

SOLUTION OF THE CROSS-TALK PROBLEM IN CELL IMPEDANCE ANALYSIS OF CARDIAC MYOCYTES

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Abstract

Membrane capacitance is a fundamental electrical characteristic of the surface membrane of living cells. The membrane capacitance is quantitatively related to the surface area, thickness and dielectric properties of the cell membrane and thus provides valuable information about the state of the cell. A generally accepted method for measuring membrane capacitance is based on stimulation of the cell with rectangular voltage pulses and approximation of the recorded membrane current by a mono-exponential decay function. We found that in cardiac muscle cells this method provides high variability of the measured capacitance and large cross-correlation among parameters of the measured circuit. In this study we focused on the elimination of cross-correlation error between the membrane capacitance, the membrane resistance, and the access resistance of the recording set-up. We showed how the use of the standard approximation model affects the level of crosstalk between estimates of these parameters. We proposed a modified model and tested its applicability on simulated and experimental data. The results revealed that the crosstalk error can be reduced by three orders of magnitude, well below the natural variability of membrane capacitance arising from biological reasons in cardiac myocytes.

Keywords

Membrane capacitance, cardiac myocyte, impedance measurement, crosstalk

Introduction

The plasma membrane of living cells can be represented by the equivalent circuit shown in Fig. 1. Membrane capacitance C_M and membrane resistance R_M constitute the cell impedance, and the access resistance R_A characterizes the measuring interface. Impedance parameters are of interest for a variety of reasons, such as estimating complex cell morphology, secretion of hormones, or cell growth. The value of the access resistance defines the quality and fidelity of the cell impedance measurement. To study these parameters, a number of approaches based on the patch-clamp technique has been developed and successfully applied to single isolated cells. It was shown [1] that in small hormone secreting cells, membrane capacitance of about 10 pF can be estimated with a resolution of several femtofarads. This made possible to observe fusion of miniature secretory vesicles with the cell membrane. In large excitable cells, such as mammalian cardiac myocytes, recordings of subtle changes of membrane capacitance are also of interest but have not been successful yet due to the intrinsically higher current noise of large cells and due

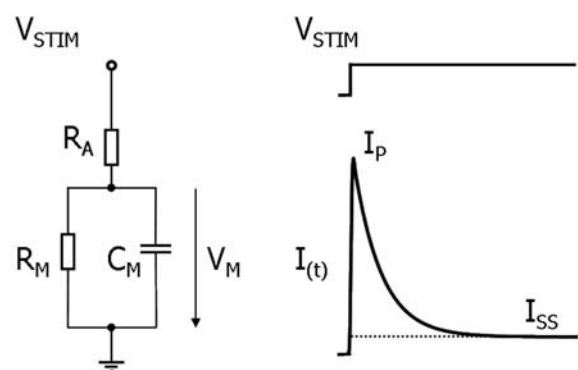


Fig. 1: The equivalent scheme of the cell recording configuration (left), and the voltage and current used for analysis (right). V_{STIM} – voltage stimulus, R_A – access resistance, V_M – voltage on the cell membrane, R_M – membrane resistance, C_M – membrane capacitance, $I(t)$ – membrane current, I_{SS} – steady state current, I_P – peak current.

to a higher variability of impedance parameters. The most robust method to measure the cell impedance is the square-wave method [2]. The bipolar voltage pulse applied to the cell elicits a transient current charging the cell membrane. The current response is approximated by a decaying exponential function to obtain the time constant, the peak current and the steady current. These three parameters are sufficient to calculate the three impedance parameters R_M , C_M , and R_A of the basic equivalent circuit. This method appears in a variety of commercial data acquisition software. However, it also has some limitations. We found that in cardiac myocytes the parameters estimated by the square wave method exhibited cross-correlations.

In this study we used computer simulations of the impedance measurement to evaluate the crosstalk in membrane capacitance and series resistance measurements. We show how to reduce the cross-correlations effectively and how the new method performs in real experiments on cardiac muscle cells.

