The influence of neuromodulators on brain state transitions in larval zebrafish

Antoine Légaré

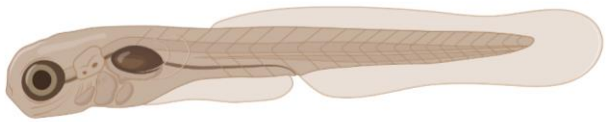
Supervision:
Paul De Koninck
& Patrick Desrosiers

CENTRE DE RECHERCHE
CERVO
BRAIN RESEARCH CENTRE

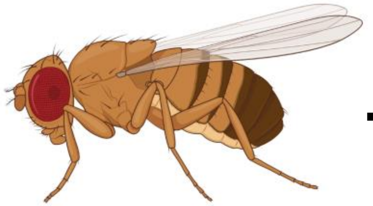

Larval zebrafish brain
>100 000 neurons
Spinal cord
Eye
Eye
Tg(*elavl3*:H2B-GCaMP6s)

Whole-brain imaging in small animals

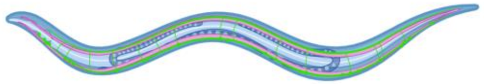
Small animal models allow us to see almost *everything*



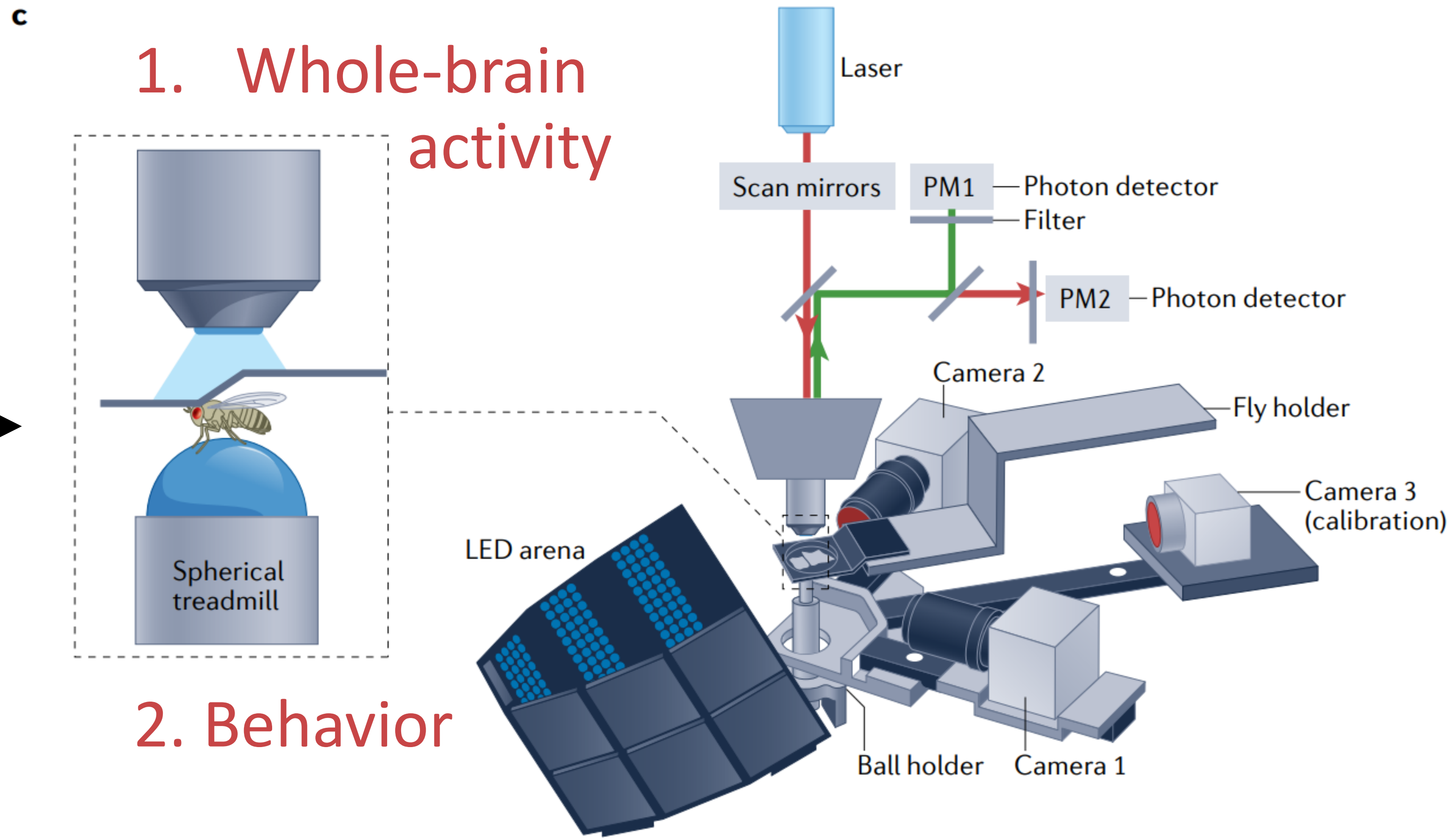
Larval zebrafish



Drosophila



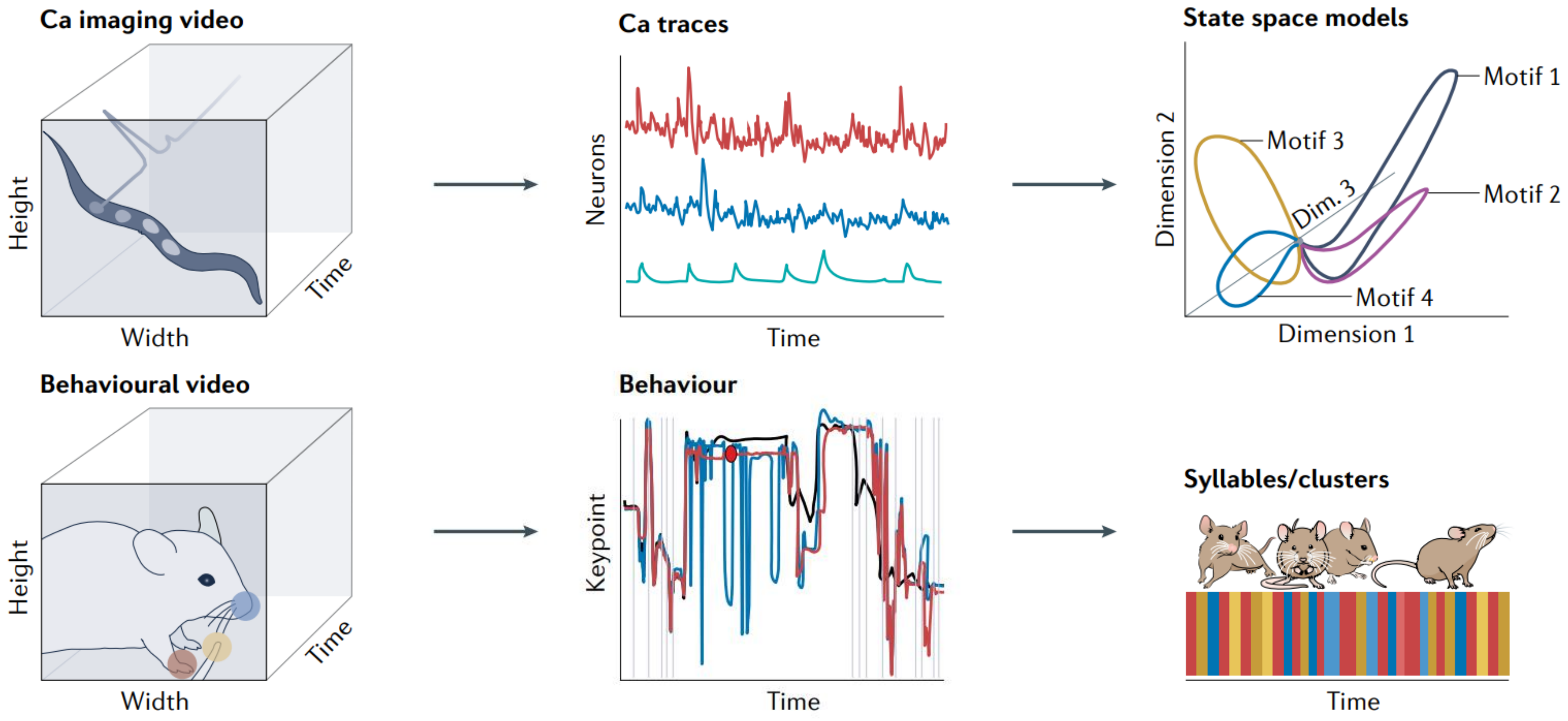
C. elegans



Lin, Albert, et al. "Imaging whole-brain activity to understand behaviour." Nature Reviews Physics (2022).

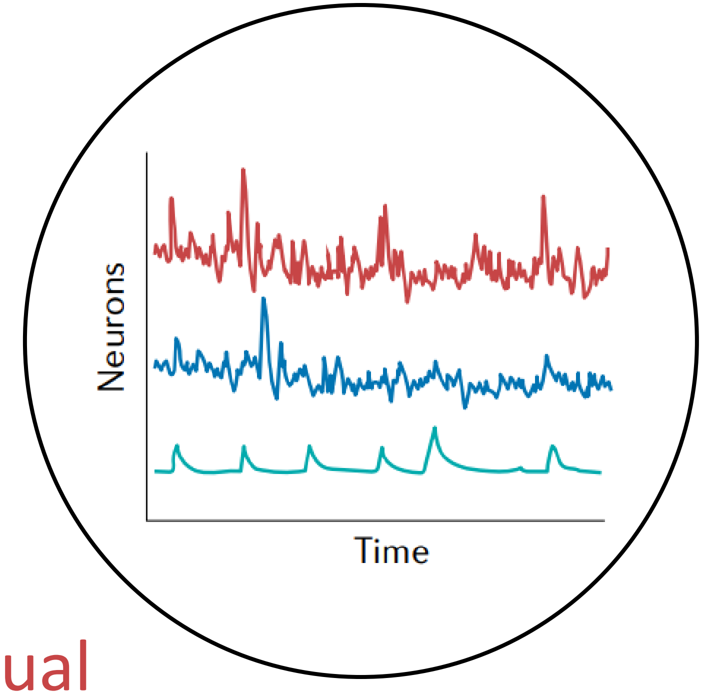
Whole-brain imaging in small animals

Small animal models allow us to see almost *everything,* but is it *too much*?

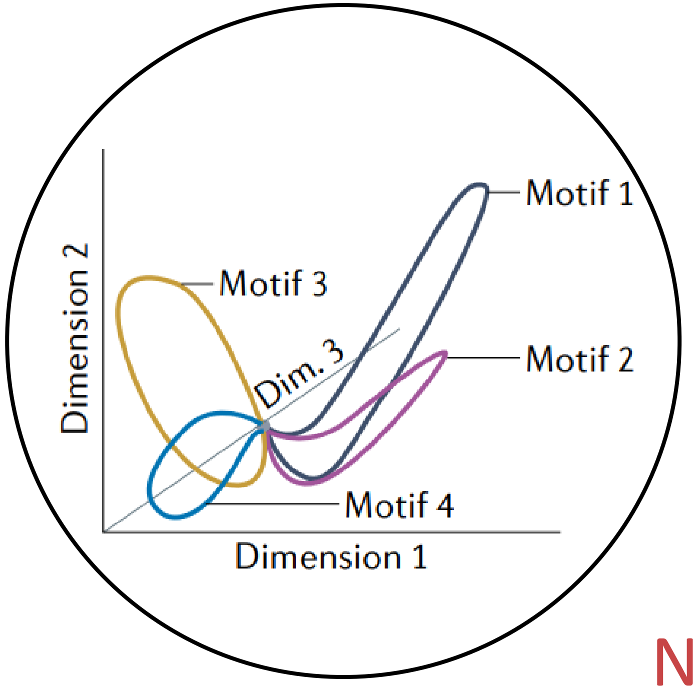


High-dimensional → Low-dimensional

Lin, Albert, et al. "Imaging whole-brain activity to understand behaviour." Nature Reviews Physics (2022).

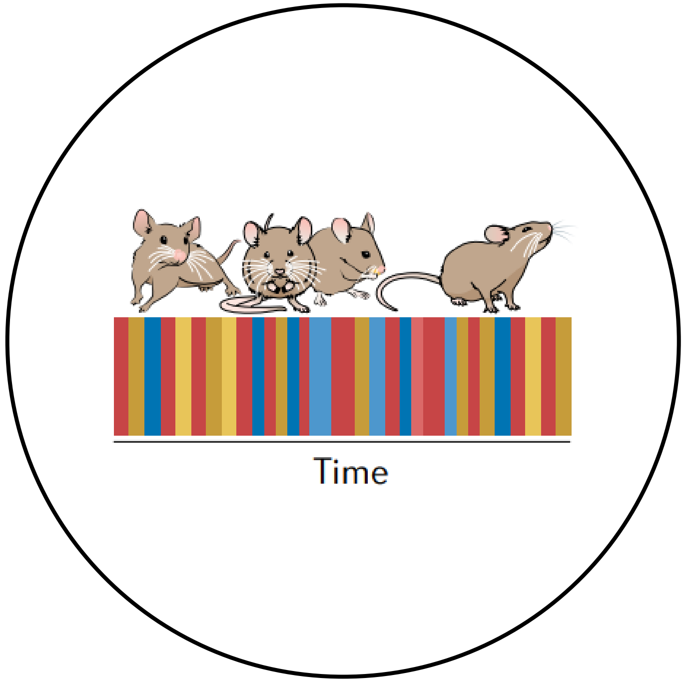


Individual neurons



Neuronal populations

Behavior



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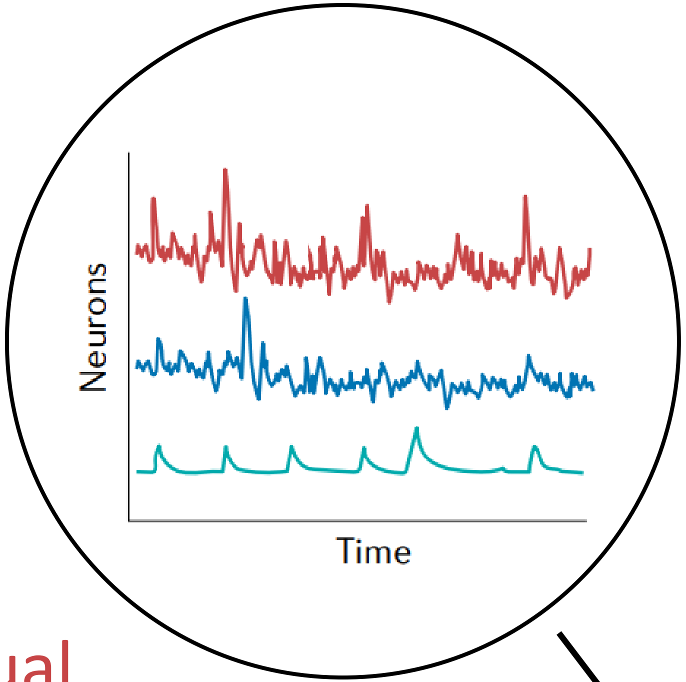
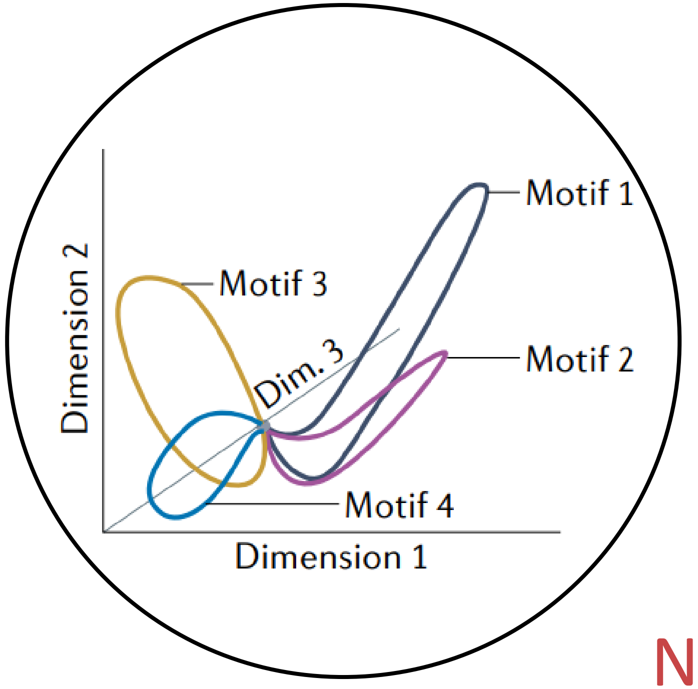


Individual neurons



Neuronal populations

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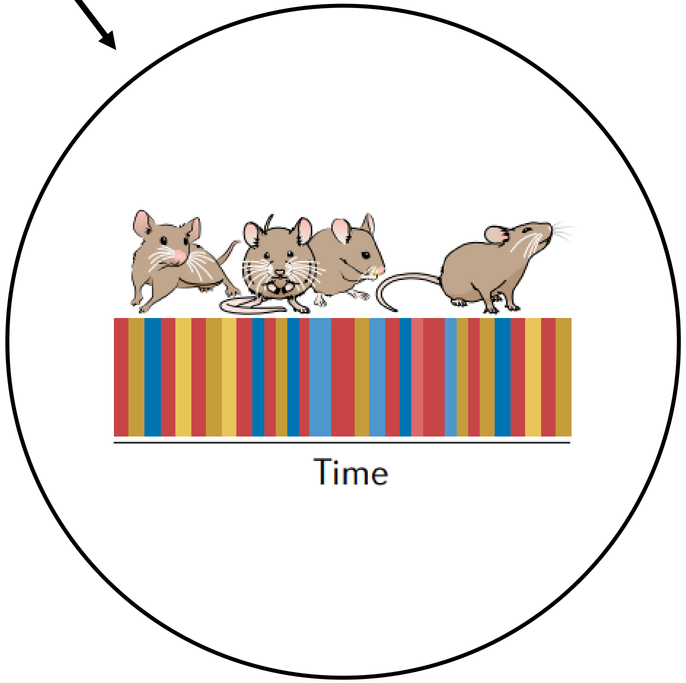

