Video Article

Recapitulation of an Ion Channel IV Curve Using Frequency Components

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URL: http://www.jove.com/video/2361
DOI: doi:10.3791/2361

Keywords: Biophysics, Issue 48, Ion channel, Kir2.1, impedance spectroscopy, frequency response, voltage clamp, electrophysiology

Date Published: 2/8/2011

Citation: Rigby, J.R., Poelzing, S. Recapitulation of an Ion Channel IV Curve Using Frequency Components. *J. Vis. Exp.* (48), e2361, doi:10.3791/2361 (2011).

Abstract

INTRODUCTION: Presently, there are no established methods to measure multiple ion channel types simultaneously and decompose the measured current into portions attributable to each channel type. This study demonstrates how impedance spectroscopy may be used to identify specific frequencies that highly correlate with the steady state current amplitude measured during voltage clamp experiments. The method involves inserting a noise function containing specific frequencies into the voltage step protocol. In the work presented, a model cell is used to demonstrate that no high correlations are introduced by the voltage clamp circuitry, and also that the noise function itself does not introduce any high correlations when no ion channels are present. This validation is necessary before the technique can be applied to preparations containing ion channels. The purpose of the protocol presented is to demonstrate how to characterize the frequency response of a single ion channel type to a noise function. Once specific frequencies have been identified in an individual channel type, they can be used to reproduce the steady state current voltage (IV) curve. Frequencies that highly correlate with one channel type and minimally correlate with other channel types may then be used to estimate the current contribution of multiple channel types measured simultaneously.

METHODS: Voltage clamp measurements were performed on a model cell using a standard voltage step protocol (-150 to +50 mV, 5mV steps). Noise functions containing equal magnitudes of 1-15 kHz frequencies (zero to peak amplitudes: 50 or 100mV) were inserted into each voltage step. The real component of the Fast Fourier transform (FFT) of the output signal was calculated with and without noise for each step potential. The magnitude of each frequency as a function of voltage step was correlated with the current amplitude at the corresponding voltages.

RESULTS AND CONCLUSIONS: In the absence of noise (control), magnitudes of all frequencies except the DC component correlated poorly ($|R|<0.5$) with the IV curve, whereas the DC component had a correlation coefficient greater than 0.999 in all measurements. The quality of correlation between individual frequencies and the IV curve did not change when a noise function was added to the voltage step protocol. Likewise, increasing the amplitude of the noise function also did not increase the correlation. Control measurements demonstrate that the voltage clamp circuitry by itself does not cause any frequencies above 0 Hz to highly correlate with the steady-state IV curve. Likewise, measurements in the presence of the noise function demonstrate that the noise function does not cause any frequencies above 0 Hz to correlate with the steady-state IV curve when no ion channels are present. Based on this verification, the method can now be applied to preparations containing a single ion channel type with the intent of identifying frequencies whose amplitudes correlate specifically with that channel type.

Video Link

The video component of this article can be found at http://www.jove.com/video/2361/

Protocol

1. Prepare Noise Function and Input Signal

1. Create a noise function containing the desired frequency components. This can be done by describing the desired frequency components in the frequency domain and then calculating the inverse fast Fourier transform. In this study, 1 - 15 kHz was used. All Fourier transforms and inverse Fourier transforms described in this study were calculated using Matlab's FFT and IFFT functions.
2. Scale the amplitude of the noise function appropriately. In this study the noise function was scaled such that the zero to peak amplitude of the noise function was 50 or 100 mV.
3. Create a stimulus file using methods appropriate for the acquisition software being used. For Clampex 8, first create a text file with the appropriate header. Below the header, insert the time increments for a single sweep in the first column. The time increments should have the same temporal spacing as the sampling interval used in the measurements. For each sweep in the voltage step protocol insert the exact voltages desired at each time step. This should include the noise function.

2. Perform Voltage Clamp Measurements

1. Create a measurement protocol within the acquisition software that is compatible with the stimulus file generated previously. In Clampex, there is a menu that allows the user to associate a stimulus file with the current protocol.

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2. Attach a model (or biological) cell to the measurement equipment.
3. Perform the experiment as scheduled. For control purposes, make sure to include periodic measurements that do not include any noise functions.

