

Quilee Simeon Thesis Proposal

zebrafish proposal v2

Whole-Brain Voltage Imaging of Ketamine-Induced Brain State Transitions in Larval Zebrafish

PhD Thesis Proposal – May 30, 2025

PhD Candidate: Quilee Simeon

Thesis Advisor: Dr. Edward S. Boyden

Collaborator: Dr. Emery Brown

Committee: Dr. Illa R. Fiete (chair), Dr. Steve Flavell, Dr. Eviatar Yemini (external)

This proposal establishes a comprehensive framework for understanding anesthetic-induced brain state transitions at high resolution, with broad implications for neuroscience, clinical medicine, and our fundamental understanding of consciousness.

Abstract

Ketamine is a dissociative anesthetic with unique neural dynamics during altered states, yet its brain-wide effects remain poorly understood at the cellular level. This proposal outlines the first study to perform whole-brain voltage imaging with single-cell, millisecond resolution *in vivo* under anesthetic-induced states. We will leverage high-speed light-sheet microscopy and genetically encoded voltage indicators (GEVIs) to capture neuronal voltage activity across the entire larval zebrafish brain during ketamine exposure. This approach uniquely bridges pharmacology, neuroscience, and clinical relevance by revealing how ketamine alters brain state transitions at high spatiotemporal resolution.

Behavioral responsiveness will be assessed using noxious heat stimulation with a near-infrared (NIR) laser to induce tail-flick reflexes in head-embedded fish (Dempsey et al., 2022). By comparing neural dynamics before, during, and after bath application of ketamine (0.2% v/v subanesthetic vs 0.8% v/v anesthetic doses), we will uncover the spatiotemporal structure of brain-wide activity across drug-induced transitions (Zakhary et al., 2011). Our analysis will incorporate predictive modeling of neural dynamics as a significant novel methodological contribution, using spectral analysis, oscillatory synchrony measures, low-dimensional embeddings, and neural network-based stability assessments.

This work will provide fundamental insights into anesthetic mechanisms at the whole-brain, cellular level, bridging molecular pharmacology with systems neuroscience of consciousness. Such knowledge could inform new anesthesia monitoring strategies and advance our understanding of brain-wide dynamics in altered arousal states.

Introduction & Background

Understanding the neural basis of anesthetic-induced unconsciousness remains a central challenge in neuroscience and medicine. General anesthesia represents a controlled, reversible loss of consciousness that provides a unique window into the neural correlates of consciousness (Brown et al., 2011). Ketamine presents a particularly compelling case study due to its unique mechanism of action and distinct neural signatures compared to traditional GABAergic anesthetics.

Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, produces fundamentally different electroencephalogram (EEG) patterns than propofol and sevoflurane. Unlike GABAergic agents that induce sustained alpha oscillations, ketamine exhibits unique EEG characteristics including gamma-burst patterns, increased theta power, and reduced alpha/beta activity (Adam et al., 2023; Akeju et al., 2016). At anesthetic doses, ketamine introduces intermittent slow-delta oscillations while maintaining the absence of classical alpha rhythms (Bardon et al., 2025). Human intracranial studies reveal that ketamine elicits high-frequency gamma oscillations in prefrontal and hippocampal circuits (linked to antidepressant action) and a characteristic ~3 Hz rhythm in posteromedial cortex associated with dissociative effects (Tian et al., 2023). Recent work demonstrates that ketamine and dexmedetomidine cause convergent increases in cortical

oscillation phase alignment, yet induce distinct patterns of local versus long-range synchronization (Bardon et al., 2025).

Larval zebrafish uniquely enable this investigation due to their transparency, small size (~100,000 neurons), and whole-brain imaging capability. Unlike mammals where capturing every neuron's activity is infeasible, the larval zebrafish brain can be imaged nearly in its entirety *in vivo*. Zebrafish exhibit conserved responses to ketamine: dose-dependent locomotor and sensorimotor deficits paralleling mammalian effects (Bedell et al., 2020). At the molecular level, ketamine triggers gene expression changes in zebrafish brains similar to those in rodents, and recent work demonstrates that brief ketamine exposure engages evolutionarily conserved astrocyte-norepinephrine circuits in larval zebrafish, leading to long-lasting behavioral changes also observed in mice (Duque et al., 2025). This cross-species conservation reinforces the model's clinical relevance.

Our work builds upon and clearly differentiates from existing zebrafish studies. While Bedell et al. (2020) established zebrafish as a model for anesthetic action using behavioral assays, and Duque et al. (2025) demonstrated ketamine's effects on specific neuromodulatory circuits using calcium imaging, no study has captured real-time, brain-wide neural dynamics during anesthetic state transitions with single-neuron resolution. Technical breakthroughs now make this possible: Wang et al. (2023) developed remote-scanning light-sheet microscopy capable of recording ~1/3 of all larval zebrafish neurons at 200 Hz using improved GEVIs (Voltron2 and Positron2), enabling direct visualization of membrane voltage changes including action potentials at millisecond timescales.

Experimental setup schematic showing the integration of light-sheet microscopy, fluidic delivery system, and behavioral monitoring for simultaneous whole-brain voltage imaging and tail-flick assays during ketamine exposure.