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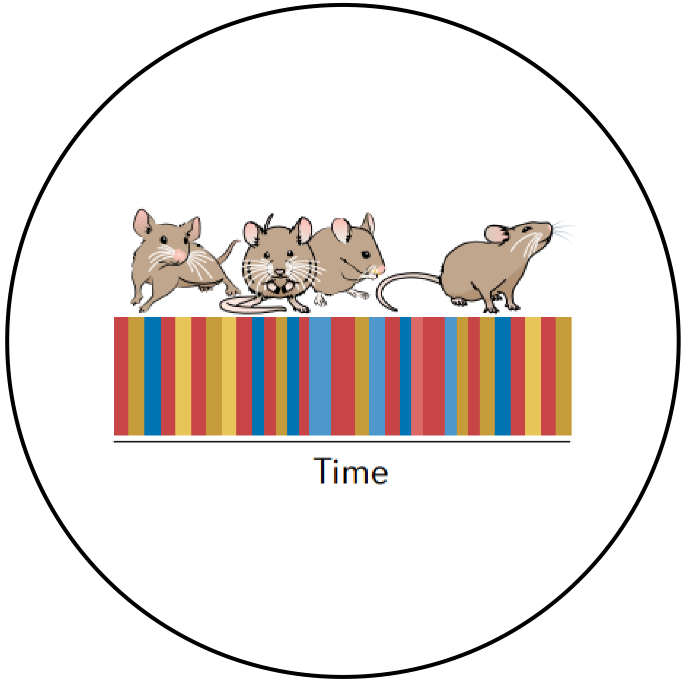
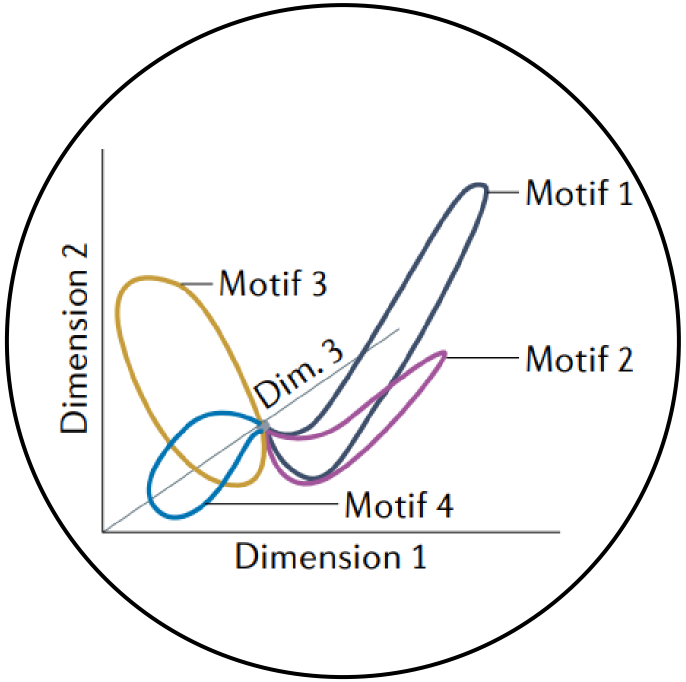
Discrete state decomposition



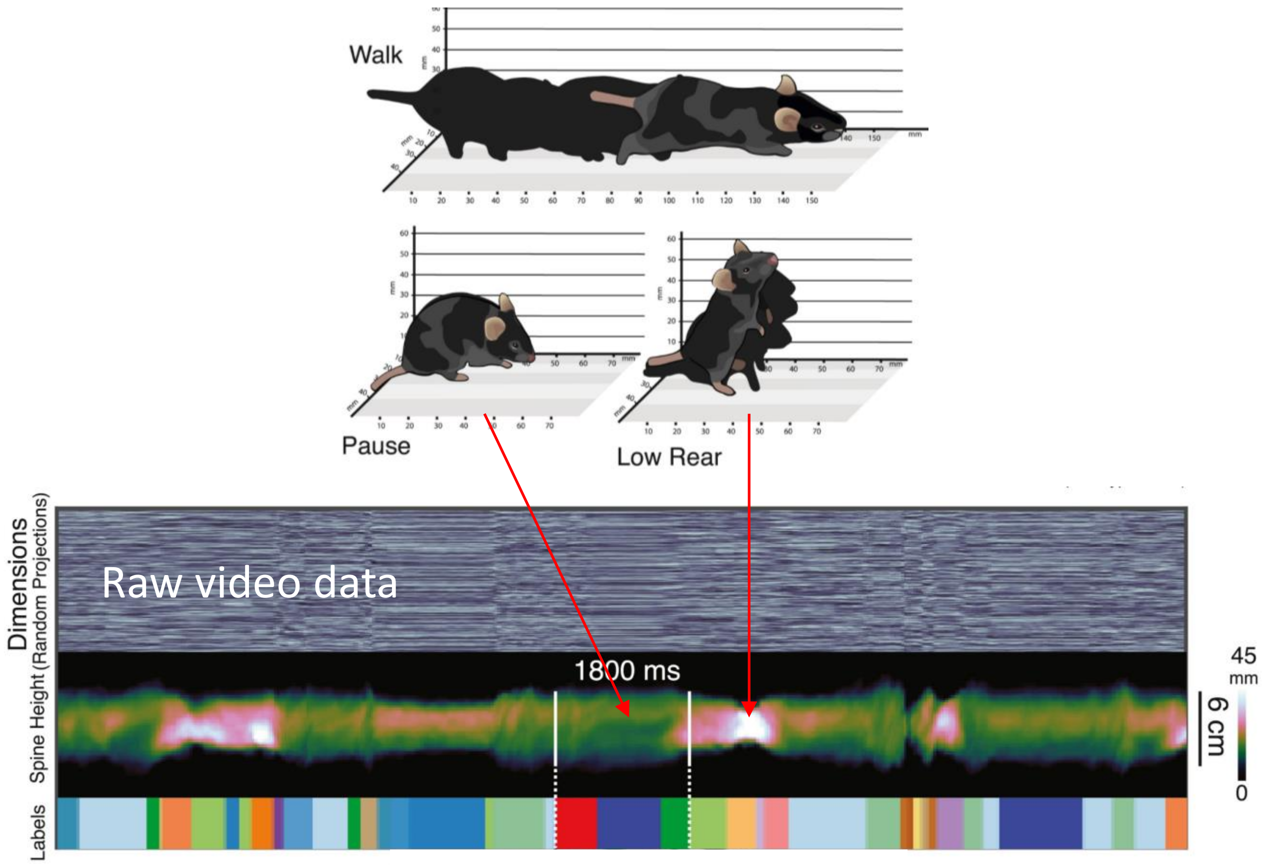
Behavior



Neuronal populations

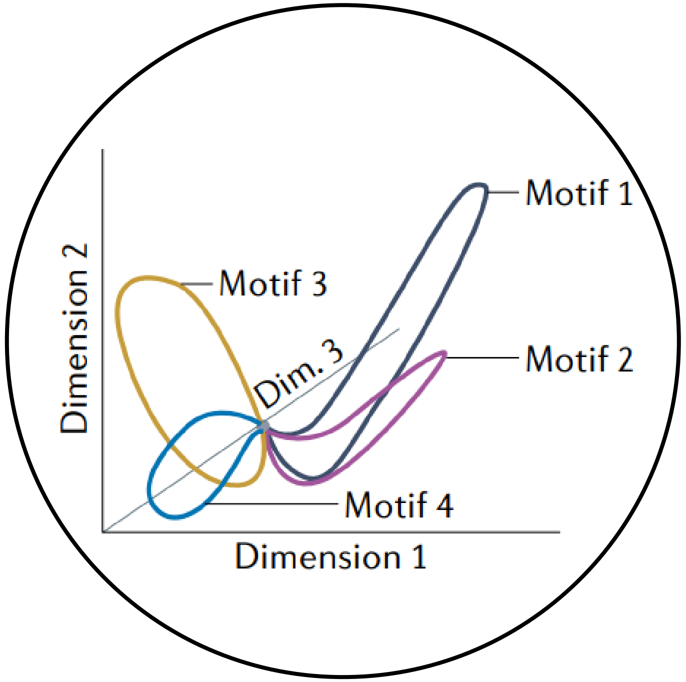
Discrete state decomposition

Markov chains have been applied to a wide array of problems in neuroscience



Behavioral states

Wiltschko *et al.* *Neuron (2015)*



Neuronal populations

Cornblath *et al.* *Communications biology* (2020)

Discrete state decomposition

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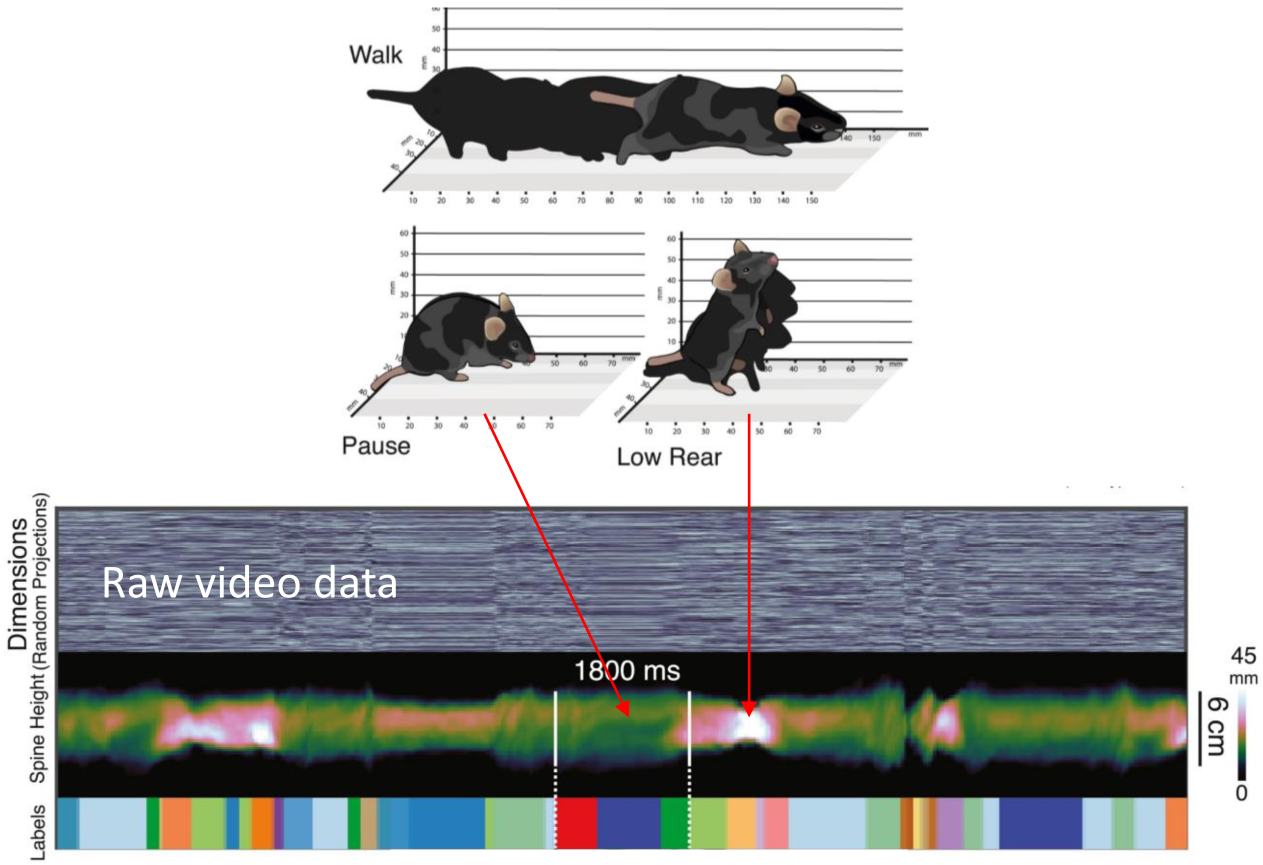

Behavioral states

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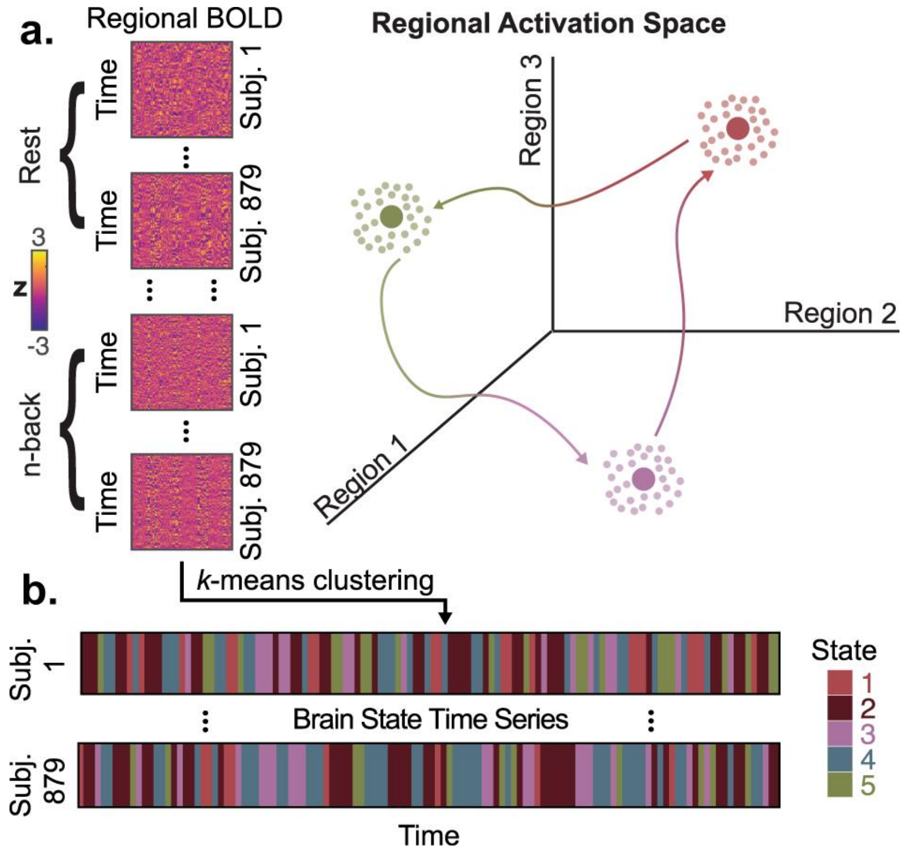
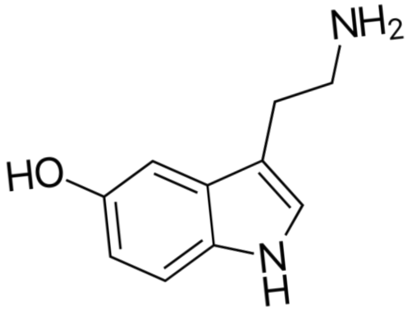
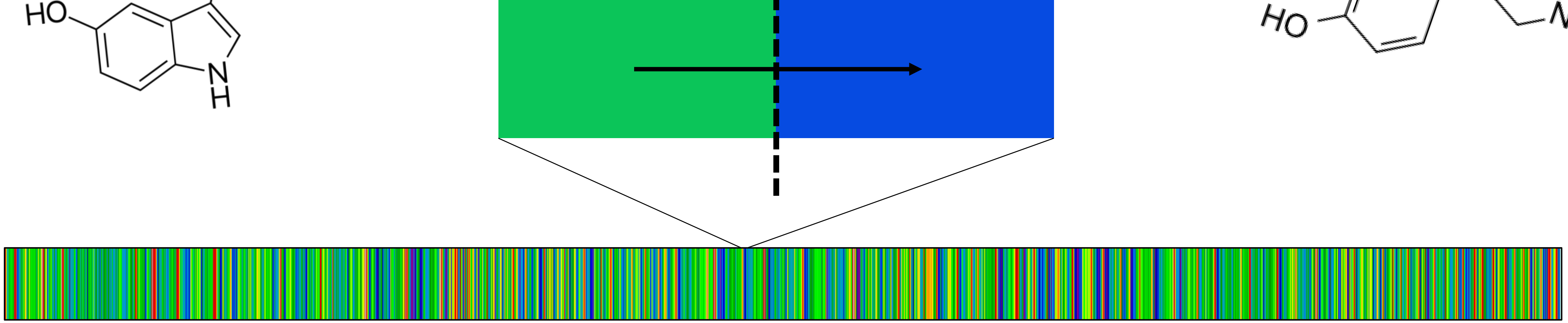


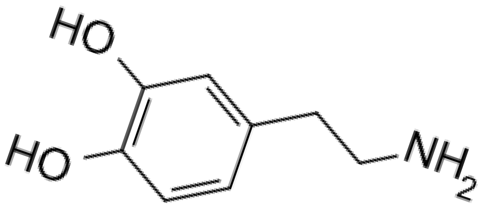
Brain states

Cornblath *et al.* *Communications biology* (2020)

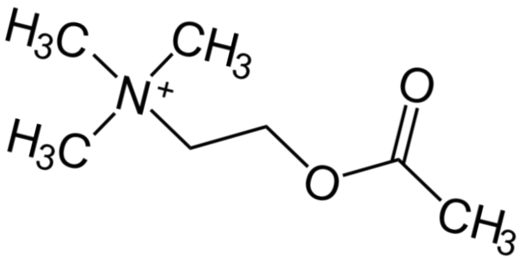
What happens at transitions?

