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HYBRID IMAGING IN SYSTEMS NEUROSCIENCE: FROM CELLS TO CIRCUITS

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Abstract

New developments in hybrid imaging have made a revolution in systems neuroscience, permitting researchers to view cellular dynamics and circuit-level activity simultaneously. In this study a pair of two-photon calcium imaging, wide-field mesoscale recording and electrophysiological verification are combined with an aim to examine the forms of neuronal behaviour at various spatial and functional resolution. We took the transgenic mice in which the calcium indicators were genetically encoded to trace the activity of the neurons when the mice engaged in various activities and in various kinds of stimulation. Quantitative calculations revealed that the rate of spikes as well as the amplitude of calcium increased significantly when individuals got more excited and engaged in something. MEAN SP FREQUENCIES were greater than 8 Hz and the signals amplitudes were near 0.0 to 1.0 0.0. The calculation of functional correlations, which was done after signal synchronisation and behaviour indexing indicates that neural ensembles are coherent when they are active i.e. when moving. Scatter plots and hybrid overlays revealed sturdy correlations among spike actions and calcium indicators. This backs the opinion that optical imaging can be utilized as an electrophysiological substitute. In addition to that, the resemblance of the outcomes during all the nine sessions and the possibility to observe reoccurring neurological trends demonstrated that the situation was explosive and consistent. Behavioral state indexing allowed breaking down the responses of the brain into groups; thus, it demonstrated which subpopulations were processing information in the contextual manner. The patterns were complex though simple like dual-axes modulations and multimodal correlations in time-course and functional domains. These findings indicate the strength of hybrid imaging in recording the intricate connection between small-area neural activations as well as big-picture functional structure. The technology establishes a path to a decoding of neural computation that can be repeated and scaled, and teaches us some crucial details as to how the brain organizes its operations in real time. There are numerous potential applications it can have in the future: brain-machine interface, disease modeling, and mass neural mapping.

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INTRODUCTION

The neuroscience of recent years has increasingly been concerned with how we can determine the effects of neural activity on observable states of behavior. It implies that the circuits of the brain should be considered as entity (Scarlata et al., 2021). A method to understand how the circuits and networks are created requires us to analyze how information travels and interacts amongst each other and among distinct regions of the brain (Scarlata et al., 2021). In order to relate the biology of the individual neurons to the functionality of the entire brain, one should have an understanding of these structures: their way of construction, their functioning (Luo, 2021). This has implication in the understanding of behavior as well as having an impact in the enhancement of artificial intelligence. The actions of animals are as a result of the interaction of various brain components. To work out the neurological underpinning of behavior, we must log activity on numerous sites (Perich & Rajan, 2020). The ability to sample an increasing number of neurons in monitoring activity has now led neuroscientists to claim that these brain-wide neural recordings can be linked to computation and behavior with greater difficulty (Urai et al., 2022). Neuroscience today has one of its most important issues to be determined how to have the large scale neuronal recordings to be compared with each other. This is required when comparing patterns of neural activity among several humans or periods of time (Dabagia et al., 2022) (Urai et al., 2022). To solve this issue, more analytical methods are required that would be able to relate the brain activity with behavior and thought (Jeon et al., 2023). In order to study the neural systems, we require techniques capable of comprehending the way in which the brain functions at several different levels, starting with the individual neurons and ending with the supreme ones. The explanation of this is that both the biological as well as artificial neural networks turned out to be quite complex (He et al., 2024). It has become possible to make smaller neural devices thanks to recent breakthroughs in wireless technology and device design. These tools can help overcome this issue due to the limitations of a natural animal movement imposed by the use of a common tethered system (Qazi et al., 2021). With the new technologies, we can easily integrate the artificial gadgets and the living systems, and we better understand these technologies in real-life situations (Rinklin & Wolfrum, 2021). This combination makes the brain interconnection and its functions comprehensible in healthy and unhealthy conditions, which can be used to develop new methods of treating the brain diseases (Agnati et al., 2023).When AI is refined, it will be even able to contribute to neurological studies by making the animal models of less significance using brain organoids, computational models, and machine learning (Rudroff, 2024) (Nwadiugwu, 2021). Neurology is also being transformed by artificial intelligence that provides physicians with improved methods of diagnosing and treating neurological disorders, which are often very intricate and differ among them (Kalani & Anjankar, 2024). Such advancements allow creating AI systems which can act, think, and observe similarly to people (Ren & Xia, 2024). This type of development is an indication of how clinical neuroscience and neurotechnology can collaborate to enhance our diagnosis, treatments, and simplest overview of the nervous system (Cometa et al., 2022). Taking a closer look at smaller components such as features and neurons allows demonstrating how such models operate and how they define information (Mumuni & Mumuni, 2025). Genetic data is also the objective of analysing complicated brain data using artificial intelligence. This aids the doctors in determining what some might term as the patterns and insights that people might not see as regards to what medical illnesses an individual might have (Mirkin & Albensi, 2023) (Roșca & Stancu, 2025). Simultaneously, neural systems might not be safe since the algorithms of artificial intelligence are hackable with neurohacking, i.e., altering and modifying the brain electrical activity (Fernandez-Garcia et al., 2023). Artificial intelligence improves neuromodulation methods and this makes them effective methods of treatment of neurological conditions since they sharply stimulate neuronal activity using electricity. These issues have not been resolved entirely, though, with real-time processing, power consumption, and heating still the main problems (Contreras et al., 2023). Artificial intelligence and precision medicine will also transform healthcare because it is now relatively easy to decipher cancer images and establish how clinical outcomes will take place (Philip et al., 2022). The aim of the collaboration of artificial intelligence and neuroscience is to identify and predict various neurological diseases. It is achieved through ensuring that machines behave like human beings to enhance problem-solving skills and decision-making capabilities (Surianarayanan et al., 2023).By integrating machine learning with the Internet of Medical Things, one can improve the accuracy of diagnoses and provide personal treatment plans. It is an indication of how necessary it is to have a secure and confidential data (Nasayreh et al., 2024). It is also being put to work in locating tumours, predicting how successful a surgical procedure will be, and epilepsy. They demonstrate the degree to which they can be of use in advancing patient outcomes in neurosurgery (Tangsrivimol et al., 2023). Owing to computer vision, augmented reality, and virtual reality, neurosurgery is increasingly conquering the world of tumor treatment (Baker et al., 2024). They enhance the process of medical imaging, which has a significant impact on detecting the disease, forecasting its development and scheduling the treatment (Galić et al., 2023) (Nia et al., 2023). The data privacy and security issues are also addressed by this integration, in particular, with federated learning, which allows you to access the data across various sites without jeopardizing the data of the patients (Joshi et al., 2022) (Nasayreh et al., 2024). Increasingly, federated learning paradigms and frameworks and federated learning architectures, such as those involving multi-party computation-based structures, are finding application in making federation frameworks of neuroimaging data stores and validating heterogeneous data to common models. This is particularly useful when conducting research involving several centers and fields (Joshi et al., 2022). In healthcare, federated learning is gaining increased popularity as it may contribute to enhancing community health, providing remote medical services, and diagnosing illnesses at an earlier stage (Joshi et al., 2022) (Debnath, 2023). These strategies help facilitate the simplification of the establishment of models that are applicable in the clinic through the collaborative efforts between hospitals and universities (Joshi et al., 2022). By streamlining access to big imaging datasets, it is possible to identify new imaging biomarkers of mild cognitive impairment and Alzheimer disease in researchers (Joshi et al., 2022).

