Identifiability analysis and parameter estimation of a single Hodgkin-Huxley type voltage dependent ion channel under voltage step measurement conditions

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Abstract

Identifiability analysis of a single Hodgkin-Huxley (HH) type voltage dependent ion channel model under voltage clamp circumstances is performed in order to decide if one can uniquely determine the model parameters from measured data in this simple case. It is shown that the two steady-state parameters (m_∞, h_∞) and the conductance (g) are not globally identifiable together using a single step voltage input. Moreover, no pair from these

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three parameters is identifiable. Based on the results of the identifiability analysis, a novel optimization-based identification method is proposed and demonstrated on in silico data. The proposed method is based on the decomposition of the parameter estimation problem into two parts using multiple voltage step traces. The results of the article are used to formulate explicit criteria for the design of voltage clamp protocols.

1. Introduction

The HH (Hodgkin-Huxley) modelling formalism of membrane currents and cell electrophysiology is one of the most widely used framework for the purpose of modelling excitable cells [1]. HH models, that are essentially nonlinear electrical circuit models, are composed of parallel voltage dependent (and possibly voltage independent) conductances, that correspond to various types of membrane currents. The dynamical descriptions of neuronal behavior, ranging from the fundamental theoretical principles [2, 3, 4] to the wide range of applications with special focus, are predominantly based on this model class.

Because of the theoretical and practical importance of HH models, a large number of papers are devoted to their parameter estimation under various conditions, applying different approaches and estimation techniques. However, the fundamental question, whether it is at least theoretically possible to determine all of the model parameters from the measured data - that is,

the question of theoretical identifiability - has not even been raised for HH models.

The concept and importance of identifiability. Once the model structure is fixed (see later Eqs. (2)-(7) in our case), one can perform parameter estimation, the quality of which is crucial in subsequent usability of the obtained model [5]. The *structural identifiability* properties of the system describe whether there is a theoretical possibility for the unique determination of system parameters from appropriate input-output measurements or not. It is important to emphasize, that *identifiability is a property of the model structure*, the analysis of which should ideally precede any model parameter estimation. Basic early references for studying identifiability of dynamical systems are [6, 7]. It has been clearly shown in the case of process systems, that prior structural identifiability analysis is an important step in the solution of model calibration problems [8]. The paper [9] solves the problem of structural parameter identifiability for chemical reaction network models.

The study and development of differential algebra methods, which are used for identifiability analysis, contributed to the better understanding of important system theoretic problems [10, 11]. The most important definitions and conditions of structural identifiability for general nonlinear systems were presented in [12] in a very clear way. Further developments in the field include the identifiability conditions of rational function state-space models [13], and the possible effect of special initial conditions on identifiability [14].

The importance of identifiability has been also stressed in the context of biological models [15, 16, 17, 18]. However, many modeling and parameter estimation studies in computational biology still continue to ignore this key

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property.

Parameter estimation and identifiability-related results of HH models. Several articles have been published which are focusing on parameter estimation problem in the case of HH based models under various assumptions. Most of the published work [19, 20, 21, 22, 23, 24, 25] is considering current clamp setup, when the voltage traces are measured in the case of known injected currents or unknown synaptic currents. In addition, a significant part of literature data assumes prior knowledge regarding the channel kinetics [19, 22, 21, 25]. The article [20] provides a survey of automated parameter-search methods for compartmental neural models, regarding also the parameters of activation and inactivation curves. The articles [26, 27, 28, 29, 30] consider voltage clamp scenarios (in this case the voltage is fixed, and transmembrane current traces are measured). In [29, 30, 31] a computationally effective global search method, differential evolution is applied.

Although the explicit identifiability properties are not addressed in the above papers, they discuss several issues, which are related to identifiability. The question whether the particular parameter values selected are the only viable parameters or just one of several possible solutions, has been addressed in [19]. The paper [32] also discusses emerging identifiability problems in the case of HH based neuronal models. In this article the authors derive 20 different computational models for the cerebellar Purkinje cell, which produce very similar outputs to current injections, and analyze their geometry in the parameter space. The article [22] considers an estimation problem of a multicompartmental model based on voltage traces, and shows that if we assume the knowledge of channel kinetics, the channel densities (in addition

intracompartmental conductances and overall strength of the presynaptic input) can be determined. Furthermore, the article shows that the proposed method leads to algorithms that are guaranteed to converge to the unique optimum. We will see later that identifiability results described in this article regarding the voltage clamp case support this observation (if channel kinetics are known, the maximal conductance can be uniquely determined).

Regarding the results corresponding to voltage clamp setup, the articles [26, 28] realized the weaknesses of the conventional estimation algorithms, which originate from the assumption of separated activation and inactivation processes, and provided improved methods for the estimation of HH models. Lee et al. in [28] proposed a new numerical approach to interpret voltage clamp experiments. Moreover, it is claimed in [28], that all channel parameters can be determined from a single appropriate voltage step, but this statement has not been proven rigorously for the whole meaningful parameter space. In addition, the numerical method proposed in [28] is based on the determination of the time and value of the maximal current during the voltage step measurement, but, as we will also show, a local maximum does not necessary appear in every case.

Aims. Because of the lack of identifiability results even in the simplest possible HH model with just a single ion channel, the primary aim of this paper is to carry out a rigorous identifiability analysis in this simplest case under voltage clamp measurement conditions. We want to show that identifiability problems may arise even in the very simple case of one HH channel with unknown kinetics and a single voltage step measurement protocol.

An additional goal of the paper is to propose a well grounded parameter

estimation method for the maximal conductance and the kinetic parameters of the channel based on the results of identifiability analysis that is able to handle the possibly appearing identifiability problems in the analyzed case.

2. Materials and Methods

In this section the model framework, the assumed measurement protocol, and the methods applied for identifiability analysis are described.

2.1. Ion channel model

We consider a simple hypothetical ion channel with one activation (m) and one inactivation variable (h). According to the most widespread notation in computational neuroscience (see for eg. [4]), the current, which is the measured variable, is simply described by

$$I = gm^{p_m}h^{p_h}(V - E) \tag{1}$$

where V [mV] is the voltage, g [nS] is the maximal conductance, and E [mV] is the reversal potential of the corresponding ion. The positive integer exponents p_m and p_h correspond to the number of independent subunits of the voltage channel protein. We will assume the simplest case in our calculations when $p_m = p_h = 1$. If p_m and $p_h \neq 1$, but their values are known, the estimation algorithm proposed in section 4 may be used with the corresponding straightforward modification.

