Neurofunctional Techniques

Lesson 2

2 October 2026

Ca^{2+} signaling

How to study the brain:

1. How to 'watch' brain activity:
   A. Functional imaging
   B. Electrophysiology
   C. Behavior
2. How to 'tell' the brain what to do:
   A. Optogenetics
   B. X-genetics
3. How to repair the brain:
   A. Genome editing
   B. X-genetics

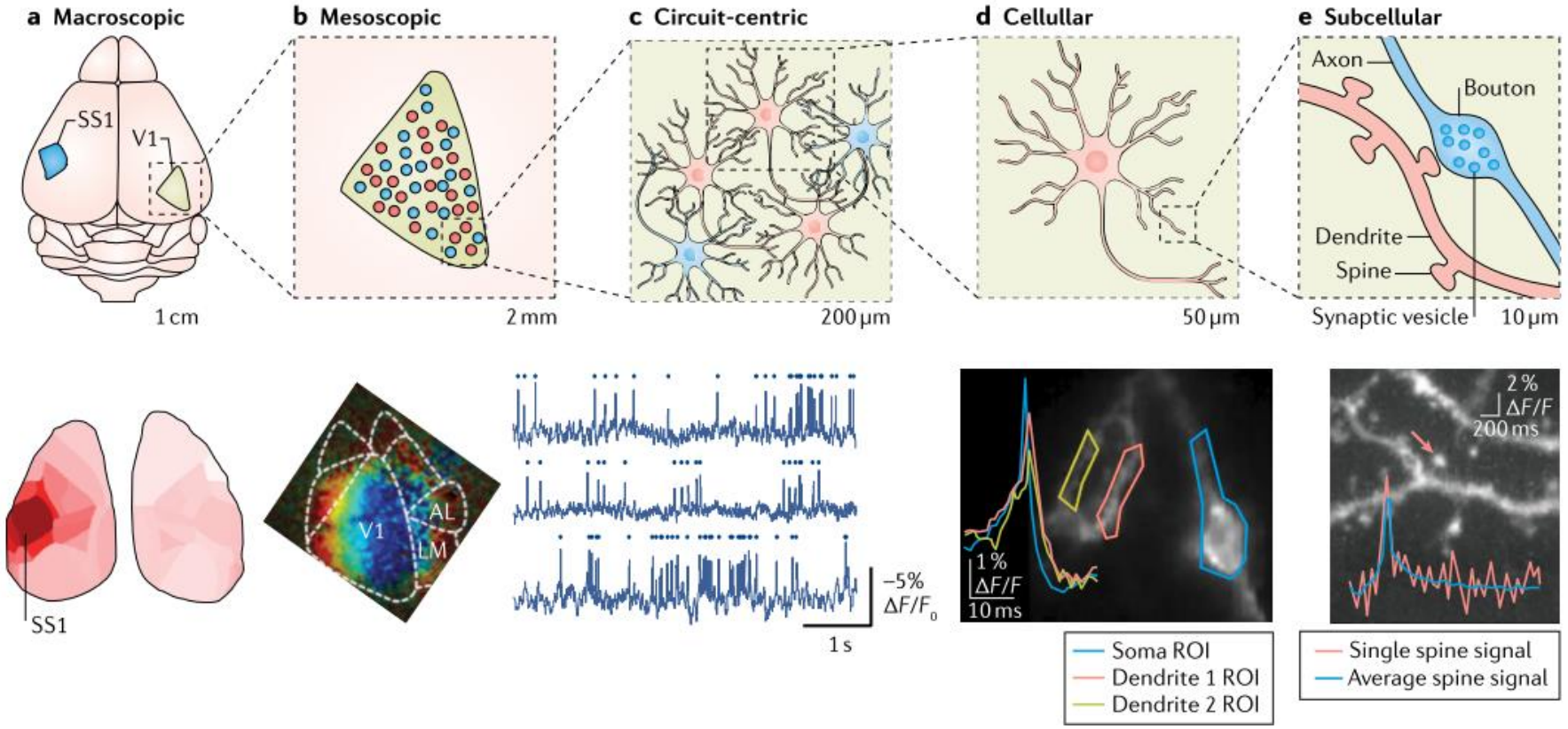
How to study the brain:

1. How to ‘watch’ brain activity:
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3. How to repair the brain:
    A. Genome editing

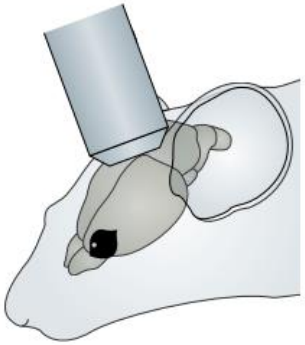
Spatial scales and level of analysis of functional imaging



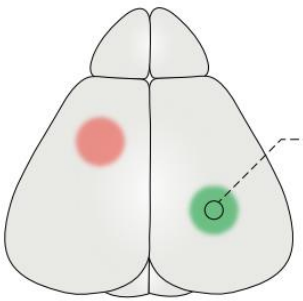
Knoepfel & Song, 2019

In vivo experimental setup configurations for different levels of functional imaging, from macroscopic to subcellular level analyses

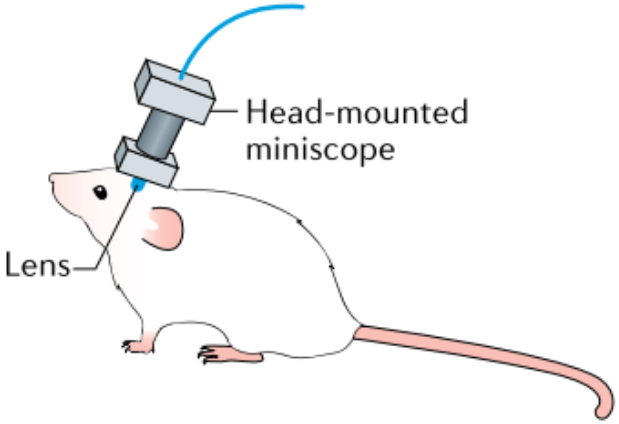
Fiber photometry
in freely moving animals
Macroscopic - mesoscopic



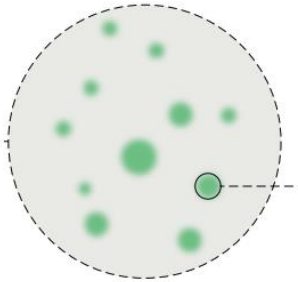
Dorsal surface of cortex



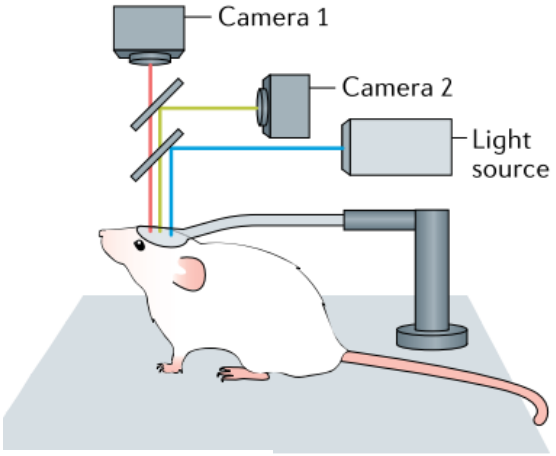
Miniscope
in freely moving animals
Circuit - cellular



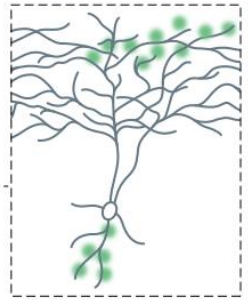
Cellular resolution



2-foton microscopy
in head-fixed animals
Cellular - subcellular

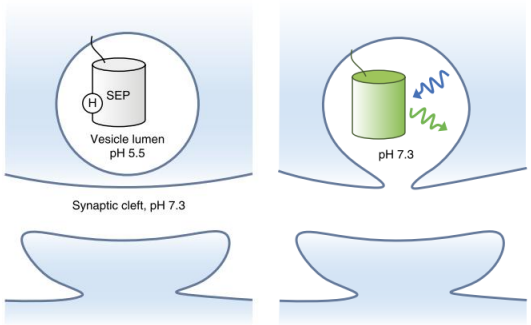


Subcellular resolution

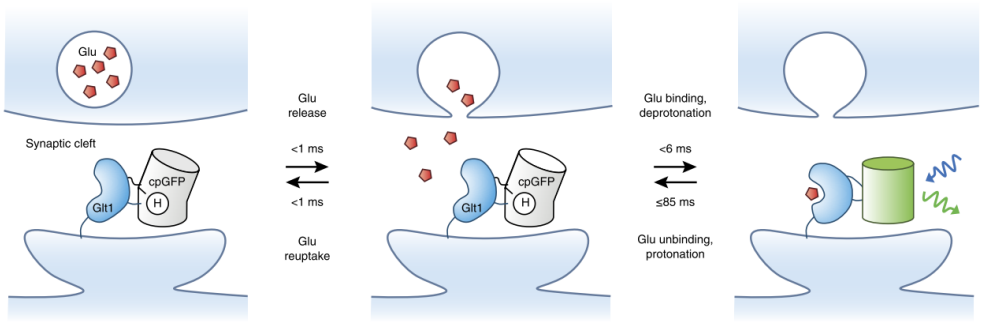


Indicators of neuronal activity

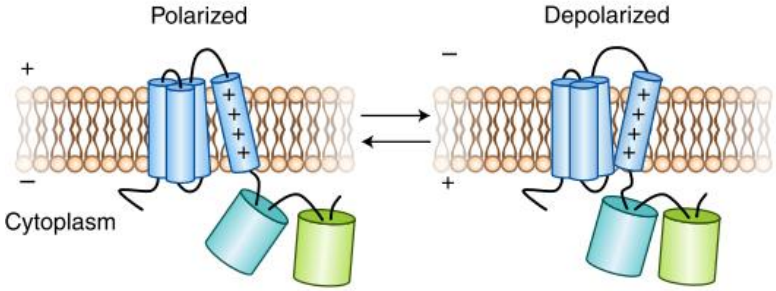
pH indicators



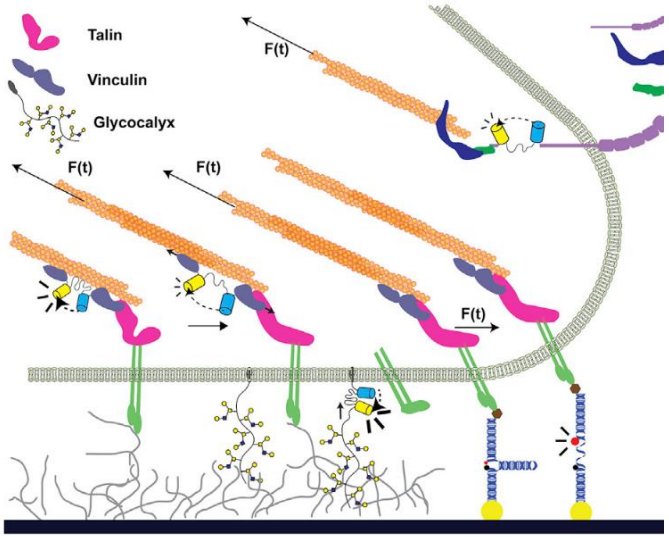
Neurotransmitter indicators (e.g. Glu)



Voltage indicators



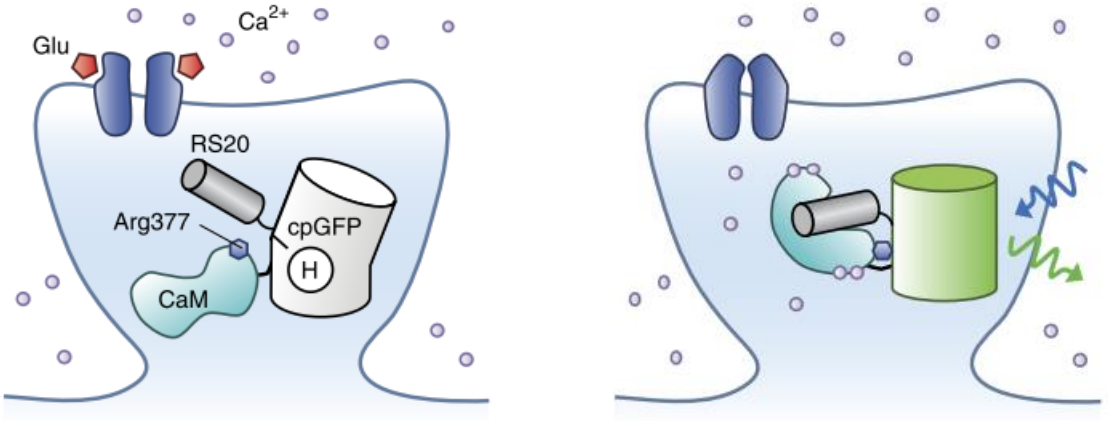
Tension indicators



- Signaling pathways (e.g. GTPases)
- Chemical reactions (e.g. Phosphorylation)
- Gene expression
- Protein-protein interactions
- Second messengers (e.g. Ca^{2+} / cAMP)

Indicators of neuronal activity

Ca^{2+} indicators



Objectives

- To understand the principles that allow Ca^{2+} imaging to provide quantitative data
- Evaluate the conditions under which Ca^{2+} imaging yields quantitative results

Ca^{2+} imaging

- Review Ca^{2+} signaling
- Ca^{2+} indicators
- Ca^{2+} binding
- Ca^{2+} diffusion
- Ca^{2+}-dependent fluorescence properties
- *In vitro* and *in vivo* imaging devises
- Key examples from the literature

Ca^{2+} signaling

Changes in $[Ca^{2+}]_i$ underlie fondamental cellular processes such as:

- **Exocytosis**
- **Muscle contraction**
- **Synaptic plasticity**
- **Cell division**
- **Gene expression**



Wide time range from µs to hrs

Effects are mediated through binding to Ca^{2+}-binding proteins (e.g. calmodulin)

With Ca^{2+} imaging we want:

1) to read out $[Ca^{2+}]_i$ (timing, amplitude, time course, location)
so that cellular process X = f($[Ca^{2+}]_i$)

or

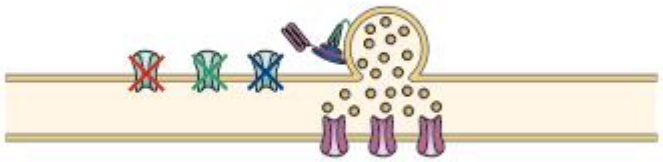
2) to use it as a proxy for activity patterns
(of synapses, dendrites, neurons/glial cells, neuronal/glial networks, brain regions...)

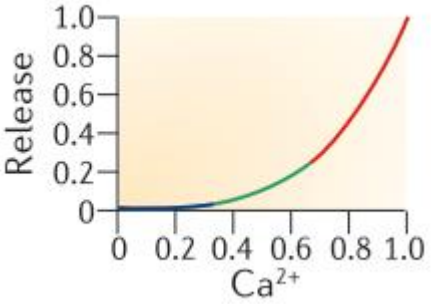
Power law relationship between Ca^{2+} entry and neurotransmitter release

$$EPSC = k*[Ca^{2+}\ influx]^n$$

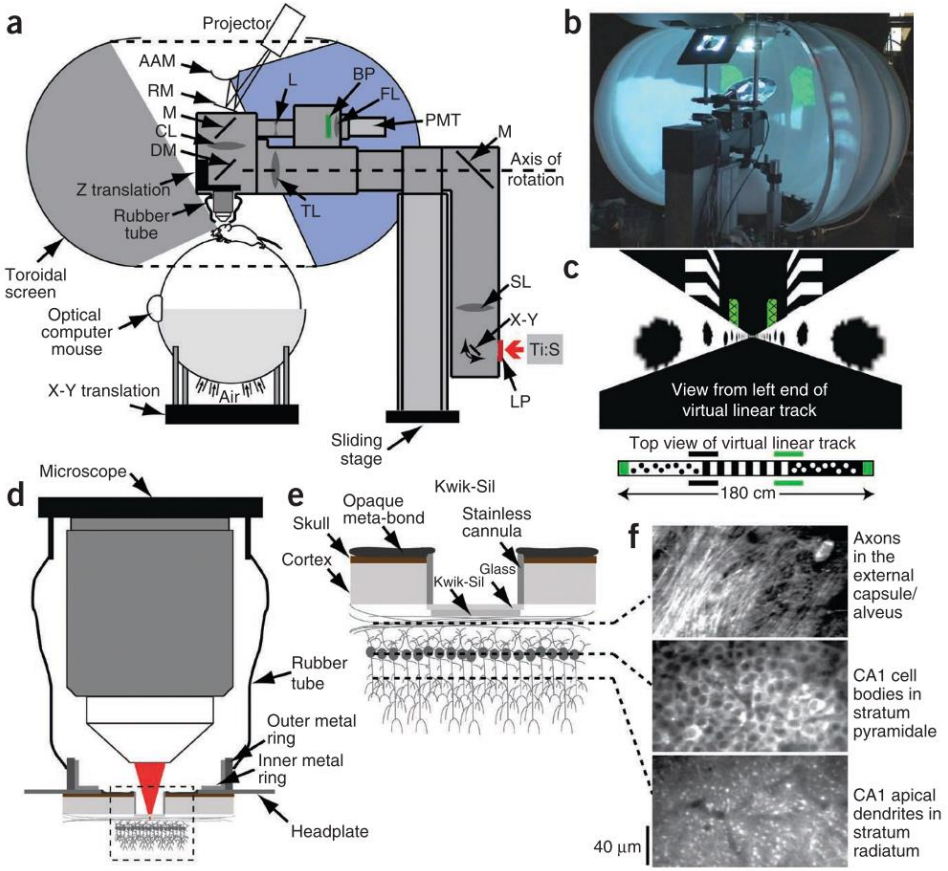
$$n = 3 - 4$$

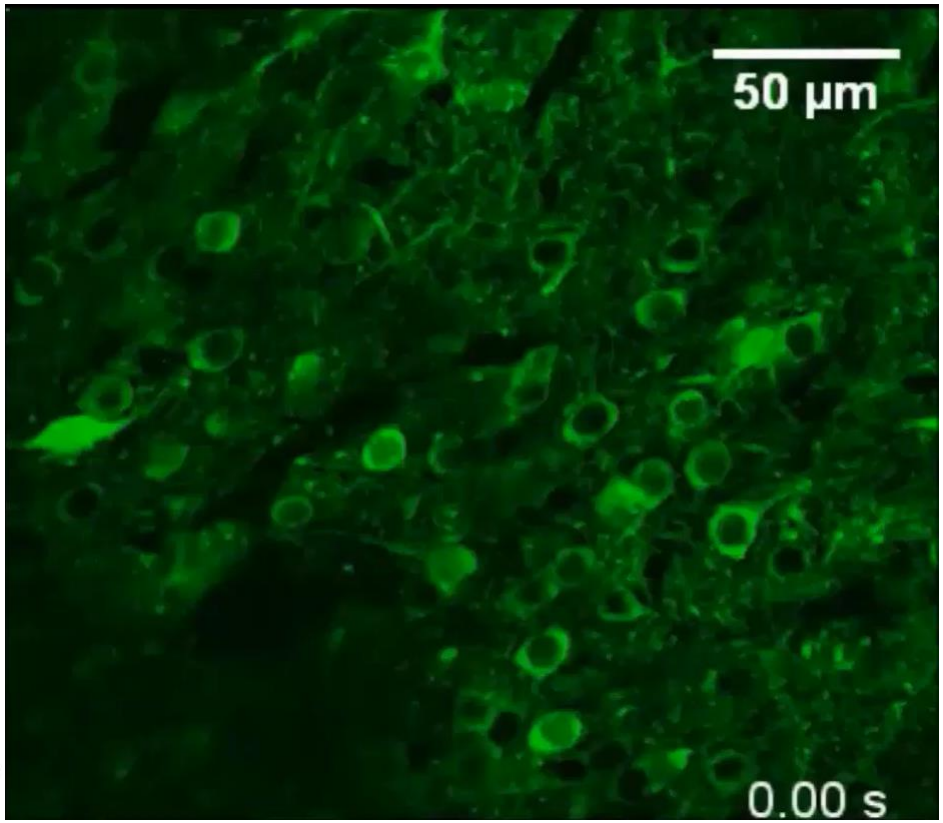
which means that doubling Ca^{2+} influx will increase the synaptic response 8- to 16-fold





Ca^{2+} imaging as a proxy for activity patterns





Ca^{2+} imaging

- **Review Ca^{2+} signaling**
- Ca^{2+} indicators
- Ca^{2+} binding
- Ca^{2+} diffusion
- Ca^{2+}-dependent fluorescence properties
- Simplified models of Ca^{2+} dynamics
- Imaging devises
- What can we do with it

Why Ca^{2+} is such an important signal?

