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Measuring complex admittance and receptor current by single electrode voltage-clamp

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Abstract

Studying the membrane properties of small excitable cells like sensory receptors in situ is often difficult. Two new techniques are described here which utilize white noise during single-electrode voltage-clamp. Cells are impaled with a single microelectrode and voltage-clamped to a given holding potential, using a time-sharing technique. The first method, based on modulating the voltage command with repeated sequences of a pseudorandom stimuli, allows measurements of cell conductance (complex admittance) in the frequency domain. The second method is designed to characterize the dynamics of the receptor current in the frequency domain. In both cases, R1-6 type blowfly photoreceptors were used as experimental models. The photoreceptor was first light-adapted to a steady light background and then clamped to the resulting potential. A pseudorandomly modulated light stimulus was then superimposed on the steady light background and the resulting receptor current was recorded. The frequency response was then calculated from the light modulation and the receptor current via fast Fourier transform (FFT). By using intracellularly applied ion channel blockers, the effects of active and passive membrane properties in modulating the transmitted signals could also be studied.

Key words: Excitable membrane; Potassium channel; Photoreceptor; White noise

1. Introduction

In a traditional 2-electrode voltage clamp, the current and voltage signals give information about the membrane conductance of the excitable cell. Because of the small size of many neurons, it is often difficult, if not impossible, to apply the traditional voltage clamp to characterize in situ membrane conductances. Suitable cell types for the 2-electrode voltage clamp are therefore few. In addition, frequency domain analysis techniques, with their obvious advantages in clarifying the dynamics of conductance changes, have been rare (but see Poussart et al., 1977).

Variations of the patch-clamp technique allow measurements to be made from isolated neurons and from neurons that are near the tissue surface. If the cells are deeper or surrounded by glia, the only suitable method for intracellular recordings in situ is the use of a single glass microelectrode. Single-electrode amplifiers based on time-sharing voltage and current clamp allow measurements of cell resistance and conductance (Wilson and Goldner, 1975; Finkel and Redman, 1984; Weckström et al., 1991; Laurent 1990, 1991; Juusola, 1993; Juusola and Weckström, 1993; Laughlin and Weckström, 1993). Recently, a method was introduced to measure cell impedance in the frequency domain with pseudorandomly modulated current injection under single-electrode current clamp (Weckström et al., 1992; Juusola and Weckström, 1993). It is also possible to measure the complex admittance of a cell in the frequency domain using pseudorandom voltage modulation under single-electrode voltage clamp. Both the complex impedance and complex admittance, which are reciprocals of each other, are extremely useful tools to determine how the passive and active properties of membranes shape the transmitted voltages in all functionally relevant frequencies. Although impedance can be measured under both current and voltage-clamp

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conditions, it is especially important to use voltage clamp when the membrane characterization includes contributions from some other current, such as the receptor current or the synaptic current, in the same neuron.

Sensory neurons differ from many other excitable cells in being able to generate voltage responses to a specific stimulus by internal transduction mechanisms. The result of sensory transduction is a receptor current generated through the cell membrane via specific ion channels gated by internal messengers. In sensory neurons, other ion channels often contribute to the modulation of the voltage response resulting from the receptor current. Hence, the membrane properties and the receptor current act in concert to encode sensory information. It has been possible to characterize receptor currents using the 2-electrode voltage-clamp technique with some large non-spiking receptor cells (e.g., in *Limulus* photoreceptors: Millecchia and Mauro, 1969). This has also been done with the whole-cell patch-clamp technique, but not in situ (e.g., Sakmann and Neher, 1983; in *Drosophila*: Hardie, 1991; Ranganathan et al., 1991; Hardie and Minke, 1992). Unfortunately the locations and sizes of many receptor cells preclude the use of double penetration or patch pipettes. So far it has been impossible to use the single-electrode voltage-clamp technique to measure the receptor currents because of their large magnitude. A method is presented that, when applied to blowfly R1-6 photoreceptors, overcomes this problem. When a photoreceptor is fully light-adapted it is depolarized, and it can be clamped to that new potential. Stimulating the receptor with pseudorandomly modulated light superimposed on the existing steady light background gives a resulting receptor current that is small enough to be fully compensated by the clamp. The frequency response of the light current can then be calculated via the fast Fourier transform (FFT) from the cross-power spectrum between the light stimulus and the receptor current.

