

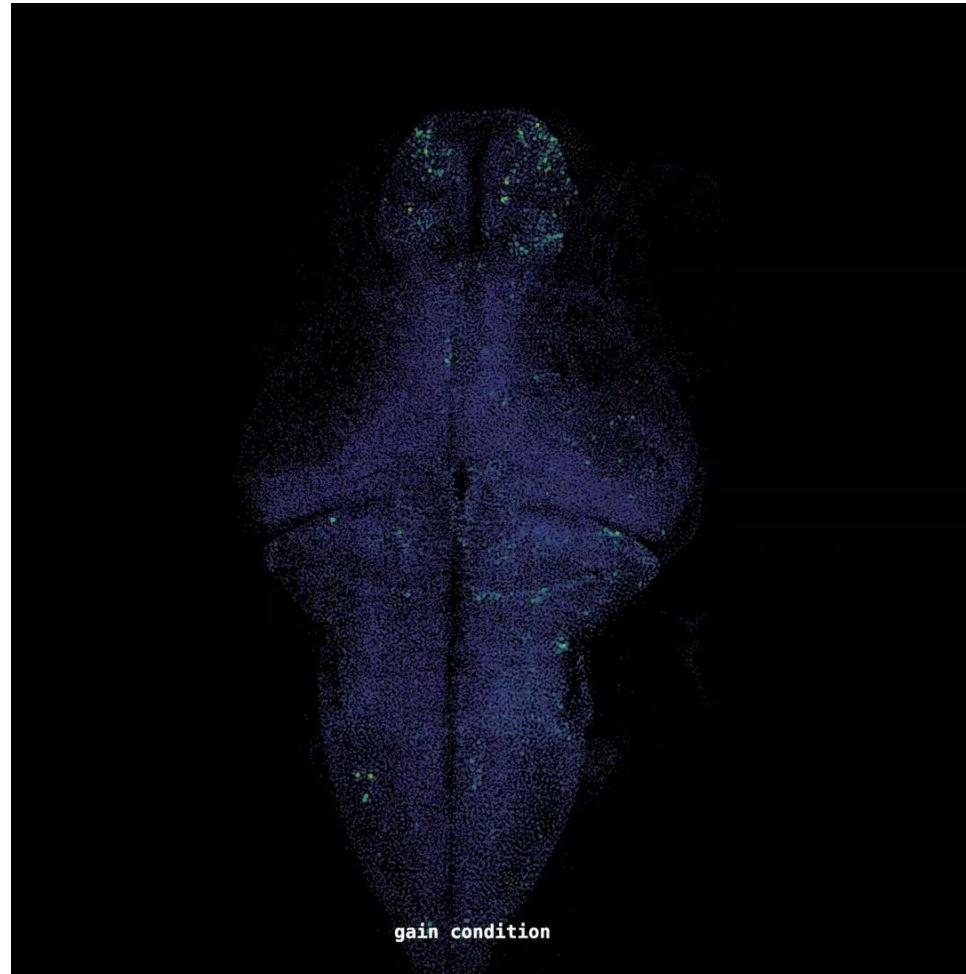
# Whole-Brain Voltage Imaging of Ketamine-Induced Brain State Transitions in Larval Zebrafish

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**Thesis Proposal**  
May 30, 2025

# Motivation

What happens to an entire vertebrate brain—neuron-by-neuron—when ketamine drives it into, and back out of, unconsciousness?

**Our goal:** map the millisecond-scale voltage dynamics of ~10-20 k neurons across the zebrafish brain while we toggle between control, sub-anesthetic and anesthetic ketamine baths.



# Background

**General anesthesia** represents a controlled, reversible loss of consciousness that provides a unique window into the neural correlates of consciousness.

**Ketamine** is a dissociative anesthetic that has both anesthetic and psychoactive properties. Intravenous induction doses of ketamine result in a rapid loss of consciousness appropriate for general anesthesia. In humans, at subanesthetic doses, ketamine produces a dissociative state, which includes gaps in memory, out-of-body experiences, and altered sensory perception.

**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*.

The larval zebrafish, can provide a simple, robust, quantitative model of drug-induced behaviors that should be easily reproduced in other laboratories.

# WHAT IS KETAMINE?

Ketamine is a dissociative anesthetic that has some hallucinogenic effects. It distorts perceptions of sight and sound and makes the user feel disconnected and not in control. It is an injectable, short-acting anesthetic for use in humans and animals. It is referred to as a “dissociative anesthetic” because it makes patients feel detached from their pain and environment.

Ketamine can induce a state of sedation (feeling calm and relaxed), immobility, relief from pain, and amnesia (no memory of events while under the influence of the drug).

It is abused for its ability to produce dissociative sensations and hallucinations. Ketamine has also been used to facilitate sexual assault.

Spectral power changes	Possible neural circuit mechanism
Ketamine increases gamma power, propofol decreases gamma power in prefrontal regions of the brain	<p>Antagonistic effects:</p> <p>The diagram illustrates the antagonistic effects of Ketamine and Propofol on neurotransmitter receptors. On the left, Ketamine is shown blocking NMDAR (green), which are coupled to GABA release. On the right, Propofol is shown blocking GABAAR (blue), which are coupled to Glutamate release. Red minus signs indicate inhibition.</p>
Ketamine and propofol <b>both</b> decrease alpha power in posterior sensory cortical regions	<p>Additive effects:</p> <p>The diagram illustrates additive effects on HCN1 channels. Ketamine (red minus sign) and Propofol (red minus sign) both inhibit HCN1 channels, leading to a reduction of <math>I_h</math>. A purple shaded area highlights the posterior sensory cortical regions.</p>
Ketamine and propofol <b>both</b> increase 3-4Hz power in posterior cingulate and isthmus cingulate cortex	<p>Additive effects:</p> <p>The diagram illustrates additive effects on HCN1 and NMDAR/GABAAR. Ketamine (red plus sign) activates HCN1 channels, while Propofol (red plus sign) activates GABAAR. Both lead to an increase of <math>I_h</math>. Red circles with Ca<sup>2+</sup> indicate increased calcium levels. A purple shaded area highlights the posterior cingulate and isthmus cingulate cortex.</p>

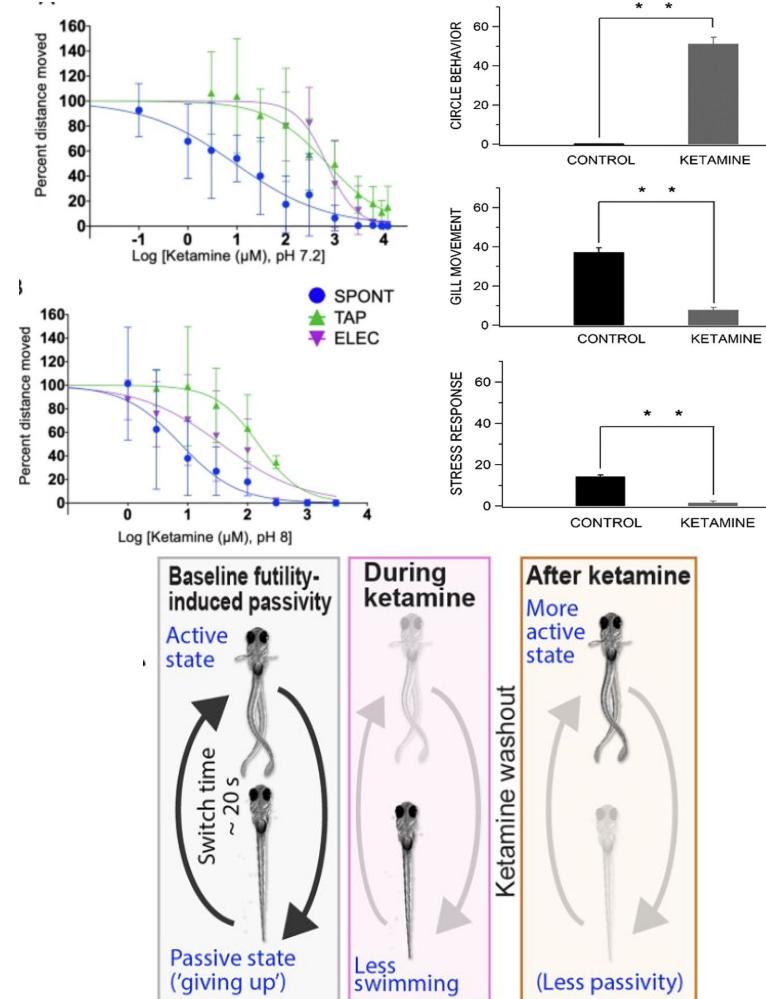


Zanos, P., & Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. Molecular Psychiatry, 23(4), 801–811. <https://doi.org/10.1038/mp.2017.255>

<https://www.dea.gov/sites/default/files/2020-06/Ketamine-2020.pdf>

# Zebrafish behavior and ketamine

- **Larval zebrafish** respond to **anesthetics** with loss loss of spontaneous movement at clinically relevant concentrations similar to mammals, making them a **highly tractable vertebrate model** for the of detection neural substrates involved in the transition from consciousness to **unconsciousness**.
- Subanesthetic doses of ketamine produced a variety of abnormal behaviors in zebrafish that were qualitatively analogous to those previously measured in humans and rodents treated with drugs that produce transient psychosis.
- Brief ketamine exposure causes long-term suppression of futility-induced passivity in larval zebrafish, reversing the “giving-up” response that normally occurs when swimming fails to cause forward movement.



# High-speed remote-scanning light-sheet microscope

Wang, Z. et al. Imaging the voltage of neurons distributed across entire brains of larval zebrafish. bioRxiv 2023.

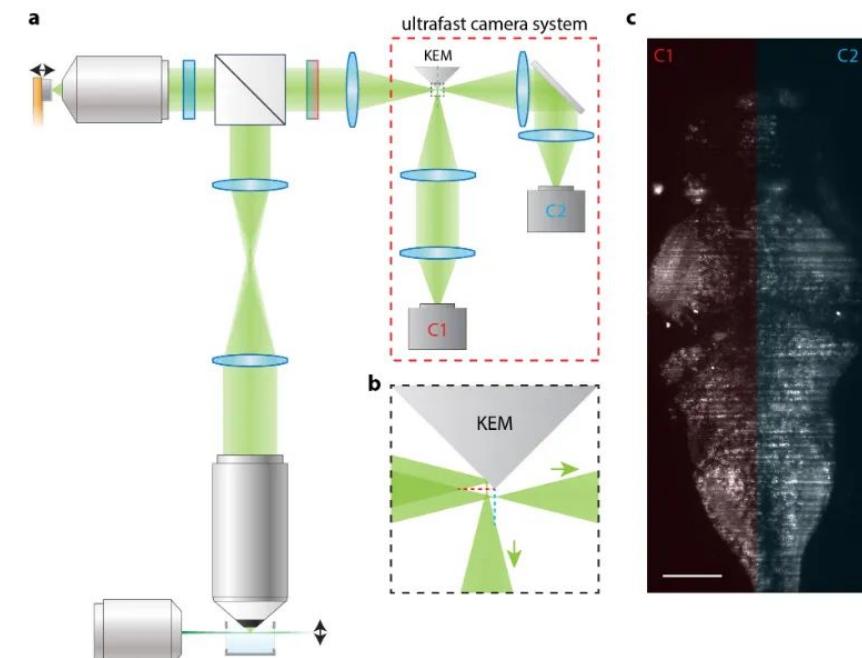
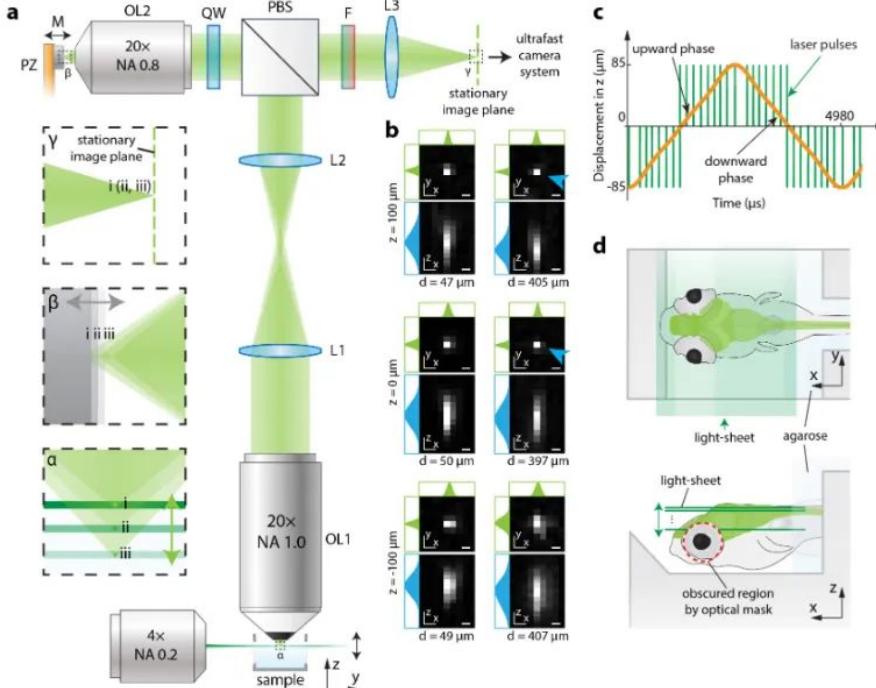


Figure 1. Remote-scanning light-sheet microscopy optimized for voltage imaging of neurons distributed across the entire larval zebrafish brain.

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

# Specific Aims

**Goal:** Capture whole-brain, single-neuron voltage dynamics in larval zebrafish during ketamine-induced state transitions.

**Aim 1:** Empirically map neural and behavioral (tail-flick) responses before, during (sub-anesthetic 0.2% v/v and anesthetic 0.8% v/v), and after ketamine bath application using high-speed light-sheet GEVI imaging.

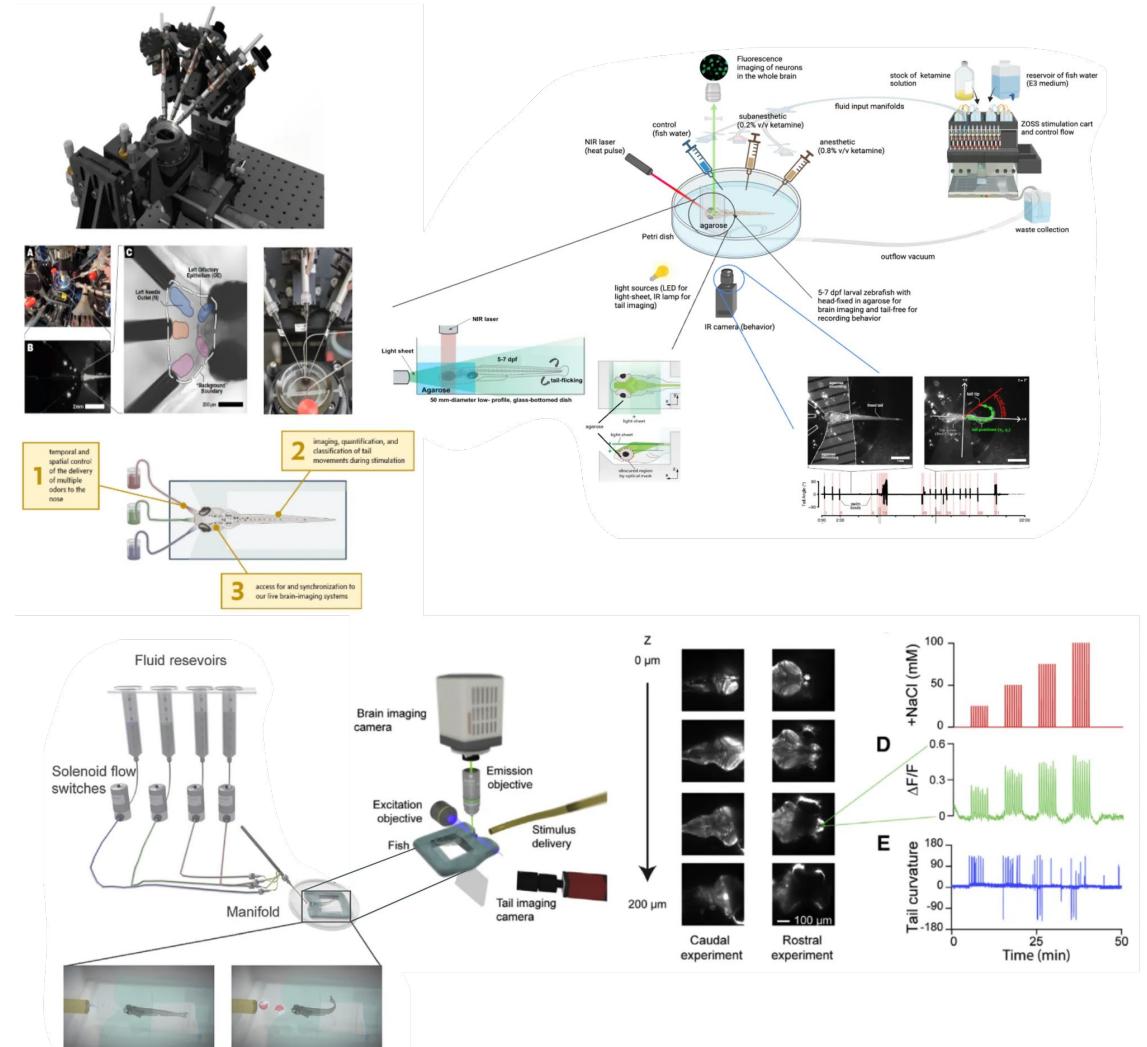
**Aim 2:** Analyze those data to extract spectral signatures (multitaper analysis), phase-locking/synchrony (PLV), low-dimensional brain-state trajectories, and dynamical predictability (neural-network forecasting).

