I-Bio Summer School – Banyuls/mer – August 2023

Unveiling temporal organisation in behaviors and circuit activity

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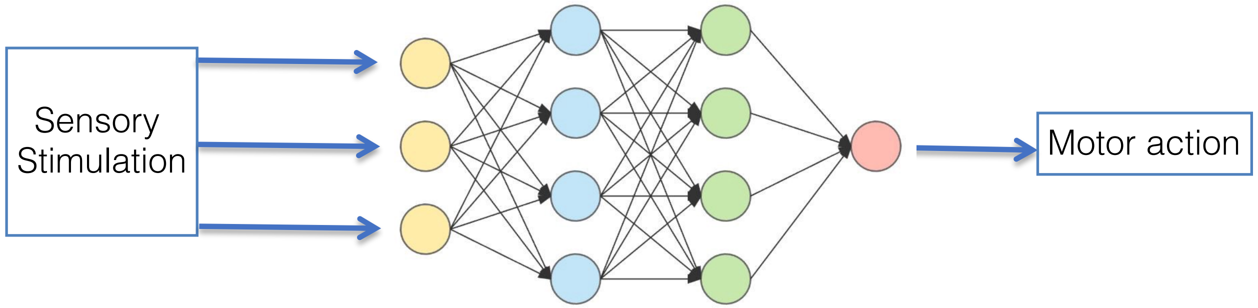
Why should we care about time in neuroscience ?



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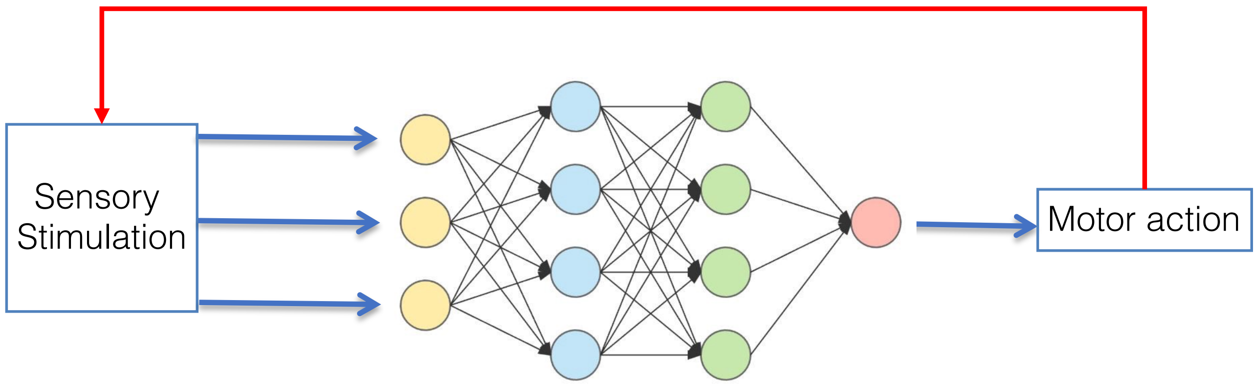

- Most actions are self-initiated / most sensory signals are reafference

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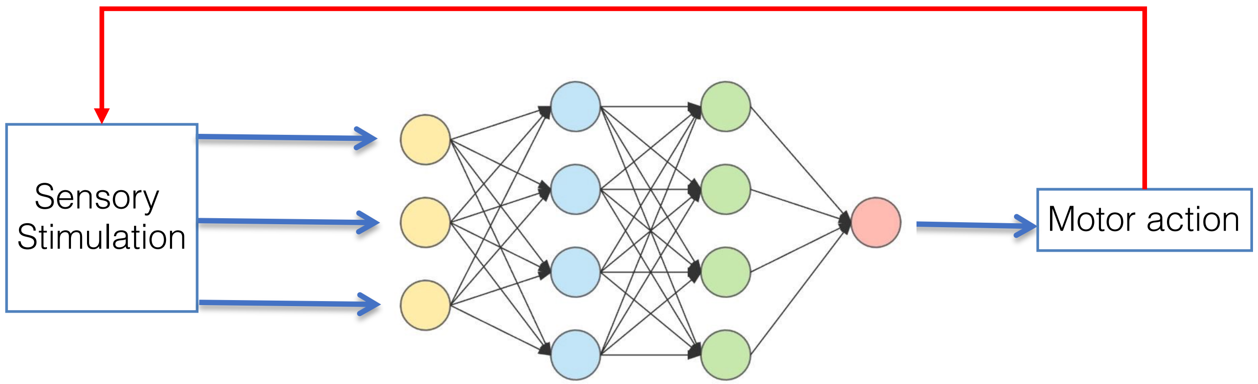
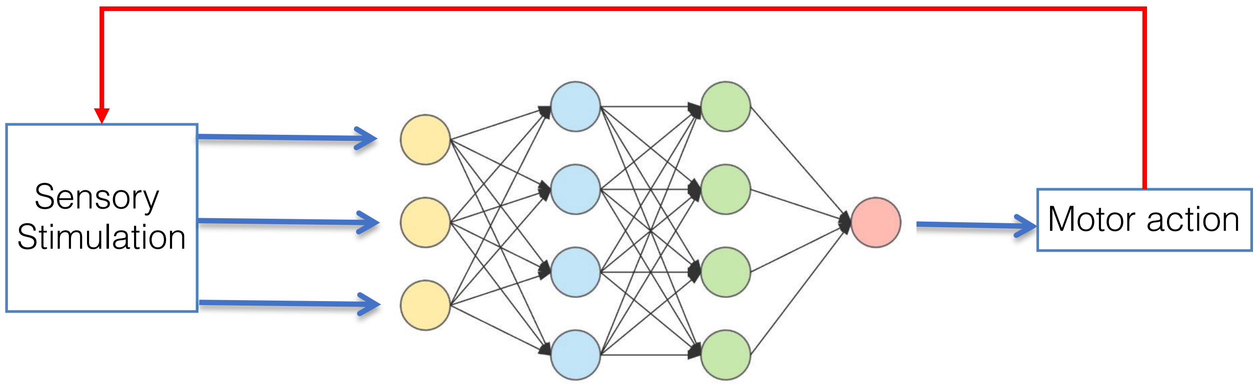


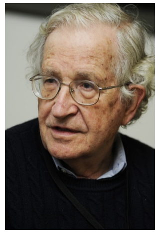
- ➢ Most actions are self-initiated / most sensory signals are reafference;
- ➢ The order in which actions are sequenced are important.

Why should we care about time in neuroscience ?

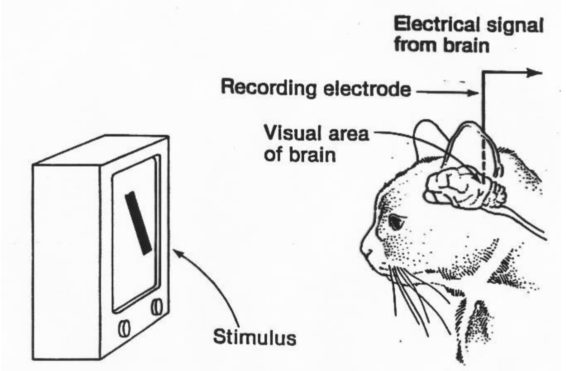


- Most actions are self-initiated / most sensory signals are reafference
- The order in which actions are sequenced is important.
- The duration of each actions can vary, but must be regulated, e.g. for cue accumulation.

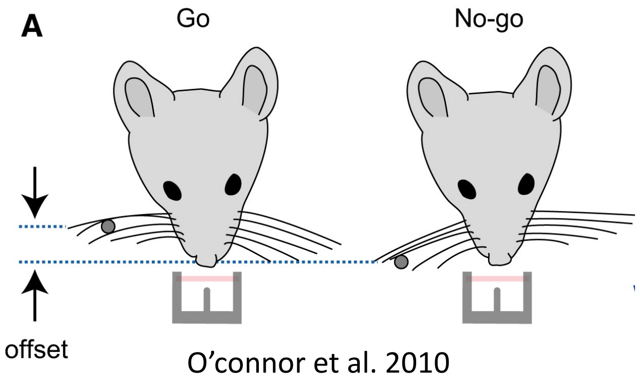
… And why have we not cared that much so far ?



“Science is a bit like the joke about the drunk who is looking under a lamppost for a key that he has lost on the other side of the street, because that's where the light is. » **– Noam Chomsky**



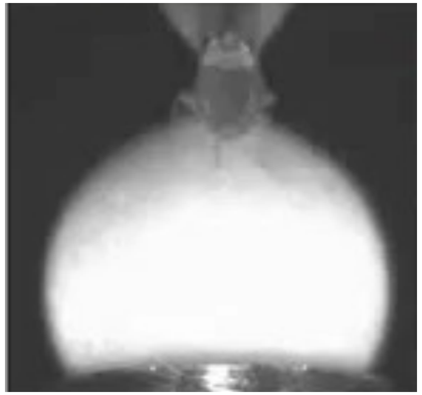
Hubel and Wiesel, 1959



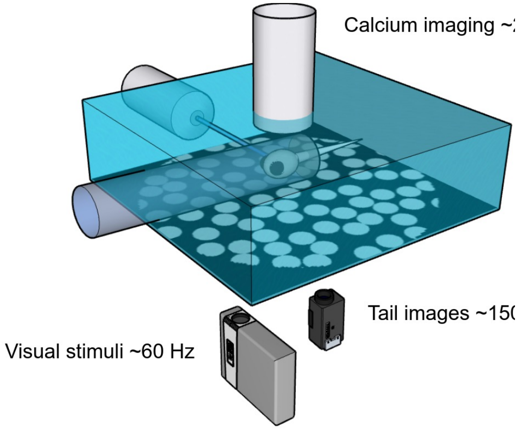
O’connor et al. 2010

New recording methods to study active interactions with the environment.

Olivia Harris' lab (UCL)



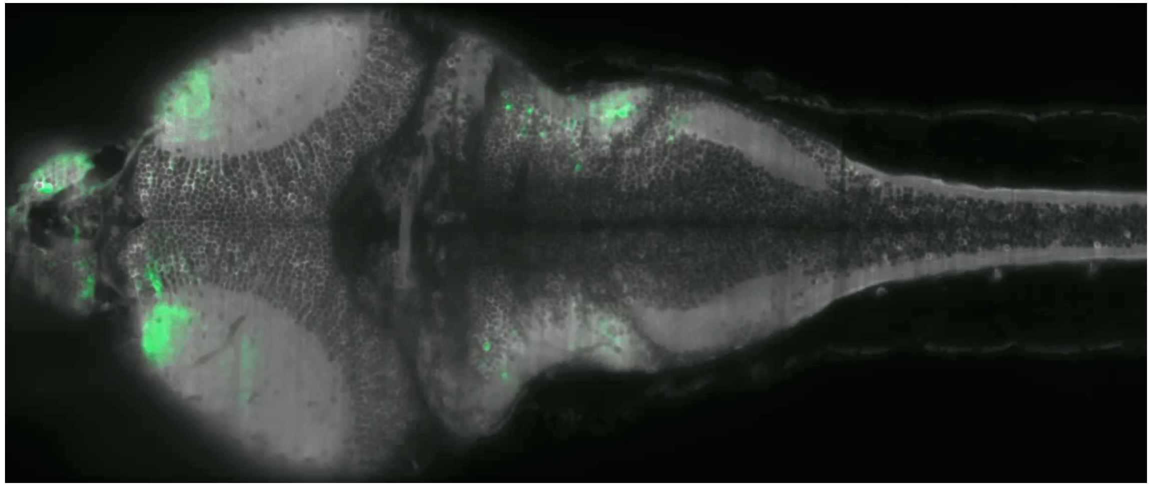
SS Kim et al., Science 2017



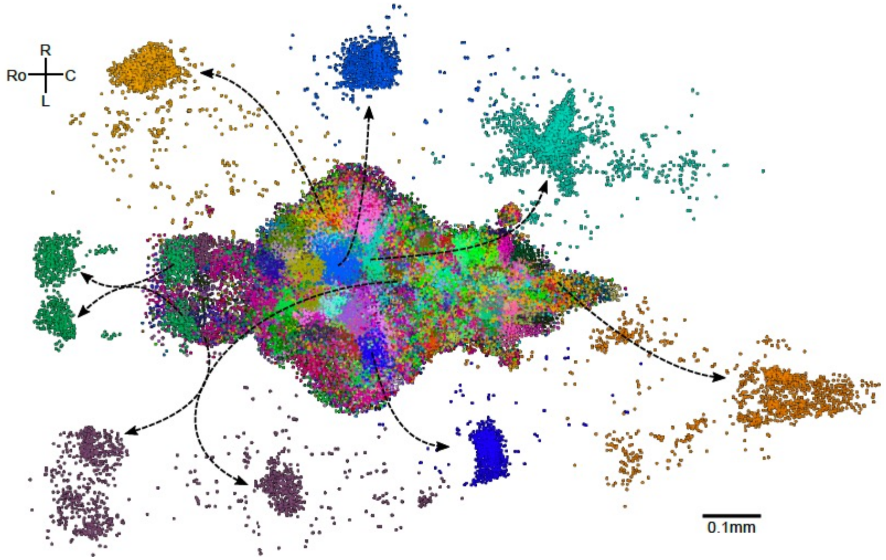
Leonardo Demarchi

Juavinett et al., Elife 2019

Spontaneous neural dynamics in extended brain circuits

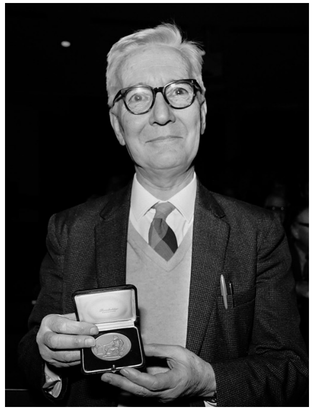


Panier et al., Front. Neuroscience 2013



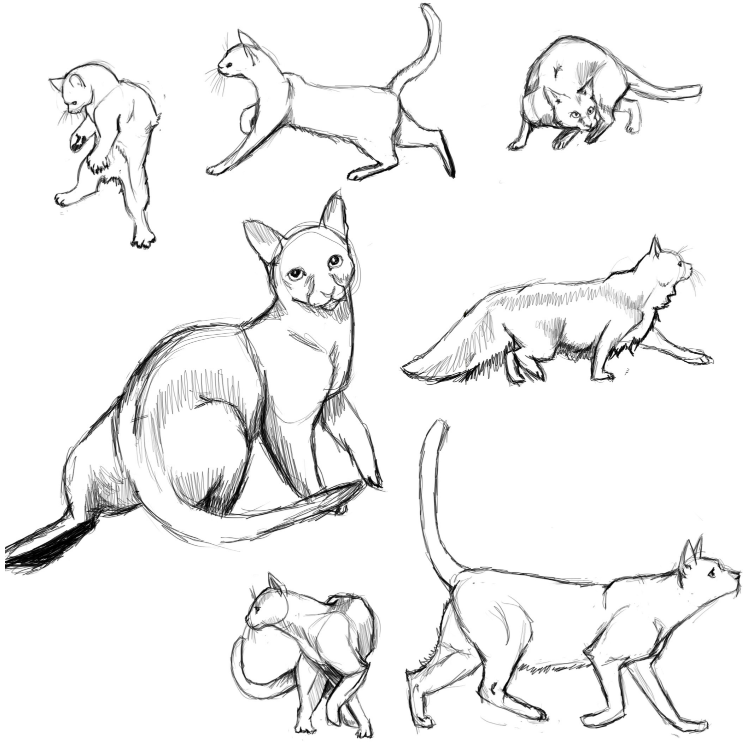
Thijs van der Plas et al, Elife 2023

How to describe/understand spontaneous behavior and its neuronal substrate
Lessons from Ethology

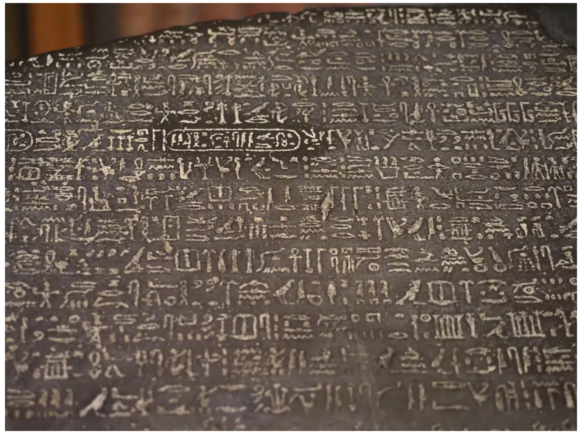


Niko Tinbergen (1907-1988) - ethologist

« Behavior is the **total** movements made by the **intact** animal in its **natural** environment»



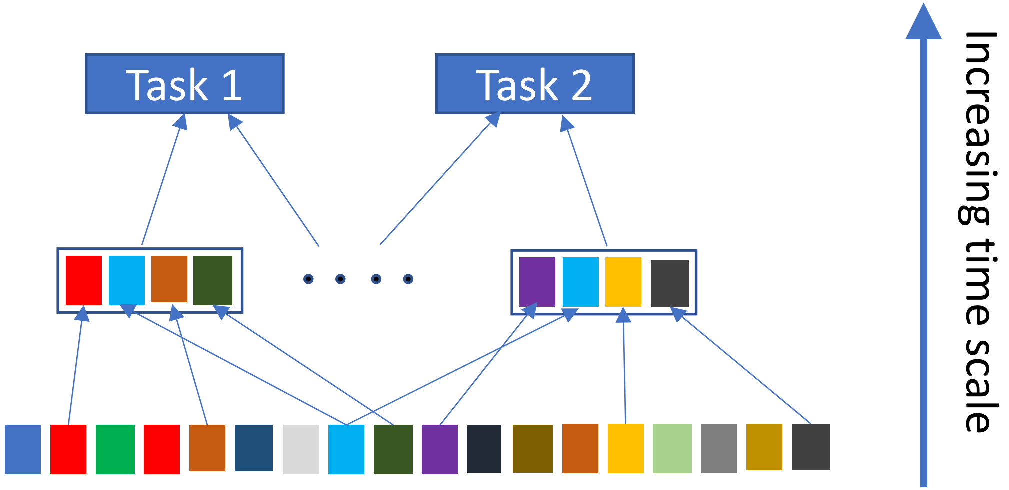
The language analogy



1) Behavior consists of elementary units, i.e. highly stereotyped **behavioral motifs**.