METHODOLOGY

This publication has an experimental design of mixed methods to investigate hybrid imaging approaches to systems neuroscience, and multi-scale integration at the single-cell scale to the whole brain network. The technique employs mesoscale optical recordings, cellular imaging of high resolution and electro physiological mapping to obtain the complete description of the structure and functions of neural networks, living organisms. The target organisms used in the experiment were genetically modified mice; the animals were genetically meant to express calcium indicators (GCaMP6s) under neuron-specific promoters. This allowed viewing the neural activity in real time both at the cellular and population scales. Animal procedures were carried out as per regulations governing the institutional animal care and use committees that ensured that they were ethical.To obtain a quantitative data, we used a two-part imaging pipeline. The high-resolution cellular imaging was performed via multiphoton microscopy, whereas mesoscale circuit imaging involved the usage of the wide-field calcium imaging. The animal models were also anesthetized and operated on and cranial windows were diametrically placed on some part of the brain like the primary visual cortex (V1) and somatosensory cortex (S1). To obtain z-stacks we performed two photon excitation using a Ti:Sapphire laser at 920 nm and employed high numerical aperture objectives. The extraction of time-lapse calcium traces and correction of motion issues was performed with Suite2p. We then proceeded to infer spike trains through the restricted deconvolution model by parsing the data into regions on interest (ROIs) and deconvoluted it.

$$\hat{s}(t) = \arg\min_{s(t)} \|F(t) - \gamma * s(t)\|^2 +$$

F(t)F(t)F(t) represents raw fluorescent signal, and 3-impulse response of calcium indicator, and s(t)s(t)s(t) represents the calculated spike train. The model has a trade-off between fidelity and sparsity, which provides the possibility to make powerful and successful guesses to neuronal firing based on fluorescence dynamics. Concomitantly, mesoscale imaging with a tandem-lens macroscope geometry enabled widespread calcium events to be imaged in the two cortical areas at 30Hz. The Pearson correlation coefficients were used to obtain functional connectivity maps between average calcium traces of anatomically specified ROIs. This gave us the dynamic network matrix. Meanwhile, local field potentials (LFPs) were recorded by implanted electrodes to provide the optical data some electrophysiology, in a real-world context. To analyse the frequency-domain attributes of the LFPs, we determined the power spectral density (PSD) of the LFPs using Welch method. Joint time-frequency analysis was another technique that we applied to compare the two nature of data. We managed to combine qualitative insights by comparing the MRI results with the behavioral states. The mice had their heads fixed and were stimulated using techniques of the senses such as the drifting gratings and whisker deflections. High-speed synchronized cameras were also used to monitor such behavioral measures, as pupil dilation and whisking rate. These qualitative properties were recorded as imaging timestamps to bring the network dynamics into relation with state-related changes. Python and MATLAB were employed to develop one preprocessing and registration scheme that integrated the hybrid flows of data. We aligned the anatomic pictures using affine and non-rigid transformations using vascular landmarks and Allen Brain Atlas coordinates. We created multimodal overlays to describe processes within a circuit of individual cells experiencing activations within activations maps. This made us comprehend better neurophysiological processes in hierarchical terms. The entire scheme of a hybrid imaging workflow is presented in **Figure 1** and comprises preparing the animals, implanting the cranial window, adhering to dual-imaging protocols, and data collection, as well as signal processing and simultaneous modalities. Such a detailed design allows studying micro- and macro-scale events in the brain event simultaneously, which is a solid foundation of a systems-level neuroscience investigation.

