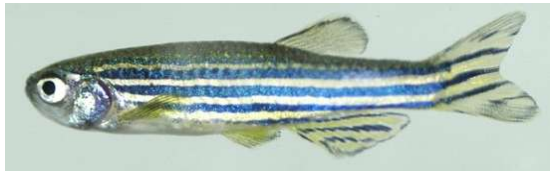
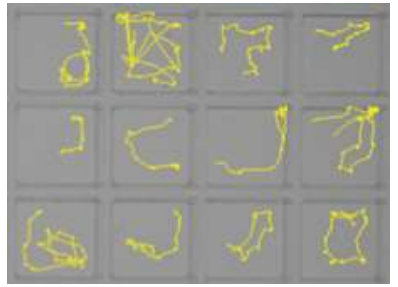
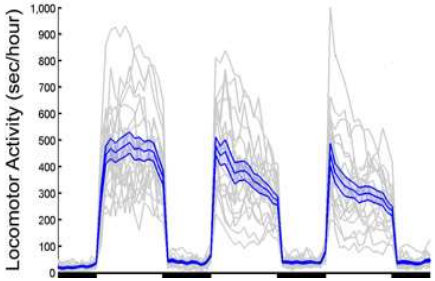
Master in Neuroscience

MOLECULAR NEUROPHYSIOLOGY -lesson 6-



Prof. G.Cellot





Zebrafish as alternative model for the study of synaptic plasticity and its behavioral correlates

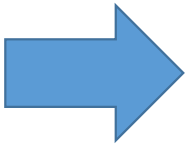
Is Zebrafish (Danio Rerio) a good alternative research model for biomedical applications?

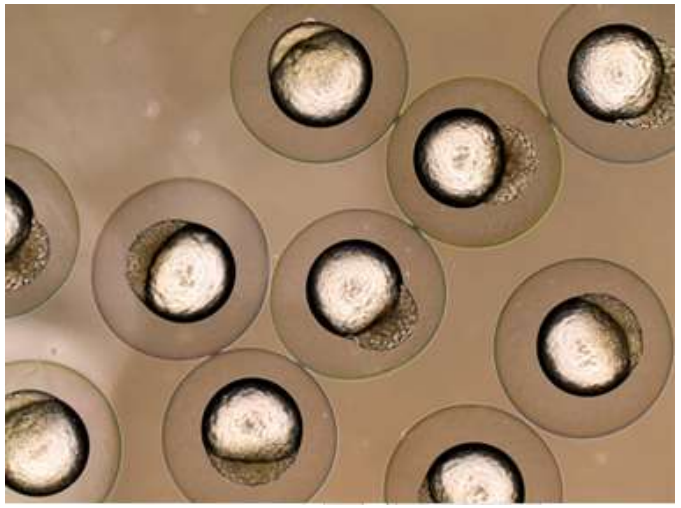
- 70% of genetic homology with humans

- Ethics in using animal for research purpose

Principle of the 3Rs (**R**eplacement, **R**eduction and **R**efinement) **OK**

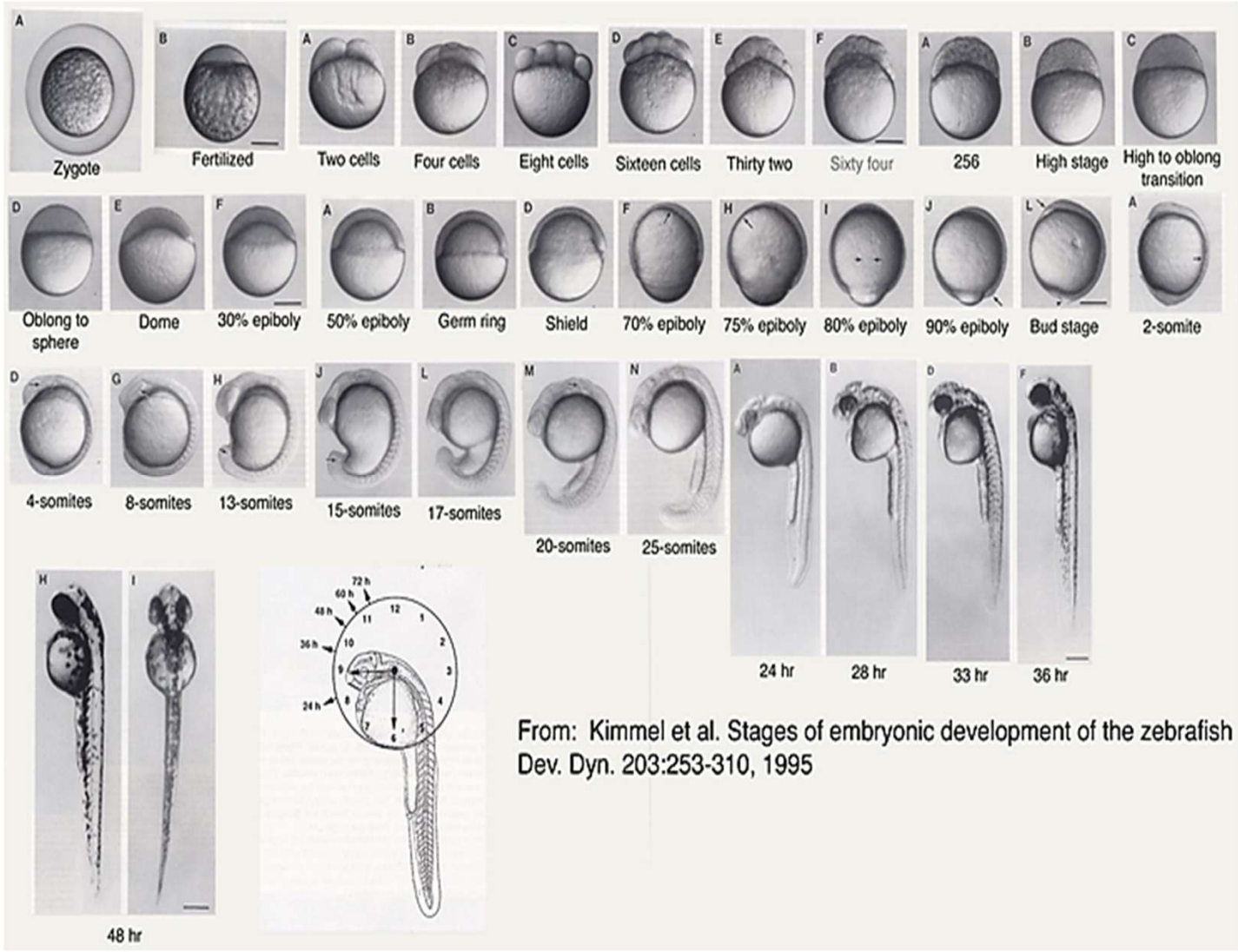
- Cost effective

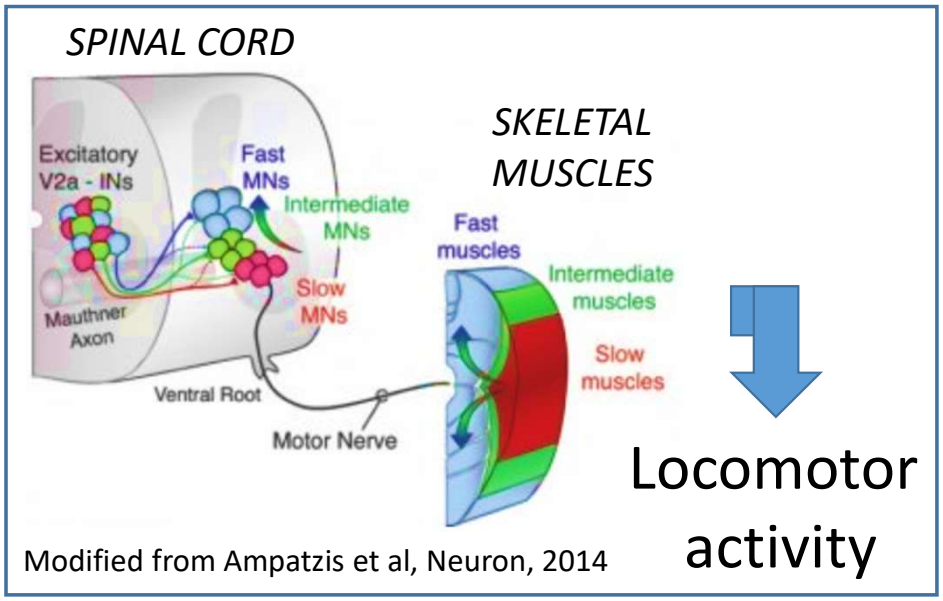




Zebrafish (Danio Rerio) as alternative research model for biomedical applications