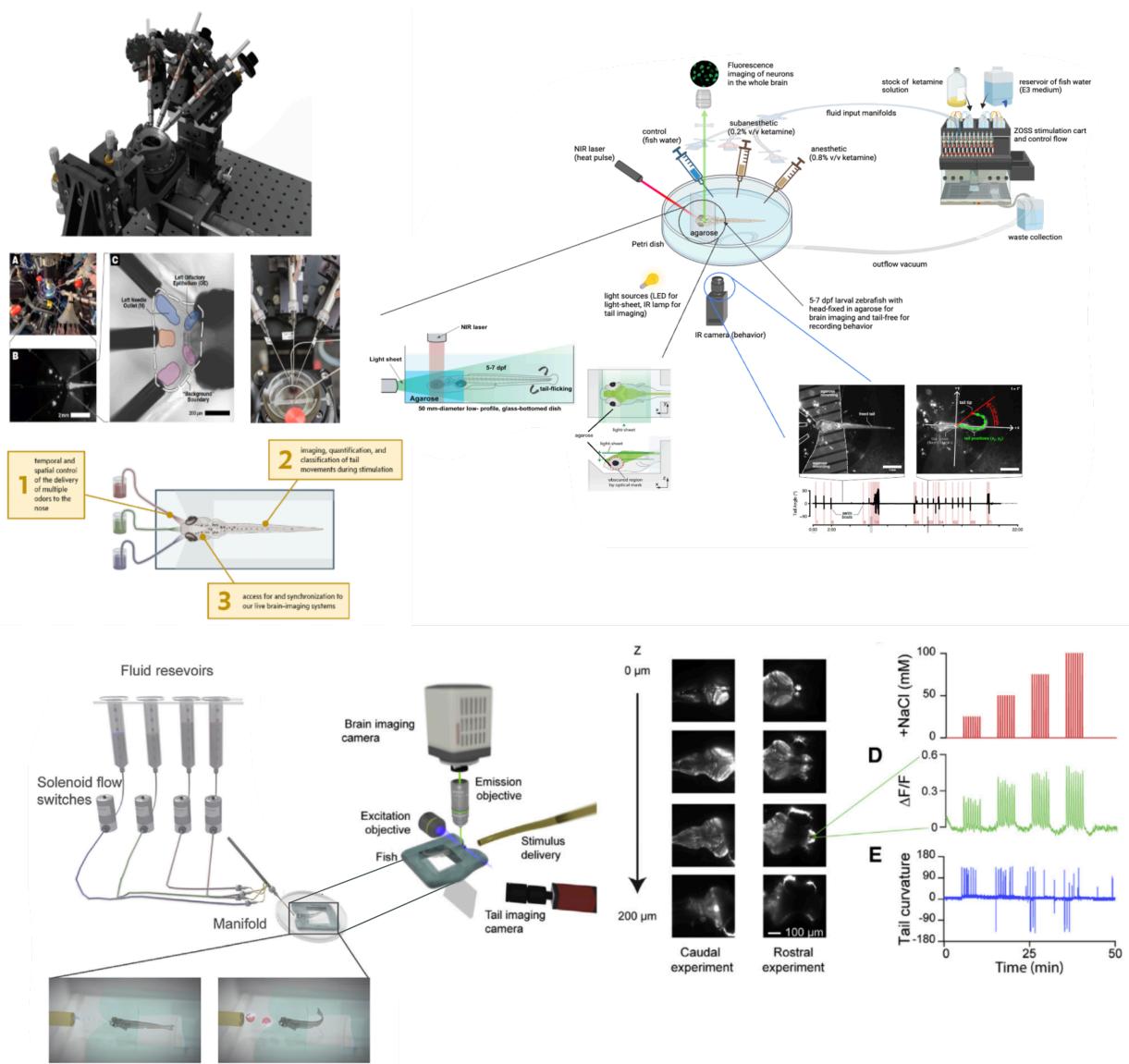


Figure 1. Adaptation of the Zebrafish Olfactory Stimulation System (ZOSS) for integrated solution perfusion, whole-brain voltage imaging and behavioral monitoring. We propose to modify the olfactory stimulation platform of the existing ZOSS apparatus developed by Swain (2024). **Top-left:** Photos, renderings and a schematic (bottom) of the olfactory stimulation platform. **Top-right:** Schematic of our intended experimental setup which involves a simplified adaption of the ZOSS rig along with equipment for brain and behavior stimulation and imaging. Our modified ZOSS setup is similar to the apparatus presented in Herrera et al. (2021) (**bottom**), but makes use of the existing equipment in the Boyden lab. Rather than precise spatiotemporal olfactory stimulation, we will use this modified ZOSS setup

to deliver bath solutions — control (E3 medium) or ketamine (0.2% or 0.8% v/v) — for our experiments. A larval zebrafish is embedded in agarose with its head in the light-sheet imaging plane and its tail free in a small chamber volume. Solution exchange is achieved through inlet and outlet tubes governed by electronically controlled valves (the ZOSS manifold). A peristaltic pump or vacuum line withdraws old solution via an outlet while fresh solution (either E3 medium or ketamine) is introduced via an inlet. An IR laser is directed at the larva's head to deliver brief noxious heat pulses, and an IR-sensitive camera beneath the chamber monitors the tail-flick reflexes. Neuronal voltage dynamics in the brain will be simultaneously recorded using the high-speed, remote-focusing light-sheet fluorescence microscope developed in-lab and described in Wang *et al.* (2023). The setup allows real-time capture of both neural and behavioral dynamics during drug exposure, enabling simultaneous analysis of responsiveness and brain activity. *Made with [Biorender.com](#) and with figures adapted from Herrera *et al.* (2021), Wang *et al.* (2023), and Swain (2024).*

Recent advances in anesthetic neuroscience suggest that unconsciousness may correspond to dynamical instability of brain networks (Eisen *et al.*, 2024). Propofol anesthesia destabilizes neural dynamics across cortex, making brain activity more chaotic and less predictable than in the awake state, yet whether ketamine—with its distinct NMDA receptor antagonism and HCN1 channel effects—produces similar network destabilization remains unknown. By observing nearly every accessible neuron's activity as the brain enters and exits ketamine-induced unconsciousness, we can test fundamental hypotheses about anesthetic mechanisms and identify network-level signatures of altered consciousness.

Specific Aims

Our overall goal is to characterize how ketamine modulates brain-wide neural dynamics during state transitions in a vertebrate brain, using larval zebrafish voltage imaging with high spatiotemporal resolution.

Aim 1: Measure and characterize brain-wide dynamics before, during, and after ketamine-induced behavioral state changes

We will utilize pan-neuronal GEVI expression in larval zebrafish and fast light-sheet imaging to record activity from thousands of neurons across the brain under

different conditions.

1.1: Conduct baseline recordings (awake state) with tail-flick behavior

Establish normative whole-brain activity patterns and tail-flick reflex responses in E3 medium without drug exposure. Characterize spontaneous neural dynamics and stimulus-evoked responses.

1.2: Record neural and behavioral responses during sub-anesthetic ketamine exposure

Image brain activity during 0.2% v/v ketamine exposure, expecting partial neural suppression and reduced but not eliminated tail reflexes.

1.3: Record neural and behavioral responses during anesthetic ketamine exposure

Capture neural dynamics during 0.8% v/v ketamine exposure, anticipating profound neural changes and loss of behavioral responsiveness.

1.4: Characterize recovery dynamics post-drug washout

Document the temporal evolution of neural activity and reflex recovery during ketamine clearance.

Hypothesis (Aim 1): Ketamine will cause dose-dependent changes in global brain synchronization, accompanied by progressive loss of responsiveness to external stimuli. We predict a shift toward lower-frequency oscillations and emergence of coherent population dynamics during anesthetic states, with specific brain regions showing differential susceptibility to ketamine's effects.