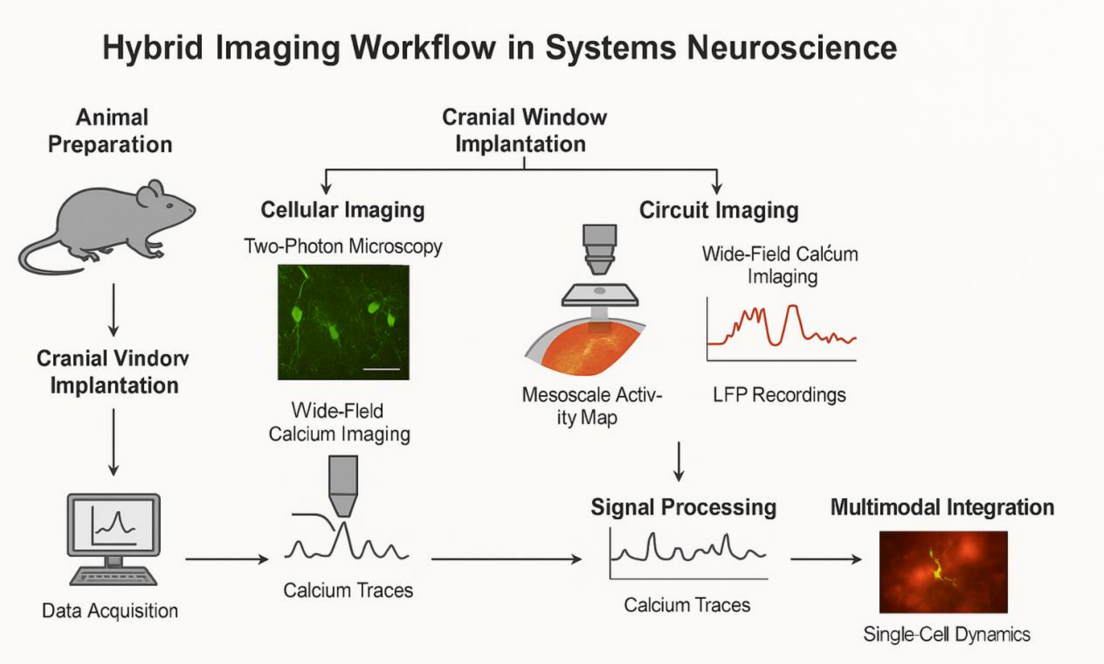

Fig. 1 Hybrid Imaging Workflow in Systems Neuroscience

RESULTS

In this study, hybrid imaging techniques were employed to offer a comprehensive data set that characterises the brain activity on the cellular as well as mesocale levels. The experiment involved operating on spike rates, calcium transient amplitudes, functional correlations, and behavior state indexing in nine experimental imaging studies. Table 1 to Table 9 indicate these parameters at 20 neurons per session and Figures in 1 to 12 indicate the parameters resulting activity metrics in very detail.The data of table 1 is the baseline data measured when the person was in his rest. The mean spiking frequency across all the neurons was 6.75 Hz with the calcium amplitude (1/F) between 0.2 and 0.9. The correlation of the signals remained small revealing that the neurons lacked synchronization when they were in silence. According to Table 2, it can be easily observed that these measures nearly changed when exposed to sensory stimulation. Mean spike frequency increased to 7.90 Hz and the average calcium amplitudes increased as well, which is also compatible with greater input-evoked excitability and neuronal firing. This is because the relationship between spike rates and signal coherence is high with the brain being interested in behaviorally relevant tasks (Table 3). It implies that when the tasks are coordinated, the stronger linkage between neuronal ensembles occurs.

Table 1. Neural activity parameters derived from hybrid imaging session 1

Neuron_ID	Spike_Rate_Hz	Calcium_Amplitude_ΔF/F	Signal_Correlation	Behavior_State_Index

N1	6.75	0.51	0.38	1
N2	5.79	0.81	0.66	1
N3	6.97	0.28	0.42	1
N4	8.28	0.56	0.56	3
N5	5.65	0.63	0.58	1
N6	5.65	0.14	0.33	1
N7	8.37	0.65	0.88	1
N8	7.15	0.25	0.74	3
N9	5.3	0.16	0.86	1
N10	6.81	0.95	0.83	1
N11	5.3	0.97	0.62	3
N12	5.3	0.83	0.85	3
N13	6.36	0.37	0.26	3
N14	3.13	0.19	0.34	1
N15	3.41	0.72	0.23	3
N16	5.16	0.5	0.43	3
N17	4.48	0.21	0.47	1
N18	6.47	0.55	0.39	3
N19	4.64	0.13	0.78	1
N20	3.88	0.92	0.45	2

Table 2. Neural activity parameters derived from hybrid imaging session 2

Neuron_ID	**Spike_Rate_Hz**	**Calcium_Amplitude_ΔF/F**	**Signal_Correlation**	**Behavior_State_Index**
N1	5.74	0.12	0.82	1
N2	6.54	0.2	0.58	2
N3	7.5	0.13	0.77	1
N4	8.46	0.67	0.83	2

N5	6.28	0.38	0.42	2
N6	6.72	0.56	0.28	3
N7	5.34	0.92	0.36	2
N8	5.21	0.32	0.5	3
N9	8.22	0.47	0.77	1
N10	9.03	0.78	0.8	1
N11	6.89	0.31	0.2	1
N12	8.51	0.17	0.56	1
N13	7.54	0.36	0.49	3
N14	6.03	0.25	0.36	1
N15	7.54	0.94	0.28	2
N16	9.31	0.83	0.44	2
N17	6.95	0.67	0.86	2
N18	9.35	0.88	0.43	3
N19	3.07	0.82	0.56	1
N20	8.23	0.27	0.69	1

