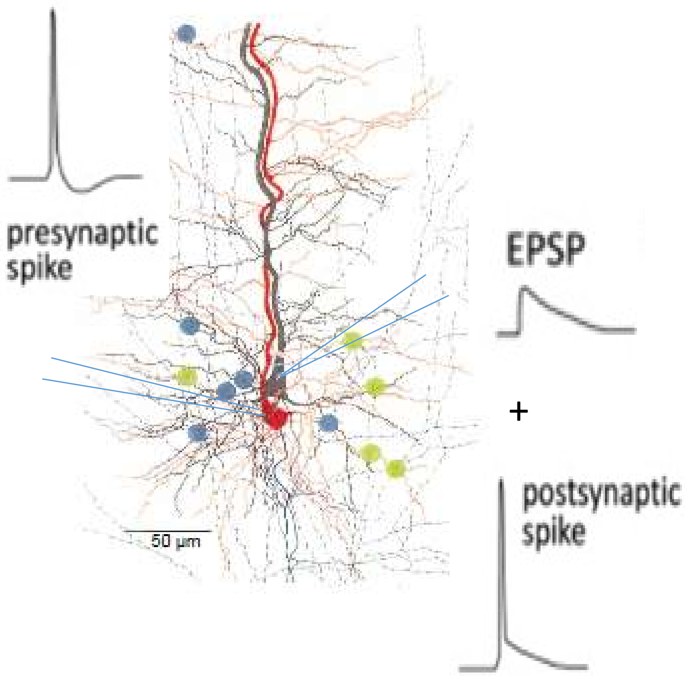
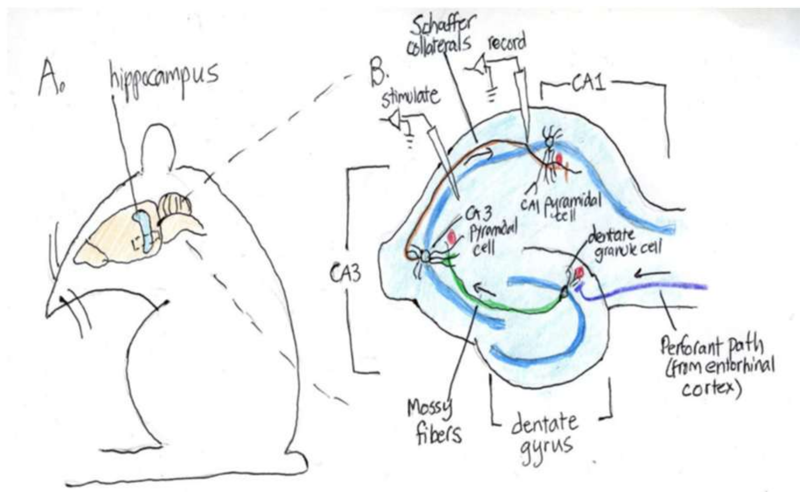
<u>Master in Neuroscience</u>

MOLECULAR NEUROPHYSIOLOGY -lesson 1-



Prof. G. Cellot



Lesson 1	**Thursday 16th October h11-13**
Lesson 2	**Friday 17th October h11-13**
~~**Lesson 3**~~	~~**Monday 20th October h11-13**~~
Lesson 4	**Tuesday 21rst October h9-11**
Lesson 5	**Wednesday 22nd October h11-13**
Lesson 6	**Thursday 23rd October h11-13**
Lesson 7	**Monday 27th October h11-13**
Lesson 8	**Tuesday 28th October h9-11**

Email contact:
giada.cellot@units.it

Next lesson of Molecular neurophysiology with Prof Lorenzon scheduled for Tuesday 4th November

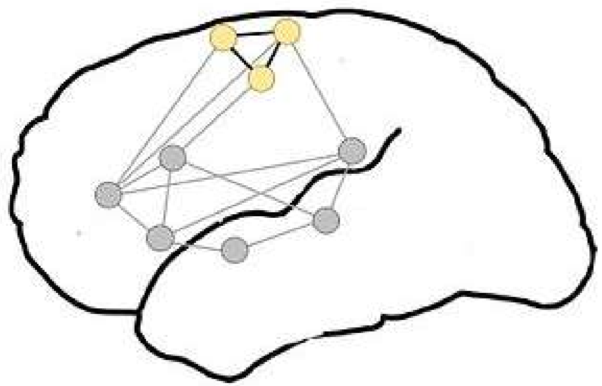
The plasticity of neuronal circuits: synaptic changes and co-related behavioral aspects

- Long term plasticity (LTP) in theory and in practice: from Hebb's postulate to experimental evidence
- Hebbian (NMDA mediated) LTP - Spike timing dependent plasticity
- Anti-Hebbian LTP
- Other forms of synaptic plasticity
- Synaptic plasticity during the development: when GABA is depolarizing
- Functional implications of hippocampal synaptic plasticity: memory and learning

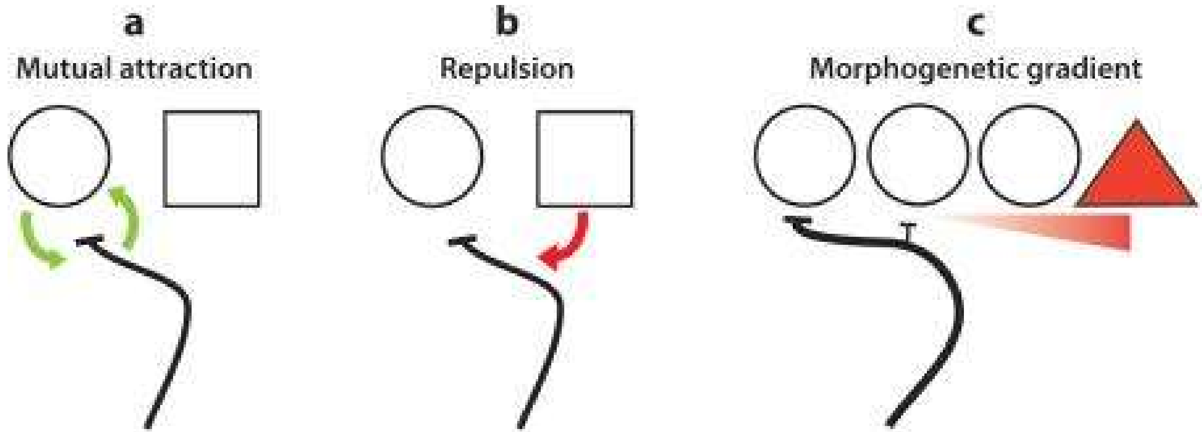
- Synaptic plasticity in the amygdala
- Associative learning: the fear conditioning paradigm
- Synaptic correlates of amygdala synaptic plasticity
- Optogenetic manipulation of synaptic plasticity: impact on behavior
- Fear extinction (anxiety disorders)

- Zebrafish as alternative model for studying synaptic plasticity

Human brain: genetically hard wired structure formed by 10^{12} of neurons and 10^{15} of synapses



SYNAPTIC SPECIFICITY



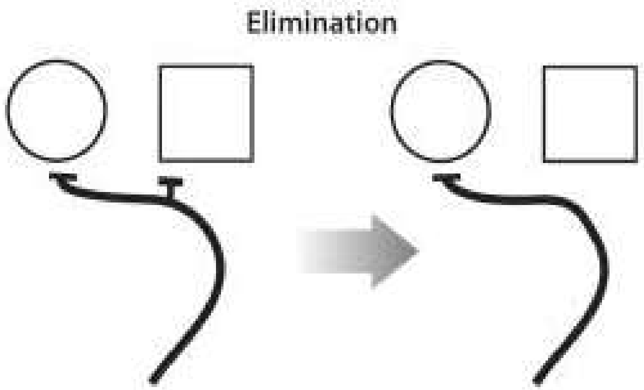
<u>Adhesion molecules</u>
(cadherins, immunoglobulin-superfamily proteins, Neurexins- Neuroligins)

<u>Graded diffusible signals</u>
(secreted by neurons/glial cells)
- BDNF, Glial Cell Line-Derived Neurotrophic Factor, class 3 semaphorins,…)

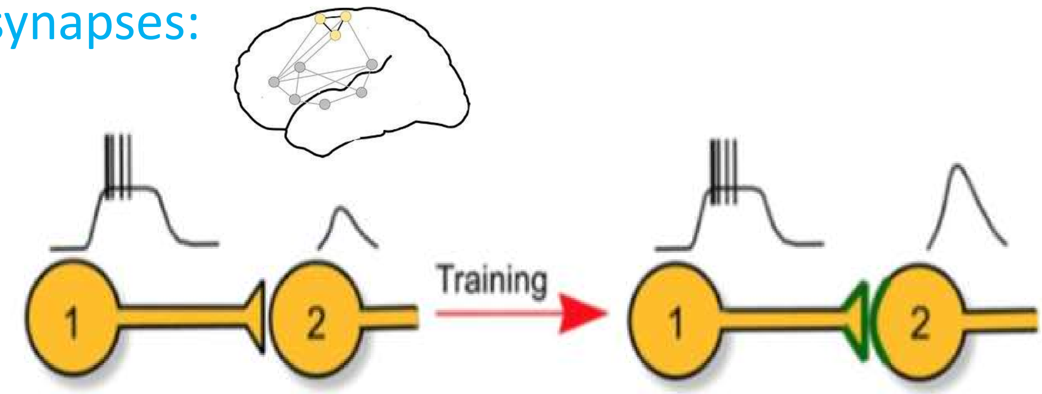
Modified from Shen & Scheiffele, 2010, doi:10.1146/annurev.neuro.051508.135302.