3. Post Experiment Analysis

1. Calculate the IV curve for an individual recording. If the recording is at steady state when the noise function is applied, the IV curve can be created using a steady state portion of the recording temporally outside the range of the noise function. If the recording was not at steady state, the noise function may interfere with calculation of the IV curve, so a second recording should be made without the noise function present.
2. For each voltage step in a recording calculate the fast Fourier transform of the portion of the recording where the noise function was inserted. Combine the Fourier transform for each voltage step into a m x n matrix, where m is the number of frequencies in the FFT, and n is the number of voltage steps. In this configuration, each row of the matrix represents the amplitude of a single frequency at all voltage steps in the experiment.
3. For each frequency (ie each row in the above matrix) correlate the row with the IV curve generated in 3.1 and record the correlation coefficient.
4. Plot the correlation coefficient vs frequency to visualize frequencies which highly correlate with the IV curve. Since the DC component is contained within the first frequency of the FFT, the correlation coefficient for this frequency should always be > 0.99.

4. Representative Results:

Representative voltage clamp measurements are shown for a model cell without (Figure 1A) and with (Figure 1B) a noise function inserted into the voltage step protocol. The IV curve was also calculated for the model cell (Figure 1C). For each sweep, in the recordings from Figure 1, the FFT was calculated over the time frame where the noise function was inserted (see red box in Figures 1A, 1B). Figures 2A and 2B show the FFTs calculated for the recordings shown in Figure 1A and 1B, respectively. Upon visual inspection, the DC component (highlighted in red) appears to mimic the shape of the IV curve. Without the noise function, all frequencies above DC appear to have amplitudes near zero (Figure 2A). When the noise function is inserted, frequencies between 1 and 15 kHz have visually noticeable amplitudes (Figure 2B). Figure 3 shows the result of correlating individual frequency amplitudes over the range of voltage steps against the IV curve. Figure 3A-C shows the correlation coefficients when the experiment was done under control conditions (no noise function) and with noise amplitudes of 50 and 100 mV, respectively. Notice in all cases, the DC component appears to correlate nearly perfectly with the IV curve. Indeed, for all recordings, the correlation coefficient for this frequency was greater than 0.99 ($R = 0.9996 \pm 1E\text{-}5$, mean ± standard deviation). When we look at Figure 3A (control conditions), there are no frequencies besides the DC component whose amplitude significantly correlates with the IV curve. Specifically, none of these frequencies have correlation coefficients greater than 0.5. Upon insertion of the lowest amplitude noise function (50 mV), these same frequencies still had correlation coefficients less than 0.5. The correlation coefficients for these frequencies also did not become greater than 0.5 when the noise amplitude was increased to 100 mV.

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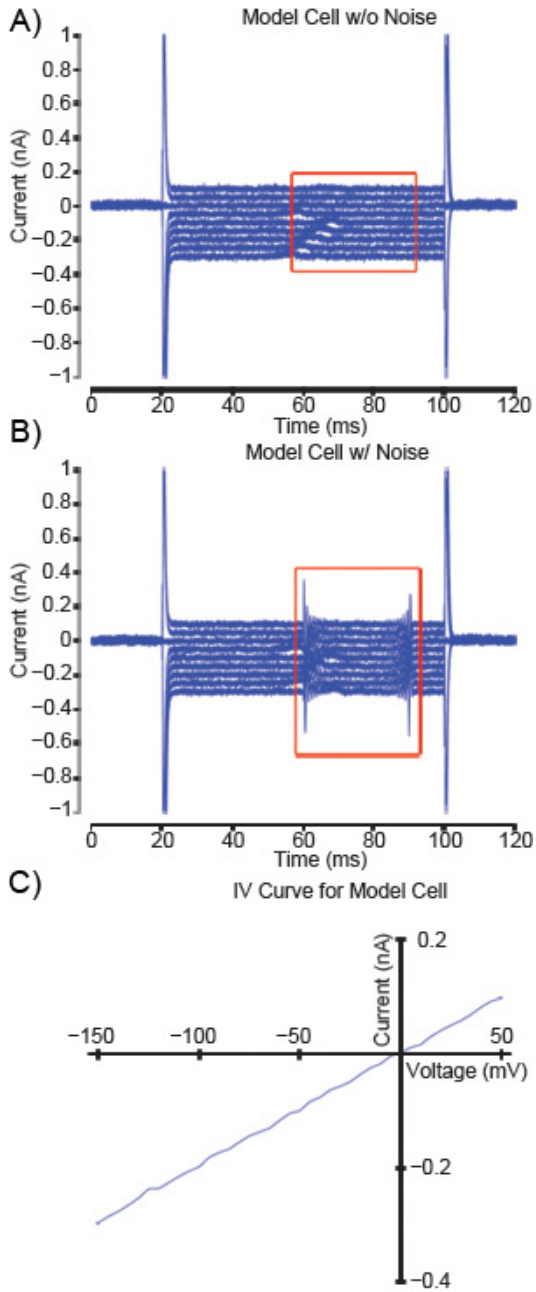