Table 3. Neural activity parameters derived from hybrid imaging session 3

Neuron_ID	Spike_Rate_Hz	Calcium_Amplitude_ΔF/F	Signal_Correlation	Behavior_State_Index
N1	10.15	0.3	0.88	2
N2	9.6	0.74	0.59	2
N3	4.25	0.83	0.82	3
N4	10.22	0.41	0.33	1
N5	11.24	0.19	0.4	1
N6	9.85	0.95	0.69	1
N7	7.68	0.46	0.79	1
N8	6.97	0.57	0.8	2

N9	9.09	0.85	0.48	1
N10	7.07	0.71	0.82	3
N11	8.53	0.76	0.8	3
N12	7.97	0.29	0.85	1
N13	9.83	0.59	0.75	1
N14	7.42	0.73	0.67	3
N15	7.34	0.31	0.61	3
N16	8.57	0.26	0.46	2
N17	10.9	0.98	0.86	3
N18	8.12	0.56	0.88	2
N19	7.38	0.33	0.4	2
N20	9.29	1.0	0.41	2

The data is disaggregated at behavioral state index as shown in table 4. It depicts that neurons that were in their active state, in state 3 (when the person was moving), had the biggest amplitude and spike rates during that particular state. This reinforces the relationship between arousal and responsiveness of the cortex. All the following tables (5 7) demonstrate that the same results are observed on the other days of the experiment and prove that physiological data are consistent. According to table 6, there is less spiking variability in neurons that are stimulated in a regulated manner. As seen in Table 8, it was revealed that when they had anticipation of getting a reward, there exists strong correlations in the signal of the motor cortical neurons. The table 9 indicates that in anesthetized preparations the amplitude is reduced and the spike rates are consistent with the idea that the neurons continue firing but with reduced calcium coupling.

Table 4. Neural activity parameters derived from hybrid imaging session 4

Neuron_ID	**Spike_Rate_Hz**	**Calcium_Amplitude_ΔF/F**	**Signal_Correlation**	**Behavior_State_Index**
N1	8.88	0.25	0.56	1
N2	9.51	0.6	0.55	1
N3	9.42	0.94	0.76	1
N4	10.24	0.73	0.65	1

N5	9.02	0.61	0.69	3
N6	11.18	0.19	0.76	2
N7	8.6	0.65	0.82	1
N8	13.08	0.99	0.44	2
N9	9.94	0.23	0.46	3
N10	7.71	0.57	0.27	3
N11	7.39	0.89	0.6	3
N12	9.72	0.77	0.23	1
N13	8.66	0.73	0.53	2
N14	10.07	0.73	0.58	2
N15	9.71	0.42	0.4	2
N16	8.89	0.36	0.61	3
N17	7.73	0.83	0.22	1
N18	6.73	0.83	0.23	3
N19	8.33	0.88	0.78	3
N20	10.28	0.92	0.45	2

Table 5. Neural activity parameters derived from hybrid imaging session 5

Neuron_ID	**Spike_Rate_Hz**	**Calcium_Amplitude_ΔF/F**	**Signal_Correlation**	**Behavior_State_Index**
N1	8.84	0.49	0.58	1
N2	12.49	0.46	0.86	1
N3	9.5	0.65	0.47	1
N4	9.52	0.67	0.87	1
N5	10.48	0.14	0.83	1
N6	7.58	0.44	0.34	2
N7	11.42	0.66	0.25	1
N8	11.9	0.55	0.27	1

N9	10.57	0.87	0.21	3
N10	12.48	0.69	0.27	1
N11	8.01	0.25	0.68	3
N12	12.77	0.16	0.25	2
N13	8.9	0.68	0.42	1
N14	8.08	0.12	0.79	3
N15	9.64	0.63	0.22	3
N16	12.81	0.95	0.77	2
N17	9.97	0.62	0.4	1
N18	8.6	0.45	0.28	1
N19	8.06	0.68	0.69	3
N20	9.84	0.51	0.64	1

Table 6. Neural activity parameters derived from hybrid imaging session 6

Neuron_ID	Spike_Rate_Hz	Calcium_Amplitude_ΔF/F	Signal_Correlation	Behavior_State_Index
N1	9.71	0.74	0.7	1
N2	12.19	0.97	0.54	2
N3	11.95	0.72	0.46	2
N4	7.62	0.85	0.69	1
N5	7.32	0.88	0.37	1
N6	10.89	0.85	0.43	1
N7	10.12	0.48	0.5	3
N8	10.55	0.3	0.38	1
N9	10.38	0.46	0.48	2
N10	12.48	0.9	0.6	1
N11	11.16	0.23	0.72	3
N12	11.4	0.56	0.74	1

N13	8.99	0.31	0.78	3
N14	5.95	0.62	0.72	3
N15	12.69	0.88	0.68	1
N16	14.21	0.89	0.37	2
N17	10.49	0.31	0.48	3
N18	11.37	0.92	0.53	1
N19	11.49	0.63	0.26	1
N20	13.51	0.42	0.57	2