NEURAL ACTIVITY CARVES CONNECTIVITY DURING THE MATURATION OF THE SYNAPTIC CIRCUIT

At specific synapses:



Synaptic elimination is driven by the lack of neuronal activity



Synaptic reinforcement is obtained by repeated activation of the synapse

SINAPTIC PLASTICITY:

<u>EXPERIENCE DEPENDENT </u>change

in the EFFICACY of synaptic communication between neurons

- *synaptic potentiation vs depression*
- *short term vs long term plasticity*

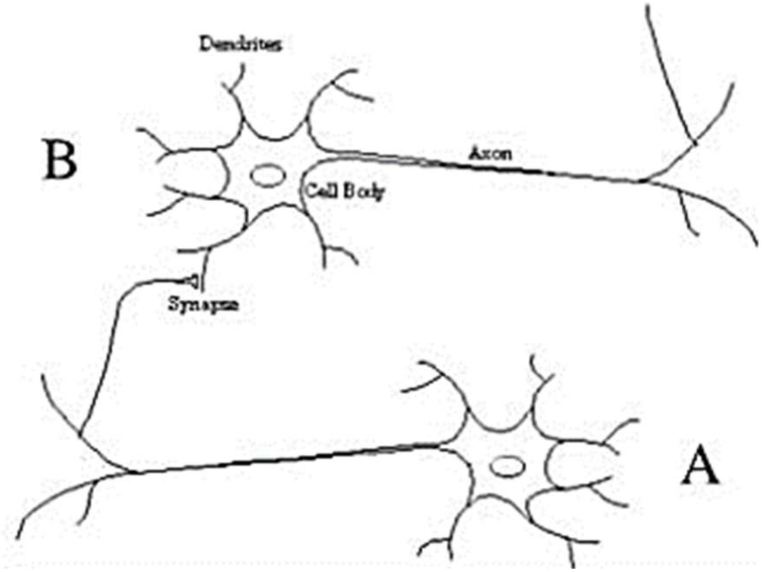
→ Long term potentiation (LTP)

A short history of Long Term Potentiation (LTP)

Hebb's postulate

"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

A short history of Long Term Potentiation (LTP)

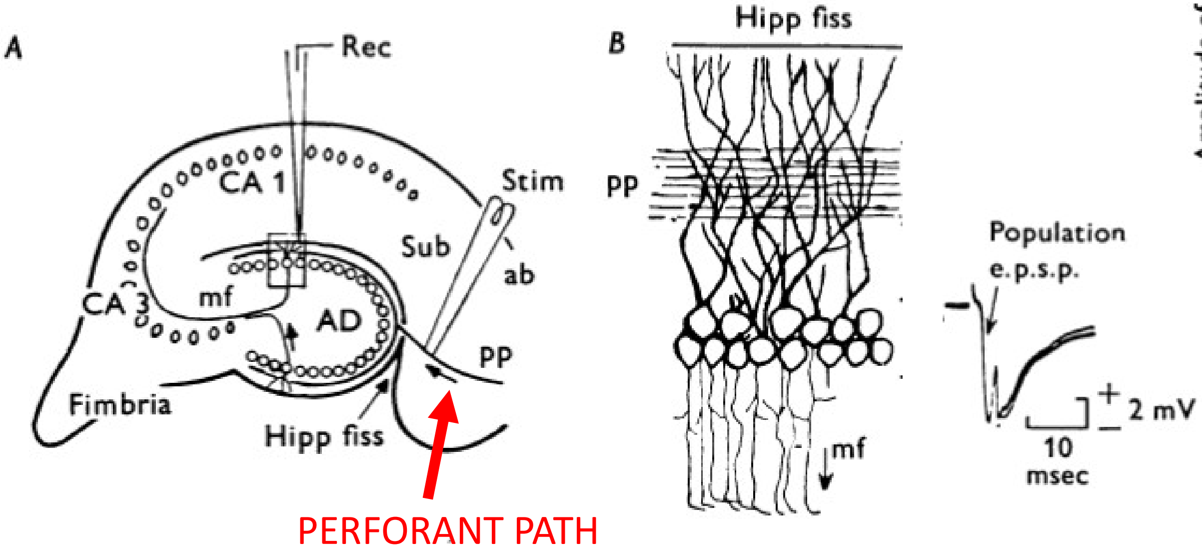
… first experimental evidence of synaptic plasticity arrived 20 years later

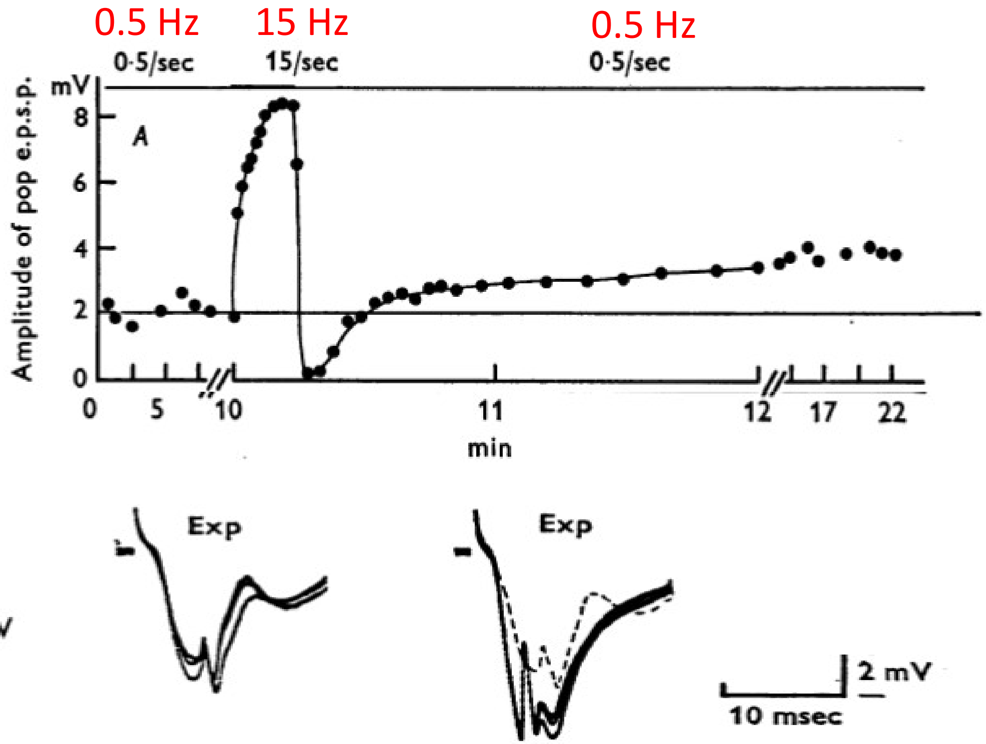
J. Physiol. (1973), **232**, pp. 331–356 331
With 12 text-figures
Printed in Great Britain

LONG-LASTING POTENTIATION OF SYNAPTIC TRANSMISSION IN THE DENTATE AREA OF THE ANAESTHETIZED RABBIT FOLLOWING STIMULATION OF THE PERFORANT PATH

BY T. V. P. BLISS AND T. LØMO
From the National Institute for Medical Research, Mill Hill, London NW7 1AA and the Institute of Neurophysiology, University of Oslo, Norway

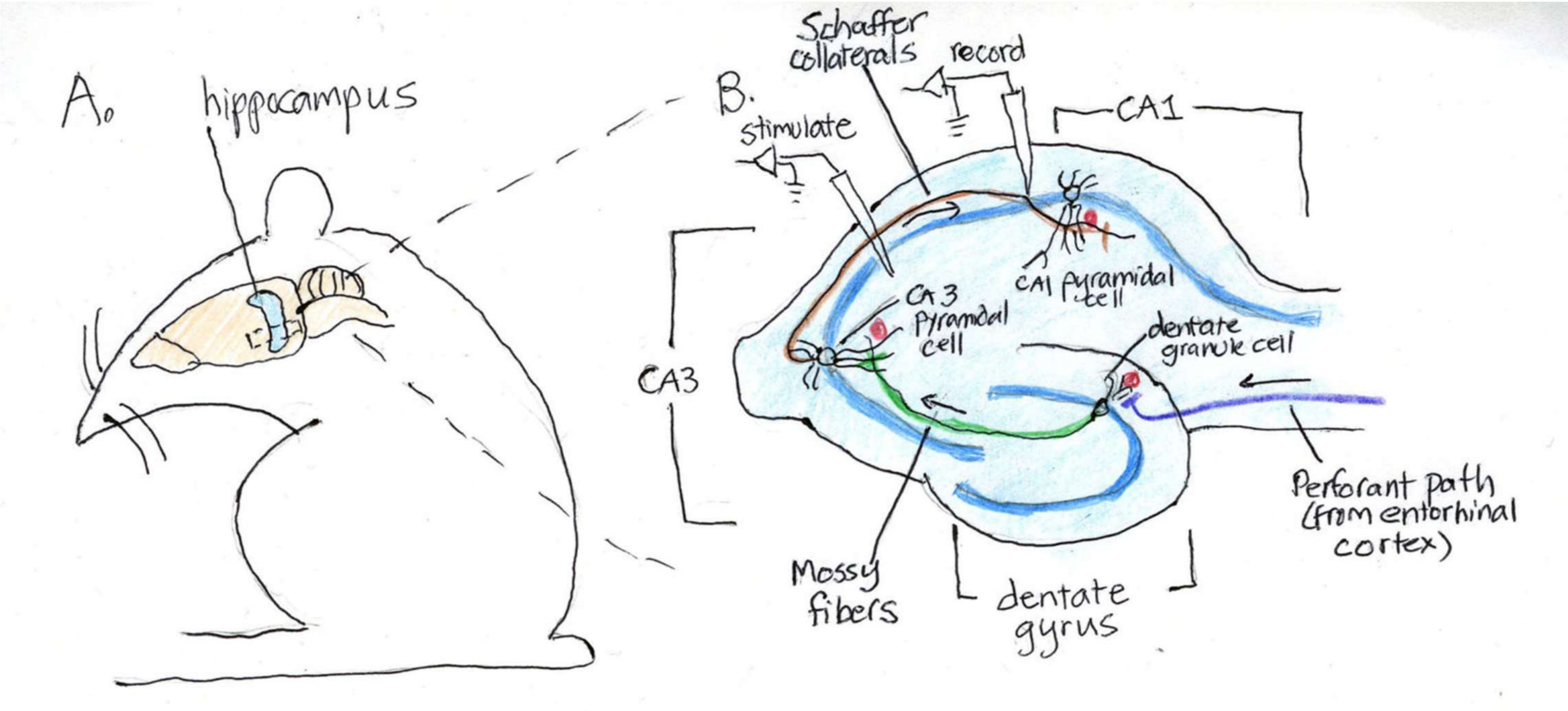
High frequency stimulation of afferent fibers to dentate gyrus produced long lasting changes in synaptic activity





The RODENT HIPPOCAMPUS as a reliable system for studying synaptic plasticity

- SIMPLE CIRCUITRY: three sequential pathways
- LAMINAR CYTOARCHITECTURE: each region with discrete cell body layers

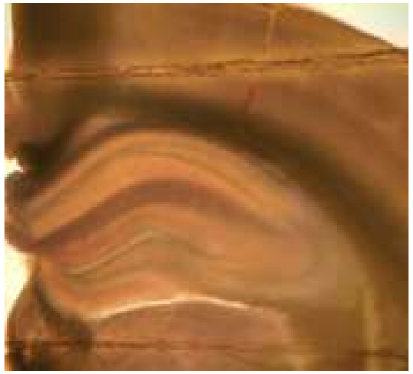


Ho et al., Science 2011, 334(6056): 623–628. doi:10.1126/science.1209236.

