Applying novel identification protocols to Markov models of I_{Na}

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Abstract

Novel protocols for whole-cell patch clamp current recordings have the potential to uniquely identify Markov model parameters. For the fast sodium current, which operates at very small time scales, measurement noise and amplifier imperfections can affect the recorded signal in non-trivial ways. We investigated the unique identification of the kinetical parameters of the fast sodium current from imperfect whole-cell patch clamp recordings.

1. Introduction

The cardiac fast sodium current I_{Na} is the driving force behind the rapid upstroke of the cellular action potential (AP) and a critical factor in achieving a healthy conduction velocity (CV) in cardiac tissue. Drugs and mutations affecting SCN5A, the gene encoding the cardiac sodium channel's alpha sub-unit, have been linked to a wide variety of heart diseases. Markov models that describe the current in detail have been shown to produce accurate representations of channel behavior. By incorporating such models in single or multi-cellular models, the effects of changes to I_{Na} can be studied at multiple scales, allowing clinical investigators to create personalized treatment strategies. By observing the differences between models of healthy and non-healthy cells, we may learn something about the underlying disease mechanisms.

Markov model parameters can be estimated using data gathered from patch clamp experiments in the whole-cell configuration [1]. By performing these experiments in expression systems, mutated and wild-type channel currents can be compared [2]. A theoretical study by Fink and Noble showed that, unlike single channel measurements, whole-cell patch-clamp data can be used to identify parameter sets that provide a locally unique best fit, even in the presence of noise.

If a Markov model recreates an observed behavior, we can use it to model "upwards" to the cell, tissue, and organ level. This works whether or not the model structure has any basis in reality. However, if we want to look "down" and learn something about the channel from a model of its current, we require a model where similarity of parameters correlates with similarity of channel behavior. Since Markov model rate equations are not commonly derived from first principles, and parameter values are obtained through numerical optimization, we require local identifiability as a necessary (but not sufficient) condition for the parameter values to have a meaningful interpretation.

In this study, we apply the type of protocol suggested by [3] in wet lab experiments and examine the practical problems that arise with a view to parameter identifiability. First, we implement a numerical identifiability check and use it to create a short step voltage protocol that can identify our model's parameters. Next, we perform patch-clamp experiments using the new protocol and examine the various sources of error. Finally, we discuss the limitations of our work and make recommendations for further studies.

2. Methods & Results

Whole-cell I_{Na} is calculated as

$$I_{Na}(V,t) = g \times O(V,t,p) \times (V - E)$$

where t is time, V is the membrane potential and E is the cell's reversal potential for sodium. The current conductance when all channels are open is given as the constant g so that all variation of conductance in time happens through changes in the fraction of open states $O(V,t,p)$ where p is the set of parameters we aim to identify. This fraction is determined using a Markov model formulation [1–3]. For our experiments, we used the model presented in [3]. This model uses the structure of [2] but has alternative formulations for the rate equations which avoid redundant parameters. A graphical representation of the model states is given in Figure 1.

Currents used in identification are elicited by applying a sequence of voltage steps and measuring the resulting current. In patch-clamp experiments, this is done by high-frequency circuitry in the amplifier that monitors V and injects current until the desired potential is reached. The capability of a protocol to find a locally unique set of parameters can be tested using simulations [3]. In a simulated

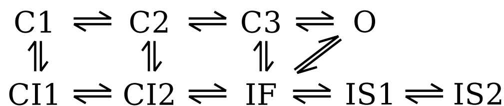

Figure 1. The Markov model structure used to simulate I_{Na}.

experiment, the voltage protocol is applied to a cell and the derivative of the model's open state(s) with respect to the parameters to identify is calculated at each point in time. A matrix of normalized parameter sensitivities is then created and tested for rank-deficiency: if each parameter has a linearly independent contribution to the open state at some point in time, the matrix should have full rank. By calculating the matrix eigenvalues and using a rank-revealing QR decomposition an identifiability score for each parameter and a cut-off score for identifiability can be defined [3]. To find the derivatives $\partial O(t)/\partial p_j$ a forward Euler simulation was run using an automatic differentiation data type. In this method, each time an operation is performed on one of the model states, both its value and the value of its derivatives to each of the parameters p_j are calculated. We implemented this in Myokit [4], our framework for computational cellular electrophysiology.

