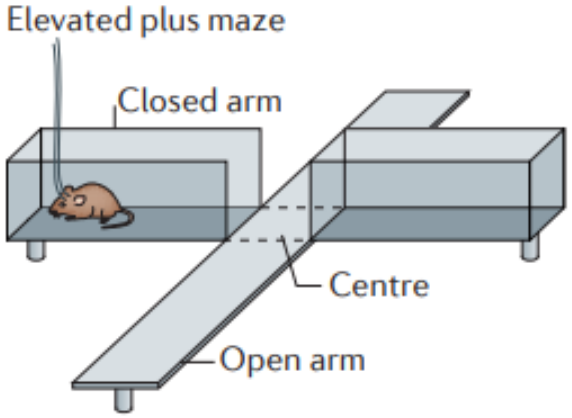
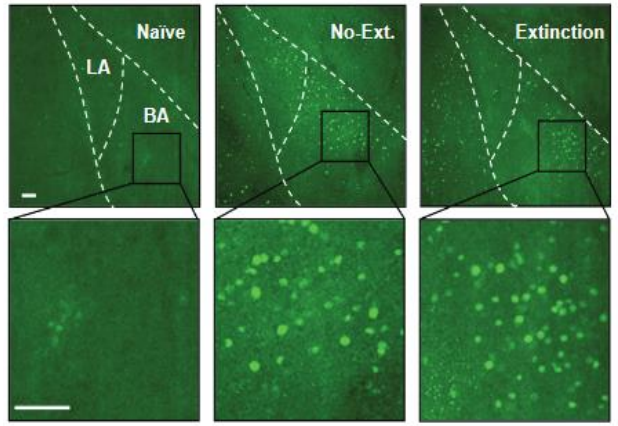
Master in Neuroscience

MOLECULAR NEUROPHYSIOLOGY
-lesson 4-



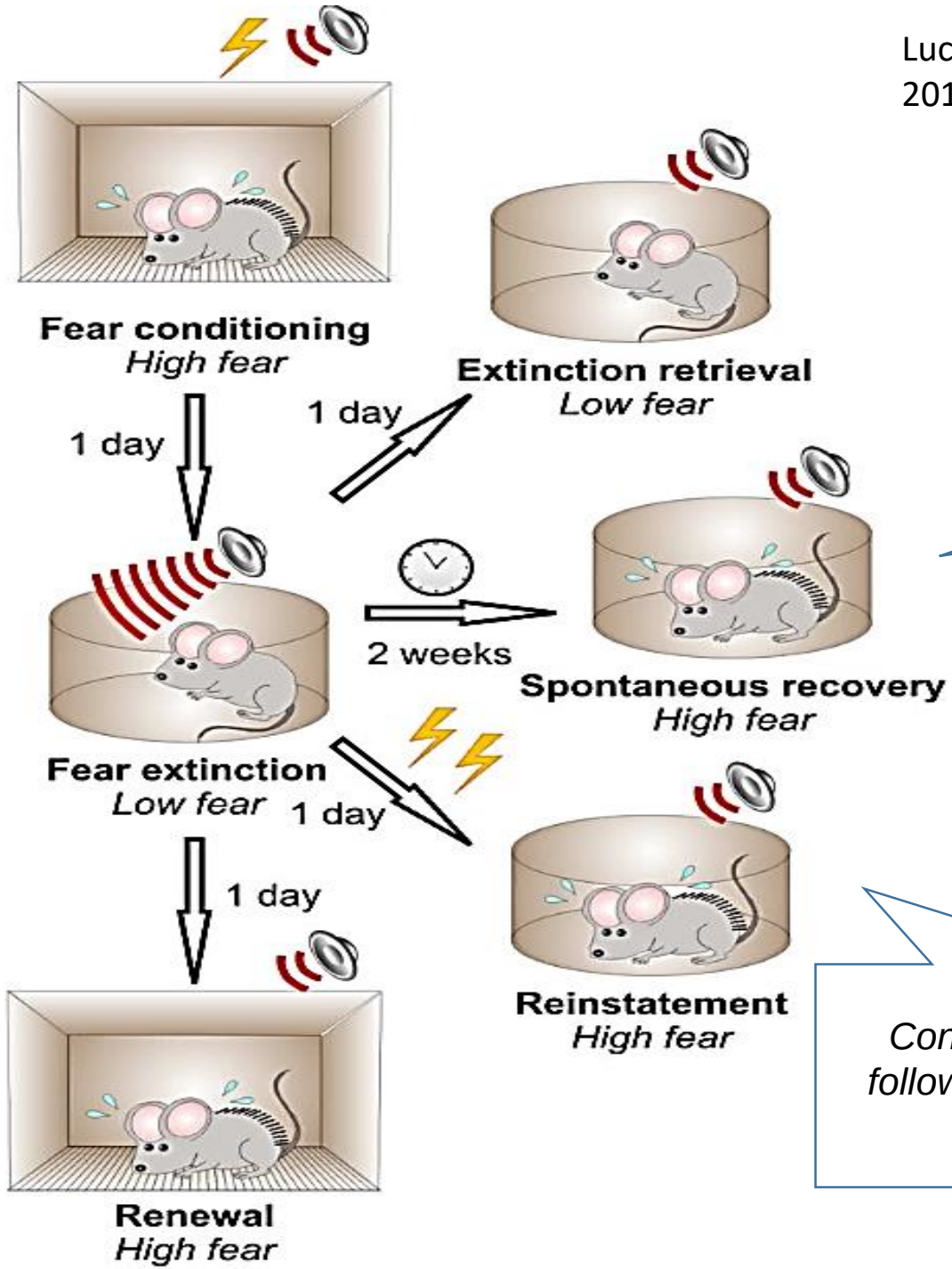
Prof. Giada Cellot

cellot@sissa.it



Depotentiation of conditioning induced plastic changes cannot be the only mechanism for fear extinction

Additional plastic changes inside and outside the amygdala might be involved



Luchkina & Bolshakov, Psycopharmacology, 2018, doi:10.1007/s00213-018-5104-4

Extinguished fear can return at extended time intervals following extinction training in a process of spontaneous recovery

*Due to **context-specificity of fear extinction**, the conditioned fear response can reappear when the context is changed*

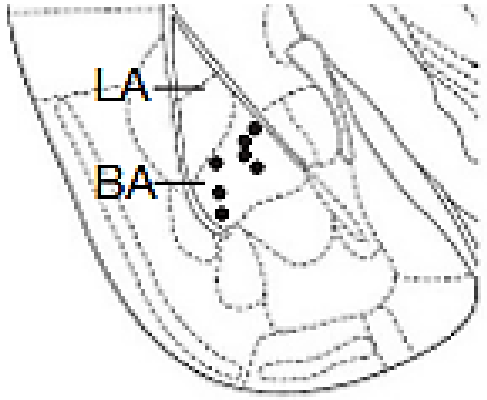
Conditioned fear can be reinstated following exposure to the unsignalled US.

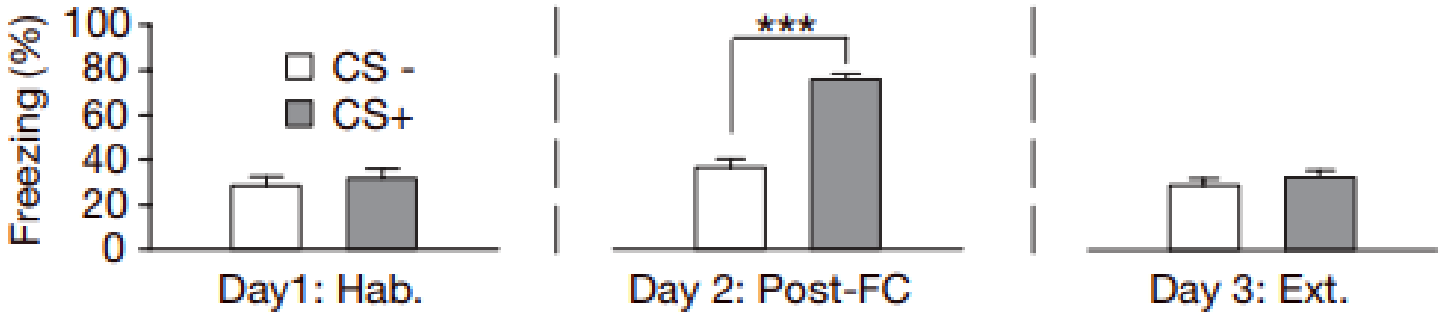
Neuronal substrates for fear extinction

- Fear extinction can be considered as a new form of learning in which extinction networks inhibit fear networks.
- Overlaps of brain areas involved in fear acquisition and extinction: **BASOLATERAL COMPLEX**

FC=fear conditioning with association of electrical foot shock with acoustic tone

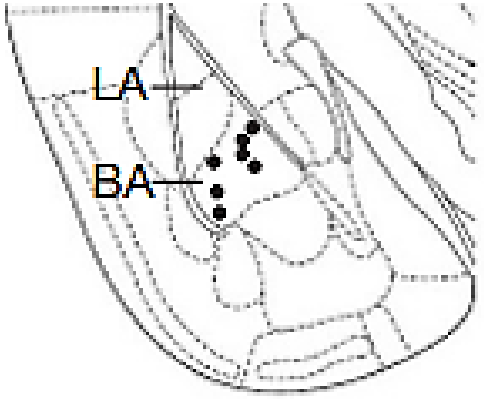
	Day 1: Hab.	Day 1: FC	Day 2: Post-FC	Day 3: Ext.
Unpaired (CTRL)	CS – 4 CS	5 CS	CS	4 CS
Paired	CS + 4 CS	5 CS–US	CS	12 CS
	Conditioning context		Extinction context	