## Aim 1

Empirically map neural and behavioral (tail-flick) responses before, during (sub-anesthetic 0.2% v/v and anesthetic 0.8% v/v), and after ketamine bath application using high-speed light-sheet GEVI imaging.

# Figure 1

**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.**



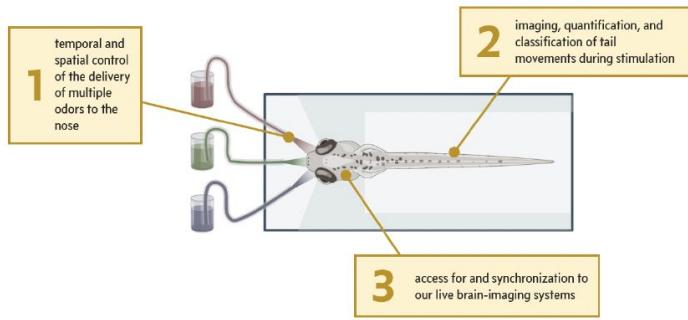


Figure 3.3: There are three primary design requirement for our olfactory stimulation platform.

Table 3.1: Our olfactory stimulation system fills a gap in existing stimulation technologies by enabling multi-directional, real-time stimulation with many odors simultaneous with whole brain imaging. Table of various reported zebrafish olfactory stimulation technologies/methods along

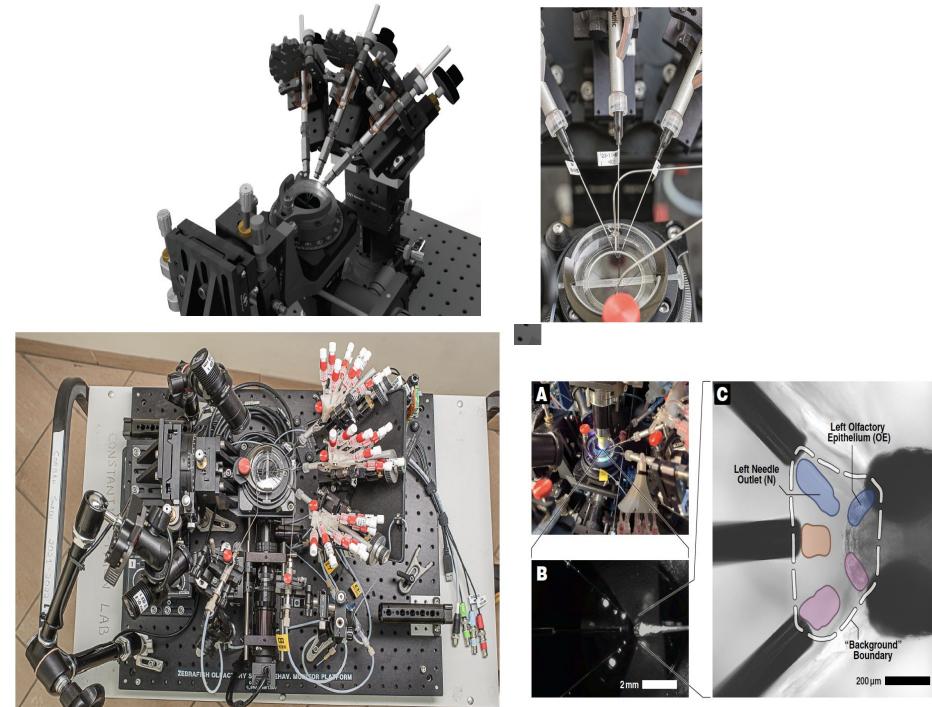
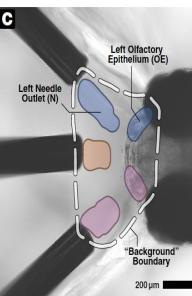
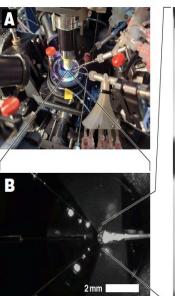
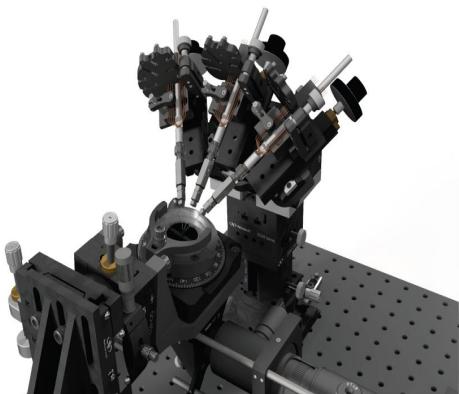
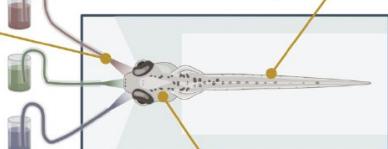


Figure 3.31: The olfactory stimulation platform enabled multi-directional olfactory stimulation and imaging of a zebrafish larva through the integration of many hardware components.

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

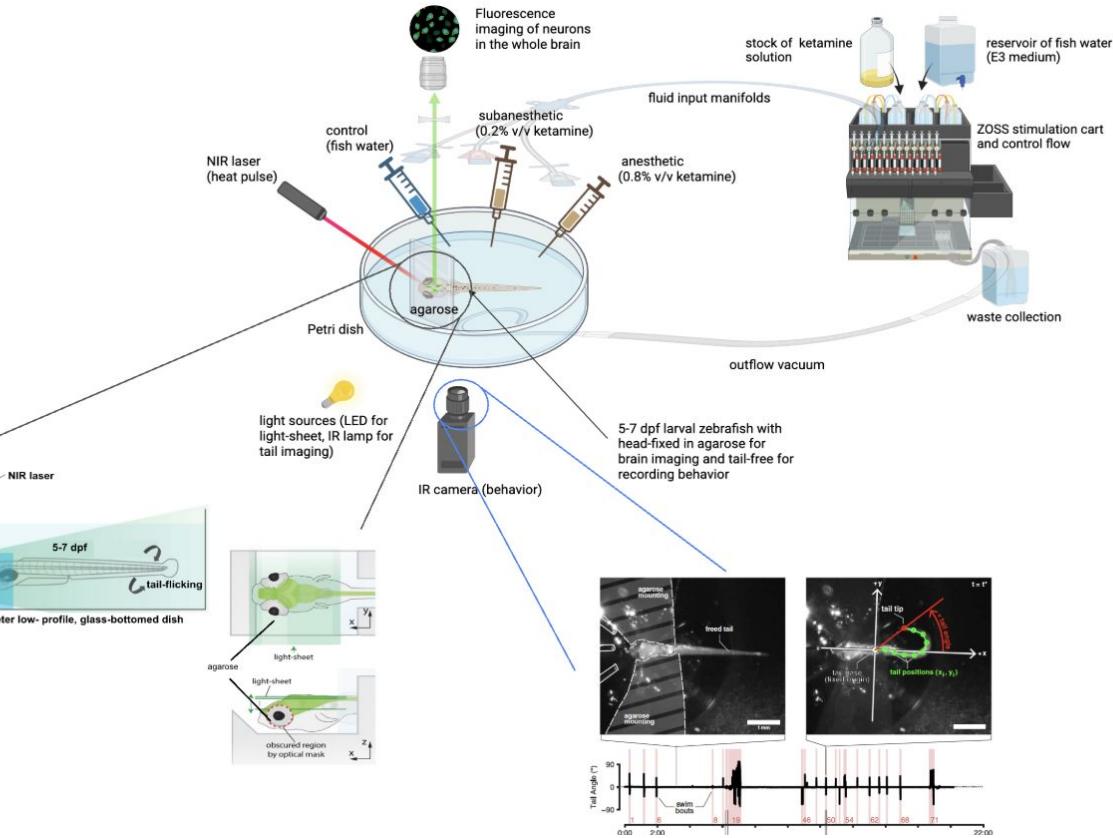


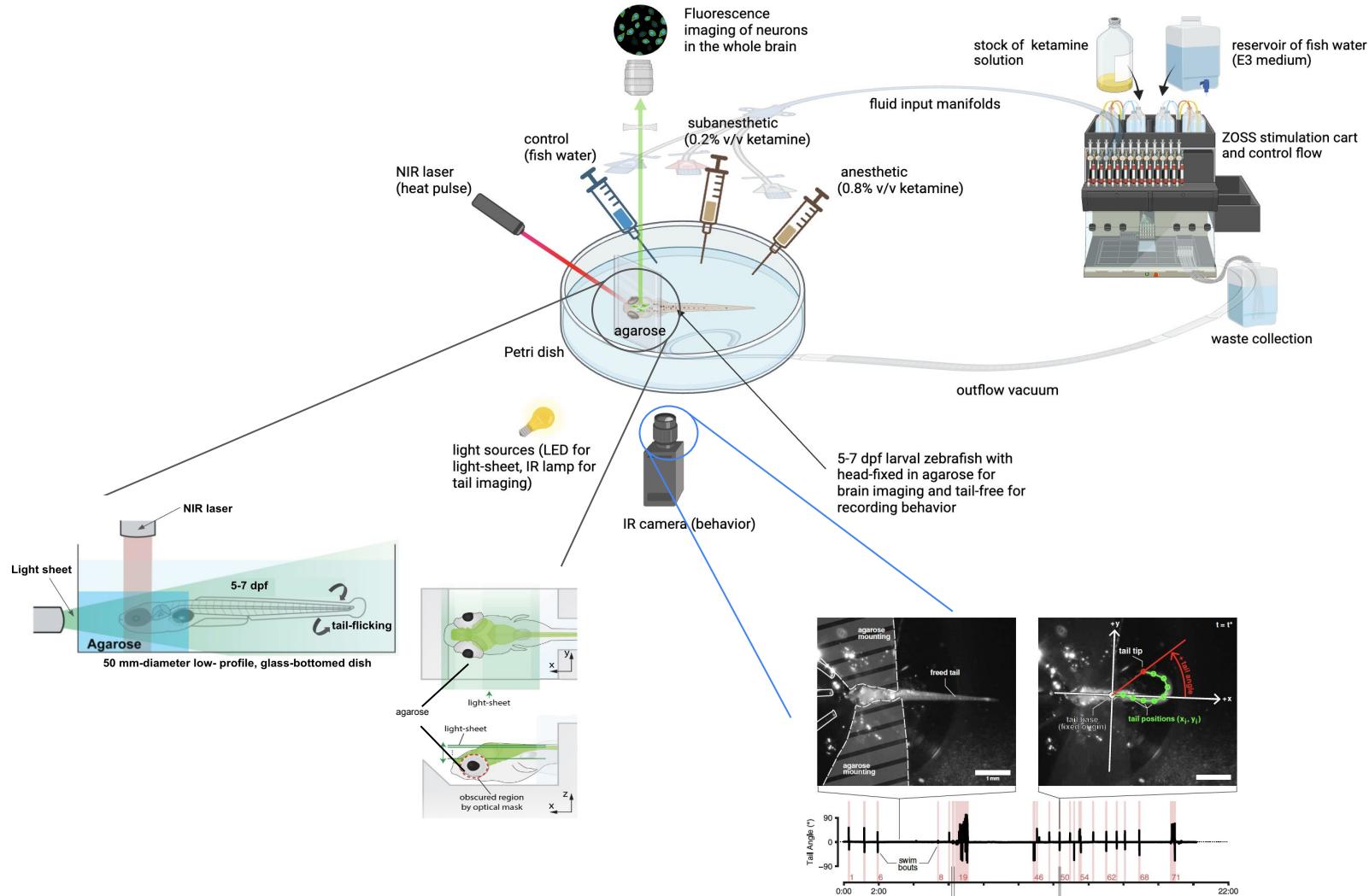
**2** imaging, quantification, and classification of tail movements during stimulation

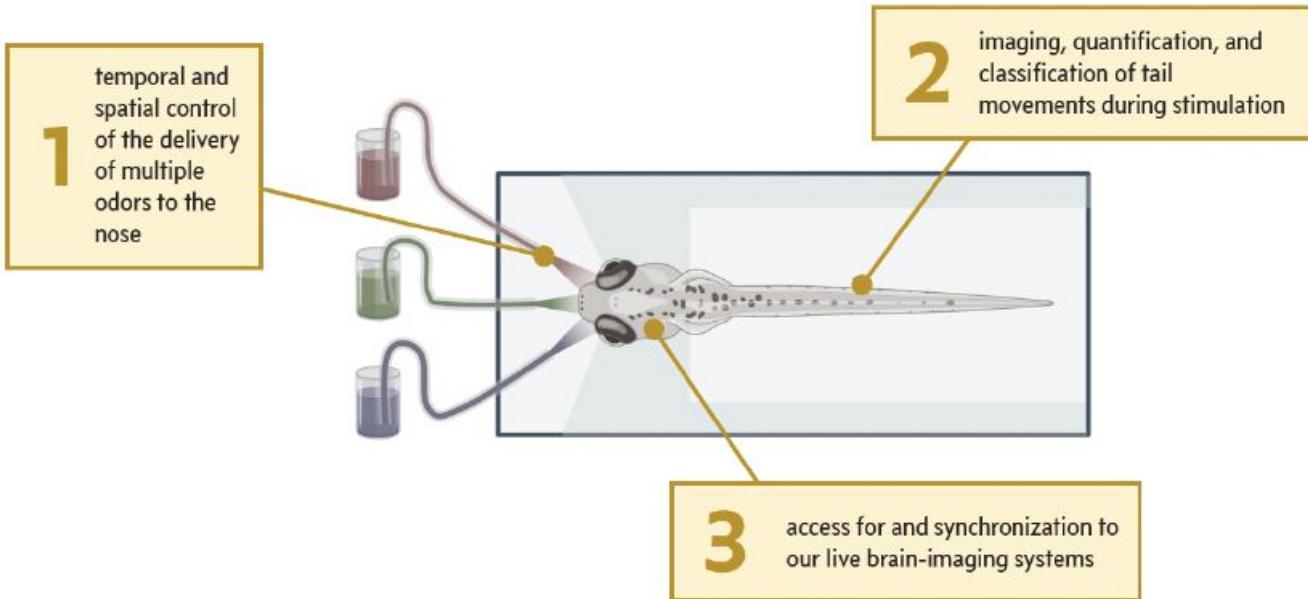


**1** temporal and spatial control of the delivery of multiple odors to the nose

**3** access for and synchronization to our live brain-imaging systems







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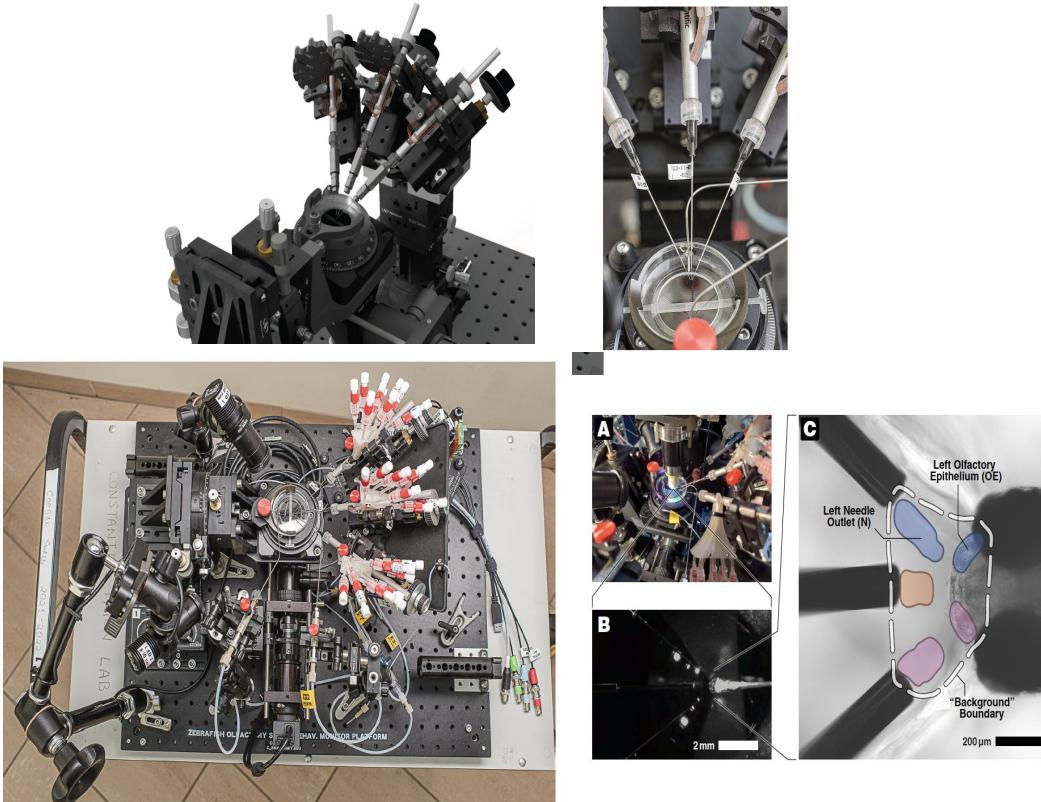


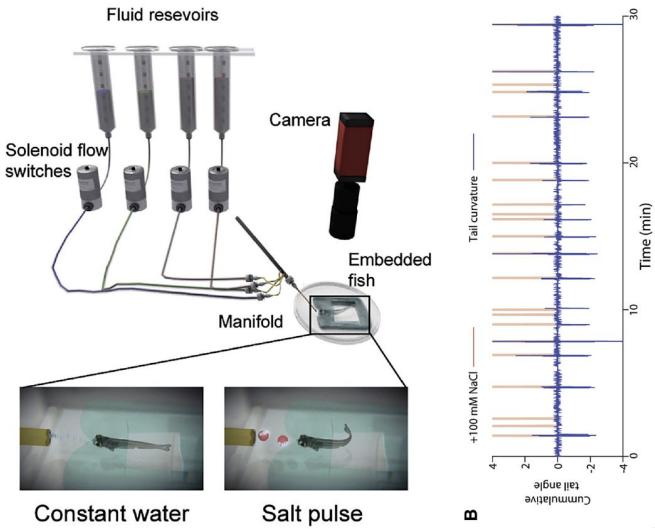
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# Experimental Design

- **Animal prep:** 5–8 dpf zebrafish expressing Voltron2/Positron2 under elavl3.
- **Imaging:** ~200 Hz volumetric remote-focus light-sheet system (Wang et al. 2023).
- **Drug delivery:** Modified ZOSS manifold for rapid bath exchanges between E3 medium and ketamine.
- **Behavioral assay:** NIR-laser-evoked tail-flick reflexes to probe responsiveness.