Figure 1. Model Cell Recordings: Voltage clamp recordings are shown for a model cell without (1A) and with (1B) a noise function included in the voltage step protocol. For the first and last 20 ms of each sweep, the potential was kept at the holding potential (0 mV). Each voltage step was 80 ms long, and the noise function was inserted 40 ms after the beginning of the step. The noise function had a duration of 30 ms and contained frequencies between 1 and 15 kHz. Voltage was stepped fro, -150 to +50 mV in 5 mV increments. An IV curve for the model cell is also shown (1C). To make the recordings easier to read, only every fifth sweep was included in 1A and 1B, but all sweeps were included in 1C.

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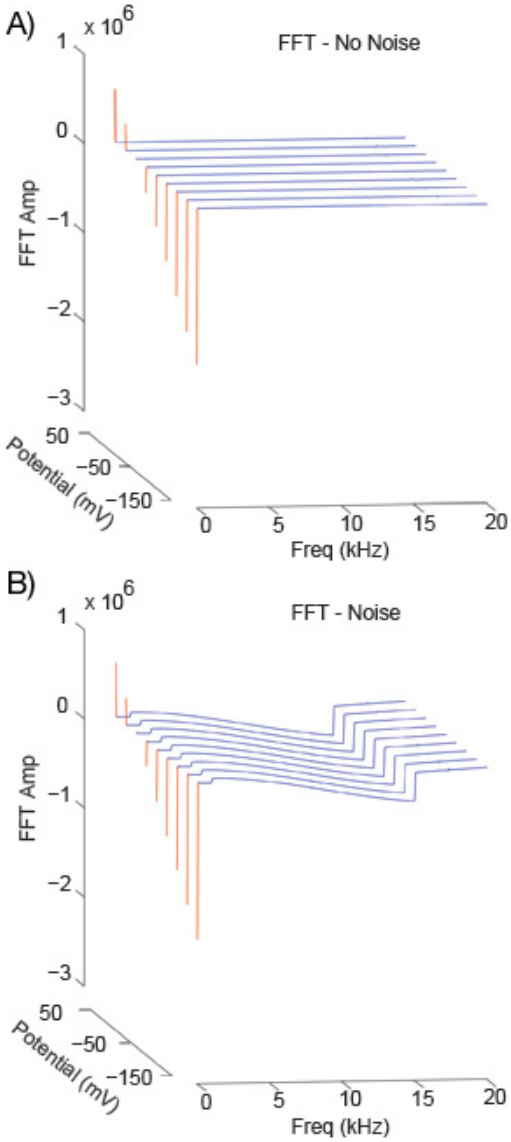

Figure 2. FFT of Recordings: The FFT was calculated for the 30 ms portion of each sweep where the noise function is to be inserted (the area bounded by the red box in Figures 1A, 1B). Figures 2A and 2B show the FFTs calculated without and with the noise function, respectively. Once again, for clarity, only the FFT from every fifth sweep is included in the figure, but all sweeps were used in future calculations.

