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

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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 study (Hyperstim, [1]), we developed flexible multi-shank probes containing multiple small electrodes to assess the effects of advanced electrical microstimulation strategies on cortical activity in both primary (V1) and higher-order visual areas (HVAs), obtained using in vivo two-photon calcium imaging. Here we present the observed range of neuronal responses in V1 to different parameters of the electrical stimuli.

CONCLUSION AND FUTURE PLANS

Here, we demonstrate the impact of various electrical stimulation parameters on visual cortical activity in transgenic mice.

After developing a pipeline for the processing and analysis of the data we found that:

- Most neurons were activated closest to the stimulating electrode where their response was also the strongest. (Figs. 10,14,18)
- We detected neurons that ceased firing after a few stimulation trials and neurons that sustained their activity until the end of stimulation (Figs. 9, 13, 17).
- We can extend the volume of activated neurons with longer pulses (Fig.11B) and higher current amplitudes (Fig. 7B) and increase the neuron density with higher frequencies (Fig. 15B).

Additionally, we have initiated the development of the experimental protocol for conducting experiments in awake head-fixed mice. (Fig.19)

REFERENCES

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

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METHODS

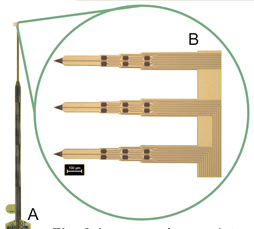

Fig. 1 Polyimide high-density electrode arrays used in the experiments have shanks with a cross-section of 20 × 70 µm² (thickness × width) (A). The shanks are 1000 µm long and are spaced at a fixed distance of 250 µm from each other. Rectangular iridium oxide electrodes (20 × 30 µm²) are arranged along the width and length of each shank, forming a grid with spacing ranging from 15 µm to several 100 µm (B).

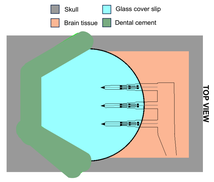

Fig. 2 A rectangular craniotomy (~4 mm x 3 mm) is prepared, centered over the visual cortex (V1) of Thy1-GCaMP6 transgenic mice anaesthetized with ketamine/xylazine. A glass coverslip with a 3 mm diameter is then carefully placed in the anterior part of the craniotomy and fixed to the skull using a small amount of light-curing dental cement.

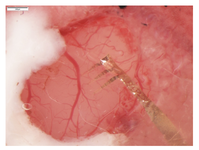

Fig. 3 Following this, the flexible polymer-based probe is slowly inserted into the brain tissue inserted at an angle of ~55° from vertical and at a speed of approximately 2 µm/s, targeting the primary visual cortex for two-photon calcium imaging. The probe was carefully released, and Dura-Gel was applied on the exposed cortex.

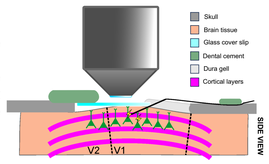

Fig. 4 A two-photon laser scanning microscope with a 20x water immersion objective was used to image calcium activity in layer 2/3 of the visual cortex adjacent to the probe (resonant scanning, FOV: 550 x 550 µm², ~31 Hz frame rate).

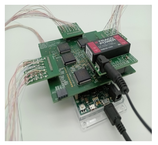

Fig. 5 The neurostimulator device used to generate pulse trains for electrical stimulation. During the inital in vivo elcetrical stimulation experiments, we investigated various stimulation parameters and observed diverse spatial and temporal activation patterns among neurons. Symmetric biphasic anodic leading pulses were used for stimulation.

DATA ANALYSIS

The aquired calcium imaging time series were converted from .mesc format to multi-page TIFF files and were provided as input to suite2p [2]. To study the spatial and temporal activation patterns, we calculated the baseline fluorescence intensity (F_0) and the baseline corrected calcium trace (dF/F_0) for each region of interest (ROI). To identify neurons (ROIs) activated by electrical stimulation, for each neuron, we compared the mean calcium activity during stimulation to the baseline (pre-stimulation) signal. We calculated the number and fraction of activated neurons and the mean calcium signal of all neurons during and after stimulation. The distance between the centre of the microelectrode and the centre of each ROI was also determined to assess distance-dependent changes.

Our typical parameter set for electrical stimulation: current amplitude: ~40 µA; number of pulses: 100; pulse width : 200 µs (one phase); frequency : 100 Hz; trial delay: 2 s; number of repeats (trials): 5. A single stimulation trial lasted approximately 1 second.

