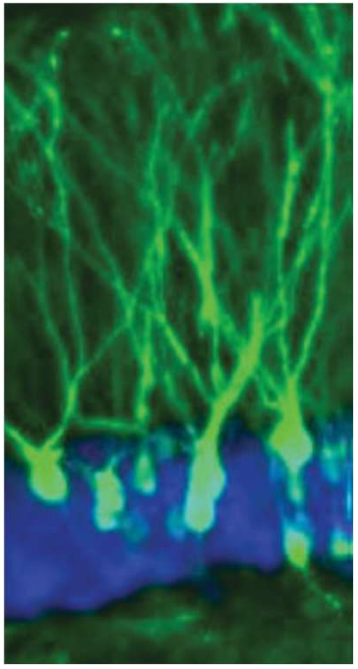
Master in Neuroscience

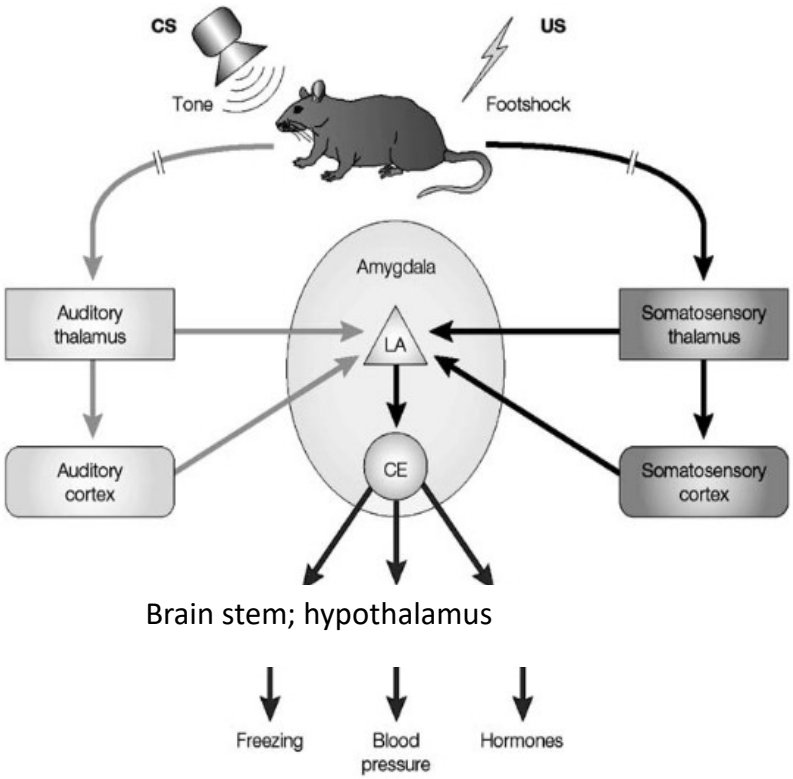
MOLECULAR NEUROPHYSIOLOGY
-lesson 4-



Prof. G.Cellot

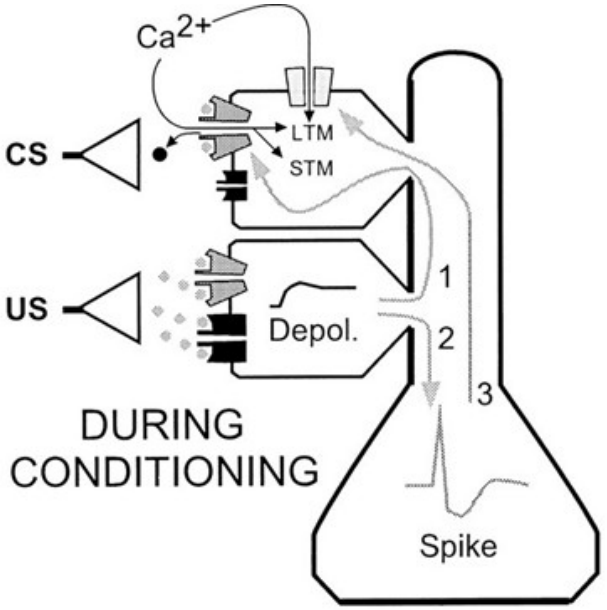
To sum up…

Synaptic plasticity in the amygdala is involved in fear conditioning

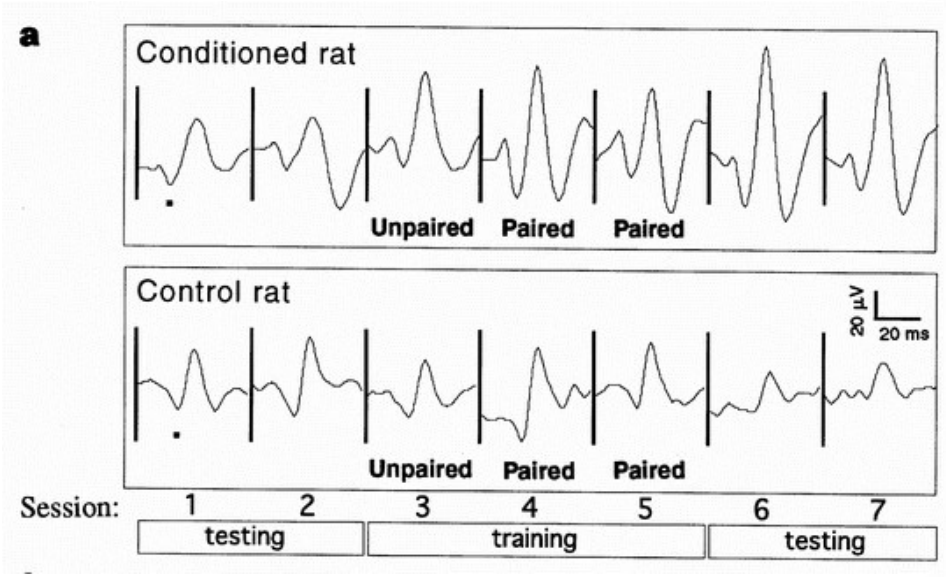


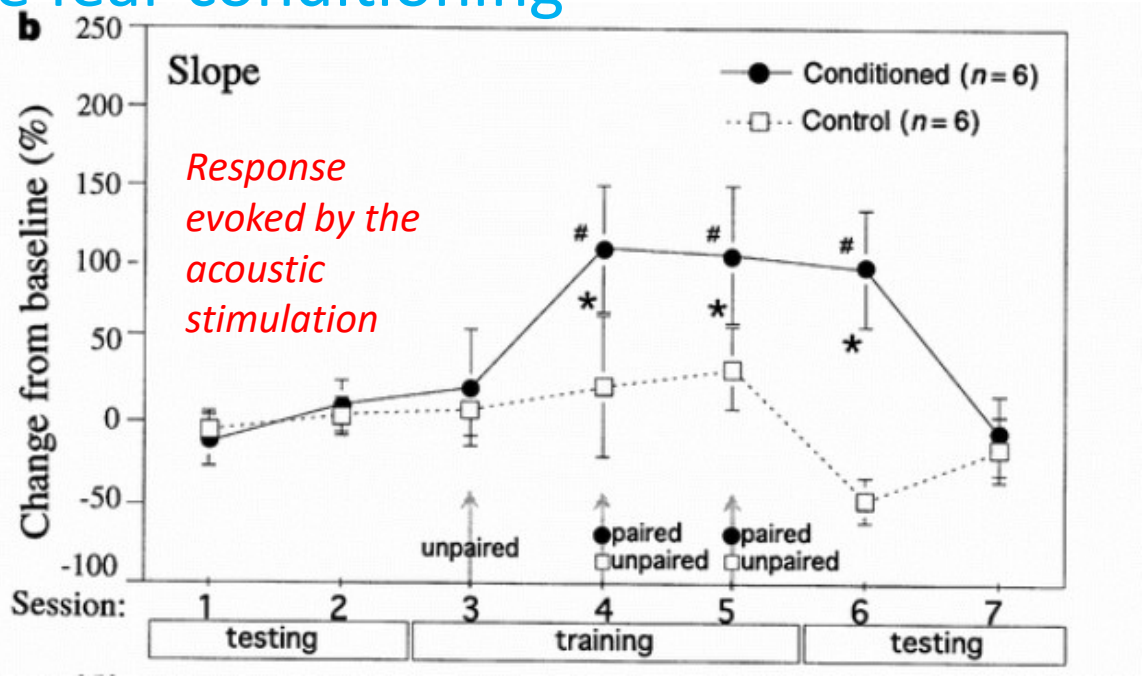