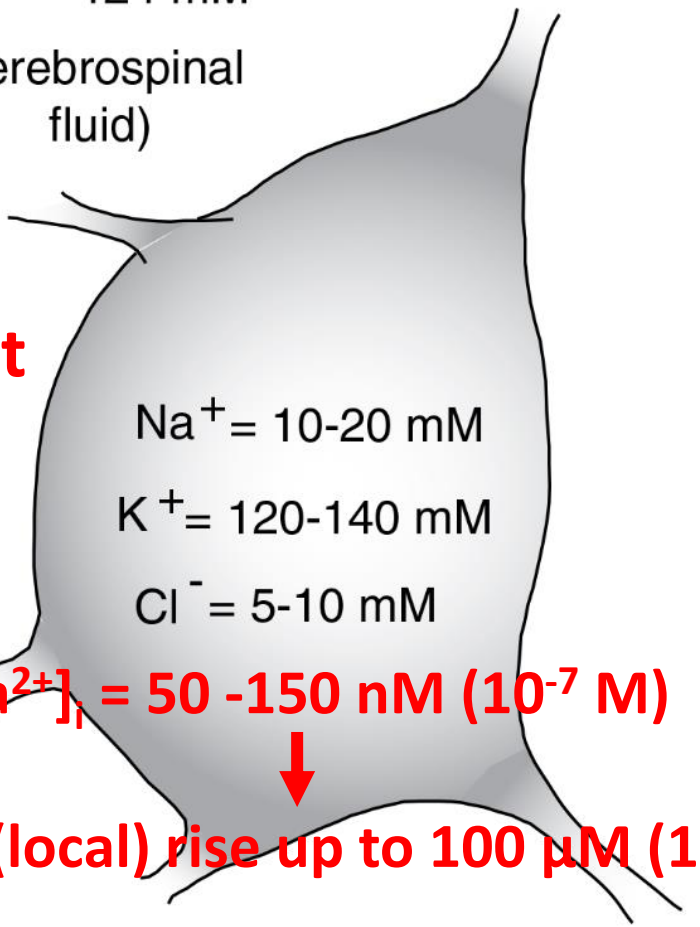
$[Ca^{2+}]_o$ = 2 mM (10^{-3} M)

Na^+ = 141 mM

K^+ = 3 mM

Cl^- = 124 mM

(cerebrospinal fluid)

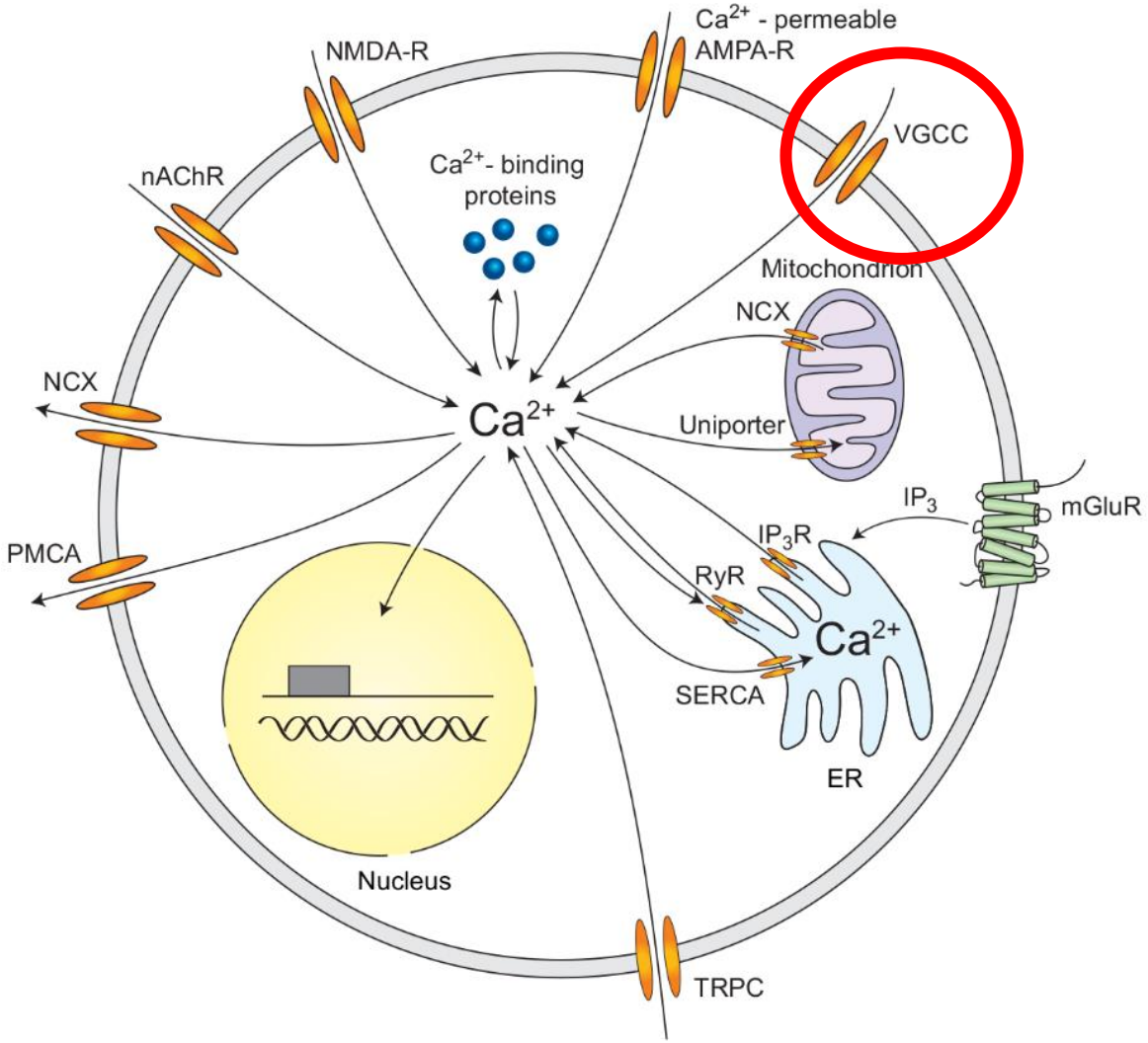


Steep concentration gradient

Transient (local) rise up to 100 µM (10^{-4} M)

Known

Neuronal Ca^{2+} signaling

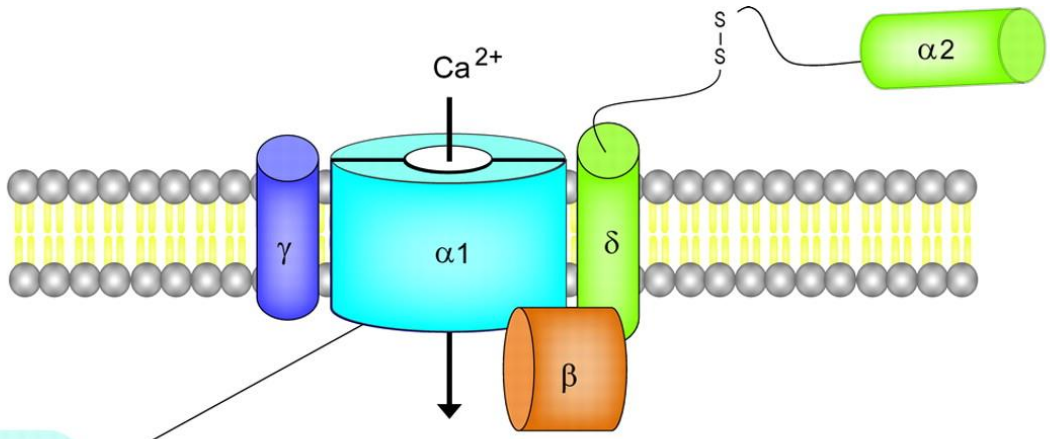


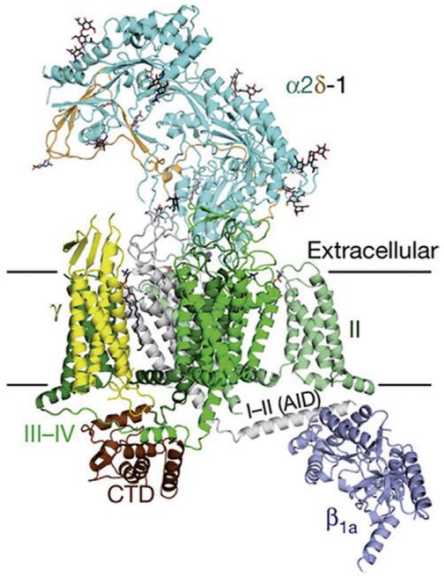
At any given moment, free $[Ca^{2+}]_i$ is determined by the balance between

- **Ca^{2+} influx**
- **Ca^{2+} efflux**
- **Ca^{2+} exchange with internal stores**
- **Ca^{2+} sequestration by endogenous buffers (Ca^{2+} -binding proteins)**

New

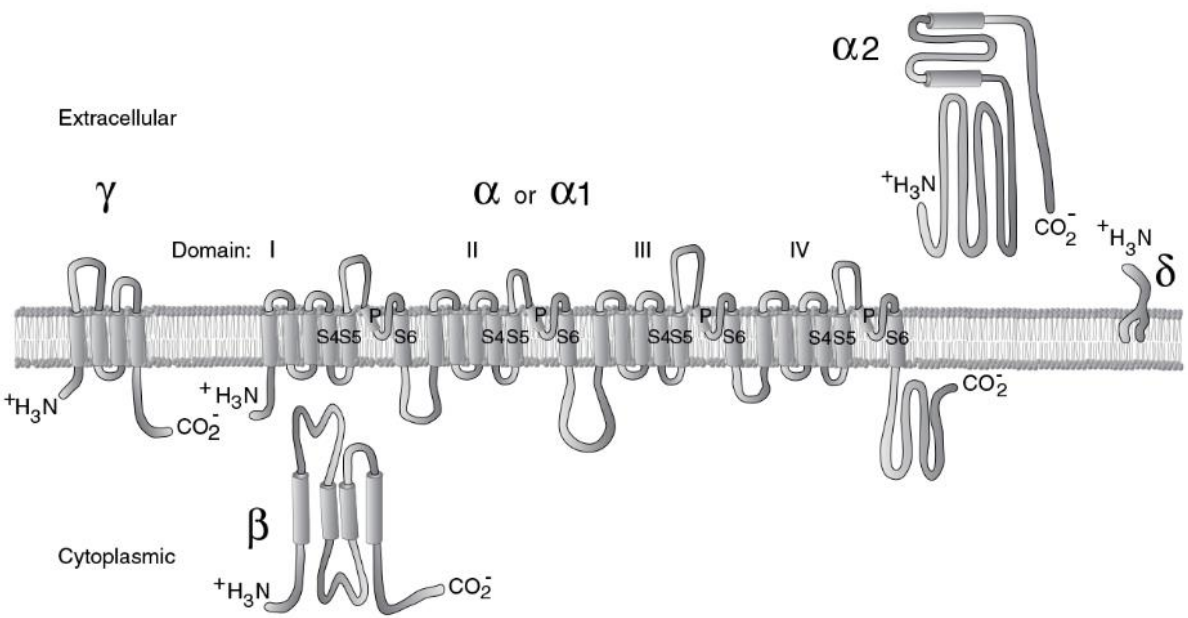
Voltage-Gated Ca^{2+} channels





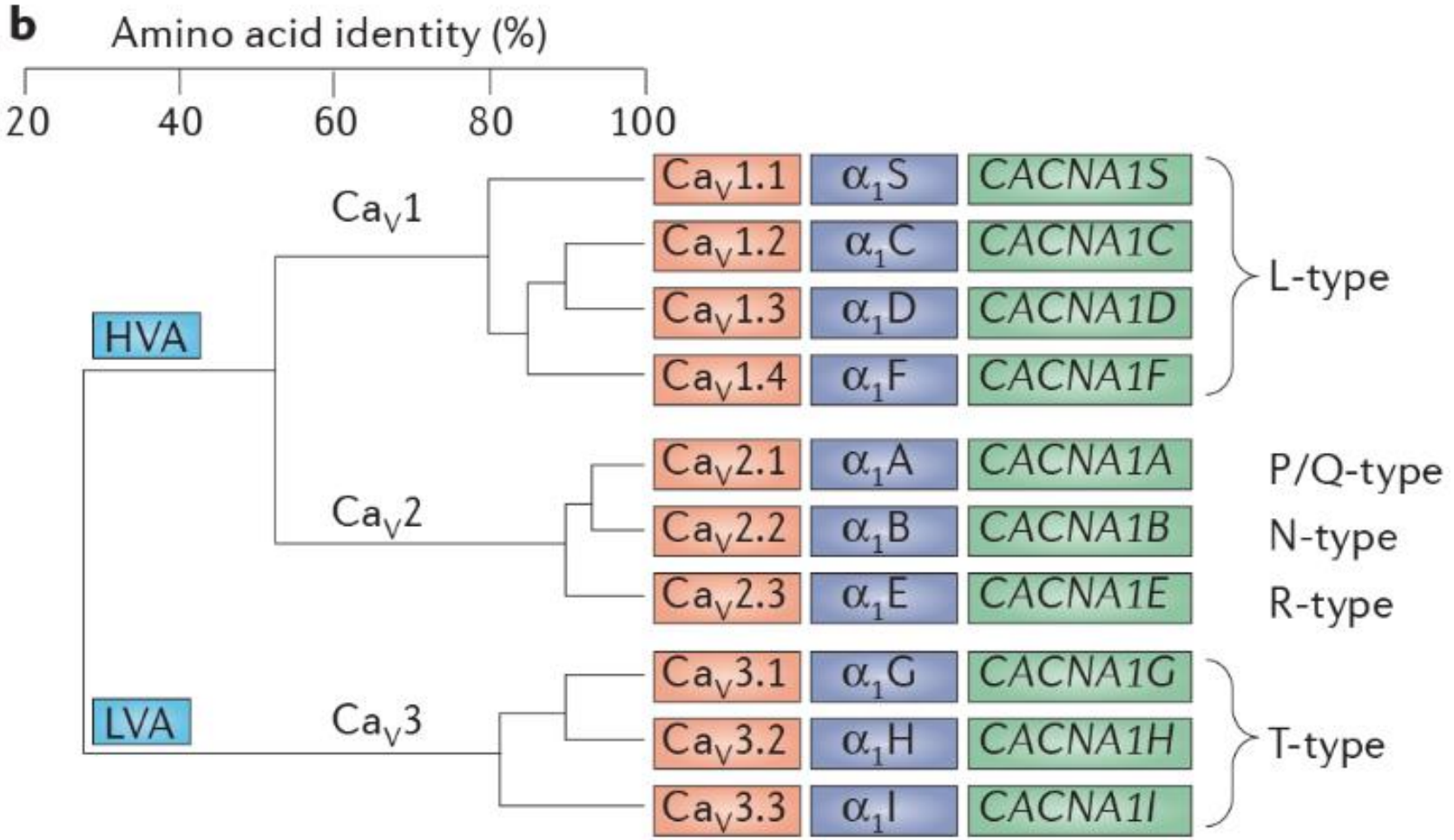
Nanou & Catterall, 2018

- L
  - $Ca_v1.1$
  - $Ca_v1.2$
  - $Ca_v1.3$
  - $Ca_v1.4$
- P/Q $Ca_v2.1$
- N $Ca_v2.2$
- R $Ca_v2.3$
- T
  - $Ca_v1.1$
  - $Ca_v1.2$
  - $Ca_v1.3$



Known

Voltage-Gated Ca^{2+} channels



Known

Voltage-Gated Ca^{2+} channels

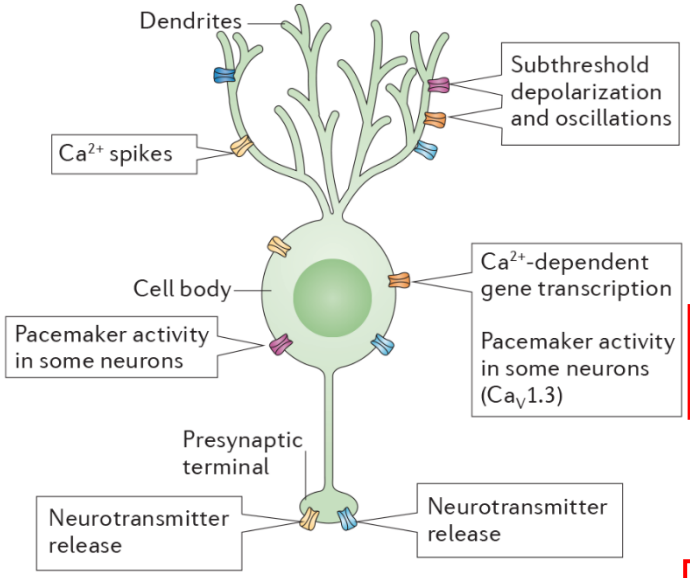


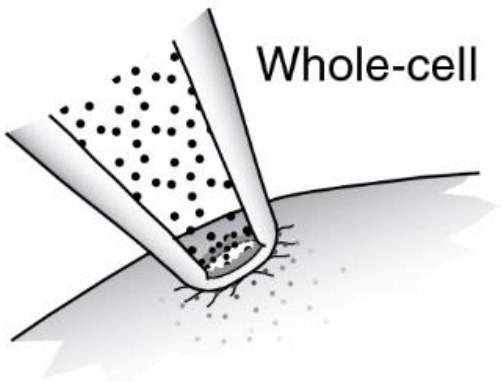


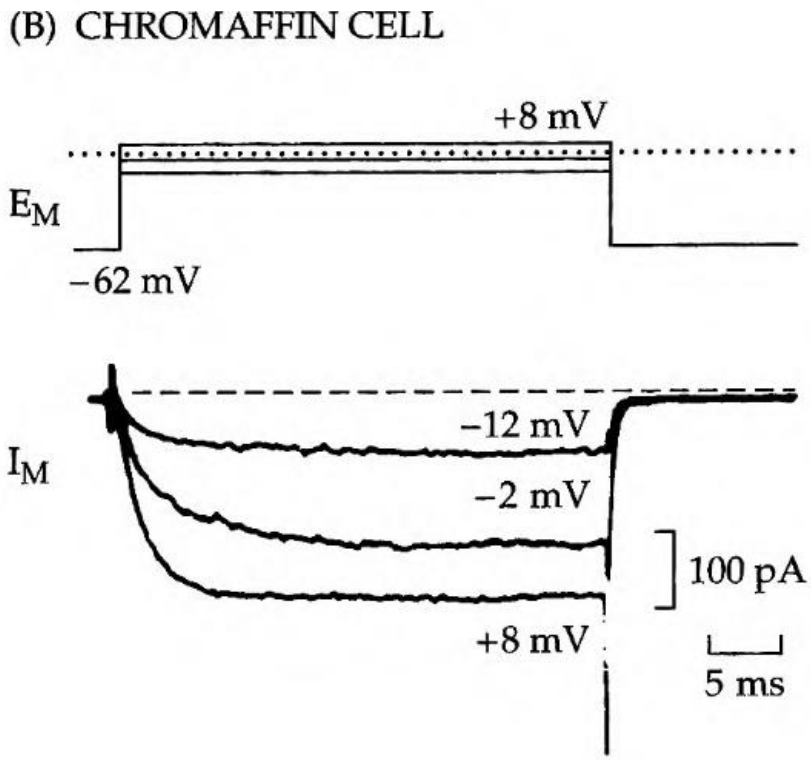
Table 7.1 Ca^{2+} channel α subunits: types, blocking agents, and physiological function

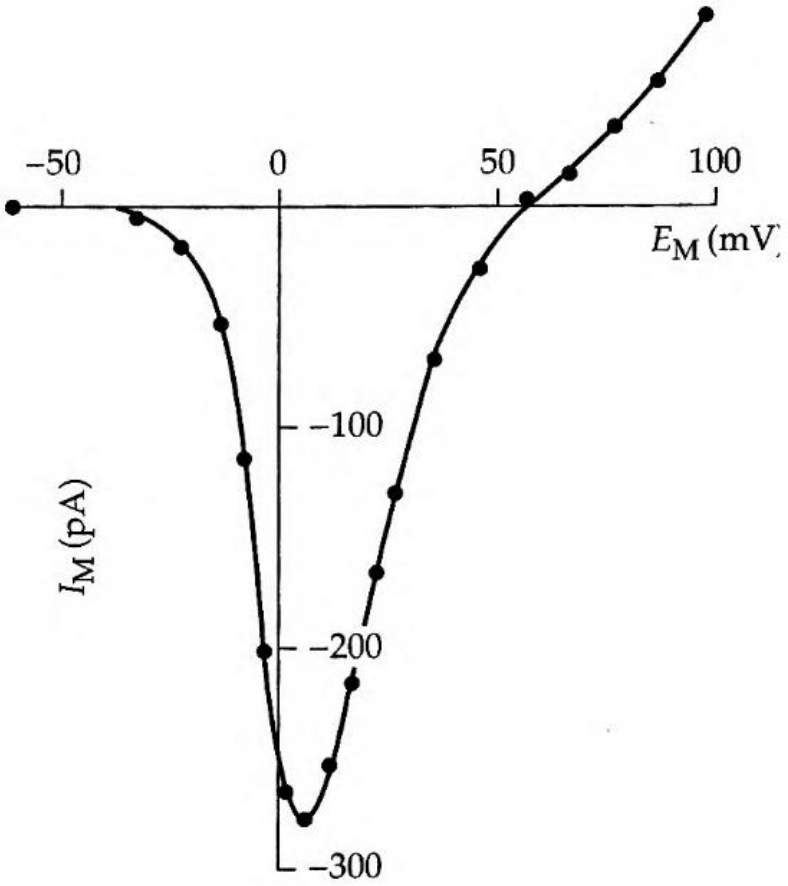
Channel	AKA	Current	Antagonists	Principal physiological function and location
$Ca_V1.1$	α_{1S}	L-type	DHPs	Excitation-contraction in skeletal muscle
$Ca_V1.2$	α_{1C}	L-type	DHPs	Excitation-contraction in cardiac and smooth muscle, dendritic Ca^{2+} signaling
$Ca_V1.3$	α_{1D}	L-type	DHPs	Endocrine secretion, hair cell (auditory) synaptic transmission, dendritic Ca^{2+} signaling
$Ca_V1.4$	α_{1F}	L-type	DHPs	Retinal synaptic transmission
$Ca_V2.1$	α_{1A}	P/Q-type	ω-agatoxin	Neurotransmitter release in CNS
$Ca_V2.2$	α_{1B}	N-type	ω-conotoxin-GVIA	Neurotransmitter release in CNS
$Ca_V2.3$	α_{1E}	R-type	SNX-482	Neurotransmitter release in CNS
$Ca_V3.1$	α_{1G}	T-type	none	Bursting and repetitive firing in CNS, low threshold Ca^{2+} signaling in dendrites
$Ca_V3.2$	α_{1H}	T-type	none	Bursting and repetitive firing in CNS, low threshold Ca^{2+} signaling in dendrites
$Ca_V3.3$	α_{1I}	T-type	none	Bursting and repetitive firing in CNS, low threshold Ca^{2+} signaling in dendrites

Known/new

Voltage-Gated Ca^{2+} channels







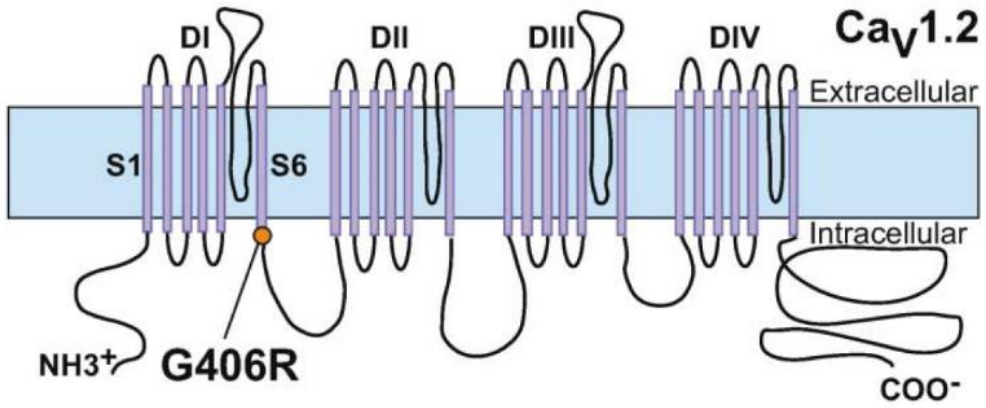
VGCCs are a broad class of channels exhibiting high Ca^{2+} selectivity

