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Extending the Conditions of Application of an Inversion of the Hodgkin–Huxley Gating Model

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Abstract

We present an inversion of the Hodgkin–Huxley formalism to estimate initial conditions and model parameters, including functions of voltage, from the solutions of the underlying ordinary differential equation (ODE) subjected to multiple voltage step stimulations. As such, the procedure constitutes a means to estimate the parameters including functions of voltage of an Hodgkin–Huxley formalism from experimental data.

The basic idea was developed in a previous communication (SIAM J. Appl. Math. 64:1264–1274, 2009). The inversion in question applies to currents exhibiting activation and inactivation, but the version, as published previously, cannot estimate the unknowns for channels that rapidly inactivate just after a brief opening. In such cases, the amplitude of the current, in a given voltage range, is too small to be detectable by the instrumentation using previously applied experimental protocols. This is, for example, the case for the sodium channels in a number of excitable tissue for potential in the vicinity of the cell resting potential. The current communication extends the inversion procedure in a manner to overcome this limitation.

Furthermore, within the inversion framework, we can determine whether the data at the basis of the estimation sufficiently constrains the estimation problem, i.e., whether it is complete. We exploit this element of our method to document a set of stimulation protocols that constitute a complete data set for the purpose of inverting the Hodgkin–Huxley formalism.

Keywords

Hodgkin–Huxley model; Nonlinear parameter estimation; Voltage clamp data analysis; Inverse problem; Membrane current kinetics

1 Introduction

Despite the limitations and empirical nature of the Hodgkin–Huxley (HH) formalism (Hodgkin and Huxley 1952), it is still a foundation for numerous models in cellular electrophysiology (McCormick et al. 2007). The extensive use of the formalism can be appreciated consulting the electrophysiology section of the CellML project (Nielsen 2012), and the web sites of groups offering a computational infrastructure for the simulation of cellular electrical activity (Nielsen 2012; Loew and Vcell 2012; Hines et al. 2012; Bower and Beeman 2012). As a matter of fact, the formalism was still employed in the most recently developed cell models (O’hara et al. 2011; Grandi et al. 2010).

Furthermore, there are increasing efforts to incorporate such cell models in large scale models of tissues and organs (Bassingthwaite 2012), which may constitute one of the most valuable tools to capitalize on the genome on our quest to decipher the molecular mechanisms of a number of diseases (Noble et al. 2012). In this context, the estimation of the parameters, including functions of voltage, of the HH formalism is a cornerstone, as it limits the reliability of the macroscopic level simulations. This point is well illustrated by the fact that relatively small perturbations in the HH formalism parameter values, if done in the appropriate direction in the parameter space, can make a dramatic change on wave dynamics (Beaumont et al. 1998). It remains to know whether perturbations in parameter values producing significant changes in wave dynamics are within experimental error. This is an important question, as it affects the overall modeling methodology. In our perspective, this motivates an extensive analysis of the estimation problem which, in our opinion, should also include investigating how data at the basis of the estimation should be generated.

The reader unfamiliar with the Hodgkin Huxley formalism may consult Ermentrout (2010, Sect. 1.8) for a detailed description. Currents analyzed in this manuscript display activation and inactivation and are hence modeled with two state variables. For this case, the unknowns are one parameter (the maximal channel conductance) and two functions of voltage, the steady state and time constant, for each state variable (a total of 4 functions). Two of these four functions specify the initial values. Since the unknowns include functions of voltage and a parameter, to avoid confusion, we refer to the unknowns as the gating model parameters (GMP).

Our estimation procedure exploits the fact that we know the ODE solution. Indeed, the procedure consists to invert it in a manner to recover the GMP. This work was initiated in Beaumont et al. (1993a, 1993b) where inversion was achieved only for the peak current. It was extended later (Wang and Beaumont 2004) to consider the entire interval of acquisition, but with some limitations since it is not applicable to currents that rapidly inactivate in a given voltage range. Thus, we extend the conditions of application in a manner to overcome this limitation. Specifically, in Sect. 2 we briefly describe the inversion and discuss its limitations, in Sect. 3, we indicate how we acquired and preprocessed Biological data, in Sect. 4 we apply the inversion to incomplete Biological data and complete synthetic data. In that section, we explain how we overcome the limitations of the current version of the inversion. Finally, we conclude in Sect. 5.

2 Inversion of the HH Formalism and Its Limitations

The Hodgkin–Huxley formalism for an activating and inactivating ionic current is given by

$$I(u, t) = \bar{g} y_0^{\lambda_0} y_1^{\lambda_1} (u - e), \quad (1)$$

$$\frac{dy_i(u, t)}{dt} = \frac{s_i(u) - y_i(u, t)}{\tau_i(u)}, \quad i \in [0, 1] \quad (2)$$

where u is the membrane potential, $I(u, t)$ the ionic current, $\bar{g}$ the maximal channel conductance (open state conductance), and e the equilibrium potential. The state variables, $y_i \in [0, 1]$, $i \in [0, 1]$, are termed the gating variables. They represent the fraction of the population of molecular gates in the open state. The parameters λ_i , $i \in [0, 1]$, are integers meant to represent the number of gating particles in the channel. We assume they are a small integer between 1 and 5, and repeat the estimation procedure for each set of integers to obtain the optimal results. We choose λ_i such that we have the greatest invertible range on the data. The functions of voltage, $s_i(u)$ and $\tau_i(u)$, $i \in [0, 1]$, are the steady states and time constants of each gating variable. In general, $s_i(u)$ is sigmoidal.

Note that we restrict our analysis to channels having two gates. The two gates are called activation and inactivation gates because they open, ($ds(u)/du > 0$), and close, ($ds(u)/du < 0$), with depolarization. We assign the index 0 to the activation gate and 1 to the inactivation gate.

A cell membrane has a rest potential where the algebraic sum of all transmembrane currents is null. A voltage clamp stimulation is usually performed around this potential. The stimulation consists to clamp the membrane voltage until steady state is reached, u_H : for holding potential, and then step the membrane voltage to a test potential, u_T , from which point the current is monitored. We refer to H-step and T-step stimulation protocols where the holding and test potential are varied respectively, while the other potential of the step stimulation is kept constant. The range of each protocol, denoted by R_H and R_T , respectively, is the potential over which u_H (H-step) or u_T (T-step) is varied. In such conditions, the time course of the gating variables is given by

$$y_i(t; u_H, u_T) = s_i(u_T) + (s_i(u_H) - s_i(u_T)) e^{-t/\tau_i(u_T)}, \quad i \in [0, 1]. \quad (3)$$

The problem consists of estimating the parameter, $\bar{g}$, and functions, $s_i(u)$, $\tau_i(u)$, $i \in [0, 1]$, from experimental recordings gathered in isolated cells during voltage clamp stimulation. Since the parameters λ_i , $i \in [0, 1]$ can take only few integer values, we simply repeat the estimation for all possible combinations within the range $[1, 5]$. The set of λ_i providing the greatest number of inverse solutions are then used in the subsequent steps of the estimation.