Defensive responses + autonomic and endocrine “setting”

The LATERAL AMYGDALA (LA) is an interface where US can modify the functional meaning of CS

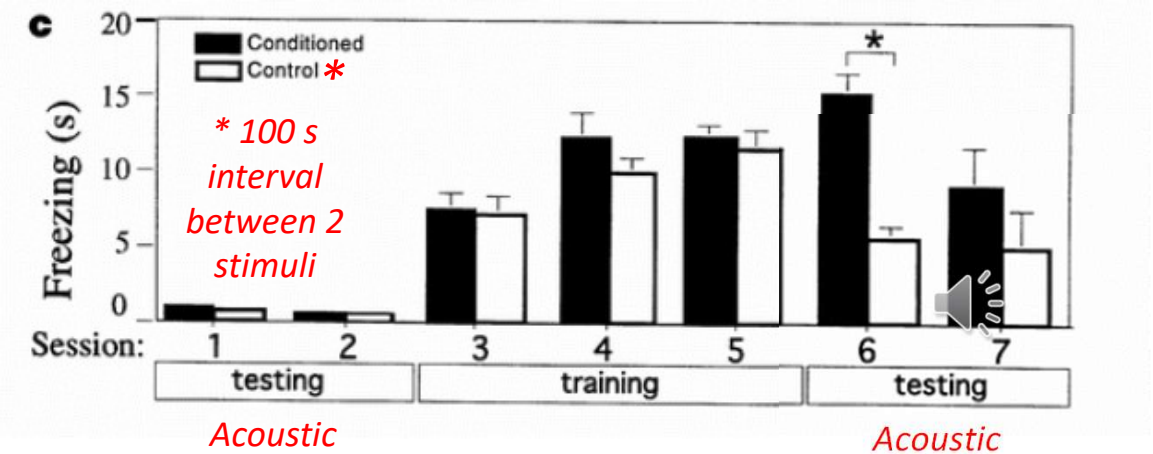


A cellular hypothesis for associative fear conditioning



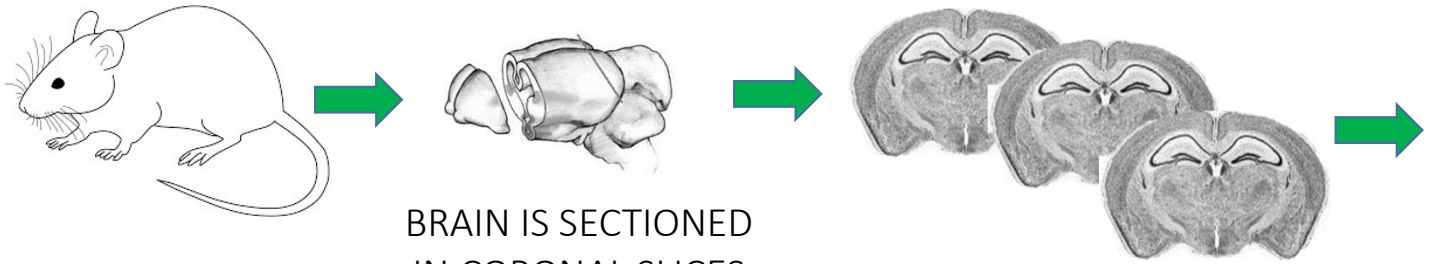


In vivo recordings in the LA showed the potentiation of the CS inputs after conditioning

