

Mechanisms behind postsynaptic expression of LTP/D

Synaptic Plasticity — Lecture 7

Neuronal Physiology and Plasticity

Aug 2018 Semester

What is phosphorylation and what is dephosphorylation?

Phosphorylation is the process of adding a phosphate group to a protein.
Dephosphorylation is the process of its removal.

It is a common regulatory mechanism, and can lead to conformational changes in the underlying protein.

What are kinases?

Kinases form a family of enzymes that transfer phosphate groups from high-energy donor molecules, such as ATP, to specific substrates. i.e. Phosphorylation!

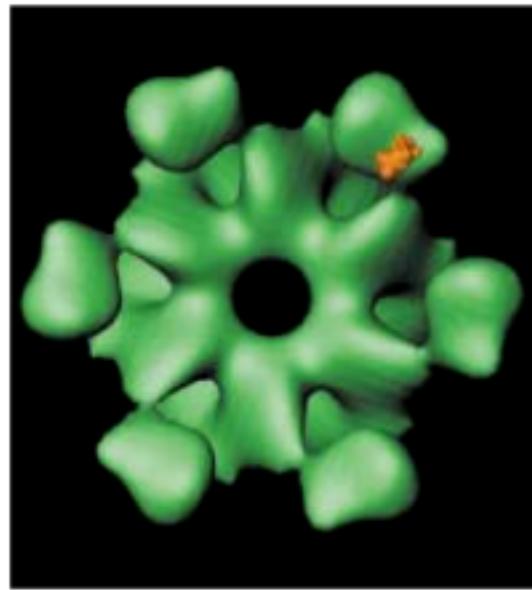
What are phosphatases?

Phosphatases are a family of enzymes that are involved in the removal of phosphate groups from their substrates. i.e. Dephosphorylation!

Proteins can have multiple sites for phosphorylation, each of which can be specific to a given kinase/phosphatase.

CaMKII: An important kinase for LTP!

Calcium/calmodulin-dependent protein kinase II

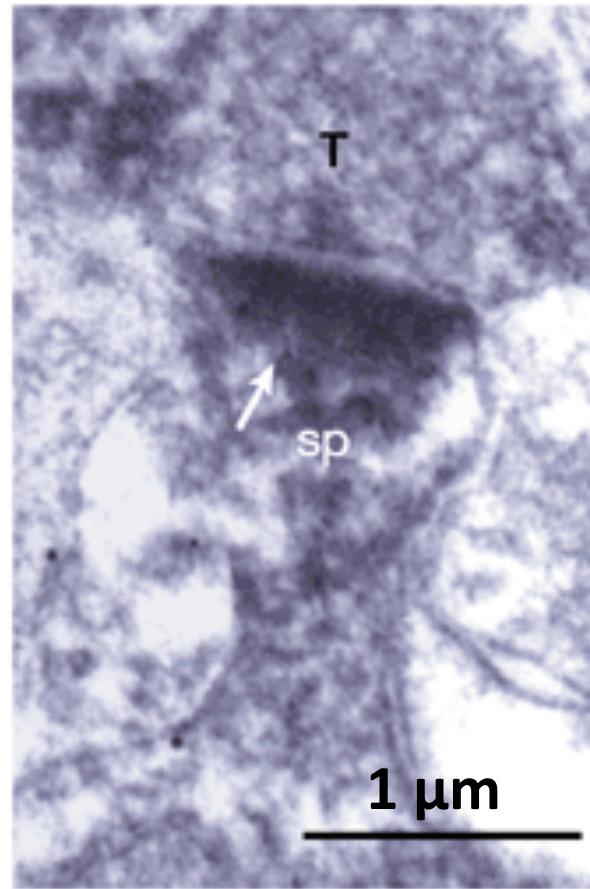


Dodecameric structure:
complex made of 12 subunits

Lisman et al. Nature Reviews Neuroscience, 2002

Three-dimensional structure of CaMKII, showing one of the hexameric rings formed by six subunits, forming a gear-like structure

CaMKII in the postsynaptic density is ideally positioned to detect local Ca^{2+} entry



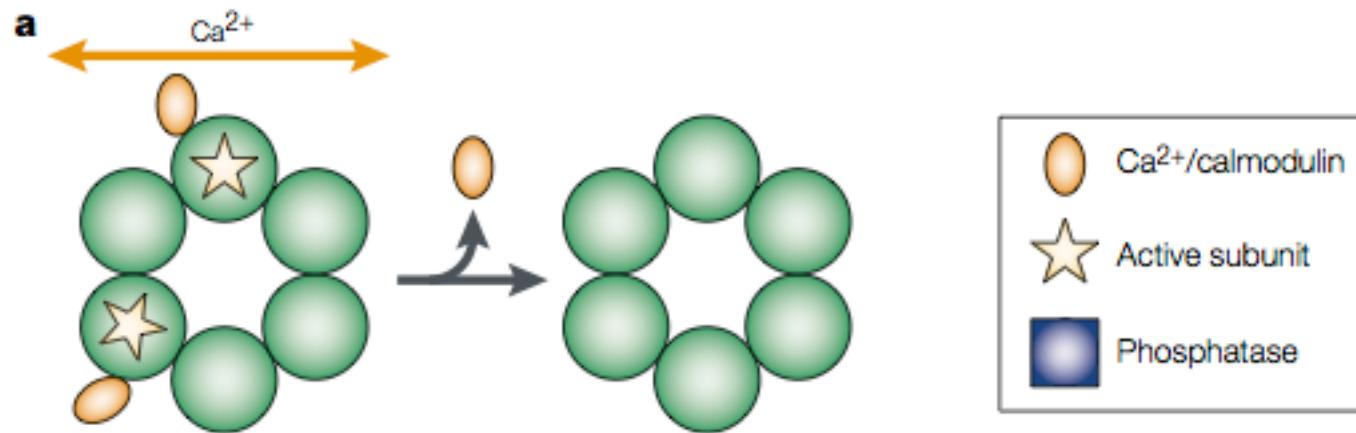
sp: Spine

T: presynaptic terminal

Lisman et al. Nature Reviews Neuroscience, 2002

What happens when calcium enters the cell during tetanus?

1. Calcium binds to calmodulin and Ca^{2+} /Calmodulin binds to CaMKII subunits to activate them

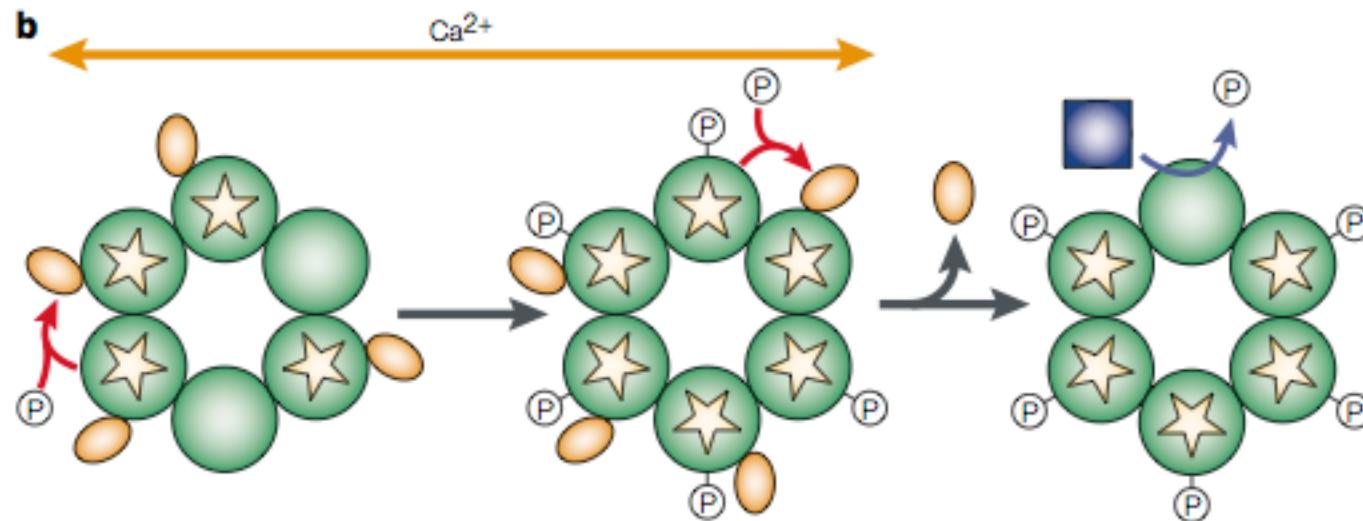


Lisman et al. Nature Reviews Neuroscience, 2002

If the duration/magnitude of calcium influx is low, nothing happens after this, and it gets inactive within 0.1– 0.2 s after calcium levels drop

Initiation and propagation of autophosphorylation

2. When two molecules of $\text{Ca}^{2+}/\text{Calmodulin}$ bind to different subunits of the same structure, conformational changes occur, making one phosphorylate the other!

