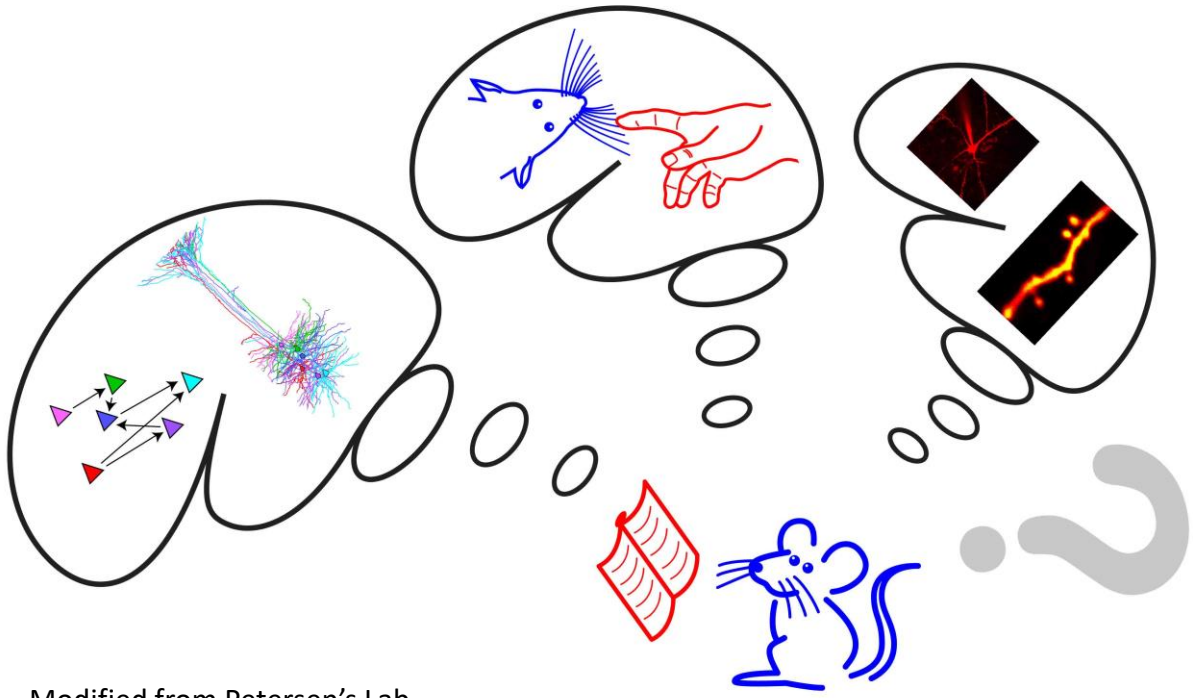
SAPIENZA
UNIVERSITÀ DI ROMA

Techniques in Neurosciences

Rocco Pizzarelli

20-05-2019

Hierarchical organization of the brain



Modified from Petersen's Lab

Synapses allow neuronal communication

Neurons are organized to form circuits

Circuits interaction is responsible for behavior

Techniques in Neurosciences research

- ✓ In the last years many molecular, optical and electrophysiological methodologies have been developed
- ✓ These new techniques fostered the study of neuronal circuits
- ✓ By combining new tools with behavioral studies **System Neurosciences** is unraveling brain functions

Electrophysiology

✓ The first experimental evidences of electrical activity go back to the experiments performed by Luigi Galvani

✓ Soon after many improvements have been introduced in the electrophysiological methodologies

✓ Electrophysiology reached the actual form with the scientific works of Bert Sakmann & Erwin Neher

