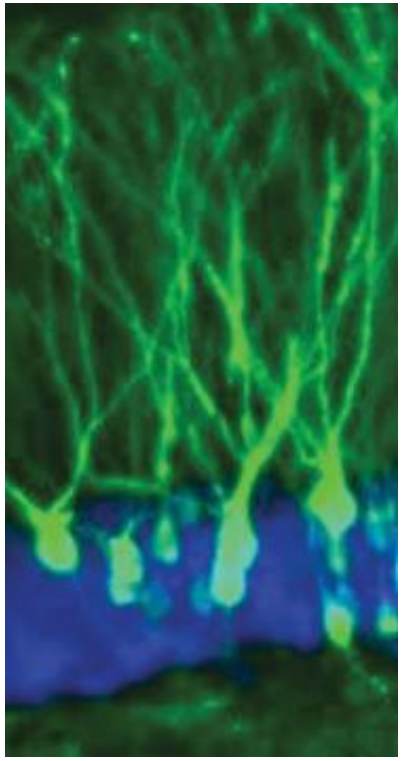
Master in Neuroscience

MOLECULAR NEUROPHYSIOLOGY

-lesson 2-



Prof. Giada Cellot

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Synaptic plasticity and memory (SPM) hypothesis

Synaptic plasticity

- Experience dependent modification in strength of neuronal communication relying on synaptic changes.
- Short/long term modifications
- LTP/LTD
- NMDA dependent/independent phenomena

Memory

- Experience dependent changes in BEHAVIOUR or the stored knowledge upon which changes in behavior are dependent.

Activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed

Martin & Morris, Hippocampus, 2002

Four criteria to validate the SPM hypothesis

DETECTABILITY: if learning involves activity-dependent synaptic plasticity, it should be possible to detect changes in synaptic efficacy following learning

ANTEROGRADE ALTERATION: Interventions that prevent the induction of synaptic efficacy changes during a learning experience should impair the animal's memory of that experience.

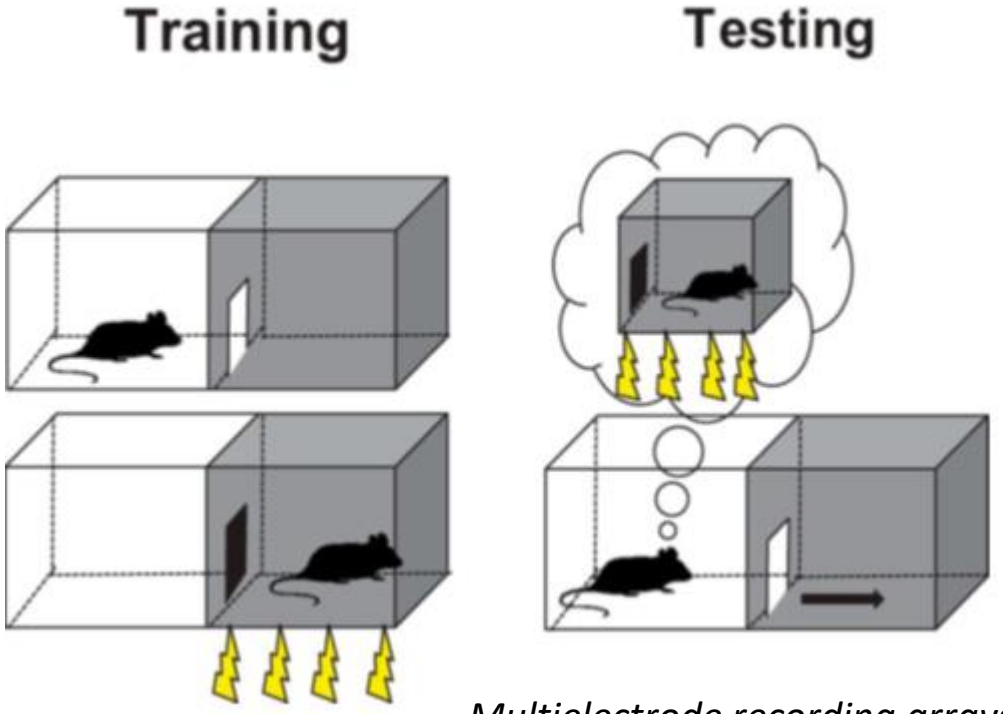
RETROGRADE ALTERATION: Interventions that alter the spatial distribution of synaptic efficacy changes induced by a prior learning experience should alter the animal's memory of that experience.

MIMICRY: if memory resides in specific distributed patterns of altered synaptic weights, the artificial creation of such a pattern should result in the creation of a 'false memory' for an event that did not happen or some aspect of knowledge or skill that had not been taught or trained.

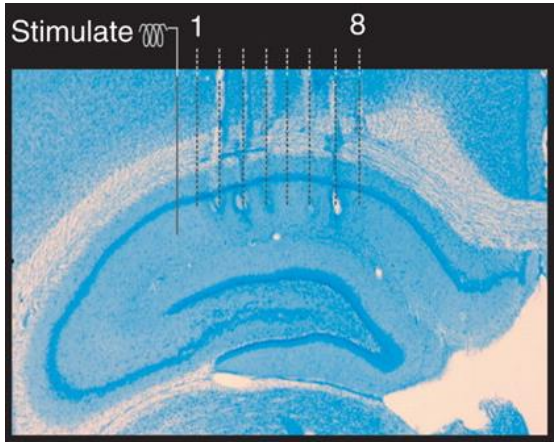
Martin & Morris, Hippocampus, 2002

DETECTABILITY: if learning involves activity-dependent synaptic plasticity, it should be possible to detect changes in synaptic efficacy following learning

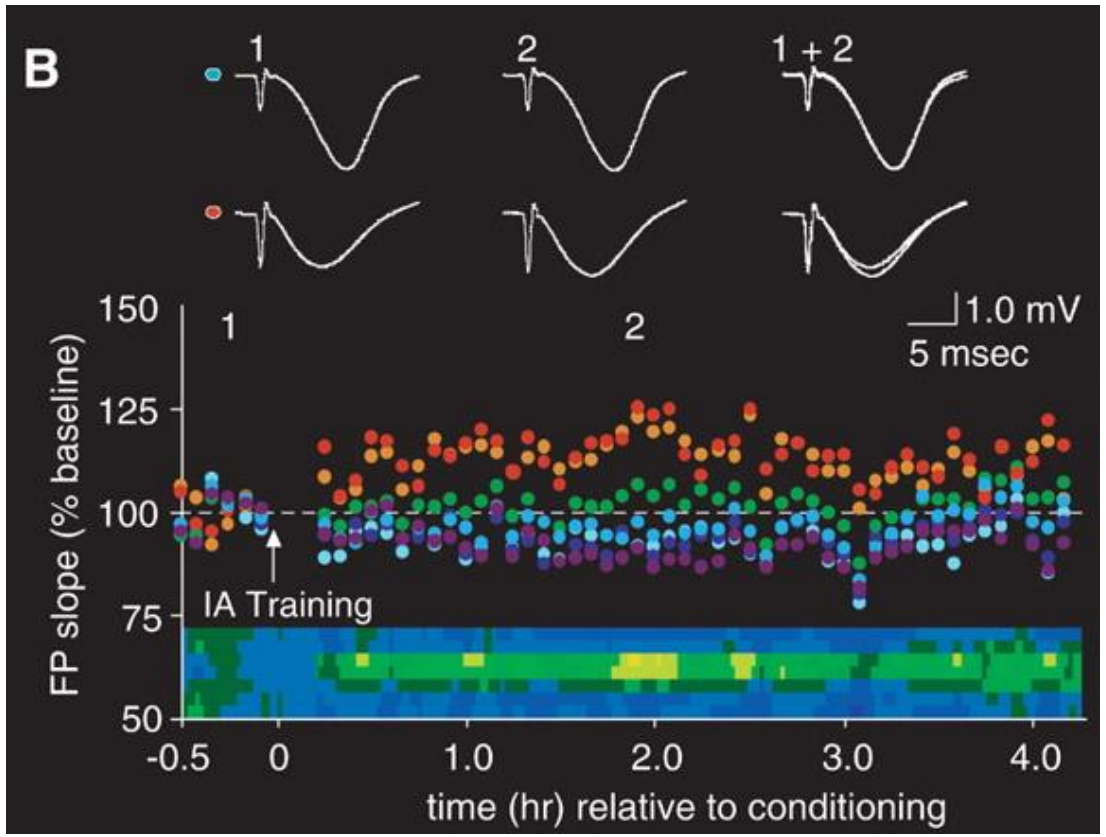
Inhibitory avoidance (IA) apparatus



Multielectrode recording arrays



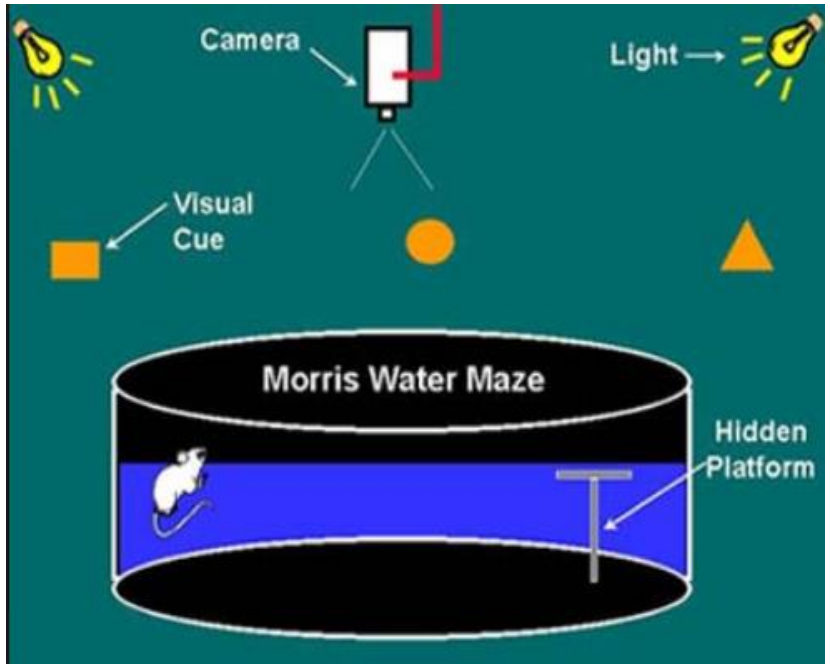
Whitlock JR, Heynen AJ, Shuler MG, Bear MF. 2006 Learning induces long-term potentiation in the hippocampus. *Science* **313**, 1093–1097. (doi:10.1126/science.1128134)

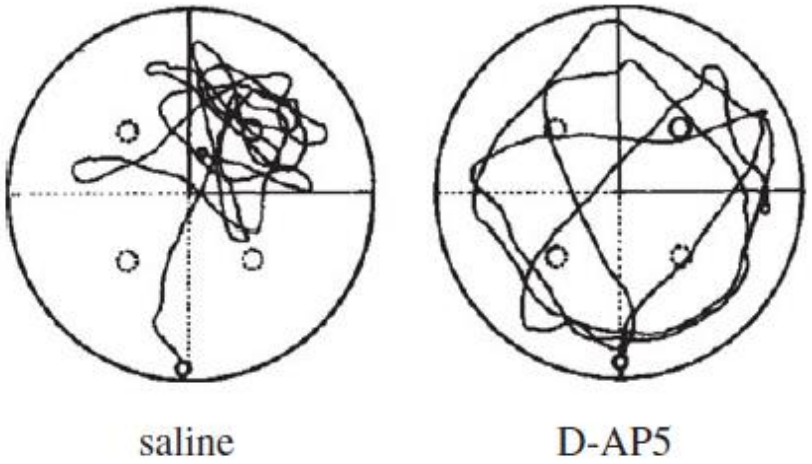


- After AI training, 2 out of 8 electrodes implanted in the CA1 region could detect an increase in the FP amplitude

ANTEROGRADE ALTERATION: Interventions that prevent the induction of synaptic efficacy changes during a learning experience should impair the animal's memory of that experience.

