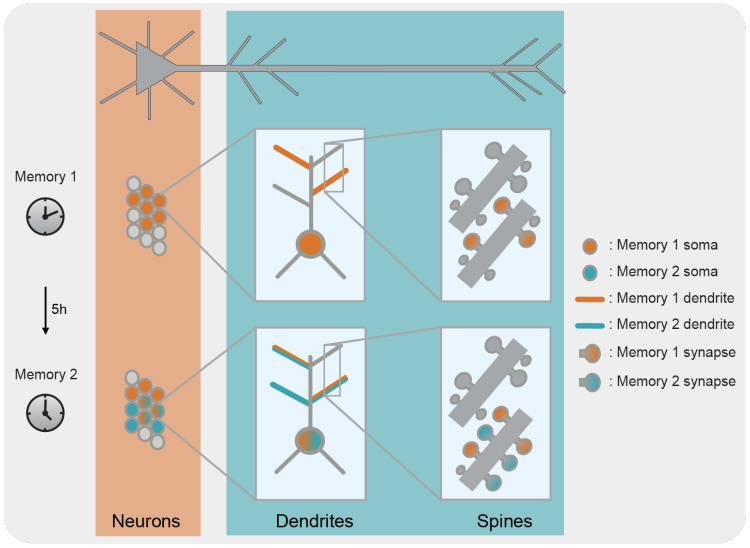
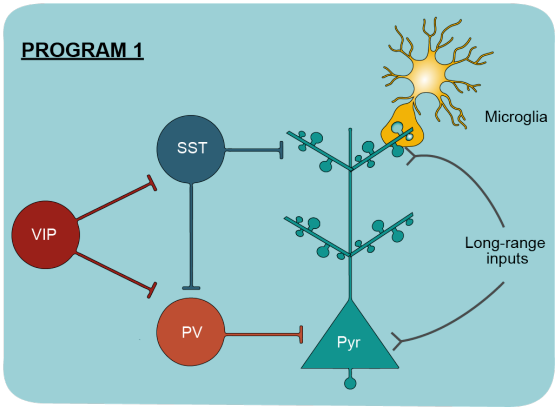
Co-allocation to Overlapping Dendritic Branches in the Retrosplenial Cortex Integrates Memories Across Time

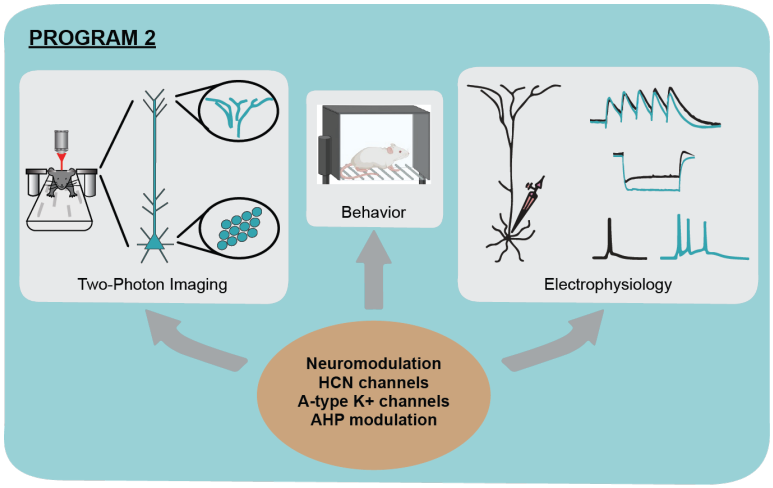
Events occurring close in time are often linked in memory, providing an episodic timeline and a framework for those memories. Recent studies suggest that memories acquired close in time are encoded by overlapping neuronal ensembles, and that this overlap is necessary for memory linking. Transient increases in neuronal excitability drive this ensemble overlap, but whether dendritic plasticity plays a role in linking memories is unknown. Here, we show that contextual memory linking is not only dependent on ensemble overlap in the retrosplenial cortex (RSC), but also on RSC branch-specific dendritic allocation mechanisms. We used miniature microscopes and activity-dependent labeling to demonstrate that neuronal ensemble overlap within the RSC underlies the linking of two contextual memories. Using longitudinal two-photon imaging of RSC dendrites, we show that the same dendritic segments are preferentially activated by two linked (but not independent) contextual memories, and that spine clusters added after each of two linked (but not independent) contextual memories are allocated to the same dendritic segments. Importantly, with a novel optogenetic tool selectively targeted to activated dendritic segments following learning, we show that reactivation of dendrites tagged during the first context exploration is sufficient to link two contextual memories. These results demonstrate a causal role for dendritic mechanisms in memory linking and reveal a novel set of rules that govern how linked, and independent memories are allocated to dendritic compartments.