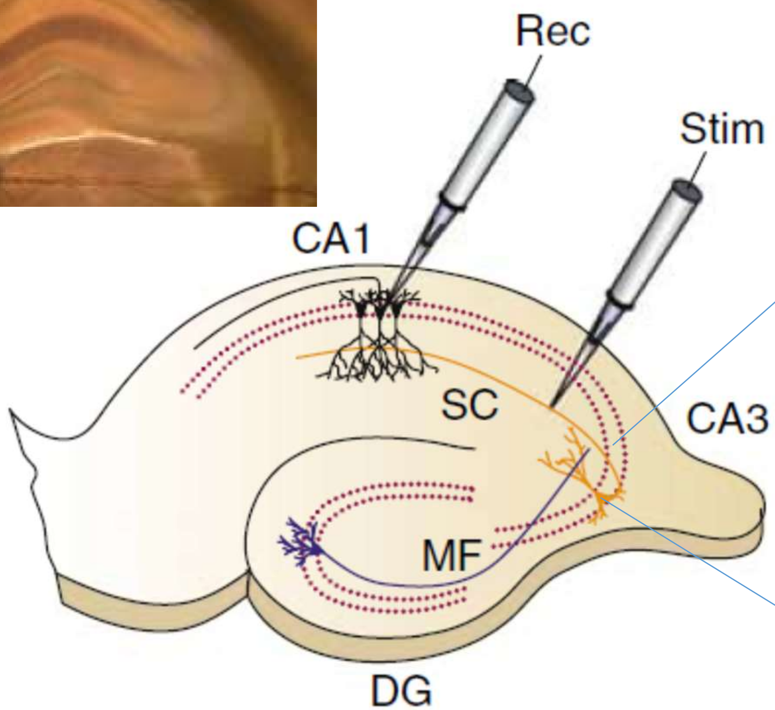
Synaptic plasticity in the hippocampus can be studied *in vitro*

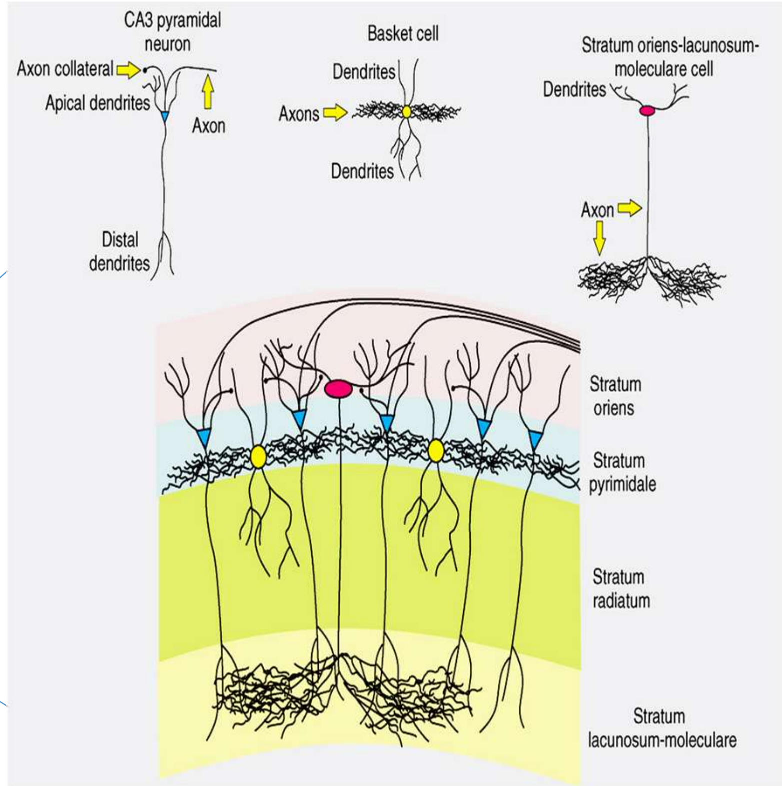
HIPPOCAMPAL SLICES

- 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

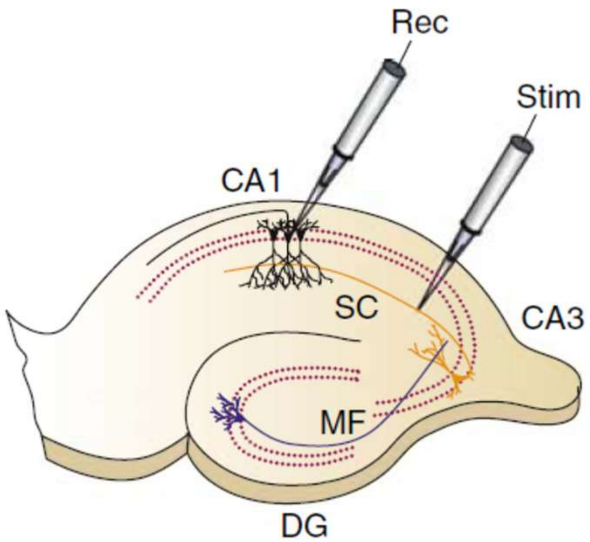


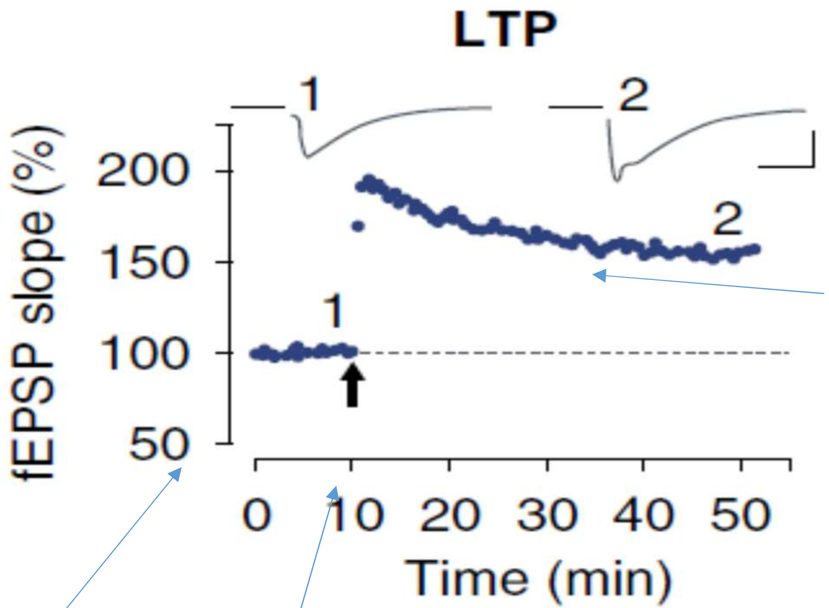


Citri & Malenka, Neuropsychopharmacology REVIEWS (2008) 33, 18–41

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

Phases of a LTP experiment





3.Post-induction responses
(low frequency stimulation 0.5 Hz)

1.Baseline responses
(low frequency stimulation 0.5 Hz)

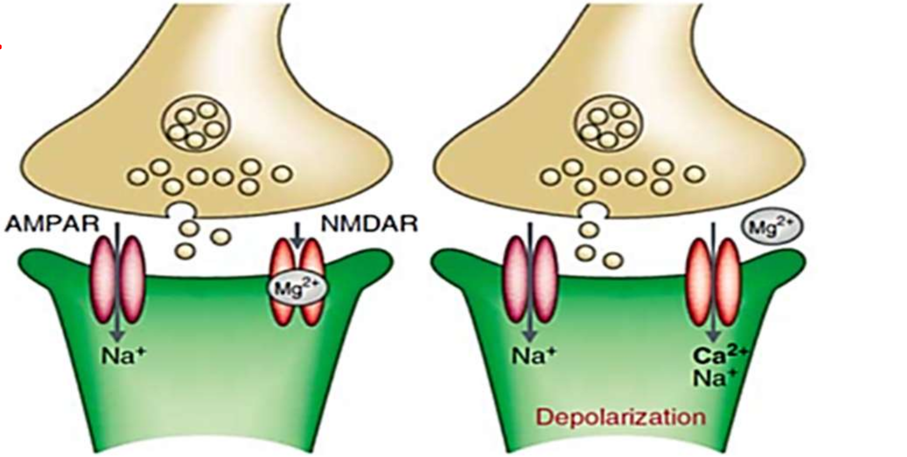
2. Protocol to induce potentiation = INDUCE STRONG STIMULATION OF SYNAPSES

- Electrical stimulation: Tetanus: 1s @100 Hz; or theta burst stimulation: interval of 200 ms)
- Chemical stimulation (cLTP): KCl, glutamate, AMPA

- LTP as an experience driven change in synaptic efficacy
- LTP can be induced in the hippocampus *in vivo* through electrical stimulation
- LTP can be induced *in vitro* in hippocampal slices

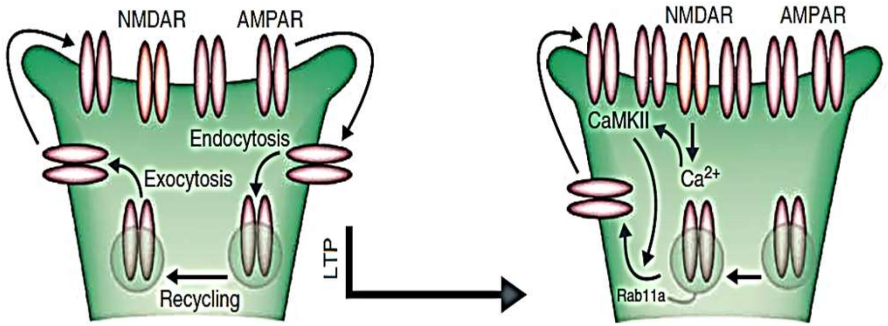
HEBBIAN LTP mechanisms

1.



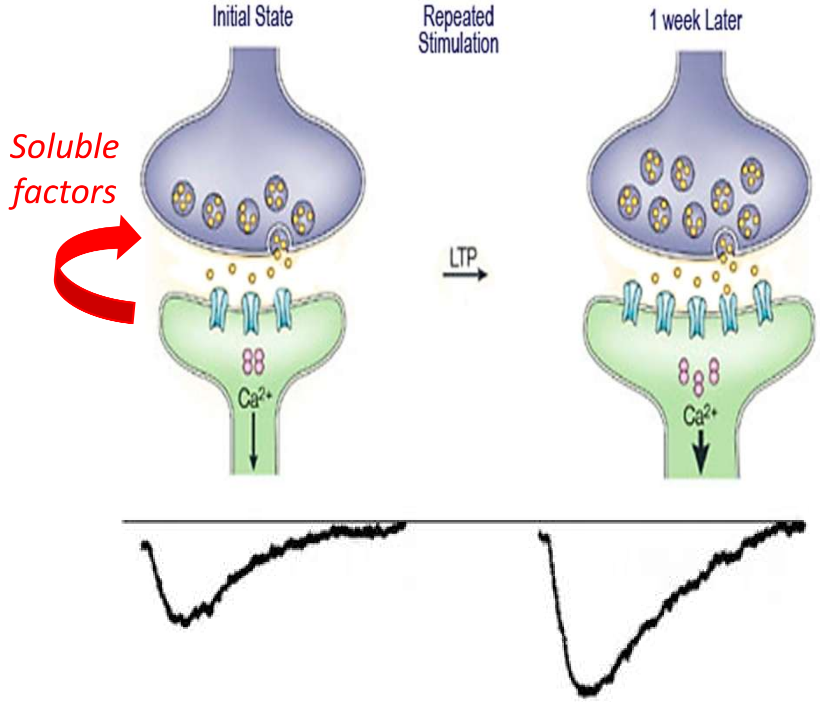
Upon repetitive stimulation, AMPA r activation leads to massive depolarization of the postsynaptic cell. This activates NMDA r

2.