2) These behavioral units are deterministically or statistically concatenated, producing **modules**.

3) Environmental cues and internal needs modulate their usage and temporal structure.



OUTLINE

I – Unveiling the temporal organization in behavior

1 – Fly grooming behavior
2 – Zebrafish navigation
3 – Mice spontaneous activity
4 – Zebrafish phototaxis

II – Dissecting the neuronal circuits organizing these behaviors

1 – Hierarchical suppression in the drosophila grooming circuits
2 – Metastable attractors explain variable timing in behavioral sequences

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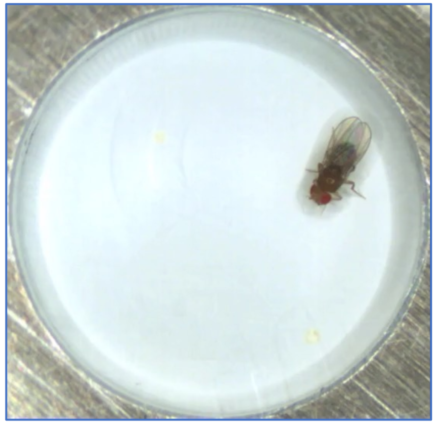
Example 1 –grooming behaviour in flies

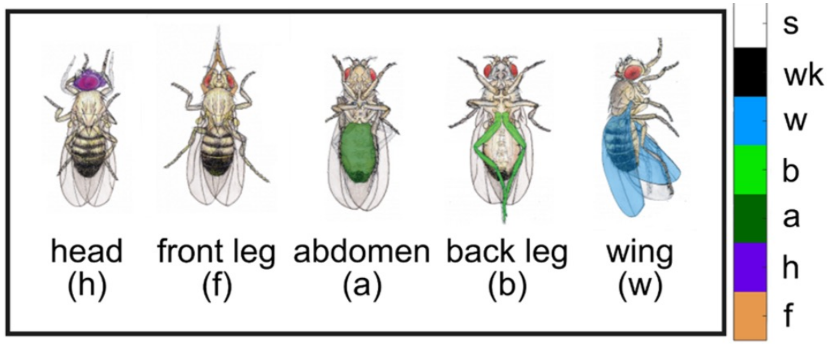


head

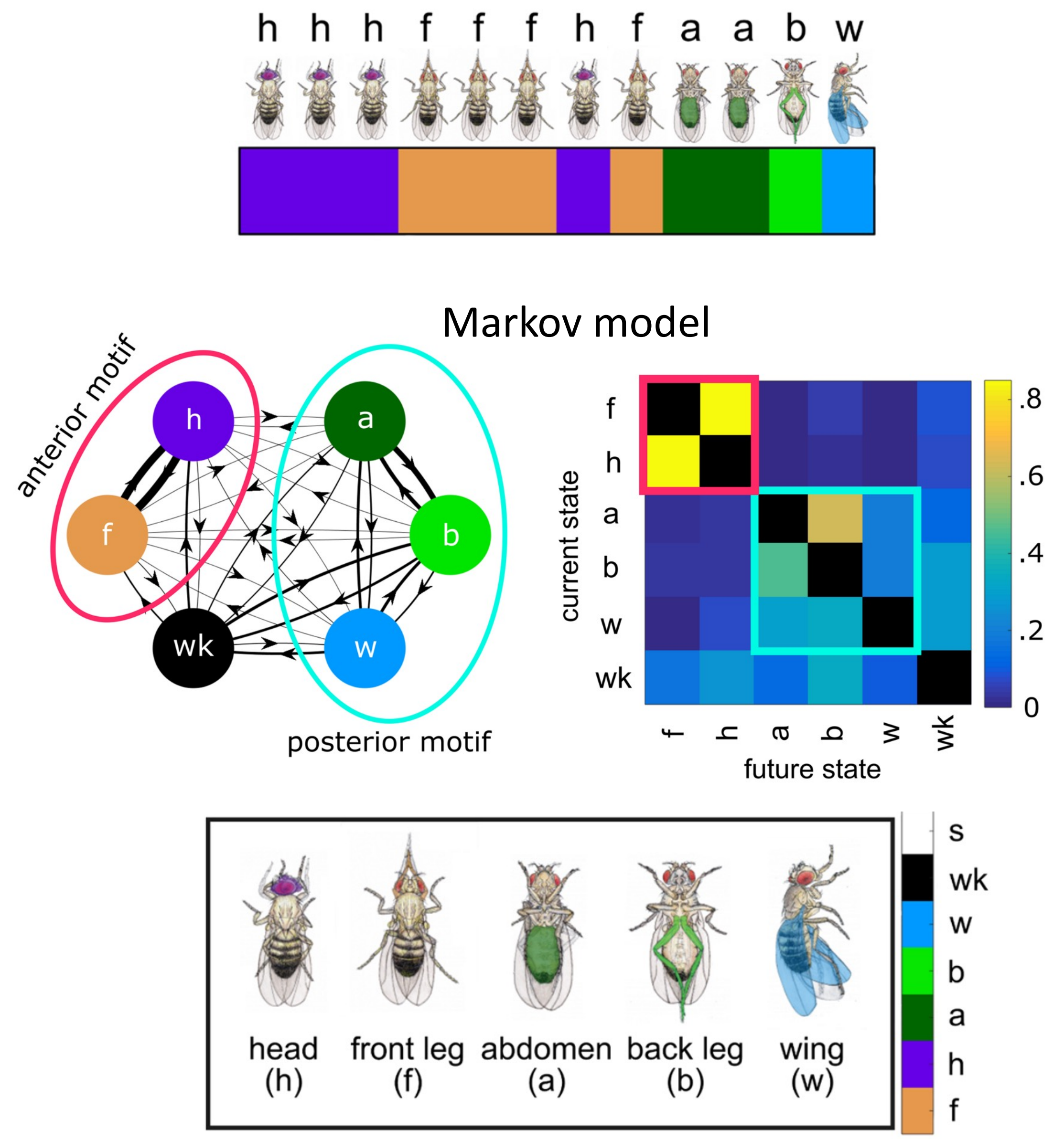
abdomen

wings

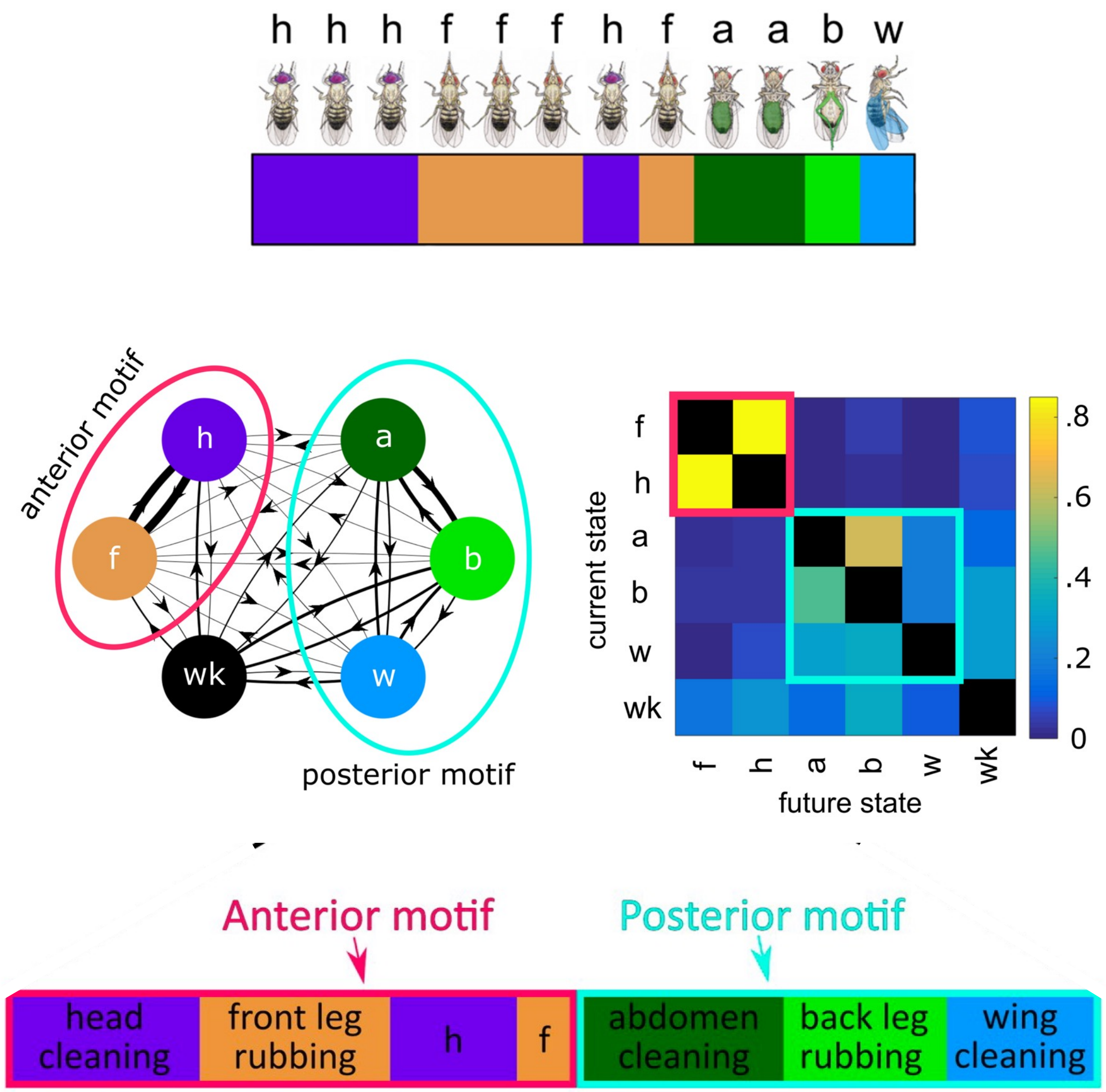
Supervized classifier

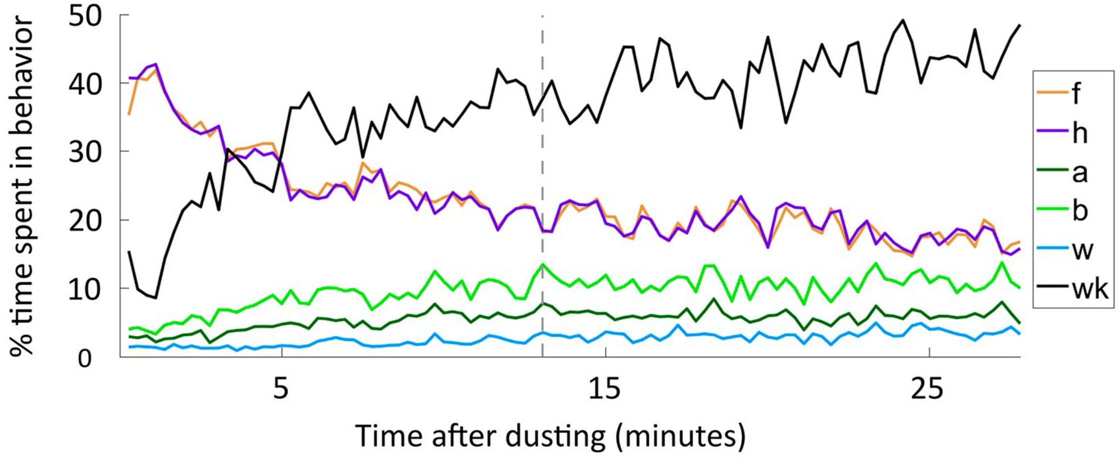


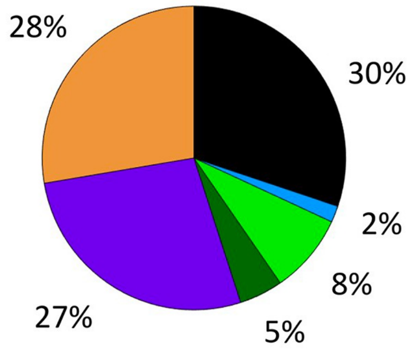
Drosophila melanogaster grooming possesses syntax with distinct rules at different temporal scales
Joshua M. Mueller Primoz Ravbar, Julie H. Simpson, Jean M. Carlson, *Plos Comp. Biol. 2019*



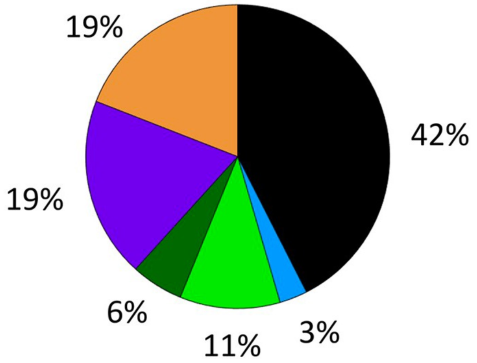
h h h f f f h f a a b w
Markov model
anterior motif
posterior motif
h
f
a
b
wk
w
current state
future state
f
h
a
b
w
wk
.8
.6
.4
.2
0
head (h)
front leg (f)
abdomen (a)
back leg (b)
wing (w)
s
wk
w
b
a
h
f


h h h f f f h f a a b w
anterior motif
posterior motif
h
a
f
b
wk
w
current state
future state
f
h
a
b
w
wk
.8
.6
.4
.2
0
Anterior motif
Posterior motif
head cleaning
front leg rubbing
h
f
abdomen cleaning
back leg rubbing
wing cleaning





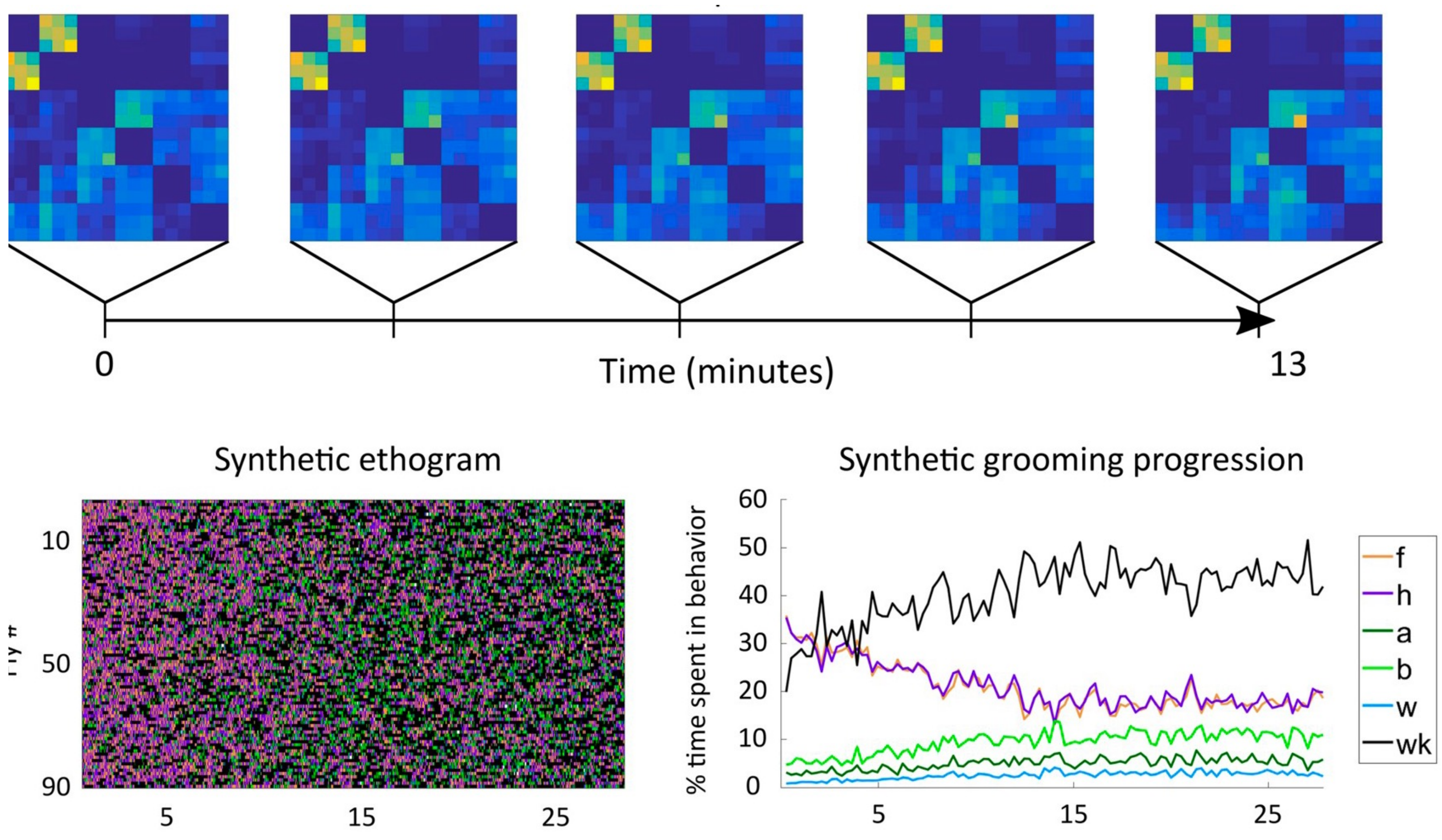
Early phase



Late phase

Anterior and posterior modules are unchanged, but their relative usage evolve with time