Morris water maze apparatus

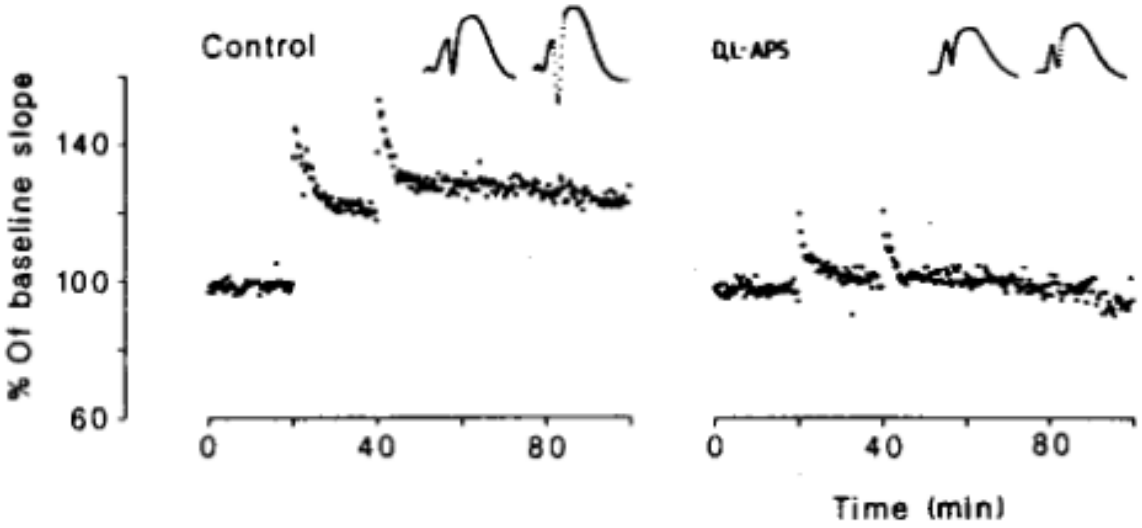




Morris et al., Nature, 1986

Application of NMDA antagonist (AP5) to hippocampus during the training impairs learning in spatial memory task

Perforant path -> Dentate gyrus



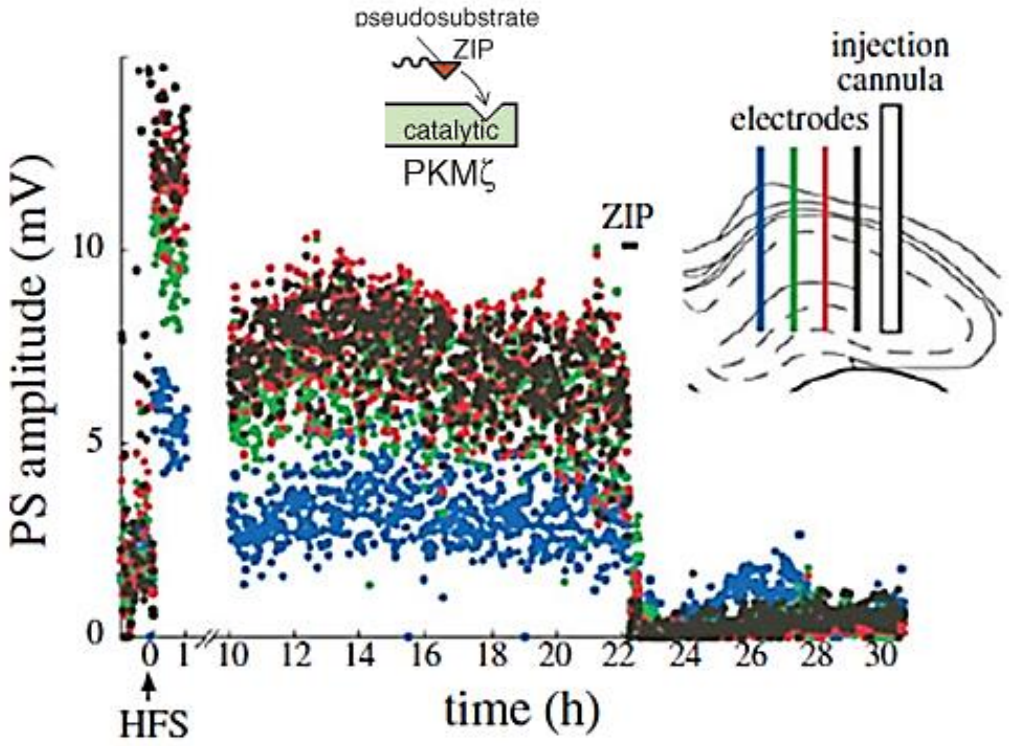
Or

the in vivo LTP induction upon high frequency stimulation of afferent fibers

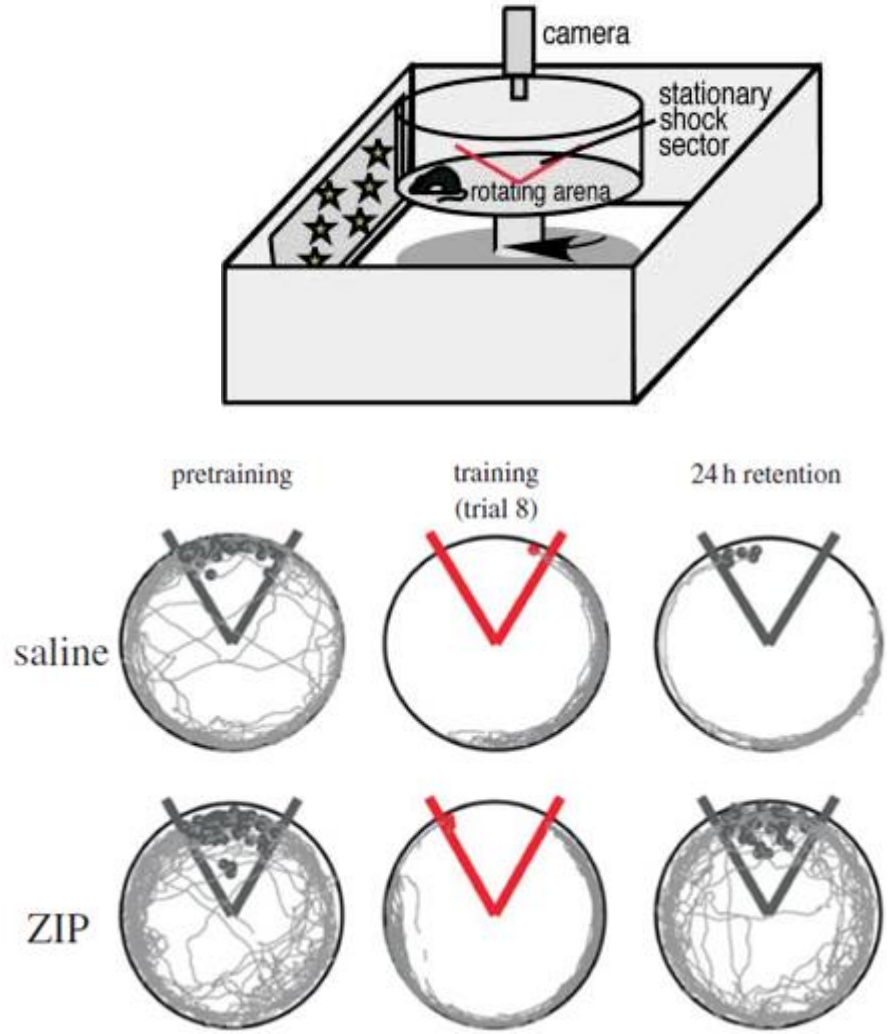
RETROGRADE ALTERATION: Interventions that alter the spatial distribution of synaptic efficacy changes induced by a prior learning experience should alter the animal's memory of that experience.

- The late phase of LTP (LTP maintenance) is supported by the activity of an atypical isoform of PKC: protein kinase Mzeta

1) Can PKMzeta inhibition by ZIP reverse the late phase of LTP in vivo?



Storage of Spatial Information by the Maintenance Mechanism of LTP
Eva Pastalkova, *et al.*
Science **313**, 1141 (2006);
DOI: 10.1126/science.1128657



Place avoidance apparatus

2) If so, does ZIP cause retrograde loss of spatial memory?

MIMICRY:

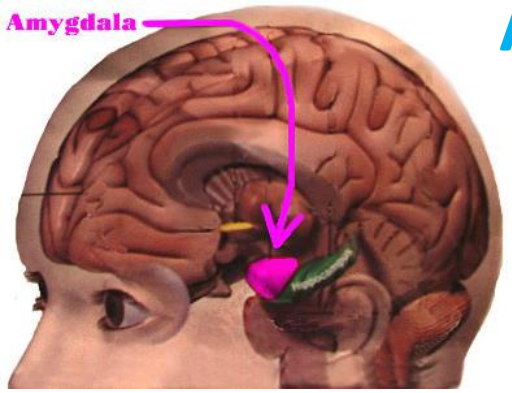
if memory resides in specific distributed patterns of potentiated synaptic contacts, the artificial creation of such a pattern should result in the creation of a ‘false memory’ for an event that did not happen or some aspect of knowledge or skill that had not been taught or trained.

How to artificially potentiate specific synapses?

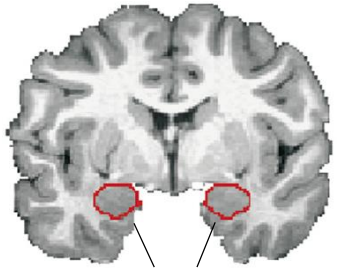
OPTOGENETICS

How to identify synapses undergoing to LTP during learning?

AMYGDALA



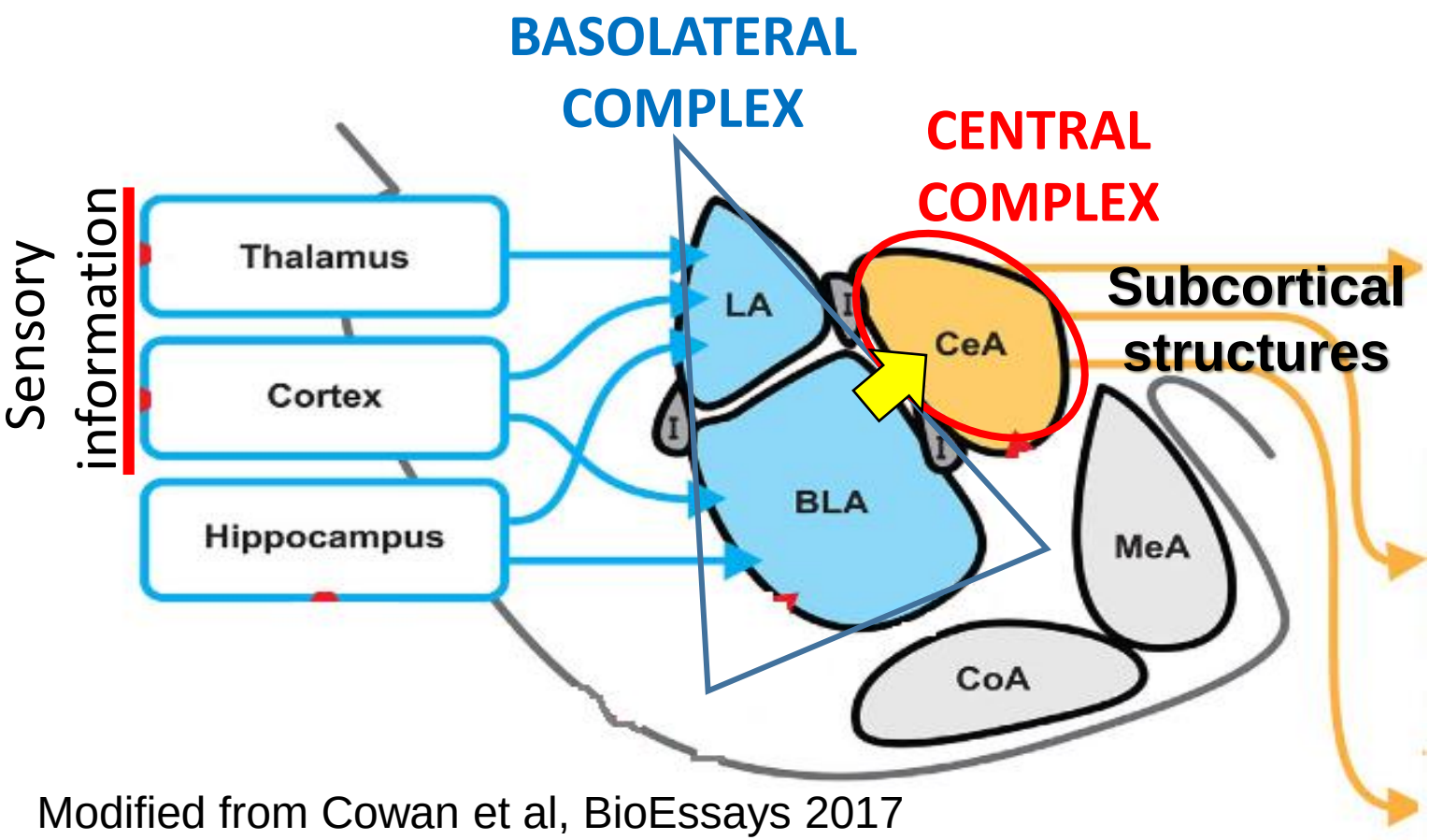
AMYGDALA



Amygdalae

- critical component of the LYMBIC SYSTEM, it plays a key role in emotions

- two almond-shaped clusters of nuclei located deep and medially within the temporal lobes of the brain in vertebrates.