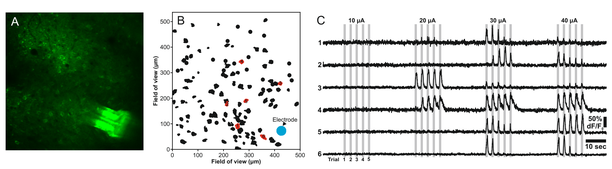

Fig. 5 Example of calcium imaging in a GCaMP6f mouse, detected ROIs and calcium traces.
(A) A sample frame of calcium imaging data obtained during electrical stimulation. (B) ROIs (black and red patches) identified by suite2p. The blue ROI corresponds to the location of the stimulating microelectrode. (C) Baseline-corrected calcium traces of six ROIs extracted from the calcium data shown in (A). Calcium traces correspond to ROIs colored red in (B). Note the diverse responses of different neurons to different current amplitudes (from 10 µA to 40 µA). Five stimulation trials were conducted for each stimulation amplitude.

RESULTS

Electrical stimulation using different current amplitudes

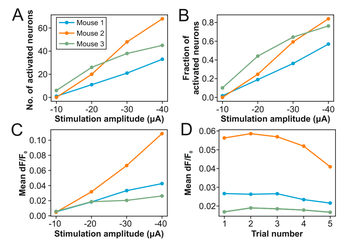

Fig. 7 Change in the number of activated neurons and mean GCaMP intensity during electrical stimulation.
Change in the number (A) and fraction (B) of activated neurons. Change in the mean df/F_0 of all identified ROIs during the stimulation period at different current amplitudes (C) and during consecutive trials (D).
For (C), all five trials, while for (D), all four current amplitudes were averaged.

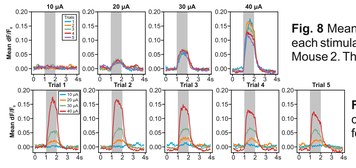

Fig. 8 Mean calcium traces (df/F_0) of all identified ROIs calculated for each stimulation trial and for each current amplitude (from left to right) in Mouse 2. The gray background indicates the stimulation period.

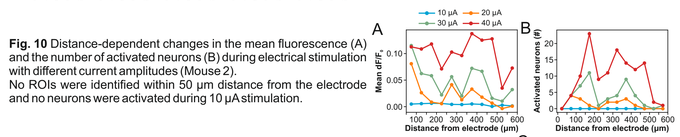

Fig. 9 Mean calcium traces (df/F_0) of all identified ROIs calculated for each current amplitude (colored traces) and for each trial (from left to right) in Mouse 2.

Fig. 10 Distance-dependent changes in the mean fluorescence (A) and the number of activated neurons (B) during electrical stimulation with different current amplitudes (Mouse 2).
No ROIs were identified within 50 µm distance from the electrode and no neurons were activated during 10 µA stimulation.

Fig. 10 (C) Distance-dependent changes in the mean fluorescence with different current amplitudes. Note that the mean distance of activated neurons increased with higher stimulating currents, supporting our observation that higher currents activate more neurons further from the electrode.

Electrical stimulation using different pulse duration

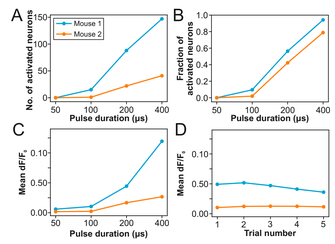

Fig. 11 Change in the number (A) and fraction (B) of activated neurons during electrical stimulation with four different pulse durations: 50, 100, 200, 400 µs. Change in the mean df/F_0 of all identified ROIs during the stimulation period (1 sec) at different pulse durations (C) and during consecutive trials (D). For (C), all five trials were averaged, while for (D), all four pulse durations were averaged.

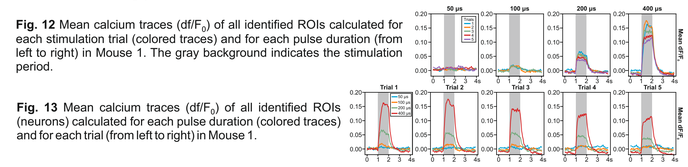

Fig. 12 Mean calcium traces (df/F_0) of all identified ROIs calculated for each stimulation trial (colored traces) and for each pulse duration (from left to right) in Mouse 1. The gray background indicates the stimulation period.

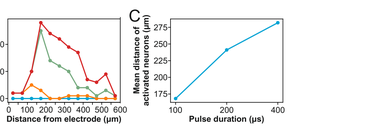

Fig. 13 Mean calcium traces (df/F_0) of all identified ROIs (neurons) calculated for each pulse duration (colored traces) and for each trial (from left to right) in Mouse 1.