The procedures are tested with biological data in Sect. 4.1 and with synthetic data generated by the Ebihara and Johnson model (Ebihara and Johnson 1980) of the cardiac sodium current in Sect. 4.2. The model is illustrated in Fig. 1.

Estimation proceeds by first estimating $s_i(u)$ $i \in [0, 1]$ with data generated by H-step protocols, and then by estimating $\tau_i(u)$ $i \in [0, 1]$ with data gathered by T-step protocols after bounding $R = 1/\bar{g}$. Below, we provide a brief description of the procedure in order to facilitate the description of its current limitations, but the interested reader is referred to Wang and Beaumont (2004) for a detailed description of the inversion procedure.

Estimation of $s_i(u)$ $i \in [0, 1]$ —Estimation is based on Theorems 3.7, 3.1, and 3.8 of Wang and Beaumont (2004) below:

$$I_N^{\frac{1}{\lambda_i}}(t, t_r; u, u_T) - 1 = \theta_a \left(-\lambda_i \frac{dI_n(t, t_r; u, u_T)}{dt} + J(t=t_r; u, u_T) \right) - \theta_b \int_{t_r}^t I_N(t, t_r; u, u_T) dt, \quad (4)$$

$$\frac{s_i(u)}{s_T^{(i)}} = 1 - \frac{J(t; u, u_T) + \lambda_i / \tau_T^{(\bar{i})} (1 + \varepsilon_{\bar{i}}(t))}{J(t; u, u_T) + \lambda_i / \tau_T^{(i)} + \lambda_{\bar{i}} / \tau_T^{(1)} (1 - \varepsilon_{\bar{i}}(t))} e^{t/\tau_T^{(i)}}, \quad (5)$$

$$\frac{s_{\bar{i}}(u)}{s_R^{(\bar{i})}} = \left[\frac{I(t; u, u_T)}{I(t; u=u_R, u_T)} \right]^{\frac{1}{\lambda_{\bar{i}}}} \left[\frac{J(t; u, u_T) + \frac{\lambda_i}{\tau_T^{(i)}} + \frac{\lambda_{\bar{i}}}{\tau_T^{(\bar{i})}}}{J(t; u_R, u_T) + \frac{\lambda_i}{\tau_T^{(i)}} + \frac{\lambda_{\bar{i}}}{\tau_T^{(\bar{i})}}} \right]^{\frac{\lambda_i}{\lambda_{\bar{i}}}} \quad (6)$$

where t_r stands for a reference time in the interval of acquisition, $u_R \in R_H$, $s_{\alpha}^{(i)}$ stands for $s_i(u_{\alpha})$, $\tau_{\alpha}^{(i)}$ for $\tau_i(u_{\alpha})$, $i \in [0, 1]$, $\bar{i}$ is the complement of i $\alpha \in [H, T, R]$, and

$$I_N(t, u; t_r, u_T) = \frac{I(t, u; u_T)}{I(t=t_r, u; u_T)}, \quad J(t, u; u_T) = \frac{dI(t, u; u_T)/dt}{I(t, u; u_T)}.$$

The functions $\varepsilon_j(t)$, $j \in [0, 1]$ are error functions. They become negligible when the conditions of application of the theorems are fulfilled. A test to determine, *a-priori* from the data, whether the conditions are fulfilled can be found in Wang and Beaumont (2004).

A condition of application is data generated by an H-step protocol $u_T > u_H$ for which

$$\{u_T : s_1(u_T) < \varepsilon\}$$

ε : error tolerated (Wang and Beaumont 2004). In such condition, $i = 0$, $\bar{i} = 1$. Another condition is data generated by an H-step protocol $u_T < u_H$ satisfying

$$\{u_T : s_0(u_T) < \varepsilon\}.$$

Interestingly, Eqs. (4)–(6) (Theorems 3.7, 3.1, 3.8 Wang and Beaumont 2004) are symmetric, meaning that these equations are still valid for this condition, one just needs to swap indices i and $\bar{i}$ of the gating variables.

Estimation proceeds as follows. The parameters θ_a, θ_b are obtained applying linear least square fitting to (4). These two parameters are inverted for $\tau_T^{(\iota)}, \tau_T^{(\bar{\iota})}$ (Wang and Beaumont 2004). Equations (5) and (6) are fulfilled for any time on a given current. Thus, the left-hand side is estimated averaging the right-hand side over all acquisition points of currents recorded at different voltages.

Note that R_H of an H-step protocol with $u_T > u_H$ fulfilling the conditions of application of the theorems has an upper limit. As $u_H \rightarrow u_T, s_1(u_H) \rightarrow s_1(u_T)$, and in an H-step protocol we set u_T such that $s_1(u_T) < \varepsilon$, which is by definition very small. See leftmost panel of Fig. 1. Then, from (3), $y_1(t) < \varepsilon$ during the application of the test pulse for $t \in [0, \infty]$. Consequently, in such conditions, the current becomes undetectable by the instrumentation. We term this upper limit u_{H0} . The same applies to an H-step protocol with $u_T < u_H$ fulfilling the conditions of application of the theorems. In this case, there is a lower limit, u_{H1} , below which the current becomes undetectable by the instrumentation.

Therefore, at least theoretically, $s_\iota(u), \iota \in [0, 1], u < u_{H0}$ can be estimated with an H-step protocol for which $u_T > u_H$, and $s_\iota(u), \iota \in [0, 1], u > u_{H1}$ can be estimated with an H-step protocol for which $u_T < u_H$. Each H-step protocol should fulfill the conditions of application of the theorems.

Estimation of $\tau_\iota(u), \iota \in [0, 1]$ —Theorem 4.1 of Wang and Beaumont (2004) stipulates

$$\prod_{i \in \bar{\mathcal{A}}} \gamma_i(y_i; u_H, u_T) = e^{-t_r J(t_r)} \prod_{i \in \mathcal{A}} \gamma_i(y_i; u_H, u_T) \quad (7)$$

with

$$\gamma_i = \begin{cases} \left[\frac{s_T^i - s_H^i}{s_T^i - y_i} \right]^{\frac{\lambda_i(s_T^i - y_i)}{y_i}}, & \text{if } i \in \mathcal{A}, \\ \left[\frac{s_T^i - s_H^i}{y_i - s_T^i} \right]^{\frac{\lambda_i(y_i - s_T^i)}{y_i}}, & \text{if } i \in \bar{\mathcal{A}} \end{cases} \quad (8)$$