Time-varying Markov model



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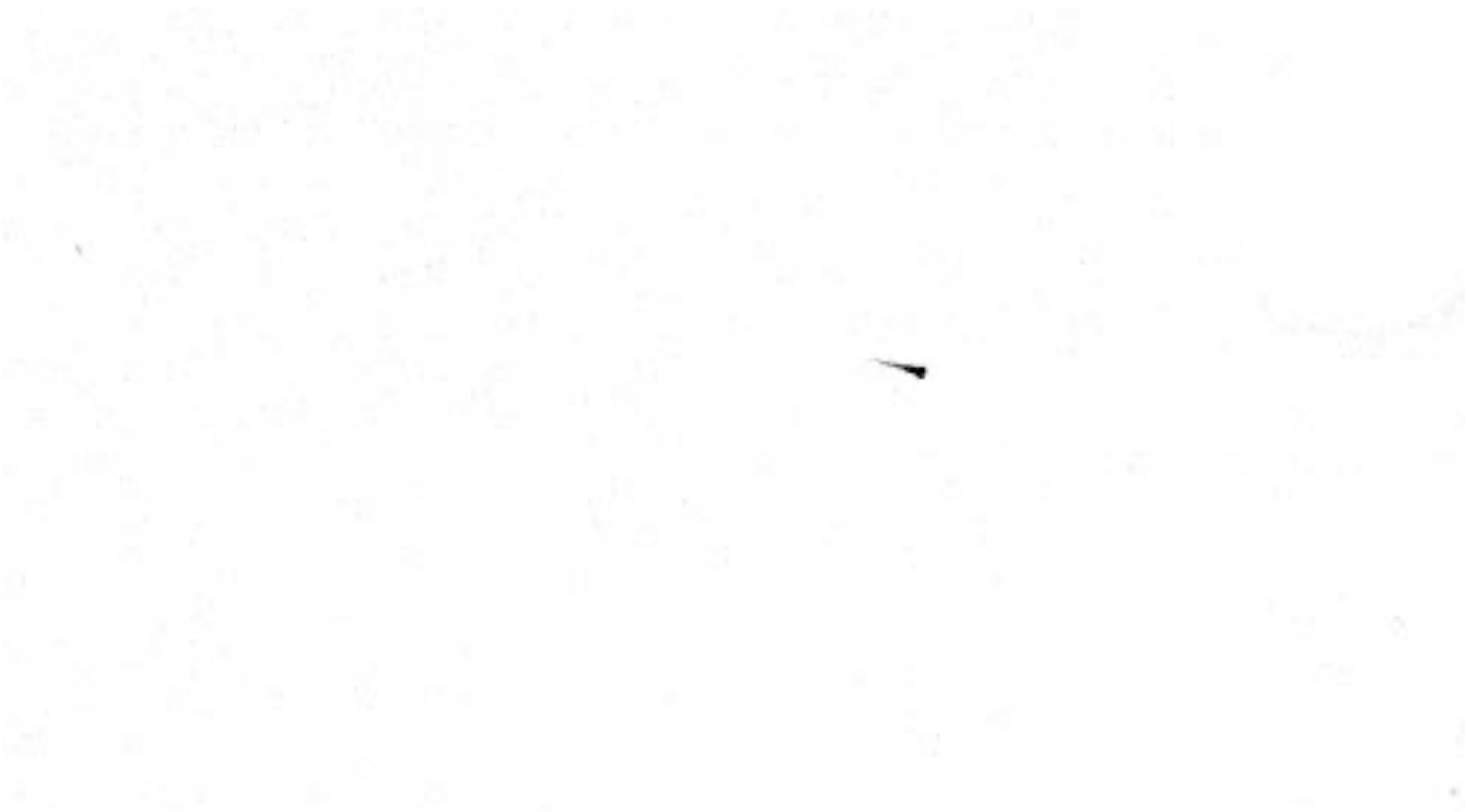
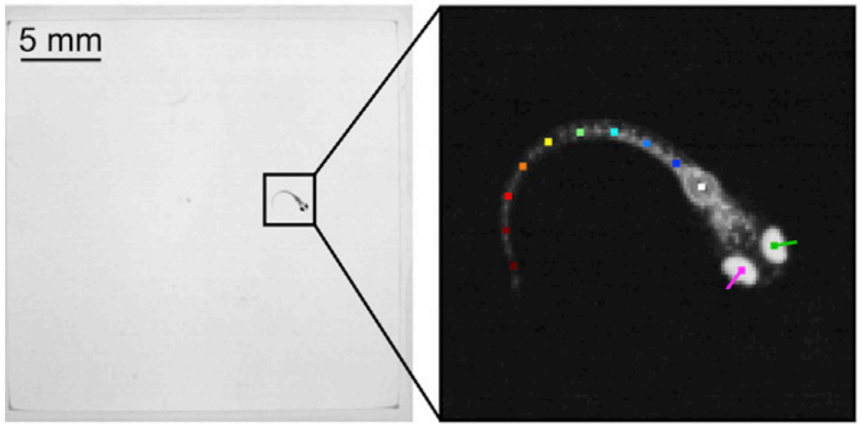
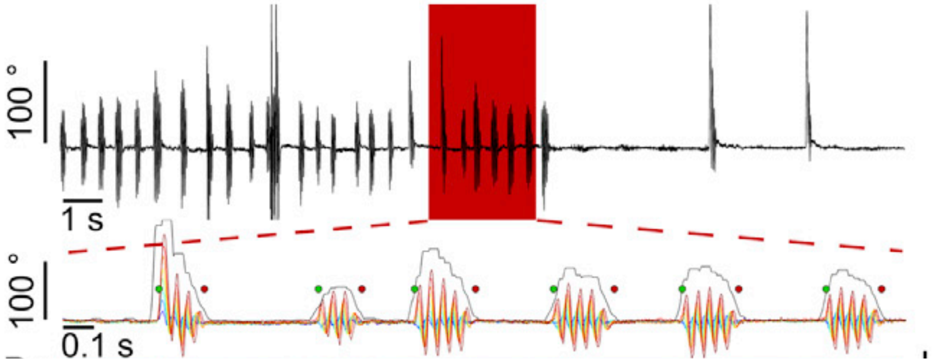
Example 2 –bout classification in zebrafish navigation

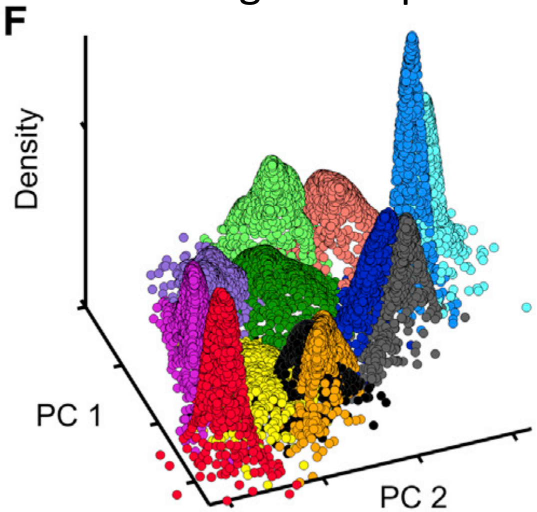






Extract 73 kinematic parameters

Clustering in PC space



Structure of the Zebrafish Locomotor Repertoire Revealed with Unsupervised Behavioral Clustering

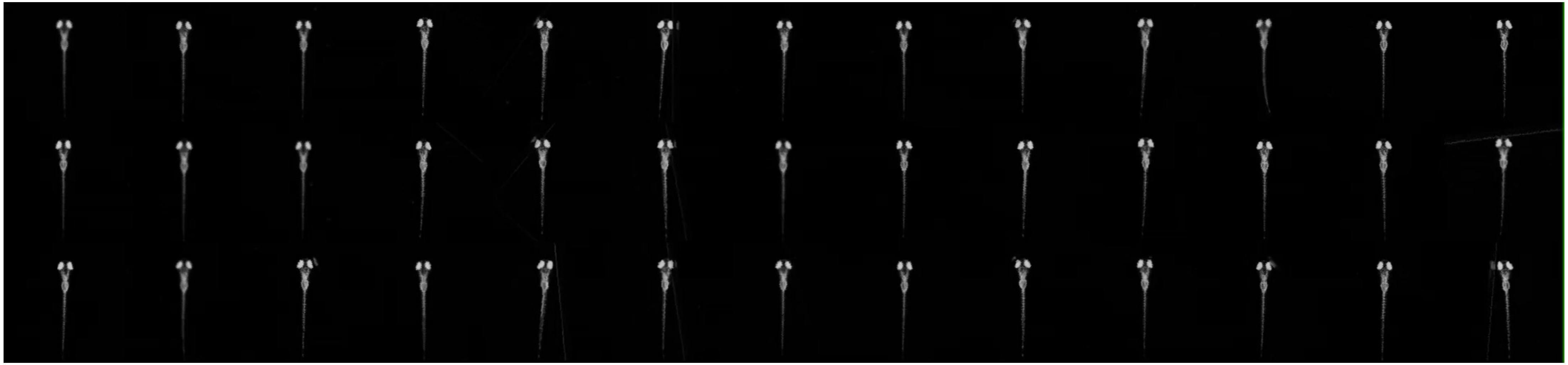
Joao C. Marques, Simone Lackner, Rita Felix, and Michael B. Orger

Current Biology 2017

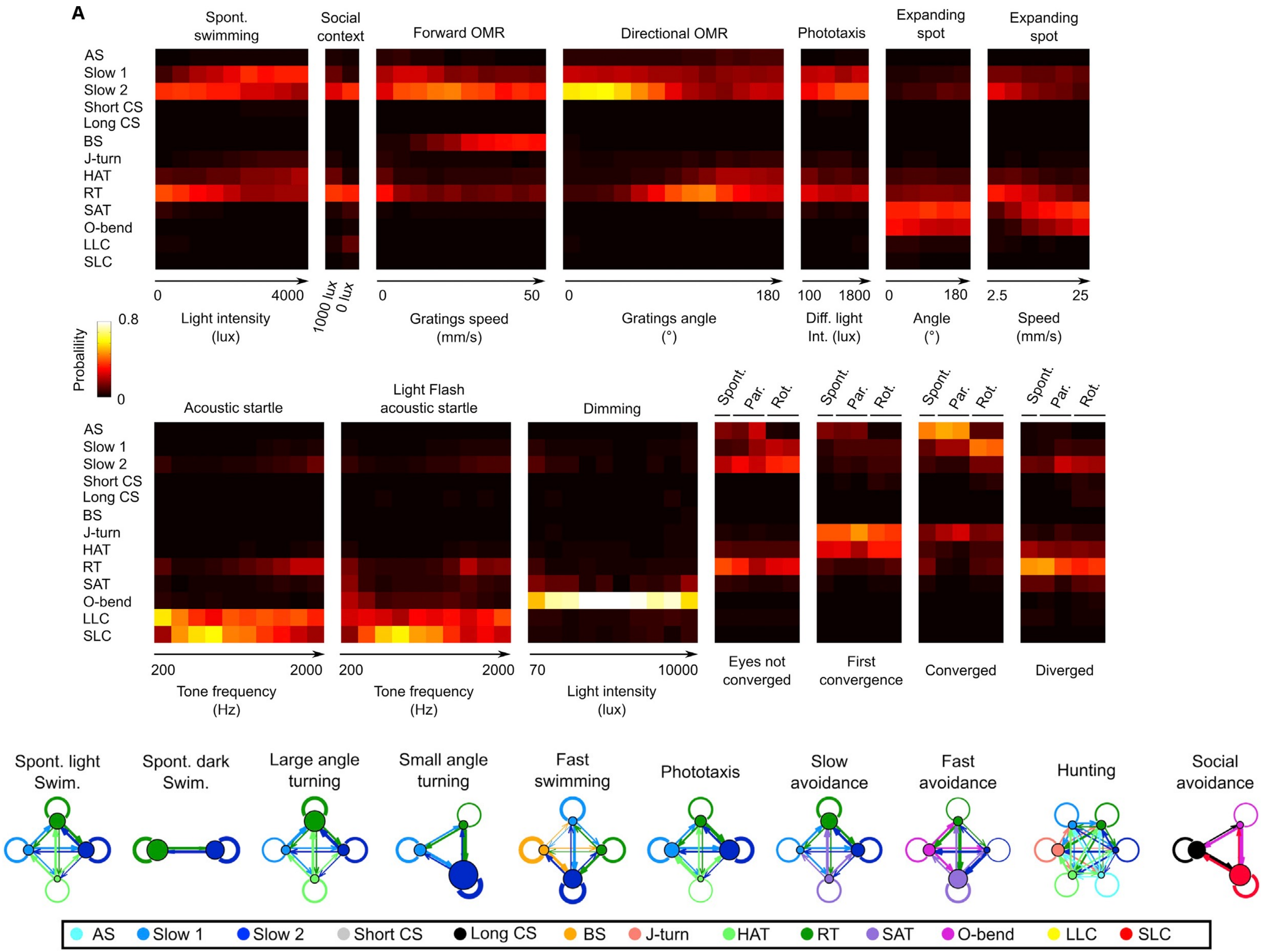
Fish #1

Fish #2

Fish #3



Rq: there is a level of subjectivity in this classification. One could decide to merge some of them.



A
Spont. swimming
Social context
Forward OMR
Directional OMR
Phototaxis
Expanding spot
Expanding spot
AS
Slow 1
Slow 2
Short CS
Long CS
BS
J-turn
HAT
RT
SAT
O-bend
LLC
SLC
0
4000
Light intensity (lux)
1000 lux
0 lux
0
50
Gratings speed (mm/s)
0
180
Gratings angle (°)
100
1800
Diff. light Int. (lux)
0
180
Angle (°)
2.5
25
Speed (mm/s)
Probalility
0.8
0
Acoustic startle
Light Flash acoustic startle
Dimming
Spont.
Par.
Rot.
200
2000
Tone frequency (Hz)
200
2000
Tone frequency (Hz)
70
10000
Light intensity (lux)
Eyes not converged
First convergence
Converged
Diverged
Spont. light Swim.
Spont. dark Swim.
Large angle turning
Small angle turning
Fast swimming
Phototaxis
Slow avoidance
Fast avoidance
Hunting
Social avoidance
AS
Slow 1
Slow 2
Short CS
Long CS
BS
J-turn
HAT
RT
SAT
O-bend
LLC
SLC

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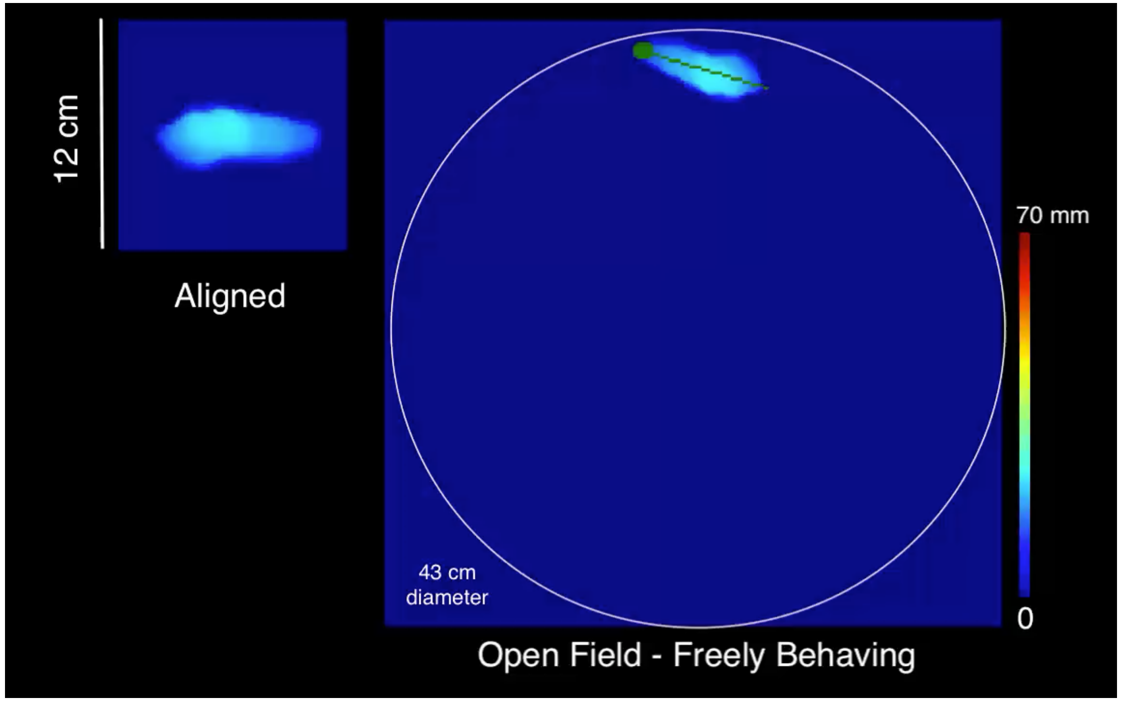
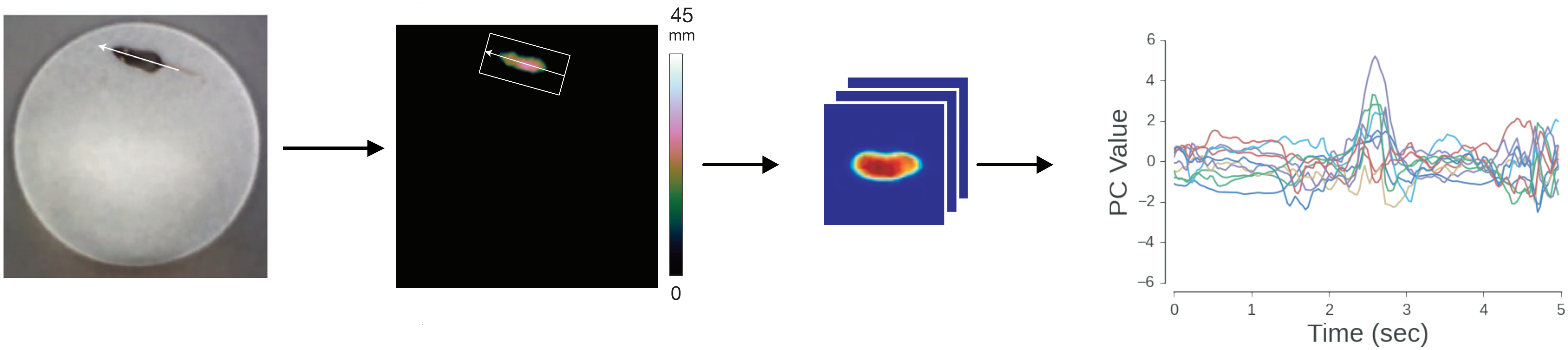
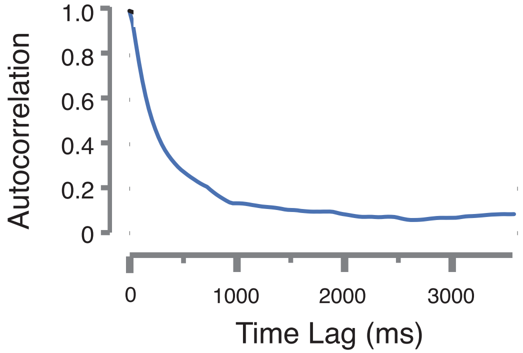
Example 3 –identification of stereotyped motifs in mouse behavior