Graphical Abstract

<u>Significance, Innovation & Impact</u>: Our brains can link and separate a wide array of memories and the mechanisms that underlie these processes are incredibly important but poorly understood. Even less is understood about the role of localized dendritic plasticity. My work is the first demonstration of the role of localized dendritic plasticity for any animal behavior. The study combines cutting-edge techniques (some of which **I developed and freely shared/supported through the open-source UCLA Miniscope forum**) with novel optogenetic tools to demonstrate the role of dendrites in memory organization. Through highly mechanistic experiments, this work is conceptually and technically innovative with the potential to change the focus of learning and memory research in the coming years.





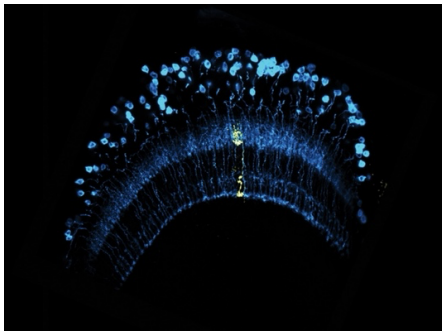
<u>Future Goals:</u> Many active dendritic processes sculpt the flow of information from the synapse to the neuronal soma. It is well established that these processes undergo localized (branch-specific) experience-dependent plasticity but **<u>almost nothing is known about the function of branch-specific dendritic plasticity</u>**. In my laboratory, I will investigate the microcircuit (Program 1) and intrinsic (Program 2) mechanisms that underlie localized dendritic plasticity necessary for memory formation and organization. I will use state-of-the-art tools such as longitudinal Miniscope and 2-photon calcium imaging, activity-dependent labeling, intersectional viral approaches, *ex vivo* electrophysiology along with novel behavioral assays to determine the mechanisms that drive localized dendritic plasticity and the role of this plasticity in regulating formation and organization of memories.

Multiplicative disinhibition and the logic of motion vision

Detecting the direction of visual motion is a prime example of neural computation. Motion vision is found in the nervous systems of virtually all sighted animals, it is important for their survival, and it requires well-defined linear and nonlinear operations; yet the whole process is of moderate complexity. Each photoreceptor of the retina is naturally ambiguous about the direction of visual motion. All it reports to downstream neuropils are changes in luminance. Theory predicts that a neuron can compute the direction of visual motion by multiplying the signals from two adjacent photoreceptors, corresponding to two image points, after delaying one of the signals relative to the other. The two signals coincide at the postsynaptic neuron only if one photoreceptor is activated prior to its neighbor—as it would be during motion in the neuron's preferred direction. I study the molecular mechanisms by which neurons delay and multiply signals using the visual system of the fruit fly as a model.

I measure the input and the output signals of the multiplicative processing step during visual stimulation: the membrane potentials of presynaptic columnar cells and those of postsynaptic T4 neurons, which respond selectively to light edges moving in one particular direction. A T4 neuron samples from multiple adjacent image points and choses the identity of its presynaptic partner at each image point depending on its preferred direction. To compute the direction of visual motion, the T4 neuron needs to detect the sequence of input signals. In contrast to all the other input neuron classes, one glutamatergic neuron was continuously active in the dark and hyperpolarized in response to light. Glutamate opened the glutamate-gated chloride channel GluClα in the postsynaptic T4 dendrite, which decreased the input resistance and short-circuited the T4 neuron. If the two points in visual space were stimulated in the correct sequence, delayed disinhibition from one input coincided with excitation from its neighbor and amplified the postsynaptic response. The multiplicative supralinearity arose from a transient increase in input resistance—a 'window of opportunity' that opened up only during visual motion in the preferred direction. The peak in resistance depended on the direction of visual motion and on the presence of GluClα. Without the glutamate receptor, T4 neurons were locked in a state of high resistance and responded strongly to visual motion not just in one but in many directions. When navigating a virtual visual environment, flies whose T4 neurons lacked GluClα struggled to compensate for their inaccurate estimate of visual motion and, unlike their parental controls, failed to stay on course.

