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Measurement of cell impedance in frequency domain using discontinuous current clamp and white-noise-modulated current injection

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Abstract. A method is decribed for the determination of cellular input impedance of non-spiking neurones. The input impedance is important when cellular geometry and the effects of voltage-dependent channels are considered. Cells are impaled with a single glass microelectrod and current is injected using a time-sharing technique. The cell's impedance is measured by randomly modulating the injected current and calculating the impedance as a transfer function between current and recorded membrane voltage. Corresponding coherence functions can also be calculated for estimating the signal-to-noise ratio, and also linearity (i.e. possible activation of voltage-dependent conductances) of the membrane.

Key words: Non-Spiking – Vision – Photoreceptor – Potassium channels – FFT

Introduction

Neural impedance is generally measured by injecting current pulses of small amplitude and studying the resulting changes in the membrane voltage. In the case of large cells two intracellular electrodes can be used. If the cell diameter is too small this is usually not practical and other recording methods must be resorted to. Isolated cells and, in some cases, cells near the surface of the tissue can be recorded using the patch-clamp techniques. Neural elements in situ, embedded deep inside barriers of glia or connective tissue and surrounded by other neurones, can at present only be recorded intracellularly with a single glass microelectrode. Injecting current through micropipettes involves a lot of problems. Multi-barrel recording and bridge balance systems do not give reliable information if the investigated neurones are so small that they necessitate the use of microelectrodes of considerable (and often nonlinear) impedance.

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To alleviate these problems a time-sharing, discontinuous (or "switched") current clamp can be used [2–4, 18]. By its proper application, cell impedance can be determined reliably within limits dictated by the amplifier and the microelectrodes and, of course, the neuronal properties (e.g. [7, 9, 10, 18]).

White-noise or frequency-sweep (ZAP) functions have previously been used to study neuronal impedance [12–14]. In this paper we describe a use of a discontinuous current clamp that involves pseudorandom (white noise) modulation of the amplitude of the injected current, and analysis of the neuronal impedance in frequency domain. The need for such a measurement system is obvious. First, if we want to know how the membrane with its passive and active (voltage-dependent) properties shapes the voltage signals transmitted along the cell membrane, we need not only just the cellular resistance and membrane time-constant, but the membrane impedance in all physiologically relevant frequencies. This kind of information is especially required if the neurones studied are non-spiking ones, i.e. responding with graded changes of their membrane potential without action potentials. That means that the signals are continuously at the mercy of the membrane impedance.

Secondly, a recently developed cable model [15, 16] analyses the membrane properties in the frequency domain, and the results of the method we describe can be used in conjunction of that model to give a better hold on those properties of the neurones that affect the signal transmission in the neurones themselves, i.e. the neuronal geometry.

Theoretical considerations

The use of single-electrode current clamp (SECC) means a time-sharing current injection and voltage recording. To give reliable information of the neurone's impedance, the user of this method must be aware of the sources of artifacts when using it. In the application of the SECC presently described, where white-noise modulation of the in-

jected current – with computerised data collection and analysis – is used, additional caveats are necessary.

We have to deal with five different frequencies that define the correctness and also limitations of the method:

f_s, the sampling frequency of our data collection system
f_f, the upper cut-off frequency of the low-pass filter used for voltage recording
f_{sw}, the switching frequency of the SECC, i.e. how fast the current injection and voltage recording are alternated
f_e, the upper cut-off frequency of the intracellular electrode in site
f_m, the upper cut-off frequency of the cell membrane

The basic rules of sampling theory dictate the relationships between f_s, f_f, and the recorded signal. The sampling frequency, f_s, must be at least two times larger than the highest frequency in the recorded signals. The frequency content in the recordings in controlled with a (usually steep) low-pass filter. Any recording also contains, in additions, in addition to the signals originating in the neurone, various kinds of noise. The f_f must be adjusted accordingly, i.e. to cut off unwanted noise but to preserve the neuronal signals.

The adjustment of the SECC switching frequency, f_{sw} to the time constant of the recording electrode and the neuronal membrane is a little trickier. Basically, f_{sw} cannot be larger than about 0.3 times the upper cut-off frequency of the recording electrode, f_e, if large electrode artifacts are to be avoided [4]. At the other extreme, the f_{sw} must be about 10 times larger than the membrane cut-off frequency. Although the estimates of membrane voltage depend on the membrane capacitance only and not on the membrane time constant per se, the average deviations of the membrane voltage from the sampled value may become intolerably large if this criterion is left unheeded. If f_{sw} is considerably smaller, the true membrane voltage at the sampling time is still recorded, but the membrane voltage may alternate so much between sampling points that the sampled voltage is not an accurate measure of current-induced voltage change. In addition, f_{sw} must be clearly higher than the sampling frequency of the recording system f_s and, of course, higher than f_f if serious sampling artifacts are to be avoided.