Erwin Neher & Bert Sakmann

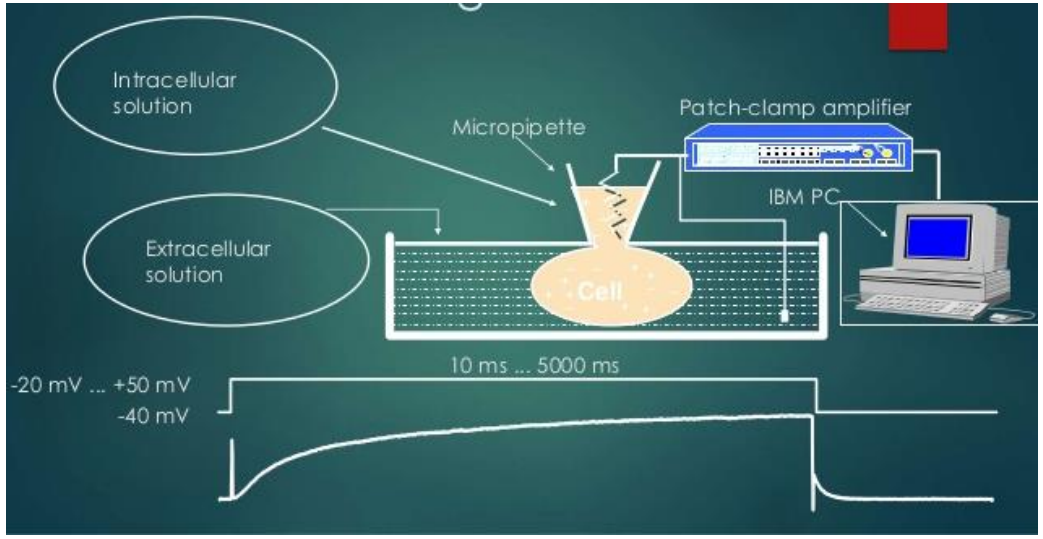
What is Electrophysiology good for?

Whit electrophysiology techniques is possible to monitor excitable cells activity

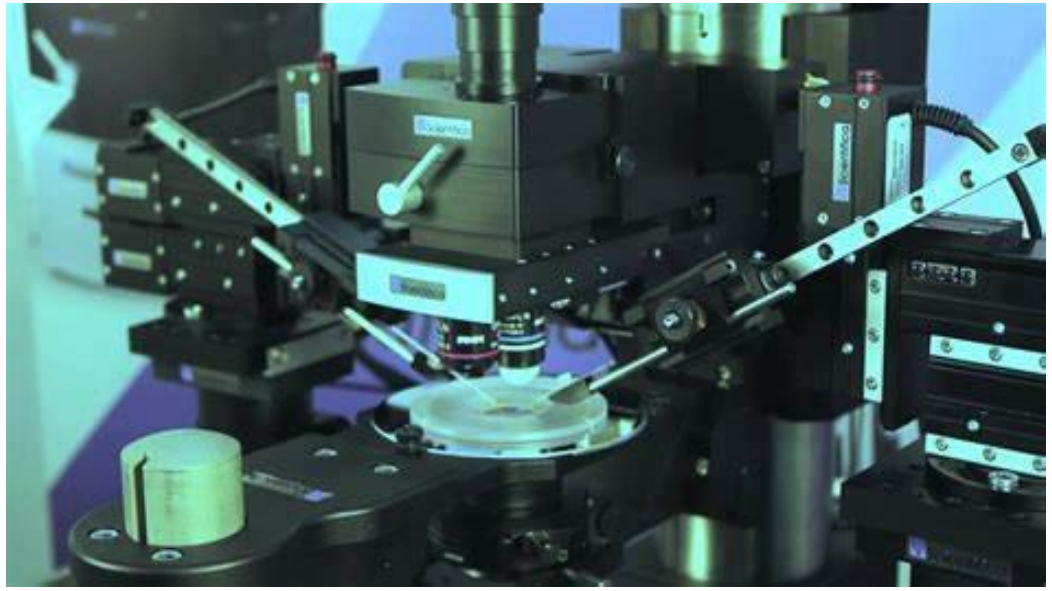
This techniques enhanced our comprehension of

- Ion channels and receptors functioning
- Synaptic transmission
- Large scale neuronal activity

How does it work?



