

zebrafish proposal v3

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

PhD Thesis Proposal – May 30, 2025

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Abstract

Understanding the neural mechanisms underlying anesthetic-induced unconsciousness remains a fundamental challenge in neuroscience and clinical medicine. This proposal outlines a groundbreaking approach by performing **the first whole-brain voltage imaging study with single-cell, millisecond resolution during anesthetic-induced brain state transitions in any vertebrate**. 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 as animals transition from consciousness to ketamine-induced unconsciousness and back to recovery.

Ketamine, an NMDA receptor antagonist with unique dissociative and rapid antidepressant properties, produces fundamentally different neural dynamics compared to traditional GABAergic anesthetics. Using a behavioral paradigm that assesses responsiveness through noxious heat-induced tail-flick reflexes, we will compare neural dynamics before, during, and after bath application of subanesthetic (0.2% v/v) versus anesthetic (0.8% v/v) ketamine doses.

Our analysis will employ cutting-edge computational approaches including multitaper spectral analysis, phase synchrony measurements, and **neural network-based predictive modeling of brain dynamics** as a significant novel methodological contribution. This represents the first

application of modern deep learning forecasting methods to whole-brain voltage data during anesthetic state transitions.

This unprecedented approach uniquely bridges molecular pharmacology, systems neuroscience, and clinical anesthesia by revealing how ketamine alters the spatiotemporal structure of brain-wide electrical activity at the fundamental level of neuronal voltage dynamics. Insights from this project will advance our understanding of consciousness, inform clinical monitoring of anesthetic depth, and potentially reveal mechanisms underlying ketamine's therapeutic effects.

Introduction & Background

The neural mechanisms underlying anesthetic-induced unconsciousness represent one of medicine's most profound unsolved problems (Brown et al., 2011). While general anesthesia is administered to millions of patients daily, the brain-wide dynamics that distinguish conscious from unconscious states remain poorly understood at the cellular level. This fundamental knowledge gap limits our ability to monitor anesthetic depth precisely, optimize drug dosing, and understand the mechanisms underlying anesthetic-induced complications.

Ketamine occupies a unique position among anesthetic agents due to its distinct mechanism of action and clinical profile. Unlike traditional GABAergic anesthetics such as propofol and sevoflurane, ketamine acts primarily as an NMDA receptor antagonist, producing fundamentally different electroencephalogram (EEG) signatures (Brown et al., 2011; Akeju et al., 2016). **Large-scale clinical studies have demonstrated that ketamine produces unique neural oscillations characterized by prominent gamma-burst patterns, reduced alpha/beta power, and the absence of sustained alpha oscillations typical of GABAergic agents** (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).

Recent human intracranial studies have provided crucial insights into ketamine's brain-wide effects. Tian et al. (2023) demonstrated 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. Similarly, Bardon et al. (2025) found that ketamine and dexmedetomidine cause convergent increases in cortical oscillation phase alignment, yet induce distinct patterns of local versus long-range synchronization. These findings suggest that ketamine fundamentally reorganizes information flow during unconsciousness, possibly explaining its unique dissociative effects.

However, existing studies are limited by the spatial and temporal resolution of available recording methods. EEG and local field potential recordings provide only macroscopic views of

brain activity, while human intracranial studies are constrained to a few electrode locations. **No study to date has captured the real-time, brain-wide neural dynamics of anesthetic-induced unconsciousness at single-neuron resolution.** This represents a critical gap, as the cellular mechanisms underlying network-level oscillations and their role in consciousness remain unknown.

Larval zebrafish (*Danio rerio*) provide an exceptional experimental platform to address this limitation. These small (~4-5 mm), optically transparent vertebrates possess conserved brain structures and neurotransmitter systems homologous to mammals, yet contain only ~100,000 neurons—enabling optical access to nearly the entire brain *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.**

Recent technological advances now enable unprecedented investigation of brain-wide neural activity in zebrafish. Wang et al. (2023) developed a revolutionary remote-scanning light-sheet microscope capable of recording membrane voltage signals from thousands of neurons throughout the larval zebrafish brain at ~200 Hz volumetric rate. **Using improved genetically encoded voltage indicators (GEVIs) such as Voltron2 and Positron2, they captured roughly one-third of all brain neurons with single-cell, single-spike resolution,** enabling direct visualization of millisecond-scale neural events across distributed brain circuits—impossible with slower calcium indicators or point electrodes.

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.**

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 central goal is to understand the neural mechanisms underlying ketamine-induced unconsciousness by addressing two distinct but complementary scientific questions about brain-wide dynamics in larval zebrafish.

Aim 1: Determine how ketamine reconfigures brain-wide oscillatory dynamics and functional connectivity patterns

Scientific Question: What are the specific changes in neural oscillations and inter-regional connectivity that characterize ketamine-induced unconsciousness across different brain states?

We will use whole-brain voltage imaging to compare oscillatory patterns and functional connectivity between awake, subanesthetic, and anesthetic states. This aim focuses on identifying the neural "signatures" that distinguish conscious from unconscious brain states under ketamine.

Sub-Aim 1.1: Map ketamine's dose-dependent effects on regional oscillatory power

Quantify changes in spectral power across frequency bands (delta, theta, alpha, beta, gamma) in distinct brain regions (forebrain, midbrain, hindbrain) using multitaper spectral analysis. This will reveal whether ketamine produces region-specific or brain-wide oscillatory changes.

Sub-Aim 1.2: Characterize ketamine's effects on cross-frequency coupling

Assess phase-amplitude coupling between different frequency bands to determine if ketamine alters the hierarchical organization of neural oscillations, which may be critical for maintaining consciousness.

