Theoretical methods for exploring the neurophysiological behavior of single neurons based on experimental data

Doctoral thesis points

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1. Introduction

Nowadays, the tools of investigation approach the state of development which enables the observation and interpretation of the detailed activity of single neurons and the identification of correlations with cognitive functions. Furthermore, the physical models of neurons are advanced, but it doesn't mean that we know how a neuron processes the incoming signals and reacts to those. The most interesting would be to see, that in case of certain inputs, how does the cell react, that is to understand the computations related to cognitive functions.

The quickly developing designs of multielectrode-arrays makes it possible to capture more precise details of neuron electrophysiology than before. Together with the advanced experimental methods, new theoretical methods are needed to process the data and get the most information out of it.

These methods, as any other method relying on a model of reality, are not perfect and in many cases the error we make by applying these are not known or considered. As an example to these, there is an ongoing discussion about the measured and calculated non-null monopole current source related to a neuron, which violates the quasistatic approximation. This phenomenon, as we investigated in this work, may be explained by understanding the limitations of the tool used to draw this conclusion. Nevertheless, even if not perfect, these methods still provide us novel information which are vital for exploration.

In the first part of our work we show results related to theoretical methods for estimating the current source density distribution (CSD) of single neurons. Thereafter, we introduce the skCSD [1] method which aims to provide CSD reconstruction on morphology level.

2. Objectives

1. Investigation of the limitations of experimental tools regarding the appearance of monopole current sources

There is the ongoing discussion about the emergence of the monopole source, which contradicts the quasistatic estimation of charge conservation. We aim to understand and explain the possible causes leading to this observation. We analyse the role of finite spatial sampling of the extracellular medium in the the measurement of monopole current sources.

2. Studying the sensitivity of sCSD method to the arrangement

One of the key assumptions of the sCSD [2] method is, that the main axis of the cell is parallel to the electrode. This is roughly manageable experimentally, for example when recording the activity of pyramidal neurons the linear probe is inserted perpendicular to the brain surface. Therefore, we investigate the distortion introduced by the violation of the previous assumption.

3. Development and validation of the skCSD method

The skCSD method aims to provide information about the fine details of the electrophysiological behaviour of an observed cell by estimating the membrane current source density distributions. Due to the high temporal and spatial resolution of the multielectrodes used for recording the extracellular potentials to the skCSD method, the resolution of the method is below ms

and few tens of μm. Definitely such a new method shall be tested in diverse situations. This goal is challenging in terms of experimental and theoretical methods as well, but the first one we leave to others.

As the first milestone we created the theoretical relationship between the continuous membrane CSD distribution and the finite sampled extracellular potentials. Second, validation of the method is vital, for this reason simulated data was generated. This has multiple benefits: the ground truth is known to compare the reconstruction with and we could construct various scenarios from simple to complex ones. As a third milestone, we applied the method on real experimental data.

3. Methods

For the investigation of our objectives we used mainly simulated and occasionally experimental data related to the electrophysiological behavior of neurons. We wrote scripts in R and Python to prepare the simulated data invoking Neuron and LFPy using toy or more realistic neuron morphologies taken from the Neuromorpho database. With this approach we generated ground truth data of arbitrary setup of electrodes and current source distribution matching our purposes. The experimental data used as proof-of-concept for the skCSD method consisted of the measurement of the extracellular potentials by 14 electrodes from a hippocampal slice. Furthermore, patch clamp recording from a nearby cell was carried out and after the experiment the morphology was reconstructed. Based on the intracellular recording of this cell after the pre-procession of the extracellular data the spike triggered average was calculated .

For the calculation of current source density distributions the tCSD, sCSD and vCSD methods were used. While the oldest, simplest and most widely used method is the tCSD, the other two methods which also estimate the CSD of neuron populations, use more advanced mathematical tool and more parameters to provide a more precise estimation. The development of the skCSD method build on the mathematical tools introduces in the kCSD method, it advances the main idea of sCSD method, namely to reconstruct the CSD of single neurons, for which the kernel trick used in the kCSD method is essential.

4. Results

We summarized our results in the following thesis points related to the three main topics:

1. The finite spatial sampling of the extracellular potentials leads to non-null values of monopole current sources related to a single neuron in measurements

Through simulations we investigated the limitations of the experimental tools and the applied mathematical methods used for observing the apparent monopole current source of a neuron. We simulated the electrophysiological behaviour of two neuron models and observed the extracellular potential at certain points in space. These points are chosen to form a grid of various inter-electrode distances. The tCSD and the vCSD methods are used to estimate the current source density distribution. The monopole, dipole and quadrupole moments are calculated and compared. As expected, the sparser the electrode grid in a certain volume is, the worse the reconstruction quality gets and the bigger the value of monopole source is.

2. The sCSD method gives stable estimation up to noticeable misalignment of the cell

We described the position of the linear cell model in the setup with the distance of the soma from the electrode (d) and with 2 angels, α marks the angle from being parallel with the electrode and the main axis of the cell, β shows the rotation. As to see the distortion due to the misalignment, we created known current source density distributions of the cell and we selected a

certain α, β and d and performed the reconstruction as if the cell was perfectly parallel with the electrode. We found that the sCSD method still performs better than the tCSD when the tilting is not too big, meaning that is experimentally easily achievable. Furthermore, the distance estimation is only slightly influenced by realistic tilting as well.

