Discovering Neuronal Firing Codes underneath Slow Waves: A Novel Approach in Rodent Models of Anesthesia and Sleep

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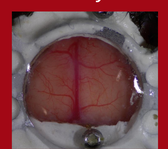
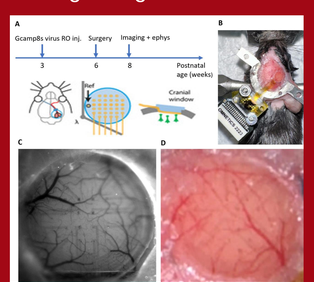
Introduction

- Slow waves are key features of general anesthesia (GA) and resemble those in slow-wave sleep (SWS).
- The similarity of neuronal firing patterns between GA and SWS remains debated.
- We introduce a novel approach combining EEGs and optical recordings in transgenic mice to capture large-scale neuronal activity.
- Our method also allows cortical electrical stimulation, enabling direct comparison of cortical states during anesthetic-induced and SWS slow waves.

Material and Methods

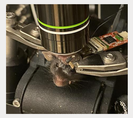
- Firing patterns of individual neurons over a large brain area
  - » Vgat-Cre/tdTomato transgenic mice + pGP-AAV-syn-jGCaMP8s-WPR (PhP.eB)
  - » 4-mm curved cranial window by glass window with ECOG
  - » Epifluorescence imaging with Bruker 2P+ Olympus 20X/1.0 920-nm excitation
- Concurrent extracellular electrophysiology recording
  - » The old method (left)
    - Bone screws anterior-posteriorly
    - Site-specific recording and intervention via a sharp electrode by penetrating the PDMS elastomer film
  - » The new method - transparent electrode array (right) with reference electrode anterior
    - Wires: Nano-meshed, Au:PEDOT/PSS
    - Parylene C embedding with glass window.



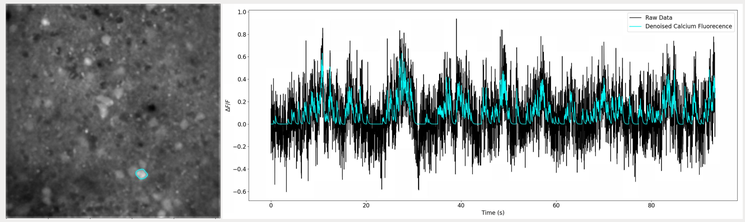


- GA
  - » Isoflurane
- SWS models
  - » Dexmedetomidine (100 mcg/g IP or 75 mcg/g RO)
  - » 24-hour sleep-deprivation

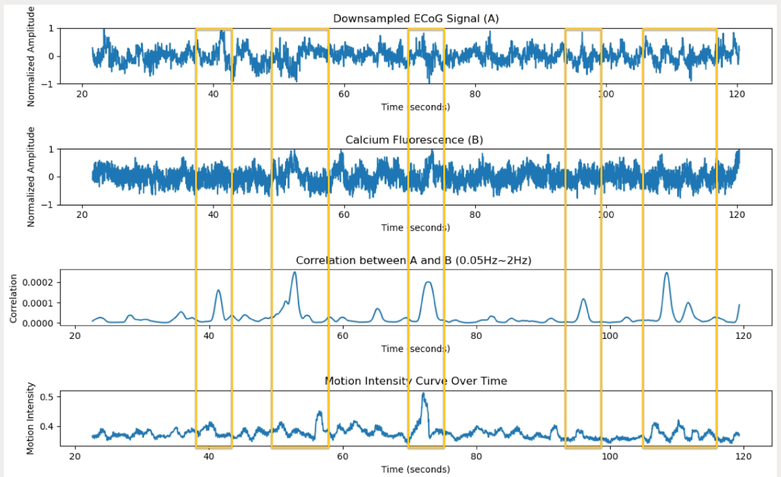
Results



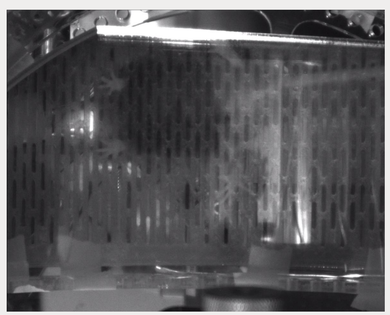
A two-photon calcium signal from a neuron.



