Calcium signalling in health and disease

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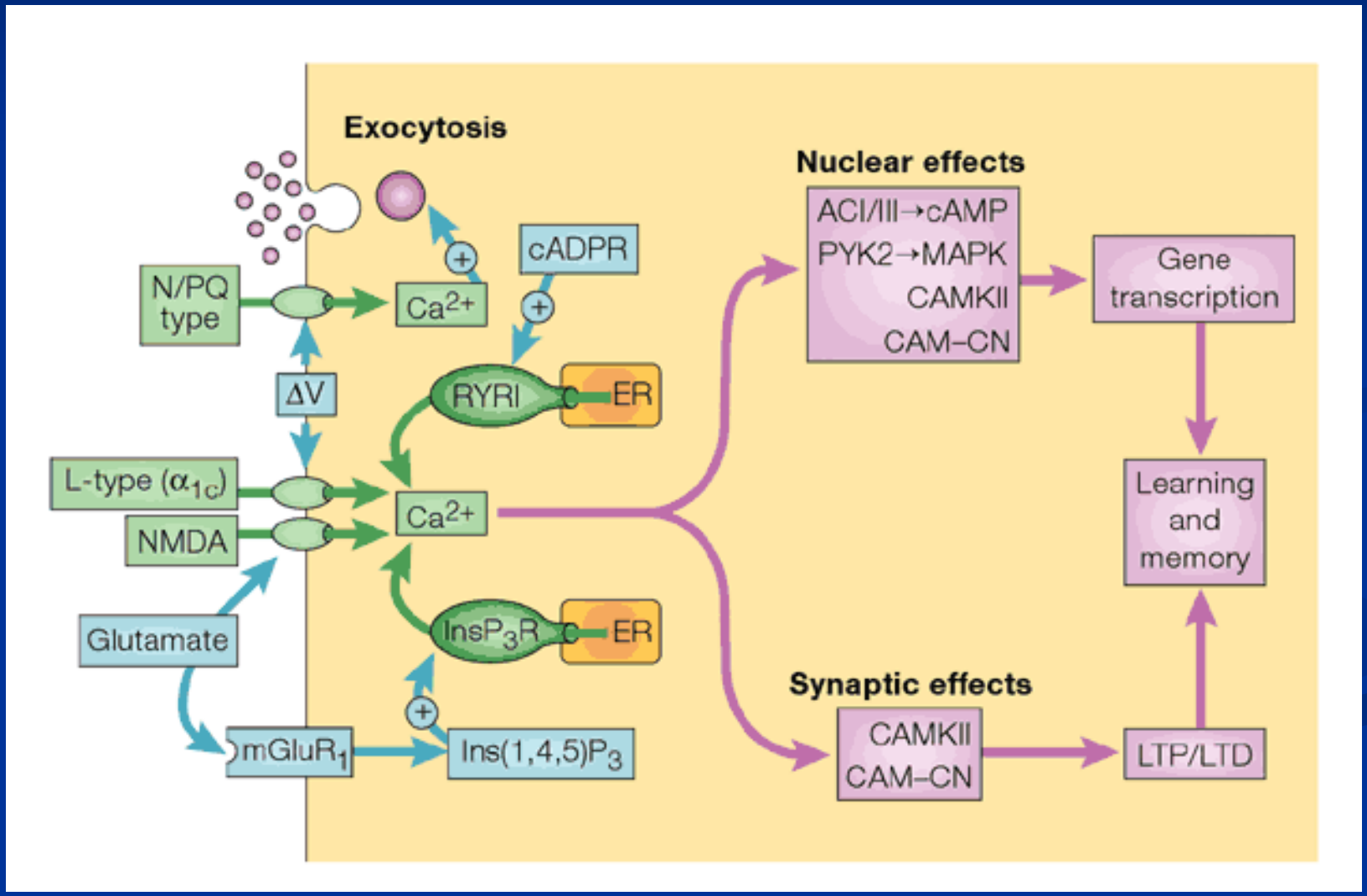
Calcium in biology

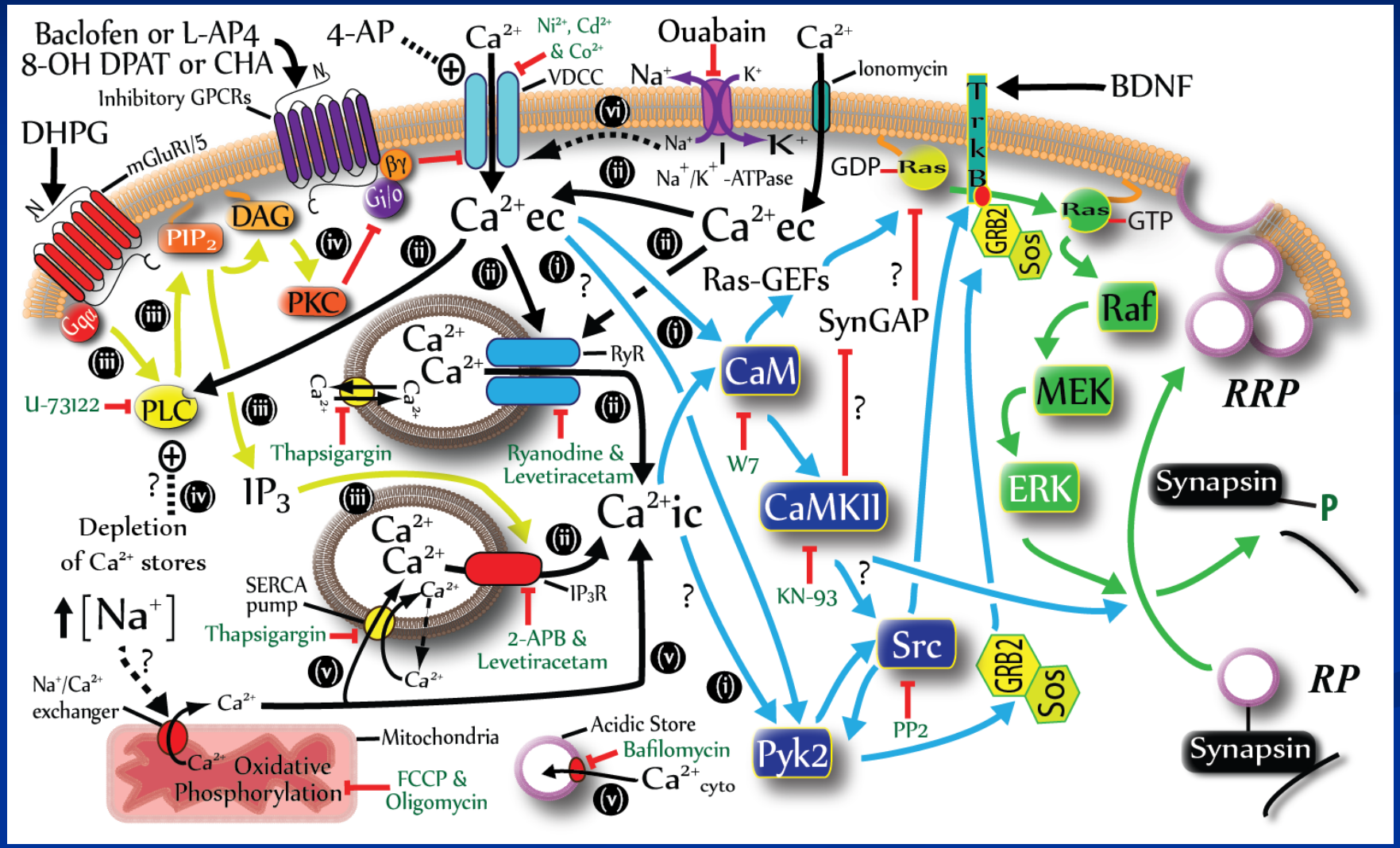
Information transfer by intracellular Ca^{2+}

Bio-functions of calcium

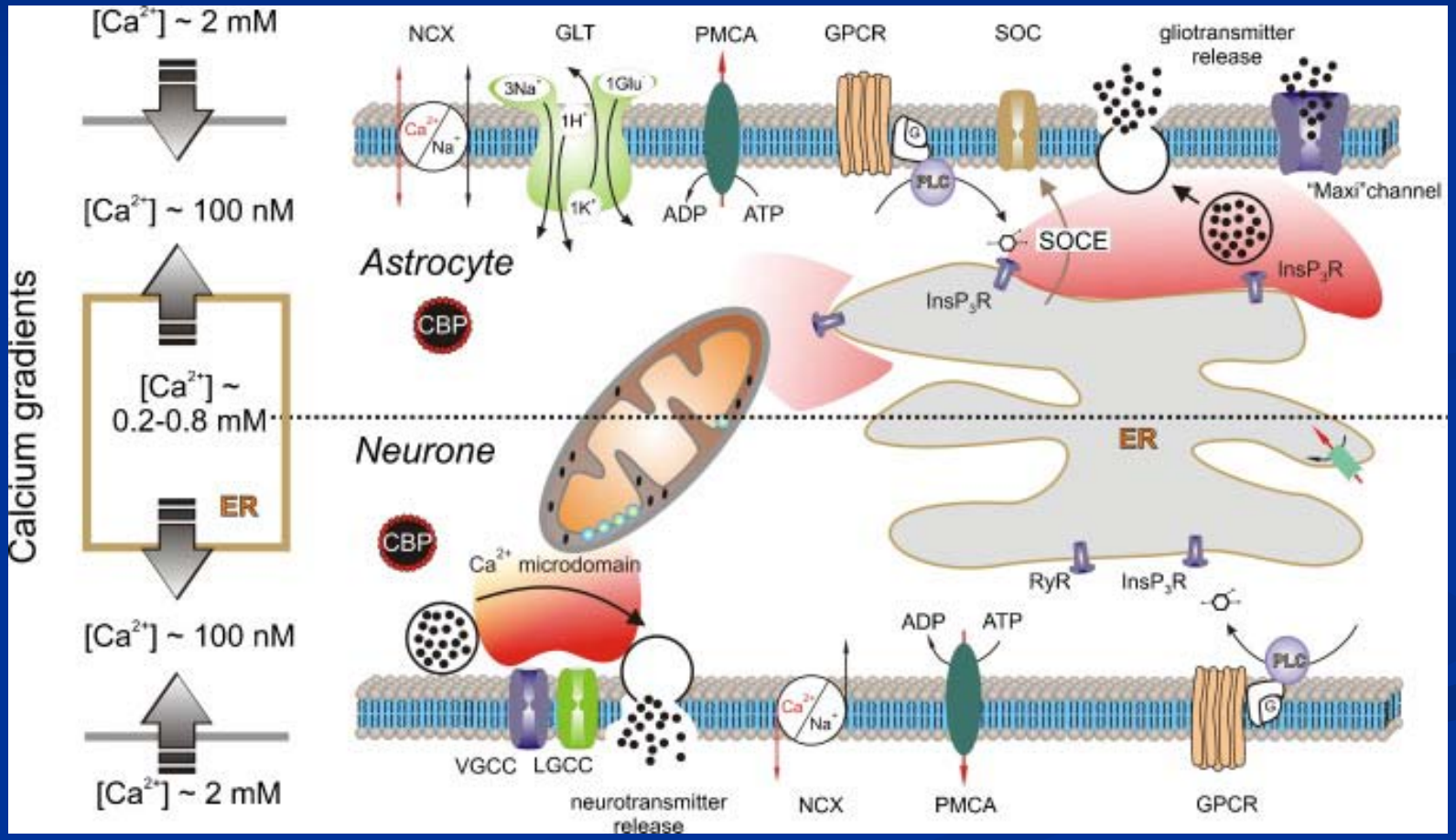
"on the path from plasma membrane to store"



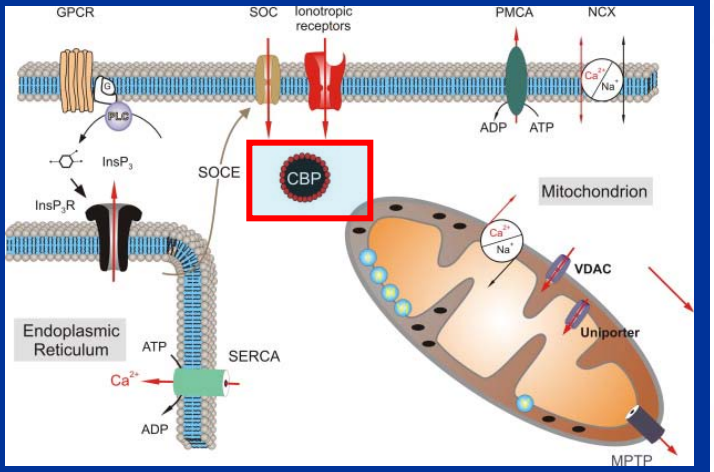
Ca^{2+}- the main "secondary messenger"

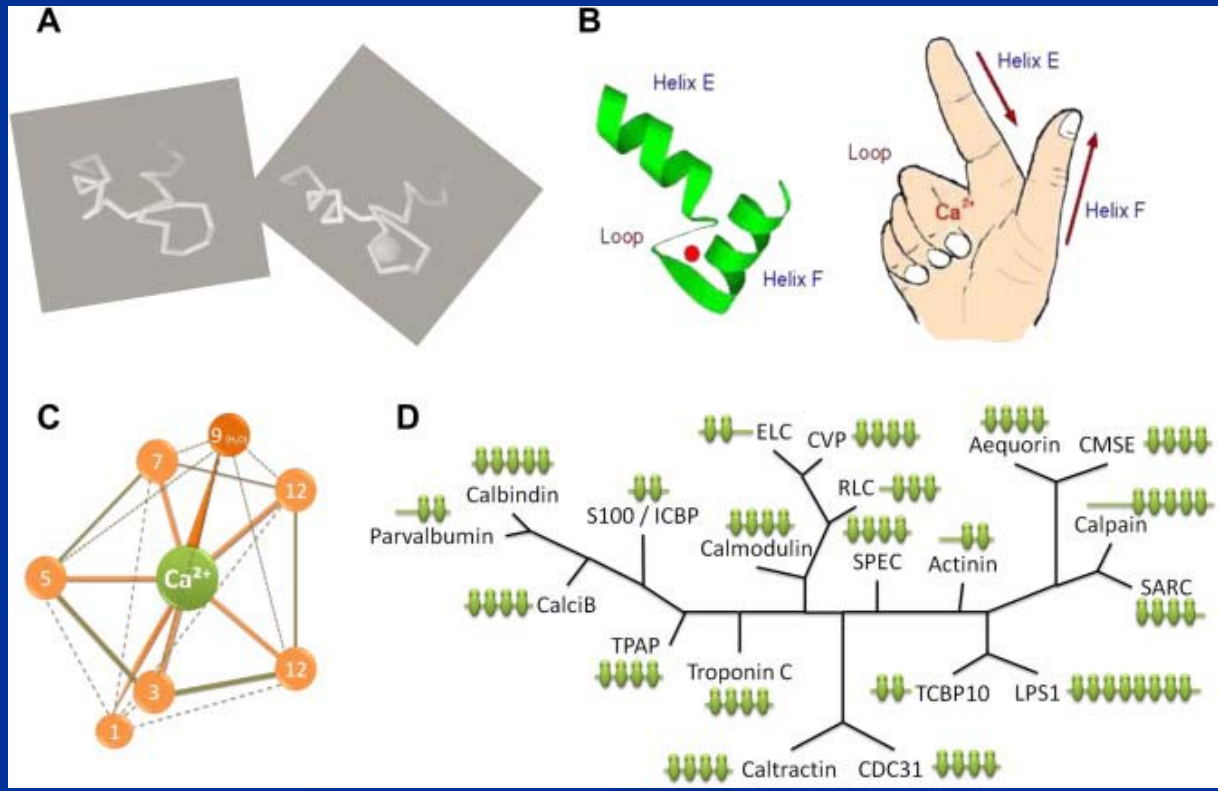


Cell signaling by calcium (neurona & glia)