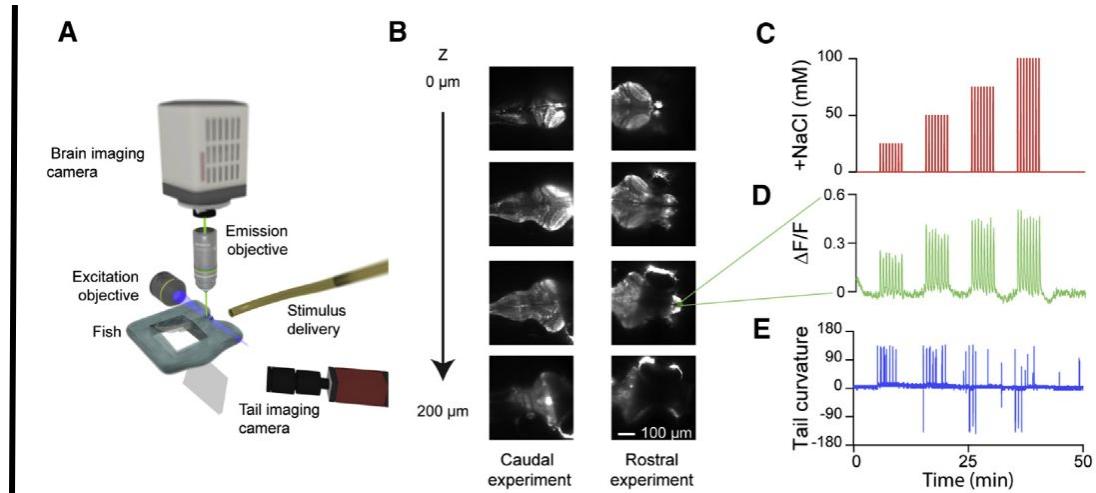
Our intended experimental setup is more like that depicted in Figure 2 and 3 from Herrera et al. (2021) Current Biology. Swain's ZOSS system has all the necessary hardware to be setup up in a more simplified configuration Herrera's.



**Figure 2. Head-immobilized Larvae Respond to Salt Concentration Increases**

(A) Schematic of preparation used to stimulate head-immobilized larvae.

(B) Sample data from an experiment where a larva is stimulated with 10-s pulses of 100 mM NaCl at random intervals.



**Figure 3. NaCl Levels Are Represented in the Olfactory System and Lateral Line**

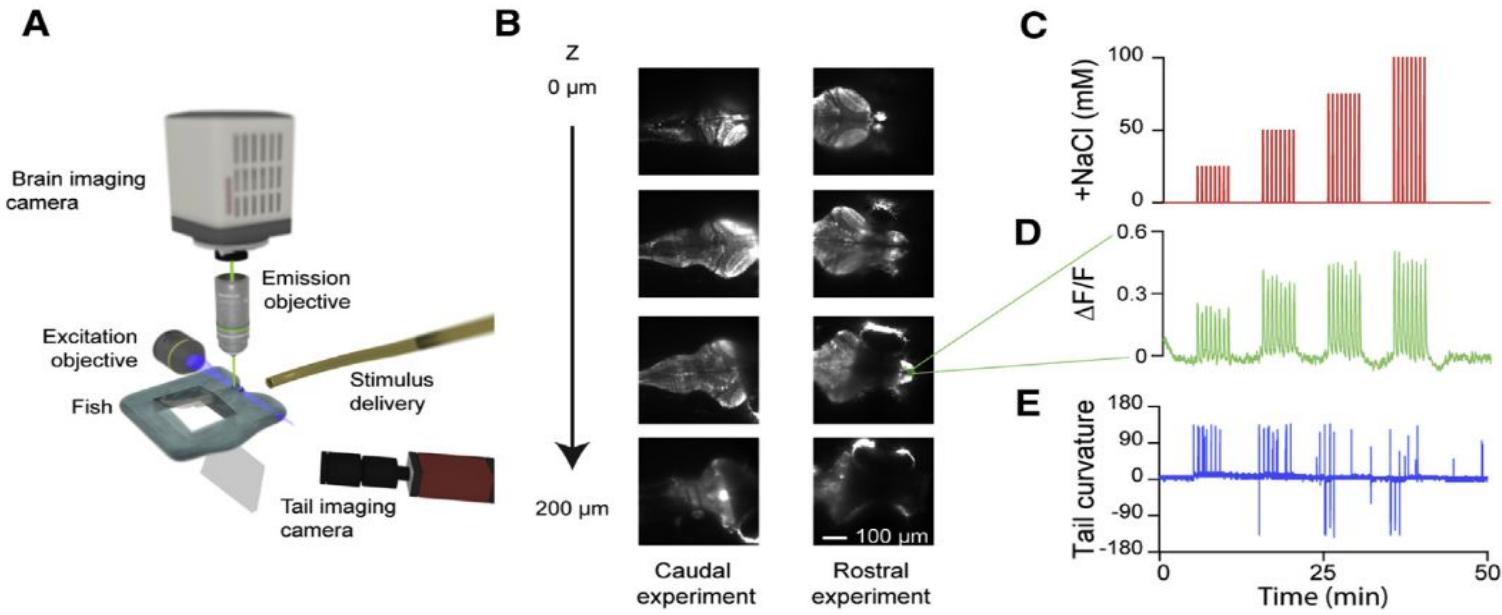
(A) Schematic of the light-sheet microscope.

(B) Sample z-slices taken within a stack of a *huc:GCaMP6S* transgenic larva. Stacks were collected at 1 Hz. Imaging experiments captured either the rostral 2/3<sup>rd</sup>s or caudal 2/3<sup>rd</sup>s of the fish.

(C) Arrangement of salt pulses during each experiment. Zebrafish experienced escalating concentrations of NaCl pulses in 5-min blocks.

(D) Example calcium signal from an activity unit in the olfactory bulb.

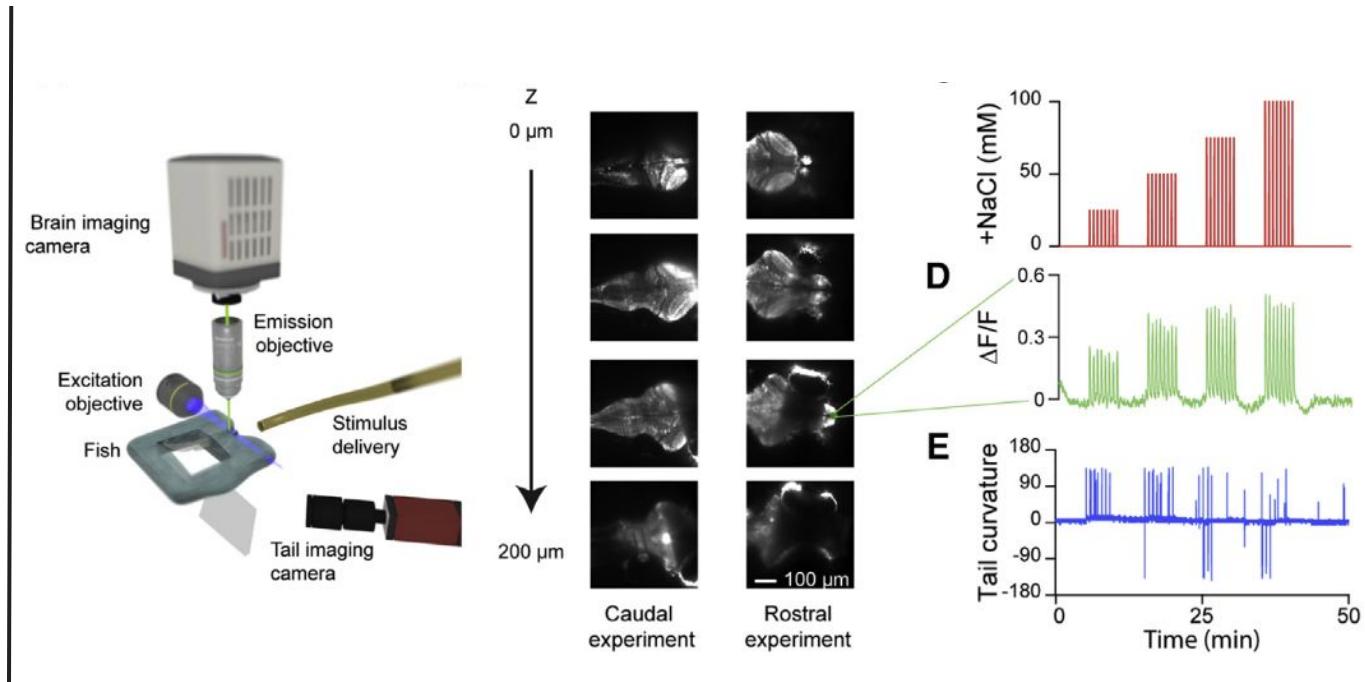
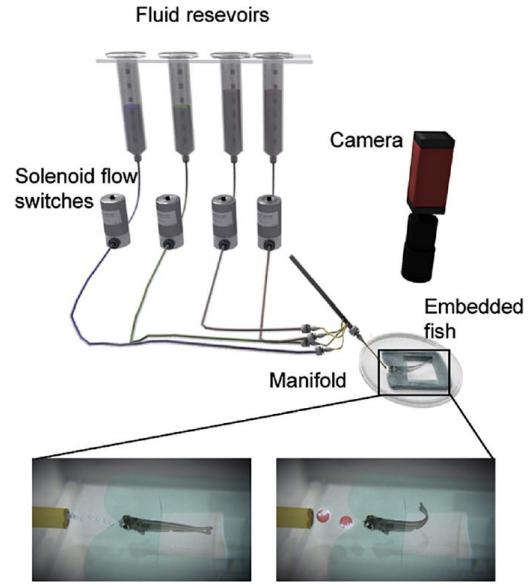
(E) Example tail curvature trace during imaging experiment.

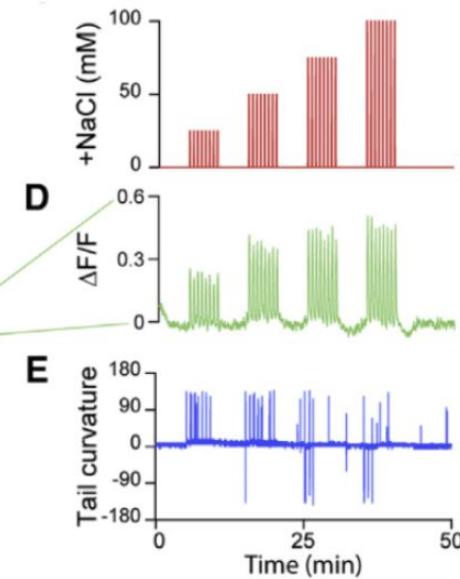
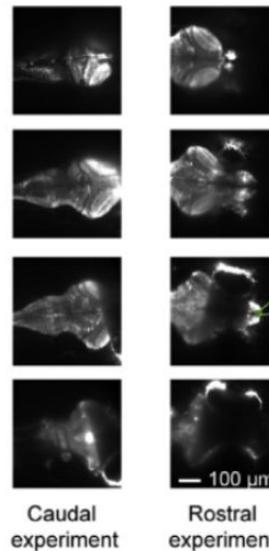
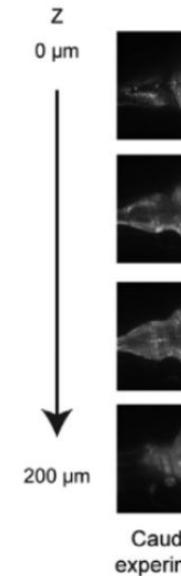
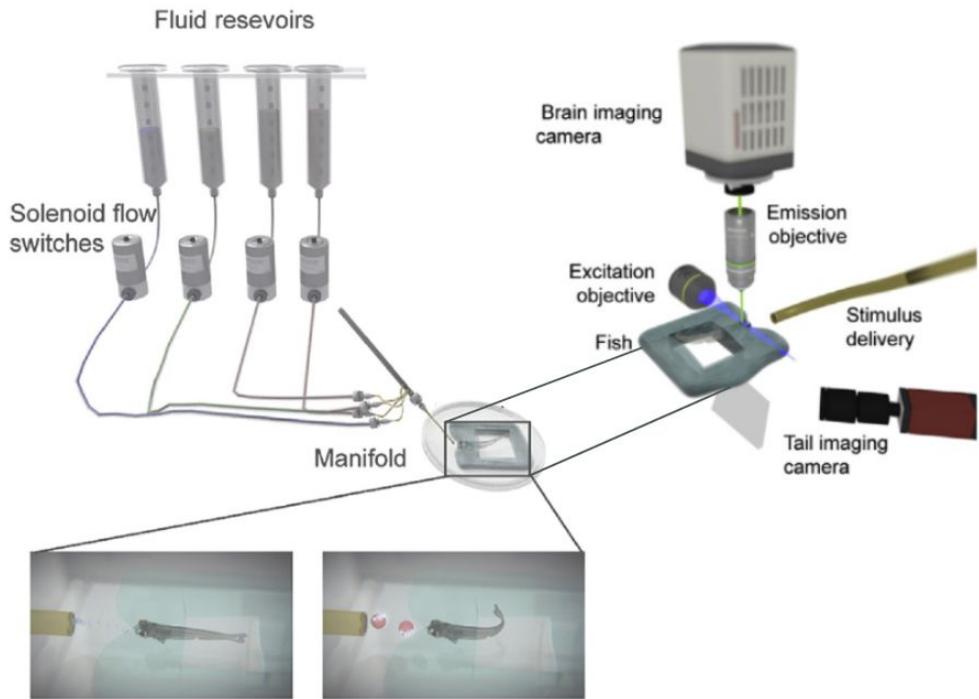


