Effect of electrical microstimulation parameters on in vivo neuronal calcium responses in the visual cortex of anesthetized mice

Eszter Nguyen[1,2], Csaba Horváth[1,3], Melinda Rácz[1,3,4], Frederik Ceyssens[5,6], Maarten Schelles[5], Michael Kraft[5,6], István Ulbert[1,2], Lucia Wittner[1,2], Richárd Fiáth[1,2]

[1] Integrative Neuroscience Group, Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences,Budapest, Hungary
[2]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary
[3]János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, Hungary
[4]Selye János Doctoral College for Advanced Studies, Semmelweis University, Budapest, Hungary
[5]Department of Electrical Engineering (ESAT), Micro- and NanoSystems (MNS), KU Leuven, Leuven, Belgium
[6]Leuven Institute for Micro- and Nanoscale Integration (LIMNI), KU Leuven, Leuven, Belgium

INTRODUCTION

Sensory neuroprostheses use electrical microstimulation through implanted neural interfaces with the aim to restore sight or hearing. Although this field has shown great progress in recent years, there are still gaps in our knowledge on how to precisely target and activate specific neurons or neuron populations by intracortical microstimulation. To improve the stimulation resolution, an evident solution would be to increase the number of stimulation sites as well as decrease the size of the electrodes. However, increasing the volume or the number of devices implanted into the brain tissue will inherently result in more tissue damage and complications. Another, less invasive way to provide more precise control of neuronal activity without increasing the number of electrodes could be the application of advanced stimulation patterns (e.g., current steering, dynamic stimulation). In this pilot study [2], we developed flexible multi-shank probes containing multiple small electrodes to assess the effects of advanced electrical microstimulation strategies on cortical activity obtained using in vivo two-photon calcium imaging. The fabricated probes were implanted into the visual cortex (V1) of Thy1-GCaMP6 transgenic mice anaesthetized with ketamine/xylazine. Here we present the observed range of neuronal responses to the different parameters of the electrical stimuli. A two-photon laser scanning microscope was used to image calcium activity in layer 2/3 of the visual cortex adjacent to the probes.

METHODS

The project aims to investigate the activity of cortical populations in both primary (V1) and higher-order visual areas (HVAs) at the level of individual neurons. To achieve this, calcium imaging experiments were conducted using transgenic mice (GCaMP6s, GCaMP6f) with genetically encoded intracellular calcium indicators. During the initial in vivo electrical stimulation experiments, we investigated various stimulation parameters and observed diverse spatial and temporal activation patterns among neurons . The polyimide high-density electrode arrays used in the experiments have shanks with a cross-section of 20 × 70 µm² (thickness × width). The shanks are 1000 µm long and are spaced at a fixed distance of 250 µm from each other (Fig. 1). Rectangular iridium oxide electrodes (20 × 30 µm²) are arranged along the width and length of each shank, forming a grid with spacing ranging from 15 µm to several 100 µm (Fig. 1B). A rectangular craniotomy (~4 mm x 3 mm) is prepared, centered over the visual cortex. A glass coverslip with a 3 mm diameter is then carefully placed in the anterior part of the craniotomy and fixed to the skull using a small amount of light-curing dental cement. Following this, the flexible polymer-based probe is slowly inserted into the brain tissue at a speed of approximately 2 µm/s, targeting the primary visual cortex for two-photon Ca2+ imaging (Fig. 2). The calcium imaging data time series obtained during the experiments were processed after the necessary file conversion. The data analysis pipeline we are currently developing is based on suite2p [1] (Fig. 8A,D).