Correlation Analysis between ECoG and Calcium Fluorescence Signals



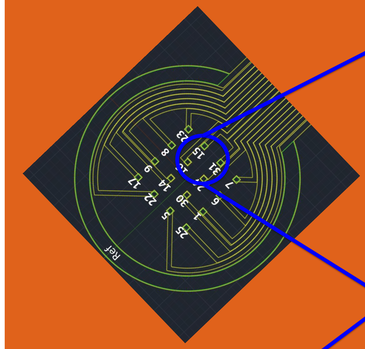
Downsampled ECoG Signal (A) is the signal obtained from the electrode in the transparent ECoG electrode array closest to the microscope observation window. **Calcium Fluorescence (B)** represents the fluorescence signal of jGCaMP8s located 150 micrometers below the brain surface. Since the decay time of the jGCaMP8s fluorescence signal is less than 500 ms, we used wavelet transform to calculate the correlation coefficient between signals A and B within the bandwidth of 0.05 Hz and 2 Hz. Meanwhile, we used the optical flow method to calculate the mouse's **motion intensity** from the behavioral camera. As indicated in the signal intervals marked by yellow boxes, there is a noticeable change in the mouse's motion intensity during periods when signals A and B are highly correlated. This suggests that the calcium fluorescence and electrophysiological signals we captured together detected movement-related events.

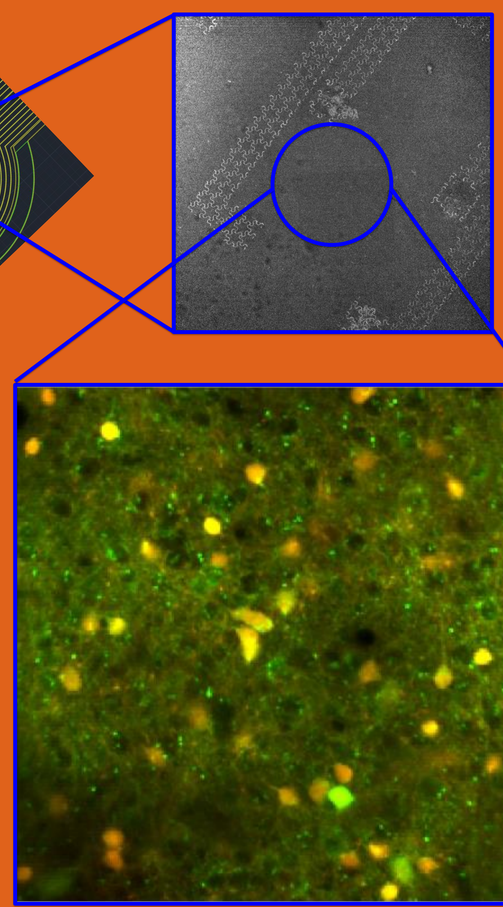


Behavioral camera, observing the activity of the laboratory rodent from a bottom-up perspective.

Calcium imaging under the new ECoG array

- Two-photon imaging





Red neurons express only tdTomato, indicating they are inhibitory neurons.

Green neurons express only jGCaMP8s, indicating they are excitatory neurons.

Yellow neurons indicate inhibitory neurons that have been infected with a virus carrying jGCaMP8s.

Conclusion

- We successfully developed a mouse model capable of simultaneously recording electrophysiological signals and calcium fluorescence signals.
- Experimental results demonstrate that our electrophysiological and calcium fluorescence signals show a strong correlation and the neuronal events can be simultaneously detected across systems.
- The next step will involve studying the difference in cortical states in GA and SWS mouse models based on electrophysiological signals and calcium signals from excitatory and inhibitory neurons.