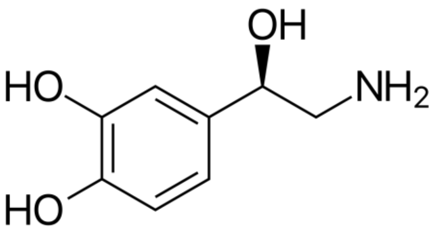






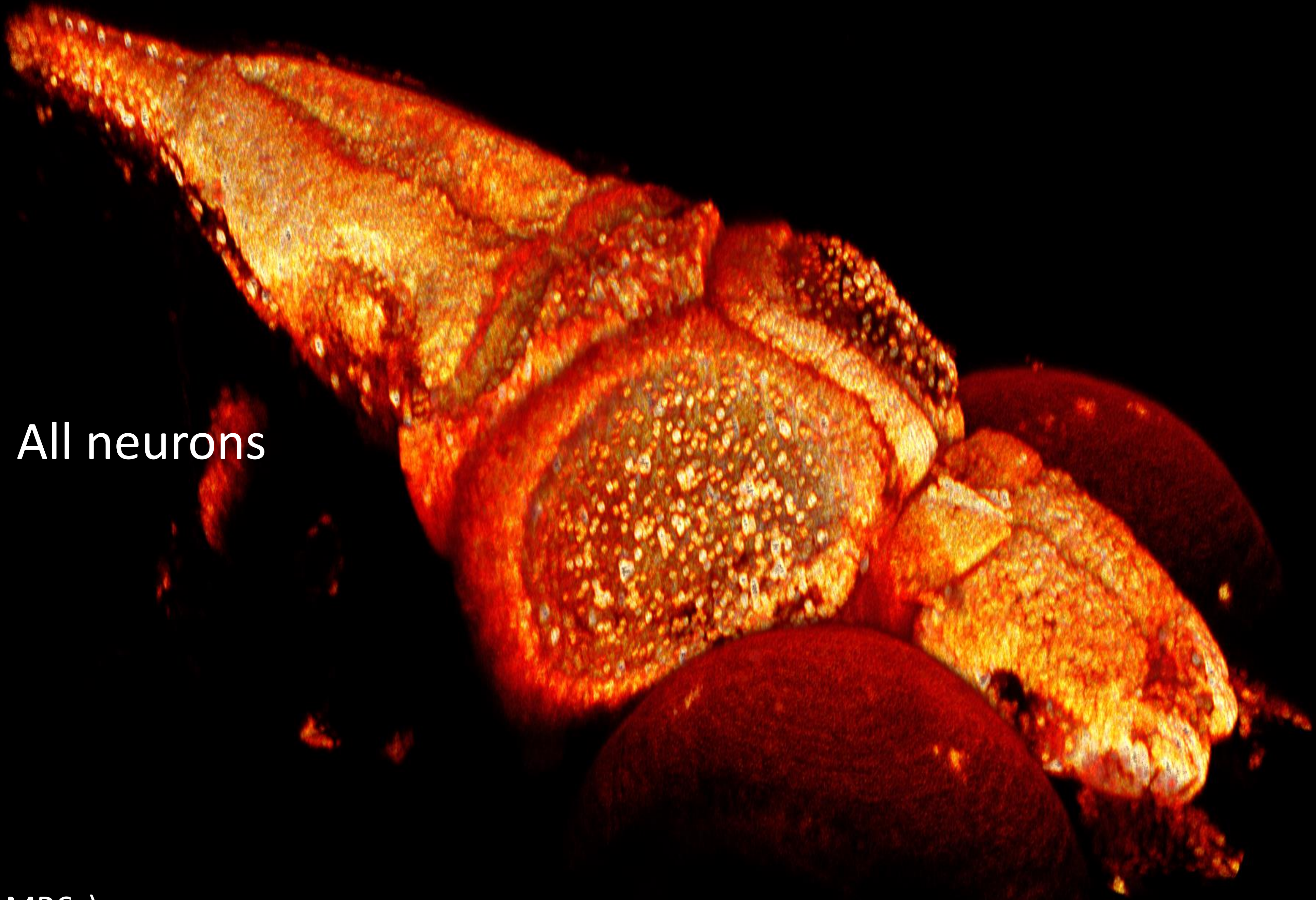
Brain states





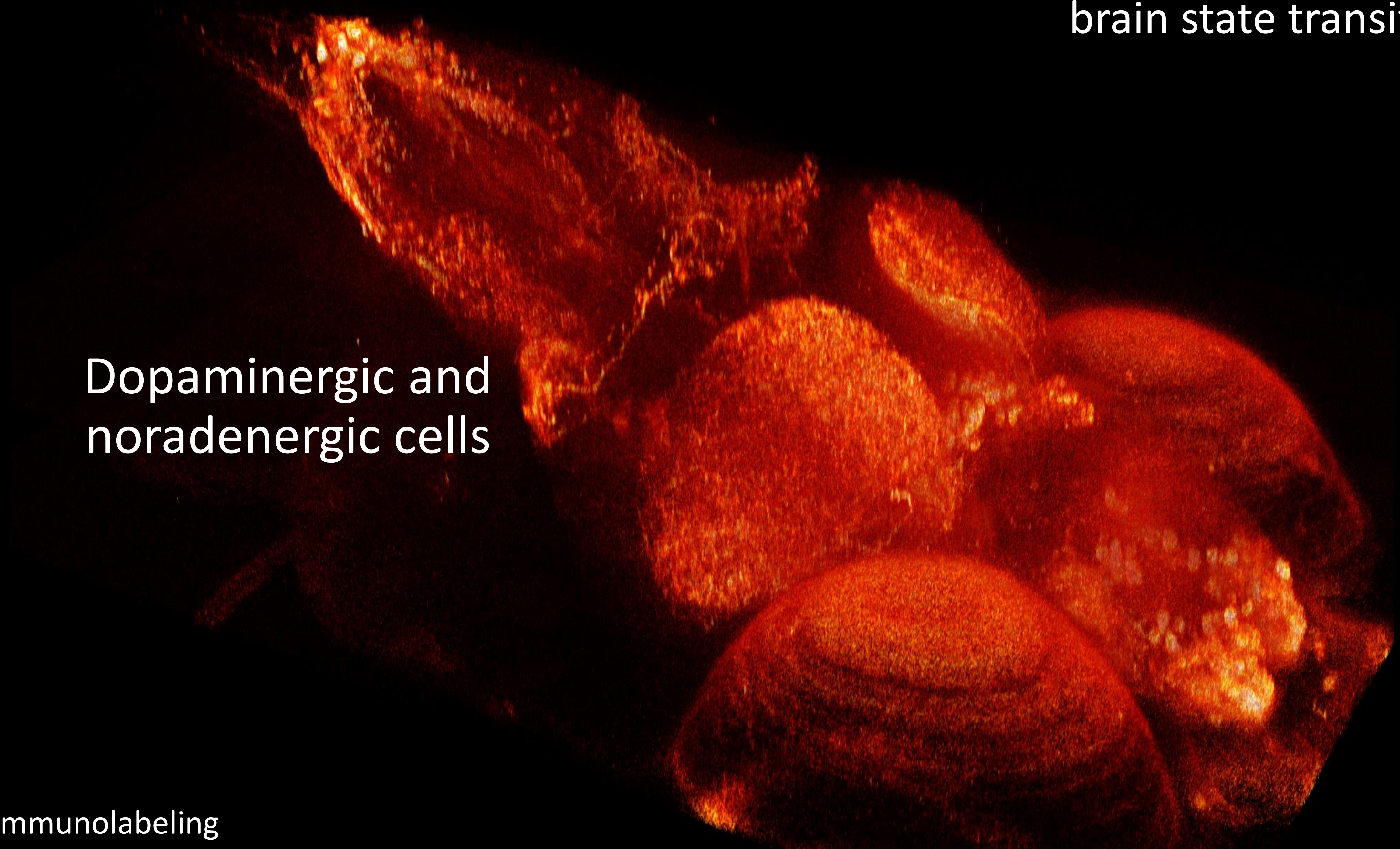
Are these state transitions the result of purely **excitatory/inhibitory** dynamics?

Or do they require a little bit more help?



Tg(*elavl3*:H2B-GCaMP6s)

If/how neuromodulators control brain state transitions

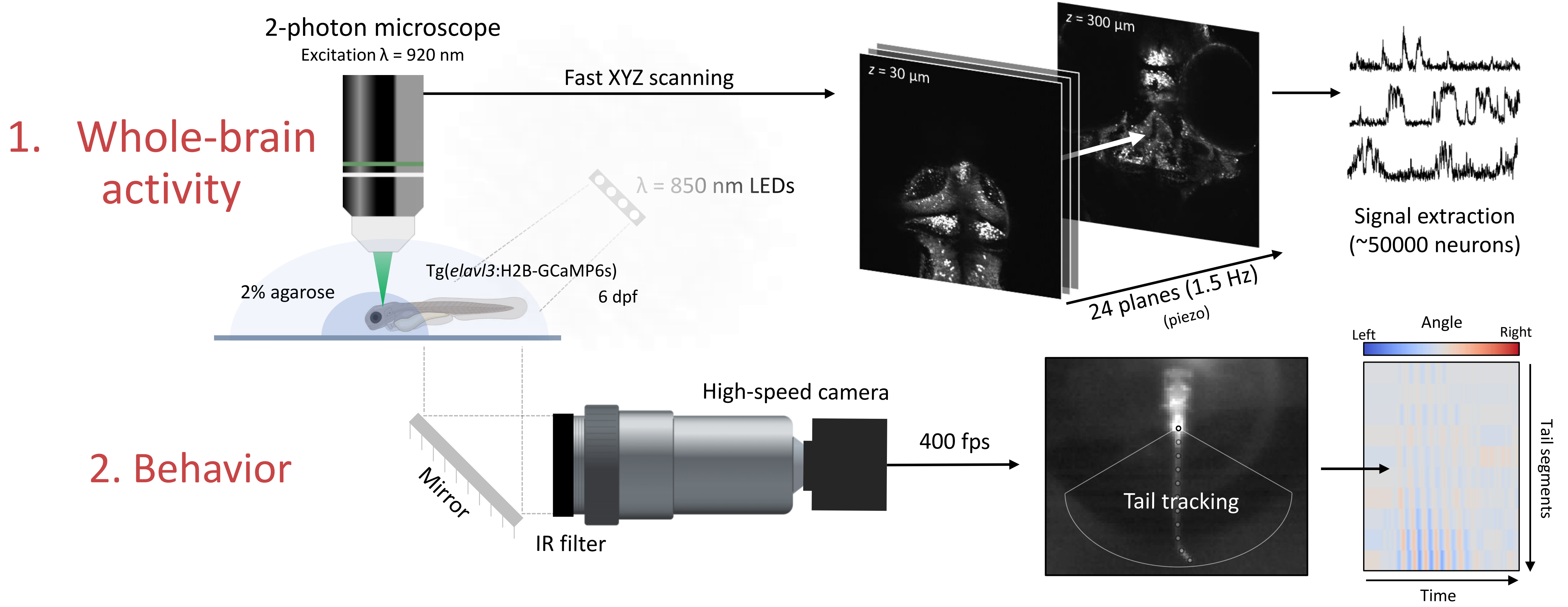


Anti-th immunolabeling

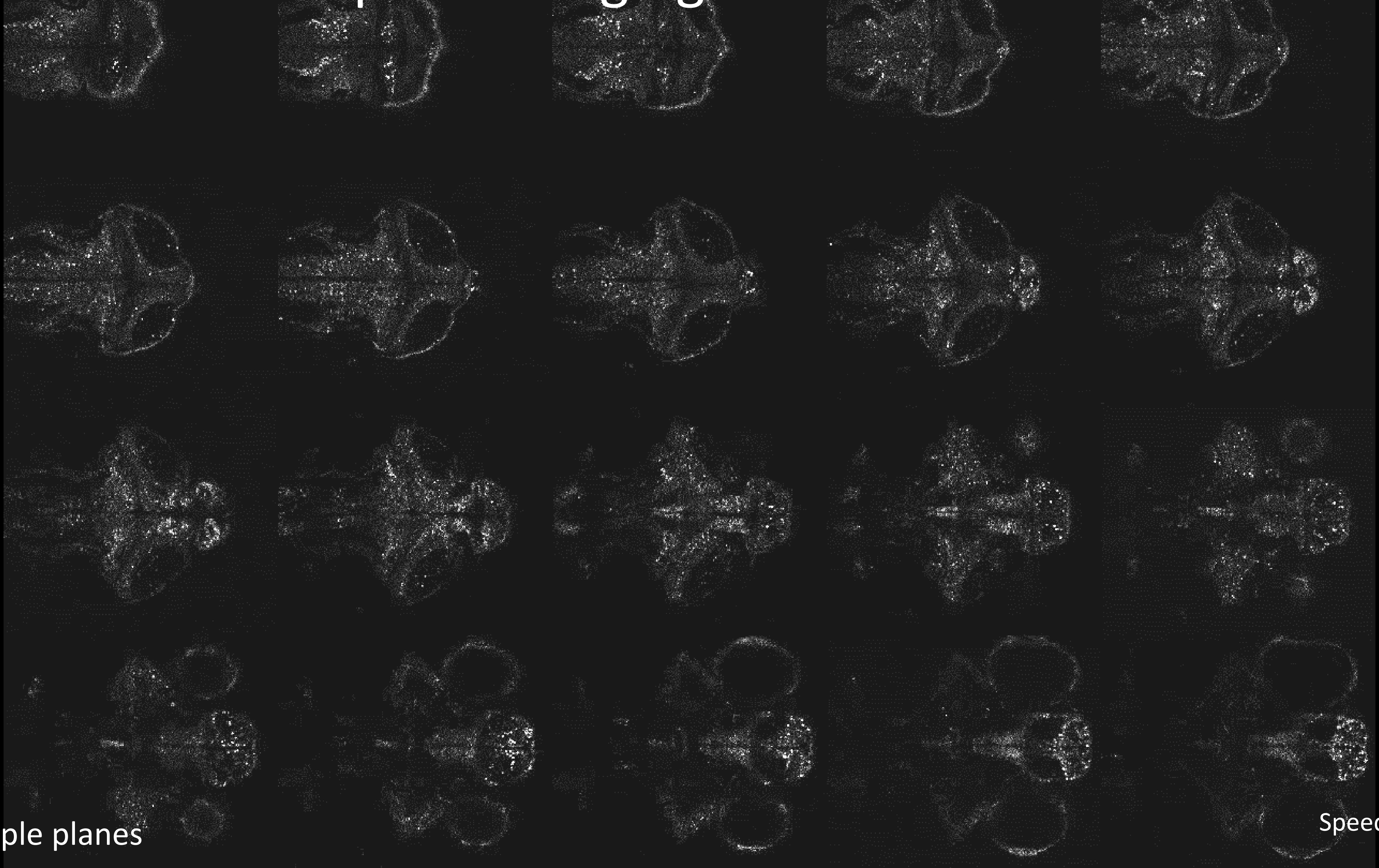
1. Measuring brain activity and behavior

Experimental setup

Core elements of our microscopy setup



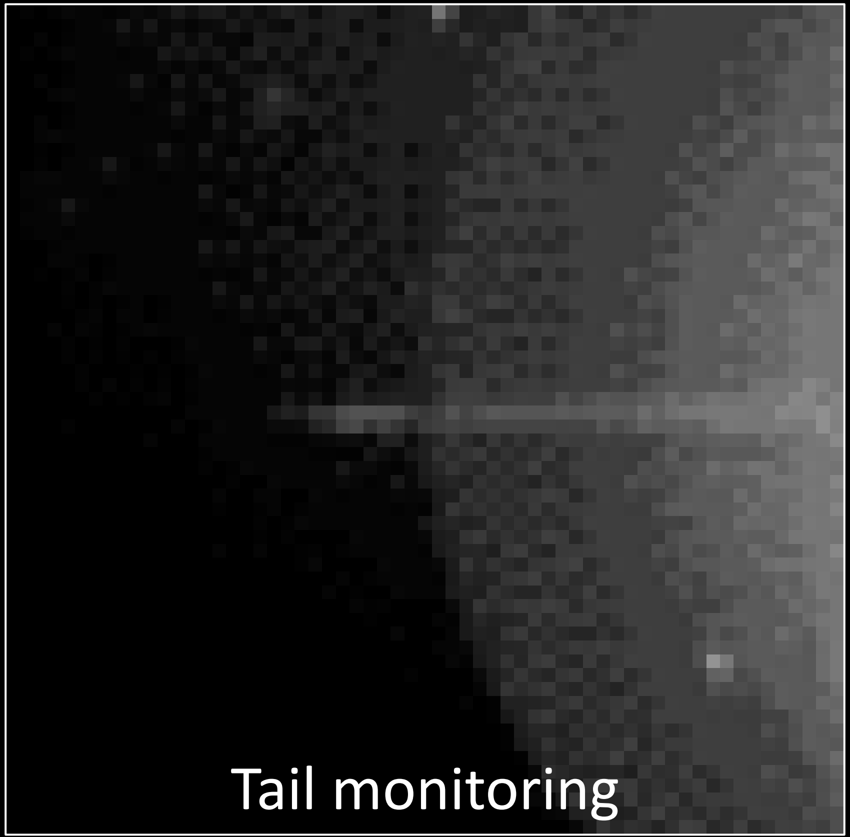
Whole-brain multi-plane imaging



20 example planes

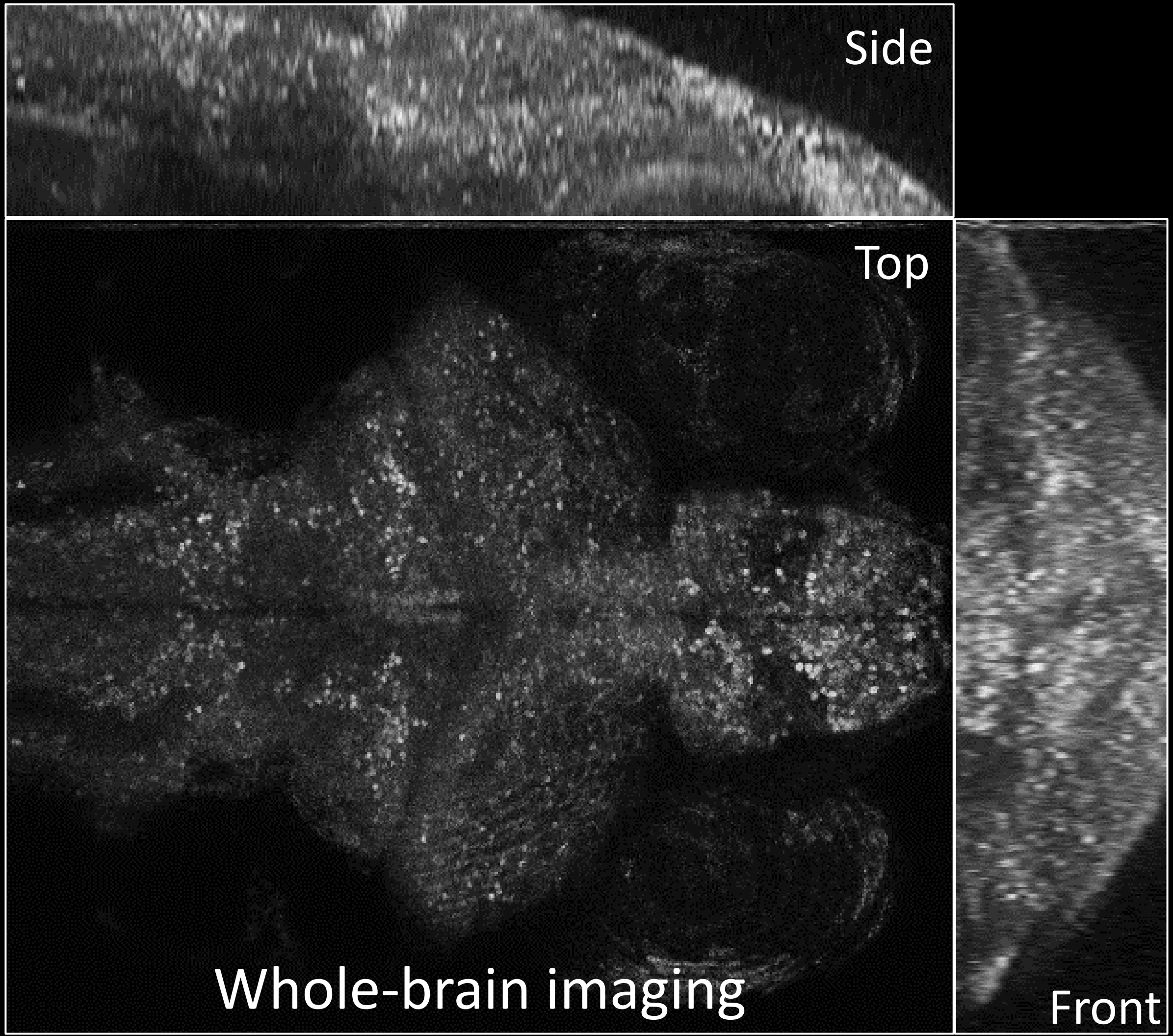
Speed 40x

Calcium imaging + Behavior



400 fps

...