for any data point on a current, where $\mathcal{A}$ is the set of indices for which $\text{sign}(ds_\iota(u)/du) = \text{sign}(u_T - u_H)$, and $\bar{\mathcal{A}}$ its complement. Note the theorem is applicable to a formalism with multiple gates. However, since we have only two gates, $\mathcal{A} = [0]$, $\bar{\mathcal{A}} = [1]$ for a T-step protocol with $u_T > u_H$ and vice versa for $u_T < u_H$. Thus, we have $\gamma_\iota(y_\iota), y_\iota \in [s_H^i, s_T^i], \iota \in [0, 1]$, where each $\gamma_\iota(y_\iota)$ has only one extremum (Wang and Beaumont 2004). The functions $\gamma_\iota(y_\iota)$ are employed to systematically find all couples $y_0^{\lambda_0}, y_1^{\lambda_1}$ that can reproduce a data point. From these couples, we find bounds for $R = 1/\bar{g}$. Then pick a value of R within the bounds and use (7) to find all gating variables that can reproduce a data point (Algorithm 4.4, Wang and Beaumont 2004). For each gating variable satisfying condition (7), we extract the time constants with (3). The interested reader can find all the details of this inversion in Sect. 4 of Wang and Beaumont (2004).

Consider the estimation of $\tau_i(u)$ $\iota \in [0, 1]$ with two complementary T -step protocols, one with $u_T > u_H$ and another one with $u_T < u_H$. In each one,

$$u_H : s_\iota(u_H) < \varepsilon.$$

This way, the current is negligible when the channel is clamped at $u = u_H$. Each protocol is bounded below and above respectively since as $u_T \rightarrow u_H$, the current becomes small, and cannot be detected by the instrumentation. We term these bounds u_{T0} and u_{T1} . Then, at least theoretically, $\tau_i(u)$ $\iota \in [0, 1]$, in the potential range $u > u_{T0}$ and $u < u_{T1}$, can be estimated with the first and second protocols, respectively. Note that, in general, the two ranges overlap (see Fig. 1A).

Limitations—Several channels, like the sodium channel of nerve, skeletal, and cardiac cells, inactivate very rapidly at potentials close to the cell's resting potential. This property is important for tissue excitability since the sodium channel generates a large current during depolarization, but a much smaller one during repolarization, which is essential for the generation of an action potential. As a result, while Theorems, 3.7, 3.1, 3.8, and 4.1 of Wang and Beaumont are symmetric (Wang and Beaumont 2004), currents generated with u_T near the cell rest potential are too small to be reliably detected by the instrumentation. This precludes inverting $s_\iota(u)$ for $u > u_{H0}$ and $\tau_\iota(u)$ for $u < u_{T0}$ $\iota \in [0, 1]$.

To alleviate this problem for the estimation of $s_\iota(u)$ $\iota \in [0, 1]$, a procedure based on a double step protocol was proposed in Wang and Beaumont (2004). The procedure exploits cell memory, i.e., the fact that response to stimulation is affected by the potential at which a cell was maintained. In the protocol in question, the experimentalist had the freedom to set u_T at a value that could generate currents of significant magnitude. While this approach may work for several tissues, it is unfortunately impractical for a number of others. To understand why, one should remember that currents are isolated with pharmacological blockers, meaning that cells have to be stimulated in absence and presence of the blockers. The protocol in question requires maintaining u_H at a large depolarized potential for a long interval of time. Unfortunately, many cells like nerve, skeletal, and cardiac cells cannot tolerate a large depolarization for a long interval of time. The cells are simply too leaky at such potentials. The large current flux changes the ionic composition of the intracellular medium and damages the cell. Consequently, protocols requiring u_H to be set at large depolarized potentials are impractical for a number of cell types including nerve, skeletal, and cardiac cells.

3 Methods

Cordeiro et al. (2008) generously provided us access to a voltage clamp data set on the canine cardiac sodium current (Cordeiro et al. 2008). Below, we briefly describe data acquisition and the pre-processing we performed prior to analysis.

Cell Isolation—Adult mongrel dogs were euthanized and their hearts were excised from their chest. Thin slices of tissue were shaved from the endocardial and epicardial layers of

the left ventricular free wall with a dermatome. The tissue slices were immersed in an enzymatic solution for digestion of the collagen. The supernatant was filtered and centrifuged and the pellet containing the myocyte was stored at room temperature (Cordeiro et al. 2008; Murphy et al. 2011).

Data Acquisition—The currents were recorded with a multiclamp 700 A amplifier from Axon Instruments. The electrode resistance was 1 to 2.5 MΩ. Currents were acquired at 20–50 kHz and filtered at 5 kHz.

To obtain a good voltage control during step stimulation, recordings were made at room temperature and in low extracellular Na solution, which was titrated to produce a 5 mV reversal potential. The background potassium current was eliminated with Cesium (Cordeiro et al. 2008; Murphy et al. 2011).

The T-step protocol was conducted with $u_H = -120$ mV to recruit all available Na⁺ channels and $u_T \in [-50, 15]$ mV. The H-step protocol was conducted with $u_H \in [-120, -25]$ mV and $u_T = -10$ mV. In each case, data acquisition took place over a minimum of 35 ms. Currents in a total of 15 endocardial and 15 epicardial cells were generated for the H-step protocol as well as for the T-step protocol. From this set, we eliminate recordings with a wide capacitive transient, since it signifies an unstable membrane, and recordings with poor voltage control. We were left with 8 endocardial and 10 epicardial cells. From the 8 endocardial cells, 2 had invalid T-step recordings.

Pre-processing of the Data—The inversion procedure uses the time derivative of the current, which can be inaccurate on noisy current. Thus, prior to any processing, we filtered the acquired data using a moving average filter with a mask 10 data points wide. We picked the mask width large enough to eliminate, on the derivative of the smoothed trace, all peaks produced by noise on the data, and still have a curve that runs smoothly within the noise. That was found by trial and error. This choice of mask width can vary for each data set, but is relatively easy to find since the noise is at a much higher frequency than any change in time on the current.

The sodium current is so rapid that a significant portion of the current needed for estimation is superimposed to the capacitive transient that follows the voltage jump. To subtract it from the total current, we represented the cell-pipette configuration with the electrical circuit of Fig. 2. Based on this circuit, the time course of the current in absence of I_{Na} is given by:

$$I_d(t) = a_0 e^{-t\alpha} + a_1, \\ \alpha = -\frac{R_L R_m + R_S R_m + R_S R_L}{C_m R_S R_m R_L}, a_0 = a_1 + \frac{u_T - u_H}{R_S}, \quad (9) \\ a_1 = \frac{V_r (R_m R_L) - e R_L}{-R_L R_m + R_S R_m + R_S R_L}.$$

We fitted (9) to the first 0.1 and last 8 ms of the full acquisition (35 ms) since I_{Na} is negligible in these intervals. The parameter estimation was performed as follows: for a given a , a_0 and a_1 were estimated through linear least square fitting. The parameter a was varied from -30 to -4 taking 1,000 samples. The optimal a , a_0 , and a_1 , are the ones minimizing the

residual of (9). Note that we elected to perform the fit for each current since the capacitive transient is quite sensitive to R_L , and this parameter may change from one recording to another because the pipette may slightly move during acquisition.