Wiltschko 2015 - Mapping Sub-Second Structure in Mouse Behavior







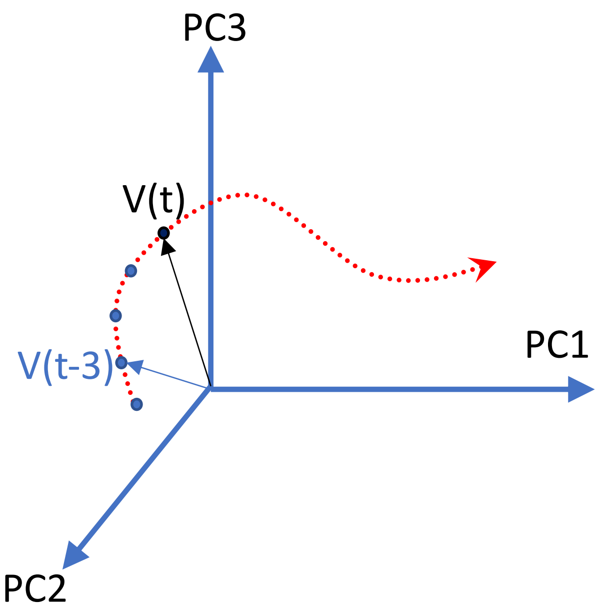
The behavioral sequence appears to be structured on sub-second time scale

AR-HMM model (auto-regressive hidden Markov model)

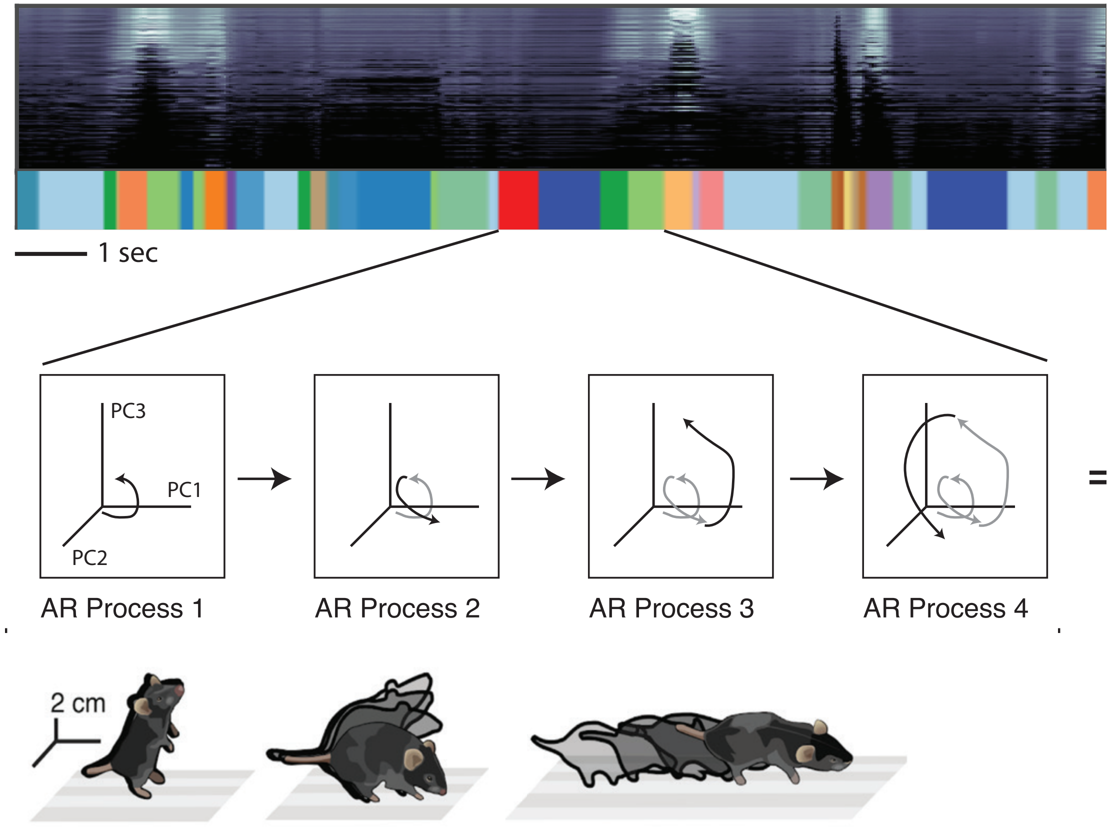
1) The behavior can be decomposed into a set of short-lived discrete behavioral motifs

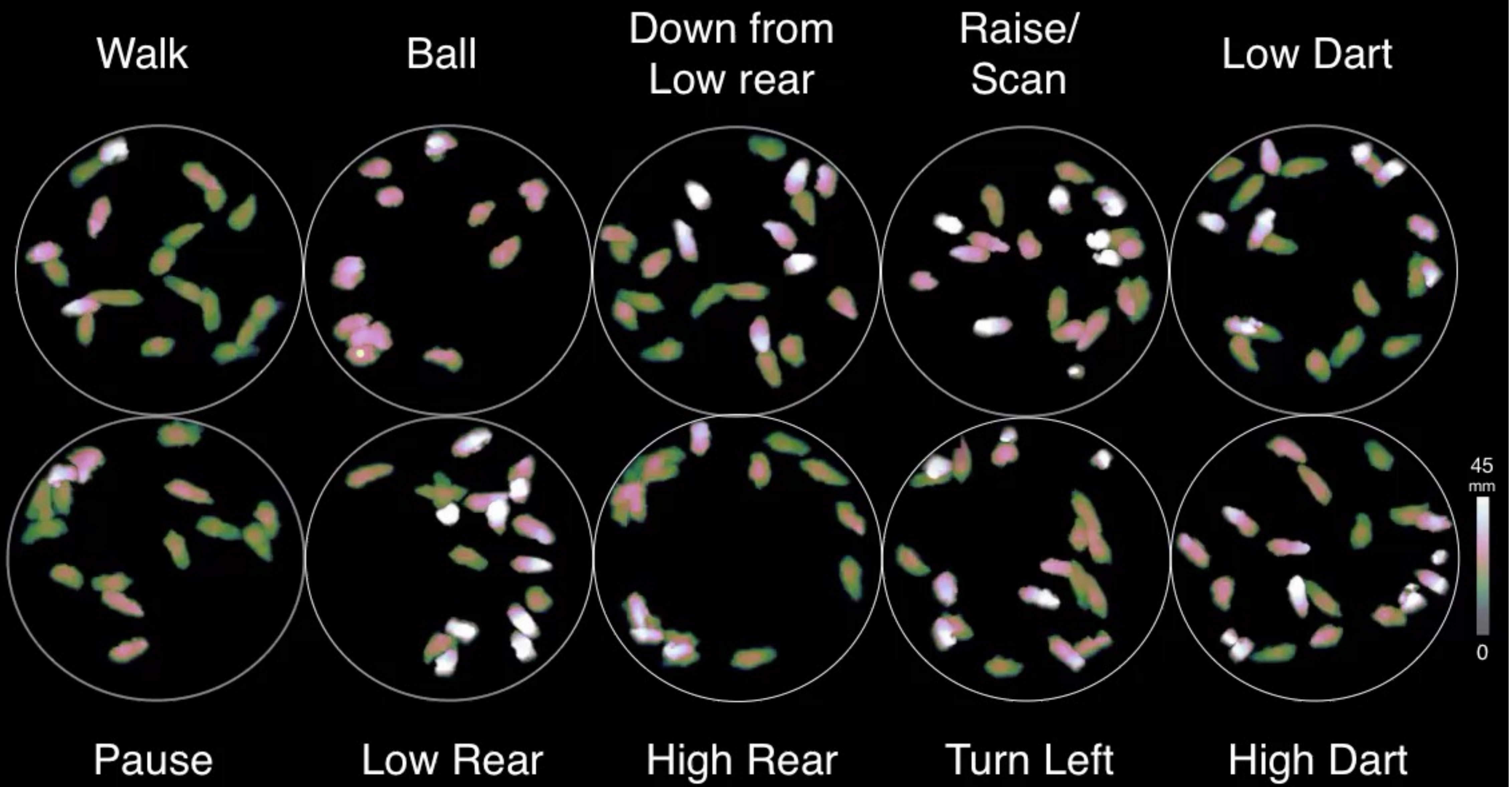
2) Each motif is characterized by its own pose dynamics captured by an AR process

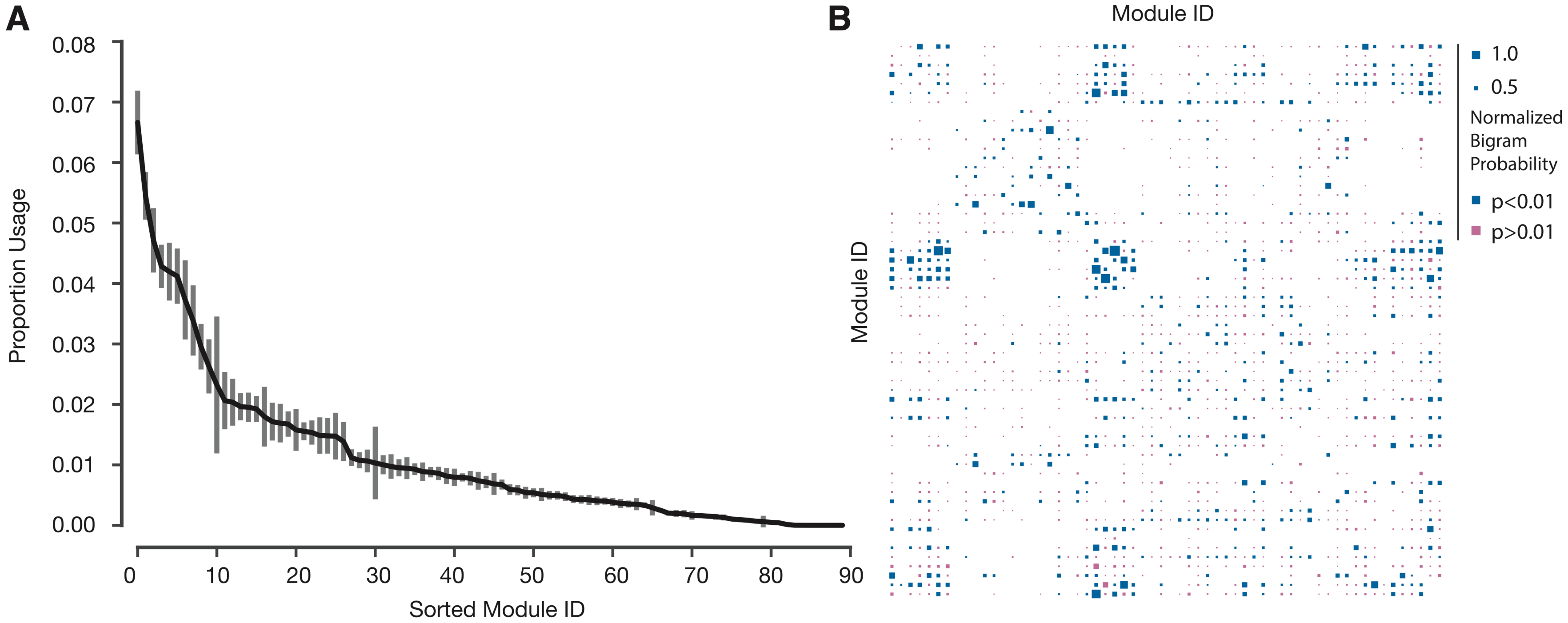
AR process: $V(t) = \alpha_1 . V(t-1) + \alpha_2 . V(t-2) + \cdots + \alpha_k . V(t-k)$



$\{\alpha_1, \alpha_2, .., \alpha_k\}$ is specific to a given hidden state, i.e. a particular behavioral motif

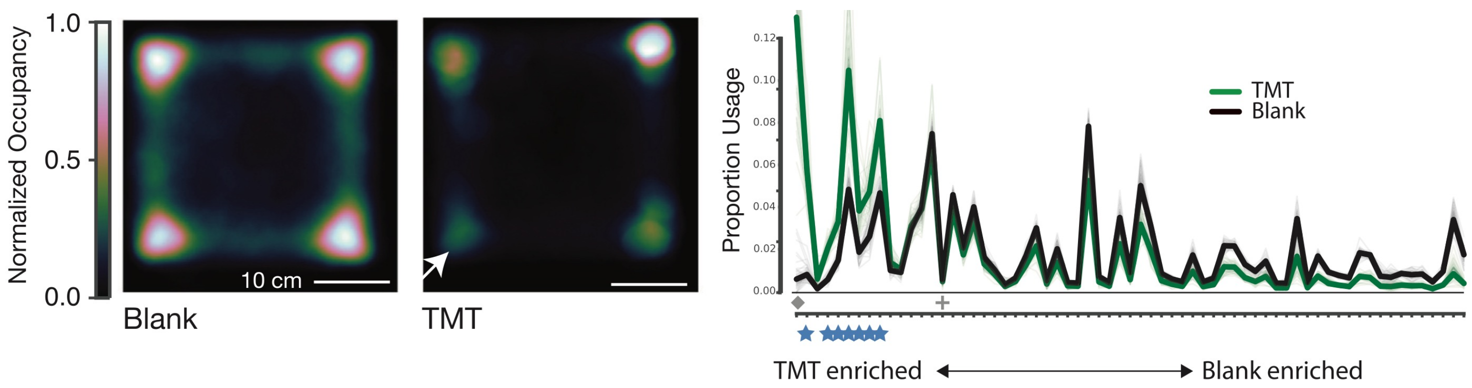

1 sec
PC3
PC1
PC2
AR Process 1
AR Process 2
AR Process 3
AR Process 4
=
2 cm


Walk
Ball
Down from Low rear
Raise/ Scan
Low Dart
45 mm
0
Pause
Low Rear
High Rear
Turn Left
High Dart

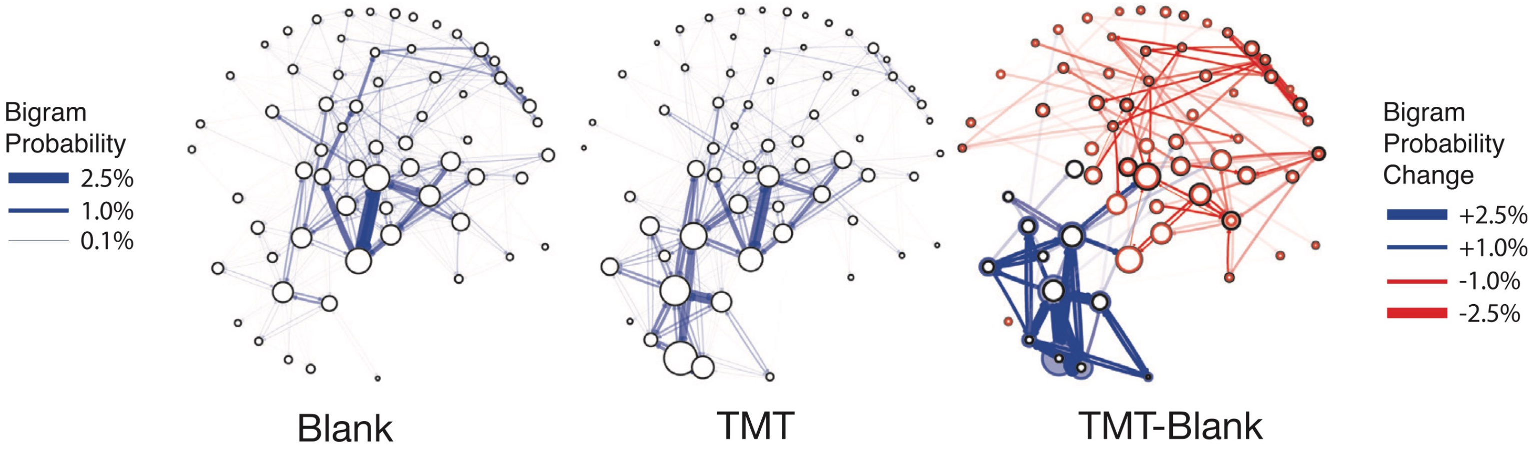


A
B
Proportion Usage
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
10
20
30
40
50
60
70
80
90
Sorted Module ID
Module ID
Module ID
1.0
0.5
Normalized Bigram Probability
p<0.01
p>0.01

When submitted to an aversive odor, the repertoire is essentially unchanged, but the transition rates and pose usage is modified.