Slideshare.net

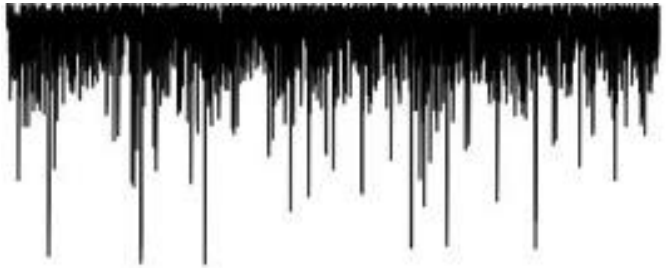


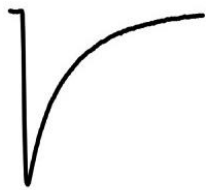
Some examples….

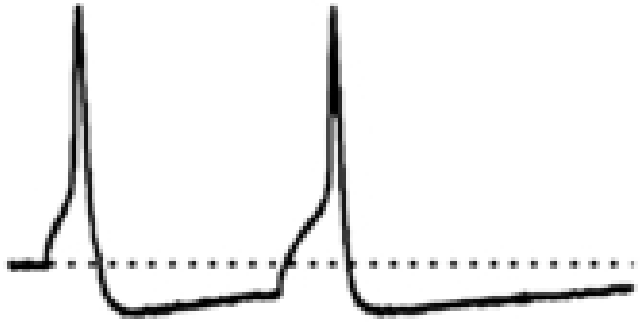
Cortical pyramidal neurons

Hugh Robinson
Cambridge

Synaptic currents





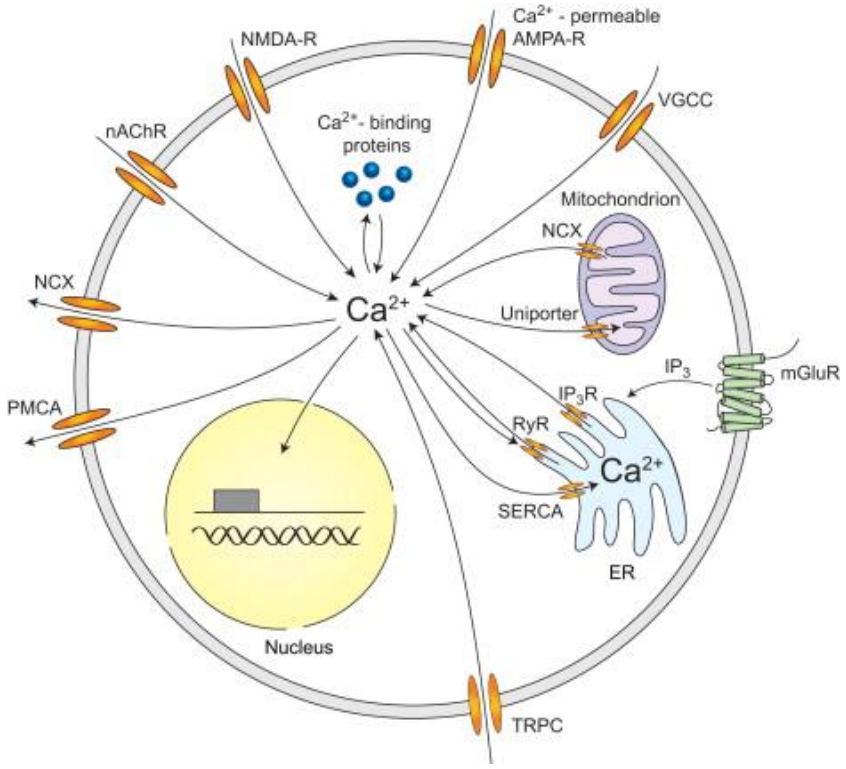


Action potentials

Calcium imaging

- ✓ Very often electrophysiology is performed together with calcium imaging
- ✓ This allow to link neuronal electrical activity with intracellular biochemical pathways
- ✓ Over the years calcium imaging has become more and more refined