Aim 2: Quantify and model neural dynamics to identify signatures of stability, predictability, and altered oscillatory structure

Building on empirical recordings from Aim 1, we will apply advanced computational methods to understand network-level mechanisms underlying observed state transitions.

2.1: Perform multitaper spectral analysis to characterize oscillatory dynamics

Compute power spectra and spectrograms to identify dominant oscillation frequencies and their temporal evolution across conditions (Prerau et al., 2017; Adam et al., 2023).

2.2: Quantify brain-wide synchronization using phase-locking values (PLV)

Assess functional coupling between brain regions and test hypotheses about ketamine-induced changes in long-range versus local synchrony (Bardon et al., 2025).

2.3: Assess brain-state stability and transitions using low-dimensional embedding methods

Apply dimensionality reduction techniques to visualize and quantify how brain states evolve in neural state space during drug exposure.

2.4: Develop neural network predictive models to evaluate stability and predictability of neural dynamics

Train data-driven models to forecast neural time series and assess whether ketamine alters the fundamental predictability of brain activity, testing hypotheses about dynamical instability during unconsciousness (Eisen et al., 2024; Lueckmann et al., 2025).

Hypothesis (Aim 2): Ketamine will destabilize whole-brain neural dynamics, reducing the predictability and stability of neural trajectories. We expect increased low-frequency power, altered phase relationships between regions, and decreased temporal coherence in neural network activity, consistent with a transition to a less ordered dynamical regime.

Significance

This project will yield insights into anesthetic-induced unconsciousness by providing the first single-neuron-resolution voltage imaging of anesthetic state transitions and the first detailed modeling of these dynamics using neural network forecasting methods. The methodological novelty lies in capturing millisecond-scale neural events across an entire vertebrate brain during pharmacologically-induced state changes—a capability previously impossible with slower calcium imaging or limited electrode recordings.

The conceptual significance extends beyond technical achievement. By identifying network-level signatures of ketamine-induced unconsciousness, we will test fundamental theories about the neural basis of consciousness and anesthetic action. Our results will have direct clinical relevance for anesthesia

monitoring and provide insights into ketamine's antidepressant mechanisms, potentially informing therapeutic applications and drug development.

This work will establish general principles of unconsciousness with potential clinical translation. If we identify conserved patterns of neural dynamics during ketamine anesthesia (e.g., specific oscillatory signatures or circuit connectivity changes), these findings could inform the development of brain-state monitors for clinical anesthesia. The ability to observe cellular-level mechanisms underlying EEG patterns will bridge scales from molecular pharmacology to clinical electrophysiology.

By integrating cutting-edge neurotechnology with classical pharmacological neuroscience, this project demonstrates the power of whole-brain imaging for understanding complex brain states. The resulting datasets and analytical frameworks will be valuable resources for the neuroscience community, enabling further investigation of consciousness, anesthesia, and brain state dynamics across species.

Research Plan and Methods

Animal Model and Transgenic Lines

Collaboration with lab members: Adam Amsterdam, Caroline Zhang, Zeguan Wang

Methods outlined here address Aim 1.1–1.4 for establishing the experimental model. We will use larval zebrafish (5–8 dpf) of the nacre or casper background for optimal optical transparency. Pan-neuronal GEVI lines using the elavl3 promoter drive expression of Voltron2 and Positron2 indicators (Wang et al., 2023), which pair fast voltage-sensing domains with bright JF585 fluorophores for far-red voltage imaging at sub-millisecond resolution. These technical breakthroughs enable single-cell, single-spike resolution across tens of thousands of neurons simultaneously (Wang et al., 2023).

Imaging Setup

Collaboration with lab members: Yuechuan Lin, Corban Swain, Zeguan Wang

Neural activity will be recorded using our custom high-speed light-sheet fluorescence microscope based on the remote-scanning system of Wang et al. (2023). The system achieves volumetric imaging at ~200 Hz across the entire larval brain with dual orthogonal illumination and high-speed sCMOS detection.

Figures 1 and S2 depicting our high-speed remote-scanning light-sheet microscope from Wang, Z. et al. Imaging the voltage of neurons distributed across entire brains of larval zebrafish. *bioRxiv* 2023.12.15.571964 (2023) doi:10.1101/2023.12.15.571964.