2. Materials and Methods

The switching single-electrode voltage clamp is based on a time-sharing control of the voltage measurement and current injection to produce a membrane potential close to that of the voltage command (Wilson and Goldner, 1975; Finkel and Redman, 1984). The method is prone to several recording artifacts, which are similar to the difficulties with the single-electrode current clamp discussed in Weckström et al. (1992). The conditions for a successful clamp are the following. (1) The sampling frequency (f_s) should be at least twice (or more if possible) that of the highest frequency component in the recorded signal. (2) The upper cut-off frequency of the low-pass filter, (f_f) (used to eliminate high-frequency noise) must be adjusted to preserve the important features of the neuronal signal. (3) The switching frequency (f_{sw}) cannot be higher than 0.3 times the upper cut-off frequency of the recording electrode f_e, but must be about 10 times that of the membrane cut-off frequency f_m. Also, f_{sw} should be significantly greater than the sampling frequency of the recording system, f_s.

In summary:

$$f_e > 3f_{sw} \text{ and } f_{sw} > 2f_s > 2f_f > f_m \quad (1)$$

2.1. Experimental animals and preparation

I used adult blowflies (*Calliphora vicina*) of both sexes. The flies were attached with beeswax onto the recording platform and grounded with an indifferent electrode (Ag/AgCl) positioned inside the head. Intracellular recordings of R1-6 photoreceptors were performed with glass capillary microelectrodes, which were moved with a piezoelectric microtranslator (Burleigh inchworm PZ-550, USA) into the retina through a small hole made in the lateral cornea and sealed with a high vacuum grease. The resistance of the microelectrodes, filled either with 3 M potassium chloride or with 50 mM potassium acetate and 0.5 M TEA, varied between 60 and 100 MΩ with the electrode in the tissue. The time constant of the electrodes (τ_e) in tissue after a dual capacitance compensation of the amplifier (SEC-1L, NPI Electronic, Germany) was 2–3 μs, giving a high cut-off frequency greater than 60 kHz.

$$f_e = 1/2\pi\tau_e \quad (2)$$

2.2. Illumination

The light source was a LED (Stanley HBG 5666X, with peak emission at 555 nm) mounted on a cardan arm. Computer-aided stimulation of cells was performed using pseudorandomly modulated light stimuli (Juusola et al., submitted). The LED was driven by a current source, the output of which was limited to the linear range of the current-light intensity relation. The contrast, c, of the light stimulus was defined as:

$$c = \Delta I / I_{mean} \quad (3)$$

where ΔI represented the SD of the intensity modulation (see Juusola, 1993) and I_{mean} was the mean background. The light output of the LED (and the mean background) was calibrated by counting after prolonged dark adaptation, the number of discrete responses (evoked by single photons) occurring during prolonged dim illumination. The pseudorandom stimulus had a gaussian intensity distribution and a flat

power spectrum up to about 250 Hz (Juusola et al., submitted).

2.3. Recording procedures

The photoreceptor admittance experiments were recorded in darkness, while the light-induced receptor current and voltage responses were recorded form a TEA-treated photoreceptor at an adapting background of 500,000 effective photons/s. The ionophoretical TEA-treatment of a photoreceptor with 8 nA depolarising pulses usually lasted 5–10 min. As TEA blocked the delayed rectifier K^+ channels, the dynamics of the photoreceptor voltage responses slowed, but the sensitivity of the cell was usually sustained (cf., Juusola, 1993). The maximum response amplitudes of dark adapted TEA-treated photoreceptors varied between 50 and 60 mV. After TEA treatment, the photoreceptor light current (receptor current) was first recorded under voltage clamp. Then the voltage responses of the same TEA-treated photoreceptor were recorded under current clamp.

The input and output signals were different during the voltage-clamp and current-clamp experiments. (1) In the admittance experiments the current responses were transmitted via a microelectrode to a high-impedance preamplifier (SEC-1L, NPI Electronic, Germany; 1/2, 1/4, 1/8 duty cycles; 1L headstage) and filtered with the corresponding voltage command at 500 Hz (VBF/23 KEMO; Cauer-type of low-pass dual-channel elliptic filter, high-frequency roll-off: 135 dB/octave). The voltage command was injected via the recording electrode with a switching frequency of up to 20 kHz. (2) In the light current experiments the resulting receptor current and the corresponding pseudorandomly modulated LED stimulus current were also low-pass filtered at 500 Hz. (3) In the TEA experiments the photoreceptor voltage response was amplified and low-pass filtered with the corresponding LED stimulus current at 500 Hz as well. During each experiment these paired input and output signals were monitored on an oscilloscope, sampled at 2 kHz, digitized with a 12-bit A/D converter (DT2821, Data Translation, USA) and stored on a hard disk or in the memory of a computer (IBM-486 compatible 33 MHz). The sampling process was initiated synchronously to the pseudorandom modulation produced by the computer, using a shift-register type pseudorandom generator, and 8-s records of both signals were obtained during each recording cycle. After a preset number of responses (usually 10–30) the average response was calculated (see also French, 1980b). The data processing was done using ASYST 4.0 (Keithley, USA) based programs (Juusola, 1993; Juusola et al., in press; Kouvalainen et al., submitted).