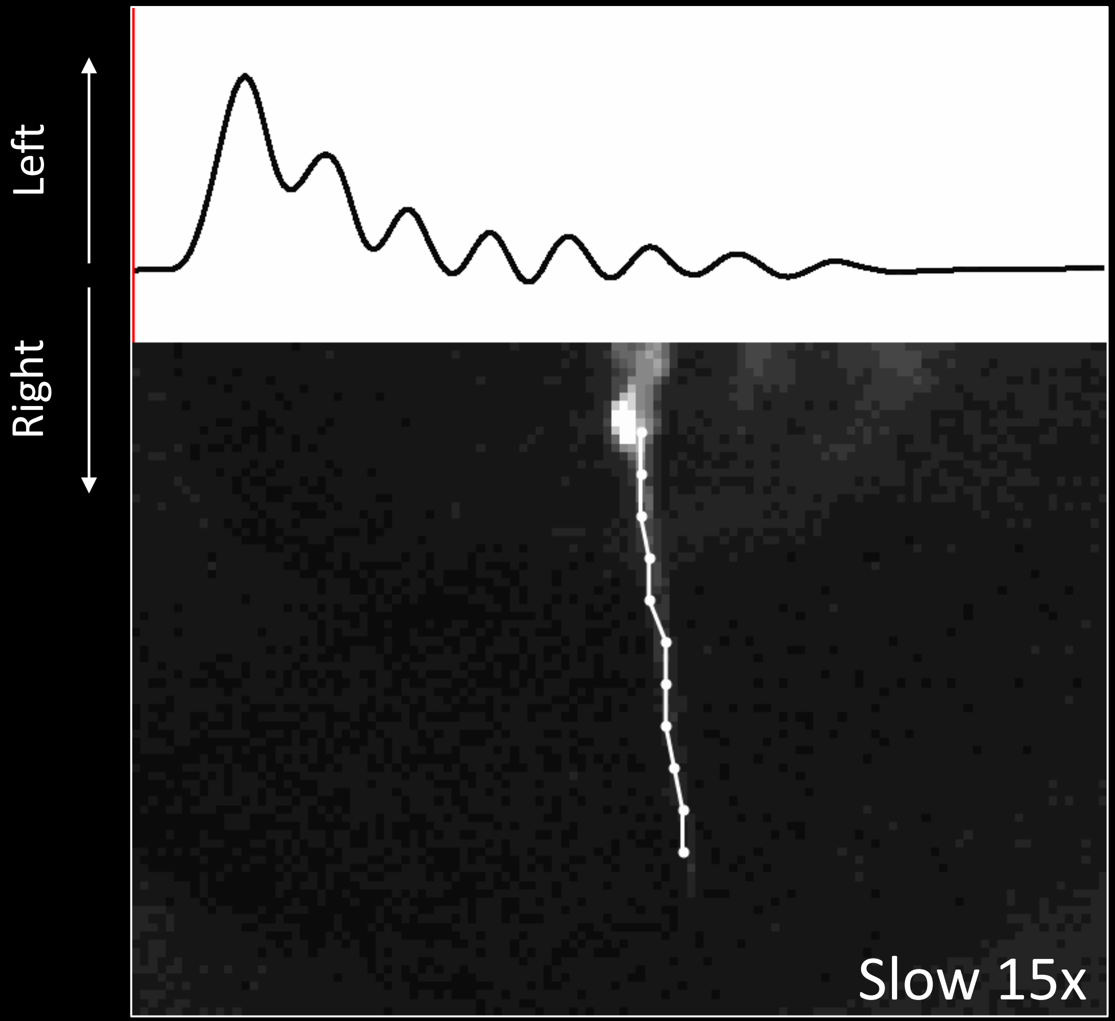
1.5 volumes/s

Speed 20x

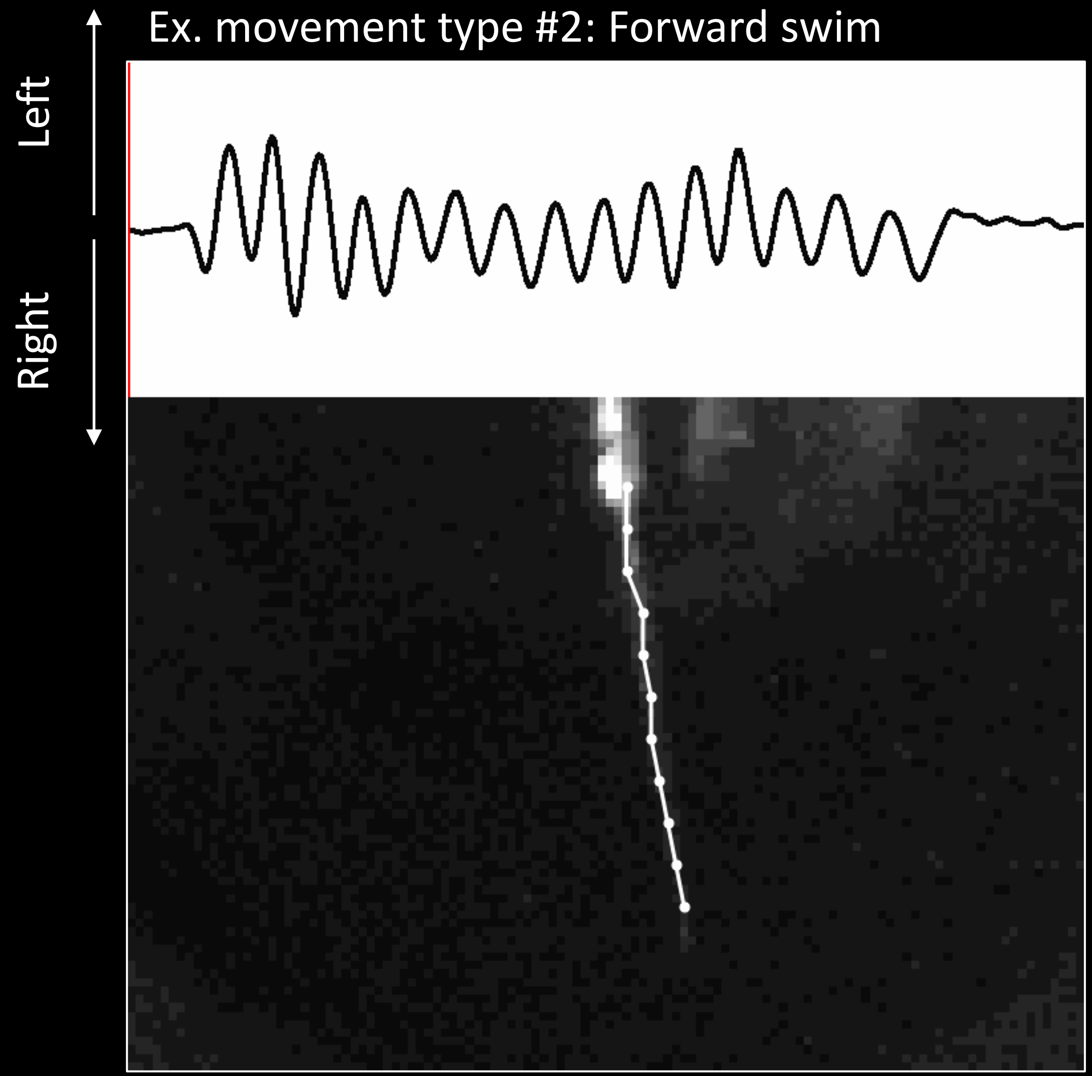
High-speed tail tracking

Fish swim at 20-50 Hz and many locomotor patterns can be distinguished

Ex. movement type #1: Large-angle turn



Ex. movement type #2: Forward swim



2. Extracting low-dimensional states

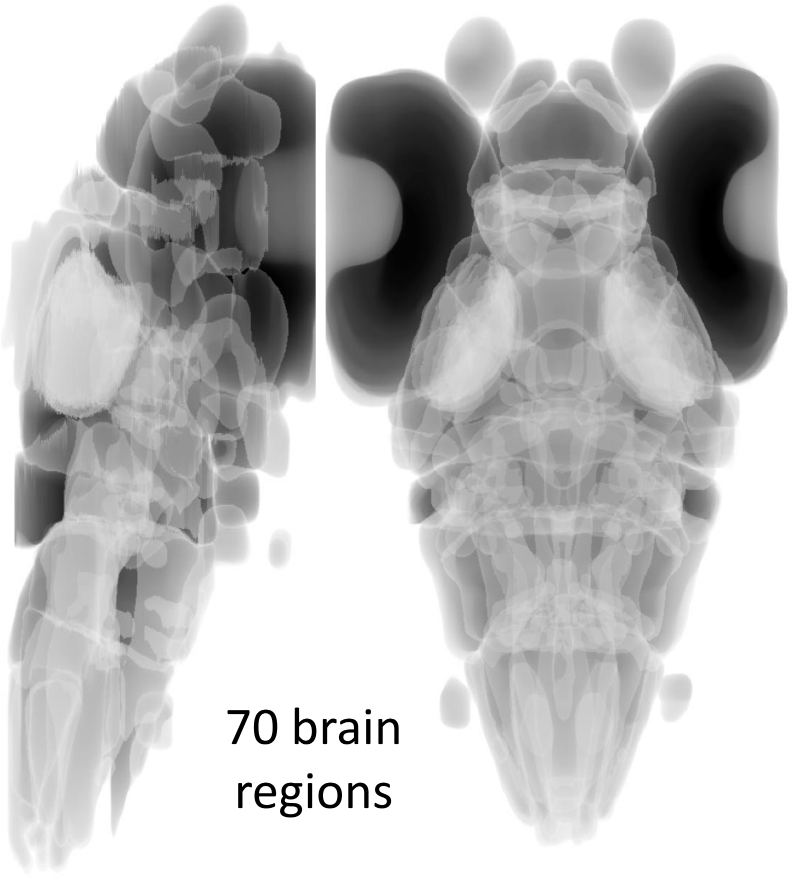
Brain atlas registration

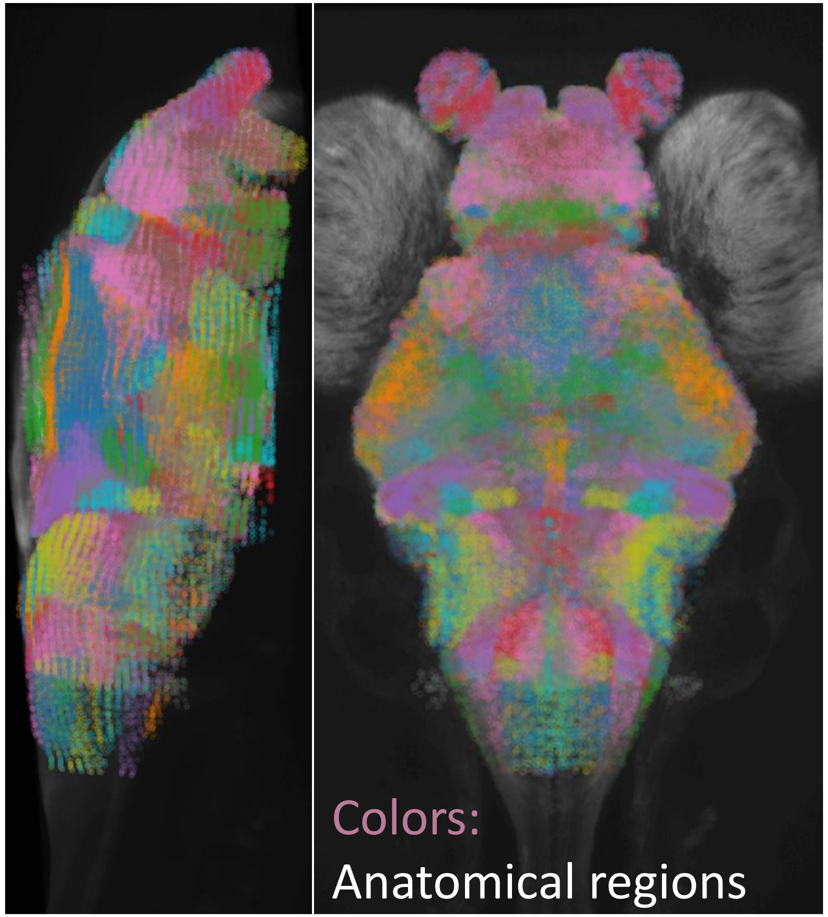
Imaging volumes are registered on a brain atlas
(*Mapzebrain, Herwig Baier Lab*)

1. Registration on atlas template brain
(ANTs Registration)

2. Mapping all segmented centroids into brain areas

Atlas template brain *In vivo* stack



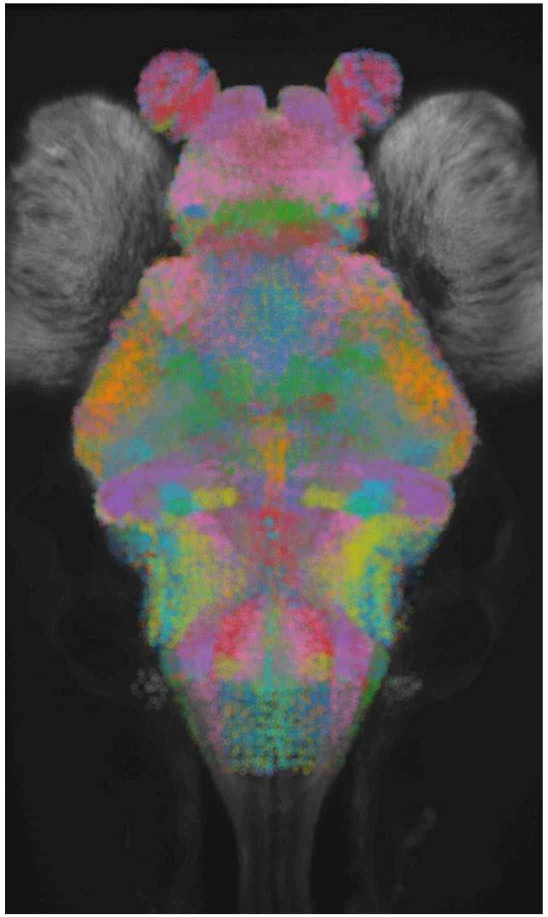


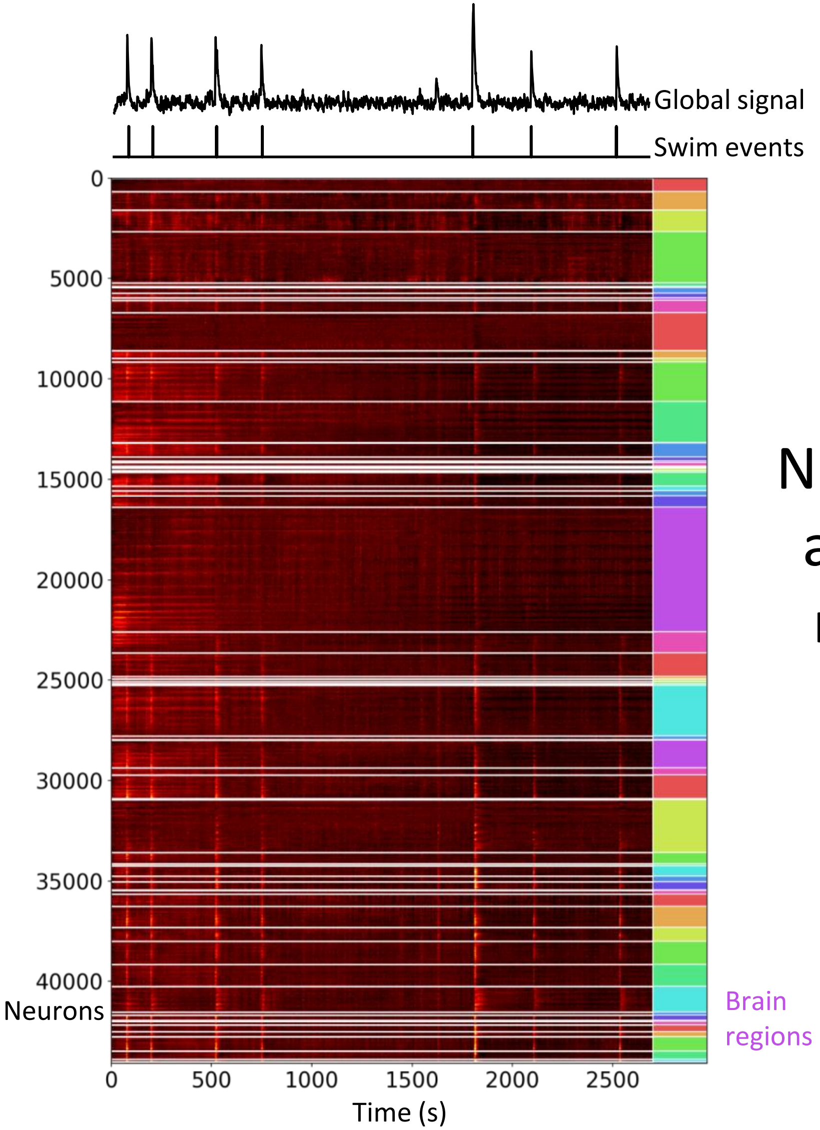
Reference volume for multi-animal comparisons