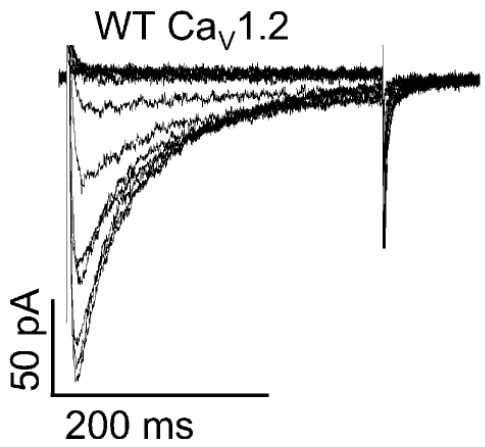
Known

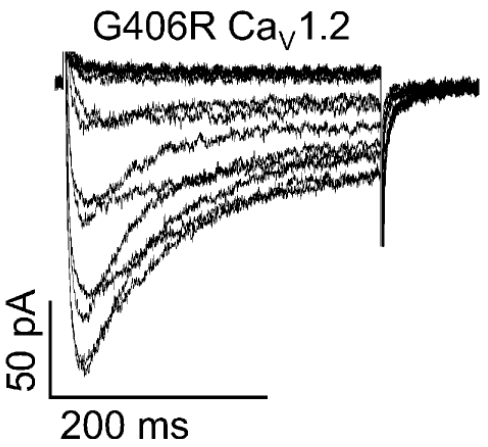
Timothy syndrome

Due to a single point mutation that reduces the inactivation of the ubiquitous $Ca_V1.2$

	DI/S6				R				
Human $Ca_V1.2$	G	V	L	S	**G**	E	F	S	K
Human $Ca_V1.3$	G	V	L	S	**G**	E	F	S	K
Human $Ca_V1.4$	G	V	L	S	**G**	E	F	S	K
Human $Ca_V1.1$	G	V	L	S	**G**	E	F	T	K
Human $Ca_V2.1$	G	V	L	S	**G**	E	F	A	K
Human $Ca_V2.2$	G	V	L	S	**G**	E	F	A	K
Human $Ca_V2.3$	G	V	L	S	**G**	E	F	A	K
Rat	G	V	L	S	**G**	E	F	S	K
Mouse	G	V	L	S	**G**	E	F	S	K
Chicken	G	V	L	S	**G**	E	F	S	K
Zebrafish	G	V	L	S	**G**	E	F	S	K
Fruitfly	G	V	L	S	**G**	E	F	S	K
Worm	G	V	L	S	**G**	E	F	S	K





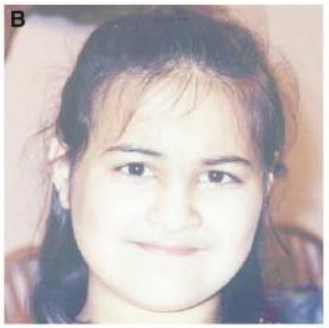


New

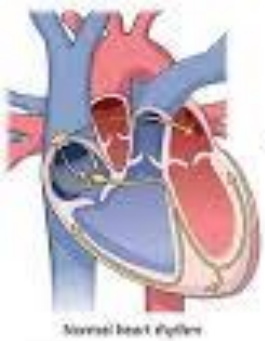
Timothy syndrome

It is a rare disease characterized by physical malformations, as well as neurological and developmental defects, including heart arrhythmias, syndactyly (webbed fingers and toes) and autism spectrum disorders



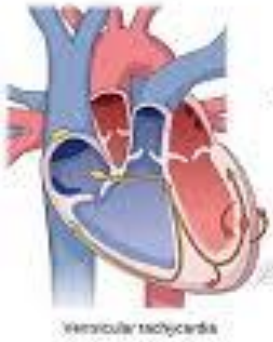


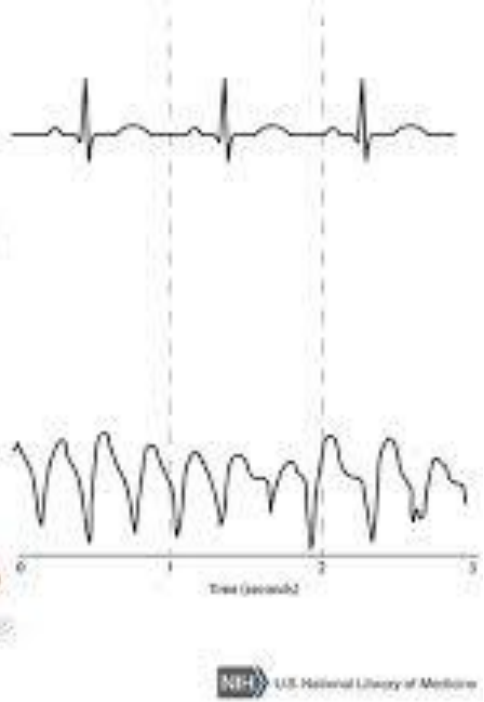












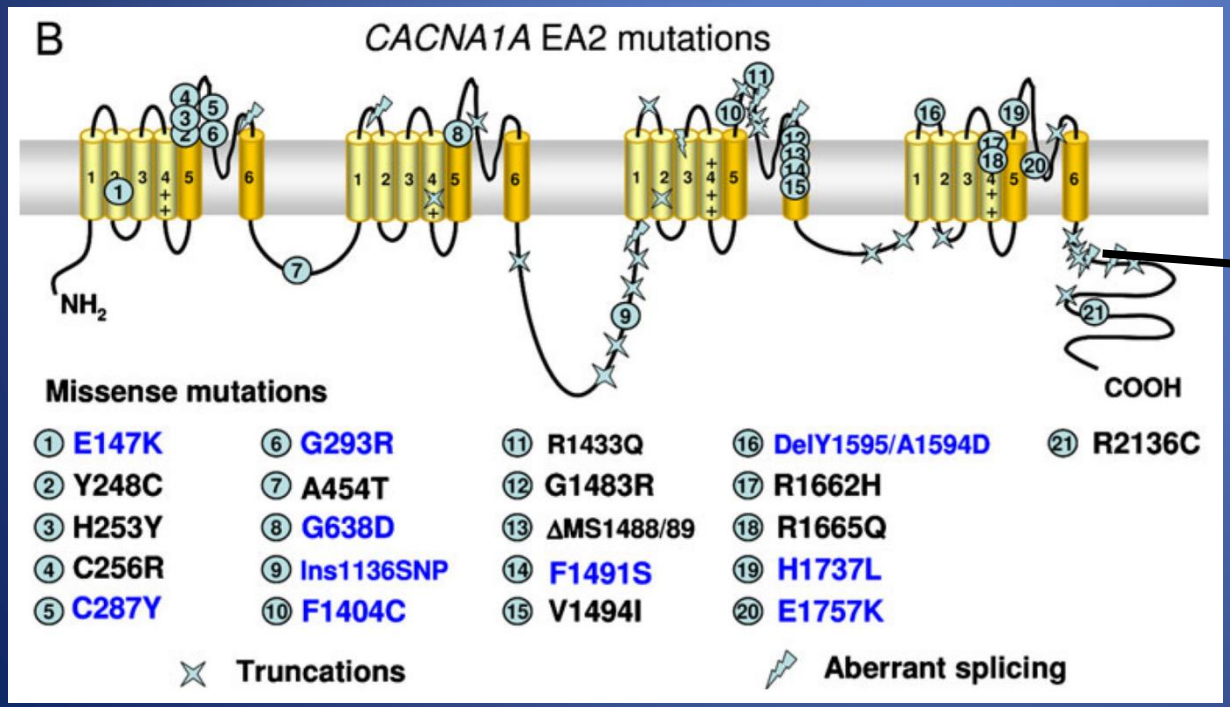
New

Episodic ataxia type II

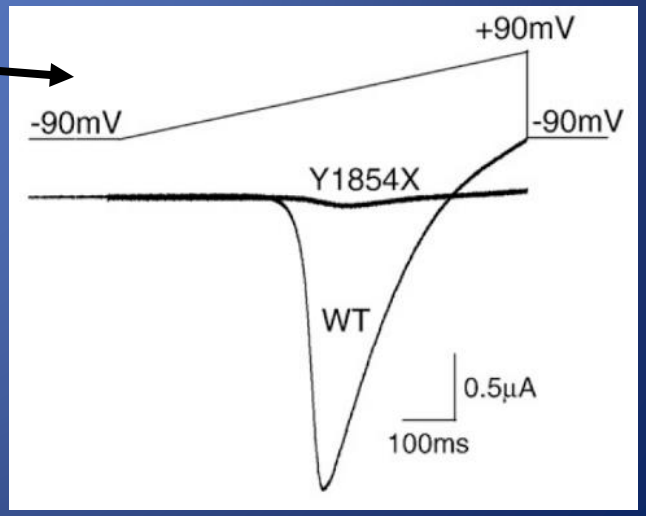


It manifests itself with bouts of incoordination and imbalance

It is due to loss-of-function mutations in $Ca_V2.1$ (over 100 mutations)