Multiplicative signal processing in T4 neurons does not depend on the well-known and rather rigid nonlinearities of voltage-gated ion channels. All it takes is the coincidence of excitation and release from shunting inhibition in a passive membrane. If the conductance decreases, but the current remains the same, then the voltage scales multiplicatively. This type of multiplicative disinhibition could constitute a canonical computation with the potential to endow a single neuron with a logic AND gate. In future research, I will investigate how different presynaptic neurons of virtually identical morphology act as temporal filters with vastly different time constants and how local motion information is integrated over time and space to inform behavioral choice.



A dye-filled glutamatergic neuron in the visual system of the fruit fly.

An animal's survival depends on its ability to adaptively forage between multiple potentially rewarding options. To guide these foraging decisions appropriately, animals learn associations between options and rewards. However, learning these associations in natural environments is often complicated by the uncertainty of rewards, and studies in both vertebrates and invertebrates have found that animals employ decision-making strategies that account for this uncertainty. One strikingly convergent strategy observed across the animal kingdom is to divide choices between options in proportion to the ratio of rewards received from each. Elegant theoretical work has identified a characteristic relationship between this ubiquitous operant matching strategy and a specific form of expectation-based learning rules that incorporate the covariance between reward and neural activity. In particular, it has been hypothesized that these covariance-based rules make use of the expectation of reward – the recency-weighted rolling average over past rewards – to learn option-reward associations. Despite this strong theoretical link between specific learning rules and matching, there has not been a mapping of these rules onto particular synapses or plasticity mechanisms in any animal. As a result, it has not been tractable to use modern neuroscientific approaches to probe these theories or manipulate and test the nature of learning rules underlying operant matching. Here, working in the relatively simple nervous system of the fruit fly, *Drosophila melanogaster*, we combine behavioral measurements, mathematical modeling, and neural circuit perturbations to map these learning rules onto specific synapses in the fly Mushroom Body (MB). Using a novel olfactory dynamic foraging task, we characterize the behavioral strategies used by individual flies when faced with unpredictable rewards and show, for the first time, that they perform operant matching (Fig.1A). We develop a novel analysis approach to identify the nature of learning rules used by the fly based on behavioral outcomes and show that operant matching depends on a covariance-based learning rule (Fig. 1B). Finally, using experiments where we bypass computation of reward expectation by training animals with direct activation of dopaminergic neurons that drive plasticity, we show that a covariance-based rule incorporating reward expectation calculated over multiple past trials (Fig. 1D) governs flies' matching behavior (Fig. 1C). Together these results provide compelling evidence for the role of reward expectation signals in the fly brain and offer the first synaptic level mapping and manipulation of learning rules underlying operant matching. The MB offers a tractable system in which we can understand and readily test the learning rules that underlie decision-making behaviors at a level of mechanistic detail that is presently unparalleled in the field of systems neuroscience. We plan to leverage this by proceeding with neural recordings of dopaminergic neurons in the MB and analysis of the electron-microscopic connectome of the fly brain to understand the mechanisms by which reward-expectations are calculated.