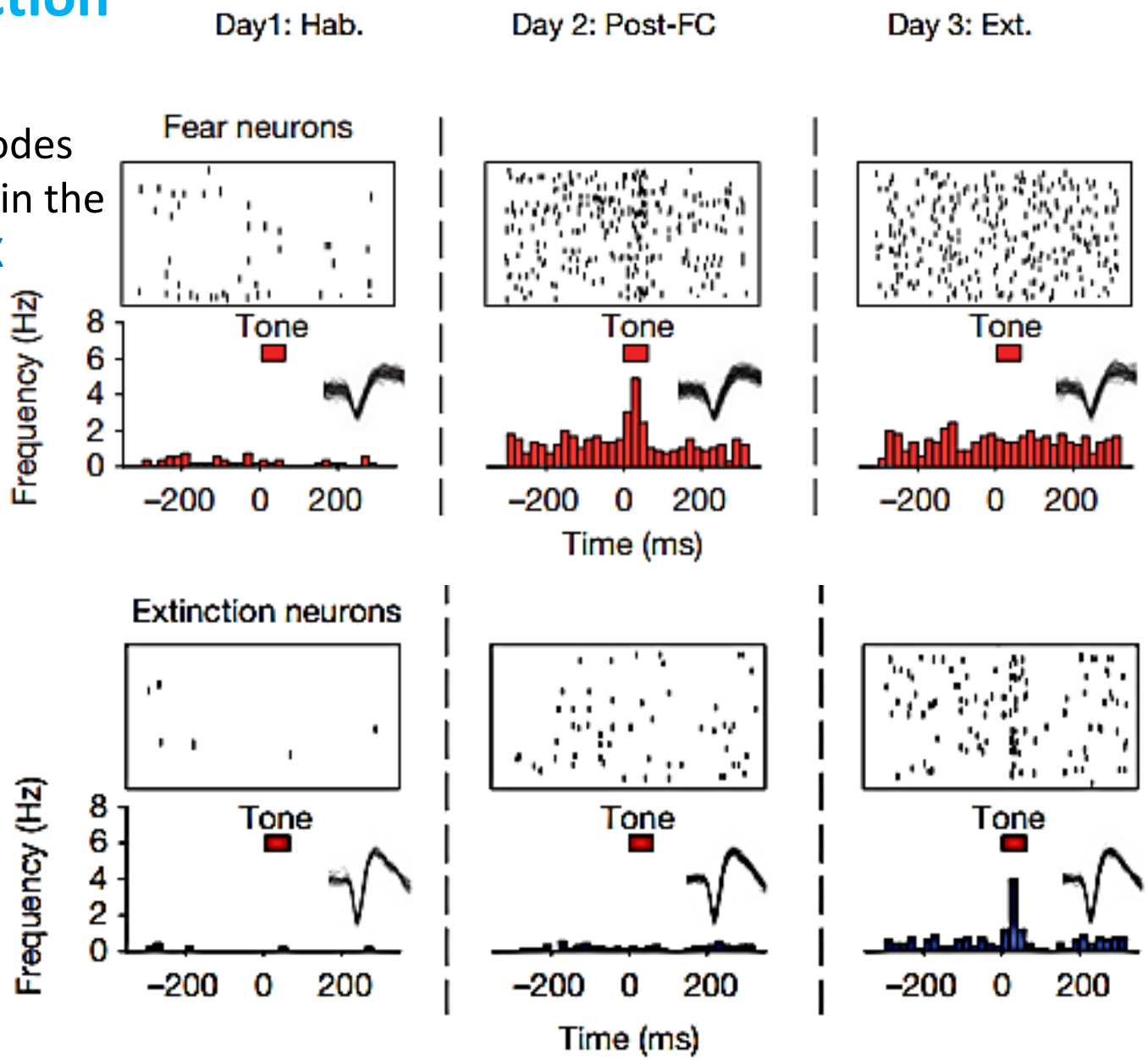


Herry et al, 2008, Nature, doi:10.1038/nature07166

Neuronal substrates for fear extinction



Chronic recording electrodes (18 channels) implanted in the **BASOLATERAL COMPLEX**



Fear neurons are potentiated and depotentiated during fear conditioning and extinction, respectively.

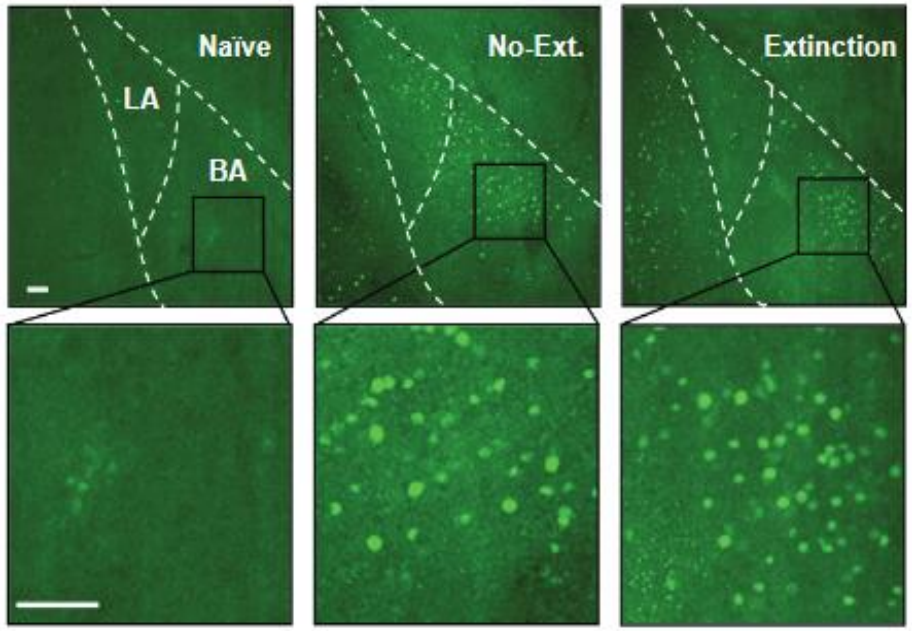
Another group of cells in the BA, **extinctions neurons**, are selectively activated during extinction procedure.

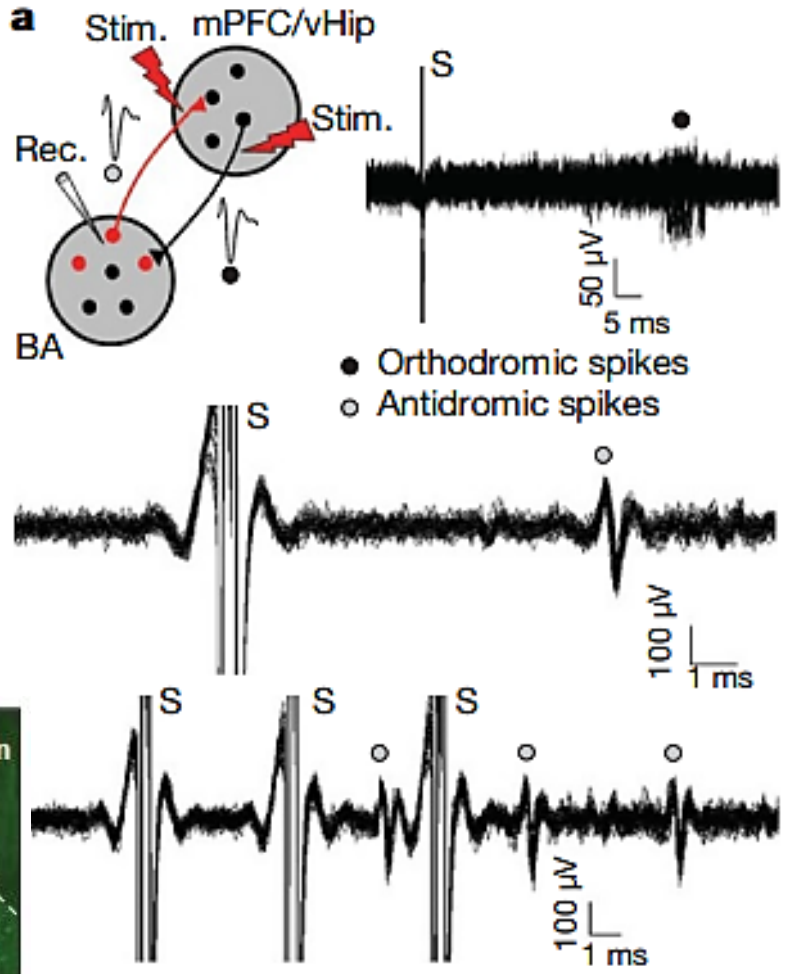
Herry et al, 2008, Nature, doi:10.1038/nature07166