Both m and h are state variables in the following nonlinear state-space

model

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)} \tag{2}$$

$$m_\infty(V) = \left(1 + exp\left(\frac{V_{1/2m} - V}{k_m}\right)\right)^{-1}, \quad k_m > 0 \tag{3}$$

$$\frac{1}{\tau_m(V)} = \left(c_{bm} + c_{am}exp\left(-\frac{(V_{Maxm} - V)^2}{\sigma_m^2}\right)\right)^{-1} \tag{4}$$

$$\frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h} \tag{5}$$

$$h_\infty(V) = \left(1 + exp\left(\frac{V_{1/2h} - V}{k_h}\right)\right)^{-1}, \quad k_h < 0 \tag{6}$$

$$\frac{1}{\tau_h(V)} = \left(c_{bh} + c_{ah}exp\left(-\frac{(V_{Maxh} - V)^2}{\sigma_h^2}\right)\right)^{-1} \tag{7}$$

where $V_{1/2m}$, k_m, $V_{1/2h}$, and k_h are the parameters of the Boltzmann functions which describe the steady state activation and inactivation values c_{bm}, c_{am}, V_{Maxm}, σ_m, c_{bh}, c_{ah}, V_{Maxh} and σ_h denote the parameters of Gauss-functions which describe the voltage dependent time-constants [1].

As described in section 1, in this paper we will consider *voltage clamp*

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[1]We have to note that the approximation of the steady state values with Boltzmann functions is not always realistic, as it is described in [26]. However, in the rest of this paper we assume that this assumption holds. It can be said that the use of Boltzmann-type sigmoid functions for the description of steady-state values in the literature is widespread, but not exclusive (see e.g. [33]). The description of the voltage dependent time constants in the literature is more diverse. In fact, the wide set of possible time constant curves corresponding to various rate constant functions is described in [26]. The applied Gauss-functions are an approximation of the skewed bell shape curves, resulting from the rate constant based description, where the rate constants depend exponentially on the voltage (see [26]).

measurement conditions, when the voltage is determined and the transmembrane currents are measured.

We have to note that because of the bifurcation structure of HH models, small estimation errors of ion channel properties based on the voltage clamp setup may imply significantly different behavior at the membrane voltage level, if the voltage is not fixed. However the description of membrane voltage dynamics is not in the scope of this paper.

2.2. Voltage step protocol

An important version of the voltage clamp method is when the voltage, which is in this case the manipulable input (u) of the system, is held piecewise constant ($V(t) = u(t) = V_k$ for $t_k \leq t < t_{k+1}$, $k = 1, \ldots, N$). Thus, during each interval, the values of m_∞, h_∞, τ_m and τ_h can be considered as time-invariant parameters in addition to g and E. This implies that the non-polynomial nonlinearities of Boltzmann and Gauss functions are naturally eliminated from the equations, and the model will fall into the class of polynomial systems, which makes the application of effective computer algebra based software tools (e.g. DAISY [34]) possible for identifiability testing. Moreover, we also point out that this way we also neglect the prior knowledge that the activation and inactivation functions are described by Boltzmann and Gauss functions.

We will denote the voltage independent nature of the above parameters shortly by suppressing the V argument, i.e. $m_\infty(V) = m_\infty$, $\tau_m(V) = \tau_m$, $h_\infty(V) = h_\infty$ and $\tau_h(V) = \tau_h$, with $V = V_0$. In this case, Eqs. (1-7) are simplified as follows:

$$I = gmh(V_0 - E) = gmh(u - E) \tag{8}$$

$$y = I, \quad u = V_0 = const.$$

$$\frac{dm}{dt} = \frac{m_\infty - m}{\tau_m} , \quad \frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \tag{9}$$

where the model parameters are $g, E, m_\infty, \tau_m, h_\infty$ and τ_h.

2.3. Structural identifiability notions and tools

In general let us consider the following class of models

$$\dot{x} = f(x, u, \theta), \quad x(0) = x_0 \tag{10}$$

$$y = h(x, u, \theta)$$

where $x \in \mathbb{R}^n$ is the state vector, $y \in \mathbb{R}^m$ is the output, $u \in \mathbb{R}^k$ is the input, and $\theta \in \mathbb{R}^d$ denotes the parameter vector. We assume that the functions f and h are polynomial in the variables x, u and θ. We remark that majority of nonlinear state-space models with smooth right-hand sides can also be embedded into the above polynomial model form (10) on the price of increasing the state space dimension [35].

2.3.1. The notions of structural and practical identifiability

The problem statement of structural identifiability analysis is to determine, whether there is a theoretical possibility for the unique determination of model parameters from measurement data. Shortly speaking, *global structural identifiability* means that

$$y(t|\theta') \equiv y(t|\theta'') \Rightarrow \theta' = \theta'' \tag{11}$$

where

$$y(t|\theta) = h(x(t, \theta), u(t), \theta) \tag{12}$$

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and $x(t, \theta)$ denotes the solution of (10) with parameter vector θ. This means that if the system outputs are identical, then the underlying parameters are necessarily the same: this is a model property, e.g. the property of (10). According to (11), a structurally non-identifiable model may produce exactly the same observed output with different parametrizations. This is clearly a fundamental obstacle of determining the true model parameters from measurements irrespectively of the applied estimation method (however sophisticated it is), even if the selected model structure is considered to be correct. The lack of structural identifiability can usually be fixed by incorporating more prior information into the modeling process e.g. in the form of model parameter constraints, by changing the input/output configuration, or by modifying the internal model structure in case of need. If (11) is valid only in a subset of the studied parameter space, then the model is called *locally structurally identifiable*.

Even if the conditions of structural identifiability are fulfilled, we are often faced with serious computational difficulties during the implementation of the actual parameter estimation procedure. These problems are usually referred to as *practical identifiability* problems, and they are most often caused by the scarcity and/or the noisiness of measurement data, by low output sensitivity to certain parameters, or simply by inappropriately designed input signals. Beside more advanced measurement technology, the results in this case can often be greatly improved using optimal experiment design techniques [16].

2.3.2. Global structural identifiability analysis using differential algebra

The following notations, definitions and conditions are mostly taken from [12]. Let us denote a differential polynomial $F(u, \dot{u}, \ldots, y, \dot{y}, \ldots)$ by $F(u, y; p)$

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where $p = \frac{d}{dt}$.

The structure (10) is *globally identifiable* if and only if by differentiating, adding, scaling and multiplying the equations the model can be rearranged to the parameter-by-parameter linear regression form:

$$P_i(u, y; p)\theta_i - Q_i(u, y; p) = 0 \quad i = 1, \ldots, d \tag{13}$$

It is visible from (13) that θ_i can be expressed as

$$\theta_i = \frac{Q_i(u, y; p)}{P_i(u, y; p)} \quad i = 1, \ldots, d \tag{14}$$

if P_is are non-degenerate. The non-degenerate condition can be fostered by ensuring that the inputs excite the system dynamics sufficiently so that the parameter vector can be determined in a numerically well-conditioned way.