Parameter identification proceeds by minimizing a loss function over all m steps in the voltage protocol. We defined:

$$S(p) = \sum_{k=1}^{m} \sqrt{\sum_{t} \left(\frac{I_k(t,p) - I_{\mathsf{ref},k}(t)}{I_{\mathsf{max},k}} \right)^2}$$

Here, for each step k, I_k is the simulated current, $I_{\mathsf{ref},k}$ is the corresponding measured current and $I_{\mathsf{max},k}$ is the maximum absolute measured current:

$$I_{\mathsf{max},k} = I_{\mathsf{ref},k}(\arg\max_t |I_{\mathsf{ref},k}(t)|)$$

Simulations of $I_k(t,p)$ were performed using Myokit's `Simulation` class. As no derivative information is available for S a particle search optimization (PSO) [5] was employed, which has been shown to work well for this type of problem [6]. A basic parallel version of PSO was implemented in Myokit that uses multiple CPU cores to calculate solutions for the independent particles.

Patch-clamp recordings were obtained from a stable culture of Chinese hamster ovary (CHO) cells, transfected transiently with wild type human SCN5A (isoform b) and GFP to identify successfully transfected cells. Experiments were performed 24 hours after transfection in the first few hours after trypsinization. Patch-clamp experiments were performed using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) and WinWCP V4.8.6 (Strathclyde Electrophysiology Software). Signals were

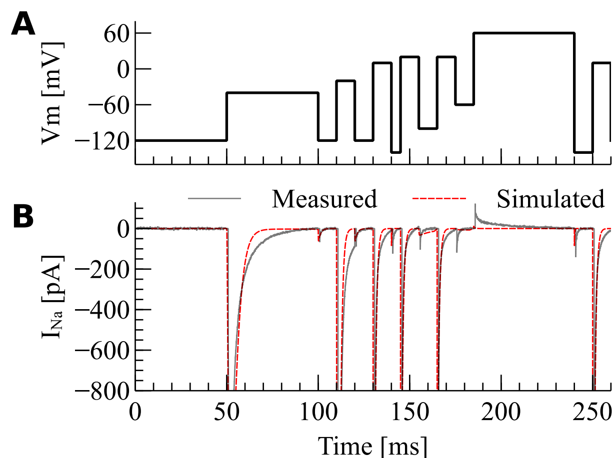

Figure 2. (A) Protocol A, designed to uniquely identify all parameters of a Markov model of I_{Na} in a single pass. (B) The response of a single CHO cell expressing wild type SCN5A. The cell's capacitance was measured as $C = 11.1\mathsf{pF}$. The simulated current based on a naive parameter fit is shown in red (dashed line).

sampled at 100kHz but filtered at 10kHz using the analog low-pass filter on the amplifier.

A voltage step protocol was defined based on the second protocol in [3] but with faster steps and voltages chosen to elicit a stronger I_{Na} current. Between runs, the cell was held at -120mV for 740ms, allowing the channels to return to a close-to-stable state while keeping total experiment time at 1s. By checking $\partial O(t)/\partial p_j$ we confirmed the protocol had the power to identify all parameters in the model. The result is shown in Figure 2 *A*.

The sodium current measured in a in a CHO cell stimulated with this protocol is shown in Figure 2 *B* and is representative of the results in the eight cells we tested. The shown data has been post-processed to remove leak current, capacitance artifacts and stochastic noise in ways that will be explained below. A simulation of the same experiment with a model fit to this data is shown in the same figure. As the figure shows, the model recreates the general features of the current but does not match it well, particularly in the decaying phase.