Sub-Aim 1.3: Quantify functional connectivity reconfiguration

Calculate phase-locking values and coherence between brain regions to map how ketamine alters information flow patterns. Based on human studies (Bardon et al., 2025), we will test whether ketamine increases long-range synchrony while disrupting local connectivity.

Sub-Aim 1.4: Identify oscillatory biomarkers of unconsciousness

Determine which specific oscillatory features (frequency bands, coupling patterns, connectivity metrics) best predict behavioral responsiveness, establishing neural correlates of ketamine-

induced unconsciousness.

Hypothesis (Aim 1): Ketamine will produce dose-dependent shifts toward low-frequency oscillations (enhanced delta/theta, reduced alpha/beta), altered cross-frequency coupling patterns, and a paradoxical increase in long-range synchrony despite reduced local connectivity. These changes will correlate with loss of behavioral responsiveness, providing oscillatory biomarkers of unconsciousness.

Aim 2: Establish how ketamine affects neural network stability and brain state transition dynamics

Scientific Question: How does ketamine alter the fundamental dynamical properties of brain networks, and what do these changes reveal about the mechanisms of anesthetic-induced unconsciousness?

We will apply dynamical systems approaches and predictive modeling to understand whether ketamine-induced unconsciousness involves network destabilization, as suggested by recent theories of anesthetic action (Eisen et al., 2024).

Sub-Aim 2.1: Characterize brain state transitions in neural state space

Use dimensionality reduction (PCA, t-SNE, UMAP) to visualize how brain activity evolves through distinct states during ketamine exposure and recovery. This will reveal whether unconsciousness corresponds to transitions into fundamentally different regions of neural state space.

Sub-Aim 2.2: Quantify network stability across conscious and unconscious states

Apply neural network forecasting models (following ZAPBench guidelines; Lueckmann et al., 2025) to assess the predictability of brain dynamics. We will test whether ketamine-induced unconsciousness involves increased chaos and reduced temporal structure in neural activity patterns.

Sub-Aim 2.3: Analyze the temporal dynamics of state transitions

Characterize the kinetics and directionality of transitions between conscious and unconscious states during ketamine onset and recovery. This includes identifying lead-lag relationships between brain regions and determining whether transitions are gradual or switch-like.

Sub-Aim 2.4: Test competing theories of anesthetic action

Compare our findings against two major theoretical frameworks: (1) increased synchronization/integration theories vs. (2) network destabilization theories. This will determine

which mechanisms best explain ketamine's unique effects compared to other anesthetics.

Hypothesis (Aim 2): Ketamine will destabilize neural network dynamics, reducing the predictability and temporal coherence of brain activity despite creating apparent oscillatory organization. State transitions will reveal discrete unconscious states separated from conscious states in neural state space, with specific brain regions leading transitions into and out of unconsciousness. This will support dynamical instability theories over simple synchronization theories of anesthetic action.

Research Plan and Methods

Animal Model and Transgenic Lines

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

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. Larvae will be incubated in 5 μ M JF585 dye for ~20 minutes before experiments to ensure uniform HaloTag labeling across all neurons.

Experimental Design Overview

To address both aims, we will collect comprehensive brain-wide voltage imaging data from larval zebrafish across four experimental conditions: (1) baseline awake state in E3 medium, (2) subanesthetic ketamine exposure (0.2% v/v), (3) anesthetic ketamine exposure (0.8% v/v), and (4) recovery following drug washout. Each experiment will include behavioral responsiveness testing via tail-flick reflexes, allowing direct correlation between neural dynamics and consciousness state. This experimental design provides the foundation for both oscillatory/connectivity analysis (Aim 1) and dynamical systems analysis (Aim 2).

