Influence of neuromodulation on brain state transitions in larval zebrafish

Antoine Légaré [1], Vincent Boily [1], Sandrine Poulin [1], Mado Lemieux [1], Patrick Desrosiers [1,3,4], Paul De Koninck [1,2]

[1] CERVO Brain Research Centre, Québec (QC). Université Laval, Québec (QC) Canada: [2] Département de biochimie, de microbiologie et de bio-informatique
[3] Département de physique, de génie physique et d'optique [4] Centre Interdisciplinaire en Modélisation Mathématique de l'Université Laval (CIMMUL)

Introduction

Whole-brain calcium imaging in transparent larval zebrafish offers an unprecedented opportunity to study **mesoscopic** neuronal dynamics at **cellular resolution**[1]. Using a recent multimodal zebrafish brain atlas, we investigated how functional connectivity across brain regions is constrained by structural pathways. We then identified spontaneously reoccurring brain states whose spatial footprints were tightly constrained by structural communities. Our goal is to link these states and their transitions to the activity of **neuromodulators**, as their influence on large-scale brain dynamics remains poorly characterized.

Methods

To investigate mesoscopic brain networks, we built a multimodal imaging system to measure whole-brain neuronal activity in 6-8 dpf transgenic larvae expressing a pan-neuronal calcium sensor[2] using resonant piezo-scanning two-photon microscopy while monitoring tail movements with a high-speed camera.

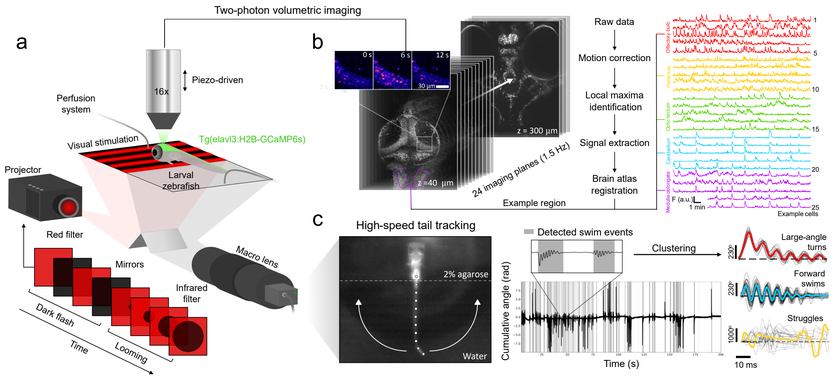


Figure 1: a) Neuronal and behavioral imaging configuration; b) whole-brain calcium imaging pipeline; c) high-speed tail tracking of head-restrained larvae and identification of distinct behavioral events.

We use a dual registration approach to map calcium imaging volumes in a brain atlas[3] and to identify neuromodulators in functional imaging planes. Larvae are stained for dopamine (DA), norepinephrine (NE) and serotonin (5HT) after imaging experiments, then their brains are re-imaged and warped onto in vivo data[4].

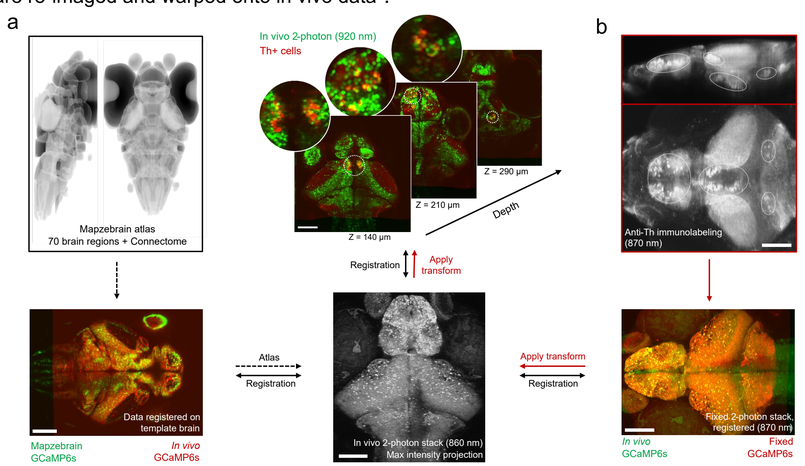


Figure 2: a) Brain atlas registration pipeline using ANTs registration; b) post-mortem brain registration pipeline; stainings are warped on top of piezo imaging planes while preserving single-cell precision (circular inset). Scale bars: 100 microns.

