Long term synaptic plasticity in the hippocampal cortex

Master 2 BIP, mention Neurosciences

Hippocampus: from cell to physiology and human pathology
20023-2024

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- Long term synaptic plasticity: what for, how and where?
- Long term potentiation: Bliss & Lomo 1973
- Induction and expression mechanisms of hippocampal LTP
- Hippocampal LTP, learning and memory

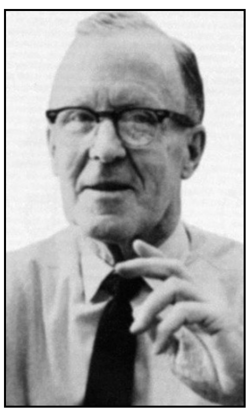
Long term synaptic plasticity: what for?

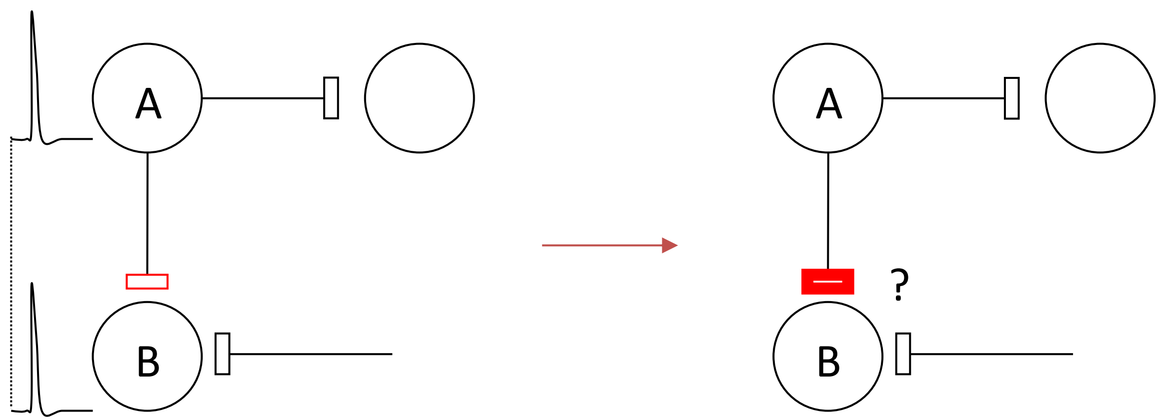
- <u>Neuronal substrate of learning and memory</u>

Lashley (1924) : "among the many unsubstantiated beliefs concerning the physiology of the learning process, none is more widely prevalent than the doctrine that the passage of the nerve impulse through the synapse somehow reduces synaptic resistance and leads to the fixation of a new habit"

Lorente de No (1938) : Anatomical demonstration of *reverberating circuits*

Hebb (1949) : *"The organization of behavior" : 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.*





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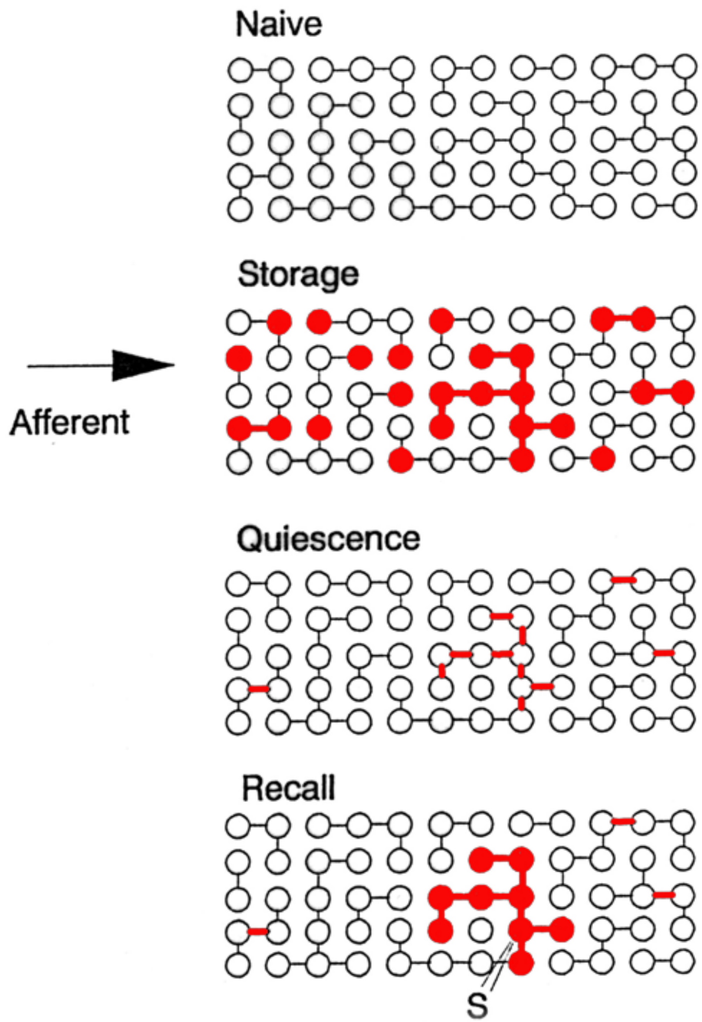
Long term synaptic plasticity and memory storage/retrieval



Long term synaptic plasticity: where?

- Invertebrate neuronal networks

Kandel & Tauc (1964): *persistant and hetero-synaptic facilitation* in the abdominal ganglion of Aplysia (Aplysia depilans)

- Vertebrate neuronal networks

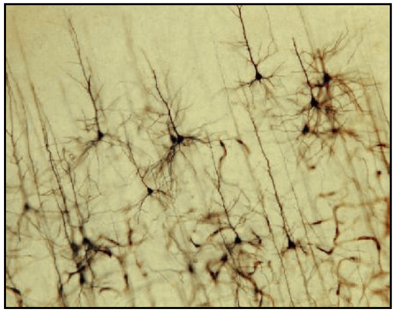
Eccles & Rall (1950): Post-tetanic potentiation at the neuromuscular junction (a few minutes)

Bliss & Burns (1968): Attempts to induce long term plasticity in the cat cortex: too complex preparation

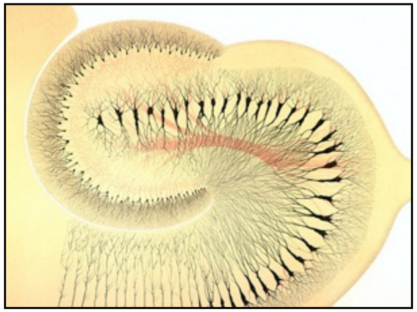
Long term synaptic plasticity: why hippocampus?

- Brain structure (more likely involved in learning and memory processes)

- Better laminar organization than neocortex



neocortex

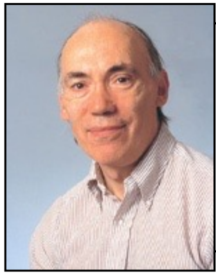


hippocampus

- The HM patient: involvement of the hippocampus in anterograde memory

  (Scoville & Milner, 1957)

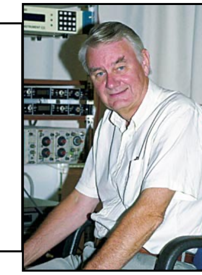
Long term synaptic plasticity: Bliss & Lømo 1973



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

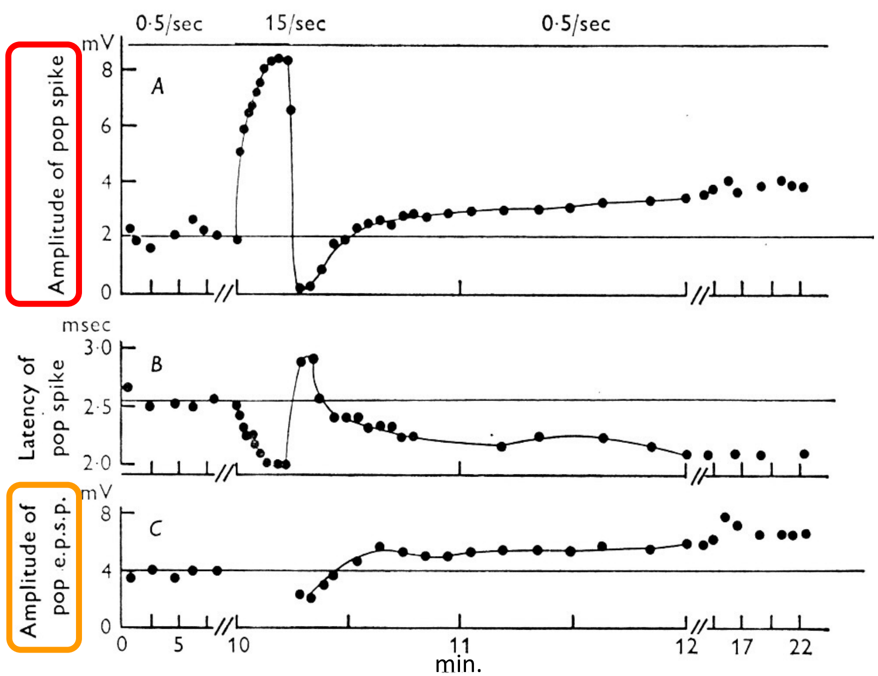
LONG-LASTING POTENTIATION OF SYNAPTIC TRANSMISSION IN THE DENTATE AREA OF THE ANAESTHETIZED RABBIT FOLLOWING STIMULATION OF THE PERFORANT PATH

By T. V. P. BLISS and T. LØMO



Long term synaptic plasticity: Bliss & Lømo 1973

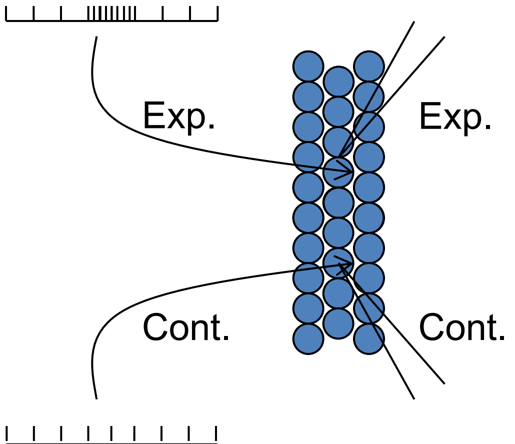
Brief period of high frequency activity

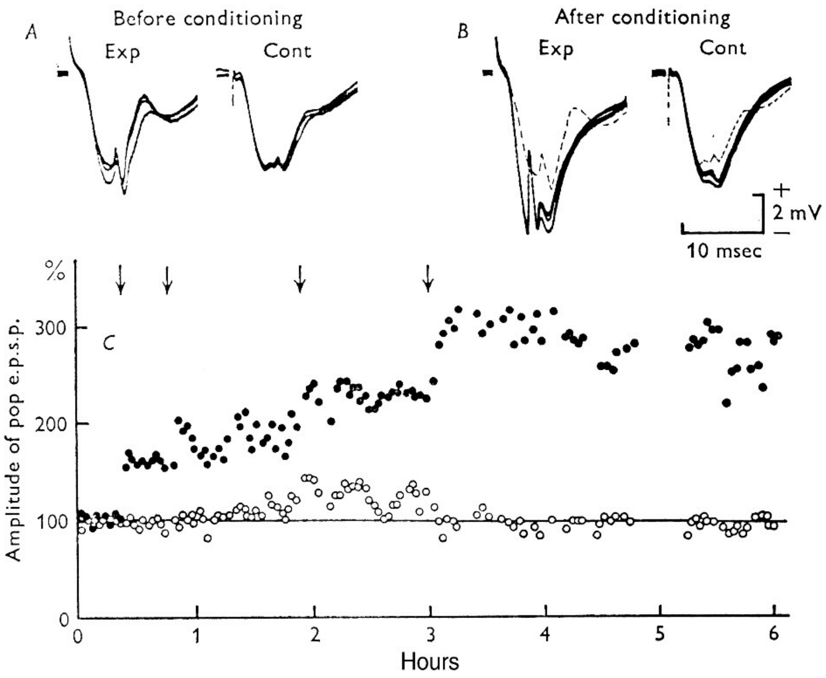


→ A brief period of high frequency afferent stimulation induces a persistent potentiation:

- of the postsynaptic response
- of the discharge of postsynaptic cells (amplitude ↗ while latency ↘)

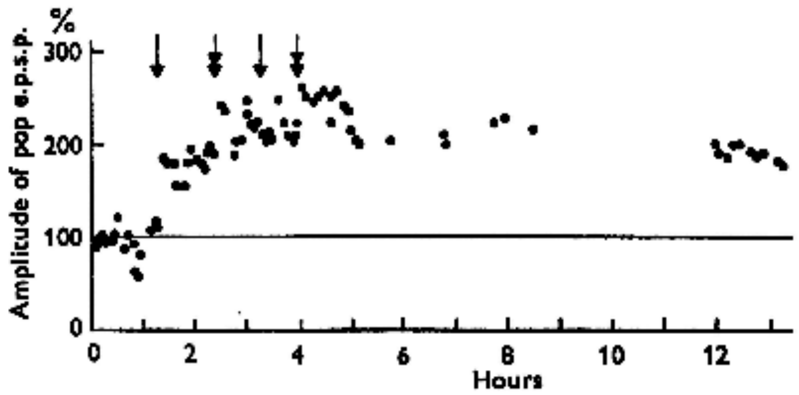
Long term synaptic plasticity: Bliss & Lømo 1973