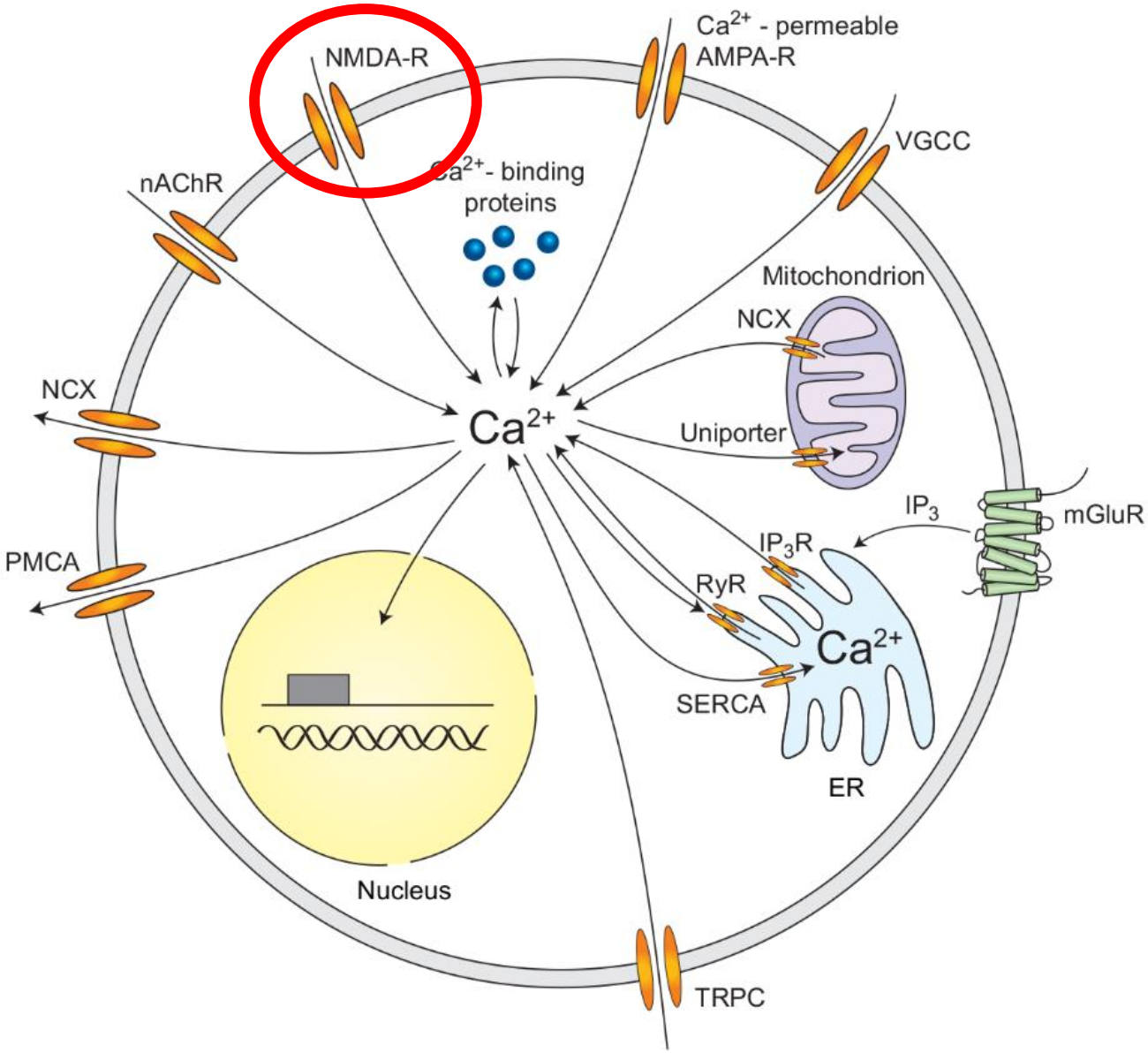


Example 1 mutation

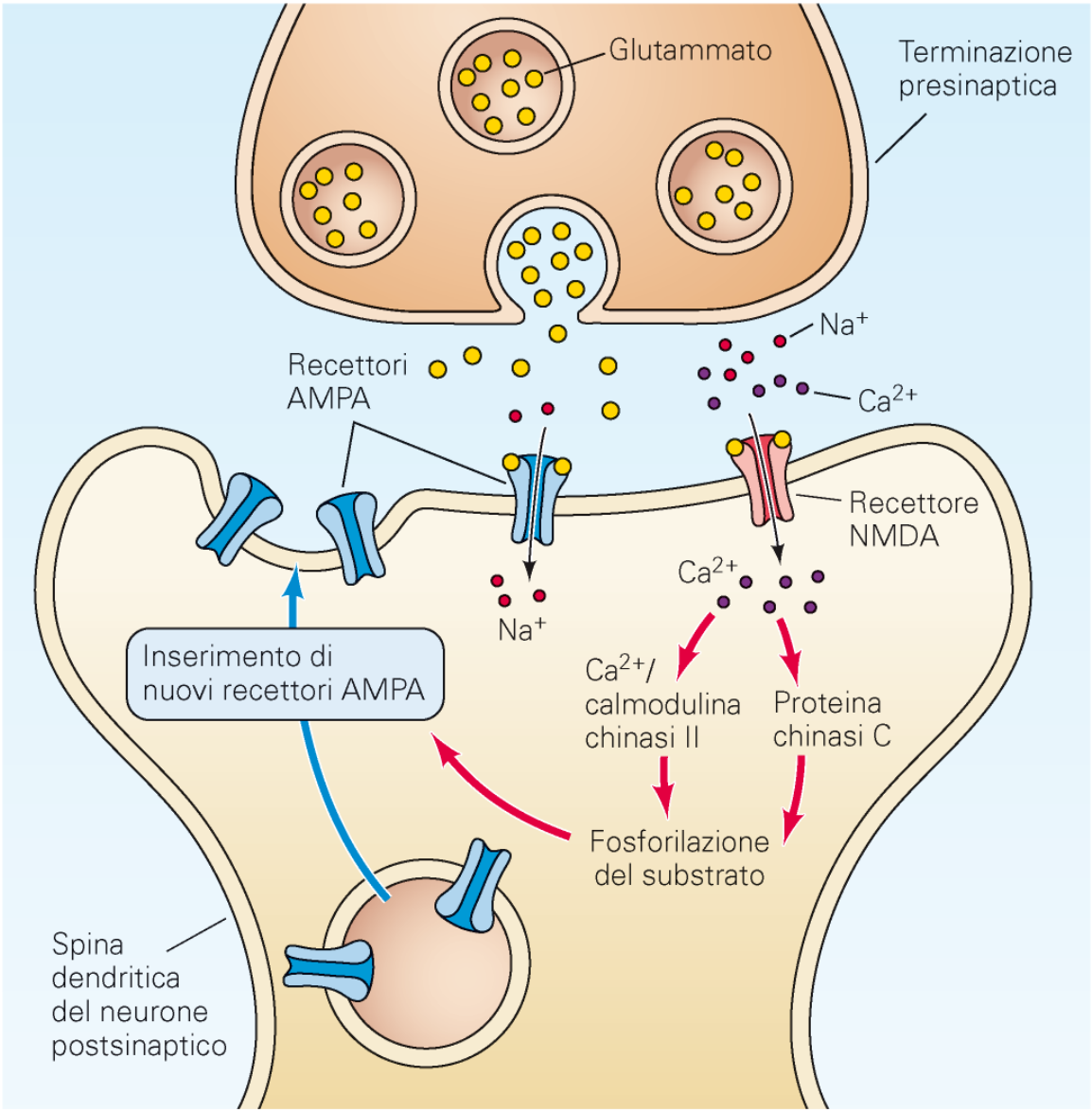


New

Neuronal Ca^{2+} signaling

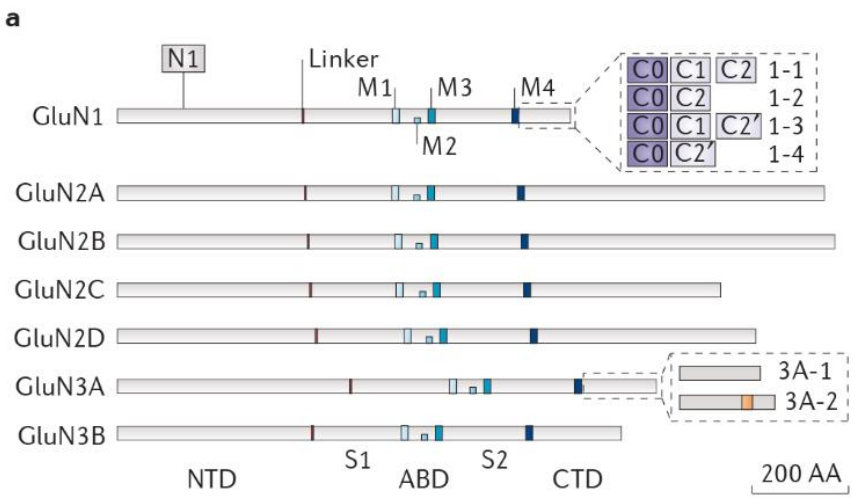


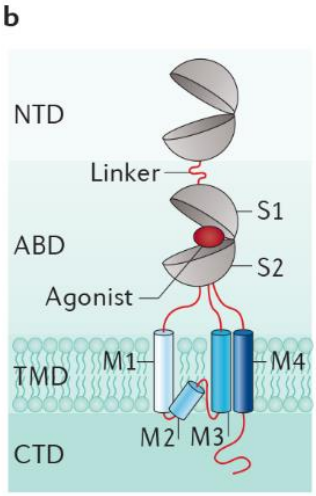
NMDA receptors

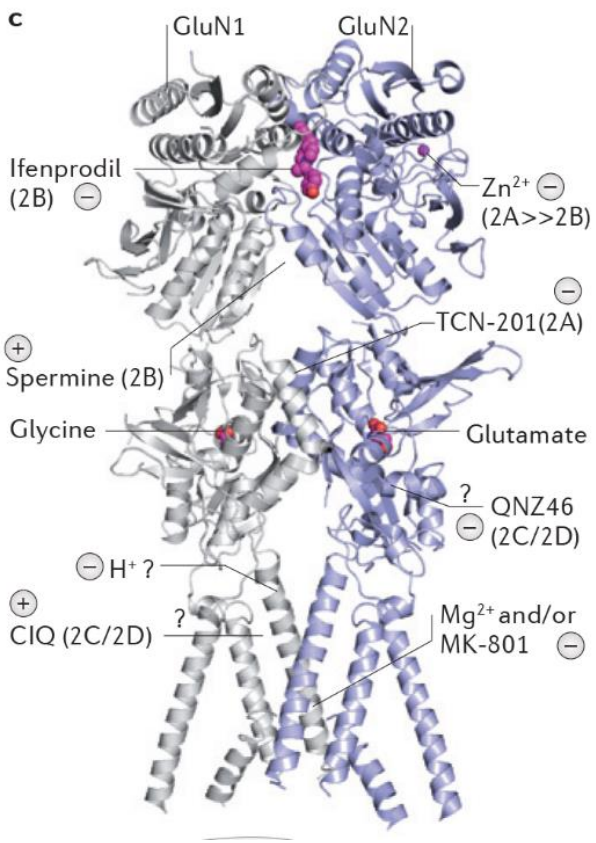


Known

NMDA receptors

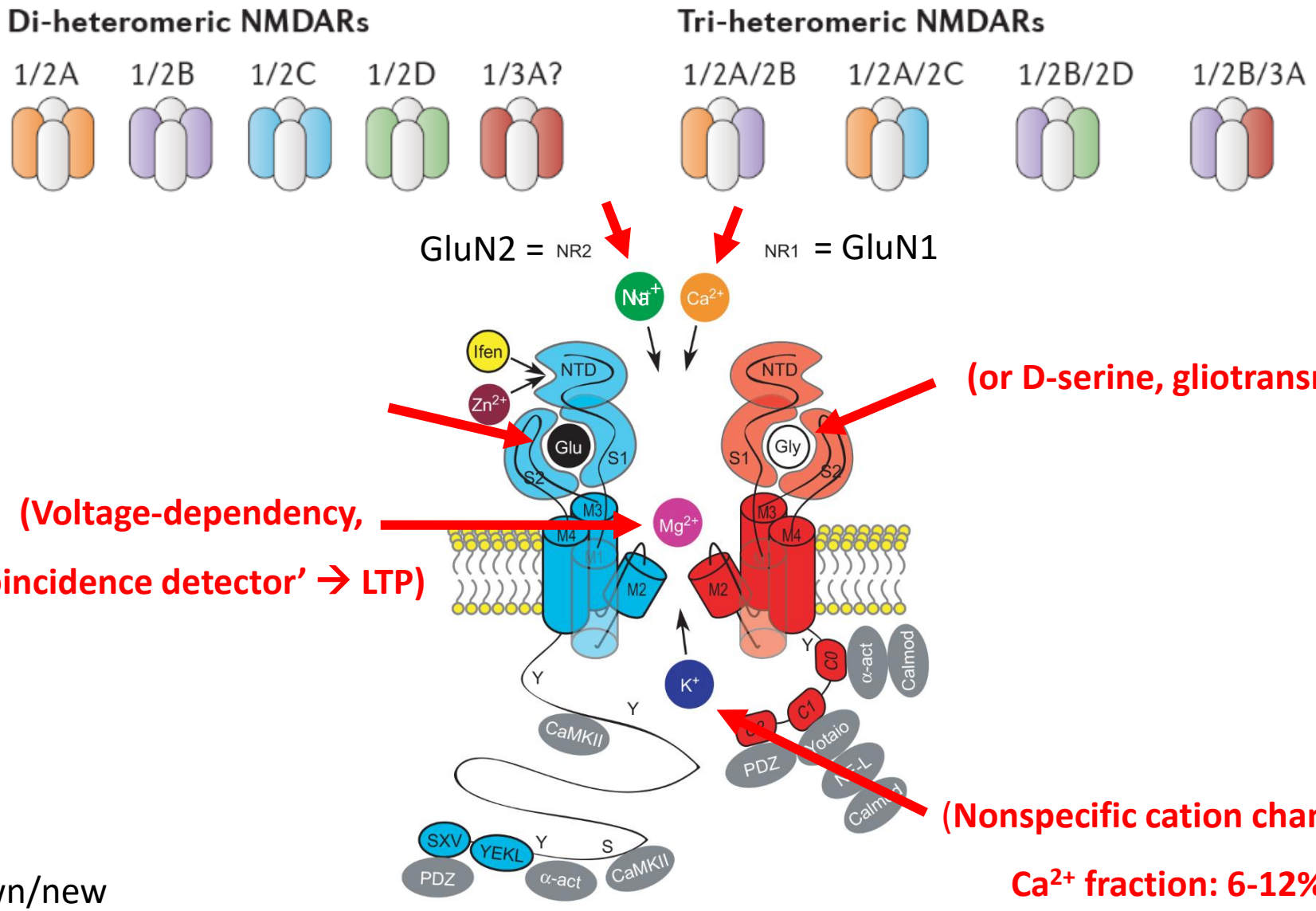






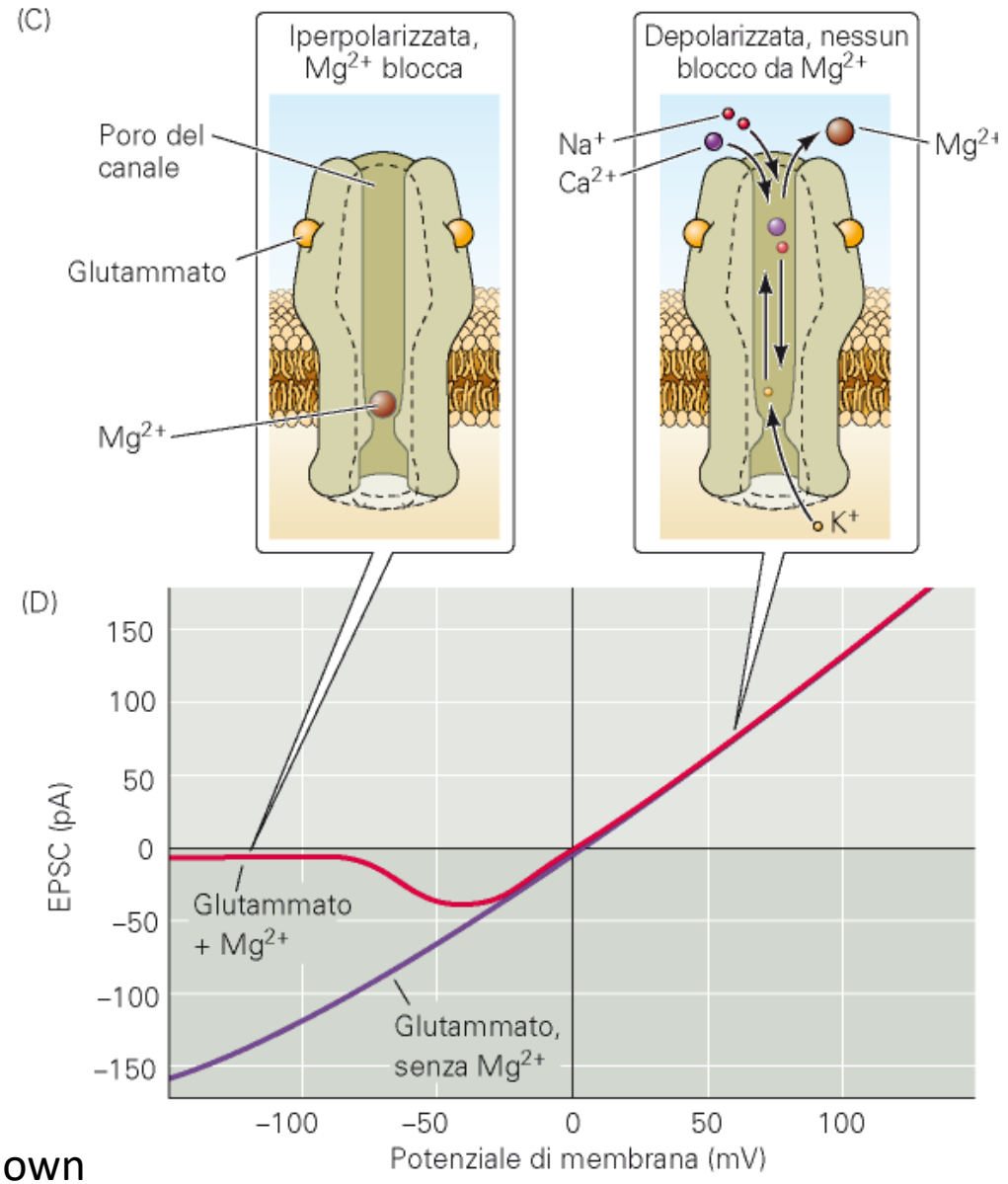
Known/new

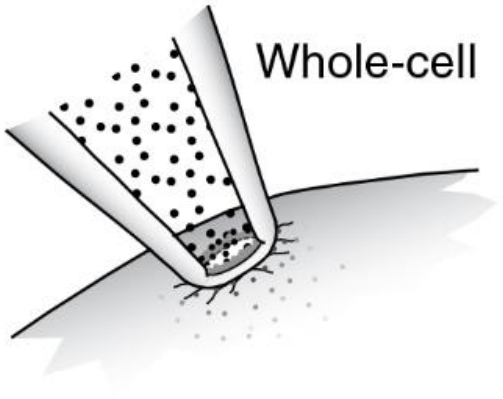
NMDA receptors



Known/new

NMDA receptors





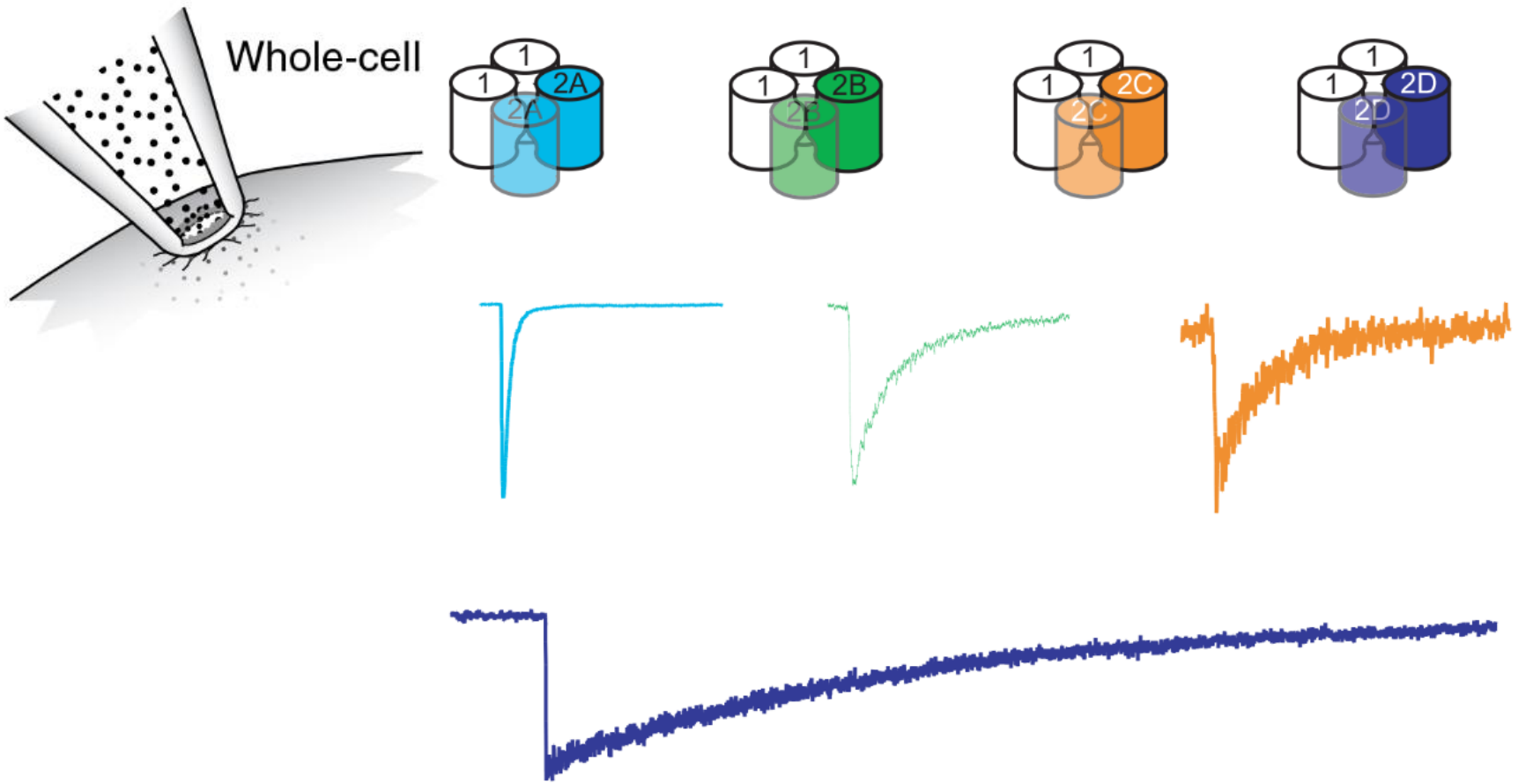
Mg^{2+}-mediated voltage-dependency, 'coincidence detector' of pre- (glutamate) and postsynaptic (depolarization) activity

↓

Hebbian-type synaptic plasticity (e.g. LTP)

Known

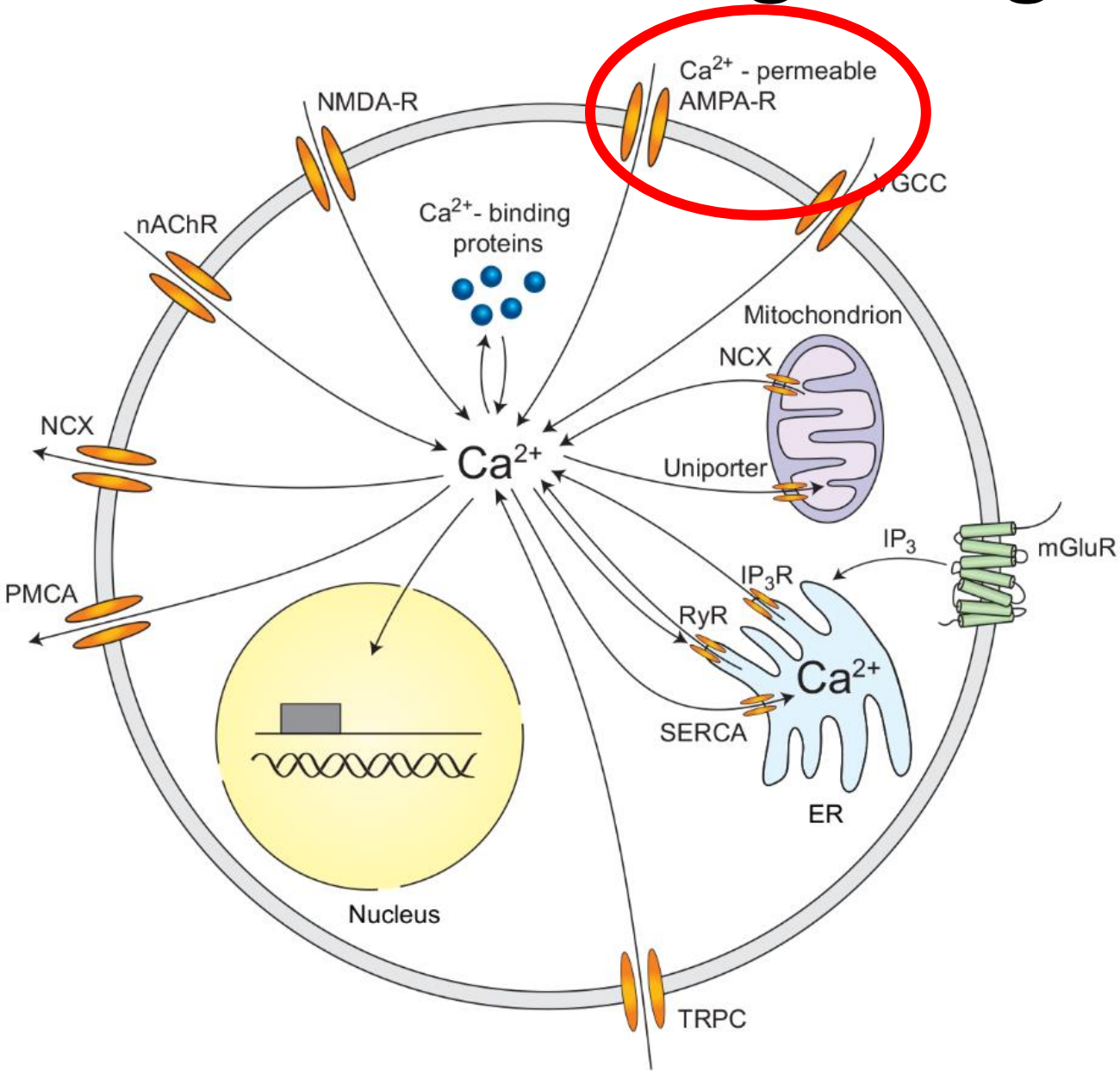
NMDA receptors



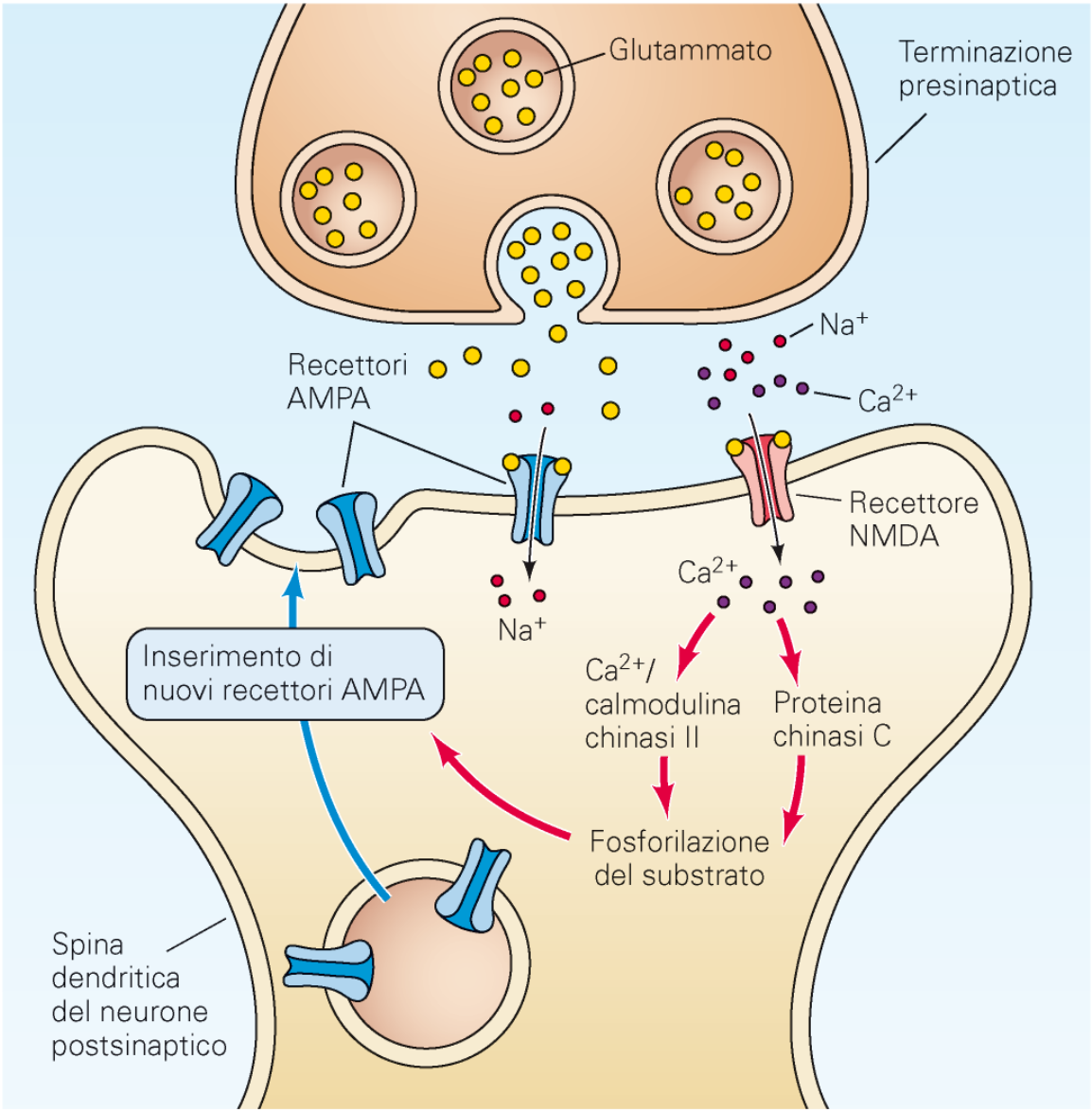
The type of GluN2 subunit determines kinetics of NMDAR currents and therefore Ca^{2+} influx