Results

Mesoscopic dynamics are structurally constrained

Mesoscopic functional connectivity, defined as the pairwise correlation of neuronal activity across brain regions, exhibits striking similarity with the underlying connectome. Correlations increase with the number of structural pathways between regions, and a linear model of structural properties explains 78.4% of the measured temporal correlations.

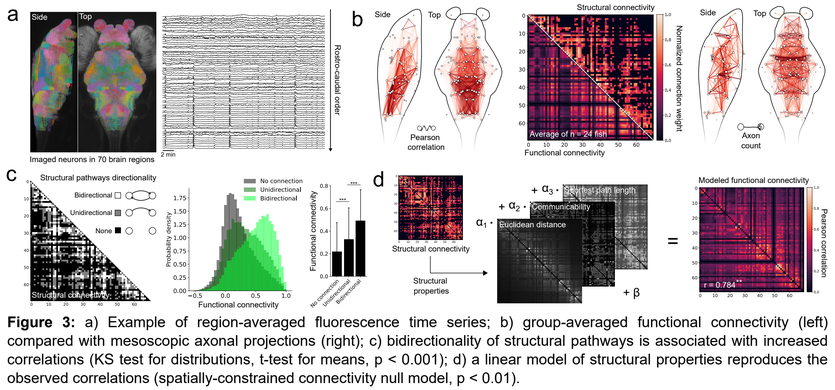


Figure 3: a) Example of region-averaged fluorescence time series; b) group-averaged functional connectivity (left) compared with mesoscopic axonal projections (right); c) bidirectionality of structural pathways is associated with increased correlations (KS test for distributions, t-test for means, $p < 0.001$); d) a linear model of structural properties reproduces the observed correlations (spatially-constrained connectivity null model, $p < 0.01$).

Unsupervised identification of recurrent brain states

To investigate transient changes in mesoscopic dynamics, we used an unsupervised clustering approach at the single frame level to identify recurrent patterns of regional activity, or brain states. We observed a rich repertoire of states with different activity configurations.

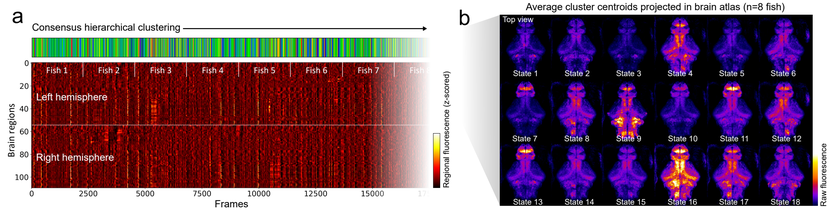


Figure 4: a) Regional time series are clustered along the temporal axis to identify recurrent states of activity across time and individuals, 30 min experiments; b) subset of cluster centroids (k = 20) registered in brain atlas, ordered by prevalence.

Temporal and spatial properties of brain states

Brain states identified using clustering are typically short-lived, but some can last over ten seconds. State transitions are stochastic but not uniform: some states are more likely to transition into each other. The coactivation patterns of individual states exhibit striking modularity which overlaps significantly with structural modules from the connectome.

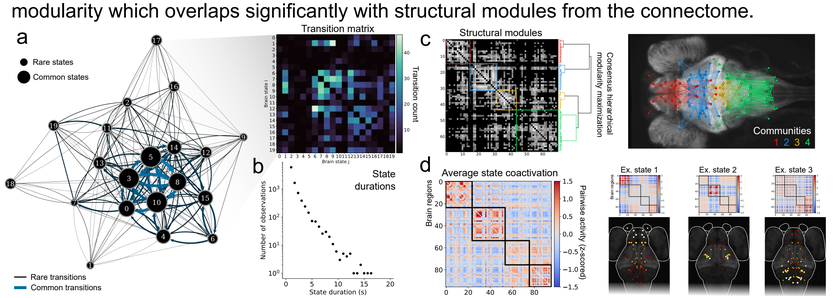


Figure 5: a) Markov graph of transitions between brain states identified through consensus clusering; b) heavy-tailed distribution of state durations; c) 4 structural communities identified using consensus modularity maximization; d) average state coactivation matrix and individual states; black boxes correspond to structural modules in c).