3. Discussion

We identified the following possible sources of error in the fit: (1) The unknown non-Markov parameters E and g. (2) Noise in the current recording. (3) Capacitative artifacts due to imperfect filtering of cell and pipette charging currents. (4) Changes in channel response due to a non-negligible charging time of the cell membrane. (5) Voltage dependent leak currents, leading to a shift in the baseline current at each step. (6) Imperfections in the protocol, due

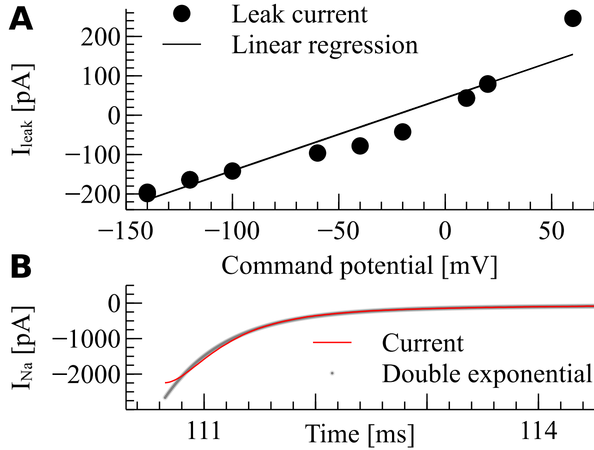

Figure 3. (A) Leak current versus command (step) potential in a small cell ($C = 11.1$pF). (B) Decay of I_{Na} is well fitted with a double exponential.

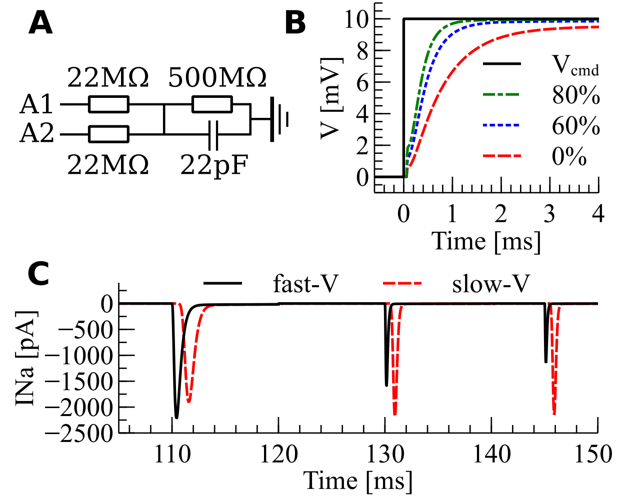

Figure 4. (A) The model cell used to inspect the effect of membrane charging time. (B) Model cell "membrane potential" directly after a jump in command potential has been applied and the effect of series resistance compensation at 60% and 80%. (C) Simulated I_{Na} response to instantaneously changing V_m and slowly changing V_m.

to a failure to adequately anticipate 1-5. In the following paragraphs, we deal with the first five points individually and examine the consequences of each for model identifiability.

While there are various methods of estimating the reversal potential E, the g of a patch-clamped cell is difficult to establish. To check the impact of estimating both parameters in conjunction with the Markov model parameters we modified our method to test not $\partial O/\partial p_j$ but $\partial I_{\mathsf{Na}}/\partial p_j$, allowing $\partial I_{\mathsf{Na}}/\partial E$ and $\partial I_{\mathsf{Na}}/\partial g$ to be calculated and included in the test.

Stochastic or non-periodic noise enters the recordings from a variety of sources. To reduce this type of noise, we made use of our protocol's short length by running it sixty times and averaging the results. This reduced noise levels to approximately $\pm$5pA. After scaling the model's g to match experimentally observed current magnitudes, we incorporated this information into the identifiability check by filtering the sensitivity matrix, removing all data from times when $I_{\mathsf{Na}}(t) < 5$pA.

A similar strategy was used to deal with incompletely compensated capacitative currents. These occur at every change in command potential and, in our data, typically obscure the next 0.5 to 1 millisecond of the current measurement. We filtered the sensitivity matrix by removing all points from the first millisecond after each step.

