Acousto voltage imaging with voltage sensitive dyes and genetically encoded sensors

Levente Cseri[1,2], Zsófia Horváth[1,2], Réka Lányi[1], Katalin Ócsai[1,4], Balázs Chiovini[3], Viktória Kiss[3], Zsolt Mezriczky[3], Anna Mihály[3], Zoltán Mucsi[1], Balázs J. Rózsa[1,3,5]

[1]BrainVisionCenter, Budapest, Hungary

[2]Department of Organic Chemistry & Technology, Budapest University of Technology & Economics, Budapest, Hungary

[3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary

[4]Department of Mathematical Geometry, Institute of Mathematics, Budapest University of Technology and Economics, Budapest, Hungary,

[5]Laboratory of 3D Functional Network and Dendritic Imaging, Institute of Experimental Medicine, Budapest, Hungary

Abstract

Two-photon Ca^{2+} imaging is a widely used tool for studying cortical network dynamics at multiple depths, that underlie higher cognitive functions. While this technique provides a high spatial resolution, its main limitation is low temporal resolution. This may be circumvented by using electrophysiological recordings; however, it reflects the activity of a neuronal population from a confined brain region only and lacks cellular specificity. To better understand the direct communication between neurons, it is essential to monitor the activity changes even at the subcellular level with a high spatiotemporal resolution. Genetically encoded voltage indicators (GEVIs), such as JEDI-2P, are emerging tools to capture changes directly in neuronal membrane potential. Combining fast acousto-optic imaging with voltage sensors enables non-invasive monitoring of fast neuronal activity at subcellular level on the millisecond timescale. Here, we present various applications that demonstrate the power of direct voltage imaging. By using the novel acousto-optical scanning technique and JEDI-2P indicator, we will show that the activity of multiple cells can be reliably recorded with a temporal resolution of up to 50 kHz both *in vitro* and *in vivo*. Certain experimental systems, such as those involving *ex vivo* human tissue, are not compatible with the expression times of virally delivered GEVIs, that may be as long as several weeks. Voltage sensitive dyes (VSDs) enable ready voltage imaging through the fast staining of cell membranes, but the currently available sensors are limited by their low fluorescence enhancement and brightness. In this work, we synthetically modified the role of the lipophilic wire part of a prominent VSD (RhoVR) and we studied its action mechanism by computational methods to identify a pathway to more efficient VSDs. These novel tools working in combination can revolutionize our knowledge of neuronal computation science.

Acknowledgements

This research was supported by the National Research, Development and Innovation Office through the grant **TKP2021-EGA-42.** Supported by the **ÚNKP-23-5-BME-460** New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund. This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (**BO/365/23/7**).