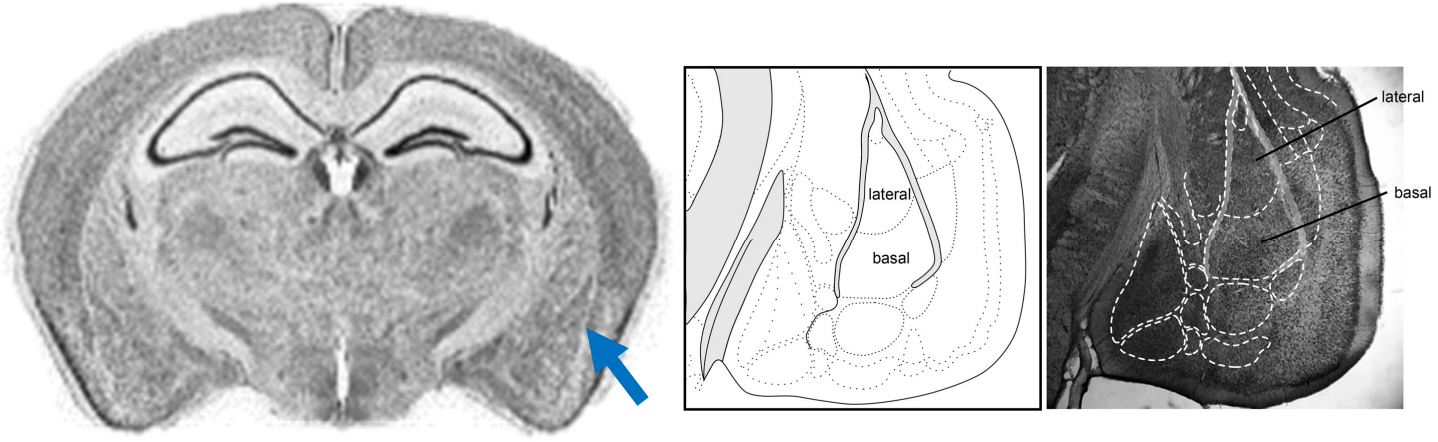


Fear conditioning induces associative long-term potentiation in the amygdala
Rogan et al., Nature, 1997

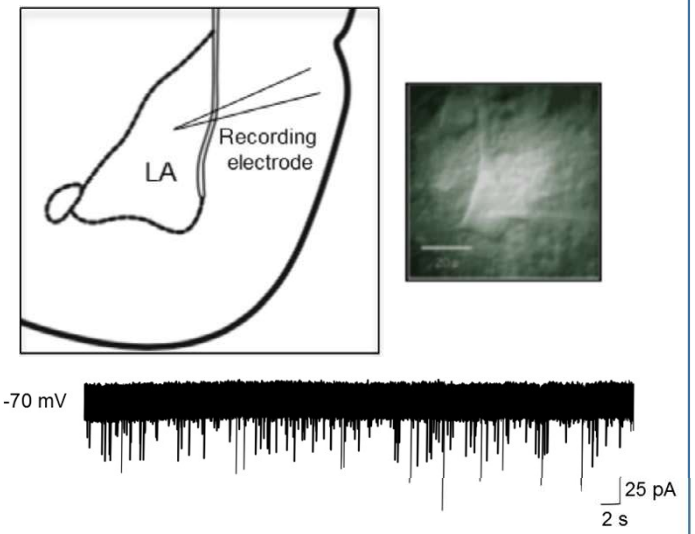
Amygdalar acute slices: a simplified in vitro model of amygdala



BRAIN IS SECTIONED
IN CORONAL SLICES



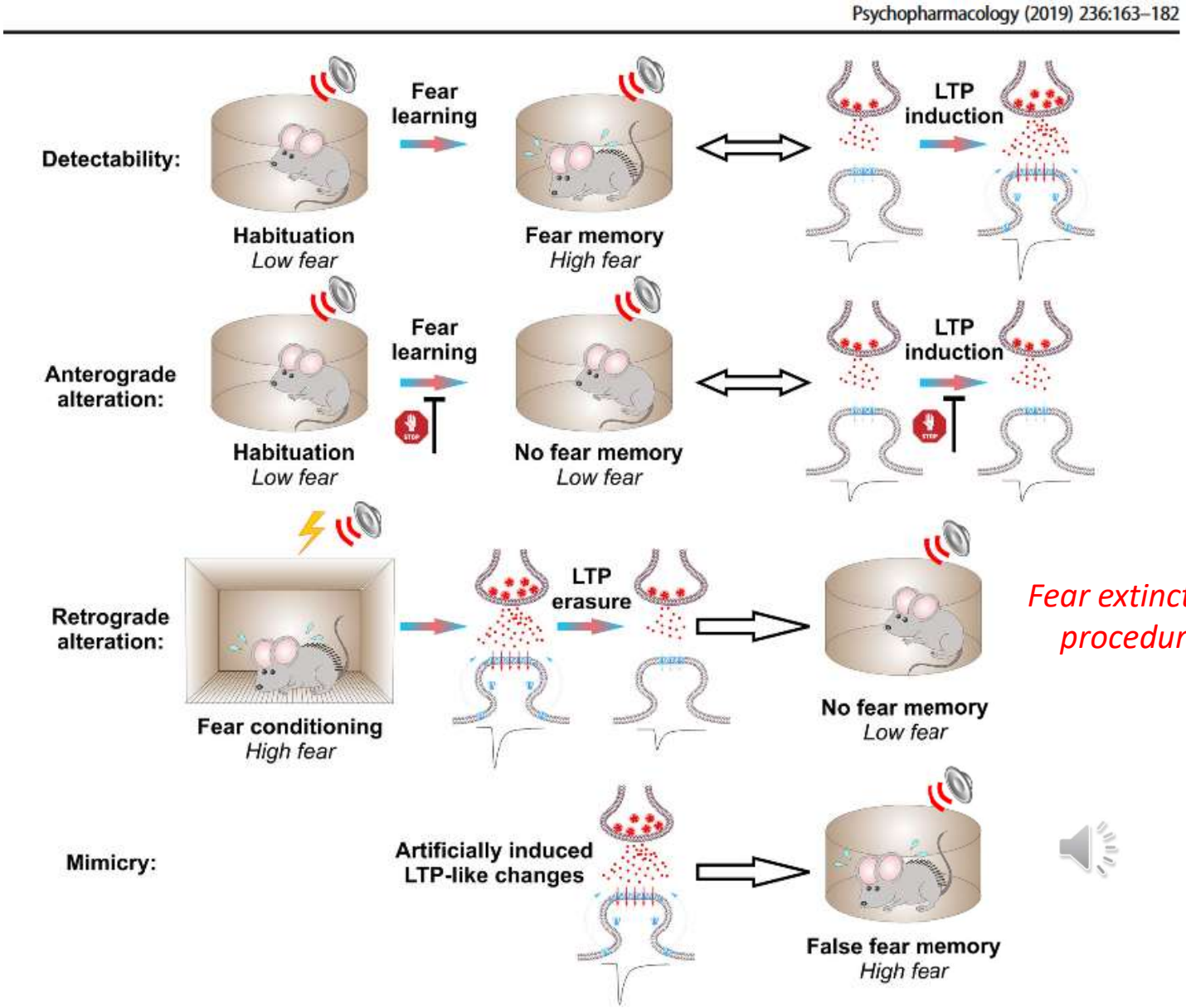
ELECTROPHYSIOLOGICAL
ANALYSIS OF
GLUTAMATERGIC
POSTSYNAPTIC CURRENTS