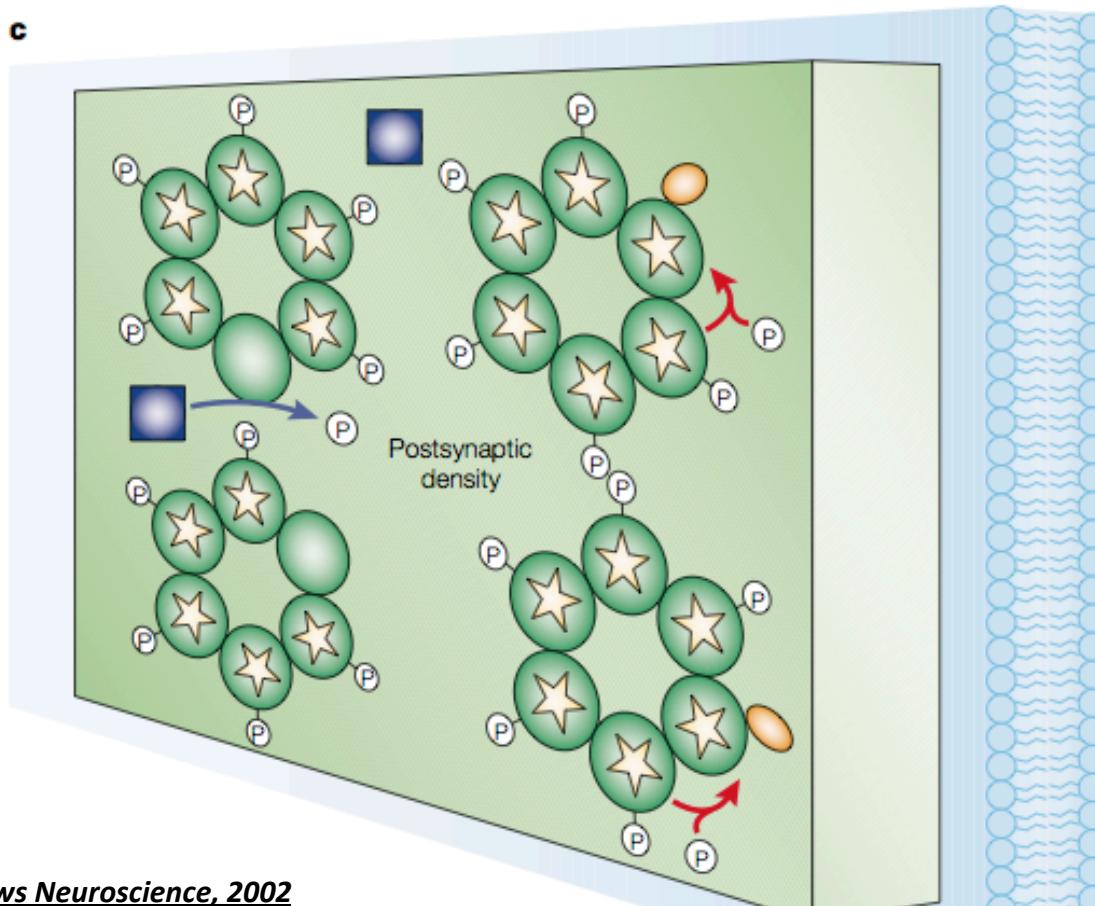


Lisman et al. Nature Reviews Neuroscience, 2002

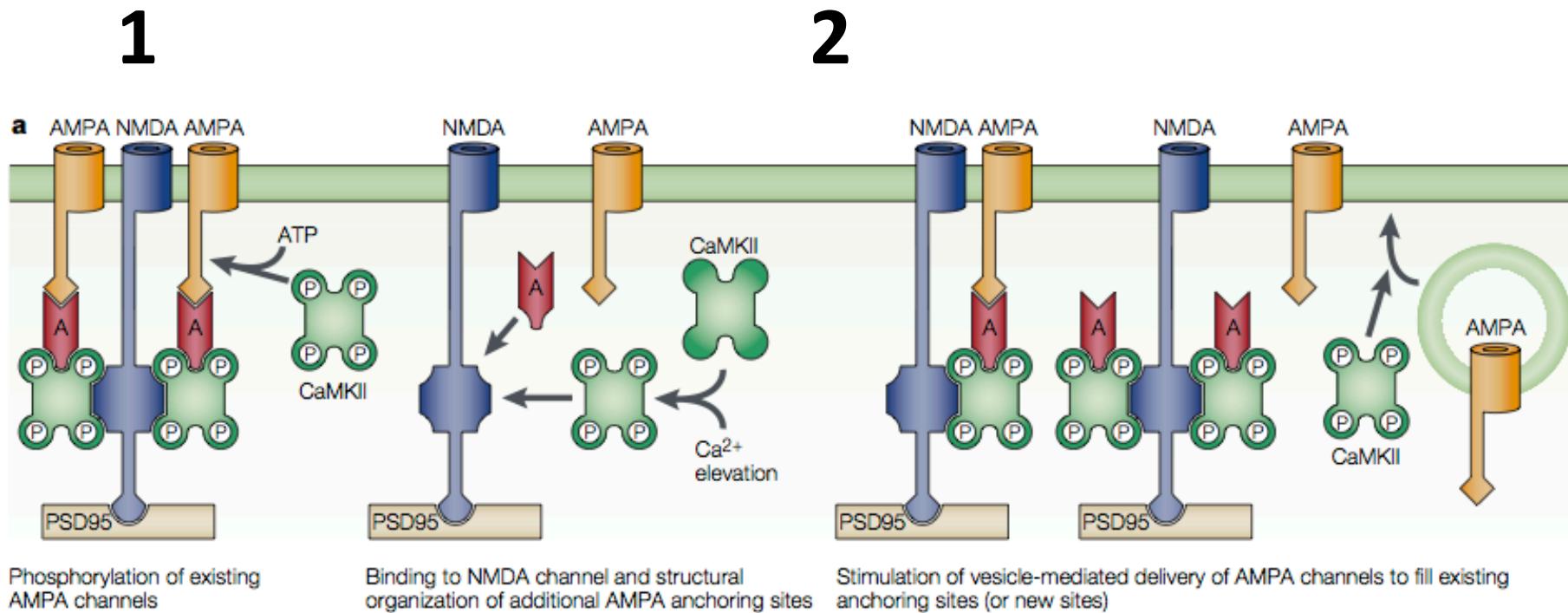
3. Phosphorylation propagates along the ring, and propagation requires lesser calcium because only one $\text{Ca}^{2+}/\text{Calmodulin}$ is required.

Persistence of phosphorylation

4. Once phosphorylated, it persists EVEN after Ca^{2+} levels are down, as long as dephosphorylation rates are lower than phosphorylation rates!



CaMKII then acts on AMPAR in two ways

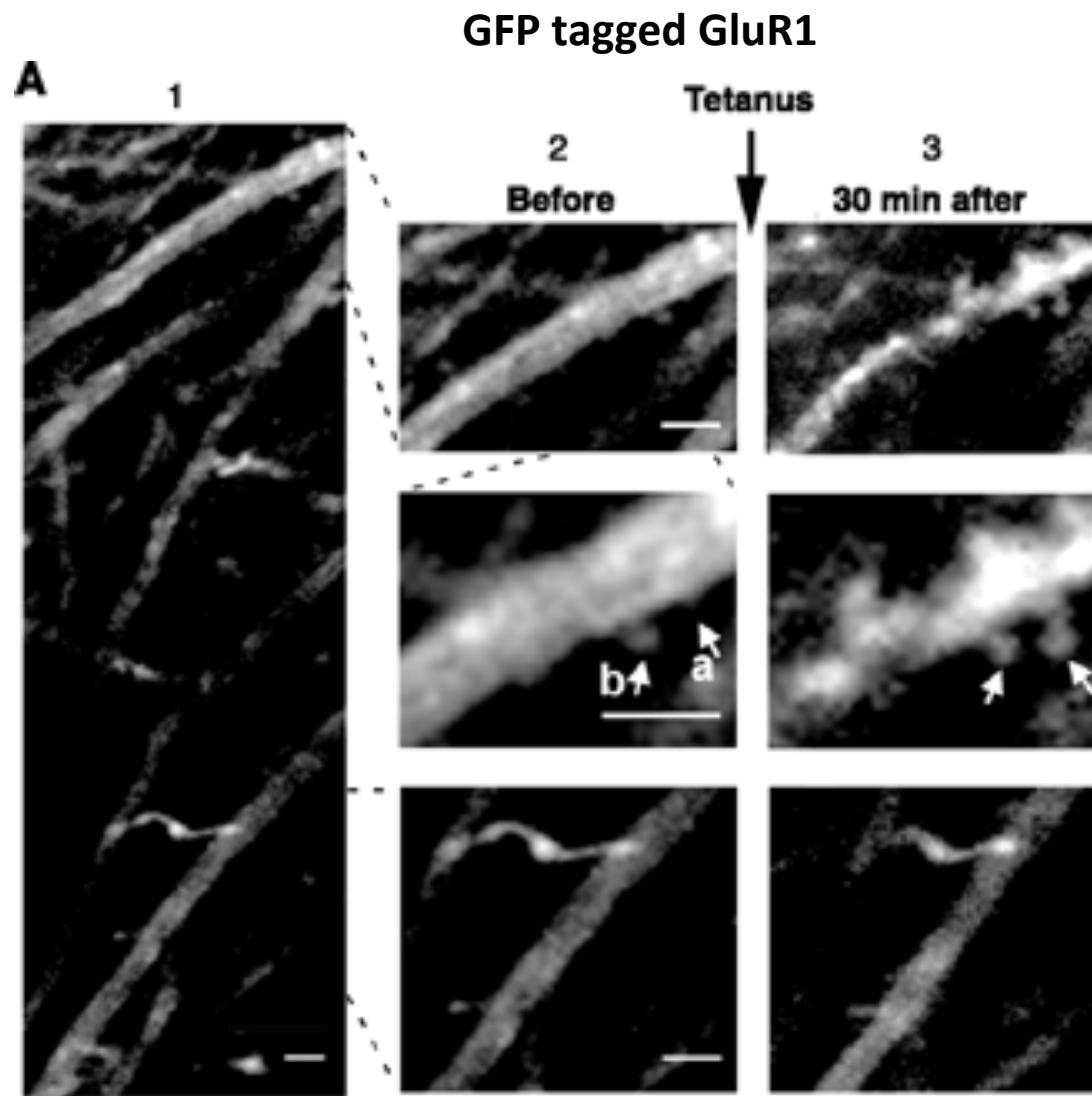


Lisman et al. Nature Reviews Neuroscience, 2002

Also see Lisman et al., Nat. Rev. Neurosci., 2012

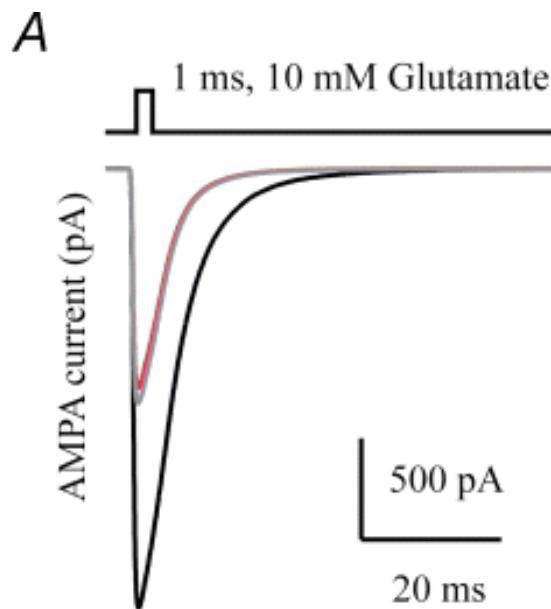
Insertion of AMPARs and increase in their conductance have been directly visualized/demonstrated!