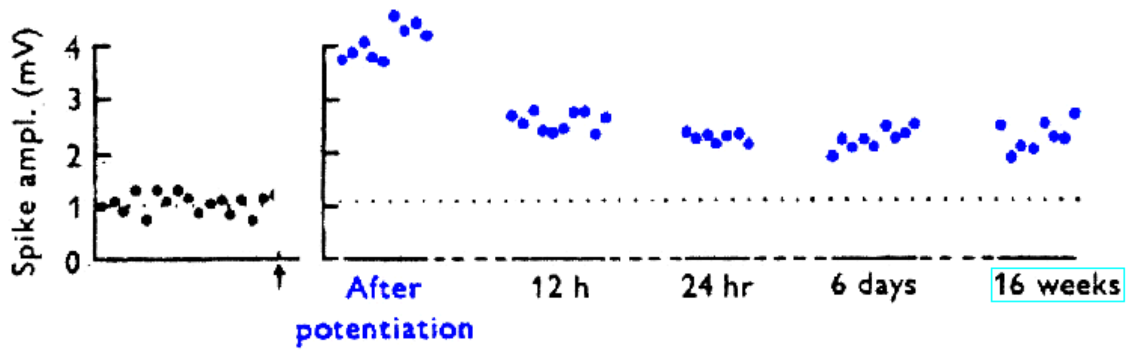


→ EPSP potentiation is:
- homosynaptic (the control pathway is not potentiated)
- cooperative (it is not an all-or-none phenomenon)

Long term synaptic plasticity: Bliss & Lømo 1973



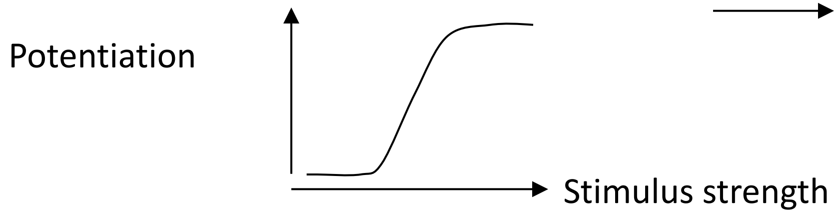
→ Long term potentiation can be saturated



→ Long term potentiation (‘**LTP’** : Douglas & Goddard, 1975) may last up to hours (or even weeks in the unanesthesized animal).

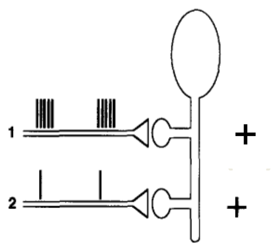
Long term synaptic potentiation: learning rules

1 - Cooperativity



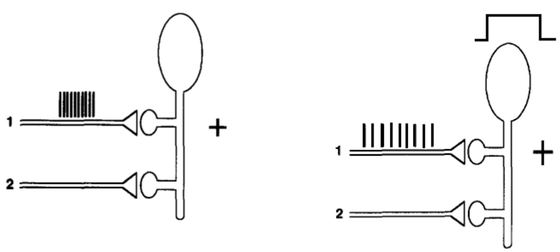
→ Threshold for LTP induction. Too weak stimuli leads to a transient (PTP) or short-lasting (STP) potentiation

2 - Associativity



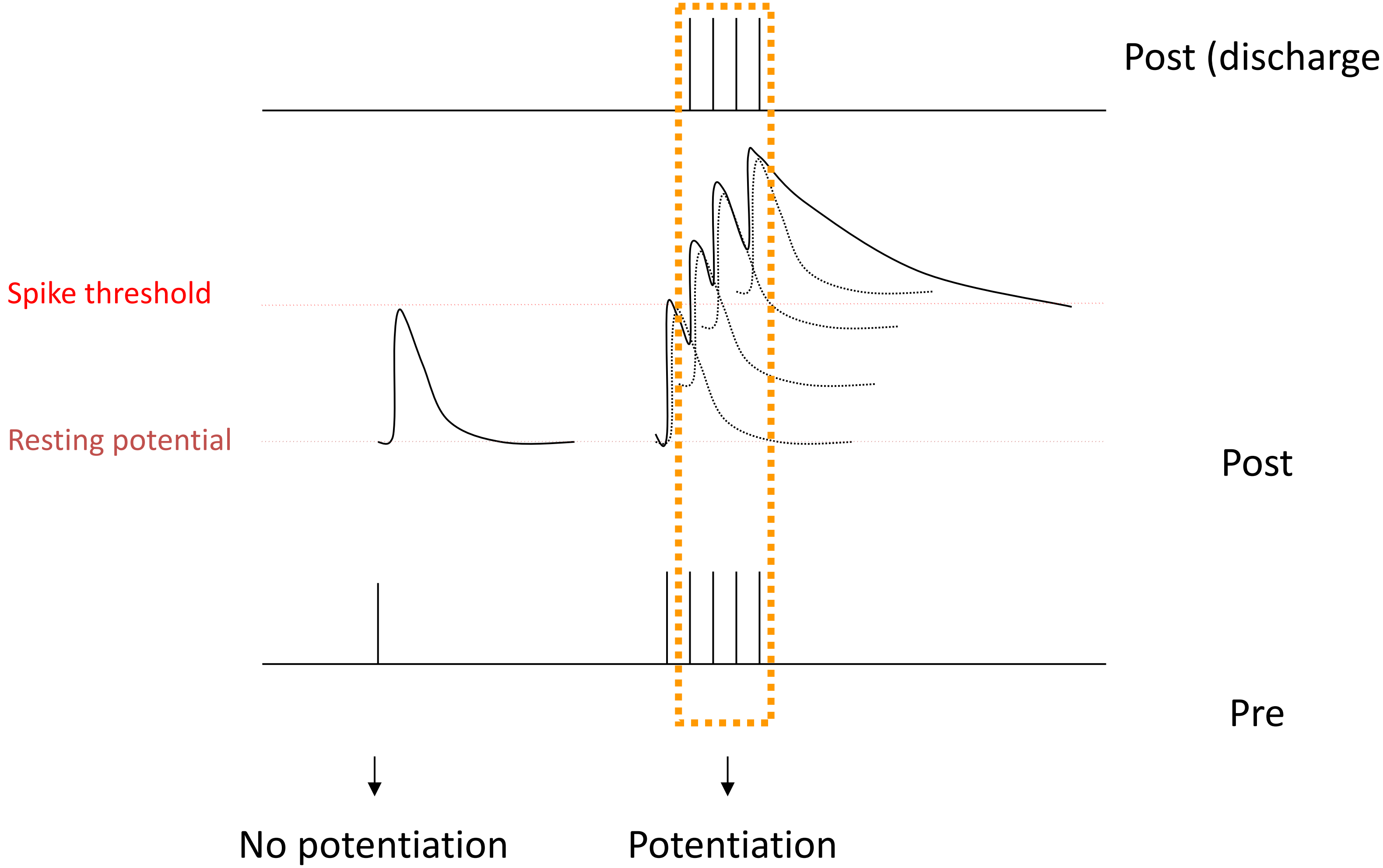
→ A weak afferent may be potentiated if it is activated simultaneously with a distinct but converging, strong afferent.

3 - Specificity



→ Inactive afferents are not potentiated (homosynaptic LTP)

Long term potentiation and Hebb's postulate



→ The coïncident discharge of pre- and post-synaptic elements induces the potentiation of the activated synapses

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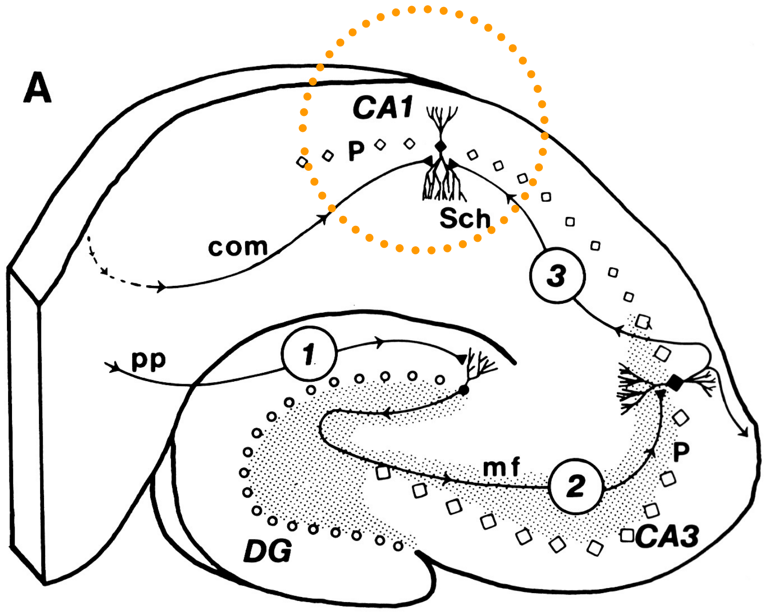
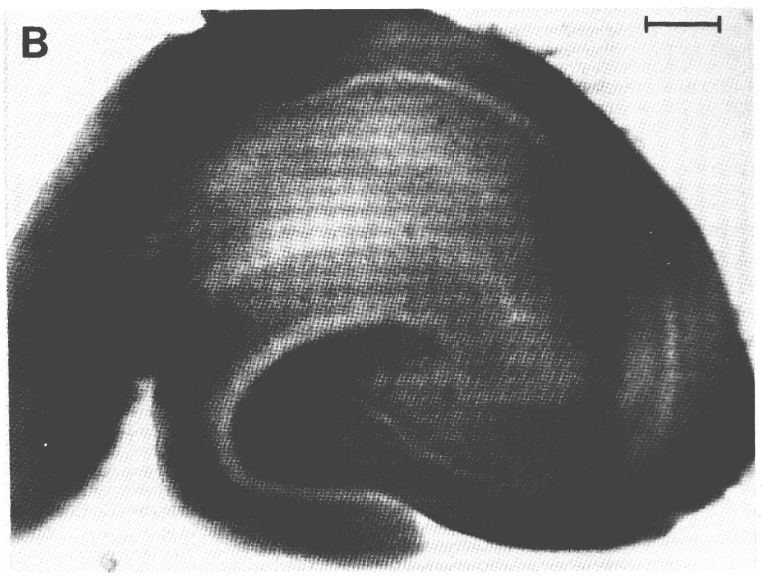
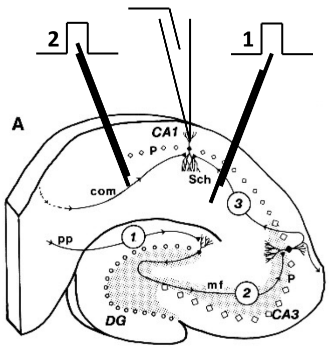
The Schaffer collateral/CA1 synapse: a prototypical synapse for studying LTP *in vitro*



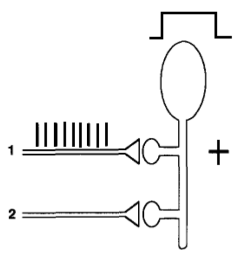


Hippocampal LTP : induction protocols



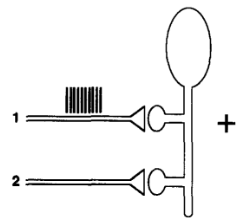
Pairing

(200-300 stim. at 1-2 Hz + depolarization of the postsynaptic cell to 0 mV)



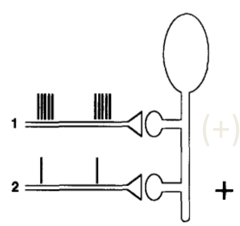
Tetanus (100Hz)
or theta burst

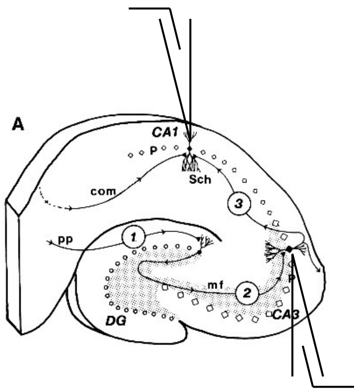
(10 bursts of 4 stim. at 100Hz every 200 ms, repeated 3 times at 20 sec intervals)



Associative stimulus

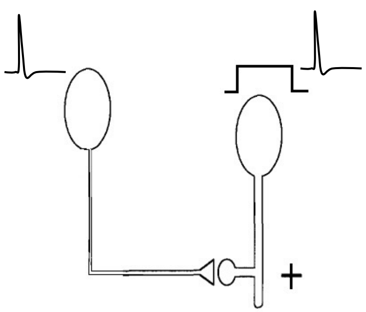
(stim. at 5Hz associated with brief tetanus on another afferent)



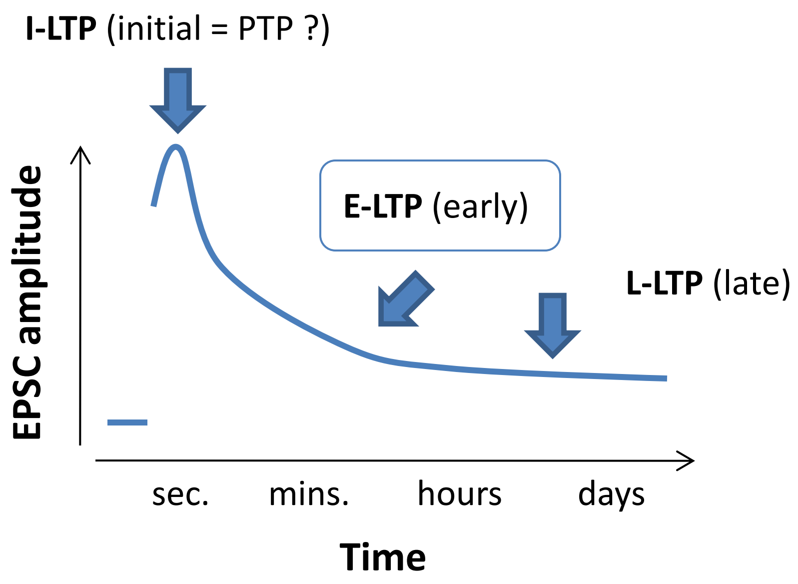


Pairing

(200-300 presynaptic spikes at 1Hz paired with postsynaptic action potentials or depolarizing steps)

