Investigation of novel implant materials for high-resolution, multiparametric imaging of cortical activity

NKFIH K 120143 – Final report

Aim of the project

The goal of our project was to create novel, flexible, minimally invasive microsystems that are capable of capturing electrophysiological signals at high spatial resolution from the superficial layer of the cortex. An explicit aim was to foster the simultaneous use of such devices with state-of-the-art neuroimaging schemes like functional magnetic resonance imaging, ultrafast ultrasound localization microscopy or two-photon microscopy. The initiative also intended to deepen several international collaborations including UT Dallas, INSERM and CNRS. To achieve the expected results several microdevices were required relying on microfabrication approaches that have not been proposed or tested elsewhere.

Results

Functional brain mapping using optical imaging of intrinsic signals and simultaneous high-resolution cortical electrophysiology with a flexible, transparent microelectrode array

In this work, we introduced a micromachined device, which facilitates parallel detection of ECoG signals and intrinsic optical signal imaging in the primary visual cortex of the cat. The 8 micron thick, 32-channel microelectrode array is composed of polyimide (PI2611) substrate and indium-tin-oxide (ITO) metallization, and can withstand the impact of surgical and recording procedure in a small cranial chamber (see Figure 1-2). Reconstruction of the functional domains through this transparent array of microelectrodes was possible using 609 nm illumination of the exposed cortical surface (see Figure 3). Based on the analysis of the recorded optical and electrophysiological data, we can conclude that power map of narrowband gamma signals recorded by our device is dependent on stimulus patterns, and shows similarities to the orientation preference map obtained from single stimulus conditions. The resulted electric orientation preference map (E-OPM) showed some structural similarity to the traditional, IOSI based orientation preference map (OPM), which needs to be confirmed by further experiments. As far as we know, this is the first in vivo demonstration of a flexible, polyimide/ITO/polyimide based microECoG array, used for the simultaneous detection of intrinsic optical signals in conjunction with cortical EEG (Zátonyi, 2018, SNB).

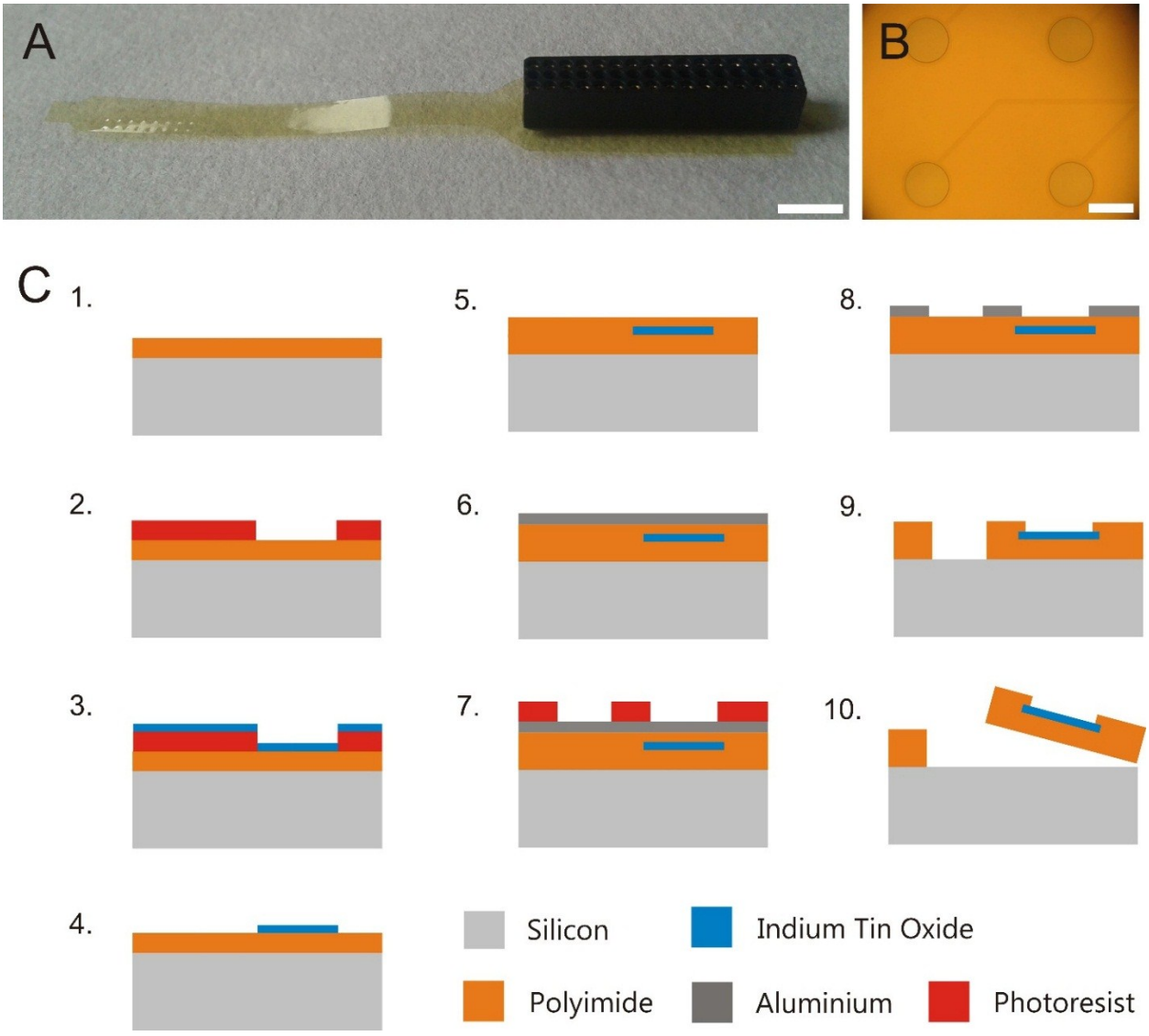

Figure 1. Perspective view of a ready-to-use transparent electrode array (a). Close view on recording sites of a polyimide/ITO/polyimide electrode array (b). Schematic view of the fabrication steps of the electrode array (c).