C



D

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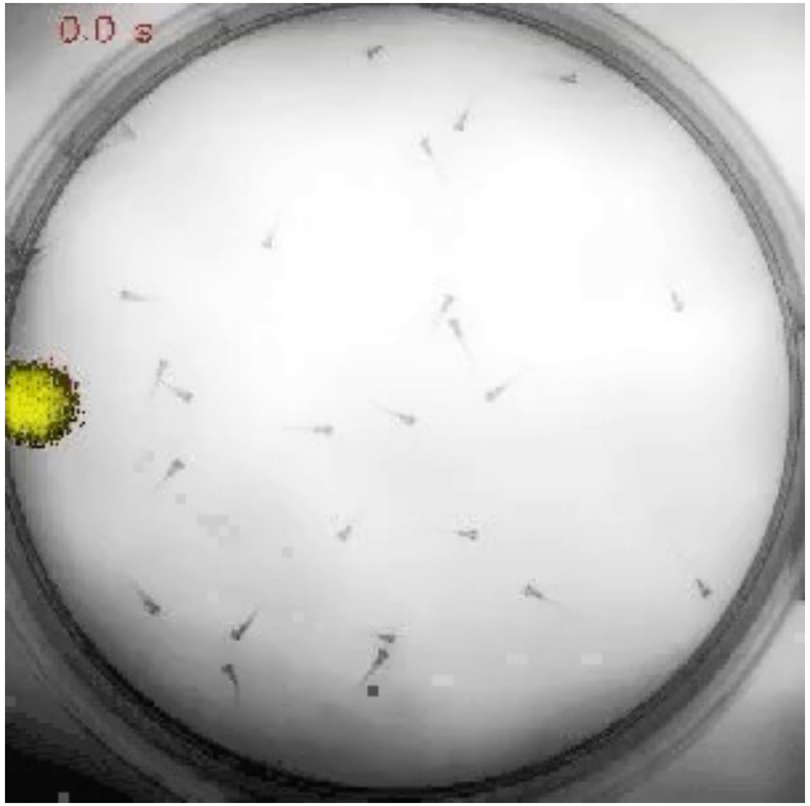
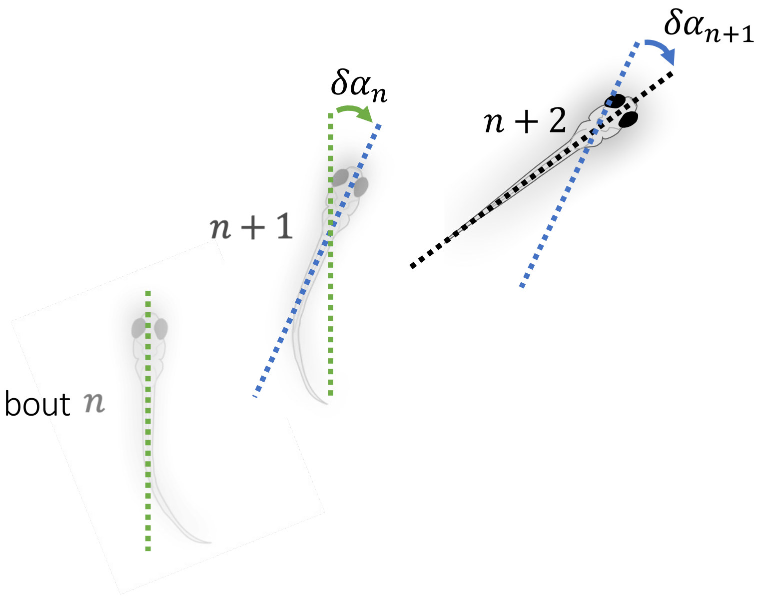
Using Markov-chain to understand phototactic strategies in zebrafish larvae

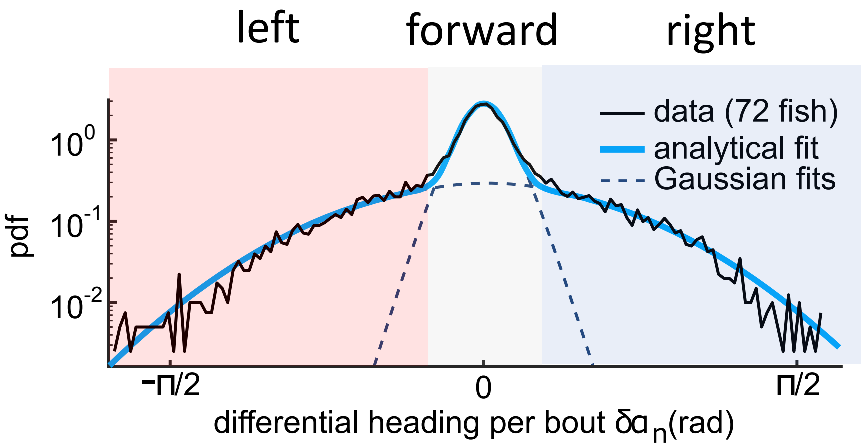


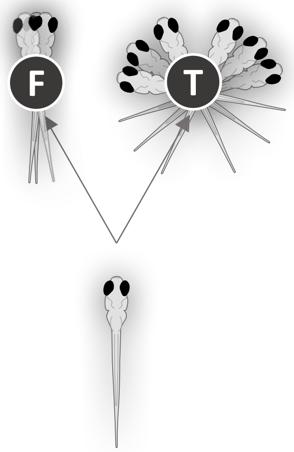
Burgess et al; 2010

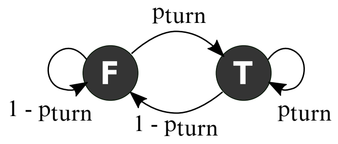
From behavior to circuit modeling of light-seeking navigation in zebrafish larvae
Sophia Karpenko et al., Elife 2020

Focus on reorientation dynamics

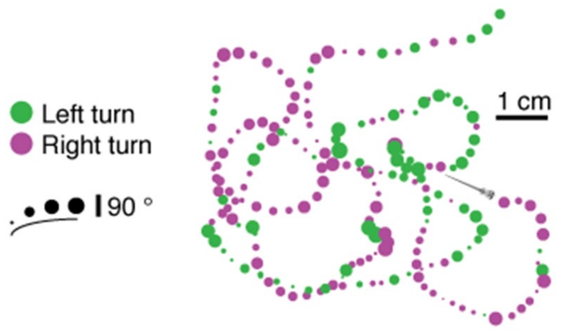


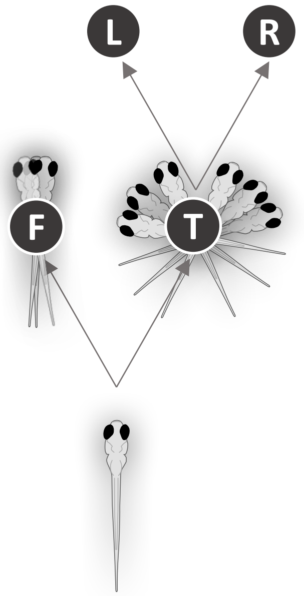


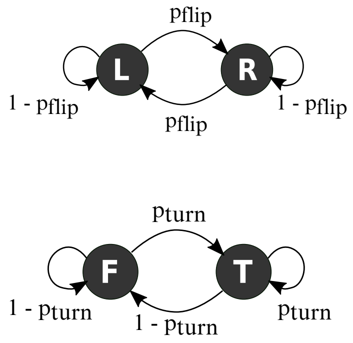




$p_{turn} \sim 0.4$







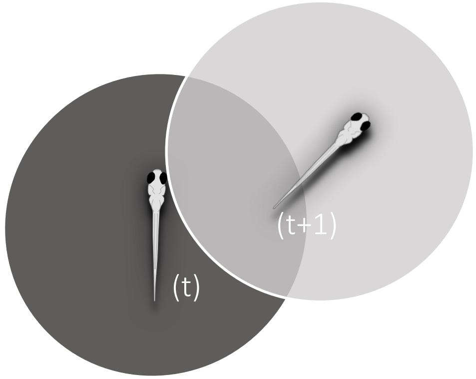
$p_{flip} \sim 0.2$

$p_{turn} \sim 0.4$

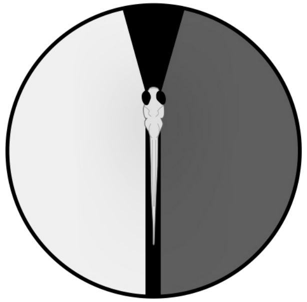
How does the light gradient impact the navigational dynamics.

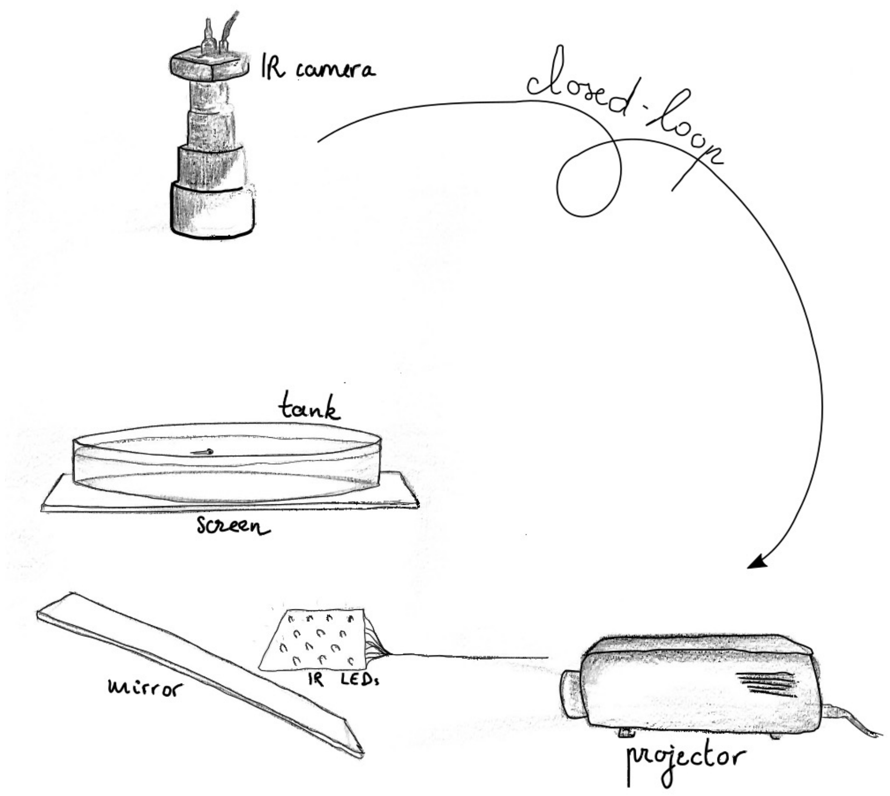
2 possible strategies

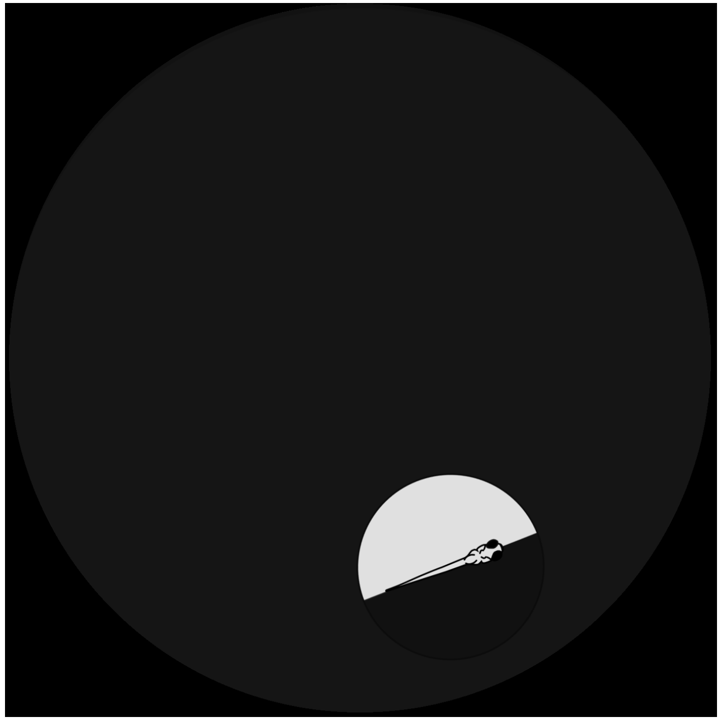
Temporal integration



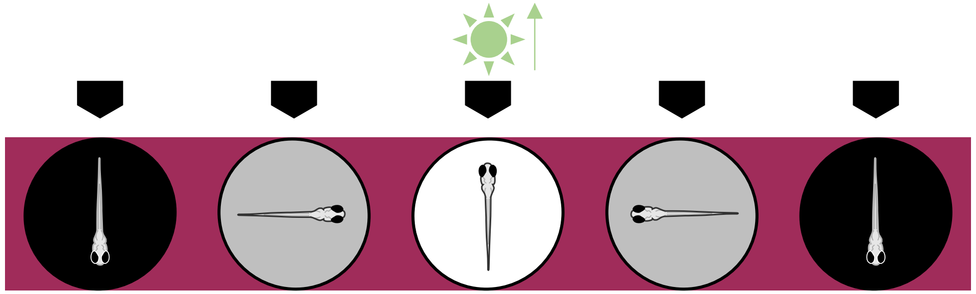
Contrast

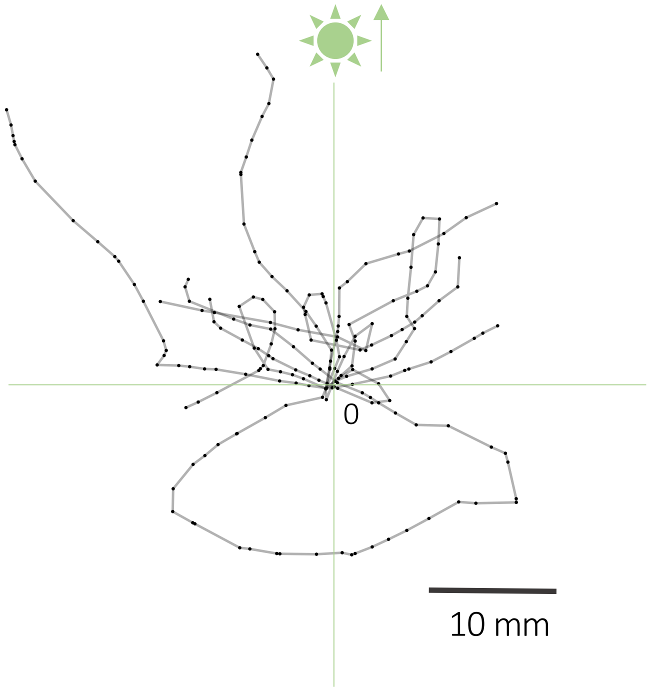


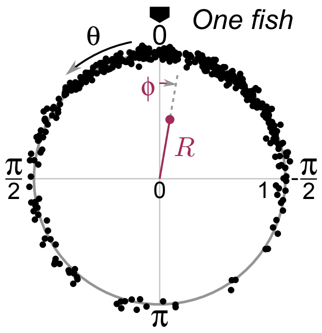

IR camera
closed-loop
tank
screen
mirror
IR LEDs
projector

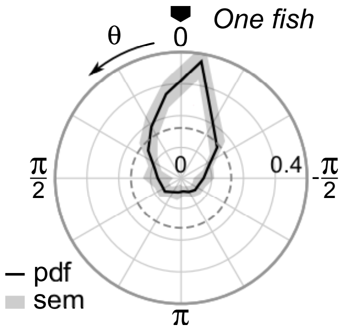


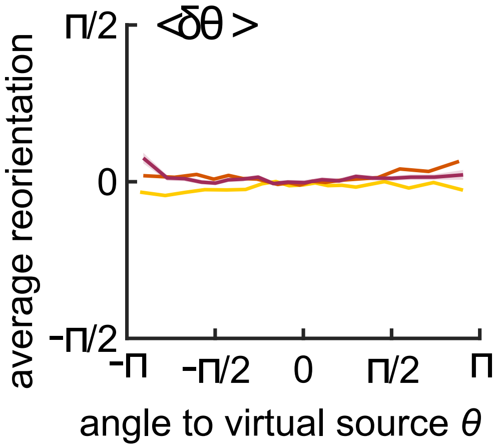
1) Temporal integration



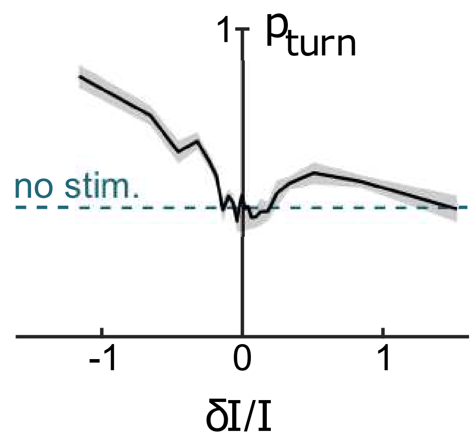




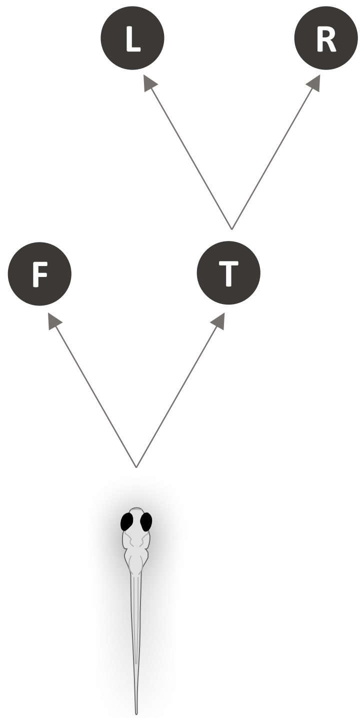


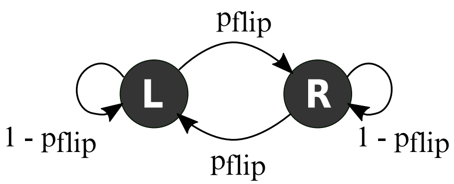


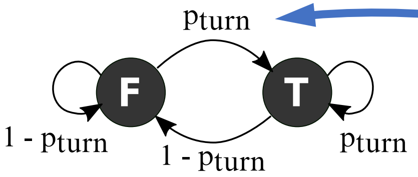
<δθ>
π/2
0
-π/2
average reorientation
-π
-π/2
0
π/2
π
angle to virtual source θ