Fig. 14 Distance-dependent changes in the mean fluorescence (A) and the number of activated neurons (B) during electrical stimulation with different pulse durations (Mouse 1). (C) Pulse duration-dependent changes in the mean distance of activated neurons relative to the location of the stimulating microelectrode. No ROIs were identified within 50 µm distance from the electrode and no neurons were activated with 50 µs long pulses.

Electrical stimulation using dynamic current steering

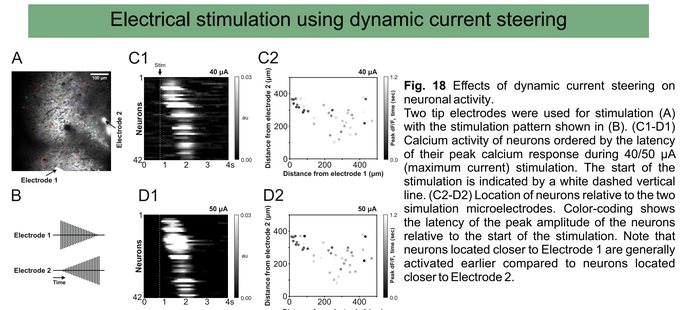

Fig. 18 Effects of dynamic current steering on neuronal activity.
Two tip electrodes were used for stimulation (A) with the stimulation pattern shown in (B). (C1-D1) Calcium activity of neurons ordered by the latency of their peak calcium response during 40/50 µA (maximum current) stimulation. The start of the stimulation is indicated by a white dashed vertical line. (C2-D2) Location of neurons relative to the two simulation microelectrodes. Color-coding shows the latency of the peak amplitude of the neurons relative to the start of the stimulation. Note that neurons located closer to Electrode 1 are generally activated earlier compared to neurons located closer to Electrode 2.

CALCIUM IMAGING IN HEAD-FIXED MICE

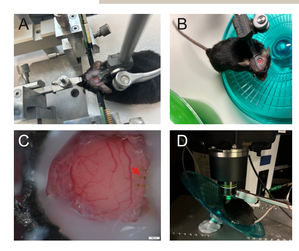

Fig. 19 Pilot experiments with awake, head-fixed mice
(A) The RODIN head-fixation implant (Cambridge NeuroTech) was attached to the skull of the mouse with a cranial window prepared over the visual cortex. (B) The awake, head-fixed mouse on a running wheel. (C) The prepared craniotomy with the implanted polymer probe (marked with a red arrow). (D) The head-fixed mouse placed under the objective of the two-photon microscope for calcium imaging.

Electrical stimulation using different frequencies

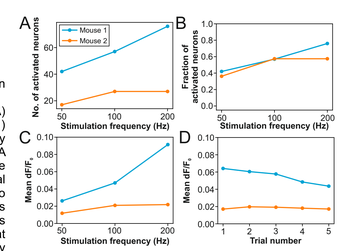

Fig. 15 Change in the number (A) and fraction (B) of activated neurons during electrical stimulation with three different frequencies: 50, 100, 200 Hz. Change in the mean (df/F_0) of all identified ROIs during the stimulation period (0.5-2 sec) at different frequencies (C) and during consecutive trials (D). For (C), all five trials were averaged, while for (D), all three frequencies were averaged.

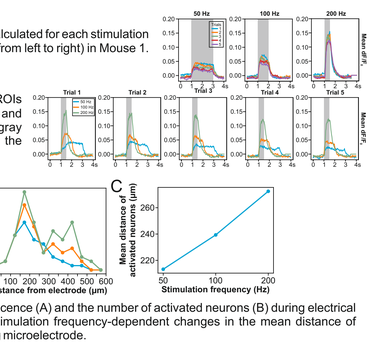

Fig. 16 Mean calcium traces (df/F_0) of all identified ROIs calculated for each stimulation trial (colored traces) and for each stimulation frequency (from left to right) in Mouse 1. The gray background indicates the stimulation period.

Fig. 17 Mean calcium traces (df/F_0) of all identified ROIs calculated for each stimulation frequency (colored traces) and for each trial (from left to right) in Mouse 1. The gray background indicates the stimulation period (only for the shortest stimulation, 0.5 se).

Fig. 18 Distance-dependent changes in the mean fluorescence (A) and the number of activated neurons (B) during electrical stimulation with different frequencies (Mouse 1). (C) Stimulation frequency-dependent changes in the mean distance of activated neurons relative to the location of the stimulating microelectrode.