2.4. Frequency domain analysis of the signal

The averaged responses, now containing virtually no stimulus-independent noise, were segmented for FFT analysis using a Blackman-Harris 4-term window with 50% overlap of the segments (Bendat and Piersol, 1971; Harris, 1978). The spectrum ($S_{R1\text{-}6}(f)$) of each segment was averaged in the frequency domain. Thereafter the corresponding photoreceptor frequency responses ($H_{Admittance}(f)$, $H_{TEA}(f)$ and $H_{light\text{-}c}(f)$) with coherence estimates and first-order Wiener-kernels (or linear impulse responses) were calculated, using the power spectra of the corresponding input and output and their cross-power spectrum (French et al., 1972; Bendat and Piersol, 1971; Marmarelis and Marmarelis, 1978; French, 1980b).

3. Results and discussion

3.1. Measuring the complex admittance of the photoreceptor membrane

Fig. 1A shows 250-ms samples of the pseudorandomly modulated voltage command and the resulting photoreceptor currents at 3 different holding potentials: at resting potential (−60 mV) and when hyperpolarized by −20 and −30 mV from resting potential. As the holding potential was made more negative, the resulting current evoked by the same voltage command was also reduced. This is presumably because hyperpolarizing the cell deactivated the voltage-dependent delayed rectifier K^+ channels (Weckström et al., 1991). These effects of hyperpolarization are also seen in the complex admittance (Fig. 1B). The capacitance of the photoreceptor membrane was constant, but the input impedance varied with the proportion of open voltage-dependent channels. Therefore, hyperpolarising the membrane increased its time constant (e.g., Hille, 1992), $\tau = R_m C_m$.

At 30 mV below the dark-adapted photoreceptor resting potential, nearly all of the voltage-dependent channels are closed (cf., Juusola and Weckström, 1993; Weckström et al., 1993) and the photoreceptor membrane acts as a simple first-order low-pass filter. When the photoreceptor is clamped to more positive potentials, voltage-dependent K^+ channels open and the membrane begins to act as a band-pass filter. Blowfly photoreceptors depolarise with background illumination. Very intense light saturates the photoreceptor membrane potential at about 20–30 mV above the dark resting potential (e.g., Juusola, 1993). At such potentials, the large admittance of the membrane provides rapid transmission of photoreceptor voltage responses (cf., Weckström et al., 1991; Juusola and Weckström, 1993).

The coherence, $\gamma^2(f)$, in Fig. 1C is a measure of both the linearity and the signal-to-noise ratio of the system (Bendat and Piersol, 1971). If the value of the coherence is unity, the system is linear and has no added noise. (Because of the time-domain averaging of the recording data, uncorrelated noise was effectively removed from these records.) The coherence then represents the linearity of the membrane admittance. The results show that the membrane behaved linearly at the three tested holding potentials. The coherence function was calculated from:

$$\gamma^2(f) = G_{xy}(f)G^*_{xy}(f)/G_{xx}(f)G_{yy}(f) \quad (4)$$

where $G_{xy}(f)$ is the cross-power spectrum between the input and output (here voltage and current), $G^*_{xy}(f)$ is its complex conjugate, and $G_{xx}(f)$ and $G_{yy}(f)$ are the input and output power spectra, respectively.

Use of a pseudorandom stimulus under voltage clamp has some advantages over to traditional step command approach. First, it reduces the non-linearities associated with changing the clamp potential by big steps from one potential to another. Indeed, white noise has a tendency to linearize non-linear systems (Spekreijse and Oostings, 1970). Thus, the complex admittance is less contaminated by non-linearities due to the voltage clamp itself than the conductances measured by the voltage-step approach. Second, analyzing the integrative properties of neurons in the frequency domain increases our understanding of neural systems. For example one can directly and reliably estimate the information carrying capacity of the membrane of a small excitable cell and compare it to theoretical predictions (cf., Van Hateren and Laughlin, 1990).