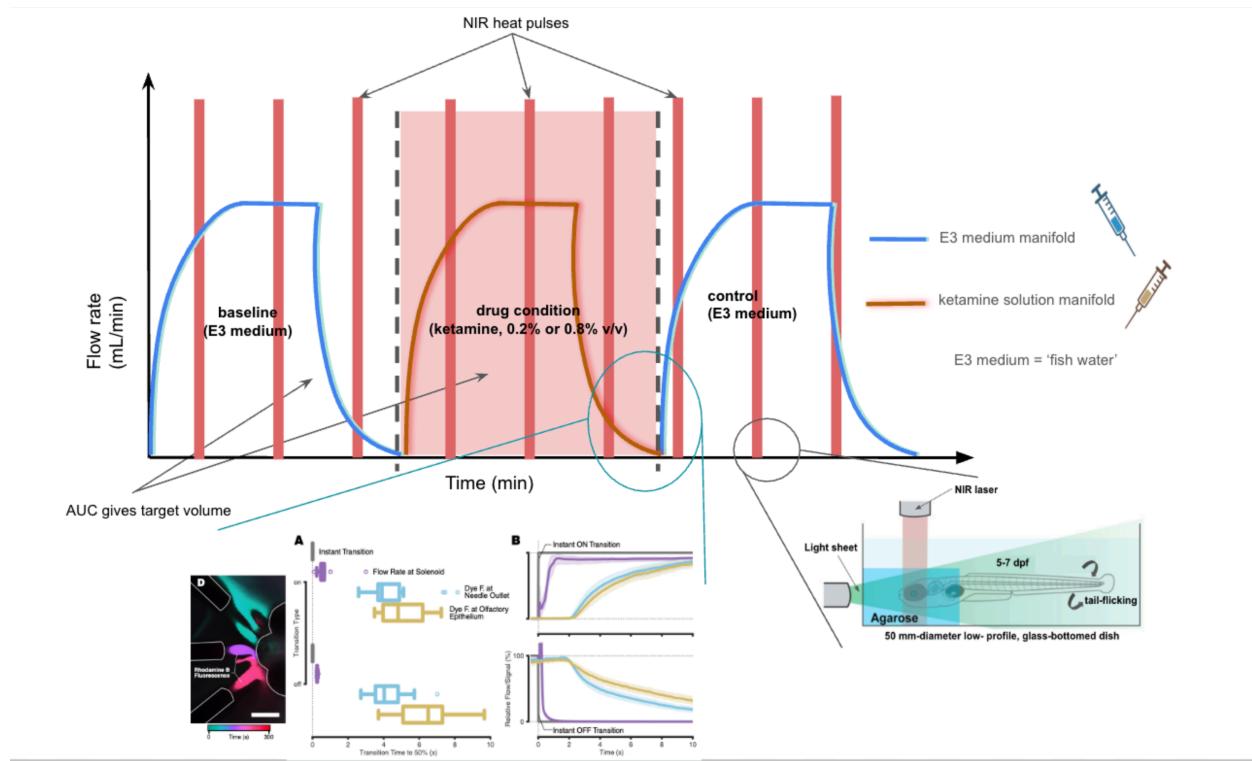


Figure 2. Temporal structure of a single experiment integrating drug delivery and behavioral probing. Fish are initially perfused with control solution (E3 medium or 'fish water') for baseline recording, followed by bath application of ketamine (0.2% or 0.8% v/v), and finally washed back into control 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)

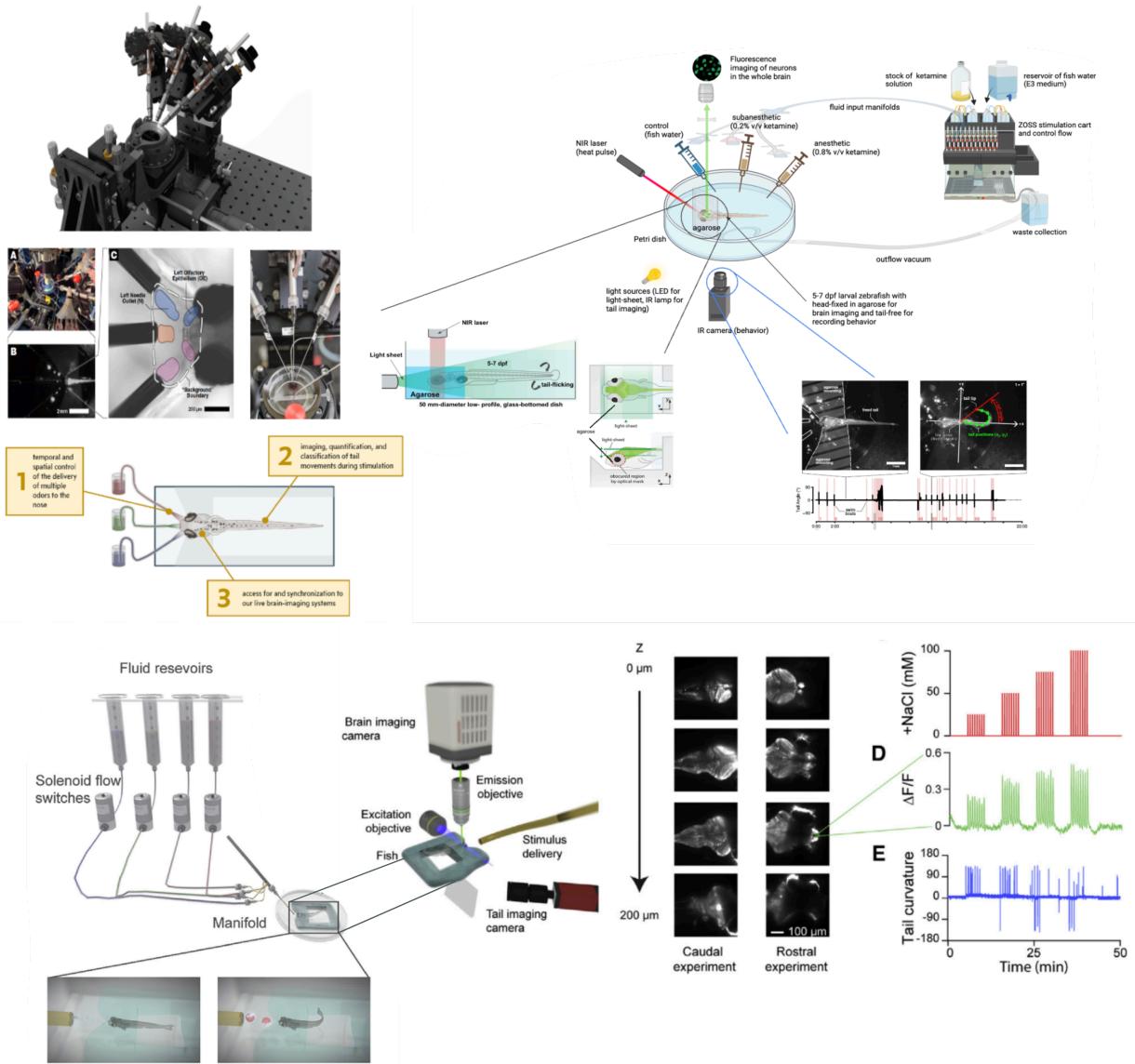
quantification to determine delays in the transition times for switching between bath solutions.
Made with Biorender.com and with figures adapted from Swain (2024) and Dempsey et al. (2022).

Experimental Setup and Drug Delivery

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

Larval Preparation: Individual larvae will be embedded in 2% low-melting-point agarose within custom imaging chambers, positioned dorsal-side up with heads immobilized but tails free for behavioral monitoring. The head-fixed, tail-free configuration enables high-resolution brain imaging while preserving the tail-flick reflex as a behavioral readout.

