Simulations of space-clamp errors in estimating parameters of voltage-gated conductances localized at different electrotonic distances

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Abstract

The ability to correct parameters of voltage-gated conductances measured under poor spatial control by a point voltage clamp could rescue much flawed experimental data, but requires a detailed understanding of errors caused by poor space clamp. We evaluated errors in simulated voltage-clamp experiments on a soma with a single (0.5 λ) cylindrical process having a patch of voltage-dependent Hodgkin–Huxley channels placed at various distances from the soma. Kinetic and steady-state parameters were obtained by fitting the current curves. Most parameter errors increased as the patch was located more distally on the neurite. © 2002 Published by Elsevier Science B.V.

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1. Introduction

Investigators using whole-cell patch or other point voltage clamp techniques risk significant error when measuring active ionic current properties in poorly space-clamped cells. Quantitative parameters derived from clamp currents may be in considerable error, even when there are no obvious signs of poor spatial control (e.g. bumps and notches in clamp records). How to identify and assess the seriousness of those errors is not always clear. Further, there is a need to develop means for correcting errors in real cells, such as neurons with extended processes, from which it is difficult to eliminate space-clamp errors by experimental intervention. In previous studies, we used simulated voltage-clamp experiments to evaluate errors in the quantitative parameters

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of voltage-dependent conductances caused by poor space clamp when channels are distributed uniformly along a cylindrical process [1,2]. In this study, we examine how the parameter errors depend on the distribution of the ion channels. In particular, how does the space-clamp error for uniformly distributed channels compare to the error for the same number of channels localized in a small patch on the cylindrical process?

2. Methods

Simulations of standard voltage-clamp experiments were run on an “electrotonically compact” neuron with a passive soma and a single cylindrical process (0.5λ at rest). A patch of a single rapidly activating, slowly inactivating Hodgkin–Huxley channel type [4] patterned after a potassium A-channel, but having voltage-independent rate constants was placed at different points along the neurite. Distances ranged from 0.05λ to 0.47λ (covering the distal tip of the neurite) from the soma. Runs were made using the NEURON simulation software [3]. Estimates for the seven kinetic and steady-state parameters were obtained by fitting the leak-subtracted current curves with the equation:

\[
I = \tilde{g}(V - V_{rev}) \frac{(1 - \exp(-t/\tau_m))^q \exp(-t/\tau_h)}{1 + \exp((V_0 - V)/\nu)}
\]

where \(\tau_m\) and \(\tau_h\) are the activation and inactivation time constants; \(q\) is the activation exponent; \(V_{rev}\) is the reversal potential; \(V_0\), the activation mid-point voltage; \(\nu\), the activation slope factor, and \(\tilde{g}\) is the maximum conductance. The space-clamp errors were evaluated by comparing the fitted parameters with the parameter values set in the model.

3. Results

As the active patch was located more distally on the neurite, the peak clamp current recorded in the soma decreased and the initial delay in the activation of the current increased. For a patch centered at 0.25λ, the clamp currents were comparable to those for a uniform distribution of channels, although smaller and with a more pronounced delay. For example, for a 100 mV command potential and a set maximum outward conductance of 10 times leak conductance, the peak clamp current was 6.7 nA occurring 18 ms after onset of the command potential, while for uniformly distributed channels the current peak was 8.5 nA occurring at 15 ms. Good fits of the current curves were obtained for a smaller range of set maximum conductances than was possible for the uniform distribution (10× vs. 100× leak for outward current; 5× vs. 20× for inward current).

Errors for the activation exponent \((q)\), shown in Fig. 1C and D, depended strongly on the location of the active patch, since \(q\) fit the initial delay in the rise of the current. Only the patch centered at 0.05λ had smaller errors for \(q\) than those for a uniform distribution. The kinetic parameters \((q, \tau_m, \tau_h)\), which were originally voltage independent, showed pseudo-voltage dependence (Fig. 1). Inward currents, which were simulated by shifting the reversal potential from −65 to +50 mV, could not be fit
Fig. 1. Errors in kinetic parameters for inward and outward currents as functions of command potential and distance of active patch from soma.
Fig. 2. Errors in activation time constant for localized and uniformly distributed channels. Dashed lines: errors for active patches centered at 0.05λ, 0.15λ, 0.25λ, 0.35λ, and 0.45λ from the soma. Solid line: Errors for channels distributed uniformly along the neurite. A. Outward current: Set maximum conductance \( \bar{g} = 10g_{\text{leak}} \)
B. Inward current: \( \bar{g} = 5g_{\text{leak}} \).

Fig. 3. Errors in steady-state parameters for outward current. Dashed lines: Errors for localized channels (same as Fig. 2). Solid line: Uniformly distributed channels. A–B. Errors expressed as the difference between measured and set values. C–D. Errors expressed as the ratio of the measured value to set value.

for voltage ranges near reversal potential due to diphasic (initially inward and then outward) currents (Fig. 1B, D and F). Errors in the activation time constant (\( \tau_m \)) for outward currents, increased sharply when the active patch was moved from 0.05 to 0.15λ and gradually thereafter (Fig. 1A). Errors for \( \tau_m \) were smaller for localized
currents than for uniformly distributed channels (Fig. 2). Errors for the inactivation time constant \( (\tau_h) \) were relatively small (Fig. 1E and F). They were less voltage dependent and less dependent on the location of the patch than errors for the other kinetic parameters.

For most of the steady-state parameters, errors for patches located at 0.15 and 0.25\( \lambda \) bracketed the errors for uniformly distributed channels (Figs. 3 and 4). The reversal potential for the outward current was the exception, with larger error for the uniform distribution than for any of the patches (Fig. 3A). The dependence of the steady-state parameter errors on the set conductance level for localized currents was similar to that for uniformly distributed channels. Errors for maximum conductance were similar for outward and inward currents (Figs. 3D and 4D).

4. Discussion

For most parameters, space-clamp errors for patches located between 0.05\( \lambda \) and 0.25\( \lambda \) corresponded to those for uniformly distributed channels. Two exceptions, \( \tau_m \) and \( V_{\text{rev}} \) (for outward current), had larger errors for uniformly distributed channels than for any of the patches. These results suggest that a detailed knowledge of the distribution of the...
ion channels may not be necessary to assess the existence and severity of space-clamp errors. Moreover, in our previous study of space-clamp errors for a uniform channel distribution, we found that for moderate conductance densities and certain voltage ranges the errors were not extreme and were amenable to correction [1]. In those cases we were able to develop a method for correcting distortions in the quantitative parameters of ionic currents caused by poor space clamp [2]. This should provide guidance to modelers who find that simulations do not match the observed behavior of the cell without modifying the values of measured ionic current parameters.

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References