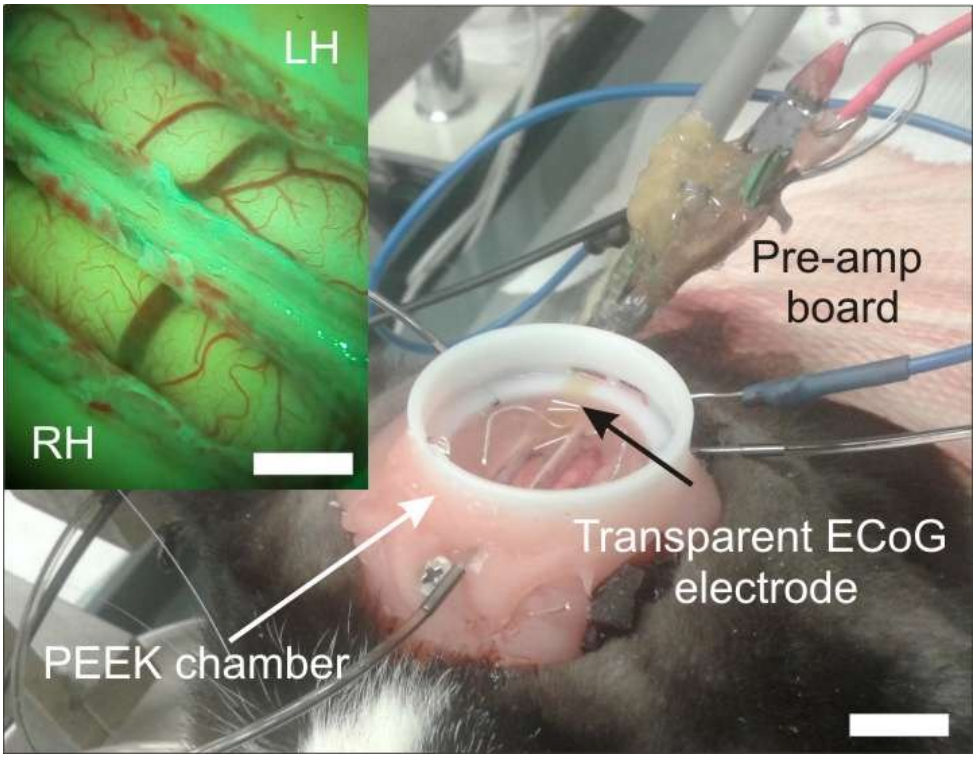

Figure 2. Recording setup mounted on the skull of an anaesthetized cat. Chamber is filled with ACSF and closed with a coverslip before optical imaging. CCD camera is not seen. The inset shows the exposed visual cortex. Right hemisphere (RH) is covered with ECoG electrode only, left hemisphere (LH) is intact.

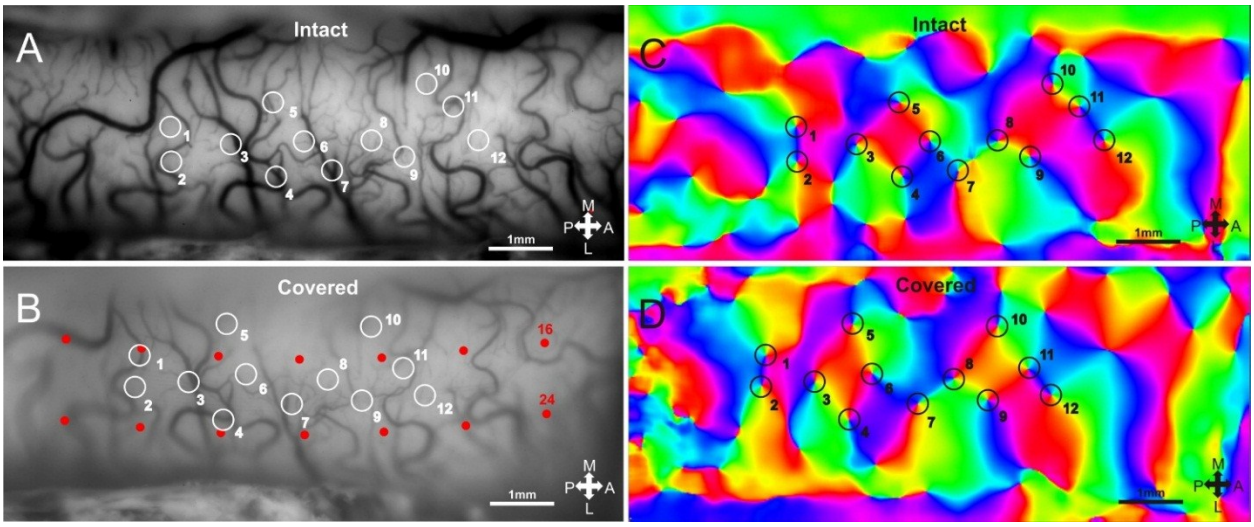

Figure 3. Vascular images of the exposed region of cat visual cortex A18 uncovered (a) and covered with an ECoG electrode (b). Orientation maps in each case are represented by (c) and (d). White and black circles with numbers and red dots show pinwheels and recording site positions of the electrode, respectively. Each numbered circle refers to the position of the same pinwheel on each picture.

In vitro and in vivo stability of black-platinum coatings on flexible, polymer microECoG arrays

Intracranial EEG (iEEG) or micro-electrocorticography (µECoG) microelectrodes offer high spatial resolution in recordings of neuronal activity from the exposed brain surface. Reliability of dielectric substrates and conductive materials of these devices are under intensive research in terms functional stability in biological environments. The aim of our study was to investigate the stability of electroplated platinum recording sites on 16-channel, 8 micron thick, polyimide based, flexible µECoG arrays implanted underneath the skull of rats (see Figure 4). Scanning electron microscopy and electrochemical impedance spectroscopy was used to reveal changes in either surface morphology or interfacial characteristics (see Figure 5). The effect of improved surface area (roughness factor = 23±0.12) on in vivo recording capability was characterized in both acute and chronic experiments. Besides the expected reduction in thermal noise and enhancement in signal-to-noise ratio (up to 39.8), a slight increase in the electrical impedance of individual sites was observed, as a result of changes in the measured interfacial capacitance. In our published paper (Zátonyi, 2018, JNE), we also presented technology processes and protocols in details to use such implants without crack formation of the porous platinum surfaces. Our findings imply that black-platinum coating deposited on the recording sites of flexible microelectrodes (20 microns in diameter) provides a stable interface between tissue and device (see Figure 6).

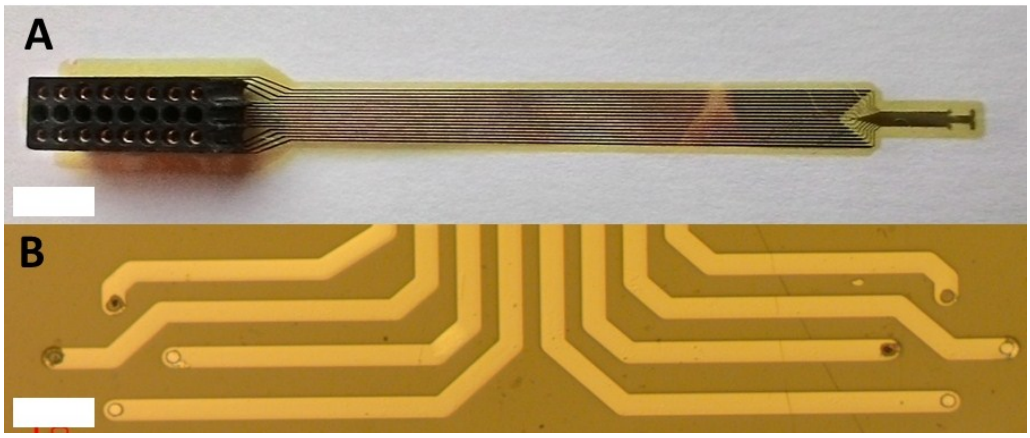

Figure 4. The ready-to-use packaged microdevice (A), close microscopic view on electrode arrays with electroplated (dark) and reference (intact Pt) recording sites (B)