In summary the following relations should hold for the listed important frequencies if the method is to be successful,

$$f_e > 3f_{sw} \tag{1}$$

$$f_{sw} > 2f_s > 2f_f > f_m \tag{2}$$

The application

We have constructed a system that satisfies the theoretical considerations presented above. A scheme of the system is presented in Fig. 1. Intracellular recordings are made from insect (non-spiking) photoreceptors using a SECC technique with SEC-1L pre-amplifier (NPI Electronics, FRG). Current injection is performed with a shift-register type pseudorandom generator, the signal of which is fed to the amplifier's current command input. This "white noise" has a power spectrum that is flat up to about 1 kHz. The sampled recorded current and sampled recorded voltage are filtered with an 80-dB/decade low-pass elliptic filter (Kemo, USA) set to 500 Hz, resampled by an A/D converter at 1 kHz

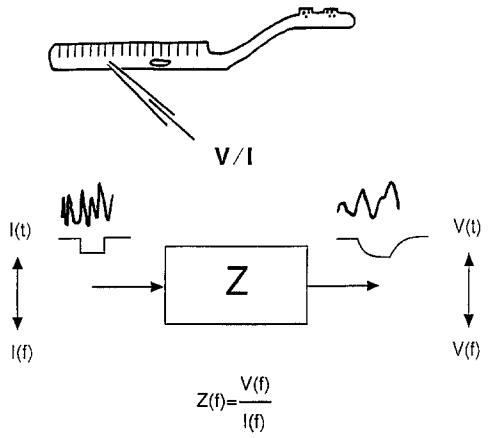

Fig. 1. A scheme of the method. The cell is impaled by a single glass microelectrode (*upper part*). Current, the amplitude of which is pseudo-sharing (switched) technique (*V/I*). The cell's impedance is then calculated from the cross-power spectrum between the current and the voltage $G_{IV}(f)$ and the input (current) power spectrum, $G_I(f)$ as $Z(f) = [G_{IV}(f)]/[G_I(f)$. For more details see text.

and stored in a μPDP 11/23+ computer. The recording electrodes have resistances of 90–150 MΩ and time constants of the order of 3 μs, when the dual capacitance compensation of the NPI amplifier is optimally tuned and when they are embedded deep in tissue (100–200 μm). This means that their high cut-off frequency is about 53 kHz, which makes it possible to use switching frequencies of up to about 16 kHz according to the criteria presented above.

The photoreceptor impedance in the frequency domain is calculated as a frequency response function [1, 11], where the injected (and also recorded) current is the input and recorded photoreceptor voltage is the output. Accordingly, the impedance is obtained by calculating (via FFT and appropriate segment and ensemble averaging) the cross-power spectrum (between current and voltage) and dividing it by the current power spectrum (Fig. 1). This method is superior to the calculation of the transfer function by a mere ratio of the output and input power spectrum, because with it it is possible to eliminate the uncorrelated noise in the output. This method of impedance calculation also makes it possible to get an estimate of the signal-to-noise ratio and linearity of the photoreceptor impedance. This is accomplished with the help of the coherence function

$$\frac{G_{IV}(f) \times G^*_{IV}(f)}{G_{II}(f) \times G_{VV}(f)} \tag{3}$$

where G_{IV} is the cross-power spectrum between current and voltage, G^*_{IV} is its complex conjugate, and G_{II} and G_{VV} stand for the power spectra of current and voltage, respectively.

The coherence function is generally a measure of both the signal-to-noise ratio of the recordings and the linearity of the system studied. To achieve a further reduction of noise (in addition to low-pass filtering) we generated the records, from which the impedance was calculated, with time-domain averaging [5, 13]. The same pseudorandom stimulus was presented ten times; the recordings were averaged and subsequently used for analysis. This procedure (plus the filtering) reduced noise to a level, where (in the linear case) the coherence function is near unity over most of the frequency range observed and the non-linearities – when present – are clearly distinguishable as a drop in the value of the coherence function.

Examples of results

The method was tested by applying it to photoreceptors of two different species, namely those of the blowfly and the cockroach, which have fast and slow-type phototrans-

duction respectively. The insect photoreceptors are very suitable for this kind of experimentation, as their membrane impedance is strongly voltage-dependent because of voltage-activated potassium channels [6, 8, 17]. Figure 2A shows the impedance of the cockroach photoreceptor with two current-induced polarization levels. The low-pass characteristics of the cell membrane are clearly seen. When the photoreceptor is hyperpolarised from the resting potential by 20 mV the high-frequency roll-off has a slope of 20 dB/decade, indicating that the cell's soma behaves very much like an ideal first-order RC filter. This fact is evidently a consequence of two phenomena. First, the membrane's voltage-activated (potassium) channels are not activated when the membrane potential is modulated around a sufficiently hyperpolarised level such as −20 mV from rest. Secondly, the photoreceptor axon evidently draws only a negligible amount of current and does not contribute to the measured impedance function. Thus the soma of the cockroach photoreceptor behaves electrically more or less like a spherical cell with a single time constant.