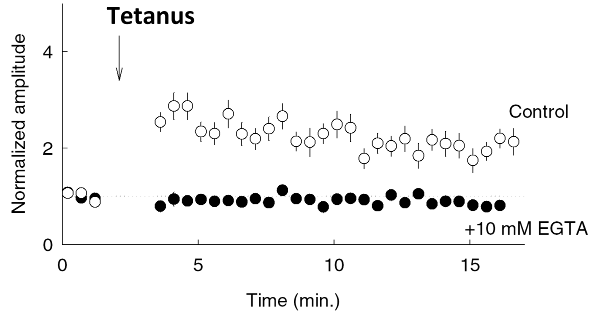


LTP can be divided into phases

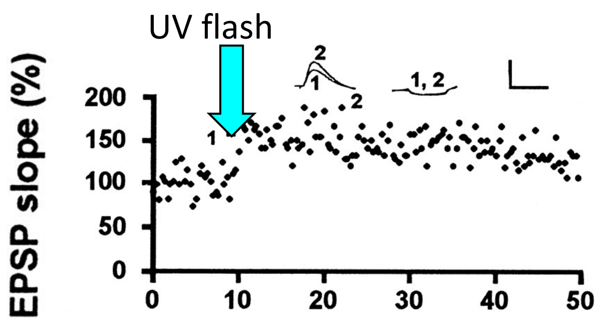


LTP induction: role of postsynaptic calcium

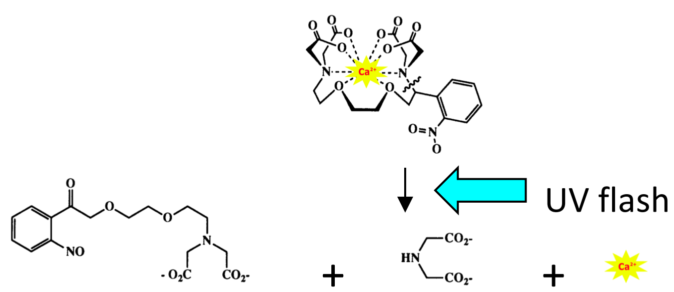
→ Intracellular injection of a calcium chelating agent in the postsynaptic cell compromises LTP induction without interfering with synaptic transmission *(Lynch et al. 1983 Nature 305: 719-721)*



→ A trasient and massive increase in postsynaptic Ca^{2+} is sufficient to induce a long term enhancement of synaptic strength *(Yang et al. 1999 J Neurophysiol 81: 781-787).*



Photolysis of nitrophenyl-EGTA.

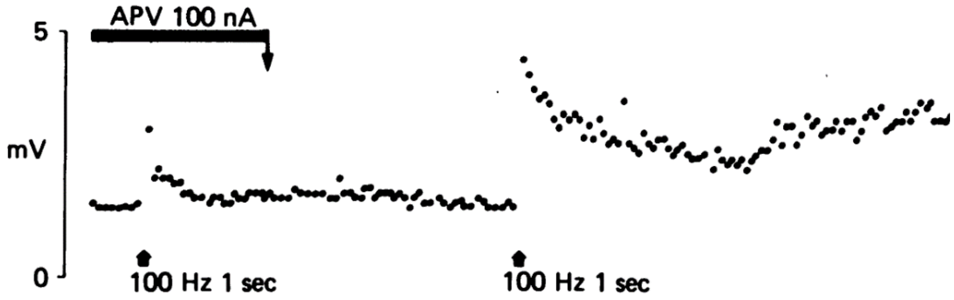


LTP induction: role of NMDA receptors

→ NMDA receptors open a Ca^{2+}-permeable conductance *(Dingledine 1983 J Physiol. 343: 385-405)*

→ Application of an NMDA receptor antagonist abolishes LTP induction without affecting synaptic transmission

APV : 2-amino-5-phosphonovalerate



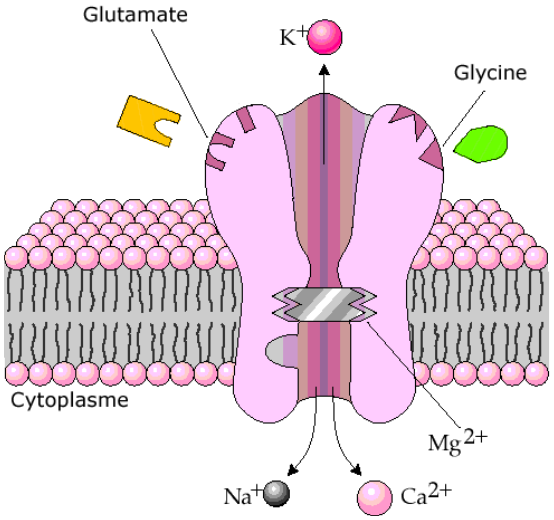
(Collingridge et al. 1983 J Physiol. 334:33-46)

NB : LTP can be induced experimentally in the presence of NMDA antagonists in specific conditions such as:

- High frequency (200 Hz) stimulation
- TEA application (K-channel blocker): 'chemical LTP'
- LTP of the mossy fiber/CA3 synapse

The NMDA receptor is a coincidence detector

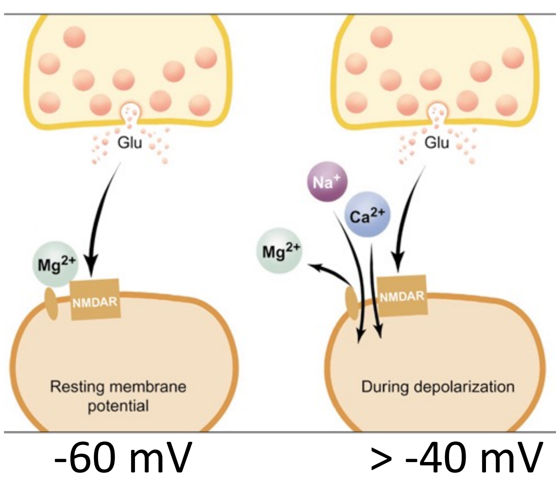
- <u>NMDA receptor</u>



→ The NMDA receptor is the main <u>synaptic</u> entry path for Ca^{2+} in neurons.

- Hetero-tetramere (GluN1, GluN2A-D, GluN3A-B)
- Non specific cation channel with high Ca^{2+} permeability: $P_{Ca^{2+}}/P_{Na^{+}} = 10.6$
- Blocked by Mg^{2+} ions in a voltage-dependent manner

→ *The NMDA receptor acts as a coincidence detector (synchronous depolarization and synaptic activity)*



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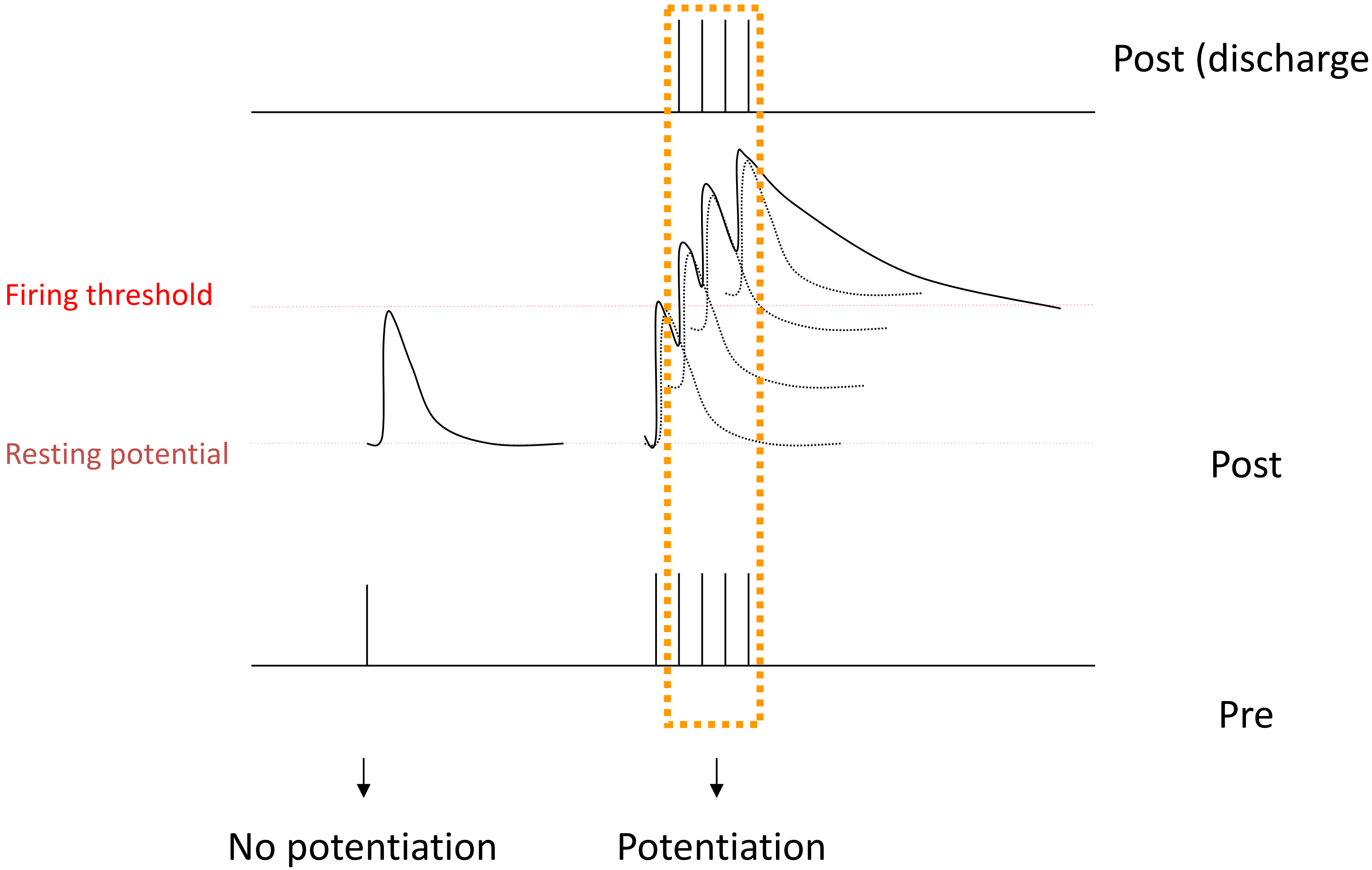
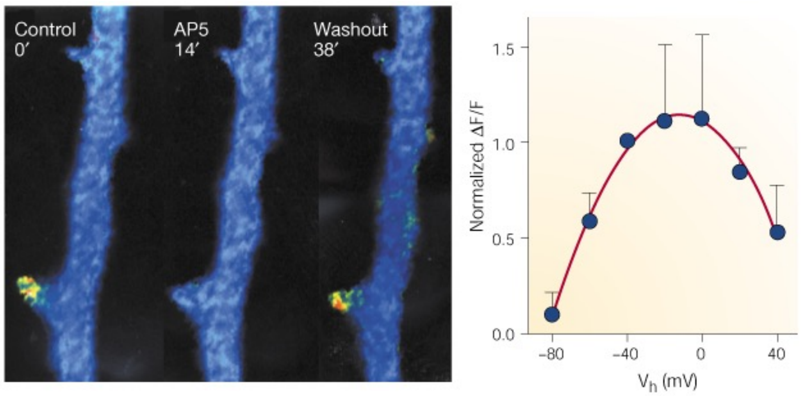
Long term potentiation and Hebb's postulate

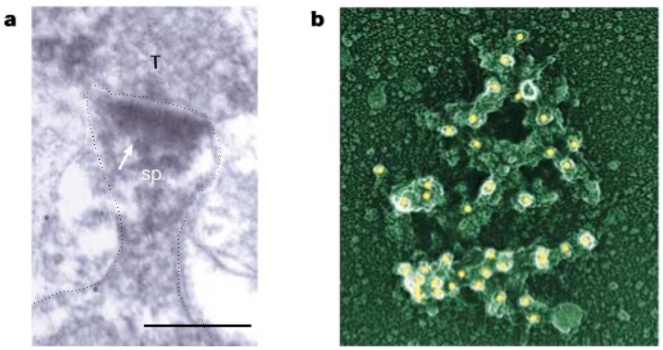


→ The coincident discharge of pre- and postsynaptic elements leads to a persistant potentiation of activated synapses.

