Final report

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Classification of cortical neurons based on their two-dimensional action potential waveforms recorded with high spatial density

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Aims of the study

The main goals of the research project were to

1. develop and use neural probes with high electrode density to record in vivo cortical activity with a significantly higher spatial resolution than before
2. analyze and classify cortical neuronal activity based on the recorded high-resolution action potential waveforms of neurons
3. share the high-resolution recordings in a public data repository

Development and validation of state-of-the-art high-density neural probes to record cortical activity with high spatial resolution

In collaboration with European universities and research institutes we developed novel planar silicon and polymer-based probes containing hundreds of small, closely packed recording sites for in vivo experiments in rodents (Raducanu et al., 2017; Dimitriadis et al., 2018; Fiáth et al., 2018a; Fiáth et al., 2018b; Fiáth et al., 2019a). Validation of the functionality of these probes was performed in anesthetized rats, and after successful testing, these devices were used to collect the high-resolution data for this research project. Mainly 128-channel (Fiáth et al., 2018a) and the linear and array type of 256-channel (Dimitriadis et al., 2018; Fiáth et al., 2019a) passive silicon probes were used to acquire data from the somatosensory and motor cortex of rats and mice anesthetized with ketamine/xylazine.

Classification of cortical single unit activity using deep learning and FPGAs

Spike sorting is the main first step during the processing and analysis of extracellular neural recordings. However, traditional spike sorting algorithms are unable to process the large amount of data provided by recently developed high-channel-count (>64 channels) neural probes, therefore new approaches and solutions are needed. Artificial intelligence-based methods are well suited to solve such complex problems. In our research group, we developed and applied deep learning algorithms to detect and sort extracellular action potentials (spikes) in high resolution cortical recordings obtained during this project (Rácz et al., 2020; Rokai et al., 2021). The performance of these machine learning-based spike sorters were comparable to methods developed recently for high density recordings. Furthermore, in collaboation with the University of Szeged, we also developed a Field Programmable Gate Array (FPGA)-based method for multichannel spike sorting which might be used to help neural probe positioning by detecting and sorting relevant single units real-time, during experiments with the 128-channel silicon probe (Schaffer et al., 2021). To validate the spike sorting system, hybrid ground truth

data was generated based on our high-density cortical measurements collected during the research project. The results of this study have demonstrated the advantages of dense spatial sampling of spiking activity: the accuracy of spike sorting is better if the spike waveform of individual single units is captured by multiple recordings sites simultanenously compared to the case when the spikes of a single neuron is detected only by a single site.

Classification of cortical neurons based on their high resolution spike waveforms

In our preliminary data analysis, we incorporated unsupervised machine learning algorithms (hierarchical clustering and k-means clustering) to classify a small set of cortical neurons (~200) based on the spatiotemporal features of their multichannel spike waveforms (two posters presented at scientific conferences in 2018, see below). Using hierarchical clustering on the features extracted from the high-resolution spike waveforms (e.g., spike duration, spatial spread of the action potential, propagation direction of the spike, peak-to-peak amplitude) we could identify eight neuron clusters where one of the clusters contained the narrow spiking putative interneurons.

Currently, we have a collection of about 10.000 sorted single units recorded from the cortex of anesthetized rats. The spike waveform of about a third of these single units was located at the center of the shank of the 128-channel silicon probe making them potential candidates to investigate their high-resolution multichannel spike waveform in more detail. The number of these center neurons (~3000) significantly exceeds the number of single units planned for the project (~1000). We extented the investigations described above to this larger set of neurons but we are still in the phase of data analysis. The results are planned to be published in the near future.

Unfortunately, based on the preliminary analysis of the in vivo mouse cortical recordings, it appears that the spatial resolution of the 128-channel silicon probe is not sufficient to distinguish between different subtypes of cortical neurons (especially interneurons) using spatial features of the spike waveform of single units; the spike waveforms of mouse neurons, because of their smaller size compared to rat neurons, were only recorded by a low number of recording sites simultaneously and the waveforms were relatively similar compared to each other. A higher resolution provided by the 256-channel probes might show finer details of the spike waveforms, however, the limited cortical coverage of these probes makes these investigations complicated, thus my focus was and will be on the analysis of cortical recordings collected from rats.