✓ Calcium (Ca^{++}) is a very versatile ion involved in nearly every cellular function

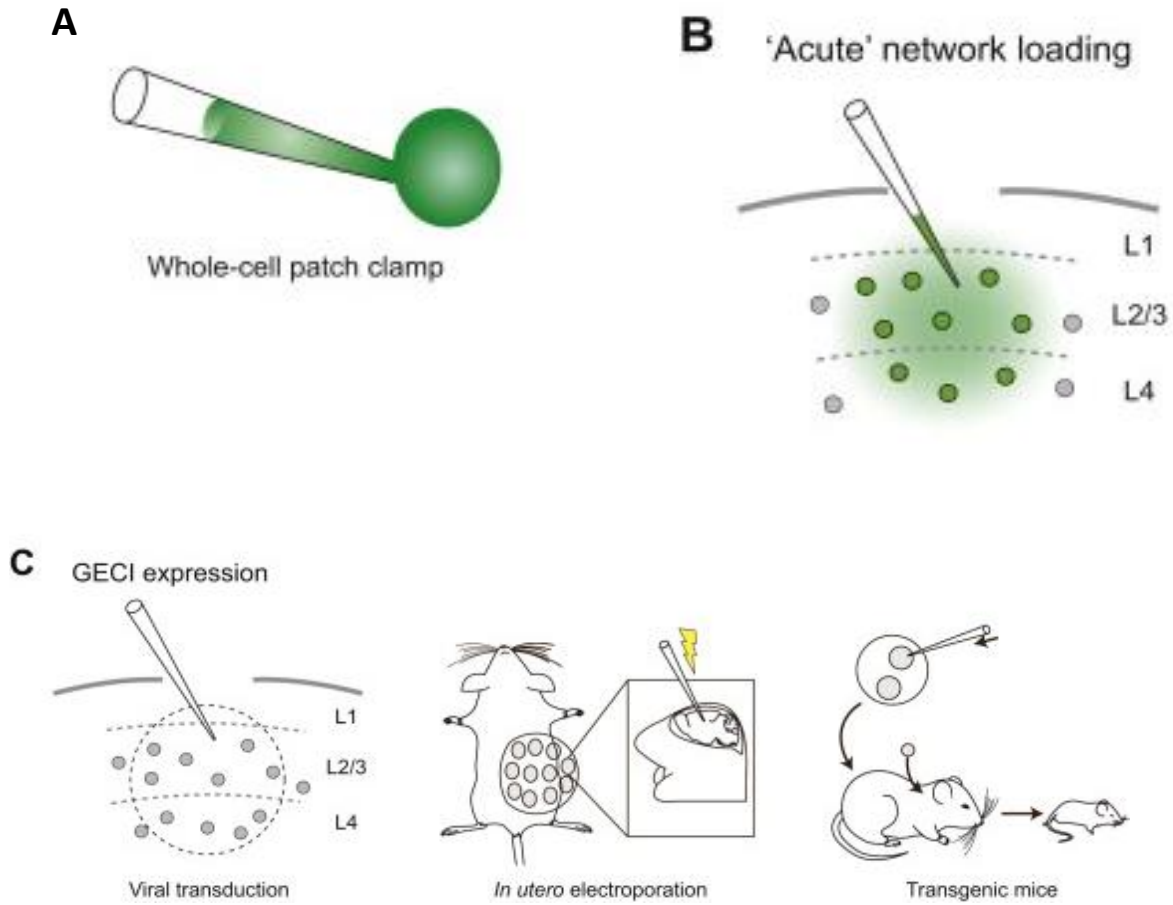


✓ Obtaining information about Ca^{++} dynamics is useful for the understanding of cellular processes