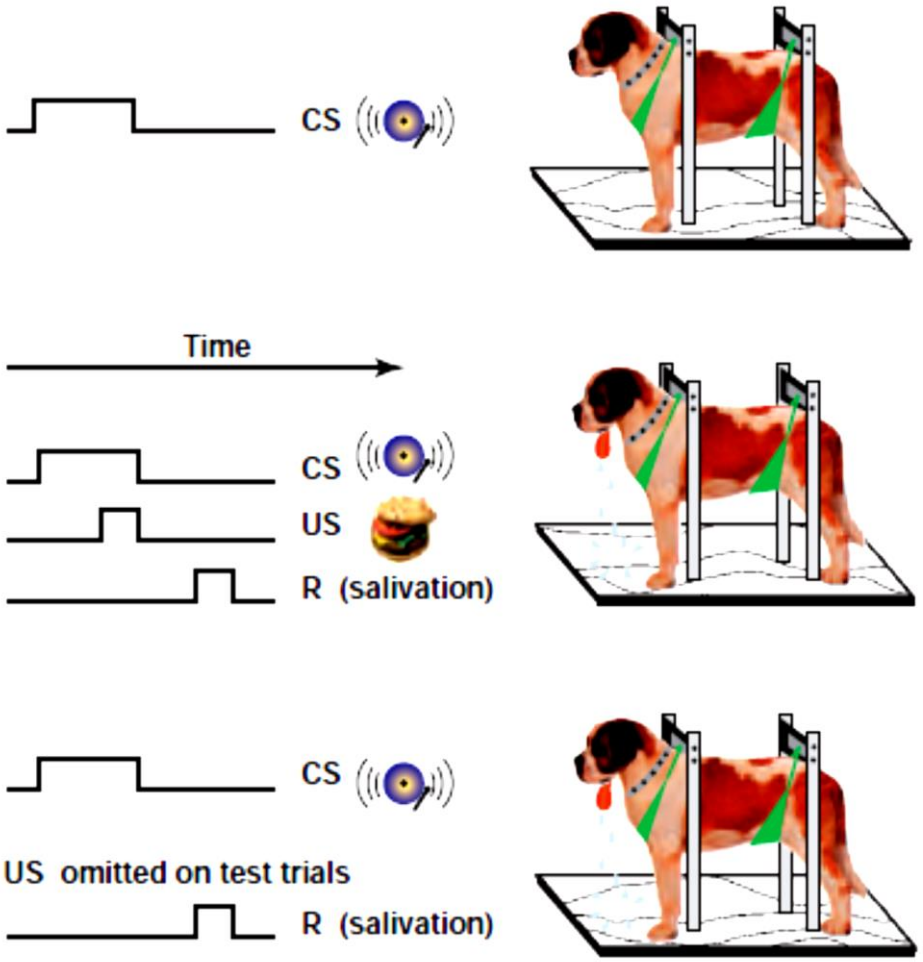


EXPRESSION OF THE EMOTIONS

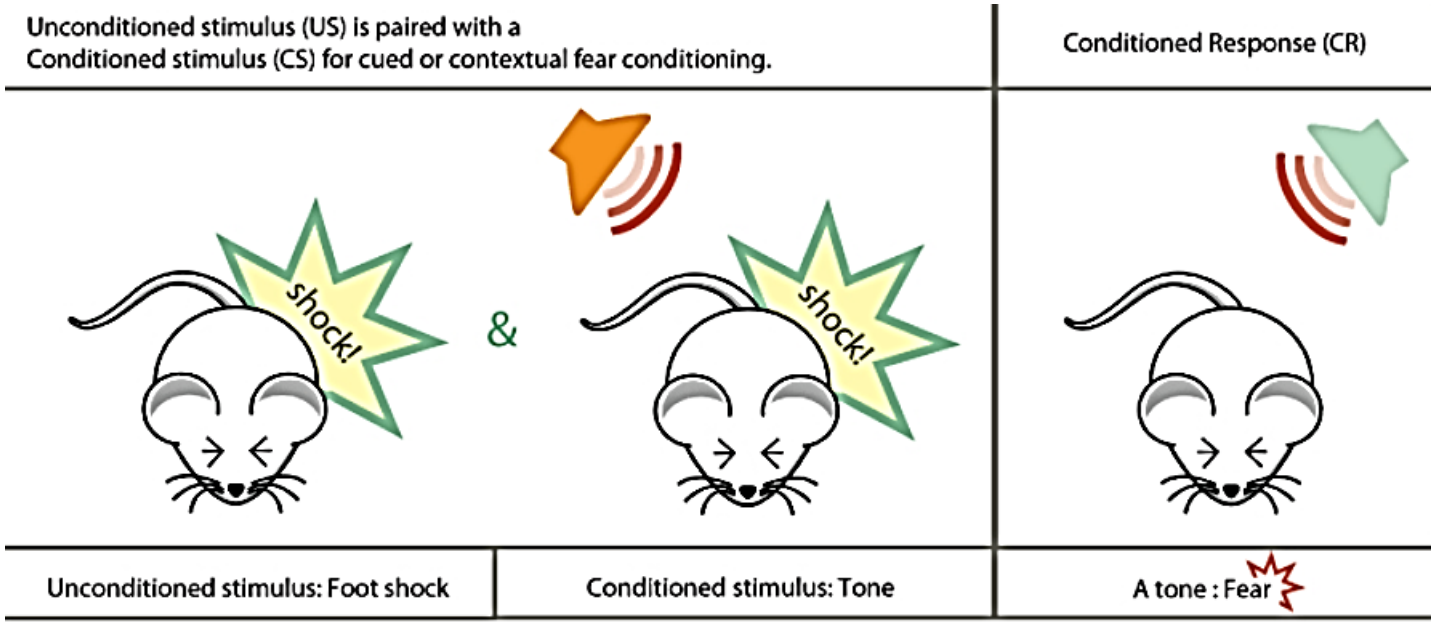
Modified from Cowan et al, BioEssays 2017

Behavioral correlates of amygdala synaptic plasticity

FEAR CONDITIONING: a form of **associative learning** in which the subject comes to express fear responses to a neutral conditioned stimulus (CS) that was previously paired with an aversive unconditioned stimulus (US).



The classical conditioning of Pavlov (modified from Rachlin, 1991)

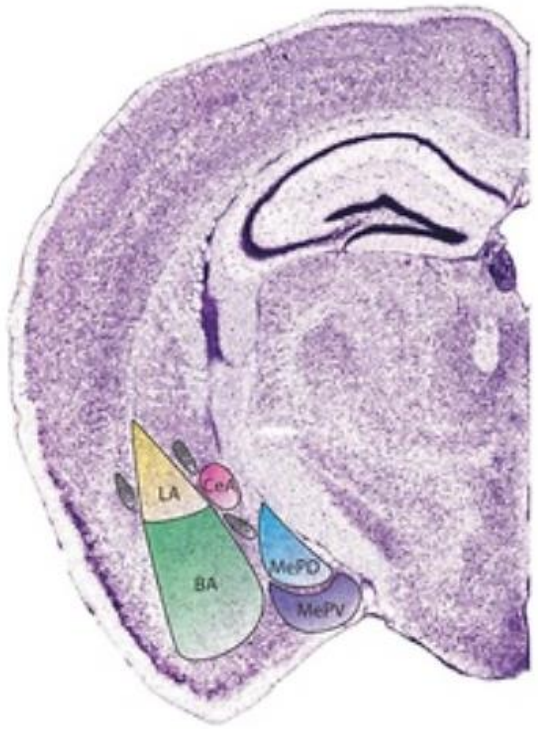


As result of the pairing, the CS acquires the capacity to elicit behavioral, autonomic and endocrine responses

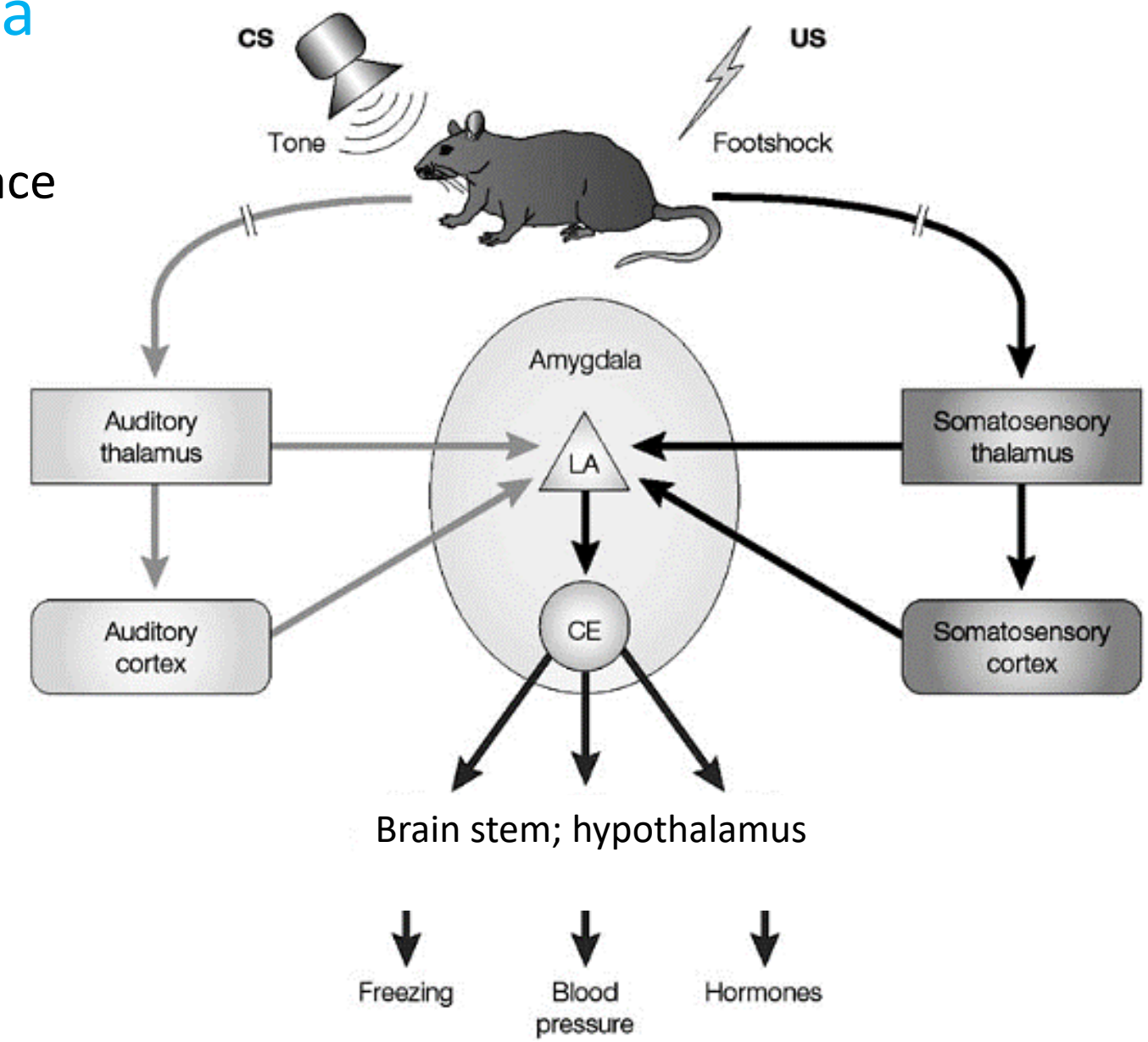
The US is able to induce modifications in the brain on how CS is processed