Freely accessible dataset of high spatial resolution cortical recordings

A freely accessible dataset consisting of high-resolution wideband recordings and spike waveforms (~1 TB of data of 109 cortical recordings containing 7126 sorted single units) was uploaded to the GIN (G-Node Infrastructure; https://gin.g-node.org/) public data repository. The dataset is packaged in the Neurodata Without Borders: Neurophysiology version 2.0 format (NWB:N 2.0; www.nwb.org), a data standard for neurophysiology. The manuscript describing the dataset was submitted to the journal Scientific Data to increase the visibility of this high-resolution dataset. Thanks to this project, the principal investigator gained a lot of experience

in sharing research data, in using standardized data formats and in creating, handling and annotating large datasets. As sharing of scientific data and open science becomes increasingly important, this skillset will be very useful in the near future.

Novel methodology-related findings based on the collected high-density cortical recordings

The high-density recordings obtained during the project also provided novel findings related to important methodological aspects of in vivo electrophysiological experimentation. High density extracellular recordings provide a richer spatial information about the spike waveforms of single units, the spikes of a typical neuron might be recorded by more than a dozen adjacent recording site. This high spatial resolution increases the accuracy and reliability of spike sorting, thus allowing a more precise evaluation of the quality of neural recordings. Using high density cortical recordings, we studied various methodology-related questions. For example, we have found that a very slow insertion (2 μm/s) of silicon probes into the brain tissue significantly improved the single unit yield and signal quality compared to implantations with faster speeds, and more neurons remained intact in the proximity of the probe (Fiáth et al., 2019b). As a positive effect, higher quality data obtained by slower probe insertions might also decrease the number of research animals used in a study. Furthermore, we investigated the effect of the recordings site position on the quality of high-density recordings, focusing mainly on sites located at the edge or in the center of the silicon shank (Fiáth et al., 2021). Cortical recordings (~150 data files) obtained with high-density 128- and 256-channel silicon probes from anesthetized rats during this postdoctoral project as well as recordings from Neuropixels and NeuroNexus silicon probes were used for this study. Our findings show that edge sites outperform center sites, the former recorded significantly more large amplitude samples, both in the positive and negative range. Although the single unit yield was similar between site positions, the difference in spike amplitudes was noticeable in the range corresponding to high-amplitude spikes. Furthermore, the advantage of edge sites slightly decreased with decreasing probe shank width. These results might aid the design of novel neural implants in enhancing their recording performance by identifying more efficient recording site placements.

Deviation from the work plan

One of the aims of the project was to simultaneously record the activity of the same neuron with a high-density silicon probe and with a juxtacellular electrode, in vivo. Unfortunately, this technique proved to be very difficult to accomplish (however, the risks were known during the planning of the project). Because the gains of this approach would have been relatively small compared to the work invested (since it was mainly done earlier by the group of Adam Kampff, see Neto et al. 2016 J Neurophysiol; Marques-Smith et al. 2018 bioRxiv), I stopped pursuing this direction. Instead, in the research group, we started to record in vitro cortical activity from rat and mouse brain slices using the developed high-density 128-channel silicon probe. We could successfully record the spikes of ~10 single units simultaneously by inserting the probe into the brain slice. Comparison of in vivo and in vitro high-resolution recordings is in progress and will be finished outside the timeframe of this research project. We used also another high-density silicon probe with protruding recording sites developed for in vitro bain slice recordings

to compare in vitro electrophysiological recordings obtained with our high-density probes designed for in vivo recordings (Meszéna et al., 2019).