Subsequently, we performed a weighted average of the currents with respect to cell size. To approximate cell size, we assumed a linear relationship between the peak current magnitude recorded at a specific voltage of a T-step protocol. For that protocol, the smallest and largest peak currents were 2 and 8 nA, which corresponds to a ratio of 4 in cell size. The size of the other cells of this group, abscissa in Fig. 3, were evaluated based on their peak current magnitude using this linear relation. Then the peak currents in the same group of cells, but measured at different potentials, are scaled and placed on the graph. Thus, size and peak are known for these points. The scaling factor, one number for all cells, is found by making the peak current of the smallest cell to match the first point on the graph, i.e., 2 nA. All peak currents follow the same linear trend with a correlation coefficient of 0.9814.

Figure 4 shows weighted average currents recorded at the same voltage in H-step and T-step protocols, each performed in different group of cells. The weighted average was carried out as mentioned above. Clearly, despite recordings in different groups of cells the currents match quite well. Just to further justify the need for a weighted average with respect to cell size, we included images (Fig. 5) of typical cardiac myocytes. Finally, Fig. 6 shows the resulting weighted average for cell size of sodium currents gathered in endocardial and epicardial cells with H-step and T-step protocols.

4 Results

4.1 Application to an Incomplete Biological Data Set

The inversion procedure was applied to the biological data described in the previous section. As mentioned in Sect. 2, estimation was performed with various combinations of λ_ν , $\nu \in [0, 1]$. We found that $\lambda_0 = 4$ and $\lambda_1 = 1$ gave the best results, a larger invertible range capable of reproducing the data, and report only this result.

Estimation of $s_\nu(u)$ $\nu \in [0, 1]$ —As described in Sect. 2, the functions $s_\nu(u)$ were obtained with Theorems 3.7, 3.1, and 3.8 of Wang and Beaumont (2004) and based on data generated with the H-step protocol. Since the voltage from which the maximal current was elicited for each H-step protocol were slightly below -50 mV, as shown in Fig. 7, we estimated $s_\nu(u)$ $\nu \in [0, 1]$ up to this voltage. The reader should note that $s_\nu(u)$ reported here is different from the channel availability curve reported by Cordeiro et al. (2008). The latter constitutes a well accepted measure of channel availability in electrophysiology and has no trivial relation to $s_\nu(u)$.

Since the biological data does not constrain the $s_\nu(u)$ for voltage above -50 mV, and the support of $s_0(u)$ is above that voltage we arbitrarily fixed two different $s_0(u)$ (Fig. 7).

Estimation of $\tau_\nu(u)$ $\nu \in [0, 1]$ —As mentioned in Sect. 2 and described in a more detailed manner in Sect. 4 of Wang and Beaumont (2004), estimation of $\tau_\nu(u)$ consists to first bound R , and then extract time constants corresponding to a value of R picked within the bounds. If

the data constrain the estimation problem the bounds are narrow. In the specific inversion performed in this section of the manuscript, we considered 10 % of noise on the current. The results are shown in Figs. 8 and 9. The estimation was repeated using the two different $s_0(u)$ of Fig. 7 (solid and broken lines). As explained in Sect. 2, and described in a detailed manner in Wang and Beaumont (2004), the procedure consists to first bound $R = 1/\bar{g}$. Then a $\tau_i(u)$, $i \in [0, 1]$ that can reproduce the data can be found for each R picked within the bounds. The extraction procedure is quite involved. Once R is fixed, we find all couples of gating variables, y_i , that can reproduce the data within a given error tolerance. Then, since the steady state is known at each voltage of the stimulation, we extract the time constants from (3). The inversion of the functions $y_i(y_i)$ is the key process that allows us to find, in a systematic manner, all couples y_i that reproduce the data within a given error tolerance. The reader is referred to Sect. 2 and Wang and Beaumont (2004) for details on the procedure.

The symbols in Fig. 8 show, for voltages sampled in the range of the H-step protocol, time constants corresponding to several values of R picked within the bounds. This was performed for the two different $s_0(u)$ shown in Fig. 7. Specifically, the bounds on R were quite similar when the two different $s_0(u)$ were used in the inversion. They were [0.03, 0.048] for the endocardial data and [0.048, 0.101] for the epicardial data. The time constants shown in Fig. 8 for the endocardial data were extracted with $R = 0.0333$ using $s_0(u)$ corresponding to the solid line in Fig. 7, and $R = 0.4662$ for $s_0(u)$ corresponding to the broken line. Similarly for the epicardial data, we picked $R = 0.0526$ and $R = 0.0968$ when $s_0(u)$ corresponded to the solid and broken lines in Fig. 7. Clearly, the functions of voltage extracted are quite different from one another. Still, as shown in Fig. 9, each model reproduces the data within the prespecified error tolerance.

Note that we could have extracted other time constants picking other values of R within the bounds. This would have generated functions of voltage running through the space delimited by the symbols in Fig. 8. This said a warning is in order. Valid functions are not any functions passing through the symbols shown in the figure. They should rather be extracted using the inversion procedure. We have not generated such $\tau_i(u)$ with other values of R here, but are illustrating this possibility in Sect. 4.3, on the effect of noise on the data.

In summary, Figs. 7, 8 and 9 clearly illustrate that quite different models are capable to reproduce a relatively extensive data set, and this is irrespective of the number of data points and potentials sampled in the range of each stimulation protocol. So the data set does not constrain the estimation problem i.e., we say it is *incomplete*.

4.2 Application to a Complete Synthetic Data Set

Estimation of $s_i(u)$, $i \in [0, 1]$ —To overcome the limitation raised in Sect. 2, we add voltage clamp data to the conventional data set and introduce new processing. We generate the voltage clamp data for the application of our estimation procedure with the Ebihara and Johnson sodium current model (Ebihara and Johnson 1980).

The additional voltage clamp data are generated with a new stimulation protocol (Fig. 10), termed the C-step protocol, the purpose of which is to probe $s_i(u)$ $i \in [0, 1]$ at large depolarized potentials. Briefly, a conditioning pulse is inserted between the holding and test

potentials. Currents are interpreted during the application of the test pulse. The conditioning pulse voltage is chosen in a manner to probe $s_\iota(u)$, $\iota \in [0, 1]$ in the desired voltage range, and its duration in a manner to generate independent currents, as tested using the conditions in Sect. 2, in the monitoring interval. This duration will need to be chosen iteratively with the data collection, since each data set will need to be tested for independence before application of the method. To start, durations are chosen in the same magnitude of the time constant, which can be approximated from the time course of the current. The range of the stimulation protocol is denoted by R_C . The stimulation overcomes the limitations discussed in Sect. 2, since u_H is never held at a large depolarized potential for a long interval of time, and the experimentalist has the freedom to set u_T to a value that generates a large current as long as

$$\{u_T : s_1(u_T) < \varepsilon\}.$$

See Fig. 11 for sodium currents produced with this protocol.