1
p turn
no stim.
-1
0
1
δI/I



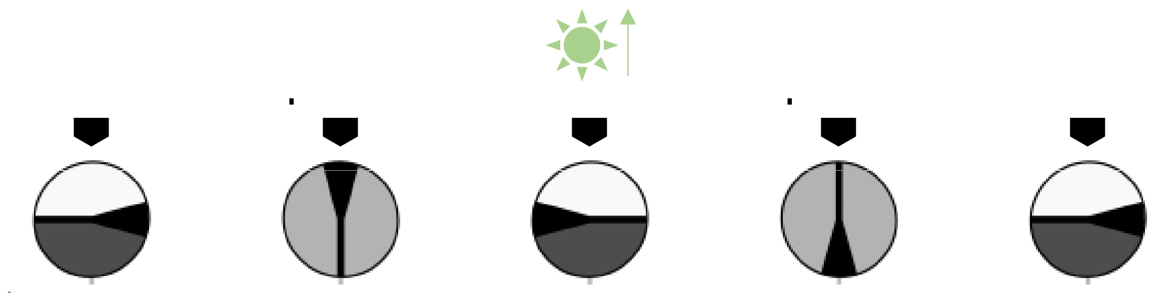


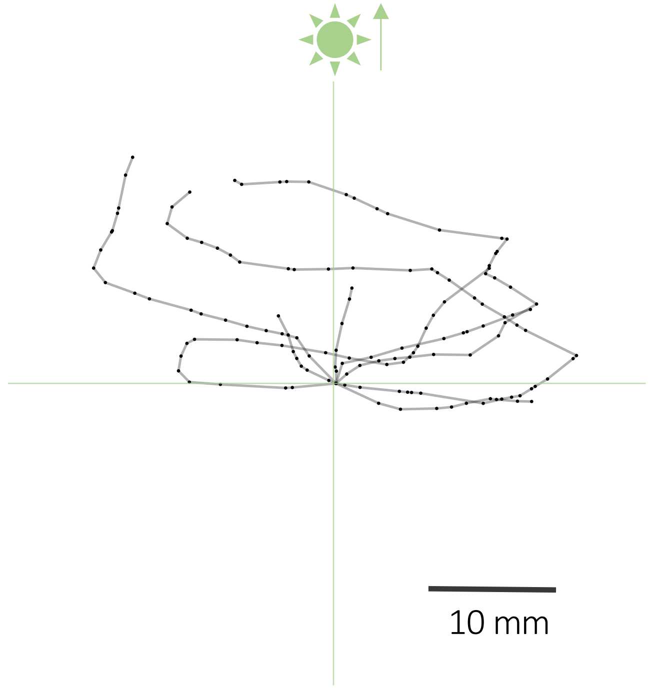


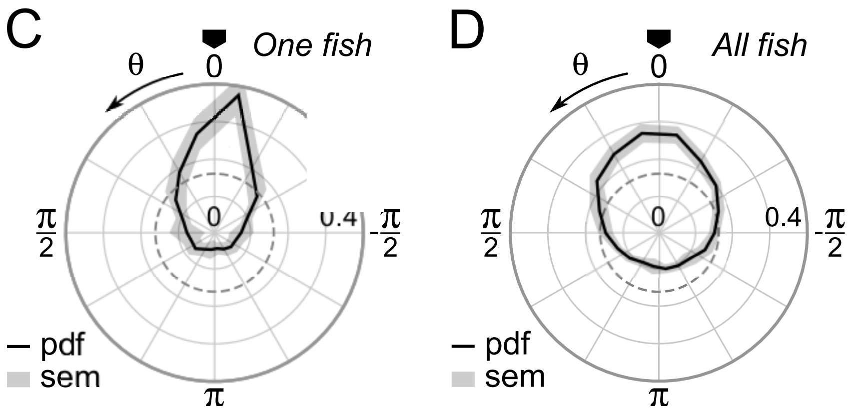
Non-directional bias

$$\frac{\delta I}{I} < 0$$

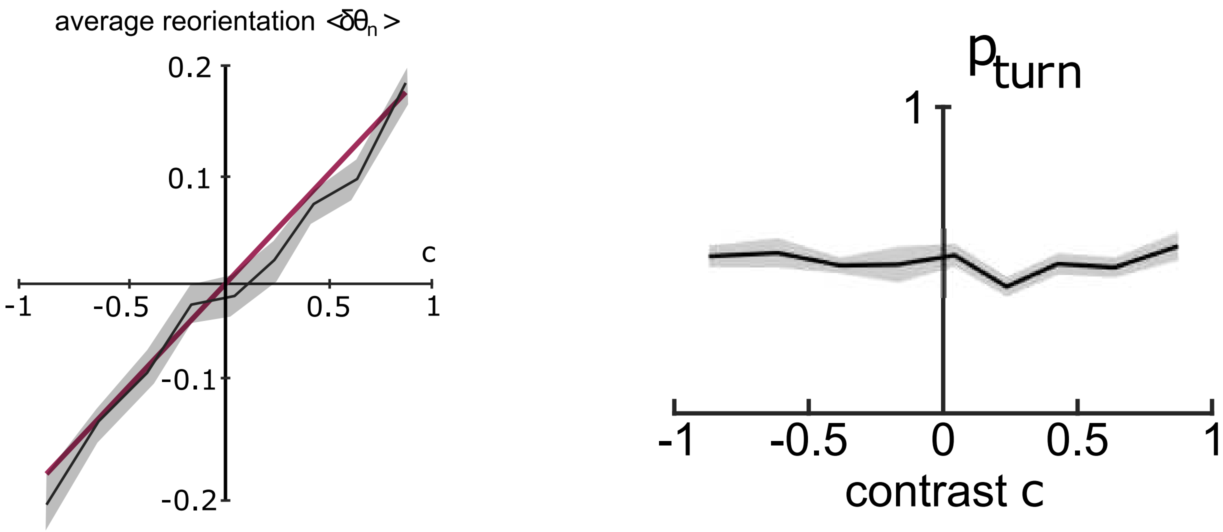
2) Binocular comparison



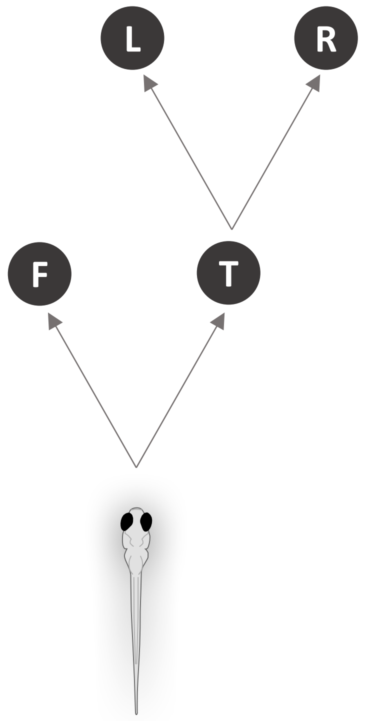


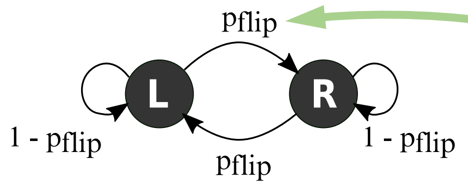


N = 47 fish



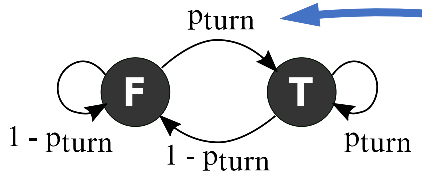
average reorientation <δθn>
0.2
0.1
-0.1
-0.2
c
-1
-0.5
0.5
1
pturn
1
-1
-0.5
0
0.5
1
contrast c





Directional bias

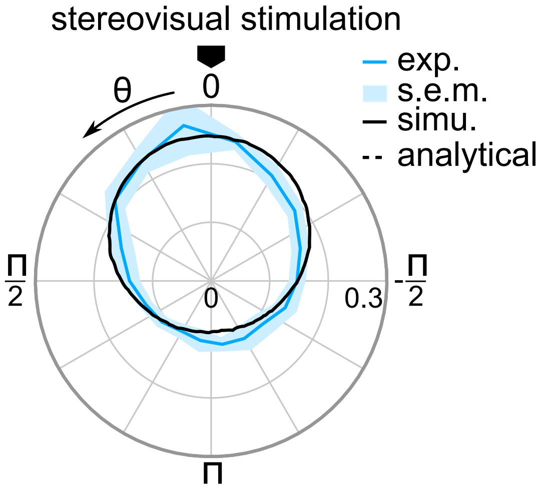
$$c = IL - IR$$

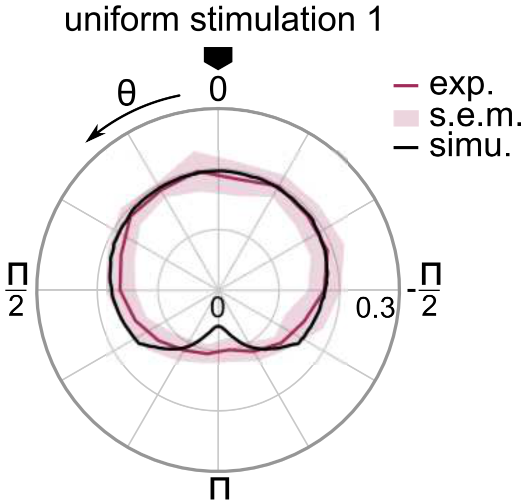


Non-directional bias

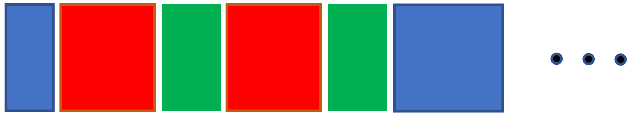
$$\frac{\delta I}{I} < 0$$

Simulated phototaxis

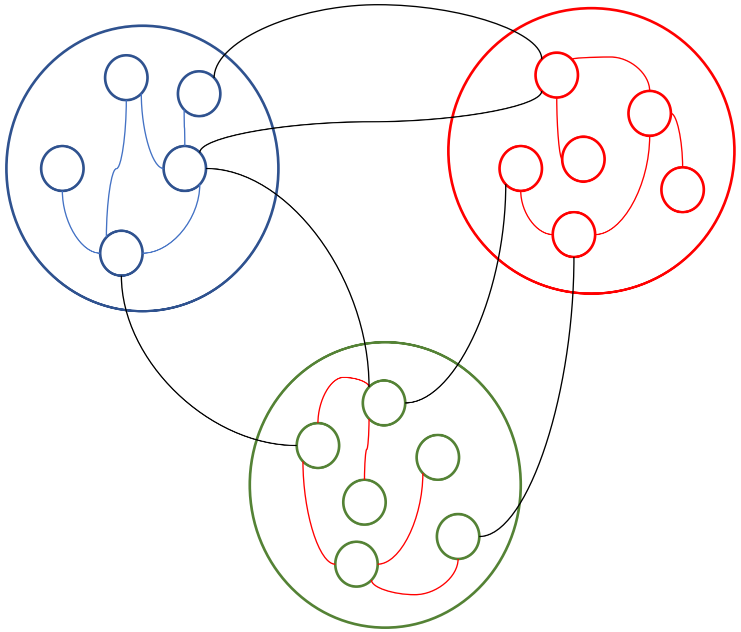




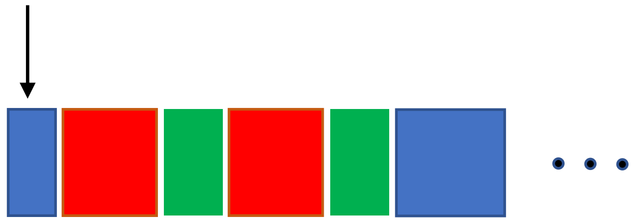
Neuronal substrate of self-initiated behavioral sequences



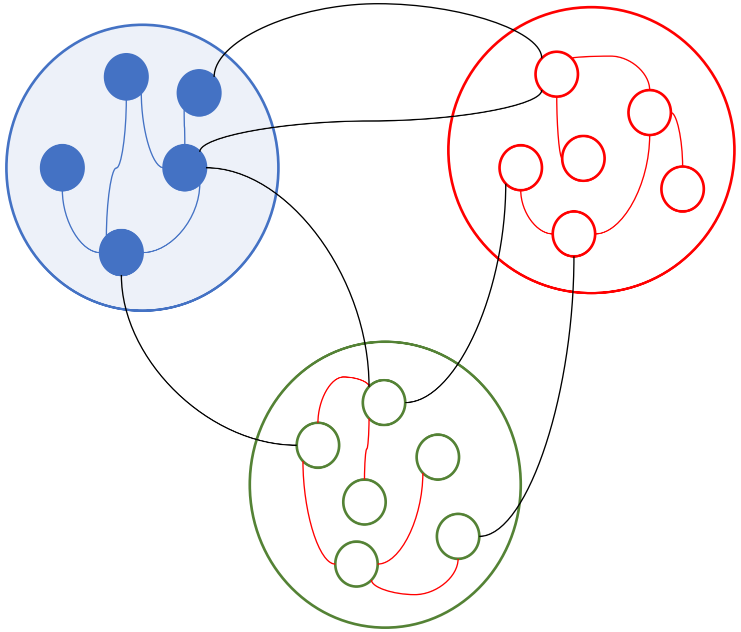
Sequence of self-initiated actions



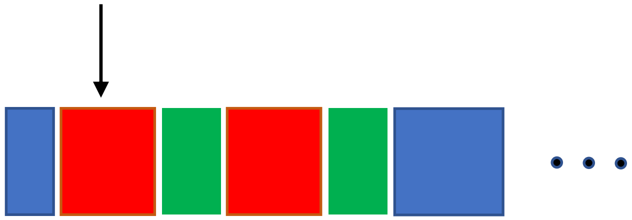
Neuronal substrate of self-initiated behavioral sequences



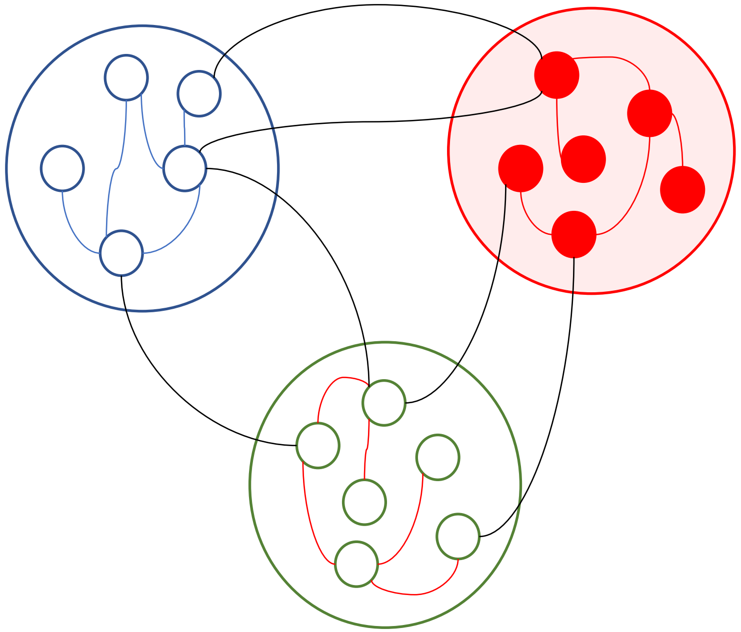
Sequence of self-initiated actions



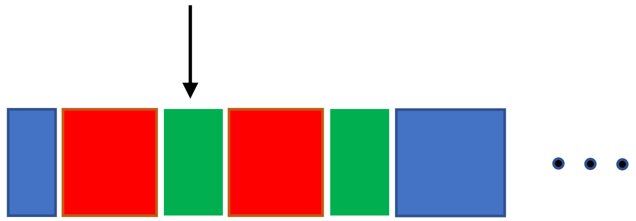
Neuronal substrate of self-initiated behavioral sequences



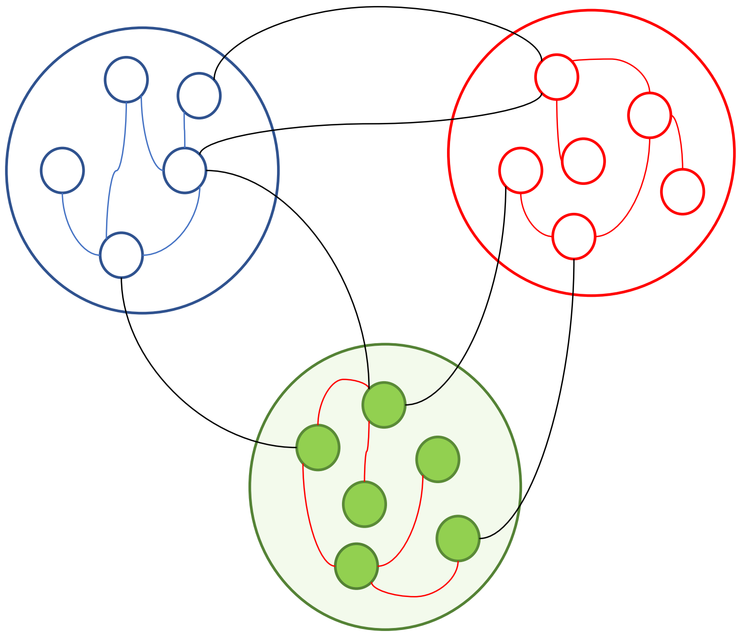
Sequence of self-initiated actions



Neuronal substrate of self-initiated behavioral sequences



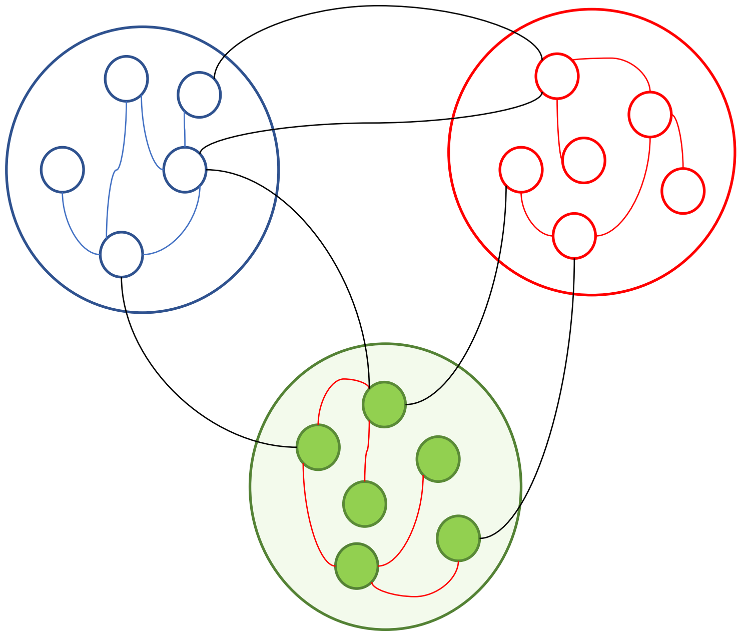
Sequence of self-initiated actions



Neuronal substrate of self-initiated behavioral sequences

1) How priority order is implemented

2) How to understand the variability in behavioral state persistence.