Visualization of AMPAR insertion

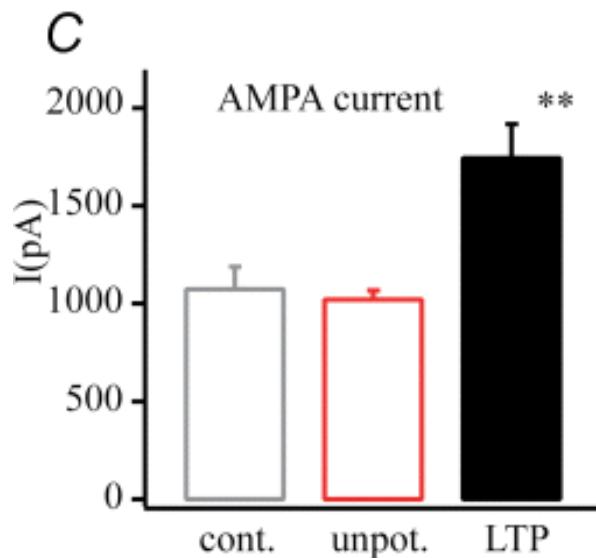


Shi et al., Science, 1999

AMPA currents from outside out patches

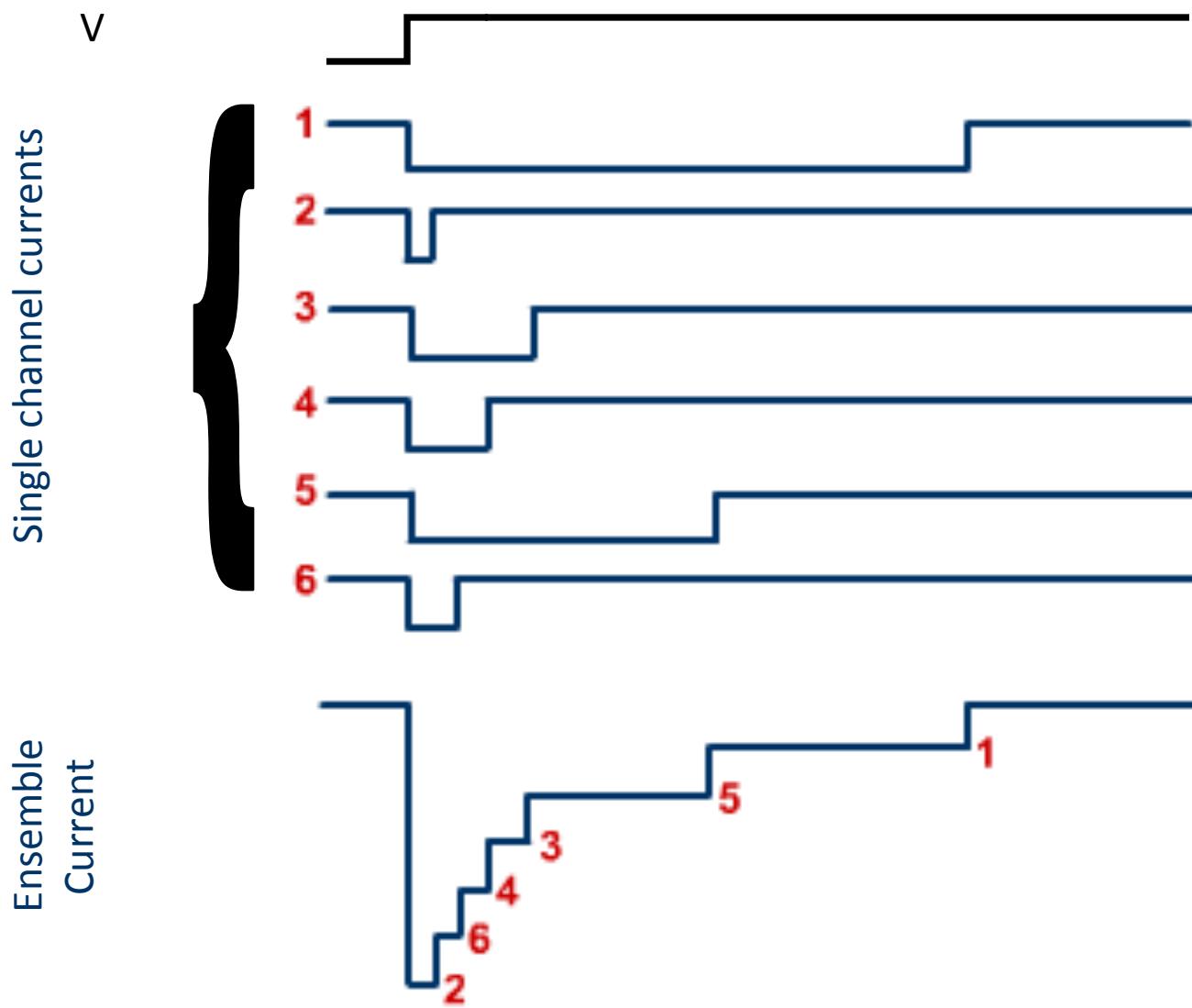


LTP through 100 Hz tetanus.



Nonstationary fluctuation analysis

Total recorded current is a sum of currents generated by individual channels



Non-stationary fluctuation analysis

A method by which properties of single channels currents that underlie a recorded macroscopic current can be deducted, even under conditions where single channel properties cannot be measured directly.

Non-stationary fluctuation analysis

Experimentally observed macroscopic current of N homogenous ion channels is:

$$I(t) = N \gamma P_o(t) (V(t) - V_{REV})$$

where $I(t)$ is the total measure current, P_o is the probability that the channel is open, γ is the single channel conductance, and V_{REV} is the reversal potential of the channel.

The variance of this current is given as:

$$\sigma_I^2 = \mu_I i - \mu_I^2 / N$$

Alvarez et al., *Advan Physiol Educ.*, 2002.

where i is the single channel current; $i = \gamma (V - V_{REV})$

Non-stationary fluctuation analysis

$$\sigma_I^2 = \mu_I i - \mu_I^2 / N \Rightarrow \text{Parabola}$$

$$\text{Slope at } \mu_I=0: \left. \frac{\partial \sigma_I^2}{\partial \mu_I} \right|_{\mu_I=0} = i = \gamma(V - V_{REV})$$

Initial slope is a measure of single channel conductance

The parabola reaches an extremum when:

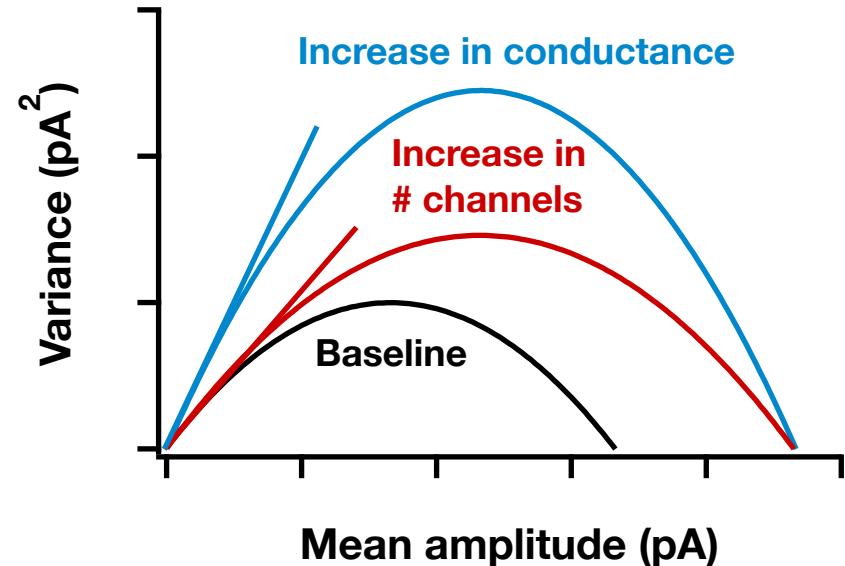
$$\frac{\partial \sigma_I^2}{\partial \mu_I} = i - \frac{2\mu_I}{N} = 0$$