Drug Delivery System: We will employ a modified Zebrafish Olfactory Stimulation System (ZOSS; Swain, 2024) for precise temporal control of ketamine exposure. This computer-controlled manifold system enables rapid switching between E3 medium and ketamine solutions (0.2% or 0.8% v/v) with complete bath exchange within ~1-2 minutes. **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; Bedell et al., 2020).



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).*

Behavioral Assessment: Noxious infrared heat pulses (1450 nm laser, 200 ms–1 s duration) will be delivered to the larva's head every 30 seconds to evoke tail-flick reflexes as a proxy for consciousness. An infrared-sensitive camera will record tail movements, providing quantitative measures of responsiveness throughout the experiment.

High-Speed Voltage Imaging

Neural activity will be recorded using our custom remote-scanning light-sheet fluorescence microscope based on the system of Wang et al. (2023). The system uses dual orthogonal illumination (561 nm excitation) and high-speed sCMOS detection to capture whole-brain volumes at ~200 Hz. Each volume spans 50–100 z-planes (forebrain to hindbrain) with ~0.6 × 0.6 × 3 µm resolution, yielding fluorescence time series from ~10,000–20,000 neurons simultaneously.

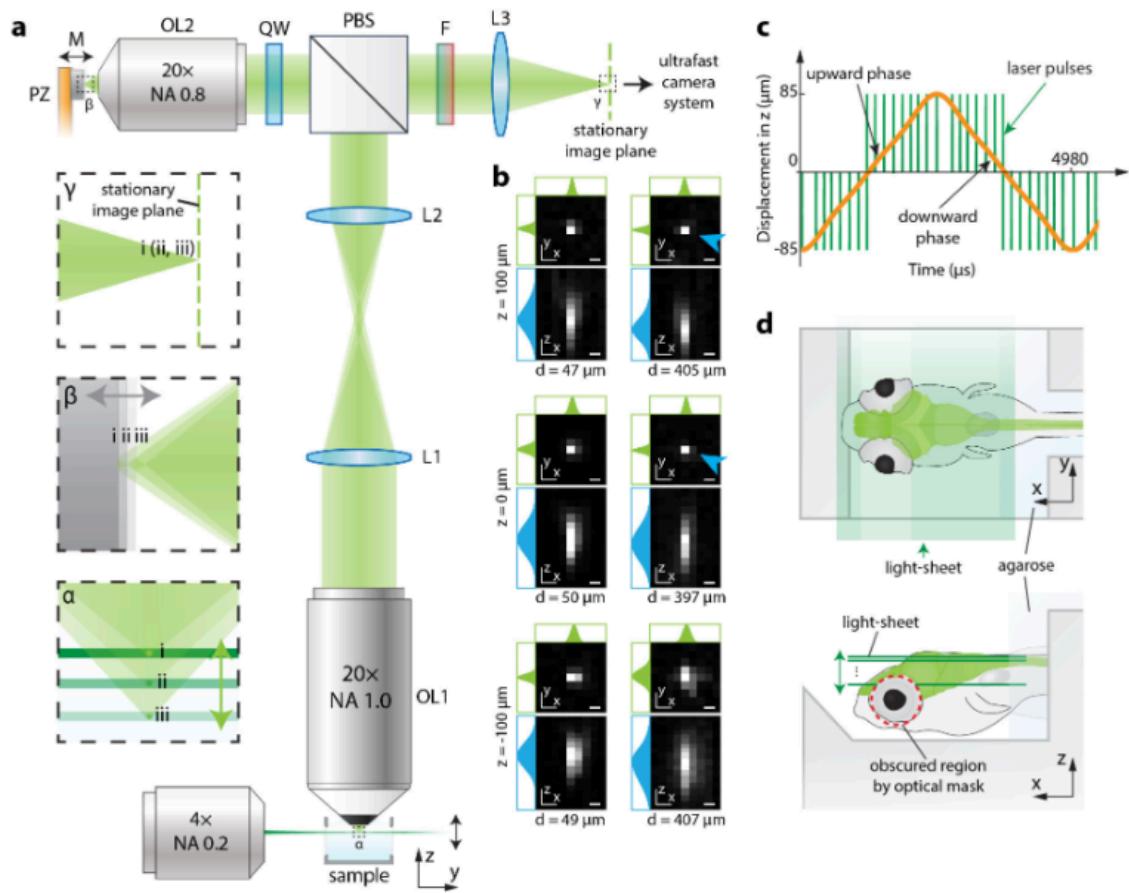


Figure 1. Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

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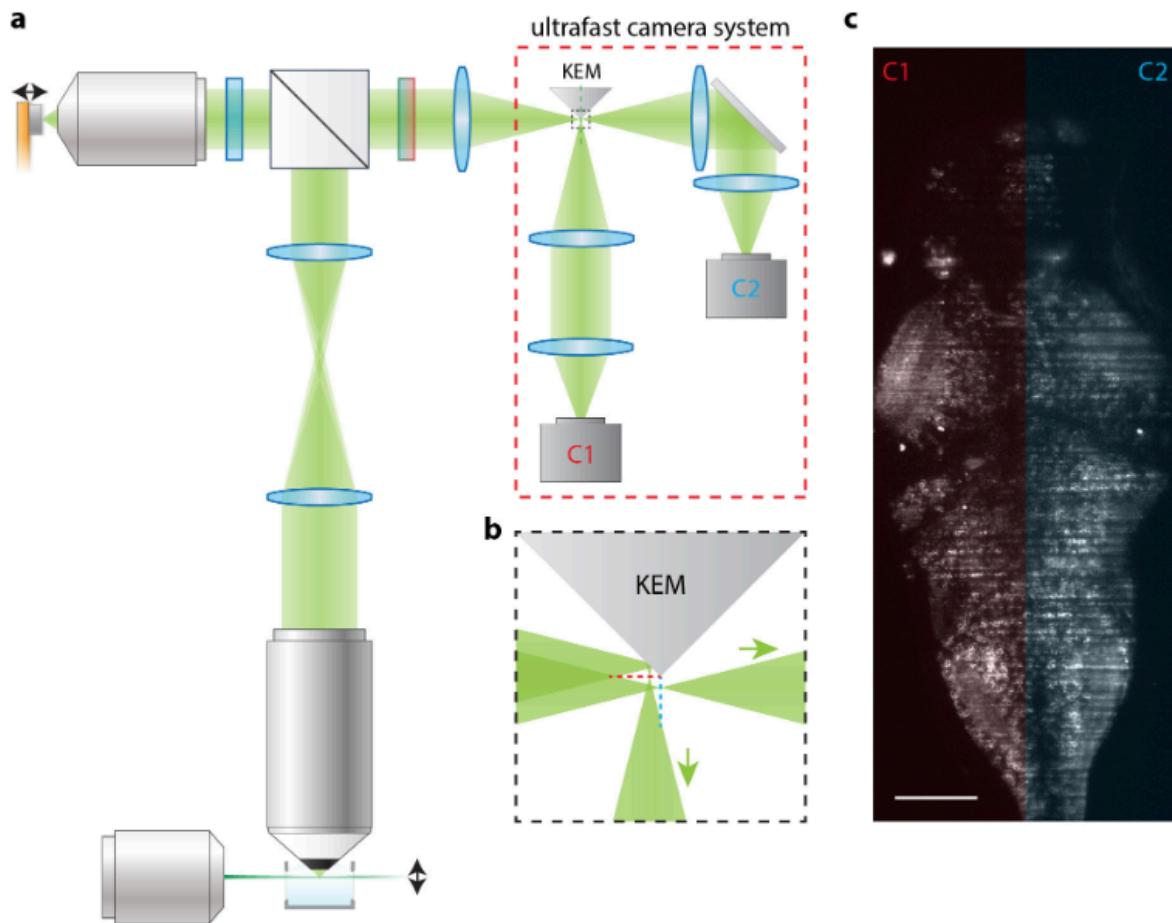


Figure S2. Overview of the ultrafast camera system. (a) Images from the high-speed light-sheet

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Figures 1 and S2 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.

Data Analysis and Modeling

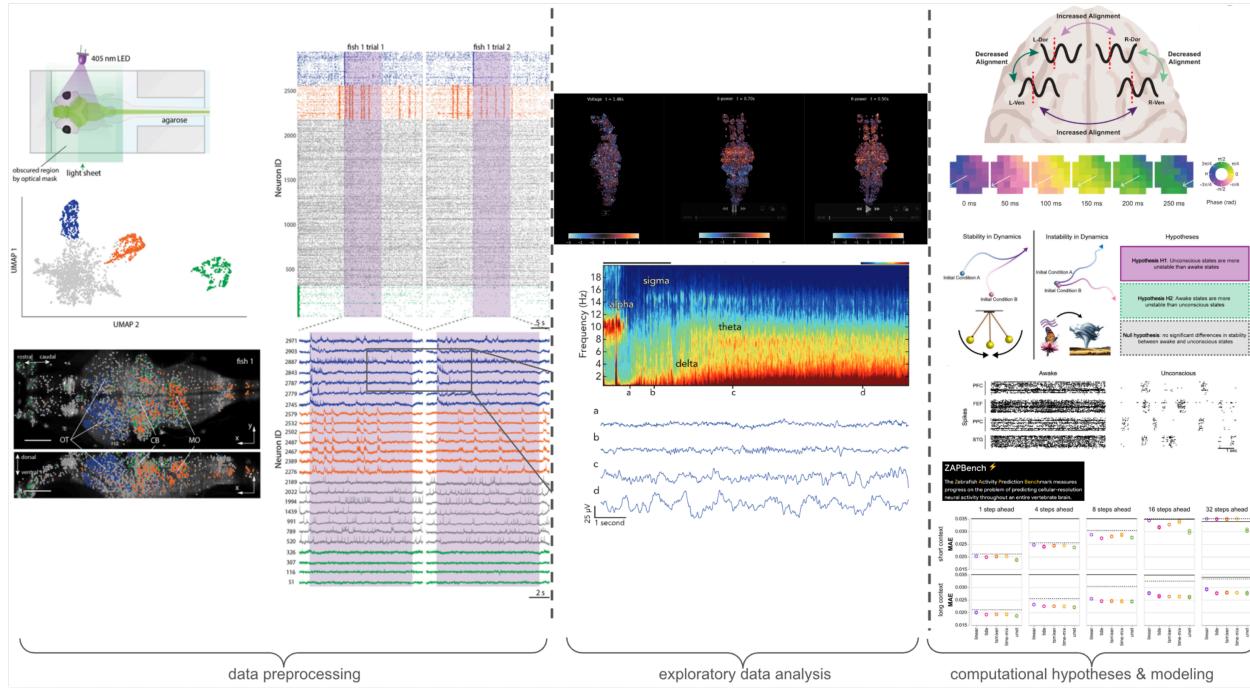


Figure 3. Multiscale analysis and modeling pipeline for whole-brain voltage imaging of ketamine-induced brain state transitions in larval zebrafish. This figure outlines the proposed computational pipeline for data processing, signal analysis, and neural predictability modeling.

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., 2025) 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 Lueckmann et al. (2025).