Synaptic plasticity in the amygdala

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



Disruption of LA prevents fear conditioning



Defensive responses + autonomic and endocrine "setting"

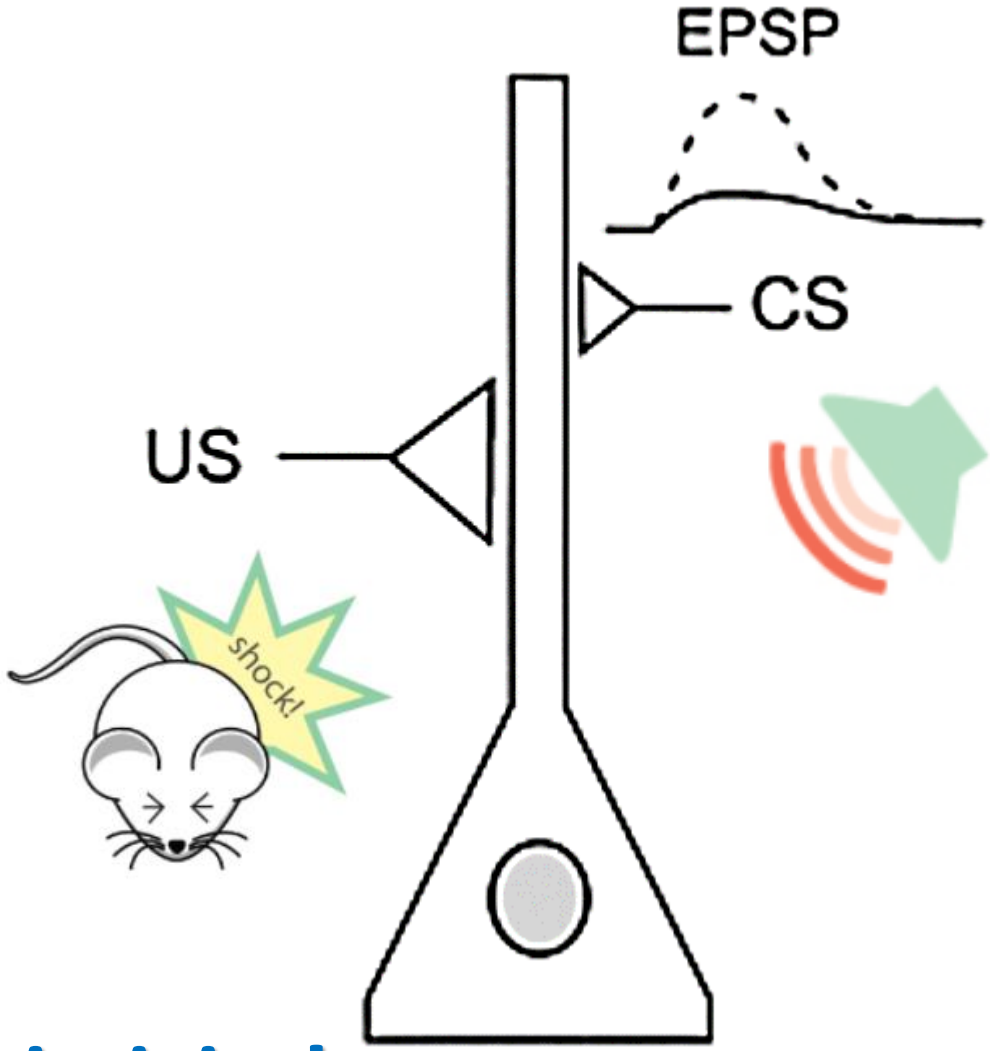
Sigurdsson et al., Neuropharmacology, 2006

A cellular hypothesis for associative fear conditioning

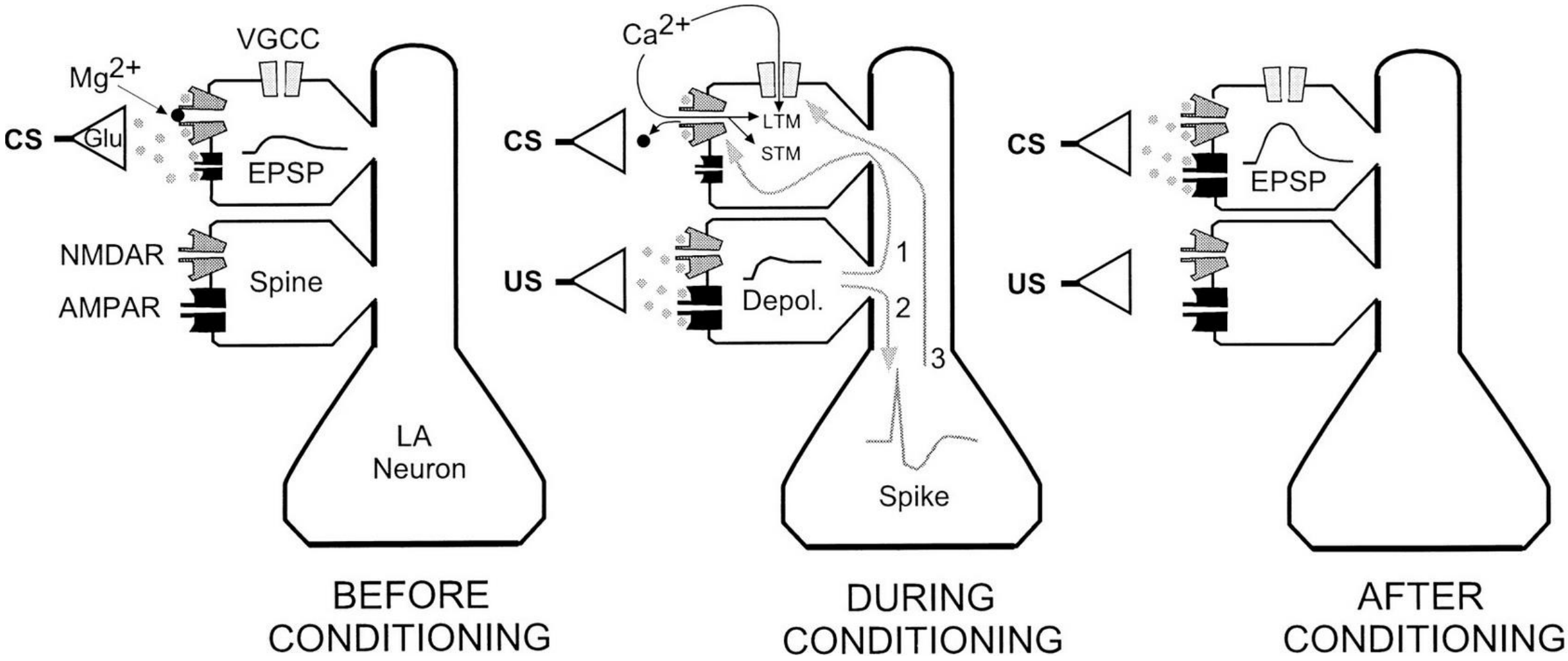
Fear conditioning is mediated by an increase in the strength of synapses that transmit CS information to principal neurons in the LA

- Prior to conditioning, the CS inputs are relatively weak and as a result the CS is unable to elicit fear responses. In contrast, the US inputs are stronger and capable of eliciting robust responses in LA neurons.

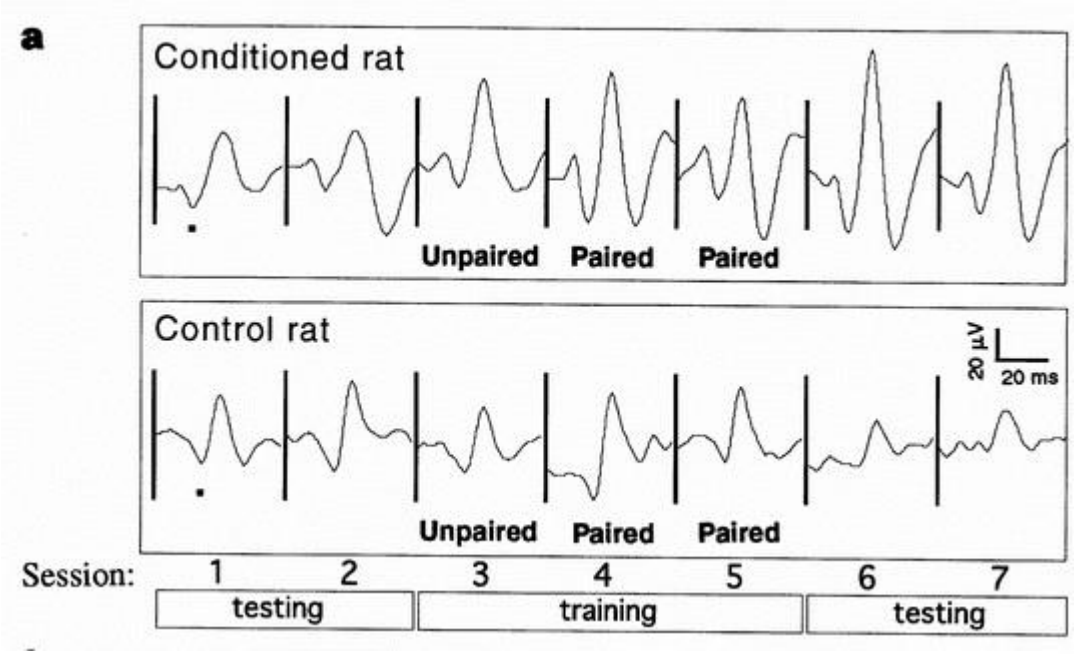
- During fear conditioning the CS inputs are active during strong postsynaptic depolarization caused by the US. As a result, the CS inputs become potentiated, making the CS more effective at driving LA neurons, which in turn can drive downstream structures that control fear responses