- LA neurons are innervated by glutamatergic synapses, whose afferent fibers come from the thalamus and cortex
- LA neurons expressed both AMPA and NMDA receptors
- LTP can be induced by high frequency stimulation of afferent fibers
- It is prevented by application of NMDAr antagonists: HEBBIAN LTP

The SPM hypothesis applied to amygdala circuitry

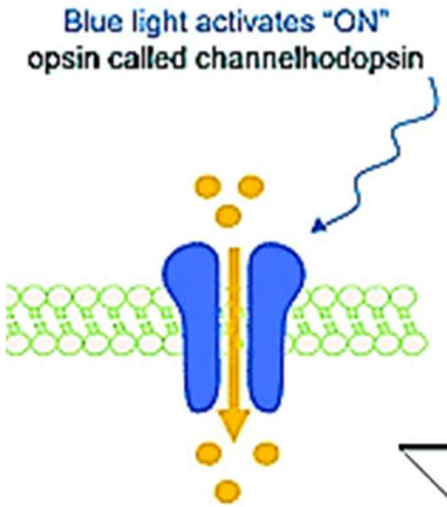
Mimicry in the amygdala is potentially easier to induce as the circuits underlying associative learning are localized in a specific nucleus (LA)



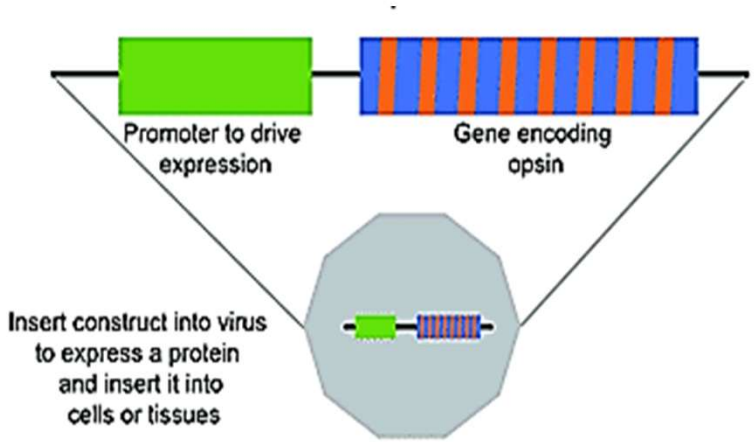
Fear extinction procedure

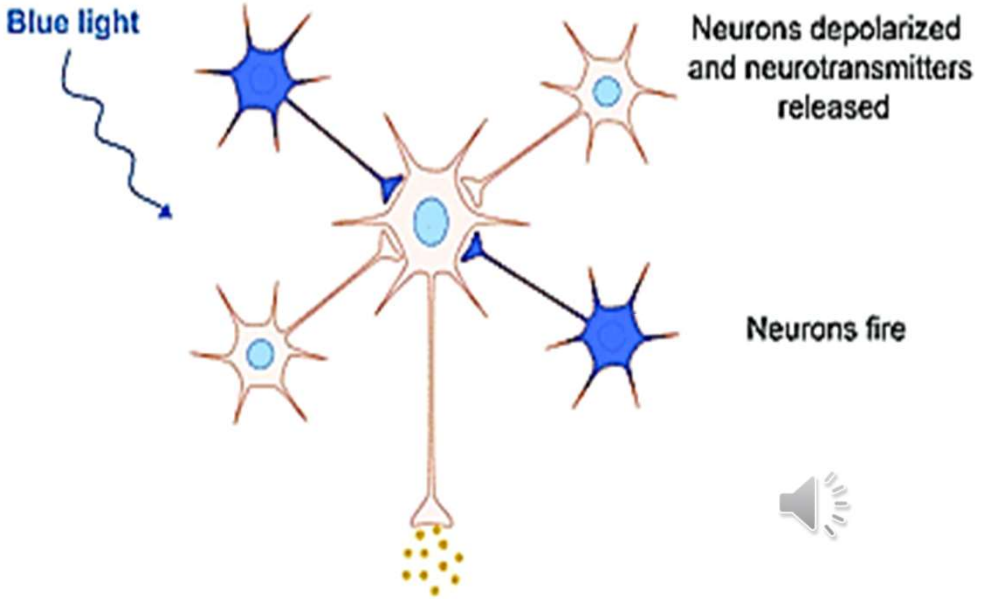
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the lateral amygdala to create ‘false’ memory ?

OPTOGENETICS: The light activated channelrhodopsin (ChR2) is transfected through viral infection to activate a specific cell population.