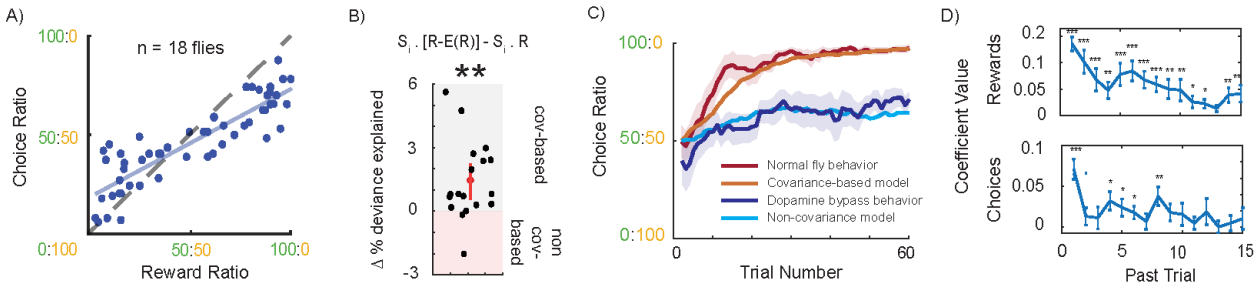


Figure 1: (A) Ratio of choices made by flies (18 flies, 3 blocks of different reward probabilities each) between the two available options, is approximately equal to ratio of rewards received from these options, following the operant matching strategy. (B) Explanatory power (percentage deviance explained) of a model that uses a covariance-based rule to predict fly behavior is greater than a non-covariance-based model (Wilcoxon signed-rank test: p = 0.0018). (C) Dopamine bypass experiment that prevents calculation of reward expectation modifies normal behavior such that manipulated behavior more closely resembles that predicted by non-covariance learning rule. (D) Reward expectations are calculated using up to 15 trials worth of past reward information; estimated using logistic regression.

Learnable latent embeddings for joint behavioral and neural analysis

Graduate Student

Neuroscience and the life sciences in general are generating increasingly large datasets, from recording millions of neurons over long timescales, RNAseq databases with thousands of gene expressions, over cell cultures exhibiting complex interactions, to behavioral recordings of animals over the spans of weeks, months, or even years. There is a great variety of recording sources, like video, audio, images, behavior, gene expressions, connectomes, neural activity, *etc*. Likewise, experiments in neuroscience use stimuli from a variety of modalities, ranging from vision, language, audio to olfaction and proprioception.

Despite these differences between recording setups and experiments, analysis goals are often shared. **We want to infer, probe and understand the "hidden causes", or latent variables and their dynamics when studying biological systems**. These dynamics can have different underlying structure, and unroll on different time scales. Up to date, there is no unifying inference framework for non-linear system identification under these constraints.

Towards this goal, we built a **contrastive learning framework for estimating Consistent EmBeddings of high-dimensional Recordings using Auxiliary variables (CEBRA; under revision at Nature)**. CEBRA is a nonlinear representation learning algorithm that can be used for mapping behavioral actions to neural activity, based on time information as well as continuous, discrete or mixed labels associated with data points. It produces consistent latent spaces for hypothesis- or discovery-driven analysis.

The algorithm maps raw signals from a given input modality (e.g., 2P imaging or electrophysiology, or kinematic data) into a low-dimensional embedding space. Within this embedding space, neighborbood between points is informed by the similarity of one or more auxiliary variables. The algorithm is highly flexible with respect to the choice of these variables; within a single inference framework, we can learn embedding spaces shaped based on time information only (neural activity close in time will be mapped to close points in embedding space), or leverage behavior (neural activity recorded along with the same behavior will be mapped close in the embedding space). Within these usage modes, we provided theoretical guarantees on the identifiability of the algorithm, and demonstrated that these guarantees also hold empirically. We were able to demonstrate the effectiveness of CEBRA under realistic conditions, and showed consistent and highly decodable embedding spaces across recording modalities (calcium and electrophysiology datasets), across sensory and motor tasks, and in simple or complex behaviors across species (rats, mice, primates).

CEBRA is one instantiation of a type of learning algorithms narrowing the "identifiability gap" that exists between discovery-driven analysis and exploration and the precise hypothesis testing that follows. In my future research group, **my north star objective is to propel data driven scientific discovery by closing the identifiability gap, fully automating the repetitive components of scientific inference and moving towards automated data science in neuroscience.** Developing novel scientific inference tools will make conclusions more reproducible and robust, and in experiments requiring animal experimentation, improve alignment with animal ethics by making more effective use of data, thereby reducing the amount of animal experiments. Narrowing the identifiability gap has the potential of drastically increasing our capabilities for scientific discovery, coming up with and testing new complex hypotheses of brain function and other biological systems, and innovating within various other areas (e.g., cell biology) within the life sciences.