2.3.3. Structural identifiability analysis using Taylor series expansion of the output

Consider again the nonlinear model structure in (10). The well-known paper [36] gives the following condition for global structural identifiability based on the Taylor series expansion of the system output. Let

$$c_k(\theta) = \lim_{t \to 0+} \frac{d^k}{dt^k} y(t, \theta) \tag{15}$$

Then a sufficient condition of global structural identifiability is

$$c_k(\theta_1) = c_k(\theta_2), \ k = 0, 1, \ldots, k_{max}, \implies \theta_1 = \theta_2 \tag{16}$$

where k_{max} is a positive integer (small enough for the symbolic computations to remain tractable). It is important to remark that the lack of global solvability of c_k for the system parameters in the case of a given k_{max} value

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is generally not enough for proving non-identifiability, since the inclusion of higher derivatives (new c_k-s) may result in the solvability of the corresponding system of nonlinear equations.

3. Identifiability Results

In this section the obtained results corresponding to structural identifiability properties of ion channel models under voltage step measurement conditions, and the proposed parameter estimation method based thereon are described.

3.1. Identifiability analysis using differential algebra

The identifiability analysis described in section 2.3.2 requires the elimination of the differential (state) variables m and h from the model Eqs. (8)-(9) and then finding the parameter groups that can be determined from the resulting equations. For convenience, let us introduce the following parametrization:

$$
\begin{aligned}
&x_1 = m, \ x_2 = h \\
&p_1 = \frac{1}{\tau_m}, \ p_2 = m_\infty, \ p_3 = \frac{1}{\tau_h} \\
&p_4 = h_\infty, \ p_5 = g, \ k_1 = u - E
\end{aligned}
\tag{17}
$$

It can be seen that the physical system parameters are trivially computable, if $p_1, \ldots, p_5$ are given. In general, we assume that k_1 is known (this means that we assume known reversal potential), and we are searching for the largest subset in $\{p_1, \ldots, p_5\}$ that is globally identifiable. Using Eq. (17), the state

and output equations of the simple model can be written as

$$\dot{x}_1 = p_1(p_2 - x_1), \ \dot{x}_2 = p_3(p_4 - x_2) \tag{18}$$

$$y = k_1 p_5 x_1 x_2 \tag{19}$$

To get a pure input-output relation, we have to eliminate the state variables from Eqs. (18)-(19). For this, the time-derivative of y is taken that gives

$$\dot{y} = (-p_1 - p_3)y + k_1 p_5 p_3 p_4 x_1 + k_1 p_5 p_1 p_2 x_2. \tag{20}$$

By taking the second derivative of y with respect to time, the following equation is obtained

$$\ddot{y} = (-p_1 - p_3)\dot{y} - k_1 p_5 p_1 p_3 p_4 x_1 - k_1 p_1 p_1 p_2 p_3 x_2$$
$$+ 2k_1 p_1 p_2 p_3 p_4 p_5 \tag{21}$$

It can be observed that both Eq. (20) and Eq. (21) depend linearly on x_1 and x_2, therefore the state variables can be expressed from them and substituted to the original output equation (19) in a straightforward way. This property is often called *algebraic observability* [10, 34]. The expression and substitution results in the following lengthy input-output relation

$$0 = (-a_0 - a_1 a_5 - a_1 a_3)y - (a_1 a_4 - a_1 a_2)\dot{y}$$
$$- 2a_1\ddot{y} + (a_2 a_5 + a_3 a_4)y\dot{y} + (a_2 + a_4)\dot{y}\ddot{y} + (a_3 + a_5)y\ddot{y}$$
$$+ a_3 a_5 y^2 + a_2 a_4 \dot{y}^2 + \ddot{y}^2 + a_1^2 \tag{22}$$

where $a_0, \ldots, a_5$ are defined as

$$a_0 = (p_3^2 - p_1 p_3)(k_1 p_1^2 p_2 - k_1 p_1 p_2 p_3)^2 p_4 p_5,$$
$$a_1 = 2k_1 p_1 p_2 p_3 p_4 p_5, \ a_2 = 2p_1 + p_3$$
$$a_3 = p_1^2 + p_1 p_3, \ a_4 = p_1 + 2p_3, \ a_5 = p_1 p_3 + p_3^2 \tag{23}$$

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The coefficients in Eq. (22) define the following set of equations for the nine coefficients $c_i, i = 1, ..., 9$

$$-a_0 - a_1 a_5 - a_1 a_3 \;=\; c_1 \tag{24}$$

$$-a_1 a_4 - a_1 a_2 \;=\; c_2 \tag{25}$$

$$-2a_1 \;=\; c_3 \tag{26}$$

$$a_2 a_5 + a_3 a_4 \;=\; c_4 \tag{27}$$

$$a_2 + a_4 \;=\; c_5 \tag{28}$$

$$a_3 + a_5 \;=\; c_6 \tag{29}$$

$$a_3 a_5 \;=\; c_7 \tag{30}$$

$$a_2 a_4 \;=\; c_8 \tag{31}$$

$$a_1^2 \;=\; c_9 \tag{32}$$

The solvability of Eqs. (24)-(32) with respect to the parameters $p_1, \dots, p_5$ can be checked by e.g. Buchberger's algorithm (see, e.g. [14]). Using this method, the following parameter-pairs can be shown to be globally identifiable: (p_1, p_2), (p_1, p_4), (p_1, p_5), (p_2, p_3), (p_3, p_4), (p_3, p_5). The following parameter combinations turned out to be locally identifiable (with 2 possible solutions for each): (p_1, p_3), (p_1, p_2, p_3), (p_1, p_3, p_4), (p_1, p_3, p_5).

For comparison, the identifiability analysis technique based on the Taylor series expansion of the output has been applied, too, that is described in the following subsection.

3.2. Structural identifiability analysis using the Taylor series method

To keep the original physical parameters (or their simple transformations), let us use the previously defined parametrization (17) of the ion chan-

nel model.

The solution of the state equations (18) is easy to give with zero initial condition:

$$x_1(t) = -p_2 e^{-p_1 t} + p_2 \tag{33}$$

$$x_2(t) = -p_4 e^{-p_3 t} + p_4 \tag{34}$$

From this, the output and its successive derivatives are given by

$$y(t) = k_1 p_2 p_4 p_5 (1 + e^{-(p_1+p_3)t} - e^{-p_1 t} - e^{-p_3 t})$$

$$\dot{y}(t) = k_1 p_2 p_4 p_5 (-(p_1+p_3)e^{-(p_1+p_3)t} + p_1 e^{-p_1 t} + p_3 e^{-p_3 t})$$

$$\ldots \tag{35}$$

$$y^{(k)}(t) = k_1 p_2 p_4 p_5 ((-1)^k (p_1+p_3)^k e^{-(p_1+p_3)t} +$$

$$+ (-1)^{k+1}(p_1^k e^{-p_1 t} + p_3^k e^{-p_3 t})), \ k \geq 1.$$

From Eq. (35), the coefficients $c_k(\theta)$ can be computed as

$$c_0(\theta) = 0$$

$$\ldots \tag{36}$$

$$c_k(\theta) = k_1 p_2 p_4 p_5 ((-1)^k (p_1+p_3)^k +$$

$$+ (-1)^{k+1}(p_1^k + p_3^k)), \ k \geq 1.$$

By the symbolic solution of (36), the following parameter pairs were found to be globally identifiable: (p_1, p_5), (p_1, p_2), (p_3, p_2), (p_3, p_5), (p_1, p_4), (p_3, p_4). The pair (p_1, p_3) was found to be locally identifiable with 2 possible solutions as well as the triplets (p_1, p_3, p_5), (p_1, p_3, p_4), (p_1, p_3, p_2).

