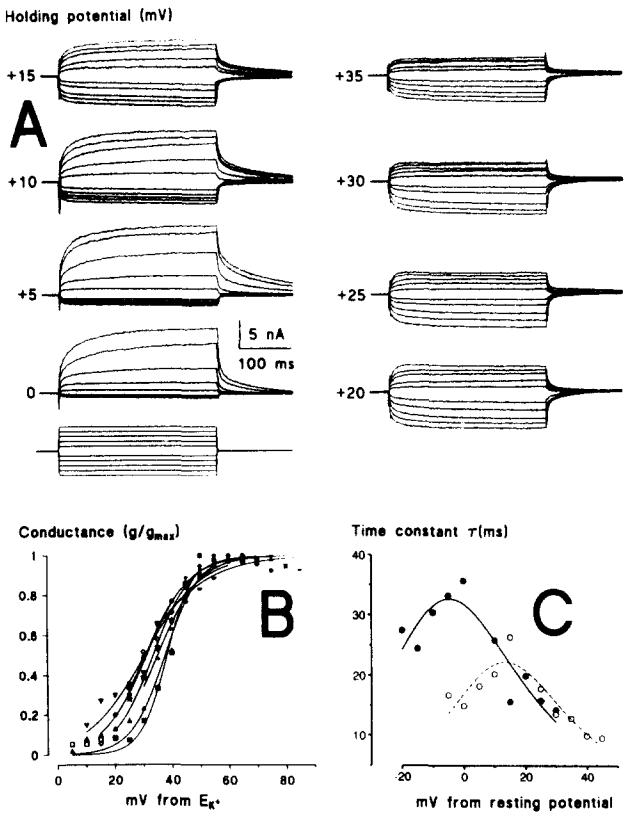

Fig. 3. The voltage dependency of a blowfly photoreceptor membrane studied by single electrode voltage clamp. A: a family of (leak-subtracted) voltage-clamp currents obtained at different holding potentials at and above resting potential, thus simulating the effect of light adaptation. The voltage command steps produced both activation and relaxation currents. B: the voltage-dependent relative K^+ conductance (scaled to max. conductance for each curve) in function of step voltage, obtained at different holding voltages (A). The continuous lines represent the best fit to an equation $\alpha/(\alpha+\beta)$, where α and β are the (voltage-dependent) rate constants of the first order channel opening kinetics [10, 19] of the form $\alpha=k_1 \cdot \exp(V/V_1)$, k_1 and V_1 being fitted constants. C: the time constants of relaxation and activation of the total rectifying K^+ conductance. Solid circles: holding potential 5 mV above the dark resting potential; open circles: holding potential 20 mV above the dark resting potential. Continuous and dashed lines: best fits to the equation $1/(\alpha+\beta)$. When held at 5 mV above resting potential the contamination from the fast channel type [19] makes the activation and relaxation somewhat asymmetrical.

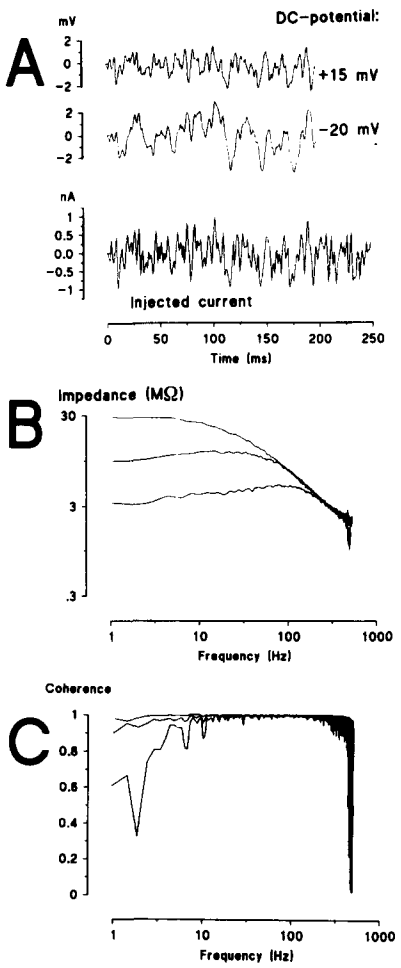

Fig. 4. Determination of the photoreceptor impedance in frequency domain using discontinuous current clamp and white noise modulated current injection at different steady depolarisation levels. A Samples of the current and voltage at −20 mV below and 15 mV above dark resting potential. The voltage modulation is smaller and slower at 15 mV above the resting potential because of the increased activation of the K^+-conductance. B The photoreceptor input impedance (absolute value) at membrane potentials of −20, 0 and +15 mV from resting potential. Note how the activation of the voltage-dependent K^+-conductance changes the impedance function from a pure first order low-pass filter to a band-pass filter. C The coherence functions for the input impedance. The impedance is linear (the coherence function is near unity) for the most part of the studied frequency range regardless of the DC-potential. The lowest trace in low frequencies represents the coherence for the impedance at +15 mV above resting potential, where the signal-to-noise ratio begins to deteriorate in frequencies below 10 Hz.

These results demonstrate that in sensory cells and neurons with graded potentials apparently outwardly rectifying K^+ channels may have a dual function in modifying long-lasting responses into both depolarising and hyperpolarising direction, and in this way crucially modifying the cells' response kinetics. In insect photoreceptors the need for this type of gain regulation is obvious, as it augments the transmission of biologically relevant fast signals [11–14]. These kind of membrane properties are by no means limited to the insect photoreceptors, but voltage-dependent conductances are wide-spread in non-spiking cells [1, 4, 15, 16] and can plausibly be proposed to have similar functions.

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