3.2. *Measuring the receptor current and the membrane potential in a TEA-treated photoreceptor*

TEA was ionophoretically injected (for 5 min) into a light-adapted blowfly photoreceptor via a glass microelectrode. TEA caused a 5 mV increase in the steady depolarisation of the cell by blocking part of the resting voltage-dependent outward K^+ current. After TEA treatment, the photoreceptor was clamped to its steady potential (26 mV above the dark resting potential) and pseudorandom light stimulation with a mean of 0.32 contrast was delivered. Immediately after the light-current recording was finished, the amplifier was changed to current-clamp mode and the voltage response of the same TEA-treated cell was measured with the same pseudorandomly modulated light stimulation. The corresponding frequency responses of the light current and the voltage response of the same TEA-treated photoreceptor are shown in Fig. 2A. It is clear that the light current had a higher 3 dB cut-off frequency than

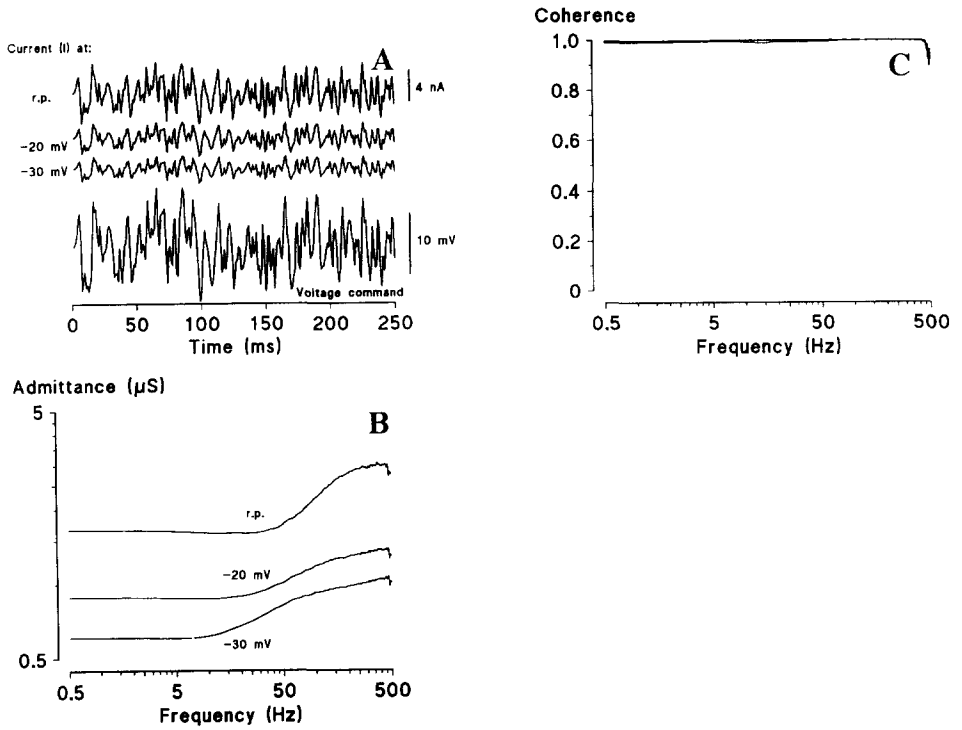

Fig. 1. Determination of photoreceptor admittance in the frequency domain using discontinuous voltage clamp and a pseudorandomly modulated voltage command on different holding potentials. A: 250-ms samples of the voltage command (bottom trace) and the resulting currents at dark resting potential (r.p.), and when hyperpolarized by −20 and −30 mV. B: the corresponding complex admittance at membrane potentials of 0, −20 and −30 mV relative to rest. C: the coherence functions of the complex admittance functions. The complex admittance was linear (the coherence function is near unity) at each of the tested membrane potentials.

the voltage responses in the TEA-treated membrane. Therefore a membrane in which voltage-sensitive K^+ channels were blocked, gave low-pass filtering of the light current. The phase function of Fig. 2B also demonstrates how the photoreceptor voltage response (at least when the cell was TEA-treated) declined more rapidly than the light current at high frequencies. This further indicates that the voltage responses was slightly slower than the corresponding receptor current. The 180° phase difference was due to polarity difference between the photoreceptor light current (measured under voltage clamp) and the corresponding voltage response (measured under current clamp).