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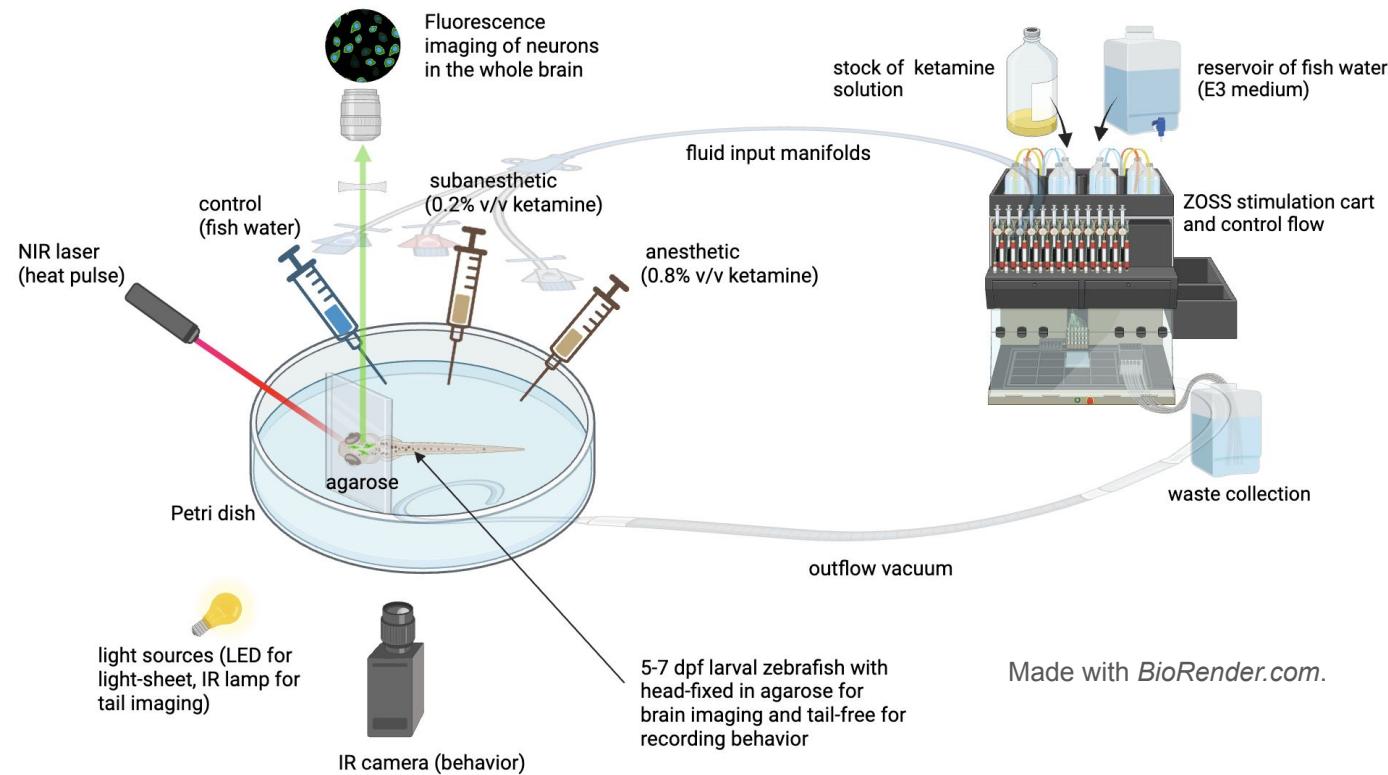
- (A) Schematic of the light-sheet microscope.
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Adapted from Herrera, K. J., Panier, T., Guggiana-Nilo, D., & Engert, F. (2021). Larval zebrafish use olfactory detection of sodium and chloride to avoid salt water. *Current Biology*: CB, 31(4), 782-793.e3.  
<https://doi.org/10.1016/j.cub.2020.11.051>

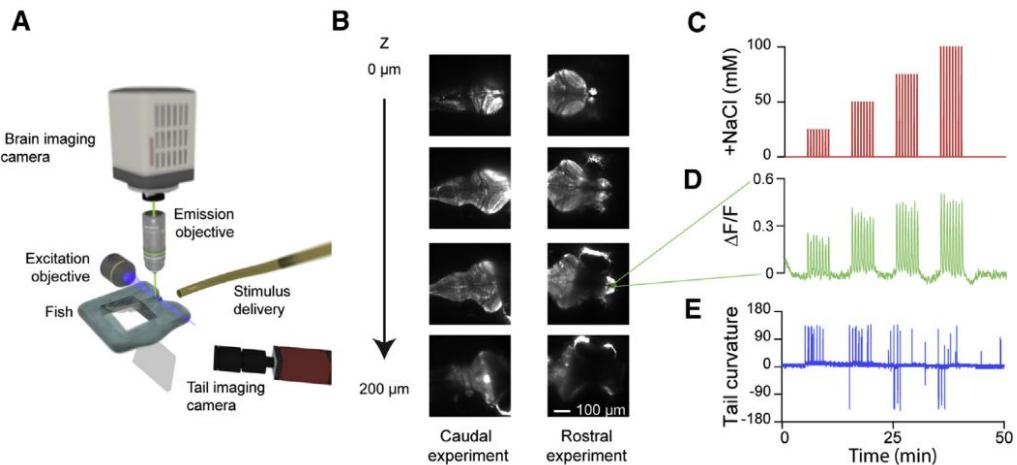
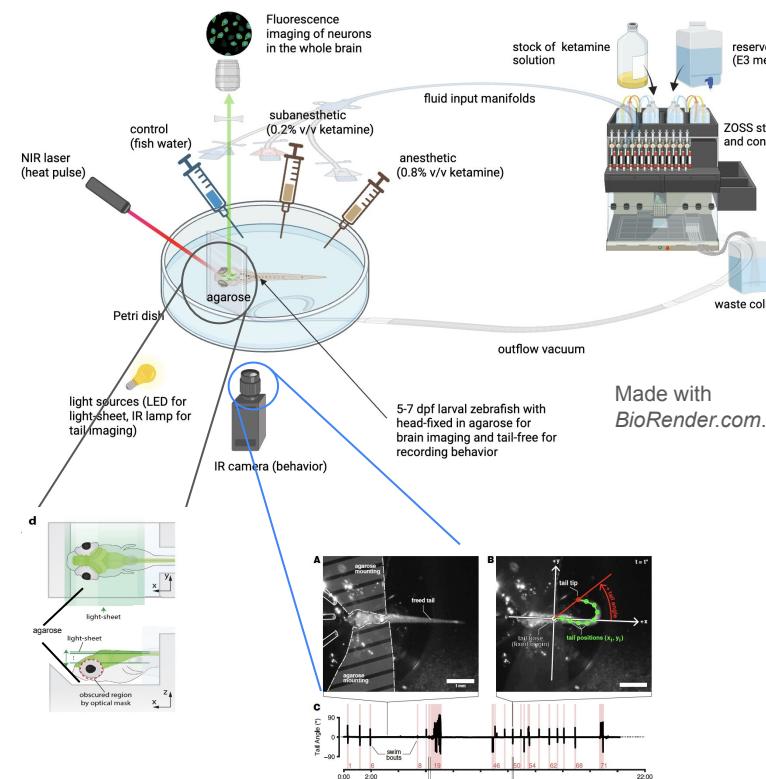




Mockup diagram of our intended modified usage of Swain's ZOSS equipment for delivering immersion volumes of the solutions for our experiment (E3 medium for the control/baseline and ketamine doses – subanesthetic or anesthetic – for the experimental condition.



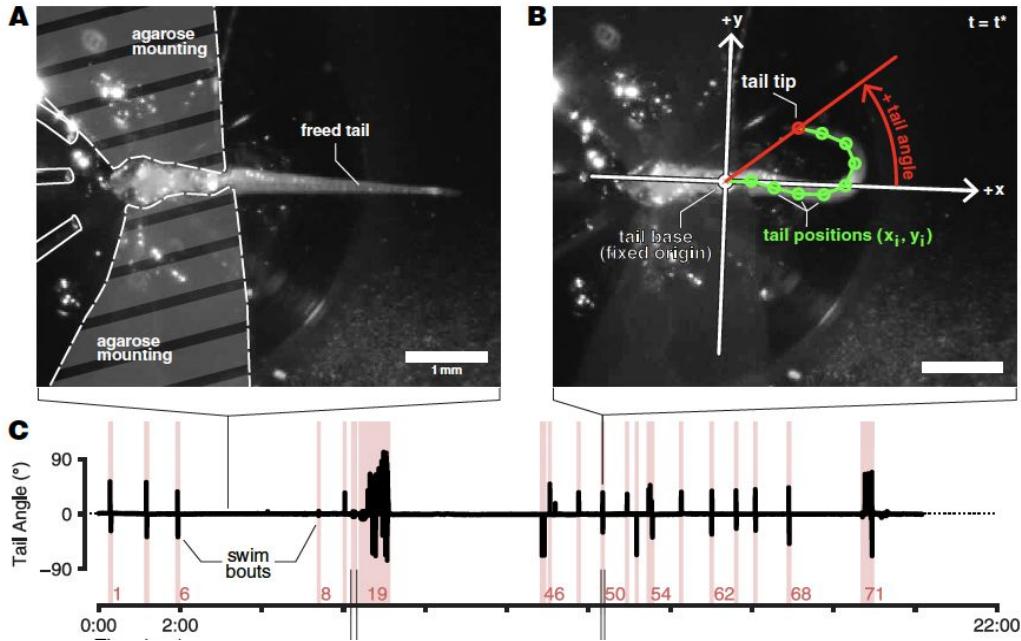
Our intended simplified adaptation of Swain's ZOSS for our experiment (*left*) is very much like the platform used by Herrera et al. (2021) Current Biology (*right*). Similarities are how we intend to perform volumetric light-sheet imaging of larval zebrafish head (embedded in agarose) simultaneously with tail imaging (free in solution). Differences are that we intend to do voltage rather than calcium imaging (but we may use GCaMP fish as a contingency plan), and rather than a salt pulse stimulus, we intend to full volume of the control or drug bath to immerse the fish without continuous perfusion, and we intend to deliver a periodic heat pulse stimulation to the head to test for behavioral state transitions like loss of consciousness during conditions.



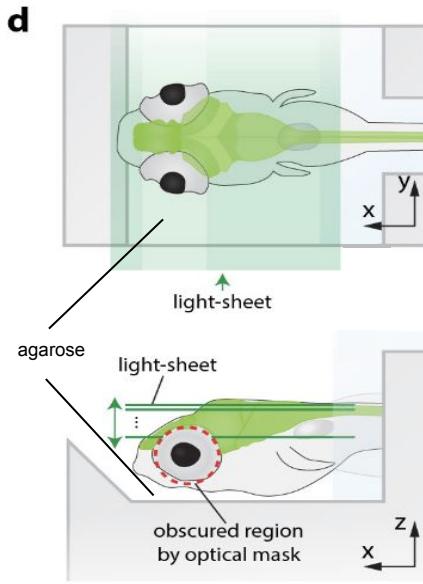
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Adapted from Wang, Z., Zhang, J., Symvoulidis, P., Guo, W., Zhang, L., Wilson, M. A., & Boyden, E. S. (2023). Imaging the voltage of neurons distributed across entire brains of larval zebrafish. In bioRxiv (p. 2023.12.15.571964). <https://doi.org/10.1101/2023.12.15.571964>

# Overall experimental strategy

Step	What we plan	Why it is promising	Where the risk sits
<b>Mount fish (head in agar, tail free)</b>	Keeps brain optically stable while preserving a behavioral read-out (tail flick).	Widely used; matches setups in Herrera <i>et al.</i> 2021 and Swain's ZOSS platform.	Tail tracking can be obscured by drug-induced muscle tone changes. Pilot whether tail contrast remains high after >5 min ketamine.
<b>Bath-immersion dosing (0.2 % vs 0.8 % v/v)</b>	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 <sup>-1</sup> so the whole dish exchanges in <60 s.
<b>Voltage imaging (Positron2, 200 Hz LS scope)</b>	Gives the millisecond-scale data that were missing from calcium-only papers.	We 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).
<b>Behavioral probe = periodic NIR heat pulse</b>	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).

# Figure 2

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

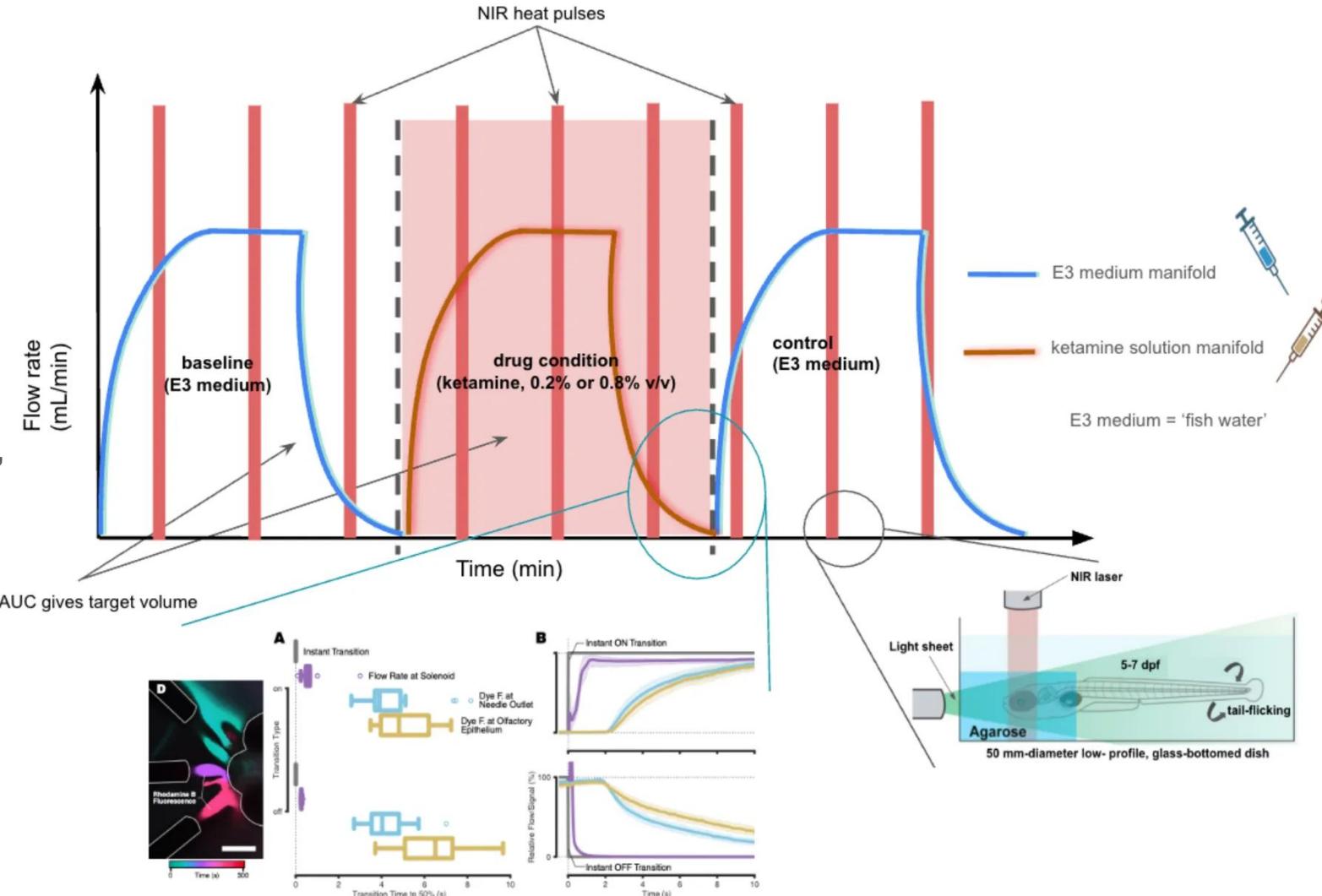
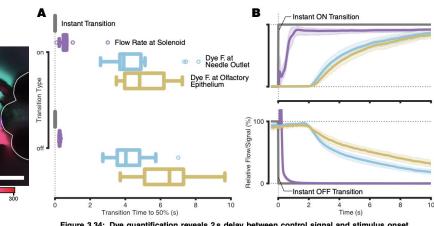


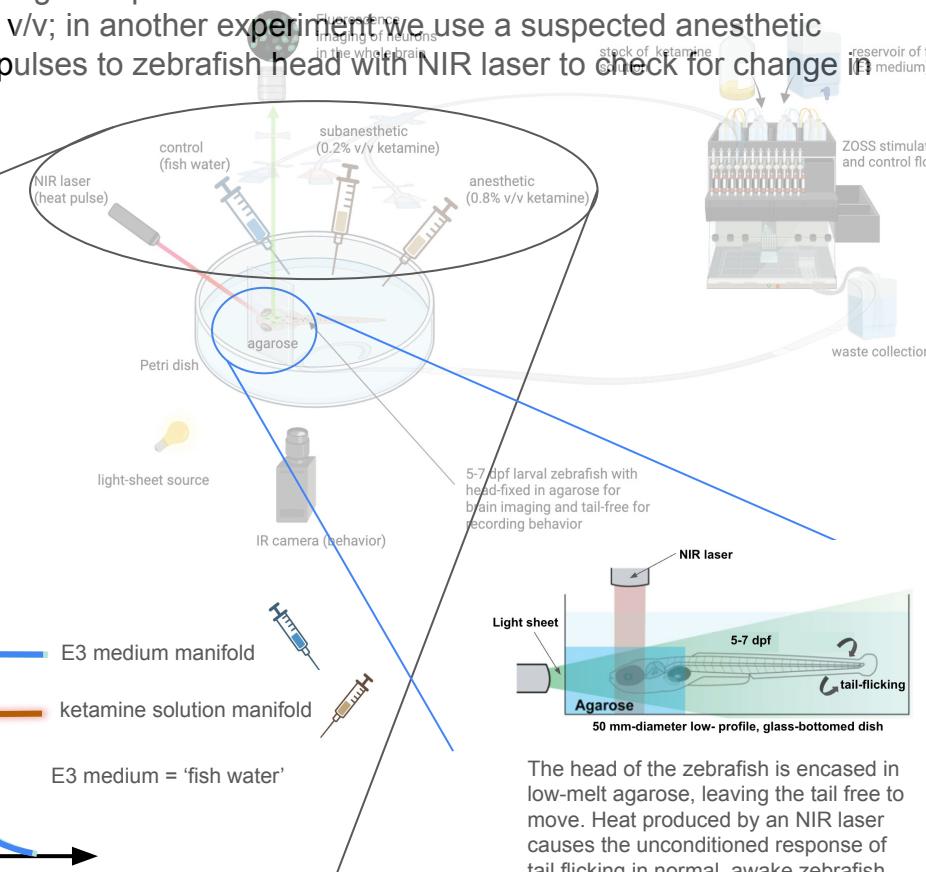
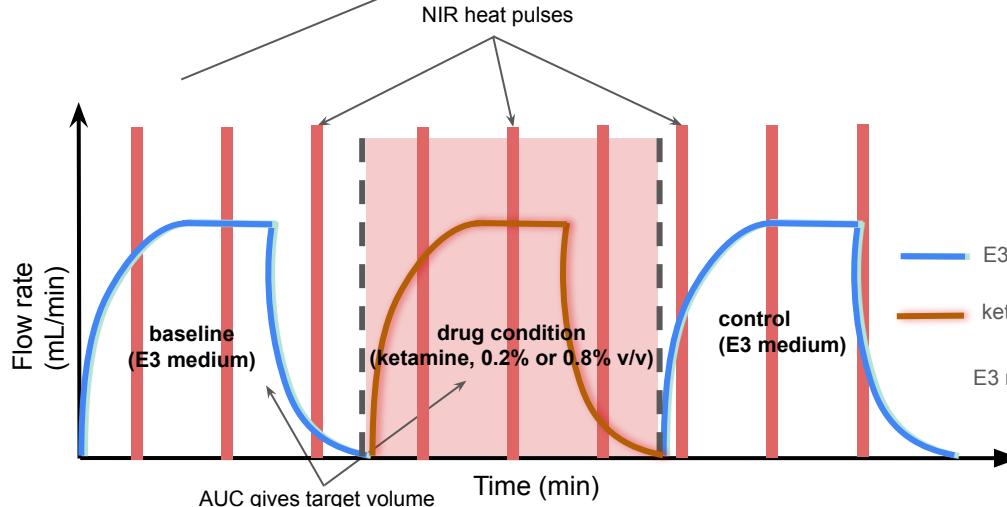
Figure depicting the manifold control signal time-courses for our proposed ketamine zebrafish experiments. The time-course of each active line is shown for each fluid manifold. The colored lines represent ketamine dose (brown) or E3 medium control (blue) signal. Gray dashed lines mark intermediate transition phases when the outflow vacuum is turned on simultaneously with manifolds to withdraw the previous solution while delivering the next. Pink shaded region represents the ketamine immersion condition: in one experiment we use a suspected subanesthetic concentration of 0.2% v/v; in another experiment we use a suspected anesthetic concentration of 0.8% v/v. Red bars represent regularly spaced heat pulses to zebrafish head with NIR laser to check for change in behavior (tail-flick response) like loss of consciousness (LOC).

