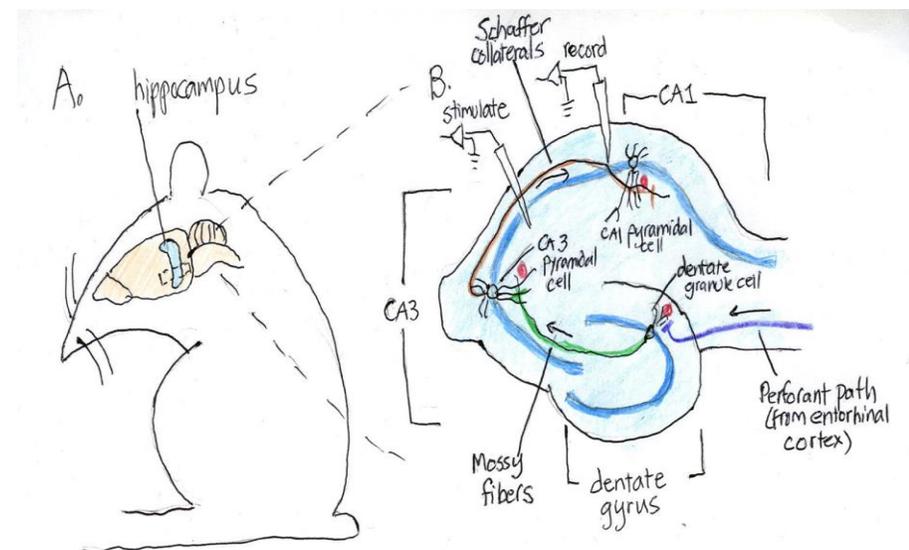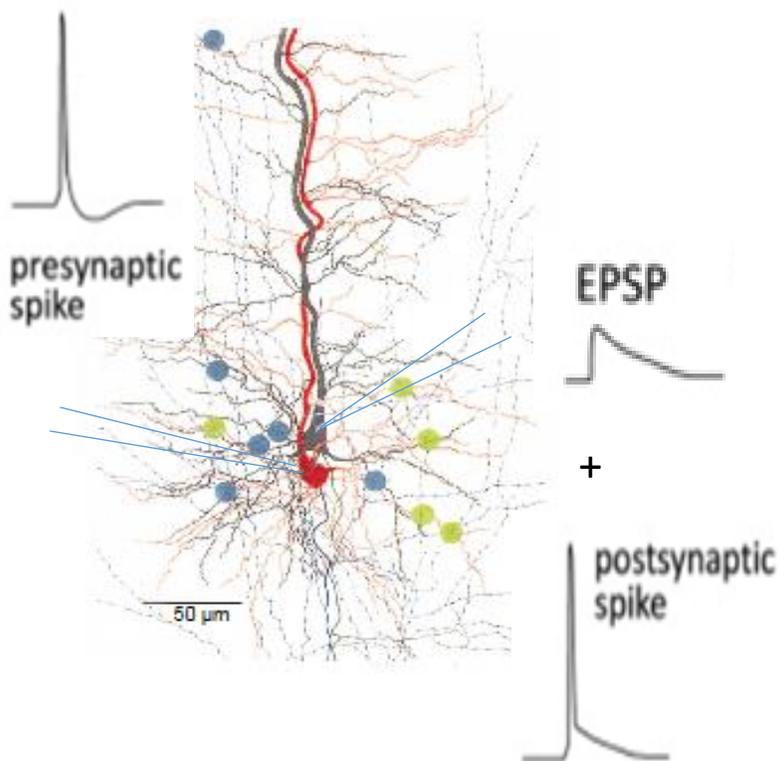# *MOLECULAR NEUROPHYSIOLOGY -lesson 1-*

Prof. Giada Cellot

cellot@sissa.it

The plasticity of neuronal circuits: synaptic changes and co-related behavioral aspects

➢ Hebbian (NMDA mediated) LTP - Spike timing dependent plasticity
➢ Anti-Hebbian LTP

➢ Functional implications of hippocampal synaptic plasticity: memory and learning
➢ Optogenetic manipulation of synaptic plasticity: impact on behavior

➢ Synaptic plasticity in the amygdala
➢ Associative learning: the fear conditioning paradigm

➢ Synaptic plasticity during the development: when GABA is depolarizing

Lesson 1	November 19th h9-11
Lesson 2	November 26th h9-11
Lesson 3	December 3rd h9-11
Lesson 4	December 10th h9-11

NEURAL ACTIVITY CARVES CONNECTIVITY DURING THE MATURATION OF THE SYNAPTIC CIRCUIT

At specific synapses:
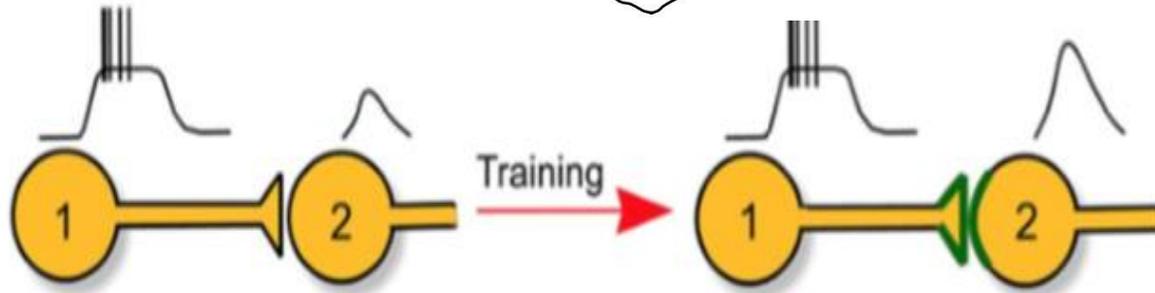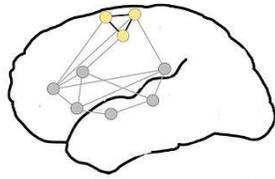
Synaptic reinforcement is obtained by repeated activation of the synapse

SINAPTIC PLASTICITY: EXPERIENCE DEPENDENT change in the EFFICACY of synaptic communication between neurons ⟹ Long term potentiation (LTP)

"When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased"

D. O. Hebb, 1949

CELLS that FIRE TOGETHER, WIRE TOGETHER

# Synaptic plasticity in the hippocampus can be studied *in vitro*

HIPPOCAMPAL SLICES

→ Composed of defined populations of identifiable neurons

→ Suitable for investigation (electrophysiology)

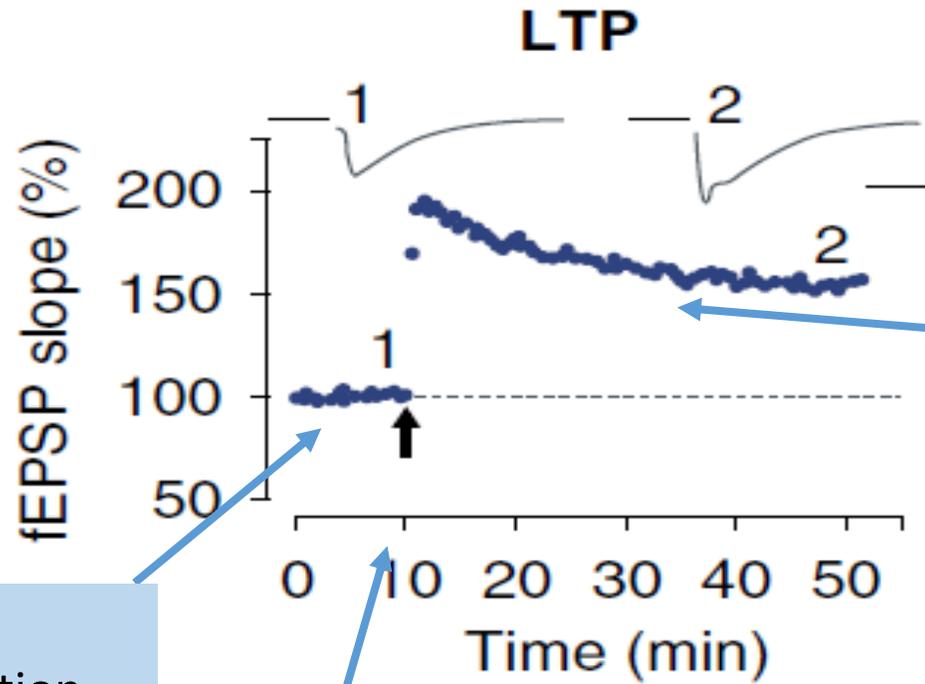→ Easy pharmacological manipulation, limitation of animals used in research

Brain is dissected in 300-400 μm thick slices that can be recorded for hours in oxygenated artificial cerebrospinal fluid

