Image Analysis of GCaMP Activity in Neurons Surrounding Intra-cortical Neuronal Probe

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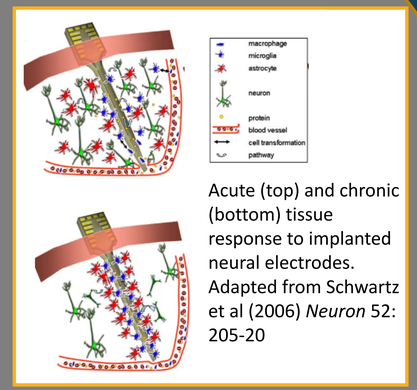
Introduction:

- Intra-cortical brain-machine interfaces (BMI) have shown great potential to help restore functional control to people with paralysis and limb loss by decoding neural signals as commands for computers and prostheses.
- BMI technology has shown to be able to hear neurons up to 140 microns away (Buzsaki 2004), but is limited by declining signal quality due to neuronal death and glial scarring.



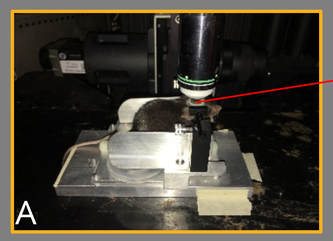
Acute (top) and chronic (bottom) tissue response to implanted neural electrodes. Adapted from Schwartz et al (2006) *Neuron* 52: 205-20

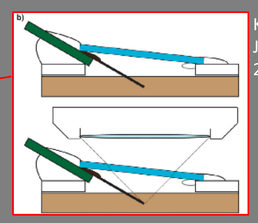
- The interaction between the surrounding neurons and the interface is captured by genetically encoding a calcium indicator known as GCaMP for the calcium to optically fluoresce through its various processes. The fluorescence of GCaMP is then captured via Two-photon excitation microscopy.
- The exact relationship between the interface and the neurons of interest in a geometric capacity is analyzed by various programs including ImageJ and MATLAB.

Objective

- To be able to determine the dynamics of neuronal death in relation to intracortical electrodes. A better understanding of this dynamic is critical to designing strategies to extend the lifespan of neural electrodes.
- Optimize and validate a strategy to study the dynamic behavior of neurons over time after a neural probe implantation.
- Optically convey the relationship between the neurons and the interface by showing the difference in calcium activity from baseline to the injection of the general anesthetic known as Isoflurane
- Obtain an accurate count of all neurons within the surrounding area across the entire depth that the Two photon microscope allows us to see

Methods: Data acquisition



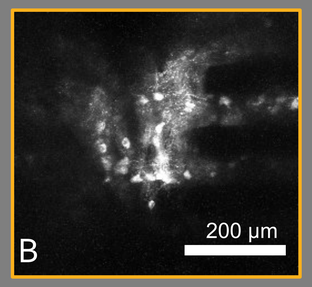


Kozai et al (2015) J Neurosci Meth. 268: 46-55

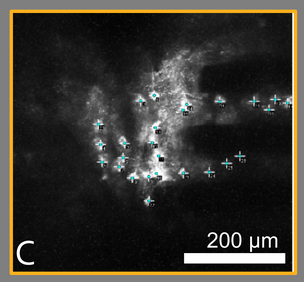
A) Picture of a C57BL6/J mouse head-fixed by a custom built awake recording frame, which attaches to an aluminum headplate that is cemented to the animal's cranium post-craniotomy. Non-functional 4-shank silicon electrodes are implanted at a 30° angle to accommodate the microscope objective

Methods: Image Pre-Processing and Mask Generation

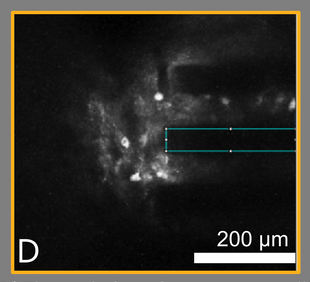
Data was obtained after a craniotomy on C57BLBL/J MOUSE transfected with AAV-Syn-GCaMP6f. In vivo imaging was conducted with a two-photon laser tuned at a wavelength of 920 nm. Image analysis utilized ImageJ, Microsoft Excel, MATLAB.