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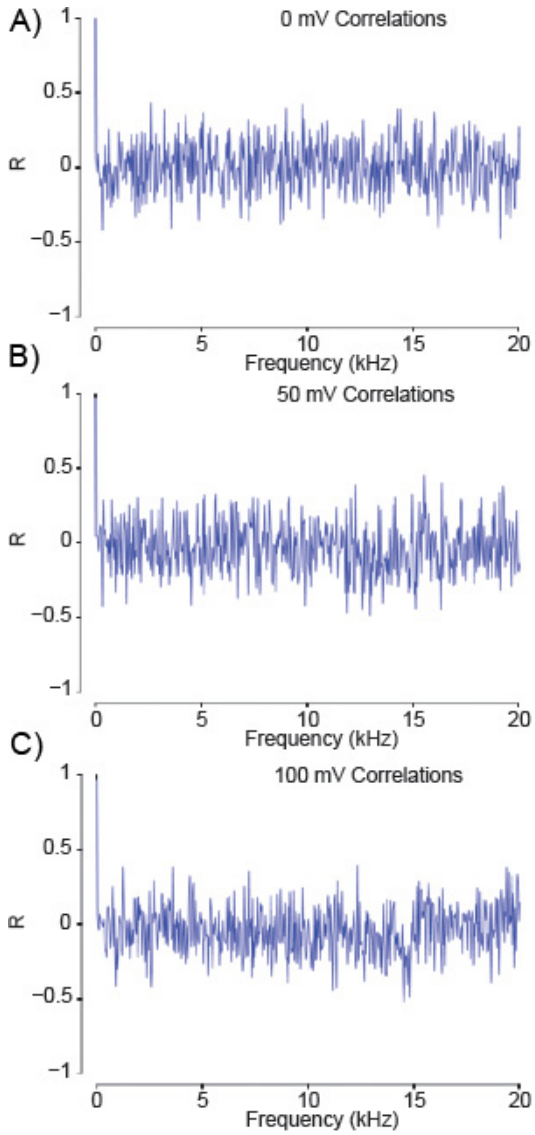

Figure 3. IV Frequency Correlations: The results of correlations between the IV curve of a recording and the amplitude of individual frequencies over the range of voltage steps is shown. Figures 1A - 1C show the correlation coefficient for frequencies from 0 to 20 kHz under control conditions, and in the presence of 50 or 100 mV noise functions, respectively.

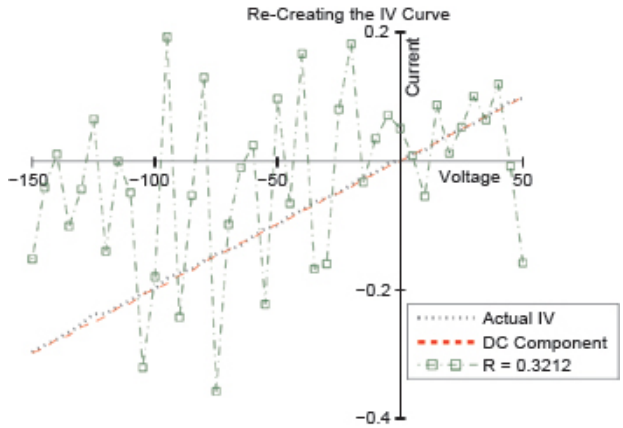

Figure 4. Re-Creation of the IV Curve: The IV curve for the model cell (same as Figure 1C) and the magnitude of two frequencies were overlaid. The first frequency was the DC component (R = 0.995), and the second was a randomly chosen frequency with low correlation (R = 0.3212). The frequency amplitudes were scaled to approximately the same amplitude as the IV curve.

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Discussion

There are technical obstacles that presently prevent researchers from measuring multiple ion channel types simultaneously with the intent of later determining how much current should be attributed to each channel type. Because of this limitation, ion channels are usually studied individually using techniques such as voltage, current, and action potential clamp.[1] To study individual channel types, heterologous expression systems are often used.[2] When working with cells isolated from tissue, such as cardiomyocytes, other means must be used to block various ion channels. For example, sodium channels can be inactivated by a slow depolarizing voltage ramp,[3] inward rectifying potassium channels can be blocked with extracellular BaCl2,[4] and calcium channels can be blocked using verapamil.[5]

One method used that partially overcomes this limitation is to measure current flux through two channels types simultaneously, then repeat the measurement after selectively blocking one channel type with an appropriate agent. Subtraction of the two measurements can then be used to estimate the amount of current attributable to the channel type that was blocked.[6] However, there are two major limitations to this technique. First, chemical agents have not been identified that can selectively block each ion channel, and some widely used drugs have non-specific interactions with other channel types.[5,7] Second, it cannot be determined from this technique whether one channel is modulated by another channel. For example, heterogeneous expression of NaV 1.5 and Kir 2.1 has been shown in guinea pig ventricles, and it has been suggested that a synergistic relationship exists between the two channels, such that higher Kir 2.1 expression in the right ventricle depresses conduction velocity.[8] Presently, this cannot be verified.