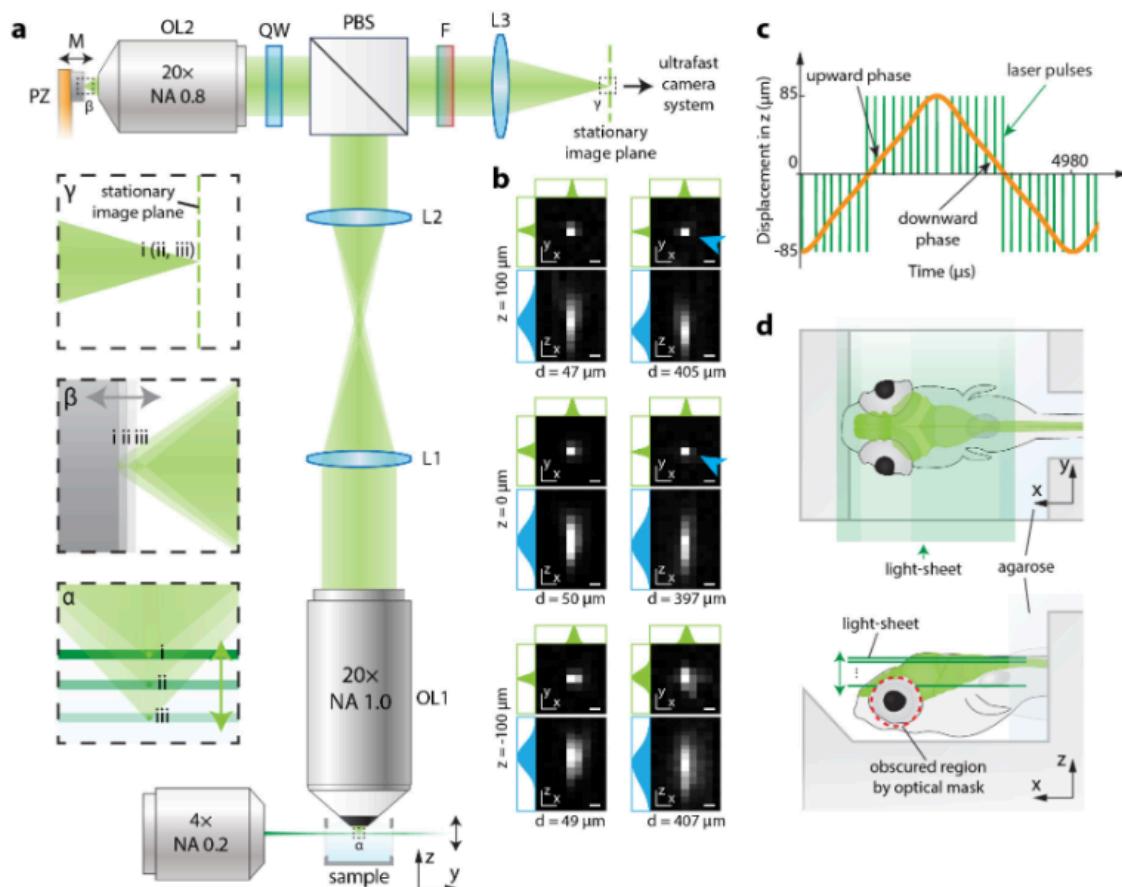


Figure 1. Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

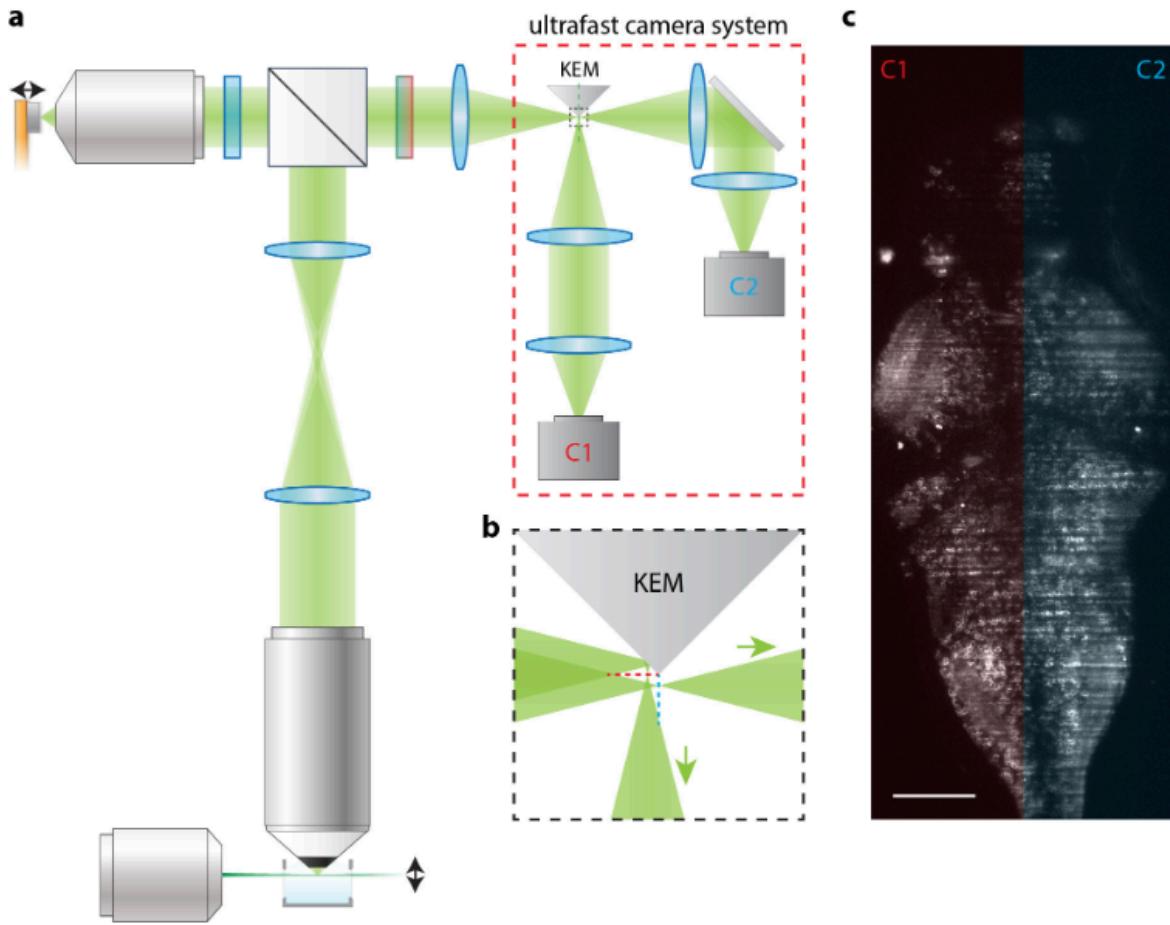


Figure S2. Overview of the ultrafast camera system. (a) Images from the high-speed light-sheet

Drug Delivery System and Behavioral Assay

Methods for drug delivery and behavioral monitoring align with Aim 1.2–1.3

We will employ a modified Zebrafish Olfactory Stimulation System (ZOSS) for rapid solution exchanges between E3 medium and ketamine solutions (Swain, 2024). Two ketamine concentrations will be tested: 0.2% v/v (sub-anesthetic) and 0.8% v/v (anesthetic), based on established zebrafish protocols (Zakhary et al., 2011).

Behavioral responsiveness will be assessed using NIR laser-evoked tail-flick reflexes as a proxy for consciousness (Dempsey et al., 2022). Each larva will be embedded in agarose with head fixed and tail free, allowing simultaneous brain imaging and behavioral monitoring.

Experimental timeline showing the temporal structure of solution switching, drug delivery, behavioral probing, and neural recording phases during a single experiment.