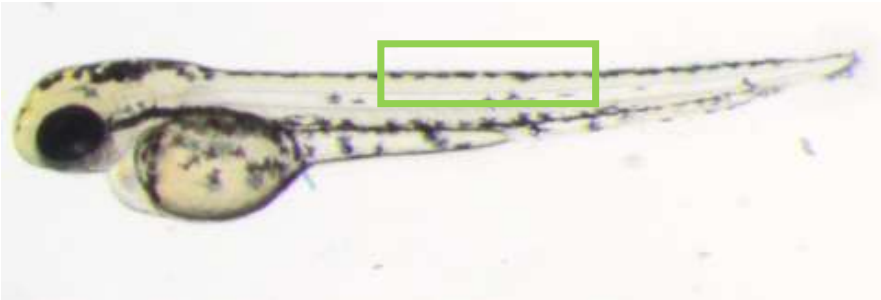
- Extra-uterine fertilization
- Quick development
  - *Genetic manipulation for transgenic lines*
- 2-3 years of lifespan
  - *Studying aging, neurodegeneration,…*
- Optical transparency
  - *Accessibility of the nervous system for functional techniques*

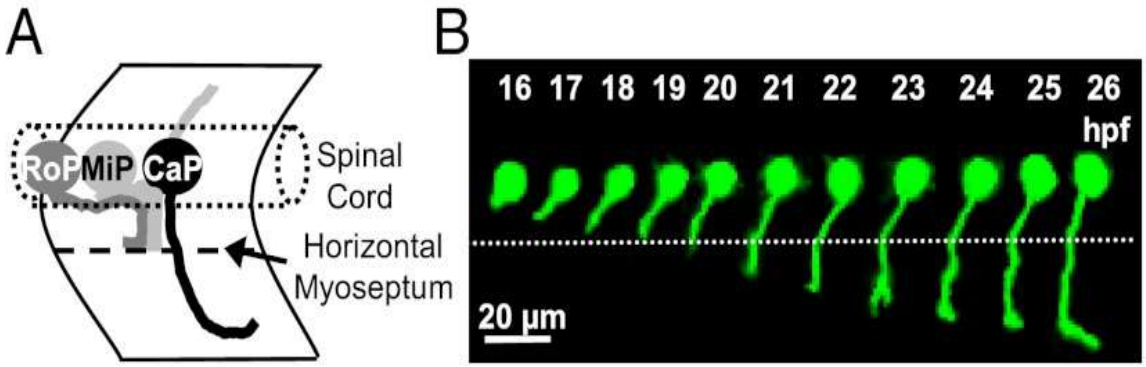




Using zebrafish in neurophysiology to correlate behavior with neuronal function

- Spontaneous behavior
- Independent on sensory stimulation
- Correlating with neuro-muscular synapses

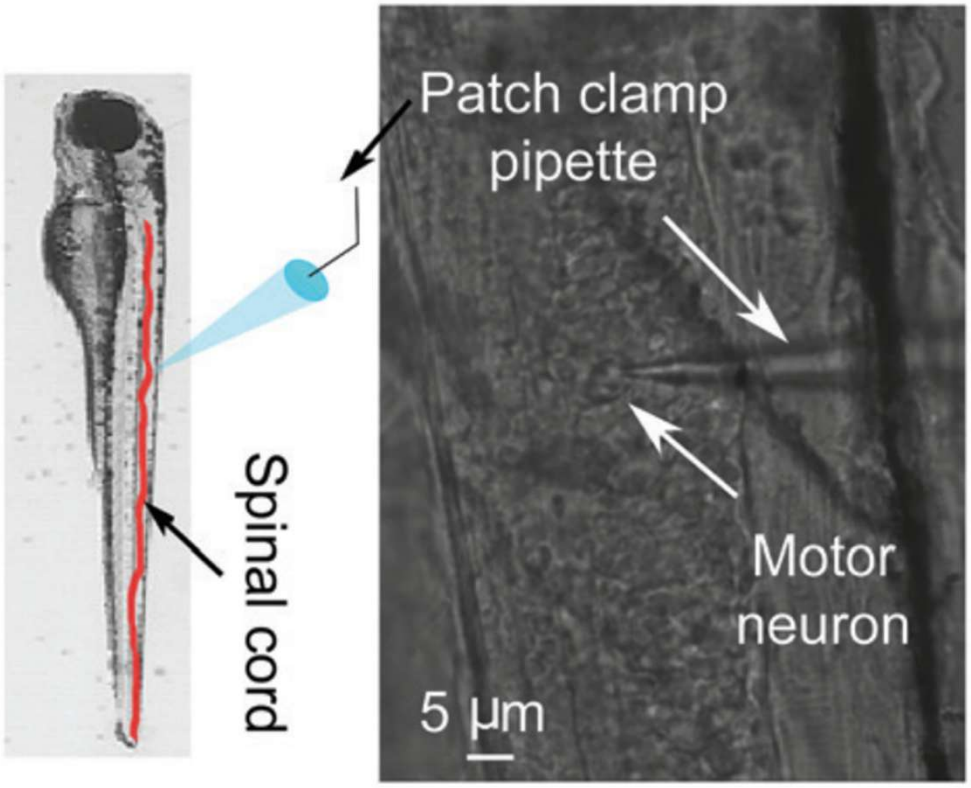




Plazas et al, PNAS 2013, DOI: 10.1073/pnas.1213048110

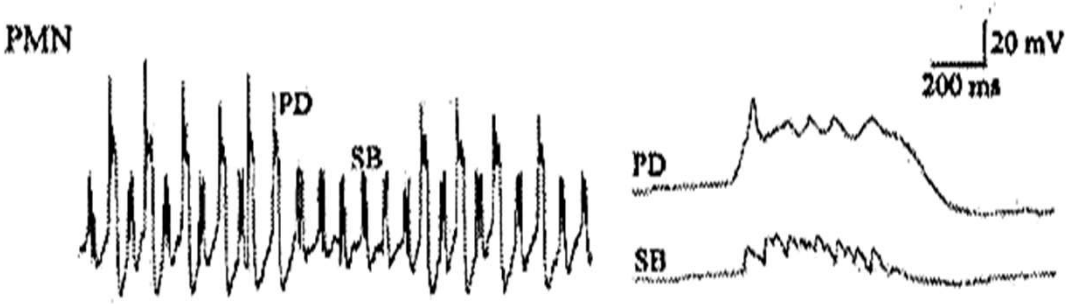
Using zebrafish in neurophysiology to correlate behavior with neuronal function

Electrical correlates of coiling behavior



Cellot et al, Nanoscale Horizons, 2020

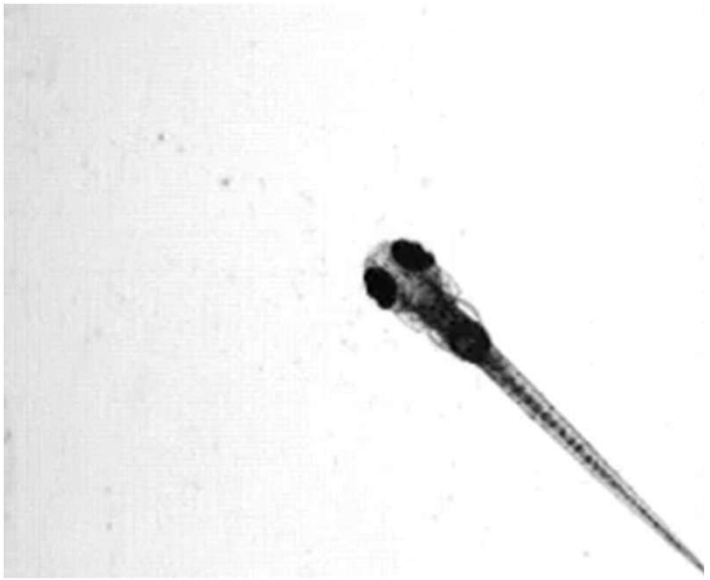
- Based on GJ dependent depolarizations
- Glycine depolarizing action