Grienberg & Konnerth 2011

In order to be visualized Ca^{++} has to be bound to fluorescent probes

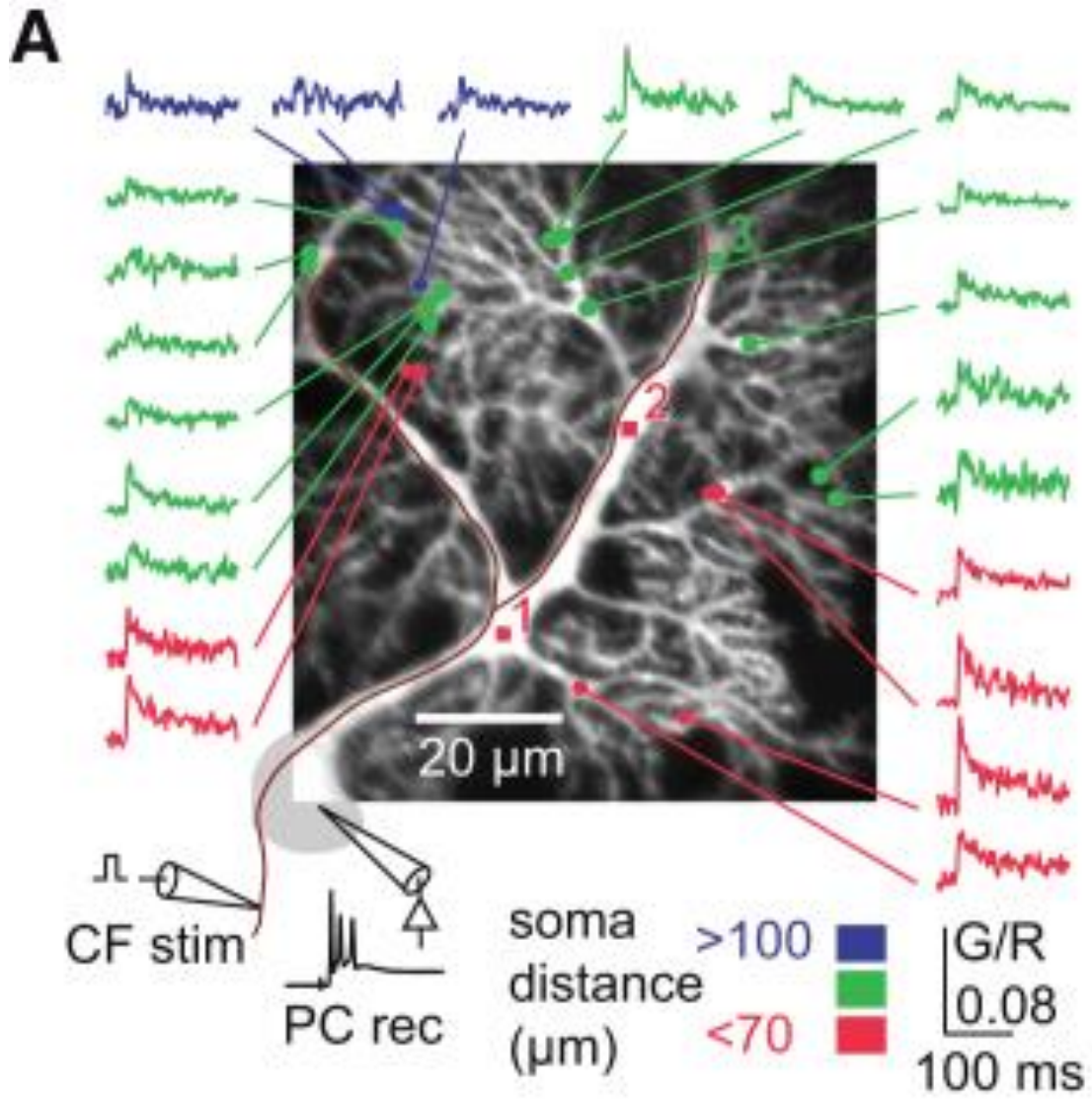
Different ways of loading Calcium indicators