Reproducibility of spontaneous brain states

We measured very stable functional networks and states in 6-8 dpf larvae, replicated across 2-photon and light-sheet modalities[5] (not shown). Individual larvae could be recognized across consecutive imaging days using functional connectivity fingerprinting[6] (n = 9 fish).

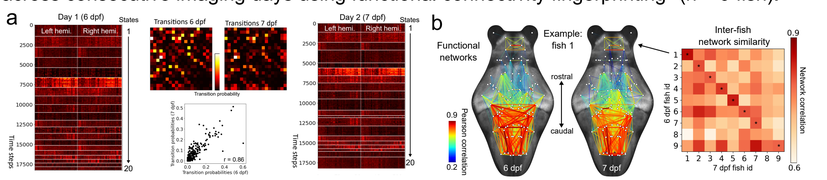


Figure 6: a) Similar brain states and transitions are measured on consecutive days; b) top view of a functional network on consecutive days (left), identity recovery using functional connectivity (right, 8/9 fish identified, max correlation criterion).

Future directions

Predicting behavior from internal states

Some states are directly associated with ongoing motor activity. Linear regression with tail tracking to retrieve motor-correlated cells highlights a spatial domain similar to the main motor state. Our next goal is to study if some internal states can predict upcoming behavior.

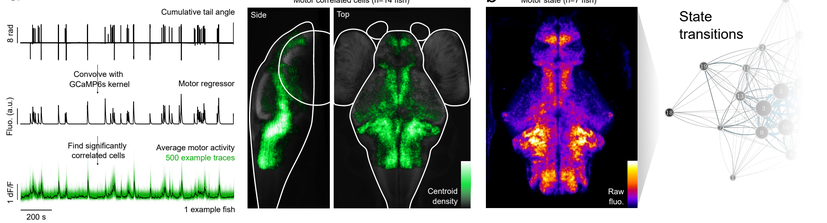
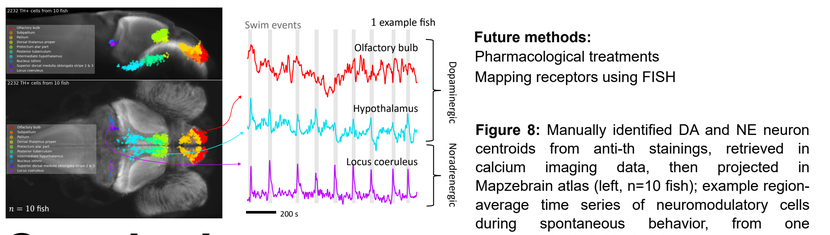


Figure 7: a) Behavioral regressors are generated by identifying swim events in the cumulative trace angle signal, then convolving with a slow GCaMP kernel. Significantly correlated cells are identified using a temporal permutation test ($p < 0.01$); b) average motor state projected in a zebrafish brain atlas.

Influence of neuromodulation on state transitions

Using immunofluorescence to retrieve neuromodulators, our preliminary data shows DA and NE cells are unsurprisingly active during swim events. Our next goal is to study their more subtle influence on internal state transitions[7], sensory processing, and behavior.



Future methods:
Pharmacological treatments
Mapping receptors using FISH

Figure 8: Manually identified DA and NE neuron centroids from anti-th stainings, retrieved in calcium imaging data, then projected in Mapzebrain atlas (left, n=10 fish); example region-average time series of neuromodulatory cells during spontaneous behavior, from one representative individual (right).

Conclusion

We have combined mesoscopic functional and structural measurements in the larval zebrafish brain to replicate many observations from the human neuroimaging literature, including the well-known connectome fingerprinting of individuals. Our state-based approach is inspired by recent efforts to understand mesoscopic networks at a finer temporal scale, revealing multiple recurrent patterns of activity with rich temporal and spatial features. By expanding our experimental framework, we will study how these internal state fluctuations are guided by neuromodulatory signalling.

Funding:
A.L. is supported by a studentship from the *Unifying Neuroscience and Artificial Intelligence in Quebec* Center

References: **1**. Bezel, R.F. (2020), Network Neurosci. **2**. Vladimirov et al. (2014), Nat. Methods. **3**. Kunst et al. (2019), Neuron. **4**. Lovett-Barron et al. (2017), Cell. **5**. Chen et al. (2018), Neuron. **6**. Finn et al. (2015), Nat. Neurosci. **7**. Marques, et al. (2020), Nature.