Extracting recurrent brain states

Step 1: Extracting region-averaged neuronal activity

Cells mapped in atlas

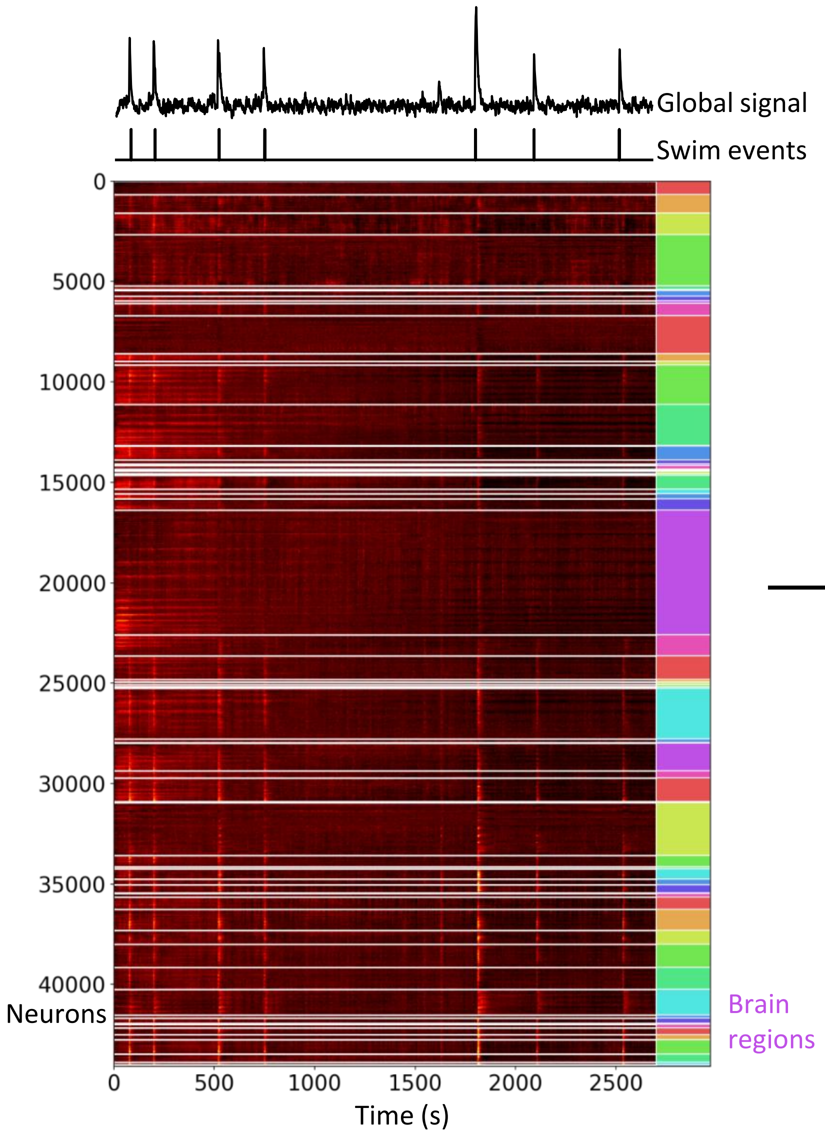




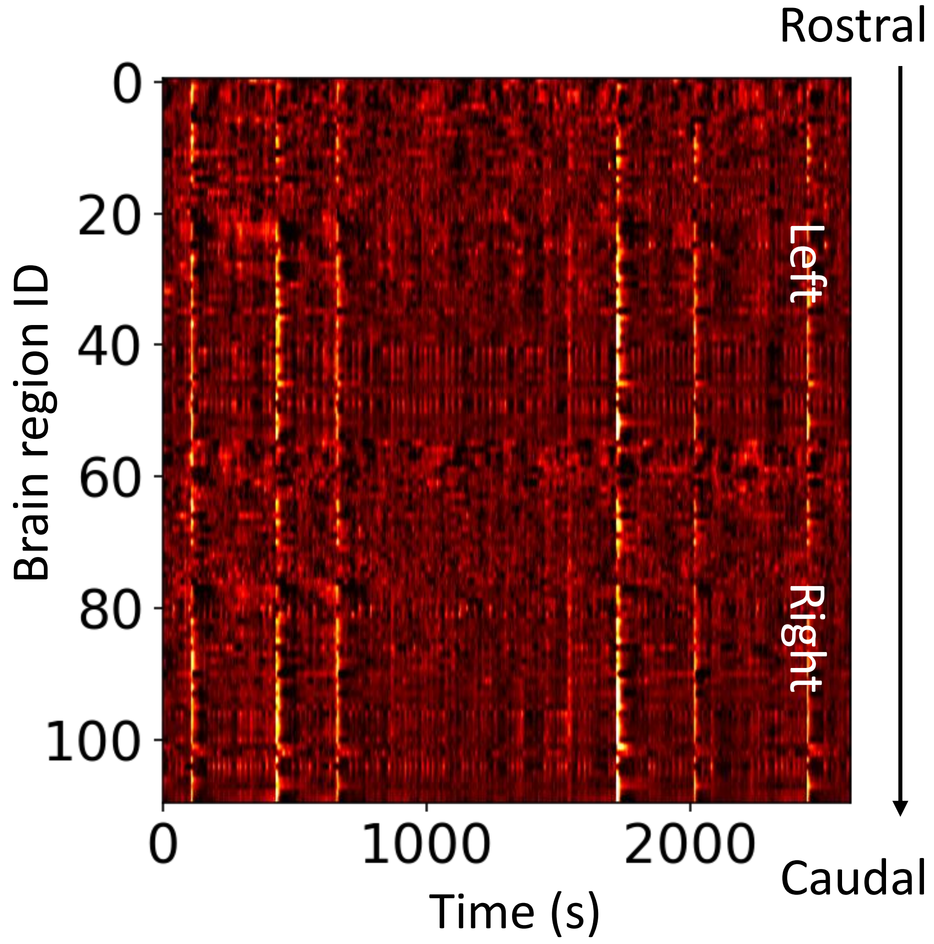
Neuronal activity matrix

Extracting recurrent brain states

Step 1: Extracting region-averaged neuronal activity

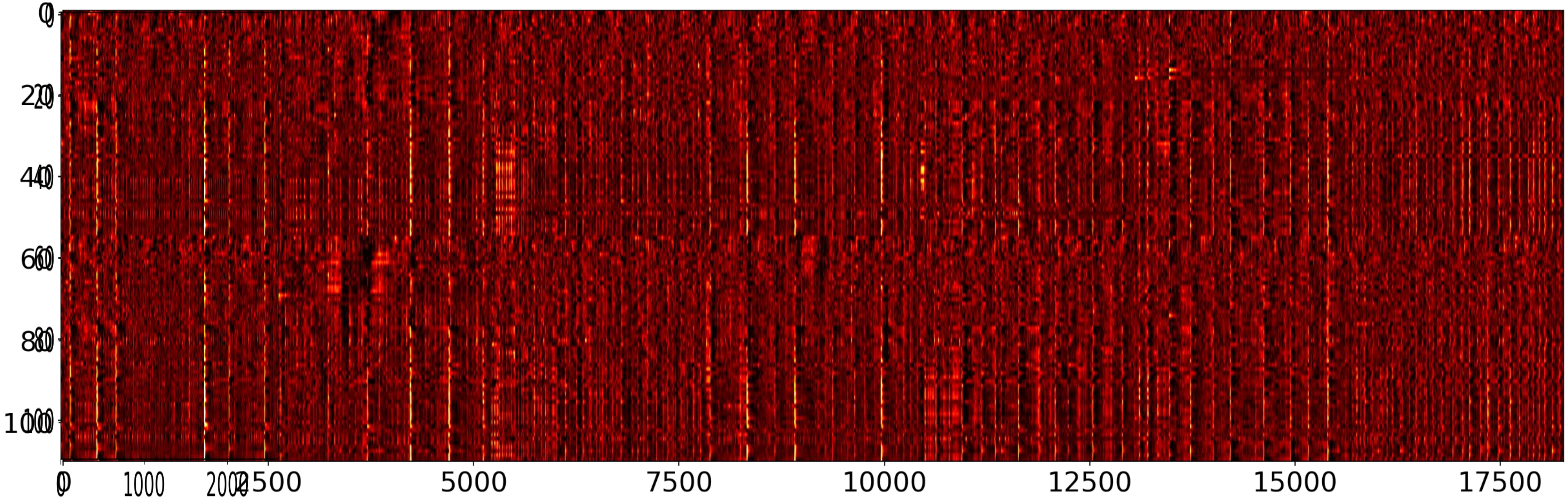


Regional average



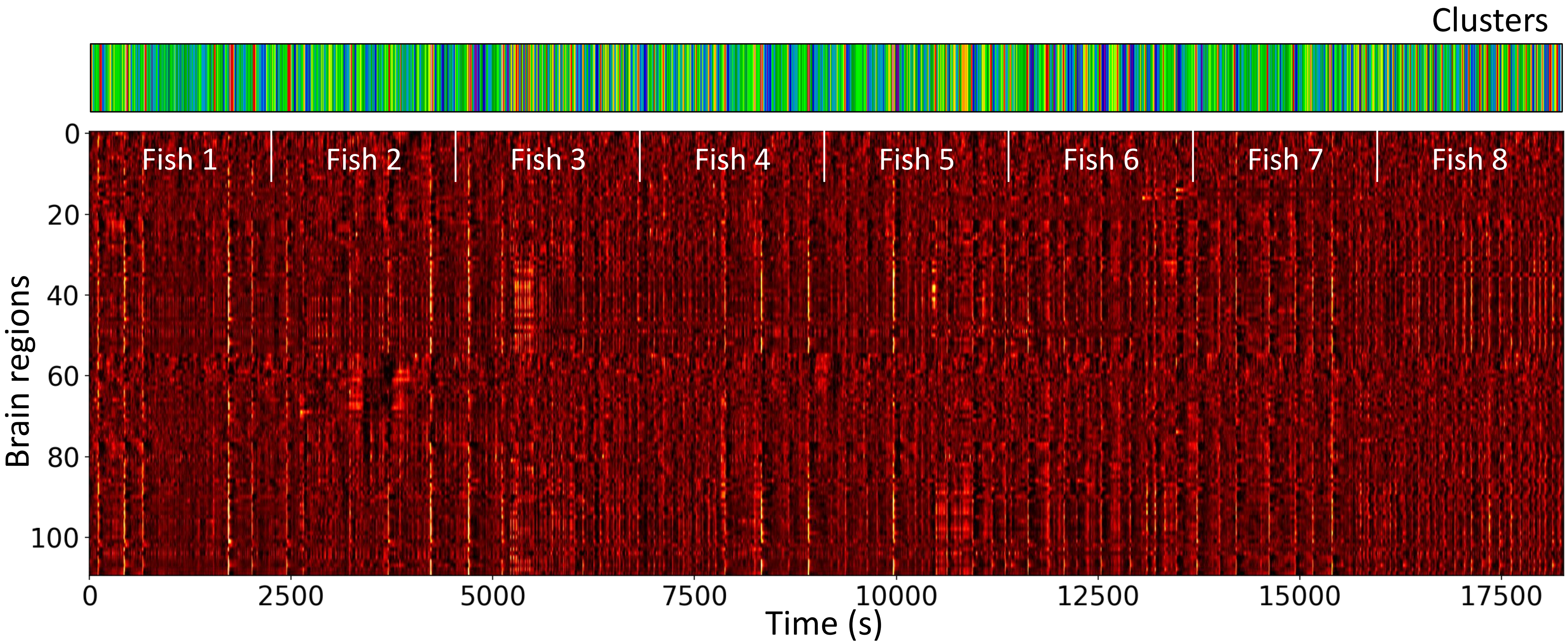
Extracting recurrent brain states

Step 2: Consensus clustering along temporal axis



Extracting recurrent brain states

Step 2: Consensus clustering along temporal axis



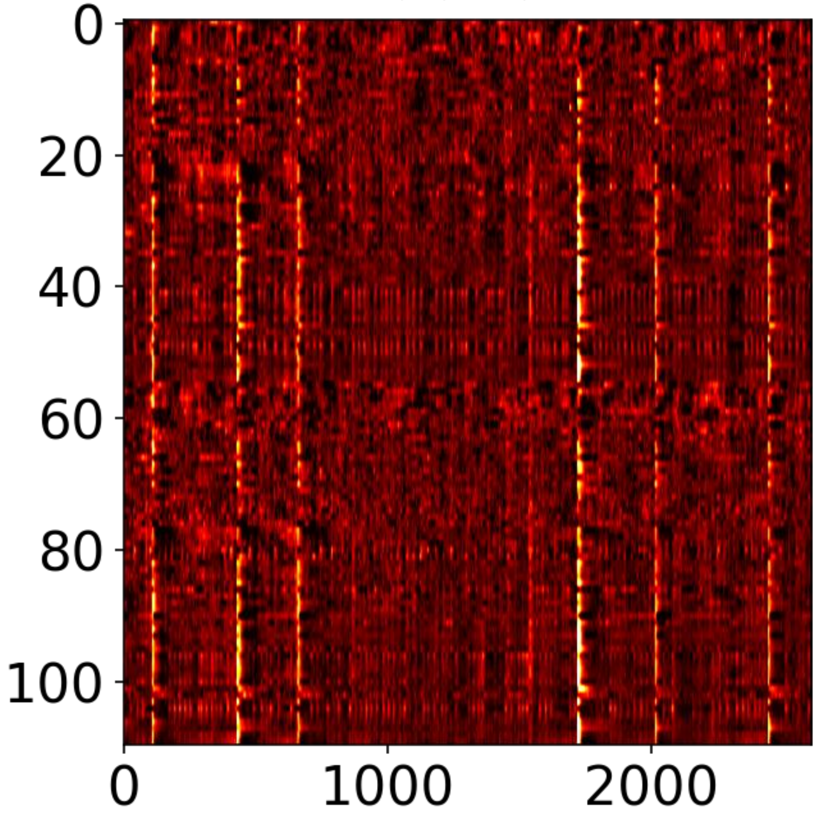
Assumption: Instantaneous snapshots of regional activity are reproducible across time and individuals

Consensus clustering

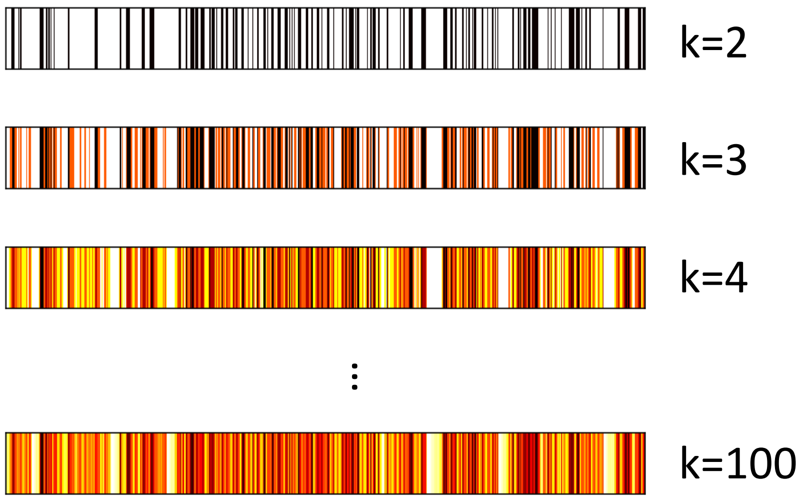
Most clustering algorithms typically yield different outputs on repeated runs

Consensus clustering **averages results** across multiple runs

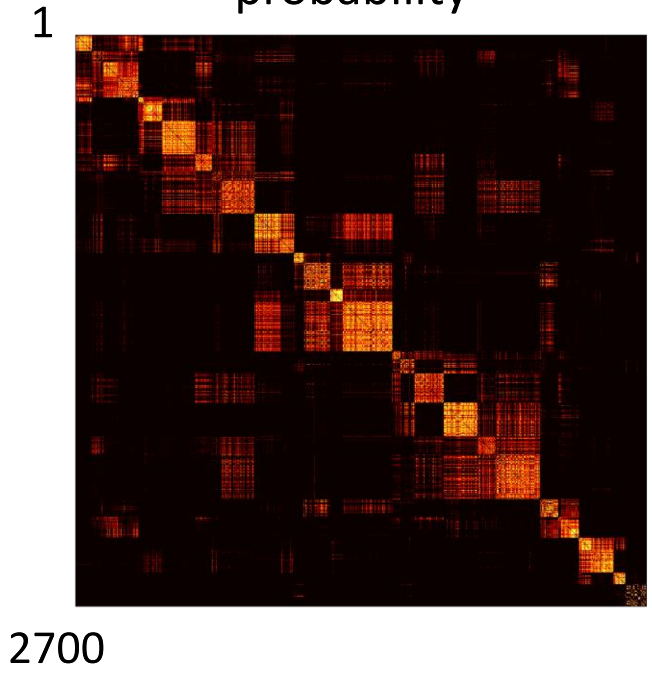
1. Data matrix



2. Run k-means *N* times



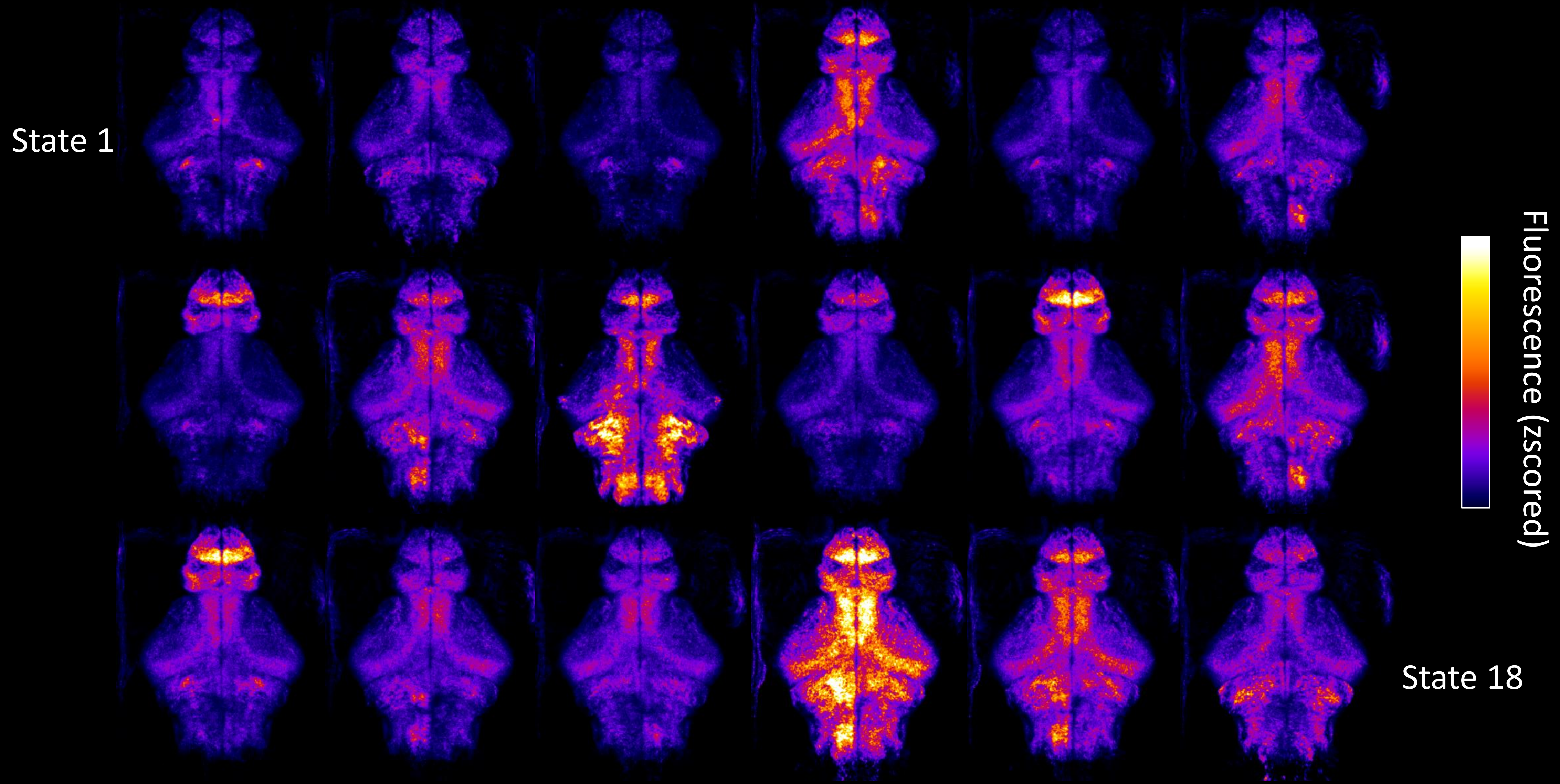
3. Compute coassignment probability



4. Hierarchical clustering

What do these clusters actually look like?