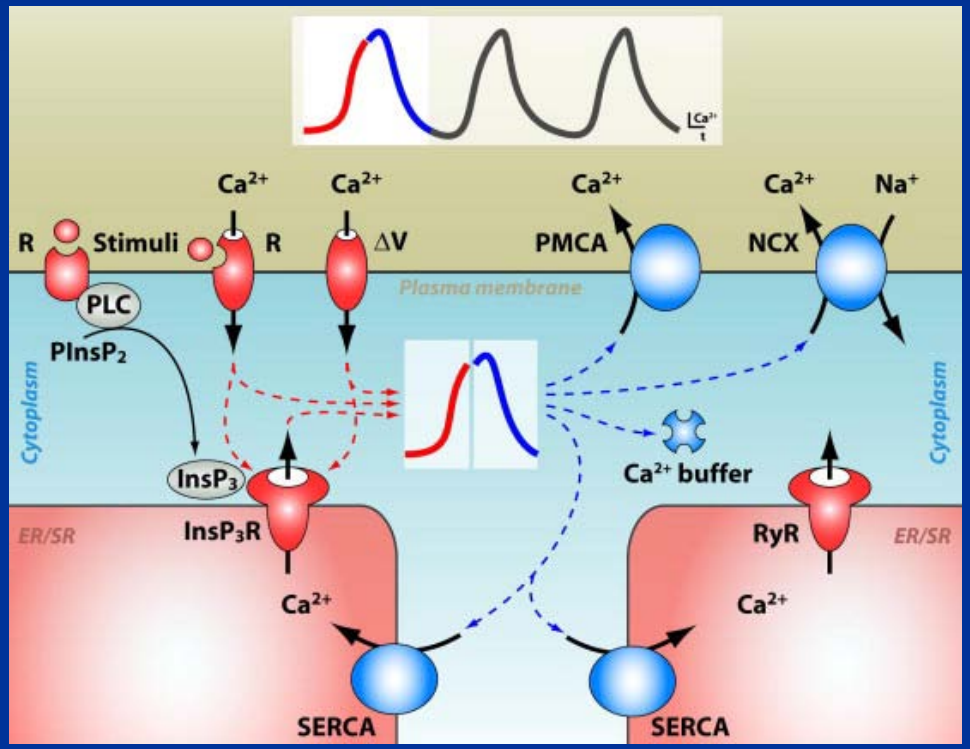


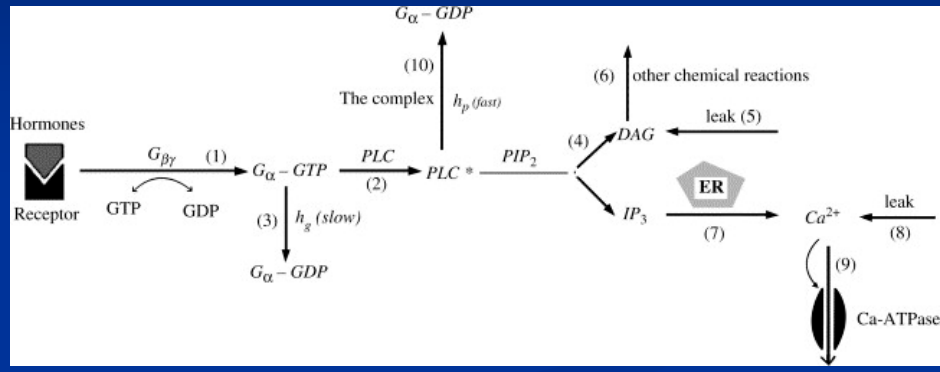
Calcium binding proteins





Secondary messengers and Ca^{2+} - oscilations / *cross-talk* of the plasma membrane and Ca^{2+} stores





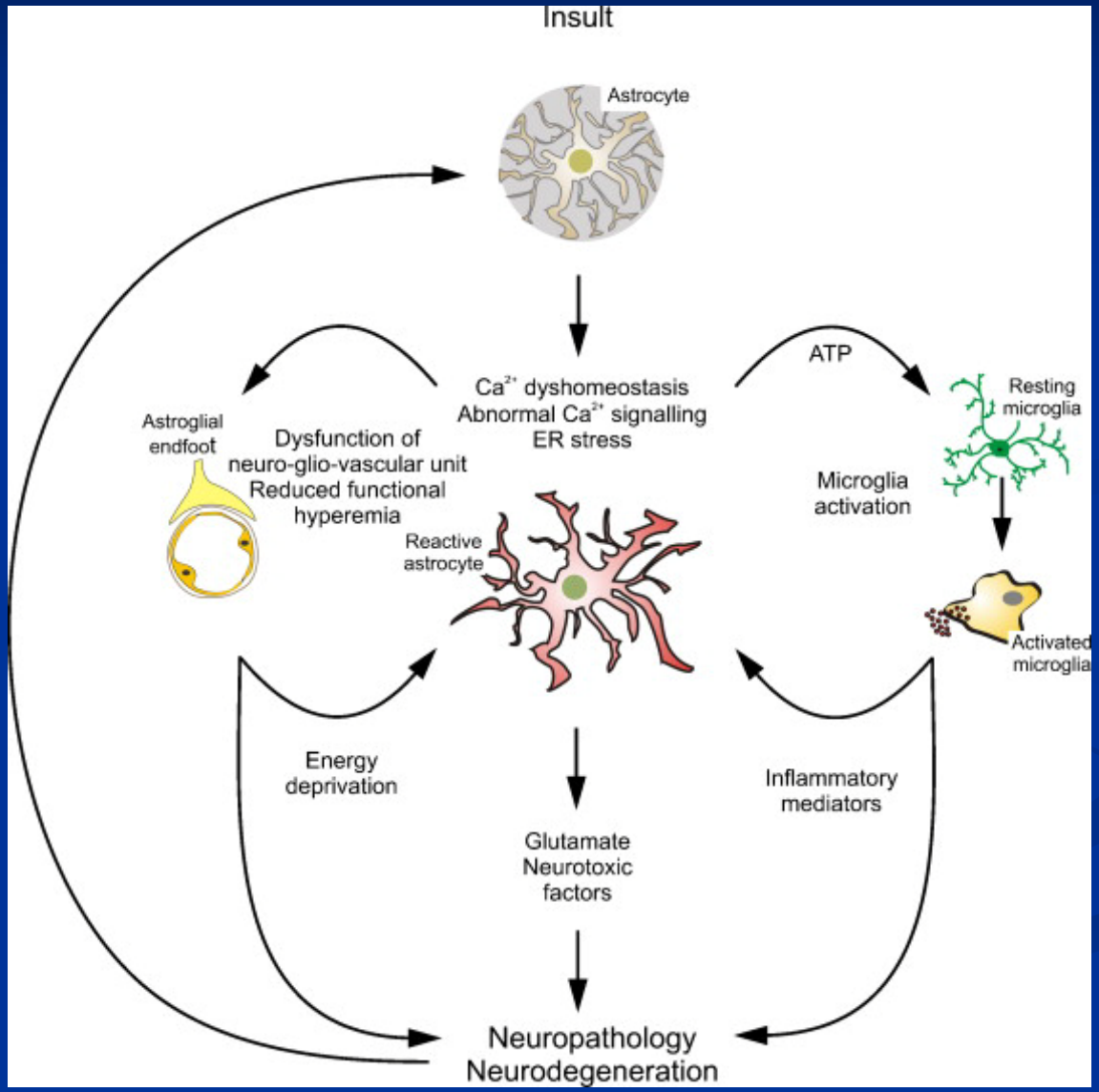
$$\frac{d[G_\alpha - GTP]}{dt} = k_g[G_\alpha - GDP] - 4k_p[G_\alpha - GTP]^4[PLC] - h_g[G_\alpha - GTP],$$

$$\frac{d[DAG]}{dt} = k_d[PLC^*] - h_d[DAG] + l_d,$$

$$\frac{d[Ca^{2+}]_i}{dt} = \rho\left\{k_c\frac{[IP_3]^3}{K_S^3 + [IP_3]^3} - h_c[Ca^{2+}]_i + l_c\right\},$$

$$\frac{d[PLC^*]}{dt} = k_p[G_\alpha - GTP]^4[PLC] - h_p[PLC^*],$$

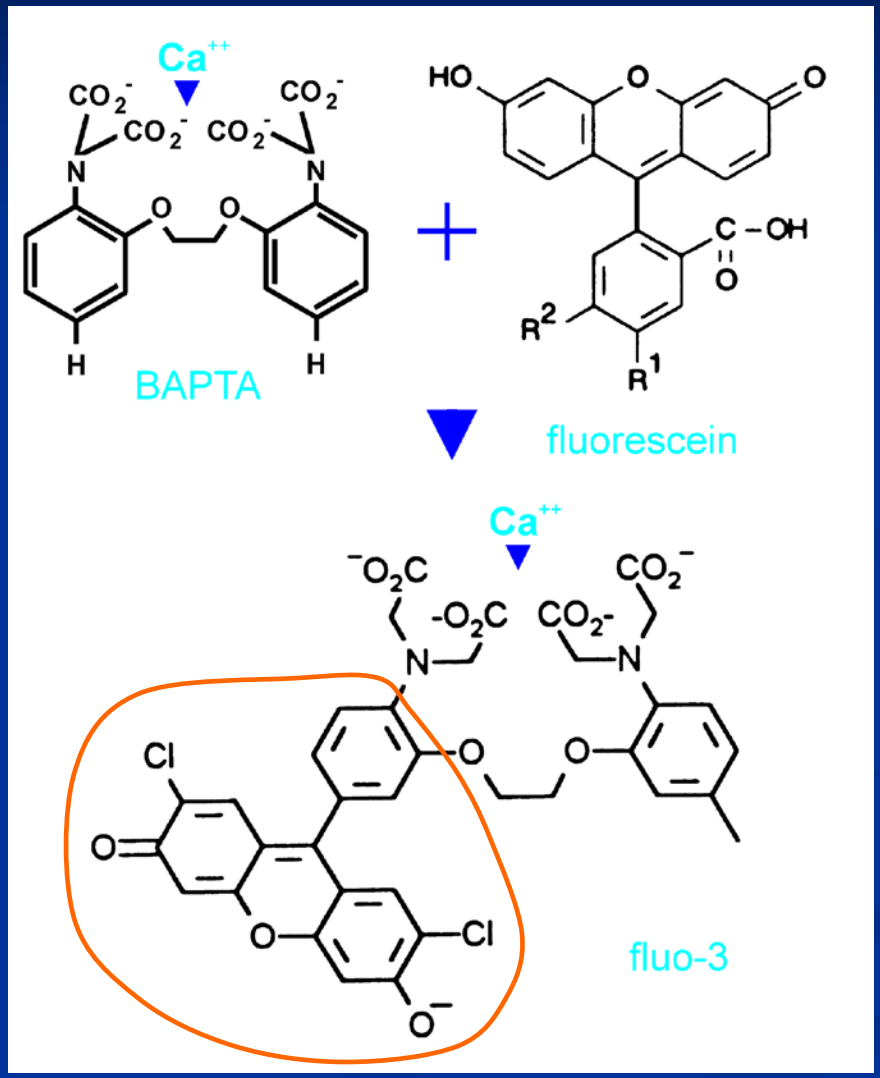
Calcium & neurodegeneration



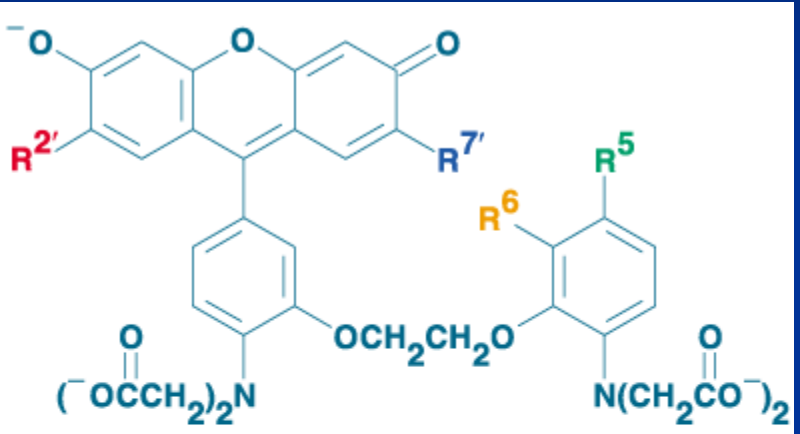
Calcium fluorescent probe

Measurement of intracellular Ca^{2+}

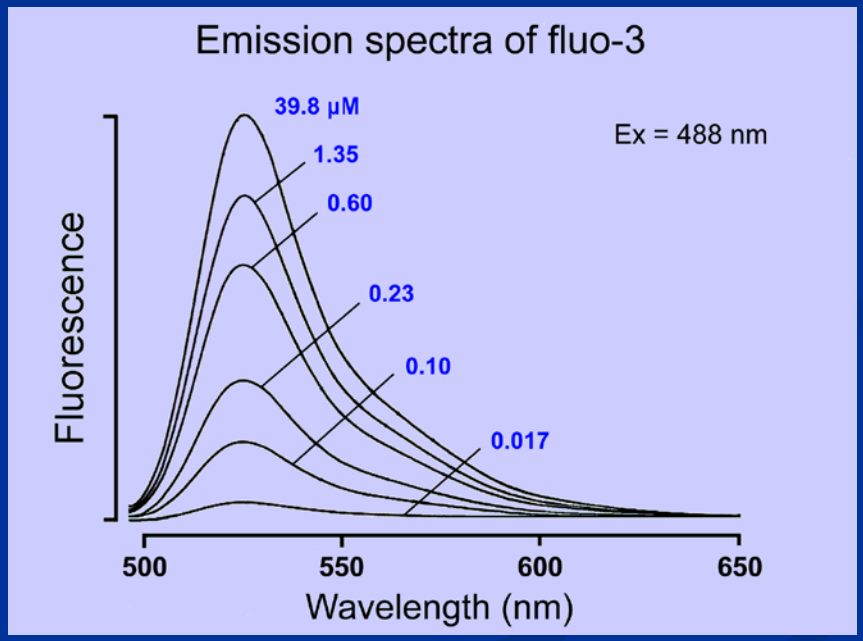
From Ca^{2+} bufer to Ca^{2+} indicator (sintesis of fluo-3)