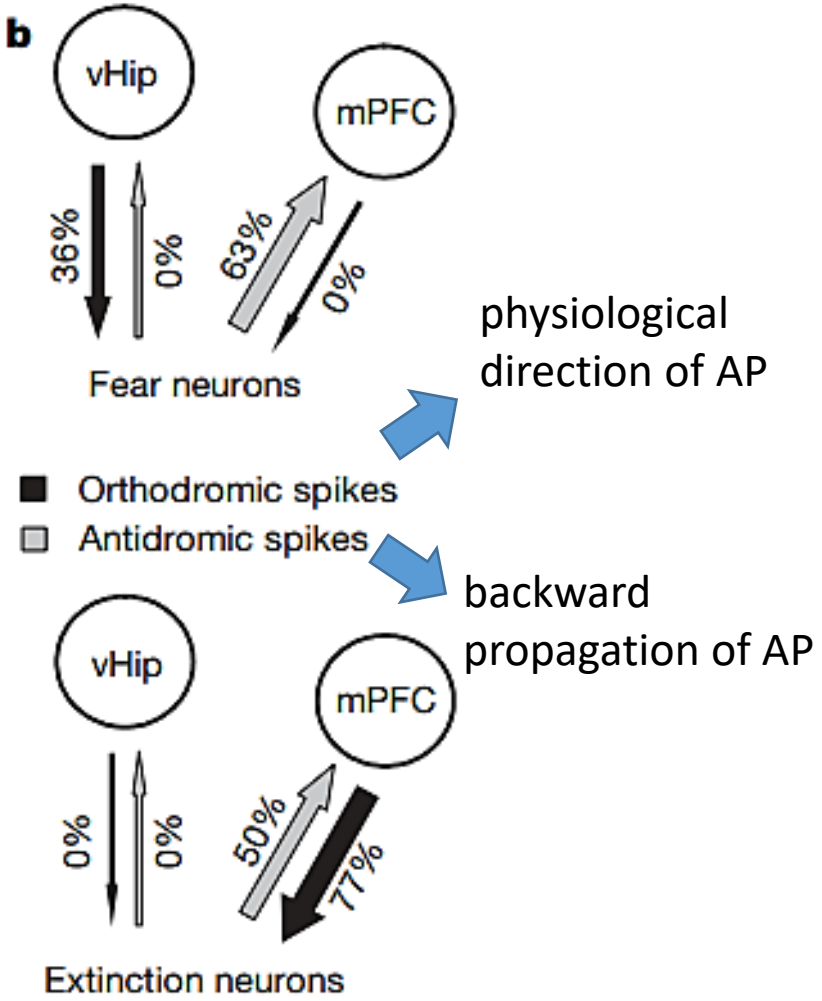
Neuronal substrates for fear extinction

Although c-fos expression studies show that **fear and extinction neurons** are not segregated in different regions of BA, they form part of discrete neuronal circuits.

c-fos expression







Orthodromic spikes vs **Antidromic spikes**

high failure rates — ability to follow 200 Hz frequency stimulation

variable latency (>0.3 ms jitter) — stable latency (<0.3 ms jitter)

Herry et al, 2008, Nature, doi:10.1038/nature07166

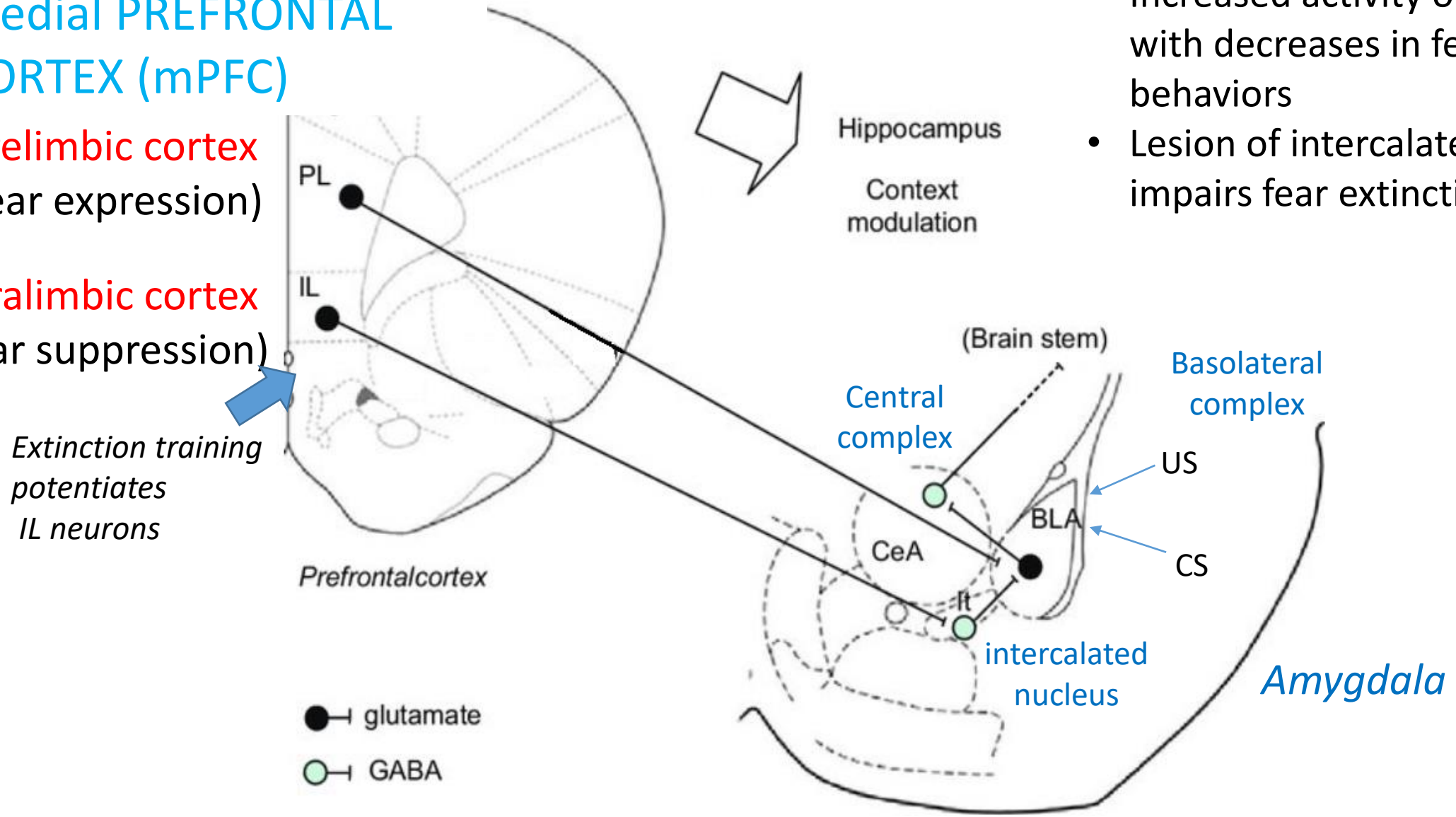
The medial PreFrontal Cortex (mPFC) exerts a top-down control of limbic regions

Medial PREFRONTAL CORTEX (mPFC)

prelimbic cortex
(fear expression)

infralimbic cortex
(fear suppression)

- Increased activity of IL correlates with decreases in fear related behaviors
- Lesion of intercalated nucleus impairs fear extinction