Leakage currents occur through the cell membrane and various parts of the set-up. Ideally, this would lead to a current linearly dependent on V. We tested this assumption by fitting the decaying phase of the current at each step with a double exponential:

$$I_{\mathsf{decay}}(t) = I_{\mathsf{leak}} + b_1 \exp(-c_1 t) + b_2 \exp(-c_2 t)$$

where t was the time since the instantaneous jump in voltage occurred. Biexponential decay of I_{Na} has been shown in several publications [7, 8] and is illustrated for our data in Figure 3 *B*. When plotted against voltage in Figure 3 *A* the leak current shows a strong linear component which was consistent between runs. Some of the non-linearity observed is likely due to non-zero stationary I_{Na} (i.e. window, late or persistent I_{Na}), yet non-linear elements and "memory effects" in the experimental setup can also not be discounted. Because separating intrinsic from external non-linearity proved difficult, we chose to "correct" the signal by estimating the leak current individually for each voltage step and subtracting it from the signal. As may be expected, incorporating uncertainty in the baseline level of individual steps into the identifiability check resulted in an unidentifiable model. A better strategy may be to test each cell twice, adding a channel blocker on the second run and subtracting the results.

The cell membrane has a considerable capacitance, which may lead to a significant delay before the voltage specified by the protocol is reached. To investigate this effect and the amplifier's ability to compensate for it (so-called "series resistance compensation") we performed experiments on the model cell circuit shown in Figure 4 *A*. In these experiments, the cell (without ion channels) was represented as a parallel membrane capacitance ($22pF$) and resistance ($500M\Omega$). The patch clamp amplifier is connected at terminal `A1` via an access resistance of $22M\Omega$. An estimate of the voltage seen by the cell membrane is obtained by connecting a second amplifier in voltage record-

ing mode to terminal A2.

Figure 4 *B* shows the model cell's "membrane potential" after a 10 mV step in the command potential. Series-resistance compensation at 60% and 80% levels improves the response time but cannot fully eliminate it, while still higher levels lead to oscillations in the circuitry. Based on these qualitative results, we updated our simulations of the patch clamp experiment to separate command potential V_{in} from the membrane potential: $\dot{V} = A \times (V_{\mathsf{in}} - V)$. Here, a value of $A = 3\mathsf{ms}^{-1}$ gave a response similar to that seen in the model cell experiments. The simulated difference in current response to our protocol for $A = 3$ is illustrated in Figure 4 *C*. These results are in line with similar experimental findings [9] and clearly demonstrate to need to account for membrane charging time in I_{Na} voltage-step protocol simulations. Adding A to the parameters tested in our identifiability check revealed that A can be identified alongside the original parameters.

With a modified, noise-aware identifiability check in place, we re-tested the possibility of uniquely identifying the model given in [3]. To eliminate issues due solely to our protocol we implemented the first protocol given in [3], which is is essentially a sequence of well established activation, inactivation, deactivation and reactivation experiments. This revealed two new issues with the model: Firstly, the occupancy of the second slow inactivation state IS2 stayed consistently low, even after the long inactivating steps it is meant to account for. Consequently parameters relating exclusively to this state were unidentifiable which we resolved by dropping this state from the model. Secondly, two of the four parameters describing activation were unidentifiable, which could only be resolved by setting their values to fixed multiples of the two identifiable ones. The resulting model showed no significant difference in current response when stimulated with either the full or the shortened protocol and both protocols could fully identify its parameters.

A parameter estimation routine run with the reduced model and updated formulation for V produced only a small improvement over the original results. This suggests that, rather than being the result of unidentifiable parameters the failure to match the data is due to other issues with either the model or the post-processed experimental results. Manually tweaking model parameters to create a slower current decay created a significant non-zero current at the end of certain voltage steps. Since this would have been removed by the leak correction method, it is likely that this is, at least in part, to blame.

4. Conclusions

Novel, rapid protocols to uniquely identify I_{Na} Markov model parameters can be designed to account for a noise threshold, capacitance artifacts and membrane charging time. Leak currents can not be dealt with numerically, but should be eliminated experimentally, for example by subtracting a signal with blocked I_{Na} . While the methods described here can only be used to locally verify a model's identifiability, the new models created this way can be used to design new, more realistic checks of identifiability, leading to an iterative and hopefully convergent process of continuous improvement.

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