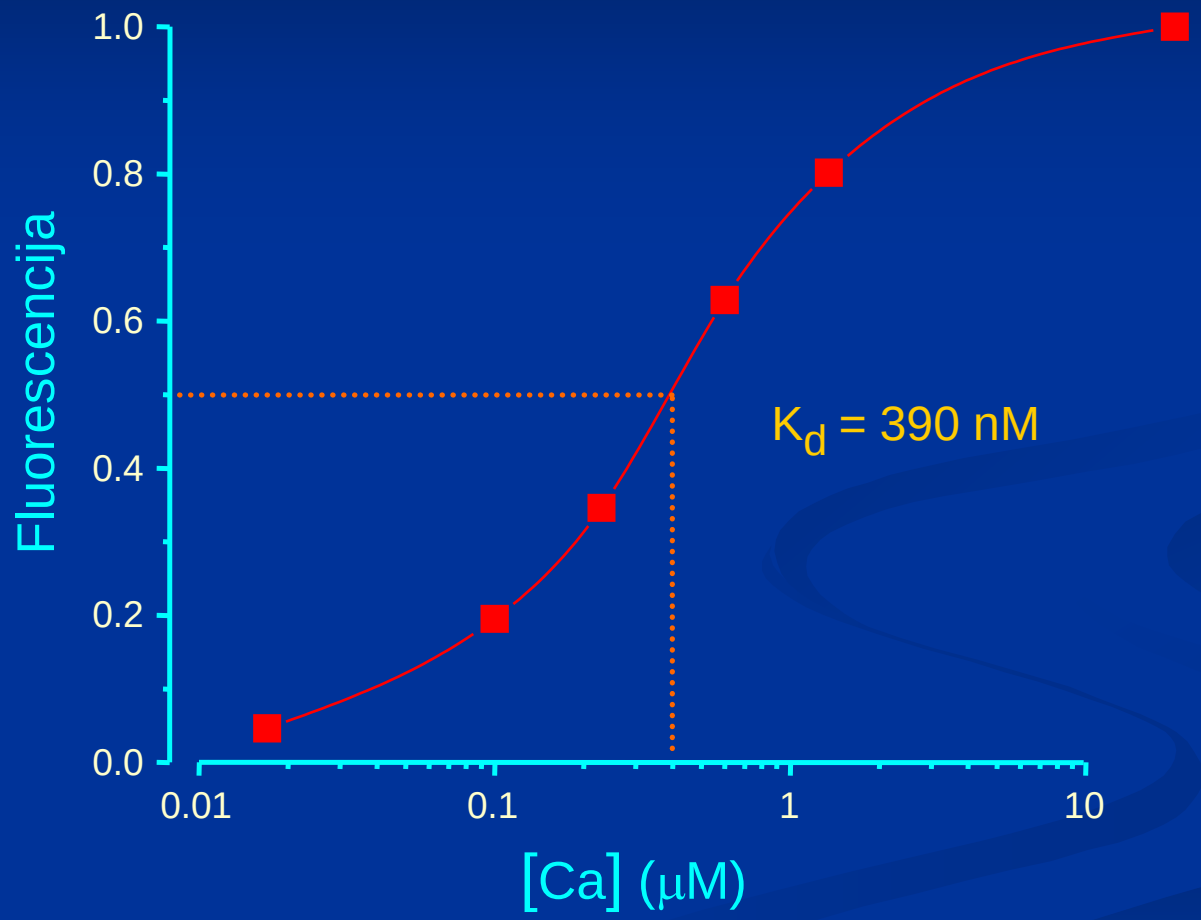
Family of Fluo-indicators



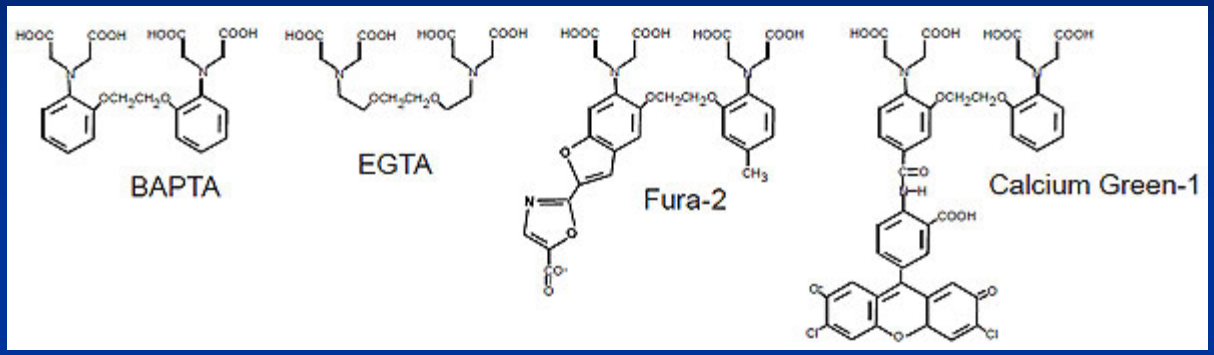
Indicator	$K_d(Ca^{2+})$	$R^{2'}$	$R^{7'}$	R^5	R^6
Fluo-3	0.39 μM	Cl	Cl	CH_3	H
Fluo-4	0.35 μM	F	F	CH_3	H
Fluo-5F	2.3 μM	F	F	F	H
Fluo-5N	90 μM	F	F	NO_2	H
Fluo-4FF	9.7 μM	F	F	F	F

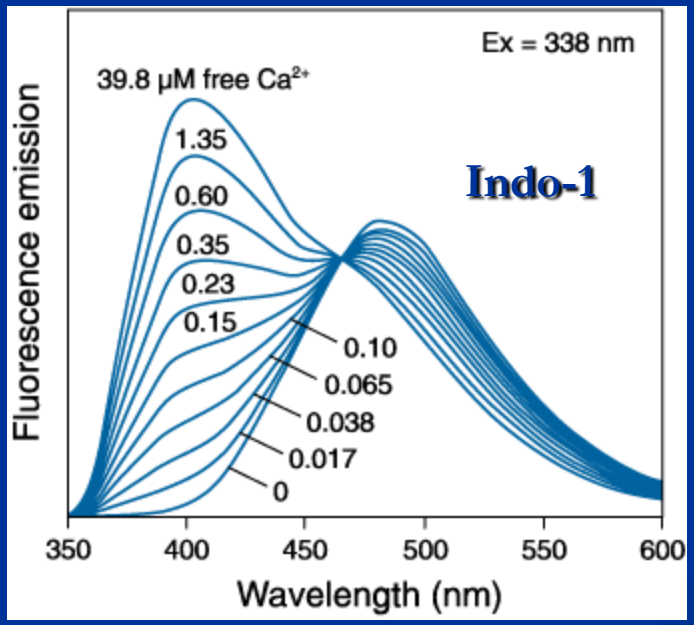


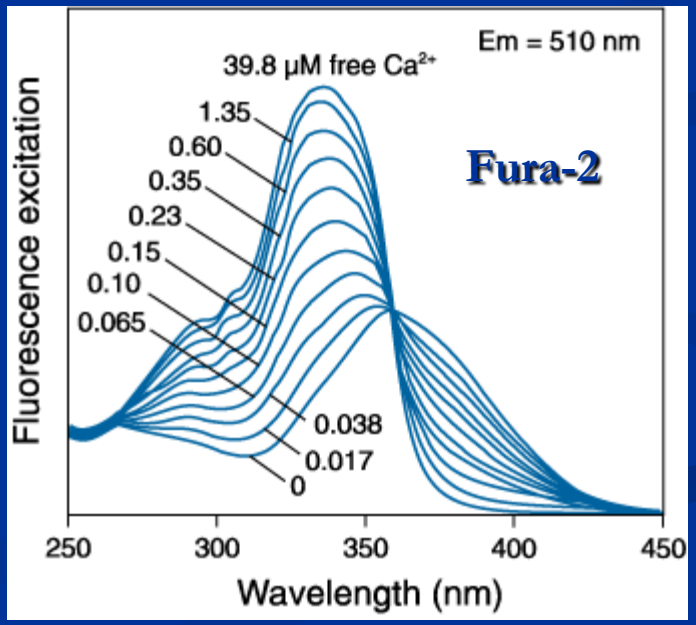
Intensity of fluo-3 fluorescence *vs*. Ca^{2+} concentraction



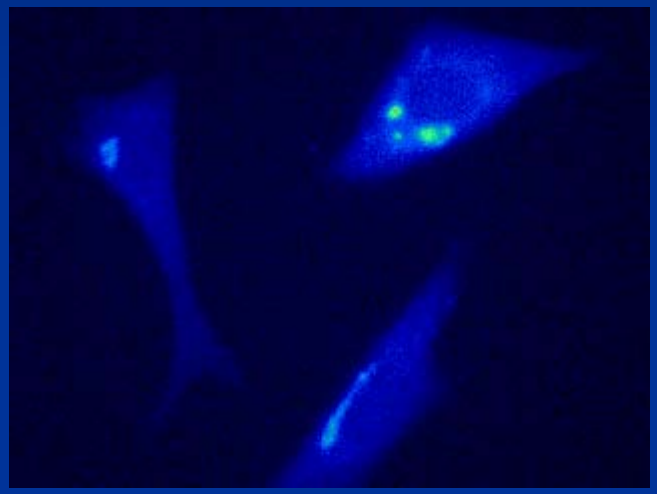
Raciometric dyes

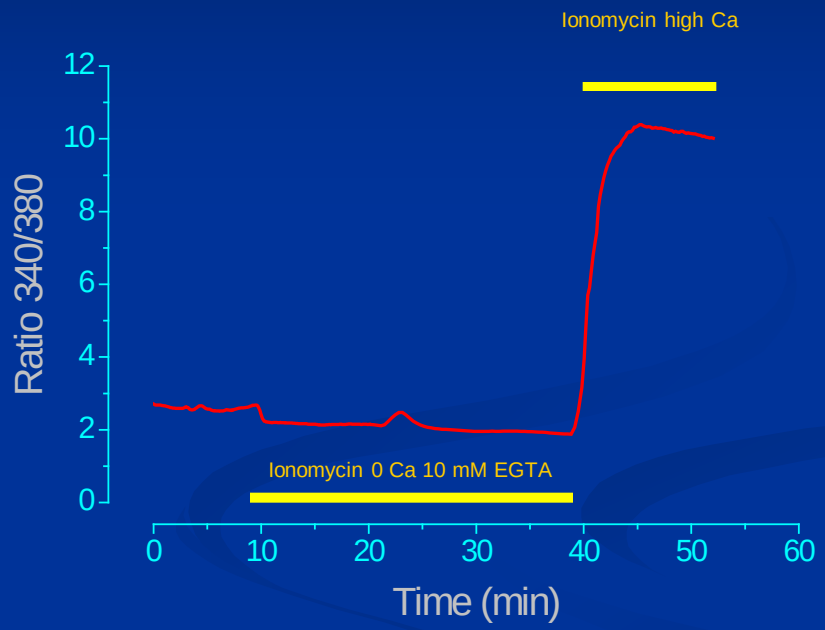






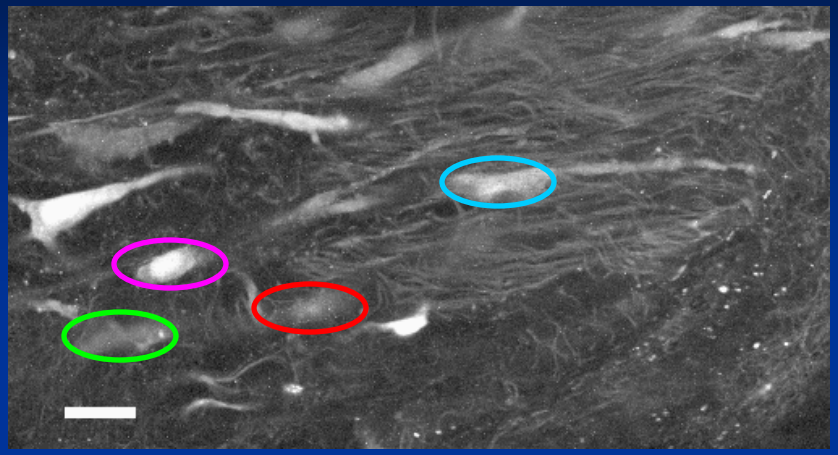
In vivo calibration of the Ca^{2+} signal

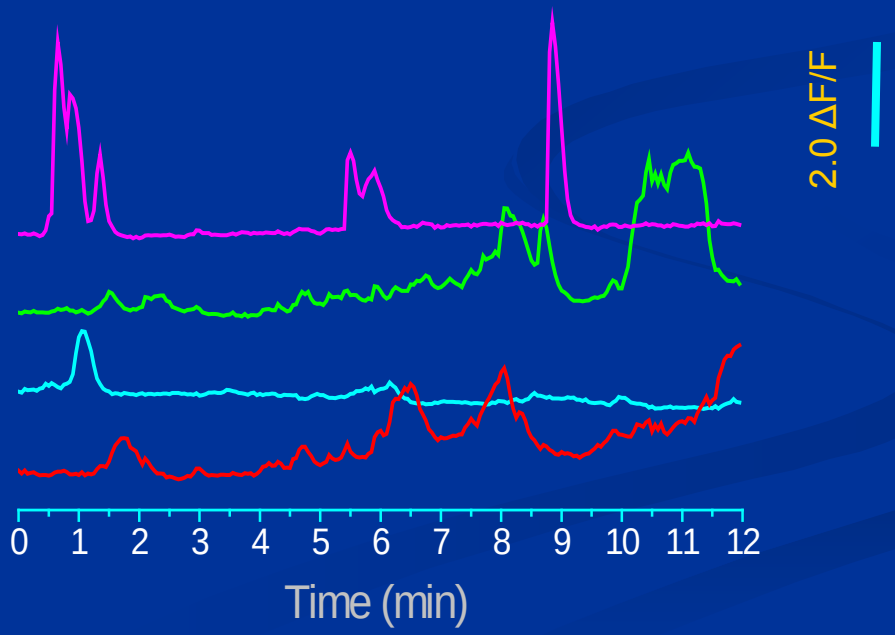




$$[Ca]_{in}(t) = K_d \frac{F_0^{380}}{F_{max}^{380}} \cdot \frac{R(t) - R_{min}}{R_{max} - R(t)}$$