Canteras et al, 2010 DOI: 10.1007/7854_2009_7

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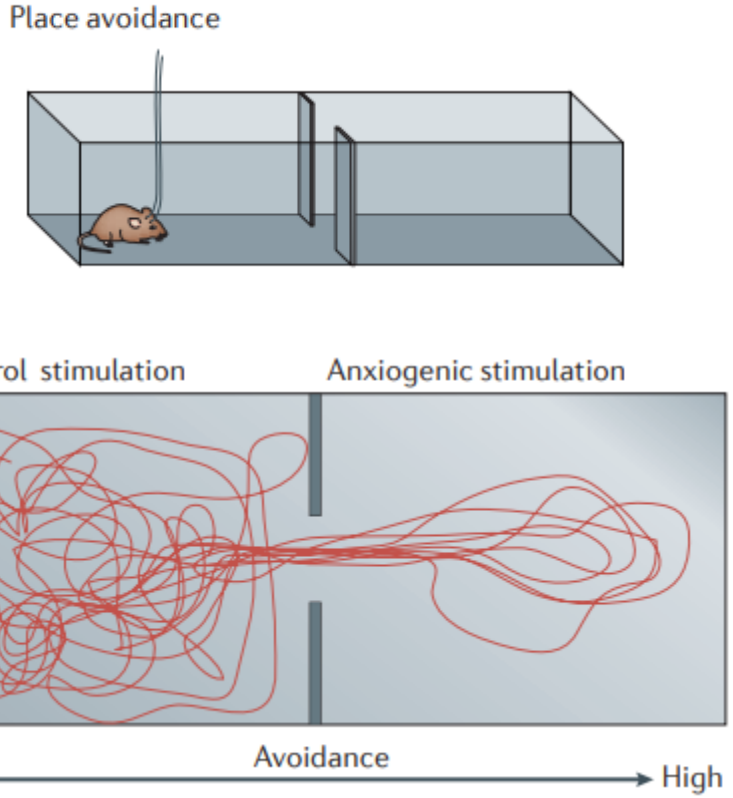
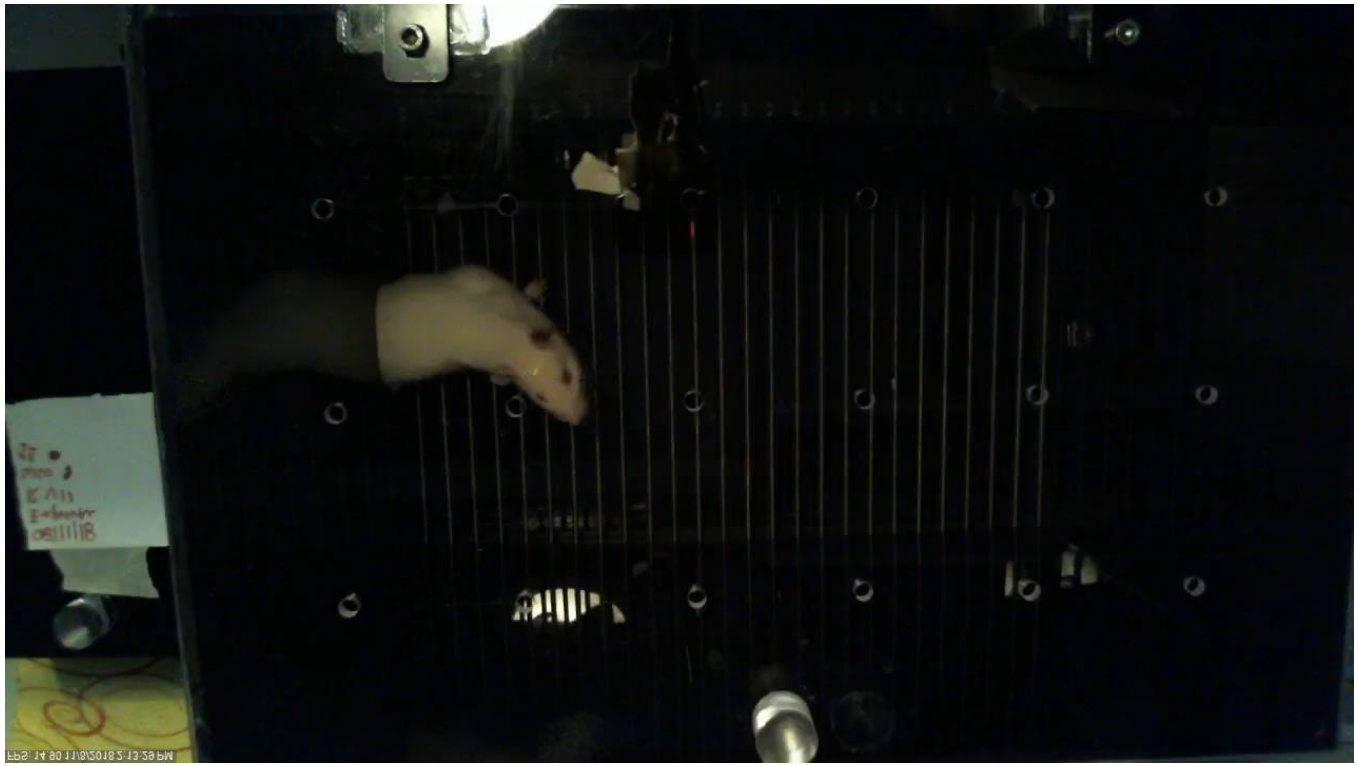
Anxiety related behaviors

FEAR: response to discrete and acutely threatening stimuli

ANXIETY: response to vague, potential threats

Almost overlapping neuronal circuits



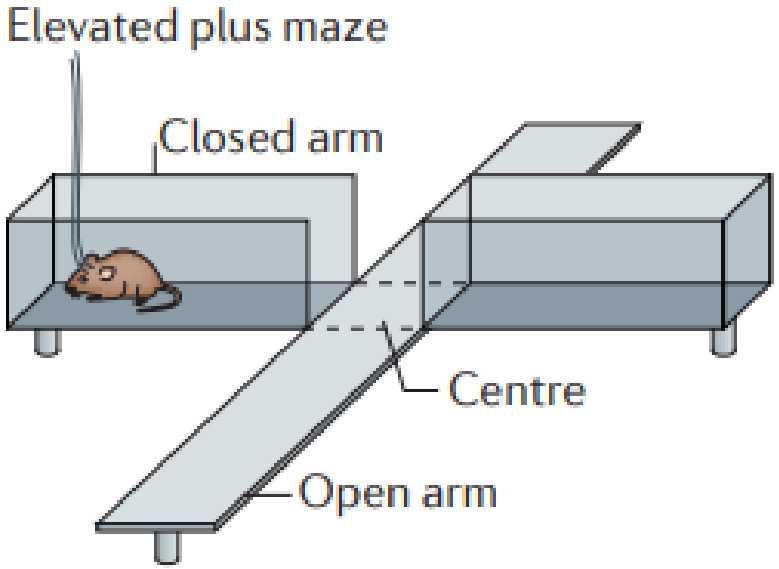


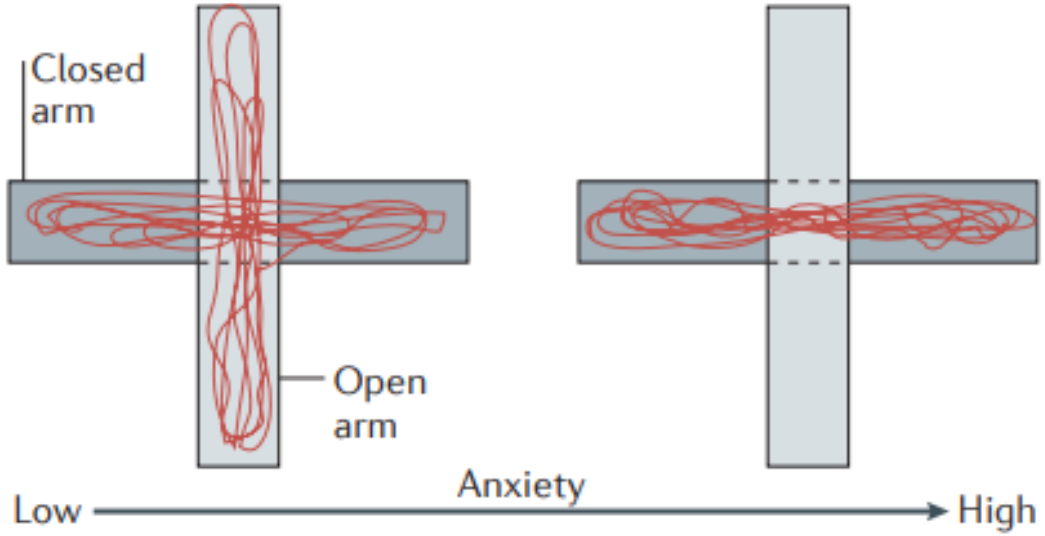
Tovote et al, 2015, Nat Neurosci, 16(6):317-31. doi: 10.1038/nrn3945.

Courtesy of AF Biagioni, Lab. Prof. Ballerini

Pati et al, Nanoscale, 2023, DOI: 10.1039/d3nr04490d

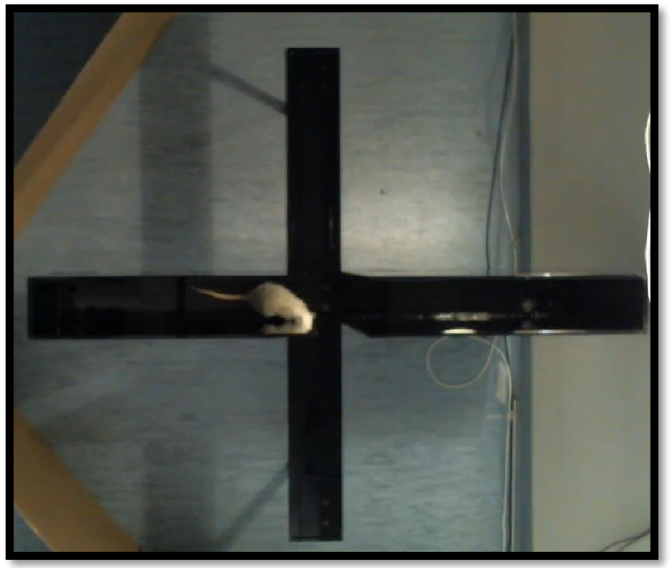
Anxiety related behaviors



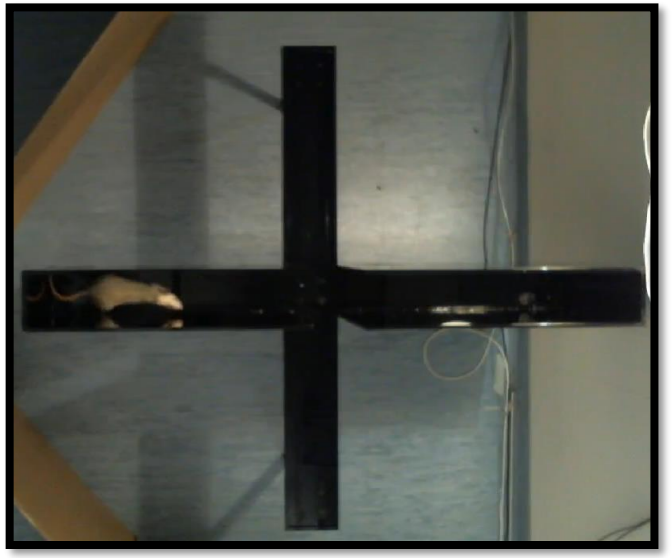


Tovote et al, 2015, Nat Neurosci, 16(6):317-31. doi: 10.1038/nrn3945.