The coherence function in Fig. 2C shows that both the photoreceptor light current and the voltage response behaved approximately linearly when evoked by a pseudorandom light contrast stimulus. The linearity of photoreceptor voltage responses to pseudorandomly modulated contrasts has been found previously (French, 1980b; Juusola et al., in press), but the data of Fig. 2 show the first frequency domain measurements of the receptor current in blowfly photoreceptors.

Since the system was approximately linear, we could get good estimates of both the receptor current and voltage impulse responses. The first-order Wiener kernels (i.e., linear impulse responses) were calculated by the inverse FFT (Bendat and Piersol, 1971). Fig. 2D shows that the light-current impulse (here inverted) reached its peak amplitude in about 12 ms, but the impulse response of the TEA-treated photoreceptor was delayed by ~ 3 ms. Also the decay of the voltage response was slightly slower than the receptor current.

The results demonstrate that it is possible to measure the receptor current from small sensory cells using a single-electrode voltage-clamp and pseudorandom stimulus modulation. With little effort corresponding voltage responses could be obtained from the same cell under current clamp (Fig. 2A–D). Using this approach allows one to study the effects of specific ion channels on the receptor currents and voltage response.

Both the methods introduced here have a range of possible applications. They can be applied to any small sensory cell that produces graded potential responses. One could, for example, use these methods to study phototransduction in mutant strains (e.g., *Calliphora* and *Drosophila*) in the frequency domain. Moreover, these methods could be extended to spiking receptor cells. If the voltage-dependent Na^+ channels are first blocked, these methods may allow to determine the dynamics of subthreshold potentials.

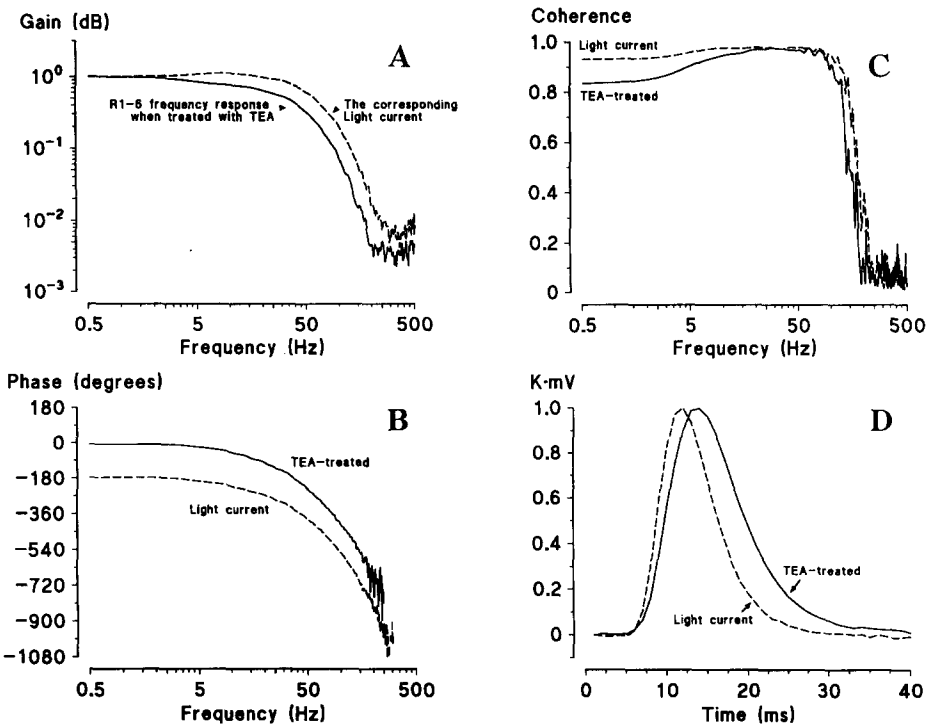

Fig. 2. Analysis of the receptor current (i.e., light current; dashed line) and the voltage responses (continuous line) of a TEA-treated photoreceptor. A: the gain parts of the frequency responses. The high-frequency content of the voltage response was lower than for the light current. The vertical bars indicate the corresponding 3 dB cut-off frequencies. B: the phase functions of the frequency responses. There was a 180° phase shift between current and voltage. Note how the phase of the voltage responses declined more rapidly than the light current phase at high frequencies. C: the coherence functions show that both the voltage response and the light current were nearly linear processes when modulated with a pseudorandom stimulus. D: linear impulse responses calculated via the inverse FFT. The light current was faster than the photoreceptor response.