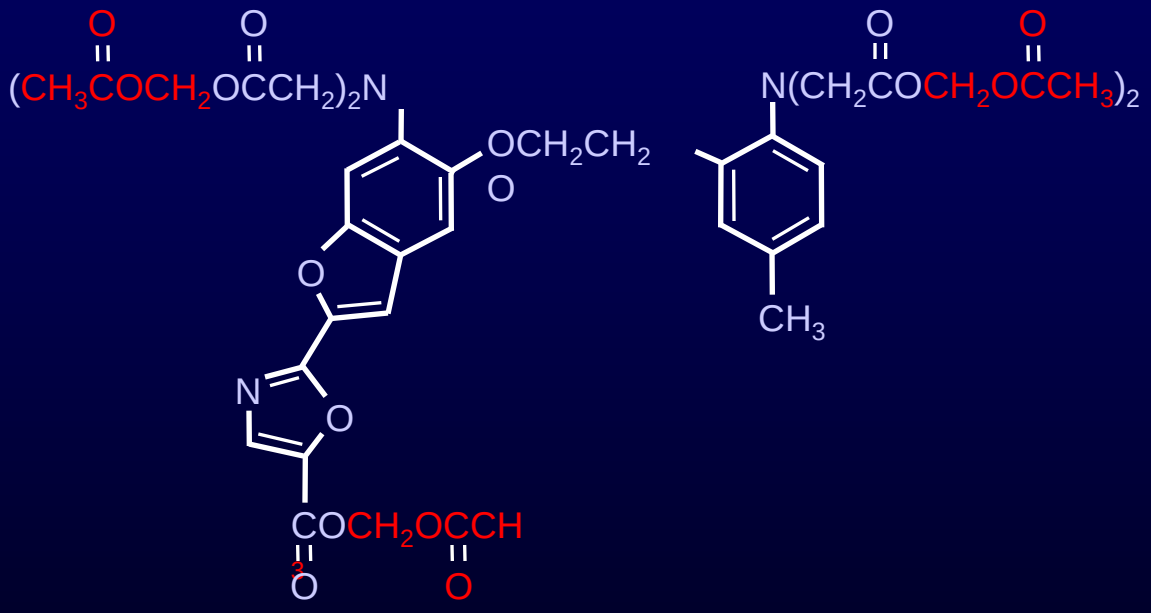
Space & time dynamics of Ca^{2+} in a multicellular system





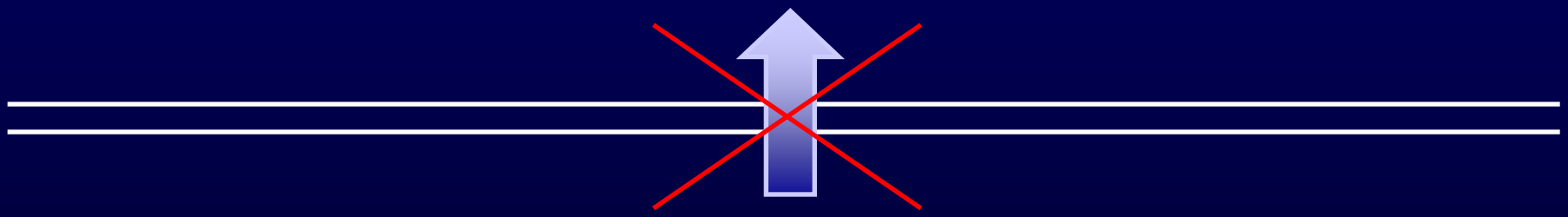
Probe entrance into the cell (1)

(Roger Y. Tsien)

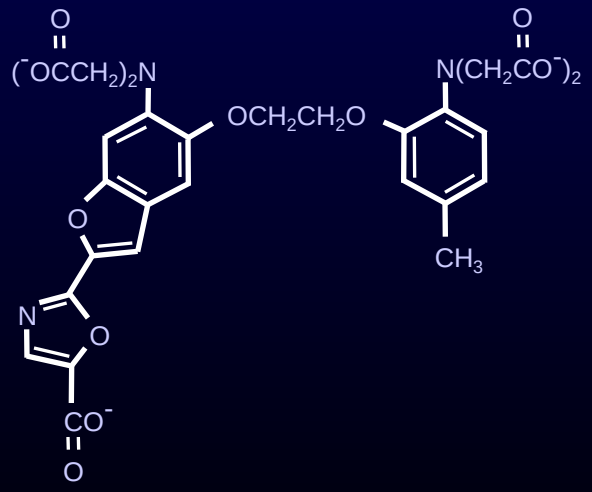


Probe entrance into the cell (2)