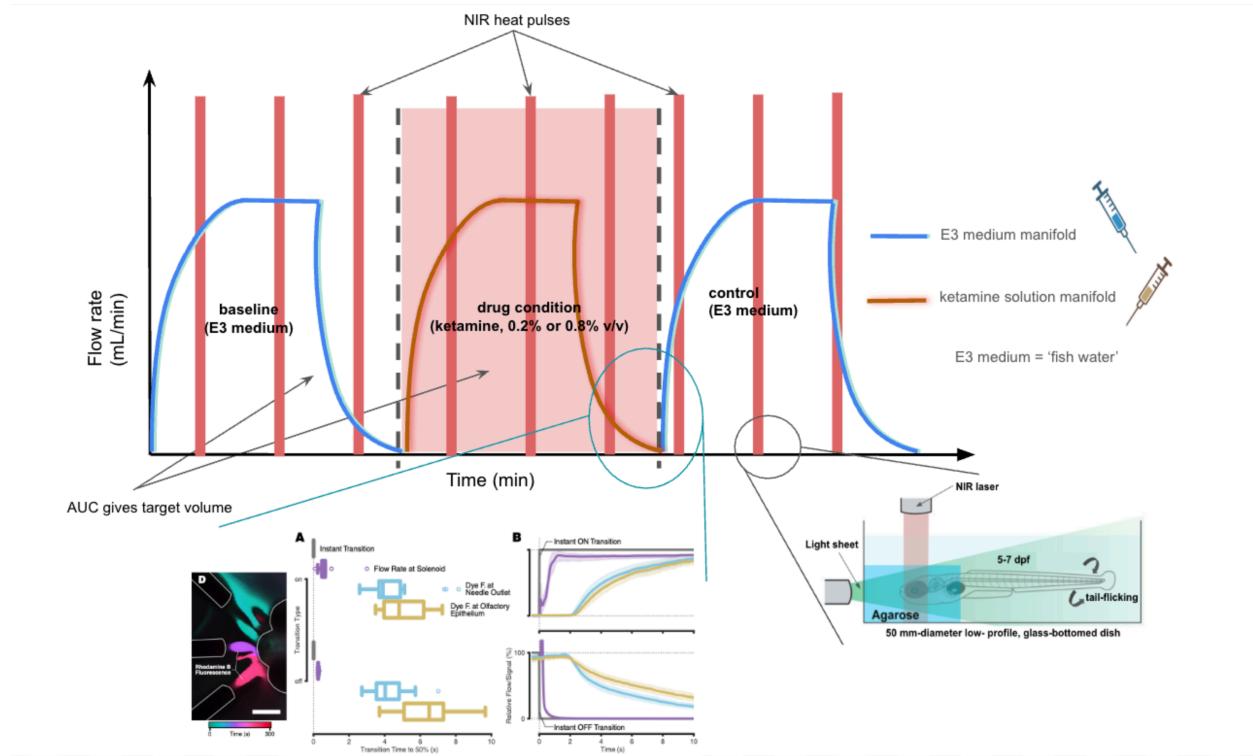


Figure 2. Temporal structure of a single experiment integrating drug delivery and behavioral probing. Fish are initially perfused with control solution (E3 medium or ‘fish water’) for baseline recording, followed by bath application of ketamine (0.2% or 0.8% v/v), and finally washed back into control medium. A fluidic manifold controls switching between reservoirs, achieving near-complete bath exchange within ~1-2 minutes. Red vertical bars indicate periodic IR heat pulse stimuli (e.g. ~5 seconds each, spaced ~1 minute apart) to the larva’s head used to assess behavioral responsiveness (tail-flick reflex) throughout baseline, ketamine, and recovery phases. This structure mimics anesthesia induction and emergence phases, allowing each fish to serve as its own control. **Main figure:** Blue and brown traces indicate the flow of control E3 medium and ketamine solution, respectively, as a function of time. The transition phases (dashed lines) involve simultaneous inflow of new solution and removal of old solution. In each trial, after an initial baseline period in E3, ketamine is introduced (pink shaded region) and maintained for a set duration (e.g. 5–10 min) before switching back to E3 for

washout. Two ketamine dose conditions are planned: a sub-anesthetic dose (0.2% v/v ketamine) and a higher anesthetic dose (0.8% v/v), each tested in separate trials. **Bottom-right inset:** The head of the zebrafish is encased in low-melt agarose, leaving the tail free to move. Heat produced by an NIR laser causes the unconditioned response of tail flicking in normal, awake zebrafish. **Bottom-left inset:** We will validate the capacity and timing of our modified ZOSS setup to deliver and withdraw the desired bath concentrations and volumes by performing synthetic dye (e.g. rhodamine B) quantification to determine delays in the transition times for switching between bath solutions. *Made with [Biorender.com](#) and with figures adapted from Swain (2024) and Dempsey et al. (2022).*

Data Analysis and Modeling

Methods for spectral analysis, PLV computation, state embedding, and predictive modeling align with **Aim 2.1–2.4** (Figure 3).

Preprocessing and Signal Extraction

Raw voltage imaging data will be processed using the VolPy pipeline (Cai et al., 2021) for motion correction, cell segmentation (enhanced with Cellpose; Stringer et al., 2025), and spike detection. This yields single-neuron voltage traces and spike times for subsequent analysis.

Spectral and Synchrony Analysis

Addressing Aim 2.1–2.2: We will apply multitaper spectral methods to characterize oscillatory content across frequency bands (Prerau et al., 2017; Adam et al., 2023). Phase-locking values will quantify inter-regional synchronization, testing hypotheses about ketamine's effects on cortical phase alignment (Bardon et al., 2025). All analytical approaches are supported by established methodologies from anesthesia EEG research (Adam et al., 2023; Eisen et al., 2024).

Dynamical Systems and Predictive Modeling

Addressing Aim 2.3–2.4: Low-dimensional embeddings will visualize brain state trajectories during drug exposure. Neural network models (following ZAPBench benchmarks) will assess the predictability of neural dynamics, testing whether

ketamine destabilizes brain activity patterns (Lueckmann et al., 2025; Eisen et al., 2024).

Data analysis pipeline overview.