Citri & Malenka, Neuropsychopharmacology REVIEWS (2008) 33, 18–41
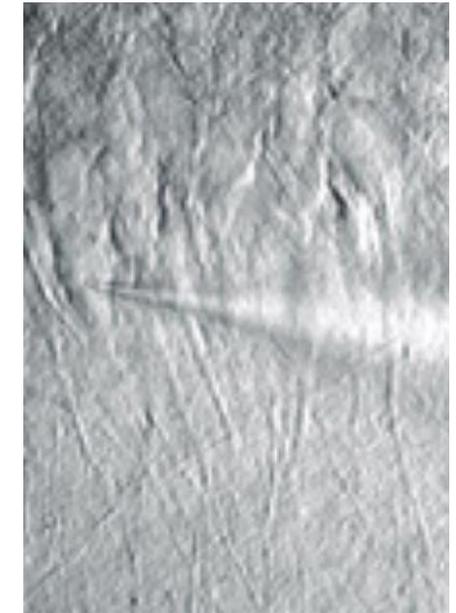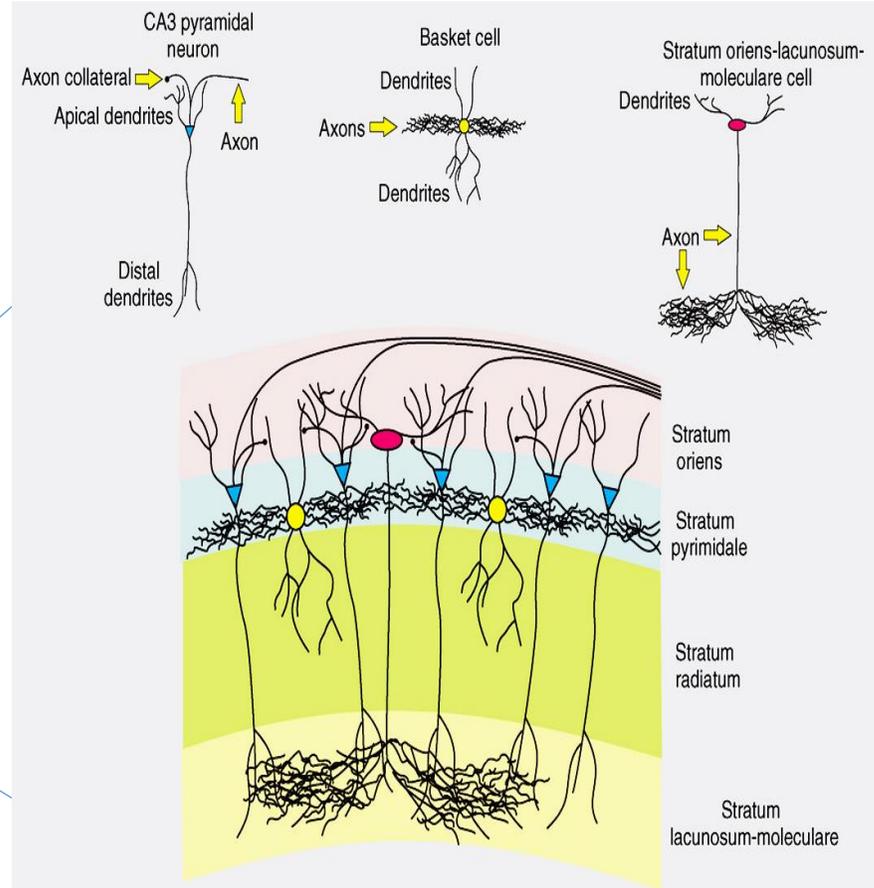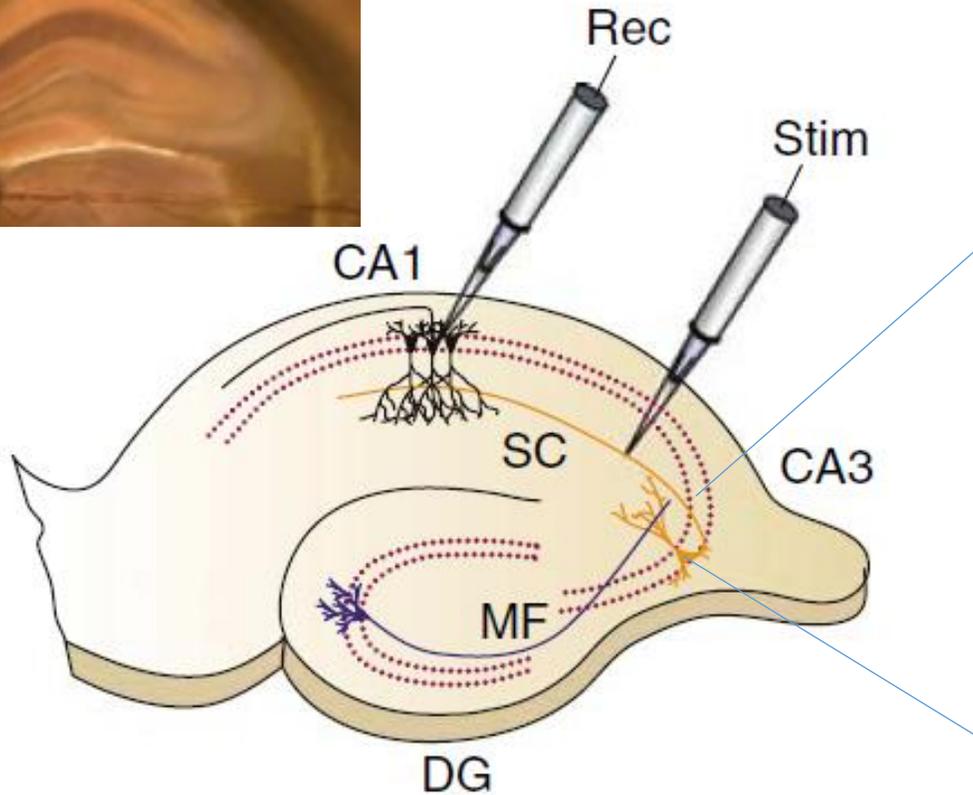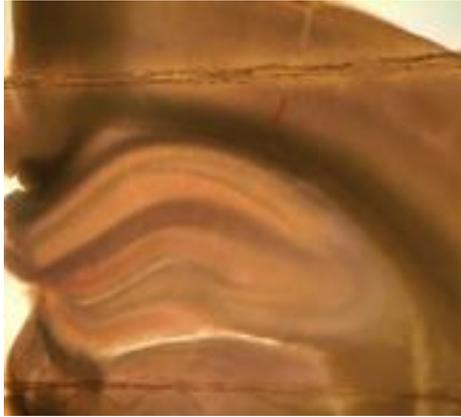
Phases of a LTP experiment
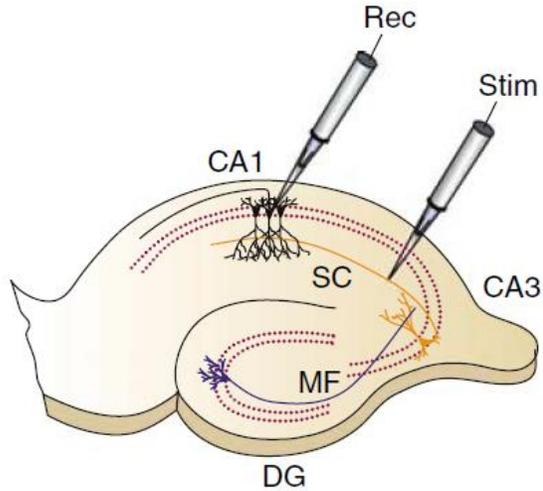
1.Baseline responses
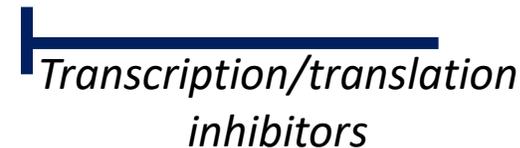(low frequency stimulation
0.5 Hz)

2. Protocol to induce potentiation = INDUCE STRONG STIMULATION OF SYNAPSES (Electrical stimulation: Tetanus: 1s @100 Hz; or theta burst stimulation: interval of 200 ms)
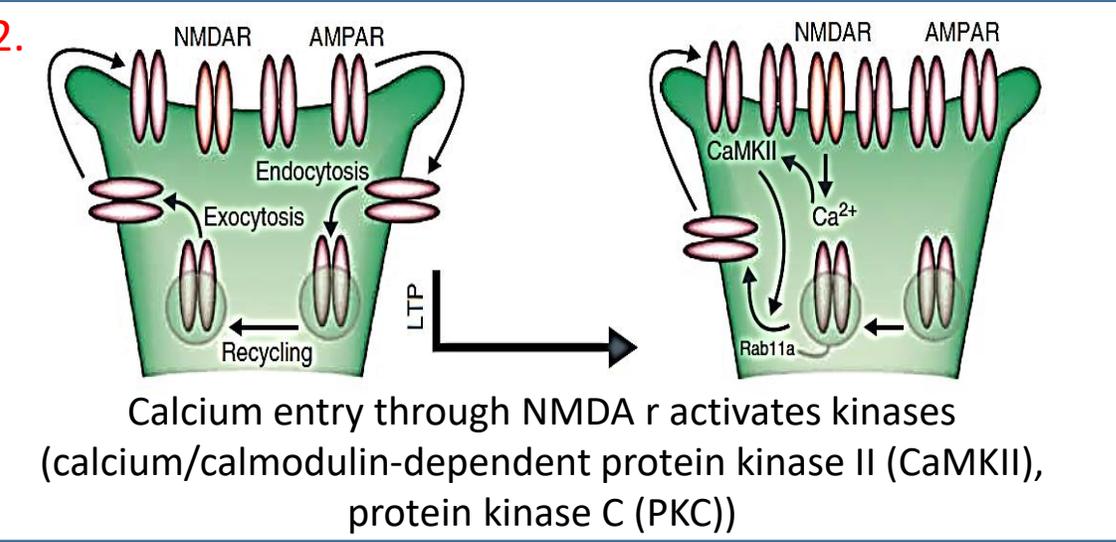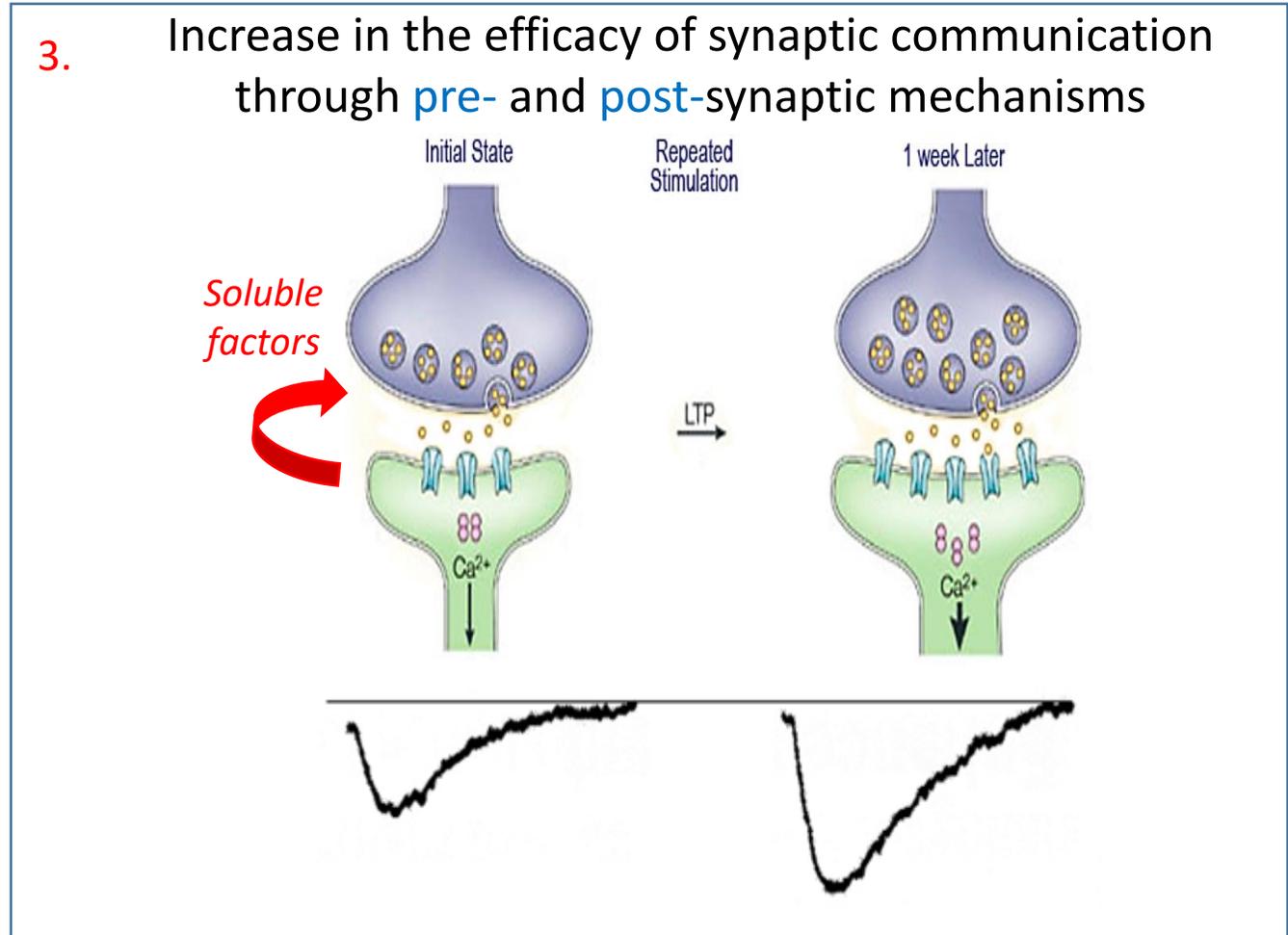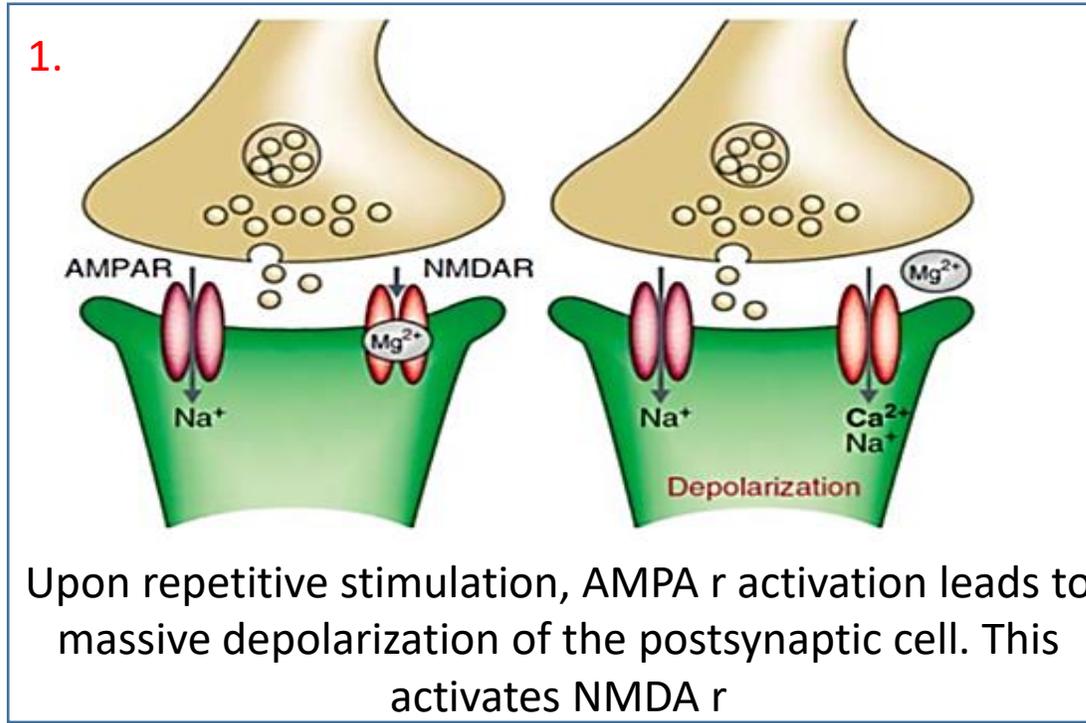
3.Post-induction responses (low frequency stimulation 0.5 Hz)