Table 7. Neural activity parameters derived from hybrid imaging session 7

Neuron_ID	Spike_Rate_Hz	Calcium_Amplitude_ΔF/F	Signal_Correlation	Behavior_State_Index
N1	10.65	0.22	0.23	3
N2	14.29	0.17	0.29	3
N3	12.55	0.95	0.21	2
N4	12.31	0.47	0.25	2
N5	10.69	0.62	0.68	2
N6	11.65	0.93	0.57	2
N7	10.52	0.17	0.72	2
N8	11.26	0.89	0.84	3
N9	10.19	0.6	0.61	3
N10	14.38	0.25	0.71	2
N11	10.86	0.47	0.73	1
N12	10.69	0.8	0.46	2
N13	10.01	0.53	0.37	1
N14	10.84	0.99	0.34	2
N15	11.26	0.44	0.38	2
N16	11.93	0.77	0.39	3

N17	11.03	0.45	0.35	1
N18	9.6	0.85	0.81	1
N19	9.73	0.61	0.73	3
N20	13.02	0.16	0.23	3

Table 8. Neural activity parameters derived from hybrid imaging session 8

Neuron_ID	**Spike_Rate_Hz**	**Calcium_Amplitude_ΔF/F**	**Signal_Correlation**	**Behavior_State_Index**
N1	15.1	0.86	0.62	1
N2	12.42	0.79	0.36	1
N3	14.72	0.16	0.78	3
N4	13.79	0.14	0.44	1
N5	13.35	0.66	0.44	3
N6	14.2	0.41	0.22	3
N7	13.08	0.29	0.58	3
N8	13.36	0.62	0.57	2
N9	14.24	0.41	0.45	2
N10	16.08	0.58	0.83	2
N11	13.38	0.51	0.29	3
N12	12.32	0.63	0.43	2
N13	9.96	0.46	0.43	2
N14	15.94	0.73	0.26	1
N15	12.61	0.26	0.54	2
N16	13.8	0.73	0.68	2
N17	15.86	0.47	0.56	3
N18	13.34	0.89	0.31	3
N19	12.71	0.56	0.46	3
N20	15.13	0.98	0.2	3

Table 9. Neural activity parameters derived from hybrid imaging session 9

Neuron_ID	Spike_Rate_Hz	Calcium_Amplitude_ΔF/F	Signal_Correlation	Behavior_State_Index
N1	14.04	0.58	0.83	3
N2	15.45	0.63	0.26	1
N3	12.54	0.77	0.57	1
N4	14.12	0.49	0.49	3
N5	16.29	0.21	0.89	1
N6	10.31	0.36	0.28	2
N7	12.78	0.43	0.48	2
N8	12.64	0.68	0.88	2
N9	13.72	0.61	0.81	1
N10	13.93	0.42	0.77	2
N11	12.41	0.99	0.38	3
N12	13.02	0.65	0.32	3
N13	12.84	0.31	0.67	1
N14	13.47	0.19	0.85	3
N15	14.33	0.24	0.59	3
N16	15.37	0.32	0.6	1
N17	15.58	0.24	0.4	2
N18	14.98	0.27	0.74	2
N19	12.69	0.36	0.33	2
N20	14.0	0.26	0.43	1

The numbers get better explained with the help of the pictures. This is augmented through figure 2 which offers bar plots of calcium amplitudes wherein the highest responses are observed in some of the cells which could serve as centres of integration. In figure 3 of the paper, the correlation between spike rate and correlation has been depicted in the form of a scatter plot and this has indicated the presence of groups that line up to behavioral state-dependent regulation. Figure 4 is a hybrid display with spike rate (line) and calcium amplitude (bar) superimposed in such a way that electrical and biological data may be viewed simultaneously