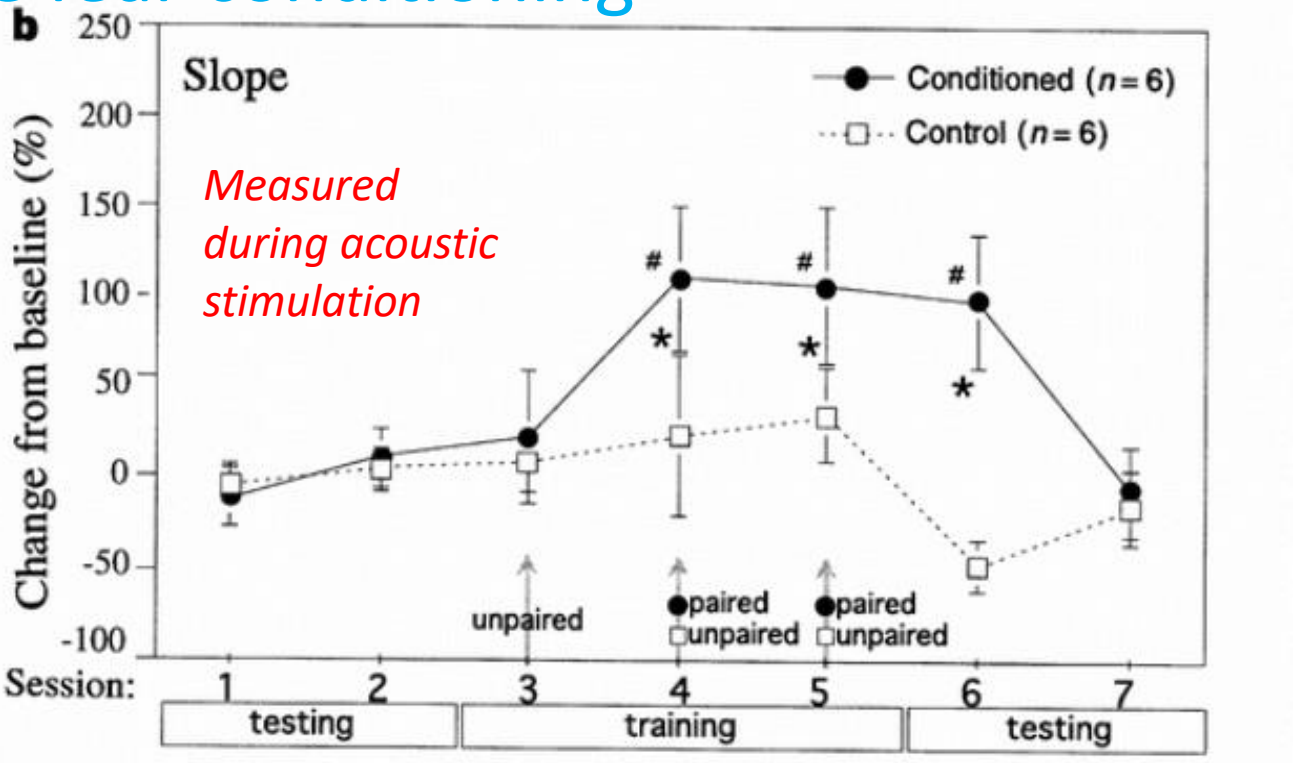


A property of LTP is associativity!

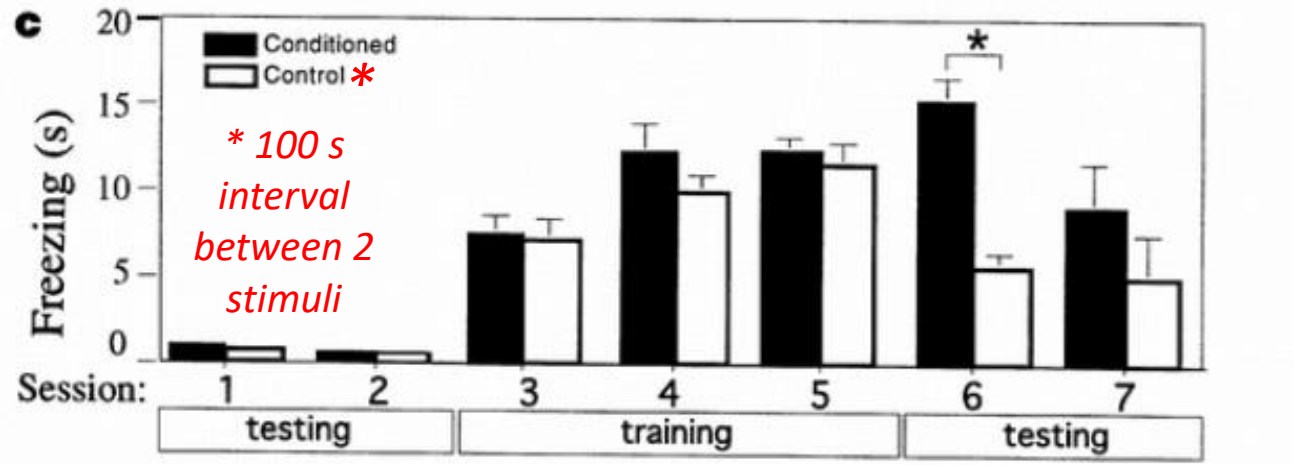

VGCC
Mg2+
CS
Glu
EPSP
NMDAR
AMPAR
Spine
LA
Neuron
BEFORE
CONDITIONING
Ca2+
LTM
STM
CS
US
Depol.
1
2
3
Spike
DURING
CONDITIONING
CS
US
EPSP
AFTER
CONDITIONING

A cellular hypothesis for associative fear conditioning





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

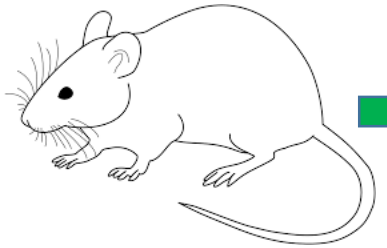


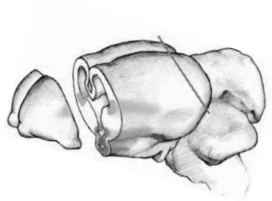
Fear conditioning induces associative long-term potentiation in the amygdala
Rogan et al., Nature, 1997

Acoustic stimulus only

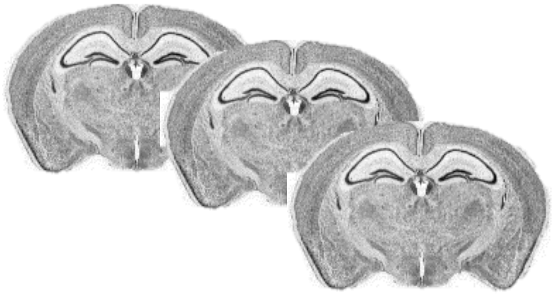
Acoustic stimulus only

Amygdalar acute slices: a simplified in vitro model of amygdala

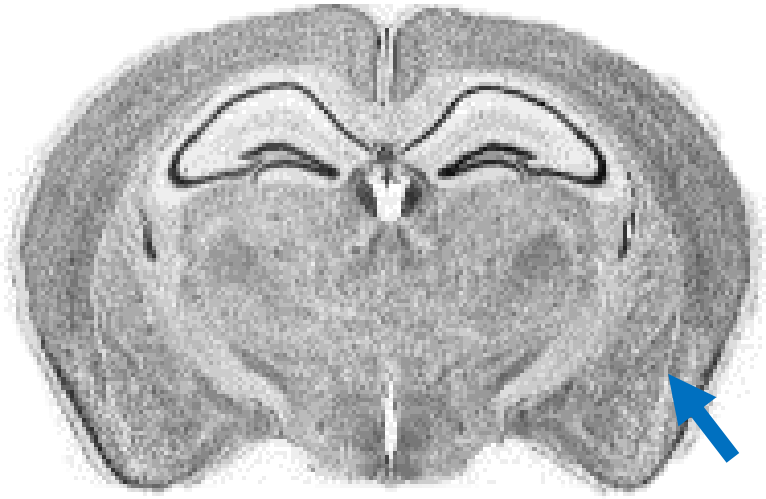


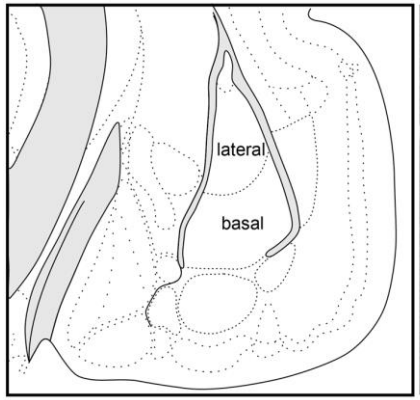


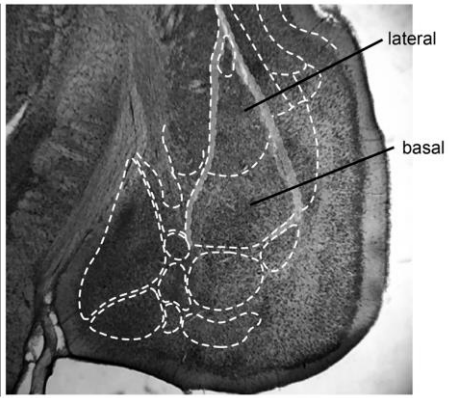
BRAIN IS SECTIONED
IN CORONAL SLICES

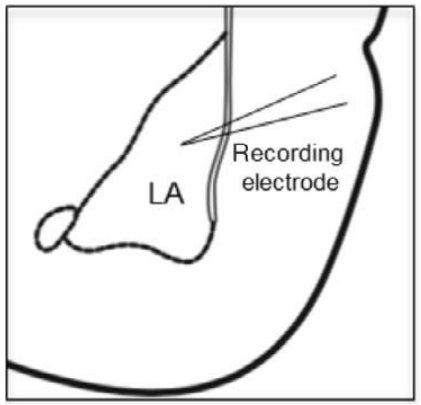


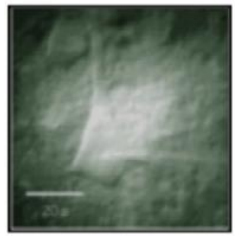
ELECTROPHYSIOLOGICAL
ANALYSIS OF
GLUTAMATERGIC
POSTSYNAPTIC CURRENTS







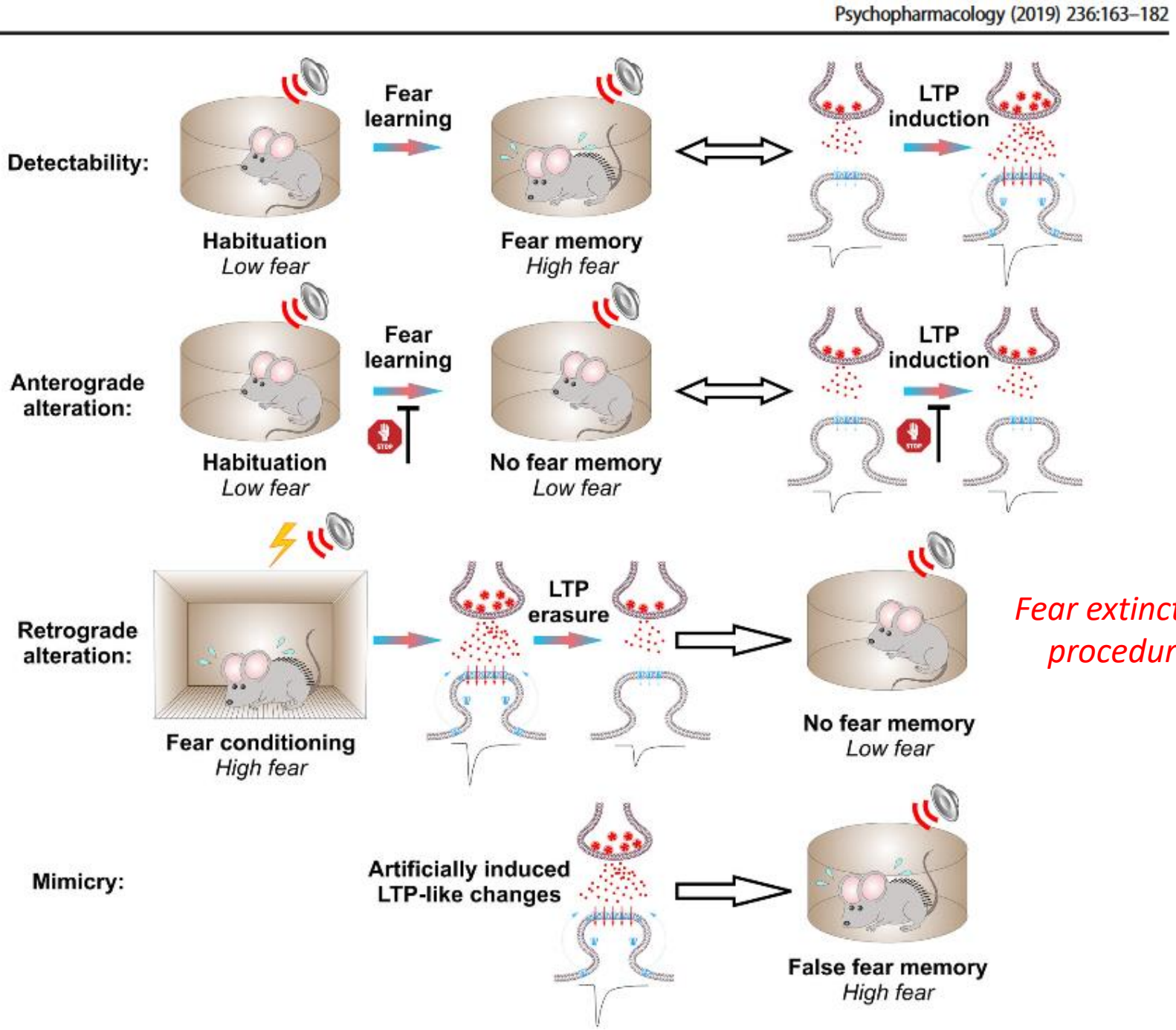




- LA neurons are innervated by glutamatergic synapses, whose afferent fibers come from the thalamus and cortex
- LA neurons expressed both AMPA and NMDA receptors
- LTP can be induced by high frequency stimulation of afferent fibers
- It is prevented by application of NMDAr antagonists: HEBBIAN LTP