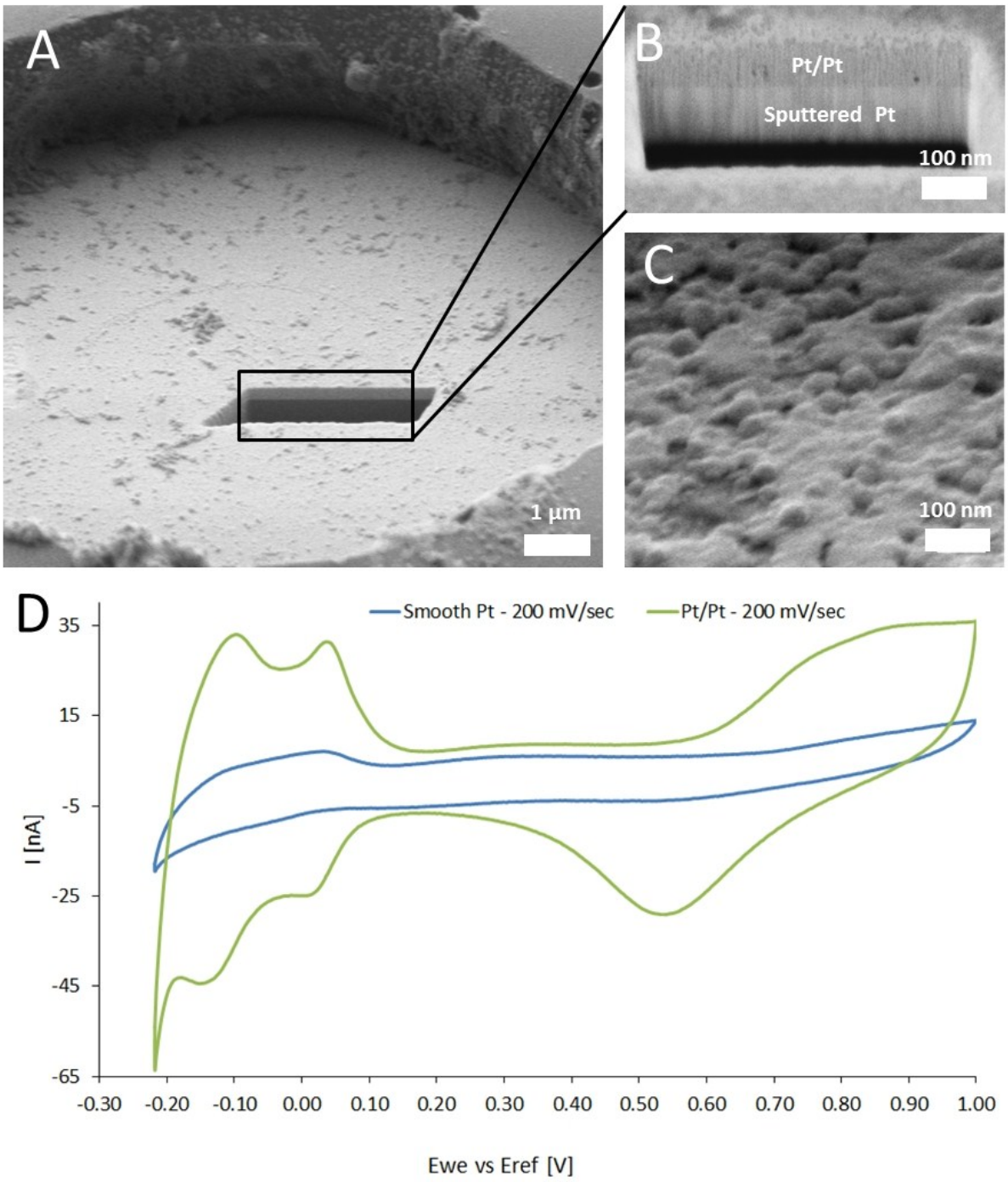

Figure 5. (A) Electron micrograph of the electroplated recording sites. (B) Cross-section view of the porous platinum/sputtered platinum multi-layer revealed by focused ion beam analysis. (C) Representative surface quality of the porous platinum formed by electroplating. (D) Cyclic voltammetry curves of sputtered (blue) and electroplated (green) platinum surfaces.

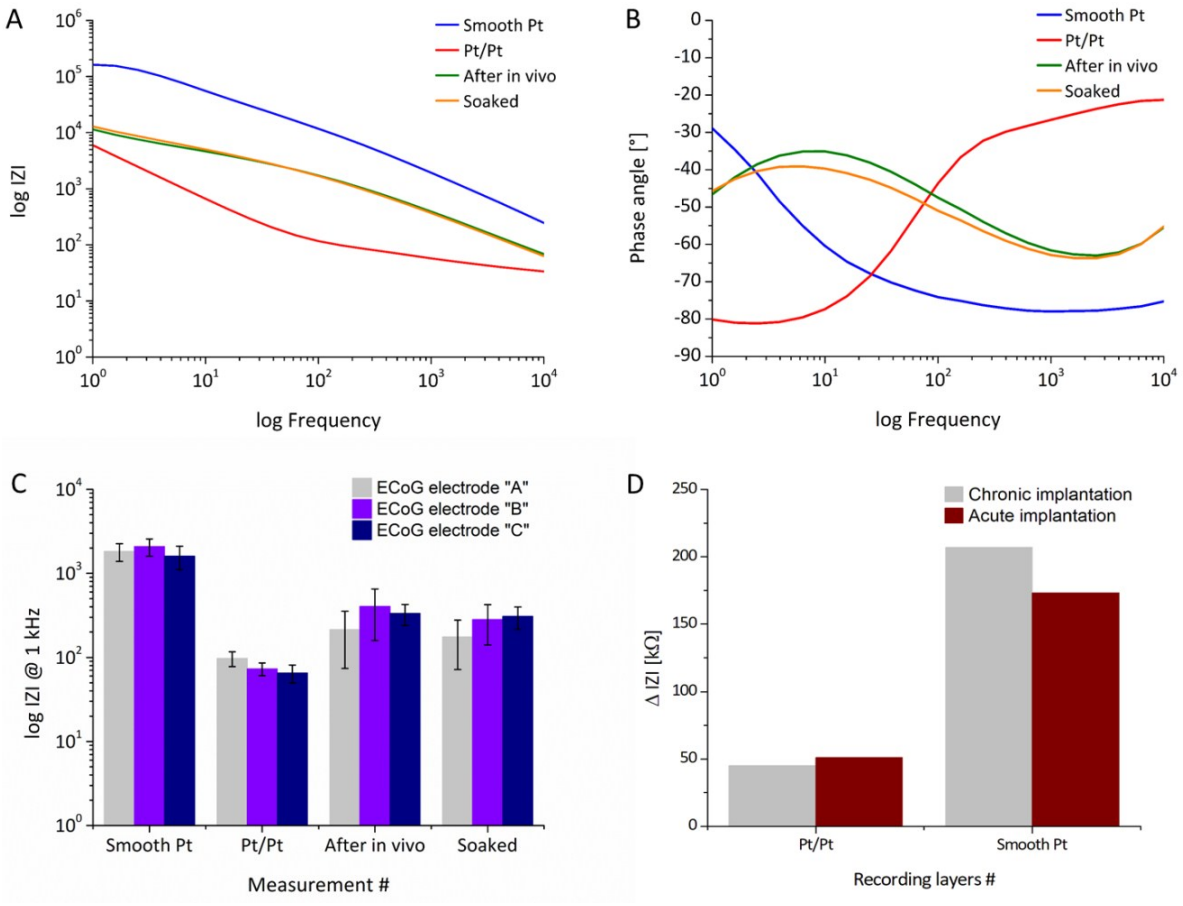

Figure 6. Representative Bode magnitude (A) and phase (B) plots derived from electrochemical impedance spectroscopy of an ECoG electrode along its lifecycle (C) Variation of impedance on three different but functionally identical ECoG electrode arrays (denoted as electrode „A", ECoG electrode „B", ECoG electrode „C") (D) Variation of impedance were also evaluated both in acute and chronic implantations.