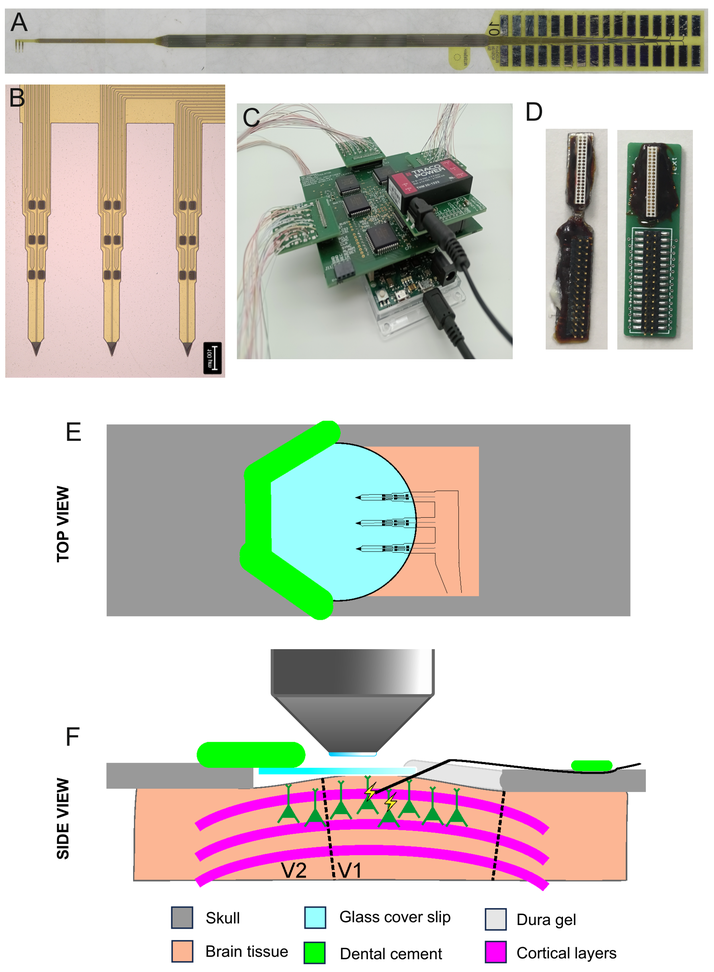

Figure 1. The flexible, polymer-based probe and the neurostimulator used for in vivo calcium imaging experiments in mice. (A) Photograph showing the realized probe with the implantable part (left), the cabling (middle) and the connector interface (right). (B) Stereomicroscopic image of the three shanks and microelectrodes (black rectangles and triangles) of the probe. (C) The neurostimulator device used to generate pulse trains for electrical stimulation. (D) Adaptor printed circuit board used to connect the probes to the neurostimulator. Top (E) and side (F) views of the experimental design for in vivo calcium imaging and stimulation.

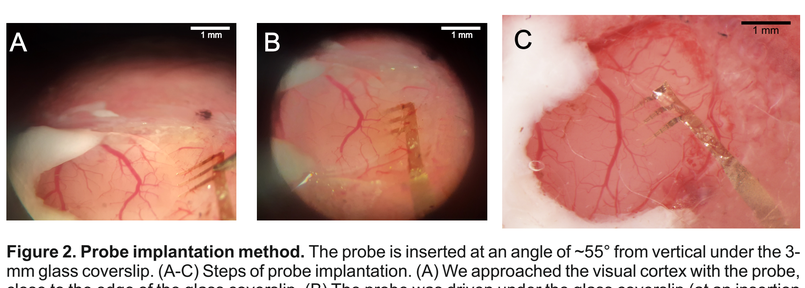

Figure 2. Probe implantation method. The probe is inserted at an angle of ~55° from vertical under the 3-mm glass coverslip. (A-C) Steps of probe implantation. (A) We approached the visual cortex with the probe, close to the edge of the glass coverslip. (B) The probe was driven under the glass coverslip (at an insertion speed of 2 µm/s). (D) The probe was carefully released, and Dura-Gel was applied on the exposed cortex.

CONCLUSION AND FUTURE PLANS

Here, we demonstrated the impact of varying stimulation parameters on visual cortical activity under deep ketamine/xylazine anesthesia (Figs. 3-8). Our findings, based on the mouse strains GCaMP6s & GCaMP6f, indicate that higher current amplitudes and stimulation frequencies lead to the activation of a greater number of neurons within the calcium imaging field of view (Figs. 3, 6 and 8C). Different pulse trains also resulted in distinct temporal activation patterns (Fig. 8D). In addition, longer stimulation durations at certain parameters showed relatively stable activation patterns at different times (Fig. 7). Currently, we are in the process of developing a processing and analysis pipeline to allow a more in-depth calcium imaging data analysis. Additionally, we have initiated the development of the experimental protocol for conducting experiments in awake, head-fixed mice.

This project (HYPERSTIM) has received funding from the HORIZON EIC Pathfinder Grant under grant agreement No101071015. R.F. was supported by the Bolyai János Scholarship of the Hungarian Academy of Sciences and by the New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund (ÚNKP-23-5-PPKE-128).. M. R. is thankful for the SE 250+ Doctoral Scholarship for Excellence.