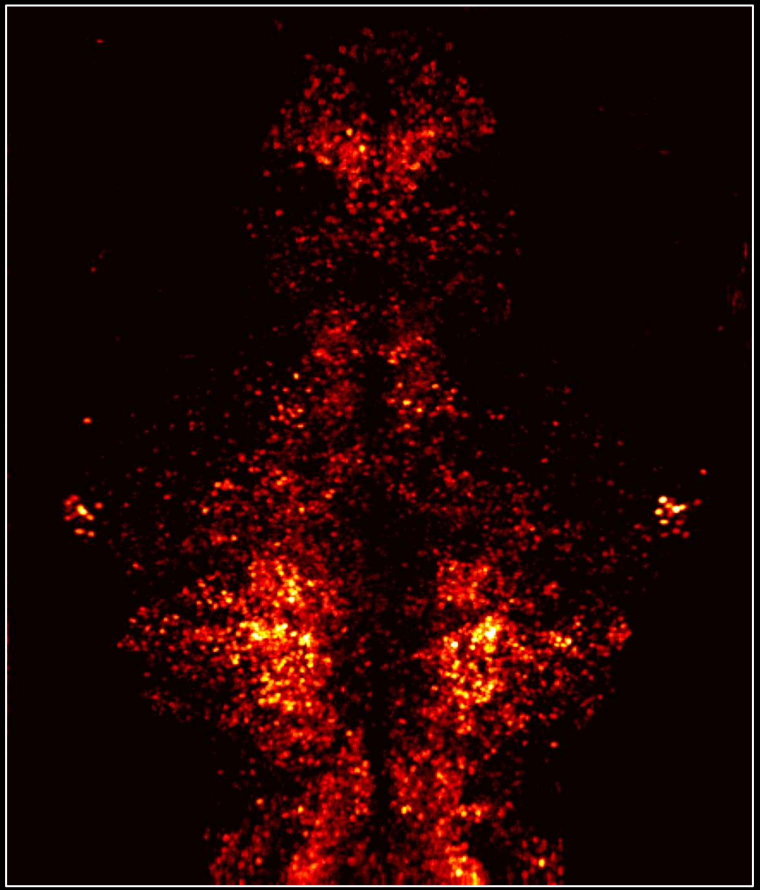
Spontaneous brain states Ordered by prevalence



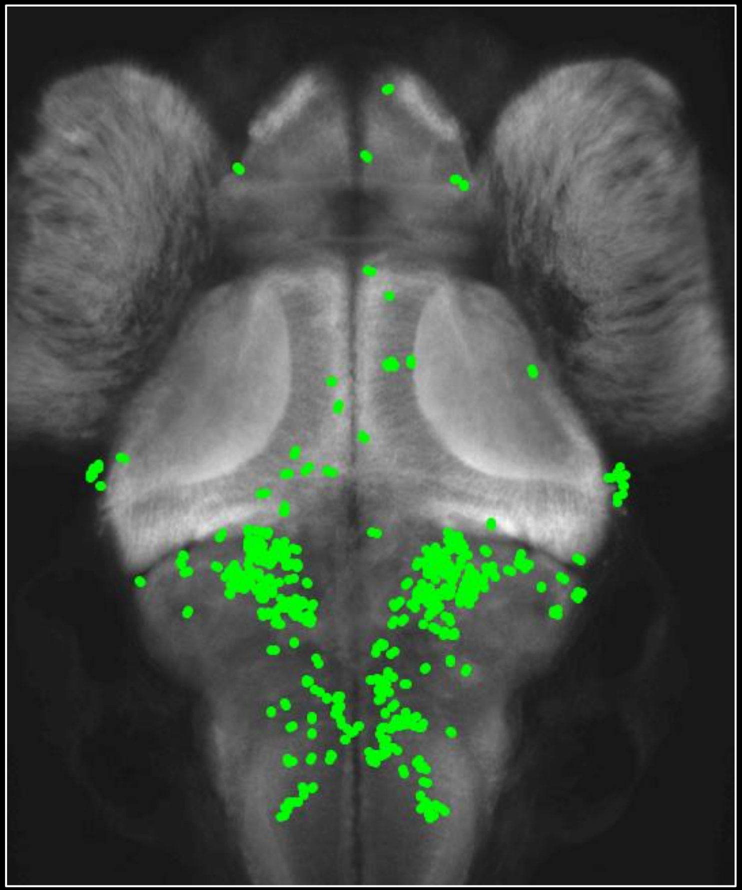
Average states projected in *Mapzebrain atlas* (n = 7 fish)

Motor state validation

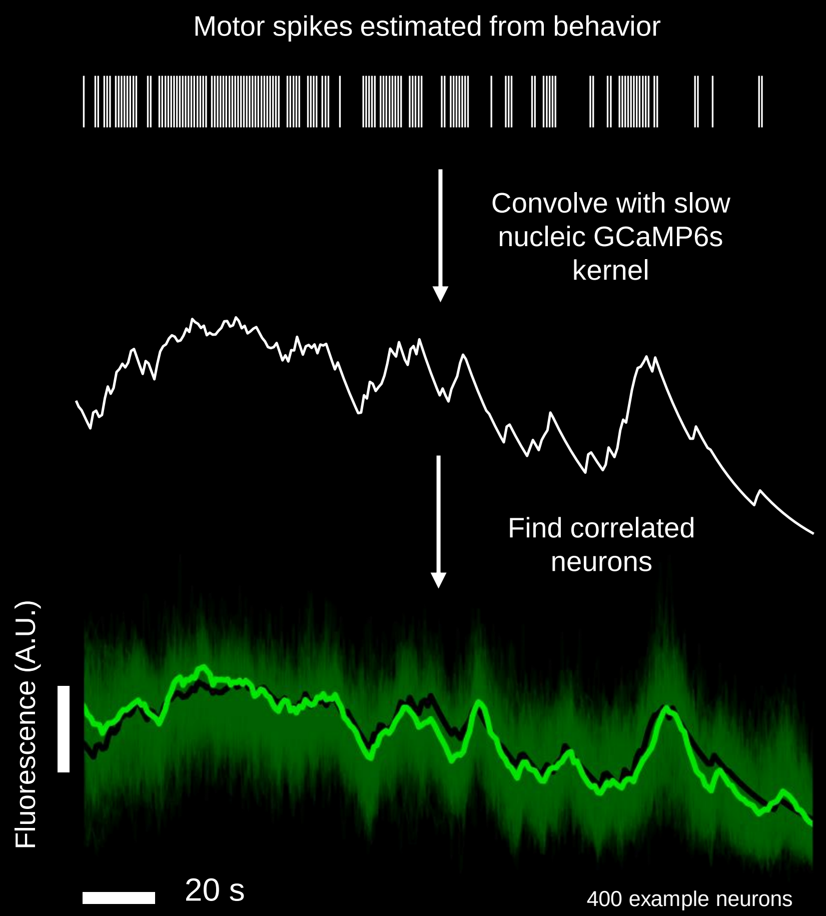
State 8 can be recovered independently through behavioral regression



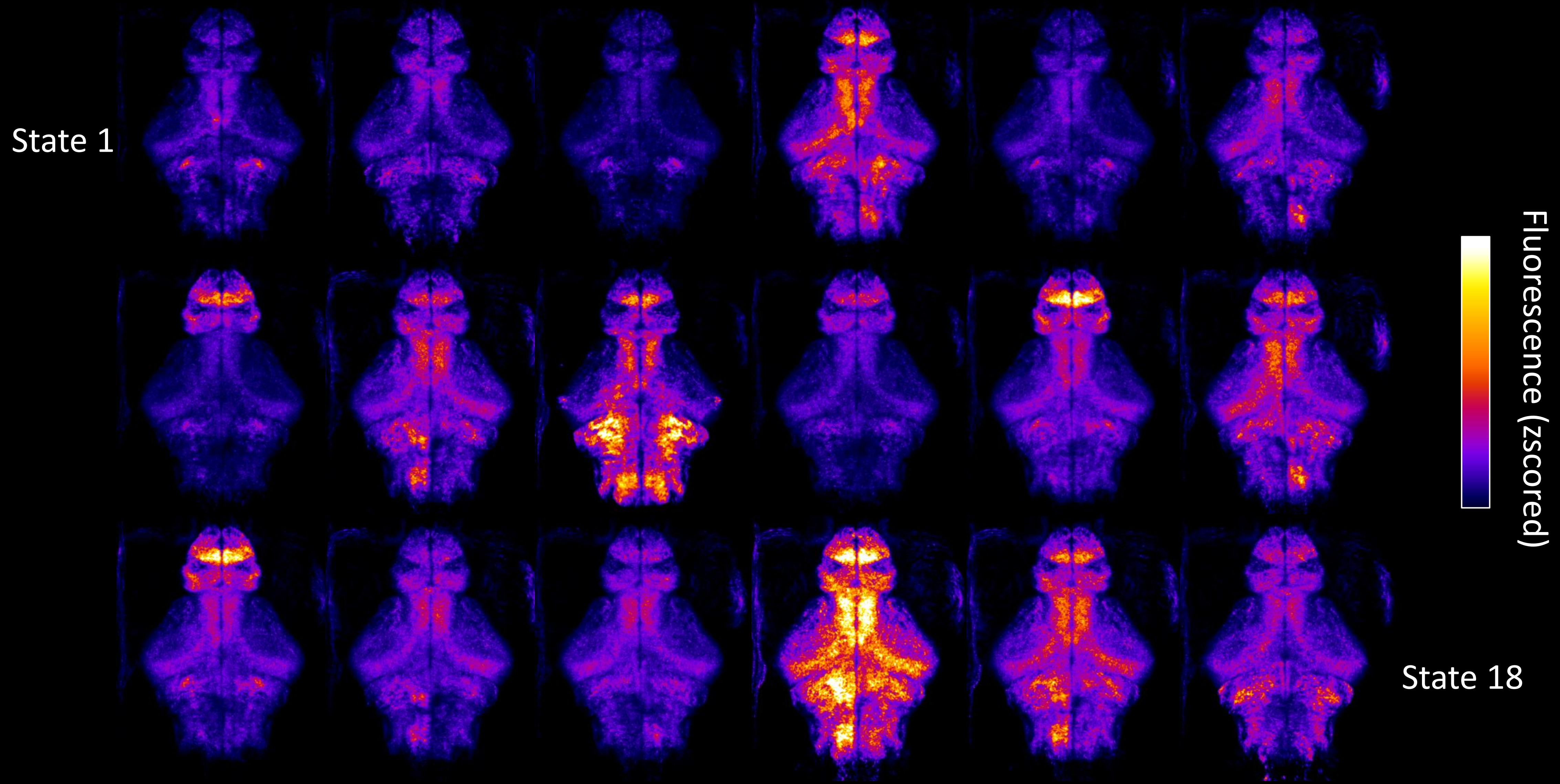
State 8



Tail-correlated neurons +
significant overlap across fish



Spontaneous brain states Ordered by prevalence

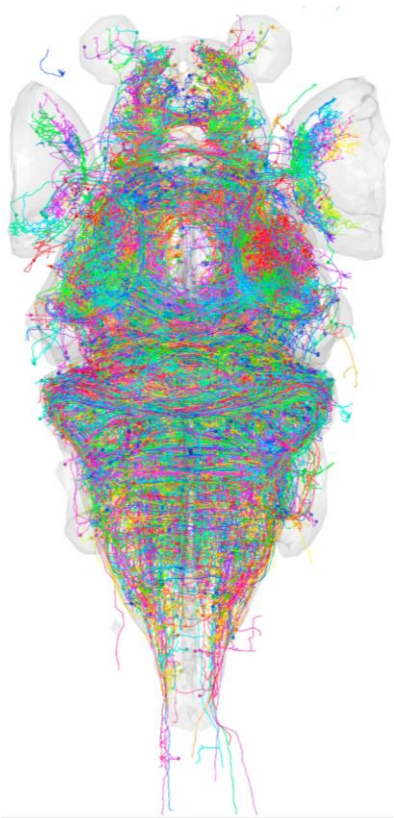


Average states projected in *Mapzebrain atlas* ($n = 7$ fish)

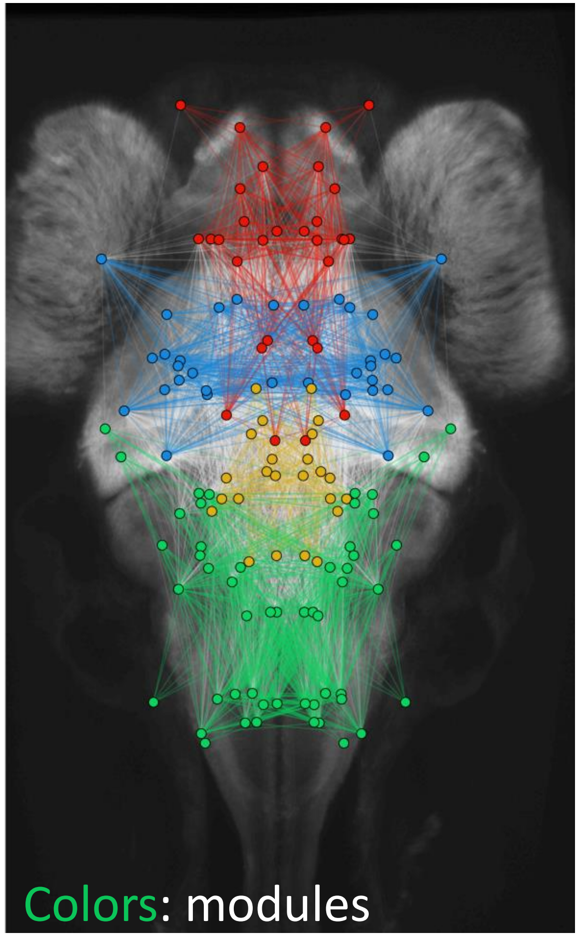
3. Properties of brain states

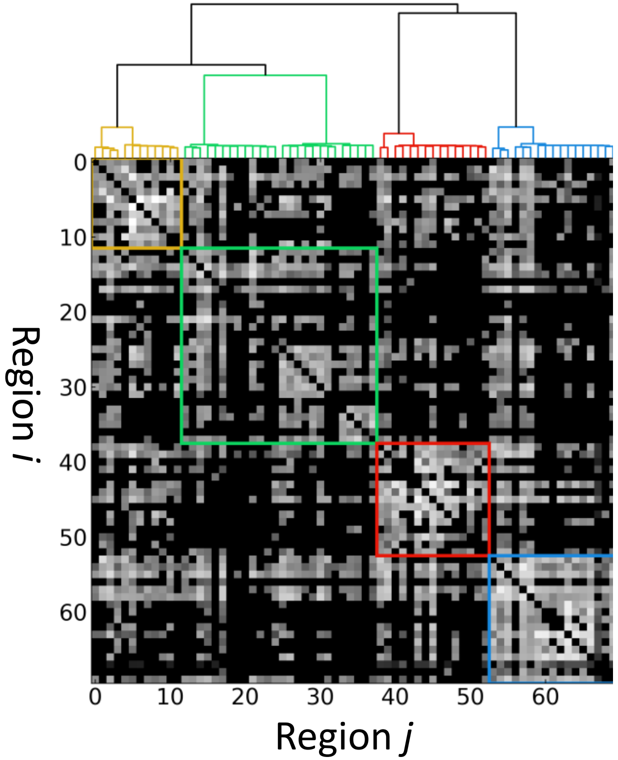
Spatial properties of states

Modules/communities: Groups of strongly interconnected brain regions



Connectome



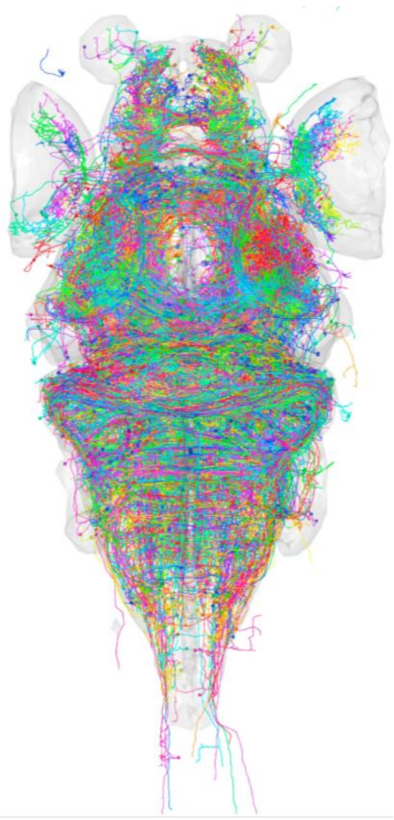


4 principal modules identified through hierarchical community detection

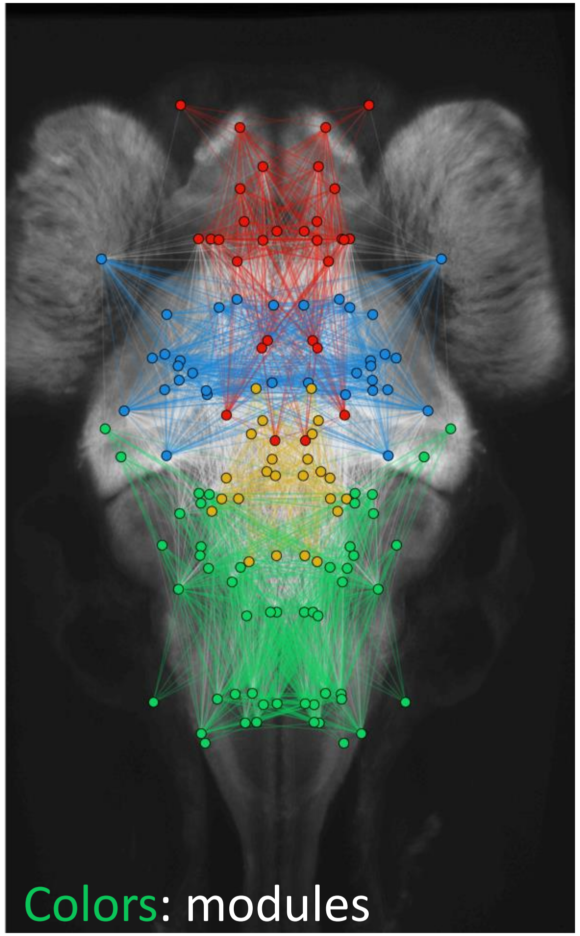
Kunst *et al.*
Neuron (2019).