which occurs when $\mu_I = \frac{Ni}{2}$

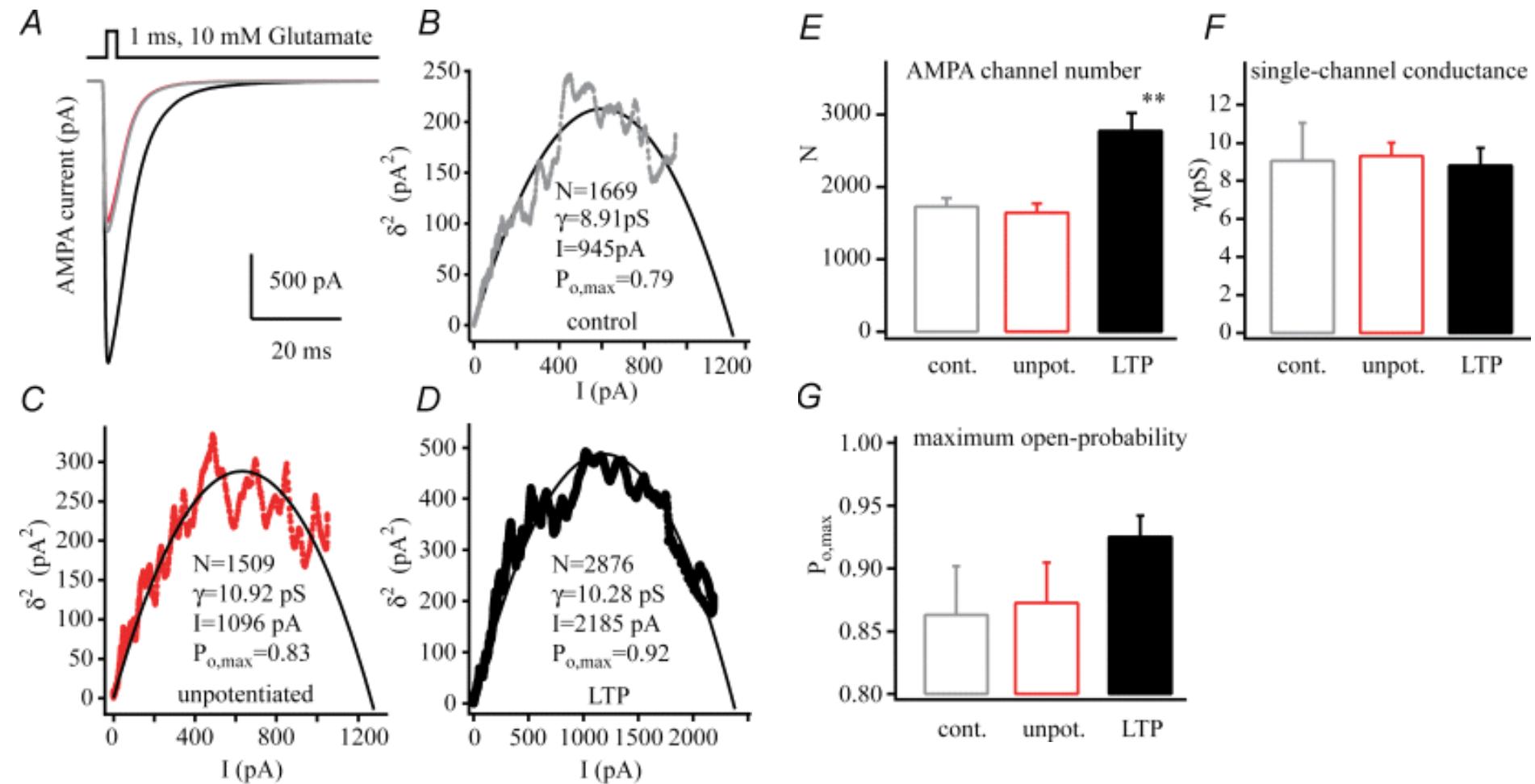
Number of channels can be estimated from the point at which the parabola reaches its maximum

Open probability can be obtained from the average current amplitude at steady state, I_{max} :

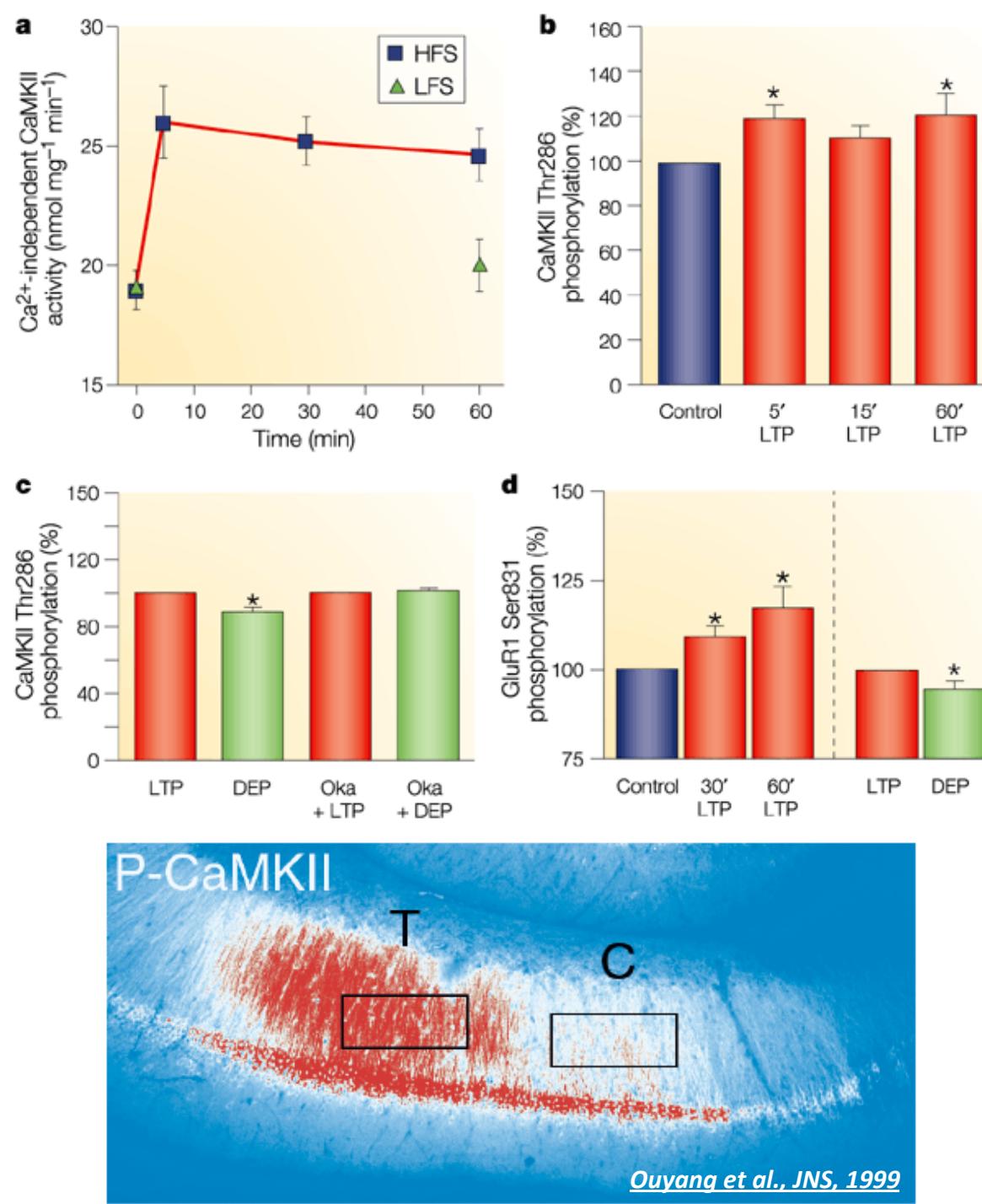
$$P_O = \frac{I_{max}}{iN}$$



Non-stationary fluctuation analysis on AMPAR currents



**How to show the involvement of
CaMKII?**



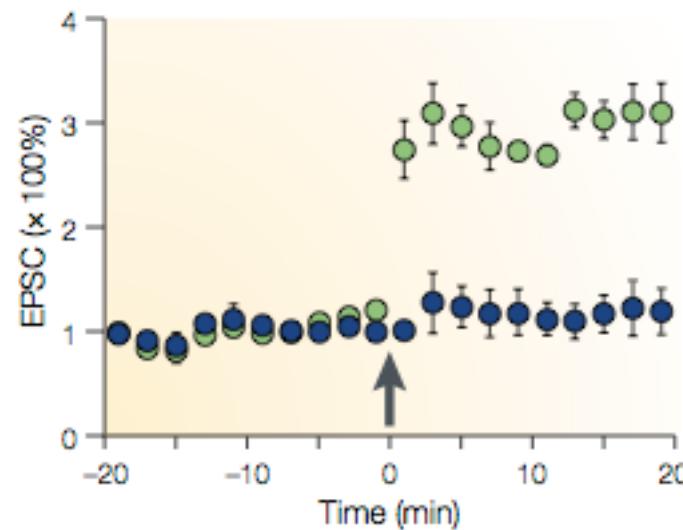
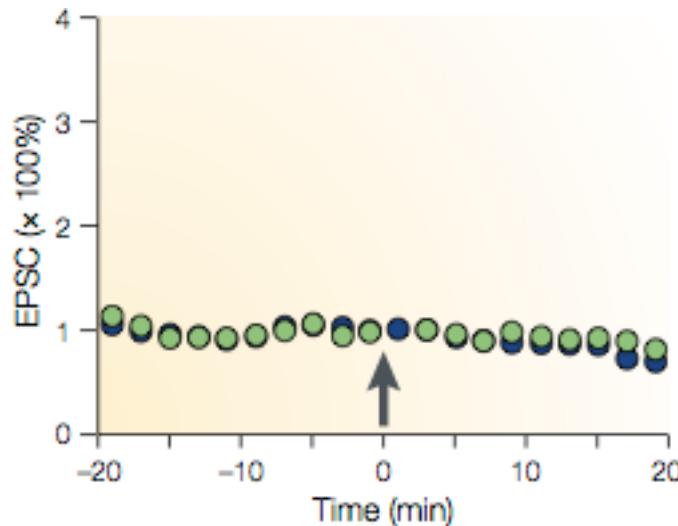
Phosphorylation of specific sites on CaMKII and GluR1