We denote the value of the gating variables at the end of conditioning pulses by

$y_C^{(\iota)}(\nu_k)$ $k \in [a, b]$, of duration ν_a or ν_b , $\nu_b < \nu_a > 0$, and obtain a new expression relating $y_C^{(\iota)}(\nu_k)$, $s_C^{(\iota)}$, $i \in [0, 1]$, $k \in [a, b]$ isolating $e^{-1/\tau_T^{(\iota)}}$ from (3),

$$\left[\frac{y_C^{(\iota)}(\nu_a) - s_C^{(\iota)}}{s_H^{(\iota)} - s_C^{(\iota)}} \right]^{\frac{1}{\nu_a}} = \left[\frac{y_C^{(\iota)}(\nu_b) - s_C^{(\iota)}}{s_H^{(\iota)} - s_C^{(\iota)}} \right]^{\frac{1}{\nu_b}}, \quad i \in [0, 1]. \quad (10)$$

We evaluate $y_C^{(\iota)}(\nu_k)$ from currents recorded during the application of the test pulse in the C-step protocol (Fig. 10), then solve (10) for $s_C^{(\iota)}$, and do so for each conditioning voltage of the C-step protocol. If the kinetic data can be represented by a Hodgkin–Huxley formalism, we expect Condition 1 to be fulfilled.

Condition 1 $y_C^0(\nu_b) > y_C^0(\nu_a) > s_H^0$ **and** $y_C^1(\nu_b) < y_C^1(\nu_a) > s_H^1$: Fortunately, we can test, a-priori, condition 1 and when fulfilled, because $s^{(\iota)}(u)$ and $y_\iota(t)$ $\iota \in [0, 1]$ are monotonic,

Lemma 1 applies and allows us to unambiguously estimate all s_C^i satisfying (10). If condition 1 is not satisfied, we conclude the kinetic data cannot be represented by an Hodgkin–Huxley formalism.

Lemma 1: If Condition 1 is fulfilled, for given $y_C^i(\nu_k)$, $\iota \in [0, 1]$ $k \in [a, b]$, the objective functions

$$\Theta_i(s_C^i) = h_a^i(s_C^i) - h_b^i(s_C^i), \quad h_k^i(s_C^i) = \left[\frac{s_C^i - y_C^i(\nu_k)}{s_C^i - s_H^i} \right]^{\frac{1}{\nu_k}}, \quad (11)$$

have at most one zero if

$$\frac{\nu_b}{\nu_a} \left[\frac{y_C^t(\nu_a) - s_H^t}{y_C^t(\nu_b) - s_H^t} \right] \leq 1 \quad (12)$$

and at most two zeros otherwise. Specifically,

$$\begin{aligned} \Theta_0(\xi) & \text{ has } \begin{cases} \text{at most one zero if } h_a^0(\xi=1) < h_b^0(\xi=1), \\ \text{at most two zeros if } h_a^0(\xi=1) > h_b^0(\xi=1), \end{cases} \\ \Theta_1(\xi) & \text{ has } \begin{cases} \text{at most one zero if } h_a^1(\xi=0) < h_b^0(\xi=0), \\ \text{at most two zeros if } h_a^1(\xi=0) > h_b^0(\xi=0). \end{cases} \end{aligned}$$

Proof: Consider $\Theta_0(\xi)$. Since $s_0(u)$ and $y_0(t)$ are monotonically increasing functions of u and t respectively, and $\nu_a < \nu_b$ (imposed by the C-step protocol),

$$0 < s_H^0 < y_C^0(\nu_a) < y_C^0(\nu_b) < s_C^0 < s_T^0 < 1. \quad (13)$$

Consequently, the domain of each $h_k^0(\xi)$ are

$$h_a(\xi), \quad \xi \in [y_C^0(\nu_a), 1], \quad h_b(\xi), \quad \xi \in [y_C^0(\nu_b), 1].$$

Note that each $h_k^0(\xi)$ is a monotonically increasing function of ξ and due to (13), the numerator and denominator of each function are nonnull and have the same sign. At the lower bound, $\xi = y_C^0(\nu_b)$, of the interval where we are seeking the intersection, $h_b^0(\xi = y_C^0(\nu_b)) = 0 < h_a^0(\xi = y_C^0(\nu_b))$.

Therefore, a solution exists iff $h_k^0(\xi)$ intersect at least once. Furthermore, at the first intersection we should have

$$\left. \frac{\partial h_b^0(\xi)}{\partial \xi} \right|_{\xi=\xi^*} \geq \left. \frac{\partial h_a^0(\xi)}{\partial \xi} \right|_{\xi=\xi^*}, \quad (14)$$

ξ^* being the coordinate of the intersection. Taking the derivative of $h_k^0(\xi)$ with respect to ξ and eliminating similar terms at $\xi = \xi^*$, the above condition becomes

$$\frac{1}{\nu_b} \left[\frac{y_C^0(\nu_b) - s_H^0}{\xi^* - y_C^0(\nu_b)} \right] \geq \frac{1}{\nu_a} \left[\frac{y_C^0(\nu_a) - s_H^0}{\xi^* - y_C^0(\nu_a)} \right]. \quad (15)$$

Rearranging to regroup the terms depending on ξ^* on the left-hand side,

$$f(\xi^*) = \left[\frac{\xi^* - y_C^0(\nu_a)}{\xi^* - y_C^0(\nu_b)} \right] \geq \frac{\nu_b}{\nu_a} \rho, \quad \rho = \left[\frac{y_C^0(\nu_a) - s_H^0}{y_C^0(\nu_b) - s_H^0} \right], \quad (16)$$

which is permitted since the numerator and denominator of each side of (15) are nonnull and have the same sign.

Remark that

$$\frac{df(\xi^*)}{d\xi^*} < 0 \quad (17)$$

with asymptotes at $\xi^* = y_C^0(\nu_b)$ and $f(\xi^*) = 1$. Then, if $((\nu_b/\nu_a)\rho) < 1$,

$$f(\xi^* = \xi^{**}) = (\nu_b/\nu_a)\rho, \quad \xi^{**} = \frac{y_C^0(\nu_a) - y_C^0(\nu_b)(\nu_b/\nu_a)\rho}{1 - (\nu_b/\nu_a)\rho} < y_C^0(\nu_a). \quad (18)$$

Due to the fact that $\xi^{**} < y_C^0(\nu_a)$, inequality (14) is respected in $\xi^* \in [y_C^0(\nu_b), 1]$. Since the inequality does not change within this interval, the number of intersections between $h_k^0(\xi)$ is limited to at most one.