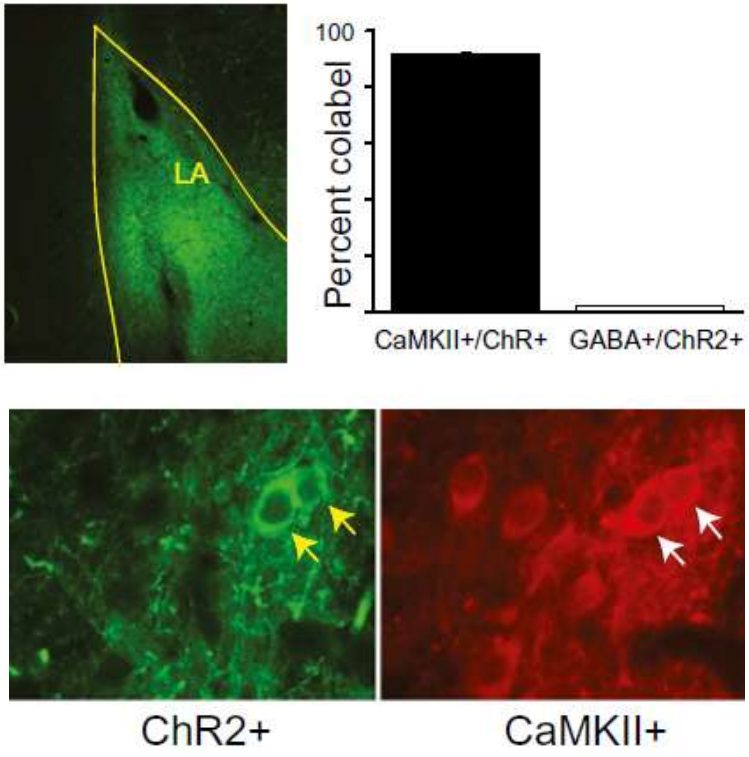
sensory photoreceptors (cloned from unicellular green algae)

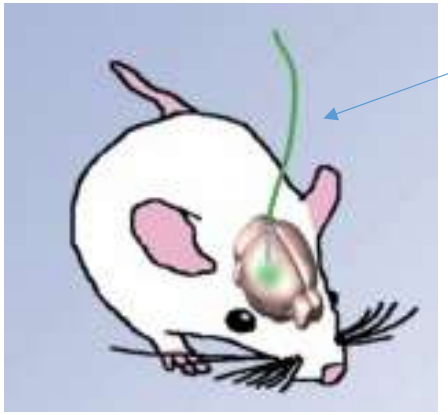




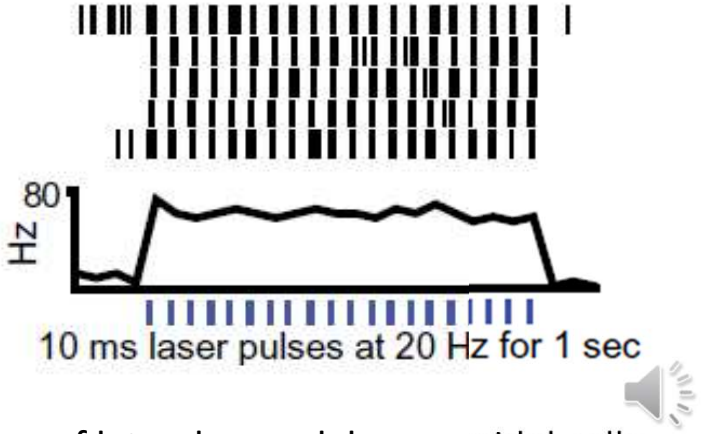
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the AMYGDALA to create 'false' memory ?

LA pyramidal neurons were transfected in vivo with a fusion protein of ChR2 and green fluorescent protein (GFP) expressed under the promoter of CaMKII.





Optical fiber + recording electrodes cable



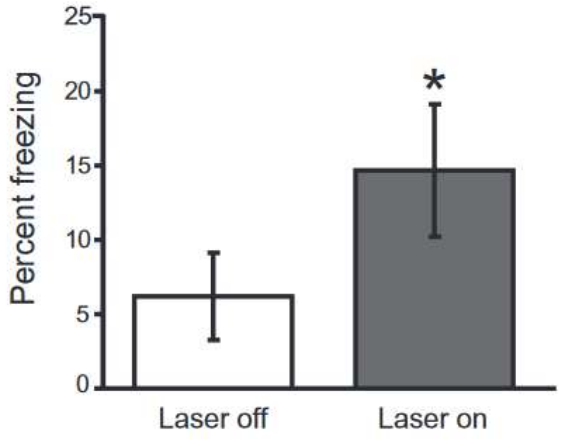
Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Johansen et al, PNAS, 2010

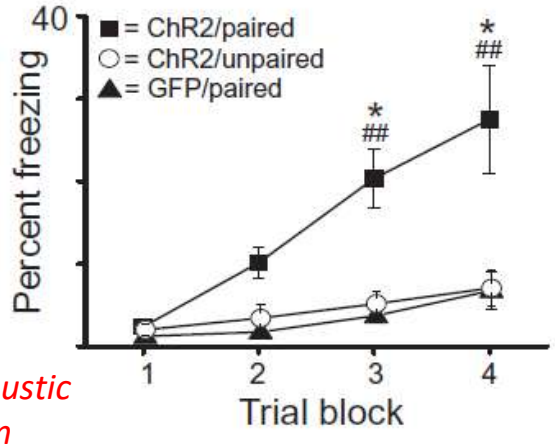
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the lateral amygdala to create 'false' memory ?