The SPM hypothesis applied to amygdala circuitry

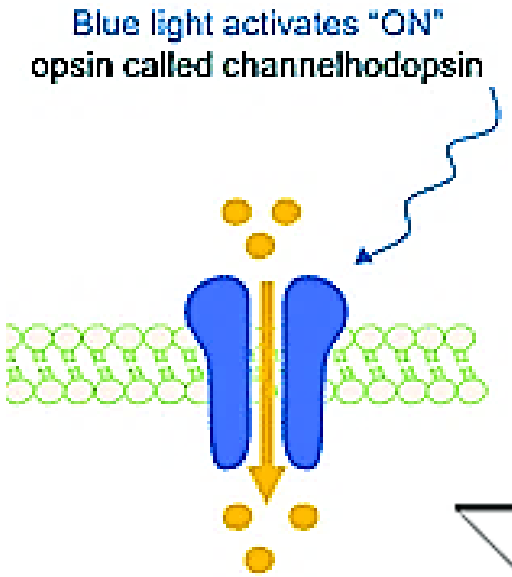
Mimicry in the amygdala is potentially easier to induce as the circuits underlying associative learning are localized in a specific nucleus (LA)



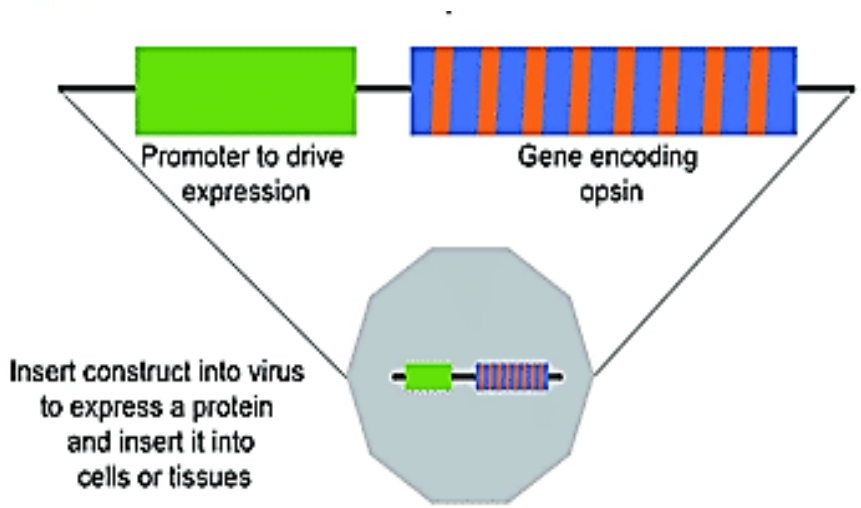
Fear extinction procedure

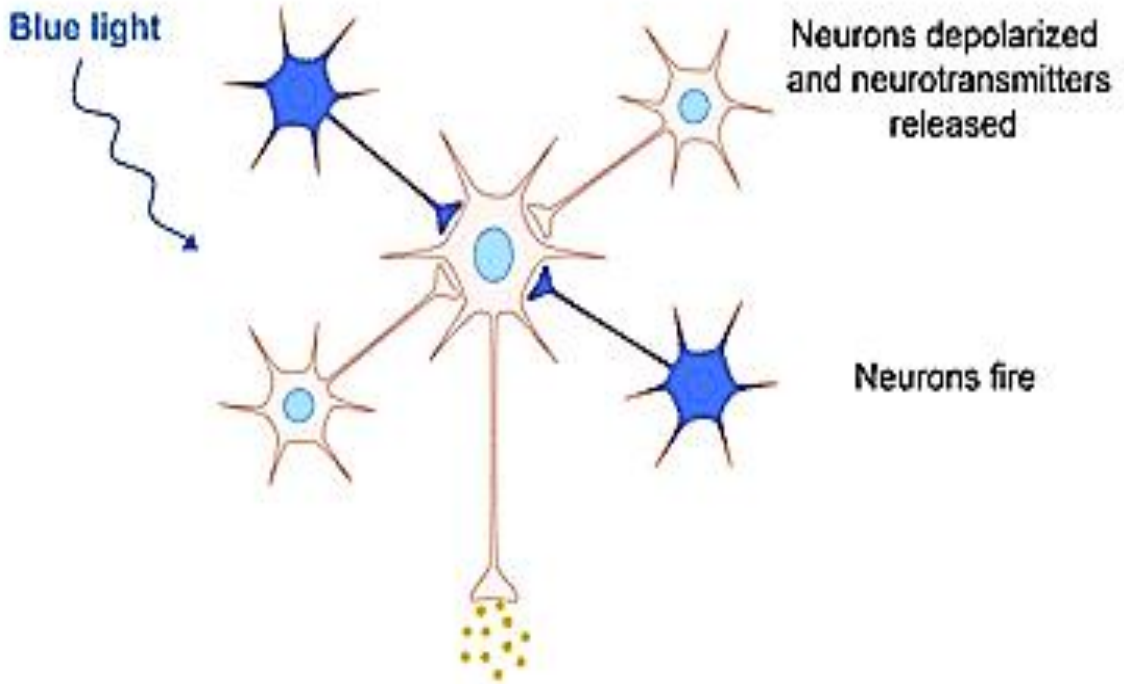
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the lateral amygdala to create ‘false’ memory ?

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



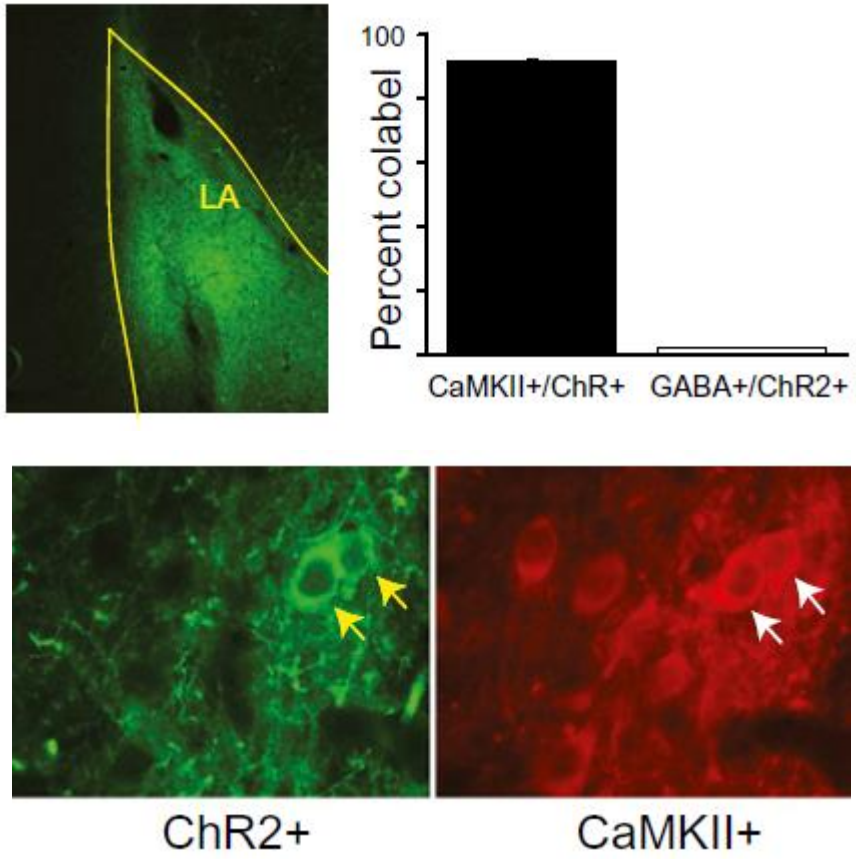
sensory photoreceptors (cloned from unicellular green algae)

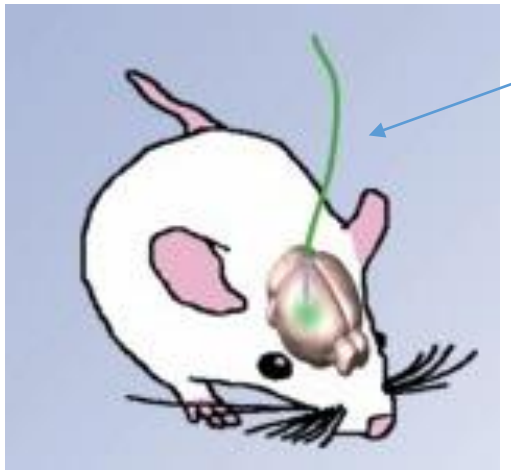




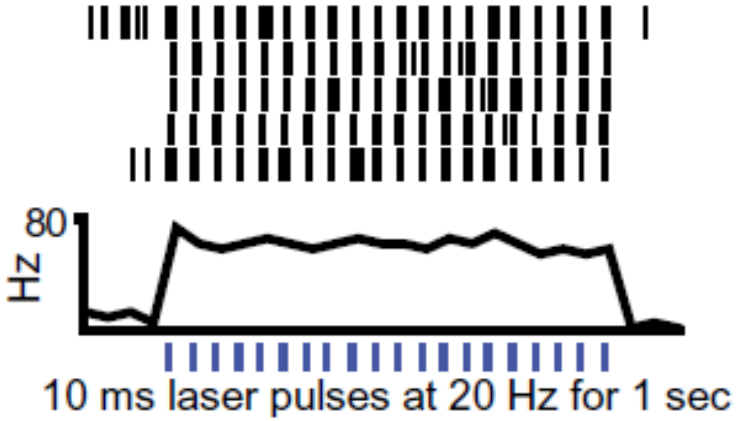
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the AMYGDALA to create 'false' memory ?

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

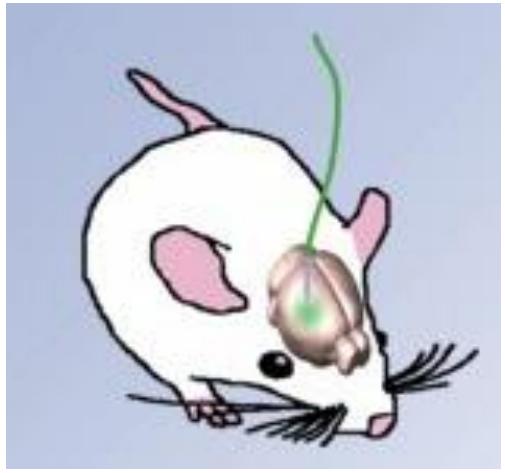


Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Johansen et al, PNAS, 2010

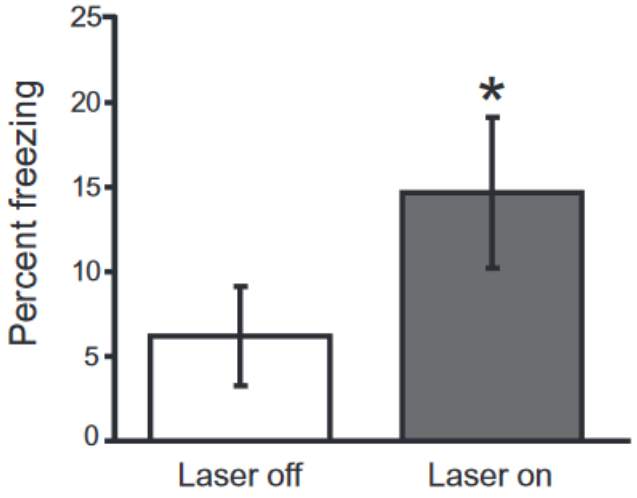
MIMICRY: Is it possible to artificially potentiate excitatory synapses of the lateral amygdala to create 'false' memory ?