Oka: Okadic Acid, a Phosphatase inhibitor

CaMKII is necessary and sufficient for LTP

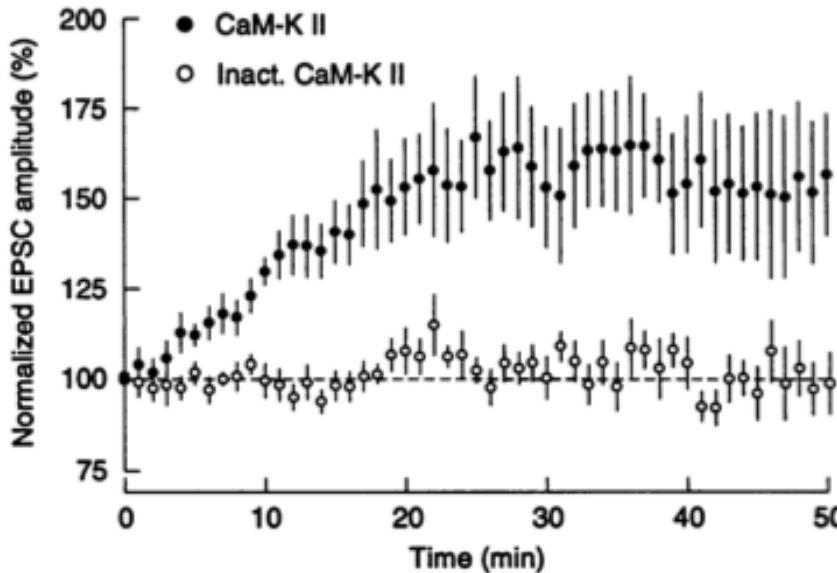
CaMKII inhibitory peptide in pipette

Necessity



Lisman et al. Nature Reviews Neuroscience, 2002

Sufficiency

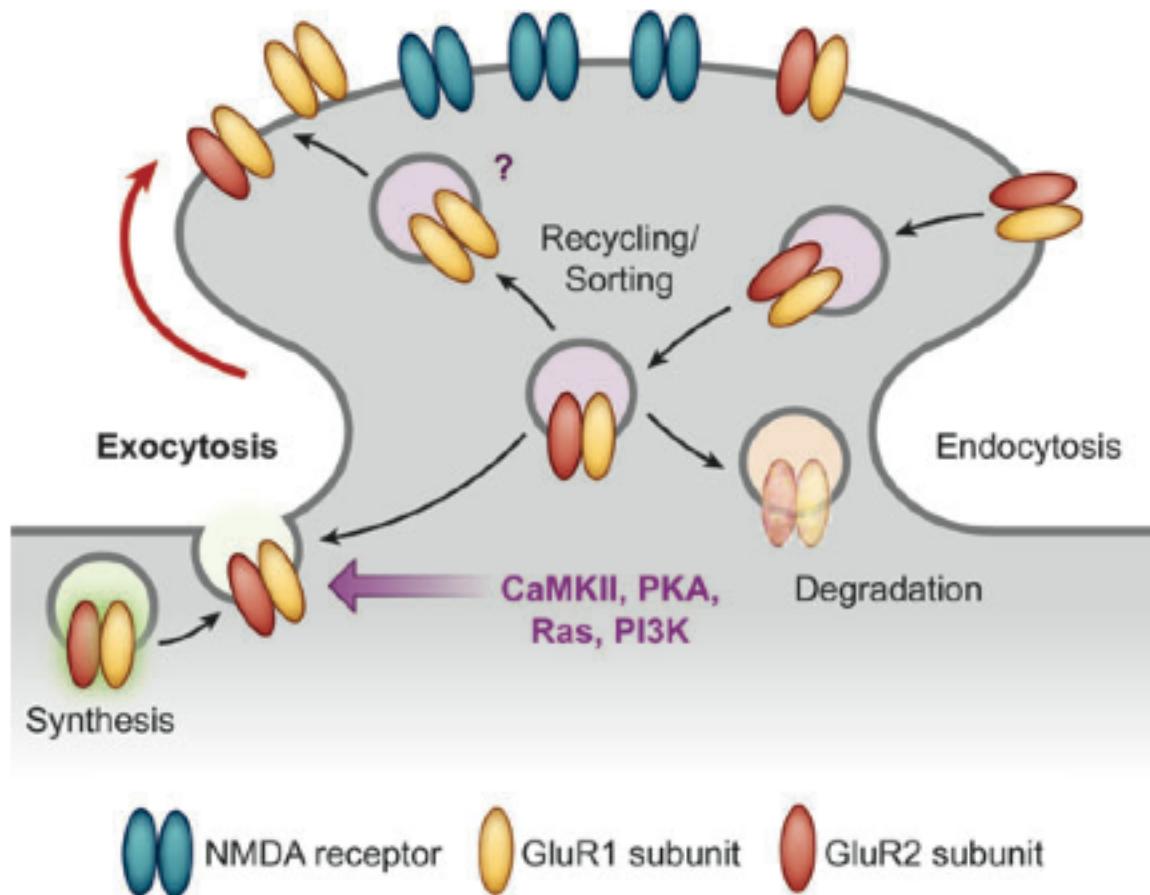


Also see Hayashi et al., Science, 2000 for a direct link between CaMKII and GluR1. Also see Lisman et al., Nat. Rev. Neurosci. 2012.

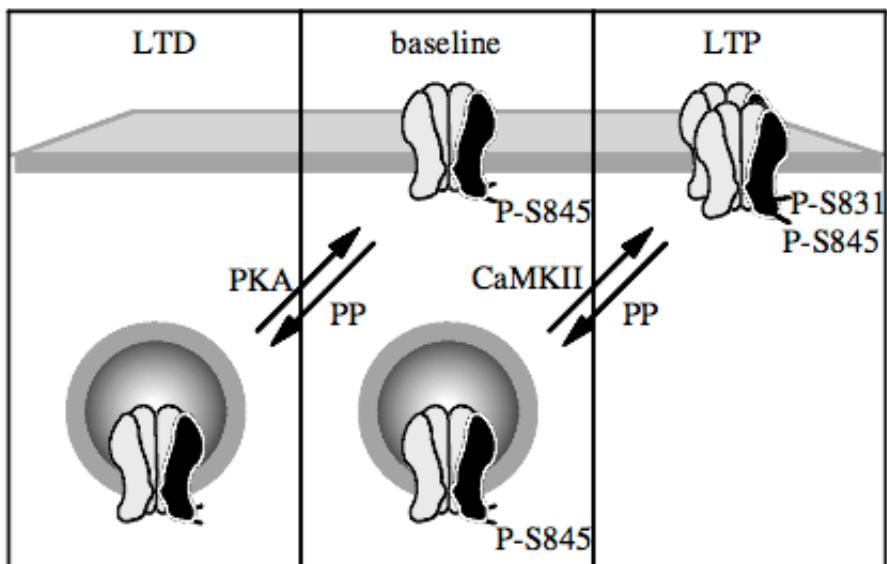
Lledo et al., PNAS, 1995

Surprise, surprise! CaMKII is not the only one that can do that!