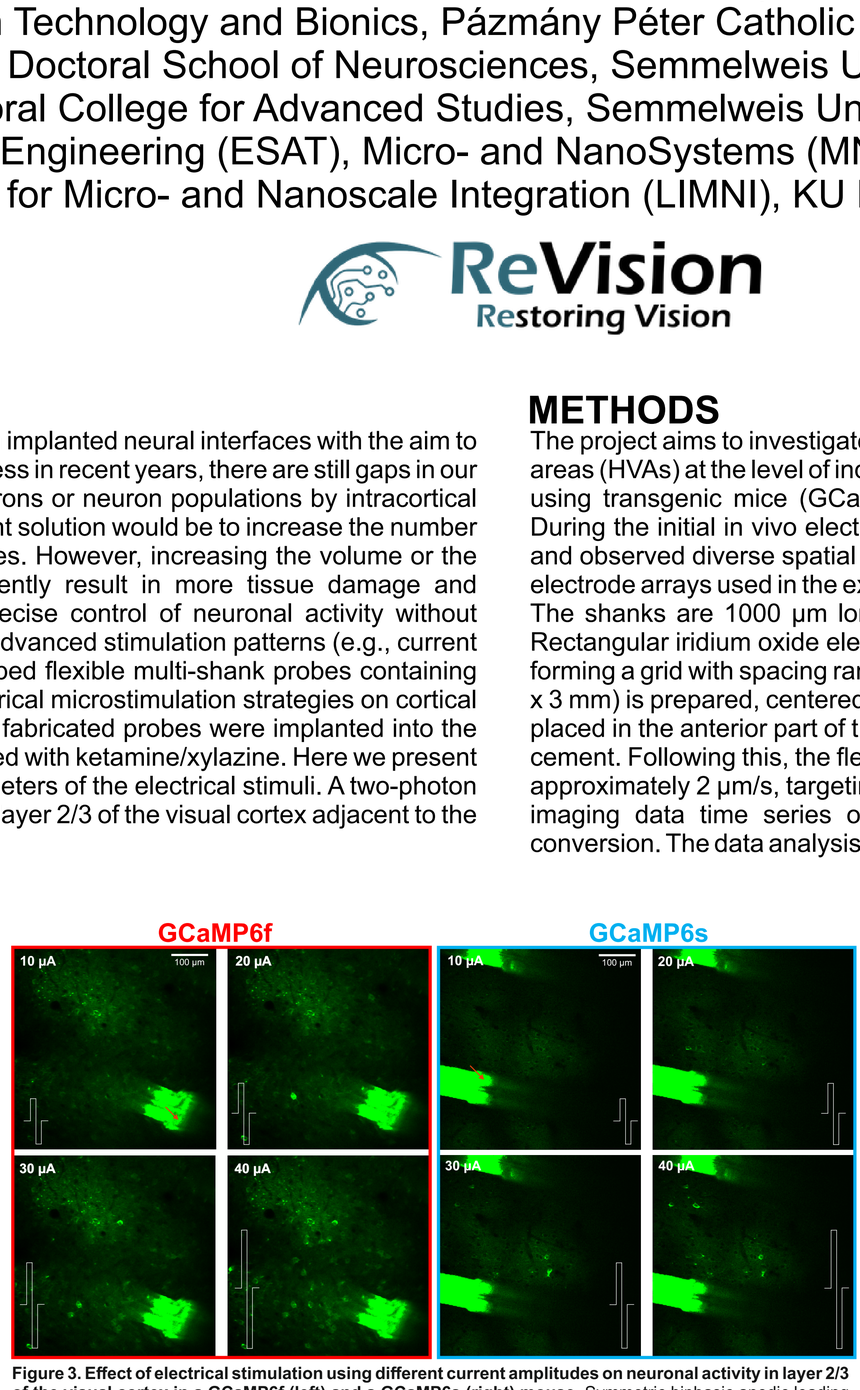

Figure 3. Effect of electrical stimulation using different current amplitudes on neuronal activity in layer 2/3 of the visual cortex in a GCaMP6f (left) and a GCaMP6s (right) mouse. Symmetric biphasic anodic leading pulses were used for stimulation at a rectangular microelectrode (red arrow). The parameters used for stimulation were as follows. Number of pulses (**NP**): 100; Pulse width (**PW**) : 200 µs (one phase);Frequency (**F**): 100 Hz; Interphase interval (**IPI**): 0 µs; Trial delay (**TD**): 2 s; number of repeats (**NR**, trials): 5. In the frames, a small segment of one or two probe shanks is visible in the imaged plane, appearing highly fluorescent. Frames were acquired approximately 500 ms after the onset of stimulation. It is worth noting that the number of activated neurons increases with higher current amplitudes.