3.1 The skCSD method is developed for the estimation the current source density distribution on the morphology of a single neuron

In order to describe the relationship between the current source distribution and the measured extracellular potentials, kernel functions were introduced. These are created from basis functions spanning the potential space and the CSD space.
The morphology of the cell is mapped to a loop along which the CSD basis functions were distributed. The method has three parameters: the number and width of the Gaussian-like basis functions and a regularization parameter. The best set of parameter is to be found either by cross-validation error or by tuning it on simulated data.

3.2 The skCSD method is able to capture simple and more complex CSD patterns in simulated setups

Taking a ball-and-stick neuron model and a laminar probe parallel to the cell, we studied the basic viability of this method. We showed that introducing more electrodes to cover the same area leads to increased spatial resolution of the method allowing reconstruction of higher Fourier modes of the CSD or or more

detailed information about synaptic inputs generating the measured potentials.

The Y-shaped model was used to show, that the method is able to distinguish between synaptic inputs on different branches and to study the effect of noise on the performance in case of different numbers of electrodes.

The ganglion cell model was our most complex and biologically realistic model, the reconstruction was able to reveal the propagating current source density patterns. Furthermore we gained useful insights for further experimental application. With a variation of electrode distributions we draw conclusion about the more and less optimal ways of arranging the experimental setup.

3.3 The skCSD method gives meaningful reconstruction on real experimental data

To examine the experimental feasibility of the skCSD method we analyzed data from a patch clamp electrode and a linear probe with 14 working electrodes recording signals simultaneously from a hippocampal pyramidal cell in an in vitro slice preparation. As there is no ground truth data available in this case, the optimal width of the basis functions and the regularization parameter were selected using the L1 error and simulated data. The method was applied on the spike triggered average potentials revealing the formation of the action potential.

5. Conclusions

We proposed, that the discrete spatio-temporal sampling of the extracellular potential and the approximations of the mathematical tools to calculate the current source density distribution contribute to the observation of monopole current sources. Simulation containing a 3D electrode grid recording the extracellular potentials of a spiking neuron was used as a tool to support our conclusion. As the finite spatial sampling is shown to be a contributor to the appearance of the monopole sources, the quasistatic estimation, which is the core assumption of many methods, may still hold.

The sCSD method aims to estimate the CSD distribution of single cells on a linear cell model. This method uses innovative assumptions about the experimental setup and even about the cell-electrode distance estimation for the current source density distribution. In this thesis we presented the study of cases, when a key assumption of the sCSD method are not satisfied, as this can help to understand the limitations of the method and the validity of the result. The sCSD method performed well, it gave meaningful results with reasonable RMSE even when $\alpha = 20°$. In experiments higher precision of the setup is easily reachable.

The developed skCSD method aims to estimate the current source density along the morphology of single neurons based on extracellular recordings and the morphology. We validated the method on several simulational and also an experimental data.

Testing the reconstruction against the known CSD (the ground truth) shows a clear transition from poor to faithful reconstruction when the electrode distribution becomes dense

enough to capture the fine detail of the CSD profile to be reconstructed.

Using the Y-shaped morphology we showed that the synaptic inputs activating different dendrites can be separated. The skCSD provides meaningful information about the membrane CSD in cases, when the interpolated potential and standard, population CSD analyses, are not informative. Furthermore the reconstruction is not sensitive to a specific selection of electrode placement and even significant additive noise is not prohibitive for the reconstruction.

Our study, assumed realistic cell morphology of the ganglion cell and commercially available MEA designs, as well as realistic cell activity, showed that it is feasible to reconstruct the distribution of the current sources in realistic, noisy situations.

The skCSD method performed adequately for the proof of concept experimental data, even if the nature of the experiment allowed only the reconstruction of the general features of the spike-triggered average spatio-temporal current source density distribution patterns.

6. Author's publications

Related to the thesis

[1] Cserpán D, Meszéna D, Wittner L, Tóth K, Ulbert I, Somogyvári Z, Wójcik D: Revealing the Distribution of Transmembrane Currents along the Dendritic Tree of a Neuron with Known Morphology from Extracellular Recordings eLife 2017;6:e29384 DOI: 10.7554/eLife.29384

[2] Somogyvári Z, Cserpán D, Ulbert I, Érdi P: Localization of single cell current sources based on extracellular potentials patterns: the spike CSD method, European Journal of Neuroscience 36, Issue 1, November 2012.

Other publications

[3] Kántor O, Cserpán D, Völgyi B, Lukáts A, Somogyvári Z: The Retinal TNAP. In: Fonta C., Négyessy L. (eds) Neuronal Tissue-Nonspecific Alkaline Phosphatase (TNAP). Subcellular Biochemistry, vol 76. Springer, Dordrecht. 2015

[4] Zátonyi A, Borhegyi Z, Srivastava M, Cserpán D, Somogyvári Z, Kisvárday Z, Fekete Z: Functional brain mapping using optical imaging of intrinsic signals and simultaneous high-resolution cortical electrophysiology with a flexible, transparent microelectrode array, Sensors and actuators B-chemical 27: pp. 519-526. (2018)

[5] Zátonyi A, Borhegyi Z, Cserpán D, Somogyvári Z, Srivastava M, Kisvárday Z, Fekete Z: Optical Imaging of Intrinsic Neural Signals and Simultaneous MicroECoG Recording Using Polyimide Implants. Proceedings 1: Paper 610. 4 p. (2017)