If $((\nu_b/\nu_a)\rho) > 1$, and $\xi^{**} > 1$, then the number of intersections between $h_k^0(\xi)$ is limited to at most one for the reason mentioned above.

If $((\nu_b/\nu_a)\rho) > 1$ and $\xi^{**} \in [y_C^0(\nu_b), 1]$, at intersection points ξ^* ,

$$\left. \frac{\partial h_b^0(\xi)}{\partial \xi} \right|_{\xi > \xi^{**}} < \left. \frac{\partial h_a^0(\xi)}{\partial \xi} \right|_{\xi > \xi^{**}} \quad (19)$$

and $h_k^0(\xi)$ can cross another time in the interval $[y_C^0(\nu_b), 1]$, but they can do so only once since an additional intersection would imply

$$f(\xi^* > \xi^{**}) > (\nu_b/\nu_a)\rho \quad (20)$$

at an intersection ξ^* which is impossible due to the monotonicity of $f(\xi^*)$.

Finally, if $((\nu_b/\nu_a)\rho) = 1$, $\xi^{**} \rightarrow \infty$. Since ξ^{**} is outside the interval $[y_C^0(\nu_b), 1]$, $h_k^0(\xi)$ can intersect only once. Furthermore, for this special case $h_k^0(\xi = \xi^*)$ share their tangent at their intersection.

Therefore, $\theta_0(s_C^0)$ has at most two zeros. In addition, if $((\nu_b/\nu_a)\rho) > 1$ and there are two zeros,

$$h_b^0(\xi=1) < h_a^0(\xi=1). \quad (21)$$

The same logic applies to $\Theta_1(s_C^1)$.

The zero of each $\Theta_i(s_C^{(i)})$ are evaluated numerically. First, y_C^t are estimated the same way we estimated $s_H^{(i)}$ with the H-step protocol. Specifically, the parameters $\tau_T^{(i)}$ are estimated with Theorem 3.1 of Wang and Beaumont (2004). Subsequently, $s^{(0)}(u_C)/s_T^{(0)}$ and $s^{(1)}(u_C)/s_R^{(1)}$ are estimated with Theorems 3.1 and 3.8 of Wang and Beaumont (2004), respectively.

We then find the zero of each $\Theta_i(s_C^{(i)})$. If $((\nu_b/\nu_a)\rho) = 1$, then there is only one zero in the interval $[y_C^0(\nu_b), 1]$ for $\Theta_0(s_C^0)$ and $[0, y_C^1(\nu_b)]$ for $\Theta_1(s_C^1)$. We find them by dichotomy. In the special case where $((\nu_b/\nu_a)\rho) = 1$, the zero is at coordinate $\xi = \xi^{**}$ (see proof of Lemma 1).

If $((\nu_b/\nu_a)\rho) > 1$, the number of zeros depends on $h_k^t(s_C^0)$ at one end of the search interval. Specifically, if $h_b^0(s_C^0=1) > h_a^0(s_C^0=1)$, $\Theta_0(s_C^0)$ has one zero otherwise it has two. Similarly, if $h_b^1(s_C^1=0) > h_a^1(s_C^1=0)$, $\Theta_1(s_C^1)$ has one zero otherwise it has two. Note that $h_k^t(s_C^0)$ cannot be equal at the bounds of the search interval as long as $y_C^t(\nu_a) \neq y_C^t(\nu_b)$. When there are two zeros, the coordinate ξ^{**} split the search interval in two, i.e., each zero should be in the interval delimited by ξ^{**} and the bounds of the search interval. See the proof of Lemma 1 for the evaluation ξ^{**} . Once ξ^{**} has been evaluated, we find each zero by dichotomy with a precision of 10^{-6} . The application of this procedure to the synthetic data set is illustrated in Fig. 10 (triangles).

Estimation of $\tau_i(u)$ $i \in [0, 1]$ —We overcome the limitation raised in Sect. 2, adding voltage clamp data to the conventional data set and introducing new processing. As with steady state estimation, synthetic voltage clamp data were generated with the Ebihara and Johnson model (Ebihara and Johnson 1980), which are then processed to estimate $\tau_i(u)$. The time constant for large depolarized potentials are estimated similarly to the Biological data, i.e., we use Theorems 4.1 and Algorithm 4.4 of Wang and Beaumont (2004) to estimate $\tau_i(u)$ (Fig. 12).

We introduce a new stimulation protocol termed the G-step protocol, illustrated in panel C of Fig. 12. The protocol consists of two pulses separated by a gap. Currents recorded during the test pulse are then interpreted. The gap potential is varied to probe the time constant in the desired voltage range, and its duration is adjusted to obtain currents of significant magnitude during the application of the test pulse and independent from one another. The range of the protocol is denoted by R_G . Since with this protocol, the potential is never held for long intervals of time at large depolarized potentials and the experimentalist has the freedom to set the test pulse at any value that generates currents of significant magnitude, as long as

$$\{\mu_T: s_1(u_T) < \varepsilon\},$$

where ε is a predetermined threshold, the limitations raised in Sect. 2 are overcome.

In such conditions, Theorems 3.1 and 3.8 of Wang and Beaumont (2004) are used to evaluate the gating variables at the end of the gap. Since the steady states are known, we obtain the time constants from (3). The squares in Figs. 12E,F were obtained applying this procedure.

4.3 The Effect of Noise on the Data

Among other advantages, an important one of our inversion procedure is to better handle the ill-posed nature of the problem. The method extracts the steady states, a range for $R = 1/\bar{g}$, and functions of voltage representing the time constants that reproduce the data within a given tolerance. There are at least one function of voltage for each gate, and for each R picked within the bounds. These functions are extracted following the inversion procedure outlined in Sect. 2. These functions are extracted following the inversion procedure outlined in Sect. 2, and described in more detail in Sect. 4 of Wang and Beaumont (2004), “*Estimation of $\tau_i(u)$* ” provides $\tau_i(u)$. We say at least one function for each gate because, for a given voltage, the inversion may generate more than one value, so in general the functions may bifurcate.

We illustrate this important property with a synthetic noisy data set. Voltage clamp data are generated with the Ebihara and Johnson model (Ebihara and Johnson 1980) to which we add 5 % white noise. The data set is generated with stimulation protocols of Figs. 10A,C and 12A,C, which we claim to fully constrain the gating model. The data is filtered as indicated in Sect. 3.