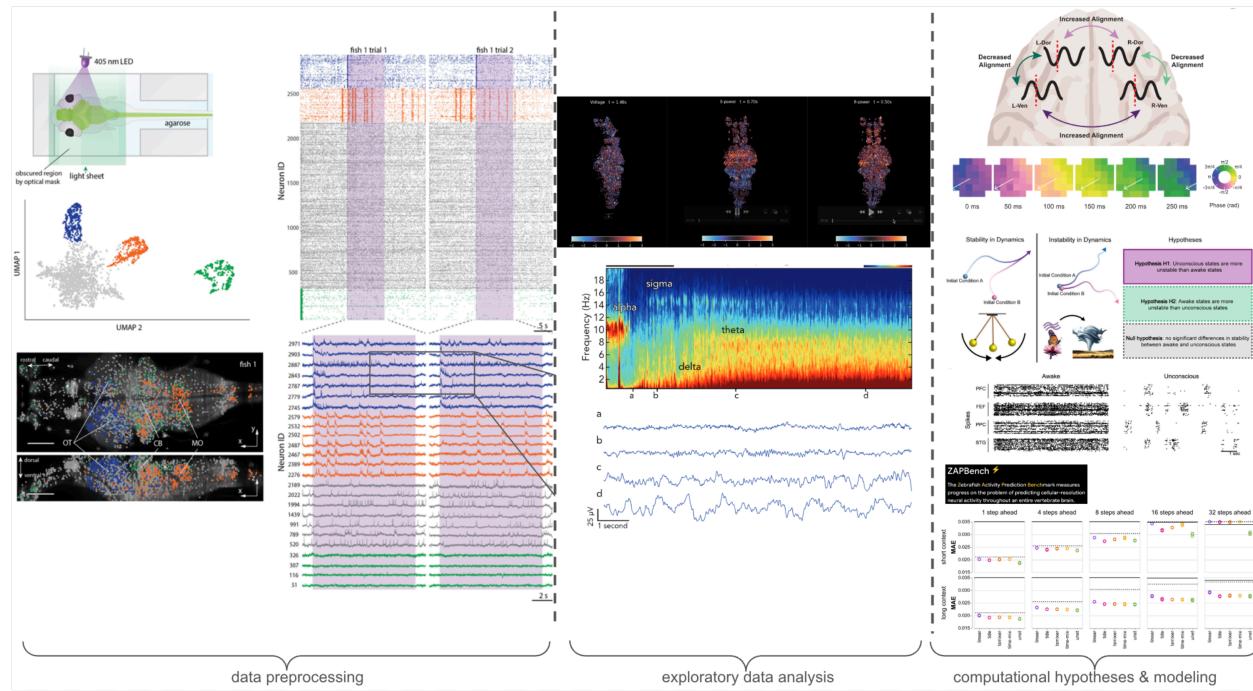


Figure 3. Multiscale analysis and modeling pipeline for whole-brain voltage imaging of ketamine-induced brain state transitions in larval zebrafish. This figure outlines the proposed computational pipeline for data processing, signal analysis, and neural predictability modeling. **Left panel:** Raw voltage imaging data are acquired at ~200 Hz using a custom-built remote-focusing light-sheet microscope that captures neural dynamics across nearly the entire larval zebrafish brain at single-cell resolution (adapted from Wang et al., 2023). After preprocessing—including motion correction, image registration, cell segmentation (e.g., with Cellpose), and spike inference using VolPy—single-neuron voltage traces and spike rasters are extracted. The layout and example traces mirror the pipeline described in Wang et al. and align with our expected dataset structure. **Middle panel (top):** Exploratory analysis on published Wang et al. (2023) datasets illustrates how band-specific power can be computed for each neuron over time using multitaper spectral analysis and visualized across space, effectively rendering dynamic brain-wide maps of frequency-specific activity (e.g., delta and

theta power). These analyses are inspired by work in sleep neurophysiology, where frequency bands correlate with brain state . **Middle panel (bottom):** As in Prerau *et al.* (2017), we treat each neuron's voltage trace as a 1D time series analogous to EEG/LFP recordings and compute its spectrogram using the multitaper method, which offers robust estimation of time-varying power distributions across frequency bands . **Right panel (top):** Inspired by Bardon *et al.* (2025), we will compute phase-locking values (PLV) and other synchrony metrics to quantify how ketamine modulates inter- versus intra-hemispheric oscillatory coordination. Their work revealed ketamine-induced increases in interhemispheric alignment of low-frequency activity in PFC. We aim to replicate analogous analyses in zebrafish using neuron-resolved voltage data. **Right panel (middle):** From Eisen *et al.* (2024), we borrow frameworks to assess dynamical (in)stability across brain states. Diagrams illustrate the concept of state trajectory divergence under anesthesia and the corresponding loss of attractor stability. Our proposal adapts these analyses to determine whether ketamine drives zebrafish neural dynamics into unstable regimes . **Right panel (bottom):** We incorporate methods from the ZAPBench benchmark (Lueckmann *et al.*, 2024) to evaluate neural predictability. Neural networks (e.g., transformers or RNNs) will be trained to forecast future population activity from past data under each condition (control, sub-anesthetic, anesthetic). Model performance (e.g., prediction error) serves as a proxy for neural stability and structure in the underlying dynamics. Together, this integrative framework bridges high-speed imaging with spectral analysis, synchrony quantification, and predictive modeling to uncover how ketamine alters the spatiotemporal structure and computational stability of vertebrate brain dynamics. Figure constructed using [BioRender.com](#) and adapted from Wang *et al.* (2023), Prerau *et al.* (2017), Bardon *et al.* (2025), Eisen *et al.* (2024), and the Lueckmann *et al.* (2024).

Expected Outcomes

Results will directly address our stated hypotheses and sub-aims. We anticipate first-of-their-kind observations of single-cell voltage dynamics during anesthetic states, revealing how ketamine reshapes neural oscillations, synchrony, and predictability at the whole-brain level. These novel outcomes will bridge theoretical frameworks with practical clinical monitoring advances.

Specifically, we expect:

- **Aim 1 outcomes:** Dose-dependent changes in neural firing patterns, with systematic suppression of tail reflexes correlating with specific brain-wide activity signatures
- **Aim 2 outcomes:** Altered spectral content toward lower frequencies, modified inter-regional synchrony patterns, and reduced predictability of neural dynamics under ketamine

Integration of findings will reveal how ketamine's NMDA receptor antagonism manifests as network-level changes, potentially identifying biomarkers for anesthetic depth and mechanisms of ketamine's therapeutic effects.

Contingency Plan

We have developed comprehensive alternative strategies to address potential experimental challenges and ensure project success:

Validation with Simpler Systems

We may validate brain-imaging experiments using calcium-indicator expressing zebrafish lines and/or simpler microscope systems (scanning confocal) before proceeding to state-of-the-art voltage imaging with light-sheet microscopy. This staged approach would ensure our protocols work robustly before applying the most advanced techniques.

Drug Dosing Adjustments

If our initial ketamine concentrations don't produce expected behavioral differences, we will adjust accordingly. For example, if 0.2% fails to induce measurable effects, we will incrementally increase to 0.3-0.4% until partial loss of responsiveness is observed. All dosing adjustments will be guided by literature precedent and pilot testing to ensure fish safety.

Alternative Drug Delivery Approaches

Instead of our modified ZOSS system, if it proves unreliable or overly complicated, we could opt for simpler manual pipetting procedures for drug/control solution exchanges, as described in published protocols (Herrera et al., 2021). While less

automated, this approach would still achieve the necessary solution switches for our experiments.

Primary Issue: If the modified ZOSS system proves unreliable or overly complicated for consistent drug delivery.

Contingency Solution: Implement simpler manual pipetting procedures for drug/control solution exchanges, as described in published protocols. While less automated, this approach allows precise control over bath composition and timing. We would pre-warm solutions to 28.5°C and use gentle manual exchange techniques to minimize mechanical disturbance during imaging.