Transparent, low-autofluorescence microECoG device for simultaneous Ca2+ imaging and cortical electrophysiology in vivo

Multimodal neuroimaging approaches are beneficial to discover brain functionalities at high spatial and temporal resolution. In our work, a novel material composition of microECoG device relying on Parylene HT and indium-tin-oxide (ITO) is fabricated that facilitates two-photon imaging of Ca2+ signals and concurrent recording of cortical EEG (see Figure 7). Long-term stability of the interfaces of the transparent microdevice is confirmed in vitro by electrochemical and mechanical tests. The outstanding optical properties, like high transmittance and low auto-fluorescent are proven by fluorimetric measurements.

Spatial resolution of fluorescent two-photon imaging through the microECoG device is presented in transgenic hippocampal slices, while concurrent recording of Ca2+ signals and cortical EEG is demonstrated in vivo. Photoartefacts and photodegradation of the materials are also investigated in detail to provide safety guidelines for further use in two-photon in vivo imaging schemes. Two-photon imaging of Ca signals can be safely performed through the proposed transparent ECoG device, without significant distortion in the dimensions of detected neuronal structures or in the temporal signaling. In chronic use, we demonstrated that fluorescent Ca signals of individual neurons can be clearly recorded even after 51 days (see Figure 8). Our results give a firm indication that highly transparent microECoG electrode arrays made of Parylene HT/ITO/Parylene HT multilayer are excellent candidates for synergetic recording of optical signals and EEG from intact brains with high resolution and free of electrical and optical artefacts (Zátonyi, 2020).

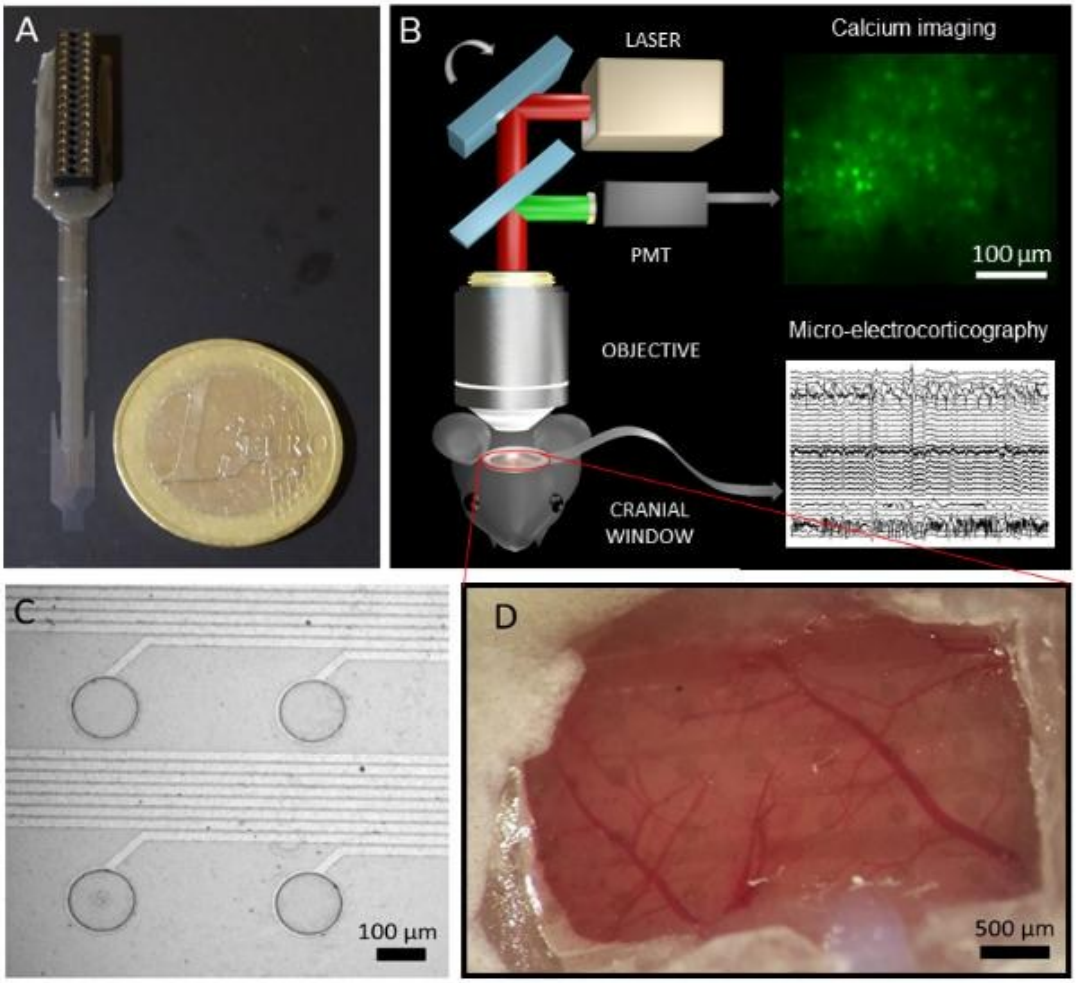

Figure 7. (A) Perspective view of the transparent microECoG array made of low-autofluorescent Parylene HT substrate. (B) Schematic figure representing the experimental design for simultaneous Ca^{2+} imaging and electrophysiology in vivo using our transparent microelectrode array. (C) Close view of recording sites and wires made of sputter-deposited indium-tin-oxide. (D) Epidurally implanted transparent microECoG array prepared for two-photon imaging in freely moving mouse.