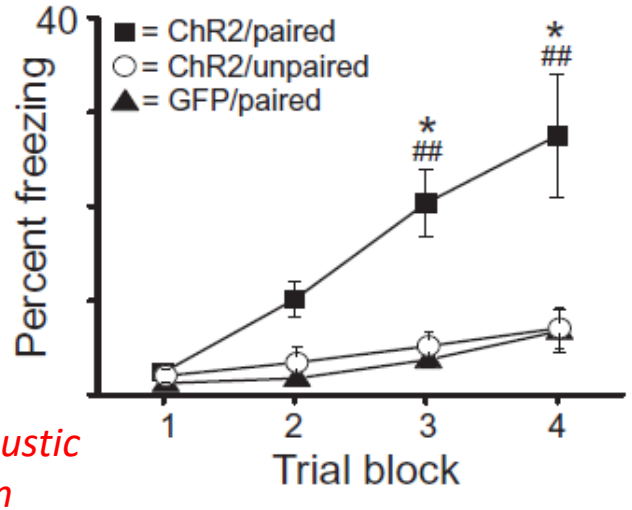
Behavioural tests of fear learning

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



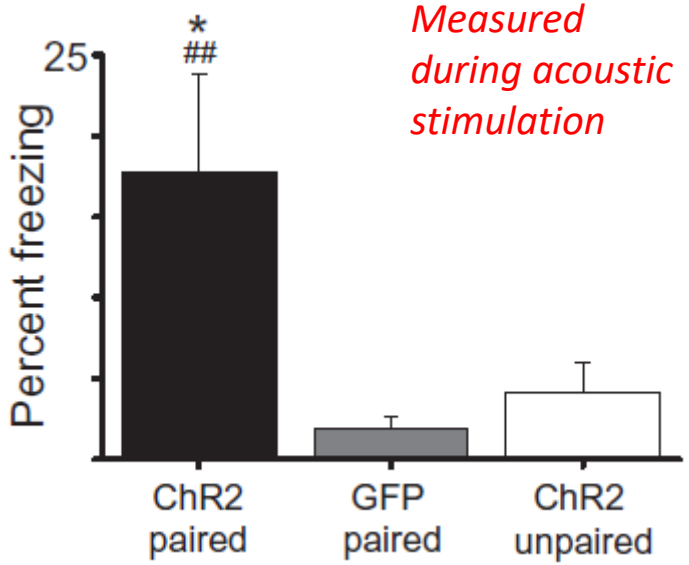
During training





Measured during acoustic stimulation

Long term memory (measured after 24 hours)



Measured during acoustic stimulation

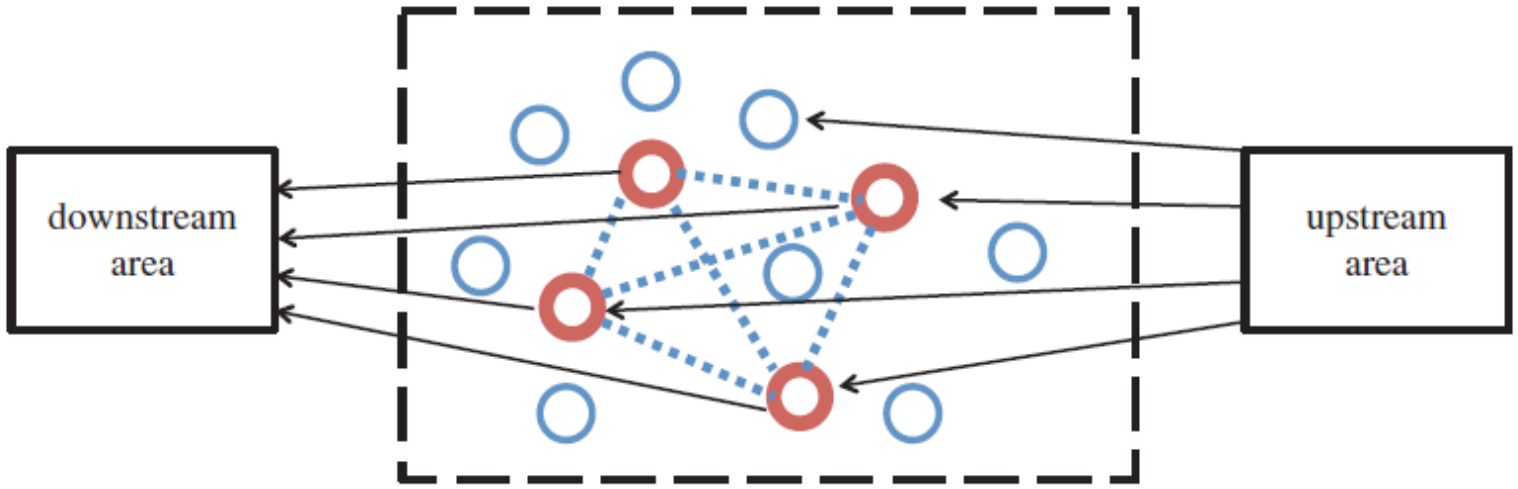
Optogenetic tools allow to instruct plastic changes that result in learned behaviour

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.

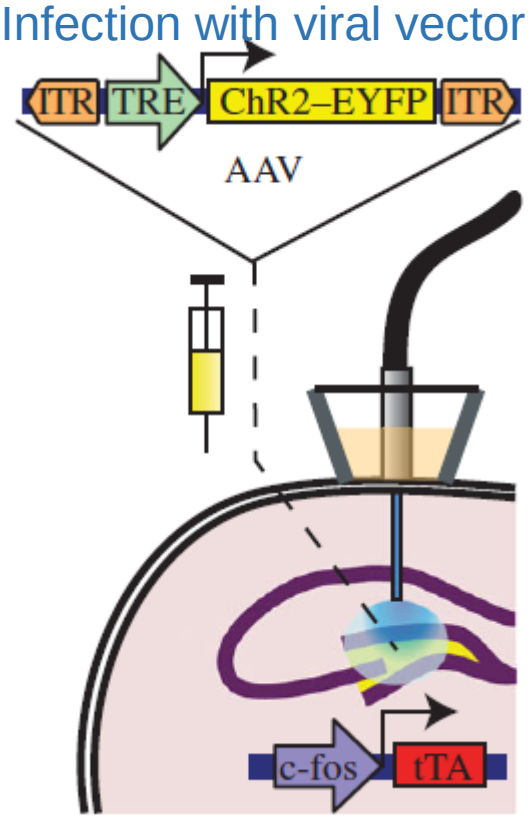
Richard Semon, 1904



- 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

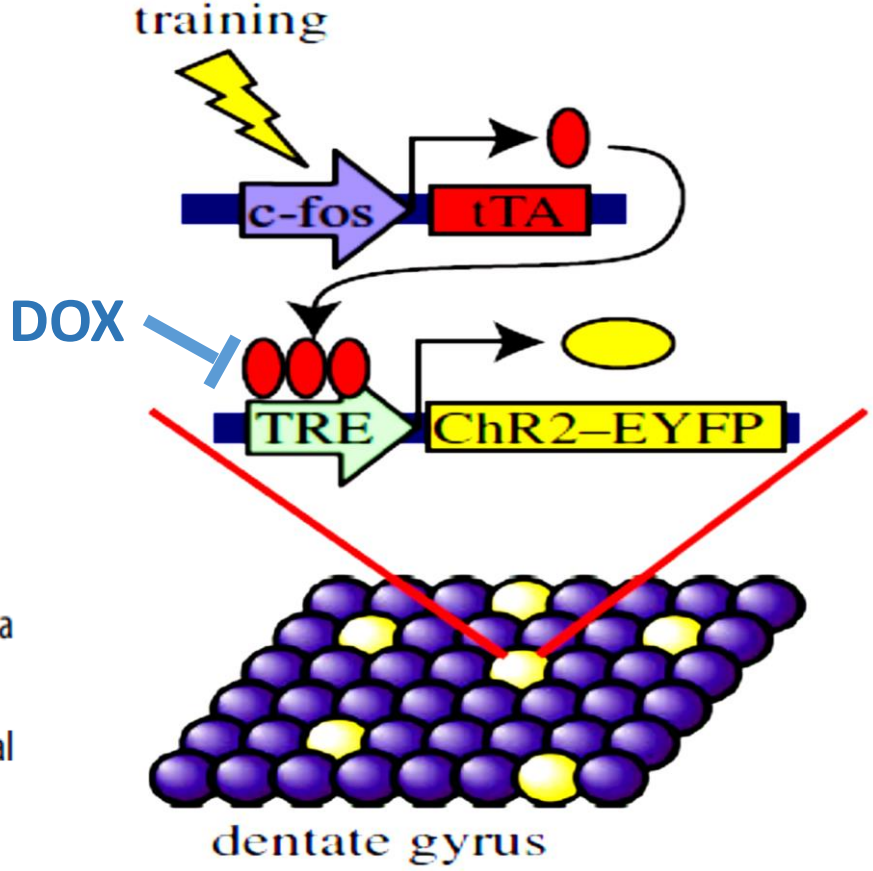
Is it possible to artificially potentiate excitatory synapses of the HIPPOCAMPUS to create a 'false' memory ?

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



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

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

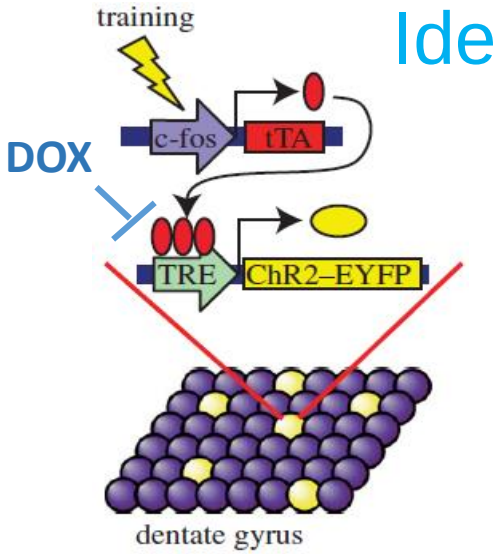


c-fos–tTA transgenic mouse

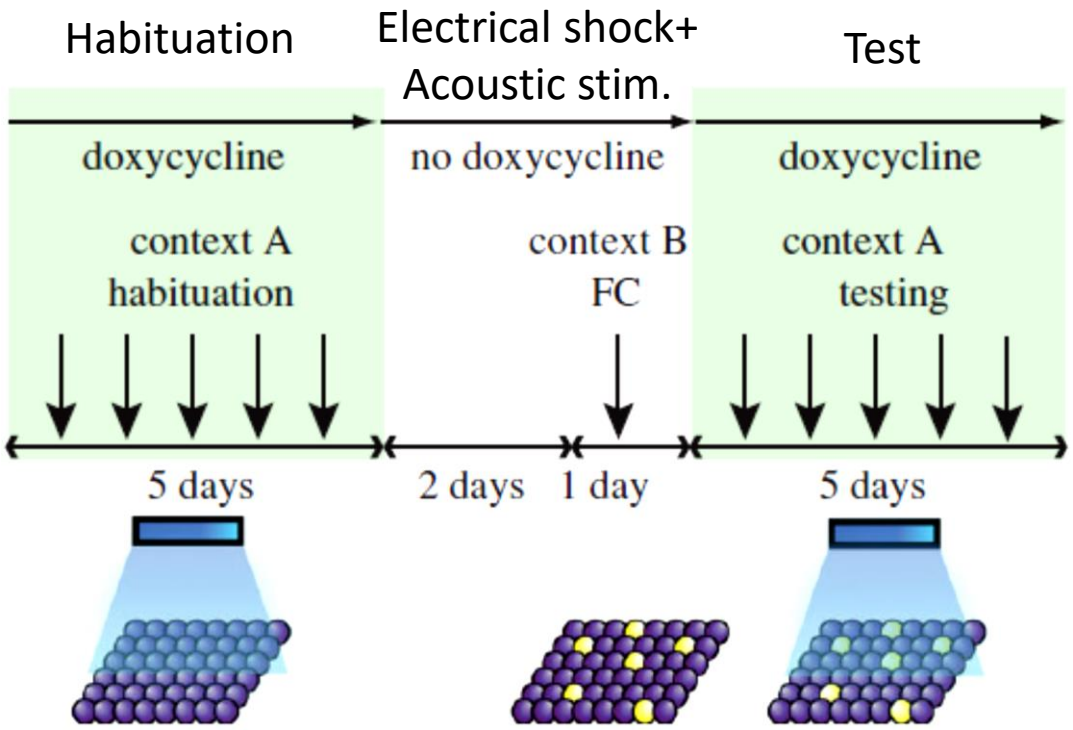
- ✓ c-fos (PROMOTER): a member of immediately early genes, marker of the recent neuronal activity
- ✓ (tTA): **tetracycline transactivator**