Method

Impedance measurements were performed using Axopatch-200B patch-clamp amplifier (Molecular Devices, USA). The output current was low-pass filtered by the Axopatch 200B built-in 10 kHz 4-pole Bessel filter, and digitized at 100 kHz by a 16-bit data acquisition system Digidata 1320A (Molecular Devices, USA). Square wave voltage stimuli were generated by the Digidata 1320A 16-bit D/A converter. The square wave voltage stimulus had peak-to-peak amplitude of 20 mV (± 10 mV around the cell holding potential). Its period T was set according to the time constant τ of the cell to fulfil the condition $T = 24 \times \tau$ [2]. Current responses were recorded with pClamp 9 software (Molecular Devices, USA) and stored in Axon Binary Files format for off-line analysis in FluctuationAnalyzer [3] written in Matlab (Mathworks, USA).

Cardiac myocytes were enzymatically isolated from left ventricles of male Wistar rats (250 g) as described in [4]. All experiments were performed at room temperature (24 - 25°C). The standard bath solution contained (in mmol/l): 135 NaCl, 5.4 CsCl, 1 CaCl₂, 5 MgCl₂, 0.33 NaH₂PO₄, 10 HEPES (pH 7.3) and 10 μ M TTX to block sodium currents. The composition of the pipette solution was (in mmol/l): 135 CsMetSO₃, 10 CsCl, 1 EGTA, 3 MgSO₄, 3 Na₂ATP, 0.05 cAMP, 10 HEPES (pH 7.3).

Recording pipettes were pulled from borosilicate glass capillaries using Sutter Micropipette puller (Sutter Instruments, USA). The pipette resistances were typically ~ 3 M Ω . Pipettes were coated with Sylgard 184 (Dow Corning, USA) to decrease pipette capacitance and fluctuations of access capacitance. All

measurements were performed after achieving a sufficiently high resistance of the seal between the recording pipette and the cell membrane (above 10 G Ω). The formation of the seal was monitored and controlled by the procedure used in Mason et al. [5]. Simulations of experiments were performed in Matlab.

Analysis of the equivalent circuit

The current response of a cell, elicited by a bipolar square wave voltage stimulus, is dominated by large decaying currents relaxing to a steady-state component (Fig. 1). The standard impedance analysis method [6] uses approximation of each current transient by a single-exponential function (eq. 1):

$$I(t) = (I_P - I_{SS}) \cdot e^{\left(\frac{-t}{\tau}\right)} + I_{SS}, \quad (1)$$

where t is time, $\tau = C_M R_M R_A / (R_M + R_A)$ is the current decay time constant, $I(t)$ is the time dependent membrane current, I_{SS} is the steady state membrane current amplitude, and I_P is the peak current amplitude. The circuit parameters are directly related to the I_{SS} , I_P and τ through eqs. 2 - 4:

$$R_A = \frac{V_{STIM}}{I_P} \quad (2)$$

$$R_M = \frac{V_{STIM}}{I_{SS}} - R_A \quad (3)$$

$$C_M = \tau \left(\frac{1}{R_A} + \frac{1}{R_M} \right) \quad (4)$$

It should be noted that the fast rising current response component (not well resolved in Fig. 1) is deformed by the low pass Bessel filter included in the signal path of the patch clamp amplifier. The filter causes a small time delay and distortion from the exponential time course in the measured current response. Therefore, to estimate the peak current amplitude I_P correctly, a proper adjustment should be applied [2].

Moreover, approximation of the decaying current responses by eq. 1 is not accurate as it does not consider the time dependence of C_M impedance and its impact on the current flowing through the resistive path. According the standard model (eq. 1), the current through the capacitive branch of the circuit is estimated by a simple subtraction of I_{SS} from $I(t)$. However, at the time of the voltage change ($t=0$), the membrane capacitance poses no resistance to the current flow, which is then limited only by R_A , thus $V_M = 0$. At later

times, V_M rises exponentially to the point when the capacitor is fully charged and the current flows only through the resistive path (conducting branch) and thus it is limited by $(R_A + R_M)$ only. Since R_M and C_M are in parallel and the voltage V_M increases exponentially, the current through the resistive branch also increases exponentially (Fig. 2). Not considering this fact introduces a systematic error in estimate of τ that increases with I_{SS} amplitude (decrease in R_M) and translates to apparent cross-correlation among parameters.