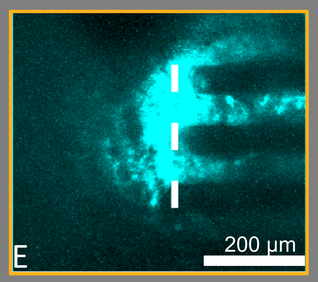
B) 542 um X 542 um X 120 um images were collected every 9.1 seconds from awake animal. Images were compressed in ImageJ to take the maximum z-projection over time to see the most amount of the neurons over 100 imaging frames. Each frame in the z-projection is the maximum projection over 15:10 minutes of imaging with images collected at 0.11Hz. We optimized these parameters to maximize our ability see activity in all neurons that were actively undergoing Ca++ transients.



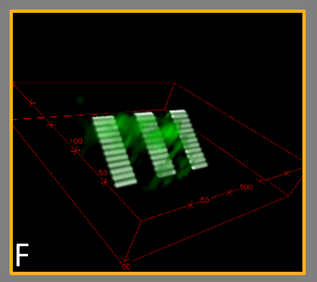
C) The neurons were then counted over the entire depth using the ROI Manager tool in ImageJ



D) The probe's surface was manually identified by contrast. The angle of the probe was estimated by changes in the probe's surface profile with depth.

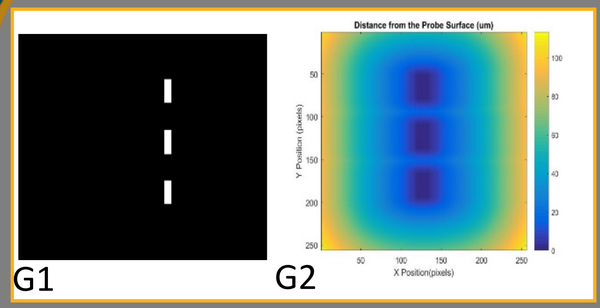


E) A mask of the probe's surface (white bars) was generated for each z-slice

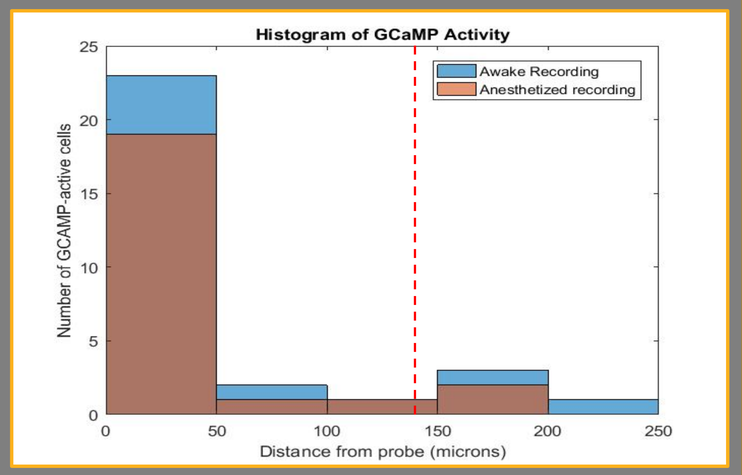


F) 3D rendering of the image mask verify accurately masking the probe

Image Analysis and Preliminary Results



G1 &G2) The distance transform of the probe in relation to the position of the neurons.



H) A histogram depicting the difference in GCaMP activity between awake mouse and Isoflurane treated

Conclusions

We have developed a method to count the number of active neurons as a function of distance from a chronically implanted neural electrode. We have shown that in both awake and anesthetized recording, this method can detect active neurons within 140um of the electrode device, which suggests that these neurons could be detected electrophysiologically. Future studies will combine this analysis with electrophysiology to define the link between electrophysiological performance of electrode devices with the geometric position of neurons over time post implant. This will enable researchers to develop next-generation neural technologies with better long-term viability.

Acknowledgements

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