OUTLINE

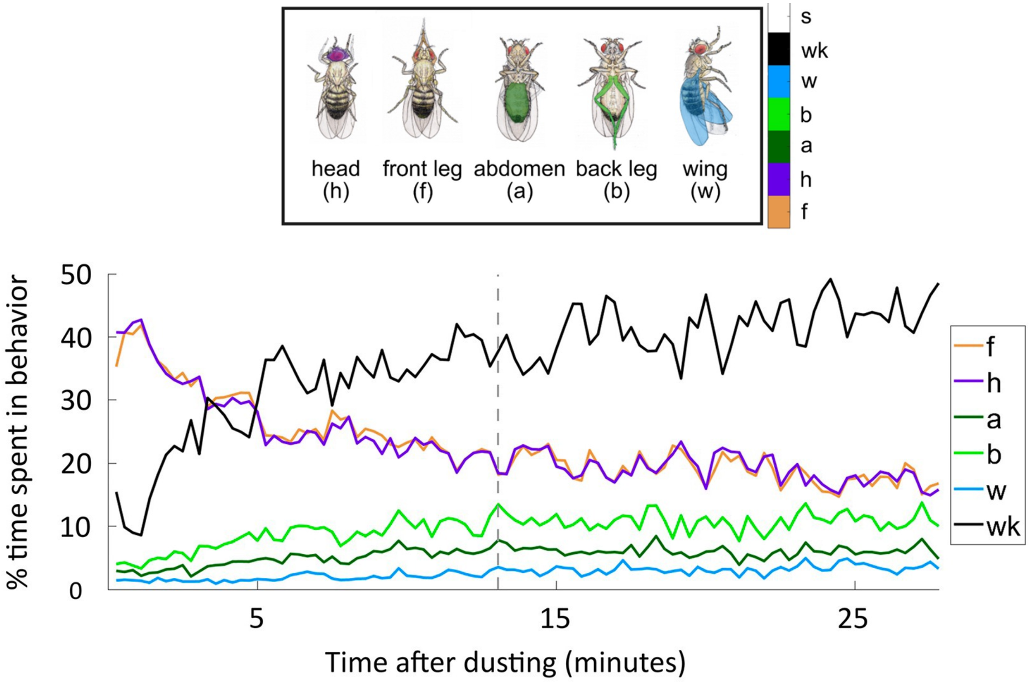
I – Unveiling the temporal organization in behavior

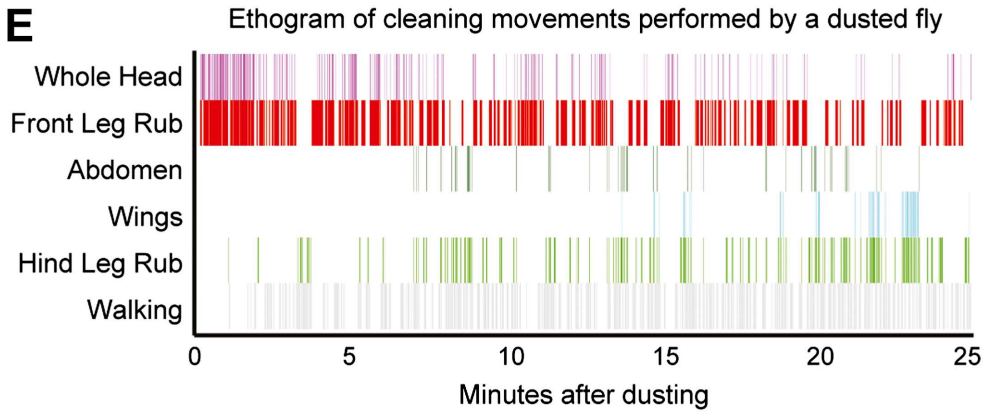
1 – Fly grooming behavior
2 – Zebrafish navigation
3 – Mice spontaneous activity
4 – Zebrafish phototaxis

II – Dissecting the neuronal circuits organizing these behaviors

1 – Hierarchical suppression in the drosophila grooming circuits
2 – Metastable attractors explain variable timing in behavioral sequences

How motor programs are organized in order to generate the observed behavioral sequence ?



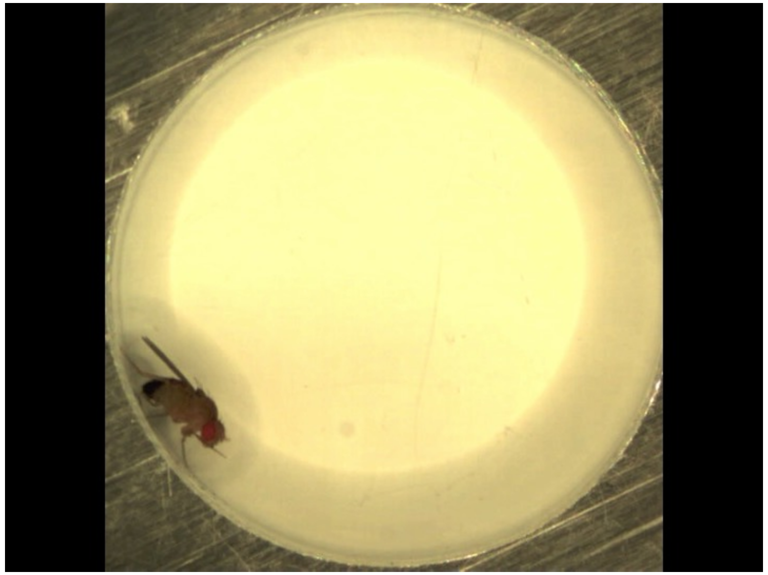


Priority order : eyes > antennae > abdomen > wings > thorax

A suppression hierarchy among competing motor programs drives sequential grooming in *Drosophila*
Andrew M Seeds, Primoz Ravbar, Phuong Chung, Stefanie Hampel, Frank M Midgley, Brett D Mensh, Julie H Simpson. Elife 2014

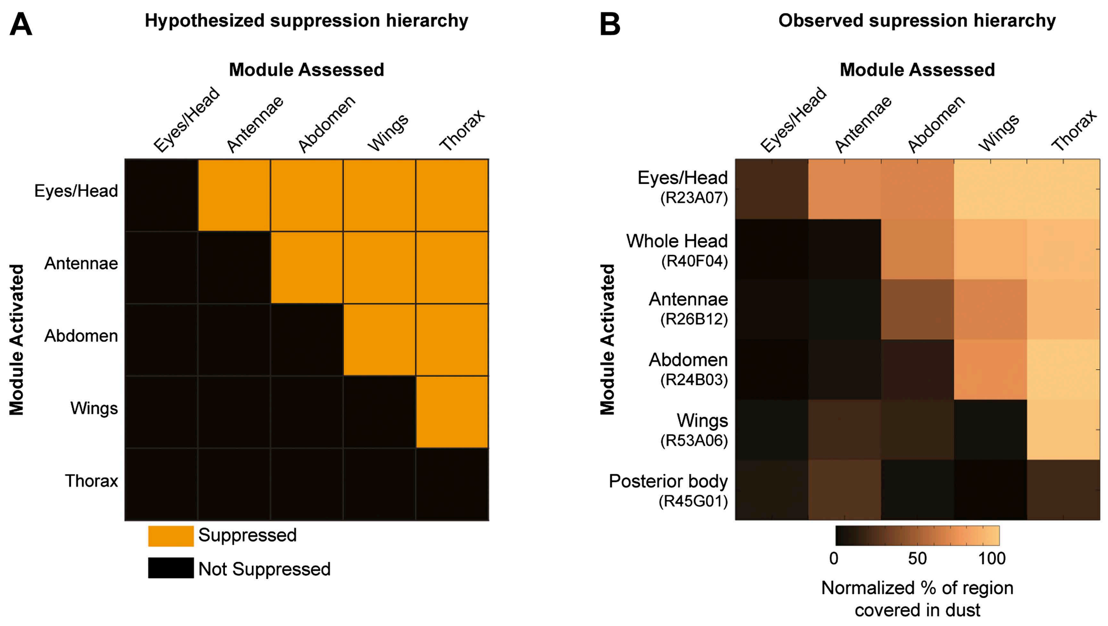
Optogenetic activation of specific grooming programs

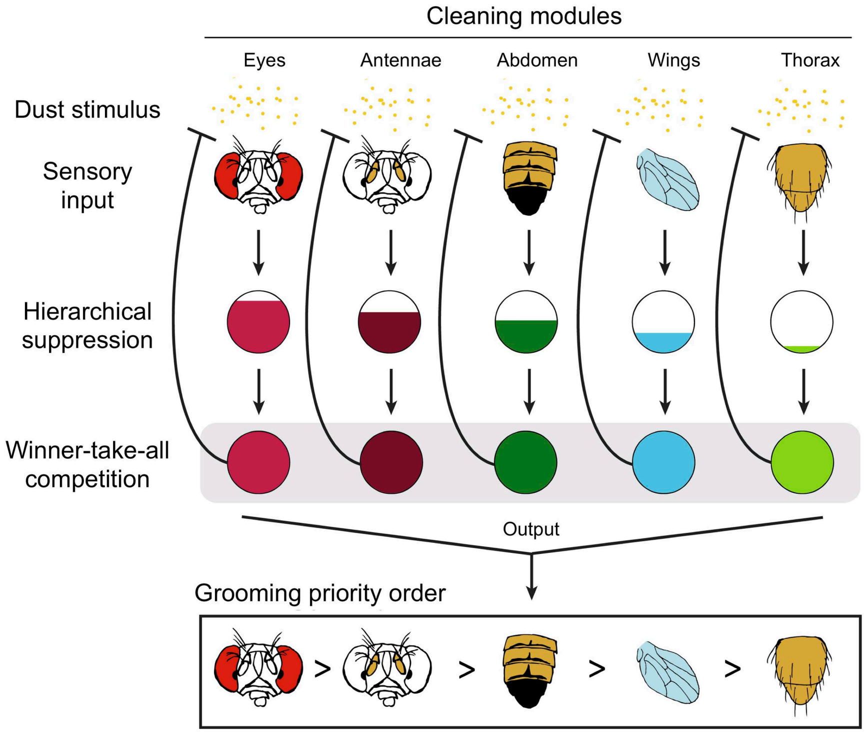
temperature-sensitive cation channel dTrpA1 (*UAS-dTrpA1*) in different enhancer-driven GAL4 expression patterns in the nervous system and screened for grooming phenotypes

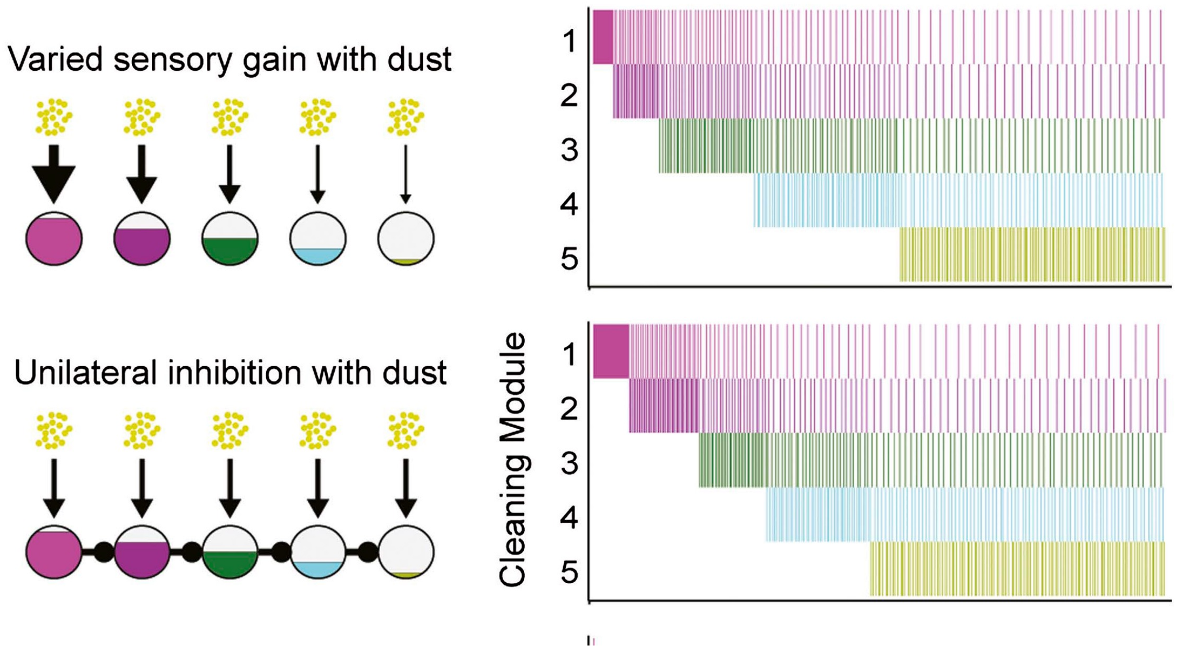


Eyes and head grooming

Abdomen grooming


A
Hypothesized suppression hierarchy
Module Assessed
Eyes/Head
Antennae
Abdomen
Wings
Thorax
Module Activated
Eyes/Head
Antennae
Abdomen
Wings
Thorax
Suppressed
Not Suppressed
B
Observed supression hierarchy
Module Assessed
Eyes/Head
Antennae
Abdomen
Wings
Thorax
Module Activated
Eyes/Head (R23A07)
Whole Head (R40F04)
Antennae (R26B12)
Abdomen (R24B03)
Wings (R53A06)
Posterior body (R45G01)
0
50
100
Normalized % of region covered in dust


Cleaning modules
Eyes
Antennae
Abdomen
Wings
Thorax
Dust stimulus
Sensory input
Hierarchical suppression
Winner-take-all competition
Output
Grooming priority order
>


Varied sensory gain with dust
Unilateral inhibition with dust
Cleaning Module
1
2
3
4
5

OUTLINE

I – Unveiling the temporal organization in behavior

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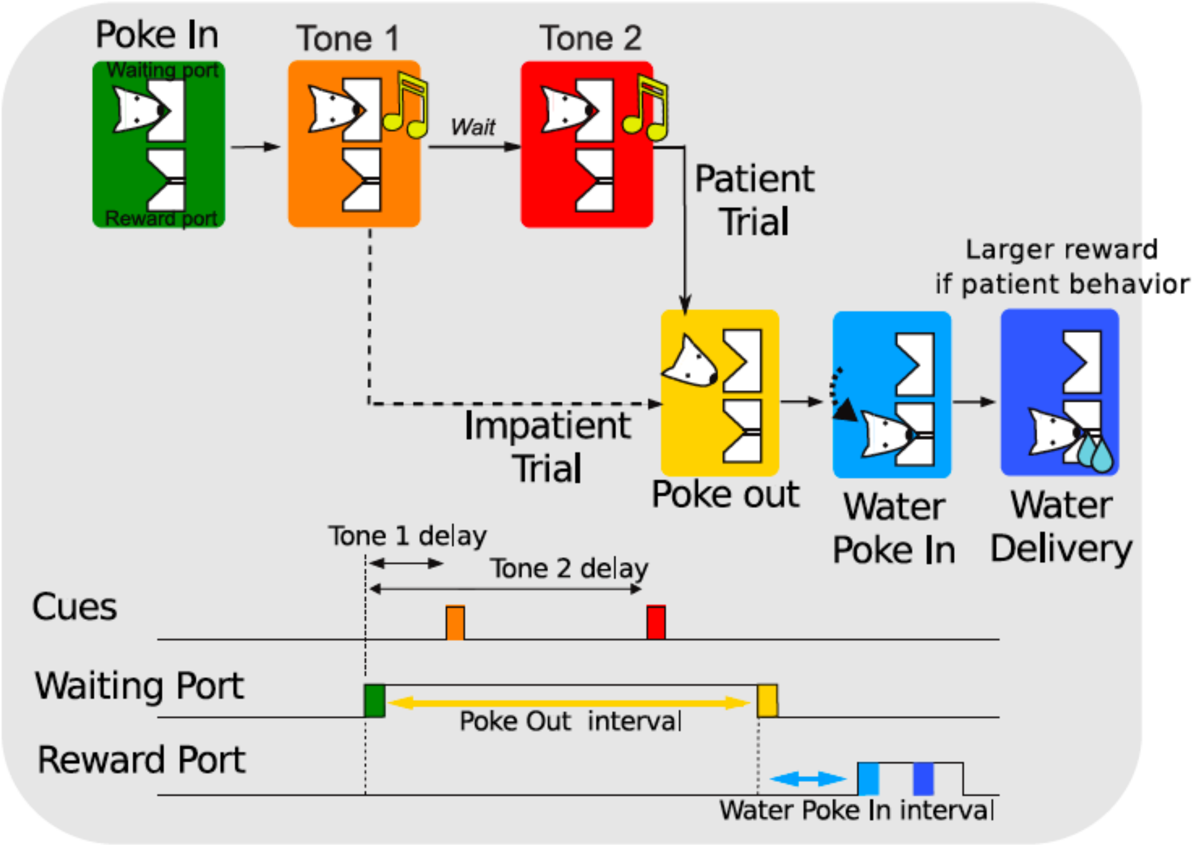
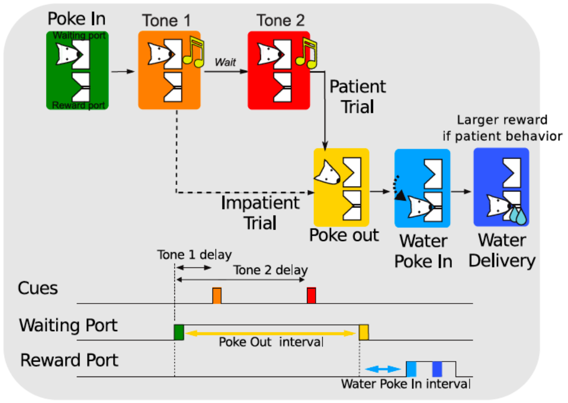
1 – Hierarchical suppression in the drosophila grooming circuits
2 – Metastable attractors explain variable timing in behavioral sequences