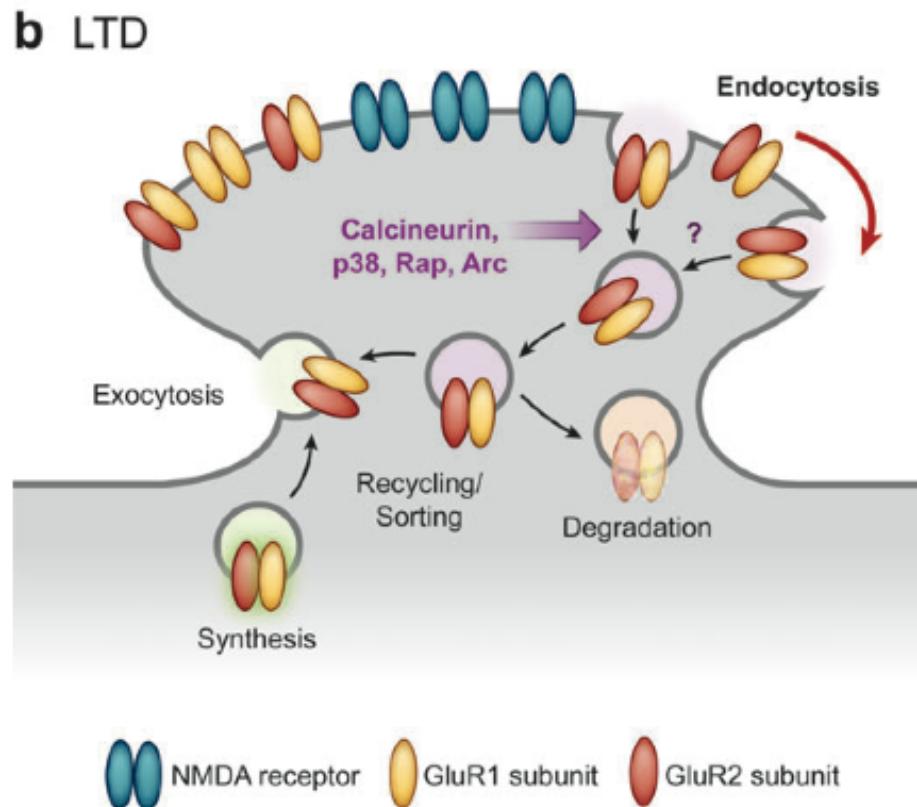
a LTP



LTD involves internalization of receptors or reduction of AMPAR conductance



Bear, PTRSL, 2003

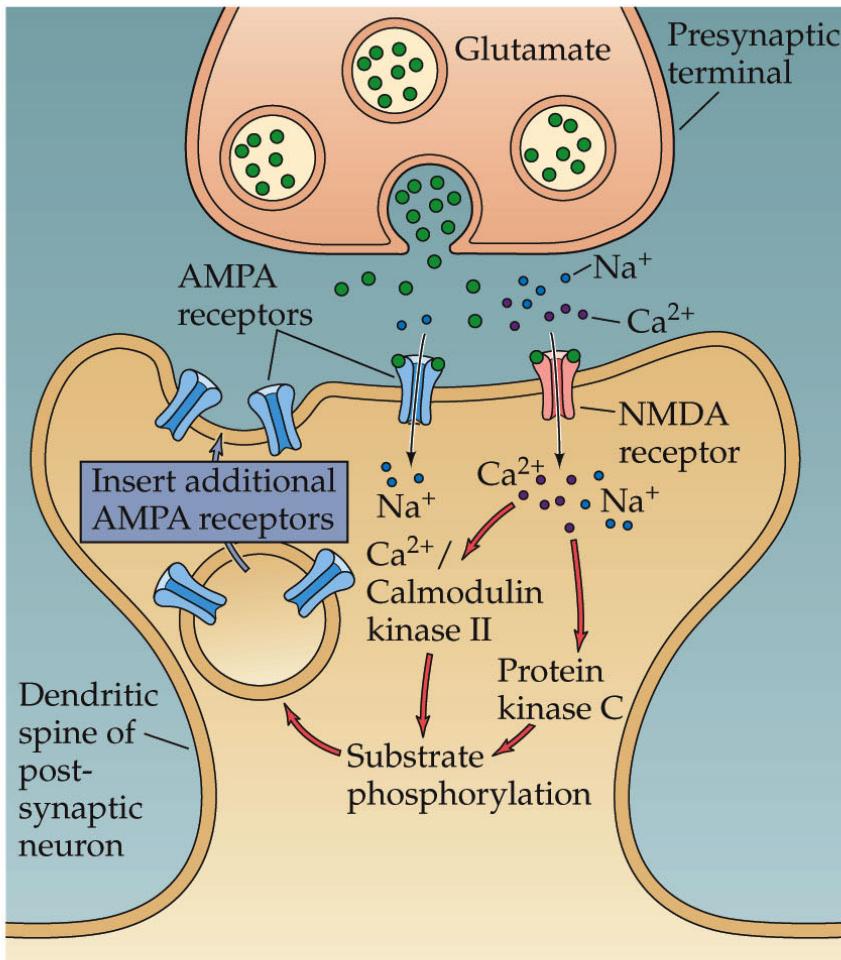


Dephosphorylation is through protein phosphatases

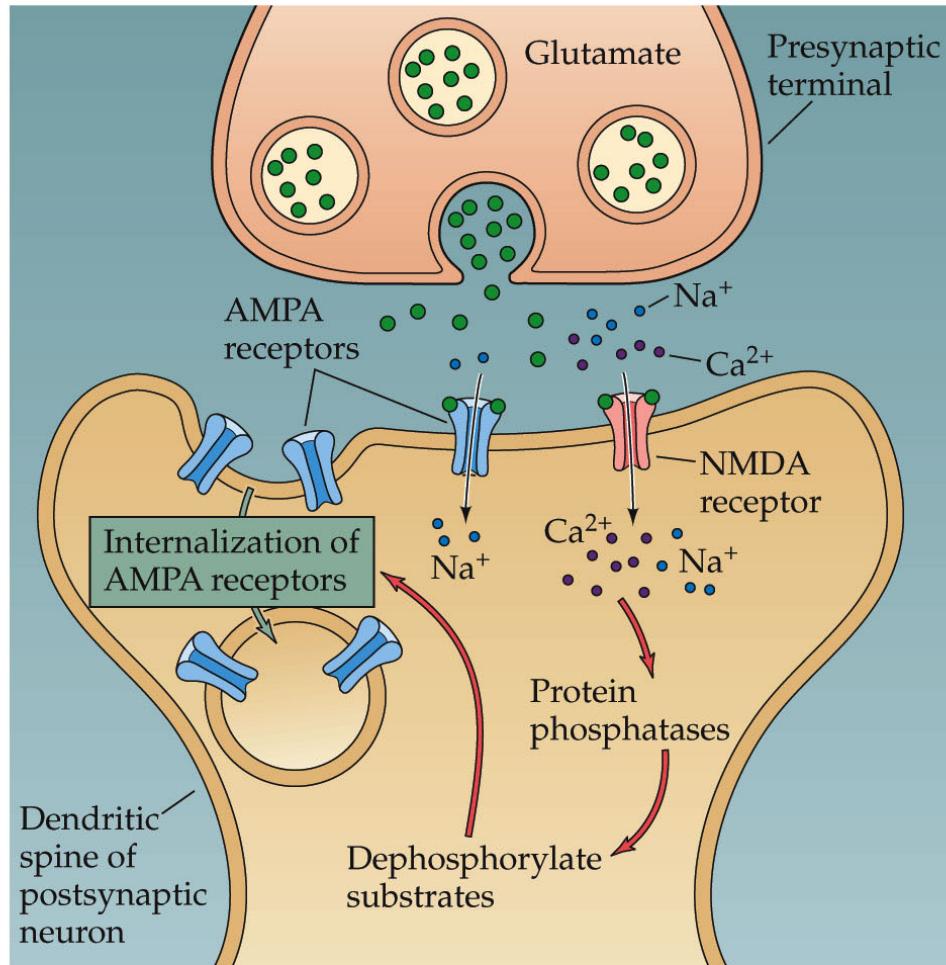
Different synapses, different rules!

Hippocampus

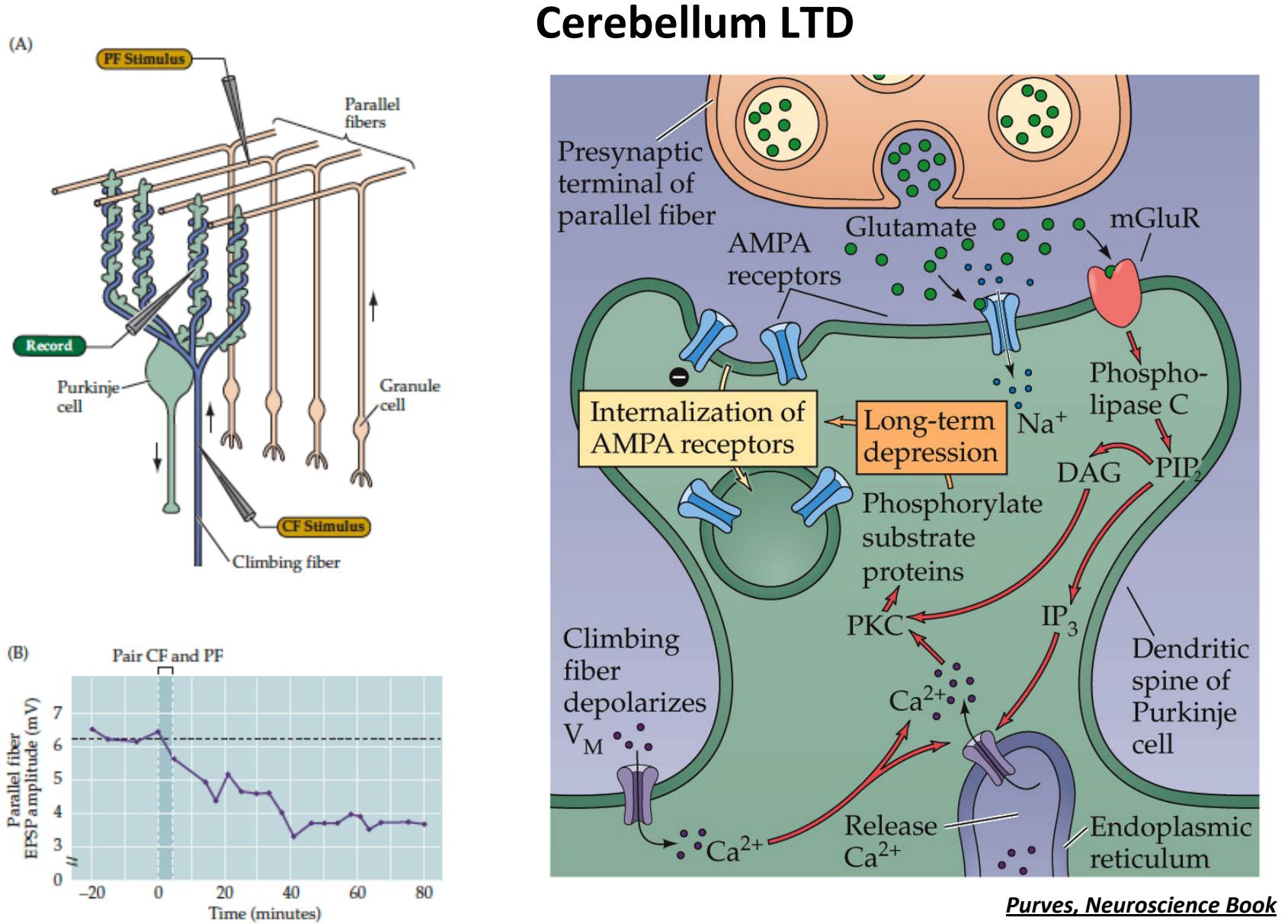
LTP



LTD

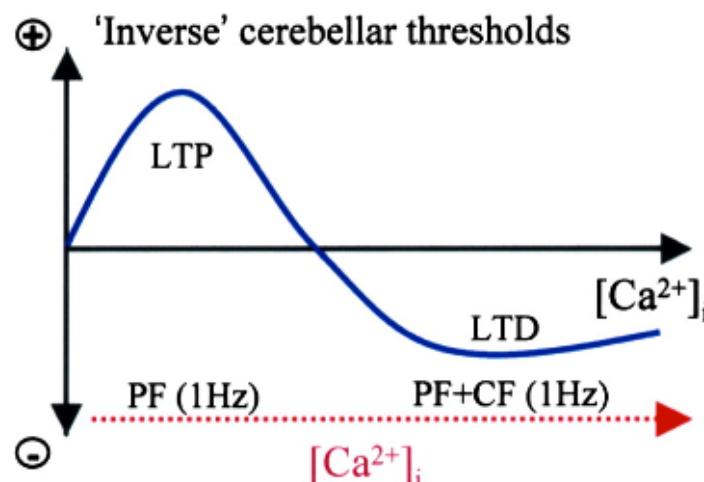
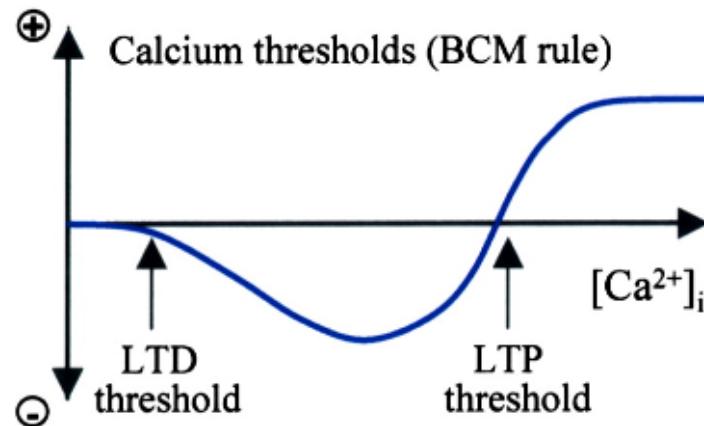


Different synapses, different rules!