New

Neuronal Ca^{2+} signaling

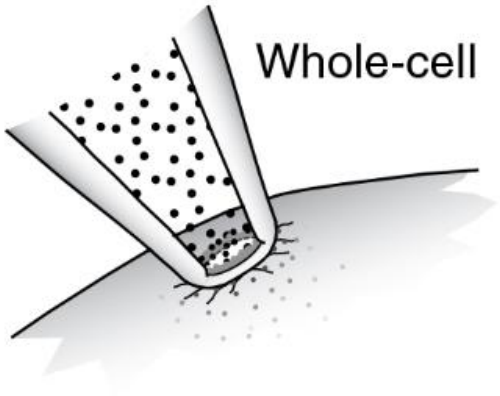


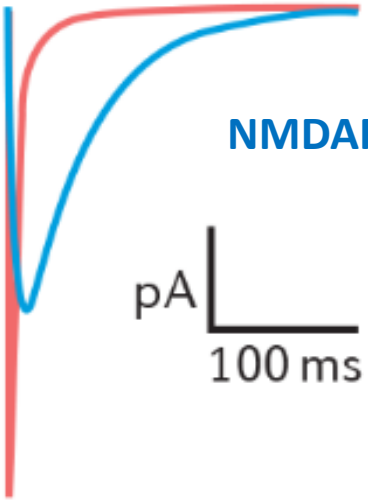
AMPA receptors



Known

AMPA receptors

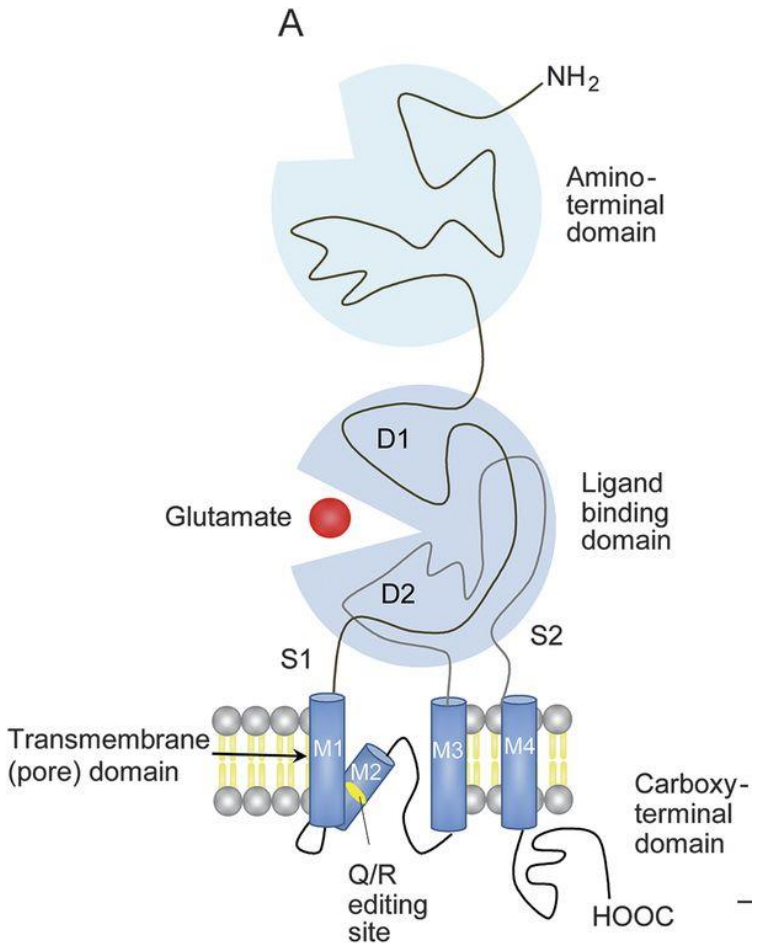


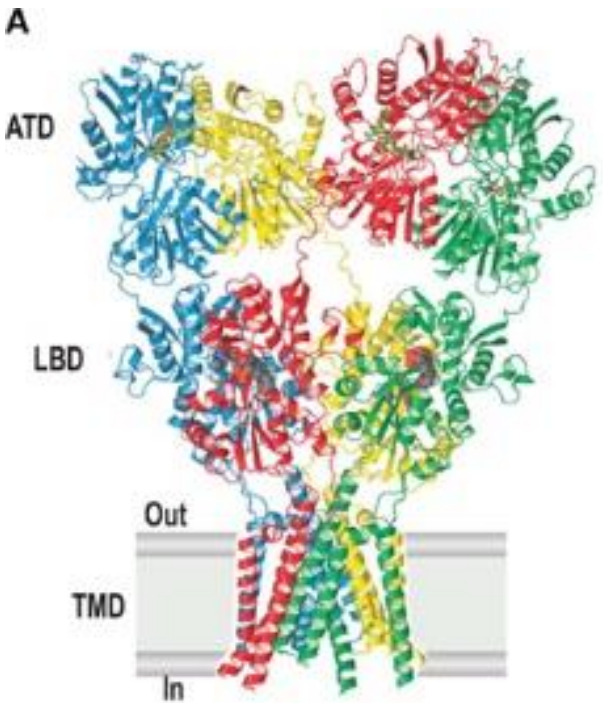


The kinetics of AMPARs are faster than those of the fastest NMDARs

Known/new

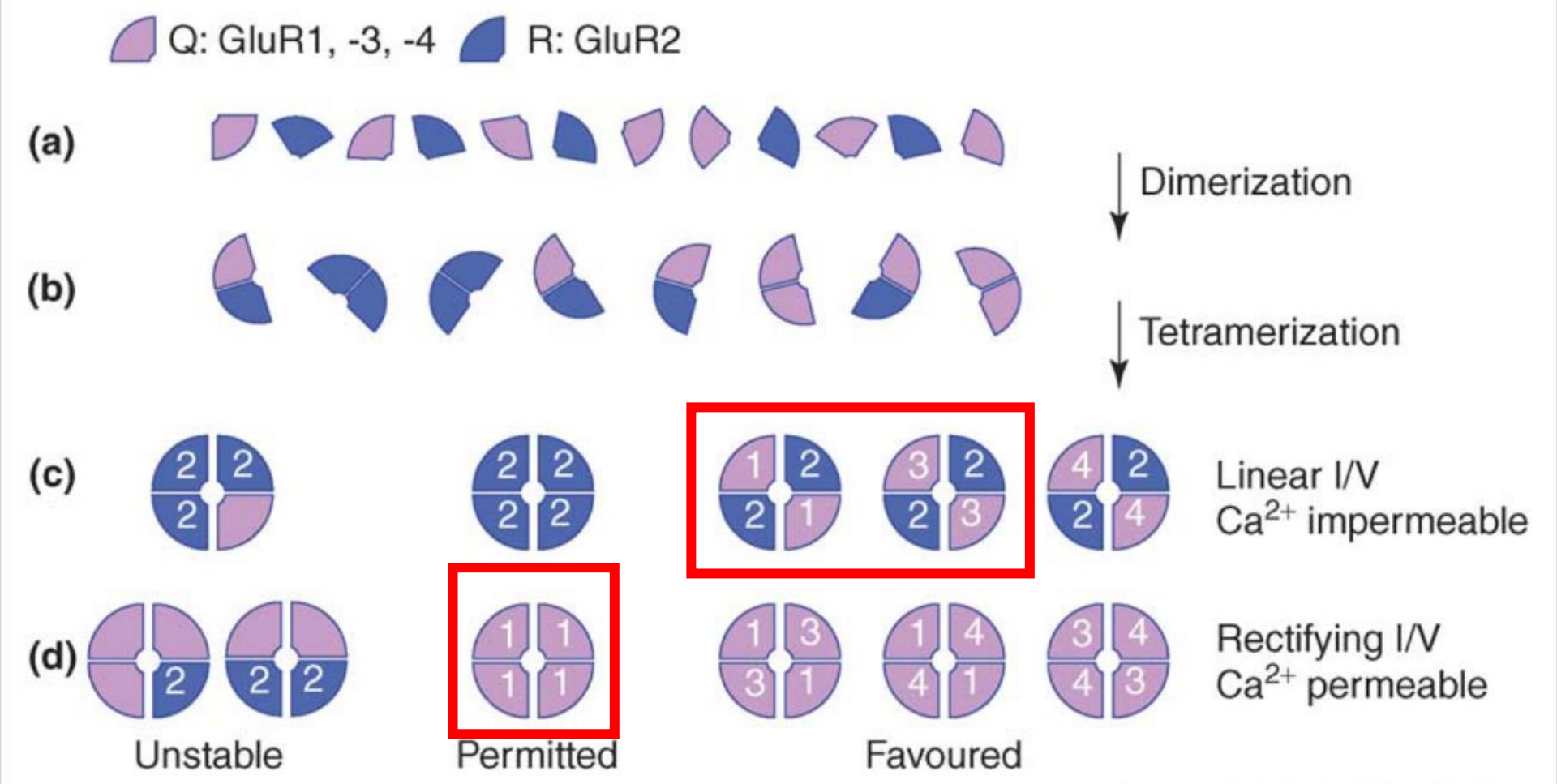
AMPA receptors





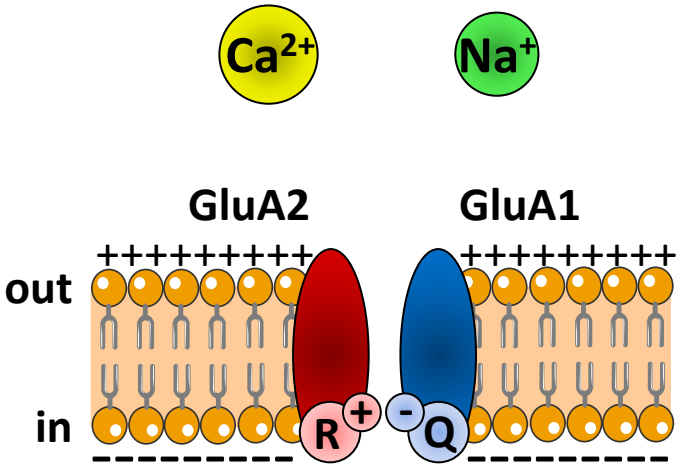
Known

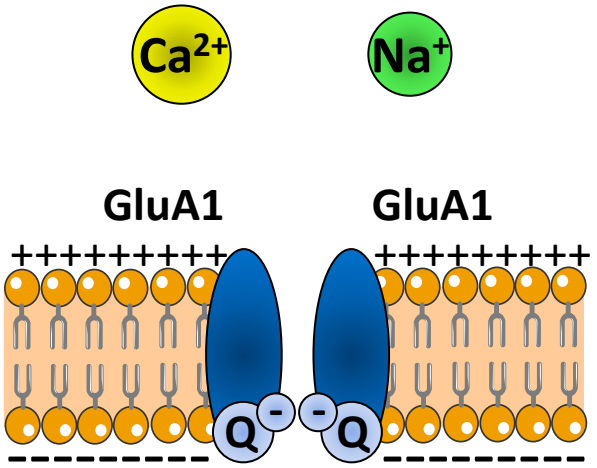
AMPA receptors



Known/new

AMPA receptors and Ca^{2+}-permeability





Ca^{2+} impermeable

Predominant form

Ca^{2+} permeable

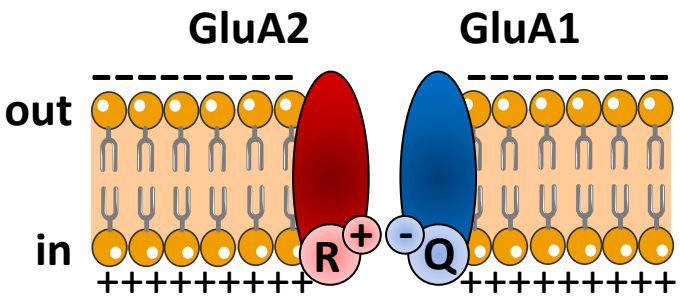
Early stages of development

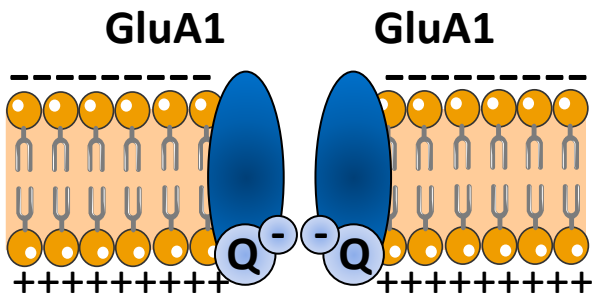
Synaptic plasticity

Some interneurons

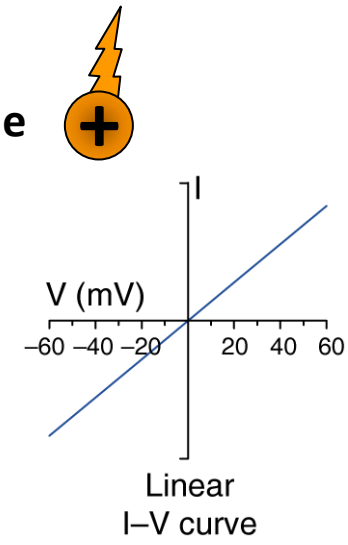
New

AMPA receptors and inward rectification

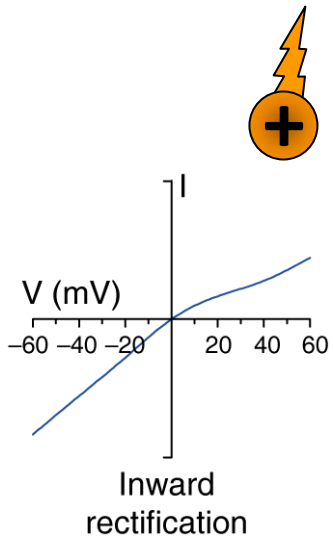




Spermine



Linear
I–V curve

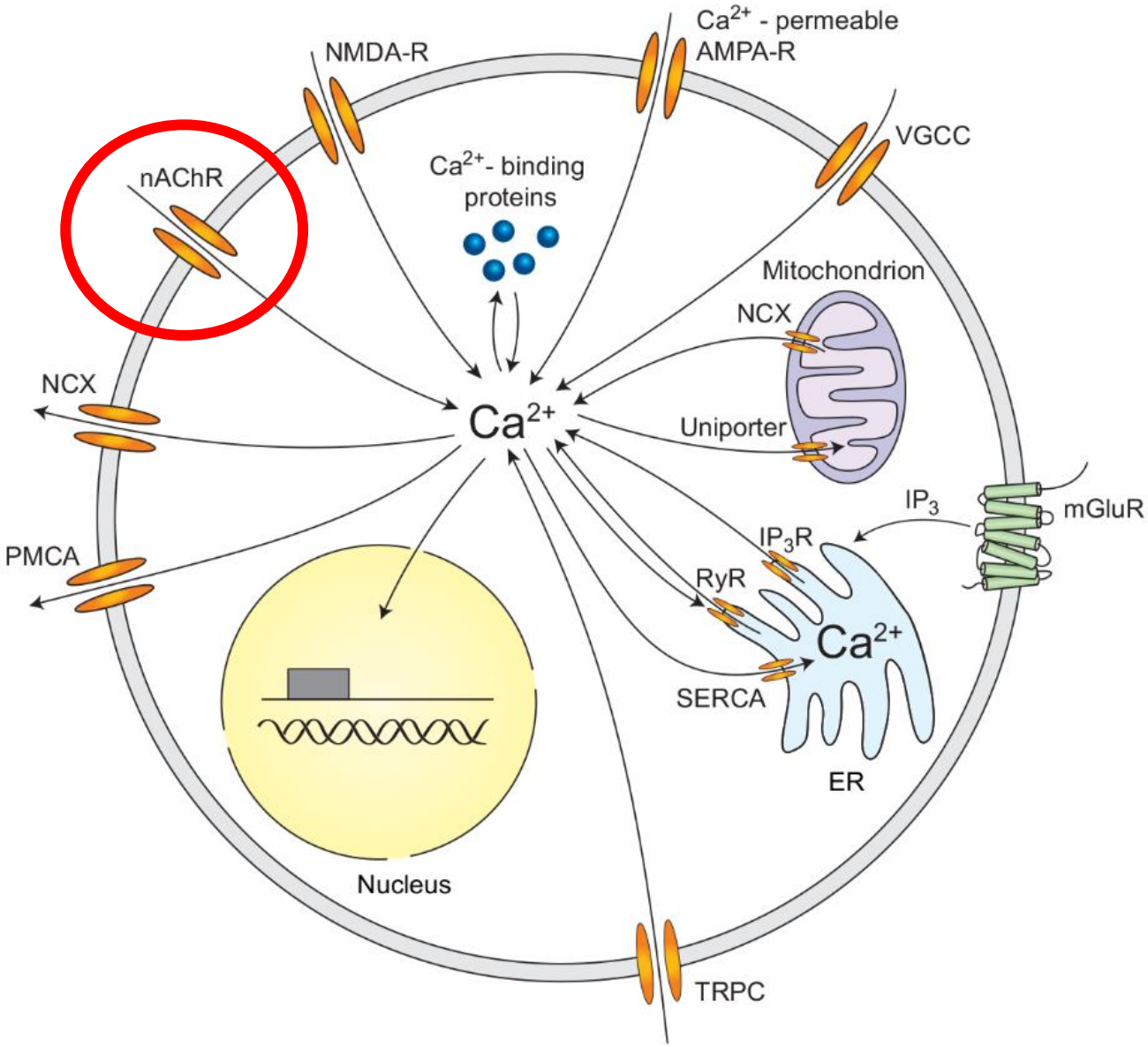


Inward
rectification

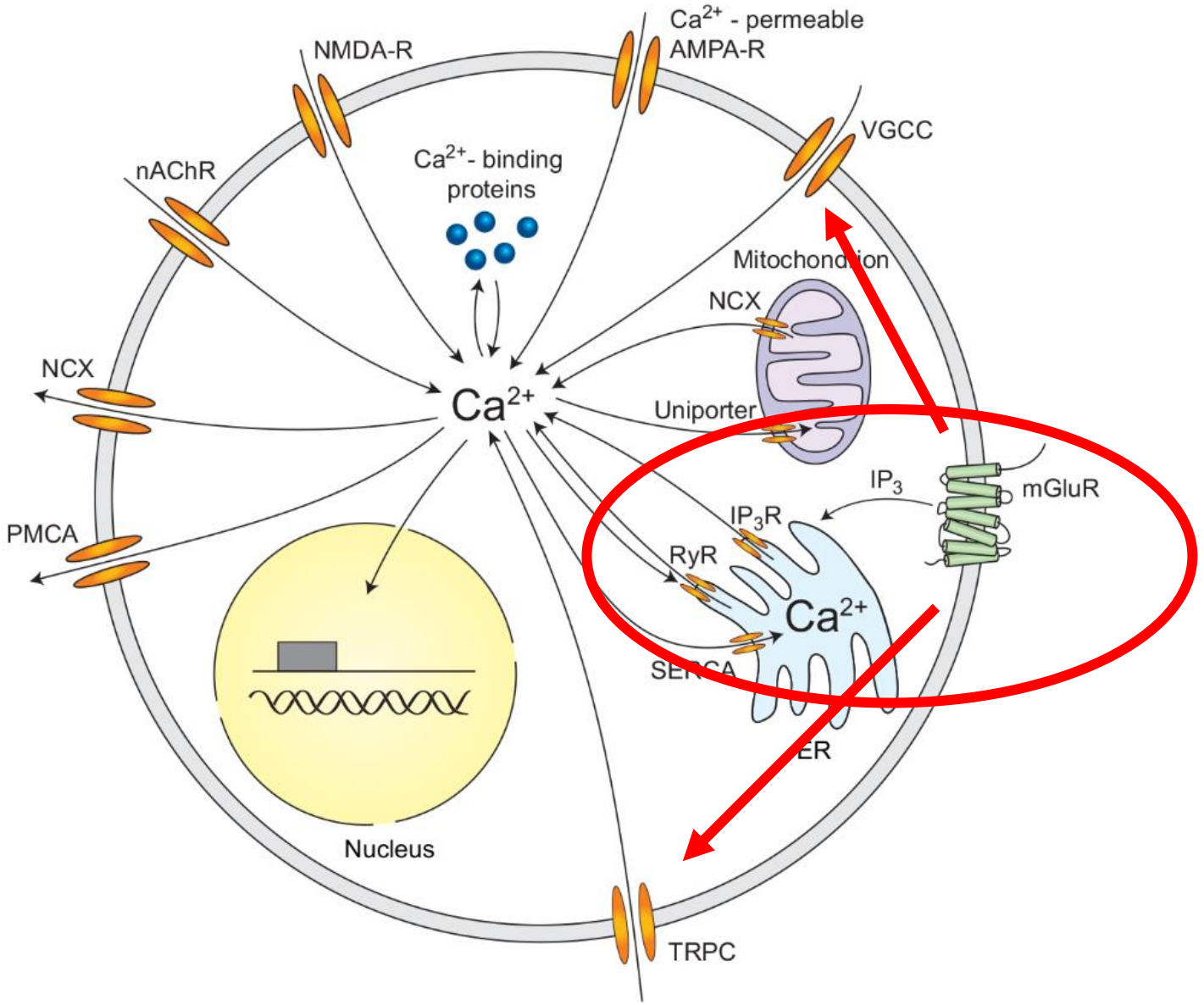
GluA2-lacking AMPARs are Ca^{2+}-permeable