3.3. Discussion of identifiability results

First we have to emphasize again, that the determination of the identifiability properties of a model is an important model-analysis result, which

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should precede the parameter estimation in ideal case. If identifiability problems arise in a model with an assumed input-output configuration, this will lead to the lack of unique global extremum regarding the optimization problem corresponding to parameter estimation. In this case, the parameter estimation process either has to be completed with additional measurements corresponding to different input-output configurations (regarding neuronal models, one may e.g. consider using both voltage clamp and current clamp data), or reinterpretation of the measurement results is needed, taking into account additional assumptions regarding model properties (see later in subsection 4).

Comparing the results in sub-sections 3.1 and 3.2 above one can observe, that the two methods gave exactly the same globally and locally identifiable parameter combinations. We remark that the necessary computations for both methods were performed using the freely available Sage symbolic computation software environment (see. e.g. [37], [38]).

The maximal number of identifiable parameters (i.e. the limits of structural identifiability) in the case of a single voltage step measurement were well-observable from the results of the differential algebra method. Moreover, it is visible from Eq. (22) that this method (if successful) finally gives us such a dynamical description that is linear in the transformed model parameters (i.e. a regression form model). This theoretically allows us to construct such an objective function for the parameter estimation that is convex in the transformed parameters (e.g. such a one that is a quadratic function of the prediction error). However, it is often not practically feasible to compute the required higher derivatives of the measured system output.

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On the other hand, the smaller set of nonlinear equations in the case of the Taylor series method was much more easily tractable with symbolical software. Furthermore, it can be seen from the closed form of Eqs. (36) that neither (p_2, p_4, p_5), nor any pair from these three parameters can be globally identifiable.

To check and support our former calculations, we also used the differential algebra software DAISY [34]. Firstly, the output of DAISY showed that the model is algebraically observable, which is in good agreement with our results regarding the elimination of differential variables. Secondly, according to the identifiability results of the analysis, the parameters m_∞, h_∞ and g (i.e. p_2, p_4, p_5) are not globally identifiable. Moreover, no pair from these three parameters are identifiable. This fact also matches the results of sub-sections 3.1 and 3.2 where we couldn't show that these three parameters (or any two of them) are identifiable under voltage clamp measurement conditions, assuming a single voltage step.

The above results are well understandable in the case of steady state, when $m = m_\infty$ and $h = h_\infty$, because in this case only the product of the three parameters appears as output in $y = I = gmh(V - E)$. However, the dependence also holds during the transient period. In Appendix A a possible scenario is described to demonstrate that the described non-identifiability properties may cause problems in the case of a realistic voltage step protocol.

The local identifiability of (p_1, p_3) implies that both voltage dependent time constants can be attempted to be estimated at each voltage value (locally), if the other parameters are known. This fact will be exploited later in section 4 during the construction of the proposed parameter estimation

method.

4. Parameter Estimation

In this section we propose a parameter estimation method based on the results of the identifiability analysis. The main idea of the method is based on the decomposition of the parameter estimation problem into two consecutive steps as follows

1. estimation of conductance, activation and inactivation parameters from the steady-state current values of multiple voltage clamp traces,
2. estimation of the voltage dependent time constants based on the entire current response.

The main motivation of the decomposition of the parameter estimation process is to handle the possibly arising identifiability problems (there may be certain model parametrizations and protocols in the case of which g, m_∞ and h_∞ can not be uniquely determined from a single voltage step) described in section 3.3. With the application of steady state currents, the three parameters, between which identifiability problems (interdependence) may arise can be estimated separately from other parameters (time constants). As we will see in the next subsection, with the utilization of prior information, these issues can be addressed. Furthermore, as we will see later in sections 4.1.1 and 4.2, this solution is a computationally efficient estimation, that improves the overall computational performance.

The properties of the proposed method are investigated in the case of data originating from simulation (in other words, using in silico data). If experimental data were used, we did not know what the exact solution was,

and therefore the error could not be estimated. With simulated data, we are able to characterize the component of error arising from the numerical approach, and obviate the effects of experimental noise. Moreover, the structural identifiability results are independent of the measurement data source.

4.1. Analysis of the proposed method

4.1.1. Estimation of conductance and activation parameters

As it has been shown in the previous sections, the parameters g, m_∞ and h_∞ are not globally identifiable using a single voltage step input. We can circumvent this problem by using multiple voltage steps, and by utilizing the prior knowledge that the voltage dependence of the steady state values of activation and inactivation functions are described by Boltzmann-functions (see Eqs. (3) and (6)).

In the first step of the method, we will analyze only the steady state currents in the case of n distinct measured input voltage values (V_i, $i \in \{1, ..., n\}$). In this case, the following set of nonlinear algebraic equations hold

$$I_i = gm_{\infty i}h_{\infty i}, \quad i = 1, \ldots n. \tag{37}$$

The activation and inactivation functions are given by

$$m_{\infty i} = \left(1 + exp\left(\frac{V_{1/2m} - V_i}{k_m}\right)\right)^{-1}, \quad h_{\infty i} = \left(1 + exp\left(\frac{V_{1/2h} - V_i}{k_h}\right)\right)^{-1} \tag{38}$$

The unknown variables to be determined using eqs. (37) and (38) are g, $V_{1/2m}$, k_m, $V_{1/2h}$ and k_h. Additionally, it is known that $k_m > 0$ and $k_h < 0$.

The objective function for the parameter estimation is defined in a stan-

dard way as

$$W_1(\theta_1)_{VC} = \sum_{i=1}^{n} (I_i - gm_{\infty i}h_{\infty i}(V_i - E))^2, \tag{39}$$

where $\theta_1 = [g\ V_{1/2m}\ k_m\ V_{1/2h}\ k_h]^T$, and $m_{\infty i}$ and $h_{\infty i}$ are given by Eq. (38). For the optimization process we used the efficient, gradient-free Nelder-Mead simplex algorithm to minimize the error [39]. The maximum iteration number was 1000, the tolerance of the objective function was 10^{-8}, and the tolerance of the parameter values was 10^{-3}.