Behavioural tests of fear learning

Acoustic tone (CS) paired with optical stimulation of infected LA neurons

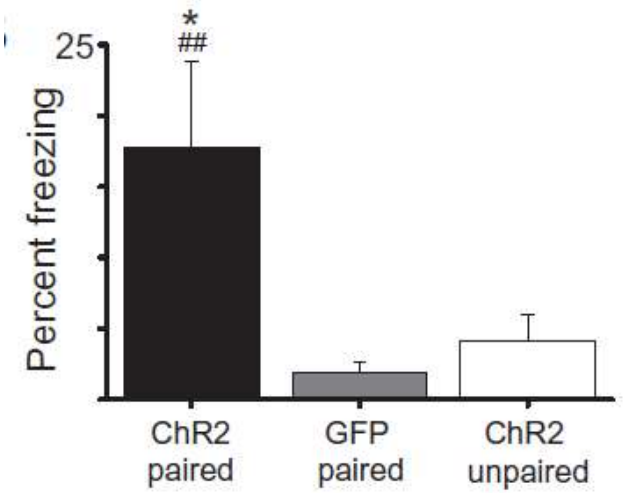
During training





Measured during acoustic stimulation

Long term memory (measured after 24 hours)



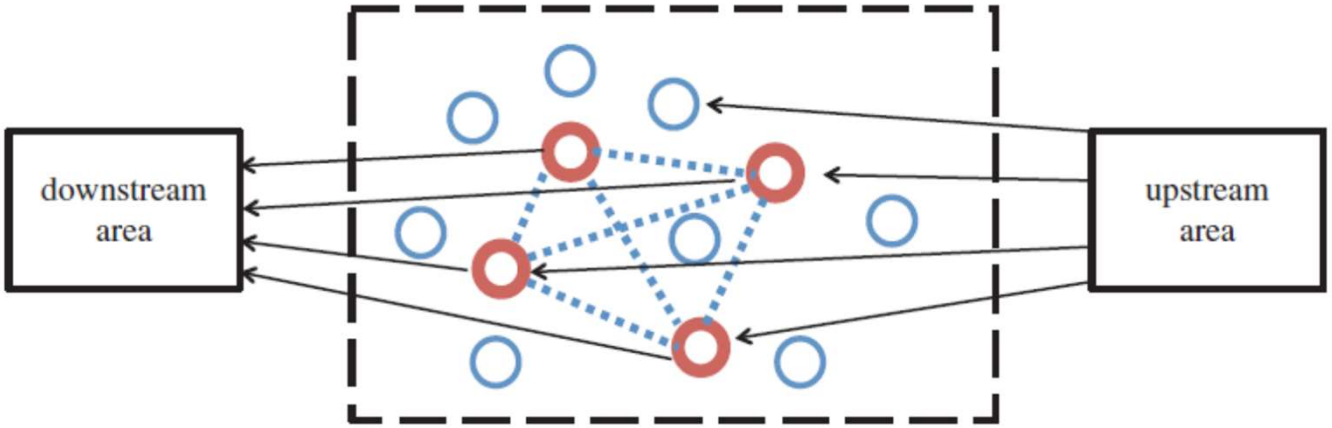
Optogenetic tools allow to instruct plastic changes that result in learned behaviour

Johansen et al, PNAS, 2010

What about **MIMICRY** in the hippocampus?

'Engram' theory of memory: An experience activates a subset of cells that undergo persistent chemical and/or physical changes to become an engram. Subsequent reactivation of this engram induces memory retrieval.

Richard Semon, 1904

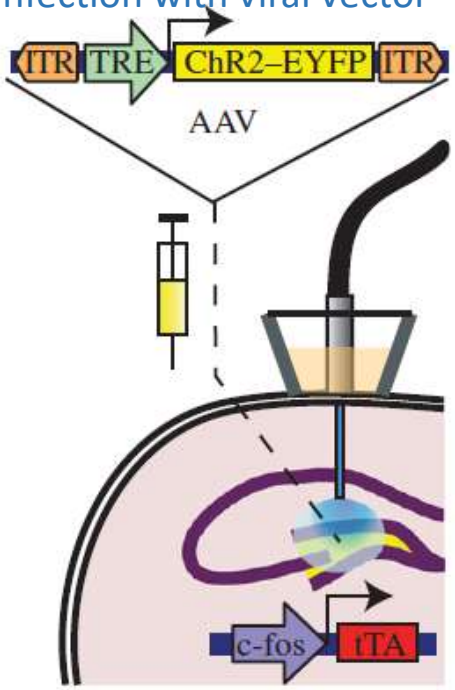


- The subpopulation of neurons that are active during the generation of a memory and whose activation can induce a recall of that memory (ENGRAM) is more sparse in the hippocampus

Is it possible to artificially potentiate excitatory synapses of the HIPPOCAMPUS to create a 'false' memory ?

Neurons that were active during memory task expressed channelrhodopsin and could be artificially re-activated through an implanted optical fiber

Infection with viral vector

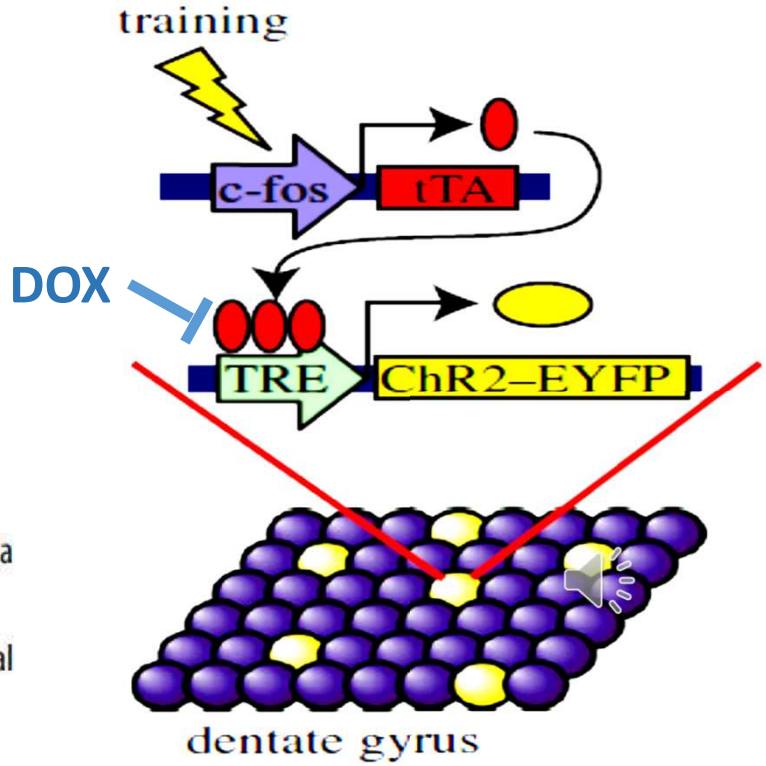


- ✓ **tetracycline-responsive element** (TRE) site (PROMOTER)
- ✓ **channelrhodopsin-2** (ChR2)–enhanced yellow fluorescent protein (EYFP)