Behavioral Readout Alternatives

If we cannot use the tail-free paradigm due to motion artifacts during light-sheet imaging, we would need to paralyze fish for imaging. In this case, we would assess consciousness using published techniques measuring motor neuron activity in the spinal cord, which correlates with fictive movements (Ahrens et al., 2012). This approach has been validated for assessing motor responsiveness in paralyzed preparations.

Primary Issue: If tail movement causes prohibitive motion artifacts for light-sheet imaging, requiring complete animal paralysis.

Contingency Solution: Implement published techniques for assessing consciousness in paralyzed preparations by monitoring spinal motor neuron activity patterns. Motor neuron firing correlates strongly with fictive tail movements and provides a reliable proxy for behavioral responsiveness (Ahrens et al., 2012). We would identify motor neurons in our voltage imaging data and track their activity patterns as a surrogate behavioral measure.

Technical and Analytical Robustness

Primary Issue: If initial analysis approaches fail to reveal clear differences between conditions.

Contingency Solutions:

- 1. Enhanced Analytical Sensitivity:** Implement more sophisticated time-frequency analysis methods, including wavelet-based approaches and advanced non-linear dynamics metrics.

2. **Expanded Sample Sizes:** Increase experimental N to ensure adequate statistical power for detecting subtle effects.
3. **Alternative Ketamine Dosing:** Adjust concentration ranges based on preliminary results to optimize the dynamic range of observable effects.

Data Quality and Interpretation

Primary Issue: If results are ambiguous or difficult to interpret in the context of existing literature.

Contingency Solutions:

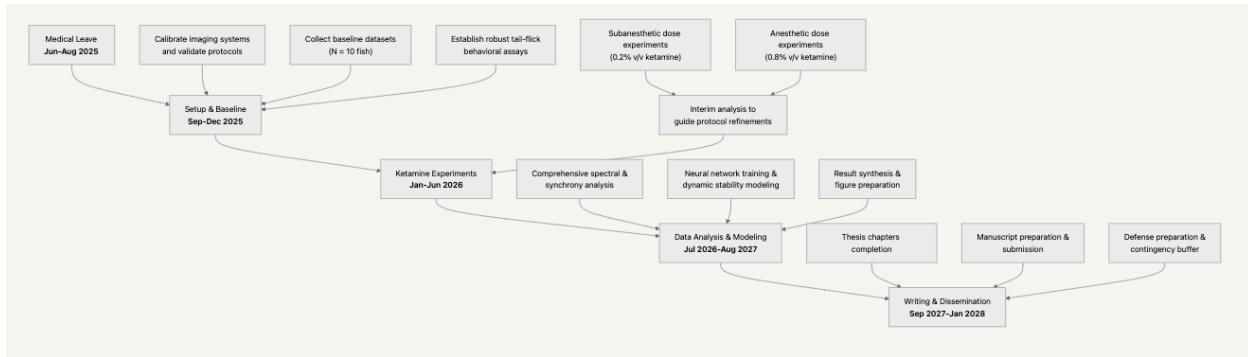
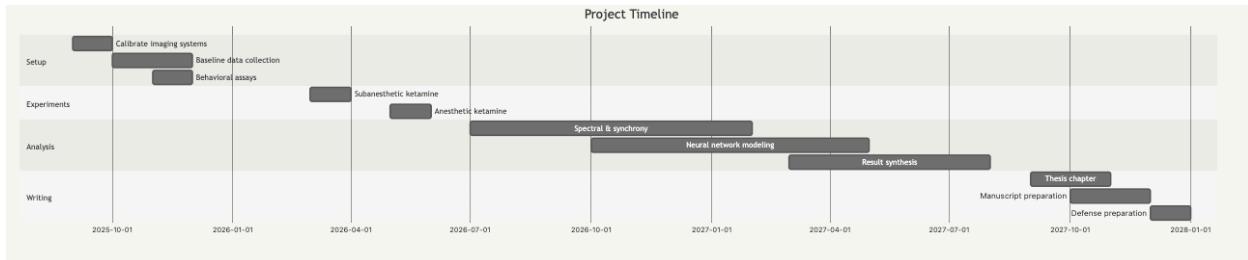
1. **Comparative Studies:** Include positive control experiments with well-characterized anesthetics in zebrafish (e.g., MS-222 tricaine) to establish baseline expectations for our analytical approaches.
2. **Expert Collaboration:** Leverage ongoing and potential collaborations with anesthesia researchers (e.g., with labs of Dr. Emery Brown and Dr. Earl Miller) to ensure proper interpretation of complex neural dynamics patterns.
3. **Literature Integration:** Maintain flexibility in interpretation by regularly updating analysis strategies based on emerging findings in anesthesia neuroscience.

This comprehensive contingency planning ensures that we can adapt our approach while maintaining scientific rigor and achieving our core objectives of understanding ketamine's brain-wide neural effects.

Timeline

We anticipate ~24 months of active research from Fall 2025 through Summer 2027, with additional buffer time through early 2028 for contingencies and dissertation writing.

Overview



Milestones

Jun–Aug 2025 – Medical Leave & Preparation

Focus on experimental design finalization and analytical pipeline development using existing data. Literature review on anesthetic mechanisms and zebrafish imaging. Animal protocol amendments submitted for approval.

Milestone: Animal use protocol approved; analysis pipeline validated on prior data.

Sep–Dec 2025 – Setup & Baseline Data Collection

Hands-on experimentation resumes with imaging setup calibration and baseline condition establishment. Baseline neural activity data collection and tail-flick reflex assay validation (target $N \approx 10$ fish).

Milestone: Baseline dataset collected with stable, reproducible neural dynamics and reflex readouts.

Jan–Feb 2026 – Preparatory Analysis & Adjustment

Preliminary baseline analyses for power spectra, synchrony measures, and reflex statistics. Parameter optimization before ketamine introduction. ZOSS trial runs with low-dose ketamine.

Milestone: All systems verified for ketamine experiments; baseline characterization completed.

Mar–Apr 2026 – Subanesthetic Ketamine Experiments

Data collection under 0.2% v/v ketamine (target ~5-6 fish). Expected partial neural suppression and reduced tail reflexes without complete unresponsiveness. Interim analysis for trend identification.

Milestone: Subanesthetic dataset collected; preliminary analysis confirms detectable differences from baseline.

May–Jun 2026 – Anesthetic Ketamine Experiments

High-dose experiments at 0.8% v/v ketamine (target ~5+ fish). Expected complete unresponsiveness and distinct brain activity patterns. Cross-condition pattern comparisons.