No anxious animal



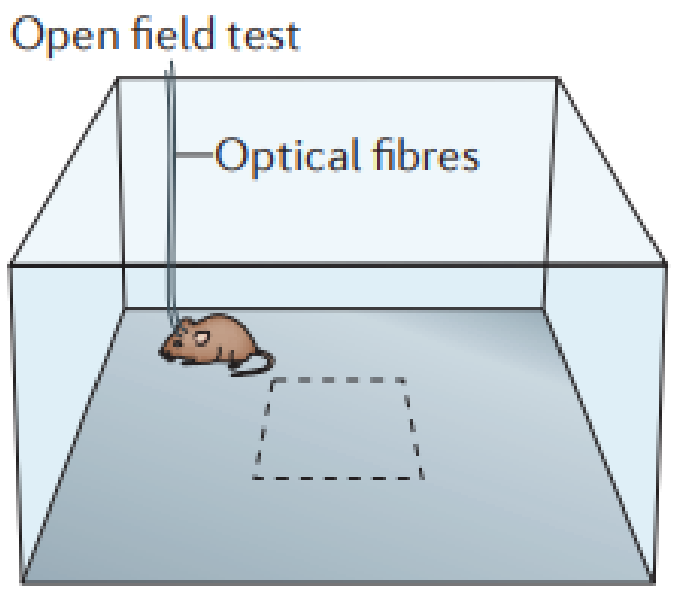
Anxious animal

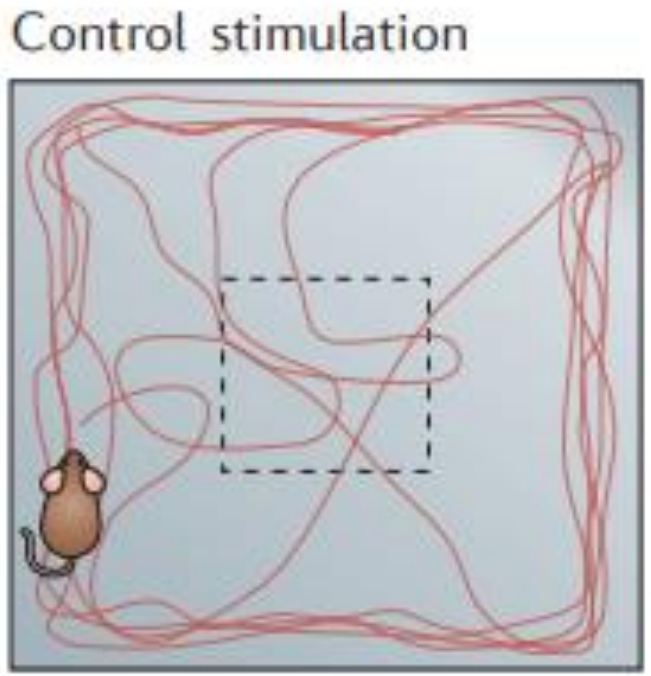


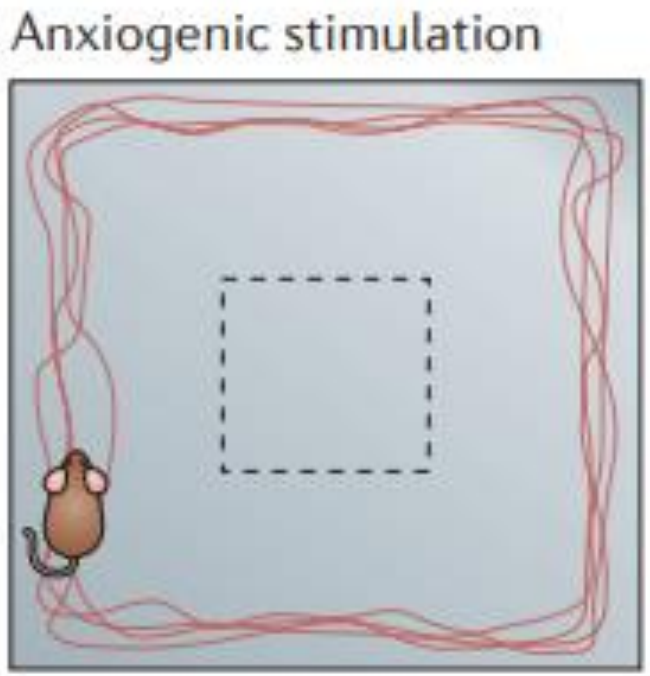
Courtesy of AF Biagioni, Lab. Ballerini

Pati et al, Nanoscale, 2023, DOI: 10.1039/d3nr04490d

Anxiety related behaviors







Tovote et al, 2015, Nat Neurosci, 16(6):317-31.
doi: 10.1038/nrn3945.

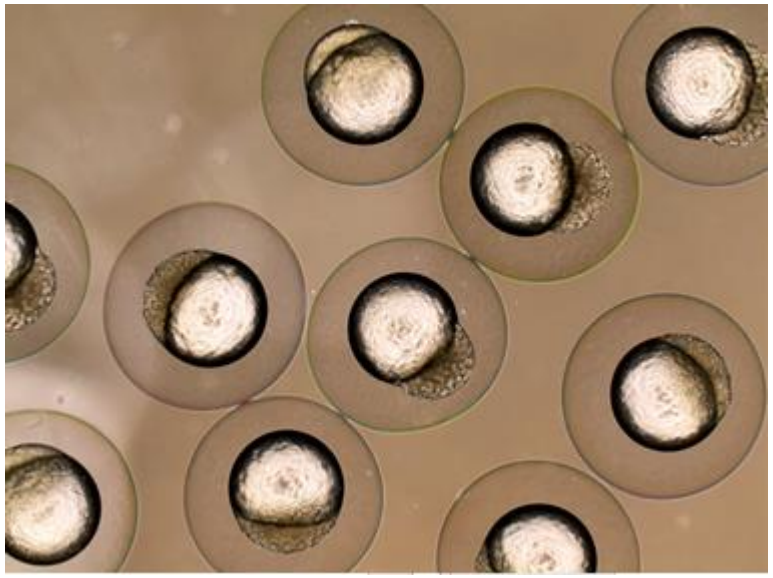
Is Zebrafish (Danio Rerio) a good alternative research model for the study of synaptic plasticity and its behavioral correlates?

- 70% of genetic homology with humans

- Ethics in using animal for research purpose: Directive 2010/63/EU

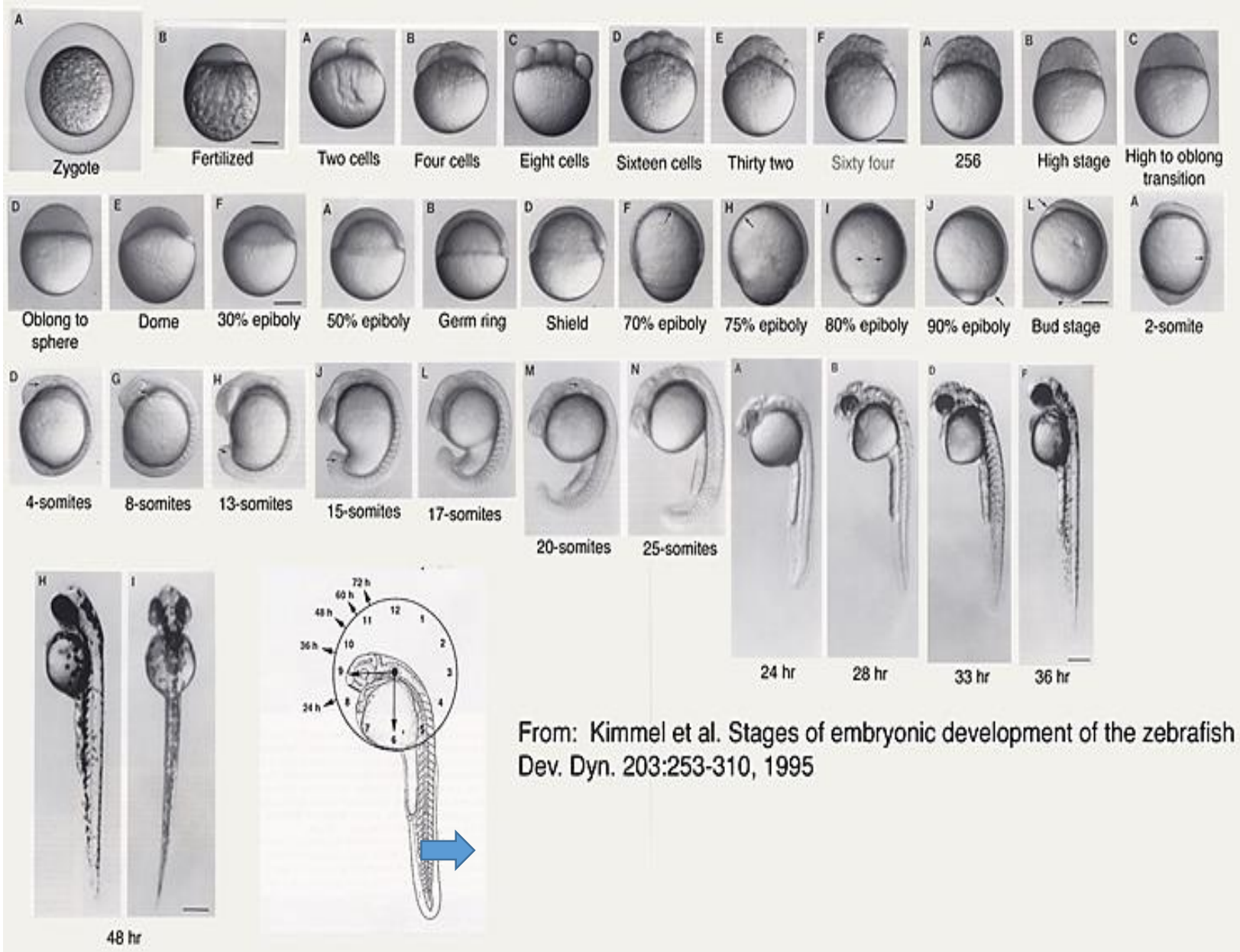
Principle of the 3Rs (**R**eplacement, **R**eduction and **R**efinement) **OK**