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

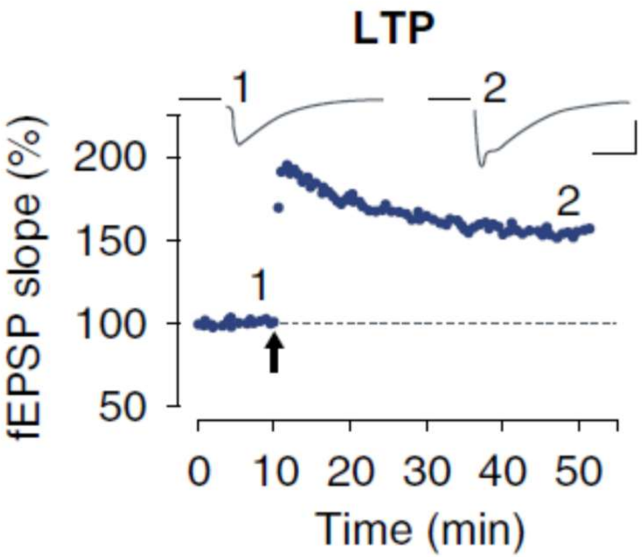
3. Increase in the efficacy of synaptic communication through pre- and post-synaptic mechanisms



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

Modified from Citri & Malenka, Neuropsychopharmacology REVIEWS (2008) 33, 18–41

Time course of LTP



- Rapid onset (seconds ÷ few minutes)
- Decay: - it can last hours (experimental limitation in acute slices) or days in alive animals

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

Potentiation is downregulated after few seconds

Kinase inhibitors

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

Potentiation is downregulated after 5-6 hours

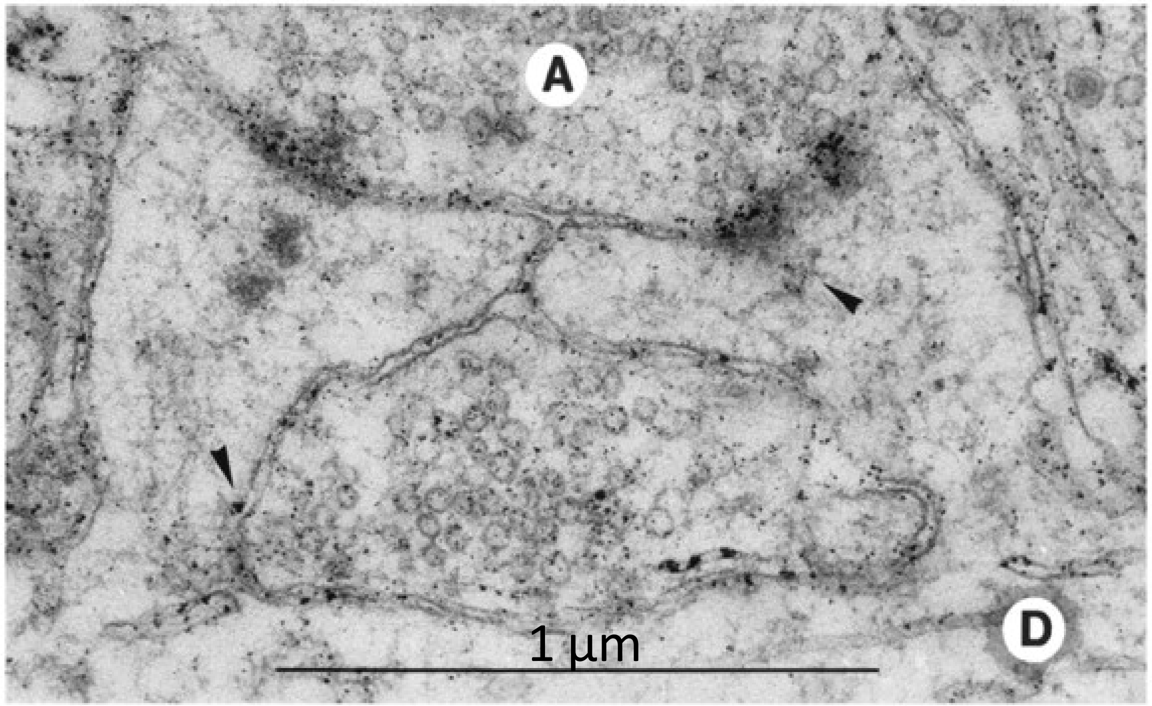
Transcription/translation inhibitors

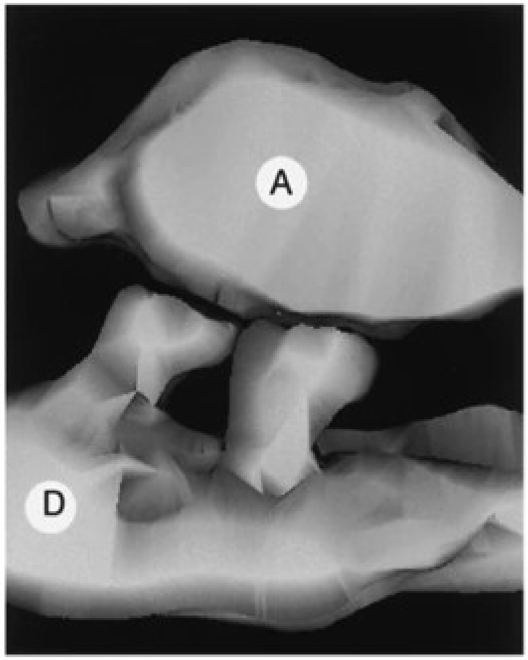
LTP induced morphological modification of synapses

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

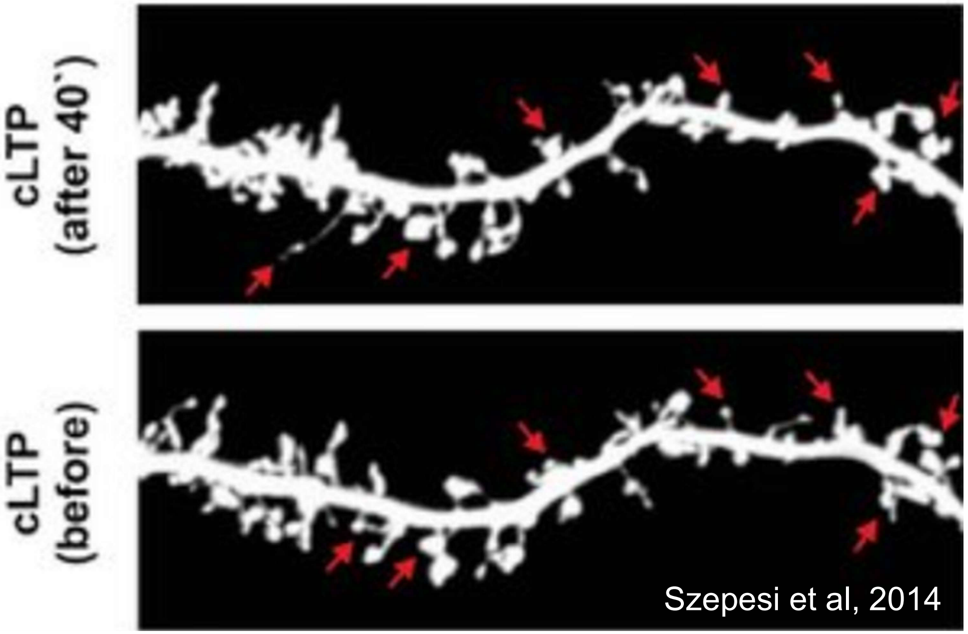




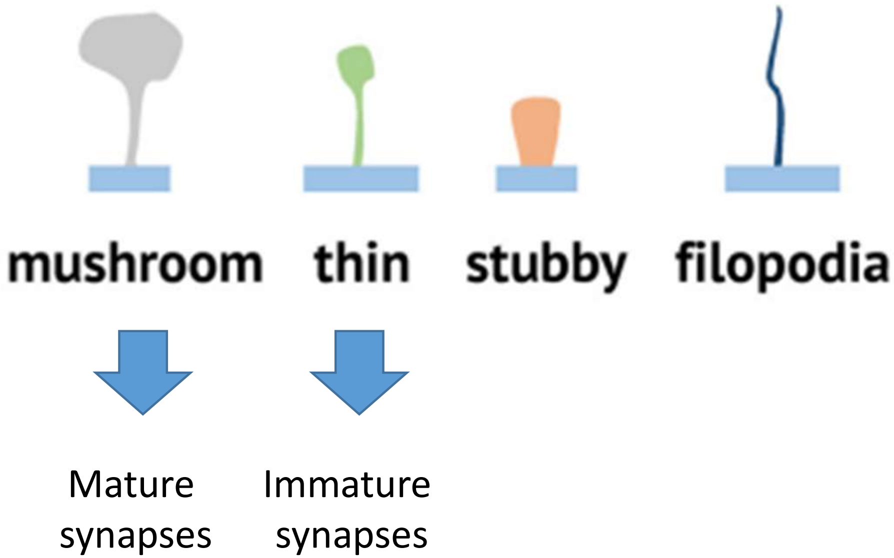
Toni et al, Nature, 1999

DENDRITIC SPINES

Hippocampal cultures exposed to chemical LTP



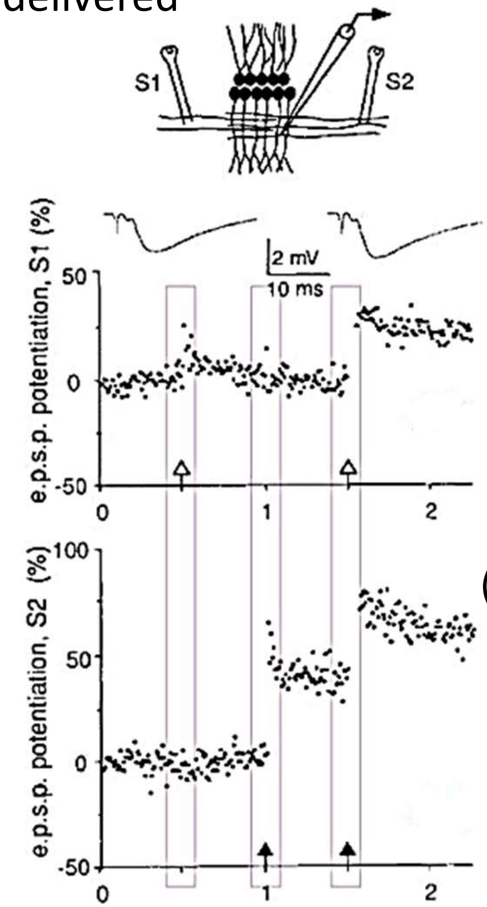
Classification
based on head /neck size



Pchitskaya & Bezprozvanny, Front Syn Neurosci, 2020
doi: 10.3389/fnsyn.2020.00031