Publication and dissemination activity

During the three years of the project, eleven peer-reviewed journal articles, two preprints and two poster presentations were published related to the topic of the research, and another three manuscripts are either under review or in preparation. The cumulative impact factor of the published papers is 63.087. Out of the eleven articles, eight were published in journals ranked Q1 (seven are ranked D1). The principal investigator of the project is the first author of five publications. In total, five BSc, four MSc and one TDK work was completed in the topic of this project at the Faculty of Information Technology and Bionics of the Pázmány Péter Catholic University (PPKE ITK) and at the Faculty of Science of the Eötvös Loránd University. Parts of the results of the supported research were used as course material at the PPKE ITK and presented by the principal investigator of this project („Applications of neural microsystems" organized by Zoltán Fekete, http://neuromems.hu/lectures/; Lecture 4: Multimodal sensor/stimulator arrays relying on integrated signal processing units). The public high-density dataset is available at the GIN data repository: https://gin.g-node.org/UlbertLab/High_Resolution_Cortical_Spikes. A MATLAB-based open source software suite was developed and made available online on GitHub (https://github.com/fiath/SDEMI). The software is used to visualize, process and analyze the high-density recordings obtained during the project.

Future project related research plans

The principal investigator gained a lot of experience with high-density recordings and installed a high-density Neuropixels electrophysiological recording system in the lab during the project to continue research on this topic.

In collaboration with the Insitute of Experimental Medicine, we also collected high-density spike waveforms with the 128-channel silicon probe from the medial septum of mice. This dataset was used to classify septal neurons based on their firing patterns and to examine theta rhythm generation. A manuscript was prepared from the results which is available as a prerprint (Kocsis et al., 2021). One of my future aims is to examine the spatiotemporal features of the spike waveforms of these septal neurons and compare these to our findings obtained in the cortex.

Several undergraduate and graduate students as well as the principal investigator are still analyzing the large dataset collected during this three year project. We have found several interesting findings by analyzing in vivo and in vitro high-spatial-resolution spike waveforms of cortical neurons (including, for example, different patterns of action potential backpropagation and spatial spread of spikes, or laminar differences in spike features of neurons). However, more time is needed to thoroughly analyze and publish these findings. Thus, these analyses will still continue after the project ended. The importance of this topic is further supported by several recent publications about neuron classification based on high-

density electrophysiology (see, for example, Buccino et al., 2018 J Neurophysiol; Jia et al., 2019 J Neurophysiol).

List of publications

Peer-reviewed journal articles

Raducanu BC, Yazicioglu RF, Lopez CM, Ballini M, Putzeys J, Wang S, Andrei A, Rochus V, Welkenhuysen M, van Helleputte N, Musa S, Puers R, Kloosterman F, van Hoof C, Fiáth R, Ulbert I, Mitra S. Time Multiplexed Active Neural Probe with 1356 Parallel Recording Sites. (2017) SENSORS 17: 2388. doi: 10.3390/s17102388. Impact factor (IF): 2.475, Scimago Journal Rank (SJR): Q2

Fiáth R, Raducanu BC, Musa S, Andrei A, Lopez CM, van Hoof C, Ruther P, Aarts A, Horváth D, Ulbert I. A silicon-based neural probe with densely-packed low-impedance titanium nitride microelectrodes for ultrahigh-resolution in vivo recordings. (2018a) BIOSENSORS & BIOELECTRONICS 106: 86-92. doi: 10.1016/j.bios.2018.01.060. IF: 9.518, SJR: D1

Fiáth R, Hofer KT, Csikós V, Horváth D, Nánási T, Tóth K, Pothof F, Böhler C, Asplund M, Ruther P, Ulbert I. Long-term recording performance and biocompatibility of chronically implanted cylindrically-shaped, polymer-based neural interfaces. (2018b) BIOMEDICAL ENGINEERING/BIOMEDIZINISCHE TECHNIK 63: 301-315. doi: 10.1515/bmt-2017-0154. IF: 1.007, SJR: Q3

Fiáth R, Raducanu BC, Musa S, Andrei A, Lopez CM, Welkenhuysen M, Ruther P, Aarts A, Ulbert I. Fine-scale mapping of cortical laminar activity during sleep slow oscillations using high-density linear silicon probes. (2019a) JOURNAL OF NEUROSCIENCE METHODS 316: 58-70. doi: 10.1016/j.jneumeth.2018.08.020 IF: 2.214, SJR: Q2