- 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*



From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

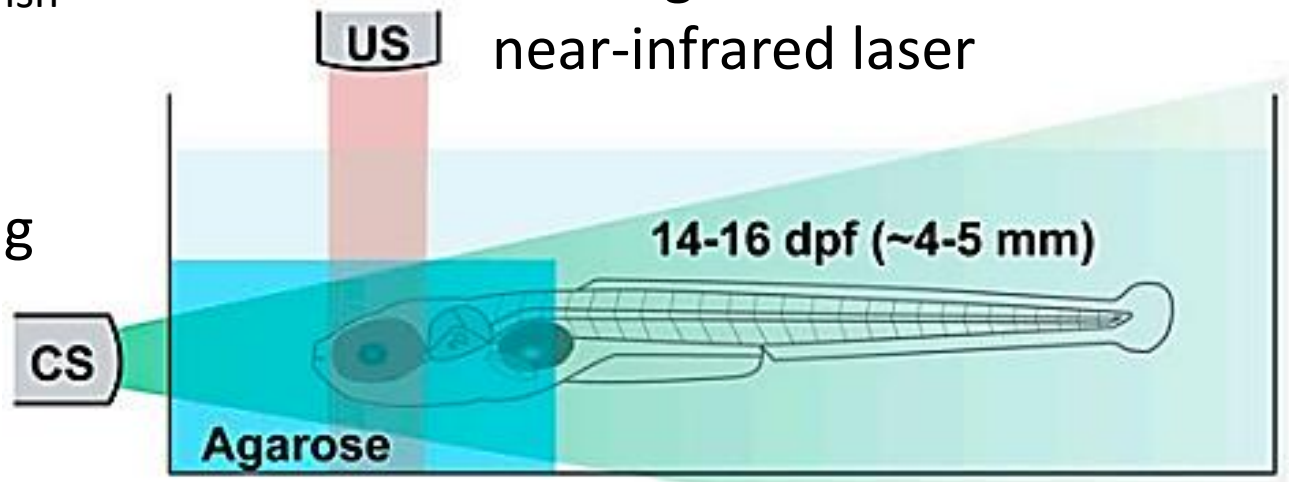
Investigating associative learning induced brain changes in in vivo zebrafish

Cued aversive conditioning

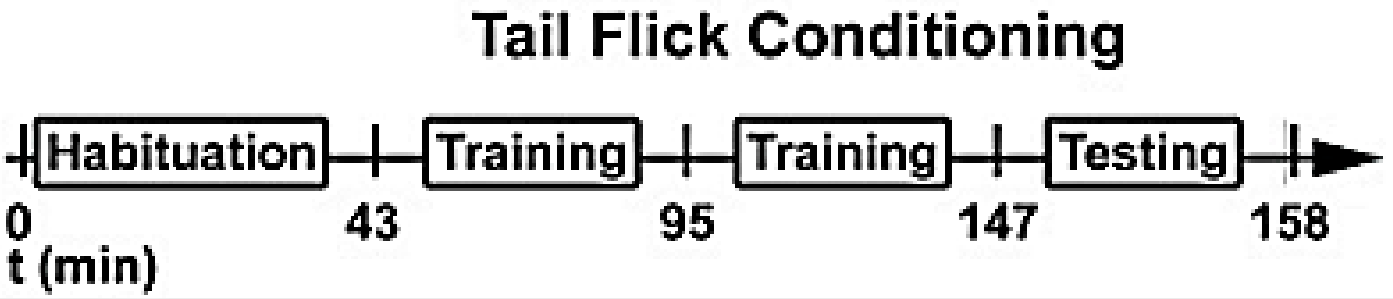
15 dpf zebrafish

Heating from a near-infrared laser

Light from a light-emitting diode (LED)