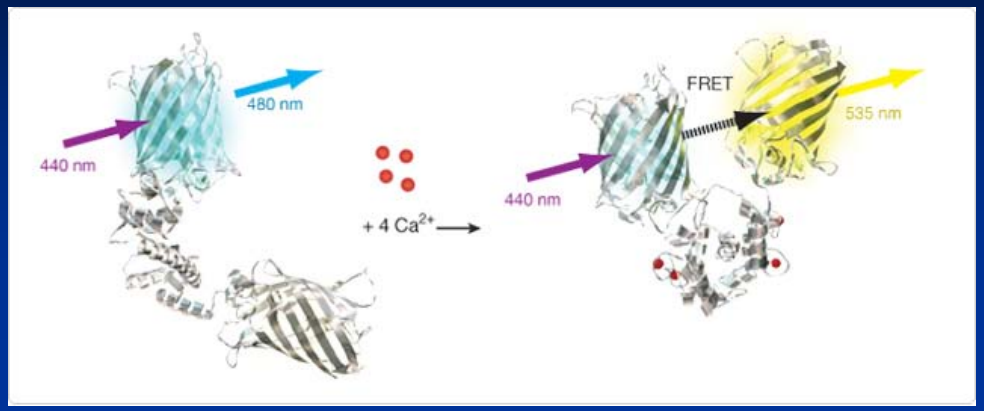
Ext

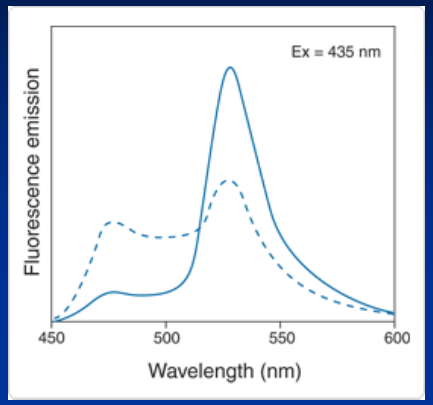


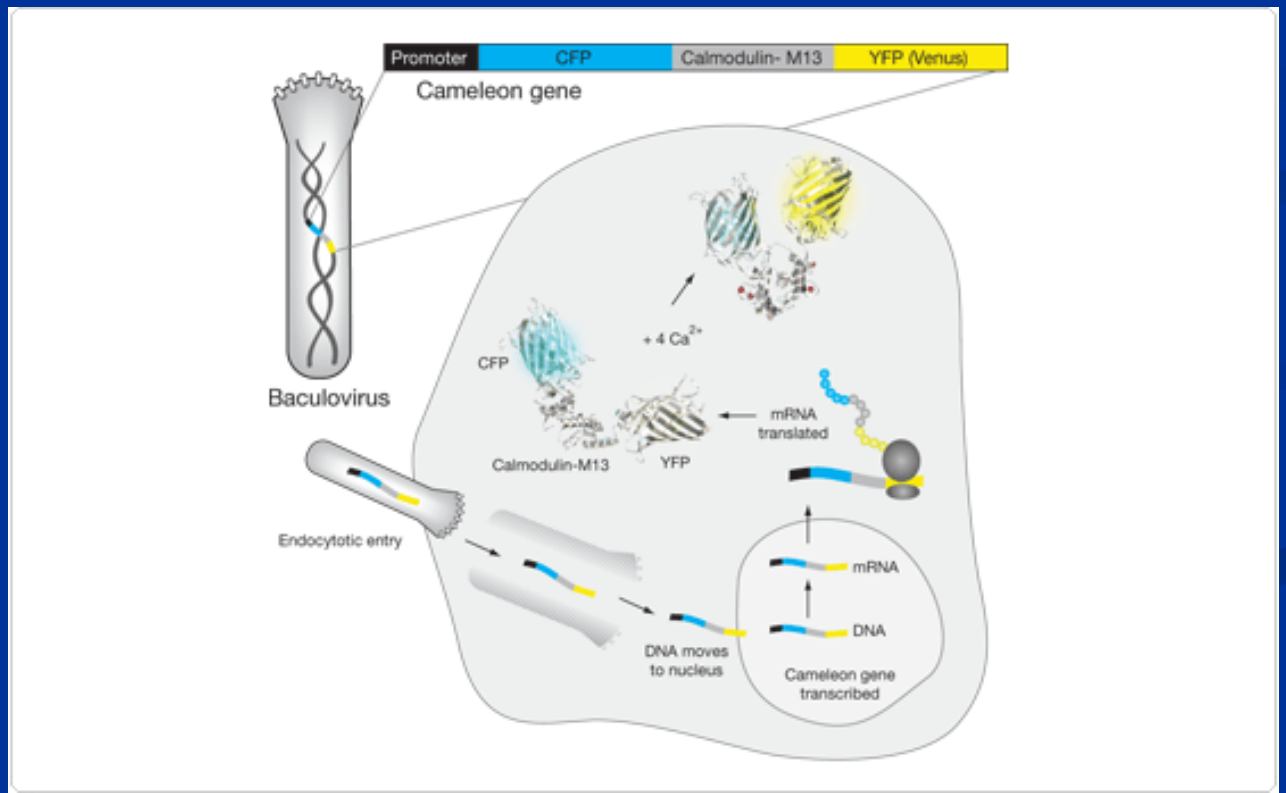
In



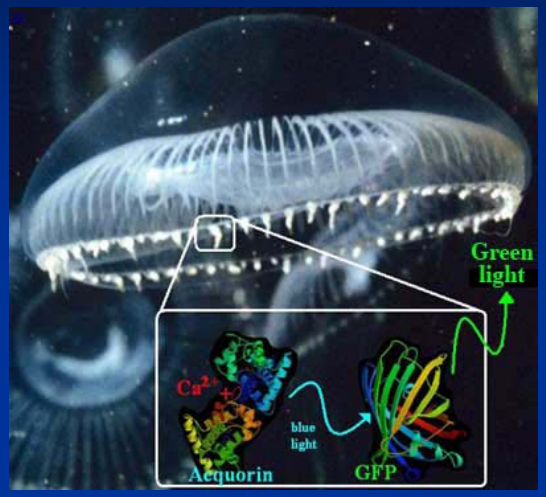
Ca^{2+}-sensitive proteins

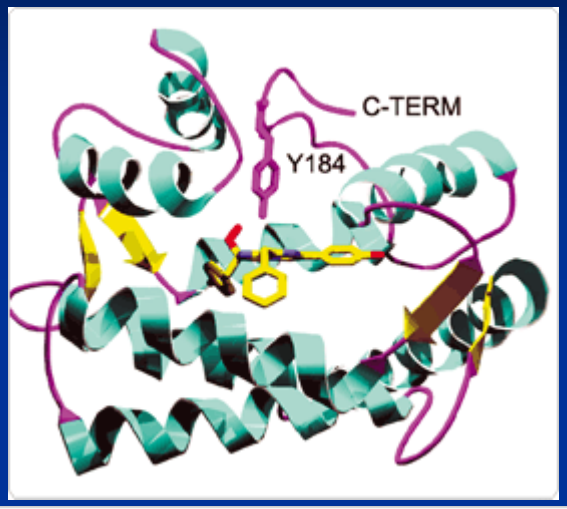


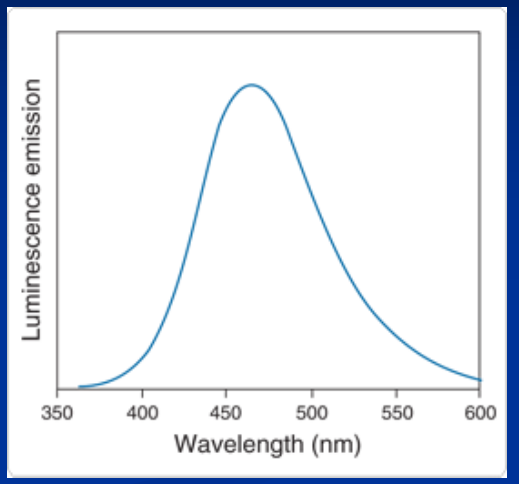


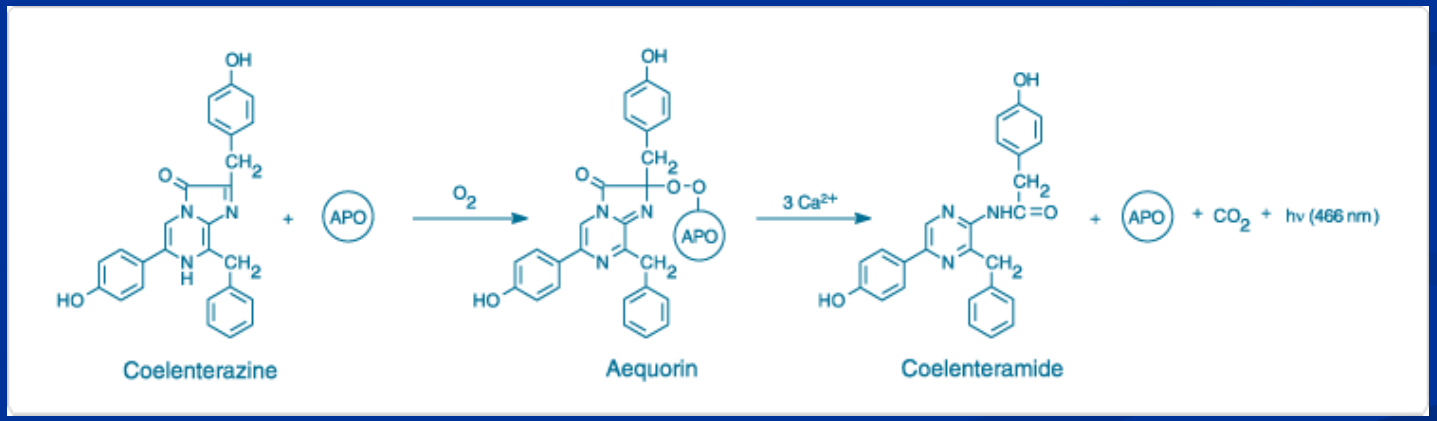


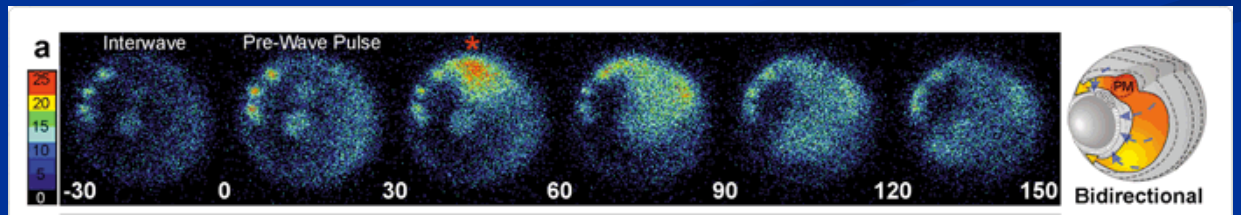
Aequorin


Green light
Ca^{2+}
blue light
Aequorin
GFP

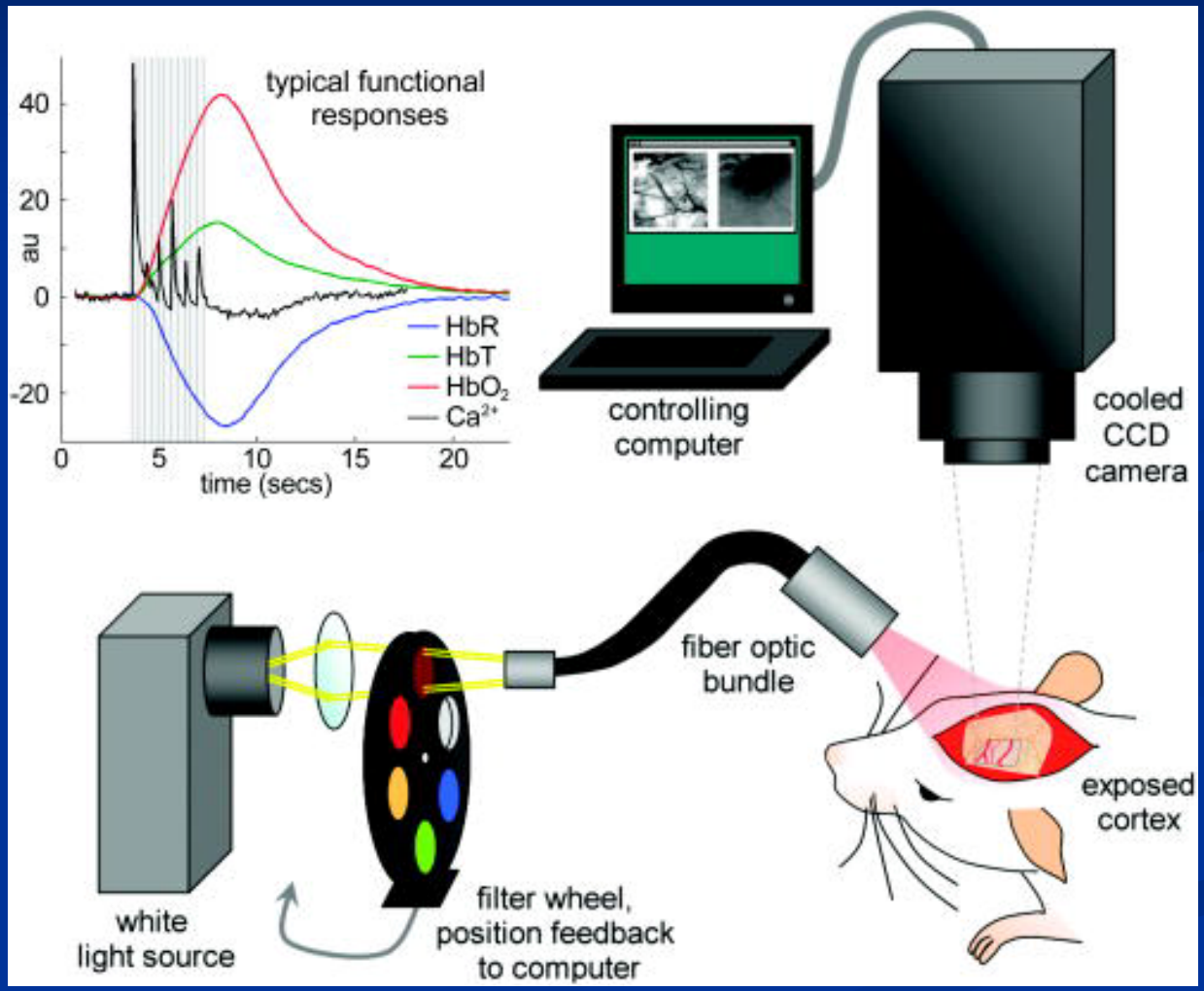

C-TERM
Y184


Luminescence emission
350
400
450
500
550
600
Wavelength (nm)


OH
CH_2
O
N
H
HO
APO
O_2
O-O
$3\ Ca^{2+}$
NHC=O
CO_2
hv (466 nm)
Coelenterazine
Aequorin
Coelenteramide


a
Interwave
Pre-Wave Pulse
25
20
15
10
5
0
-30
0
30
60
90
120
150
PM
Bidirectional

Videomicroscopy *in vivo*



Experimental study cases

Amyotrophic lateral sclerosis (ALS)

Lou Gehrig
(1903-1941)

- Late-onset neuromuscular disorder
- Death of large motor neurons in spinal cord and brainstem
- sALS - sporadic and fALS - familial (5-10%) forms.
- 15-20% familial ALS -> mutant form of Cu^{+2}/Zn^{+2} SOD (SOD1)
- Transgenic animal models – mice and rat with mutant hSOD1

Effect of ALS IgGs on astrocytes in culture

CASE 1

- Milena Milošević, *Centar za lasersku mikroskopiju*
- Robert Zorec, Matjaž Stenovec, *Institut za Patofiziologiju, Laboratorija za Neuroendokrinologiju-Molekularnu ćelijsku fiziologiju, Ljubljana, Slovenija*

- Zorica Stević, *Institut za Neurologiju, Klinički centar Srbije*
- Vladimir Petrušić, Ljiljana Dimitrijević, *Institut za viruslogiju, vakcine i serume -"Torlak", Srbija*

Humoralna imunost u ALS – poznati efekti ALS IgG

- Transfer to mouse induces degeneration of motor neurons, increase in calcium containing organelles (*Pullen et al. 2004*)
- Apoptotic cell death in hybrid motor neuron cell line (*Alexianu et al. 1994*) and human neuroblastoma cells (*Yi et al. 2000*)
- Activates caspase-3 pathway and induces selective apoptosis of neurons in rat mixed primary spinal cord cultures whereas astrocytes are less susceptible (*Demestre et al. 2005*)
- Increased P/Q type Ca^{2+} currents in Purkinje cells (*Llinas et al., 1993*), or decreased Ca^{2+} currents of cultured granule cells (*Zhainazarov et al., 1994*).
- Cultured hippocampal neurons: increased frequency, but not amplitude of the spontaneous and miniature glutamatergic currents; partly independent of external Ca^{2+} (*Andjus et al. 1997*)
- Modulate calcium transients (*Andjus et al., 1996*)
- Enhance mobility of acidic vesicles in cultured astrocytes (*Stenovec et al., 2011*) by affecting calcium homeostasis

M&M

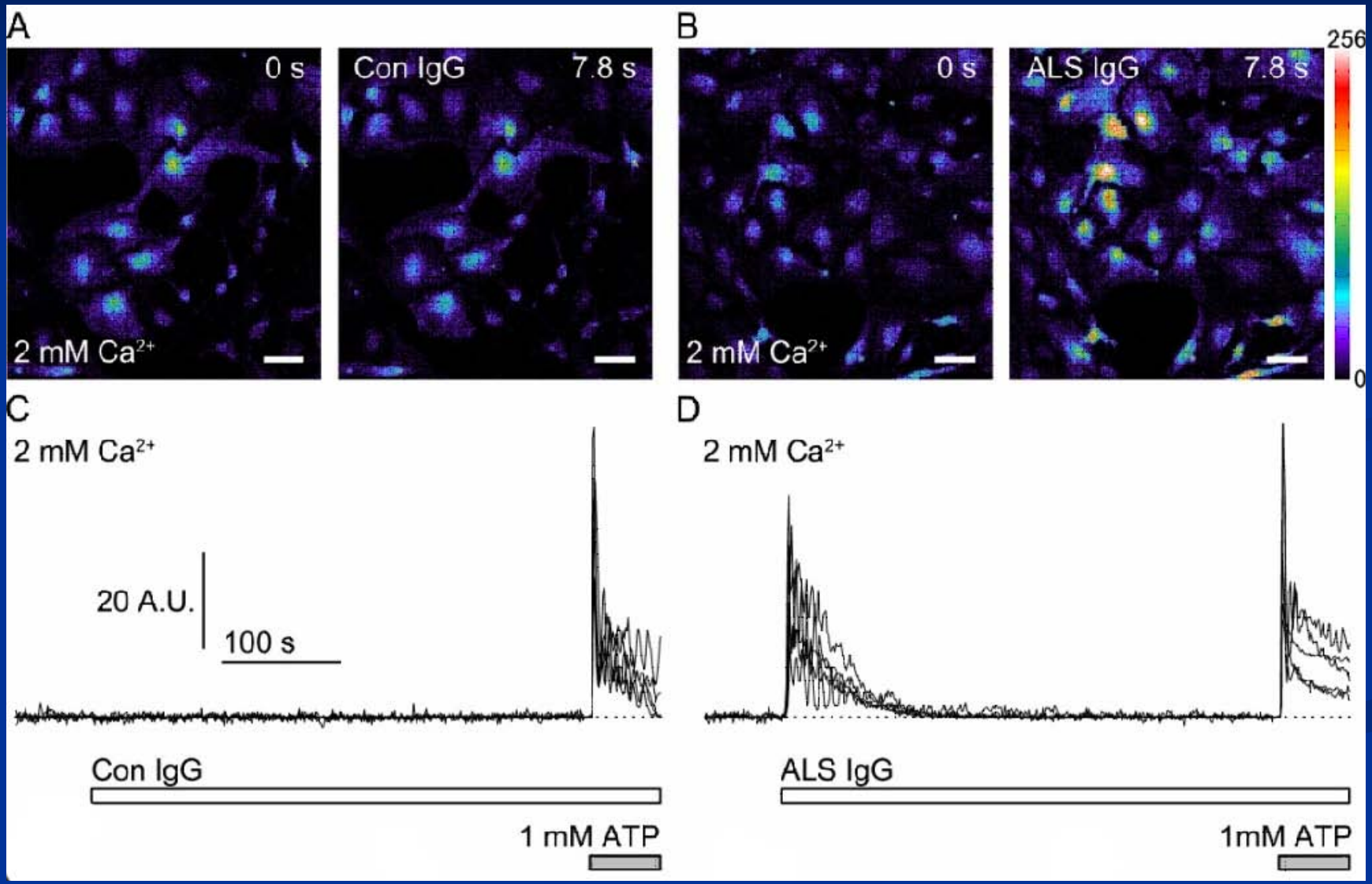
- Primary cortical astrocytic culture from Wistar P2
- Laser scanning confocal microscopy of astrocytes loaded with fluo-calcium indicators Fluo-3 AM and Fluo-4 AM
- Relative change in intracellular calcium concentration calculated from the absolute change in the indicator's intensity normalized by the basal fluorescence:

$$I_t = \frac{\Delta F}{F_0} = \frac{F_t - F_0}{F_0}$$

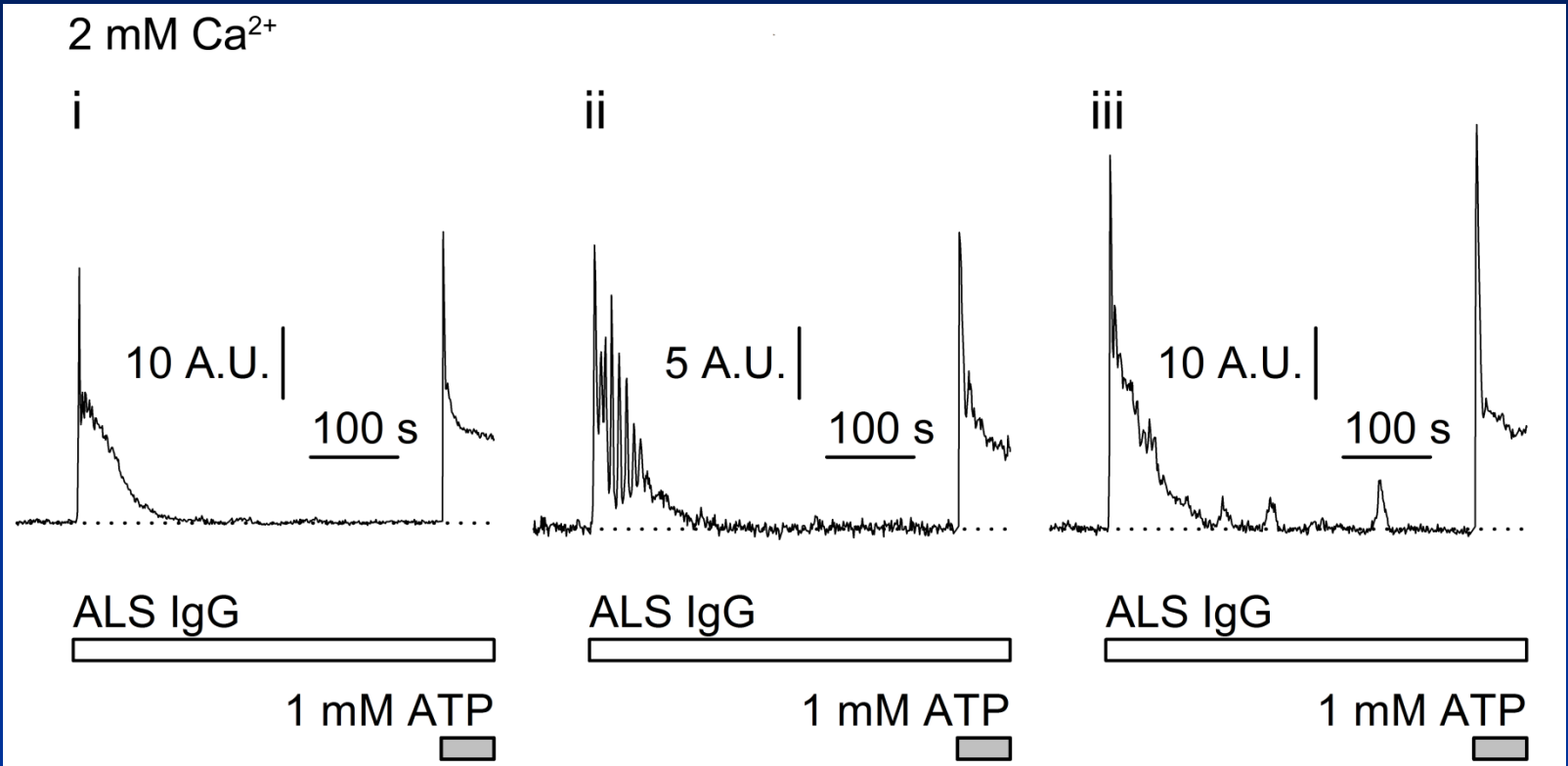
- Signal parameters: peak amplitude, time to peak, and time integral