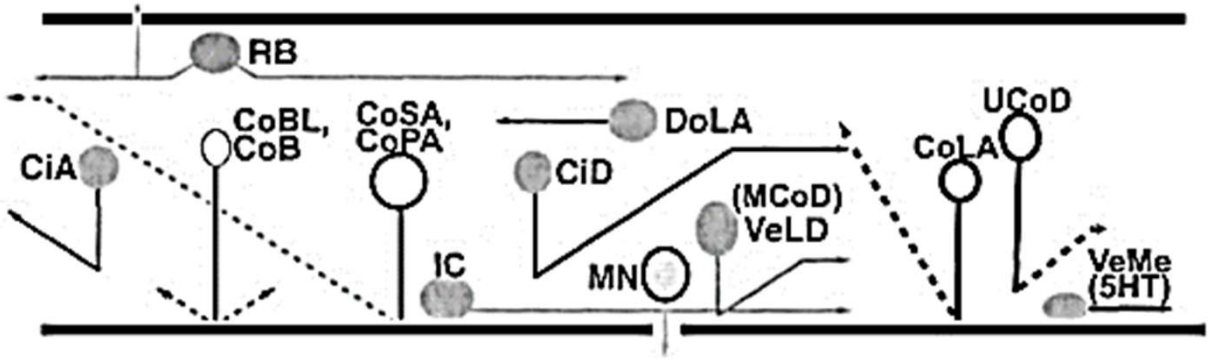


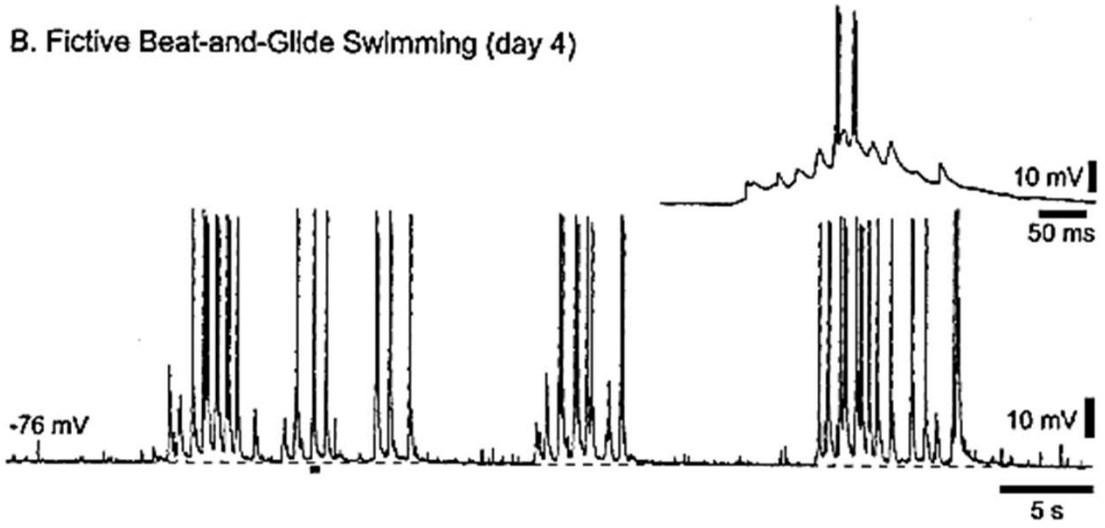
Drapeau et al, Progress in Neurobiology,2002

Using zebrafish in neurophysiology to correlate behavior with neuronal function

4 days:
Beat and glide swimming



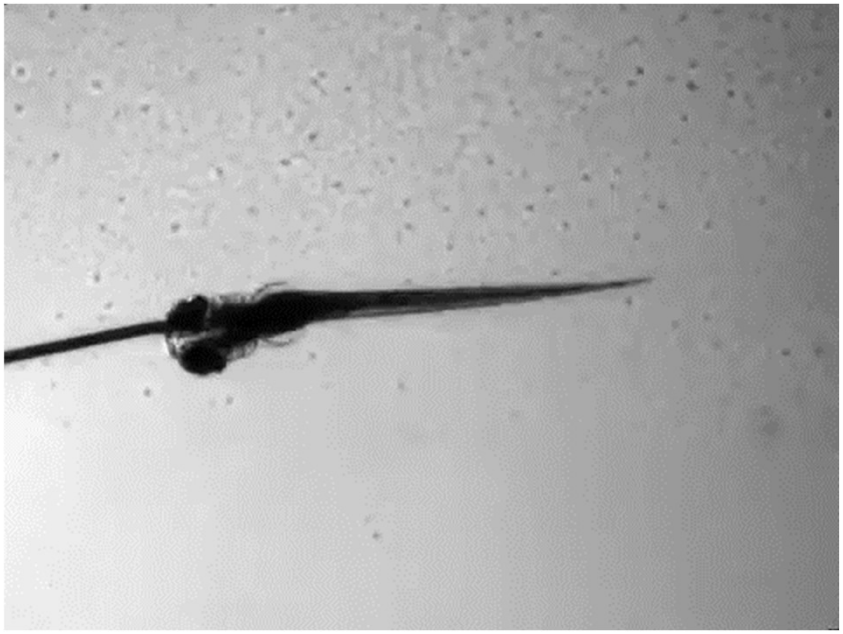


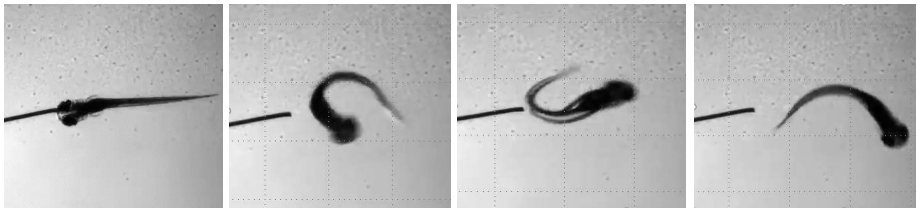


Maturation of inhibitory inputs to contralateral spinal network

Drapeau et al, Progress in Neurobiology,2002

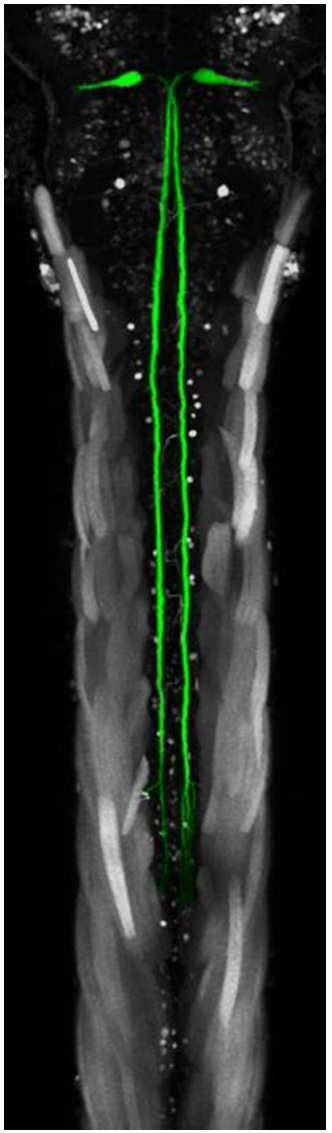
Using zebrafish in neurophysiology to correlate behavior with neuronal function





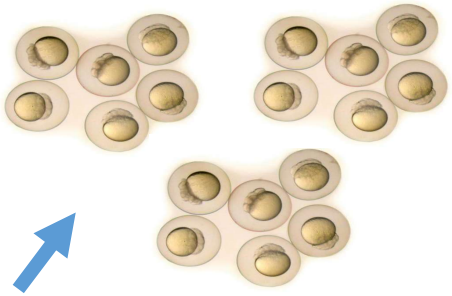
Touch response (C-start) = ESCAPE REFLEX

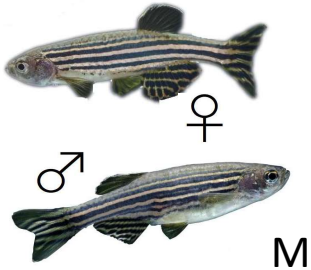
- Sensory modulation of the behavior via Mauthner cells (M-cells, reticolo-spinal cells in the hindbrain)
- Activated by visual, tactile or acoustic stimuli
- Laser ablation of M-cells decreases and delays escape response



