Assessing cortical behavioral correlates with simultaneous EEG and widefield optical imaging

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Abstract

The interplay of cortical function and behavior holds the potential to be a critical investigative avenue for developmental, neurological, and behavioral disorders. Various dynamic components (neural, glial, and vascular) underly this interplay and are key targets for therapeutic development. We present a multimodal approach combining simultaneous widefield optical imaging (WOI) and electroencephalography (EEG) aimed at assessing the cortical function of freely behaving rodents (mice and rats) during behavioral assessment. This approach was developed to allow for the assessment of the spatiotemporal cortical correlates of behavior, both global and regional, that underly numerous disorders. Here, we wirelessly measured cortical activity through a cranial window for WOI and from three skull electrodes for EEG. The recording device, <15% of body weight, is mounted to the heads of the animals and transmits data over Wi-Fi. While EEG provides high temporal resolution recordings of global cortical activity, WOI captures mesoscale neural dynamics via genetically encoded calcium indicators and vascular fluctuations through intrinsic optical properties. Our custom MATLAB pipeline is tailored to correlate EEG spectral features with surface-level calcium and hemodynamic transients and align those cortical signals with assessment specific animal behavior characteristics. Potential behavioral assessments include; open field, object recognition, elevated zero maze, and 3-chamber social tests. Our preliminary findings (n=1) demonstrate the utility of this approach by simultaneous EEG, neural fluctuation, and hemodynamic response. This project aims to enhance the interpretability of the interplay between cortical activity and behavior and provide a comprehensive evaluation of cortical state dynamics to support the development of novel therapeutics.

Introduction

The dynamic interplay between cortical networks and behavior offers a promising investigative window into developmental, neurological, and behavioral disorders. Disruptions in this interplay—spanning neural, glial, and vascular components—are implicated in a wide array of conditions, including autism spectrum disorder, epilepsy, traumatic brain injury, and neurodegenerative diseases. In particular, understanding how mesoscale cortical activity and hemodynamics relate to behavior is key to developing mechanistically informed therapeutics. Mesoscale optical methods such as wide-field optical imaging (WOI) have emerged as powerful tools to capture cortex-wide activity patterns in behaving rodents. Wide-field calcium imaging has revealed that cognitive and motor behaviors engage not only focal regions but distributed, large-scale cortical dynamics. Additionally, pairing calcium imaging with the intrinsic optical properties of hemodynamic signals (e.g., changes in cerebral blood volume, CBV) provides a non-invasive assessment of neurovascular coupling across the cortical surface. Parallel to optical approaches, electroencephalography (EEG) remains a robust method for capturing global cortical oscillations with high temporal resolution. Therefore, the combination of optical imaging offers insight into the spatiotemporal synchrony of cortical activity and behavior. While mesoscale optical imaging and EEG are not individually novel techniques, combining them in freely moving animals has been technically challenging due to systems requiring wired connections or multiple signal specific devices. Thus, the ability to directly relate global cortical activity, large-scale cortical dynamics, and behavior concurrently has been limited. We present preliminary evidence for the first integration of neural (genetically encoded calcium indicators), vascular (intrinsic hemodynamic properties), and electrophysiological signals and the potential to comprehensively evaluate the cortical and behavioral mechanisms underlying neurological disorders.

Materials

General

- Thy1-Gcamp6f mice
- 1x Arduino microcontroller
- 1x 3.7 V Li-ion battery
- MATLAB
- Elegoo 3D printer
- Solder and iron
- Silver wire
- Liquid Electrical Tape

Optical

- 1x 470nm LED
- 1x 530nm LED
- 1x 500 nm long-pass filter

EEG

- 3 x Stainless steel screws
- 1x Circuit amplifier
- Pin & Socket heads
- 3 x Resistors

Methodology

System Overview

Illustrated in Figure 1A, the custom designed system consists of a fully wireless widefield optical imaging platform designed for freely moving rodent experiments. The system is built around the Arduino microcontroller, which integrates a CMOS image sensor, and onboard BLE/Wi-Fi. The module is housed in a tailored 3D-printed housing, designed in Tinkercad, optimized for minimal weight and center-of-mass offset when mounted on the animal's head. The imaging module is powered by a lightweight 3.7 V Lithium polymer battery.

Illumination and Optical Configuration

Illumination is provided by a dual-LED array (470 nm for GCaMP excitation and 530 nm for CBV reflectance) driven by control pins on the board. The LEDs are synchronized with frame capture to alternate excitation wavelengths on successive frames, producing a time-interleaved Ca/CBV sequence. As shown in Figure 1B, The camera rests above an ultra-thin 500nm long-pass filter to prevent signal contamination from the excitation LED.

EEG Configuration

The device acquires amplified differential biopotentials from three stainless-steel screw electrodes implanted into the skull (active, reference, and ground), Figure 1C.

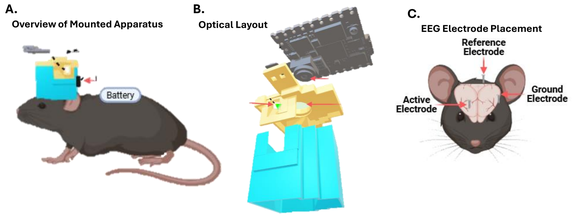
Wireless Communication and Control

All data acquisition and control routines are implemented by **custom** micropython scripts that utilize the boards Bluetooth and Wi-Fi connections. The microcontroller continuously streams compressed image frames over Wi-Fi using an MJPEG encoding pipeline, with metadata packets marking frame number, LED state, and timestamp.