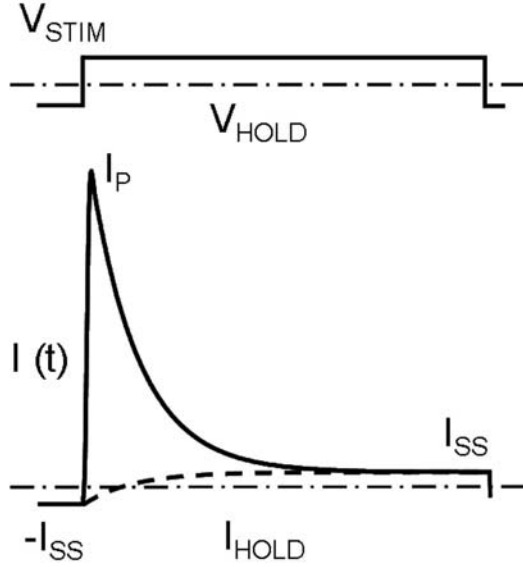


Fig. 2: The principle of the cell current response correction. V_{STIM} – voltage stimulus, V_{HOLD} – holding voltage, $I(t)$ – the whole circuit current response, I_{HOLD} – holding current. Dashed line – the correct exponentially increasing current through the resistive path of the equivalent circuit. Dotted line – the steady state current. The correct membrane capacitance charging current corresponds to the difference between currents shown as solid and dashed lines.

We solved this problem by describing the time course of the current response to bipolar pulse exactly:

$$I(t) = I_P \cdot e^{-\frac{t}{\tau}} + 2I_{SS} \left(\frac{1}{2} - e^{-\frac{t}{\tau}} \right), \quad (5)$$

The eq. 5 allows us to correctly estimate the capacitance charging current and enables a better reconstruction of the transient current around its peak, which should result in better estimation and lower crosstalk of impedance parameters.

Impedance analysis – a simulation

To estimate the level of parameter crosstalk between C_M and R_A we simulated an experiment using eq. 6, according to Sigworth et al. [7].

$$I(t) = V_{STIM} \left[\frac{1}{R_A} \cdot e^{-\frac{t}{\tau}} + \frac{1}{R_M + R_A} \left(\frac{1}{2} - e^{-\frac{t}{\tau}} \right) \right] \quad (6)$$

The cell equivalent circuit (Fig. 1) was modelled as a typical cardiac myocyte ($C_M = 100$ pF, $R_M = 500$ M Ω) and $R_A = 5$ M Ω . The stimulus was a rectangular voltage waveform of 20 mV amplitude and 20 ms period applied for 30 periods. The access resistance was increased stepwise by 1 M Ω for 10 stimulus periods, while other circuit parameters were kept constant. The crosstalk was characterized by the amplitude of a false change in the C_M estimate (Fig. 3).

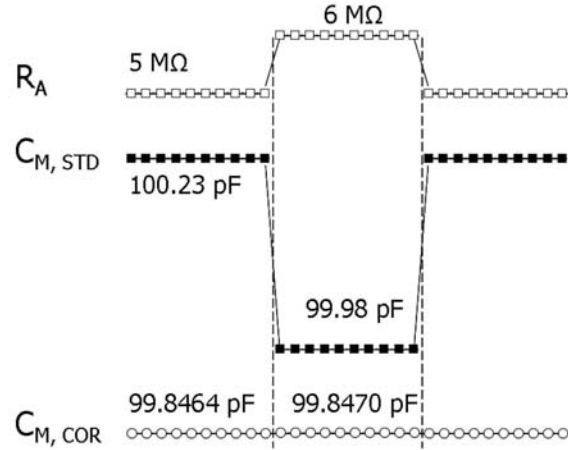


Fig. 3: Comparison of the time courses of membrane capacitance estimates in a simulated cell using the standard and corrected models. The stepwise increase of R_A by 20% infiltrates into C_M estimates in the standard model ($C_{M, STD}$) but not into the corrected model ($C_{M, COR}$). Each point represents the parameter estimate obtained from a single measurement period.