1. **Early-LTP:** kinase dependent LTP

Kinase inhibitors

Potentiation is downregulated after few seconds

2. **Late-LTP**: protein synthesis dependent LTP

Transcription/translation inhibitors

Potentiation is downregulated after 5-6 hours

HEBBIAN LTP mechanisms

1.

Upon repetitive stimulation, AMPA r activation leads to massive depolarization of the postsynaptic cell. This activates NMDA r

2.

Calcium entry through NMDA r activates kinases (calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC))

3. Increase in the efficacy of synaptic communication through pre- and post-synaptic mechanisms

Soluble factors

LTP INDUCTION: <u>always</u> postsynaptic
LTP EXPRESSION: pre- or/and postsynaptic

LTP induced morphological modification of synapses

DENDRITIC SPINES: tiny protrusions from dendrites, form functional contacts with neighboring axons of other neurons (Smith et al, 2014)

- Actin cytoskeleton
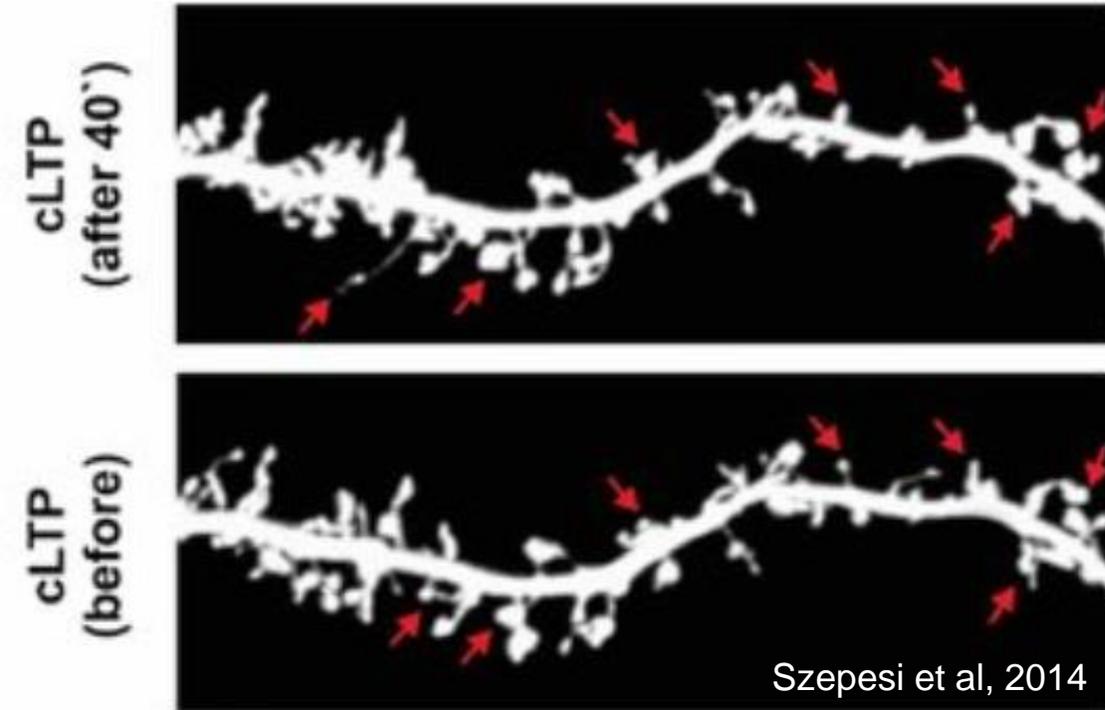- POST SYNAPTIC DENSITY (PSD): high density packed ion channels, receptors, kinases anchored by scaffolding proteins
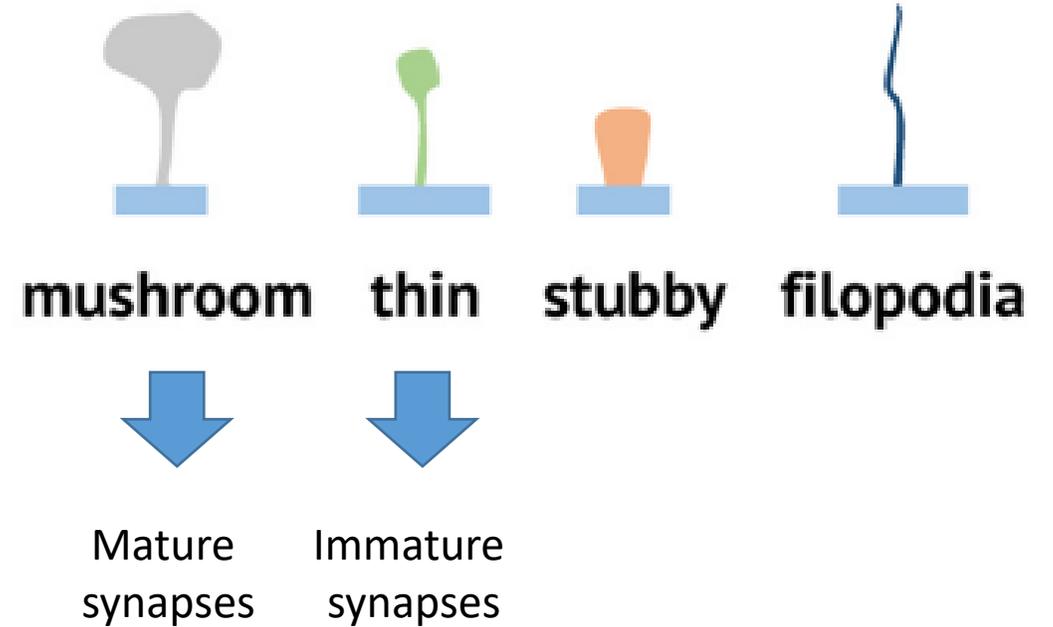
Acute hippocampal slice after TBS
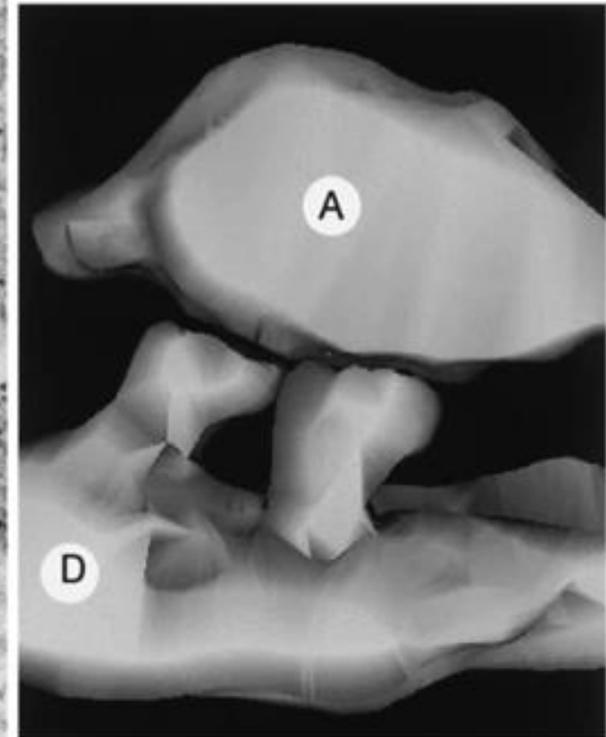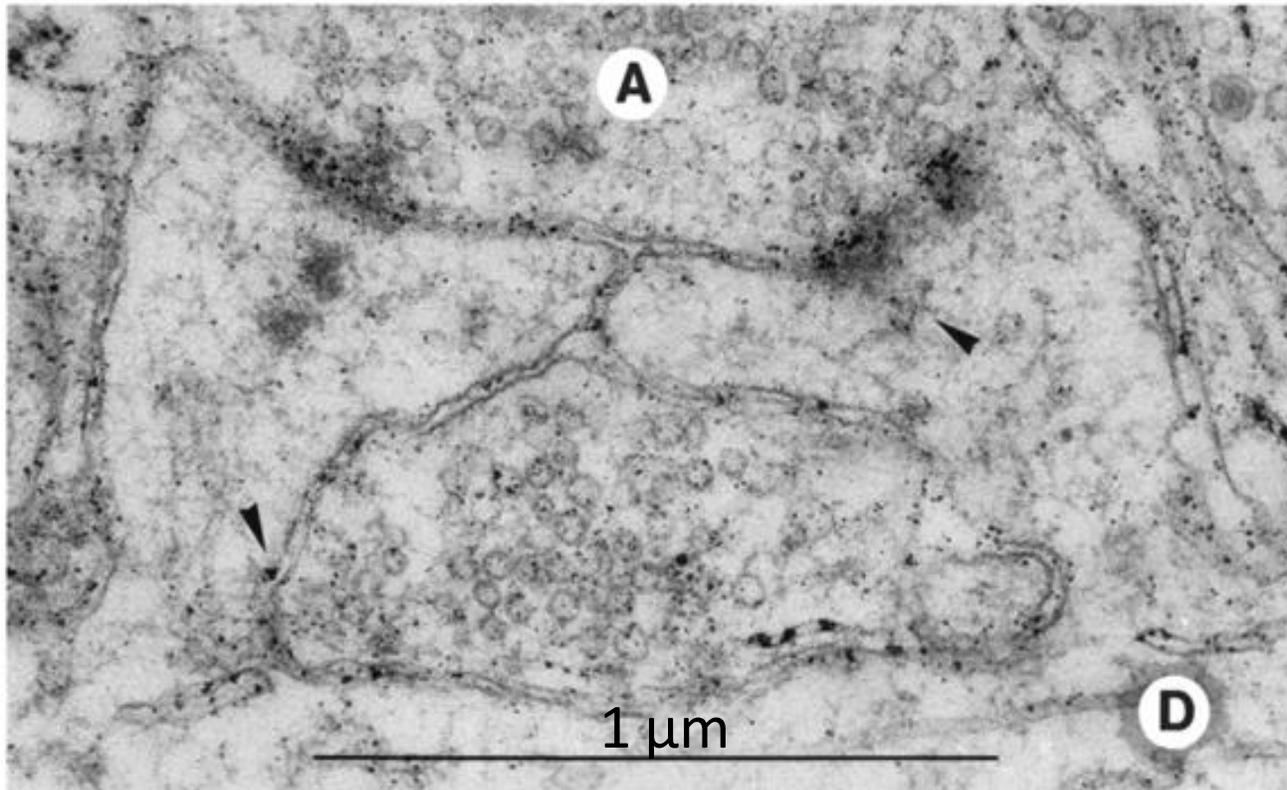
Toni et al, Nature, 1999