We analyzed the convergence of the optimization for the following realistic parameter values: $g = 67\ nS$, $V_{1/2m} = -31.93\ mV$, $k_m = 13.03$, $V_{1/2h} = -44.35\ mV$, $k_h = -5.14$. Our results showed that the convergence properties of the algorithm to the global optimum strongly depend on the number of input voltage traces (n). The results of simulation experiments suggest that in order to obtain correct parameter estimation results, a lower bound for n is around 10, if the selected input voltage values cover their possible range in an equidistant way. The estimation results show that if only significantly less voltage steps with the corresponding steady state current values are available, the optimization problem will be badly conditioned, and the convergence properties deteriorate.

According to the simulation and optimization results, we observed that a sufficient (but not necessary) condition for the convergence to the global optimum in every case is that the initial parameter values for optimization should be in the approximately $\pm 25\%$ neighborhood of the true values. This can be regarded a realistic assumption, according to [28] and [26], which conclude that the estimation error of conventional algorithms (which are simply based on the fitting of exponentials to the current trace, and assume that

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activation and inactivation are separated in time and $m = h = 1$ at the maximum of the current curve) is in this range. This means that the conventional algorithms can be used to determine initial values for the optimization.

The proposed method is based on steady-state currents, and as a consequence, it works well only if there is a voltage interval present, where both the steady state activation and inactivation variables are different from zero. If this intersection interval is narrow, the convergence properties of the optimization can be significantly deteriorated by measurement noise. Without noise, the proposed method with 10 voltage steps ranging equidistantly from -80 to -8 mV still converged to the nominal parameters for e.g. in the case of activation/inactivation characteristics depicted in Fig. 1. However, a one order of magnitude higher number of iterations (i.e. a few thousand) was needed to find the nominal parameters compared to the better conditioned cases.
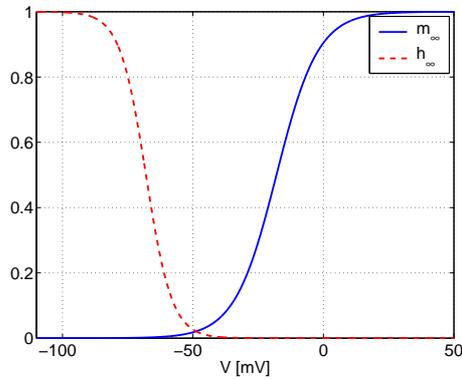
Figure 1: Activation and inactivation curves (m_∞ and h_∞), with narrow intersection (V-type curves)

We have to note that the basic Nelder-Mead simplex algorithm does not

handle constrains on the parameter values. In contrast, we have explicit constraints on the maximal conductance and the slope factor of the Boltzmann functions in our case, namely $g > 0$, $k_m > 0$ and $k_h < 0$. According to our experience, the appropriately tuned simplex based optimization usually does not result in parameter values that violate these constraints. Moreover, the simplex method's ability to effectively decrease the objective function value in the first few iteration steps is exceptionally good (see Fig. 2), and that was the main reason for choosing it. We note that there are other derivative-free optimization methods that can handle constraints, e.g. the freely available Asynchronous Parallel Pattern Search (APPS) algorithm [40]. The optimization did not require very high computational performance due to the static nature of the problem. The longest required computation time of the simplex based optimization was about 45 sec on a typical dual core desktop PC with 2 GB RAM [2]). This case corresponds to the activation functions with very narrow nonzero intersection depicted in Fig. 1 - dominantly the typical estimation time of conductance and activation parameters was about 2-3 sec. The evolution of the objective function during the optimization process in the case of a typical estimation scenarios of activation parameters is depicted in the left plot of Fig. 2.

4.1.2. Estimation of voltage dependent time constants

After the estimation of g and the parameters of the Boltzmann functions, our next task is to determine the time constants at the particular voltages defined by the applied voltage steps. In this case the global estimation of c_{bm},

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[2]In this case the maximal number of iterations was increased to 5000

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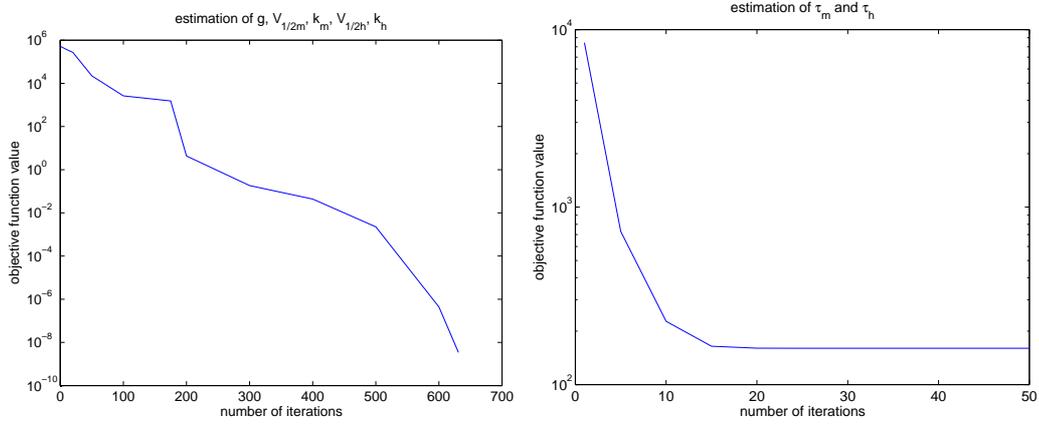
Figure 2: Demonstration of convergence properties: The evolution of the objective function during the global estimation of conductance and activation parameters (left plot), and during the estimation of time constants at a certain voltage level (right plot). In the case of the left plot, the error fell below the tolerance limit defined for the objective function in 4.1.1, which means that the value of the objective function is practically zero - this corresponds to the steady state in this case. In the right plot, the tolerance limit for the parameters was reached.

c_{am}, V_{Maxm}, σ_m, c_{bh}, c_{ah}, V_{Maxh} and σ_h in Eqs. (2)-(7) is also possible, but not needed, because the results of the identifiability analysis have shown that at a particular voltage value τ_m and τ_h are identifiable, which means that we can estimate τ_m and τ_h, locally at particular voltage values without the prior knowledge of their Gaussian-type voltage dependence. If we perform a series of such local estimations of τ_m and τ_h, we have to estimate only 2 parameters at the same time instead of 8.

For the identification of $\tau_m(V_i)$ and $\tau_h(V_i)$ at a certain voltage we can either use the method proposed in [28] (if a local maxima is present, which is the necessary condition of this method), or, similarly to [26], we can simply

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perform the minimization of the following objective function (we have chosen this latter possibility in this paper):

$$W_2(\theta_2)_{VC} = \frac{1}{N}\|I_{tot}^m - I_{tot}^s(\theta_2)\|_2 \qquad (40)$$

where θ_2 is the parameter vector (including $\tau_m(V_i)$ and $\tau_h(V_i)$), N is the number of data points in the measurement record, and I_{out}^m and I_{out}^s denote the measured and model computed total output current (as a discrete time sequence). The state trajectories, which determine the computed current can be determined either by explicit solution of the differential equations, as described in [28].