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References

Bendat, J.S. and Piersol, A.G. (1971) Random Data: Analysis and Measurement Procedures. Wiley, New York.

Finkel, A.S. and Redman, S.J. (1984) Theory and operation of a single electrode voltage-clamp. J. Neurosci. Methods, 11: 101–127.

French, A.S. (1980a) Coherence improvement in white noise analysis by the use of a repeated random sequence generator. IEEE Trans. Biomed. Eng., 27: 51–53.

French, A.S. (1980b) Phototransduction in the fly compound eye exhibits temporal resonances and a pure time delay. Nature, 283: 200–202.

French, A.S., Holden, A.V., and Stein, R.B. (1972) The estimation of a frequency response function of a mechanoreceptor. Kybernetik, 11: 15–23.

Hardie, R.C. (1991b) Whole cell recordings of the light induced current in dissociated *Drosophila* photoreceptors: evidence for feedback by calcium permeating the light-sensitive channels. Proc. R. Soc. Lond. B, 245: 203–210.

Hardie, R.C. and Minke, B (1992) The *trp* gene is essential for a light-activated Ca^{2+} channel in drosophila photoreceptors. Neuron, 8: 643–651.

Harris, F.J. (1978) On the use of the windows for harmonic analysis with the discrete Fourier transform. Proc. IEEE, 66: 51–84.

Van Hateren, J.H. and Laughlin, S.B. (1990) Membrane parameters, signal transmission, and the design of a graded potential neuron. J. Comp. Physiol. A, 166: 437–448.

Hille, B. (1992) Ionic Channels of Excitable Membranes, 2nd edn., Sinauer, Sutherland, 607 pp.

Juusola, M. (1993) Linear and nonlinear contrast coding in light adapted blowfly photoreceptors. J. Comp. Physiol. A, 172: 511–521.

Juusola, M., and Weckström, M. (1993) Band-pass filtering by voltage-dependent membrane in an insect photoreceptors. Neurosci. Lett., 154: 84–88.

Juusola, M., Kouvalainen, E., Järvilehto, M. and Weckström, M., (in press) Contrast gain, signal-to-noise ratio and linearity in light-adapted blowfly photoreceptors, J. Gen. Physiol.

Laughlin, S.B. and Weckström, M. (1993) Fast and slow photoreceptors: a comparative study of the functional diversity of coding and conductances in the *Diptera*. J. Comp. Physiol. A, 172: 593–609.

Laurent, G. (1990) Voltage-dependent nonlinearities in the membrane of locust nonspiking local interneurons, and their significance for synaptic integration. J. Neurosci., 10: 2268–2280.

Laurent, G. (1991) Evidence for voltage-activated outward currents in the neuropilar membrane of locust nonspiking local interneurons. J. Neurosci., 11: 1713–1726.

Marmarelis, P.Z. and Marmarelis, V.Z. (1978) Analysis of Physiological Systems: The White Noise Approach, Plenum Press, New york.

Millecchia, R. and Mauro, A. (1969) The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. J. Gen. Physiol., 54: 331–351.

Poussart, D., Moore, L.E. and Fishman, H. (1977) Ion movements and kinetics in squid axon. I. Complex admittance. Ann. NY Acad. Sci., 303: 355–379.

Ranganathan, R., Harris, G.L., Stevens, G.F. and Zucker, C.S. (1991) A *Drosophila* mutant defective in extracellular calcium-dependent photoreceptor deactivation and rapid desensitization. Nature, 345: 230–232.

Sakmann, B. and Neher, E. (1983) Single-Channel Recording, Plenum, New York, 503 pp.

Spekreijse, H. and Oostings, H. (1970) Linearizing: a method for analysing and synthesizing non-linear systems. Kybernetik, 7: 22.

Weckström, M., Kouvalainen, E. and Juusola, M. (1992) Measurement of cell impedance in frequency domain using discontinuous current clamp and white-noise-modulated current injection. Pfluegers Arch., 421: 469–472.

Weckstrom, M., Hardie, R.C. and Laughlin, S.B. (1991) Voltage-activated potassium channels in blowfly photoreceptors and their role in light adaptation. J. Physiol., 440: 635–657.

Wilson, W. and Goldner, M.M. (1975) Voltage clamping with a single microelectrode. J. Neurobiol., 6: 411–422.