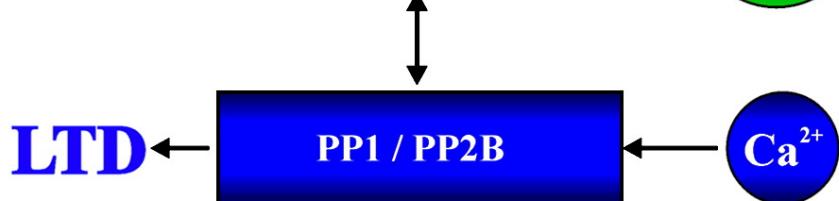
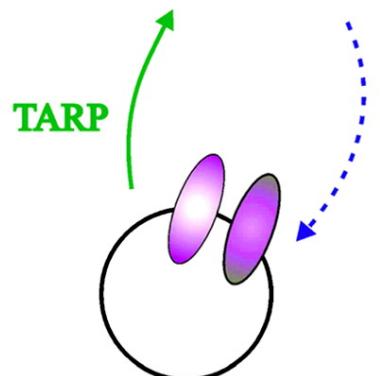
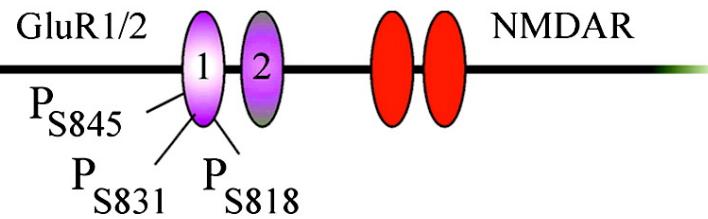


Different synapses, different rules!

Induction in hippocampal vs. cerebellar synapses

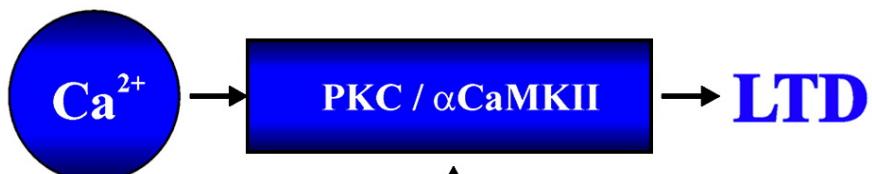
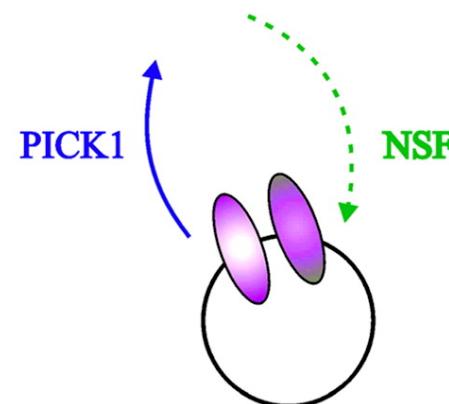
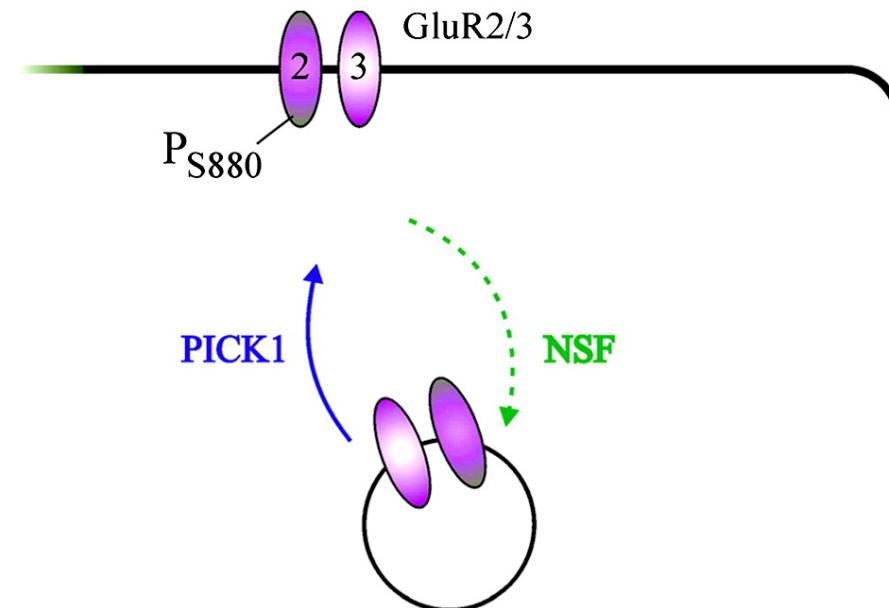


Different synapses, different rules!



Hippocampus

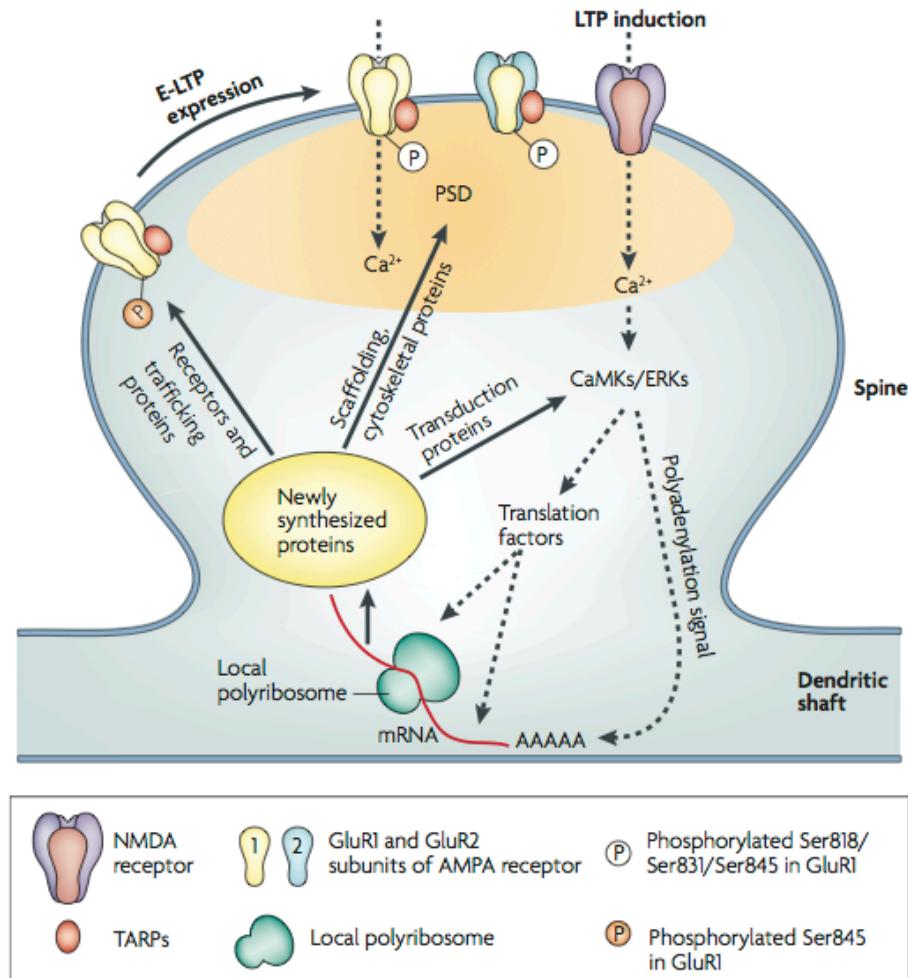
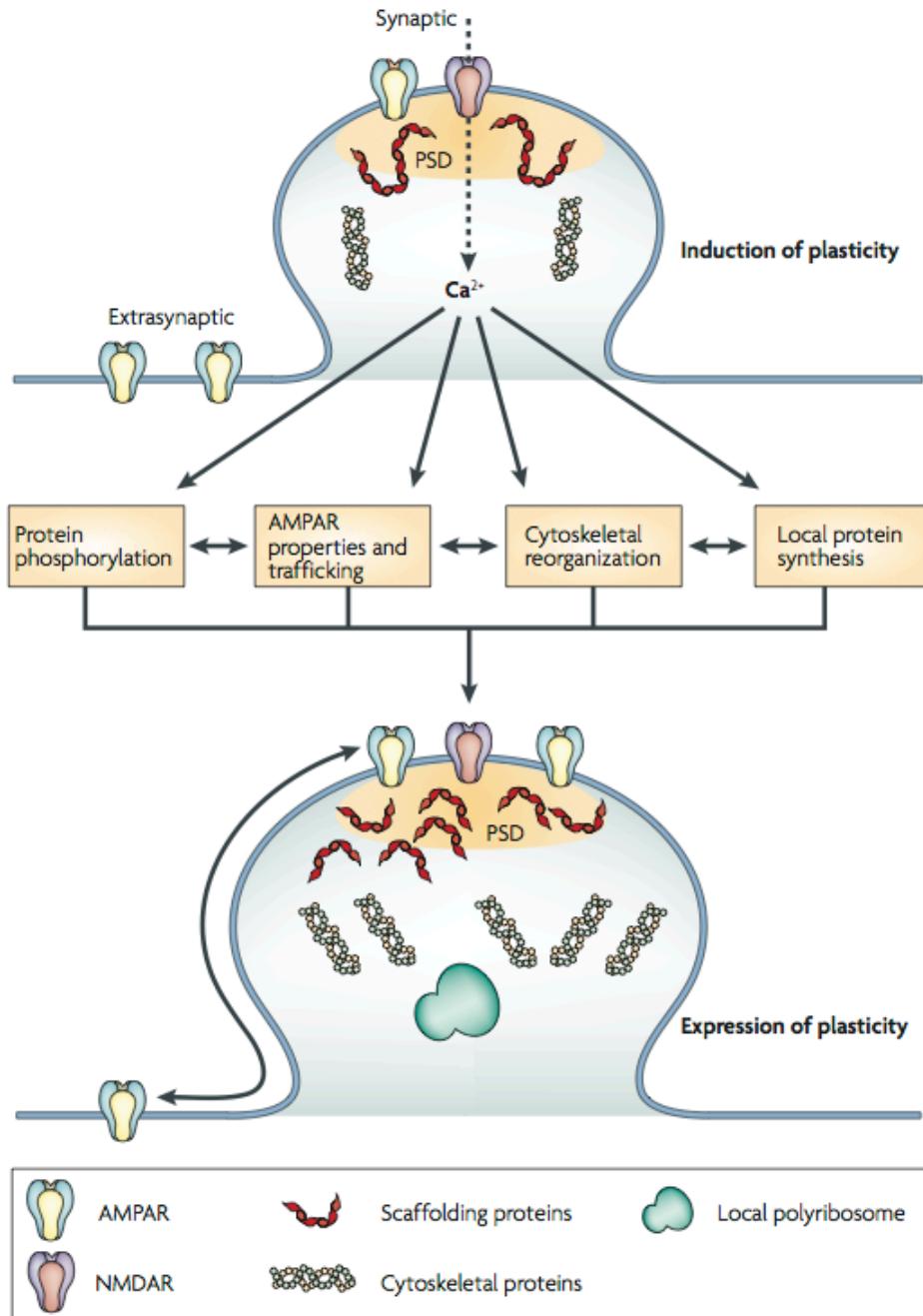
Pyramidal cell