To evaluate the change of C_M with maximal precision, no noise was added to simulations. With the standard model (eq. 1), the change in R_A from 5 M Ω to 6 M Ω resulted in C_M estimate changed by 0.25 pF. With the corrected model, the change of C_M estimate was only 0.6 fF, that is, the crosstalk was less by three orders of magnitude.

Impedance analysis – an experiment

In real experiments, the access resistance may vary in time. In patch-clamped cardiac myocytes it may assume rather large stepwise changes or vary with dynamics that might be confounded with fluctuations of membrane capacitance. It is thus of immanent interest to minimize the crosstalk between these two parameters. To see the difference in results obtained with the standard and the corrected model, we analysed the same record of membrane current responses to voltage stimulation using both models. The real parameters recording obtained on cardiac ventricular myocyte is shown in Fig. 4. Panel A shows the parameters C_M and R_A estimated by the standard fitting method. Fluctuations of these parameters in time are strongly correlated over a broad bandwidth (panel A bottom). This indicates the presence of crosstalk in parameter estimates. Panel B shows the same data estimated by the corrected model. We see almost no correlation between the membrane capacitance and the access resistance, which indicates good separation of these quantities. By virtue of suppressing the R_A/C_M crosstalk it may be possible to interpret the subtle changes in membrane capacitance seen at the end of the presented record as changes due to biological reasons.

Discussion

This study identified a new model dependent source of cross-correlation among cell impedance parameters. In the standard model (eq. 1), the description did not consider the drop of voltage on the cell membrane during the flow of the charging current, which produced an error in estimation of the capacitive charging current. This error is more prominent in large cells like cardiac myocytes due to their lower membrane resistance. With our correction for the time-dependent current increase through the resistive path, we reduced the effect and suppressed the artificial fluctuation from the membrane capacitance due to this type of cross-talk. In effect, this approach allowed disclosing fluctuations of biological origin and their correct interpretation.

Generally, however, the membrane resistance of excitable cells like cardiac myocytes is nonlinear, voltage dependent, and time dependent. Therefore, especially at large and slow stimulating voltage waveforms, the variation in membrane resistance may become substantial and start to infiltrate into the voltage dependency of membrane capacitance. In such cases, specific measures should be applied to suppress the interfering ionic conductance or to set the holding potential or the amplitude of the voltage waveform properly, if possible.

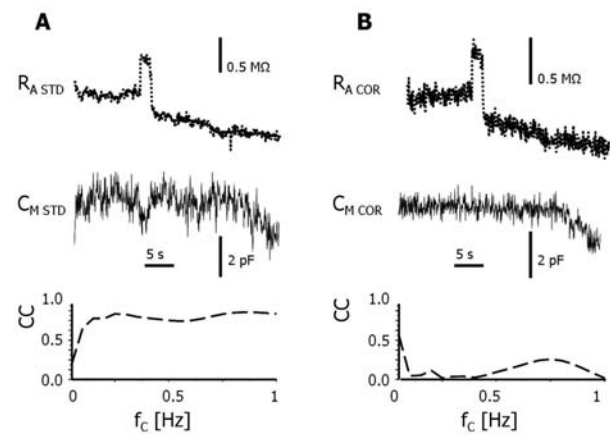


Fig. 4: Comparison of the time courses of access resistance and membrane capacitance estimates from the same current trace recorded in an isolated patch-clamped cardiac muscle cell, obtained using the standard and the corrected model. The record of R_A (top) shows a sudden change by more than 10%, which transpired to C_M estimate (middle) in the standard model (A) but not in the corrected model (B). The bottom panels show the cross-correlation coefficient (CC) between R_A and C_M traces estimated at various bandwidths (f_c – the corner frequency of the high-pass filter).

Conclusions

We have tested an improved model of membrane impedance with a correction accounting for development of the membrane potential accompanying charging of the membrane capacitance. In simulated noise-free data, this model reduced the error due to crosstalk between R_A and C_M estimates by three orders of magnitude. This result was confirmed in real records of cell impedance parameters obtained in isolated cardiac muscle cells. The low crosstalk error is of advantage in recording the intrinsic membrane impedance variability in cardiac muscle cells arising from their natural activity.

Acknowledgement

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