Data Processing and Analysis

All optical and EEG processing is performed in **MATLAB** using custom lab functions, adapted from James et al. 2022..

Figure 1.



Results

The data below used the same device to independently capture the optical or EEG signals from two different animals. The optical data (Figures 2-3), was captured from an awake Thy1-GCaMP6f mouse sans EEG electrodes. The EEG data in Figure 4 was captured from an anesthetized (isoflurane 2%) wild type mouse sans calcium dependent fluorescent indicators.

Figure 2.

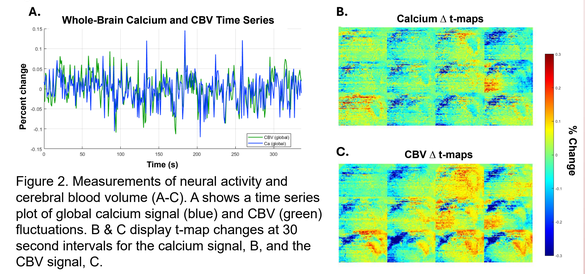


Figure 2. Measurements of neural activity and cerebral blood volume (A-C). A shows a time series plot of global calcium signal (blue) and CBV (green) fluctuations. B & C display t-map changes at 30 second intervals for the calcium signal, B, and the CBV signal, C.

Figure 3.

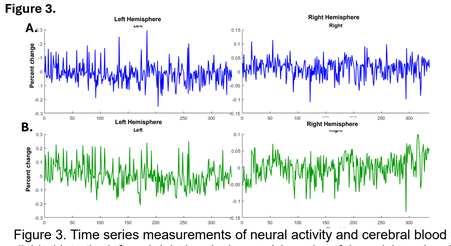
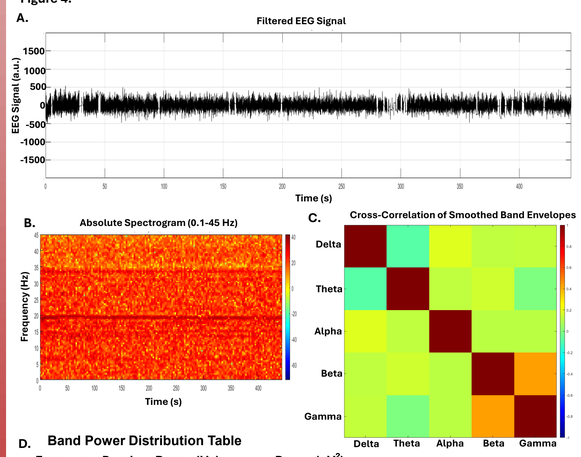


Figure 3. Time series measurements of neural activity and cerebral blood volume (A & B) divided into the left and right hemispheres. A is a plot of the calcium signal and B is a plot of the blood volume fluctuations.

Figure 4.



D. Band Power Distribution Table

Frequency Band	Range (Hz)	Power (μV^2)
Δ (Delta)	0.5 – 4	614.54
θ (Theta)	4 – 8	789.12
α (Alpha)	8 – 13	868.44
β (Beta)	13 – 30	3,264.50
γ (Gamma)	30 – 45	1,421.03

Figure 4. EEG summary visualization following artifact suppression and band-limited filtering (0.1–45 Hz; A-D) from an anesthetized mouse. A is the cleaned EEG trace across the 450 second recording. The absolute spectrogram, B, displays time–frequency power dynamics with residual harmonic peaks after soft Gaussian FFT attenuation at 20 Hz and 35 Hz. C is a cross-correlation matrix illustrating the temporal and cross-band relationships of normalized EEG power envelopes (Δ 0.5–4 Hz, θ 4–8 Hz, α 8–13 Hz, β 13–30 Hz, γ 30–45 Hz). D provides a table of the measured power for each frequency band.

Conclusion

The wireless imaging–EEG apparatus maintained synchronization and stable data transmission across modalities, demonstrating our ability to capture real-time neurovascular and electrophysiological coupling with minimal motion artifacts.

Optical Imaging

- Simultaneous dual-wavelength imaging provided mesoscale maps of neural (Ca) and vascular (CBV) activity.
- Whole-brain mean traces showed temporally aligned fluctuations between Ca and CBV signals, reflecting neurovascular coupling.
- Δt-maps revealed spatially distinct but overlapping cortical activation patterns across time windows.
- Left–right hemisphere analysis indicated comparable signal amplitudes, suggesting stable bilateral coverage.

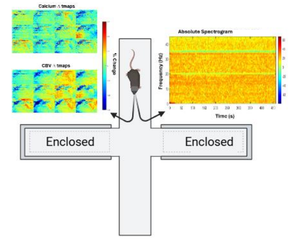
EEG Recordings

- The device successfully captured broadband cortical activity (0.1–45 Hz) over 7 minutes.
- Clear frequency-band structure detected — Δ, θ, α, β, γ bands — confirming reliable signal quality.
- Spectrogram reveals persistent low-frequency power typical of anesthetized cortical activity, with no evidence of signal drift or dropout.
- We can quantify inter-band coupling strength - showing modest positive relationships between adjacent frequency ranges and weaker coupling between distant bands for our anesthetized animal.

Future Directions

The next steps involve running additional trials to improve reproducibility and strengthen statistical power. The device will be applied alongside behavioral assays to directly link cortical calcium, hemodynamic, and EEG signals with distinct behavioral states, Figure 5. By integrating these modalities, the platform will enable a comprehensive analysis of brain–behavior relationships. Ultimately, this approach holds significant therapeutic potential for identifying circuit-level biomarkers and advancing translational neuroscience research.

Figure 5.



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