LTP induction: the calcium-calmodulin protein kinase II (CaMKII)

- Subcellular localization





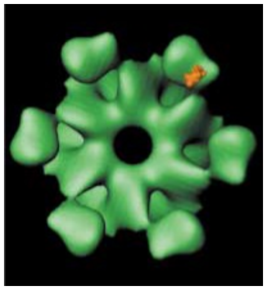
→ Local Ca^{2+} transient upon activation of NMDA receptors

→ Very high expression in the postsynaptic density in dendritic spines

→

LTP induction: the calcium-calmodulin protein kinase II (CaMKII)

● Structure / Function

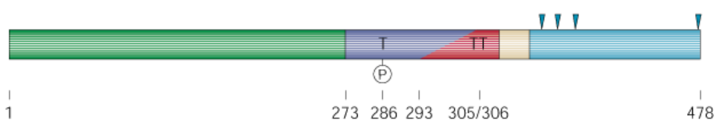


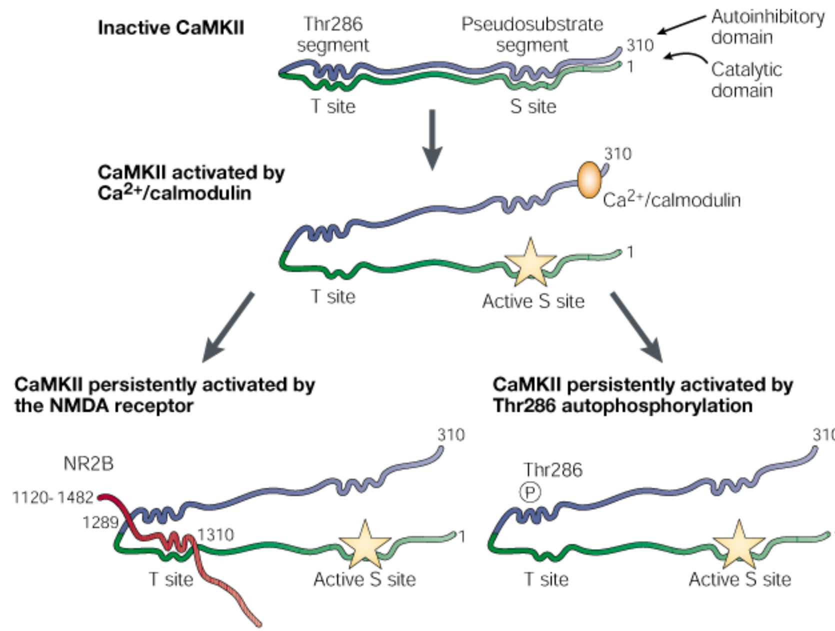
Dodecamere of α (and β) isoforms

Functional domains:

catalytic **auto-inhibitory** **auto-assembly**

inhibitory CaM binding





LTP induction: the calcium-calmodulin protein kinase II (CaMKII)

weak Ca^{2+} signal

↓

No auto-phosphorylation

(<1 sec.)

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strong Ca^{2+} signal

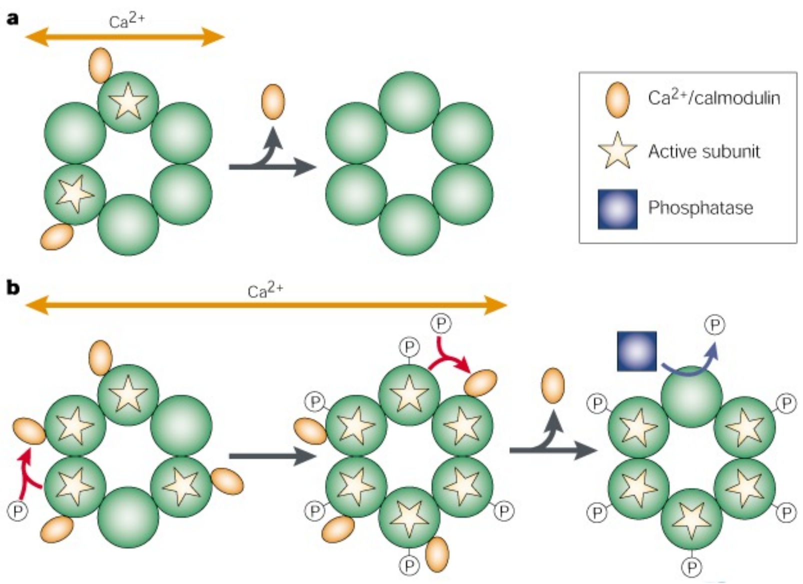
↓

auto-phosphorylation

↓

prolonged activation

(several minutes)



→ CaMKII acts a a <u>temporal integrator of calcium signals</u> in the postsynaptic density

(Miller & Kennedy ***1986*** *Cell 44: 861-870)*

LTP induction: the calcium-calmodulin protein kinase II (CaMKII)

How to convincingly demonstrate CaMKII plays a central role in LTP induction?

« See it » (show CamKII is activated upon LTP)

« Block it » (show that blocking CamKII prevents LTP induction)

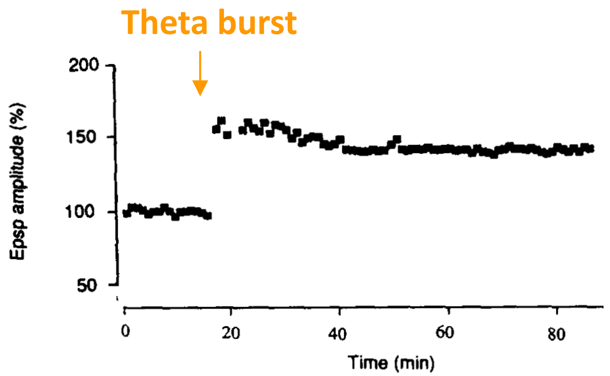
« Move it » (show that activating the enzyme directly triggers LTP)

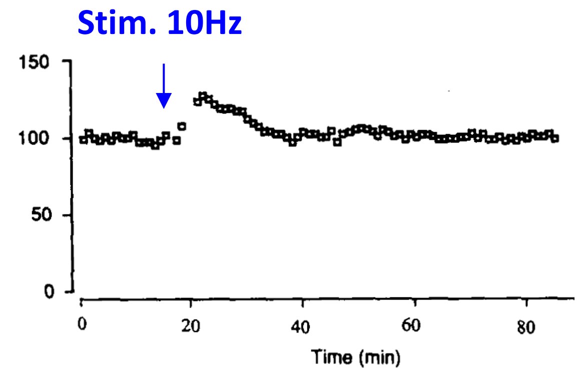
(quotation from Robert Malinow… originally from Richard Tsien !)

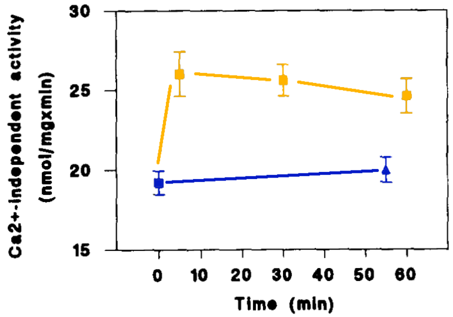
Involvement of CaMKII in LTP induction

« See it »

- The autonomous activaty of CaMKII increases during LTP induction and persits



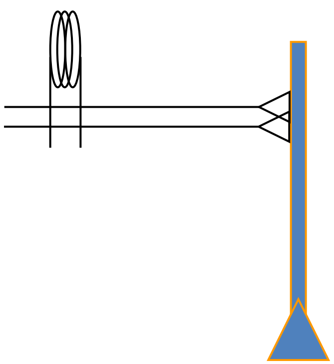


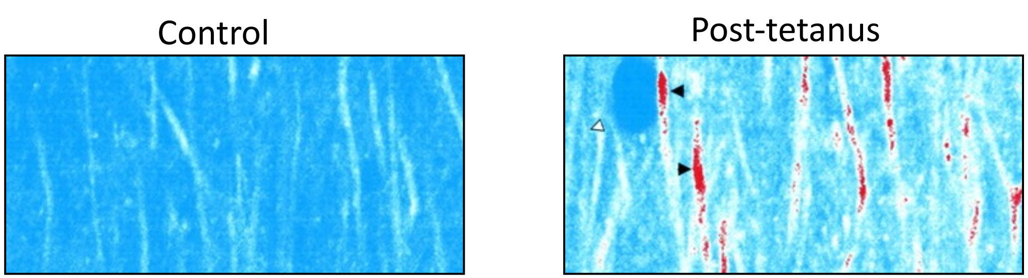


(Fukunaga et al. ***1993*** *J. Biol. Chem. 268: 7863-7867)*

- Autophosphorylated CaMKII increases in dendrites after LTP induction

→ Anti-P286-CamKII immunostaining



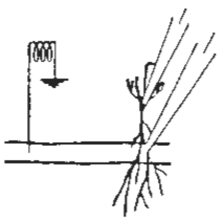


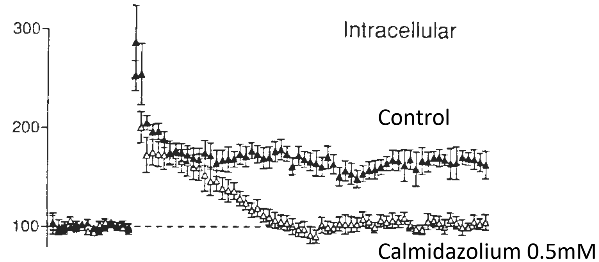
(Ouyang et al. ***1999*** *J Neurosci 19: 7823-7833)*

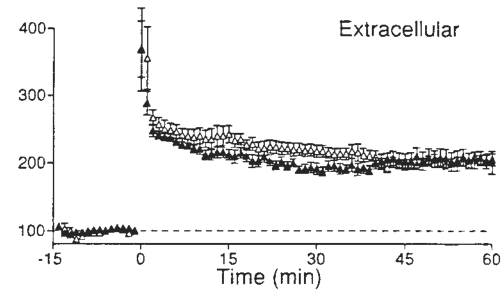
Involvement of CaMKII in LTP induction

« Block it »

- Postsynaptic injection of a CamKII inhibitor specifically blocks LTP induction

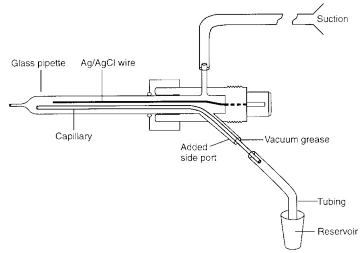


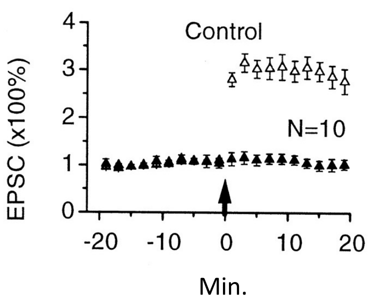


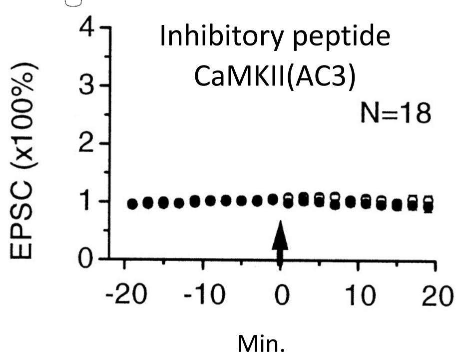


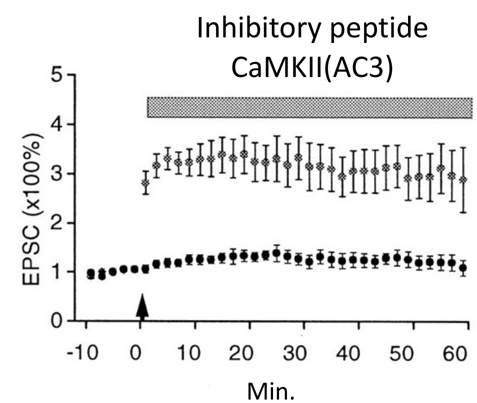
(Malenka et al. ***1989*** *Nature 340, 554 – 557)*

- Postsynaptic injection of an inhibitory peptide against CaMKII prevents LTP <u>induction</u> but not its <u>expression</u>









(Otmakhov et al. ***1997*** *J Neurosci 17: 5357-5365)*

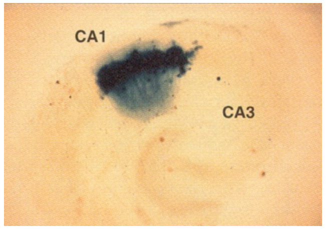
Involvement of CaMKII in LTP induction

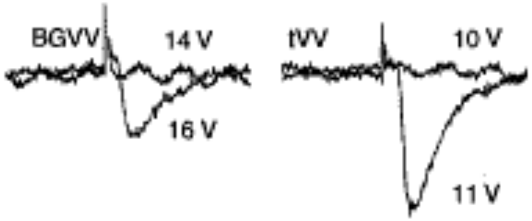
« Move it »

- Mutating the autophosphorylation site of CaMKII (T286A) abolishes LTP induction

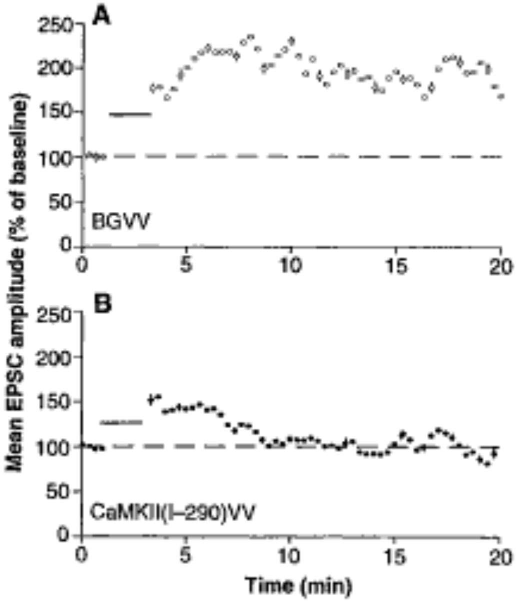
(*Giese et al.* **1998** *Science 279: 870-873)*

- Overexpression of a constitutively active form of CaMKII mimics LTP and occludes its induction