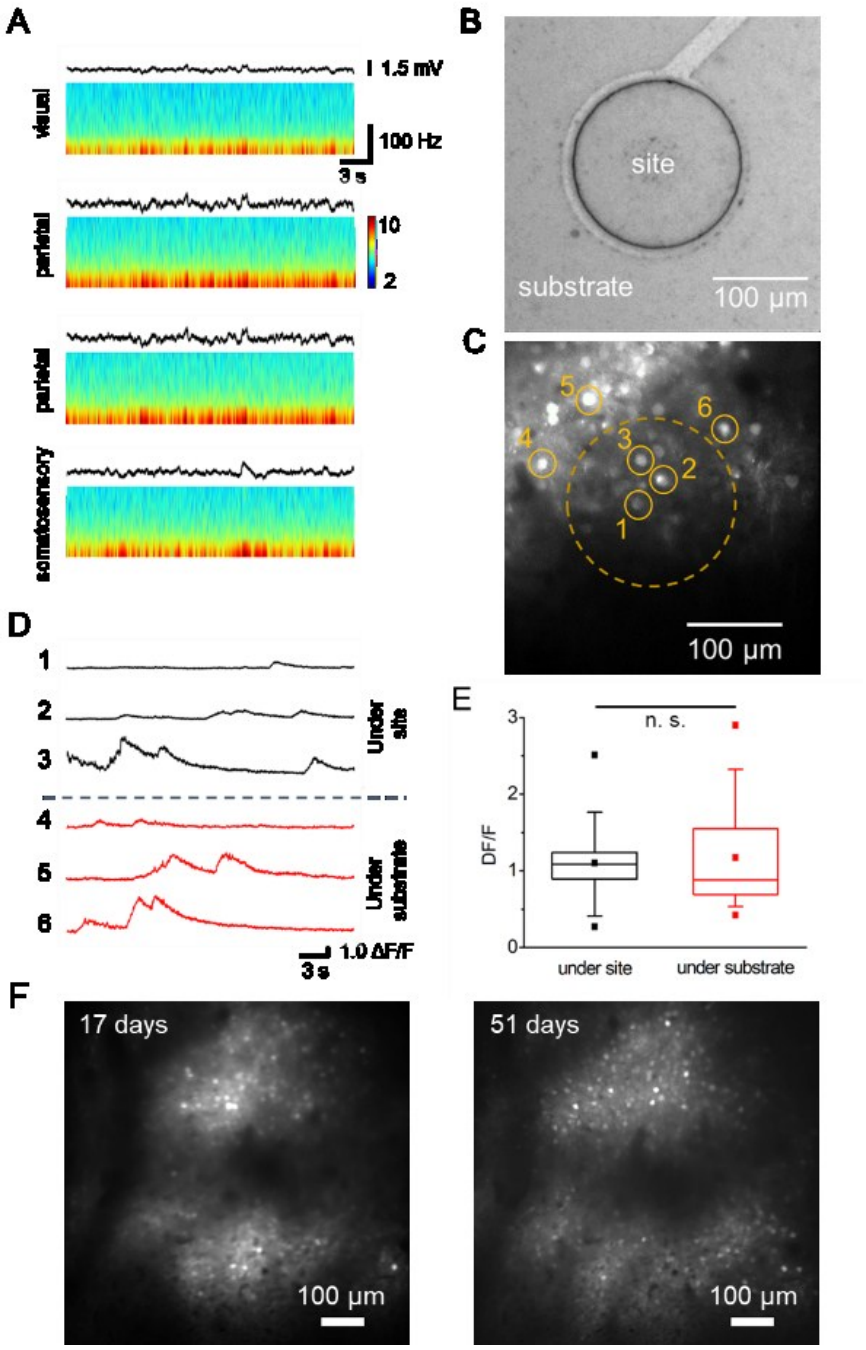

Figure 8. In vivo Ca^{2+} imaging. (A) Examples of ECoG recordings which show spontaneous activity of the tissue and heat maps of the traces. (B) A contact site of the electrode array. (C) Two-photon image at the depth of 250 µm from the surface (L2/3). GCaMP 6f expressing neurons were visible under the substrate and also the contact site. Some observed cells are denoted by solid orange circles. Dashed circle marks the position of contact site. (D) Thirty seconds long Ca^{2+} curves registered from the cells are shown on panel C (identically numbered). (E) Statistical comparison of highest Ca^{2+} peaks (expressed in ΔF/F) were produced by neurons under the site and the surrounding substrate. (N = 25 (cells under the surrounding substrate were randomly selected); Mann-Whitney U test: $p > 0.5$). (F) Images of the entire injected area in V1 region 17 (left) and 51 (right) days after the electrode implantation.

Application of a flexible polymer microECoG array to map functional coherence in schizophrenia model

Anatomically, connections form the fundamental brain network, functionally the different types of oscillatory electric activities are creating a temporarily connected fraction of the anatomical connectome generating an output to the motor system. Schizophrenia can be considered as a connectome disease, in which the sensory input generates a schizophrenia specific temporary connectome and the signal processing becomes diseased showing hallucinations and adverse behavioral reactions. In this work, flexible, 32-channel polymer microelectrode arrays fabricated by the authors are used to map the functional coherence on large cortical areas during physiological activities in a schizophrenia model in rats (see Figure 9) (Fedor, 2020). We have fabricated a flexible microECoG array and elaborated a protocol to use to characterize connectome diseases in rats using this device (see Figure 10). Customized method to analyze the functional coherence between different cortical areas during visually evoked potential is also determined. We implemented an R-based method to analyze ECoG data.

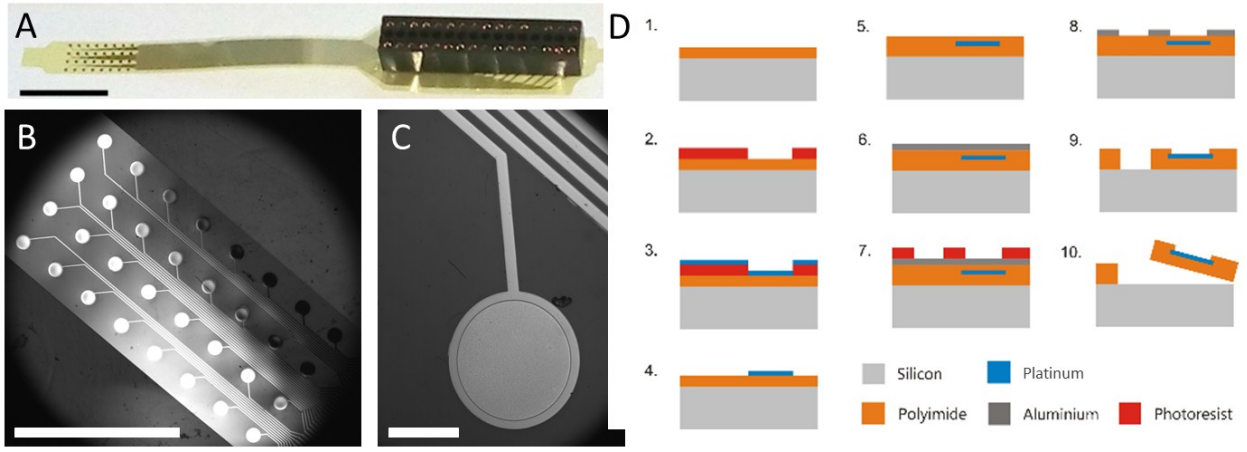

Figure 9. (a) Photo of the ready to use microECoG array. Close microscopic view on a 32-channel sensor array (b) and on a platinum recording site (c). Scale bars show10 mm, 0.5 mm and 150 µm, respectively. Schematics on the manufacturing process of the microdevice (d).