Data Preprocessing

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 supporting both ai

Oscillatory and Connectivity Analysis

Methods for Aim 1: Oscillatory dynamics and functional connectivity

Spectral Characterization: We will apply multitaper spectral analysis (Prerau et al., 2017) to compute robust power spectral densities across 0.1–50 Hz for both single neurons and population signals. Time-resolved spectrograms will reveal dynamic changes in oscillatory content during state transitions. We will quantify power in standard frequency bands and assess cross-frequency coupling using phase-amplitude coupling metrics to address Sub-Aims 1.1 and 1.2.

Functional Connectivity Analysis: Phase-locking values and coherence will quantify inter-regional synchronization, testing hypotheses about ketamine's effects on brain-wide connectivity patterns (Bardon et al., 2025). We will create connectivity matrices comparing baseline vs. ketamine conditions and develop metrics to distinguish local vs. long-range

connectivity changes (Sub-Aim 1.3). Machine learning approaches will identify which oscillatory features best predict behavioral state (Sub-Aim 1.4).

Dynamical Systems and Predictive Modeling

Methods for Aim 2: Network stability and state transitions

State-Space Analysis: Dimensionality reduction techniques (PCA, t-SNE, UMAP) will visualize brain state trajectories during drug exposure and recovery (Sub-Aim 2.1). We will quantify state space geometry, transition velocities, and the separability of conscious vs. unconscious brain states.

Predictive Modeling and Stability Assessment: Neural network models (following ZAPBench benchmarks) will assess the predictability of brain dynamics (Lueckmann et al., 2025; Eisen et al., 2024). We will train LSTM and transformer models to forecast future brain activity, with prediction accuracy serving as a proxy for dynamical stability (Sub-Aim 2.2). Analysis of transition kinetics and regional lead-lag relationships will characterize the temporal dynamics of consciousness changes (Sub-Aim 2.3). Comparative analysis against theoretical predictions will test competing theories of anesthetic action (Sub-Aim 2.4).

Expected Outcomes and Integration

Results will directly address our stated hypotheses and provide complementary insights into ketamine's neural mechanisms. We anticipate **first-of-their-kind observations of single-cell voltage dynamics during anesthetic states**, revealing both the patterns of neural change and the underlying dynamical principles.

Specifically, we expect:

- **Aim 1 outcomes:** Clear oscillatory biomarkers distinguishing conscious from unconscious states, including enhanced delta/theta power, reduced alpha/beta activity, altered cross-frequency coupling, and paradoxical long-range synchrony increases. These patterns should correlate strongly with behavioral responsiveness, providing neural signatures of ketamine-induced unconsciousness.
- **Aim 2 outcomes:** Evidence that ketamine destabilizes brain networks despite creating apparent oscillatory order. State-space analysis should reveal discrete unconscious brain states, predictive modeling should show reduced forecast accuracy under ketamine, and transition analysis should identify leading brain regions and critical dynamical changes during loss of consciousness.

Integration of findings will establish how ketamine's unique oscillatory signatures (Aim 1) relate to fundamental changes in network stability and brain state organization (Aim 2).

This dual perspective will reveal whether ketamine's distinctive neural effects result from specific oscillatory reconfigurations, dynamical destabilization, or both mechanisms working together. The combined results will advance theories of anesthetic action while providing practical biomarkers for clinical monitoring.

Contingency Plan

We have developed comprehensive alternative strategies to address potential experimental challenges:

Alternative Drug Delivery Approaches

If the modified ZOSS system proves unreliable, we will implement 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 with pre-warmed solutions and gentle exchange techniques.

Alternative Imaging Modalities

If voltage imaging encounters difficulties (low signal amplitude, excessive phototoxicity), we will pivot to calcium indicators as backup. **Transgenic larvae expressing pan-neuronal GCaMP6f can be imaged with the same light-sheet microscope** using 488 nm excitation. While calcium signals are slower, they would still capture overall activity changes and oscillatory dynamics during ketamine exposure.

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. This staged approach would ensure our protocols work robustly before applying the most advanced techniques.

Behavioral Readout Alternatives

If tail movement causes prohibitive motion artifacts, 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.

Drug Dosing Adjustments

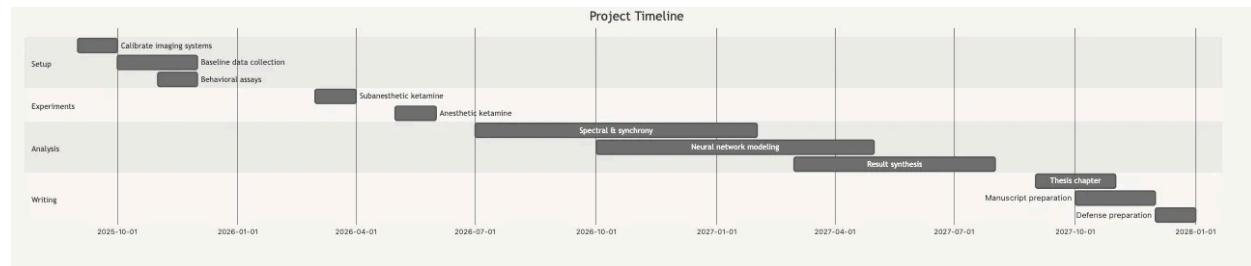
If our initial ketamine concentrations don't produce expected behavioral differences, we will adjust accordingly. **For example, if 0.2% fails to induce measurable effects, we will incrementally increase to 0.3-0.4% until partial loss of responsiveness is observed.** All dosing adjustments will be guided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

Technical and Analytical Robustness

If initial analysis approaches fail to reveal clear differences between conditions, we will implement more sophisticated time-frequency analysis methods, expand sample sizes for adequate statistical power, and include positive control experiments with well-characterized anesthetics to establish baseline expectations for our analytical approaches.