$$SIgG = \sum_{i=s}^{e} \left(I_i \times \Delta t \right)$$

ALS IgGs evoke calcium transients in cultured rat astrocytes

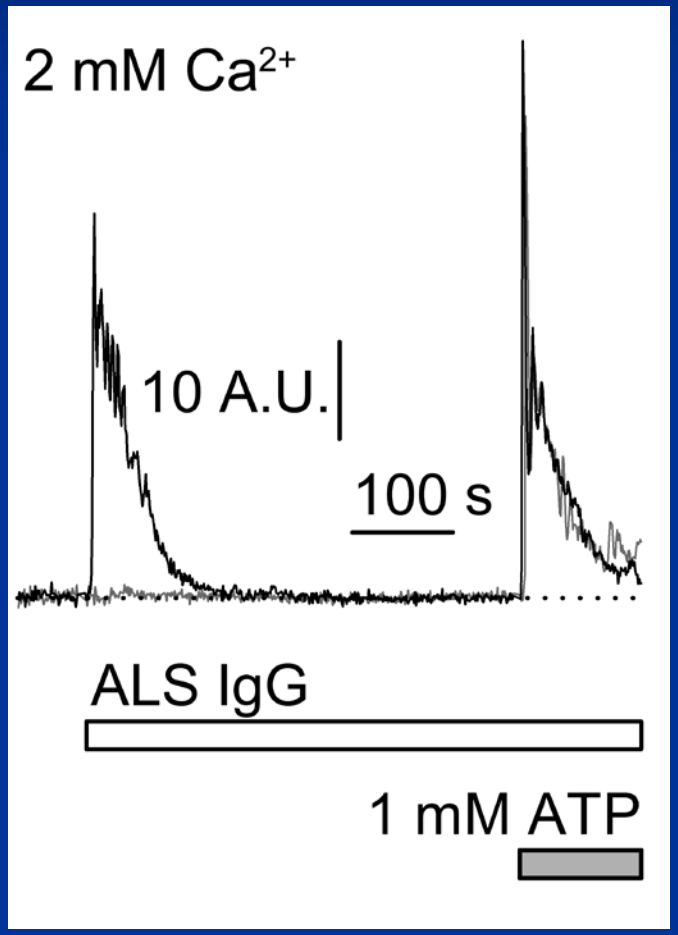


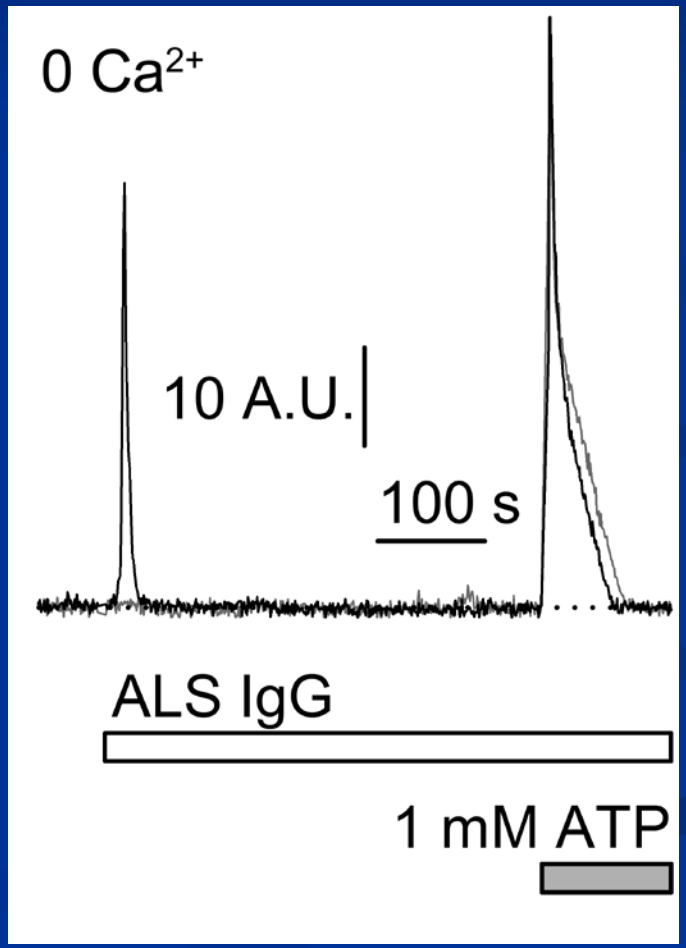
Various types of transients



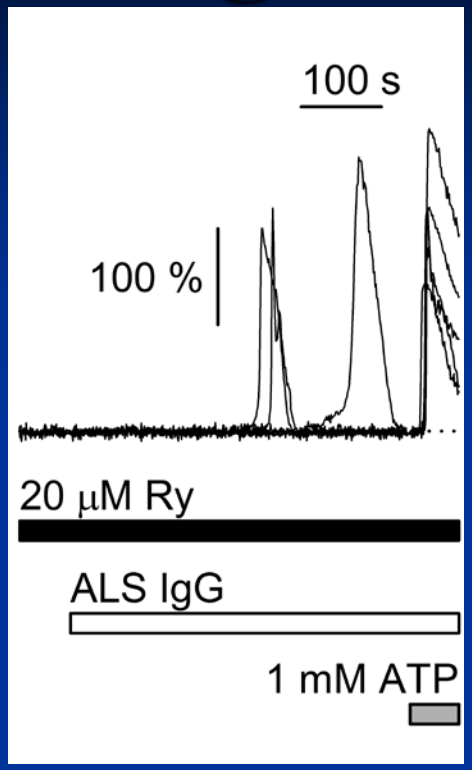
(i) single transients
(ii) high frequency bursts
(iii) repetitive calcium transients with variable peak amplitude

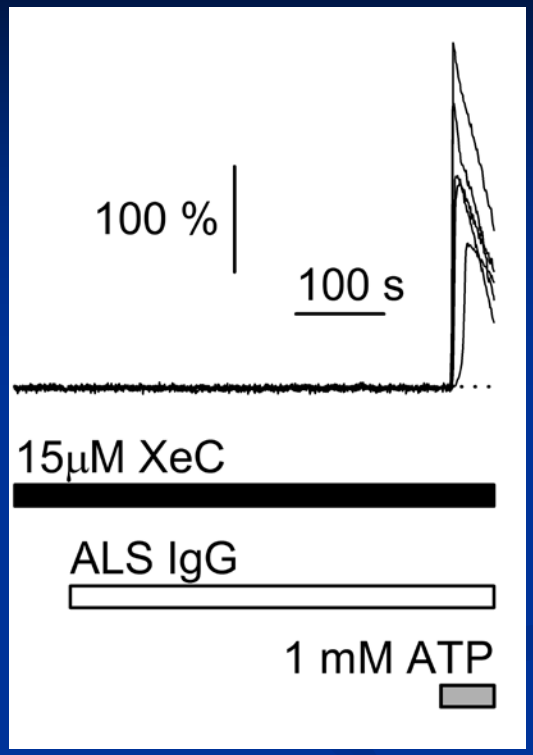
Extracellular Ca^{2+} shapes calcium transients

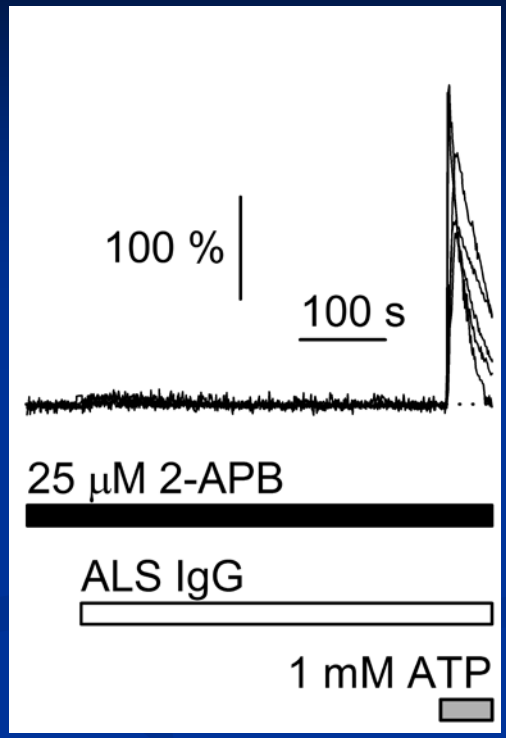


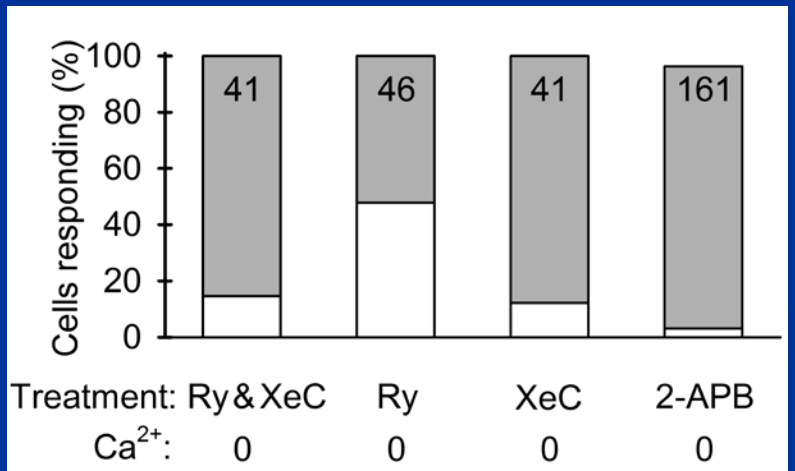


Uloga intracelularnih depoa Ca^{2+}



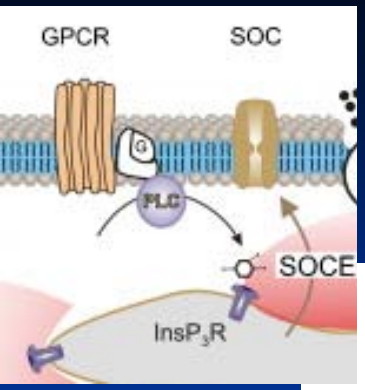




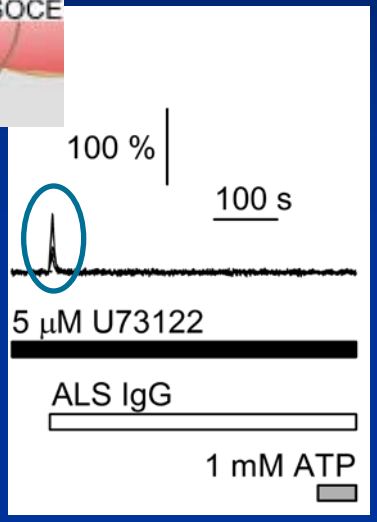


Calcium transients are abolished by selective blockers of **IP3-sensitive** but not of **Ryanodine-sensitive** receptors on the endoplasmic reticulum membrane

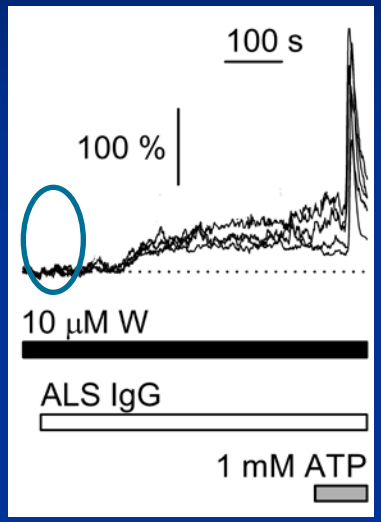
Enzymes and/or plasma membrane structures involved in ALS IgG-evoked calcium transients

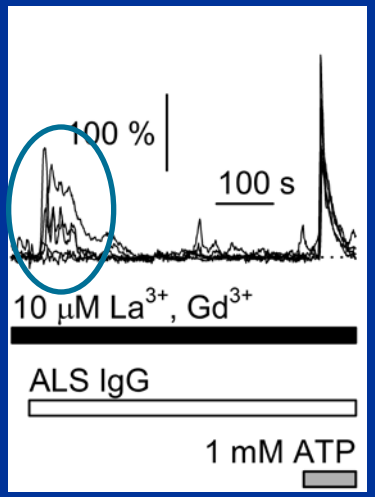


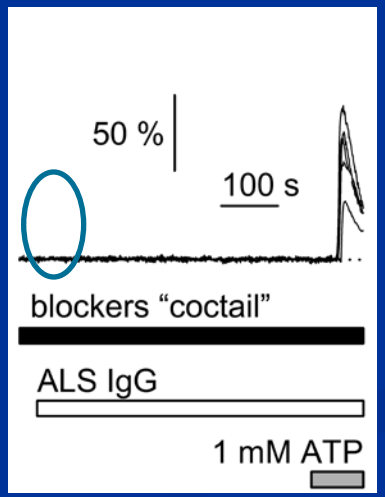
@PLipazaC



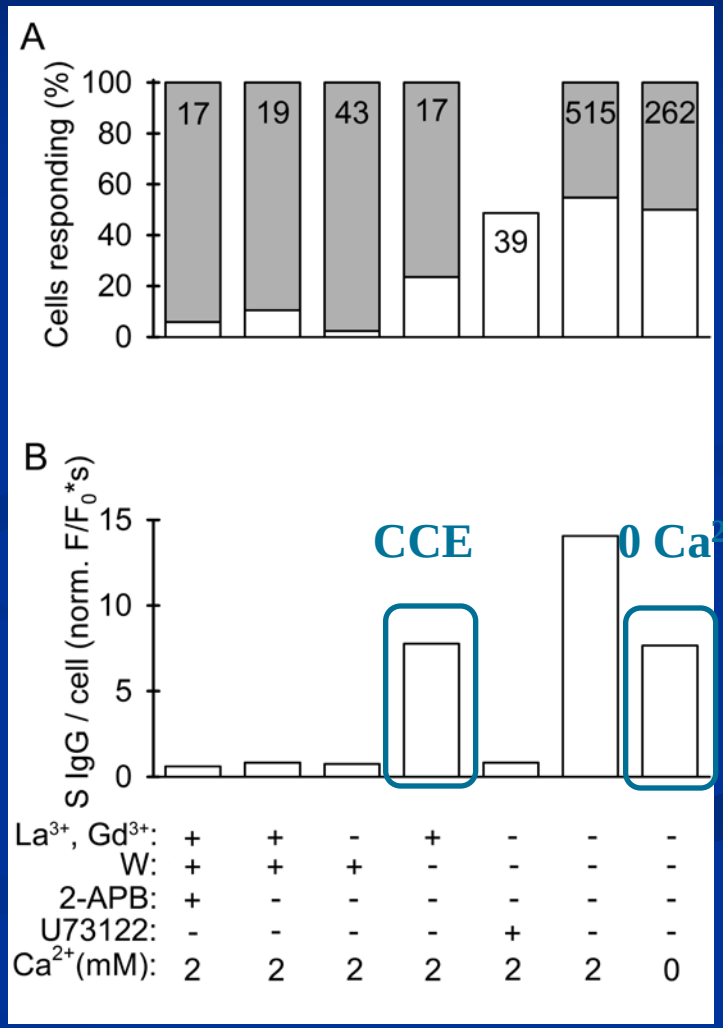
@IP3Kinase







@SOCE-memb. ch.



CONCLUSION 1

- IgG from sALS patients, but not from non-ALS controls, evoked complex calcium transients in ~50% of treated astrocytes
- The probability to evoke calcium transients by ALS IgG did not dependent on extracellular calcium
- ~60% of calcium involved in these responses originates from intracellular organelles, while the remaining ~40% of calcium originates from the extracellular space
- ALS IgG-evoked Ca^{2+} transients depend on IP_3R, while RyR is not involved
- The influx of extracellular calcium through SOCE channels prolongs the responses.
- Inhibition of PLC diminishes, while the inhibition of PI3K completely prevents ALS IgG evoked calcium response.