Fiáth R, Márton A, Mátyás F, Pinke D, Márton G, Tóth K, Ulbert I. Slow insertion of silicon probes improves the quality of acute neuronal recordings. (2019b) SCIENTIFIC REPORTS 9: 111. doi: 10.1038/s41598-018-36816-z IF: 3.998, SJR: D1

Meszéna D, Kerekes BP, Pál I, Orbán G, Fiáth R, Holzhammer T, Ruther P, Ulbert I, Márton G. A silicon-based spiky probe providing improved cell accessibility for in vitro brain slice recordings. (2019) SENSORS & ACTUATORS B – CHEM 297: 126649. doi: 10.1016/j.snb.2019.126649 IF: 7.1, SJR: D1

Yuste R, Hawrylycz M, AallingN, Arendt D, Armananzas R, Ascoli G, Bielza C, Bokharaie V, Bergmann TB, Bystron I, CapognaM, Chang Y, Clemens A, de Kock C, DeFelipe J, Dos santos SE, Dunville K, Feldmeyer D, Fiáth R, Fishell G, Foggetti A, Gao X, Ghaderi P, Güntürkün O, Hall VJ, Helmstaedter M, Herculano S, Hilscher M, Hirase H, Hjerling- Leffler J, Hodge R, Huang J, Huda R, Juan Y, Khodosevich K, Kiehn O, Koch H, Kuebler E, Kühnemund M,

Larrañaga P, Lelieveldt B, Louth EL, Lui J, Mansvelder H, Marin O, Martínez-Trujillo J, Moradi H, Goriounova N, Mohapatra A, Nedergaard M, Němec P, Ofer N, Pfisterer U, Pontes S, Redmond W, Rossier J, Sanes J, Scheuermann R, Serrano Saiz E, Somogyi P, Tamás G, Tolias A, Tosches M, Turrero Garcia M, Aguilar-Valles A, Munguba H, Wozny C, Wuttke T, Yong L, Zeng H, Lein ES. A community-based transcriptomics classification and nomenclature of neocortical cell types. (2020) NATURE NEUROSCIENCE 23: 1456-1468. doi: 10.1038/s41593-020-0685-8 IF: 20.071, SJR: D1

Rácz M, Liber C, Németh E, Fiáth R, Harmati I, Márton G, Ulbert I: Spike Detection and Sorting with Deep Learning. (2020) J NEURAL ENG 17: 016038. doi: 10.1088/1741-2552/ab4896 IF: 4.141, SJR: D1

Schaffer L, Nagy Z, Kincses Z, Fiáth R, Ulbert I: Spatial information based OSort for real-time spike sorting using FPGA. (2021) IEEE TRANS BIOMED ENG 68: 99-108. doi: 10.1109/TBME.2020.2996281 IF: 4.424, SJR: Q1

Fiáth R, Meszéna D, Somogyvári Z, Boda M, Barthó P, Ruther P, Ulbert I: Recording site placement on planar silicon-based probes affects signal quality in acute neuronal recordings. (2020) SCIENTIFIC REPORTS 11: 2028. doi: 10.1038/s41598-021-81127-5 IF: 3.998, SJR: D1

Rokai J, Rácz M, Fiáth R, Ulbert I, Márton G. ELVISort: ELVISort: Encoding Latent Variables for Instant Sorting, an Artificial Intelligence-Based End-to-End Solution. (2021) J NEURAL ENG (accepted manuscript) doi: 10.1088/1741-2552/abf521 IF: 4.141, SJR: D1

Horváth C, Tóth LF, Ulbert I, Fiáth R. Dataset of cortical activity recorded with high spatial resolution from anesthetized rats. SCIENTIFIC DATA (submitted)