Spatial properties of states

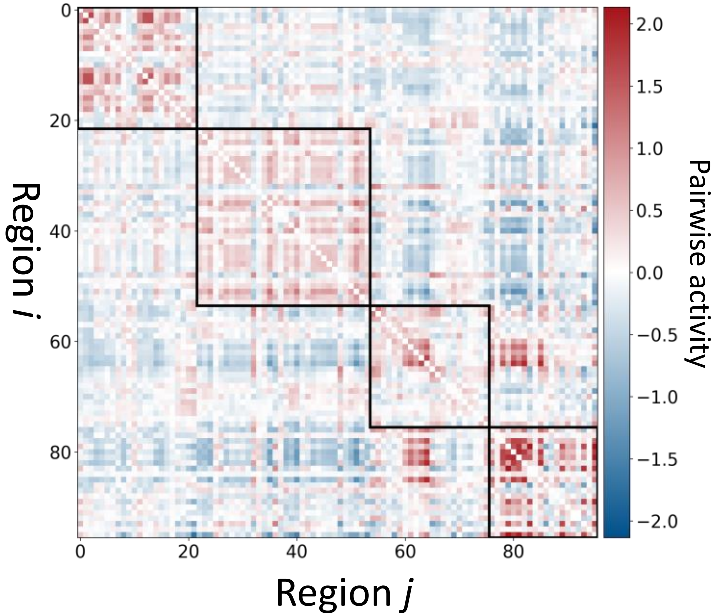
Coactivation is driven by strong recurrent connectivity