Besides the algorithmic and technical innovation, I find it crucial to develop methods with a concrete analysis problem in mind (in my case, understanding the changes in latent space dynamics across trials during sensorimotor adaptation) and in close collaboration with experimentalists with additional domain knowledge. At NeuroMEETS, I therefore hope to engage with other researchers to present CEBRA, discuss the future of neural data analysis, and the new ways in which we can merge machine learning and computational neuroscience to help our understanding of the brain.

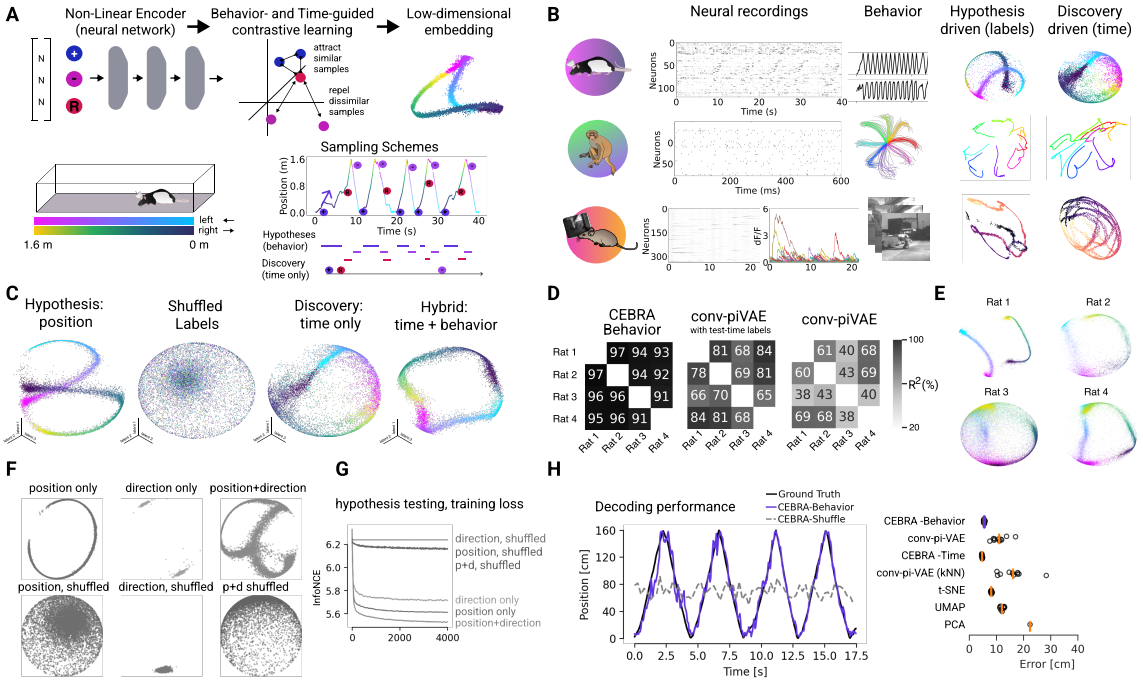
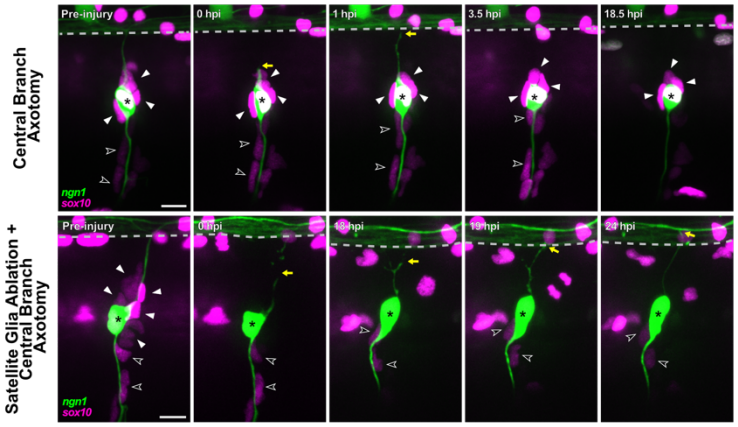


Figure 1: CEBRA: (A) Model overview & sampling schemes. (B) Embeddings obtained for different recording modalities. (C) hypothesis vs. discovery-driven usage with controls. (D) Consistency and (E) embeddings across subjects. (F) Hypothesis-driven application and (G) metrics. (H) Decoding position from the embedding.