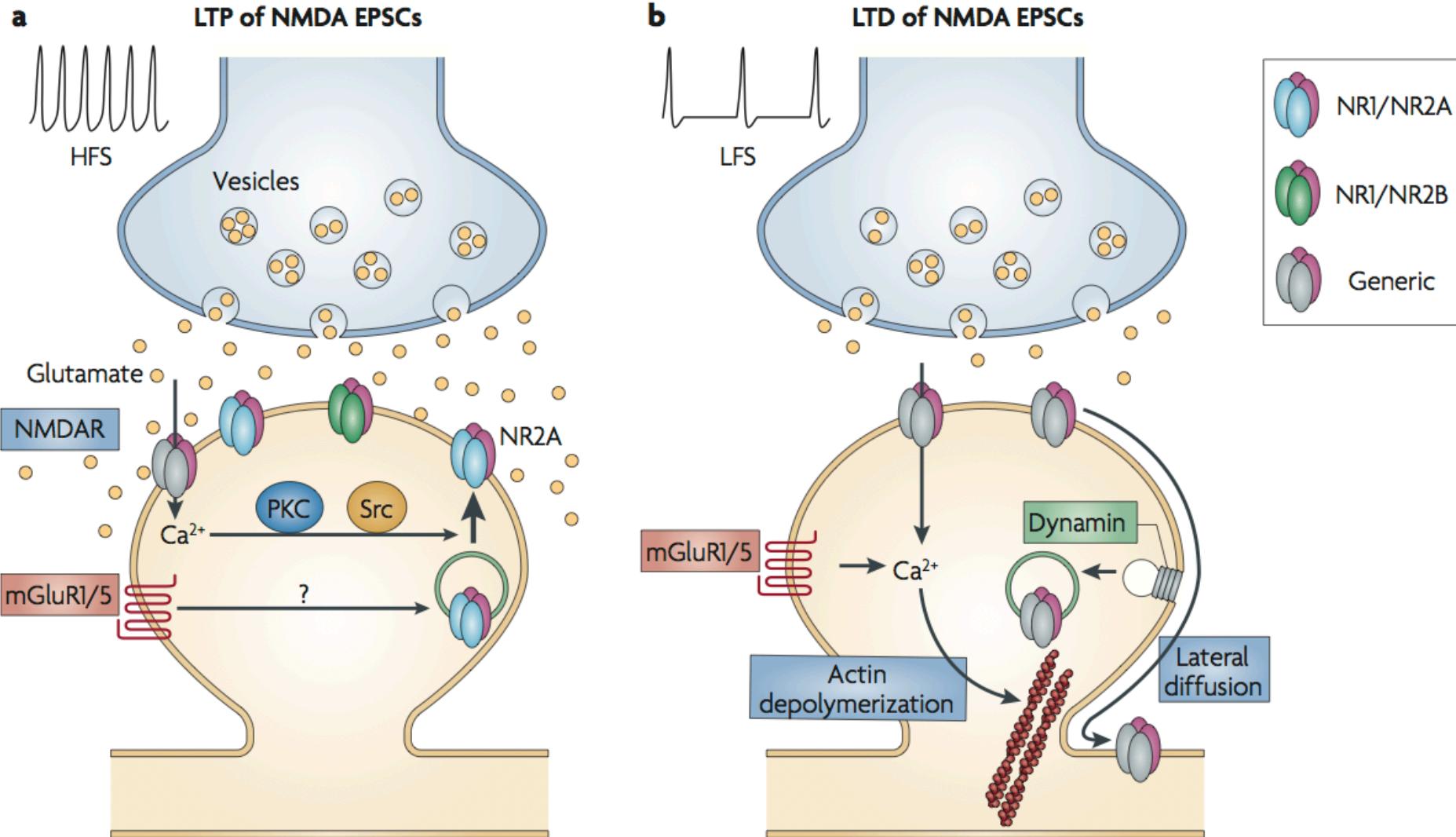
Cerebellum

Purkinje cell

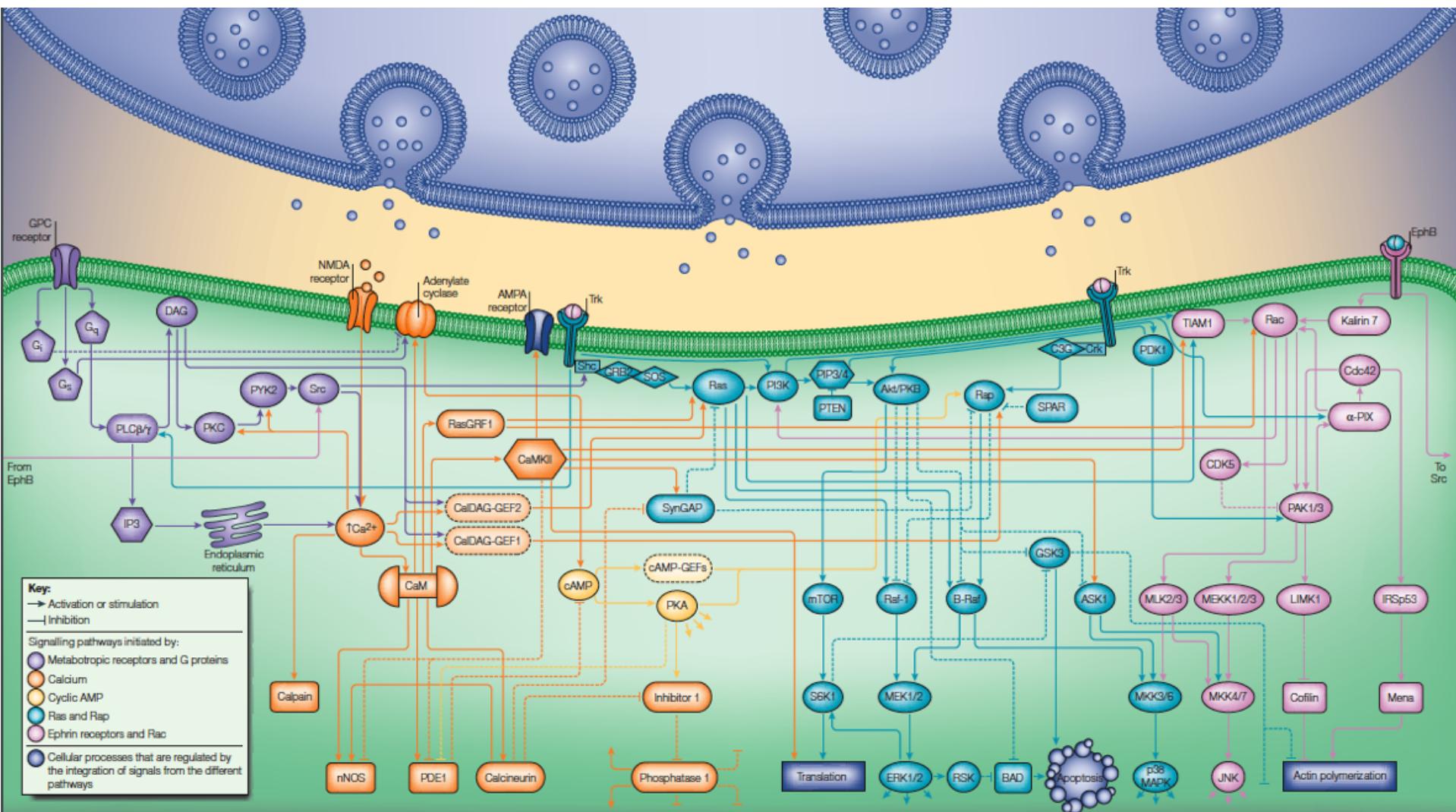
Other players



NMDAR trafficking also is known!!!



Postsynaptic pathways: some of them!



What did we learn today?

AMPAR: Phosphorylation by various kinases and dephosphorylation by various phosphatases regulate plasticity postsynaptically

LTP is expressed by an increase in conductance and/or by insertion of new receptors, while LTD is through a decrease in conductance and/or by internalization of receptors. NMDAR trafficking is also known!

Other postsynaptic changes are associated with cytoskeletal reorganization.

Synapses, protocols and signaling mechanisms are unique — **DO NOT GENERALIZE**