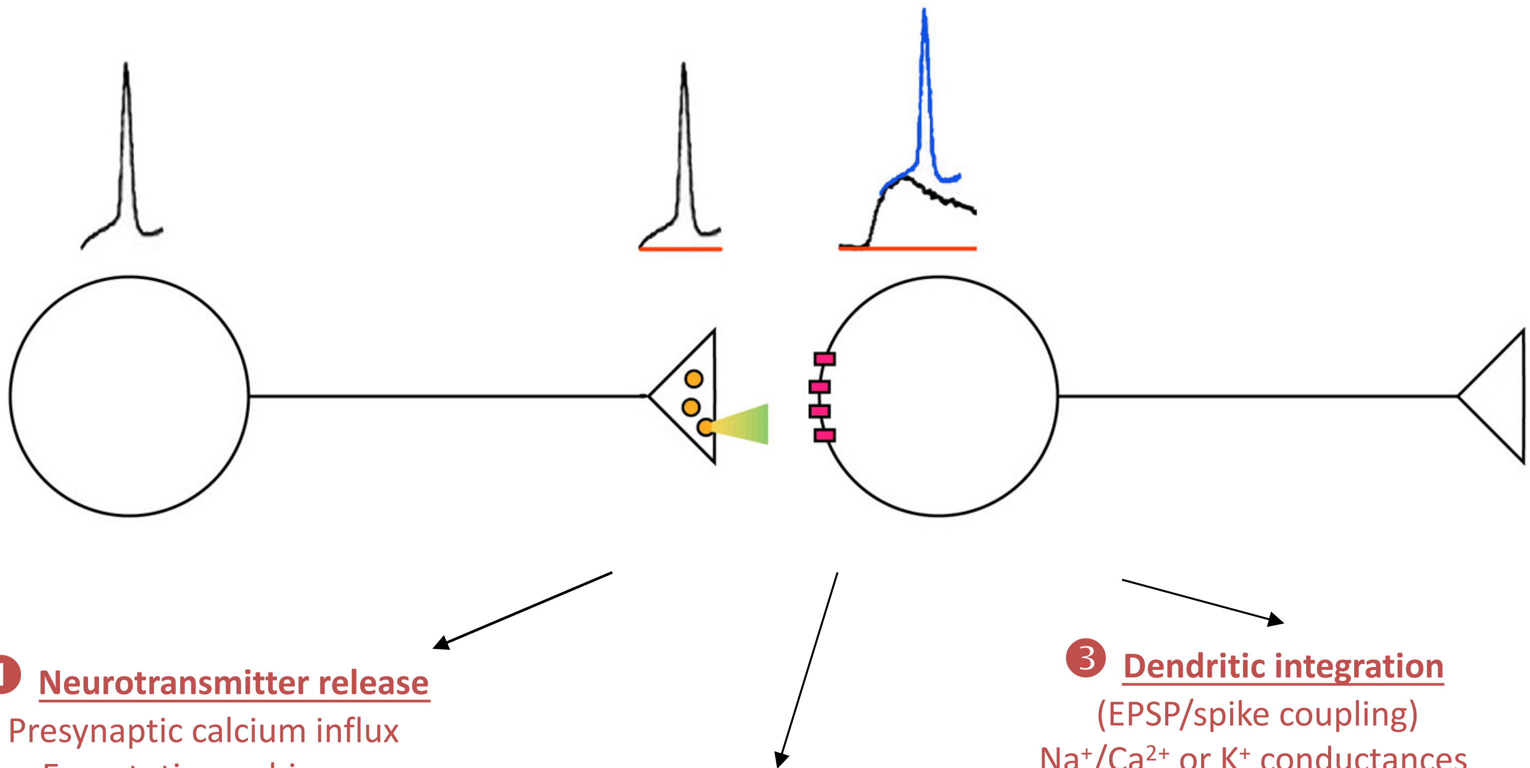
$CaMKII_{1\text{-}290}$: viral vector based transduction (Vaccinia virus)



(*Pettit et al.* **1994** *Science 266: 1881-1885)*

→ Postysnaptic activation of CaMKII is necessary and sufficient to induce homosynaptic LTP

Expression locus of long term potentiation



❶ **Neurotransmitter release**

Presynaptic calcium influx

Exocytotic machinery

Amount of neurotransmitter/vesicle

❷ **Postsynaptic response**

Affinity, availability and/or number of postsynaptic receptors

❸ **Dendritic integration**

(EPSP/spike coupling)

Na^{+}/Ca^{2+} or K^{+} conductances

Inserm

Possible loci of LTP expression

Quantal parameters

pre
- n : number of functional synapses
- p : mean probability of release of one vesicle of neurotransmitter
- m : mean quantal content = n . p = average number of released vesicles

post
- q : quantal size = amplitude of the postsynaptic response to the release of one single vesicle

Scenario	Affected parameters
Increased neurotransmitter release	
Formation of additional synapse	n/m
Increased release probability	p/m
Increased amount of neurotransmitter/vesicle	*q (if postsynaptic receptors are not saturated)*
Postsynaptic response	
Increased sensitivity or number of postsynaptic receptors	q

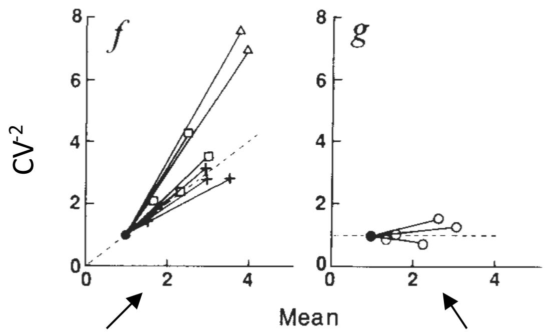
Binomial model and quantal analysis of synaptic transmission in the CNS

- A cell receives *n* synapses from an afferent or a group of afferents (n release sites)
- Each site releases at most one quantum (vesicle) with a mean probability *p* (identical at all sites)
- The postsynaptic effect of the release of a quantum is *q*
- The probability that the cell receives x quanta is:

$$p_x = C_x^n \, p^x \, . \, (1-p)^{(n-x)}$$

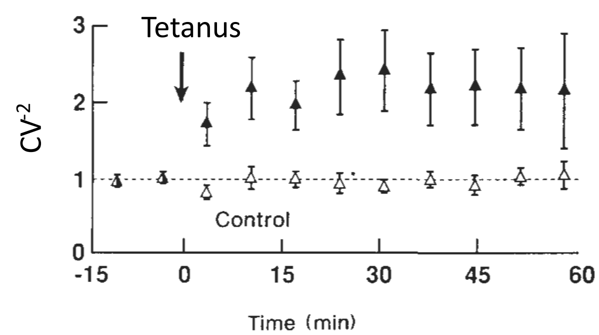
CV^{-2}= mean^2/variance = $np/(1-p)$

→ If LTP was expressed presynaptically, it should be associated with an <u>increase in CV^{-2}</u>



Presynaptic modifications (A1R agonist)

Postsynaptic Modifications (increasing driving force)



(Malinow & Tsien Nature 1990)

Poisson's law and quantal analysis of LTP

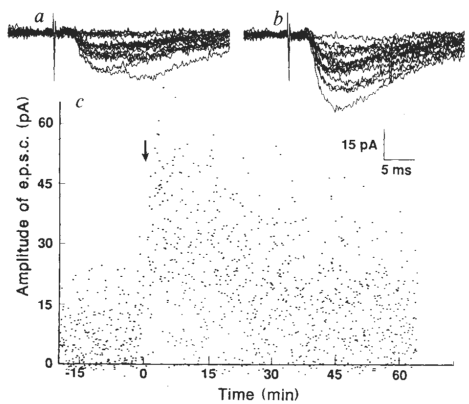
- Particular case of the binomial model when n is large and p is very low
- Probability law:

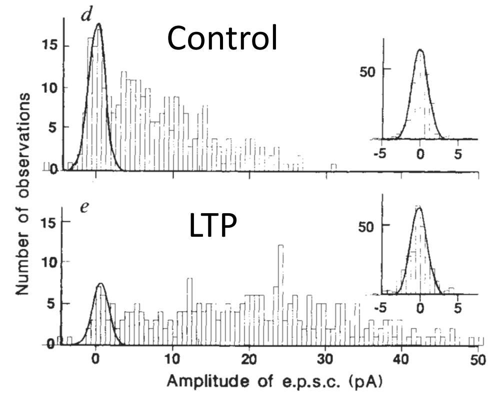
$$p_x = \frac{e^{-m}\, m^x}{X!}$$

m=n.p= quantal content

p_0 = probability that no quanta is released
$= e^{-np}$

⟶ If LTP was expressed presynaptically, it should be associated with <u>a reduction of p_0</u>.



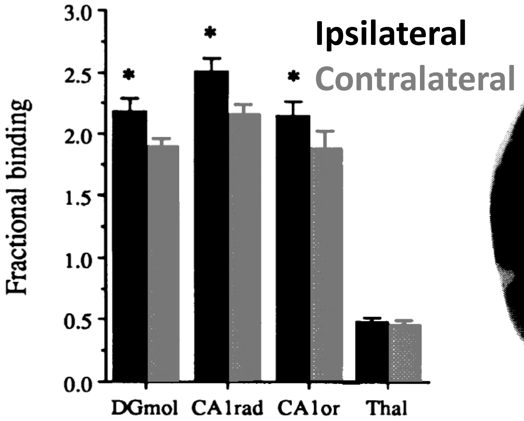


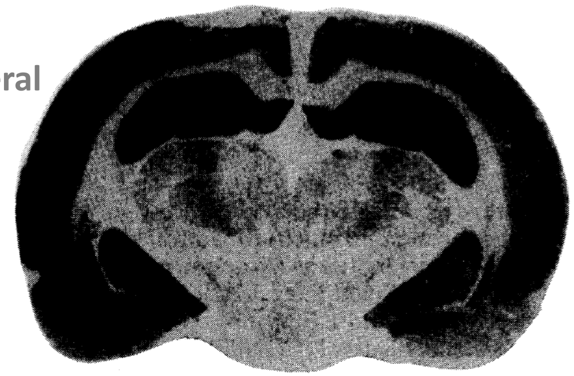
(Malinow & Tsien Nature 1990)

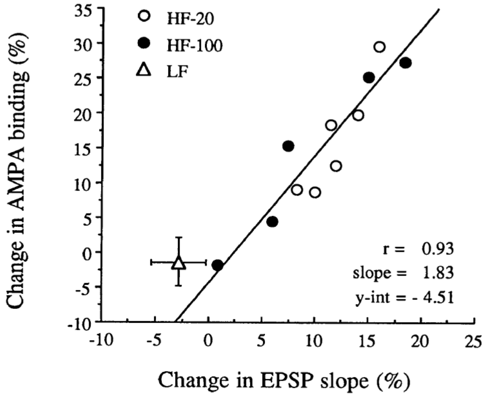
Increased number of AMPA binding sites during LTP expression

Autoradiographical detection of radioactive AMPA binding

LTP induced on the perforant path *in vivo,* in just one hippocampus

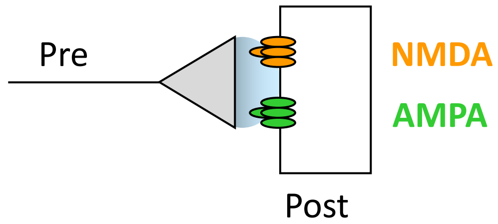






(Maren et al., PNAS 1993)

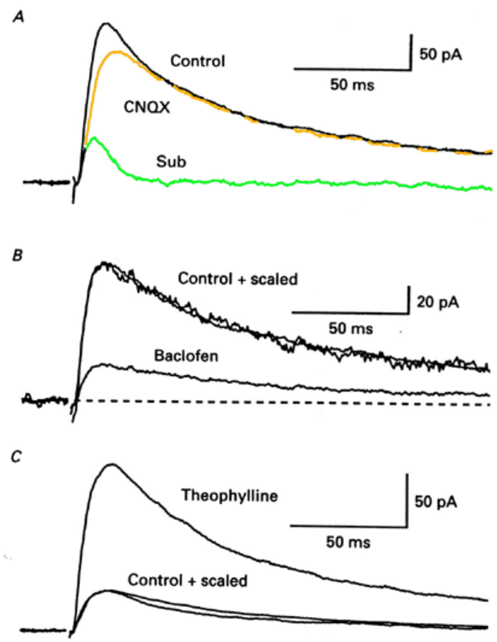
Compared plasticity of AMPA et NMDA-receptor mediated EPSCs



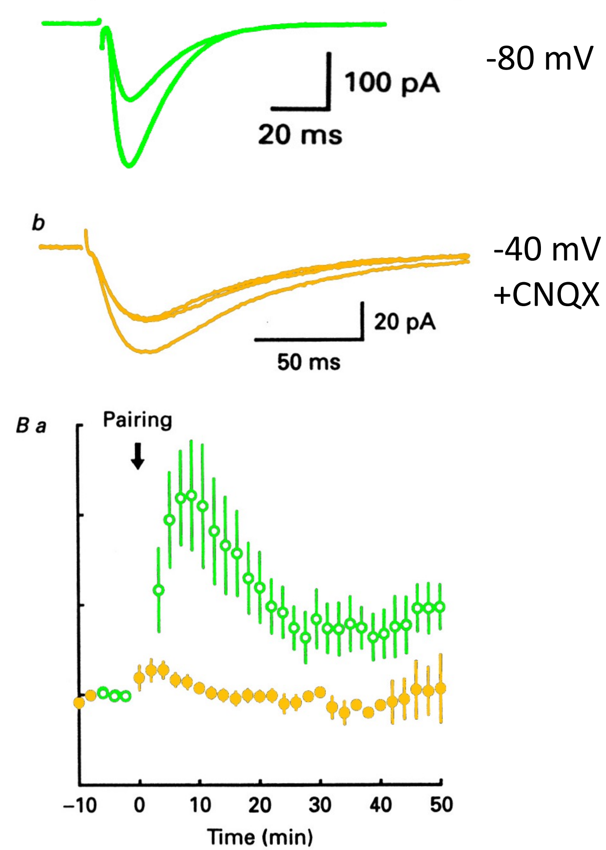
Presynaptic modulation of release probability affects similarly the AMPA and NMDAR-EPSCs…

Presynaptic GABABR activation
↓ p

Presynaptic A1R blockade
↑ p



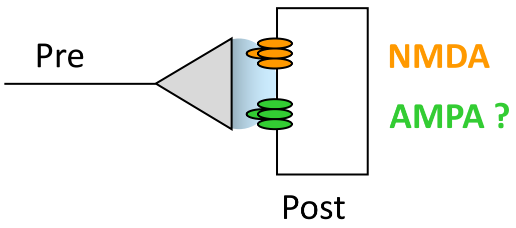
… but LTP does not !