The convergence to the global optimum (i.e. to the nominal parameters) and the remaining error depend on the value of the voltage steps, but in this case also on the holding potential. Previously, the holding potential had no role in the case of the estimation of θ_1, because we only analyzed the values of the steady state currents. Now the input data of the parameter estimation process is the whole current trace, and the initial values of the activation and inactivation variables. The comparison of the results in the case of several protocols is depicted in Fig. 3. The parameters of the particular voltage step protocols are described in Table 1, while the interpretation of protocol parameters is depicted in Fig. 4. The evolution of the objective function during the optimization process is shown in the right plot of Fig. 2 in the case of a typical estimation scenarios of time constants.

In Fig. 3, the reason for the significant deviances of the inactivation time constant in the low voltage ranges is that the holding potential and the value of the voltage step only caused a small change in the steady state value of the inactivation variable (see the relevant values in the range of -90 / -60 in

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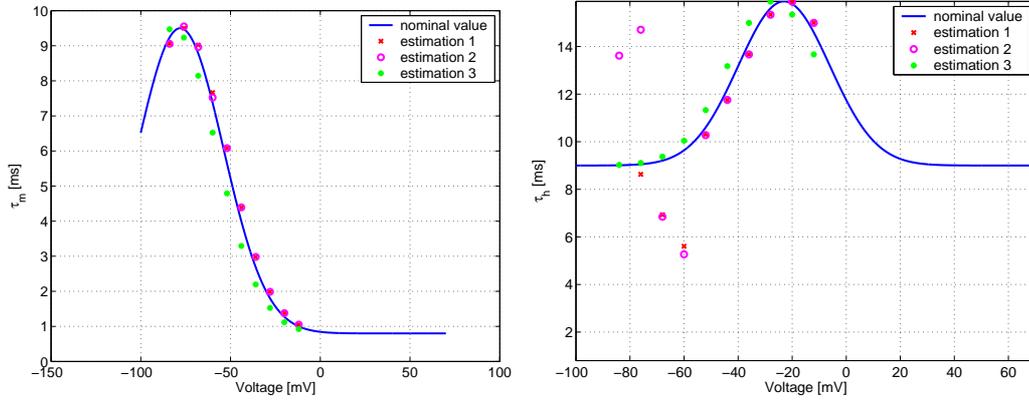
Figure 3: Results of the parameter estimation process for $\tau_m(V_i)$ and $\tau_h(V_i)$ at various voltage step protocols

Table 1: Different voltage step protocols for the estimation of τ_m and τ_h

	estimation 1	estimation 2	estimation 3
V_{hold} [mV]	-92	-68	-20
V_{base} [mV]	-94	-94	-88
$interval$ [mV]	8	8	8
$step_{num}$	10	10	10

Appendix A in Fig. 7 [3]. If the difference between V_{hold} and the corresponding voltage step is larger, we get more reliable results (for example in the case of estimation 3, which uses a higher V_{hold} of -20 mV gives better results in the lower voltage ranges). This means that if possible, it is worth to complete

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[3]In the example detailed in Appendix A, the same activation/inactivation characteristics were used, as defined in 4.1.1)
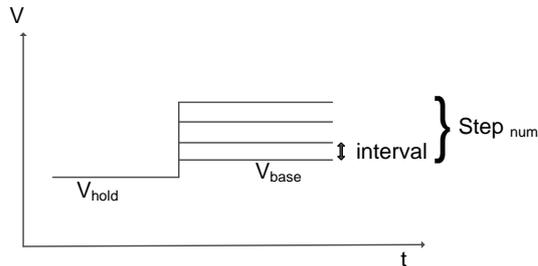
Figure 4: Interpretation of VC protocol parameters V_{hold}, V_{base}, $interval$ and $step_{num}$

the voltage step protocol with both a lower, and a higher holding potential.

4.2. Comparison with other methods

The above proposed parameter estimation algorithm was compared with two other local parameter estimation methods developed for voltage-clamp based estimation. The method of Lee at al., proposed in [28], and the method of Willms [26] have been chosen for this purpose.

4.2.1. Comparison with other local methods

Initial information. The method proposed in [28] is based on the analytical expression of the derivative of the current (which has to be zero in the extremum). Therefore, this method assumes that there is a local extremum in every current curve (as it is shown Appendix A, this is not always the case), which can be determined accurately. The other apriori information that is needed for the application of this method is the same as for our proposed approach (i.e. known steady state currents and an interval where at least one of the activation or inactivation curve is non-zero).

The 'full trace' method described in [26] simultaneously estimates m_∞, h_∞ and τ_m, τ_h from a given trace. Here it is assumed, that the maximal con-

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ductance value of the channel is known (or it has been estimated efficiently prior to the estimation of m_∞, h_∞ and τ_m, τ_h).

Computational efficiency and accuracy. For the comparison of estimation accuracy and computational time, the benchmark problem proposed in [28] was used. The parameters and equations of the hypothetical ion channel, used as benchmark problem can be found in appendix B.

The steady state activation and inactivation values (m_∞ and h_∞) were estimated at every 10 mV from -50 to 50 mV (except at 0 mV, where no current flows, due to $E = 0\ mV$). In the case of all algorithms, the corresponding error function was minimized with the Nelder-Mead simplex method. The stopping conditions of the simplex based optimization were the same as in section 4.1.1. The estimated and nominal values of the activation/inactivation functions and of the voltage dependent time constants are depicted in figures 5 and 6.

Table 2 summarizes the results of the comparison. In the table the required total computational time T_C (corresponding to the estimation of activation/inactivation parameters and time constants) is indicated together with the mean error of the activation/inactivation characteristics (E_{act}) and voltage dependent time constants (E_τ). The estimation algorithms were run in MATLAB on a standard dual-core desktop PC (3.2 GHz, 2 GB RAM).