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.



Timeline Gantt Chart

Detailed Timeline with Specific 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 ≈ 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

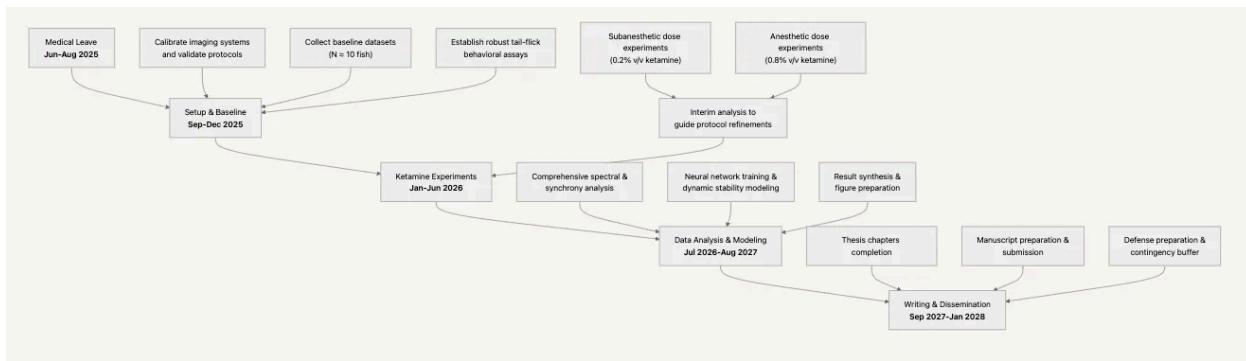
PhD thesis chapter writing integrating background, methods, and results. Manuscript preparation for publication focusing on network synchrony changes. Thesis defense scheduled for mid-2027.

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.



Milestone Flowchart

Significance

This project represents multiple converging innovations that will advance both fundamental neuroscience and clinical anesthesia:

Methodological Novelty

This project will yield unprecedented insights into anesthetic-induced unconsciousness by providing the **first single-neuron-resolution voltage imaging of anesthetic state transitions** in any vertebrate 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.

Conceptual Advances

The conceptual significance extends beyond technical achievement. Aim 1 will establish the first comprehensive map of oscillatory and connectivity changes during ketamine-induced unconsciousness, testing fundamental theories about how anesthetics alter information integration in neural networks. Aim 2 will provide the first direct test of competing dynamical theories of consciousness, determining whether unconsciousness involves network destabilization or alternative mechanisms. Together, these aims will establish cellular-level foundations for macroscopic EEG phenomena and integrate pharmacology, systems neuroscience, and consciousness research through two complementary but distinct scientific perspectives.

Clinical Relevance

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.

Broader Impact

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. This work will establish larval zebrafish as a premier model for consciousness research while demonstrating the power of combining cutting-edge imaging with modern computational approaches.

The ability to observe cellular-level mechanisms underlying EEG patterns will bridge scales from molecular pharmacology to clinical electrophysiology. The successful completion of this research will provide unprecedented insight into how pharmacological agents alter the fundamental electrical dynamics of brain networks, bridging the gap between molecular mechanisms and conscious experience with direct relevance to millions of patients undergoing anesthesia worldwide.

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1 | Overall experimental strategy

Step	What you plan	Why it is promising	Where the risk sits
Mount fish (head in agar, tail free)	Keeps brain optically stable while preserving	Widely used; matches setups in Herrera <i>et al.</i>	Tail tracking can be obscured by drug-induced muscle tone changes. Pilot whether tail

	a behavioral read-out (tail flick).	2021 and Swain's ZOSS platform.	contrast remains high after >5 min ketamine.
Bath-immersion dosing (0.2 % vs 0.8 % v/v)	Simple and repeatable; avoids perfusion plumbing that might vibrate scope.	Good first pass—immersion works for ketamine in zebrafish at 0.2–0.8 mM (Duque <i>et al.</i> 2025).	Onset / wash-out times are slow (tens of s). If you need sharp state boundaries for dynamical analyses, consider ZOSS gentle perfusion at ~0.3 mL min ⁻¹ so the whole dish exchanges in <60 s.
Voltage imaging (Positron2, 200 Hz LS scope)	Gives the millisecond-scale data that were missing from calcium-only papers.	You already have proof-of-principle with Wang <i>et al.</i> 2023.	Photobleaching: at 200 Hz Wang lost ~20 % ΔF/F in 35 s. Either restrict each recording block to ≤60 s or adopt JF585 + 4× slower frame rate (Swain thesis, Ch 3).
Behavioral probe = periodic NIR heat pulse	Gives binary “tail-flick/no-flick”—a zebrafish LOC proxy.	Clean stimulus; you decouple it from optical paths.	Verify that the brief heat pulses themselves don't confound voltage traces (fast temperature transients can change GEVI brightness).

2 | Dosing & timing suggestions

Block	Time (min)	Solution (example)	Rationale
Baseline-1	0–1 min	E3	Establish baseline neural + tail activity.
Ketamine ramp	1–3 min	Linear switch to 0.2 % (sub-anesthetic)	Treat “ramp” frames as transition state; exclude from stability analysis.
Sub-anesth. steady	3–5 min	Hold 0.2 %	Look for dissociative γ increase (human iEEG: Tian <i>et al.</i> 2023)
Anesthetic ramp	5–7 min	Switch to 0.8 %	Should push into LOC (tail flick disappears).
Deep steadystate	7–9 min	Hold 0.8 %	Expect large-scale destabilization (compare to propofol effects in Eisen <i>et al.</i> 2024)
Wash-out	9–11 min	Back to E3	Record recovery trajectories.