GECI= genetically expressed Ca++ indicators

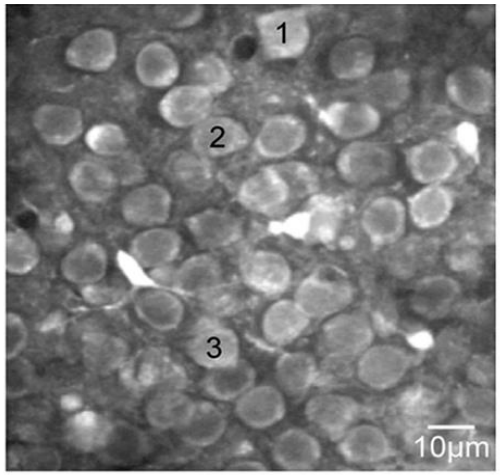
Grienberg & Konnerth 2011

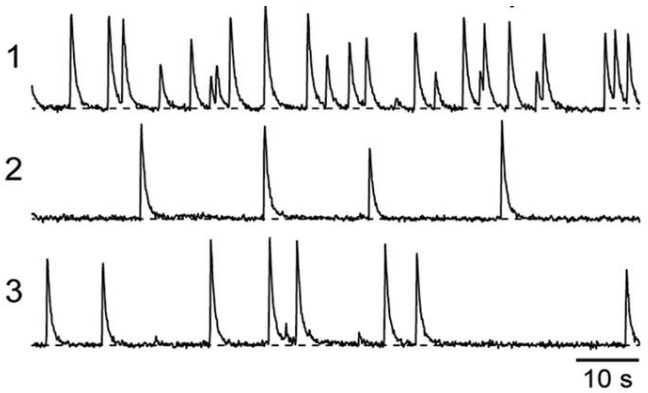
Single cell calcium dynamic

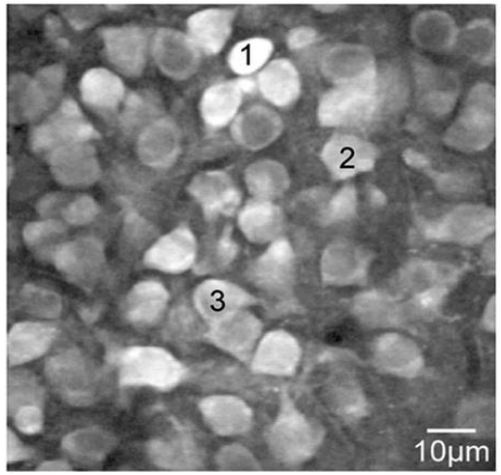


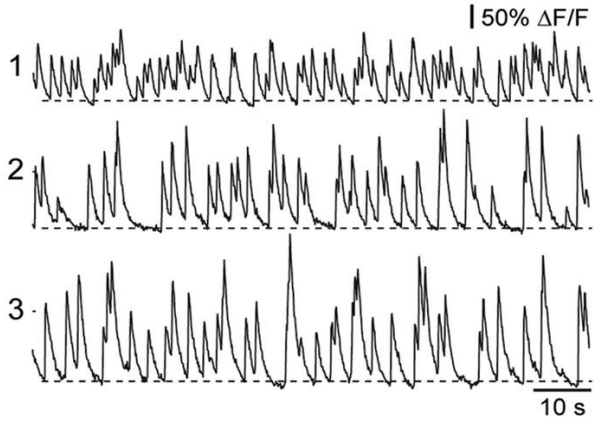
Otsu et al., 2014

Neuronal network calcium activity





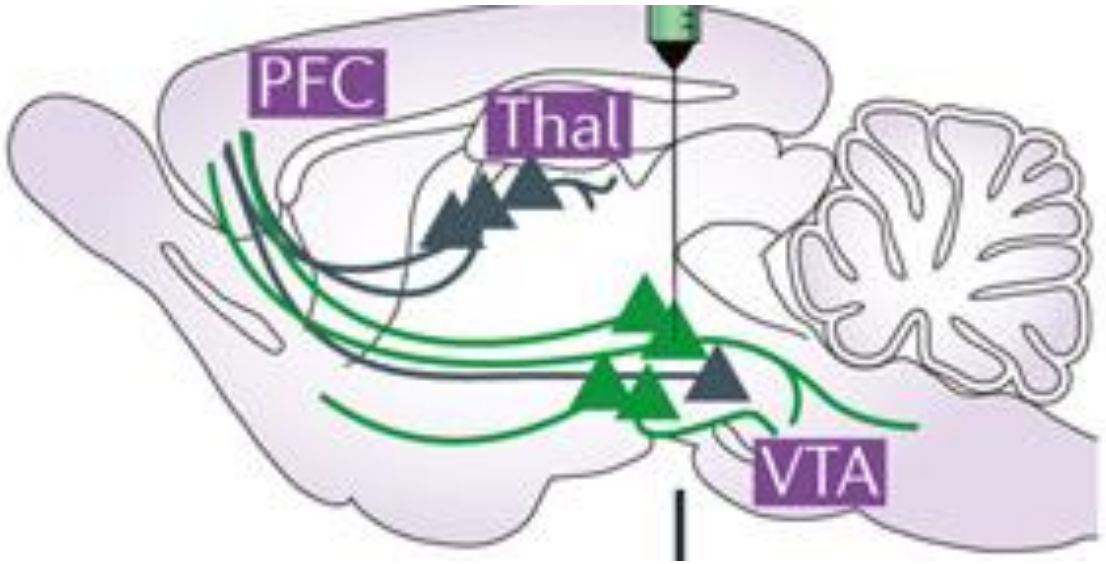




Busche 2018

Combining Electrophysiology with Ca^{2+} imaging is a very powerful approach but there are some limitations ……