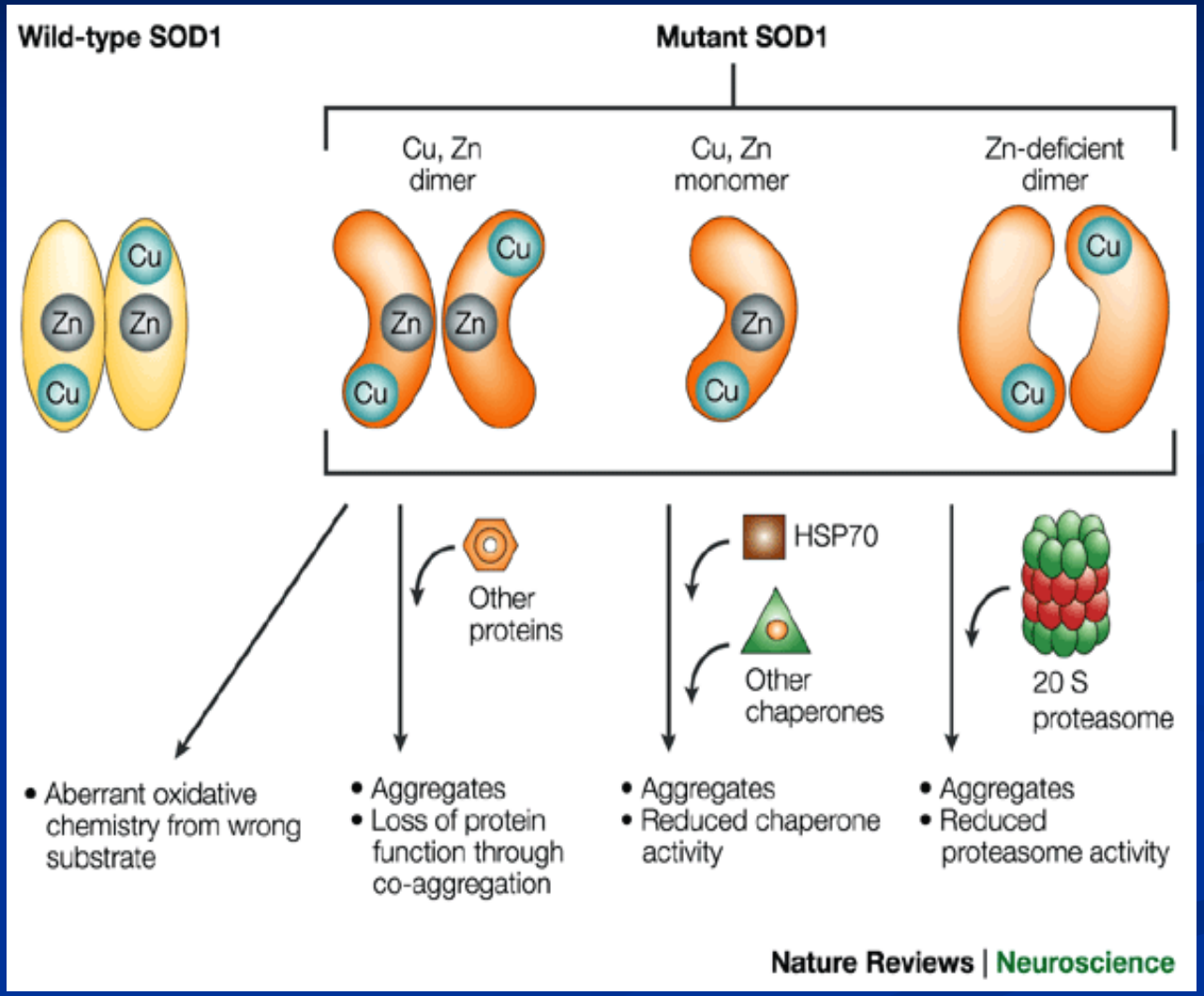
ALS IgG affect calcium homeostatic system in astrocytes by IP_3 mediated calcium release from the endoplasmic reticulum and entry of extracellular calcium through SOCE channels, with the activation of PI3K upstream of PLC.

Effects exogenous mutant SOD1

CASE 2

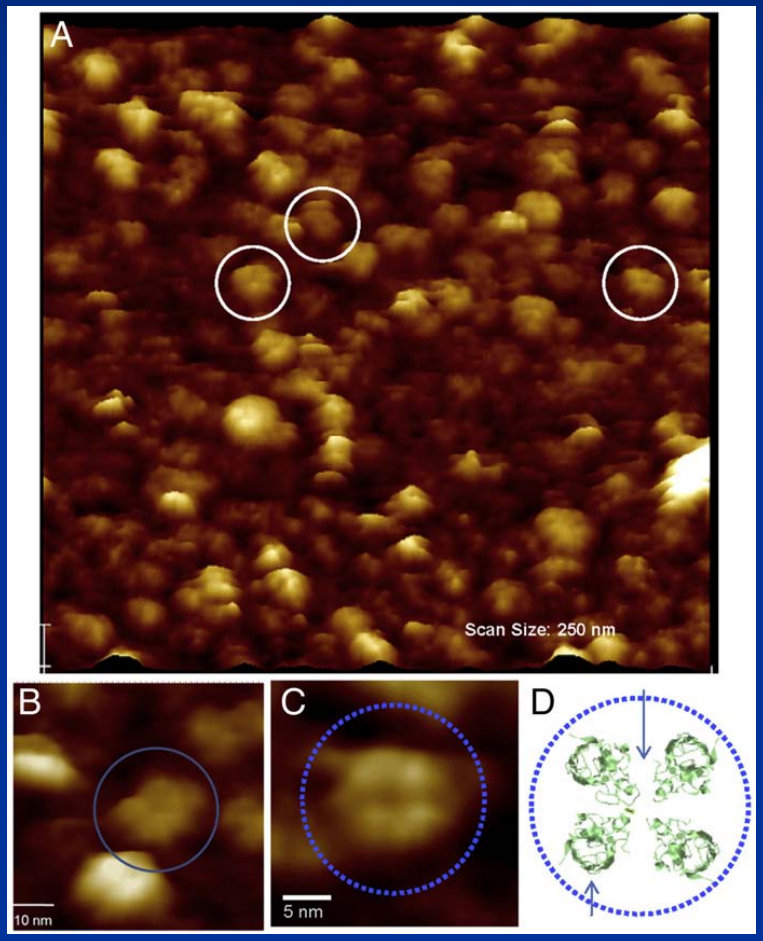
- Aleksandar Bajić, Milena Milošević, Danijela Bataveljić *Centar za lasersku mikroskopiju*
- Ljilja Nikolić *Institut za biološka istraživanja "Siniša Stanković"*
- Jean Pierre Julien *Department of Psychiatry and Neuroscience of Laval University, Quebec*

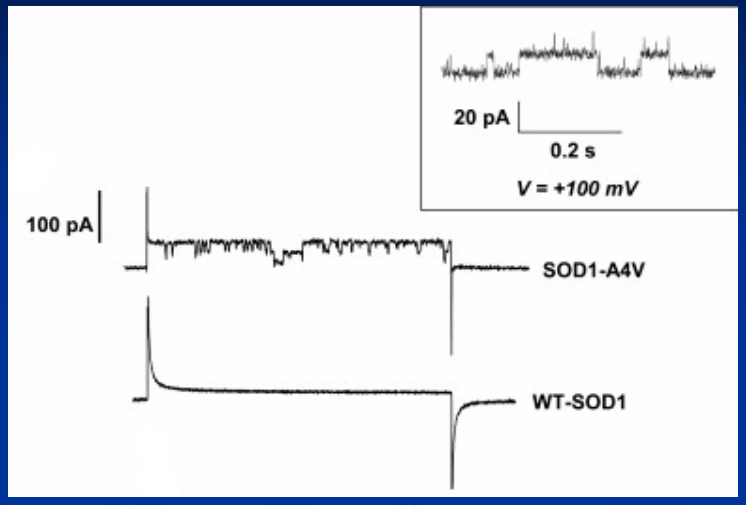
Mutant SOD1

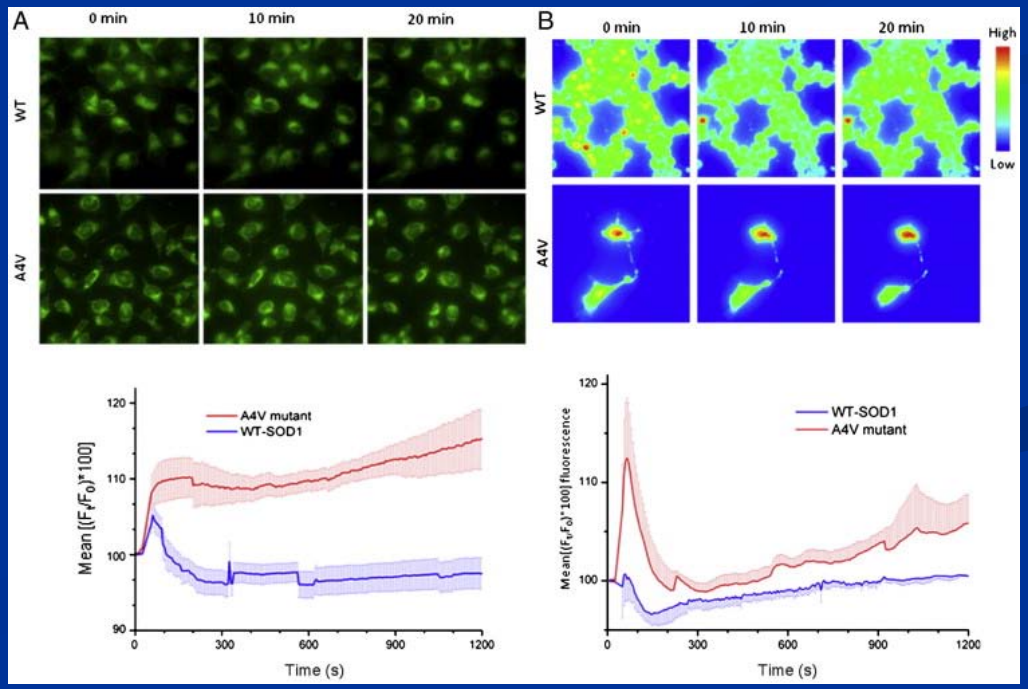

Wild-type SOD1
Mutant SOD1
Cu, Zn dimer
Cu, Zn monomer
Zn-deficient dimer
Cu
Zn
Other proteins
HSP70
Other chaperones
20 S proteasome
• Aberrant oxidative chemistry from wrong substrate
• Aggregates
• Loss of protein function through co-aggregation
• Aggregates
• Reduced chaperone activity
• Aggregates
• Reduced proteasome activity
Nature Reviews | Neuroscience

Exogenous mSOD forms tetrameric structures that are incorporated in lipid bilayers and from pores

Allen et al. 2011



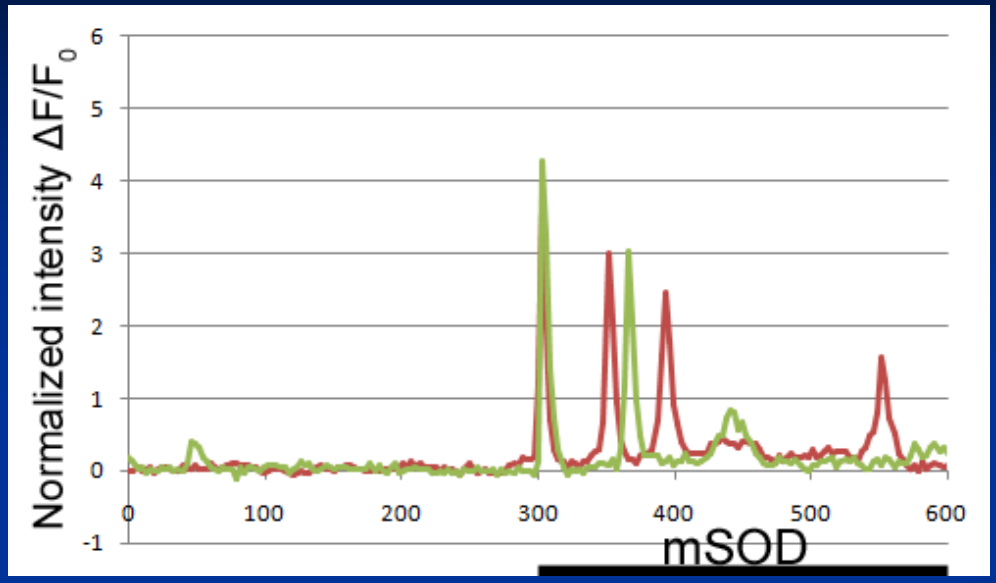


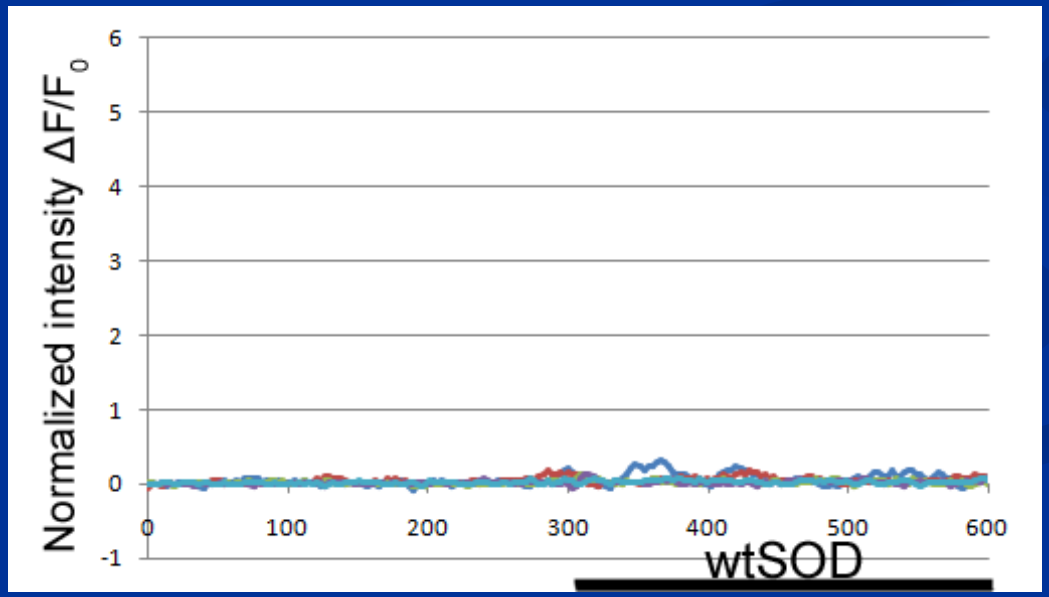


M&M

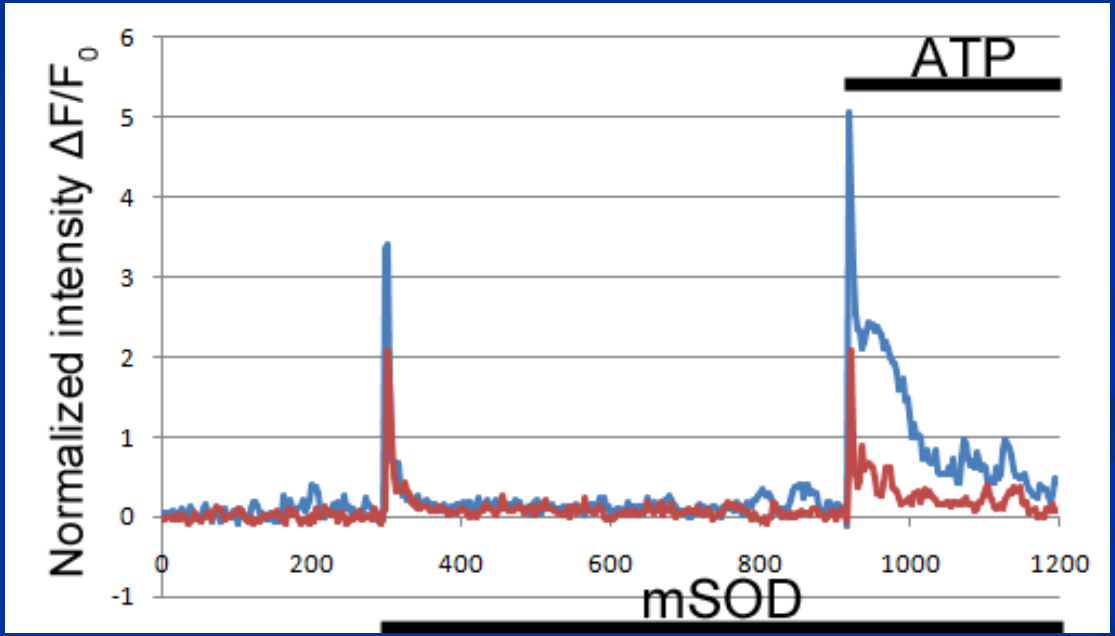
- Astrocyte cultures from postnatal 2d Wistar rats
- Laser scanning confocal microscopy with **Fluo-4 AM**
- Aplication of metalised (Cu, Zn) recombinant mSOD1 i wtSOD1 (Jean Pierre Julien, Université Laval, Quebec)
- Measured parameters: percent if cells with change in intracellular calcium and time integral of transients