New

Neuronal Ca^{2+} signaling

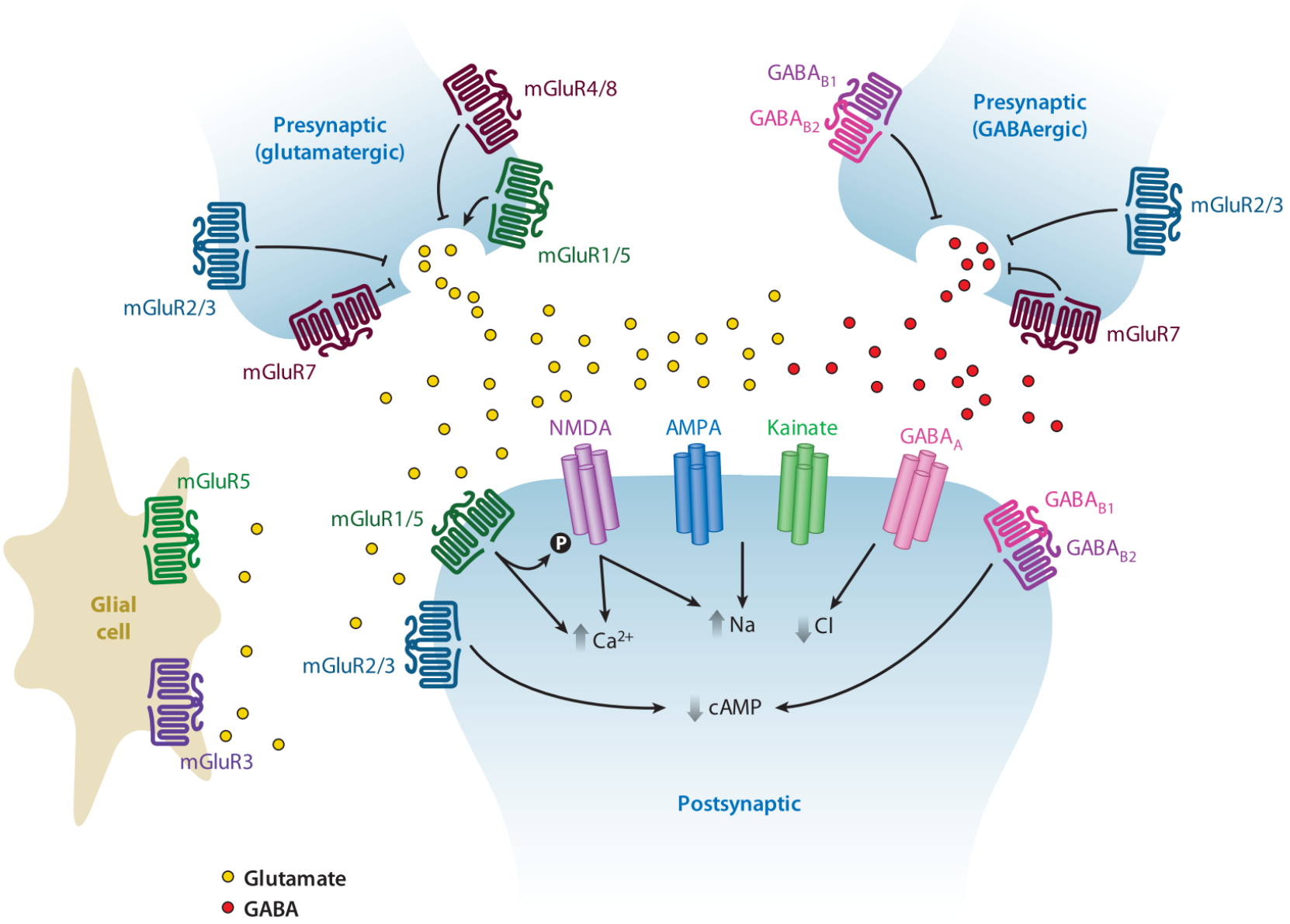


Neuronal Ca^{2+} signaling



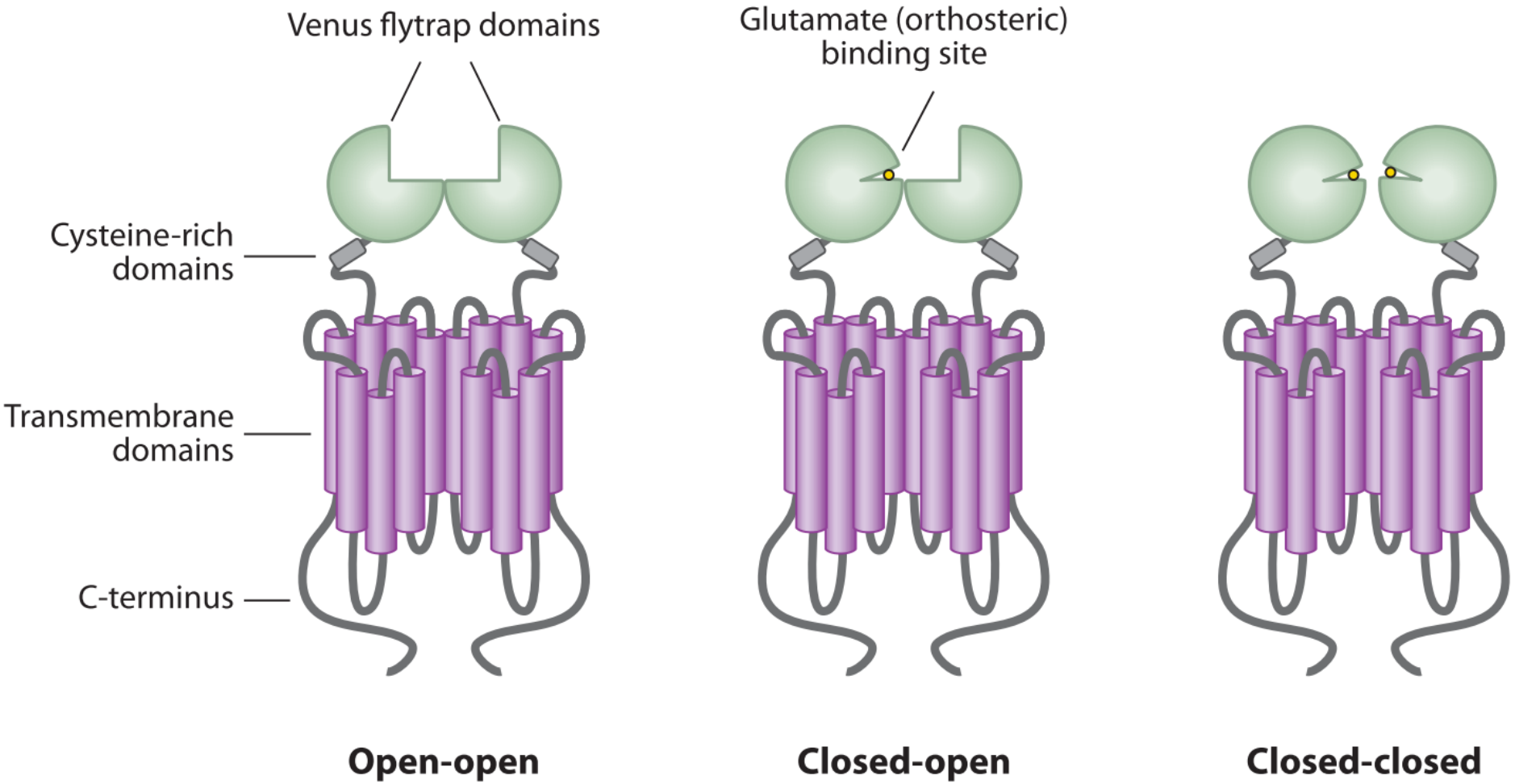
Known

mGluRs



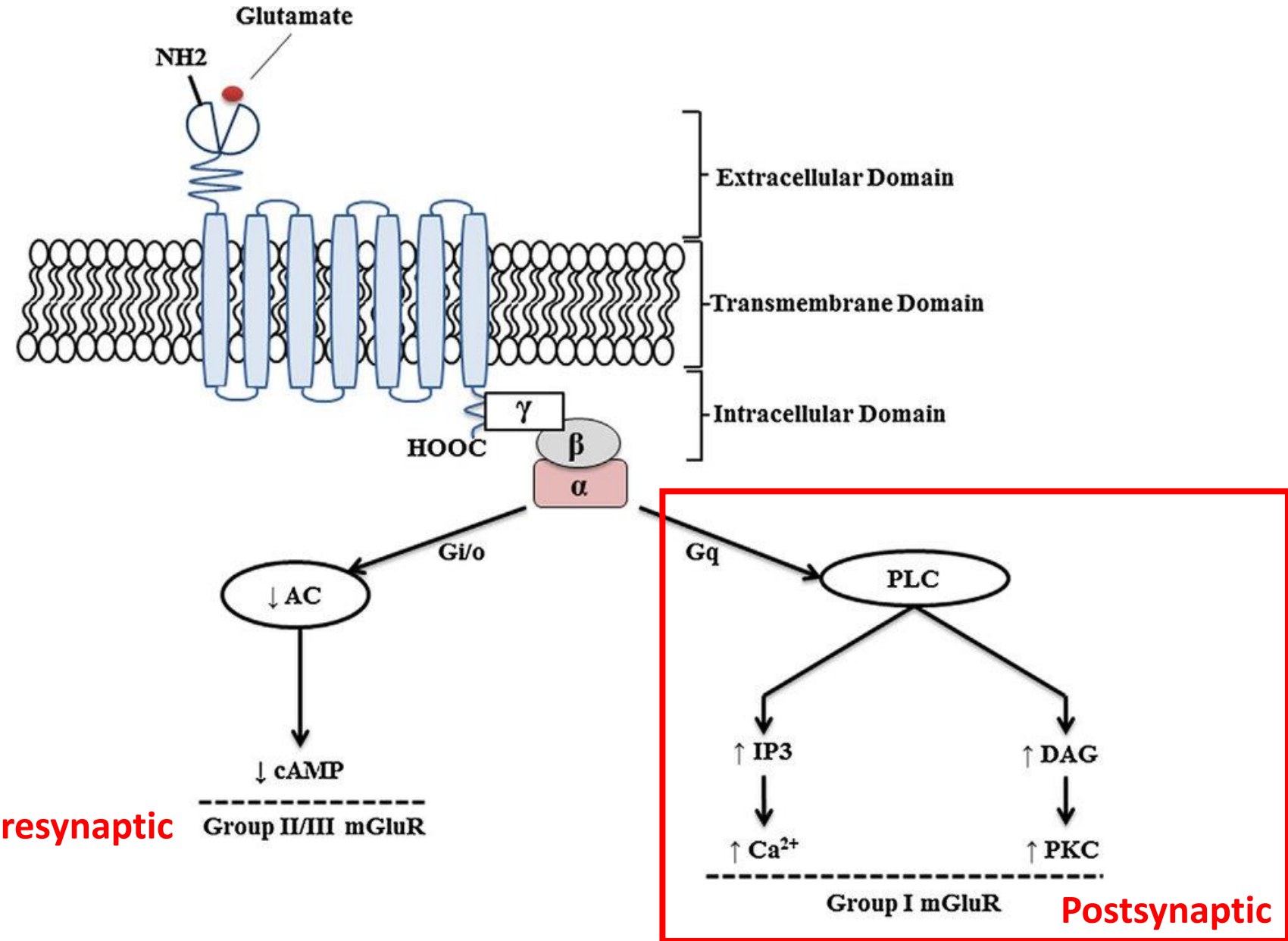
Known

mGluRs


Venus flytrap domains
Glutamate (orthosteric) binding site
Cysteine-rich domains
Transmembrane domains
C-terminus
Open-open
Closed-open
Closed-closed

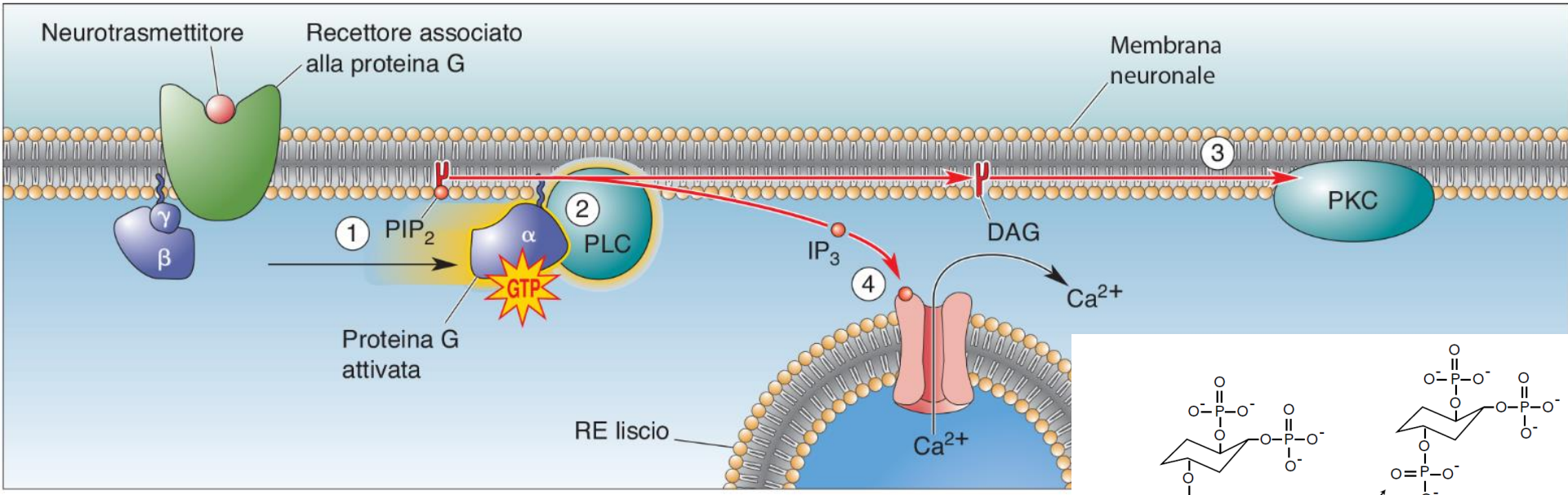
New

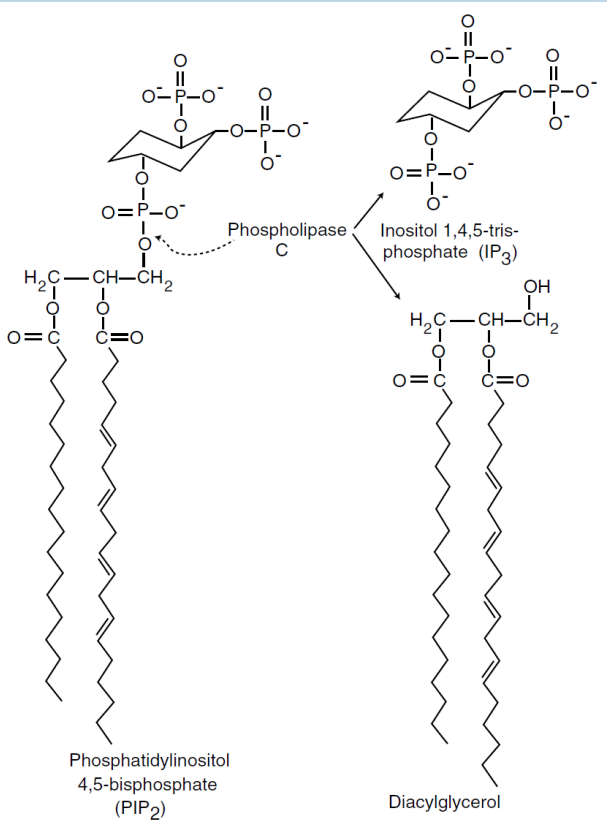
mGluRs



Known

mGluRs

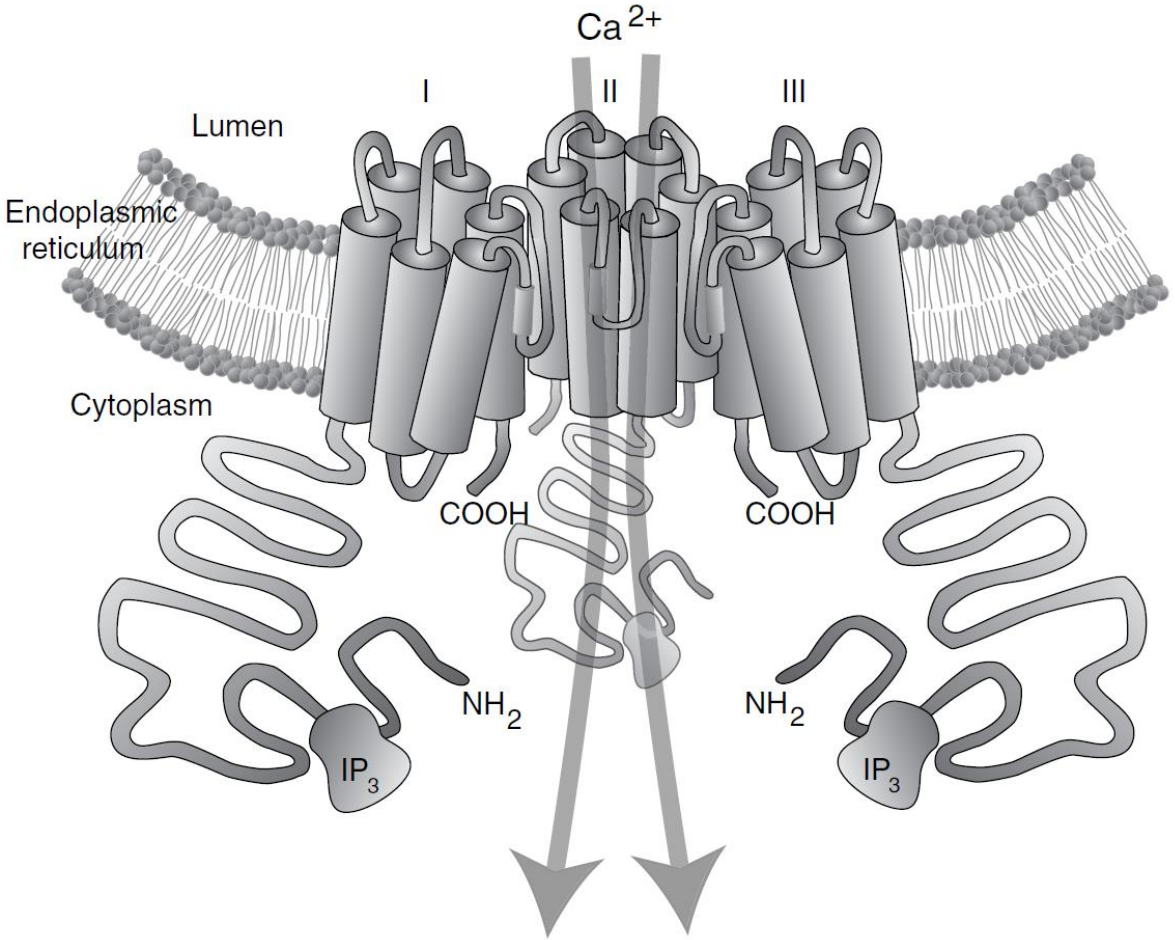




Known

IP_3 receptors

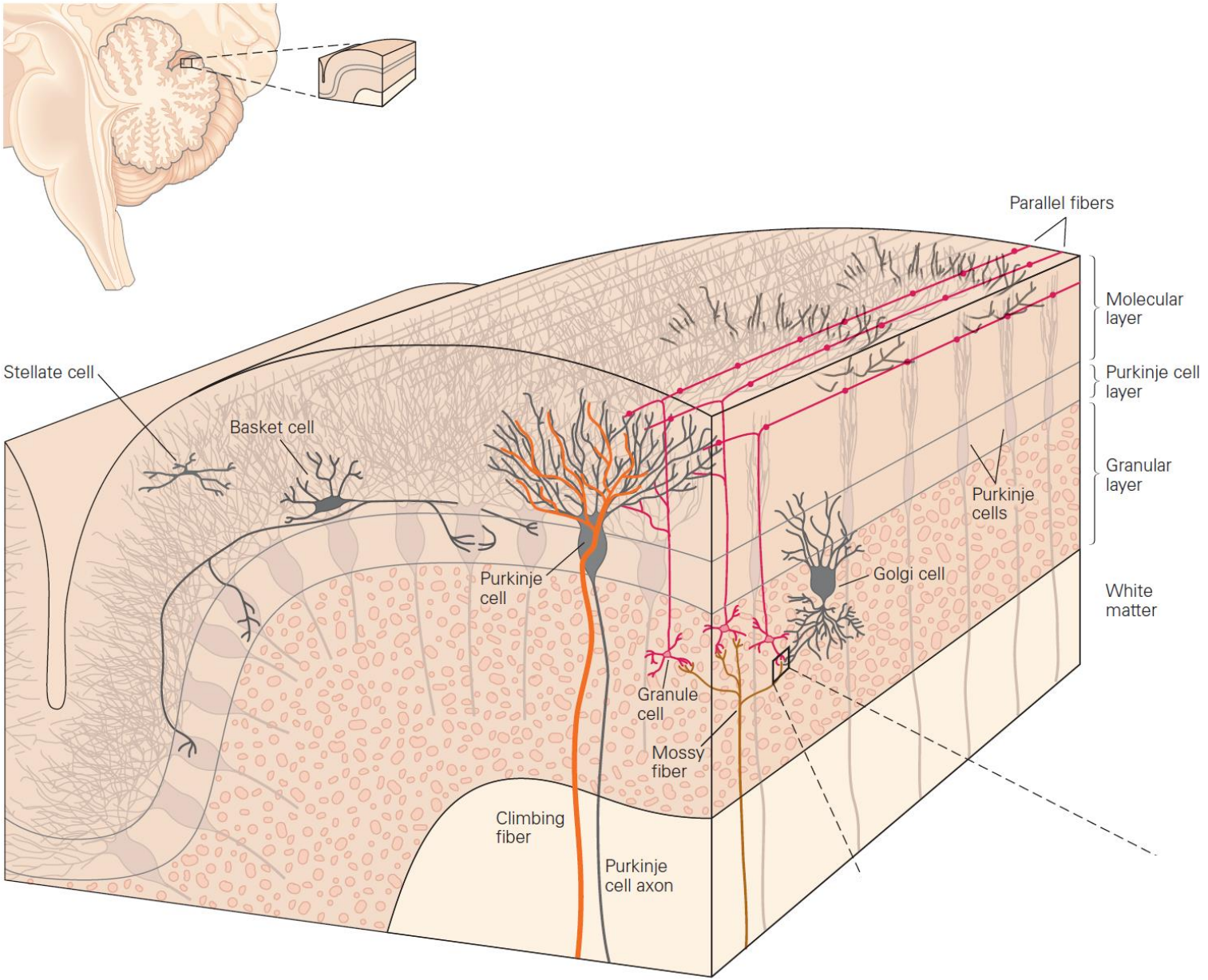
Fig. 13.3 Structure of IP_3 receptor of endoplasmic reticulum. For clarity, only three *(I–III)* of the four subunits of the tetramer are shown. The approximate positions of the IP_3 binding sites near the N-terminus of the cytoplasmic domains are indicated. (Redrawn from Foskett et al. [2007].)



- **Permeable to divalent (Ca^{2+}, Mg^{2+}) but also K^+**
- **Regulated by ATP, phosphorylation (PKA, PKC), cytoplasmic Ca^{2+} (in a biphasic manner)**

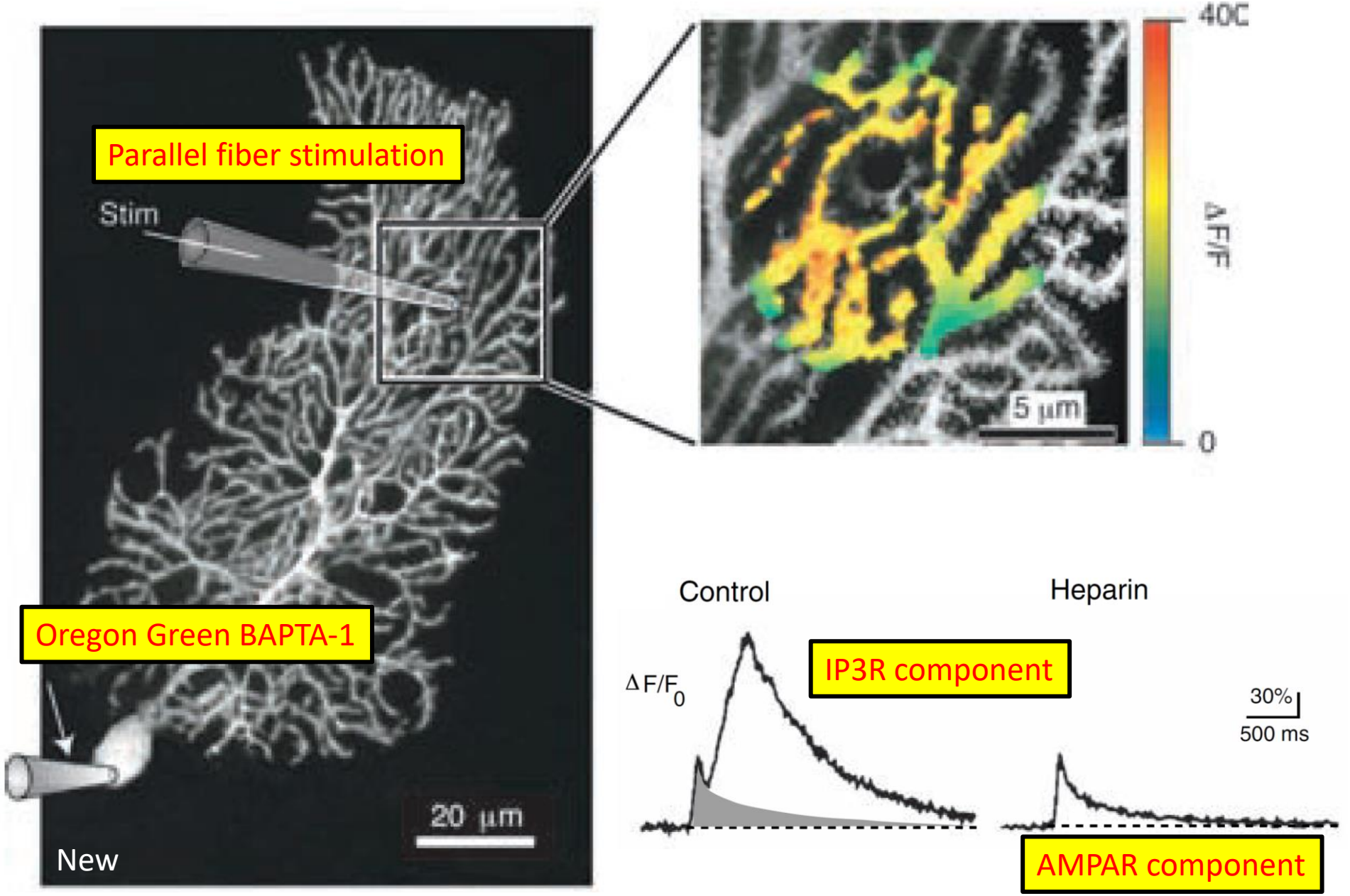
Known

mGluRs

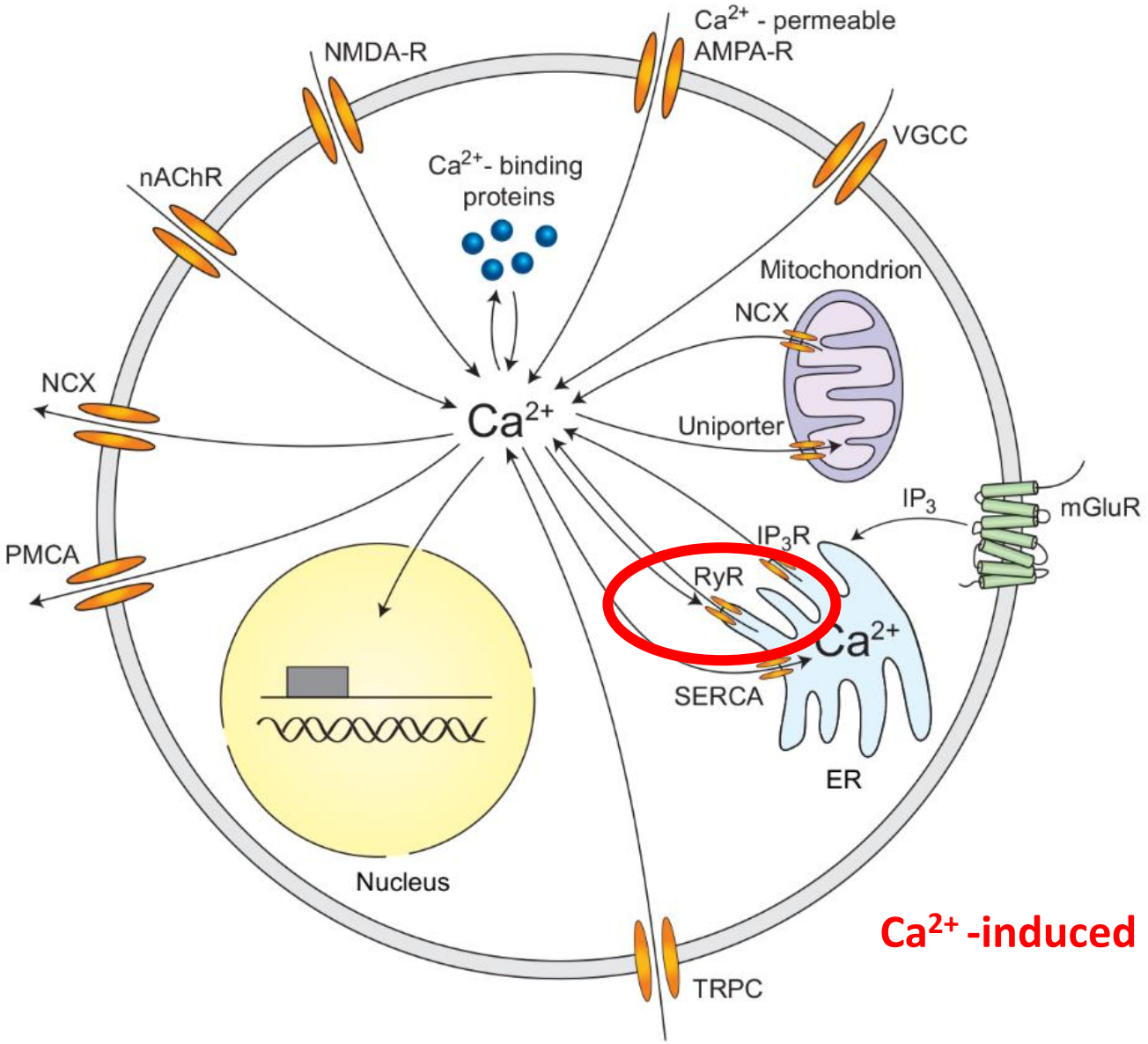

Parallel fibers
Molecular layer
Purkinje cell layer
Granular layer
White matter
Stellate cell
Basket cell
Purkinje cells
Golgi cell
Purkinje cell
Granule cell
Mossy fiber
Climbing fiber
Purkinje cell axon