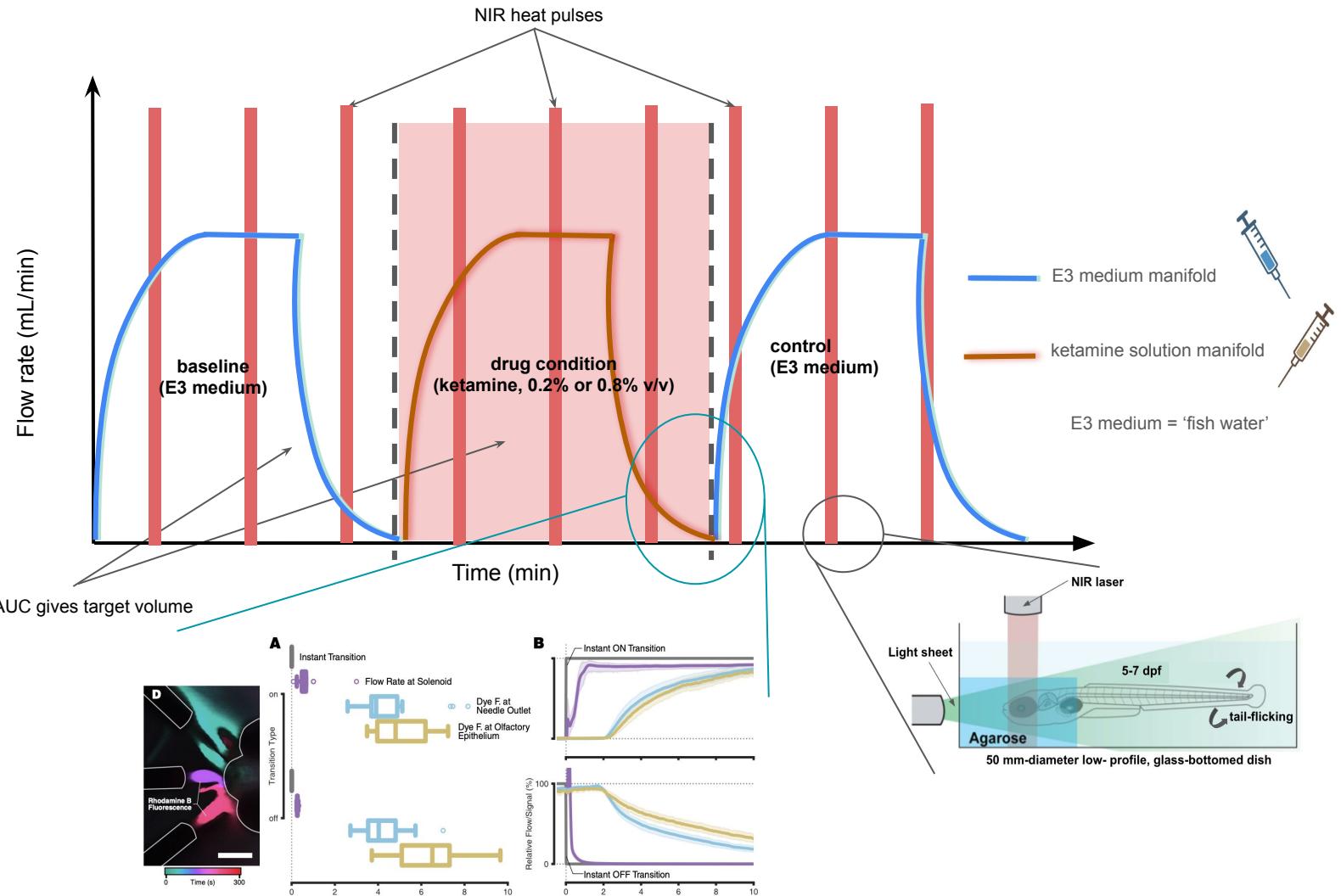


Figure 3.24: Dye quantification reveals 2 s delay between control signal and stimulus onset at the needle output and a graded increase in stimulant concentration over  $\pm 5\text{s}$ . □ Box-and-



We will validate the ability of our modified ZOSS setup to deliver the desired bath concentrations, volumes and transitions with synthetic fluorescent dye dry runs.





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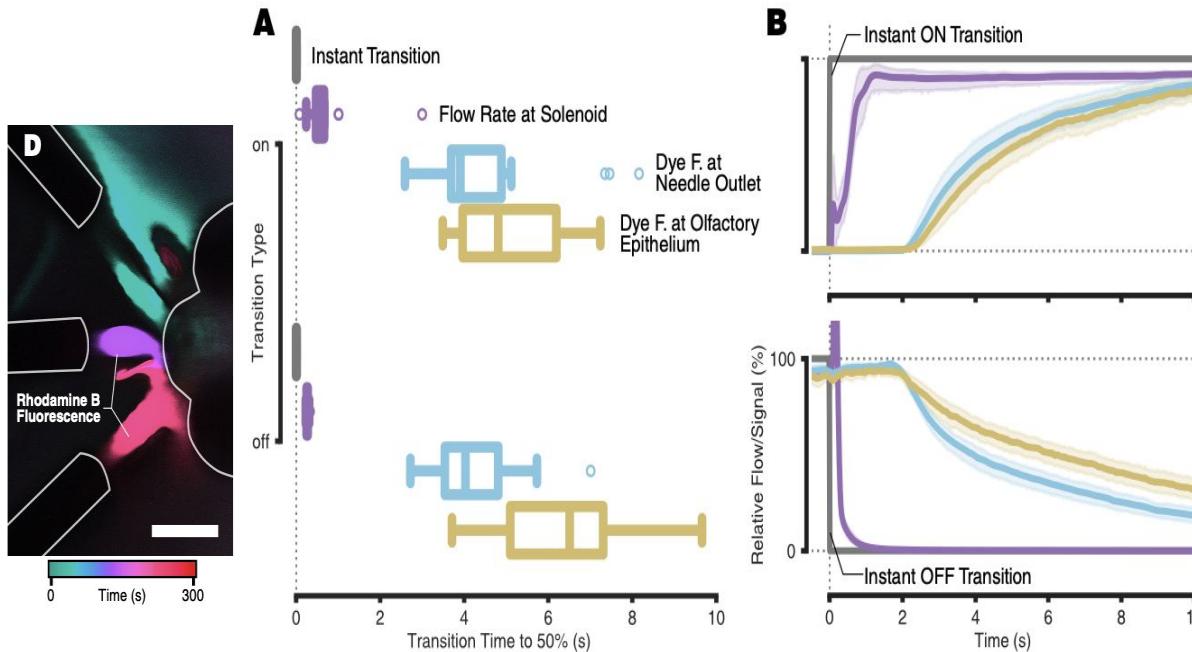
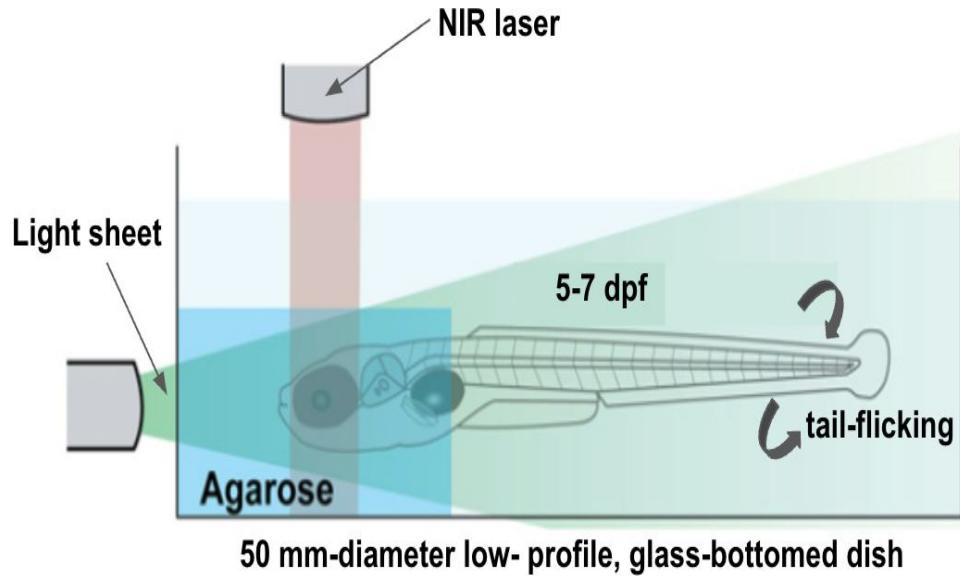


Figure 3.34: Dye quantification reveals 2 s delay between control signal and stimulus onset at the needle output and a graded increase in stimulant concentration over  $\approx$ 5 s. A: Box-and-

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

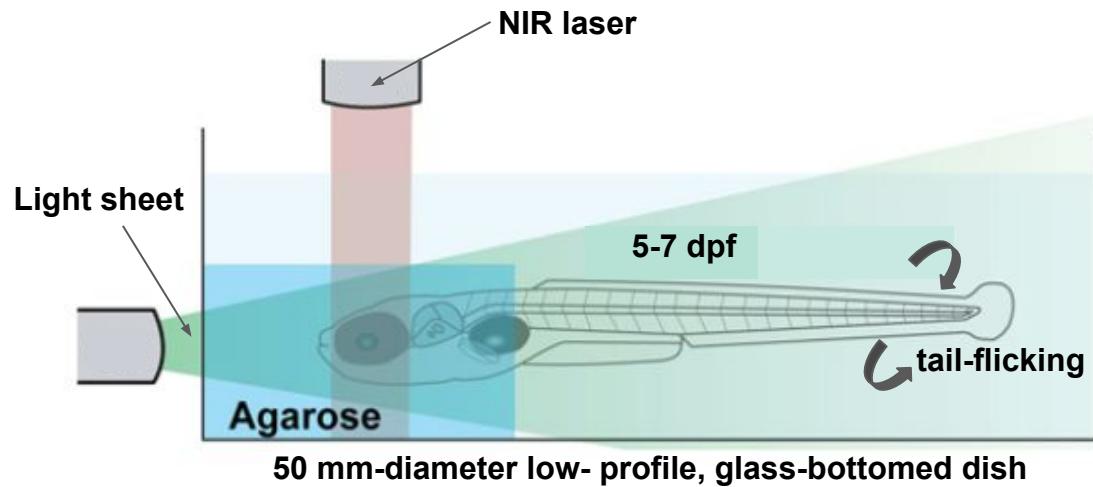
The head of the zebrafish is encased in low-melt agarose, leaving the tail free to move. Heat produced by an NIR laser causes the unconditioned response of tail flicking in normal, awake zebrafish.



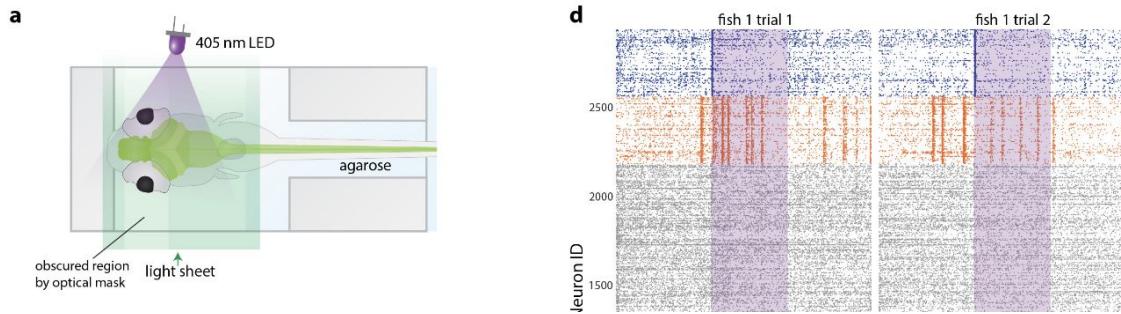
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<https://doi.org/10.1073/pnas.2107661119>