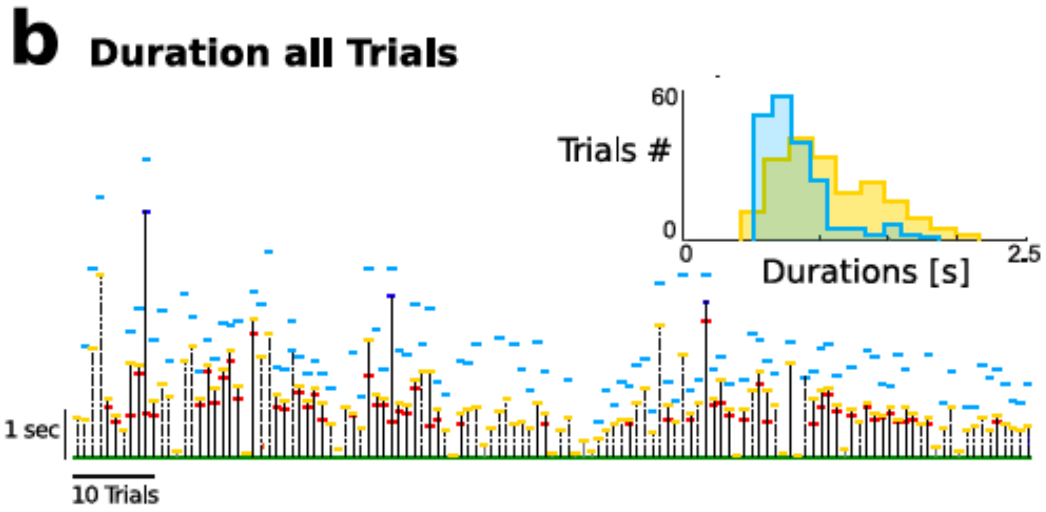
Sequences d'actions auto-initiées



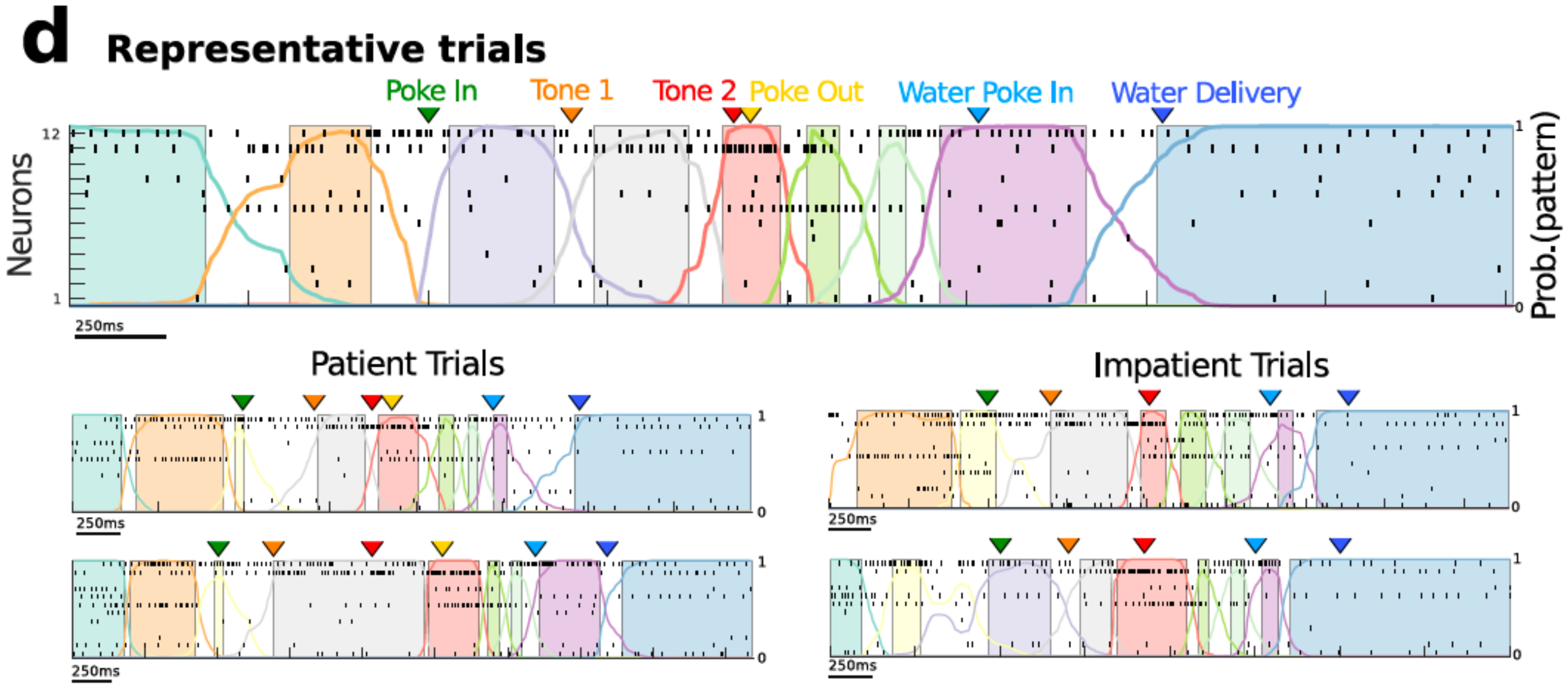
Metastable attractors explain the variable timing of stable behavioral action sequences
Stefano Recanatesi, Ulises Pereira, Masayoshi Murakamie, Zachary Mainen, Luca Mazzucato. Neuron, 2022



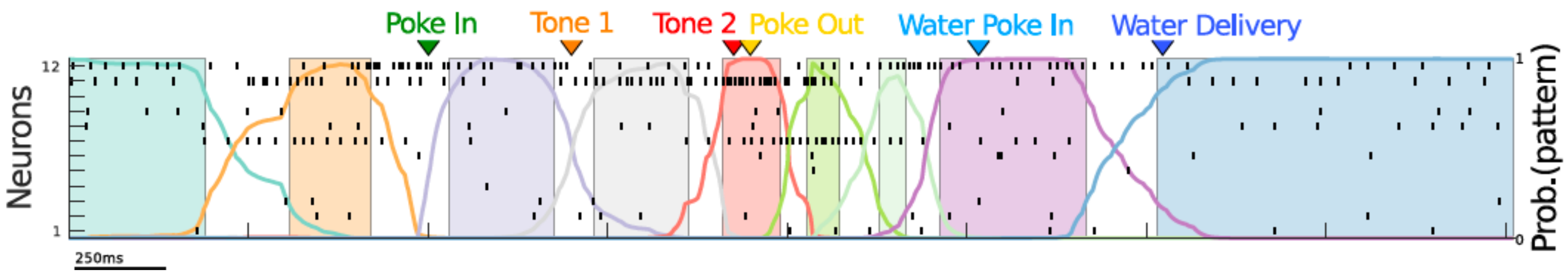
Yellow: poke in - poke-out interval
Blue : poke-out - water poke in interval



Recording from secondary motor cortex



Are the periods separating successive corresponding to specific « brain state » ,

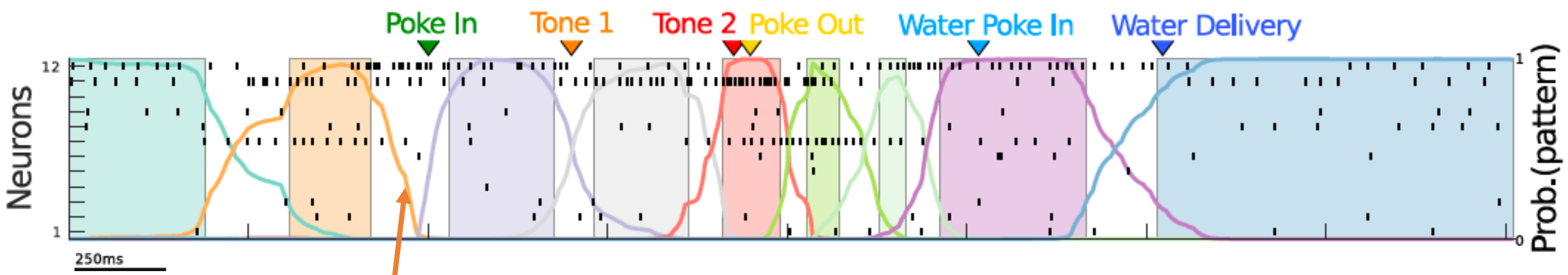


Inference of a Poisson-Hidden Markov Model

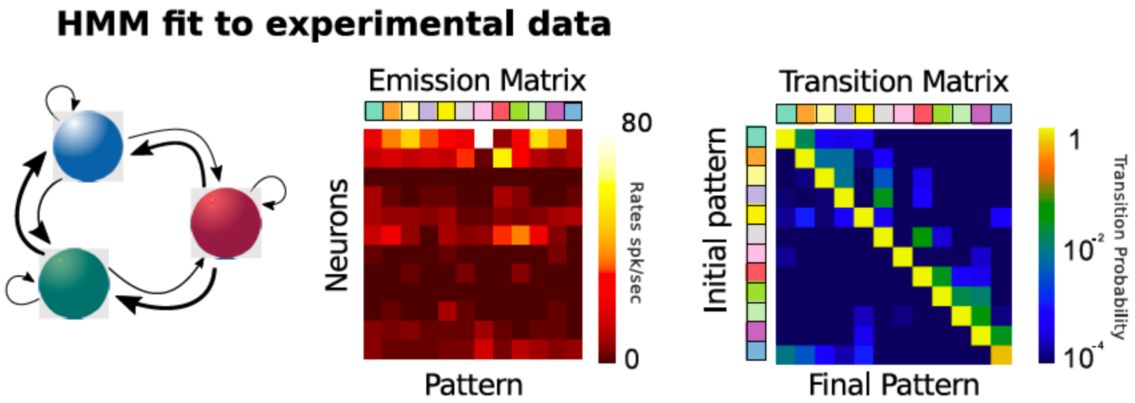
The sequence is $X(t) = [x_1(t), x_2(t), \ldots, x_N(t)]$, where $x_i(t)$ is the number of spikes (0 or 1) emitted during a short time bin [t,t+dt] (dt=5ms)

Each given state is associated with a vector of mean discharge rate $V_i = [v_{i,1}, v_{i,2}, .., v_{i,N}]$

$$\begin{cases} P\left(x_j(t) = 0 \middle| state = S_i\right) = v_{i,j}(m)\mathrm{dt} \\ P\left(x_j(t) = 1 \middle| state = S_i\right) = 1 - v_{i,j}(m)\mathrm{dt} \end{cases}$$

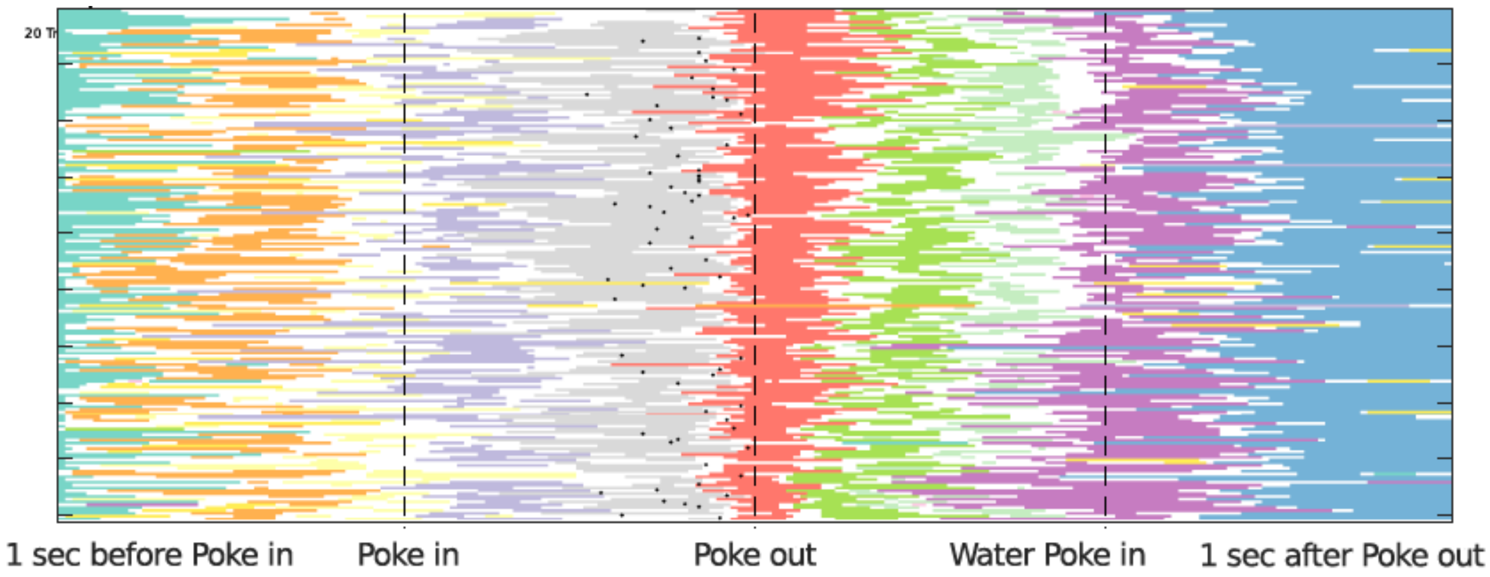


$P(S = M|X)$ Posterior probability

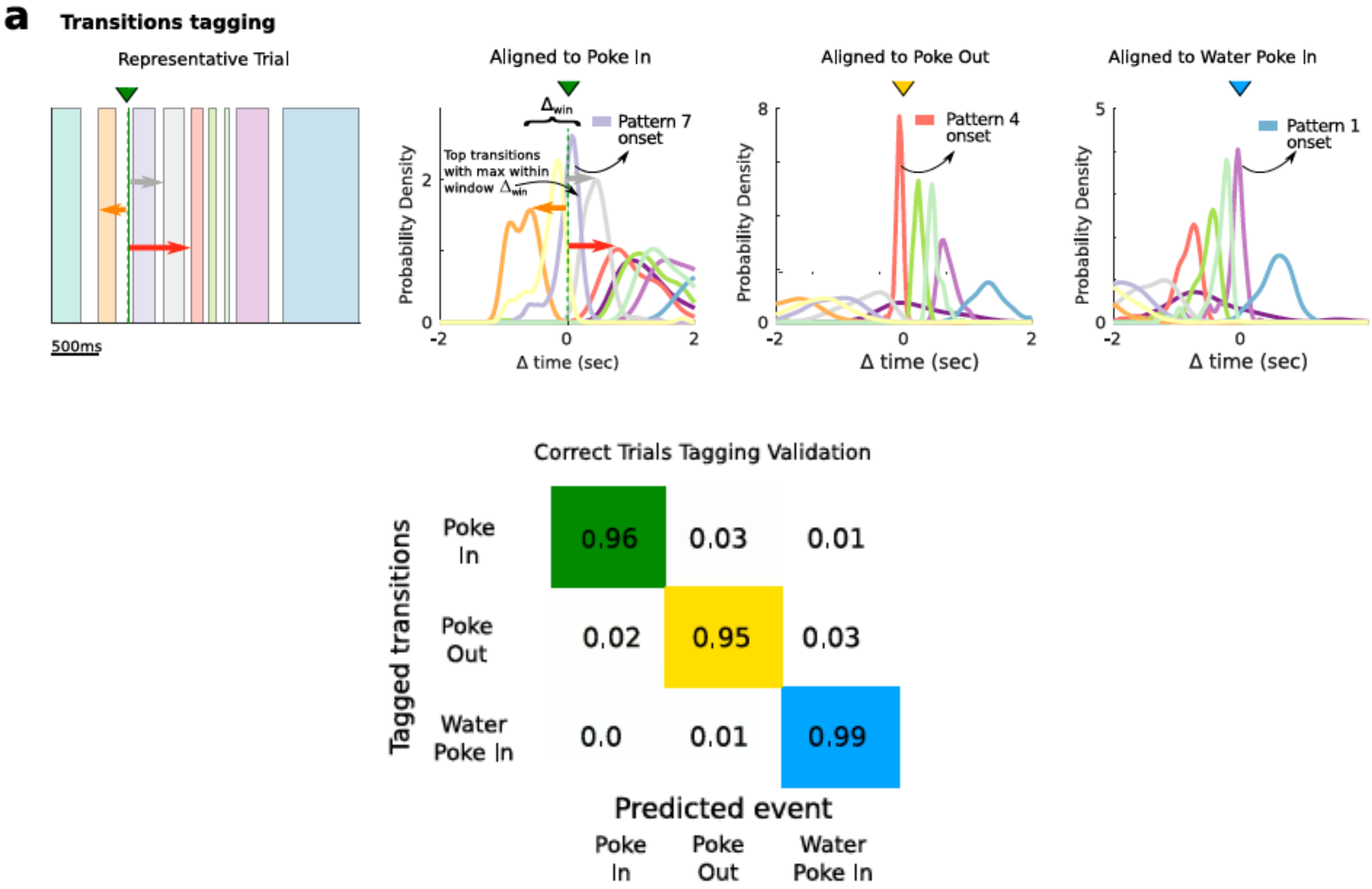


HMM allows for the unsupervized identification of persistent neural states .

States are sequenced in a precise order, but the residence time is highly variable.

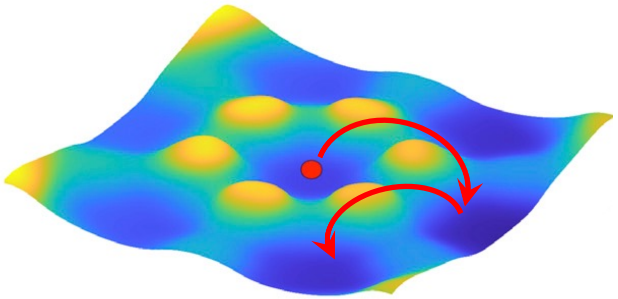


Brain states are predictive of forthcoming event time



CONCLUSION

- Behavior can be described as a hierarchically organized sequence of stereotyped motifs.
- This behavioral grammar can be discovered in an unsupervized way using dimensionality reduction + categorization.
- This approach allows one to identify how external stimuli or internal cues modulate the behavior.
- This dynamics may reflect the brain hopping dynamics through successive attractor states.



<u>One concluding remark:</u>

- The information flux for behavior is less than 8bit/s in mice.
- The possible information content of a rat's brain is ~10^{10}bit/s

=> Do we need to record that many neurons ;) ?