(Perkel & Nicoll, J Physiol 1993)

→ At the CA3/CA1 synapse, LTP cannot be explained exclusively by an increased release probability

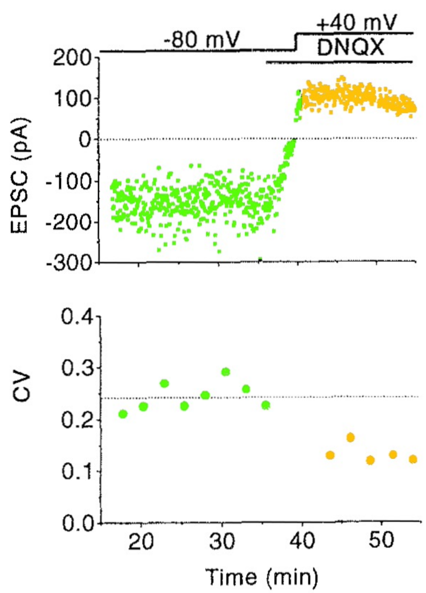
Compared variation of AMPA vs NMDA-receptor mediated EPSCs

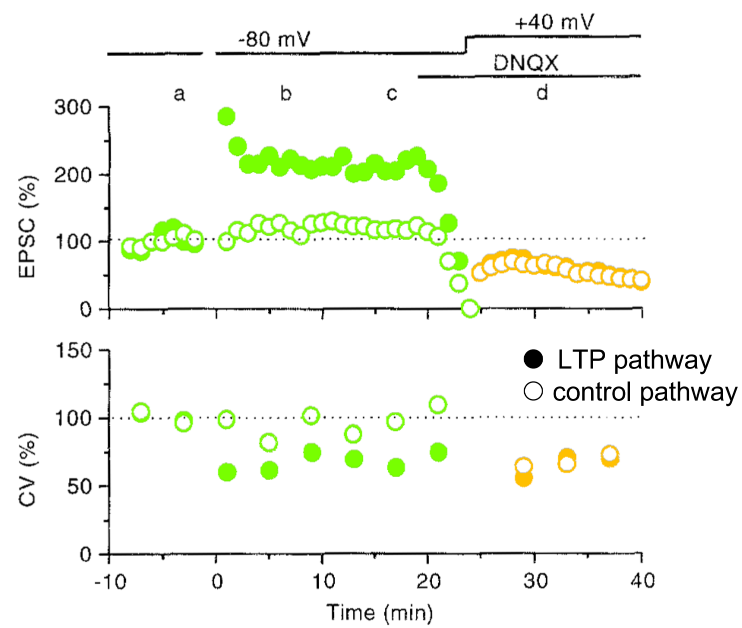


CV=standard deviation/mean = $\sqrt{(1-p)/np}$

→ -The CV of the AMPA EPSC is larger than that of the NMDA EPSC

- LTP is associated with a reduction of the CV of the AMPA but not the NMDA-R mediated EPSC





▶ distinct CV of AMPA and NMDAR EPSCs

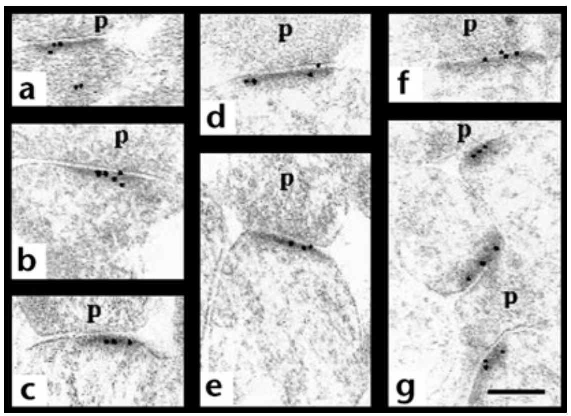
(Kullmann Neuron 1994)

Silent synapses: from concept to reality

P2 P10 5 weeks

GluN1

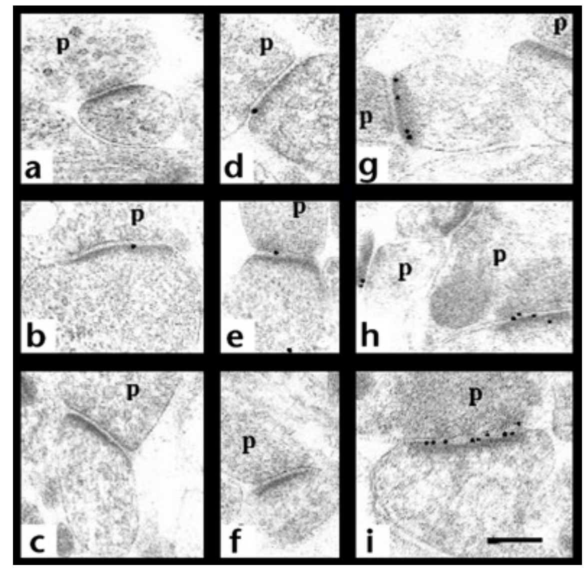
GluN2A/B



P2 P10 5 weeks

GluA1

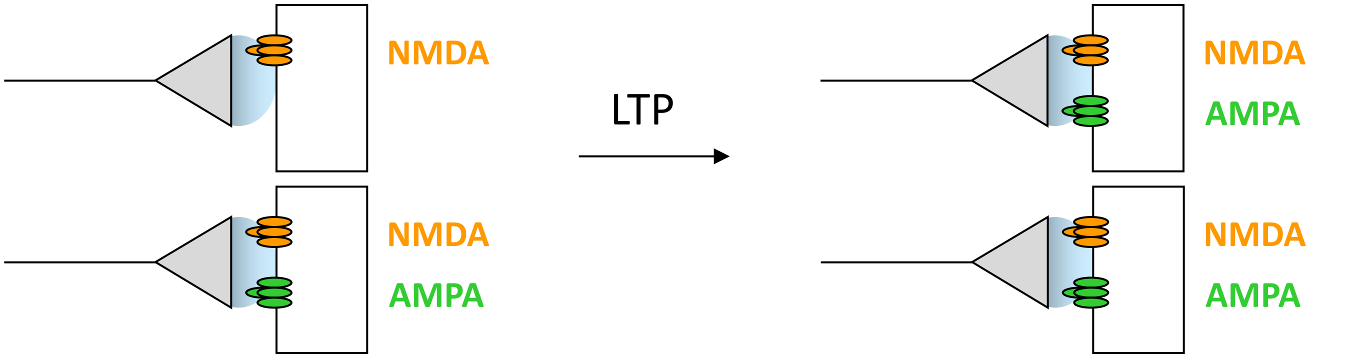
GluA2/3



(Petralia et al., Nat Neurosci 1998)

→ A fraction of synapses is devoid of AMPA receptors in the developping hippocampus

LTP and the conversion of silent synapses: reconciliation of old data and new concepts

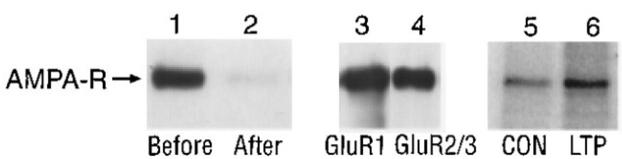


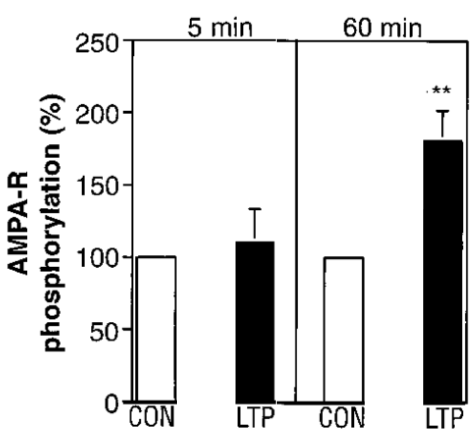
- Decreased CV of the postsynaptic response (AMPA) ✓
- Reduced frequency of synaptic failures (AMPA/NMDA) ✓
- Increased number of binding sites for ^{3}H-AMPA ✓
- Potentiation of the AMPA EPSC in the absence of a potentiation of the NMDA EPSC ✓
- CV of the AMPA EPSC larger than that of the NMDA EPSC before LTP induction ✓
- Reduced CV of the AMPA but not the NMDA EPSC during LTP ✓

LTP expression: CaMKII targets

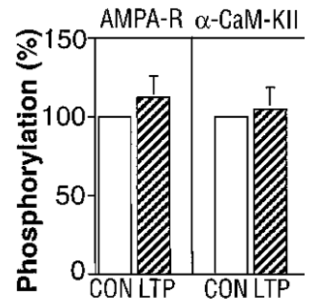
- CaMKII phosphorylates the GluA1 subunit of AMPA receptors both *in vitro* and during LTP

Immunoprecipitation avec anti-GluA
after LTP induction in the presence of ^{32}P

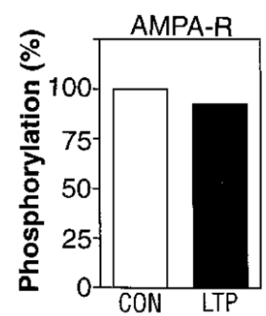




+ KN-62



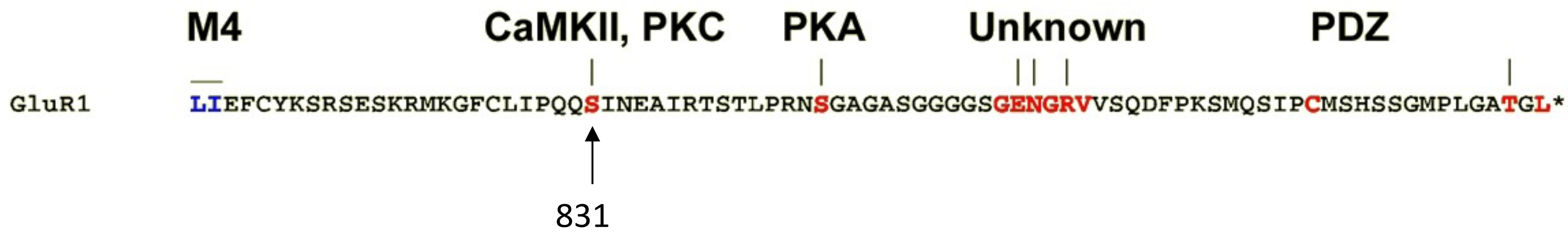
+APV

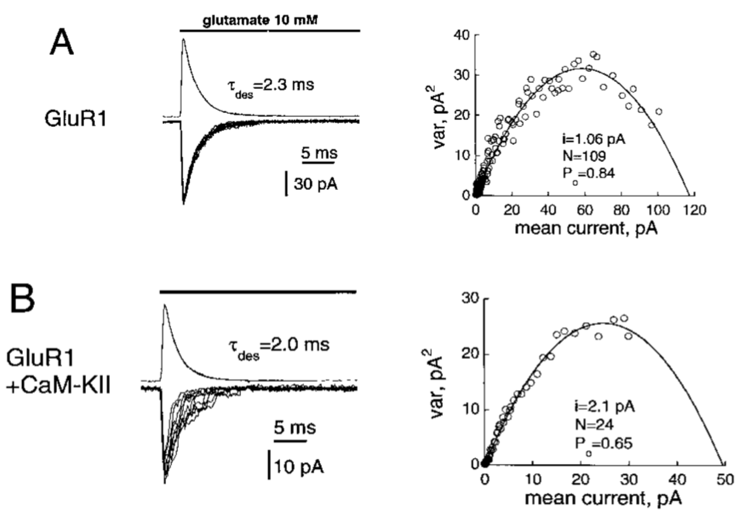


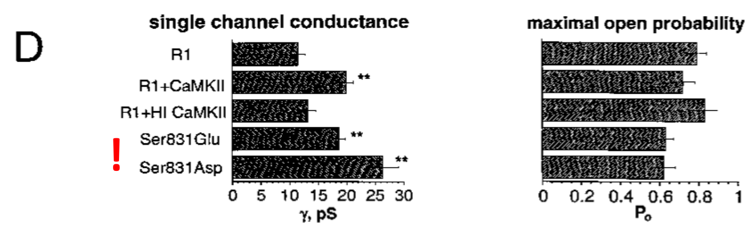
(Barria et al. 1997 Science 276: 2042-2045)

LTP expression: CaMKII targets

- Effect of GluA1 phosphorylation by CaMKII: increased unitary conductance of AMPA receptor channels