# Spreadsheet screenshot zebrafish fish lines owned by lab

			rac	loc	loc	s1	s2	owner
			k	row	pos			
1	# fish contents							
132	14 HuC:GCaMP6f (CY14); casper			7/24/24	B	3	1	GCaV HuC Ed/ ?
133	14 HuC:GCaMP6f (CY14); casper			7/24/24	B	3	2	GCaV HuC Ed/ ?
134	14 HuC:GCaMP6f (CY14); casper			7/24/24	B	3	3	GCaV HuC Ed/ ?
135	12 HuC:GCaMP6f (CY14); nacre/-; roy+/?			7/31/24	B	3	4	GCaV HuC Ed/ ?
136	12 HuC:GCaMP6f (CY14); nacre/-; roy+/?			7/31/24	B	3	5	GCaV HuC Ed/ ?
137	12 HuC:GCaMP6f (CY14); nacre/-; roy+/?			7/31/24	B	3	6	GCaV HuC Ed/ ?
139	4 HuC:H2B-jGCaMP8s Fo 2 F1 BRIGHTER; nacre; transmit 75%			7/11/23	J	4	11	H2B-jGCaM Caroline
140	2 HuC:H2B-jGCaMP8s Fo 2 F1 BRIGHTER; nacre; transmit 50%			7/11/23	J	4	12	H2B-jGCaM Caroline
141	14 HuC:H2B-jGCaMP8s F2; nacre			12/24/24	E	3	11	H2B-jGCaM Caroline
142	14 HuC:H2B-jGCaMP8s F2; nacre			12/24/24	E	3	12	H2B-jGCaM Caroline
156	15 HuC:somaGcamp7f; nacre			11/6/24	E	3	6	somaGCaM Caroline
157	15 HuC:somaGcamp7f; nacre			11/6/24	E	3	7	somaGCaM Caroline
158	14 HuC:somaGcamp7f; nacre			11/6/24	E	3	8	somaGCaM Caroline
159	14 HuC:somaGcamp7f; nacre			11/6/24	E	3	9	somaGCaM Caroline
164	10 3XUAS:Positron2-Kv F2 #20; HuC:Gal4; nacre			2/20/24	D	3	1	Positr UAS Zeguan
165	9 3XUAS:Positron2-Kv F2 #20; HuC:Gal4; nacre			2/20/24	D	3	2	Positr UAS Zeguan
166	12 3XUAS:Positron2-Kv F2 #20; HuC:Gal4; nacre			3/6/24	D	3	3	Positr UAS Zeguan
167	12 3XUAS:Positron2-Kv F2 #20; HuC:Gal4; nacre			3/6/24	D	3	4	Positr UAS Zeguan
168	1 2XUAS:Positron2-Kv FOUNDER 20 HuC:Gal4, nacre			6/21/23	C	1	18	Positr UAS Zeguan
169	9 2XUAS:Positron2-Kv F1 #20; HuC:Gal4; nacre (tested; poor)			3/6/24	D	3	6	Positr UAS Zeguan
	2XUAS:Positron2-Kv F1 #20; HuC:Gal4; nacre (untested; includes one							
170	10 2/28)			3/6/24	D	3	7	Positr UAS Zeguan
171	1 2XUAS:Positron2-Kv F1 #20-1; HuC:Gal4; nacre			2/28/24	D	4	8	Positr UAS Zeguan
172	1 2XUAS:Positron2-Kv F1 #20-2; HuC:Gal4; nacre			2/28/24	D	4	9	Positr UAS Zeguan
173	1 2XUAS:Positron2-Kv F1 #20-4; HuC:Gal4; nacre			3/6/24	D	4	10	Positr UAS Zeguan
174	1 2XUAS:Positron2-Kv F1 #20-5; HuC:Gal4; nacre			3/6/24	D	4	11	Positr UAS Zeguan
175	1 2XUAS:Positron2-Kv F1 #20-14; HuC:Gal4; nacre			3/6/24	D	3	10	Positr UAS Zeguan
176	1 2XUAS:Positron2-Kv F1 #20-15; HuC:Gal4; nacre			3/6/24	D	3	11	Positr UAS Zeguan
177	1 2XUAS:Positron2-Kv FOUNDER 9 HuC:Gal4, nacre			6/21/23	C	1	19	Positr UAS Zeguan
178	1 2XUAS:Positron2-Kv F1 #9; HuC:Gal4; nacre			4/3/24	D	3	8	Positr UAS Zeguan
179	12 2XUAS:Positron2-Kv F1 #9 (no Gal4); nacre			4/3/24	D	3	9	Positr UAS Zeguan
180	13 HuC:Positron2-Kv inj nacre (some expr?); one from 4/10 DOB			4/17/24	D	4	5	Positr HuC Zeguan
181	13 HuC:Positron2-Kv inj nacre (no expr?)			4/17/24	D	4	2	Positr HuC Zeguan
182	12 HuC:Positron2-Kv inj nacre (no expr?)			4/17/24	D	4	3	Positr HuC Zeguan
183	12 HuC:Positron2-Kv inj nacre (no expr?)			4/17/24	D	4	4	Positr HuC Zeguan
184	1 HuC:Positron2-Kv inj nacre Fo #5			4/17/24	D	4	6	Positr HuC Zeguan
185	1 HuC:Positron2-Kv inj nacre Fo #15			4/17/24	D	4	7	Positr HuC Zeguan
186	12 HuC:Positron2-KvF1 from Fo#5; nacre			9/18/24	C	4	1	Positr HuC Zeguan
187	11 HuC:Positron2-KvF1 from Fo#5; nacre			9/18/24	C	4	2	Positr HuC Zeguan
188	12 HuC:Positron2-KvF1 from Fo#15; nacre			9/18/24	C	4	3	Positr HuC Zeguan
189	12 HuC:Positron2-KvF1 from Fo#15; nacre			9/18/24	C	4	4	Positr HuC Zeguan
190	12 HuC:Positron2-KvF1 from Fo#15; nacre			9/18/24	C	4	5	Positr HuC Zeguan
191	12 HuC:Positron2-KvF1 from Fo#15; nacre			9/18/24	C	4	6	Positr HuC Zeguan
192	12 pZ95-injected HuC:Postiron(5), nacre			3/13/25	C	3	1	nYFP Positr Zeguan
193	9 pZ95-injected HuC:Postiron(5), nacre			4/3/25	R	3	9	nYFP Positr Zeguan
194	9 pZ95-injected HuC:Postiron(5), nacre			4/3/25	R	3	10	nYFP Positr Zeguan
195	9 pZ95-injected HuC:Postiron(5), nacre			4/3/25	R	3	11	nYFP Positr Zeguan
196	12 pZ96-injected HuC:Postiron(5), nacre			3/13/25	C	4	8	nYFP Positr Zeguan
197	11 pZ96-injected HuC:Postiron(5), nacre			3/13/25	C	4	9	nYFP Positr Zeguan
198	11 pZ96-injected HuC:Postiron(5), nacre			3/13/25	C	4	10	nYFP Positr Zeguan
199	12 pZ96-injected HuC:Postiron(5), nacre			3/13/25	C	4	11	nYFP Positr Zeguan
200	4 pZ96-injected HuC:Postiron(5), nacre			4/3/25	R	3	3	nYFP Positr Zeguan



Adapted from 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 of the United States of America*, 119(3), e2107661119.  
<https://doi.org/10.1073/pnas.2107661119>



**Figure 3. Imaging of activity of neurons distributed throughout entire zebrafish brains during visual stimulation.** (a) Light stimulation turns on at the lateral-right side of the fish for 10s during each

CB: cerebellum. MO: medulla oblongata. Scale bar: 100  $\mu$ m. (d) Neuron spike raster plots for Fish 1. Four distinct activity patterns are observed in each Fish: Group 1 neurons (blue) have increased activity immediately after UV stimulation onset. Group 2 neurons (orange) have multiple episodes of increased activity throughout the trials. Group 3 neurons (gray) have spontaneous activities throughout the trials. Group 4 neurons (green) have increased activity at the beginning of trial 1, perhaps due to the onset of the

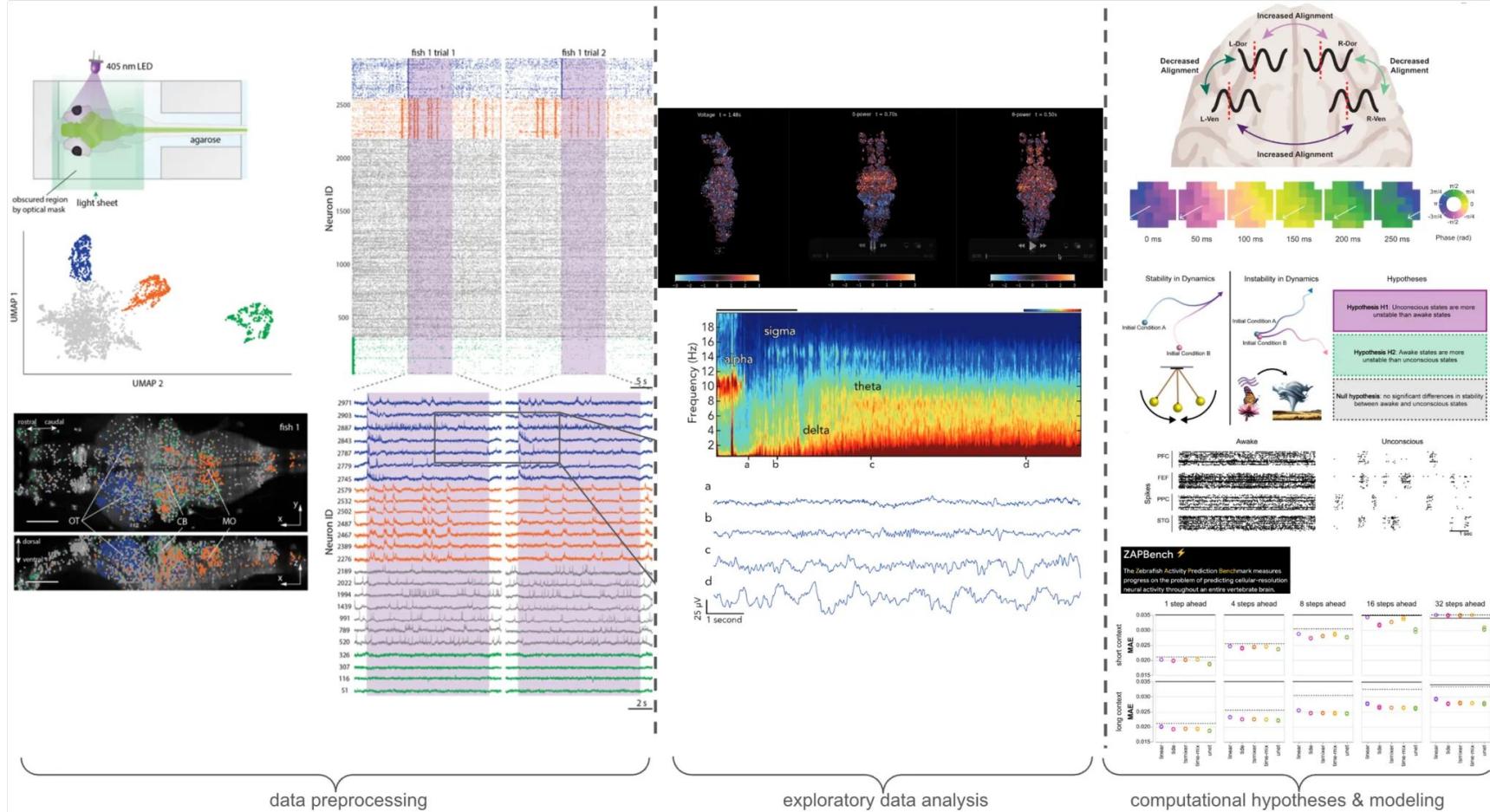
Adapted from Wang, Z., Zhang, J., Symvoulidis, P., Guo, W., Zhang, L., Wilson, M. A., & Boyden, E. S. (2023). Imaging the voltage of neurons distributed across entire brains of larval zebrafish. In bioRxiv (p. 2023.12.15.571964). <https://doi.org/10.1101/2023.12.15.571964>

## Aim 2

Analyze those data to extract spectral signatures (multitaper analysis), phase-locking/synchrony (PLV), low-dimensional brain-state trajectories, and dynamical predictability (neural-network forecasting).

# Figure 3

## Data analysis pipeline overview.



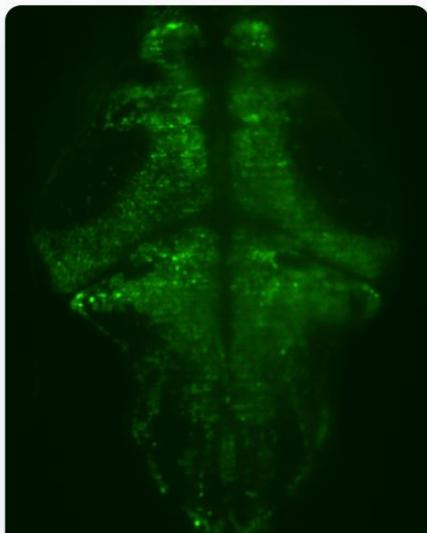
# Analysis Pipeline

**Preprocessing:** VolPy + Cellpose → motion-corrected, segmented voltage traces.

**Spectral & synchrony:** Multitaper spectrograms; PLV across regions.

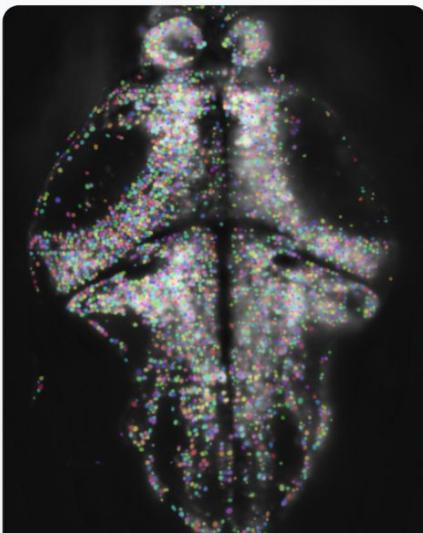
**Dynamics & predictability:** Low-dimensional embeddings of population trajectories; train models to forecast future neural states (inspired by ZAPBench and DeLASE frameworks).

# Preprocessing



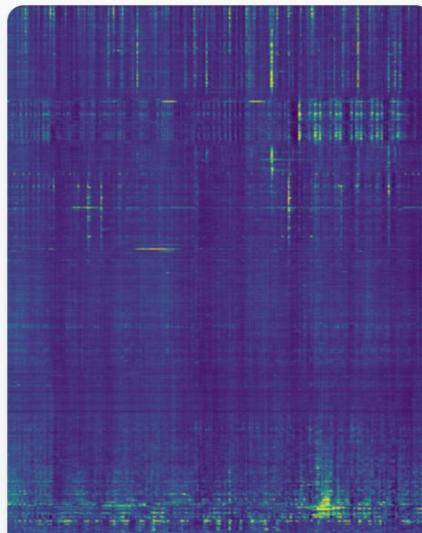
## Volumetric Activity

Whole-brain calcium activity after elastic alignment, acquired by light-sheet imaging.



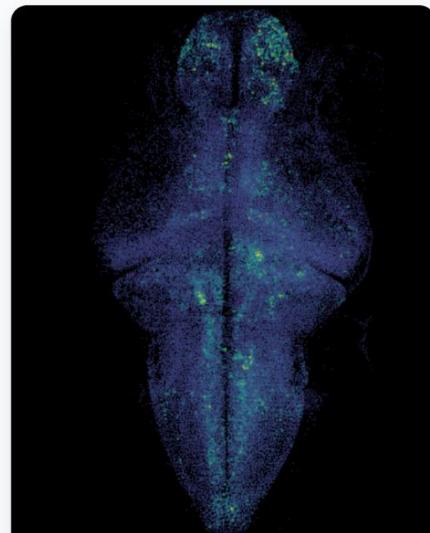
## Segmentation

Segmentation masks for ~70,000 neurons obtained by training one-shot flood-filling networks.



## Traces

Extracted activity traces per segmented neuron, sorted by similarity.

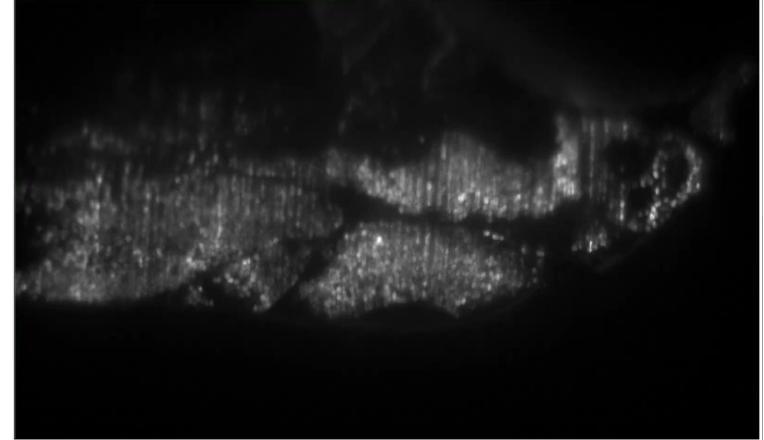


## Cell Activity

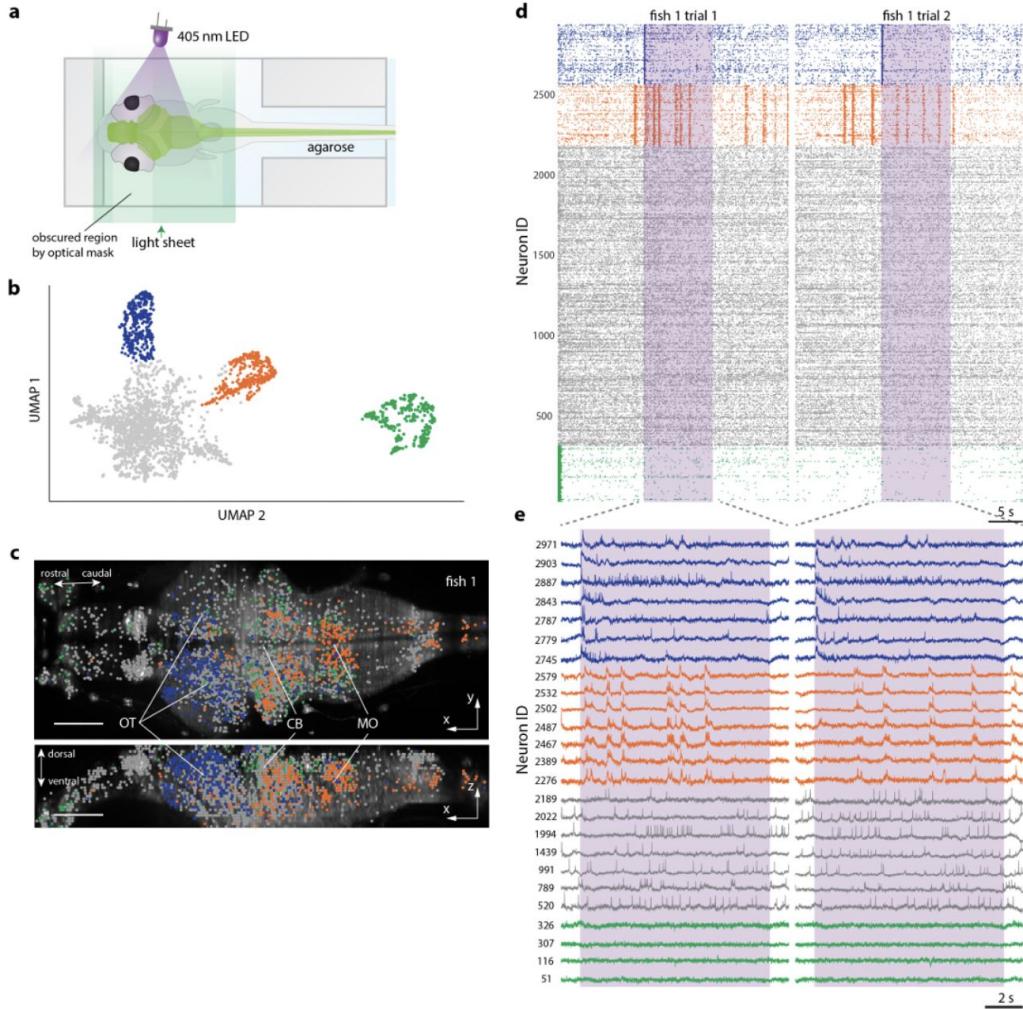
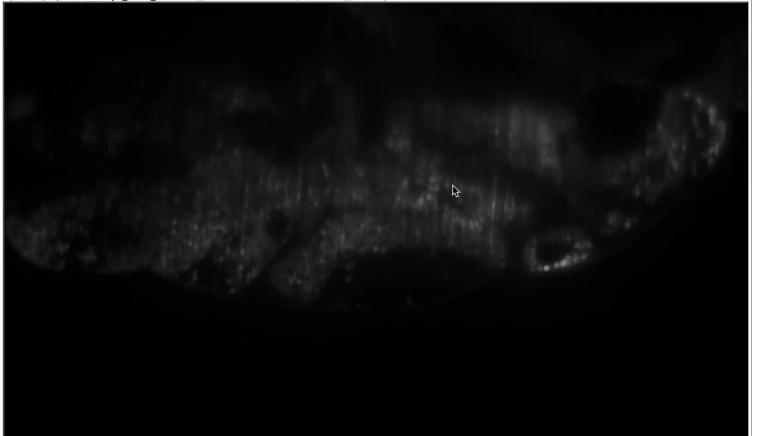
Rendering of activity traces to visualize spatial relationships.