c-fos–tTA transgenic mouse

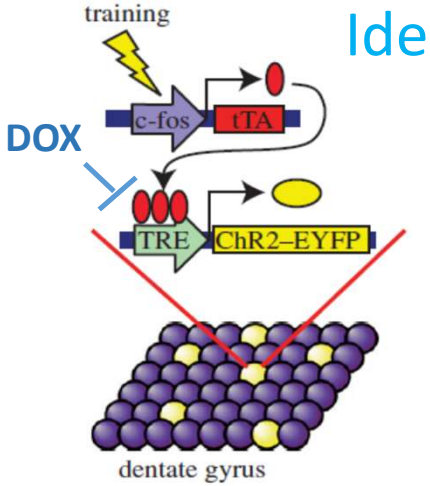
- ✓ c-fos (PROMOTER): a member of immediately early genes, marker of the recent neuronal activity
- ✓ (tTA): **tetracycline transactivator**

In the presence of doxycycline (DOX), that binds **TRE,** ChR2-EYFP is not expressed

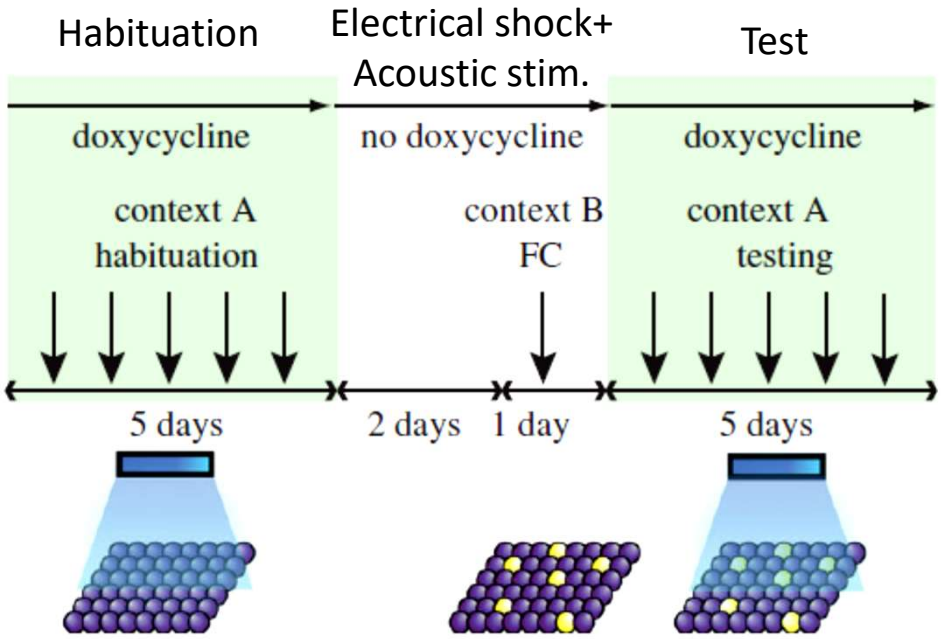


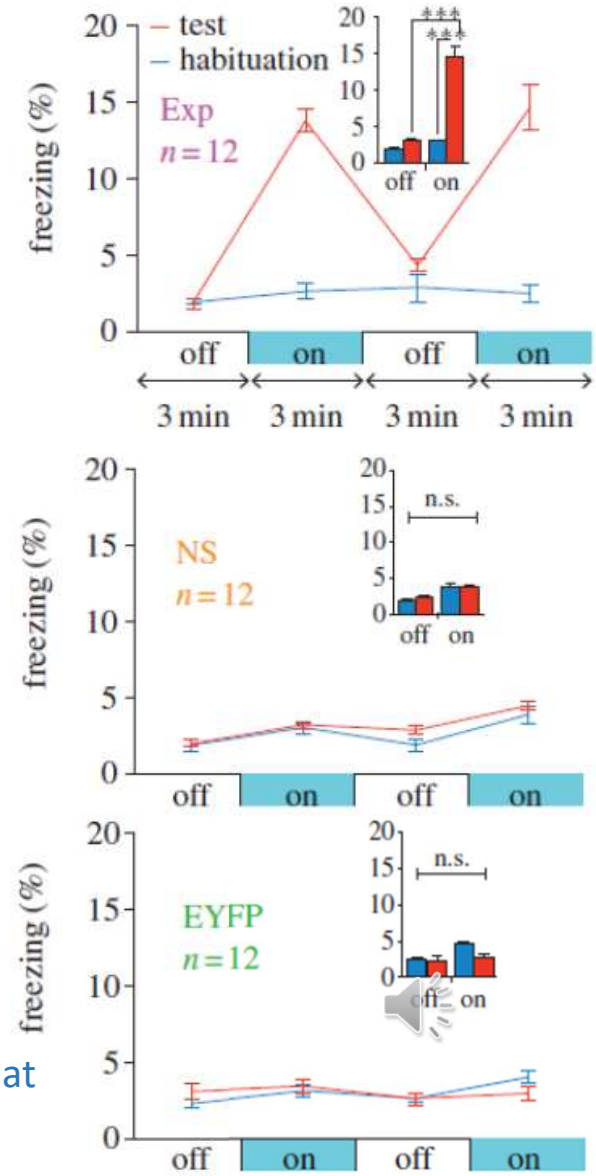
Liu X, Ramirez S, Tonegawa S. 2014 Inception of a false memory by optogenetic manipulation of a hippocampal memory engram. *Phil. Trans. R. Soc. B* **369**: 20130142.