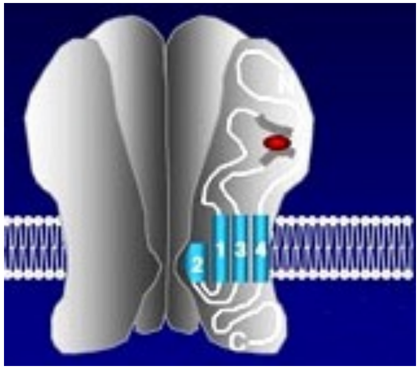




(Derkach et al. 1999 PNAS 96: 3269–3274)

Properties of AMPA receptors

● <u>AMPA receptor</u>



● Typically, in the cortex, GluA2 content is high in principal cells and lower in inhibitory interneurons

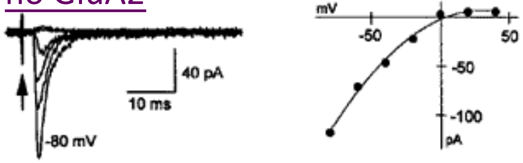
● The absence of the GluA2 subunit induces:
- higher Ca^{2+} permeability
- inward rectification
(i.e. inward but no outward currents)

● Hetero-tetramere (GluA1-4)

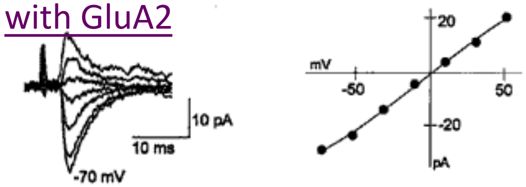
● Non specific, cation channel with low permeability for Ca^{2+}: $P_{Ca^{2+}}/P_{Na^{+}} = 0.15\text{-}2.5$

● Ca^{2+} permeability and rectification depend on its content in GluA2 subunits

no GluA2

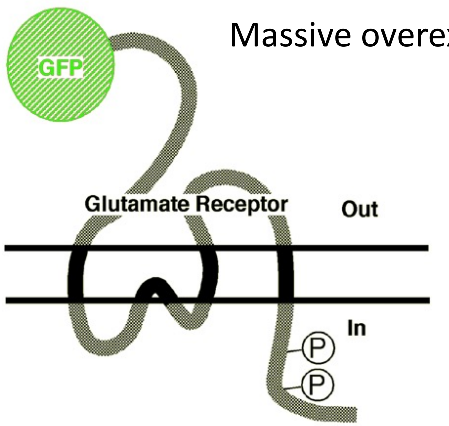


with GluA2

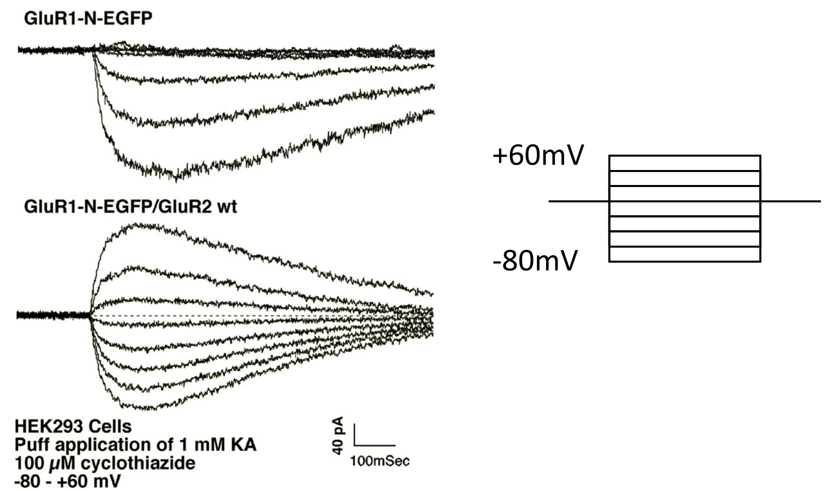


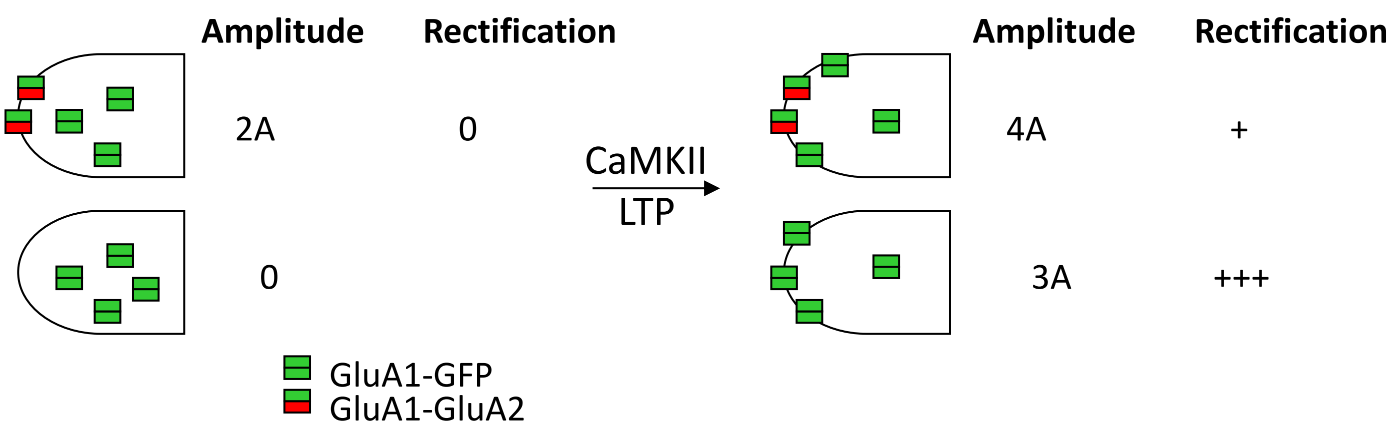
LTP expression and AMPA receptor membrane delivery

- Effects of GluA1 phosphorylation by CamKII: synaptic translocation of AMPA receptors



Massive overexpression





LTP expression and AMPA receptor membrane delivery

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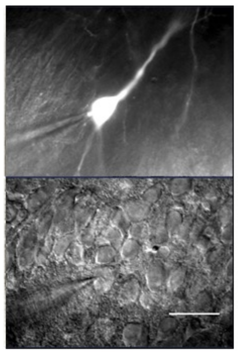
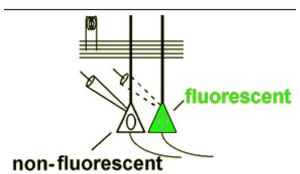
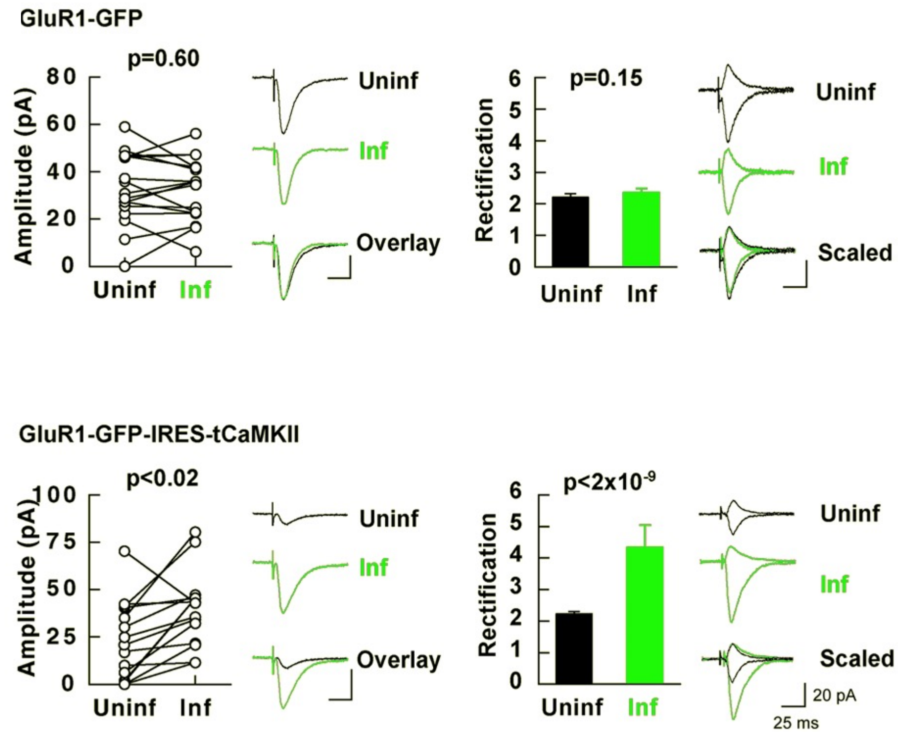
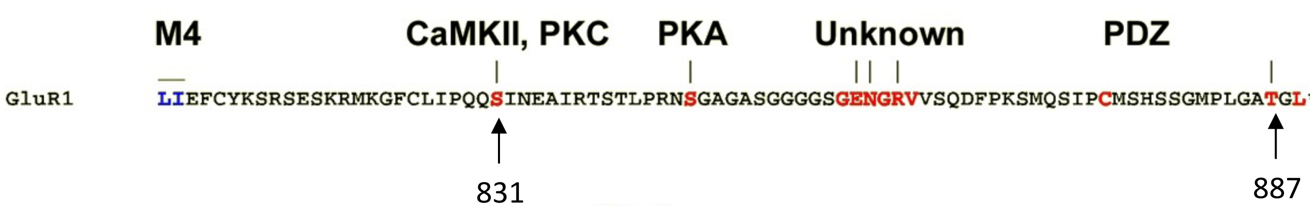






*(Hayashi et al. **2000** Science 287: 2262-2267)*



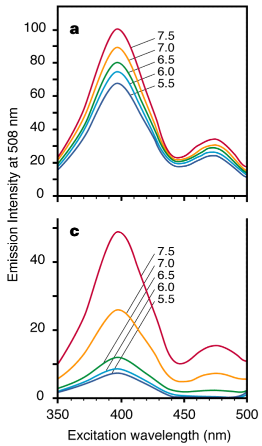
Inserm

LTP expression and AMPA receptor membrane delivery

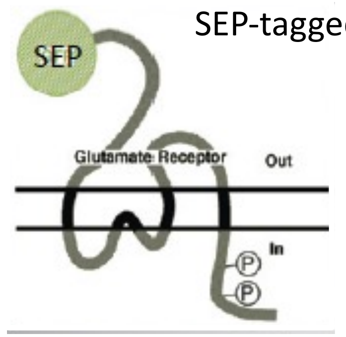
- Effects of GluA1 phosphorylation by CamKII: synaptic translocation of AMPA receptors

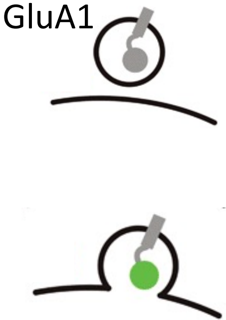
WT GFP

Super ecliptic pHluorin

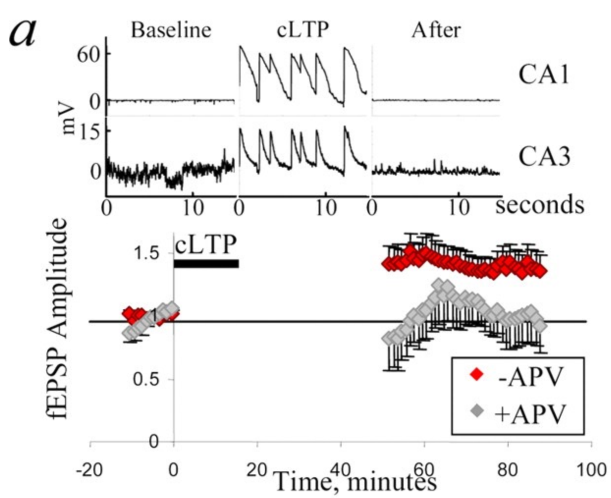


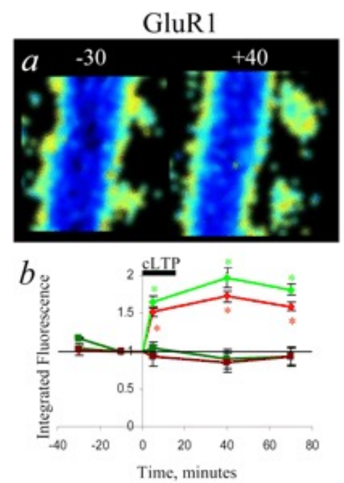
SEP-tagged GluA1





(Miesenböck et al Nature ***1998****)*



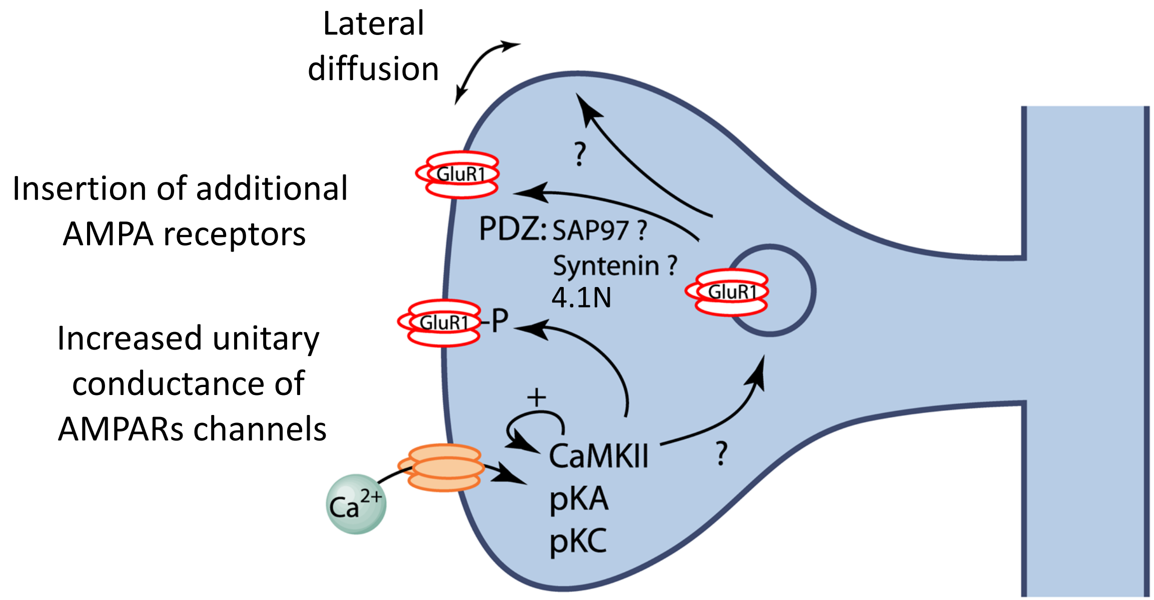


Spine volume

Spine SEP-GluA1

(Kopec et al J Neurosci ***2006****)*

LTP, αCaMKII and expression of hippocampal LTP



→ Several mechanisms converge to specifically potentiate AMPA-receptor mediated transmission

→ Other mechanisms likely contribute to LTP expression, perhaps with slower kinetics, such as spine enlargement/spliting (cf. Toni et al., Nature 1999).
(For review Lamprecht & LeDoux Nature Reviews Neuroscience 2004)

LTP expression and memory: link to GluA1-containing AMPARs

How to convincingly demonstrate memory relies on LTP-associated AMPAR modifications/exocytosis?