Behavior can be used as a macroscopic readout of the neuronal function

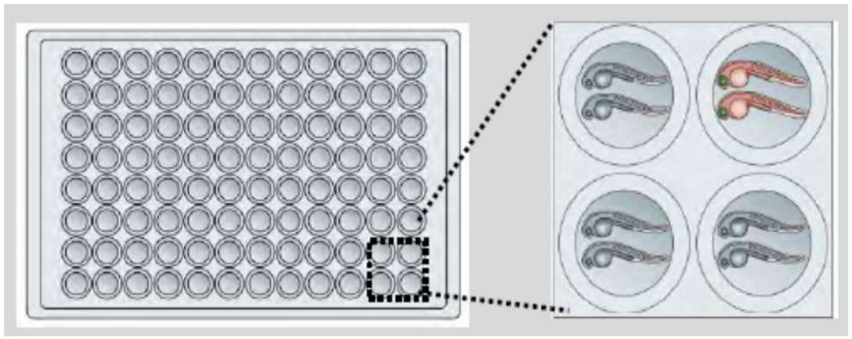
Fertilized eggs





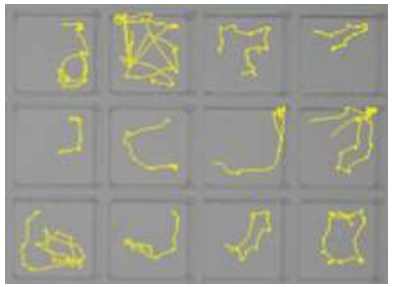
Mating

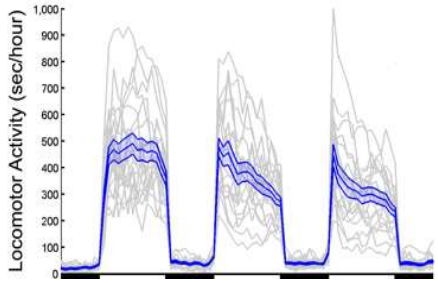
High-throughput analysis of behavior



Exposure to different drugs, (nano,micro-)materials, pollutants…

NEUROTOXICOLOGY

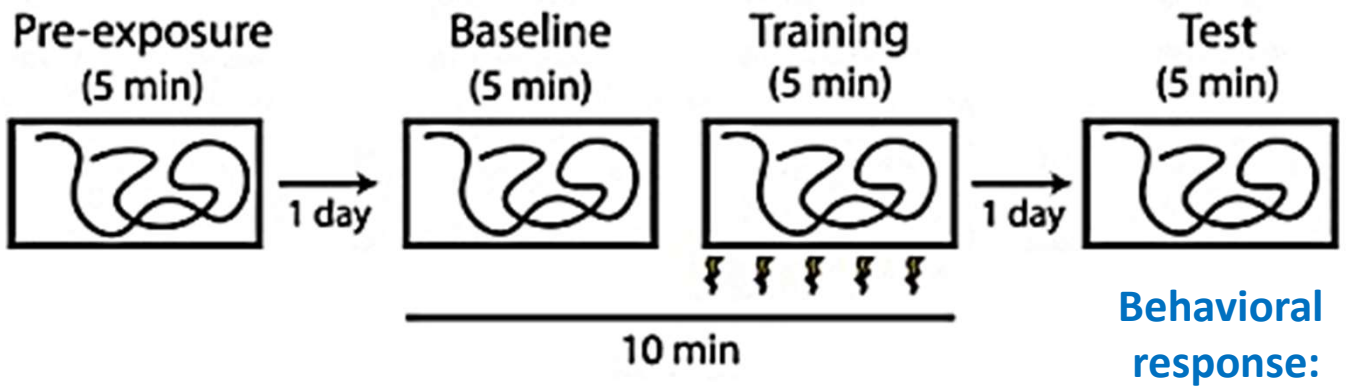




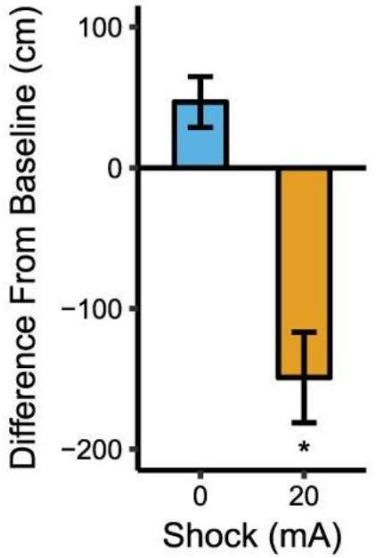
Automated analysis of locomotor behaviour

Studying complex behaviors in zebrafish: Contextual fear conditioning

Testing US (electrical shock)



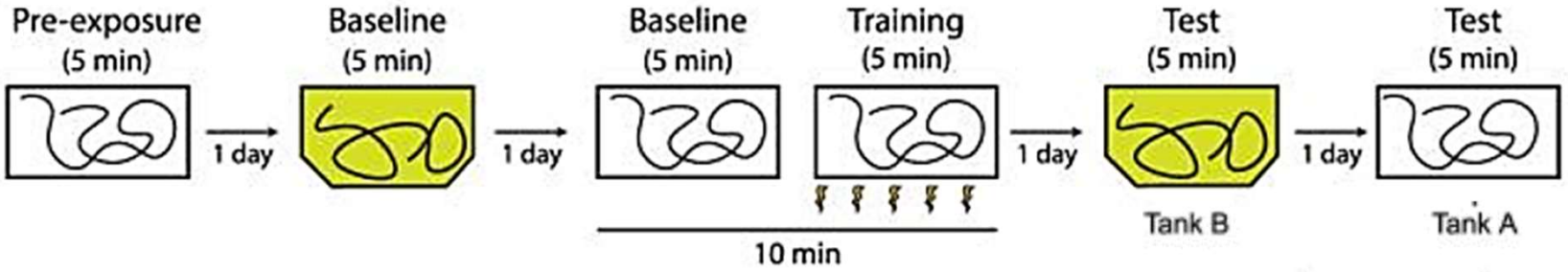
Behavioral response: FREEZING

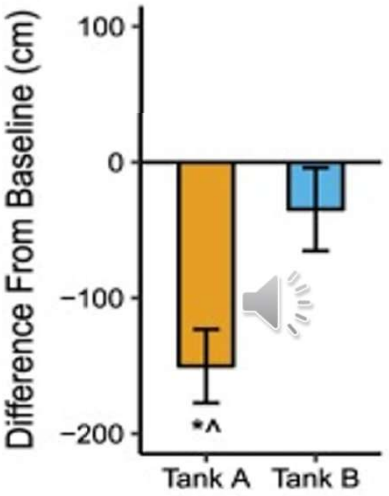


Testing US-CS association

US = electrical shock

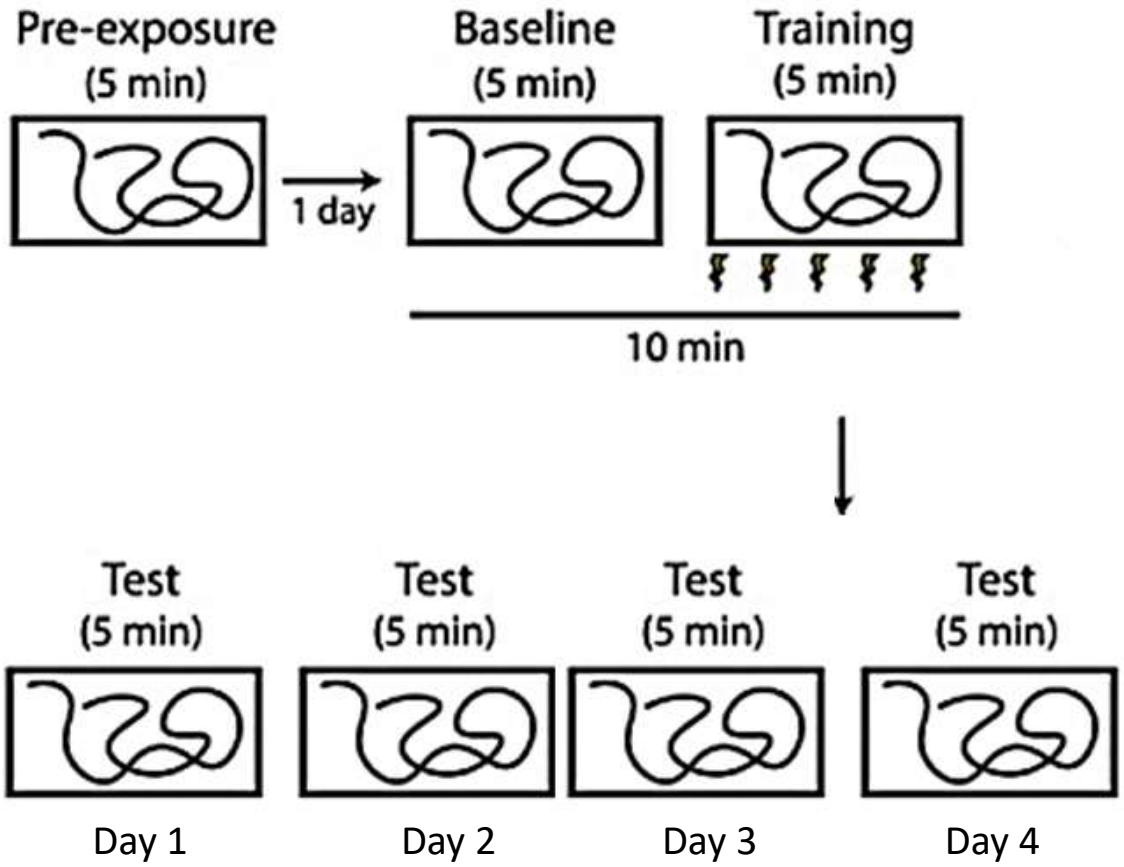
CS = context

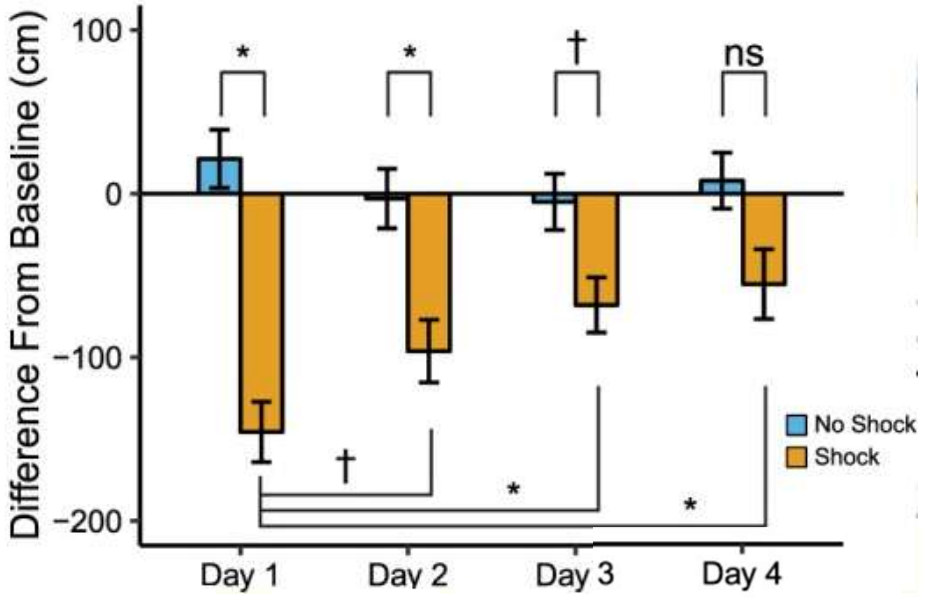




Kenney et al, Learn Mem. 2017; doi: 10.1101/lm.045690.117

Studying complex behaviors in zebrafish: fear extinction





Kenney et al, Learn Mem. 2017; doi: 10.1101/lm.045690.117

How to identify neurons involved in complex neuronal functions?