Properties of LTP

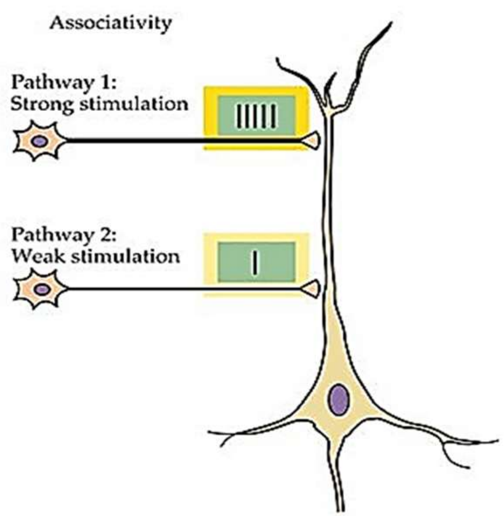
1. INPUT SPECIFICITY: LTP occurs specifically in the pathway in which the high frequency stimulation was delivered



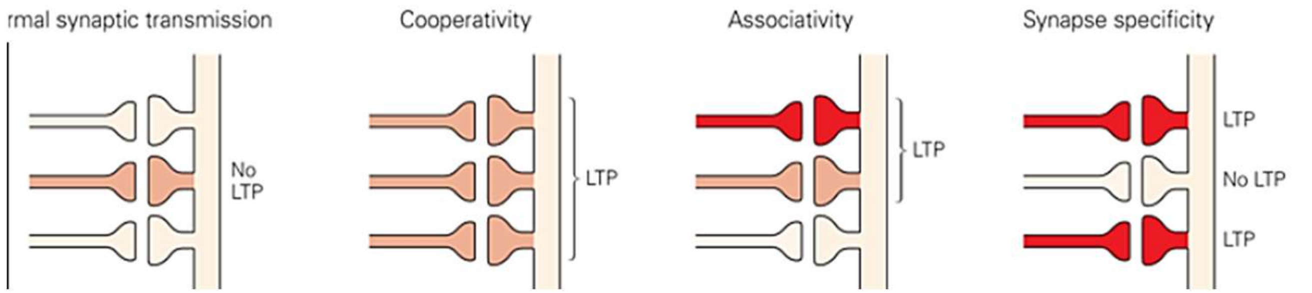
CA1 region
2 stimulation electrodes
= 2 sets of fibers
(stratum oriens or stratum radiatum

Bliss & Collingridge, 1993

2. ASSOCIATIVITY: capacity to potentiate a weak input when it is activated in association with a strong one

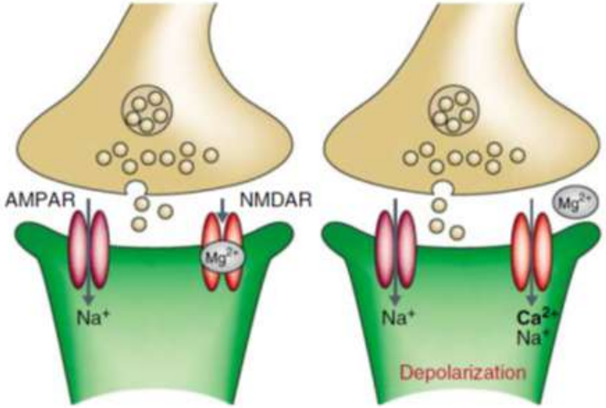


3. COOPERATIVITY: LTP can be activated by coincident activation of a critical number of synapses…

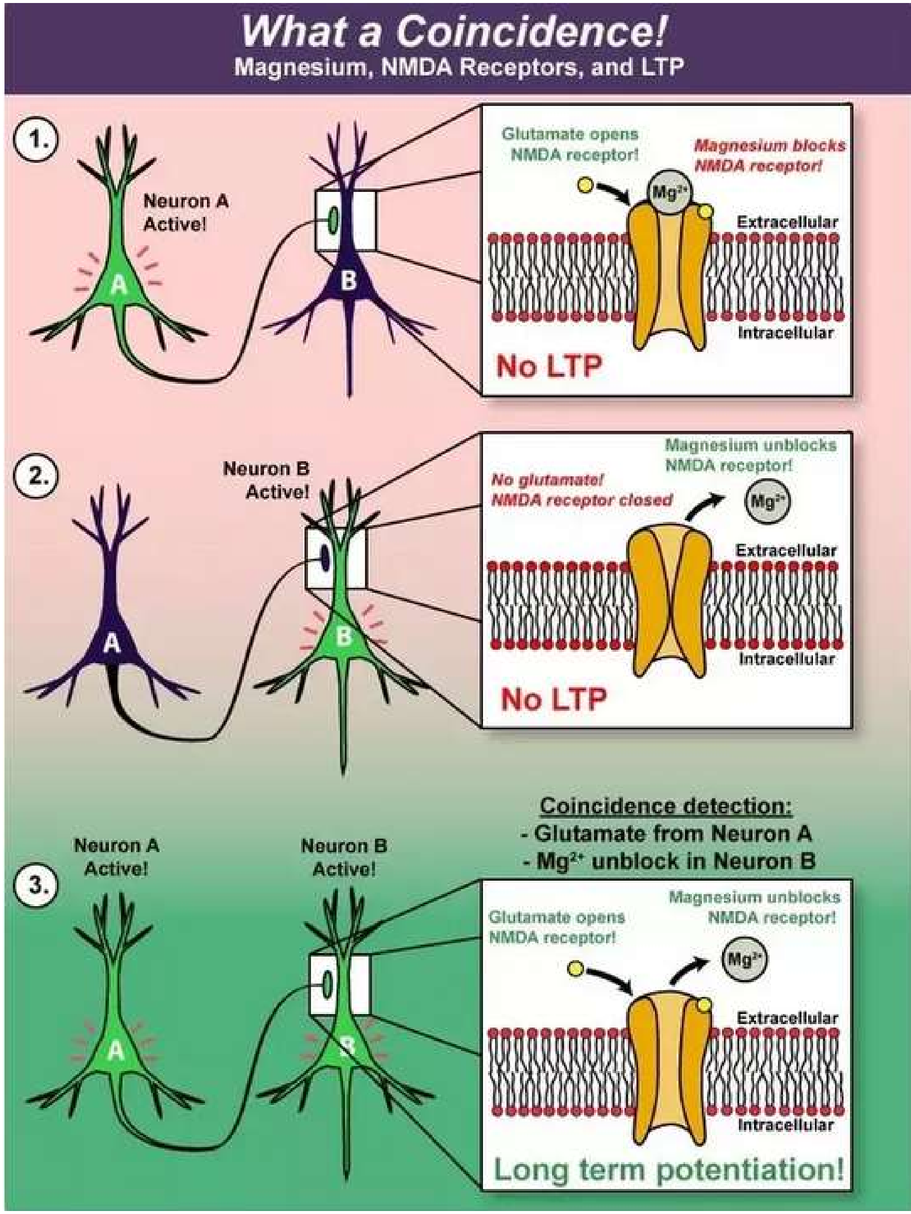


(…or by a sufficient depolarization of the postsynaptic neuron)

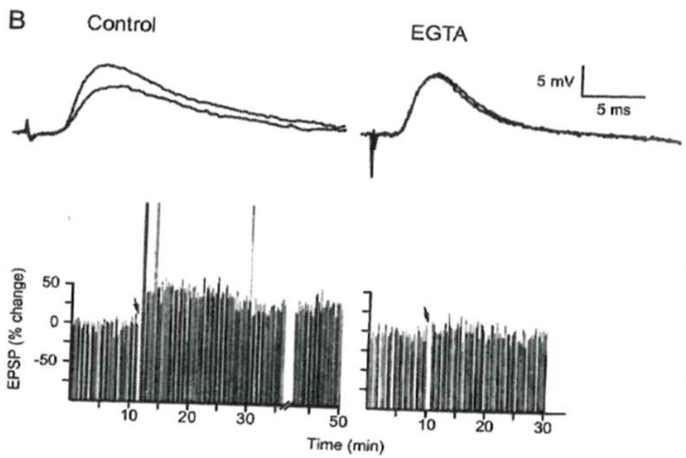
NMDA receptor as detector of coincidence



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 receptors antagonists or Buffering intracellular calcium with EGTA block the induction of LTP

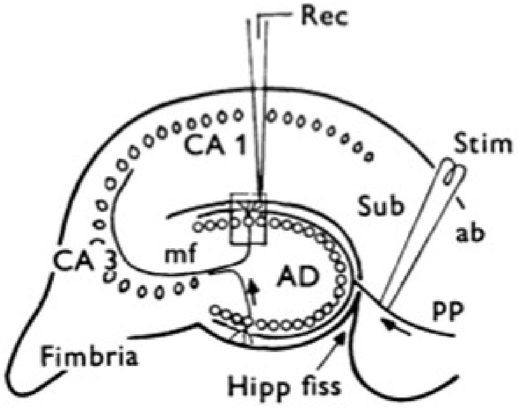


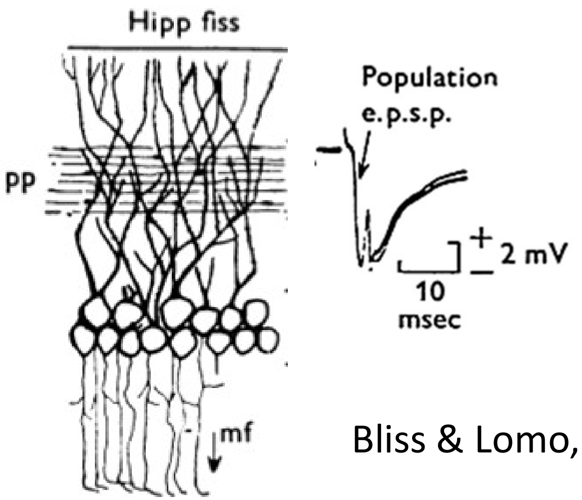
Lynch et al., 1983

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

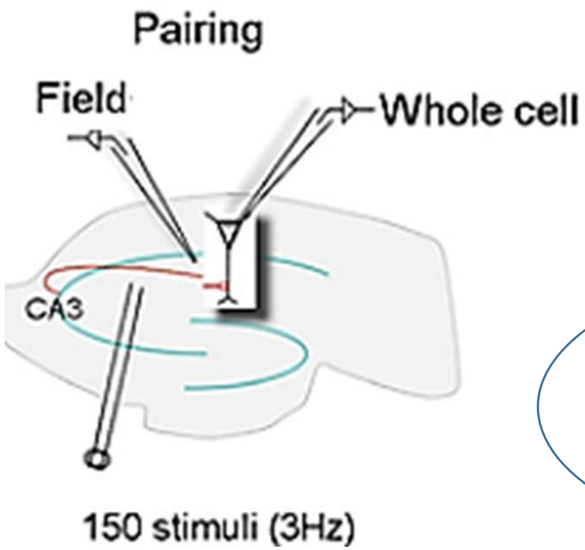
LTP can be induced by PAIRING pre- and post-synaptic NEURONS