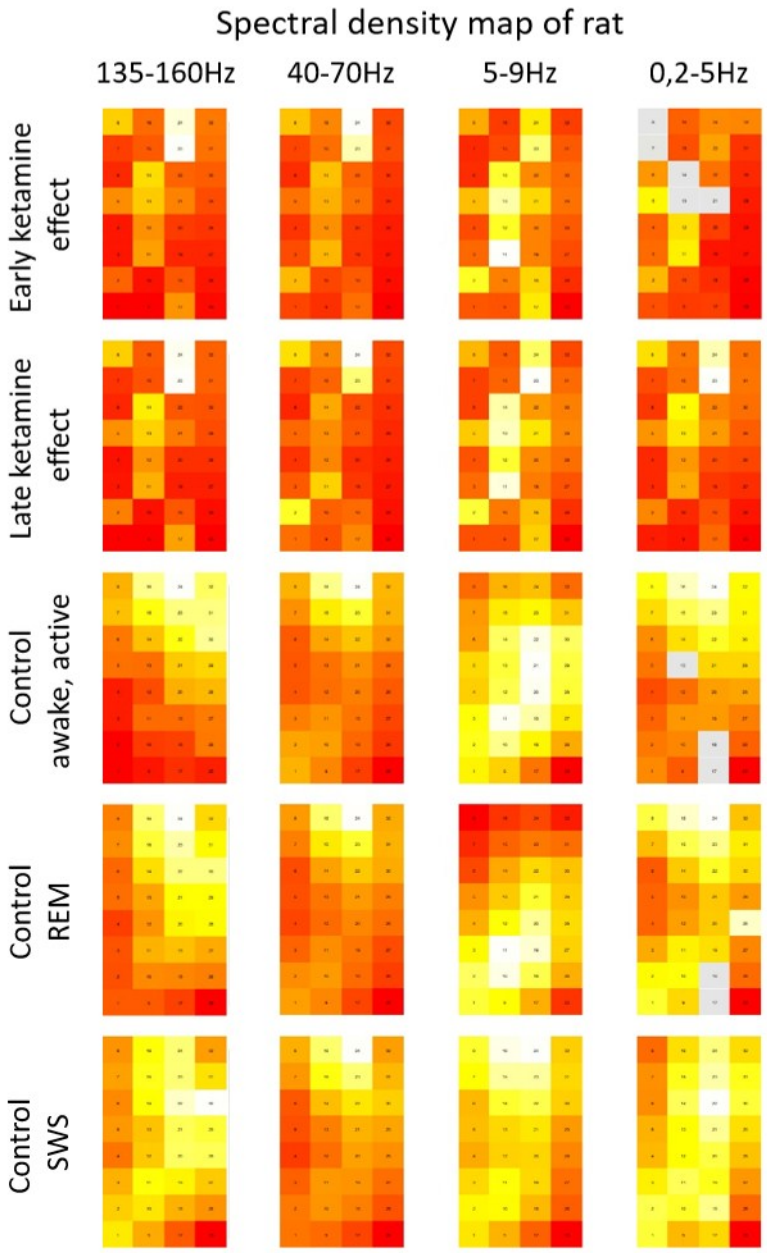

Figure 10. The power spectrum values of four EEG frequency bands (columns: 135-160 Hz, 40-70 Hz, 5-9 Hz, 0,2-5 Hz) were calculated and color-coded to show their distribution over the rat's cortical surface in different normal brain states (rows: Awake, REM sleep, Slow wave sleep) and after ketamine injection.

A softening laminar electrode for recording single unit activity from the rat hippocampus

Softening neural implants that change their elastic modulus under physiological conditions are promising candidates to mitigate neuroinflammatory response due to the reduced mechanical mismatch between the artificial interface and the brain tissue. Intracortical neural probes have been used to demonstrate the viability of this material engineering approach. In our work, we elaborated a robust technology of softening neural microelectrode and demonstrate its recording performance in the hippocampus of rat subjects (Zátonyi, 2019). The 5 mm long, single shank, multi-channel probes are composed of a custom thiol-ene / acrylate thermoset polymer substrate, and were micromachined by standard MEMS processes (see Figure 12). A special packaging technique is also developed, which guarantees the stable functionality and longevity of the device, which were tested under in vitro conditions prior to animal studies. The 60 micron thick device was successfully implanted to 4.5 mm deep in the hippocampus without the aid of any insertion shuttle. Spike amplitudes of 84 µV peak-to-peak and signal-to-noise ratio of 6.24 were achieved in acute experiments (see Figure 13). Our study demonstrates that softening neural probes may be used to investigate deep layers of the rat brain.

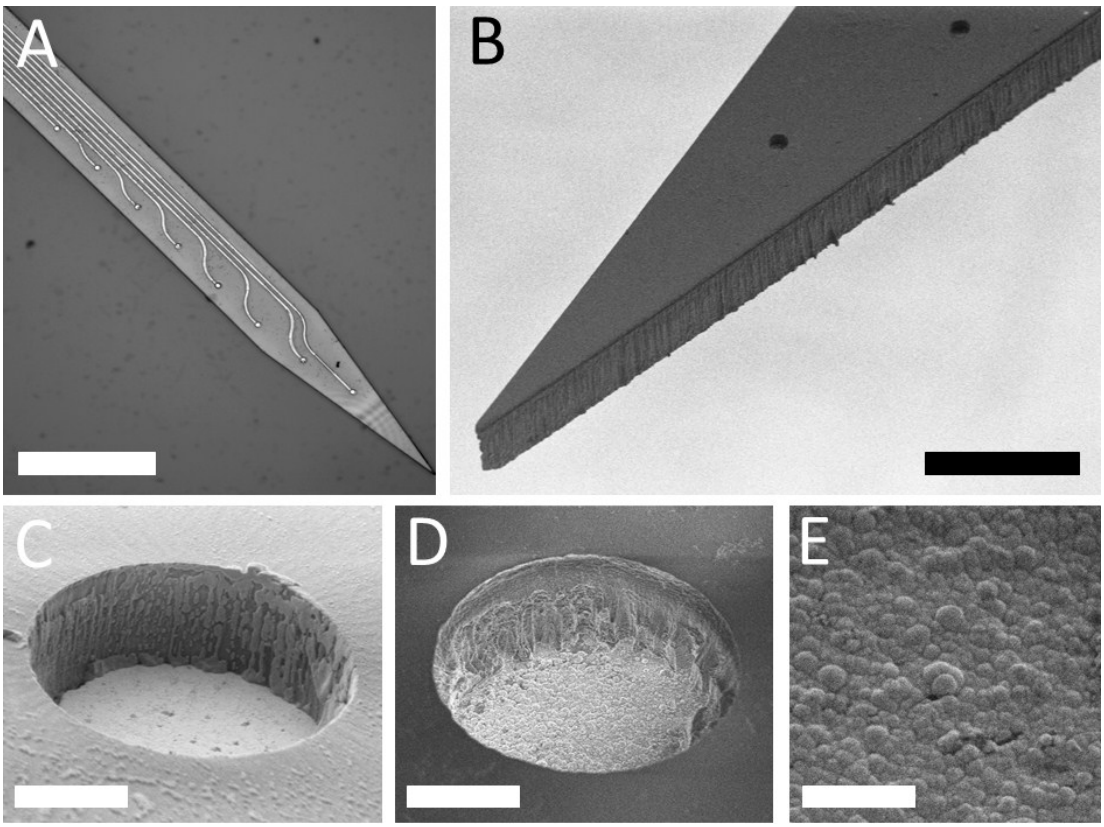

Figure 12.: Planar microscopy view of the released probe containing gold metal traces and recording sites (a). Scanning electron microscopy view on the sidewall profile (b) on a reference recording site (b) and platinum coated recording site (c) of the released probe. High magnification images of the surface morphology of platinum black coating (e). Scale bars are 750 µm, 200 µm, 5 µm, 5 µm and 1 µm respectively.