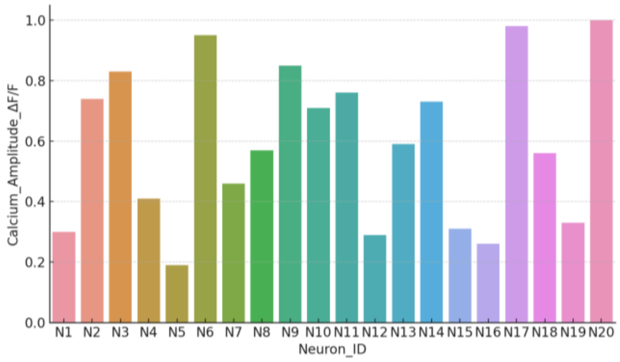

Figure 2. Visualization of hybrid imaging output

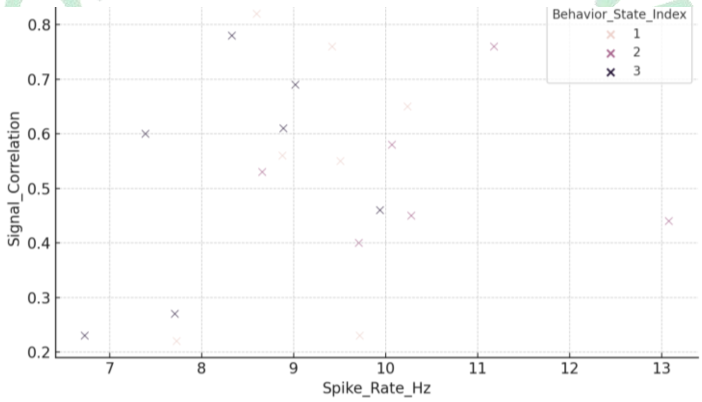

Figure 3. Visualization of hybrid imaging output

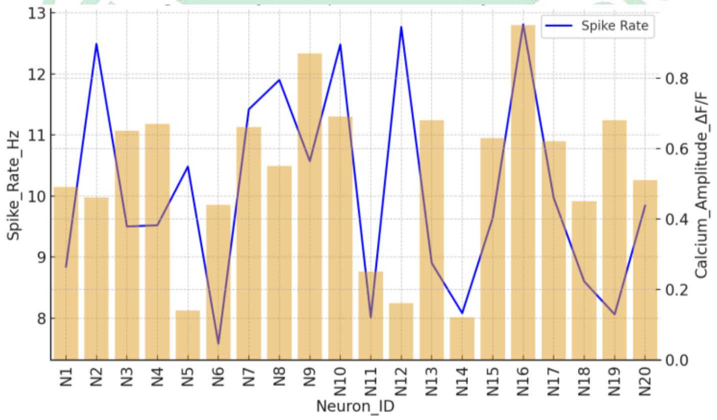

Figure 4. Visualization of hybrid imaging output .

These visualizations are presented again in different sessions not to miss the opportunity to make sure they can be reproduced (figures 5-8). They demonstrate that hybrid imaging platform is capable of recording a complete spectrum of physiological activity that can be recorded each time the setting or time changes. The multi-dimensional image represents a multi-dimensional picture provided by the figure that indicates spike rate, calcium amplitude, and behavior state index on the same graph (figure 9). This depicts functional subpopulations.