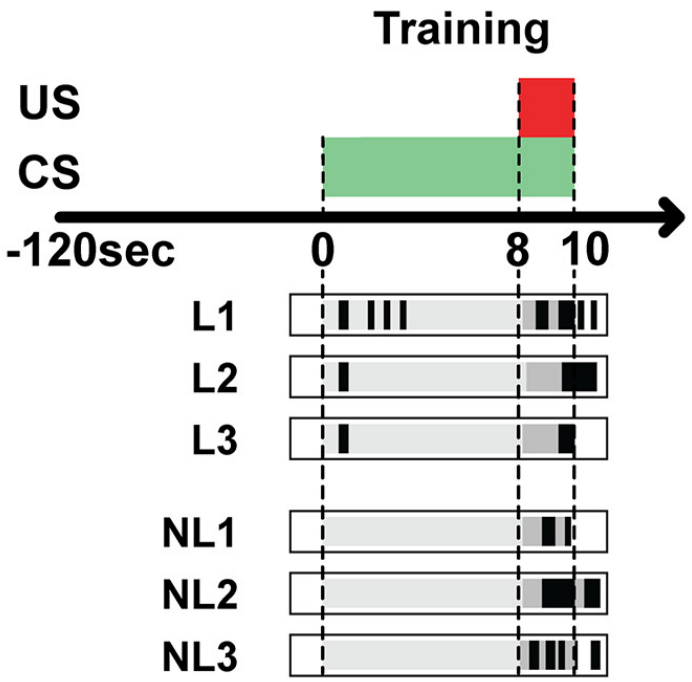


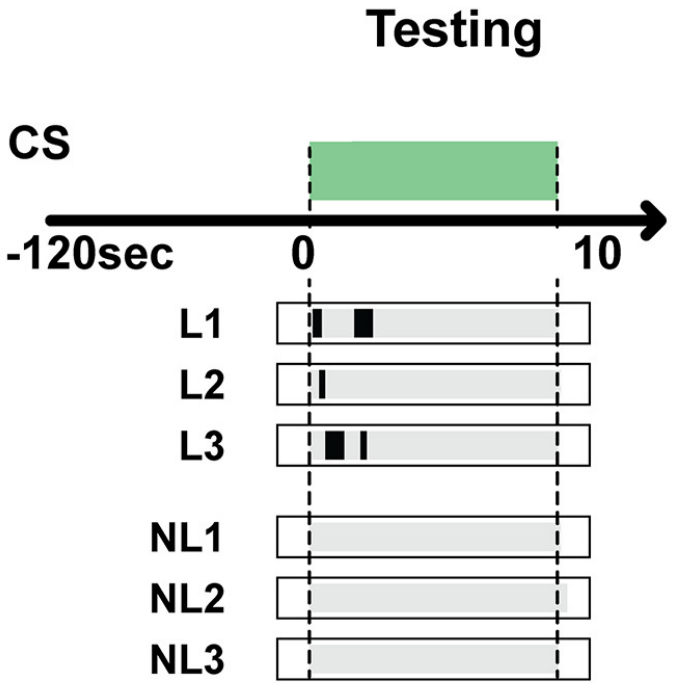
Behavioral readout: Tail-flick

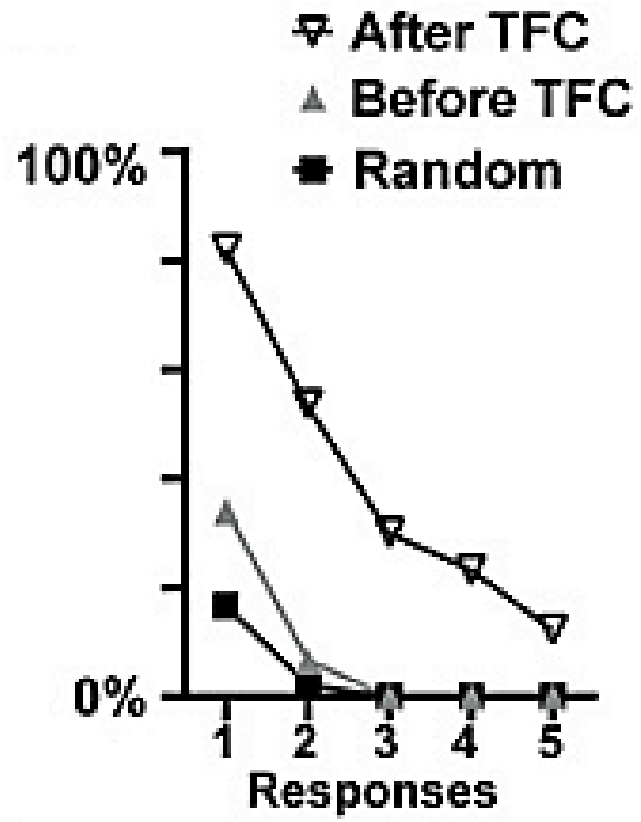


Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119

Investigating associative learning induced brain changes in in vivo zebrafish







L = Learners

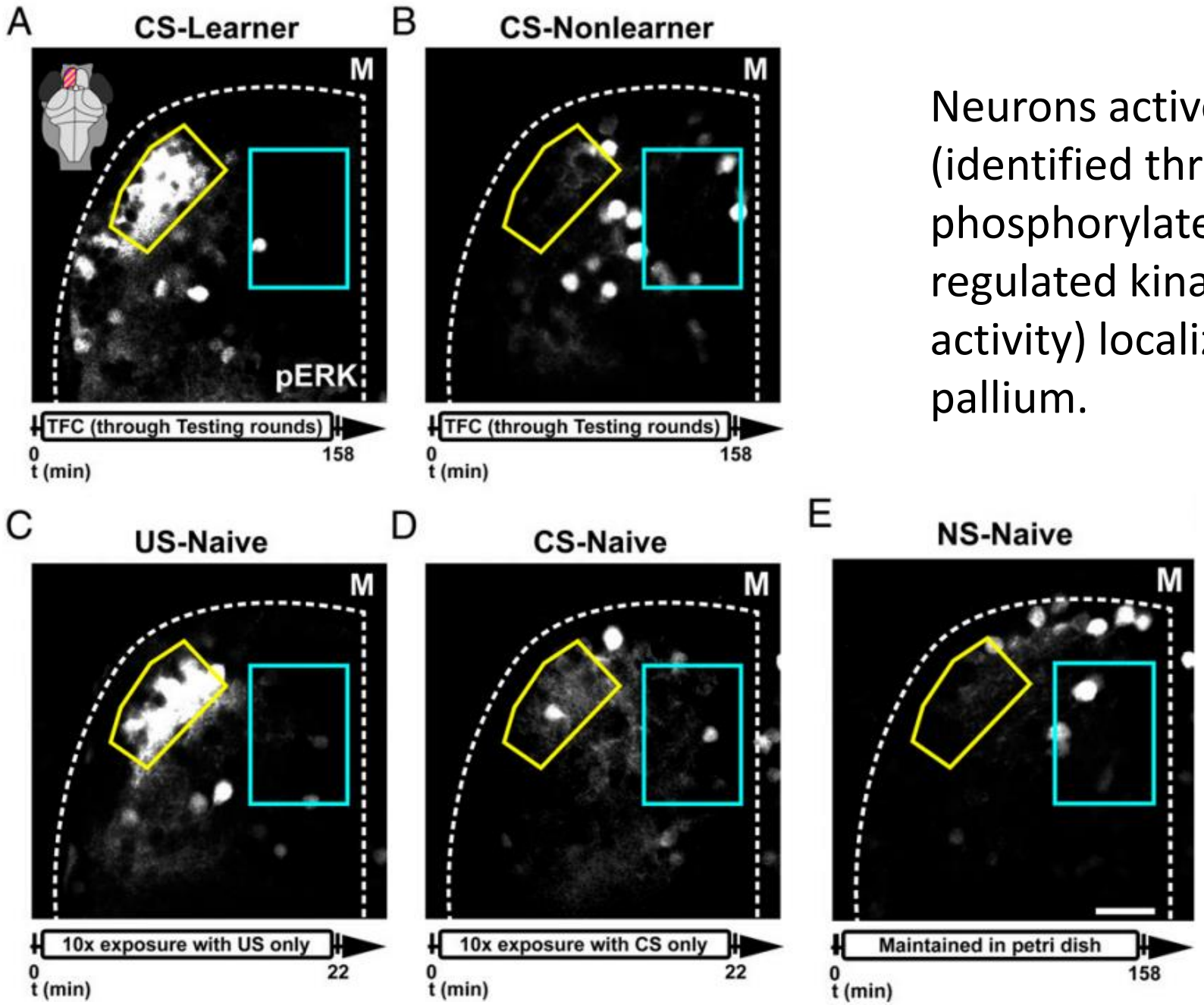
NL = Nonlearners

Tail flick conditioning is:

✓ NMDA receptor dependent

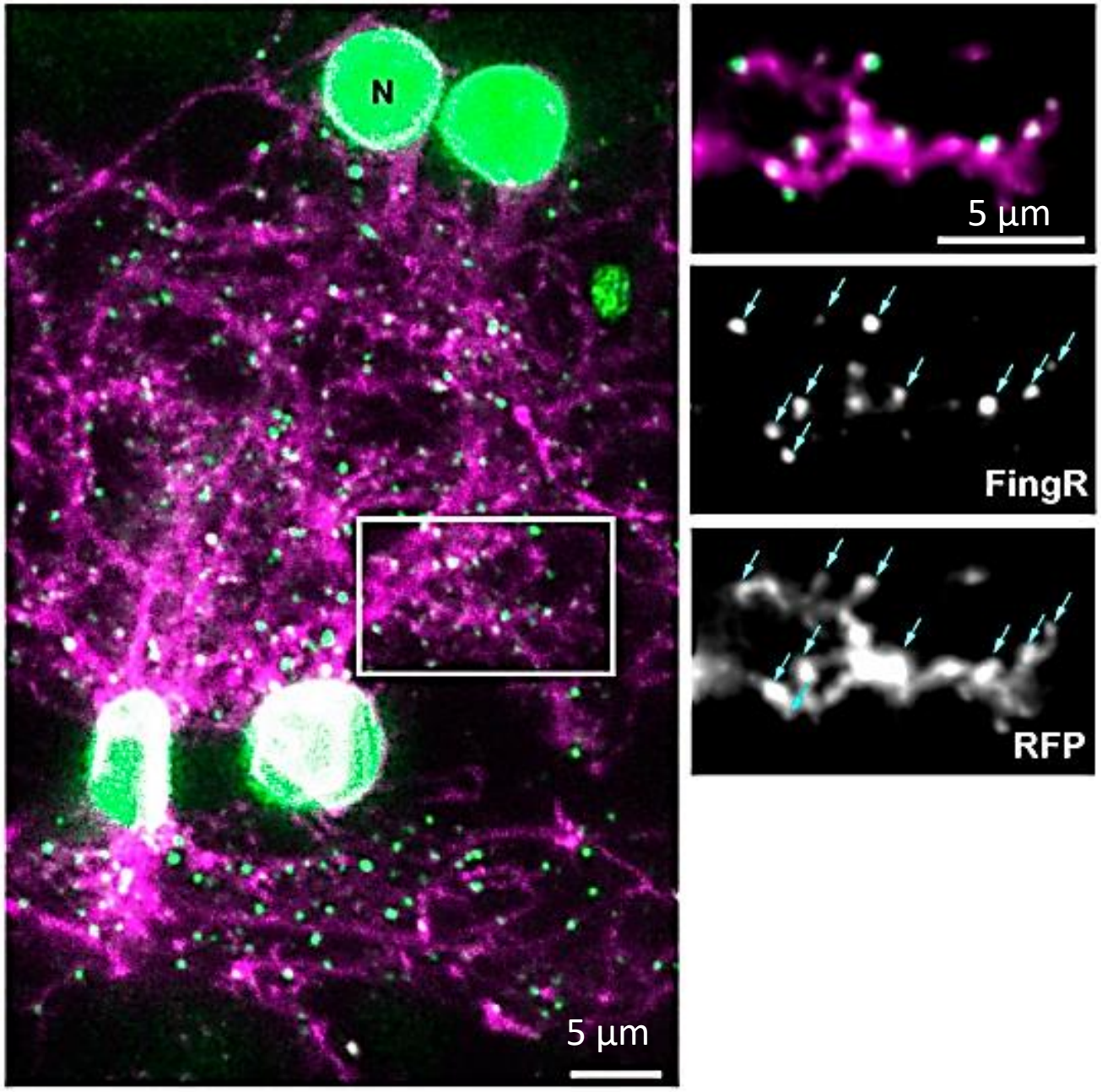
✓ Sensitive to extinction

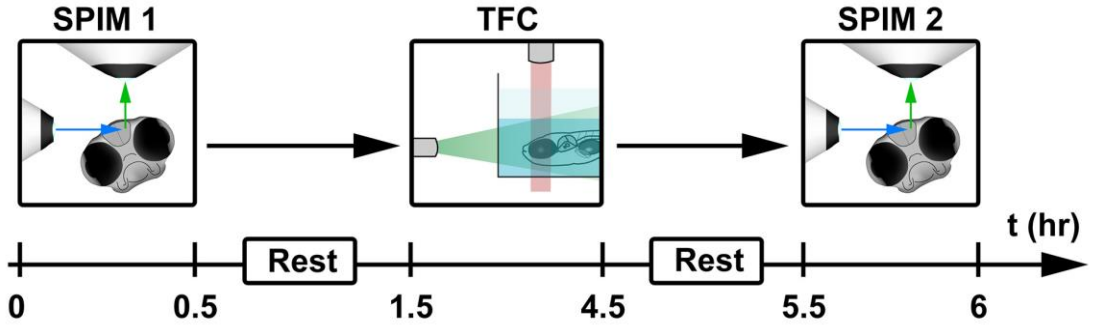
Dempsey et al., PNAS, 2022, doi.org/10.1073/pnas.2107661119