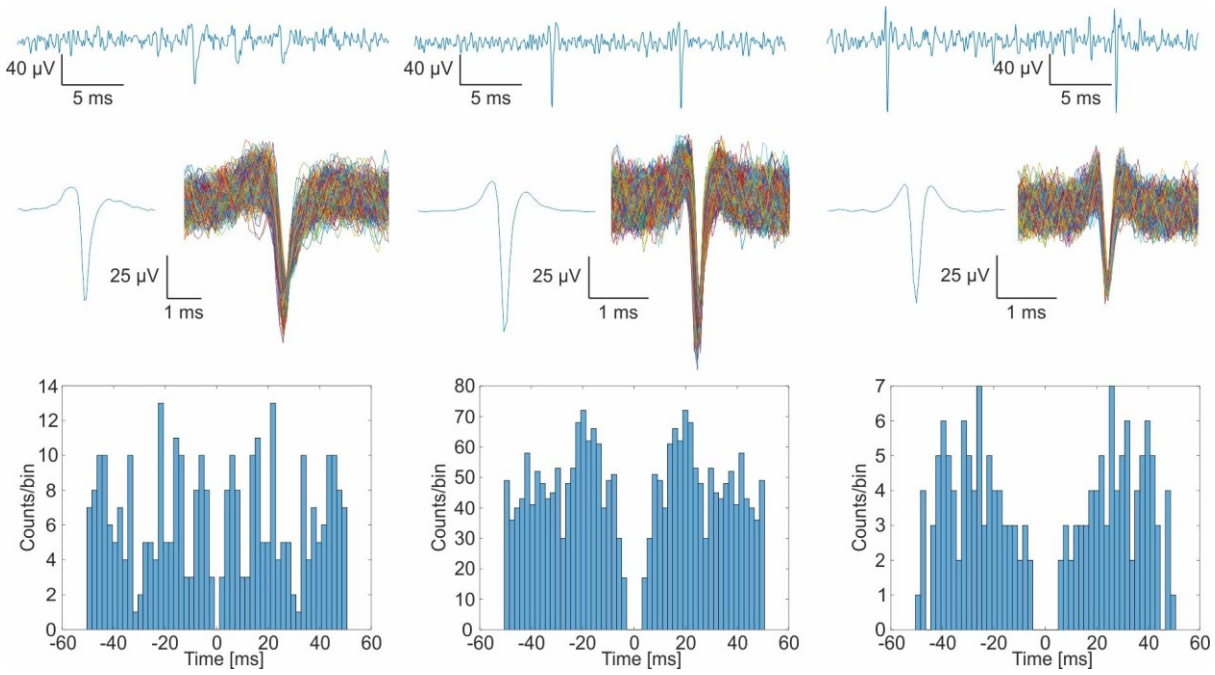

Figure 13. Representative filtered waveform of the acute test, samples of the clustered spikes from each implantation and their autocorrelograms

FMRI-compatible ECoG device to record cortical activity of primate in behavioural tasks

In the NMR spectroscope of Wigner RCP, we tested all the insulating, conductive layers of the proposed MRI compatible EEG electrdes and also investigated ferromagnetic and paramagnetic components in the selection of packaging materials. We determined the optimal layer structures of the microsensor and selected the encapsulation materials that contain the less contaminants. In cooperation with INSERM, we designed a specific ECoG array conforming the prefrontal cortex of macaques. After a successful fabrication of the devices, we started to test them in the MRI equipment of INSERM (still on brain phantom). The electrical recording quality were tested in rodents at ELTE in original experiments. In these long-term measurements (1-2 months), intracranial EEG signals were recorded and analyzed. Based on these positive experience we have already made arrangements to translate these results and prepared the first surgery of this device into a macaque monkey in Lyon (INSERM). Our MRI-compatible, polyimide based ECoG device has now been implanted into the prefrontal cortex of two rhesus macaques at INSERM (see Figure 14). Currently, we have recordings for more than two years in these subjects. The large dataset of EEG during behavioural tasks and long term stability of recording quality are being analyzed.

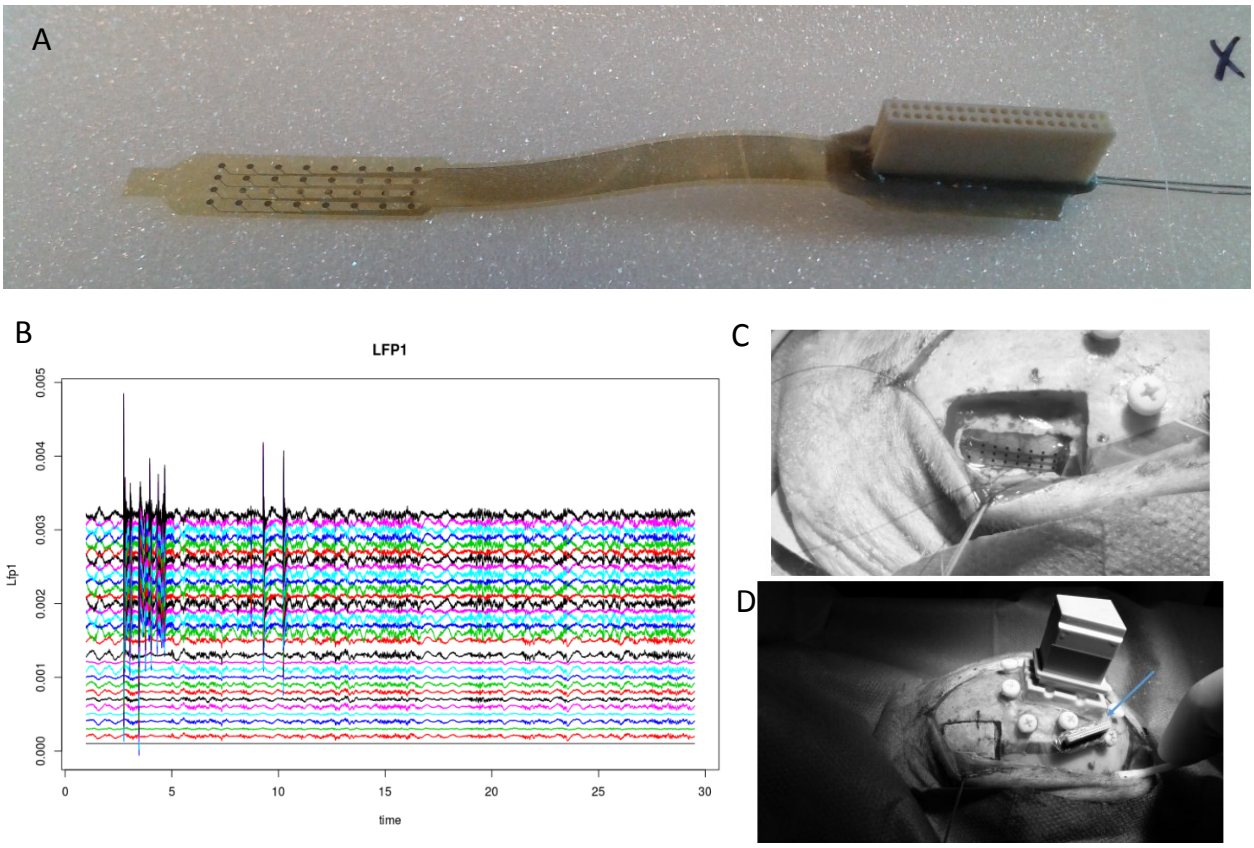

Figure 14. A) MRI-compatible ECoG device designed for macaque experiments. B) Raw ECoG data coming from the very first recordings in macaque subjects. C) Surgical placement of the ECoG devices just by the dorsal side of the sulcus principalis. D) The place of the connector of the ECoG is denoted with blue arrow on the skull.