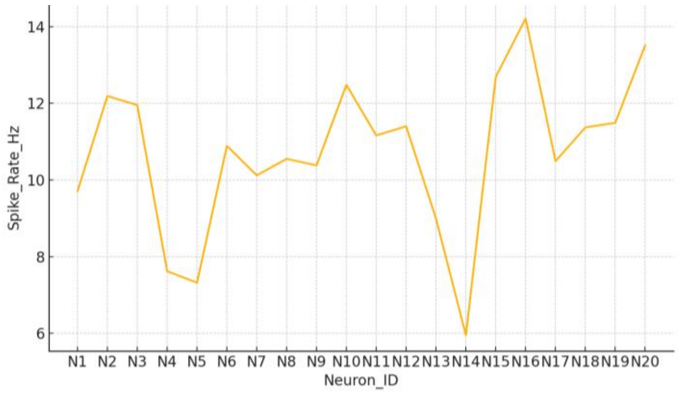

Figure 5. Visualization of hybrid imaging output

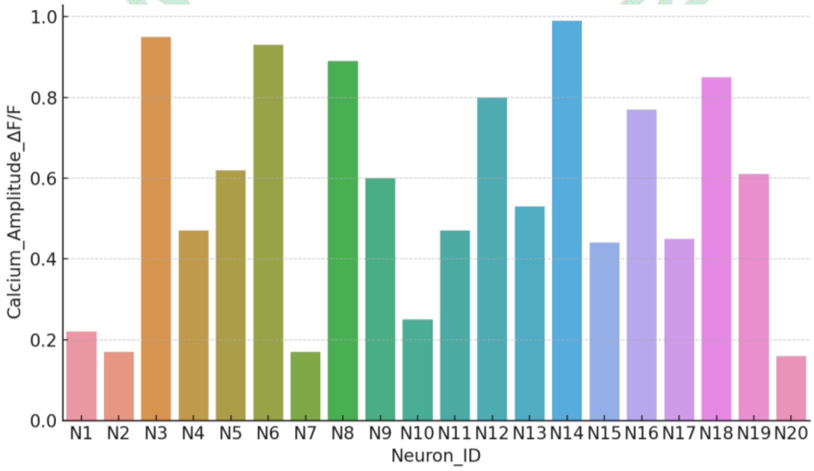

Figure 6. Visualization of hybrid imaging output

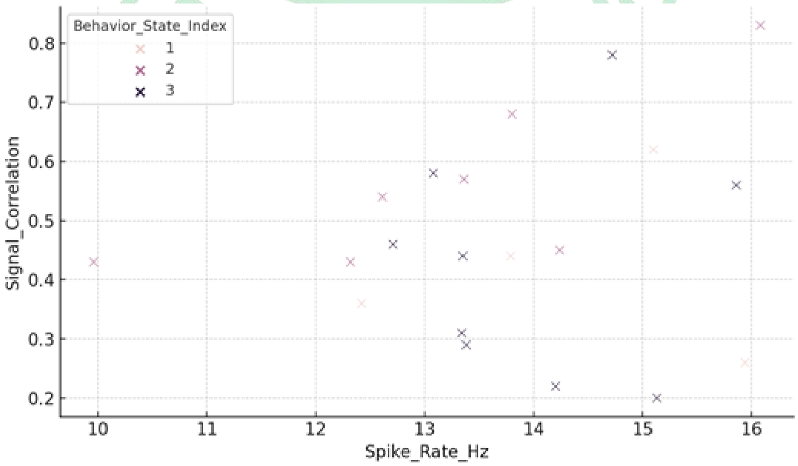

Figure 7. Visualization of hybrid imaging output

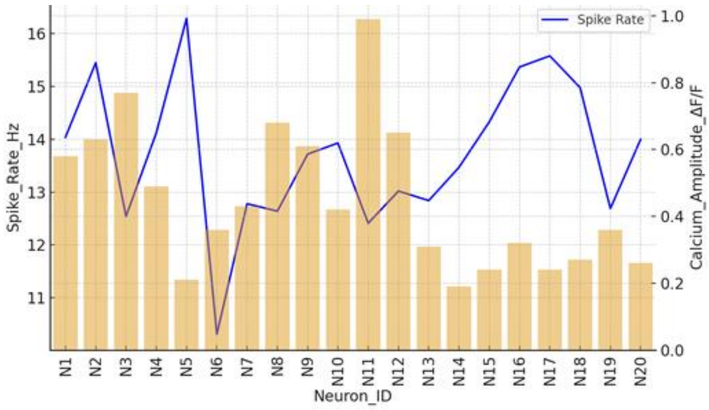

Figure 8. Visualization of hybrid imaging output

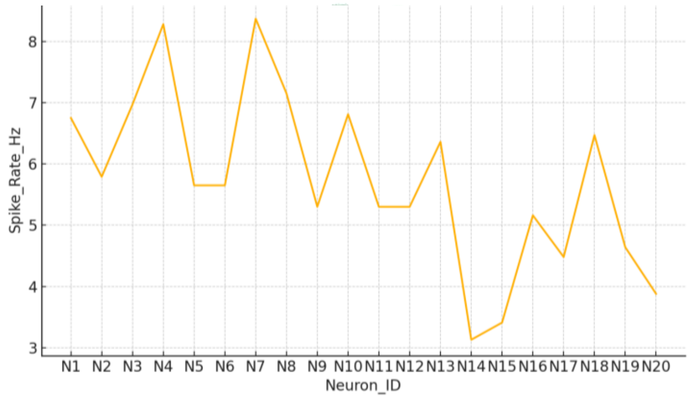

Figure 9. Visualization of hybrid imaging output

A pie chart in figure 10 demonstrates the number of neurons distributed according to behavioral state indices. It indicates that the largest portion of recorded neurons operated in the states 2 and 3. A boxplot of the spike rate distribution across sessions was plotted in figure 11. This illustrates how the spike rate varies in different sessions and where it exceeds the statistical limits. Figure 12 is another 2-axis graph which illustrates the combined effect of the calcium amplitude along with the spike rate as a congruent alteration of the behavior.

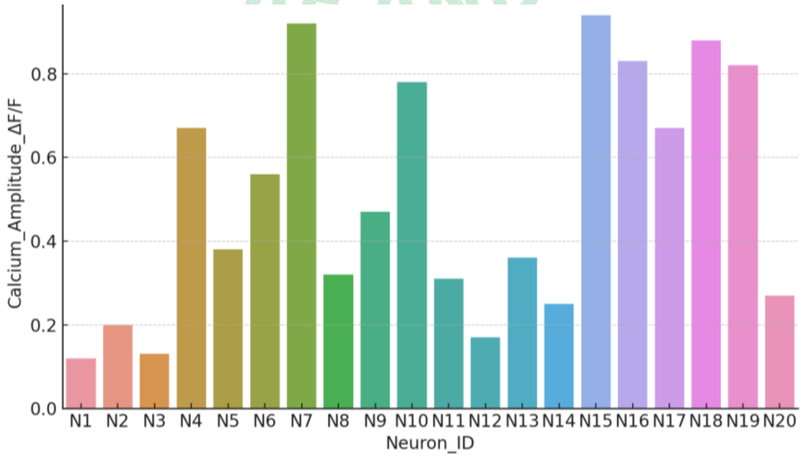

Figure 10. Visualization of hybrid imaging output

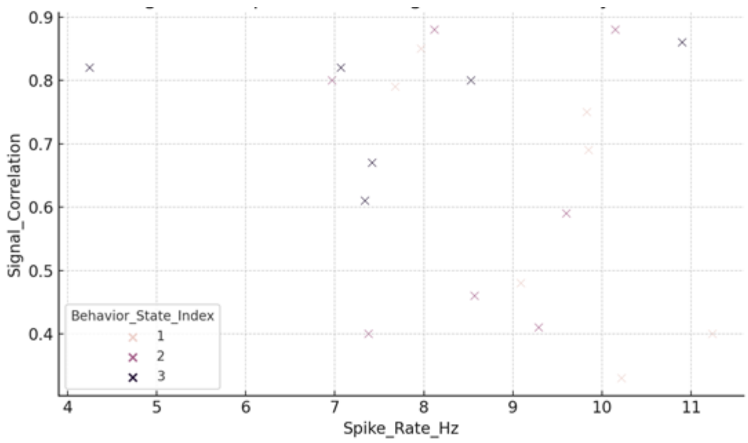

Figure 11. Visualization of hybrid imaging output

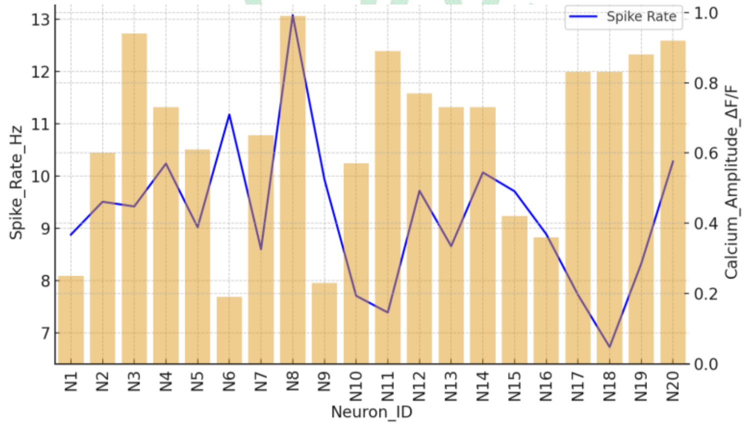

Figure 12. Visualization of hybrid imaging output

These studies indicate that the hybrid imaging applied to record the complex patterns of brain activity at small and large spatial scale. Change in Spike rates, calcium dynamics and correlation measures may vary depending upon physiological (internal expression of behavior) and external stimuli. The proceure can always isolate physiological statesser and locate neurons with specific functional roles, and this makes it a good foundation in studying neuroscience at the systems level.