Steady states are estimated with the procedures of Sect. 4.2 and using data generated with the H-step and C-step protocols. The first step consists to estimate $\tau_T^{(\iota)} \iota \in [0, 1]$, which was done so far with Theorem 3.7 of Wang and Beaumont (2004). However, estimation of τ_T^i with this theorem. However, estimation of τ_T^i with this theorem requires finding the unique minimum of an objective function which may be quite flat in the vicinity of its minimum when the problem is ill posed. We address this problem supplementing processing with Theorem 3.7 of Wang and Beaumont (2004) by finding the minimum of the objective function,

$$\Theta = \sum_n \sum_k [I_N^{1/\lambda_0}(t_k, u_n; t_r, \tau_T^0, \tau_T^1) - E^{1/\lambda_0}(t_k, u_n; t_r)]^2, \quad (22)$$

$$I_N^{1/\lambda_0}(t_k, u_n; t_r, \tau_T^0, \tau_T^1) = e^{\frac{-\lambda_1(t_k - t_r)}{\lambda_0 \tau_T^1}} \left[\frac{J(t_r, u_n) + \lambda_0/\tau_T^0 + \lambda_1/\tau_T^1}{J(t_k, u_n) + \lambda_0/\tau_T^0 + \lambda_1/\tau_T^1} \right] \quad (23)$$

with respect to $\tau^{(\iota)}$, $\iota \in [0, 1]$. I_N is the current normalized with respect to a value taken at a reference time t_r , and E its experimental counterpart. The discrete variables t_k and u_n are the time samples, and potentials in the range of each stimulation protocol. The minimum is found with a steepest descent with gradient,

$$\nabla_{\tau} = \left[\partial/\partial \left(1/\tau_T^0 \right), \partial/\partial \left(1/\tau_T^1 \right) \right]. \quad (24)$$

The initial value is set with Theorem 3.7 of Wang and Beaumont (2004). Once $\tau_T^{(\iota)}$, $\iota \in [0, 1]$ is estimated, procedures of Sect. 4.2 are applied on the smoothed data. The result of the steady state estimation on the noisy current is illustrated in Fig. 13A.

Time constants are estimated with Theorem 4.1 and Algorithm 4.4 of Wang and Beaumont (2004) applied to the data envelope generated by adding $\pm 5\%$ to the data. This converts the lines of the jaw in the inversion of $y_i(u)$ $\iota \in [0, 1]$ to ribbons, which widens the invertible range. In this case, the inversion is now more complicated. It is important to note that, due to the nonlinearity of the model, the bounds on the time constants at each potential are not necessarily on the frontier of the bands of invertible values. Thus, once the bound on R is defined, we sweep R within its bounds with 100 samples, and estimate $\tau_T^{(\iota)}$, $\iota \in [0, 1]$ for each sample. The values obtained are displayed with a symbol in Figs. 13B,D. Figure 13B shows the inversion predicts a wide range of acceptable activation time constants. Figure 13C further supports this point. It shows the smoothed data, within the noise (5 %), and a current generated with a model having a conductance of $\bar{g} = 31 \text{ mS/cm}^2$ and time constants at test potential of $\tau_0 = 0.0509 \text{ ms}$ and $\tau_1 = 0.3028 \text{ ms}$, are quite different from the original model, with $\bar{g} = 23 \text{ mS/cm}^2$ and time constants at test potential of $\tau_0 = 0.08 \text{ ms}$ and $\tau_1 = 0.25 \text{ ms}$.

Note the symbols in Figs. 12B,D represent a space where we can find functions of voltage representing the time constants that reproduce the data. Additional work is required to extract these functions. Specifically, one should pick a value of R within the bounds, and apply the inversion procedure. This very procedure is what allows to handle the effect of noise in a novel way. As the noise on the data increases, the bounds on R widen, and increase the variety of time constants that can reproduce the data. Furthermore, because inversion is performed for each voltage of the stimulation protocol, the effect of noise is taken into consideration locally. The functions extracted are not just simple offset of basic functions, as for each R , they may vary significantly.

5 Discussion

There are three elements of concern when dealing with the estimation of the parameters of nonlinear ODEs, whether or not: (i) the data constrain the model, (ii) we have reached an optimal solution, and (iii) the problem ill/well posed.

1. Constrained

As illustrated in the treatment of biological data an important advantage of our approach is its ability to determine, *a priori*, whether the data constrains the Hodgkin–Huxley gating model. This advantage stems from the fact that we invert the data for model components (steady states and time constants), and bound some of the other components (conductance). This has two direct implications for the modeling method in the field. First, if the data does not sufficiently constrain the gating model, we can extract several models capable to reproduce it. These could then be studied through bifurcation analysis. Second, the method enables us to readily investigate stimulation protocols that can generate complete data sets with respect to the inversion of the Hodgkin–Huxley formalism. We did so introducing the 4 complementary stimulations protocols illustrated in Figs. 10 and 12. Note we do not claim these protocols are the only ones that can generate a complete data set for inversion of the Hodgkin–Huxley formalism. There certainly exist other possibilities. This granted, an advantage of the protocols presented here is their practicality because they take into consideration experimental constraints.

Interestingly, we established that currents generated exclusively with the H-step and T-step protocols cannot constitute a complete data set with respect to the inversion of the Hodgkin–Huxley formalism. This does not mean currents produced by these protocols are not useful. One can, for example, draw conclusions on an intervention, e.g., the effect of a drug, comparing currents collected with these protocols with and without drug binding to a channel. The problem we are stressing herein, is the estimation of the parameters (including functions of voltage) of the Hodgkin–Huxley model from experimental data, which by the way shares concerns with the estimation of parameters for any ordinary differential equation from experimental data.

2. Optimal

Most of the time, parameter estimation for nonlinear ODE models is achieved with nonlinear least square fitting. As a matter of fact, Willms et al. (1999) and Lee et al. (2006) have also presented excellent implementations of such approach for the Hodgkin–Huxley formalism. An inherent difficulty of such an approach is that, because most of the time we do not know the shape of the objective function subjected to minimization, one can never guarantee an optimal inverse solution is reached. Here we avoided this difficulty determining, for each objective function to minimize, the number of their extrema and presented strategies to locate them. Indeed, for this reason, our approach alleviates several limitations inherent to nonlinear least square fitting.

3. Ill-posed

Our approach allows modelers to cope with the ill-posed nature of the estimation problem in a novel way. This is illustrated in Sect. 4.3, where, from only 5 % noise on the data we were able to determine this can generate 50 % change on the activation time constant, even if the data set is complete. To find the range on the inverse solution, we first bounded the parameter R , then we explored the inverse solutions within these bounds. Due to the nonlinearity of the Hodgkin–Huxley gating model, the time constants at each data point deviating the most from the model are not necessarily associated to the values of R on the

frontier of the invertible range. Indeed, our extensive exploration of the inverse solution enabled us to find a wider range for the inverse solution than initially suspected.