Polyimide based electrode array applied during the Functional ultrasound imaging of the brain of freely moving mice

We have tested PI2611-based electrode subtrates mounted in between a Doppler transducer and the skull of a mouse. We did not identified significant distortion in the image quality taken on brain vasculatura, therefore our approach is promising to combine the two modalities into a single platform. We have designed various electrode layouts to move to the first in vivo tests in freely moving subjects. After a number of successful experiments, we concluded that the spatial resolution of the ultrasound imaging is even better with our device on top of the skull, which may be attributed to the fact that the substrate helps to maintain the wetted state of the exposed skull and therefore reduces attenuation of the signal (see Figure 15).

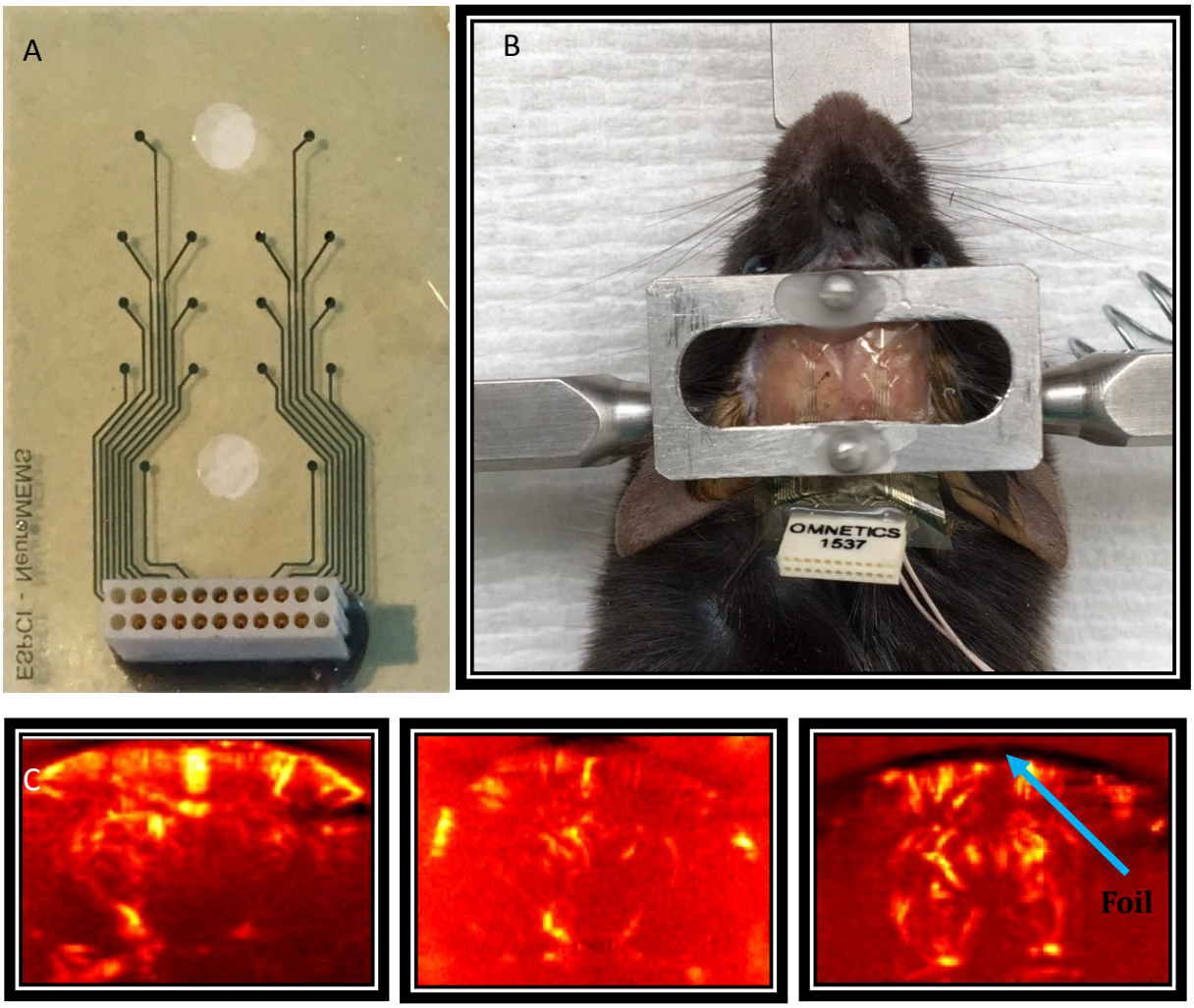

Figure 15. A) ECoG device designed to fit the fixture of the ultrasound transducer, B) Implantation of the EEG Foil on the skull with conductive gel and surgical glue. A magnetic metal frame is added to guide the ultrasonic probe secured to the skull with two anchoring screws. C) Doppler image without foil at Day 1, d) Doppler image without foil at Day 7. e) Doppler image with foil at Day 7.

Indicators

Number of PhD theses: 4 (finished and ongoing)

Number of Msc, Bsc theses: 12

Number of articles: 8

Number of D1 articles: 5

Cumulative impact factor of the articles: 24.750

List of publications

F.Z. Fedor, A. Zátonyi, D. Cserpán, Z. Somogyvári, Z. Borhegyi, G. Juhász, Z. Fekete, Application of a flexible polymer microECoG array to map functional coherence in schizophrenia model, METHODSX 7 (2020) 101117, CiteScore: 1.5

F.Z. Fedor, C. Paraczky, L. Ravasz, Z. Borhegyi, Z. Somogyvári, G. Juhász, Z. Fekete, Electrophysiological and behavioral properties of 4-aminopyridine-induced epileptic activity in mice. BIOLOGIA FUTURA (2020). https://doi.org/10.1007/s42977-020-00047-z

A. Zátonyi, M. Madarász, Á. Szabó, T. Lőrincz, R. Hodován, B. Rózsa, Z. Fekete, Transparent, low-autofluorescence microECoG device for simultaneous Ca2+ imaging and cortical electrophysiology in vivo, JOURNAL OF NEURAL ENGINEERING 17 (2020) 016062, IF: 4.141

A Zátonyi, G. Orbán, R. Modi, G. Márton, D. Meszéna, I. Ulbert, A. Pongrácz, M. Ecker, W.E. Voit, A. Joshi-Imre, Z. Fekete, A softening laminar electrode for recording single unit activity from the rat hippocampus, SCIENTIFIC REPORTS 9 (2019) 37237, IF: 3.998

A. Zátonyi, F. Fedor, Zs. Borhegyi, Z. Fekete, In vitro and in vivo stability of black-platinum coatings on flexible, polymer microECoG arrays, JOURNAL OF NEURAL ENGINEERING 15 (2018) 0453003, IF: 4.551

A. Zátonyi, Á. Szabó, F. Flóra, Á.C. Horváth, A. Pongrácz, Z. Fekete, Trendek a polimer alapú implantátumok fejlesztésében, Anyagok Világa 1 (2018) 17-33

A. Zátonyi, Zs. Borhegyi, M. Srivastava, D. Cserpán, Z. Somogyvári, Z Kisvárday, Z. Fekete, Functional brain mapping using optical imaging of intrinsic signals and simultaneous high-resolution cortical electrophysiology with a flexible, transparent microelectrode array, SENSORS & ACTUATORS B-CHEMICAL 273 (2018) 519-526, IF: 6.393

Z. Fekete, A. Pongrácz, Multifunctional soft implants to monitor and control neural activity in the central and peripheral nervous system: a review, SENSORS & ACTUATORS B-CHEMICAL 243 (2017) 1214-1223, IF: 5.667