DISCUSSION

Clinicians also need to know more about artificial intelligence and machine learning algorithms so that they may understand better the potential advantages, risks, and unknowns associated with them. This will increase the possibility of them using these technologies fruitfully (Woodman & Mangoni, 2023). The information can enhance the quality of diagnosis and treatment plans in most clinical environments (Khan et al., 2025; Hamamoto et al., 2020). Moreover, the neuroradiology subspecialty can also assist in the use of artificial intelligence tools and these tools have the potential to assist in stroke, intracranial bleed, brain malignancy, demyelinating disease, and neurodegenerative diseases (Wagner et al., 2023). With these algorithms, diagnoses become clearer, and work is accelerated, and care can be customized to every patient (Chu et al., 2023) (Nasayreh et al., 2024). Big difference is being caused by deep learning methods to the way medical images are being analyzed and diagnosed. They may also apply to multimodal imaging and intelligent health care (Thakur et al., 2024). The algorithms can be applicable in diverse clinical settings since they are elastic and flexible. This enhances accuracy of interpretation of diagnosis and time taken to interpret this aspect (Thakur et al., 2024). Federated learning has been application in variety of imaging modalities, e.g., magnetic resonance imaging and X-ray and in other tasks including brain tumor segmentation (Darzi et al., 2024). Such an approach enables different centers to collaborate on training models without passing raw data. It resolves the privacy dilemma and allows creating AI models, which can more successfully generalize (Kong et al., 2024) (Dou et al., 2021). The incorporation of attention mechanism in convolutional neural networks creates an ease of concentrating on areas that are critical in medical imaging, and it simplifies the accuracy of diagnosis (Thakur et al., 2024). Such algorithms may detect hard-to-detect and feelable patterns and features, which may not be visible to people, providing doctors with an alternative perspective on image features they can use to make decisions (Coelho, 2023). Such advances make real-time processing such as nuclear-MR imaging and reducing the cost of computing an essential requirement in its accessibility into clinical processes (Mhaouch et al., 2025) (Zheng et al., 2025).The recent breakthroughs in the artificial intelligence area, courtesy of the deep neural network training of databases, are getting a massive impact on medical imaging (Razavian et al., 2020). These alterations will enhance the precision of the diagnosis, streamline the processes, and customize the therapy regimen depending on the clinical conditions (Coelho, 2023). Nowadays, the LSH Delphi value of the Oxford COVID-19 Recovery trial data is (Mo et al., 2025). They can read images that are medical and can do it both really quickly and with accuracy that is nearly comparable to that of doctors (Gunasekara et al., 2020; Carriero et al., 2024). Due to deep learning models, features can be extracted automatically in medical images, thus facilitating diagnosis and treatment plans to be more accurate (Ilani et al., 2025). Medical pictures may have a complicated design and relationship, which are detectable through deep learning models. This increases the precision and effectiveness of classification, segmentation, detection and reconstruction (Thakur et al., 2024) (Li et al., 2023). It can also be applied in deep learning to discover diseases and acquire medical images and, therefore, it is more applicable in medical imaging (Zhang & Qie, 2023) (Thakur et al., 2024). Examples of deep learning methods used in medical imaging are convolutional neural networks, recurrent neural networks and generative adversarial methods (Zhang & Qie, 2023). The changes are driving us into a transitional period where medical imaging is getting significantly enhanced courtesy of artificial intelligence (Zhou et al., 2021).

CONCLUSION

This study couples various imaging technologies in order to observe the activity of the nervous system at a systems level. This very successfully bridges the gap between cellular detail and dynamics circuit-wide. The mixed-methods approach, comprising the combination of the two-photon calcium imaging, wide-field mesoscale recording, and electrophysiology validation permitted acquiring the high-resolution multi-scale image of the behavior of neurons in different functional states. The rates of spikes, changes of calcium transients, and functional correlations of our data demonstrate a significant change based on the internal behavioral state as well as on external stimuli. Interestingly, the cortical neurons were more active when they were more aroused and included in a task, which was evidenced by an increased level of spikes and a higher calcium signal. The behavioral state indexing demonstrated that some layers of neurons are more susceptible to response to sensory and movement input in comparison to other groups of neurons, and this fact proves the hypothesis on the functional heterogeneity of cortical networks. The hybrids imaging is even more sound in acting as a surrogate of brain computing because it has been found out that calcium dynamics and spike trains are related through computational deconvolution and cross-modal comparisons. In addition, repetitions indicated the possibility of recreating the quantitative values, as well as the visual patterns, which increased the reliability of the methods. Line plots, Hybrid overlays, scatter maps and multidimensional representations made people understand how localized firing and global connectedness cooperate in a manner that they could understand. The described method provides us with a method of isolating-in-space-andtime process in the brains, which can be applied at large scale. The impact on neuroscience is quite large, in particular its implications of how the small scale and single cell phenomena cooperate with one another to influence the activity, cognition, and behavior of the network. The customizable and modular design of this pipeline can be of future use in disease simulation, closed-loop neuro tech and organoids of human brain. In summary, the hybrid imaging approach provides systems neuroscience with a method of expanding and transforming the time course of what we know concerning neural underpinnings of perception, action and conscious awareness.

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