Two blocks \times ~2 min each at 200 Hz is ~24 k volumes—still safe before serious bleaching if you interleave 20-s dark gaps.

3 | Spectral-analysis vision

3.1 Why “EEG bands” at single-cell scale can be interesting

- Alpha/delta/burst-suppression structure is a **network** phenomenon (Adam *et al.* 2023) .
- Mapping which **cells or regions** change band power during ketamine gives spatial clues about circuit entry points (prefrontal vs posterior medial in mammals; tectum vs cerebellum in fish).

3.2 Pipeline sketch

```
Volumetric movie (Nx, Ny, Nz, T)
| VolPy → ~16 000 ΔF/F traces (cells × time)
PCA (or non-negative matrix factorization)
| keep first 50 comps (explain >70 % var)
Multitaper spectrogram on each comp
| (half-overlap 256-ms windows, ±3 Slepian tapers)
Select canonical bands
| δ(0–4) θ(4–8) α(8–12) β(13–25) γ(40–80)
Back-project band-power time-series to cells
| (solve linear system with comp loadings)
Result → Power(cell, band, time)
```

3.3 Visualizations that scale

- **Brain-render movies:** Color code cells by *dominant* band at each frame (similar to your BioRender mock-up).
- **UMAP/T-SNE** in band-power space (cells \times bands). Clusters that expand/contract between drug states flag subnetworks.
- **Stability surfaces:** Use DeLASE (Eisen *et al.*) on low-dim latent trajectories to quantify destabilization (expect positive eigenvalues under 0.8 %).

- **Cross-spectral flow:** Compute phase-locking value (PLV) between ROIs; Bardon *et al.* 2025 predict loss of nearby-ROI synchrony but *increased* homotopic synchrony under anesthetics .
-

4 | Controls & replicates

- **Temperature control** – ketamine dosing changes water heat capacity negligibly, but your NIR pulses heat locally; insert a thermistor bead near fish in pilot runs.
 - **JF585 GEVI backup** – if bleaching prevents 2-min blocks, switch to slower volume rate (50–80 Hz) with the red GEVI.
 - **GCaMP contingency** – as you wrote, keeps the project alive if voltage SNR is too low in 0.8 % ketamine.
 - **Biological N ≥ 6 fish / dose condition** to capture inter-animal variability (Duque *et al.* found notable spread at 0.5 mM).
-

5 | Analysis checkpoints

1. **Can we decode tail-flick vs no-flick from 50-ms voltage windows?**
2. **Does band-power map shift posterior-medially at LOC, mirroring human PM cortex 3-Hz oscillation (Tian *et al.*)?**
3. **Does DeLASE eigenvalue λ_{max} jump above zero only in the deep block?**
4. **Do PLV curves replicate Bardon's "within-hemi down / across-hemi up" pattern—but now across optic tectum pairs?**

If 1–2 are true in early pilots you already have a compelling *story*; 3–4 give mechanistic depth.

3–5-Minute Lightning Talk – Proposed Ketamine Brain-State Experiments in Larval Zebrafish

(~6 slides, ≈40–45 sec each)

#	Slide / Section	Key bullets & talking points	Visual cue ideas	Time
1	Hook & Goal	• “What happens to an entire vertebrate brain—neuron-by-	Catchy image of light-sheet fish	0:00–0:40

		<p>neuron—when ketamine drives it into, and back out of, unconsciousness?• Our goal: map the millisecond-scale voltage dynamics of all ~16 k zebrafish neurons while we toggle between control, sub-anesthetic and anesthetic ketamine baths.</p>	brain + ketamine vial	
2	Why zebrafish & why ketamine?	<ul style="list-style-type: none"> Larval zebrafish are transparent and let us record whole-brain voltage at 200 Hz .• Ketamine produces unique dissociative & antidepressant states and distinctive oscillations in human & primate cortex .• No study yet captures single-cell voltage during an anesthetic brain-state transition. 	2-panel: fish brain vs human EEG patterns	0:40–1:20
3	Technical edge	<ul style="list-style-type: none"> High-speed light-sheet microscope (Boyden lab) → 200 vol/s across entire brain .• ZOSS fluidics platform repurposed for rapid, software-timed bath exchange & heat-pulse reflex test .• Head embedded / tail free set-up (Herrera- 	Schematic of microscope + manifold cartoon	1:20–2:00

		style but with voltage, not calcium) .		
4	Experimental paradigm	<p>Baseline →</p> <p>Ketamine →</p> <p>Washout (see graph).• Record brain voltage + tail camera continuously.</p> <p>Deliver brief NIR heat pulses every 30 s → loss or return of tail flick = behavioral read-out of LOC.</p> <p>Two runs per fish: 0.2 % (sub-anesthetic) & 0.8 % (anesthetic).</p>	<p>Timeline graphic (blue = E3, brown = ketamine, red = heat pulses)</p>	2:00–2:40
5	Analysis roadmap	<ul style="list-style-type: none"> Multitaper spectrograms of each neuron → band-specific power maps through time (delta-gamma). Network metrics: phase alignment (Bardon 2025) and stability (DeLASE; Eisen 2024) to test Brown-Miller LOC theories. Compare spatial distribution of oscillations & stability before/after LOC. 	<p>Animated brain slices colored by band power</p>	2:40–3:20
6	Impact & next steps	<ul style="list-style-type: none"> First single-cell view of anesthetic state transitions; bridges human EEG markers to cellular circuits. Creates an open dataset to 	<p>“Roadmap” arrow; invitation to collaborate</p>	3:20–4:00

benchmark whole-brain forecasting
(leveraging ZAPBench ethos) •
Sets stage for screening other drugs
(stimulants, propofol)
and testing consciousness theories.