# Preprocessing

scape\_fish2\_vols.ome.tif kept stack kept stack  
z:25/42 t:23/81 (slice:949); 0.00x0.00 microns (1024x608); 16-bit; 3.9GB

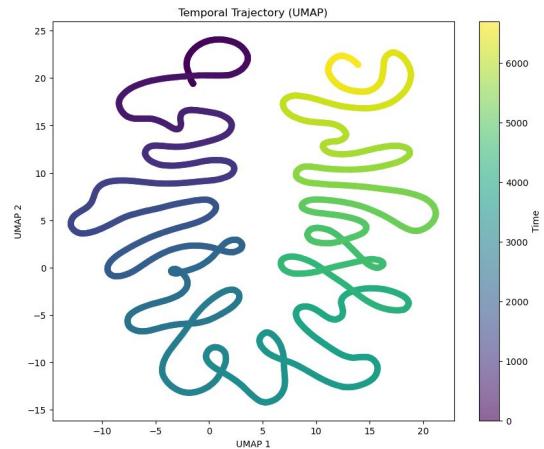


scape\_fish2\_vole.ome.tif  
1/3440 (t:1/3440 - scape\_fish2\_vole.ome); 0.00x0.00 microns (1024x608); 16-bit; 4GB

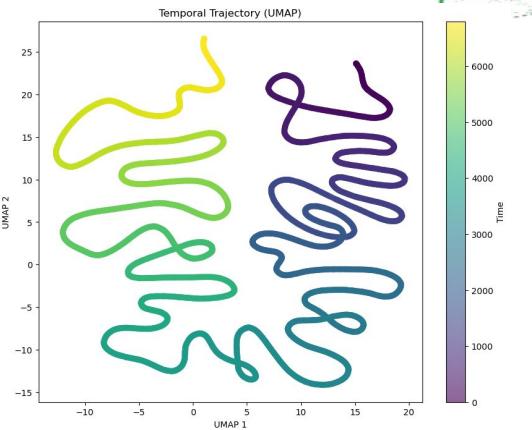


# Preliminary results

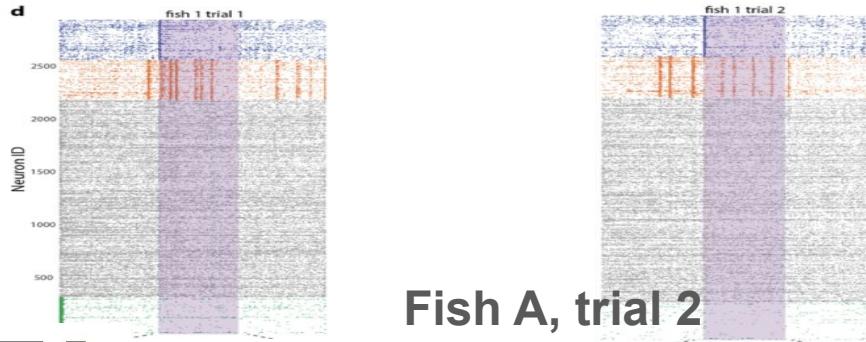
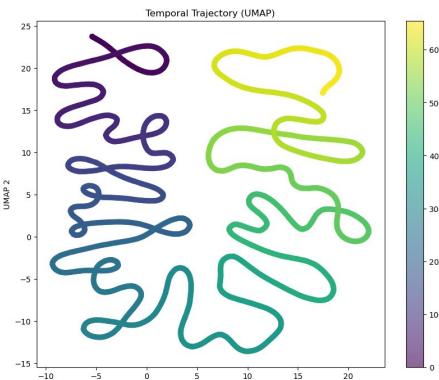
Fish D, trial 1



Fish C, trial 1

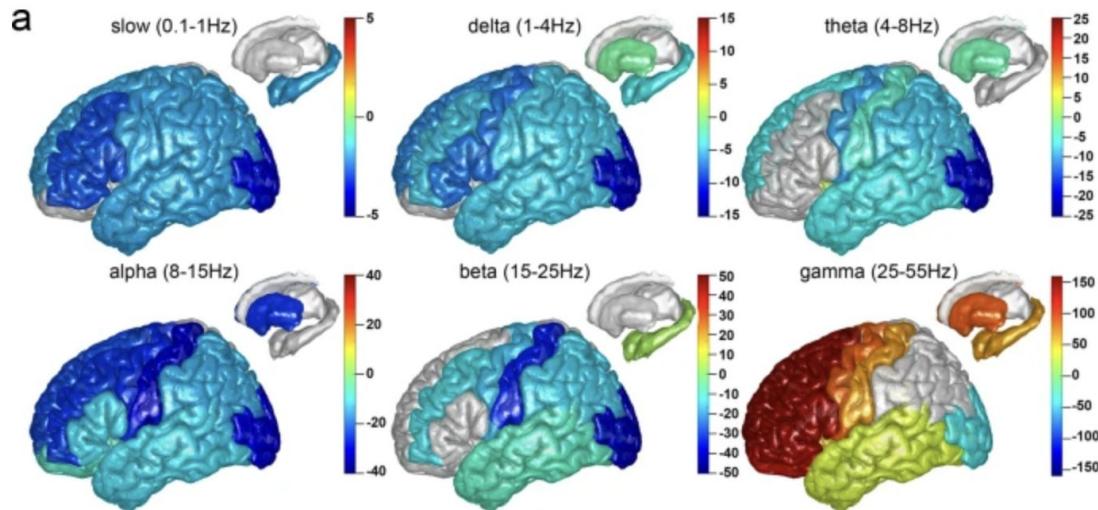


Fish A, trial 2



# Ketamine induced an increase in gamma oscillation power and a reduction of low-frequency oscillation power

**Fig. 2: Structural mapping for intracranial EEG power changes after ketamine infusion relative to baseline at 6 frequencies.**



Tian, F. et al. Characterizing brain dynamics during ketamine-induced dissociation and subsequent interactions with propofol using human intracranial neurophysiology. *Nat. Commun.* **14**, 1748 (2023).

# Spectral-analysis vision

## 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).

Volumetric movie ( $N_x, N_y, N_z, T$ )

| VolPy → ~16 000  $\Delta F/F$  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)

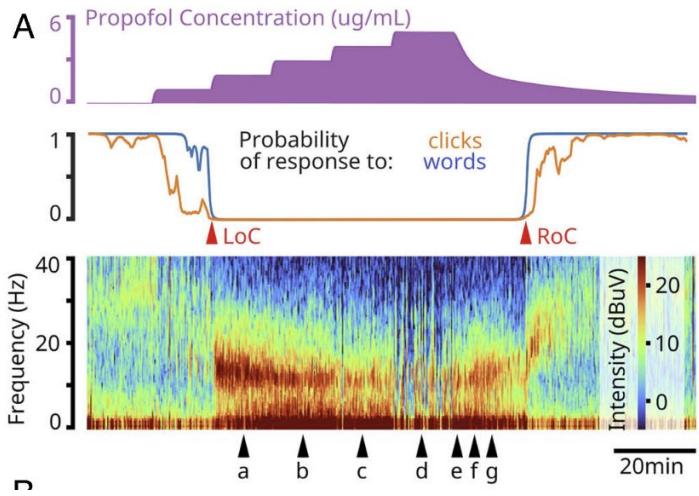
Select canonical bands

| δ(0–4) θ(4–8) α(8–12) β(13–25) γ(40–80)

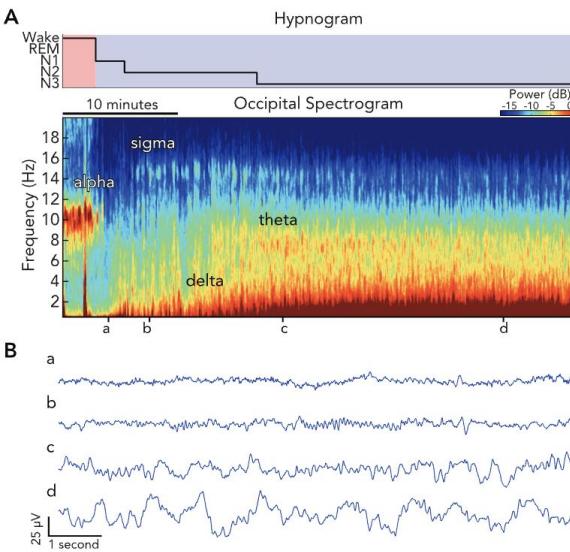
Back-project band-power time-series to cells

| (solve linear system with comp loadings)

Result → Power(cell, band, time)



**Fig. 1.** Evolution of EEG dynamics during propofol-mediated unconsciousness. (A) Top. Graph shows the target propofol effect-site concentration for a volunteer subject receiving a computer-controlled propofol infusion. The propofol infusion rate is increased in a stepwise manner to achieve 5 increasing target effect-site concentrations then the infusion is turned off. Middle. Temporal traces of the correct response probability for verbal cues (blue) and sound clicks (orange) to infer loss (LoC) and recovery (RoC) of



### NON-REM (NREM)

#### NREM Progression into Slow Wave Sleep

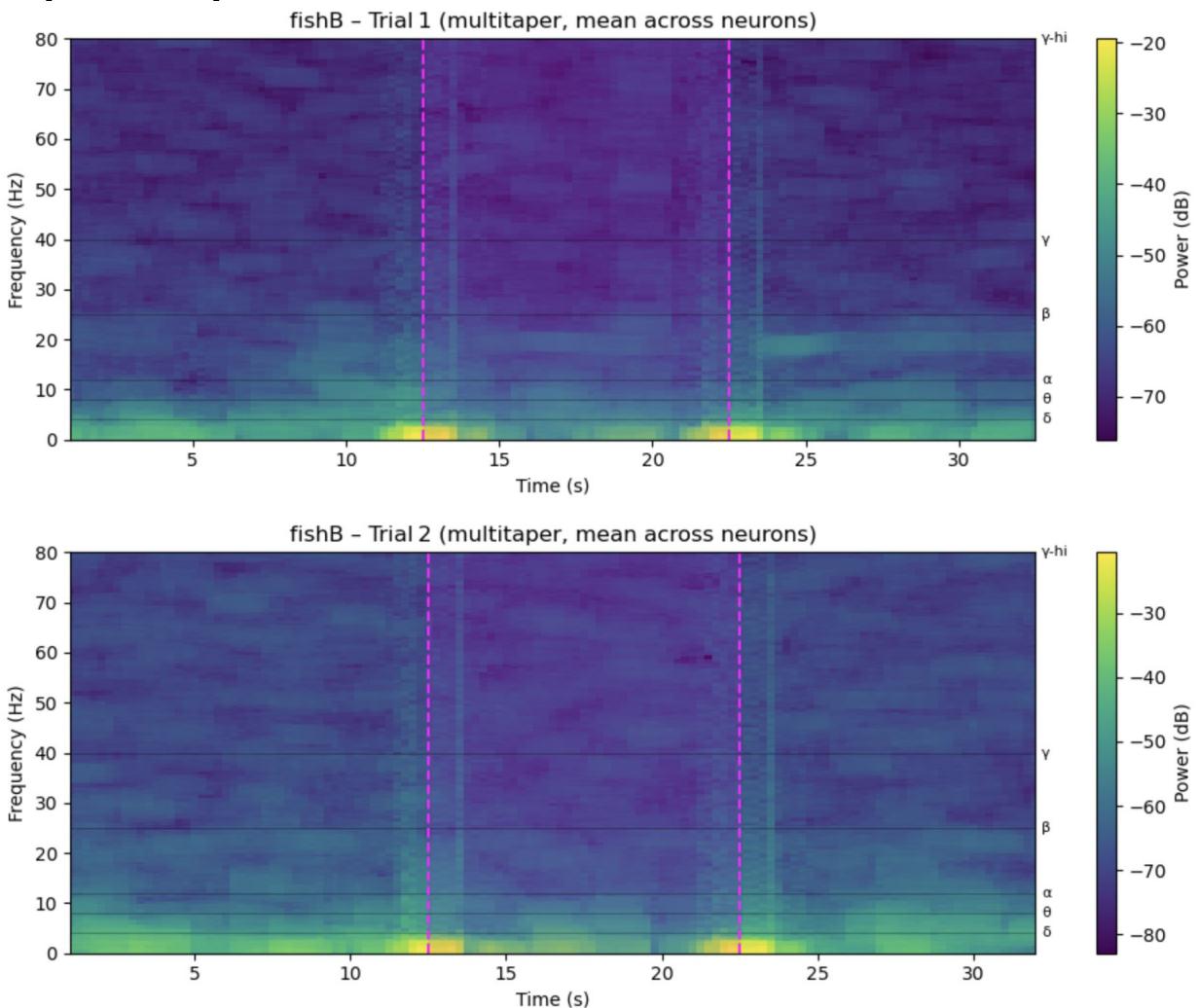
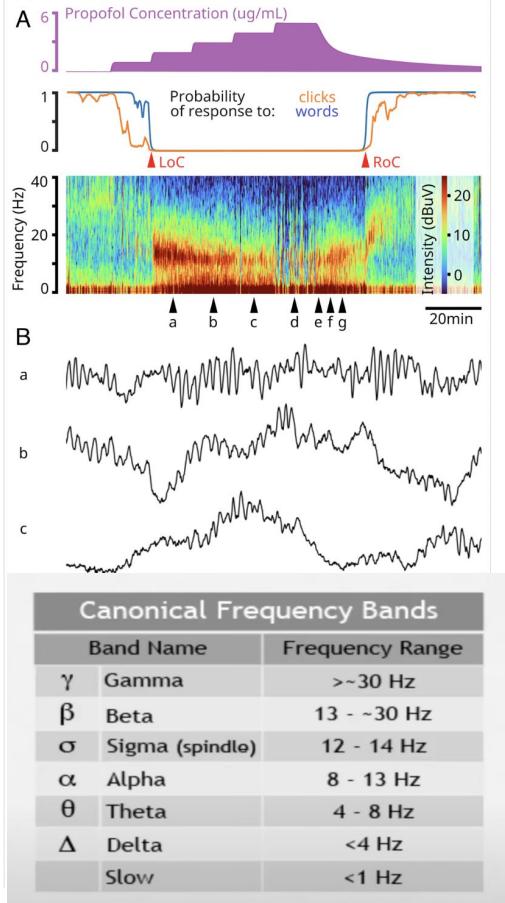
Delta	Gradual increase to high power and large bandwidth
Theta	Gradual increase in power and bandwidth following delta
Alpha	Low Presence of transient alpha corresponds to an arousal
Sigma	Rapid appearance of power following initial increase of delta Power decreases as NREM progresses
Background	Broadband background power