Traditional rodent model

Behavior

↓ Sacrifice

STAINING of
genetic markers of neural activity
(e.g. immediate early genes, c-fos)

- ✓ Only strongly activated cells can induce IEGs
- ✓ Neurons that are switched off are not detected

Zebrafish

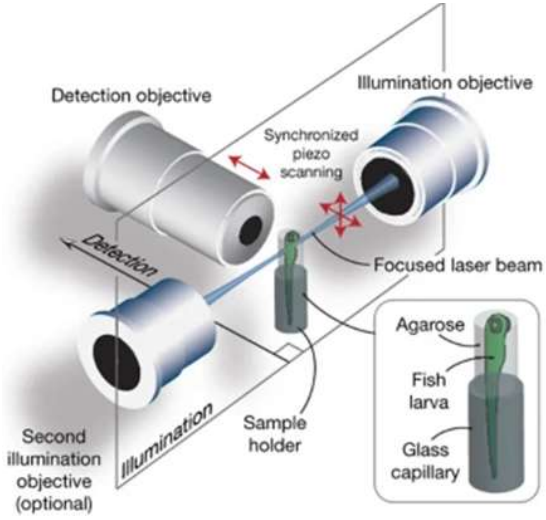
Behavior

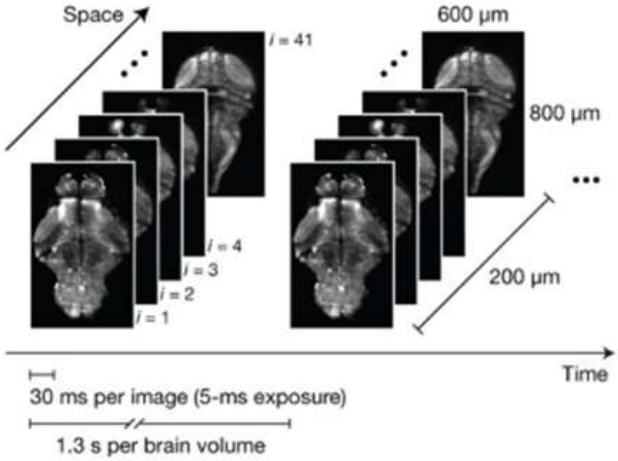
↓ Sacrifice

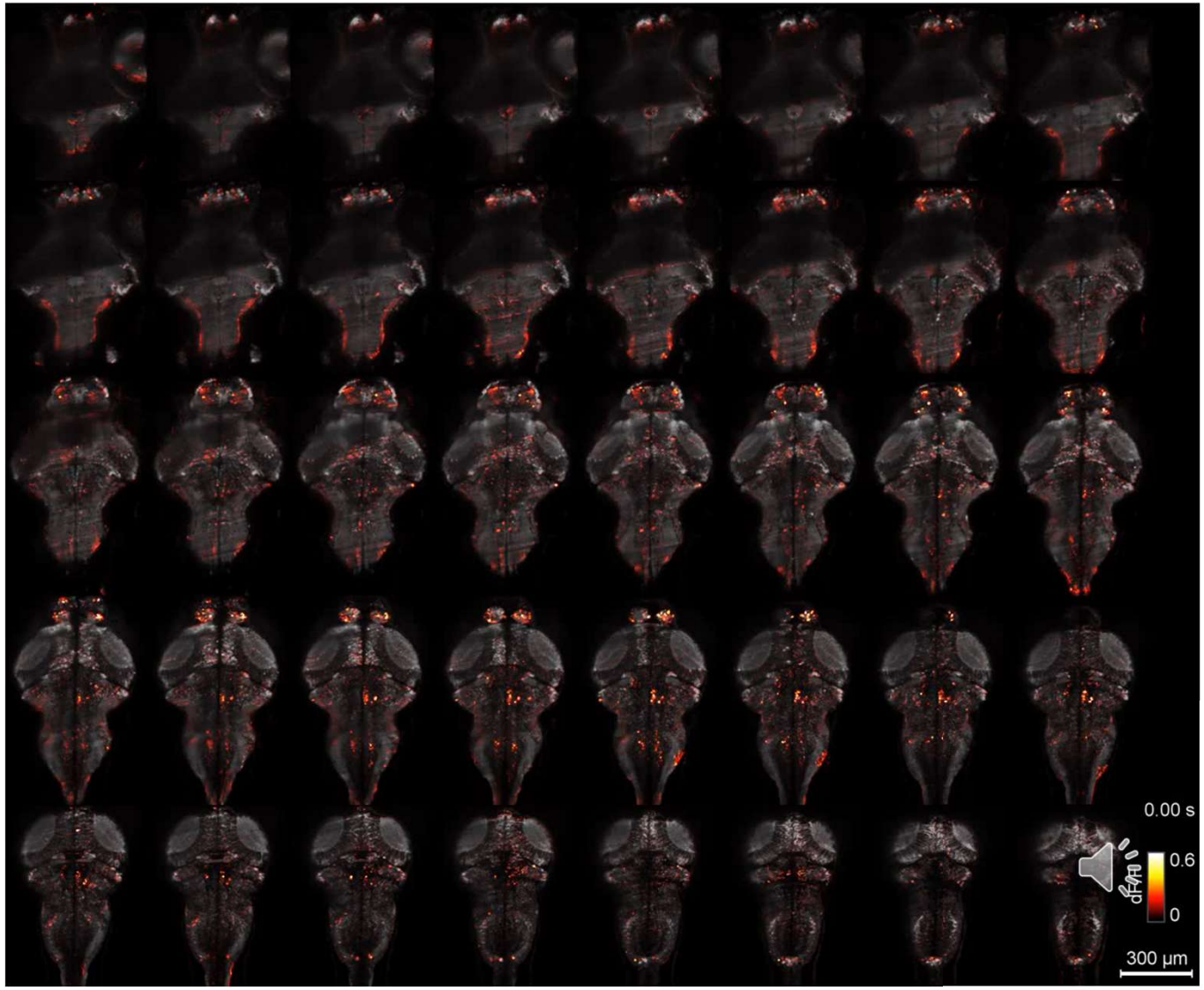
Monitoring live cell activity through genetically encoded Ca^{2+} indicators

- ✓ Not invasive
- ✓ Small brain size and transparency allow simultaneous recording from hundreds of cells
- ✓ AP resolution

Whole-brain functional imaging at cellular resolution using light-sheet microscopy.