DENDRITIC SPINES
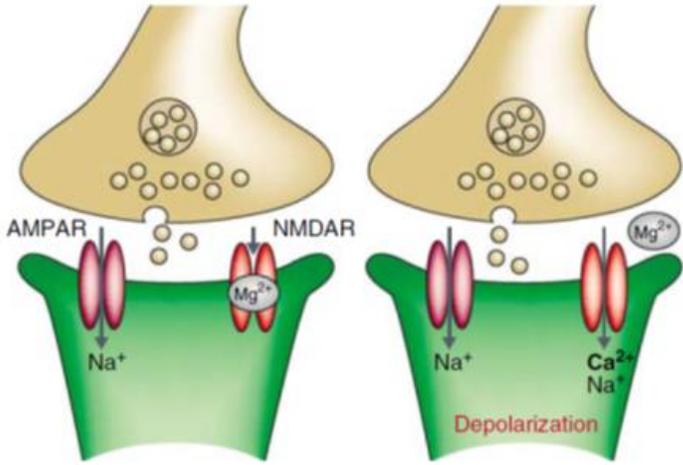
Hippocampal cultures exposed to chemical LTP

Szepesi et al, 2014

Classification
based on head /neck size

mushroom   thin   stubby   filopodia

Mature synapses   Immature synapses

# NMDA receptor as detector of coincidence

COOPERATIVITY, ASSOCIATIVITY, INPUT SPECIFICITY can be all explained on the assumption that the induction of LTP requires pre-synaptic activation with **COINCIDENT ACTIVATION** of post-synaptic cell
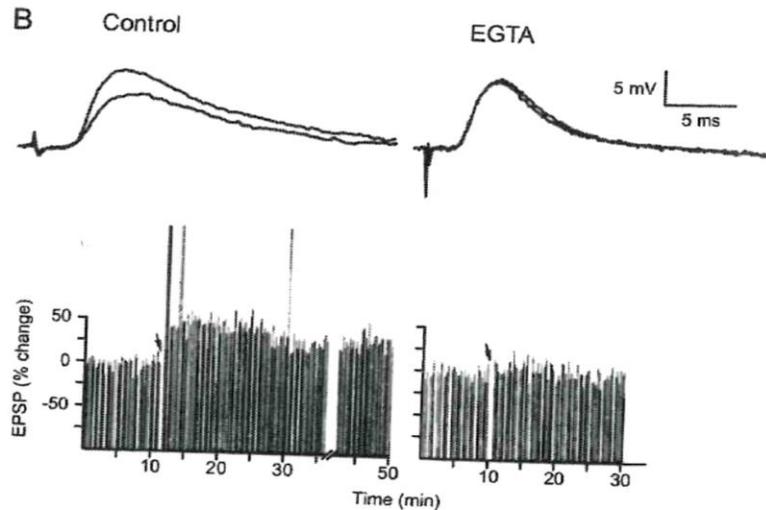
*NMDA antagonists or Buffering intracellular calcium with EGTA block the induction of LTP*

Lynch et al., 1983

Hebb's postulate:
CELLS that FIRE TOGETHER, WIRE TOGETHER

What a Coincidence!
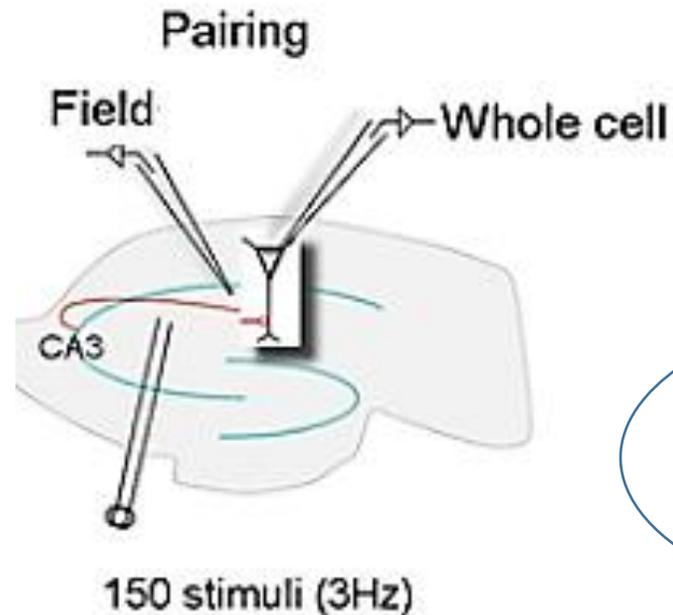Magnesium, NMDA Receptors, and LTP
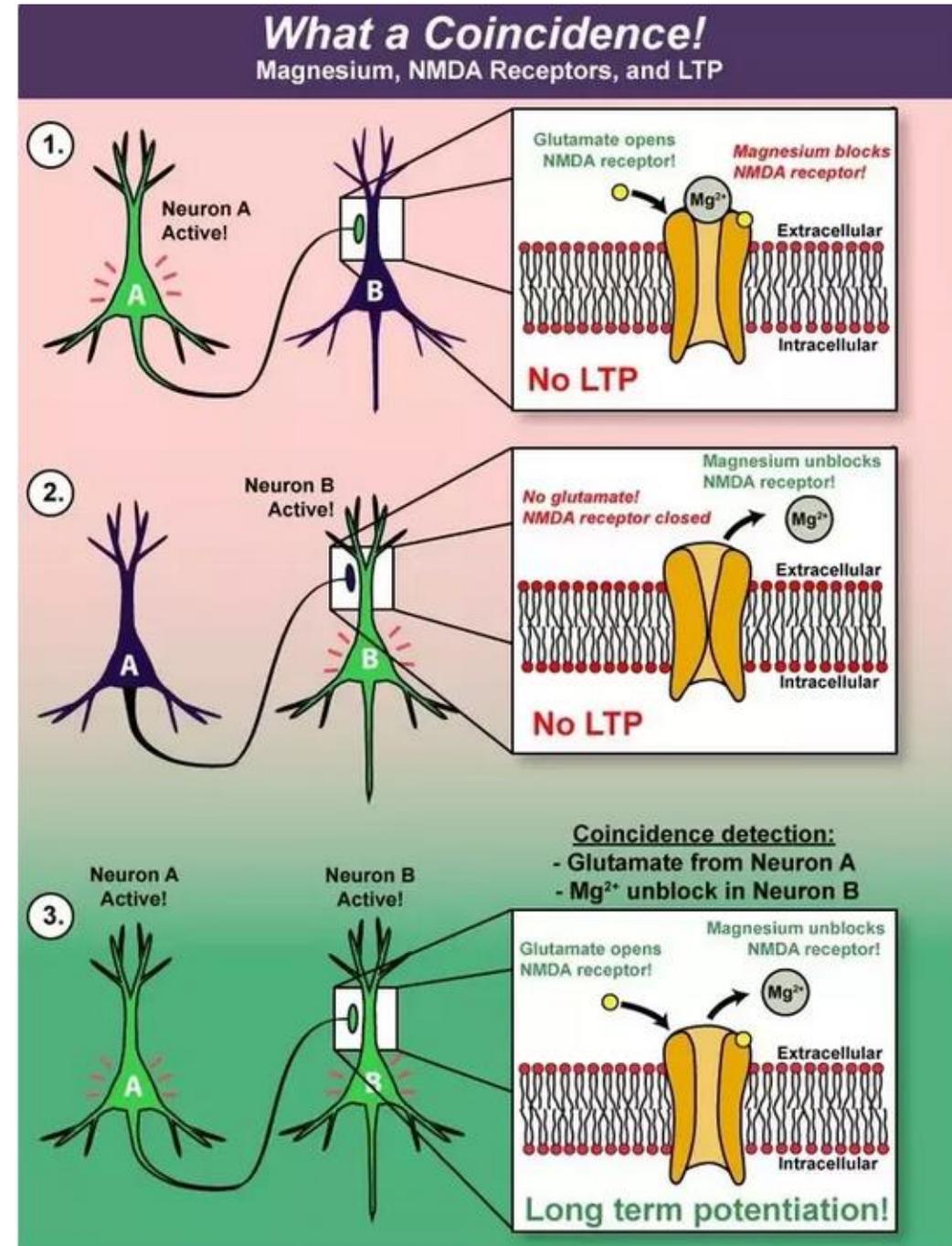
LTP can be induced by PAIRING pre- and post-synaptic NEURONS