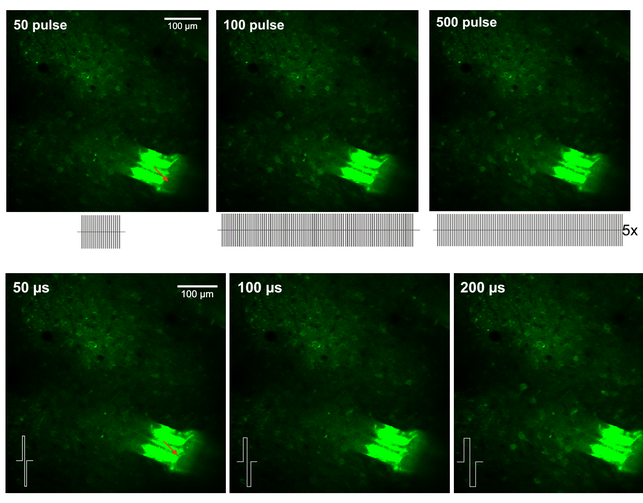

Figure 4. Effect of electrical stimulation using different number of pulses on the activity in layer 2/3 of the visual cortex in a GCaMP6f mouse. Current amplitude (**CA**): 40 µA; PW: 200 µs; F: 100 Hz; IPI: 0 µs; TD: 2 s; NR: 5. Note that the number of activated neurons and their spatial activation pattern are similar for different pulse numbers.

Figure 5. Effect of electrical stimulation using different pulse durations on neuronal activity in layer 2/3 of the visual cortex in a GCaMP6f mouse. NP: 100; CA: 40 µA; F: 100 Hz; IPI: 0 µs; TD: 2 s; NR: 5. Note that the number of activated neurons increases with longer pulse durations.

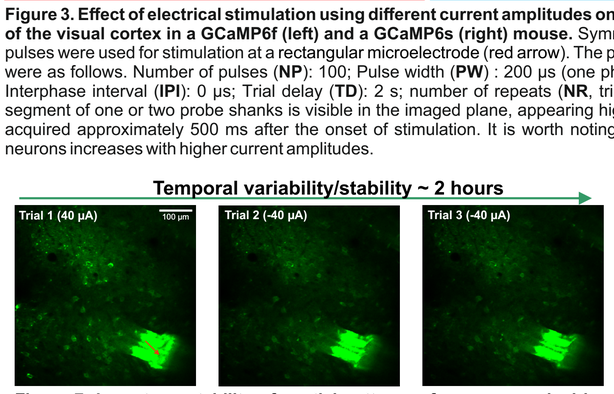

Figure 7. Long-term stability of spatial patterns of neurons evoked by intracortical microstimulation in a GCaMP6f mouse. Three stimulation trial obtained at different time points of a two-hour-long imaging session. Stimulation was performed with the same parameters (only the polarity of the current was changed once). CA: 40 (or -40) µA; NP: 100; PW: 200 µs; F: 100 Hz; IPI: 0 µs.

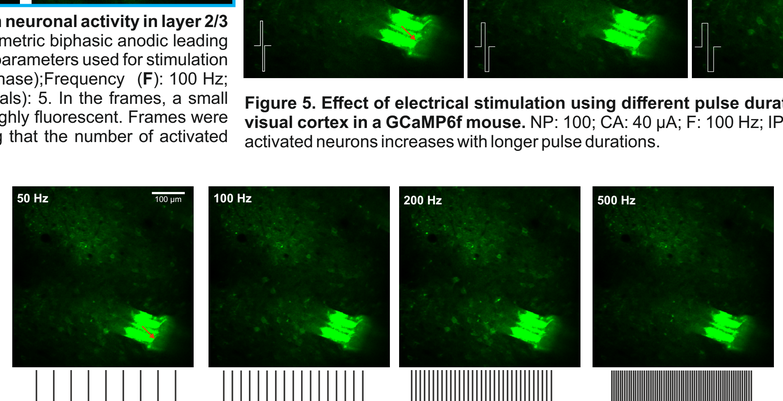

Figure 6. Effect of electrical stimulation using different stimulation frequencies on neuronal activity in layer 2/3 of the visual cortex in a GCaMP6f mouse. NP: 100; CA: 40 µA; PW: 200 µs; IPI: 0 µs; TD: 2 s; NR:5. Note that the number of activated neurons increases only slightly at higher frequencies and is significantly lower at 500 Hz due to a shorter stimulation duration.