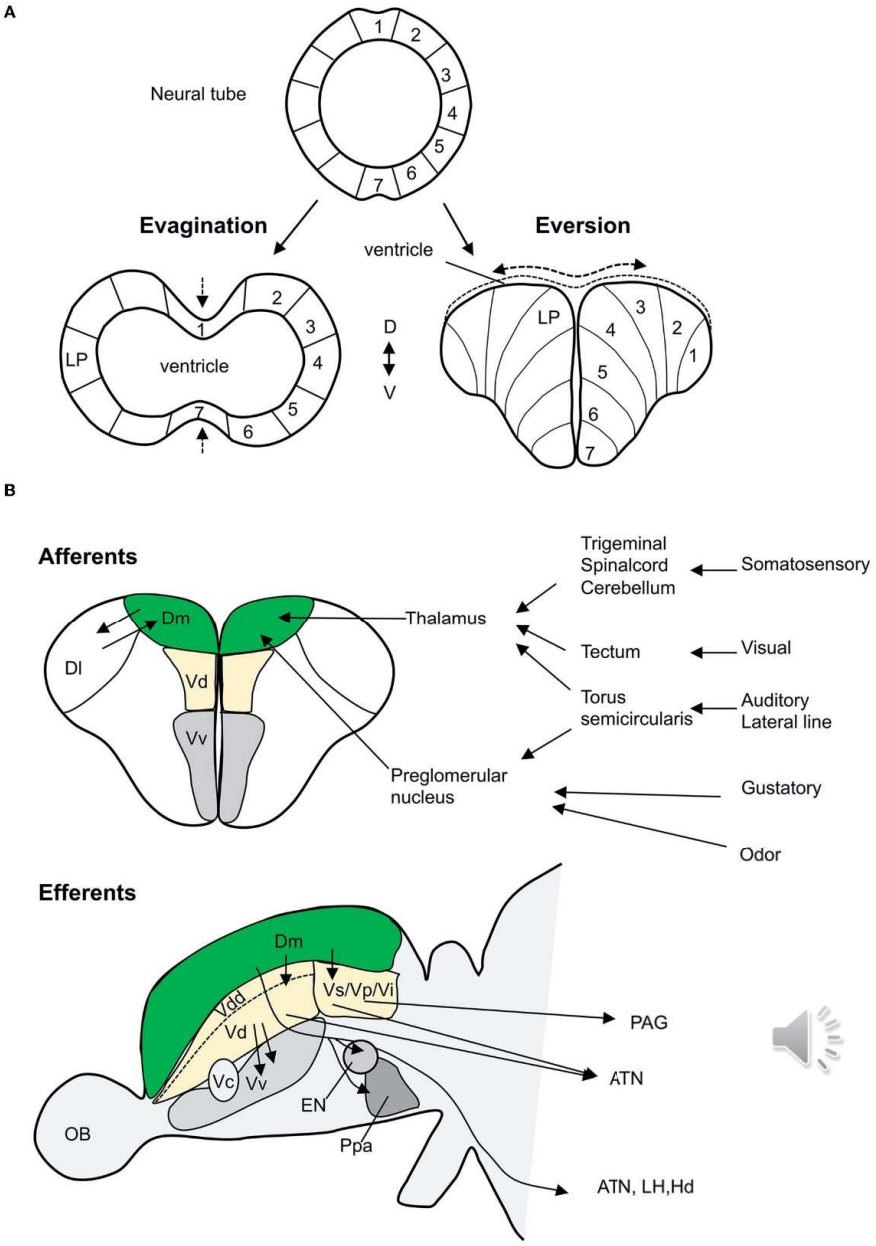
Ahrens, M. B., and Keller, P. J. (2013). Nat. Methods doi: 10.1038/nmeth.2434

Functional Anatomy of Zebrafish Amygdala

Basolateral complex of Amygdala
= Medial part of dorsal telencephalon (Dm) (also called dorsal pallium)

Central complex of the Amygdala
= Ventral telencephalon (dorsal nucleus (Vd), suppracommissural nucelus (Vs), and postcommissural nucelus (Vp))

Hippocampus = lateral telencephalon (Dl)



Lal & Kawakami, 2022, doi: 10.3389/fnana.2022.837527

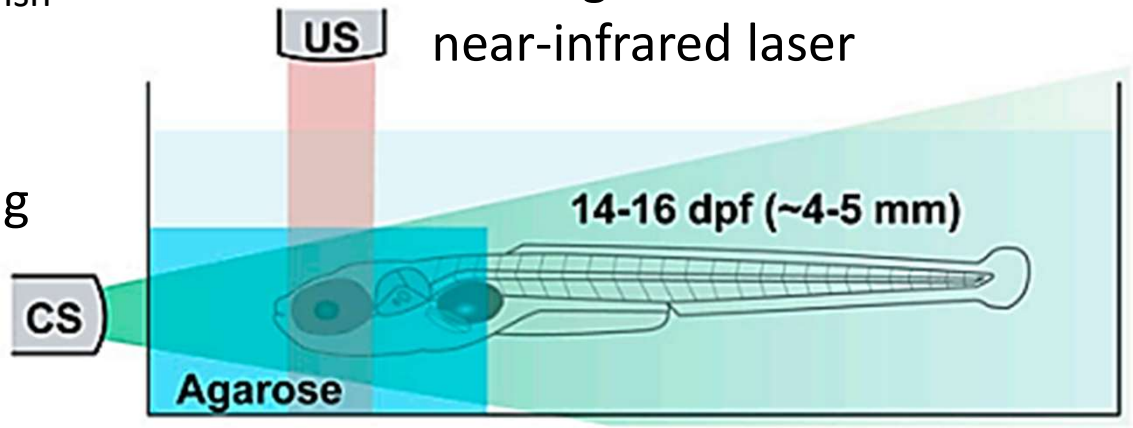
Investigating associative learning induced brain changes in in vivo zebrafish

Cued aversive conditioning

15 dpf zebrafish

Heating from a near-infrared laser

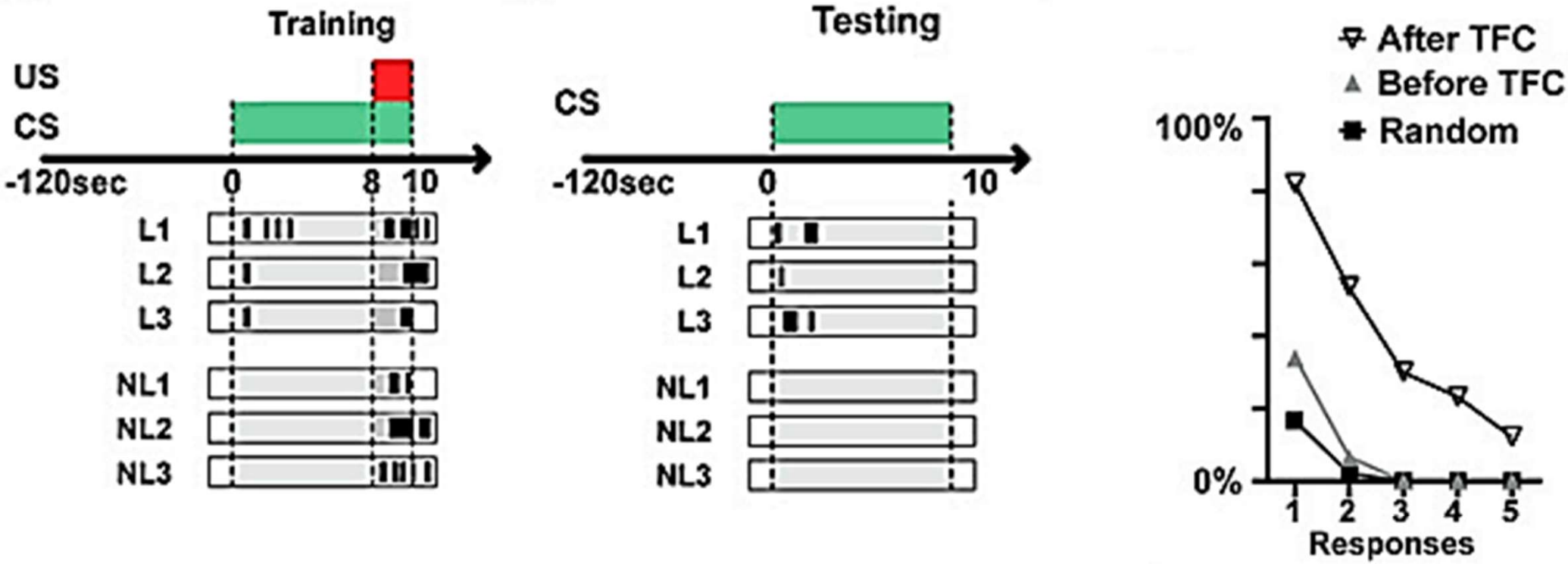
Light from a light-emitting diode (LED)