Identification of memory engrams in the DG

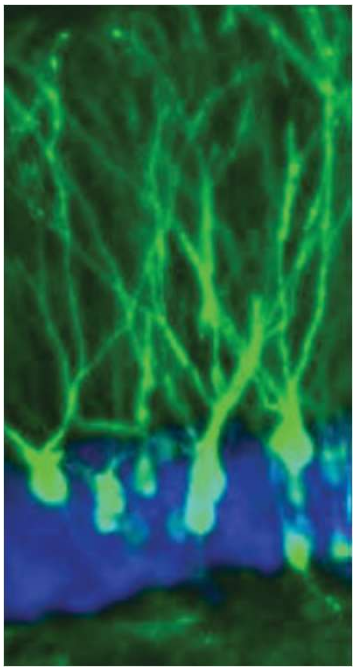


Mice were habituated in context A with light stimulation while on Dox for 5 days, then taken off Dox for 2 days and fear conditioned (FC) in context B. Mice were put back on Dox and tested for 5 days in context A with light stimulation.





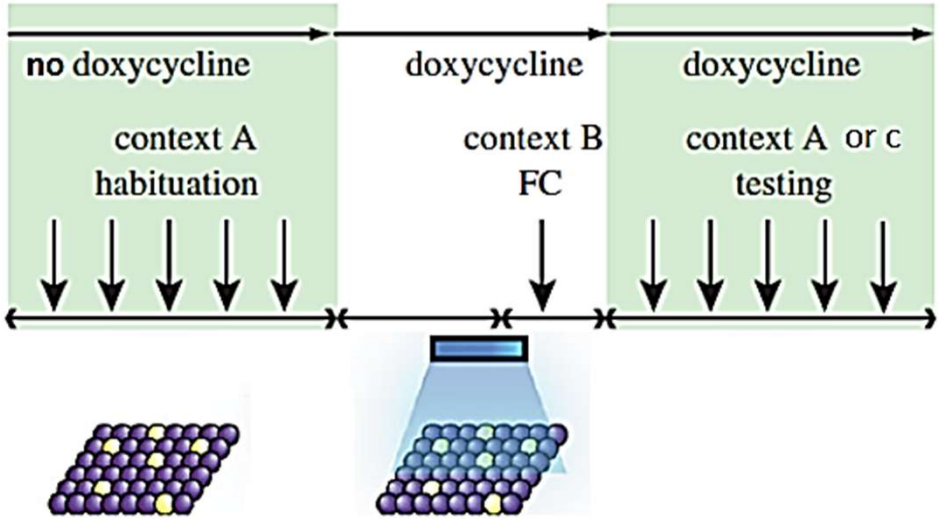
DG Granule cells expressing ChR2-EYFP



DG cells that express endogenous c-Fos during training, and therefore become labelled by ChR2–EYFP, define an active neural population that is sufficient for memory recall upon subsequent reactivation

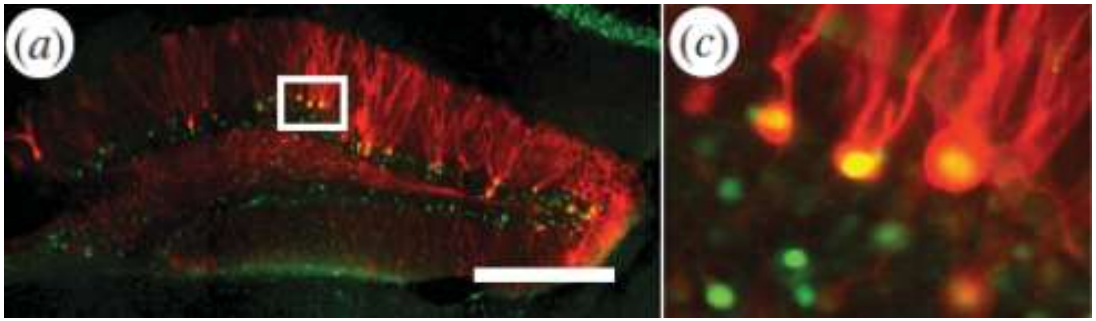
Inception of a false memory

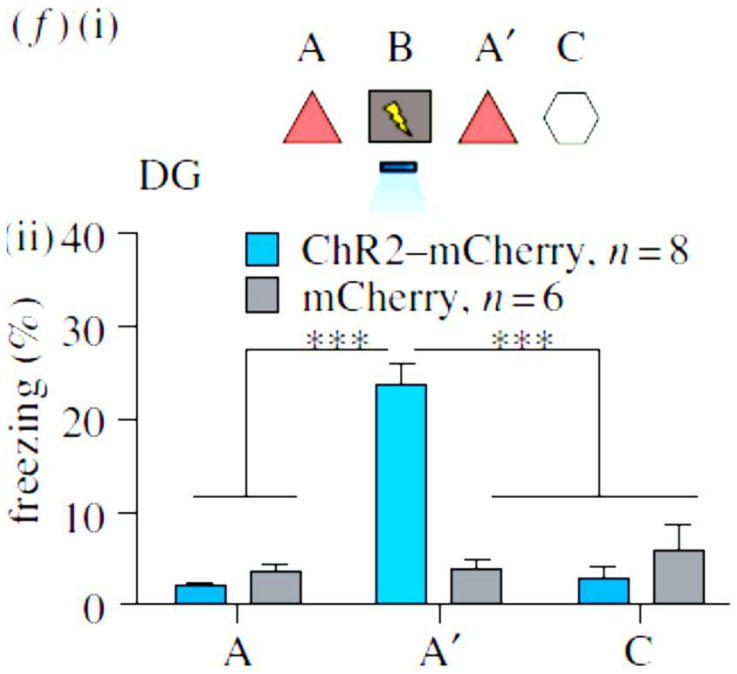
c-fos–tTA mice injected with AAV_9-TRE-ChR2-mCherry in the DG



Electrical shock+

Optogenetic stimulation of cells associated with context A





The artificial reactivation of DG cells previously activated by exposure to context A can serve as a functional CS during fear conditioning in a distinct context B and results in the formation of a false memory and related behaviour.