Preprints

George Dimitriadis, Joana P. Neto, Arno Aarts, Andrei Alexandru, Marco Ballini, Francesco Battaglia, Lorenza Calcaterra, Susu Chen, Francois David, Richárd Fiáth, João Frazão, Jesse P Geerts, Luc J. Gentet, Nick Van Helleputte, Tobias Holzhammer, Chris van Hoof, Domonkos Horváth, Gonçalo Lopes, Carolina M. Lopez, Eric Maris, Andre Marques-Smith, Gergely Márton, Bruce L. McNaughton, Domokos Meszéna, Srinjoy Mitra, Silke Musa, Hercules Neves, Joana Nogueira, Guy A. Orban, Frederick Pothof, Jan Putzeys, Bogdan C. Raducanu, Patrick Ruther, Tim Schroeder, Wolf Singer, Nicholas A. Steinmetz, Paul Tiesinga, Istvan Ulbert, Shiwei Wang, Marleen Welkenhuysen, Adam R. Kampff. Why not record from every electrode with a CMOS scanning probe? (2018) bioRxiv doi: 10.1101/275818

Kocsis B, Martinez-Bellver S, Fiáth R, Domonkos A, Sviatko K, Barthó P, Freund T, Ulbert I, Káli S, Varga V, Hangya B. Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta oscillation. (2021) bioRxiv, doi: 10.1101/2021.01.22.427736

Poster presentations

Fiáth R, Csikós V, Debreceni Á, Ulbert I. Classification of neuron types in the rat neocortex based on spatiotemporal features of action potential waveforms recorded with high spatial resolution. 11th FENS Forum of Neuroscience, Berlin, Germany 7-11 July 2018

Fiáth R, Csikós V, Debreceni Á, Ulbert I. Attempts to classify cortical neurons based on spatiotemporal features of their action potential waveforms recorded with high spatial resolution. The Brain Conferences - The Necessity of Cell Types for Brain Function, Copenhagen, Denmark, 7-11 October 2018

BSc and MSc theses related to the project

Vivien Csikós: Agykérgi neuronok akciós potenciáljainak nagy téri felbontású vizsgálata. (2017) Pázmány Péter Catholic University, Faculty of Information Technology and Bionics (PPKE ITK), BSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Adrienn Lilla Márton. A mérőelektróda agyszövetbe történő beszúrási sebességének hatása a rögzített sejtaktivitás minőségére. (2017) PPKE ITK, BSc/TDK thesis (Supervisor: Richárd Fiáth/István Ulbert)

Ádám Debreceni: Development of a software package for visualizing, processing and analyzing high-density electrophysiological recordings (2018). PPKE ITK, MSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Noémi Gulyás. Agykérgi neuronok akciós potenciáljainak vizsgálata egérben nagy téri felbontású elektrofiziológiai és optogenetikai módszerekkel. (2018) PPKE ITK, BSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Mátyás Boldizsár Vidermann. Lassú hullámú aktivitás rétegelemzéses vizsgálata egér szomatoszenzoros kéregben (2018). PPKE ITK, BSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Adrienn Lilla Márton. A mérőelektróda agyszövetbe történő beszúrási sebességének hatása a rögzített sejtaktivitás minőségére. (2018) XXXIV. Országos Tudományos Diákköri Konferencia, Biológia Szekció, PPKE ITK, 2019. április 15-17. (Supervisor: Richárd Fiáth/István Ulbert)

Adrienn Lilla Márton: Effects of insertion conditions on the quality of acute neuronal recordings and on brain tissue injury in case of silicon probe implantations (2019). PPKE ITK, MSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Mátyás Boldizsár Vidermann: Laminar analysis of cortical single unit activity during ketamine/xylazine-induced slow wave activity in mice (2020). PPKE ITK, MSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Tóth Lili Fanni: An annotated dataset of action potentials of cortical neurons recorded with high spatial resolution (2020) PPKE ITK, MSc thesis (Supervisor: Richárd Fiáth/István Ulbert)

Bálint Tamás: Nagy téri felbontású szilícium-alapú elektródok alkalmazása elektrofiziológiai kísérletekben (2020). Eötvös Loránd University, Faculty of Science, BSc thesis (Supervisor: Richárd Fiáth)