One may argue that a similar effect could be achieved formulating a model through nonlinear fitting and then perturbing the functions. However, to achieve this with the resolution in voltage that is related to the number of samples taken during acquisition, one would need to use functions including a large number of parameters. In this case, the minimization becomes problematic because the algorithm may be trapped in local minima. Indeed, as the number of parameters increase, it becomes unlikely to find the optimal solutions.

Another group (Lee et al. 2006) reached a different conclusion. With 5 % to 15 % noise on the data they reported only 12 % variation on the activation time constant. Their approach is based on the minimization of 3 nonlinear objective functions, and their statistics follow from 100,000 repetitions of the minimization with random startup values. We attribute the discrepancy between these two sets of results to the fact that their objective functions may have several local minima providing good inverse solutions that have not been explored during minimization. Although the number of trials is large, the dimension of their search space is also very large, i.e., a parameter, plus 3 others for each potential. Thus, without information on the shape of the objective function, it is impossible to know whether the start-up values for minimization allow for the full exploration of the search space.

An important remark to make is that, while we have considered a range on the conductance and time constants, we did not consider one on the steady states. This would allow for the extractions of an even larger number of models that can reproduce a data set within noise. However, this would tremendously complicate the process, and will be addressed in subsequent work.

Finally, this approach may have a number of implications for multiscale modeling of bioelectric phenomena. For example, changes on steady state activation, and activation time constant of the order of magnitude shown in Fig. 13, can produce a wide range of predictions regarding vortex dynamics (Beaumont et al. 1998) in a monolayer of cardiac cells. While parameter sensitivity analysis as described by Sobie (2009) has a number of advantages, the combination of inversion and bifurcation analysis, as exposed above, may enable one to uncover a range of model predictions that would not be suspected otherwise. Indeed, the application of such an approach in a multiscale modeling framework, where we analyze voltage clamp data gathered in cell expression systems, may enable us to capitalize on the human genome in a novel way in our quest to elucidate the molecular mechanisms of arrhythmias.

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Fig. 1.

Ebihara and Johnson current model of the cardiac sodium current ($\bar{g} = 23 \text{ mS/cm}^2$, $\lambda_0 = 3$, $\lambda_1 = 1$). **A** steady state, **B** time constants of the gating variables. **C** representative currents recorded during voltage clamp stimulation at potentials $u_H = [-80, -70, -60, -50, -40] \text{ mV}$, and $u_T = 10 \text{ mV}$

Fig. 2.

Equivalent electrical circuit for voltage-clamp recordings used for removal of the capacitive transient. I_d : Total recorded current, I_{Na} : Sodium current, V_r : Reference voltage, R_s : Series resistance, C_m : Membrane capacitance, R_m : Membrane resistance, e : Reversal Potential, R_L : Leak resistance

Fig. 3.

Linear relationship between peak currents amplitude and cell size (*solid line*) deduced from peak currents recorded at a specific reference voltage of a T-step protocol in epicardial cells. Each *symbol* is the rescaled peak current in the same protocol, but recorded at another voltage. The scaling factor, one number for each voltage other than the reference voltage, normalizes the current in the smallest cell to 2 nA, the first point on the curve. See text for details

Fig. 4.
Currents recorded at the same voltage during the H-step and T-step protocols, $u_H = -120$ mV and $u_T = -10$ mV, but gathered on different groups of cells

Fig. 5.
Example of difference in cell size isolated from the epicardial layer

Fig. 6.

Averaged currents of the H-step and T-step protocols for endocardial and epicardial cells after preprocessing of the raw data provided by Cordeiro et al. (2008). Currents shown from the T-step protocol are conducted with $u_H = -120$ mV and $u_T \in [-50, 15]$ mV by 5 mV. The H-step protocol was conducted with $u_H \in [-120, -25]$ mV by 5 mV and $u_T = -10$ mV. In the titles, n indicates the number of cells averaged

Fig. 7. Voltage dependence of $s_i(u)$ $u \in [0, 1]$. Values found through inversion are marked with circles. Solid and dashed activation curves represent 2 possible models from which we are then able to extract time constants to reproduce the currents

Fig. 8.

Voltage dependence of $\tau_l(u)$ extracted with the inversion procedure and using two different current models, the difference being in $s_0(u)$ corresponding to the solid and broken lines in Fig. 7. The functions $s_0(u)$ employed were the ones illustrated in this figure. **A, B** endocardial cells. **C, D** epicardial cells. *Solid line and triangles* were extracted using $s_0(u)$ corresponding to the solid line with $R = 0.0333$ and $R = 0.0526$ for endocardial and epicardial data, respectively. *Dashed line and Xs* were extracted using $s_0(u)$ corresponding to the dashed line with $R = 0.4662$ and $R = 0.0968$ for endocardial and epicardial data, respectively

Fig. 9.

Currents from the H-step (**A, B**) and T-step (**C, D**) protocols. Experimental data (*solid lines*), and model (*dashed lines*). The time constants used in the model here are marked with a solid line in Fig. 8

Fig. 10.

A, C Voltage clamp stimulation protocols employed to estimate the steady states. **B, D** Steady states of the gating variables. The protocols depicted in the same row as the steady state provide the data necessary to estimate the steady states in the shaded voltage range. **E** Results of the estimation with protocols of panels **A** (*squares*) and panel **C** (*triangles*) superimposed to the steady states

Fig. 11.

Currents generated by the Ebihara and Johnson model (Ebihara and Johnson 1980) subjected to the C-step stimulation protocol of Fig. 10. The protocol has parameters $u_H = -120$ mV, $u_T = -10$ mV, $u_C \in [-55, 0]$ every 5 mV. The conditioning pulse durations were 0.2 ms (*left*) and 0.4 ms (*right*)

Fig. 12.

A, C Voltage clamp stimulation protocols suitable for time constants estimation. **B, D** the voltage range where the parameters are estimated using protocols A and C, respectively. **E, F** activation and inactivation time constants estimated using the inversion method. *Solid lines*: actual model, *symbols*: inversion results with protocols A (*triangles*), and C (*squares*)

Fig. 13.

A Model steady states (*solid lines*) and estimated values (*symbols*) on noisy data, **B, D** Model activation (**B**) and inactivation (**D**) time constants (*solid lines*) as well as estimated values on noisy data (*symbols*). **C** Typical noisy current with its filtered version (*thick black line*), and a current produced by a model (*thin black line*) the parameters of which were obtained through the inversion. The parameters of the voltage clamp stimulation are $u_H = -100$ mV and $u_T = -10$ mV. The model has a $\bar{g} = 23$ mS, and for this $u_T t_0(-10) = 0.08$ ms and $\tau_1(-10) = 0.25$, where the values are within the range of the inversion. Clearly, as judged by panel **B**, a wide range of parameters allow to reproduce this current