New

mGluRs


Parallel fiber stimulation
Stim
Oregon Green BAPTA-1
20 µm
New
40
ΔF/F
0
5 µm
Control
Heparin
ΔF/F0
IP3R component
30%
500 ms
AMPAR component

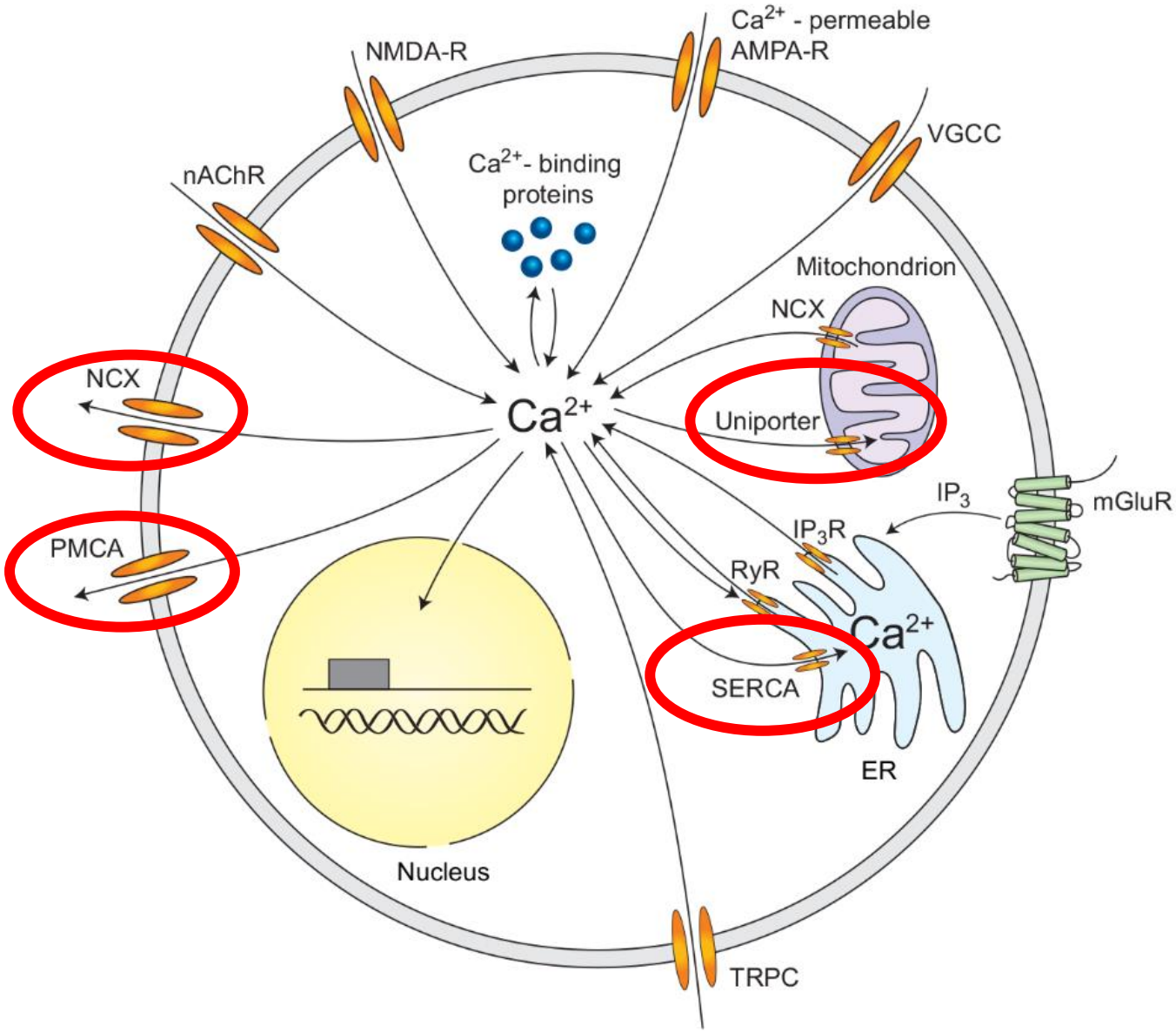
Neuronal Ca^{2+} signaling



Ca^{2+} -induced Ca^{2+} release

Known

Neuronal Ca^{2+} signaling



Known

Ca^{2+} transporters

Ca^{2+} sequestration

- **Sarco/endoplasmatic reticulum Ca^{2+} ATPase (SERCA): 2 Ca^{2+} in 1 H^+ out / ATP (high affinity but ER has low capacity → important at rest)**

- **Mitochondrial Ca^{2+} uniporter (MCU): uses H^+ concentration gradient (low affinity but mitochondria have high capacity → important upon strong stimulation or injury)**

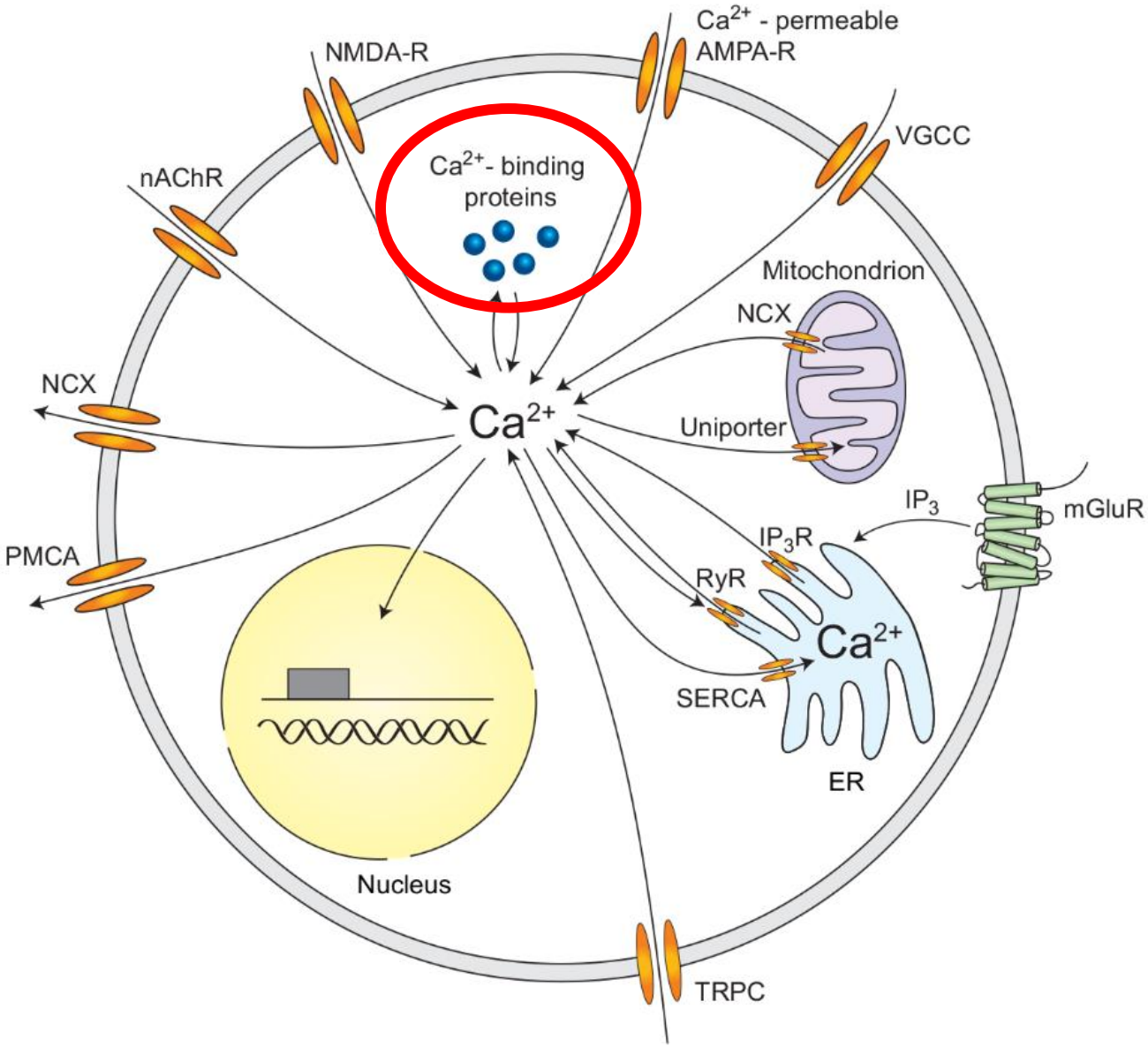
Ca^{2+} transport

- **Plasma Ca^{2+} ATPase (PMCA): 1 Ca^{2+} out 1 H^+ in / ATP (activity incresed by Ca^{2+}-calmodulin; many isoforms that cover both basal (in the 100 nM range) and elevated Ca^{2+} levels (in the µM range)**

- **Na^+/Ca^{2+} exchange protein (NCX): in neurons at rest usually Na^+ in (down the electrochemical gradient) Ca^{2+} out (against the electrochemical gradient) but it can be reversible**

Known

Neuronal Ca^{2+} signaling



New

Ca^{2+} buffering

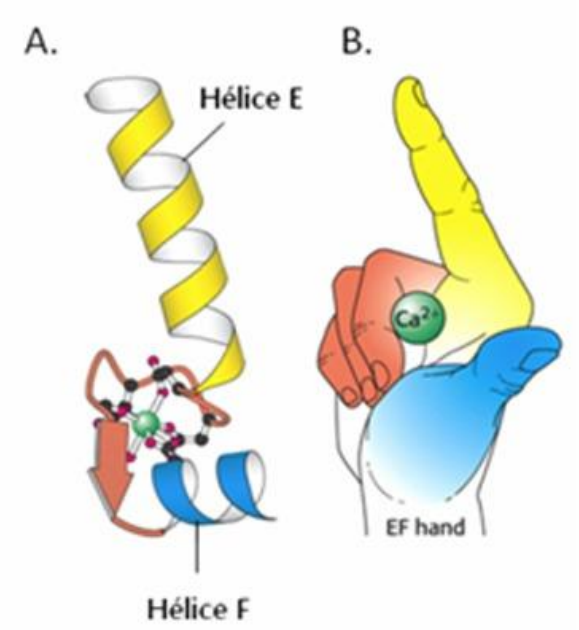
For every 100 - 1000 Ca^{2+} ions entering the neuron, 1 (on average) remains free; the remaining will be buffered (=bound to proteins) within 10 - 100 µs (we will come back to this in a more quantitative way)

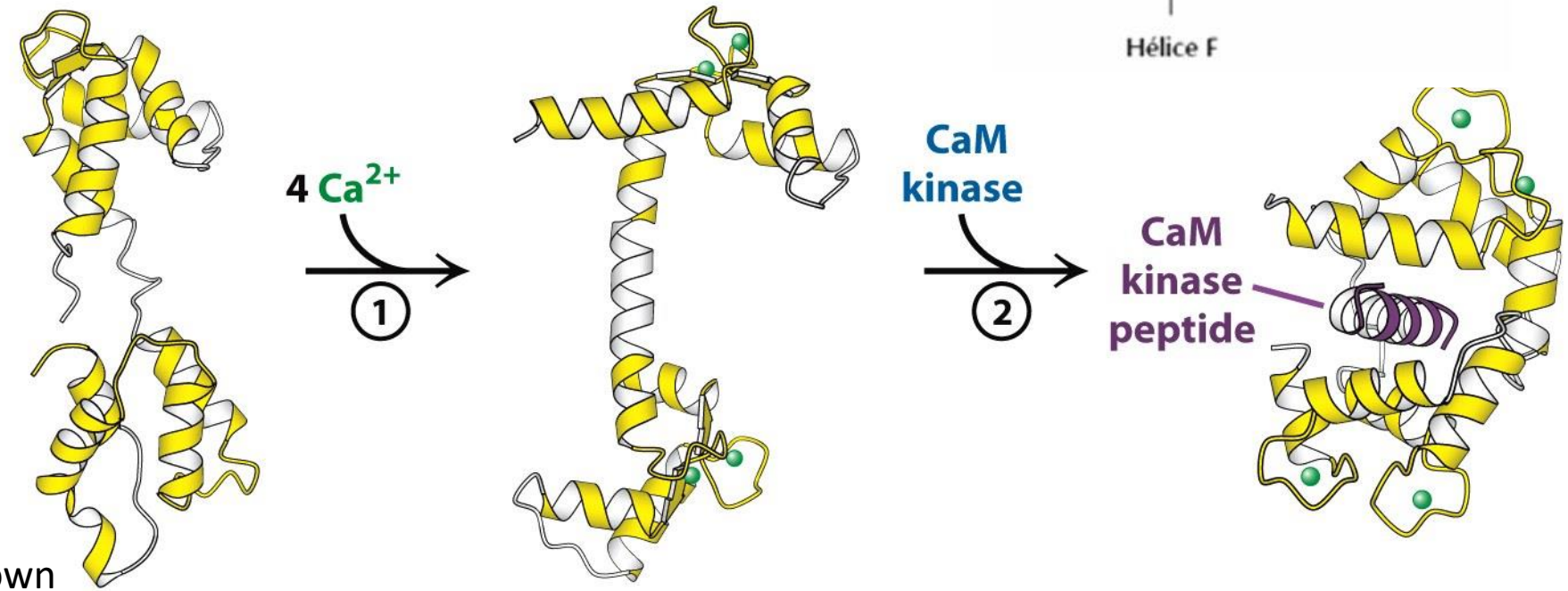
- **Ca^{2+} binding proteins:**
  - **Ca^{2+} buffers**
  - **Ca^{2+} sensor: binding of Ca^{2+} ions induces a conformational change, which allows them to interact with specific targets in a Ca^{2+}-regulated manner. However, if present at sufficiently high concentrations, Ca^{2+} sensors may also function as Ca^{2+} buffers.**

New

Prototipical example of Ca^{2+} sensor?

Calmodulin (10 µM in the squid axon)





Known

Calmodulin is ubiquitous but it is not the only EF-hand Ca^{2+} sensor protein - the neuronal calcium sensor proteins

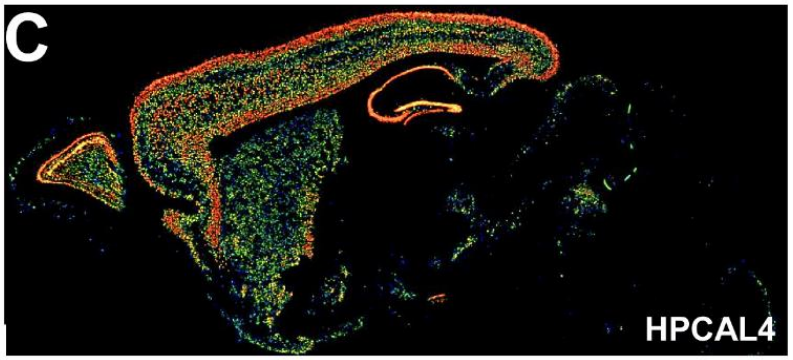
Table 1 | **Summary of the members of the NCS protein family represented in mammalian genomes**

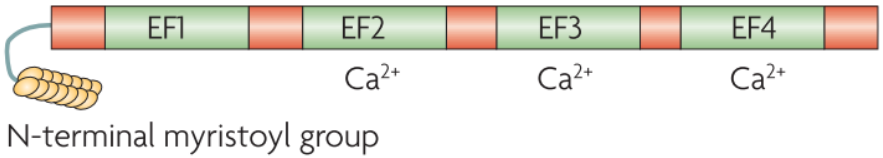
Subgroup	First appearance in evolution	Mammalian protein	Expressed human splice variants	Proposed functions
A	Yeast	NCS-1	1	Regulation of neurotransmission[16], stimulation of constitutive[79] and regulated exocytosis[18], learning[77], short-term synaptic plasticity[128], Ca^{2+} channel[129,130] and Kv4 channel[125,126] regulation, phosphoinositide metabolism[21,78,79,131], dopamine D2 receptor endocytosis[73], GDNF signalling[132], neuronal growth[127] and survival[133]
B	Nematodes	Hippocalcin	1	Anti-apoptotic[134], AMPA receptor recycling in LTD[135], MAPK signalling[136,137], learning[138]
		Neurocalcin-δ	1	Guanylyl cyclase activation[139]
		VILIP1	1	Guanylyl cyclase activation and recycling[140], traffic of nicotinic receptors[141], increase of cAMP levels and secretion[142]
		VILIP2	1	Regulation of P/Q-type Ca^{2+} channels[143]
		VILIP3	1	Unknown
C	Fish	Recoverin	1	Light adaptation by inhibition of rhodopsin kinase[43]
D	Fish	GCAP1	1	Regulation of retinal guanylyl cyclases[23]
		GCAP2	1	Regulation of retinal guanylyl cyclases[23]
		GCAP3	1	Regulation of retinal guanylyl cyclases[23]
E	Insects	KChIP1	3	Regulation of Kv4[61] and Kv 1.5[144] channels, repression of transcription[69]
		KChIP2	5	Regulation of Kv4[61] and Kv1.5[144] channels, repression of transcription[69]
		KChIP3	2	Regulation of Kv4[61] channels, presenilin processing[62] , APP processing[63], repression of transcription[66], pro-apoptotic[145], regulates ER Ca^{2+} [146]
		KChIP4	6	Regulation of Kv4 channels[100], presenilin-processing[65], repression of transcription[69]

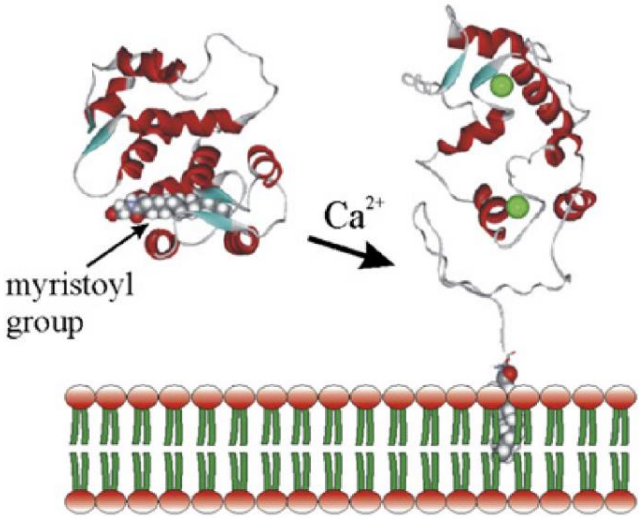
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; APP, amyloid precursor protein; cAMP, cyclic AMP; ER, endoplasmic reticulum; GCAP1, guanylyl cyclase activating protein 1; GDNF, glial cell line-derived neurotrophic factor ; KChIP1, Kv channel-interacting protein 1; Kv channel, voltage-gated potassium channel; LTD, long-term depression; MAPK, mitogen-activated protein kinase; NCS, neuronal Ca^{2+} sensor; VILIP1, visinin-like protein 1.