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

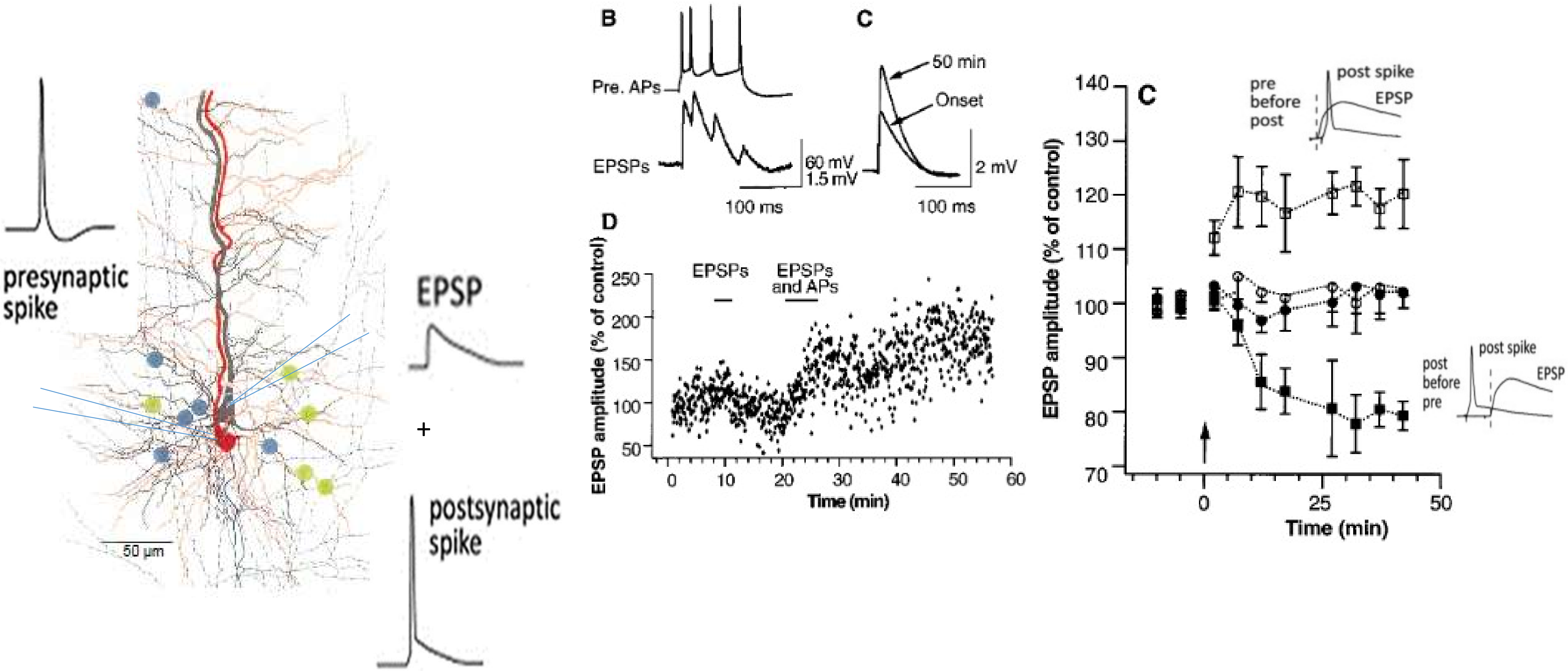


PATCH CLAMP TECHNIQUE

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

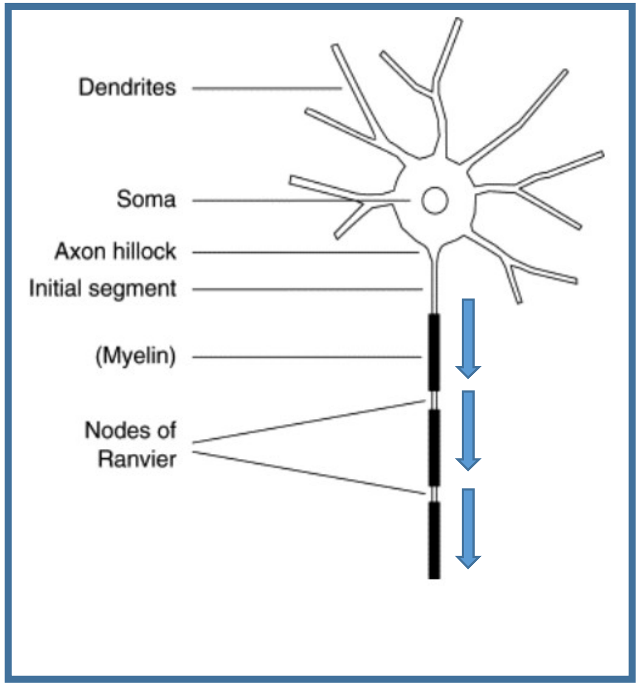
Spike Timing-Dependent Plasticity (STDP)

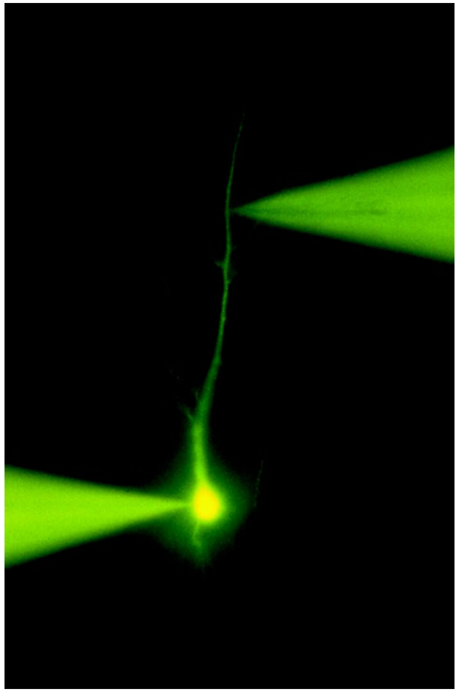
The pairing procedure becomes very efficient when postsynaptic neuron fires later respect to presynaptic cell (in a time window <20 ms)

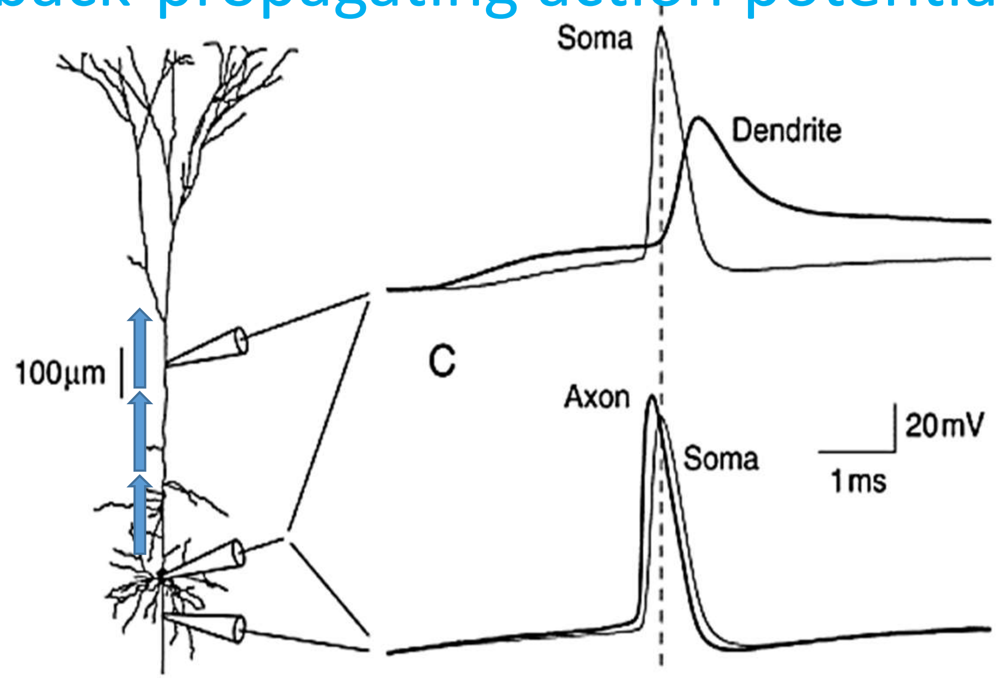


Modified from Markram et al., Science, 1997

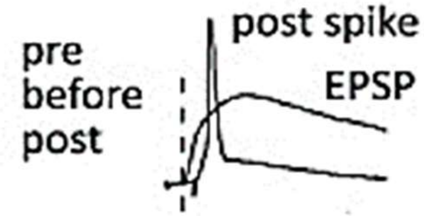
Spike timing dependent plasticity and back-propagating action potentials