With the change of the membrane polarisation level, the upper 3-dB cut-off frequency is shifted. The higher the cell's mean membrane voltage, the higher is the cut-off frequency. The explanation for this is that the voltage-dependent potassium conductance in the photoreceptor is progressively activated thus shunting the membrane and lowering the resistance, and therby changing the impedance function. As the cockroach photoreceptor's geometry does not need to be taken into account, the impedance function can be fitted simply with

$$G_{ph} = j\omega C + G_m + \frac{G_V}{1 + j\omega\tau} \quad (4)$$

where G_{ph} is the total (complex) admittance of the photoreceptor, G_m is the voltage-independent conductance, G_v is the voltage-activated conductance; $j = \sqrt{-1}$, ω is the angular frequency, C is the total capacitance, and τ stands for the relaxation time constant of the voltage-activated conductance. The fit to the data presented in Fig. 2 gives a relaxation time constant of 58 ms for the voltage-activated conductance when the photoreceptor is depolarised 5 mV from resting potential. In the same time the voltage-dependent conductance is increased to a value that is about equal to the voltage-independent conductance.

Corresponding impedance functions of a blowfly photoreceptor are shown in Fig. 2B. Basically, the same phenomenon is seen as in the case of the cockroach, but the high-frequency roll-off is not exactly an asymptote to a slope of 20 dB/decade even with the hyperpolarised membrane. This fact may be result of the different functional geometry of the fly photoreceptor cells, which allows a sizable part of the current to pass into the axon. With small depolarisation a shift in the 3-dB corner frequency takes place, very much as in the cockroach photoreceptors. The voltage-dependent conductance is more strongly activated though, and this results in the near-zero coherence in the low-frequency end, below 10 Hz (Fig. 2C). Equation (2) cannot be easily fitted to the

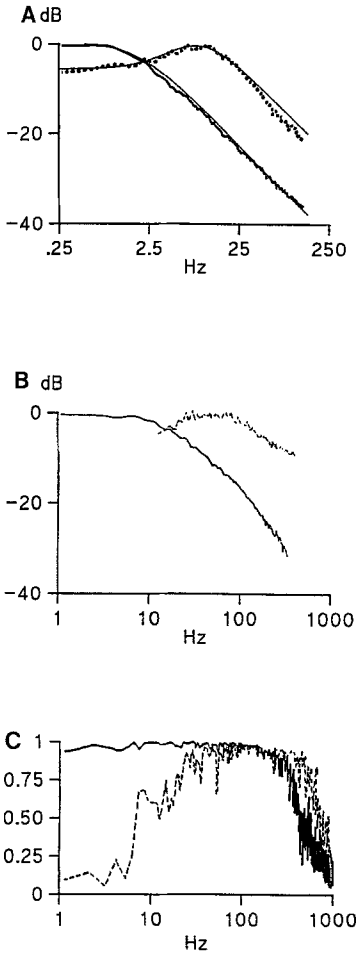

Fig. 2. A The relative value of the impedance of a cockroach photoreceptor with two levels of current induced mean membrane polarisation; *thick continuous line:* 20 mV below resting potential (which was about −65 mV); *dotted line:* 5 mV above resting potential. The peak-to-peak amplitude of the current was 0.5 nA. The *thin continuous lines* represent the best fit to Eq. (4) as explained in the text. The coherence function had values near unity over the frequency range studied. **B** The impedance of a blowfly photoreceptor. **C** The corresponding coherence function at two levels of mean membrane polarisation; *continuous line:* −10 mV below resting potential; *dashed line:* 5 mV above resting potential. Note the drastic decrease in the coherence below about 10 Hz indicating a drop in signal-to-noise ratio (and possibly a drop in linearity as well). This is a result of the activation of the powerful voltage-activated potassium conductance [17]

blowfly results. This is evident because when the photoreceptor is hyperpolarised beyond the voltage level where the potassium conductance is activated, the simple RC model is not valid. The more complex modelling of the blowfly photoreceptor impedance is beyond the scope of this paper.

The fitting of the results obtained with the present technique to existing models of graded signal transmission (e.g. [15]) is fairly straightforward in the case of the cockroach photoreceptor. If the contribution of the voltage-dependent conductances does not change significantly because of the current injection, a linear model gives a reasonably accurate estimate of the signals in each part of the cell. This holds, of course, only if we limit the study to more or less steady-state conditions.

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