Behavioral readout: Tail-flick

Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119

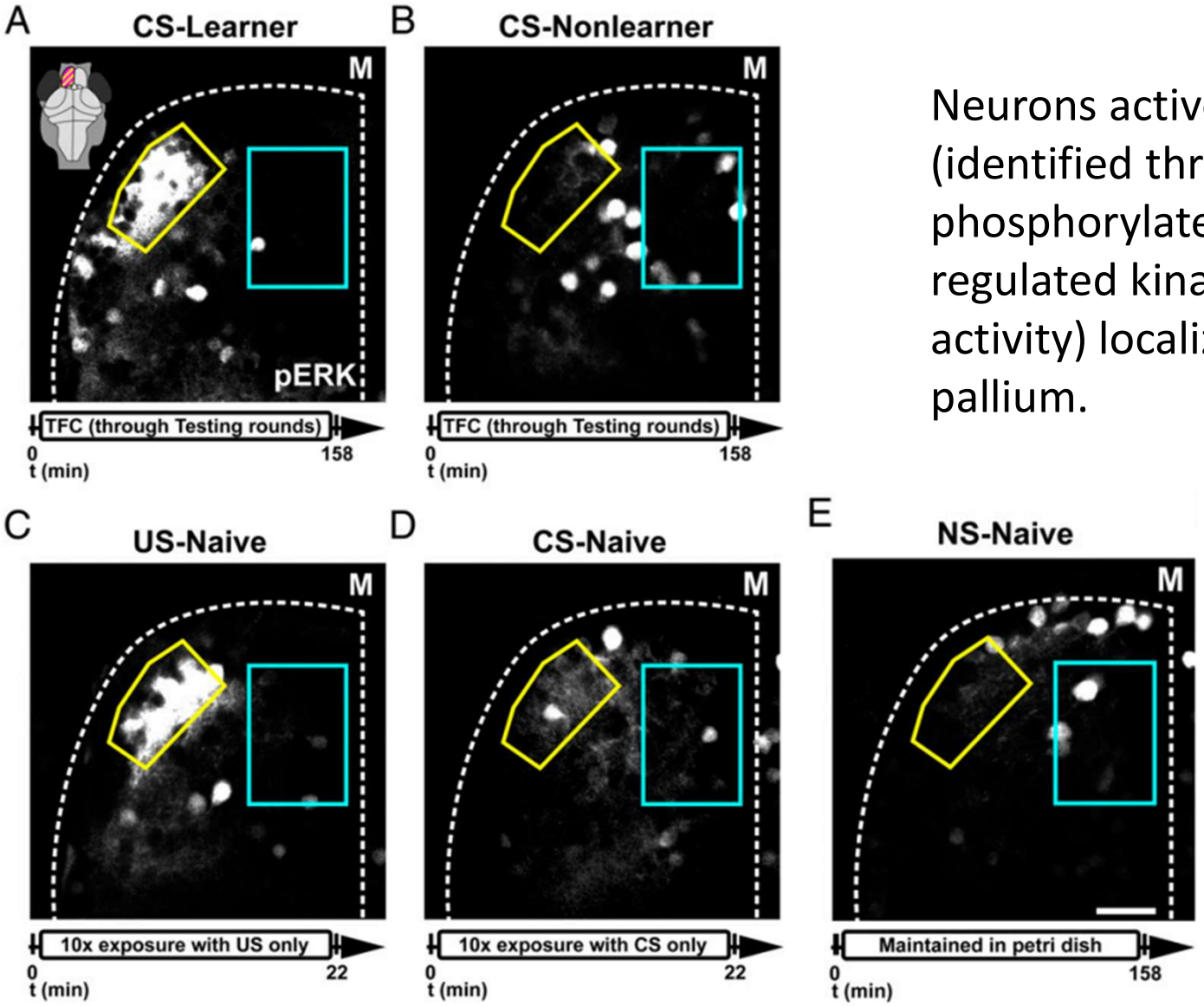
Investigating associative learning induced brain changes in in vivo zebrafish



L = Learners
NL = Nonlearners

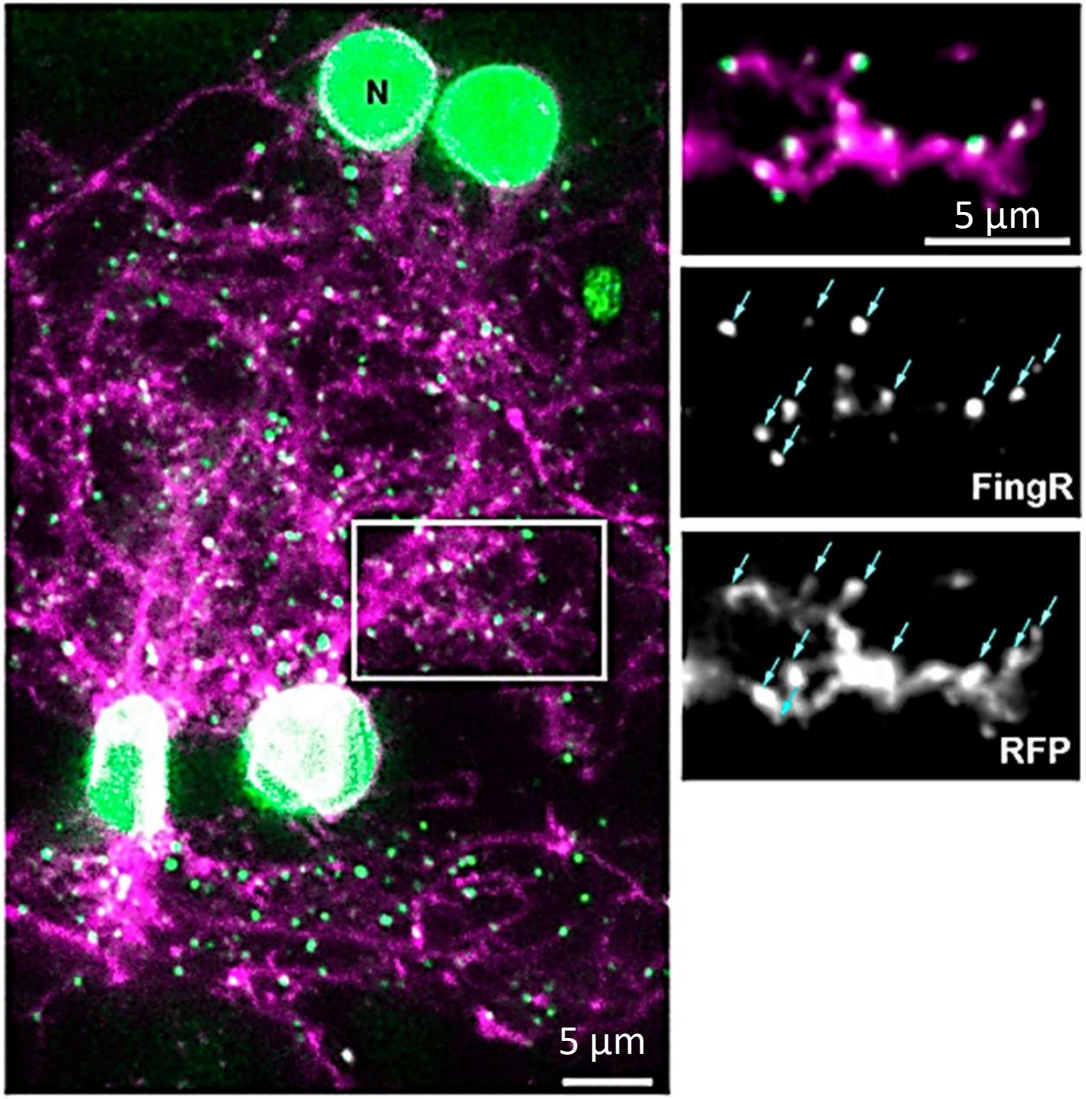
Tail flick conditioning is:
✓ NMDA receptor dependent
✓ Sensitive to extinction

Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119



Neurons active during conditioning (identified through immunostaining for the phosphorylated extracellular signal-regulated kinase, pERK, marker of neuronal activity) localize in the antero-lateral dorsal pallium.

Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119



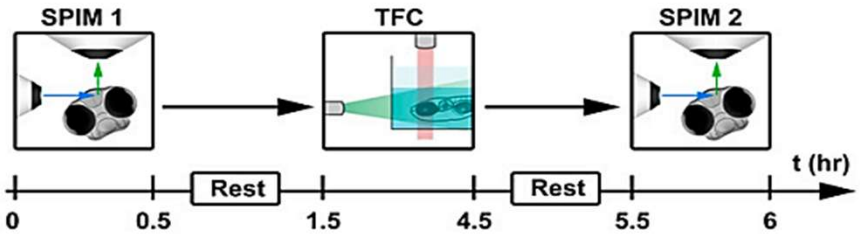
Selective plane illumination microscopy (SPIM)

Live visualization of excitatory synapses in dorsal pallium by using transgenic zebrafish co-expressing:

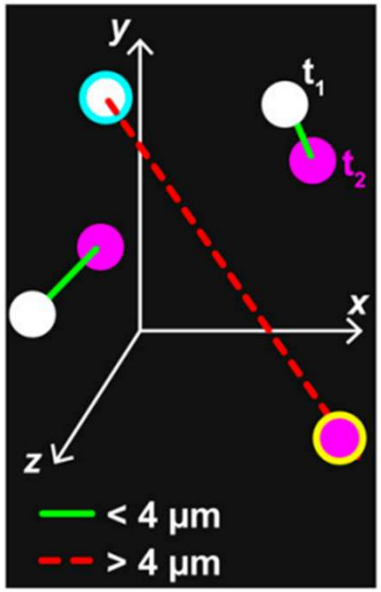
- PSD-95.FingR-GFP (green)
- membrane-targeted mScarlet (magenta)