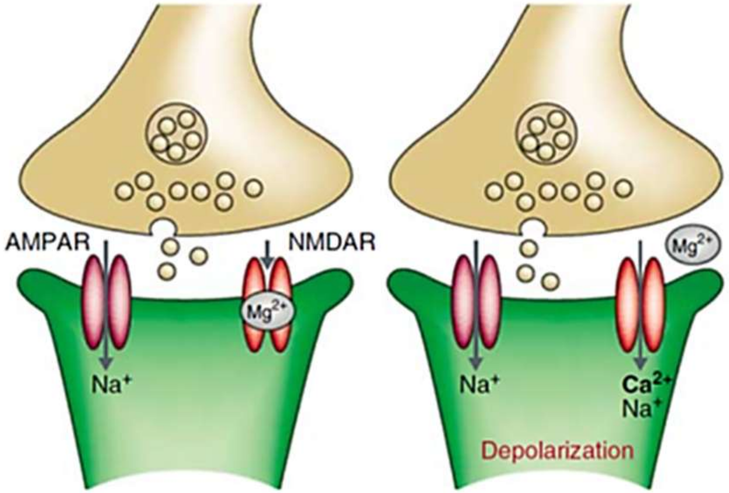




Stuart et al., Trends Neuroscience, 1997



- 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



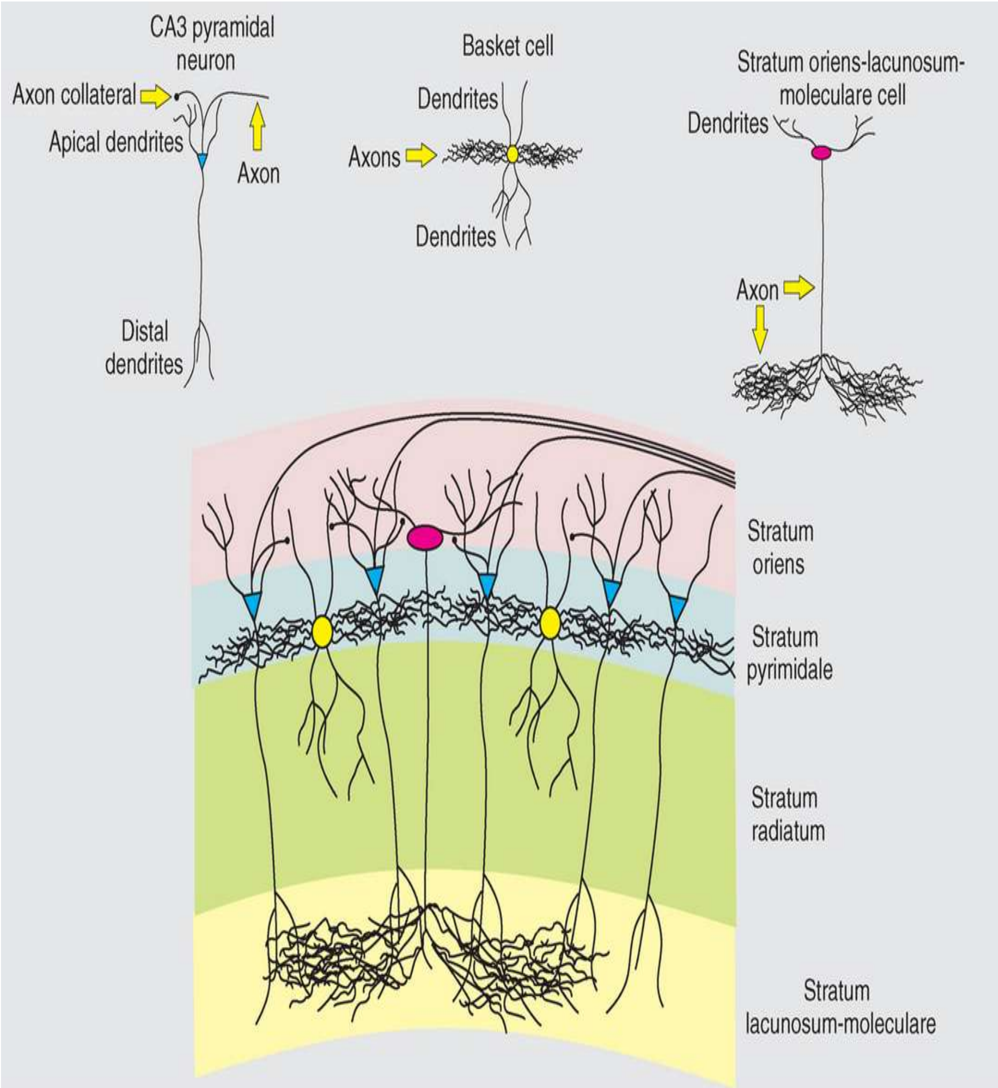
“HEBBIAN” LTP

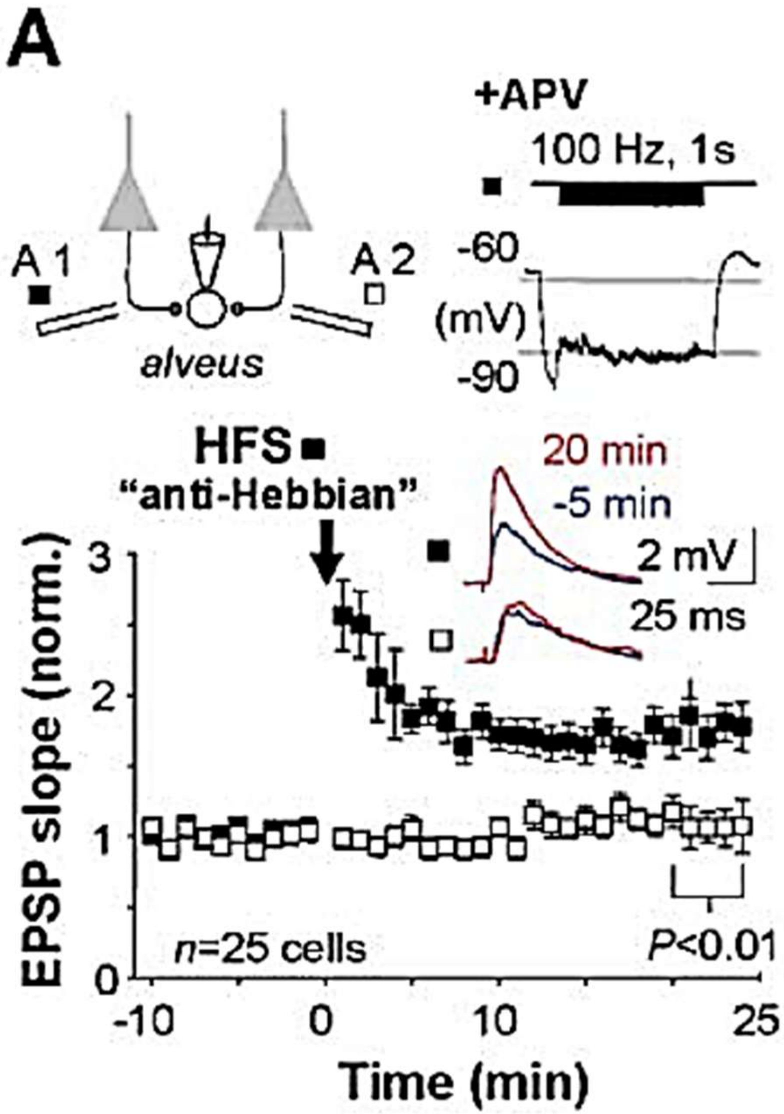
“anti-HEBBIAN” LTP

Plasticity that occurs when pre-synaptic cell firing coincides with postsynaptic neuron depolarization

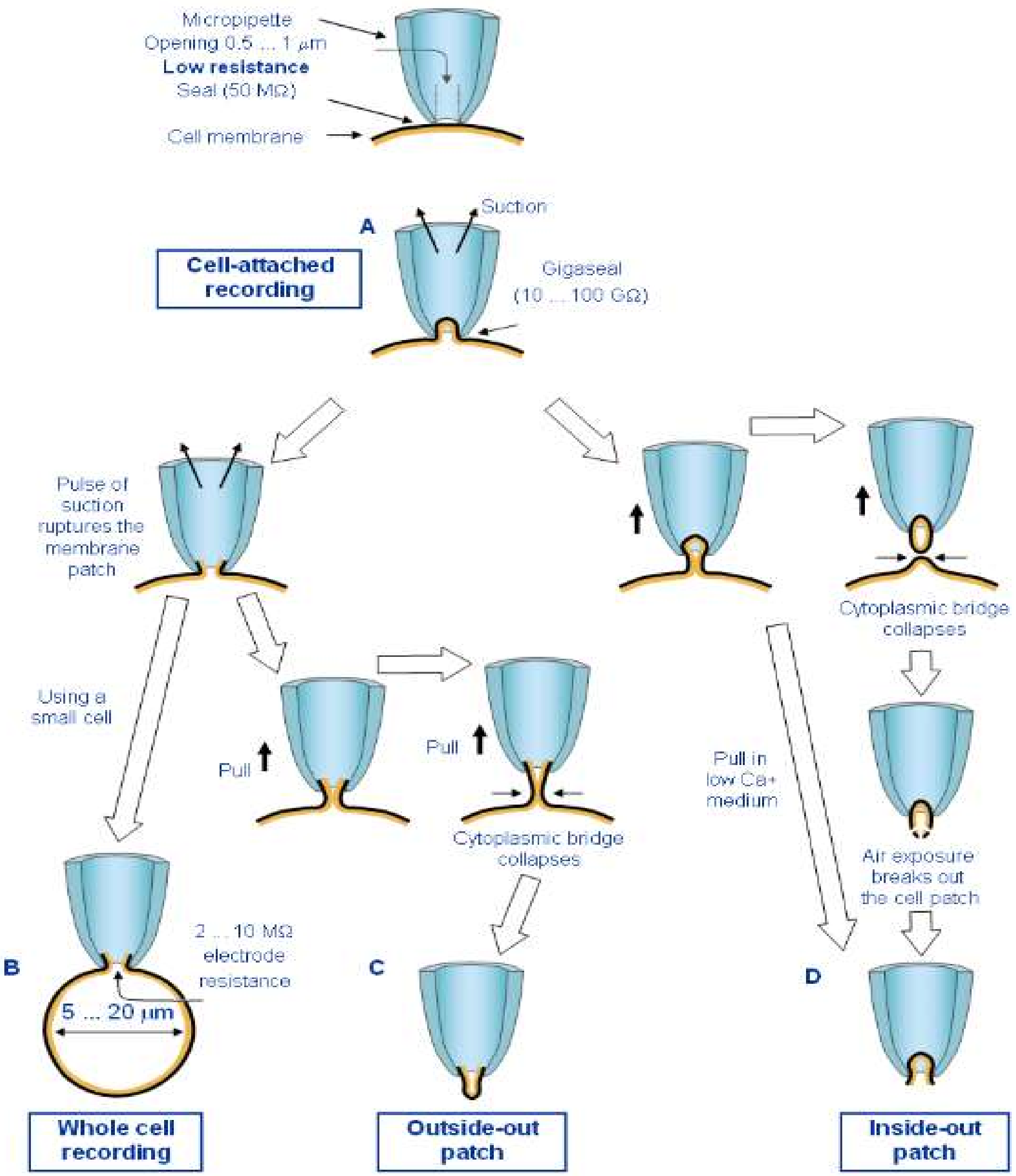
Plasticity that occurs when pre-synaptic cell firing coincides with postsynaptic neuron hyperpolarization (prevented by postsynaptic depolarization)