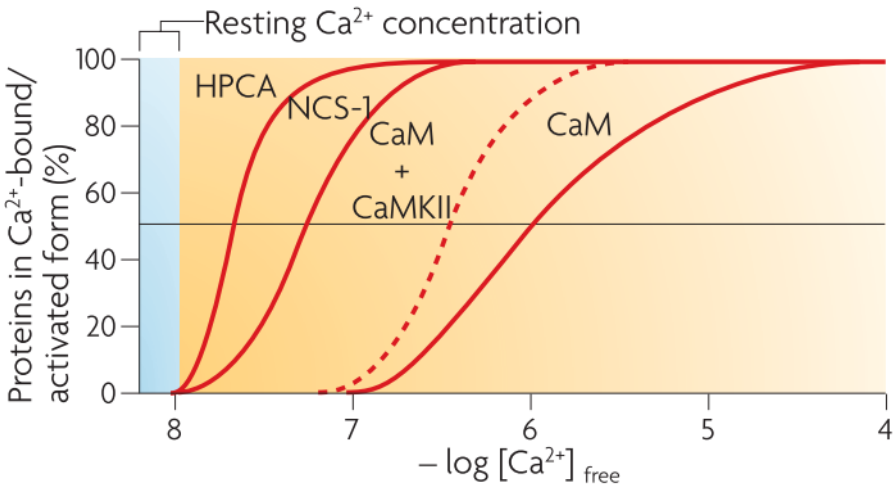
New

Calmodulin is ubiquitous but it is not the only EF-hand Ca^{2+} sensor protein - the neuronal calcium sensor proteins



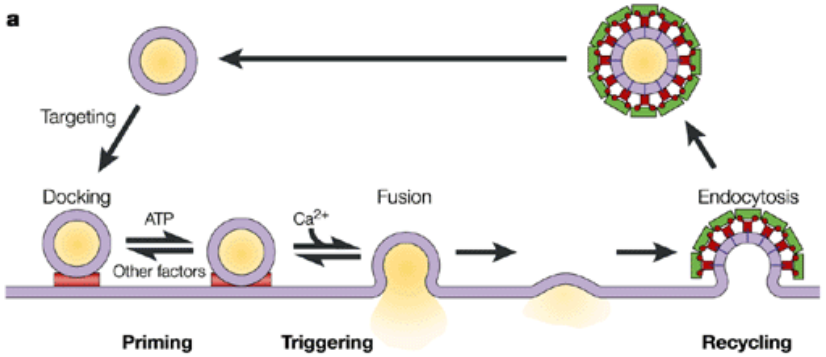


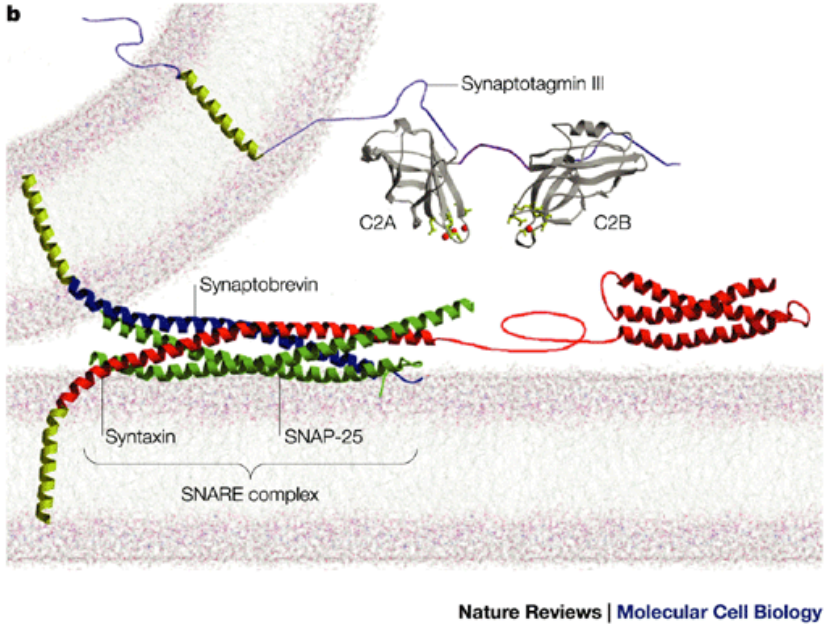


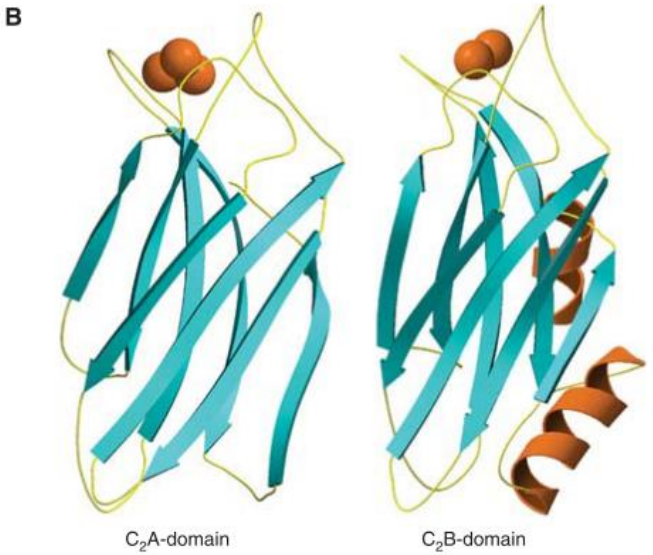


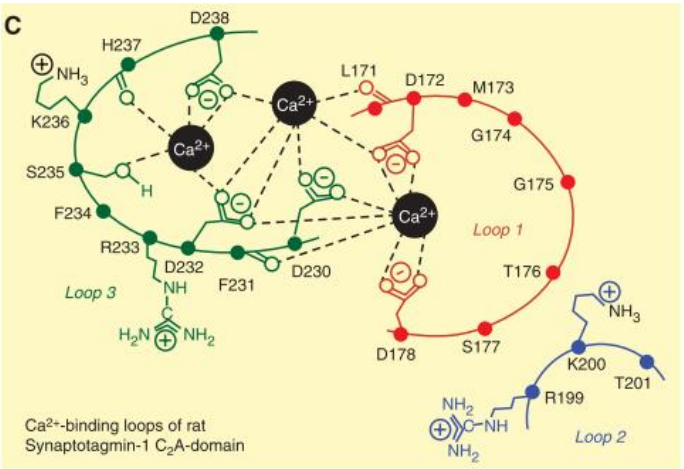
New

Ca^{2+} sensor(s) for vesicle release









Known

Examples of Ca^{2+} buffers

- **Parvalbumin (3 EF-hand domains; expressed in specific subtypes of interneurons)**

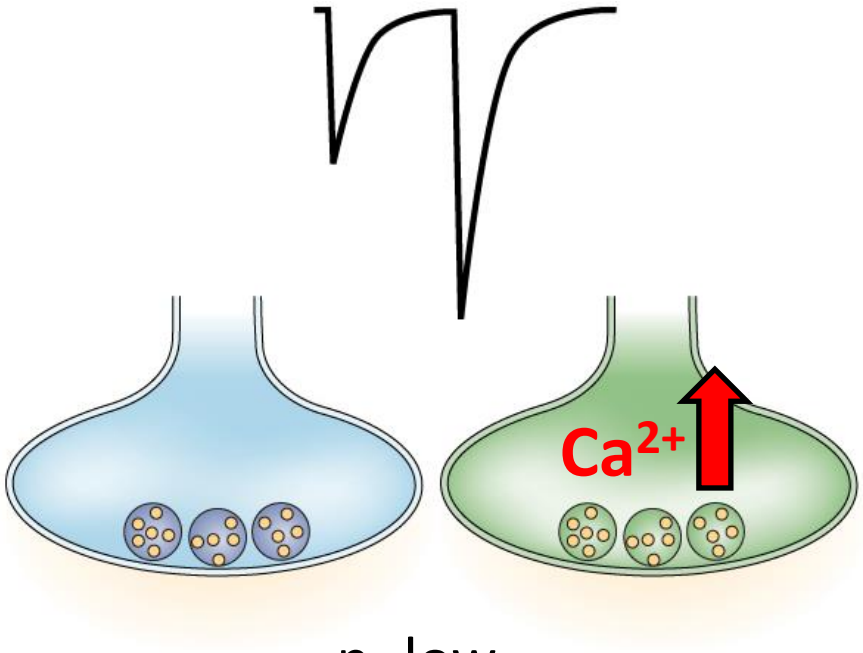


- **Calbindin (4 EF-hand domains; e.g. important for shaping neurotransmitter release)**



New

STF is due to the build-up of Ca^{2+} in the presynaptic terminal during synaptic activity



p_v low

Residual Ca^{2+} hypothesis

1. ‘Original’ residual Ca^{2+} model

(Katz and Miledi, 1968)

Problems:

$[Ca^{2+}]_{out}$ = 1 - 2 mM

$[Ca^{2+}]_{rest}$ = 50 - 100 nM

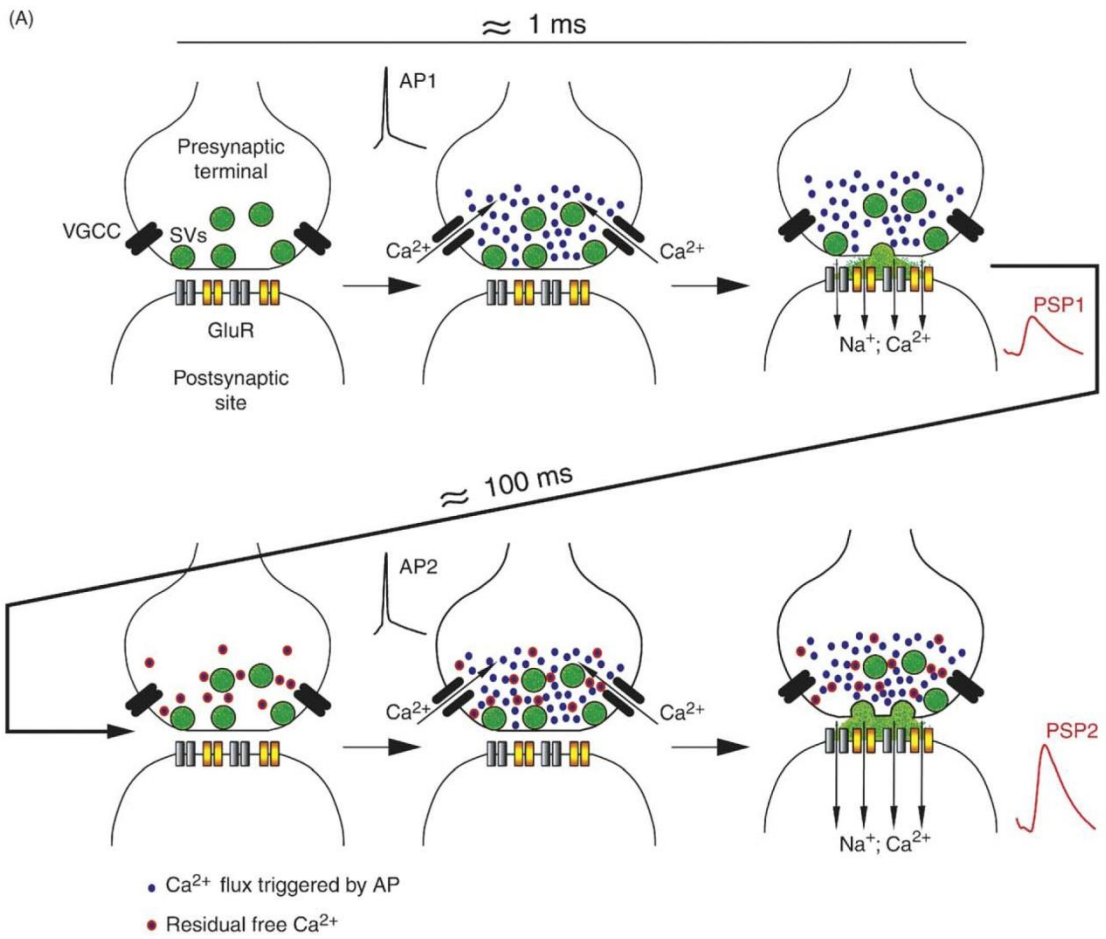
$[Ca^{2+}]_{channel\ mouth}$ ≈ 100 μM

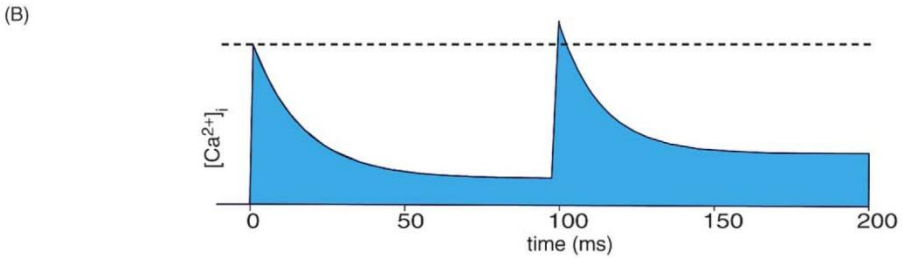
(not at equilibrium)

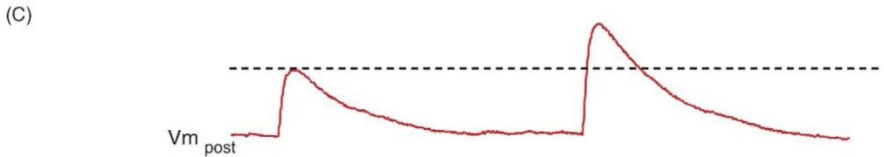
$[Ca^{2+}]_{residual}$ < 1 μM

(Ca^{2+} equilibrates throughout the bouton within 1 msec; residual Ca^{2+} lasting for tens of msec to sec)

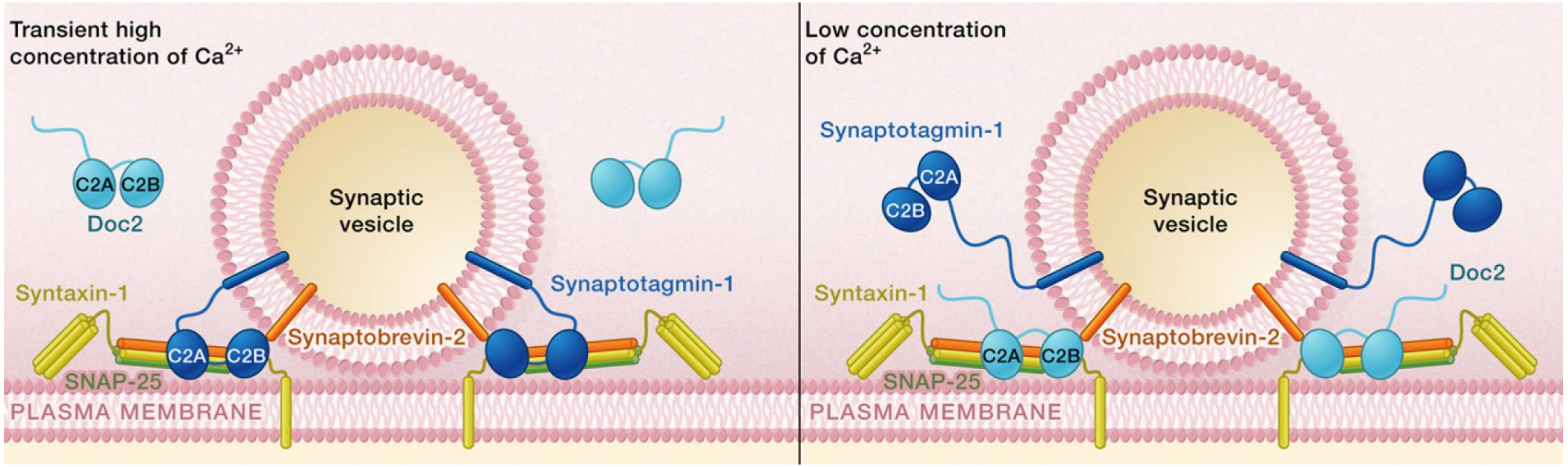
Too little even for the power law!







2. Two sensors model

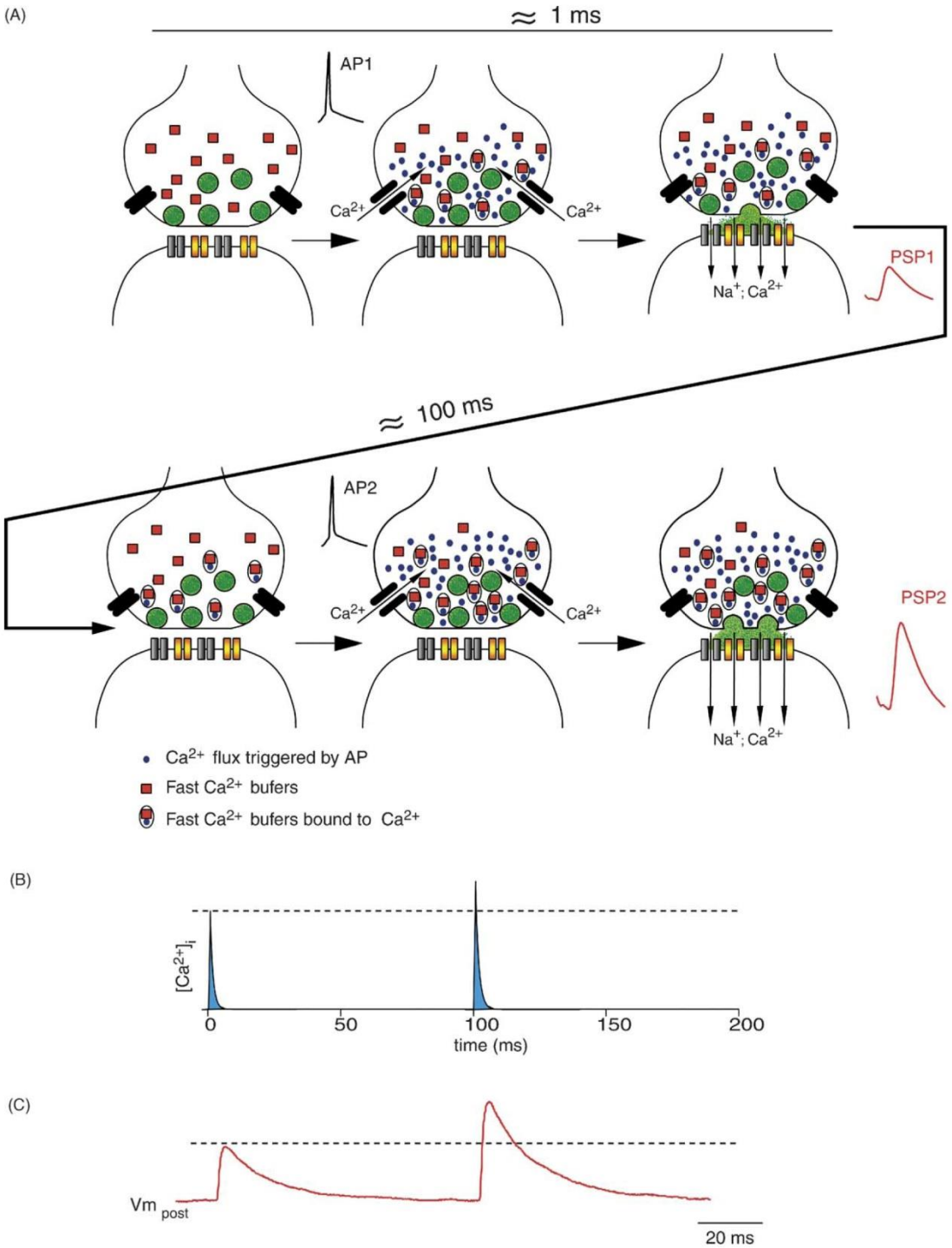


K_d ~ 10 μM

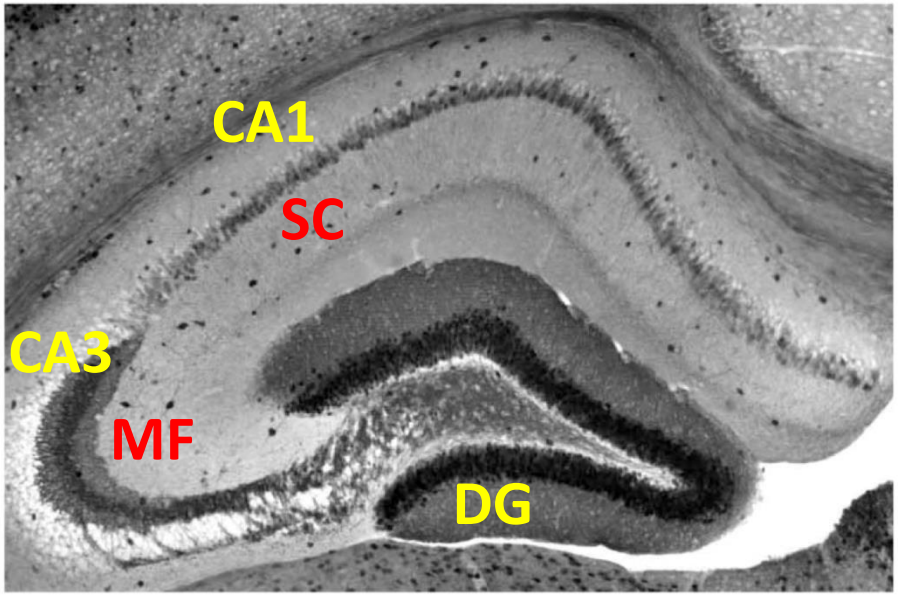
K_d < 1 μM

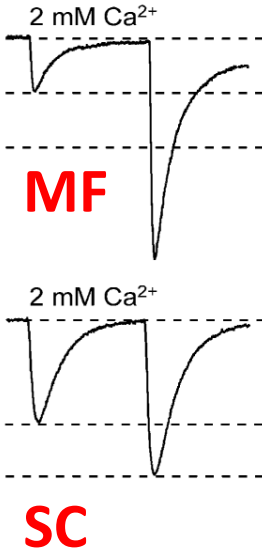
An additional high-affinity Ca^{2+} binding site (**Doc2**) with slow unbinding kinetics could operates cooperatively with the main release sensor (**synaptotagmin**). Ca^{2+} would bind to the additional site during the first AP and remain bound by the time of the second AP, thus increasing the amount of neurotransmitter release in response to the second AP .

3. Partial saturation of fast Ca^{2+} buffers model



The endogenous buffer Calbindin D_{28k} can explain the large facilitation of the Mossy fiber synapses





Blatow et al., Neuron 2003

Conclusions

- **Summary Ca^{2+} influx (old and new)**
- **Free Ca^{2+} ←→ buffered Ca^{2+}**
  0.1-1%
- **Ca^{2+} sensors vs. Ca^{2+} buffers**
- **Ca^{2+} sensor calmodulin → important for building Ca^{2+} indicators**