Not only high frequency stimulation (tetanus: 1 s @100 Hz) of the afferent fibers can be used to induce LTP, but also **PAIRING PROCEDURE** in which pre-synaptic stimuli at low frequency (1-3 Hz) are paired with postsynaptic depolarization.
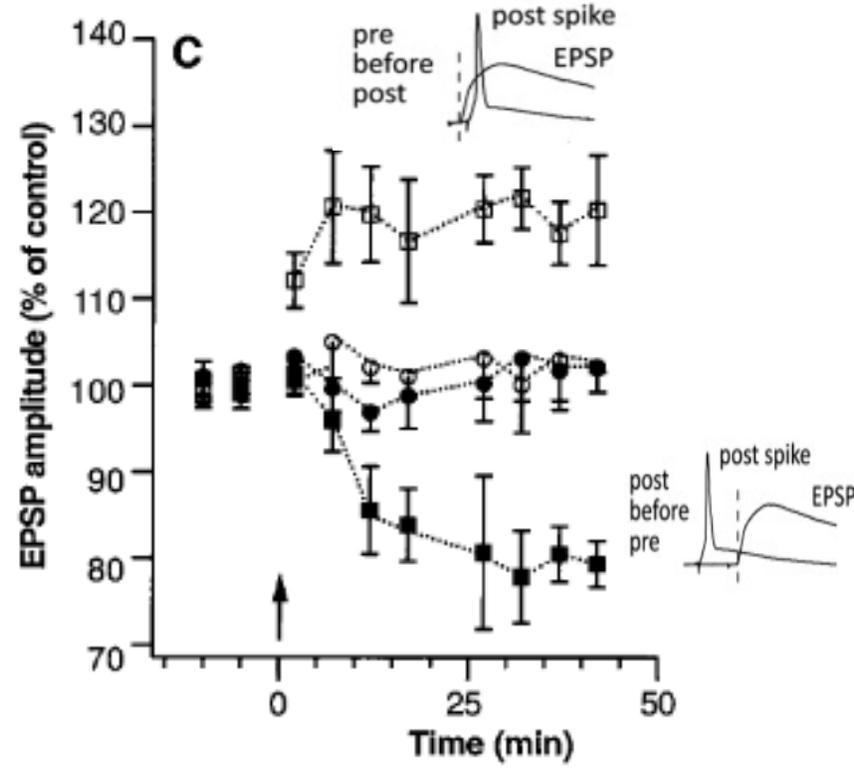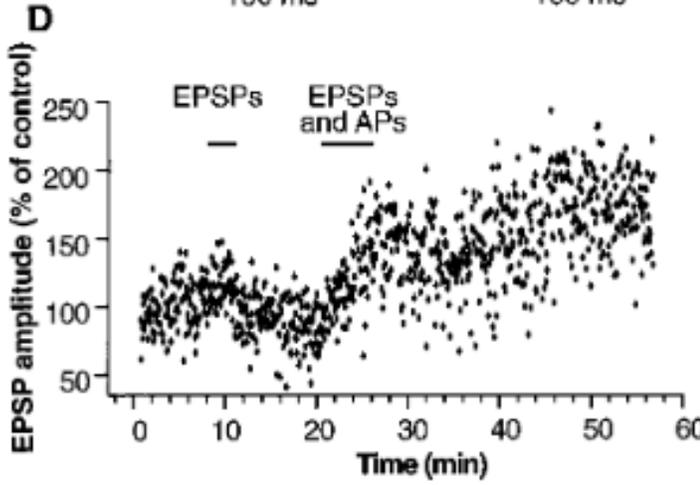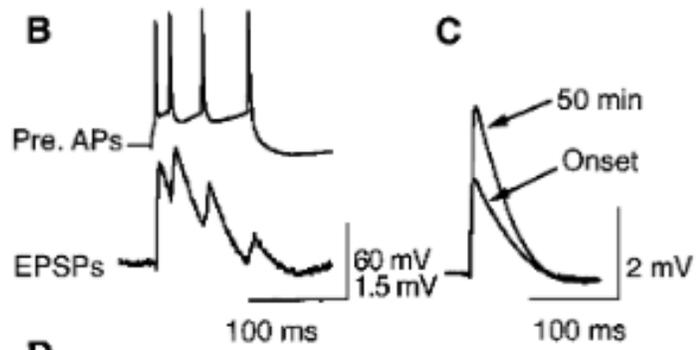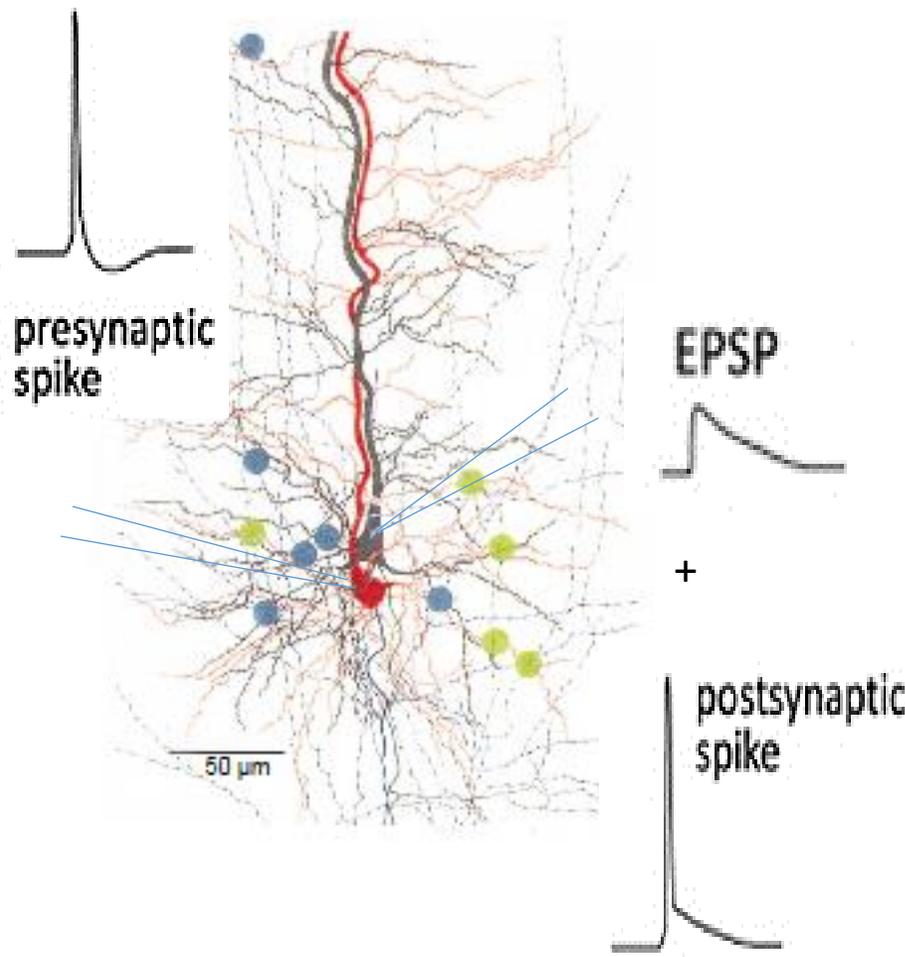

Bliss & Lomo, 1973

150 stimuli (3Hz)
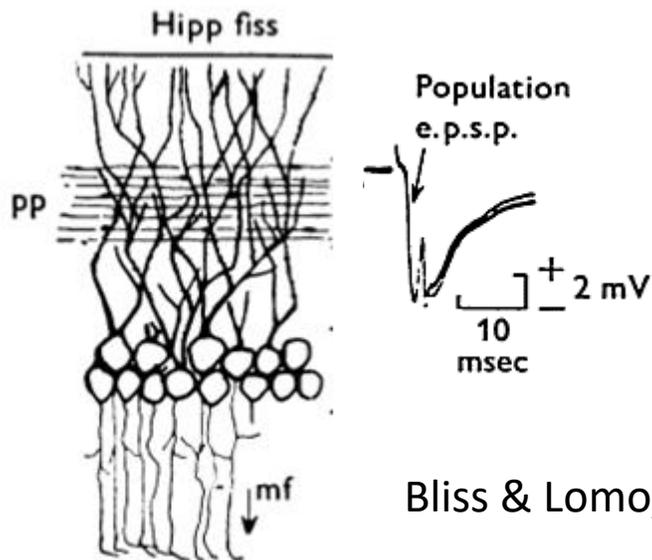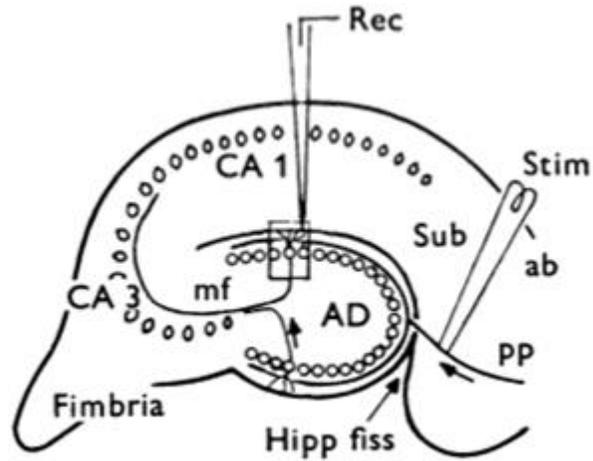
PATCH CLAMP TECHNIQUE