THE "anti-HEBBIAN" LTP

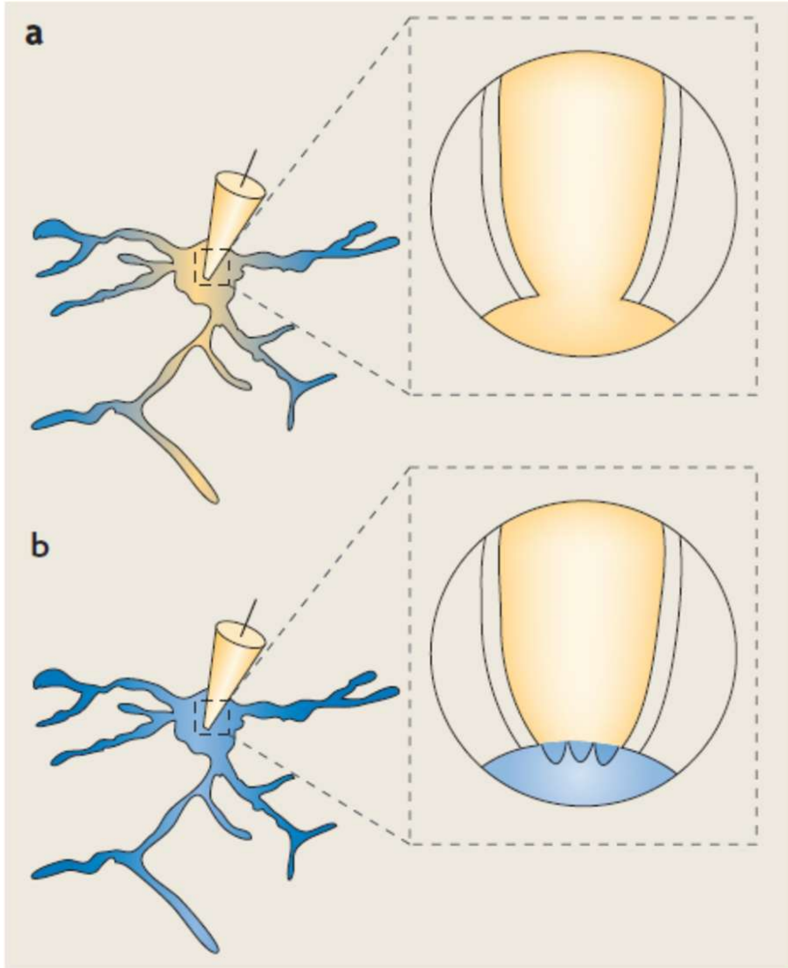




Lamsa et al., Science, 2007

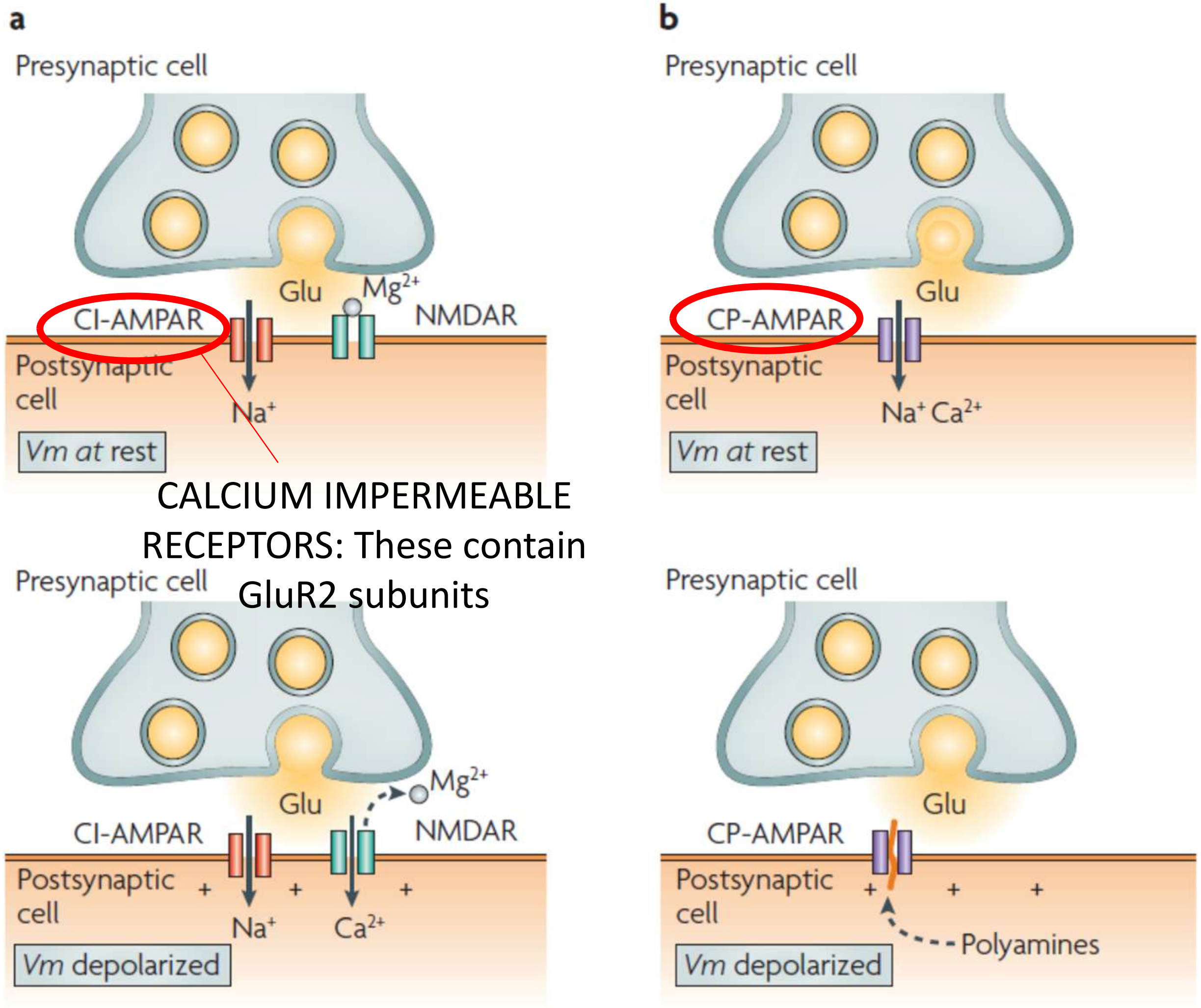


WHOLE CELL CONFIGURATION



PERFORATED PATCH CONFIGURATION
(by using antibiotics such as gramicidin)
Not to dialyze intracellular milieu

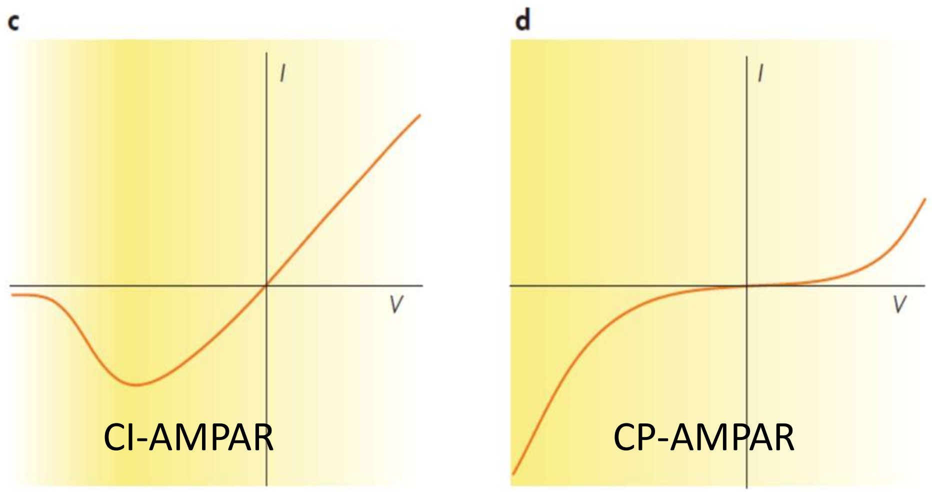
THE "anti-HEBBIAN" LTP



Kullmann & Lamsa, Nature Reviews, 2007

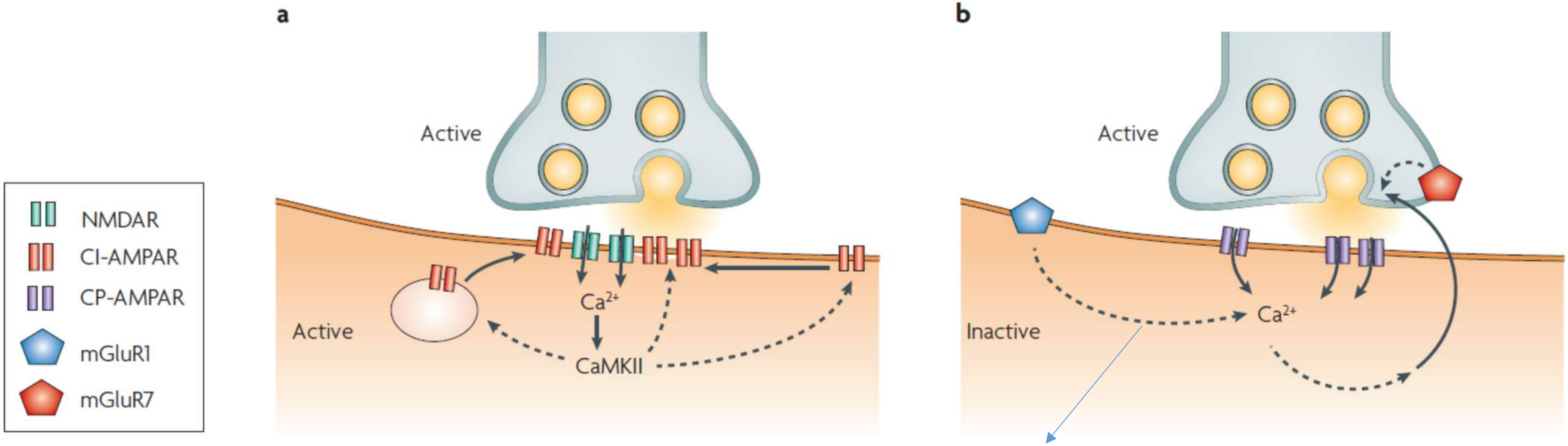
Calcium permeable AMPA receptors

- 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"



Role of metabotropic glutamatergic receptors in LTP

“HEBBIAN” LTP ⟷ “anti-HEBBIAN” LTP



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

Kullmann & Lamsa, Nature Reviews, 2007