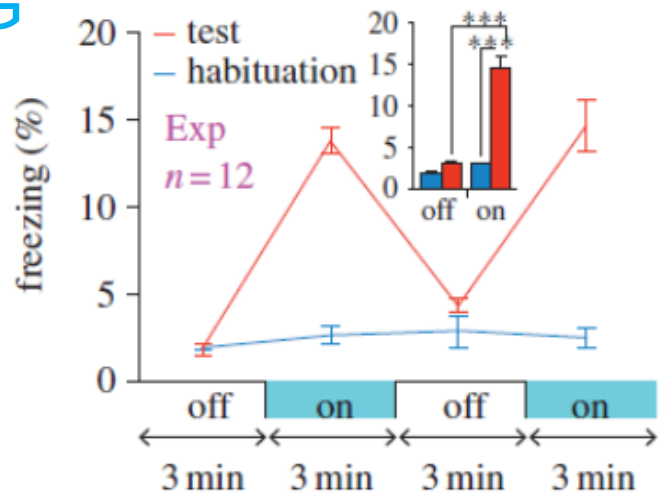
Liu X, Ramirez S, Tonegawa S. 2014 Inception of a false memory by optogenetic manipulation of a hippocampal memory engram. *Phil. Trans. R. Soc. B* **369**: 20130142.

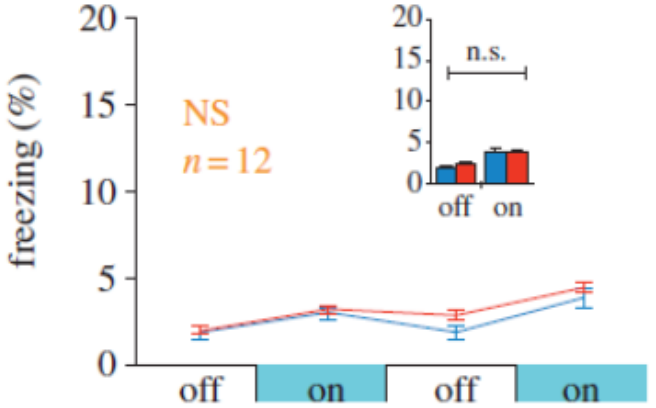
Identification of memory engrams in the DG

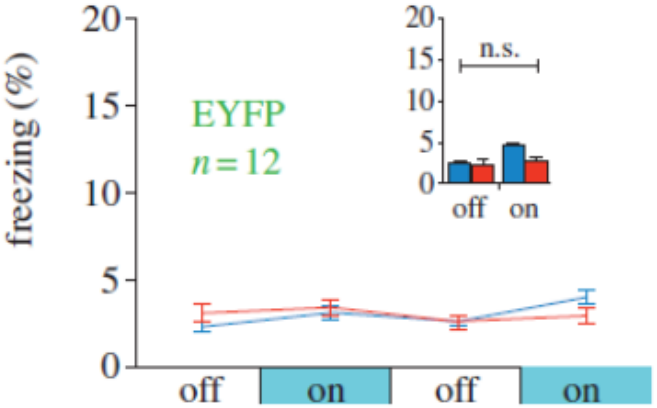


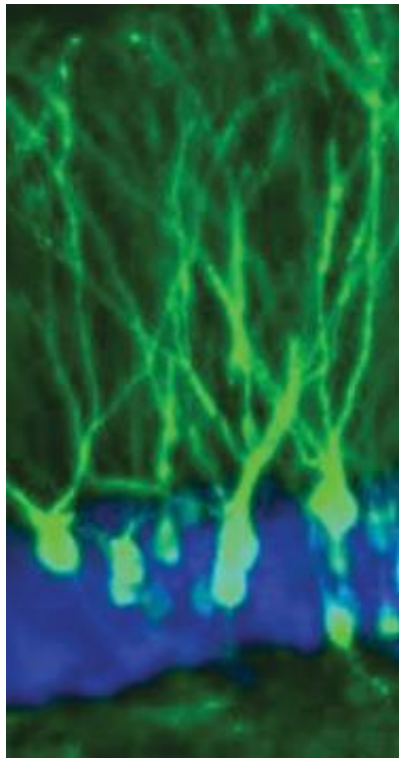
Mice were habituated in context A with light stimulation while on Dox for 5 days, then taken off Dox for 2 days and fear conditioned (FC) in context B. Mice were put back on Dox and tested for 5 days in context A with light stimulation.









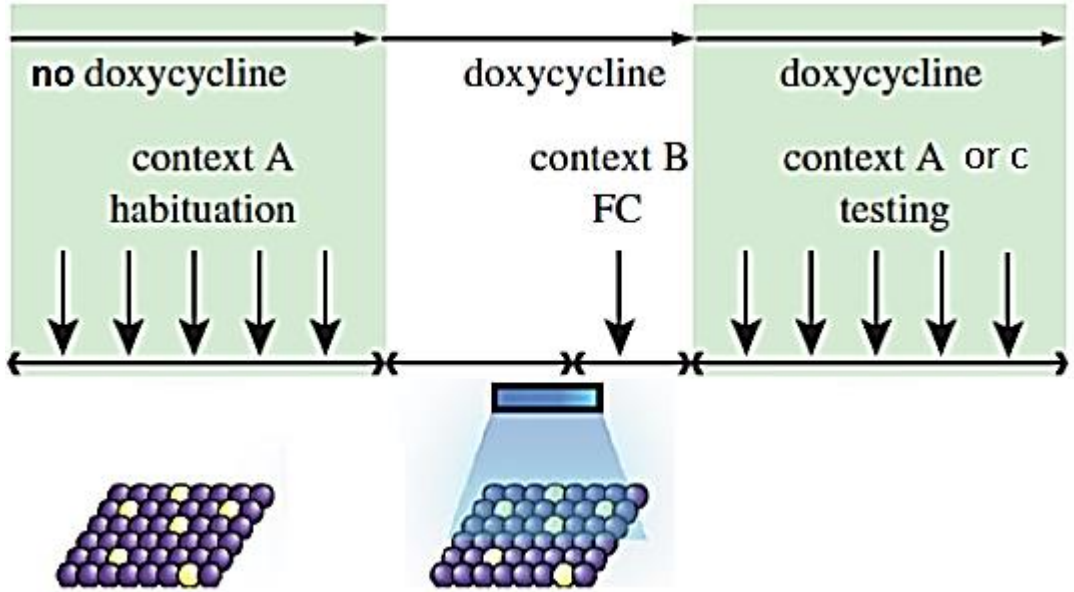


DG Granule cells expressing ChR2-EYFP

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

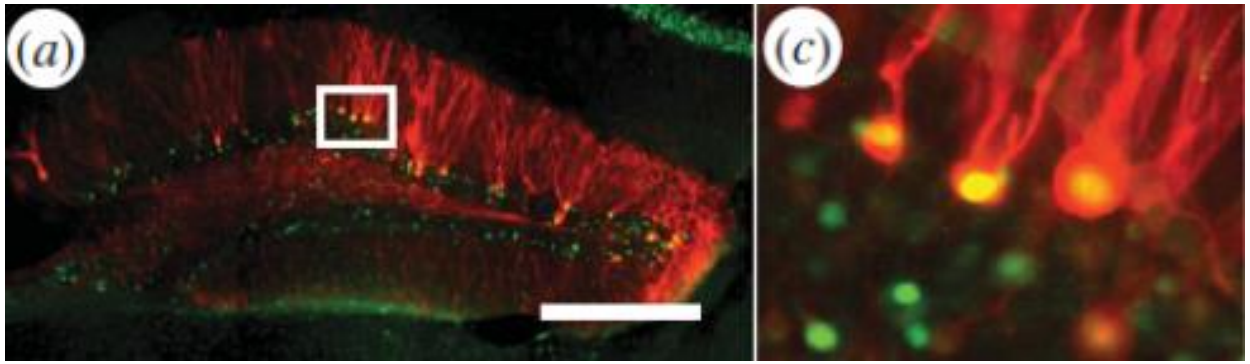
Inception of a false memory

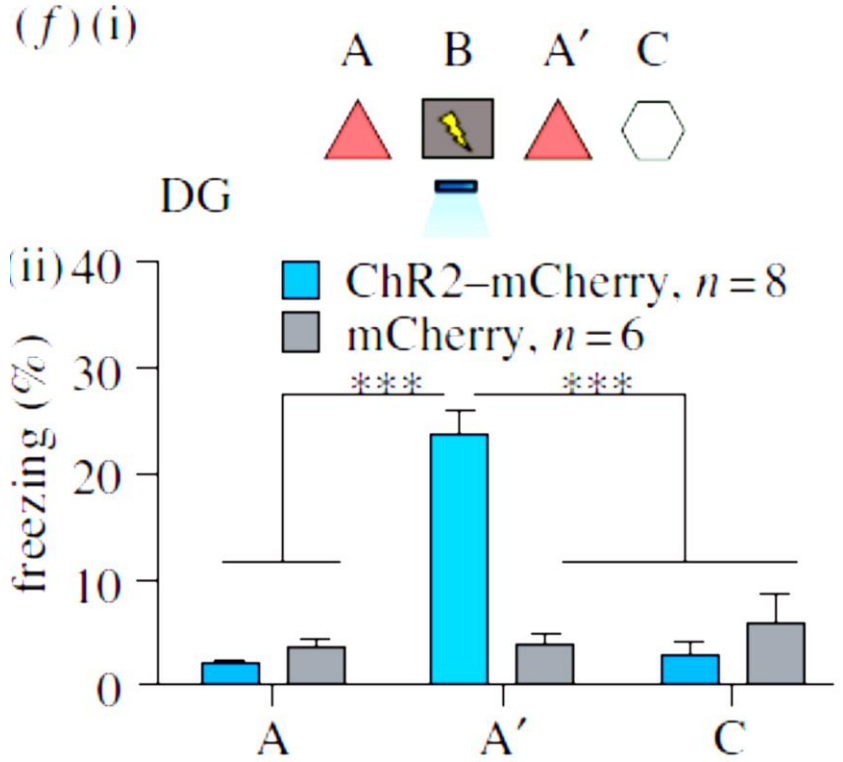
c-fos–tTA mice injected with AAV_9-TRE-ChR2-mCherry in the DG



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.