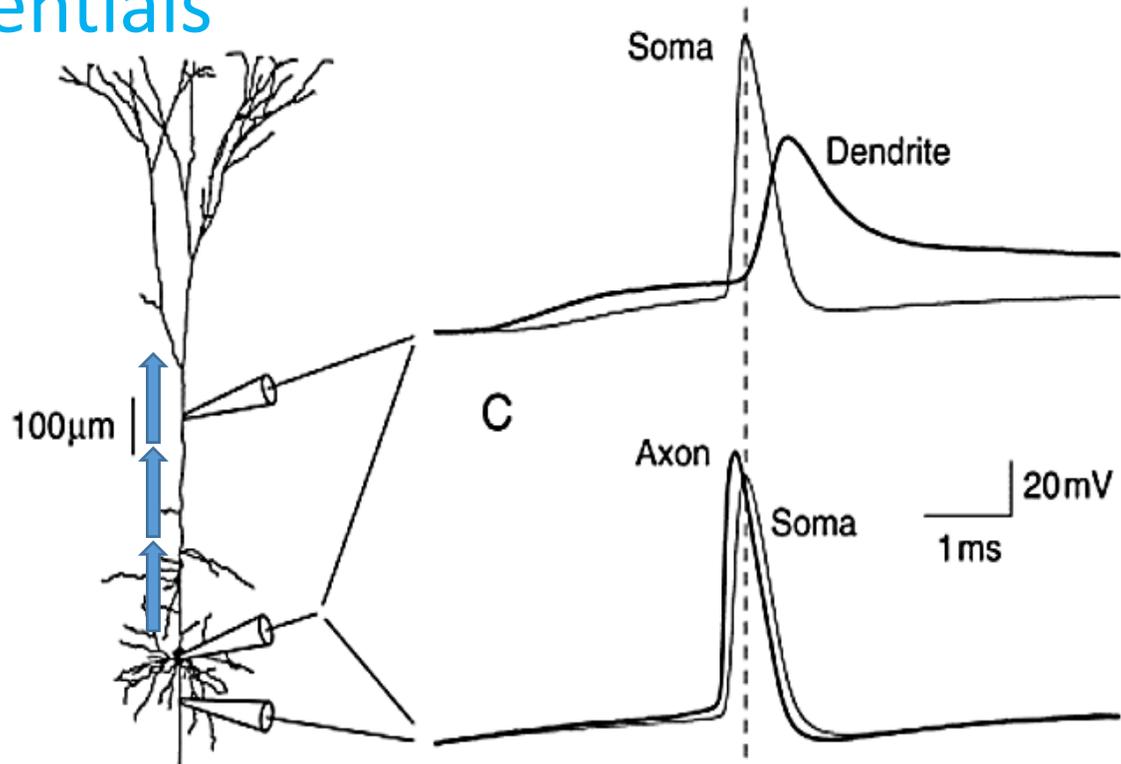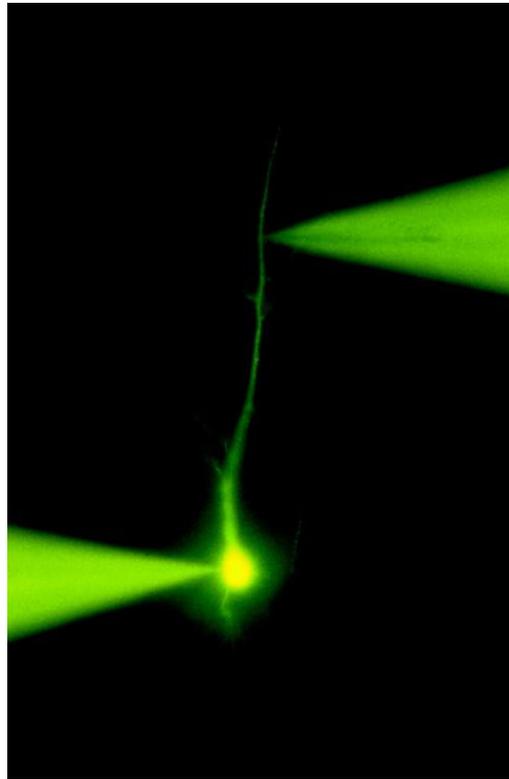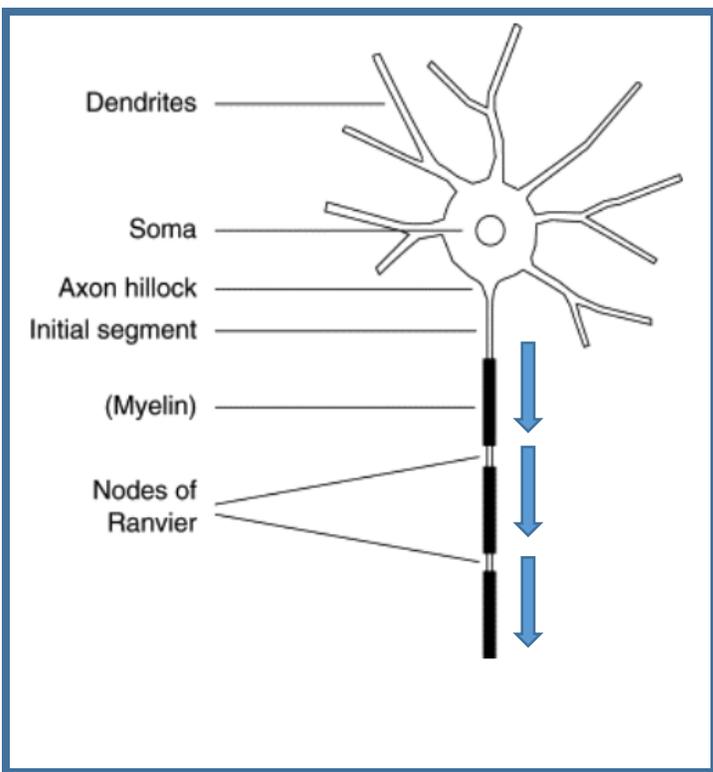It allows to set the potential of the recorded neuron at precise values

Spike Timing-Dependent Plasticity (STDP)

The pairing procedure becomes very efficient when postsynaptic neuron fires later respect to presynaptic cell (in a time window <20 ms)


Modified from Markram et al., Science, 1997

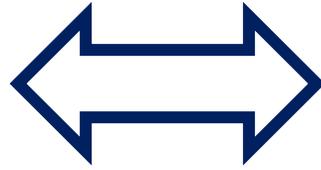# STDP and back-propagating action potentials

Stuart et al., Trends Neuroscience, 1997

- Action potentials can back-propagate in the dendritic tree, as a wave of depolarization.
- The physiological meaning of backpropagation is to have a "trace" of memory of recent cell history.
- Thus, if presynaptic firing coincides with postsynaptic AP backpropagation, LTP can occur
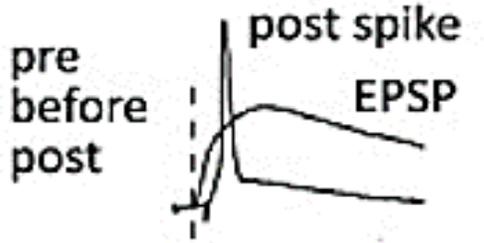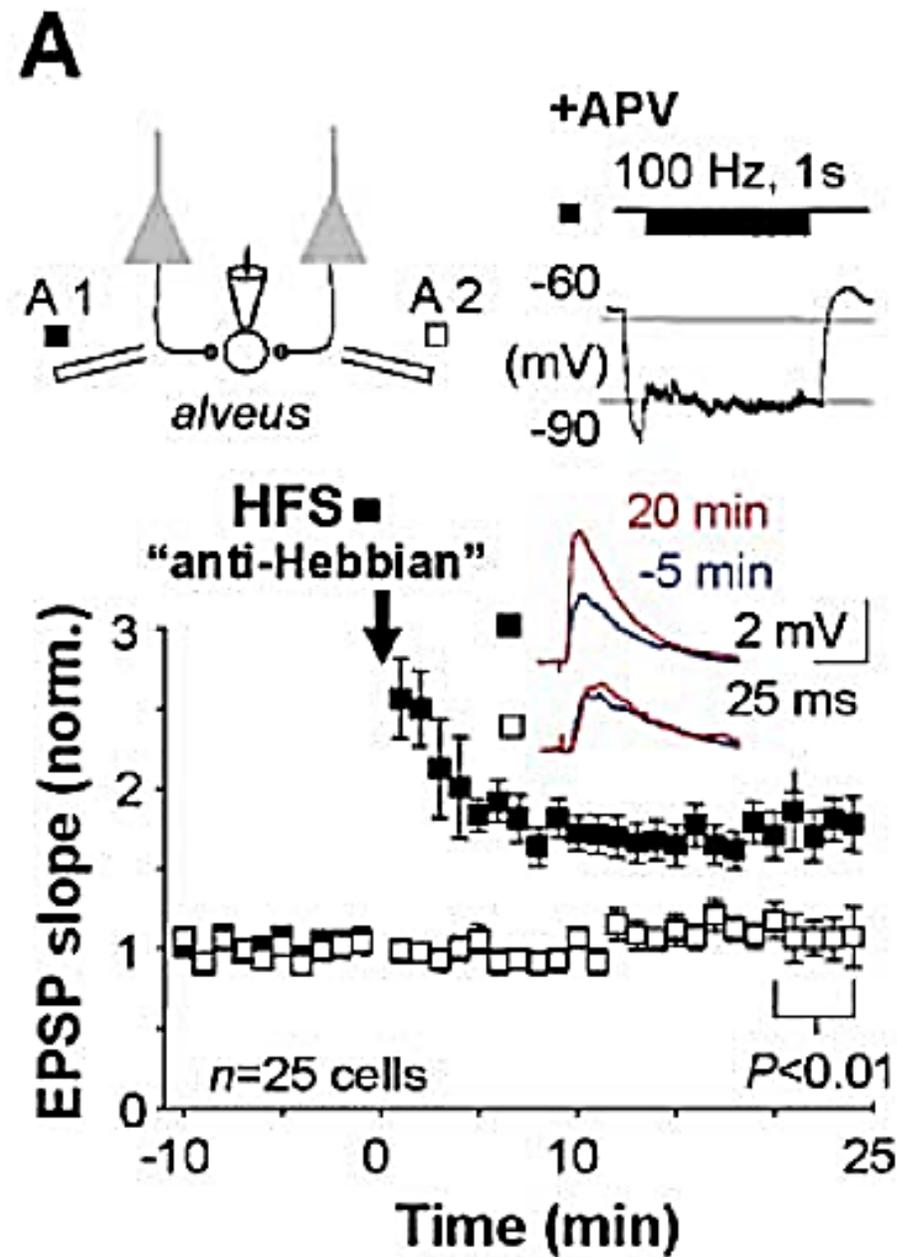
"HEBBIAN" LTP ⟷ "anti-HEBBIAN" LTP

