Óbuda University

Doktoral (PhD) dissertation theses booklet

Development of novel measurement methods for neuroscientific research and dentistry

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I. Antecedents of the research

Electrophysiology is the scientific area of observation and study of the electrical properties of the biological cells and tissues. This dissertation could be split into two parts according to this definition. Both main research projects which will be presented herein are based on electrophysiology. The aim of the first one is the simultaneous utilization of an electrophysiological measurement method and an optical imaging process for neuroscientific research, where the principal targets of observation are neurons. The simultaneous application of two-photon imaging and electrophysiological recordings within the same neural tissue region allows the observation of the bioelectrical activity of the nervous system in a high temporal and spatial resolution at the same time [1]. The utilization of implanted microelectrode arrays can be more complex and precise by the three dimensional monitoring of the morphological features near the electrodes, but the imaging laser causes photoelectrical artefacts in the electrophysiological recordings [2].

The second presented research topic is related to human dental structures. During this research an electrical property of a biological tissue, namely the electrical impedance of the human dentin has been measured, and a formula has been determined to evaluate its dependency on geometric thickness and signal frequency. Utilization of impedance measuring methods in dental researches makes the determination of electrical properties of human tooth possible. Although impedance measurement forms the basis of numerous oral diagnostic methods [3,4], limited studies are available about the impedance of human dentin.

II. Specific aims

Simultaneous electrophysiological recording and two-photon imaging *in vitro*

Simultaneous utilization of implanted MEAs for extracellular electrophysiology and two-photon microscopy for optical imaging could allow the observation of activities of individual neurons with good spatial and temporal resolution, but the imaging laser generates artefacts in the electrophysiological recordings. Special noise filtering algorithm development is required to analyse the data which were recorded in the field of view of the two-photon microscope. Our aim was to perform *in vitro* experiments on mouse neocortical slices expressing the GCaMP6 genetically encoded calcium indicator for monitoring the neural activity with two-photon microscopy around the implanted MEAs. An objective of mine was to develop a complex custom-set comb filter based algorithm which could be used for noise filtering to eliminate the artefacts caused by the imaging laser. Besides the two-photon observation of the morphology near the implanted MEA, the scope of our research was to prove that this special filtering algorithm allows the detection and the sorting of SUAs from a simultaneous two-photon imaging and extracellular electrophysiological measurement.

Simultaneous electrophysiological recording and two-photon imaging *in vivo*

Having realized the special filtering algorithm for SUA detection from simultaneous two-photon imaging and extracellular electrophysiological recordings *in vitro*, our aim was to extend our investigation onto *in vivo* experiments. To reach this goal, the scope of ours was to apply a MEMS technology based MEA which would be designed and developed in order to perform simultaneous electrophysiological recording and two-photon imaging from the same tissue region of mice brains expressing GCaMP6 genetically encoded calcium indicator. Out aim was to implant the MEA within the field of view of the two-photon imaging and perform simultaneous recordings. The previously developed algorithm was planned to improved and utilized on the recorded extracellular data to detect SUAs. The objective was to prove that combining the self-developed MEMS technology based MEA with my filtering and analyzing algorithm was capable of performing electrophysiological recording and two-photon imaging from the same tissue region at the same time.

Determination of the thickness dependent electrical impedance spectrum of the human dentin

Utilization of impedance measurement methods in dental researches makes the determination of electrical properties of human tooth possible. Although impedance measurement forms the basis of numerous oral diagnostic methods, only a limited number of studies are available focusing on the impedance of human dentin. The main goal of our experiments was to determine the thickness dependency of the impedance of the human dentin. Our model allowed the determination of a coefficient which describes the correlation between the thickness and the electrical impedance of the human dentin.