In conclusion of the comparison we can say, that if the values of the steady-state currents are known, it is suggested to use the method proposed in this article to estimate the activation/inactivation curves (which requires usually only 2-3 s). For the estimation of τ_m and τ_h, if local extremum is present in the current trace, the method proposed by Lee can be suggested,

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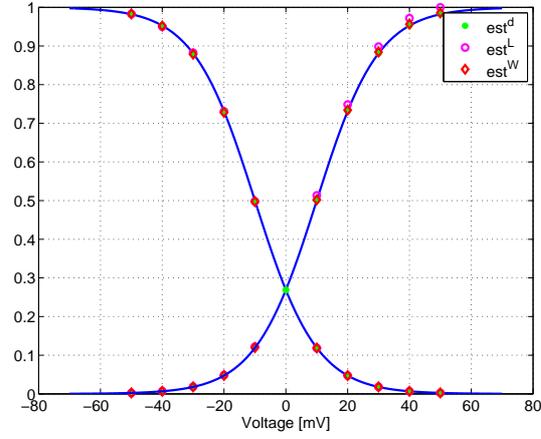
Figure 5: Estimation results of the activation and inactivation functions m_∞ and h_∞ by various methods: The decomposition method proposed by this article (est^d), the method of Lee et al. (est^L) and the method of Willms (est^W).
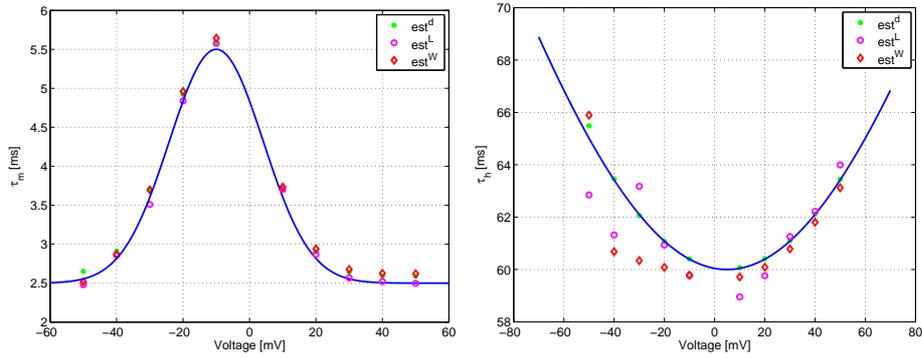
Figure 6: Estimation results of the voltage dependent time constants τ_m and τ_h by various methods: The decomposition method (proposed by this article), the method of Lee et al. and the method of Willms.

for which the previously determined m_∞ and h_∞ can be used as initial values. If no local extremum is present in the current trace, the proposed method can be used to determine τ_m and τ_h. Furthermore, as shown in section 4.1.2,

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Table 2: Comparison of the computational efficiency and accuracy

Method	T_C [sec]	E_{act} [%]	E_τ [%]
decomposition method	70	0.002	1.36
Lee	8	1.057	1.288
Willms	252	0.591	2.145

if we complete the measurements and the estimation with both a lower and a higher holding potential, the reliability of estimation results may improve significantly.

4.2.2. Relation to global methods

In this article we have assumed one ion channel. As mentioned, in this case the conventional estimation methods, which are based on fitting exponentials on the current trace (see [28] and [26]) can be used to determine useful initial conditions for the analyzed local techniques. In the analyzed cases, $\pm 25\%$ error in the initial guess ensured the convergence of local methods to the nominal values. It is however possible in general that the proposed local methods converge only to a local minimum. In this case the application of global optimization methods may be a solution. These methods require larger computational effort in general (e.g. the differential evolution - DE - algorithm, which outperforms simulated annealing and the genetic algortihm [29, 30, 31] in the estimation of HH models, requires 57 minutes in the case of 14 voltage steps assuming a potassium channel with one activation gate on a similar plattform), but they reliably find the nominal values in most cases, even when the difference of initial conditions and the nominal value is about

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one order of magnitude. In contrast, according to our results the reliability of the proposed decomposition method (which means the convergence to the nominal values) is above 95% in the case when the initial parameter values are ±25% of the nominal values, while we assume ±50% or ±75% error in the initial guess, the reliability of our algorithm is decreased to 70 and 33 % respectively.

While the proposed decomposition method needs typically about 400-800 iterations for the estimation of g, m_∞ and h_∞ (which mean iterations with low computation demand as only the computed steady-state currents are compared with the measured ones), and about 40-60 iterations for the estimation of τ_m and τ_h for each voltage step (which demand more computational effort, as in this case the whole traces are compared), the DE algoritm typicaly demands about 300 iterations (in this case, in each step whole traces are compared for multiple voltage values and multiple individuals, which explains the higher resulting running time).

Furthermore, if we consider multiple ion channels, the convergence properties of local methods significantly deteriorate, regarding both the estimation of $g/m_\infty/h_\infty$ and τ_m/τ_h.

In general, the application of global optimization methods, like the DE, can be unavoidable, if we consider multiple channels, or if the prior knowledge regarding the parameter values is limited. Although the estimation problem and the modeling assumptions are not completely identical in the two cases, our results support the findings in [31] that an input of multiple voltage steps is required for the safe determination of model parameters.

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5. Conclusions

The identifiability properties of a simple ion channel model used in Hodgkin-Huxley type neuron models were investigated in this paper using computer algebra methods. Two approaches, the differential algebraic method and the algorithm based on the Taylor series expansion of the output were applied to investigate structural identifiability. Both methods require the symbolic solution of nonlinear equations to get identifiability results. The identifiability analysis with both methods concluded that the two steady-state parameters (m_∞, h_∞) and the conductance (g) are not globally identifiable together. Moreover, no pair from these three parameters are identifiable.

Moreover, it was shown that the two methods usefully complement each other in the identifiability analysis. The differential algebra method resulted in a regression form model and an objective function that is convex in the tranformed parameters. The Taylor series expansion method clearly showed that no pair from the parameters p_2, p_4 and p_5 is globally identifiable.

Based on the results of the identifiability analysis, a novel optimization-based identification method is proposed and demonstrated on in silico data. The proposed method is based on the decomposition of the parameter estimation problem into two parts. The first step includes the estimation of the maximal conductance value and the activation/inactivation characteristics from the values of steady state currents obtained from multiple voltage step traces. The second step of the parameter estimation problem performs parameter estimation of the voltage dependent time constants. According to the results of the identifiability analysis, this step can be done locally, if the steady state values of the activation/inactivation variables corresponding to

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the actual voltage value are known.

The results of the article are used to formulate explicit criteria for the design of voltage clamp protocols which are the following.

1. The voltage steps should be long enough to ensure that the activation and inactivation variables are able to (at least approximately) reach their steady state values.

2. At least 10 voltage steps are required for the safe estimation of the investigated 5 parameters corresponding to the activation, inactivation curves and conductance values.

3. To provide a reliable estimation of the time constants in the wide voltage range, the measurements have to be completed both with a higher and a lower holding potential.

One possible generalization of the parameter estimation problem would be the addition of further ion channels of similar or different type, and the inclusion of different powers of activation and inactivation variables in the current equations. From an optimization point of view the inclusion of powers of activation and inactivation variables would lead to a mixed-integer problem.

In addition, the identifiability analysis of the kinetic description of HH models (see e.g. [26]) would be a natural extension of the work described in this paper.