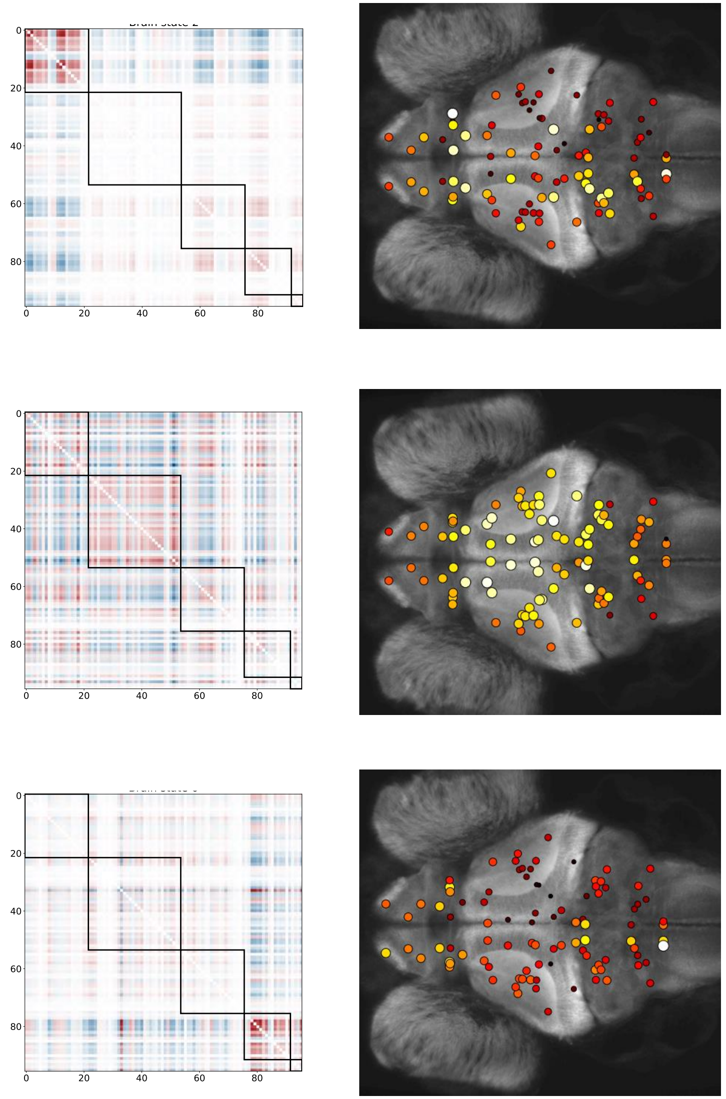


Connectome



Average state
coactivation matrix

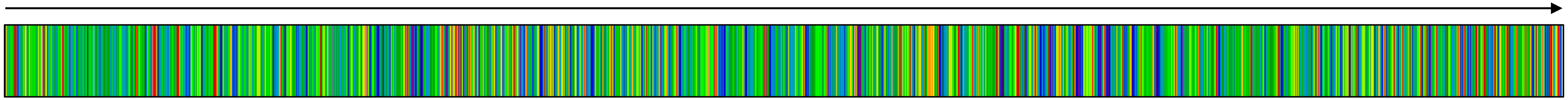


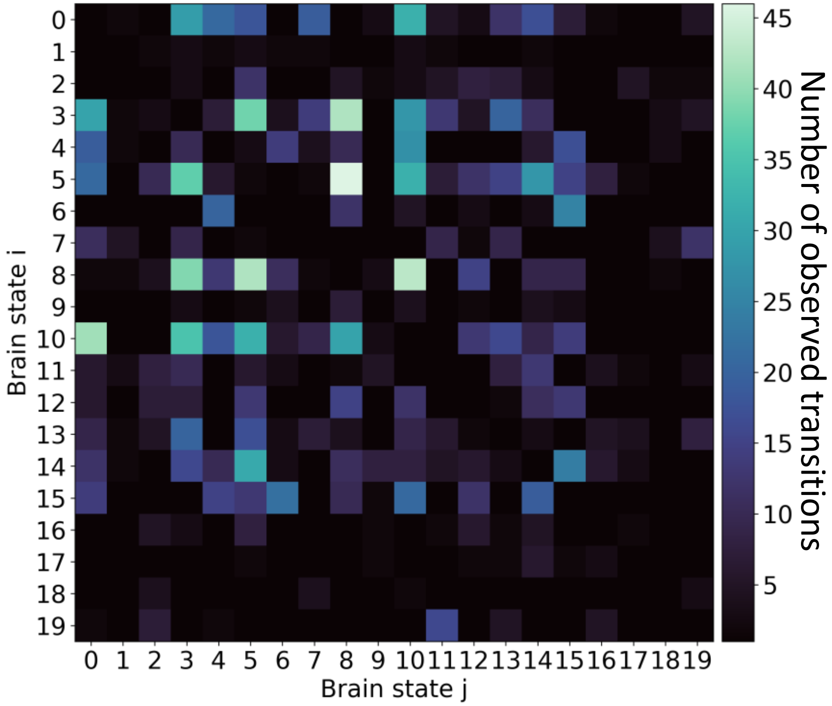


Example states

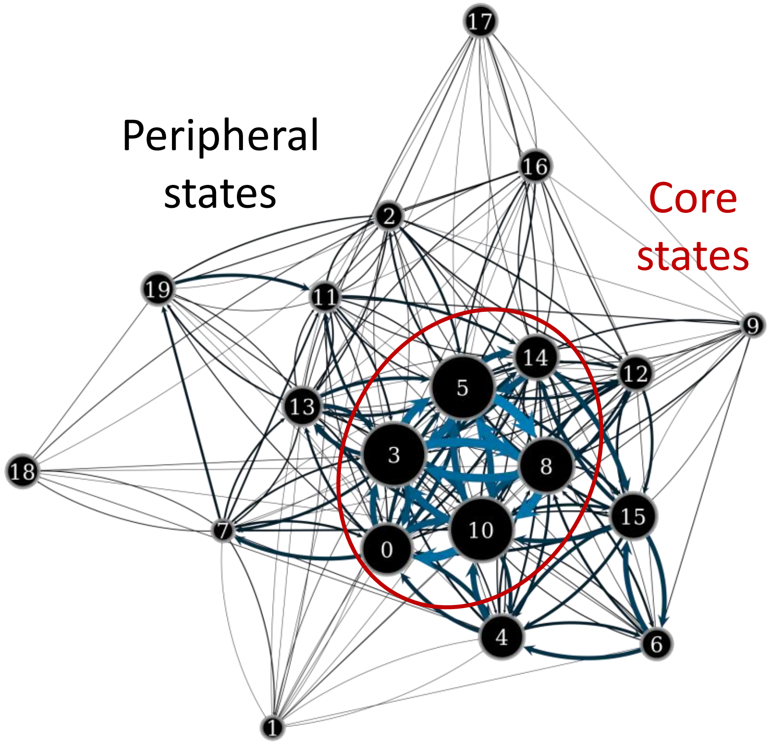
Temporal properties of states

Brain state transitions are not completely random

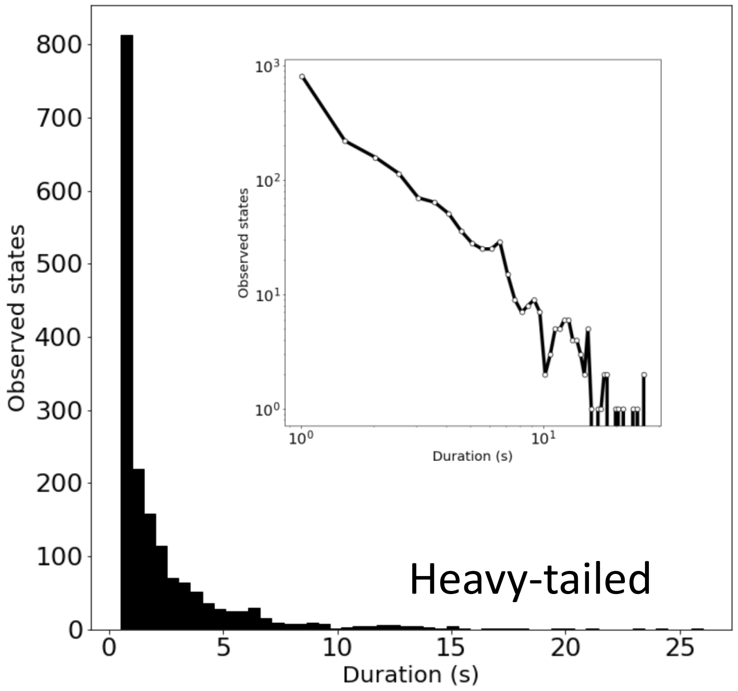




Transition matrix



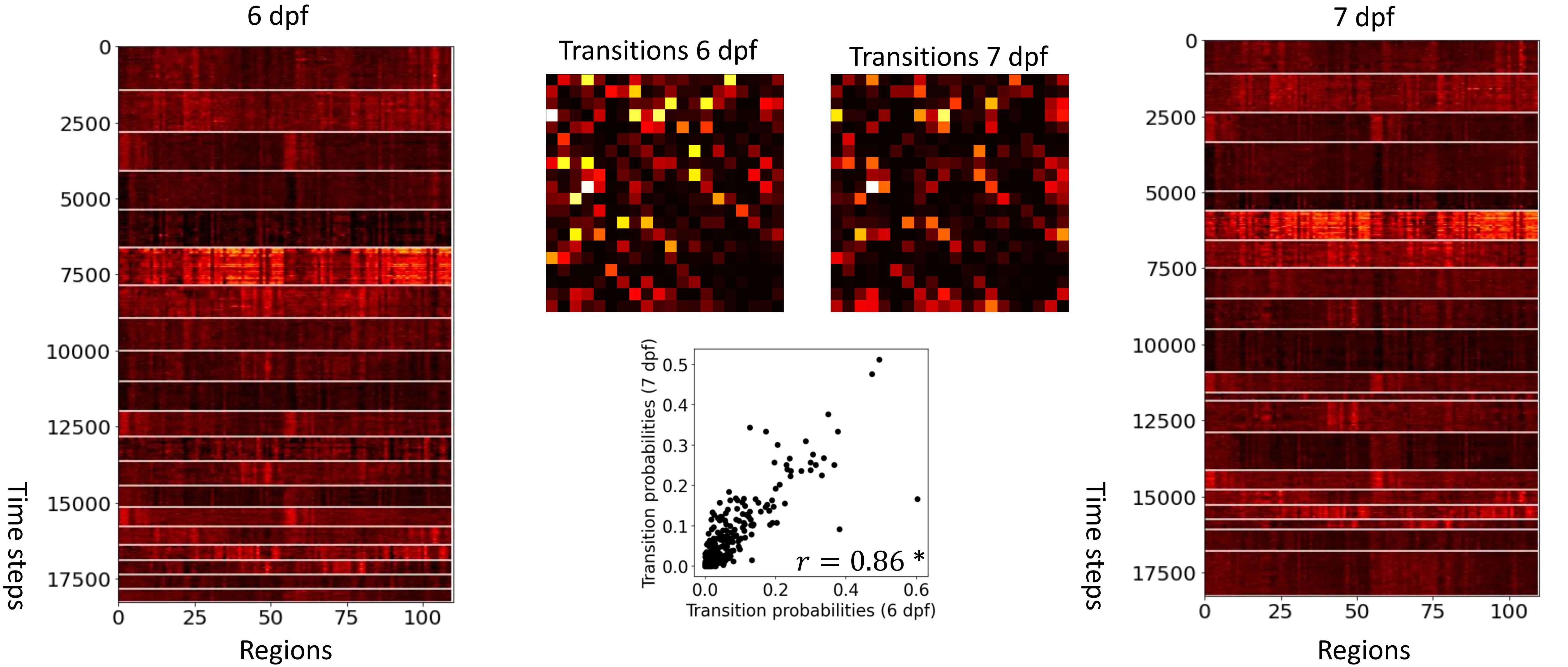
State network



State durations

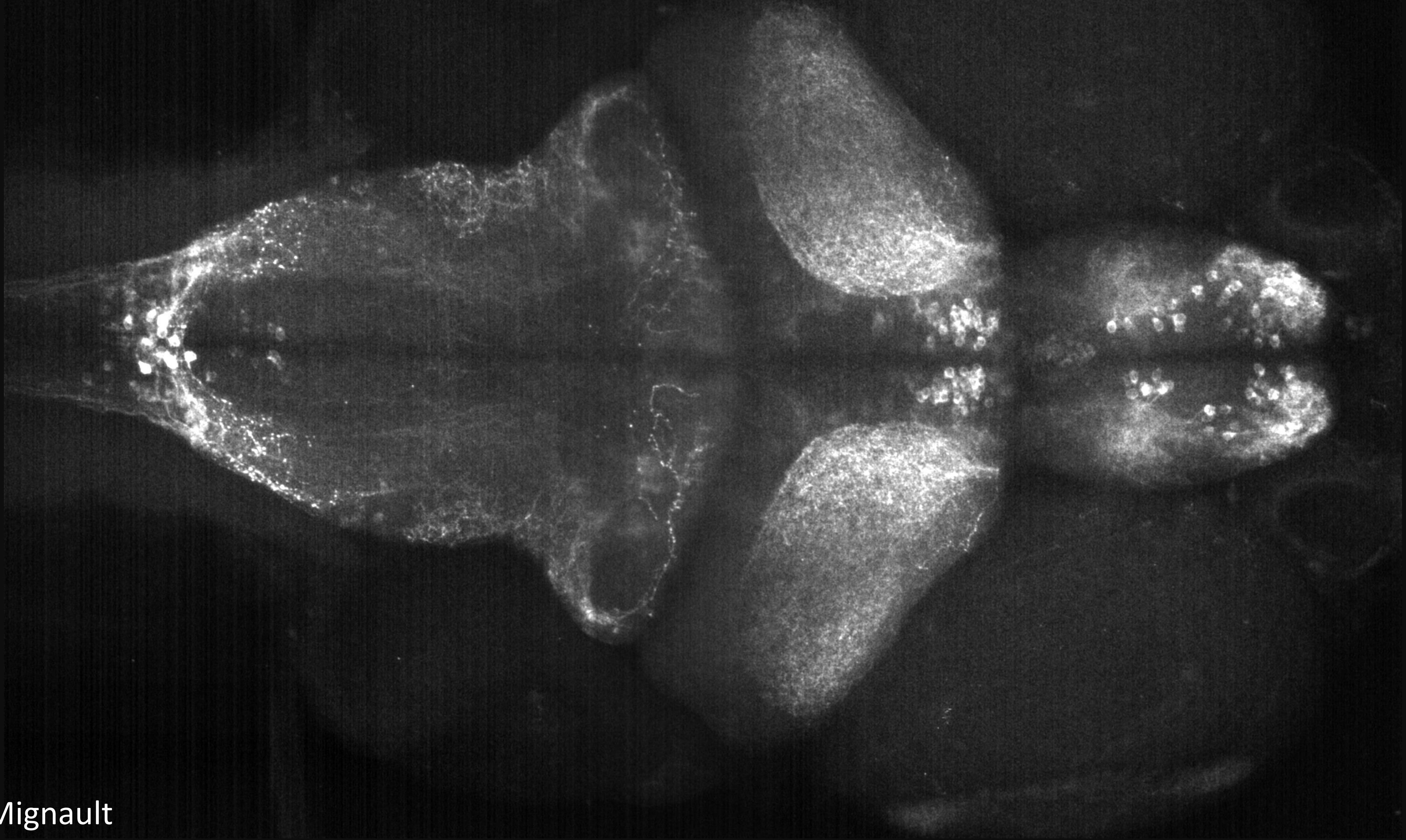
Brain states are recurrent over days

Similar states are identified in the same fish at 6 and 7 dpf



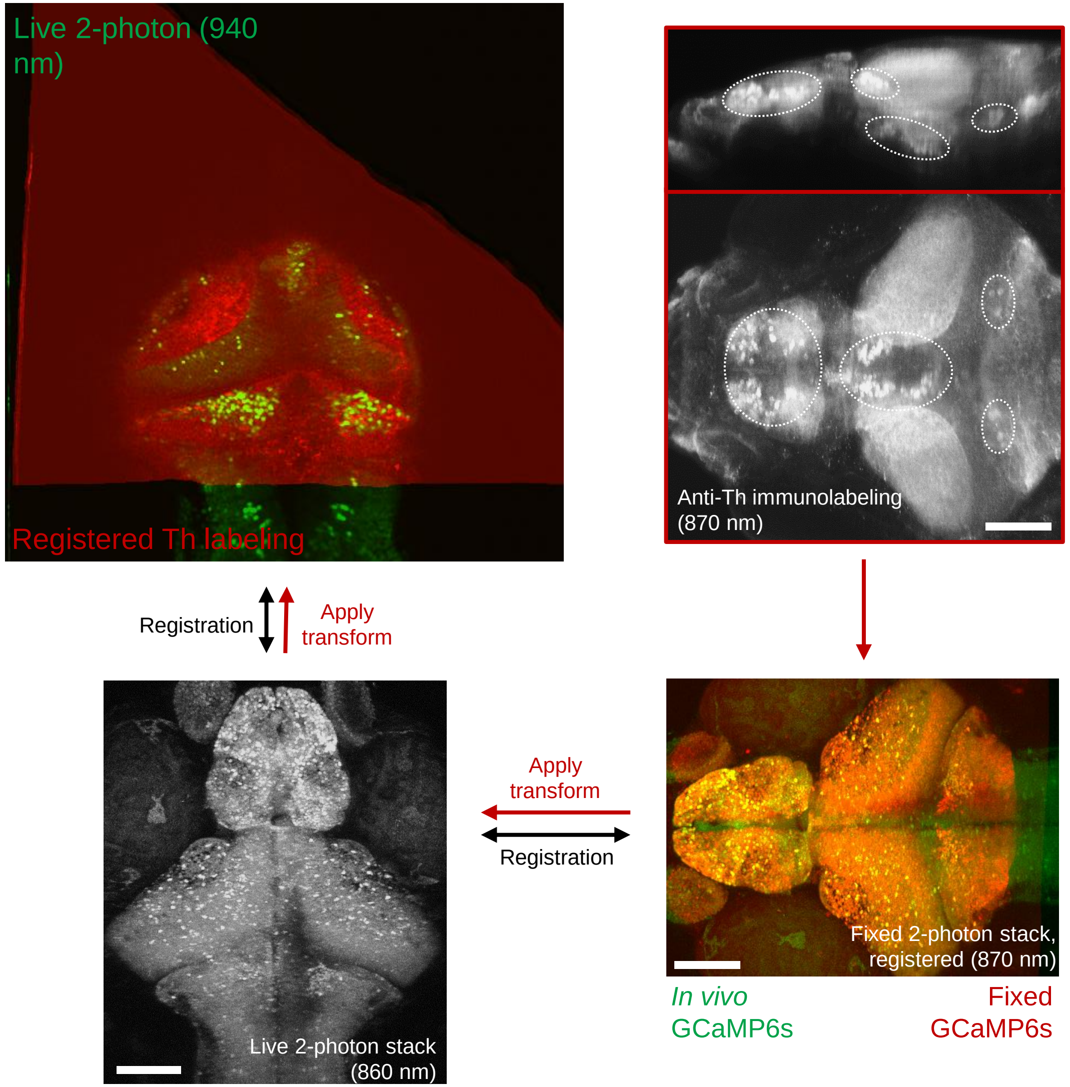
4. Adding neuromodulators to the mix

Dopaminergic & noradrenergic cells

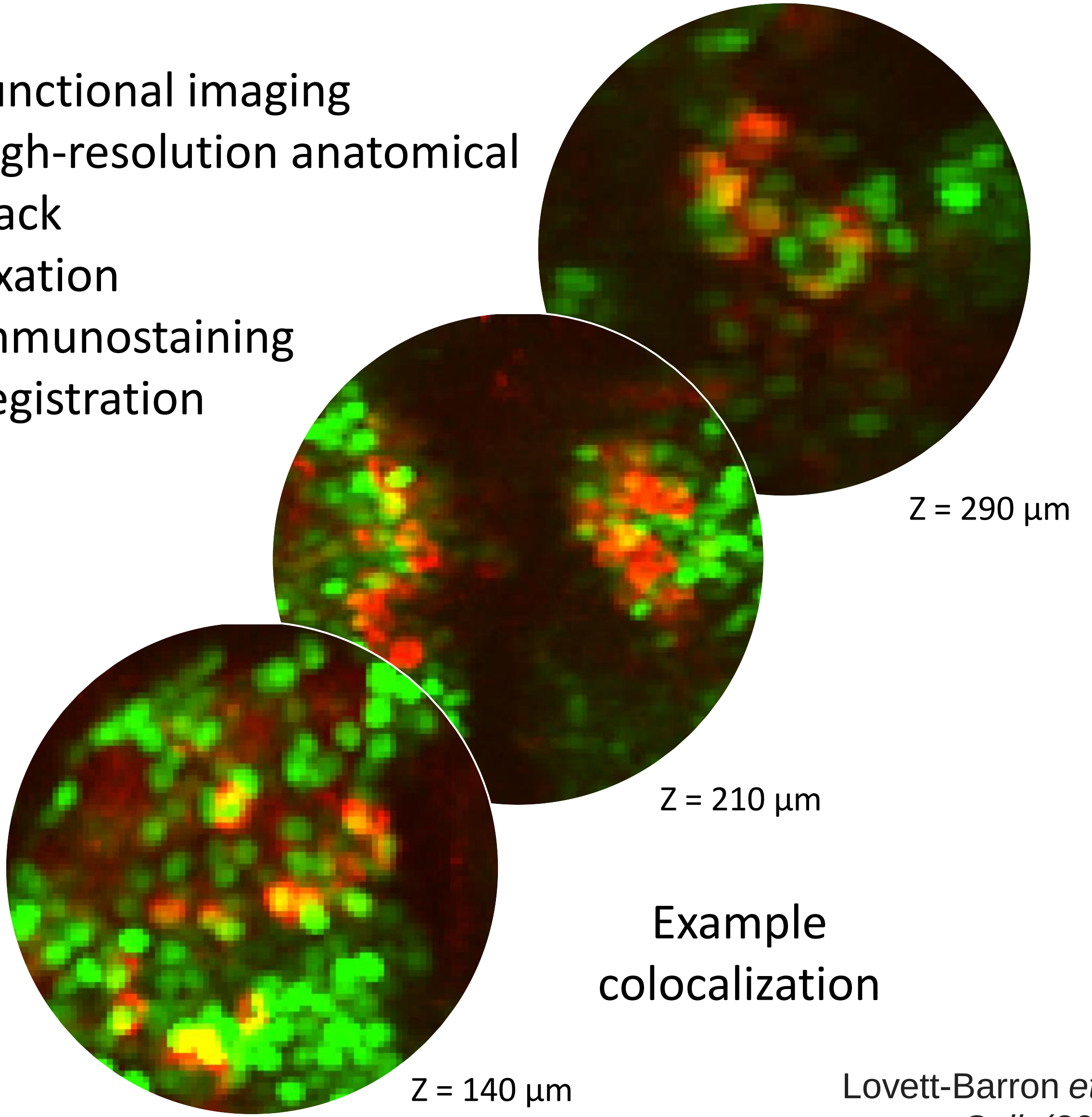


Sandra Mignault

MultiMAP registration



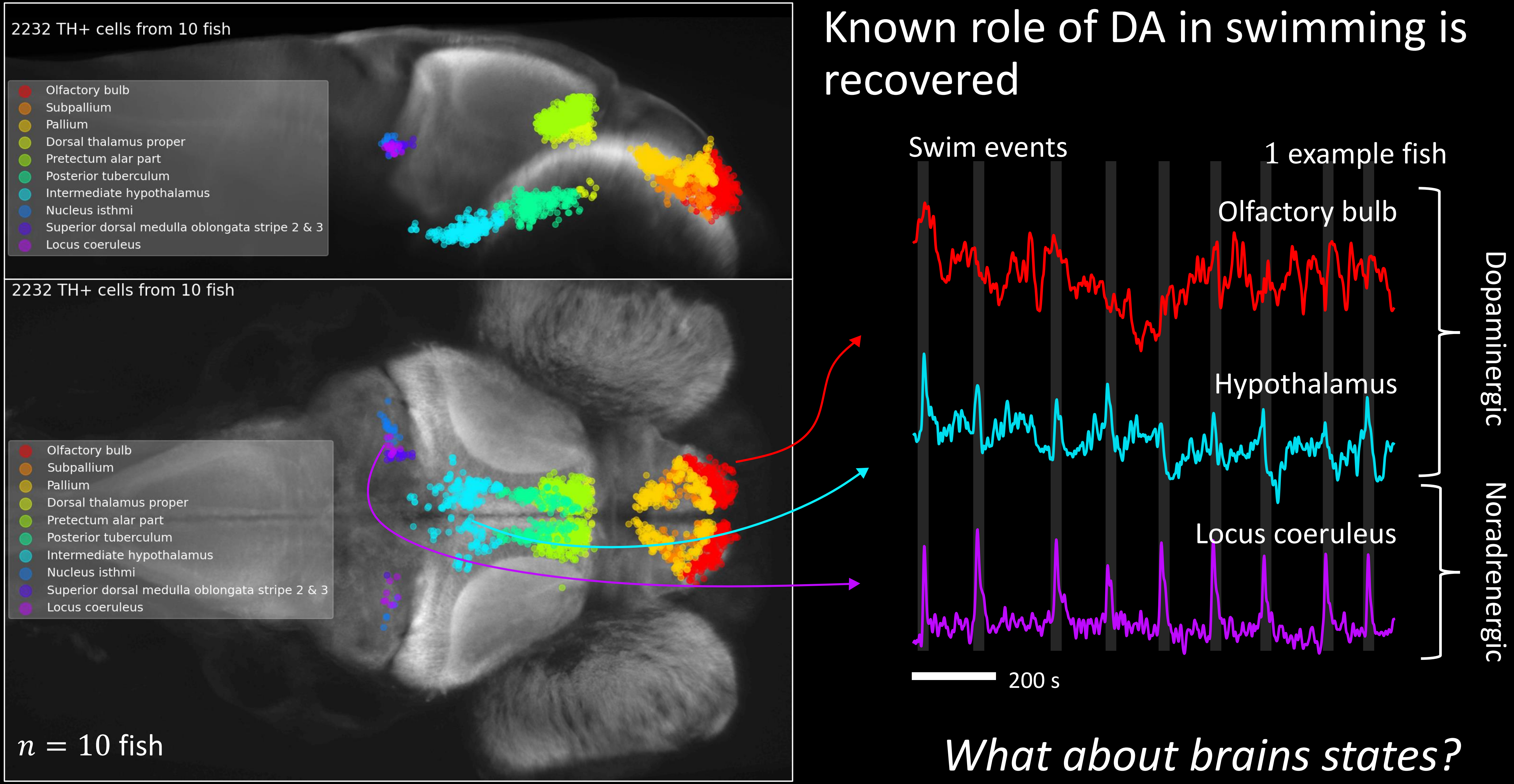
1. Functional imaging
2. High-resolution anatomical stack
3. Fixation
4. Immunostaining
5. Registration



Example colocalization

Lovett-Barron *et al.* *Cell* (2017)

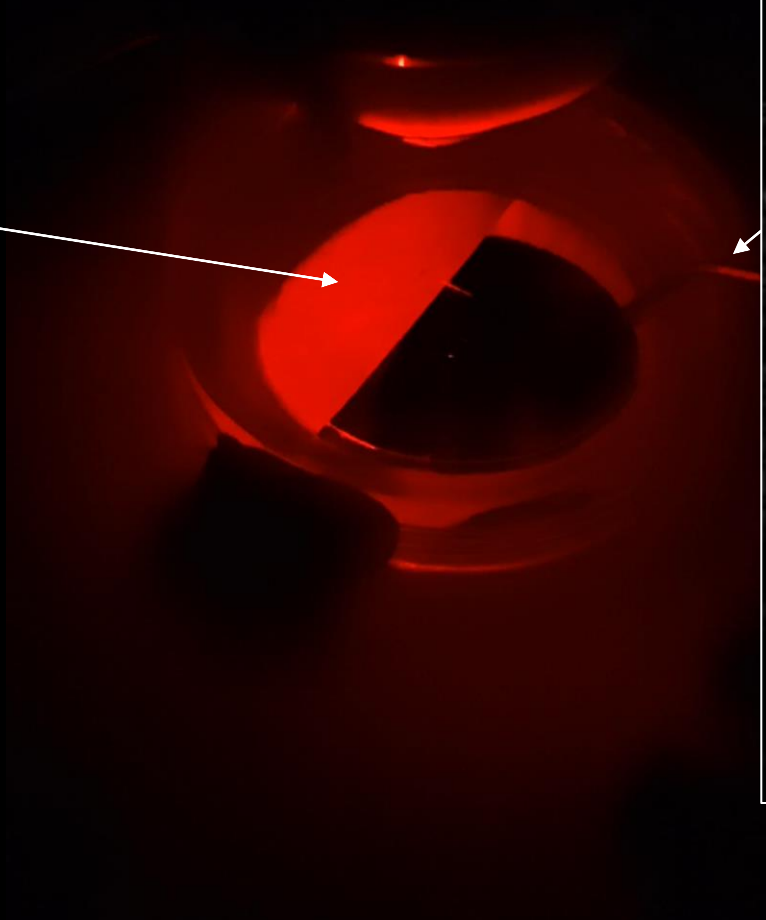
Known role of DA in swimming is recovered



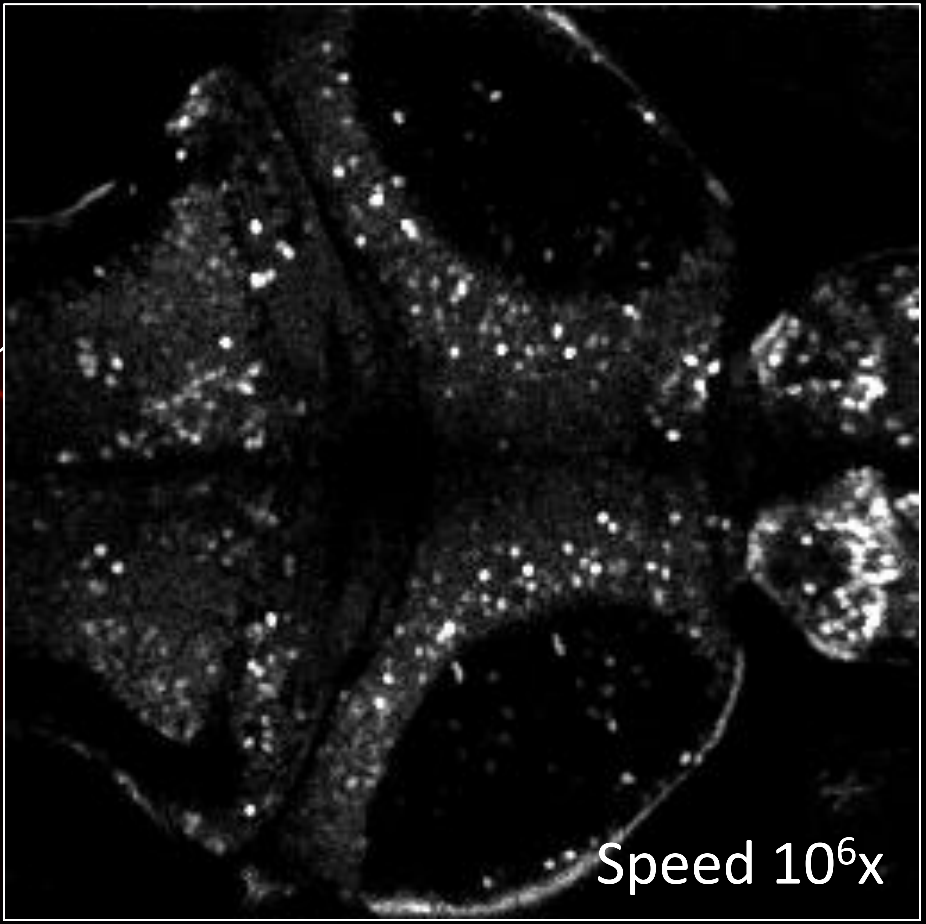
What about brains states?

Future directions

Projector



Stim. + Yohimbine

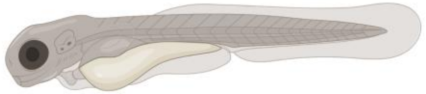


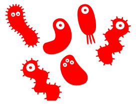
Sandrine Poulin

Antoine Légaré & Nick Benfey

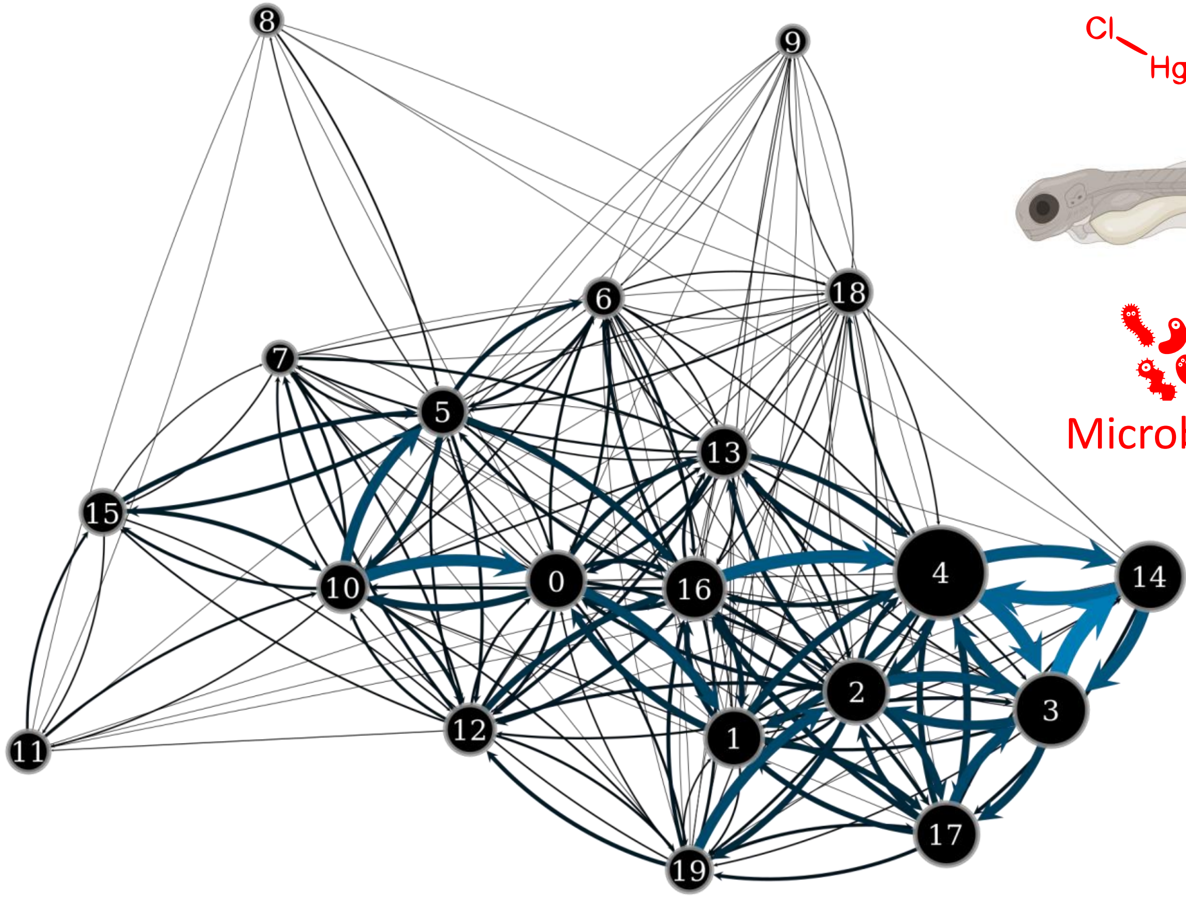
Future directions: Exposome

Methymercury

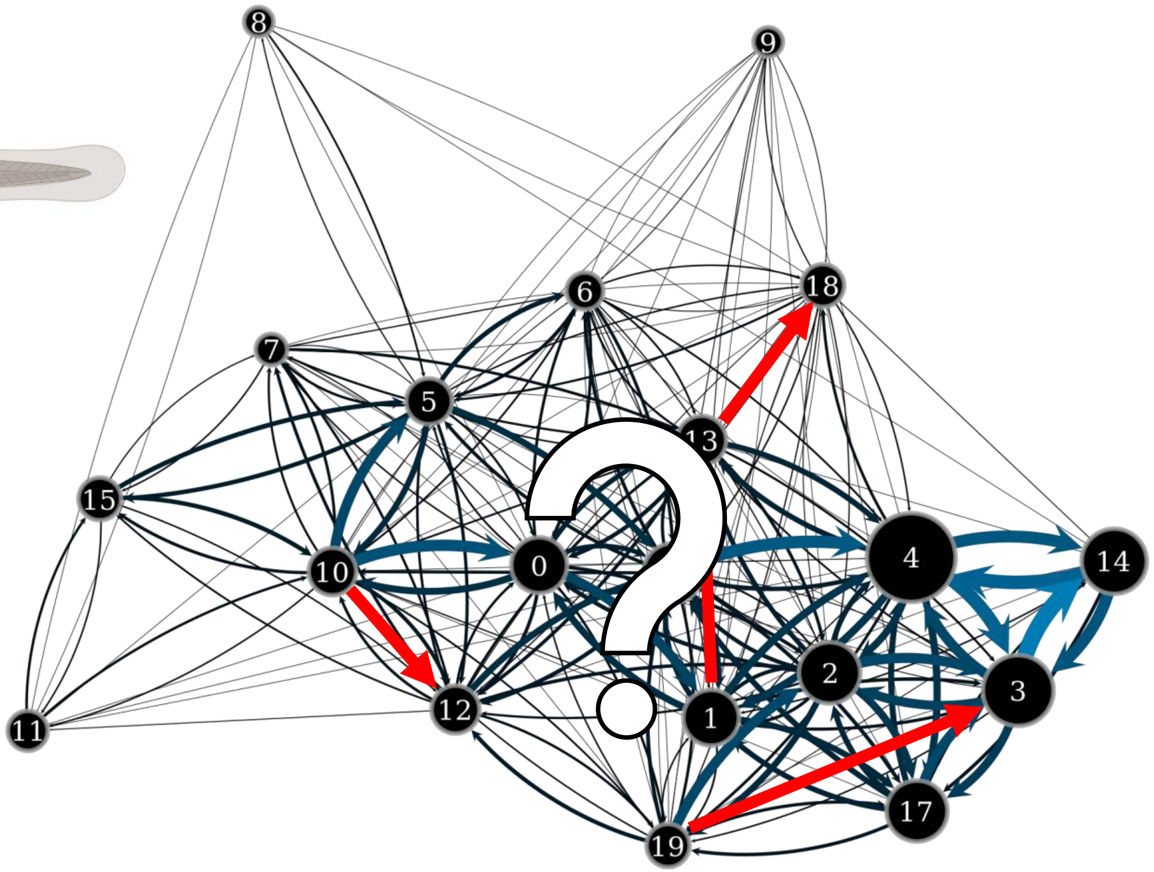




Microbiome



Healthy brain dynamics



Altered brain dynamics

PDK Lab

Key results:

- Framework to study internal state transitions
- Neuromodulators identified in pan-neuronal data
- How are both related?

Thank you!

Danio rerio
Paul De Koninck
Patrick Desrosiers
Mado Lemieux
Vincent Boily
Sandrine Poulin
Sandra Mignault

CENTRE DE RECHERCHE
CERVO
BRAIN RESEARCH CENTRE

Supp: Individuality is recovered over days

Similar states are identified in the same fish at 6 and 7 dpf