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





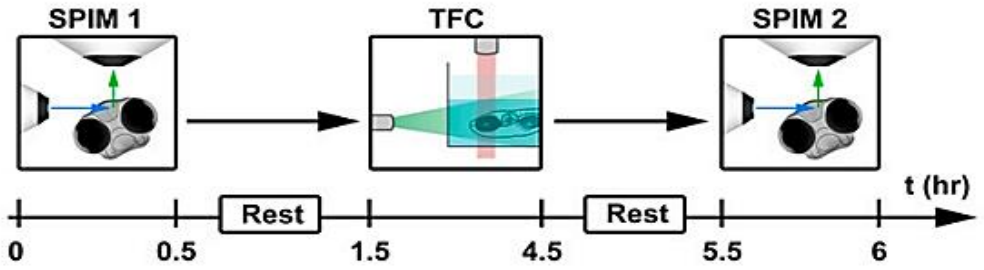
Selective plane illumination microscopy (SPIM)

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

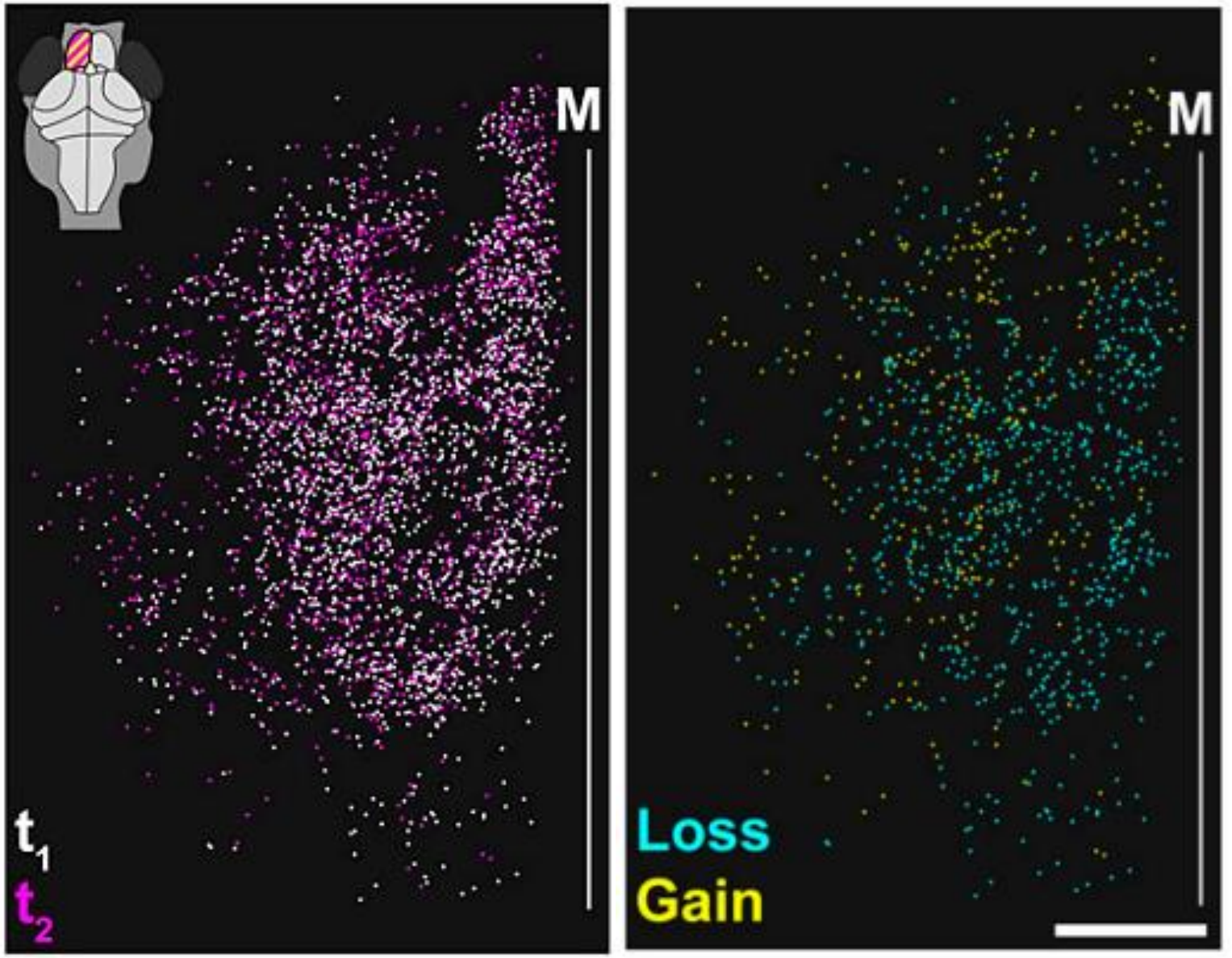
- PSD-95.FingR-GFP (green)
- membrane-targeted mScarlet (magenta)

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

A computational approach applied to in vivo observation of zebrafish brain revealed that the overall number of synapses in the dorsal pallium is not changed by conditioning…

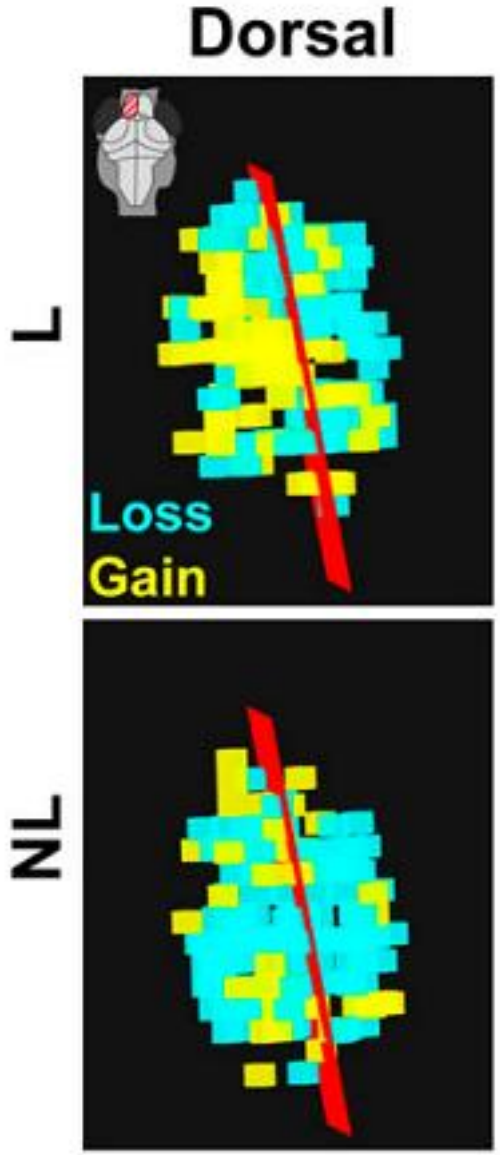


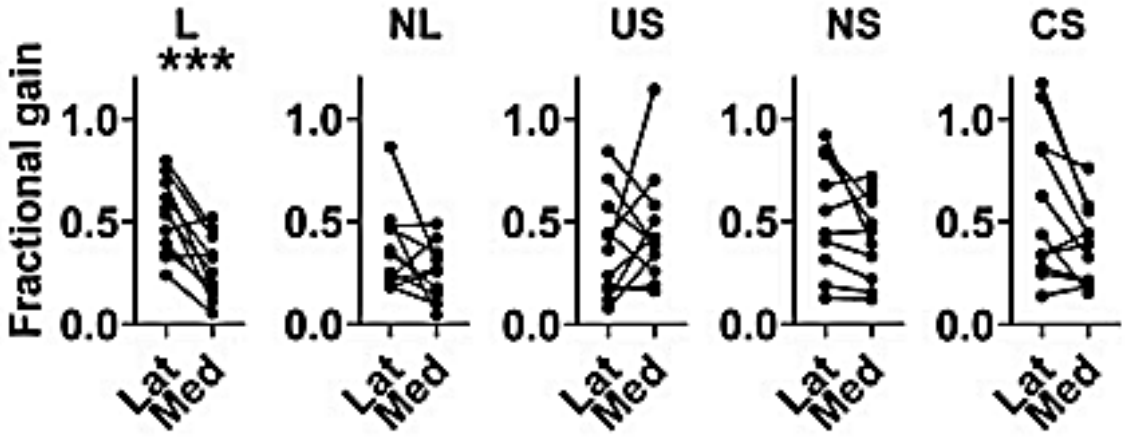
t1 **t2**



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