6. Acknowledgements

Appendix A: Example showing the lack of global identifiability

In this appendix, we show a physically meaningful example that illustrates the non-global identifiability of the ion channel model with respect to the three parameters (namely, g, m_∞, and h_∞) that often have to be estimated. Other model parameters are assumed to be known. In addition, this example also demonstrates the scenario, when a local maximum in the current trace (assumed in [28]) does not appear.

First, it will be shown that our model can produce exactly the same output for different parameter/initial condition values during the voltage step protocol. The solution of the state equation in this case is given by

$$x_1(t) = p_2 + (x_1(0) - p_2)e^{-p_1 t}$$
$$x_2(t) = p_4 + (x_2(0) - p_4)e^{-p_3 t}, \tag{41}$$

from which the output current is computed as

$$y(t) = p_5 k_1 (p_2 + (x_1(0) - p_2)e^{-p_1 t})(p_4 + (x_2(0) - p_4)e^{-p_3 t}). \tag{42}$$

Now, let us scale the model parameters with a positive scalar λ as follows: $p_2^* = \lambda \cdot p_2$, and $p_5^* = p_5/\lambda$. Furthermore, let us choose the initial values of the state variables as $x_1^*(0) = \lambda \cdot x_1(0)$, $x_2^*(0) = x_2(0)$. The output of the modified model is then

$$y^*(t) = p_5^* k_1 x_1^* x_2^*$$
$$= k_1 p_5^* (p_2^* + (x_1^*(0) - p_2^*)e^{-p_1 t})(p_4 + (x_2^*(0) - p_4)e^{-p_3 t}) \tag{43}$$
$$= k_1 \frac{p_5}{\lambda}(\lambda p_2 + (\lambda x_1(0) - \lambda p_2)e^{-p_1 t})(p_4 + (x_2(0) - p_4)e^{-p_3 t})$$
$$= y(t)$$

from which it is clear, that the scaled model generates exactly the same output as the original one. The circumstances of the above case are not very likely to hold in the case of a standard voltage clamp protocol, where the voltage is held at an other constant value (the holding potential V_{hold}) before the voltage step. The holding potential determines the initial values of the differential variables: $x_1(0) = m(0) = m_\infty(V_{hold})$ and $x_2(0) = h(0) = h_\infty(V_{hold})$. However, the scenario is not impossible, as we will show below.

Using two fictitious neurons, we will now show that the measurable current responses of a voltage step during voltage clamp measurement can be identical in the case of different parameters.

Let us suppose that both neurons to be compared here inhibit only one ion channel, and the activation and inactivation characteristics of the first neuron are described by

$$m_\infty(V) = \left(1 + exp\left(\frac{V_{1/2m} - V}{k_m}\right)\right)^{-1} \tag{44}$$
$$h_\infty(V) = \left(1 + exp\left(\frac{V_{1/2h} - V}{k_h}\right)\right)^{-1}$$

The parameter values for the two neurons can be found in Table 3. The other parameters in the case of both neurons were the following

$$h_\infty = 0.75, \ V_{Maxm} = -78mV, \ \sigma_m = 34, \ c_{am} = 8.7ms,$$
$$c_{bm} = 0.8ms, \ E = -93mV, \ V_{Maxh} = -23mV$$
$$\sigma_h = 24, \ c_{ah} = 6.9ms, \ c_{bh} = 9ms. \tag{45}$$

As it is shown in Fig. 7, the value of m_∞ is 0.35 at -40mV and it is 0.20 at -50mV. At the same time, the value of m_∞^* of the second neuron is 0.525

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Table 3: Parameters of the two neurons

No	$V_{1/2m}$	k_m	$V_{1/2h}$	k_h	g
1	-31.932 mV	13.033	-44.354 mV	-5.139	67 nS
2	-41.056 mV	10.555	-44.354 mV	-5.139	44.67 nS
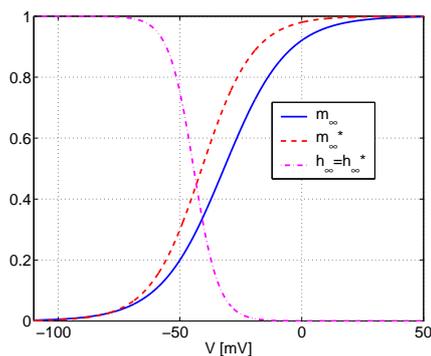
Figure 7: Voltage dependencies of the steady activation and inactivation state functions m_∞, m_∞^* and $h_\infty^* = h_\infty^*$

at -40mV and 0.30 at -50mV. The inactivation curve corresponding to h_∞ was the same in both cases. We applied a holding potential of -40 mV and a voltage step to -50 mV at t=100ms.

The comparison of trajectories of activation and inactivation variables and the output are depicted in Fig. 8. The figure clearly shows that the outputs are identical in the two cases, although the parameters of the two models are different.
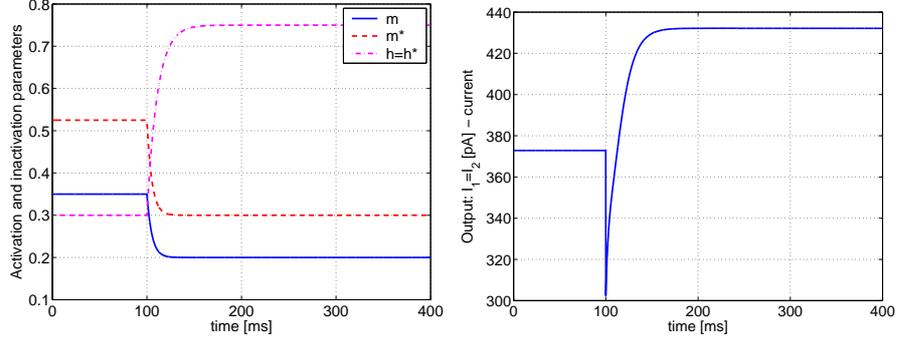
Figure 8: The activation and inactivation variables, and the output during the voltage step in the case of neuron 1 and 2. The upper index * refers to the activation and inactivation variables of neuron 2. The measured output current traces are identical in both cases.

Appendix B: benchmark example for the comparison of estimation methods

A hypothetical ion channel was considered, described by the following equations:

$$m_\infty = \frac{1}{1 + exp\left(-\frac{V-10}{10}\right)} \quad h_\infty = \frac{1}{1 + exp\left(\frac{V+10}{10}\right)} \tag{46}$$

$$\tau_m = 2.5 + 3exp\left(-\left(\frac{(-10-V)}{20}\right)^2\right) \quad \tau_h = 105 - 45exp\left(-\left(\frac{(5-V)}{160}\right)^2\right) \tag{47}$$

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)} \quad \frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)} \quad I = gmh(V - E) \tag{48}$$

and $g = 0.5mS/cm^2$ and $E = 0mV$.

The parameters were taken from [28].

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