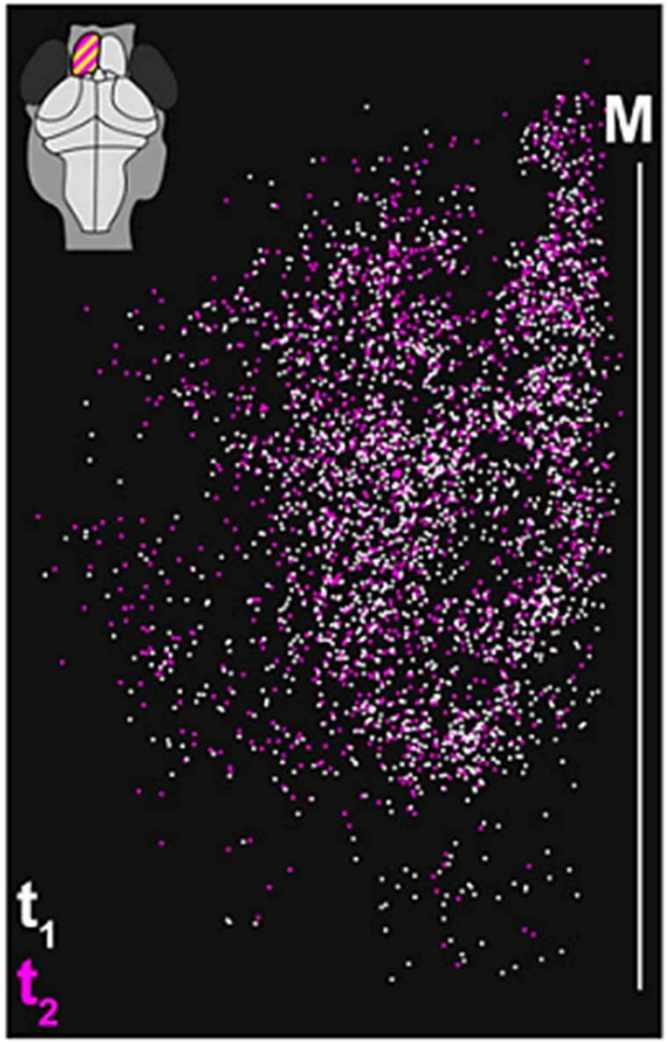
Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119

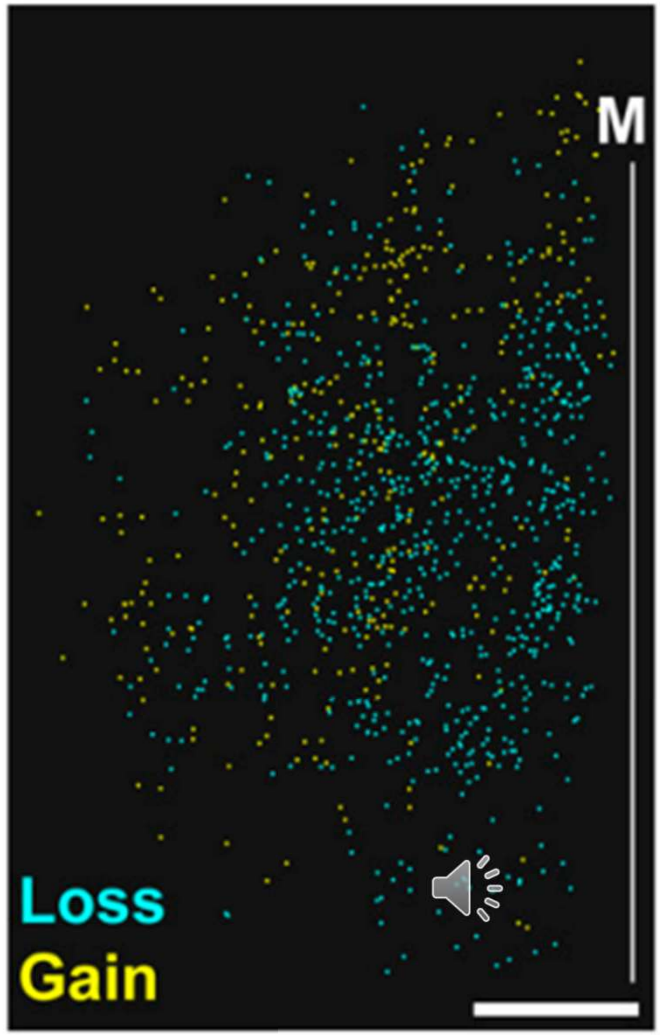
A computational approach applied to in vivo observation of zebrafish dorsal pallium during conditioning



t1 **t2**

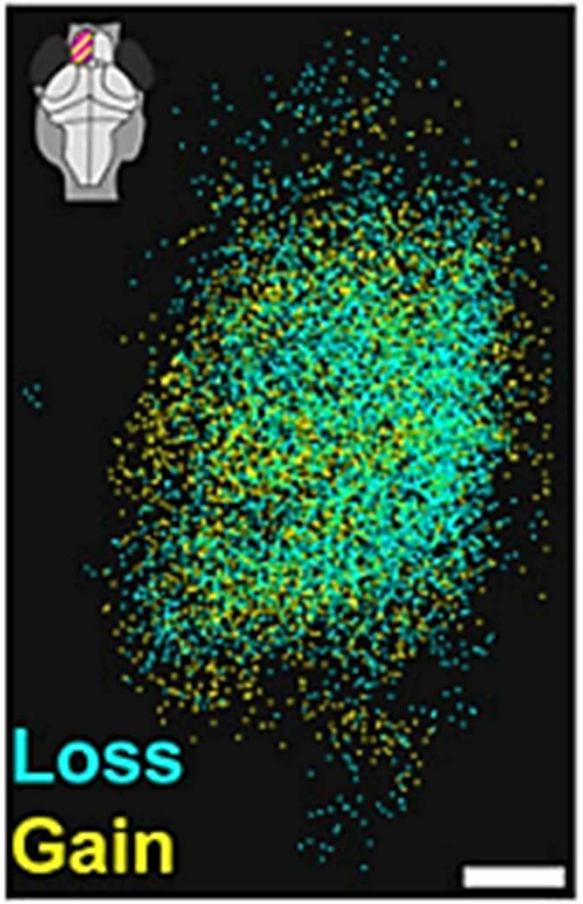


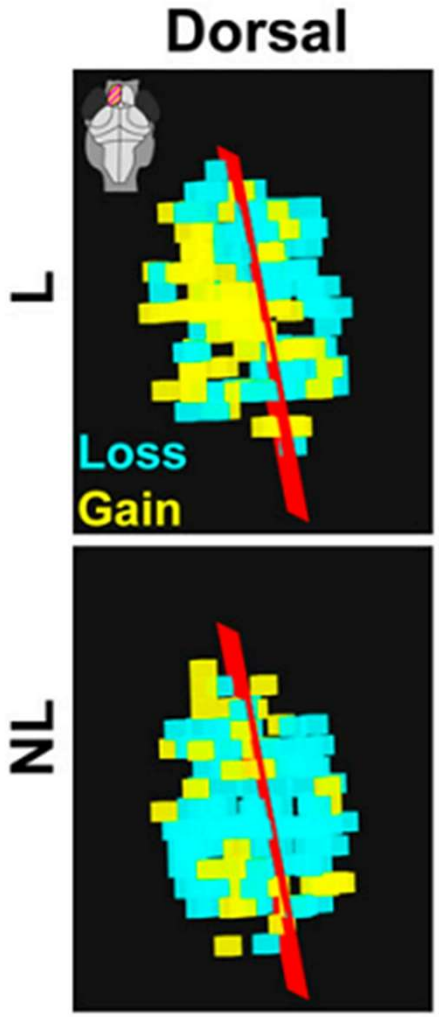


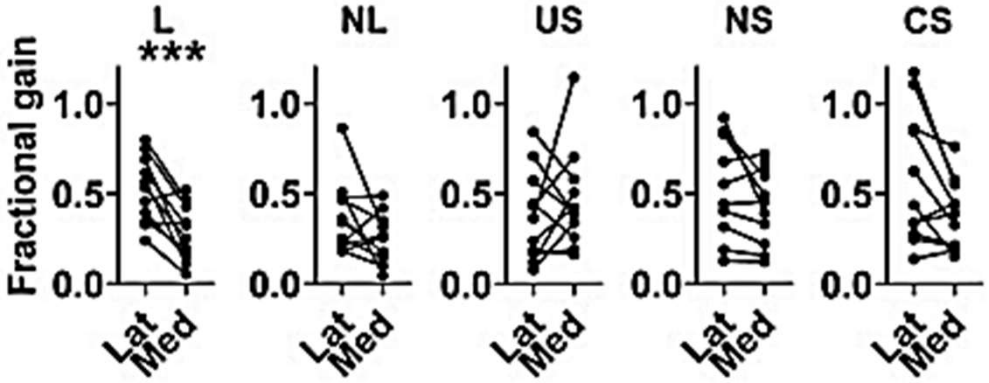


Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119

…however, their distribution across the dorsal pallium is modified

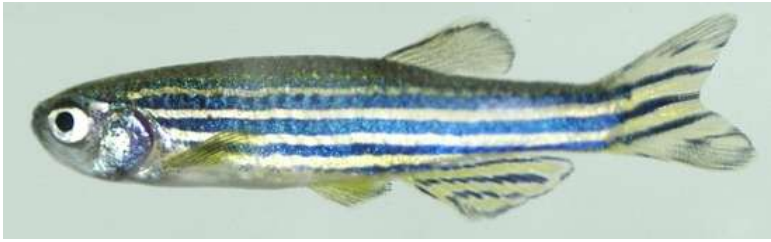






Regional differences in synapse formation in the pallium of learner fish: new synapses are formed only in the lateral pallium

Conclusions



✓ Zebrafish present several advantages for neurophysiological studies

- Multiple techniques for multiple level of resolution of neuronal functions

✓ Studying complex behavior and neuronal correlates

- genetically encoded calcium indicators
- Synaptic resolution