III. Experimental methods

a) Mouse brain slices were cut with a vibratome (VT1200s; Leica, Nussloch, Germany) for the *in vitro* experiments.

b) Mice were stabilized in a stereotaxic frame (David Kopf Instruments, Los Angeles, USA) during the *in vitro* experiments.

c) The three dimensional observation of the neural tissue was performed with two-photon microscope (Femtonics Ltd., Budapest, Hungary).

d) The electrophysiological observation of the bioelectrical activity of the neural tissue were carried out using an Intan RHD 2000 amplifier system (Intan Technologies, Los Angeles, USA).

e) The algorithm for off-line signal visualization, filtering and analysis was developed in Matlab 2017a (MathWorks Inc., Natick, MA, USA).

f) Dentin disks were cut with a dental saw (Hofer, Aathal - Seegräben, Switzerland).

g) The thickness of the examined dentin was measured with a resolution of 10 μm via a stereotaxic frame (David Kopf Instruments, Los Angeles, USA).

h) Intan RHD 2000 amplifier system (Intan Technologies, Los Angeles, USA) was applied for impedance measurement during the dental experiments

i) For the statistical analysis of the dental focus groups IBM SPSS Statistics 24 (IBM Corporation, New York, USA) software was used.

IV. New scientific results

First thesis group: Simultaneous utilization of electrophysiological recording and two-photon imaging

I.a thesis I developed a complex custom-set comb filter based filtering algorithm which was used for data analysis to eliminate the imaging laser generated artefacts from simultaneous two-photon imaging and electrophysiological measurements. *In vitro* experiments were performed on mouse neocortical slices expressing the GCaMP6 genetically encoded calcium indicator for monitoring the neural activity with two-photon microscopy around an implanted MEA and electrophysiological recordings were made from the tissue region of the optical imaging. I proved that the applied filtering is capable of eliminating the majority of the periodic photoelectric artefacts generated by the imaging laser and this method allows single unit activity detection and sorting. Publication related to the thesis point: [R1]

I.b thesis To verify the suitability of it, I have utilized the self-developed filtering algorithm on extracellular recordings from a special, MEMS technology based MEA which was developed so as to perform simultaneous electrophysiological recording and two-photon imaging from the same tissue region of mice brains expressing GCaMP6 genetically encoded calcium indicator. I proved that the filtering algorithm was suitable for SUA detection and sorting from recordings of the self-developed MEA loaded by imaging laser generated artefacts. Publication related to the thesis point: [R1]

Second thesis: Thickness-impedance coefficient of the human dentin

I observed the impedance spectrum of dentin disks prepared from human wisdom teeth in the thickness range of $0.3 - 2.3\ mm$ to reveal the correlation between the thickness and the electrical impedance of human dentin. In accordance with the results of the performed *in vitro* experiments I determined the thickness-impedance coefficient of human dentin which is

$$\frac{|Z|}{d}A = 8.356\ \Omega m$$

with the standard error of 0.605 Ωm at 1 kHz, where Z is the absolute impedance, d is the thickness and A is the measured area of the human dentin. The thickness-impedance coefficient depends on measuring frequency. The applied statistic method proved that there are significant differences at every observed frequency between the impedance values of each thickness group. Publication related to the thesis point: [R2]

V. Possibility to utilize the results

The possible utilization of the results of the first thesis group is in neuroscientific researches. The developed analyzing method may allow researchers to utilize two-photon imaging so as to reveal critical features of high density extracellular neurophysiology and vice versa. The application of simultaneous, multimodal recordings may enhance the novel findings in neuroscience and effective brain-computer interfaces.

Impedance measurement can forms the basis of several oral diagnostic methods, hence the results of the second thesis point may have significance in the field of dental researches. The determination of the thickness-impedance coefficient of the human dentin can improve measurement methods for clinical dental treatments.

VI. Bibliography

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VII. Publication related to the theses

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[R2] G. Orbán, Cs. Dobó-Nagy, I. Ulbert, G. Márton (2020): Thickness dependent electrical impedance spectrum of human dentin, INTERNATIONAL JOURNAL OF CLINICAL DENTISTRY 13: (1) p105-115. 11p.

VIII. Publication not related to the theses

[N1] T. Marek, G. Orbán, D. Meszéna, G. Márton, I. Ulbert, G. Mészáros, Z. Keresztes (2021): Optimization aspects of electrodeposition of photoluminescent conductive polymer layer onto neural microelectrode arrays, MATERIALS CHEMISTRY AND PHYSICS: (260) 124163

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Utility patents

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[P2] G. Márton, G. Orbán, I. Ulbert (2015): Carrier device for implanting a flexible implant into a biological tissue, granted by the Hungarian Intellectual Property Office, Application number: P1500592

[P3] Cs. Dobó Nagy, G. Orbán, G. Márton (2019): Equipment for measuring the thickness of the human dentin, submitted to the Hungarian Intellectual Property Office, Application number: U1900110