mSOD *vs* wtSOD – Ca^{2+} oscillations

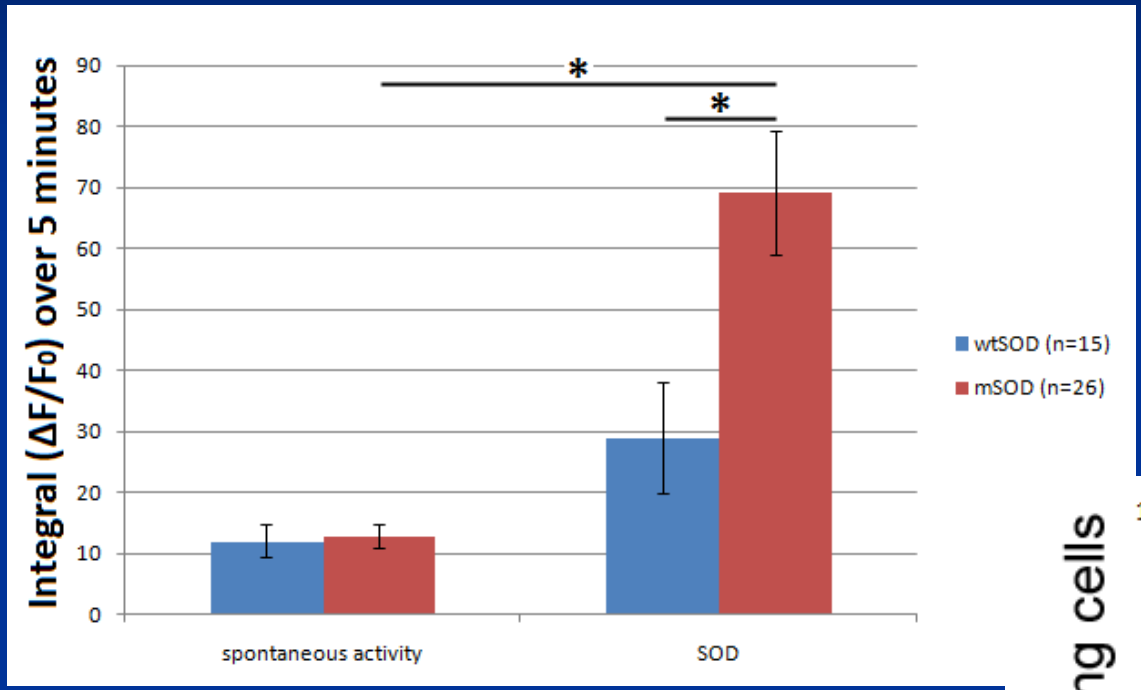


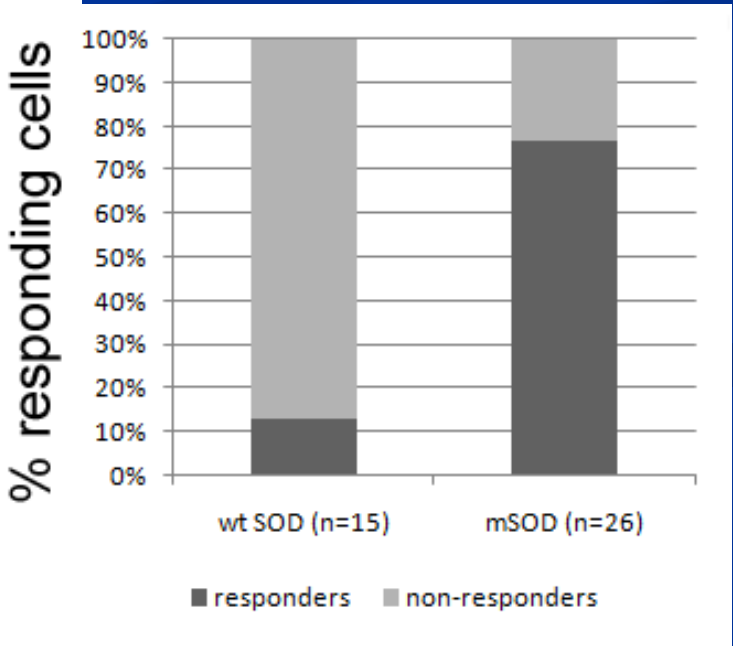


Acute Ca^{2+} response

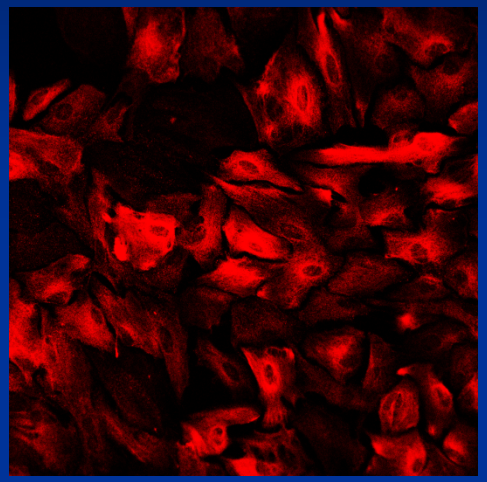


Data summary





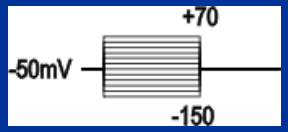
Electrophysiological exploration of SOD1 effect on astrocytes

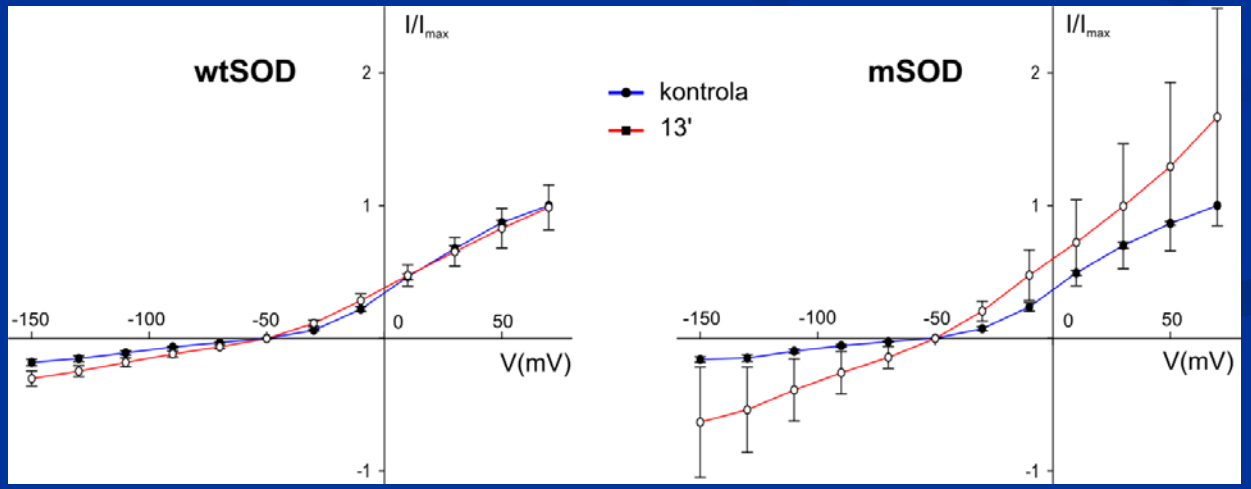


Imunocitohemija astrocita u kulturi

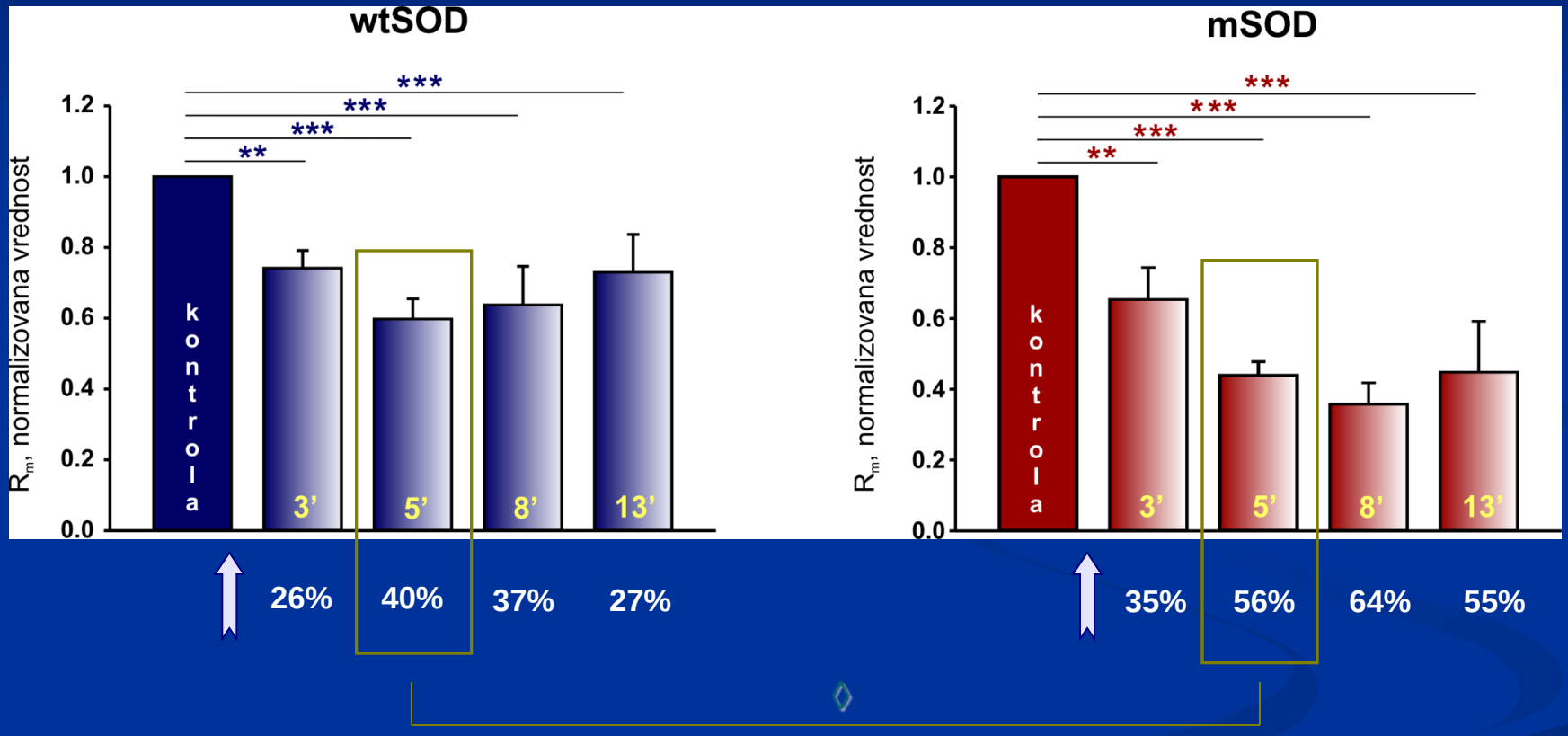
Eksploratorna elektorda

Astrocit u kulturi

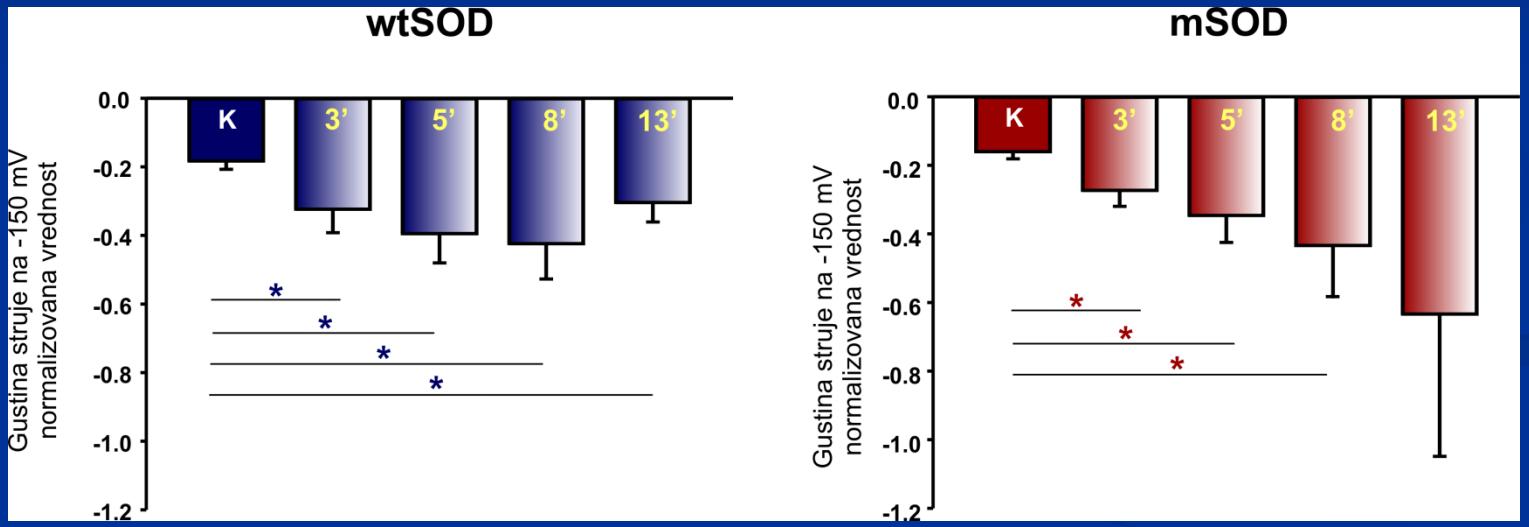




Change in membrane resistance, R_m



Change in current density at -150 mV



Thanx!

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