In this study, we suggest that impedance spectroscopy may be a useful tool for studying multiple ion channel types measured simultaneously. Although the method presented has never been used to discern currents from two channel types measured simultaneously, impedance spectroscopy has been used to study a number of other aspects of ion channel function. Goodman and Art showed using turtle auditory hair cells that current clamp protocols can be modified to tune a cell to different frequencies, and the oscillations in the transmembrane potential is due to an interplay between an inward rectifying K^+ channel and a Ca^{2+} channel.[9] Han and Frazier demonstrated that impedance can be measured in a single cell over a wide range of frequencies (100 hZ to 5 MhZ), and the increase in impedance observed when K^+ or Ca^{2+} channels were blocked could be a simple means to detect channel block in high throughput drug screens.[10] Hayashi and Fishman have used complex conductance to study kinetic properties of an inward rectifying K^+ channel.[11] Other groups have inserted a single frequency into the voltage clamp protocol of different channel types and showed that the observed frequency response agreed with the expected response for some frequencies but not others.[12,13] Millonas and Hanck suggested the reason some frequencies did not produce the expected response is the presence of multiple rate constants in the Markov model.[12] Studies such as these, as well as others, have demonstrated that there are instances when ionic currents measured from ion channels while using impedance spectroscopy do not agree with the theoretical frequency response. This is not a concern in this study because the purpose of the method in this study is to identify frequencies that correlate with the current amplitude independent of the underlying assumptions of the membrane electrical circuit. Furtheremore, the current amplitude is calculated from portions of the recordings that do not have any noise functions inserted into them. A number of other studies also present models of numerous ion channels exhibiting numerous conducting and non-conduction states all with their own rate constants.[14,15,16] Thompson et al showed that the selectivity filter of the KcsA channel has different binding sites for Na^+, Li+, and K^+, and the energetic costs of moving from one binding site to another as an ion moves through the selectivity filter is what makes the channel preferentially conduct K^+ ions through its pore.[17] In this paper we inserted a range of frequencies (noise function) into a voltage step protocol and looked for frequencies whose amplitude highly correlate with the overall current amplitude. Since strong evidence has been presented suggesting multiple rate constants play a role in conduction of ions through different channels, introduction of the frequencies associated with these rate constants may cause certain frequencies to resonate or highly correlate with the current amplitude, which would not have otherwise. The technique demonstrated in this study is performed on a model cell, which is a parallel RC circuit that is usually used to test the voltage clamp circuitry and acquisition equipment. It is not expected that any frequencies besides DC would correlate with the current magnitude, and this is shown in our data. We also show that addition of the noise function did not cause any frequencies to highly correlate with the current amplitude. These two findings are critical because they show that the measurement equipment and noise function do not by themselves cause any frequencies to correlate with the current amplitude. When future studies make measurements using membranes containing ion channels, it is expected that, depending on the channel used, frequencies that correspond to rate constants in the selectivity filter or possibly the pore will influence the frequency response of the channel and affect which frequencies have high or low correlation with the current amplitude.

Since this method is a new technique for studying ion channels, there are a number of directions future studies could follow. First, the technique should be used to characterize the frequency response of specific isolated channels. Additional work also needs to be done to calibrate the frequency amplitudes to the current amplitudes. Once multiple channels are characterized individually, multiple channel types should be measured simultaneously. The technique could also be adapted for use in action potential clamp, current clamp, and field stimulation studies. While this is a new technique, it shows what may be a powerful way to make electrophysiological measurements that were previously not possible and provide valuable new insights into the physiological role of ion channels.

Disclosures

No conflicts of interest declared.

Acknowledgements

This work was supported by the National Institutes of Health grant number R21-HL094828-01 awarded to Dr. Poelzing.

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