#### Dynamics

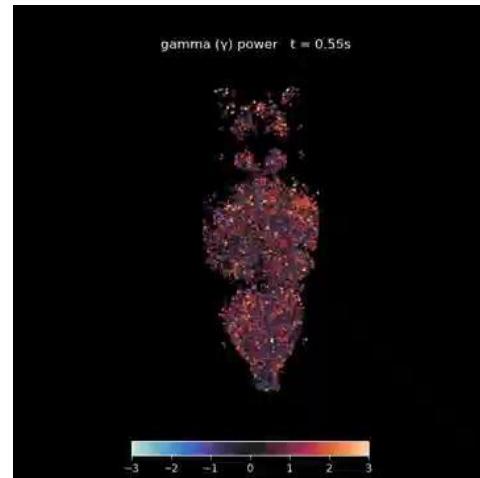
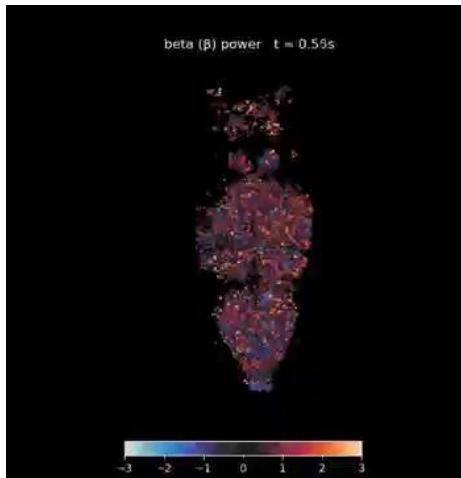
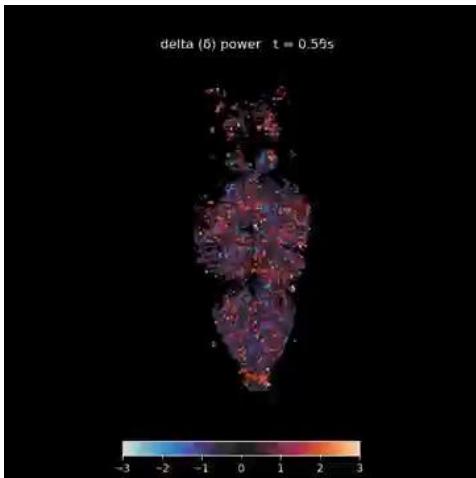
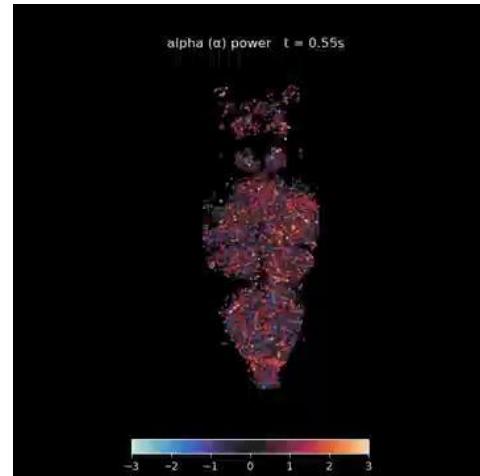
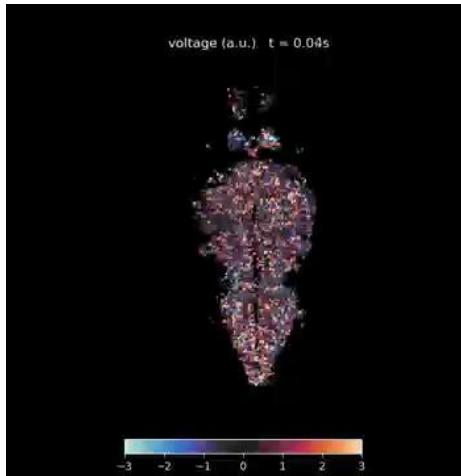
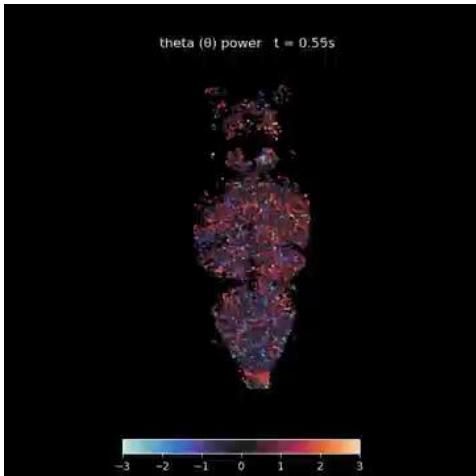
- As NREM progresses into Slow Wave Sleep:
  - The power and bandwidth of the delta and theta oscillations increase
  - Power in sigma appears (may then decrease as delta increases)
- Arousal during NREM are marked by alpha and/or a motion artifact, and a reset of NREM process to lighter levels
- During a slow lightening of NREM, there is a reduction of delta and theta power, and sigma power reappears

# Spectral Analysis $\alpha$

Fig. 1.



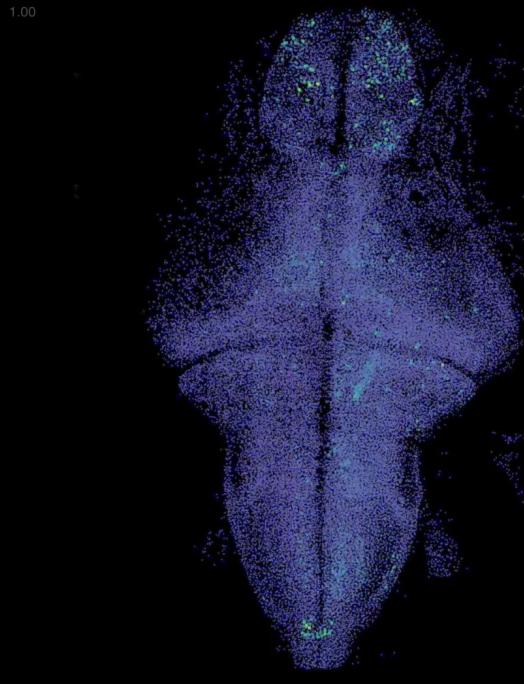
# Preliminary results



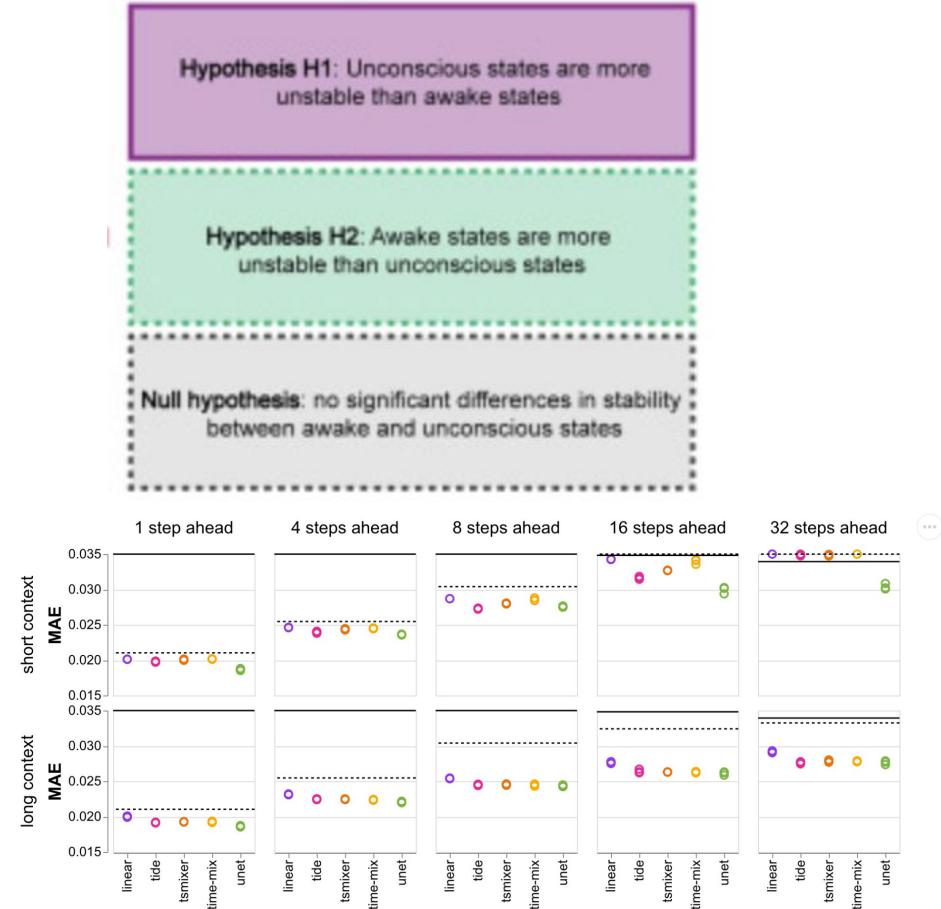
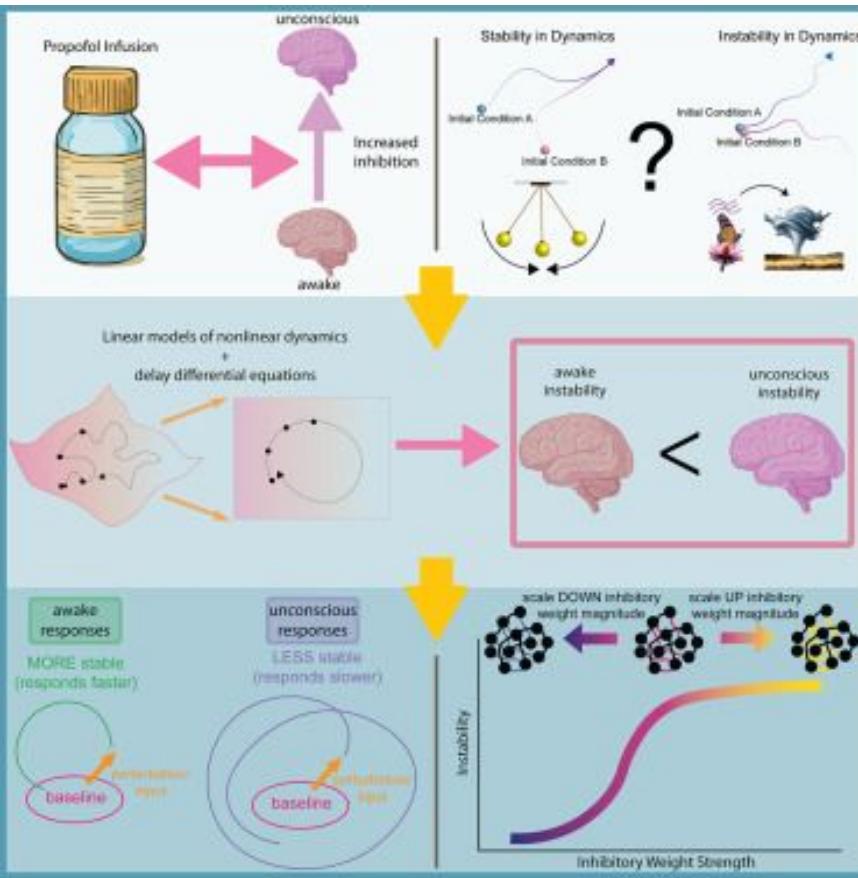
# Time-series forecasting & dynamical stability

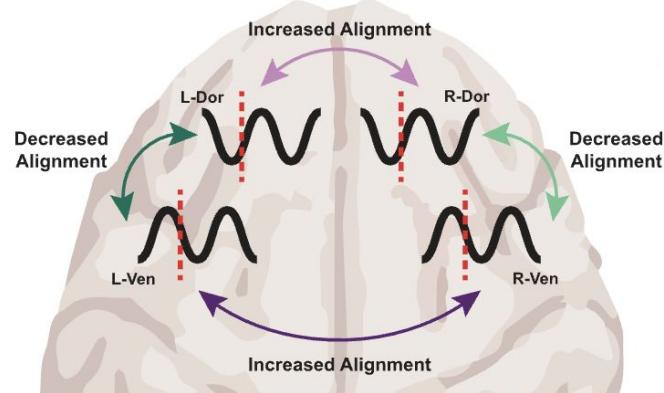
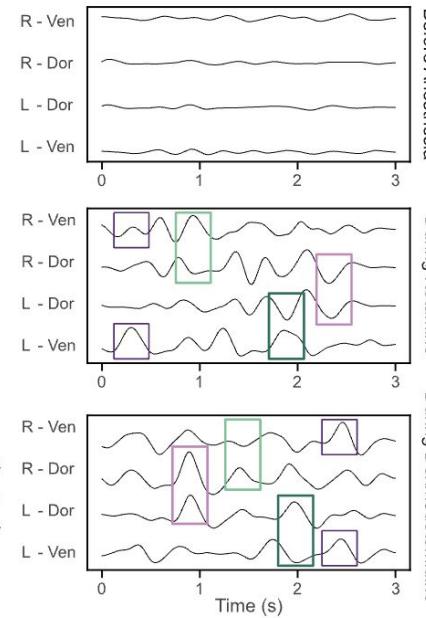
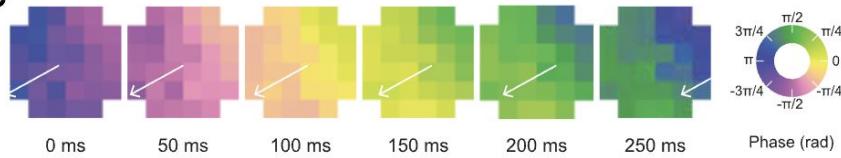
## ZAPBench ⚡

The Zebrafish Activity Prediction Benchmark measures progress on the problem of predicting cellular-resolution neural activity throughout an entire vertebrate brain.



# Does dynamical (in)stability <-> (high)/low prediction error ?



**A****B****C**

**Figure 4. Anesthetics fragment cortical activity within a hemisphere but synchronize it across hemispheres**

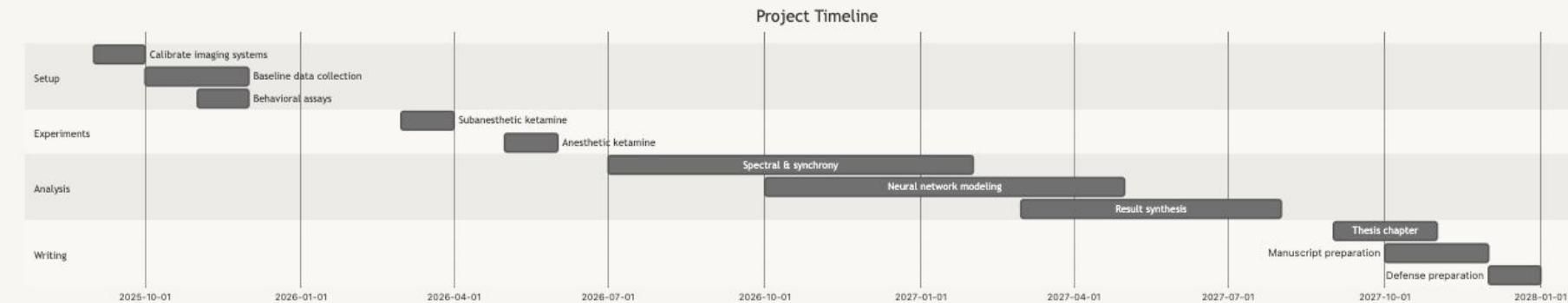
(A) Ketamine and dexmedetomidine both cause phases of low-frequency oscillatory activity to become less aligned between different regions of PFC and more aligned between homologous regions across hemispheres.

(B) Sample delta (1–4 Hz) range filtered LFP signals before anesthesia (top), with ketamine (middle), and with dexmedetomidine (bottom). Raw LFP traces shown in Figure S1A. Purple boxes show examples of increased across-hemisphere phase alignment, and green boxes show examples of decreased within-hemisphere phase alignment.

(C) Example of a traveling wave. Phase of low-frequency (1–4 Hz) LFP signal in an electrode array across time from a recording with ketamine. Each square represents one electrode contact, colored according to its instantaneous phase at the indicated time.

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.  
<https://doi.org/10.1016/j.celrep.2025.115685>

# Timeline & Contingencies



# 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.

# Milestones

## 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.

# Milestones

## Jan–Aug 2027 – Writing, Dissemination, and Defense

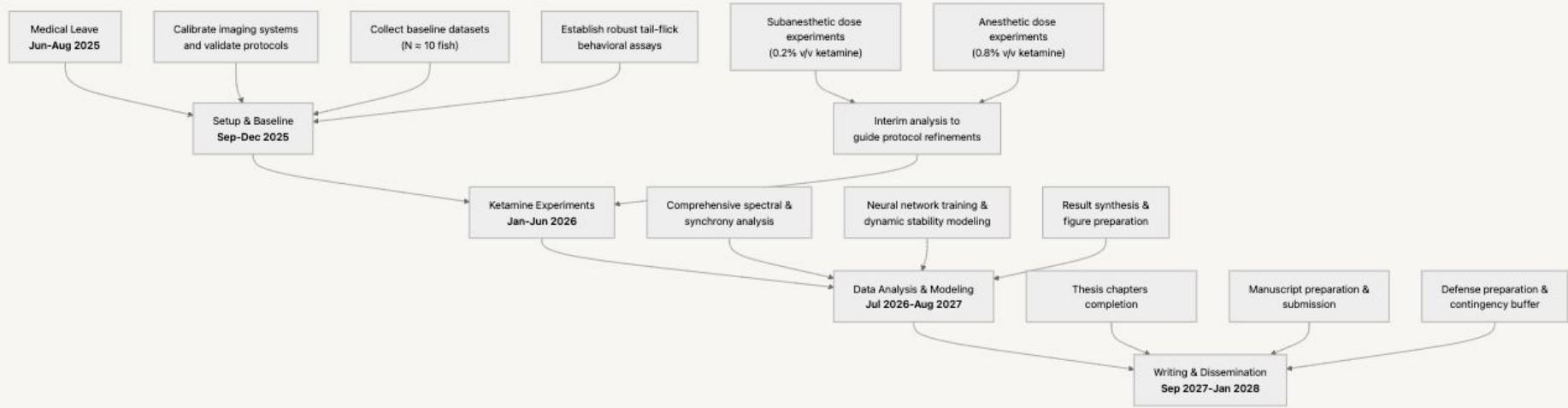
PhD thesis chapter writing integrating background, methods, and results. Manuscript preparation for publication focusing on network synchrony changes. Thesis defense scheduled for mid-2027.

**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.



# Contingency Plans

## 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.

## 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.

## 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.

# Thank You

## Collaborators

Corban Swain, PhD



Yuechuan Lin, PhD



Zeguan Wang, PhD



Lige (Caroline) Zhang

## Committee

Emery Brown, MD, PhD



Ila Fiete, PhD



Steve Flavell, PhD



Eviatar Yemini, PhD



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