Plasticity that occurs when pre-synaptic cell firing coincides with postsynaptic neuron depolarization
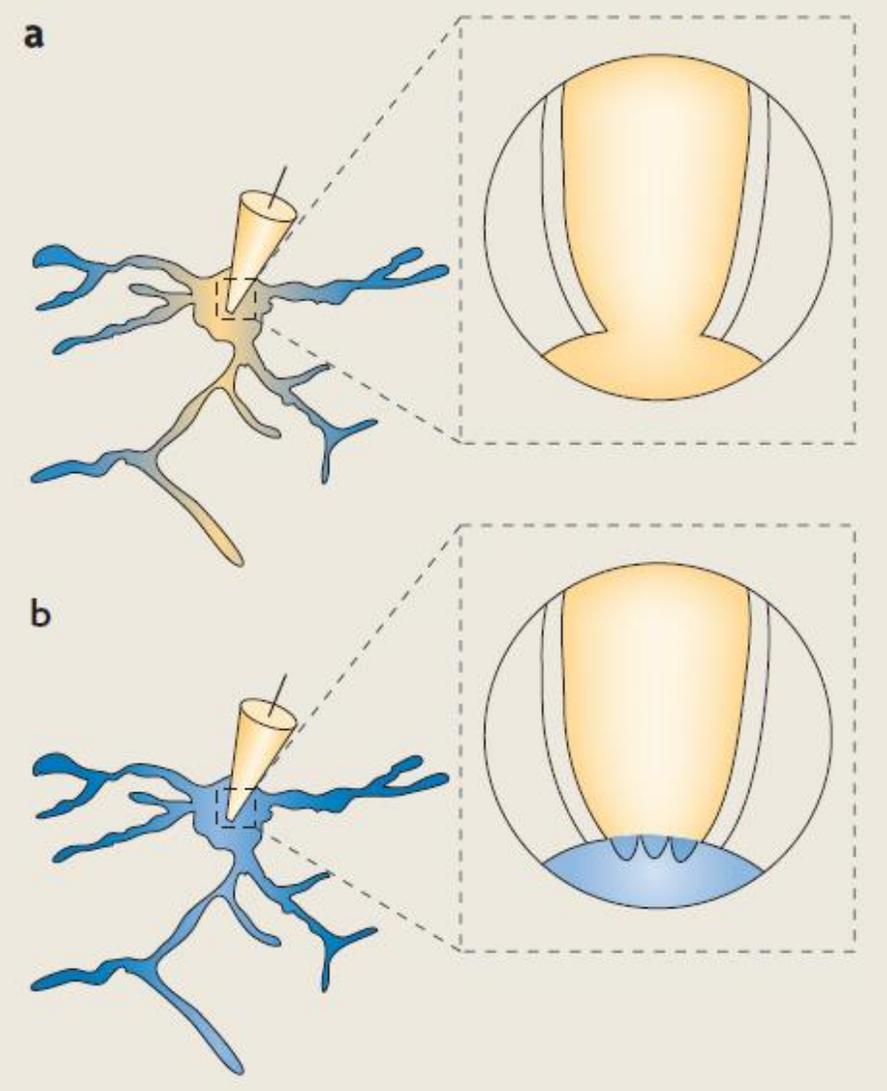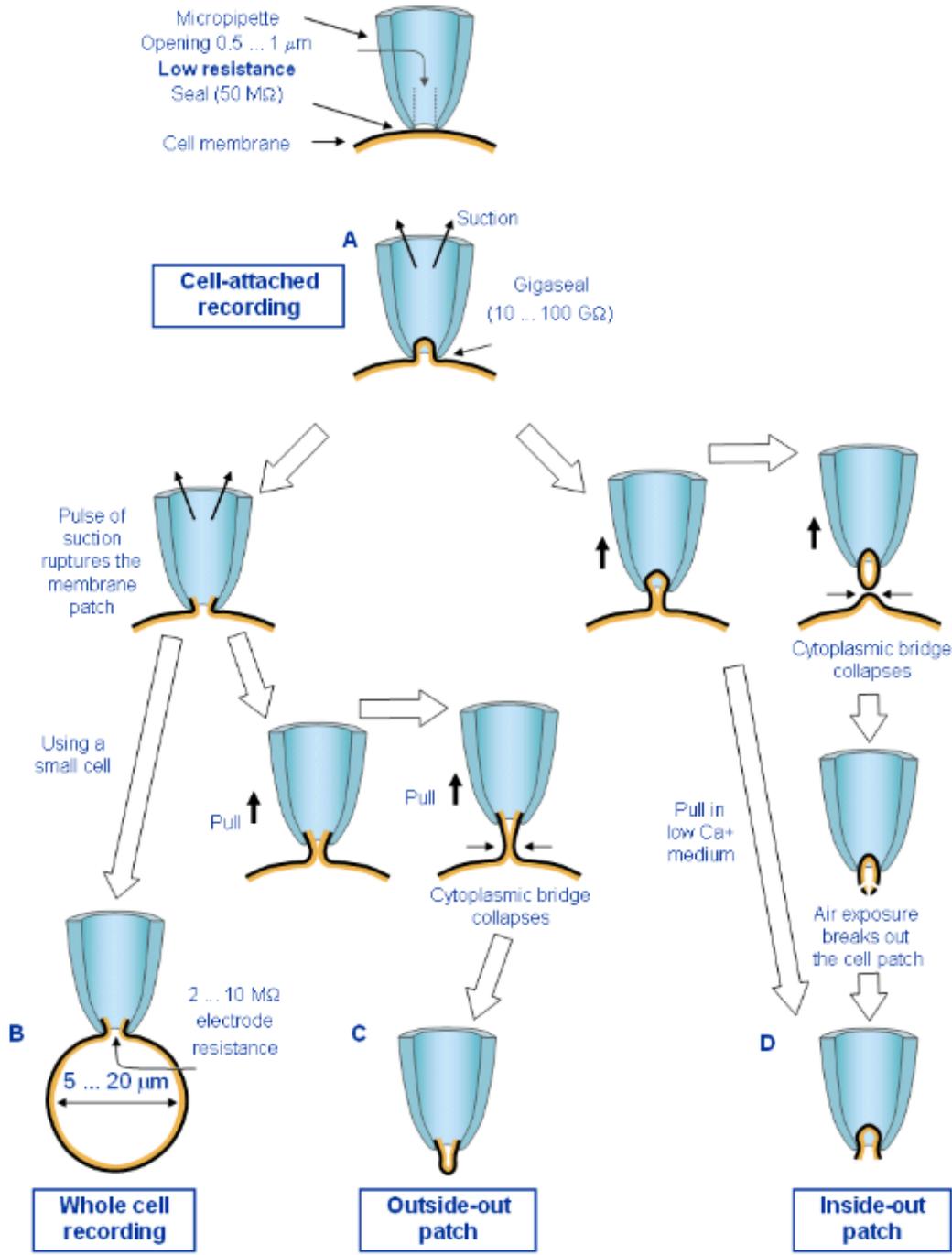
Plasticity that occurs when pre-synaptic cell firing coincides with postsynaptic neuron hyperpolarization (prevented by postsynaptic depolarization)