Across the menagerie of life, organisms exhibit an equally profound diversity in the capacity to regenerate damaged or lost tissue. Among animals, we see extreme cases such as whole-body (*Hydra, Planaria*) and limb regeneration (Echinoderms, Axolotl, lizards). While humans have the innate ability to replace cells (intestinal epithelia, blood cells, skin), we lack the extraordinary regenerative capacities exhibited by other species. This discrepancy is particularly evident in the nervous system: neurological disorders are one of the leading causes of disability and death worldwide. Researching our more regenerative relatives has led to some understanding of the cell-intrinsic and extrinsic factors that contribute to nerve regeneration, yet much remains unknown. For example, zebrafish (*Danio rerio*) have a notorious capacity to regenerate (cardiac tissue, fins, the spinal cord, motor nerves, peripheral branches of sensory nerves). Yet, like most vertebrates, they are remarkably inefficient at regenerating the central branch of sensory nerves—a crucial structure that relays peripheral sensory information to the central nervous system via the spinal cord. This conundrum presents an exciting opportunity to investigate a common phenomenon across taxa using a tractable model with critical implications in human health and well-being.

With these goals in mind, my research addresses how glia modulate sensory neuron regeneration in dorsal root ganglia (DRG) of larval zebrafish. At three days post-fertilization, the zebrafish DRG consists of 1-2 neurons with a variety of glia: satellite glial cells (SGC) ensheath the neuronal soma while Schwann cells (SC) and Schwann cell precursors (SCP) inhabit the peripheral axon branch. To probe regeneration dynamics, I utilize *in vivo* confocal microscopy and a pulsed-nitrogen laser to perform a precise central branch axotomy on the DRG sensory neuron and timelapse image for 15+ hours. Central branches often fail to regenerate into the spinal cord as expected. However, DRG glia, particularly SGCs, react to the injury by migrating dorsally to the site of neuronal axon initiation. Using quantitative cell tracking, I determined that DRG glia migrate faster, further, and more directionally after central branch axotomy. To test how this reactive behavior impacts regeneration, I removed SGCs just prior to central branch axotomy using laser ablation or via a novel transgenic tool that specifically kills peripheral glia. In these experiments, sensory neurons almost double their rate of regeneration, suggesting that DRG glia indeed inhibit central branch regeneration. Preliminary findings from these experiments also show that in some cases, SCs and SCPs aberrantly extend processes from the peripheral branch and ensheath the neuronal soma, preventing any new axon growth.



DRG of *Tg(ngn1:GFP);(sox10:NLS-Eos)* zebrafish, with a *ngn1*$^{+}$ sensory neuron (asterisk) and *sox10*$^{+}$ glia (SGCs, white arrowheads; SC and SCPs, black arrowheads) under different injury paradigms. Arrow indicates central branch tip, which fails to regenerate into the spinal cord (dashed line) after central branch axotomy (Top) but succeeds when preceded by SGC ablation (Bottom).

Using *in vivo* imaging, new genetic reagents, and a variety of cellular manipulations, my research describes novel mechanisms of how glia inhibit regeneration into the central nervous system from peripheral sensory neurons. Ongoing experiments will further elucidate the key molecular signaling pathways involved in this glial inhibition, including those responsible for damage detection, debris clearance, reactive migration, and atypical ensheathment. This work not only presents a unique extrinsic mechanism of regeneration failure; it further develops our understanding of how neuron-glia interactions change across physiological conditions and lays the groundwork for the development of new treatments of somatosensory dysfunction, disorder, and disrepair.