Milestone: Anesthetic dataset completed; initial cross-condition comparisons show major differences.

Jul–Dec 2026 – Data Analysis & Initial Modeling

Full dataset analysis including whole-brain power spectra, inter-regional synchrony mapping, and oscillatory phenomena quantification. Advanced analyses: low-dimensional embeddings and neural network model training for predictability assessment.

Milestone: Initial results obtained confirming key predictions; draft figures and summaries prepared.

Jan–Aug 2027 – Writing, Dissemination, and Defense

PhD thesis chapter writing integrating background, methods, and results. Manuscript preparation for publication focusing on network synchrony changes. Thesis defense scheduled for mid-2027.

Milestone: PhD thesis completed and defended; primary manuscript submitted.

Sep 2027–Jan 2028 – Buffer Period

Additional time for unforeseen delays, extra experiments if needed, extended analysis, or manuscript/dissertation revisions.

Milestone: All project aims fulfilled; no outstanding tasks.

References

- Adam, E., Kwon, O., Montejo, K.A., & Brown, E.N. (2023). Modulatory dynamics mark the transition between anesthetic states of unconsciousness. *Proc. Natl. Acad. Sci. U.S.A.*, 120(30), e2300058120.
- Ahrens, M.B., Li, J.M., Orger, M.B., Robson, D.N., Schier, A.F., Engert, F., & Portugues, R. (2012). Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature*, 485(7399), 471-477.
- Akeju, O., Song, A.H., Hamilos, A.E., Pavone, K.J., Flores, F.J., Brown, E.N., & Purdon, P.L. (2016). Electroencephalogram signatures of ketamine anesthesia-induced unconsciousness. *Clinical Neurophysiology*, 127(6), 2414-2422.
- Bardon, A.G., Ballesteros, J.J., Brincat, S.L., Roy, J.E., Mahnke, M.K., Ishizawa, Y., Brown, E.N., & Miller, E.K. (2025). Convergent effects of different anesthetics on changes in phase alignment of cortical oscillations. *Cell Reports*, 44(5), 115685.
- Bedell, V.M., Meng, Q.C., Pack, M.A., & Eckenhoff, R.G. (2020). A vertebrate model to reveal neural substrates underlying the transitions between conscious and unconscious states. *Scientific Reports*, 10(1), 15789.
- Brown, E.N., Purdon, P.L., & Van Dort, C.J. (2011). General anesthesia and altered states of arousal: a systems neuroscience analysis. *Annual Review of Neuroscience*, 34, 601-628.
- Cai, C., Friedrich, J., Singh, A., Eybposh, M.H., Pnevmatikakis, E.A., Podgorski, K., & Giovannucci, A. (2021). VolPy: Automated and scalable analysis pipelines for voltage imaging datasets. *PLoS Computational Biology*, 17(4), e1008806.
- Dempsey, W.P., Du, Z., Nadtochiy, A., Smith, C.D., Czajkowski, K., Andreev, A., Robson, D.N., Li, J.M., Applebaum, S., Truong, T.V., Kesselman, C., Fraser, S.E., & Arnold, D.B. (2022). Regional synapse gain and loss accompany memory formation in larval zebrafish. *Proceedings of the National Academy of Sciences*, 119(3), e2107661119.
- Duque, M., Chen, A.B., Hsu, E., Narayan, S., Rymbek, A., Begum, S., Saher, G., Cohen, A.E., Olson, D.E., Li, Y., Prober, D.A., Bergles, D.E., Fishman, M.C., Engert, F., & Ahrens, M.B. (2025). Ketamine induces plasticity in a norepinephrine-astroglial circuit to promote behavioral perseverance. *Neuron*, 113(3), 426-443.e5.

Eisen, A.J., Kozachkov, L., Bastos, A.M., Donoghue, J.A., Mahnke, M.K., Brincat, S.L., Chandra, S., Tauber, J., Brown, E.N., Fiete, I.R., & Miller, E.K. (2024). Propofol anesthesia destabilizes neural dynamics across cortex. *Neuron*, 112(16), 2799-2813.e9.

Herrera, K.J., Panier, T., Guggiana-Nilo, D., & Engert, F. (2021). Larval zebrafish use olfactory detection of sodium and chloride to avoid salt water. *Current Biology*, 31(4), 782-793.e3.

Lueckmann, J.-M., Immer, A., Chen, A.B.-Y., Li, P.H., Petkova, M.D., Iyer, N.A., Hesselink, L.W., Dev, A., Ihrke, G., Park, W., Petruccio, A., Weigel, A., Korff, W., Engert, F., Lichtman, J.W., Ahrens, M.B., Januszewski, M., & Jain, V. (2025). ZAPBench: A benchmark for whole-brain activity prediction in zebrafish. *arXiv preprint*, arXiv:2503.02618.

Prerau, M.J., Brown, R.E., Bianchi, M.T., Ellenbogen, J.M., & Purdon, P.L. (2017). Sleep neurophysiological dynamics through the lens of multitaper spectral analysis. *Physiology*, 32(1), 60-92.

Stringer, C., & Pachitariu, M. (2025). Cellpose3: one-click image restoration for improved cellular segmentation. *Nature Methods*, 22(3), 592-599.

Swain, C.N. (2024). Technological Innovation and Integration of Whole Brain Imaging, Olfactory Stimulation, and Correlative Microscopy in Larval Zebrafish. *Doctoral thesis, MIT*.

Tian, F., Lewis, L.D., Zhou, D.W., Balanza, G.A., Paulk, A.C., Zelmann, R., Peled, N., Soper, D., Santa Cruz Mercado, L.A., Peterfreund, R.A., Aglio, L.S., Eskandar, E.N., Cosgrove, G.R., Williams, Z.M., Richardson, R.M., Brown, E.N., Akeju, O., Cash, S.S., & Purdon, P.L. (2023). Characterizing brain dynamics during ketamine-induced dissociation and subsequent interactions with propofol using human intracranial neurophysiology. *Nature Communications*, 14(1), 1748.

Wang, Z., Zhang, J., Symvoulidis, P., Guo, W., Zhang, L., Wilson, M.A., & Boyden, E.S. (2023). Imaging the voltage of neurons distributed across entire brains of larval zebrafish. *bioRxiv*, 2023.12.15.571964.

Zakhary, S.M., Ayubcha, D., Ansari, F., Kamran, K., Karim, M., Lehestre, J.R., Horowitz, J.M., & Torres, G. (2011). A behavioral and molecular analysis of ketamine in zebrafish. *Synapse*, 65(2), 160-167.