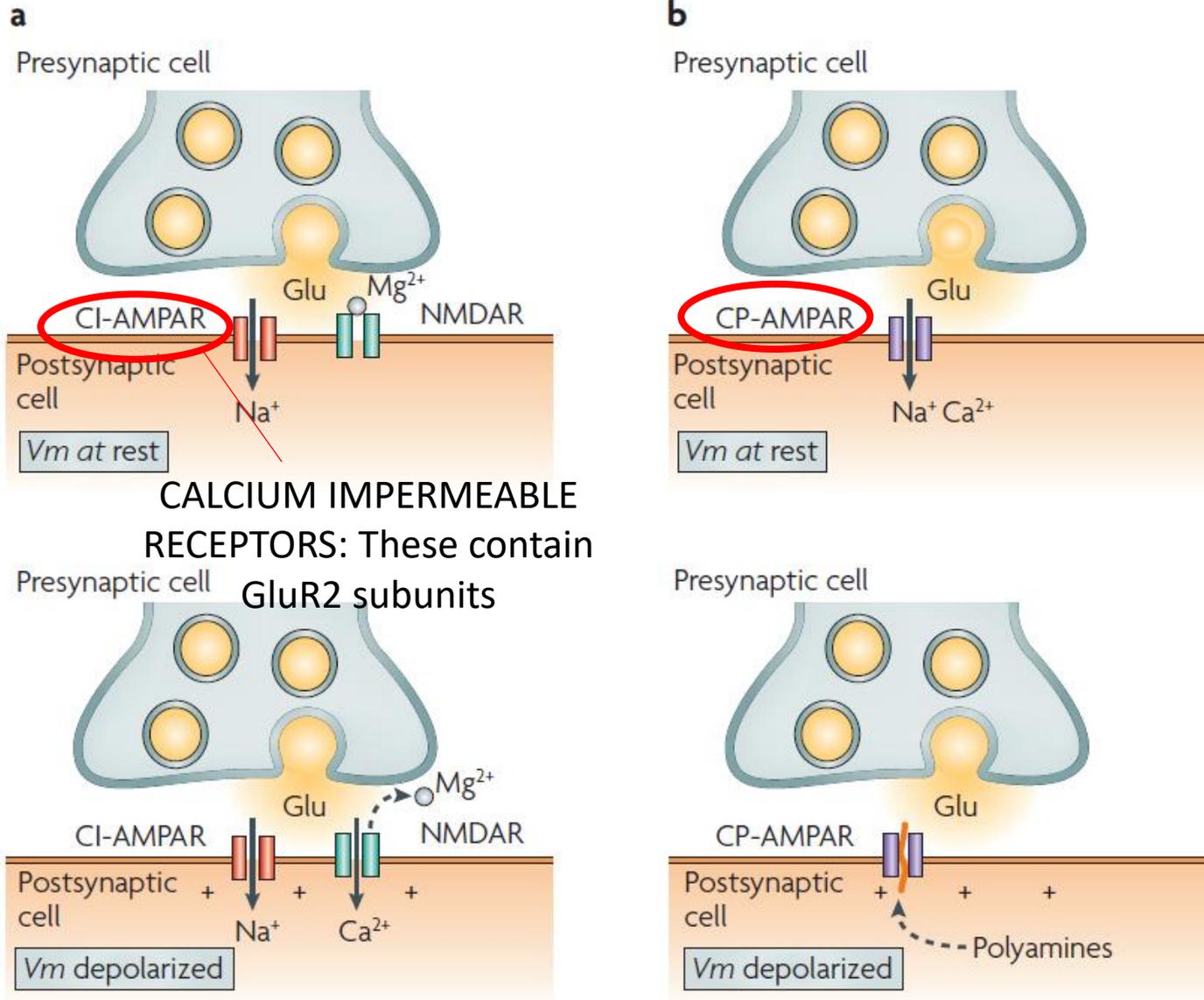# THE "anti-HEBBIAN" LTP

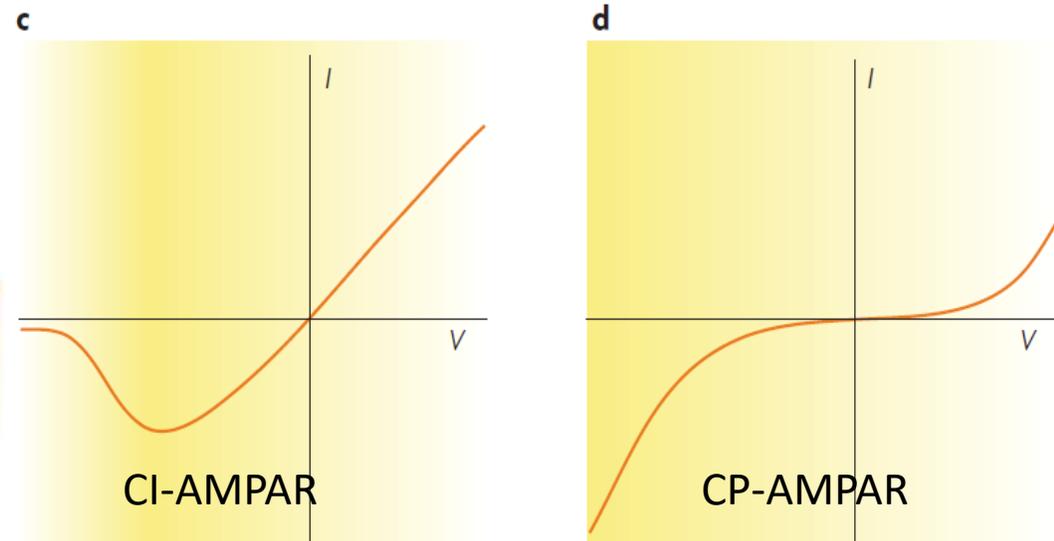Lamsa et al., Science, 2007
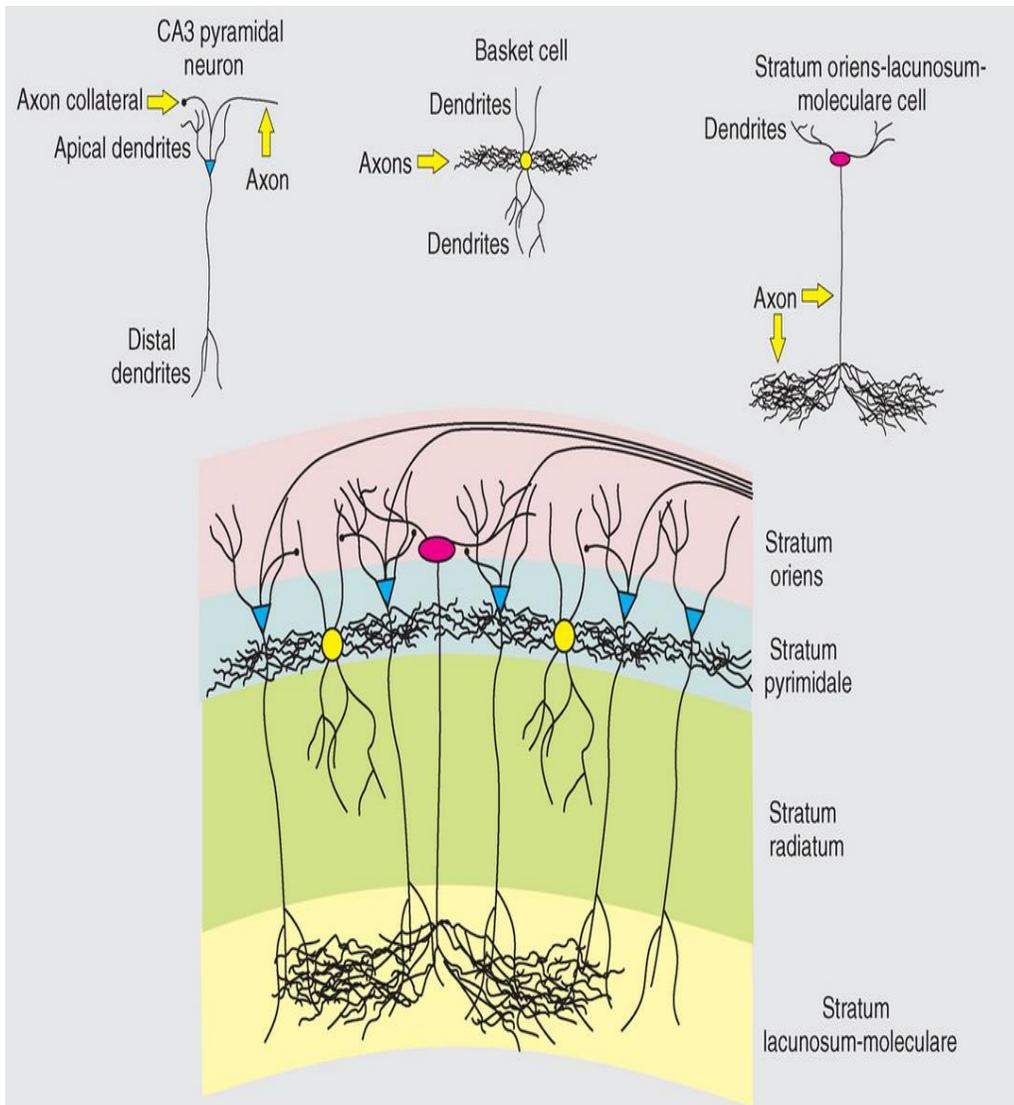
WHOLE CELL CONFIGURATION

PERFORATED PATCH CONFIGURATION
(by using antibiotics such as gramicidin)
Not to dialyze intracellular milieu
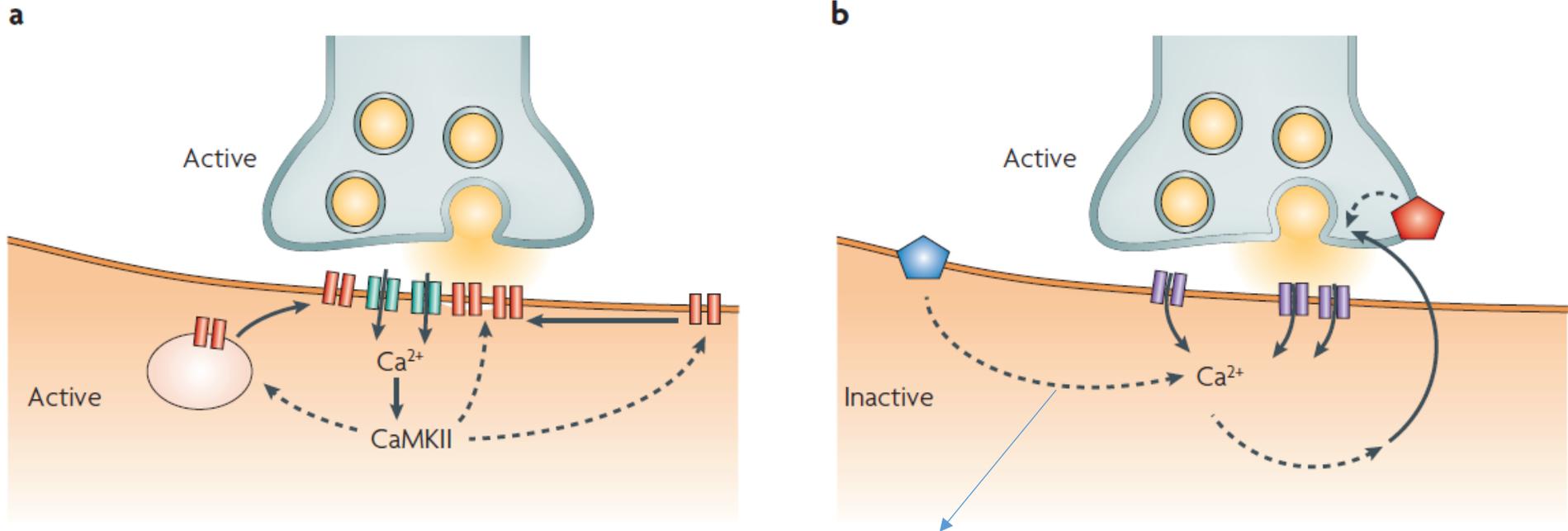
THE "anti-HEBBIAN" LTP

Calcium permeable AMPA receptors

- More abundant in interneurons
- Do not express the GluR2 subunit (-> permeable to calcium)
- At depolarized potential, the pore of the channel is blocked by polyamines (spermine, spermidine) present in the cytoplasm of neurons
- Also called "rectifying AMPAr"

a
Presynaptic cell
Glu   Mg²⁺
CI-AMPAR   NMDAR
Postsynaptic cell
Na⁺
Vm at rest

CALCIUM IMPERMEABLE RECEPTORS: These contain GluR2 subunits

Presynaptic cell
CI-AMPAR   Glu   NMDAR   Mg²⁺
Postsynaptic cell   +   +   +   +
Na⁺   Ca²⁺
Vm depolarized

b
Presynaptic cell
Glu
CP-AMPAR
Postsynaptic cell
Na⁺ Ca²⁺
Vm at rest

Presynaptic cell
CP-AMPAR   Glu
Postsynaptic cell   +   +   +
Polyamines
Vm depolarized

c   CI-AMPAR

d   CP-AMPAR

Kullmann & Lamsa, Nature Reviews, 2007

Role of metabotropic glutamatergic receptors in LTP

"HEBBIAN" LTP ⟷ "anti-HEBBIAN" LTP

Legend:
- NMDAR
- CI-AMPAR
- CP-AMPAR
- mGluR1
- mGluR7

- G-proteins
- Activation of phospholipase C
- Calcium release from internal stores