

In vitro examination of spreading depolarizations: establishing a new experimental setup and protocol

Tamás Puskás, Réka Tóth, Ákos Menyhárt, Orsolya Ivánkovits-Kiss, Ferenc Bari, Eszter Farkas

Department of Medical Physics and Informatics, Faculty of Medicine & Faculty of Science and Informatics, University of Szeged, H-6720 Szeged, Korányi fasor 9, Hungary



Introduction

Cortical spreading depolarization (SD) and the associated cerebral blood flow (CBF) response occur spontaneously in stroke, and have a detrimental role in the survival of penumbral brain tissue. Recently, we have developed an in vivo imaging technique to visualize SD, the coupled CBF response, and changes in tissue pH. The excitation and emission spectrum of the pH sensitive fluorescent dye used to monitor tissue pH falls into the high light absorption range of hemoglobin. In order to analyze the pH-sensitive dye signal free of hemoglobin interaction, we designed an in vitro setup for the investigation of the SD-related tissue acidosis in live brain slices.

Aims

To design a brain slice chamber and build an in vitro experimental setup

To establish a reliable protocol to elicit SD-s in accordance with the standards reported in the literature.

Experimental protocol:



Materials and Methods

- 1.) The designs of the slice chamber was made in Autodesk AutoCAD®, then we used COMSOL Multiphysics® to create models what ensure the laminar flow
- 2.) The chamber was fabricated from black polyphenylsulfon.
- 3.) Slice-holding platform parts made from stainless steel
- 4.) Experimental materials:

Animals: 200 g, Male, Sprague-Sawley rats (n=10)

Slices: 400 um, coronal, somatosensory cortex

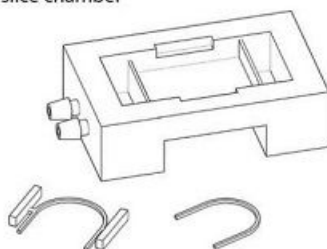
Recording temperature: 31 °C +/- 1 °C

SD elicitation: 40 nl 1 M KCl microinjection

Analysis: Mathworks Matlab®, Microsoft Excel®

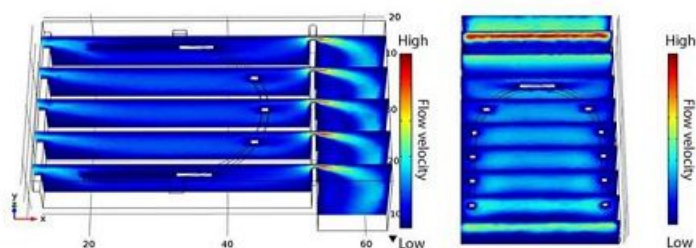
Results

Fig. 1: AutoCAD graphic design of the slice chamber



The complete 3D design was made from black polyphenylsulfon (PPSU)

Fig. 3: Flow dynamic model of the slice chamber



We created 3D flow dynamic models in the software called COMSOL Multiphysics. These models helped to find the best shape for the chamber that ensures the laminar flow in the whole experimental area.

Conclusion

In conclusion, we successfully established an in vitro brain slice setup with custom-made parts, which enables the investigation of SD in live brain slices. The quantitative analysis of recorded SDs indicates that the features of SDs meet the standard reported in the literature. Therefore, our setup is ready for the visualization of SD-related changes in tissue pH with the application of a pH sensitive dye, and is expected to be suitable for pharmacological manipulation of SDs, as well.

Funding: Hungarian Scientific Research Fund (K111923), TAMOP-4.2.2.D-15/1/KONV-2015-0024, János Bolyai Research Scholarship of the Hungarian Academy of Sciences, Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-I/13.

Fig. 2: The components of complete slice chamber

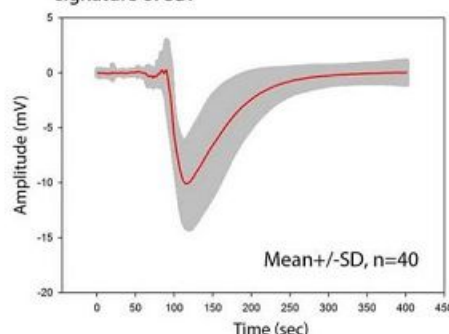


Fig. 4: Arrangement of devices for the registration of SD from the cortex



Cortical DC and injection pipettes and an ongoing SD

Fig. 5: The kinetics of the DC potential signature of SD.



Maximum amplitude of the DC shift: 11.36 +/- 3.44 mV, Duration of the DC shift at half amplitude: 3.43 +/- 1.48 min (mean +/- stdev).