« See it » (show AMPARs are exocytosed/phosphorylated during learning)

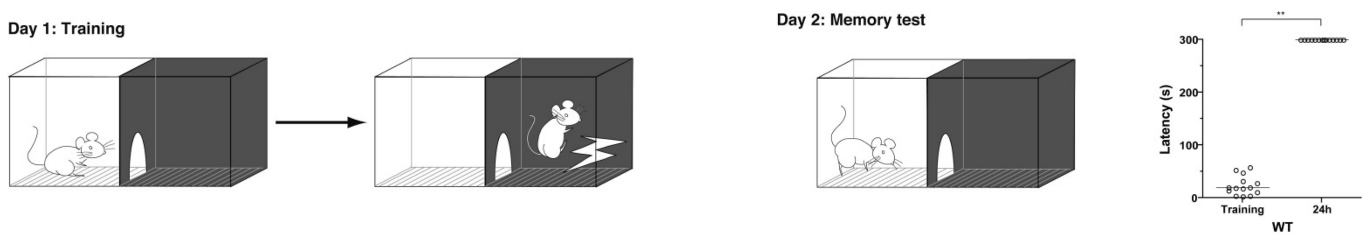
« Block it » (show that blocking AMPAR exocytosis prevents LTP and memory

« Move it » (show that inducing AMPAR exocytosis directly influence learning?)

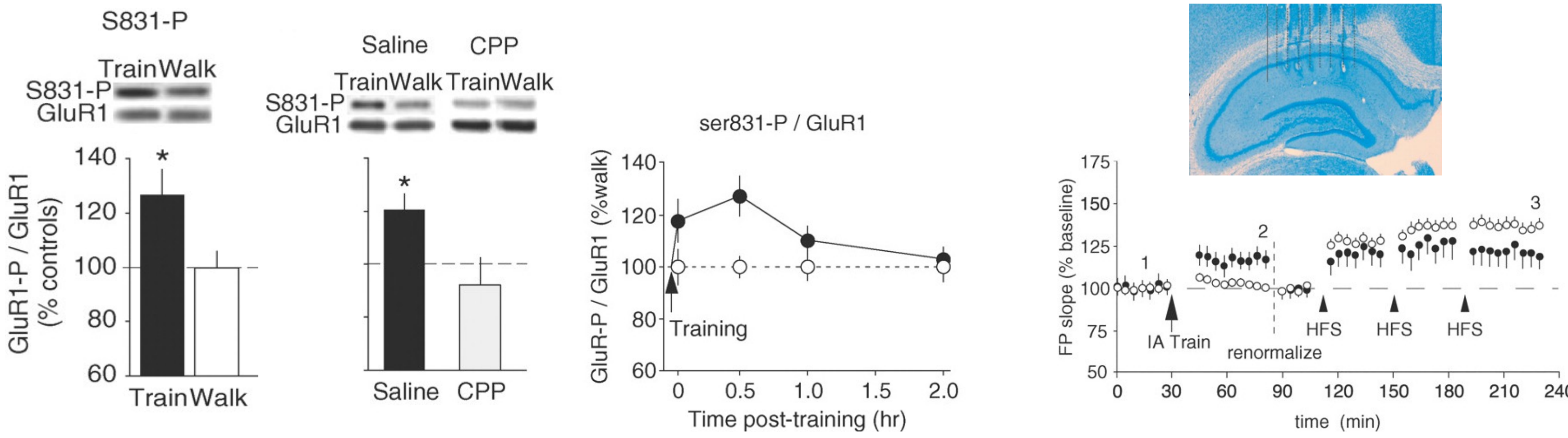
« See it »

- learning influences AMPAR traffic and synaptic function in the hippocampus

Inhibitory avoidance paradigm



IA induces GluA1 S831 phosphorylation and increased fEPSP in dorsal hippocampus



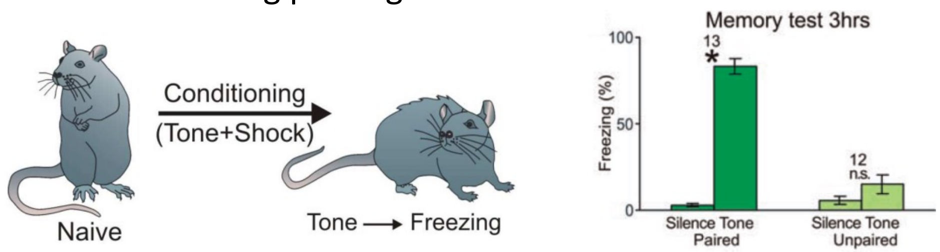
(Whitlock et al. Science 2006)

LTP, learning and memory

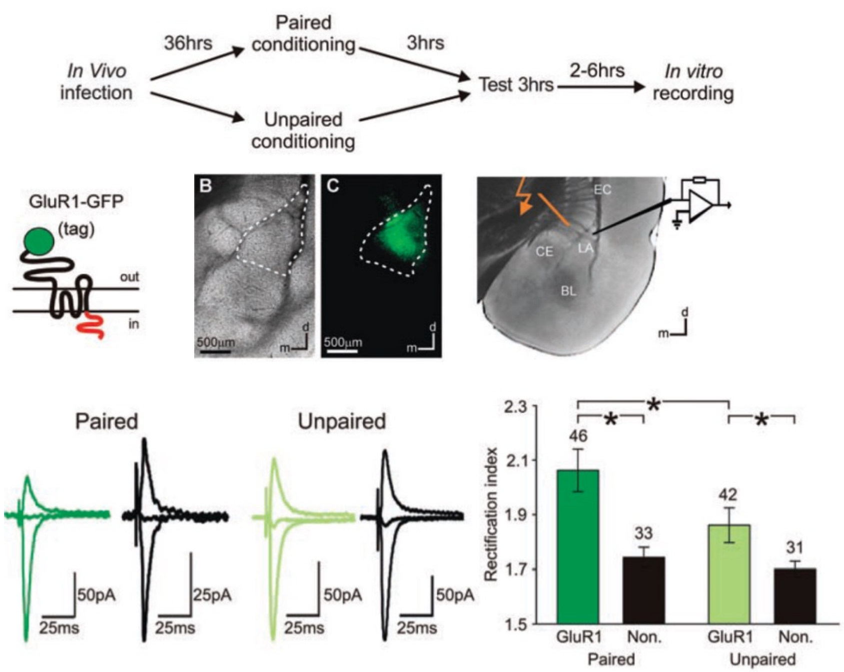
« Block it »

- blocking AMPA receptor traffic precludes learning

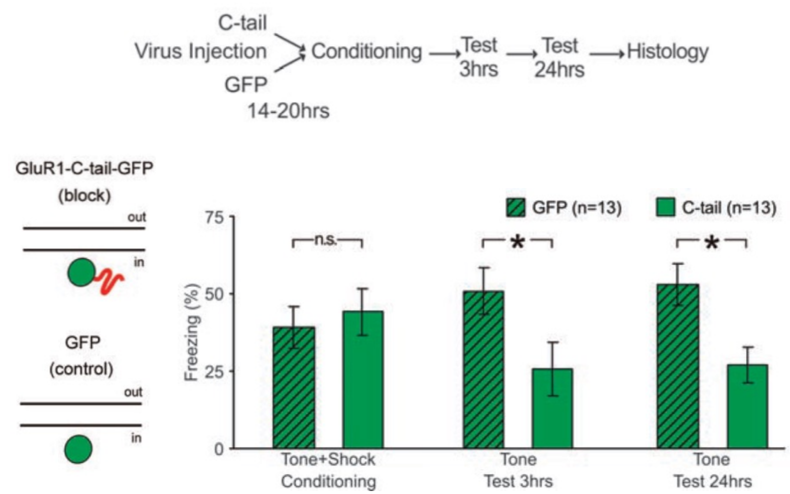
Fear conditioning paradigm



Fear training induces GluA1 synaptic incorporation



Preventing GluA1 traffic precludes fear memory



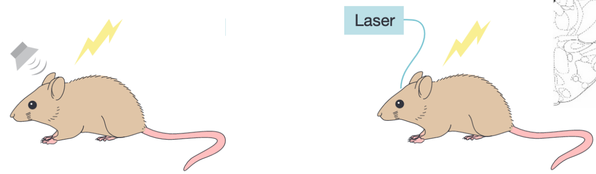
*(Rumpel et al Science **2005**)*

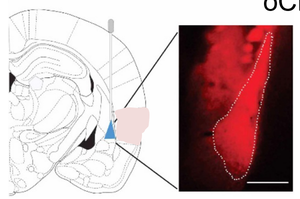
LTP, learning and memory

« Move it »

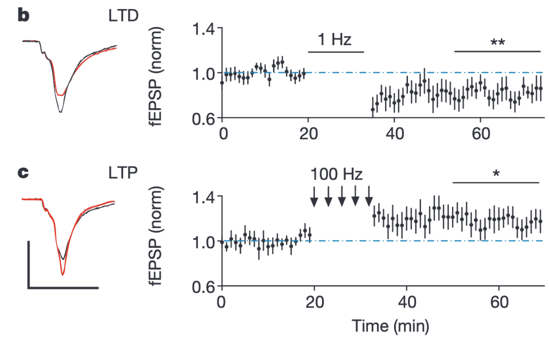
- Engineering a memory with LTD and LTP

Fear conditioning paradigm



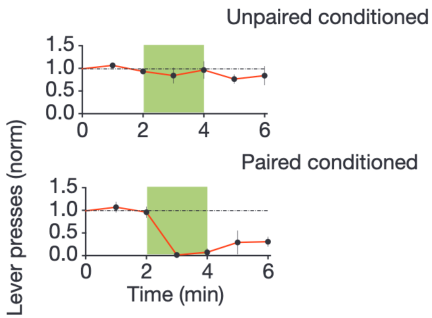


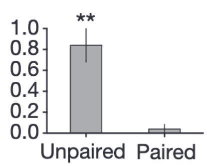
oCHIEF aud. Cx
+ mGen nucl

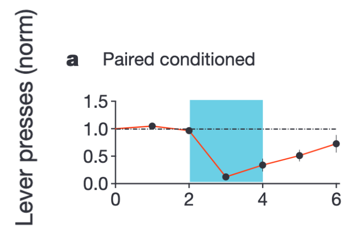


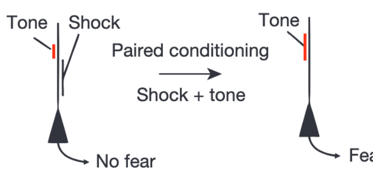
All optical LTP induces fear response

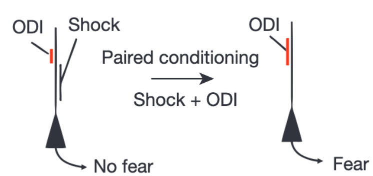
2 min 10 Hz











(Nabavi et al Nature 2014)

Inserm

Hippocampal LTP: take-home messages

- a brief period of high frequency activity triggers a long lasting increase in synaptic efficacy
- at some (but not all !) synapses, this process is induced by postsynaptic Ca influx through NMDA receptors and subsequent CaMKII activation
- at these synapses, the potentiation is largely specific of AMPA but not NMDA receptor mediated transmission
- CaMKII activation leads to GluA1 phosphorylation on S831 > increased unitary conductance
- LTP induction also promotes GluA1-contaning AMPA receptor delivery to synapses
- hippocampal LTP is induced by hippocampal-dependent learning
- preventing AMPA receptor traffic precludes learning

BUT

- some forms of hippocampal LTP are of purely presynaptic origin (e.g., mossy fiber>CA3)
- LTP at some hippocampal synapses does not involve activation of postsynaptic NMDA receptors (e.g., LTP onto GABAergic interneurons)
- LTD exists and involve mechanisms that somehow mirror those involved in LTP
- Late LTP involves other mechanisms that require de novo protein synthesis