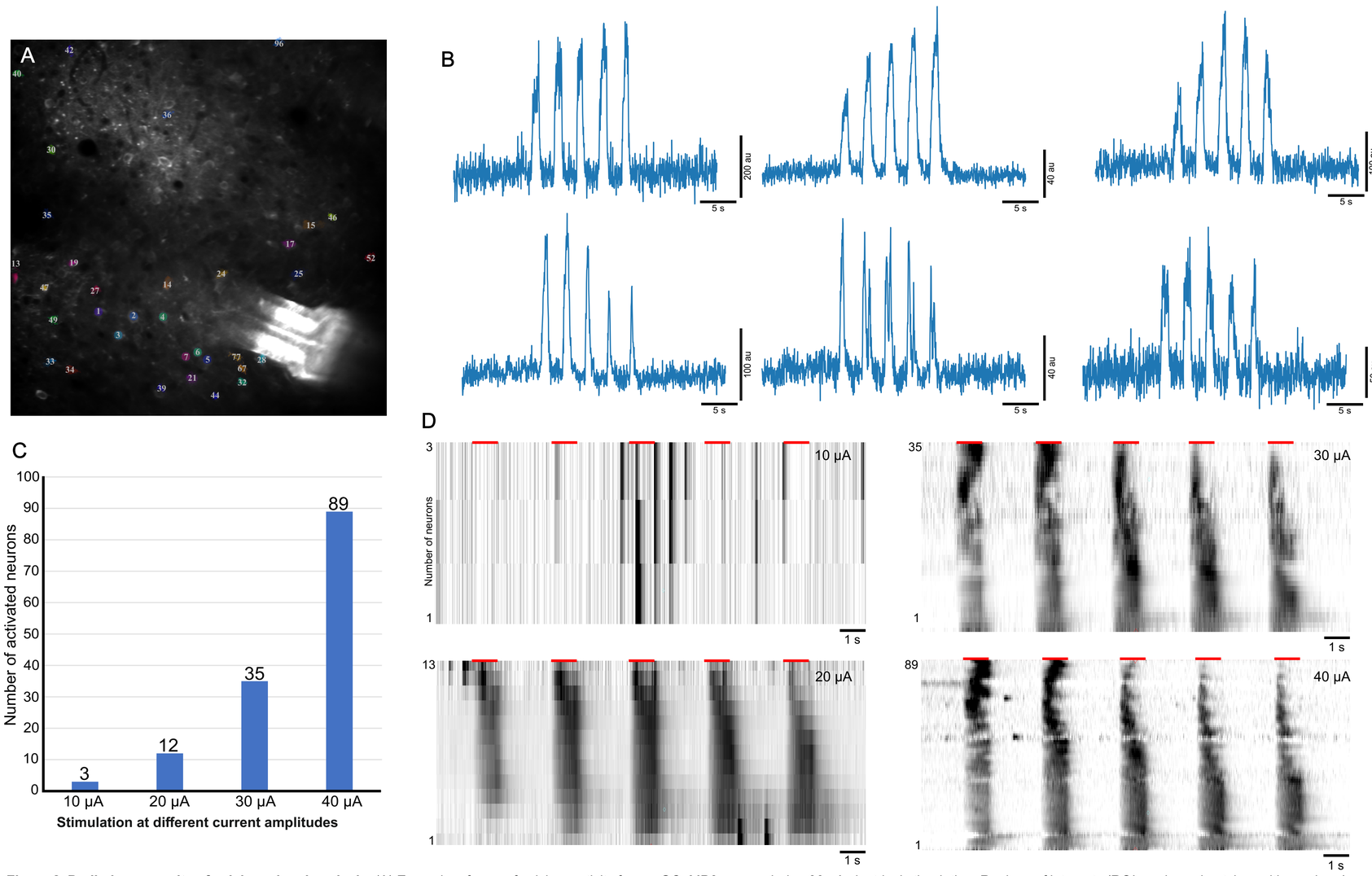

Figure 8. Preliminary results of calcium signal analysis. (A) Exemplary frame of calcium activity from a GCaMP6 mouse during 30 µA electrical stimulation. Regions of interests (ROIs, coloured patches with numbers), which represent the somas of neurons, were identified with suite2p (n = 35 neurons). (B) Sample neural calcium activity responses observed to five consecutive electrical stimuli at 30 µA. (C) Number of active cells identified at different current intensities used for stimulation. (D) Rastermaps of the calcium activity of identified neurons at different current intensities during five consecutive stimulation trials. Note the diverse temporal activation patterns of activated neurons. The red horizontal bars above the rastermaps indicate the times of simulation.

Funded by the European Union
European Innovation Council

REFERENCES

[1] Pachitariu, M., et al., Suite2p: beyond 10,000 neurons with standard two-photon microscopy. bioRxiv, 2017: p. 061507.
[2] https://hyperstim.eu/