……. it is nearly impossible to record from neurons in different brain areas



PFC= Prefrontal cortex

Thal= Thalamus

VTA= Ventral tegment Area

Optogenetic

- ✓ The term Optogenetics indicates the synergistic combination of genetic and optical methods

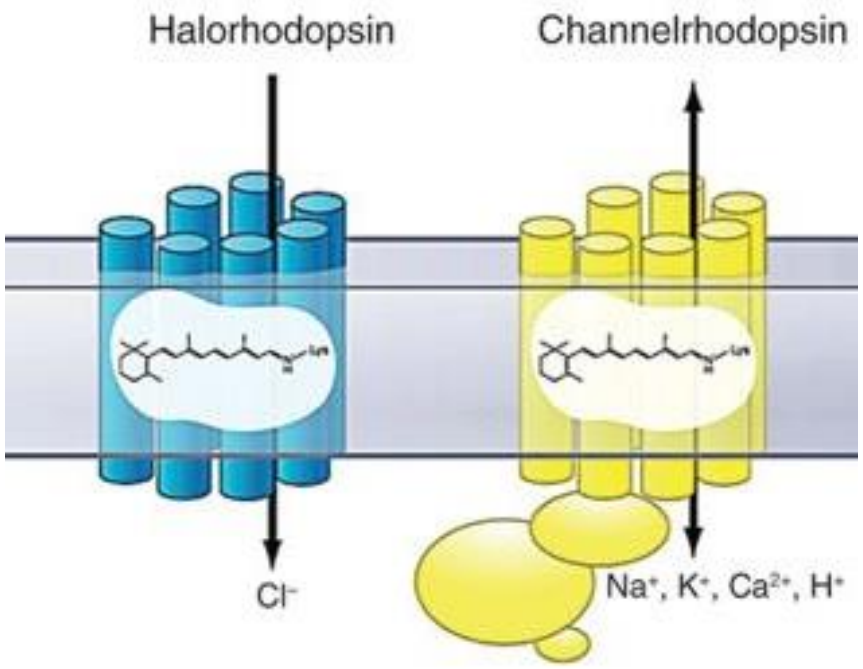
- ✓ This technology, allows to study the causal role between neural circuit and behavior and requires 3 main steps:

- ✓ **Microbial opsins-** proteins that directly when stimulated with lights elicits electrical current across cellular membranes

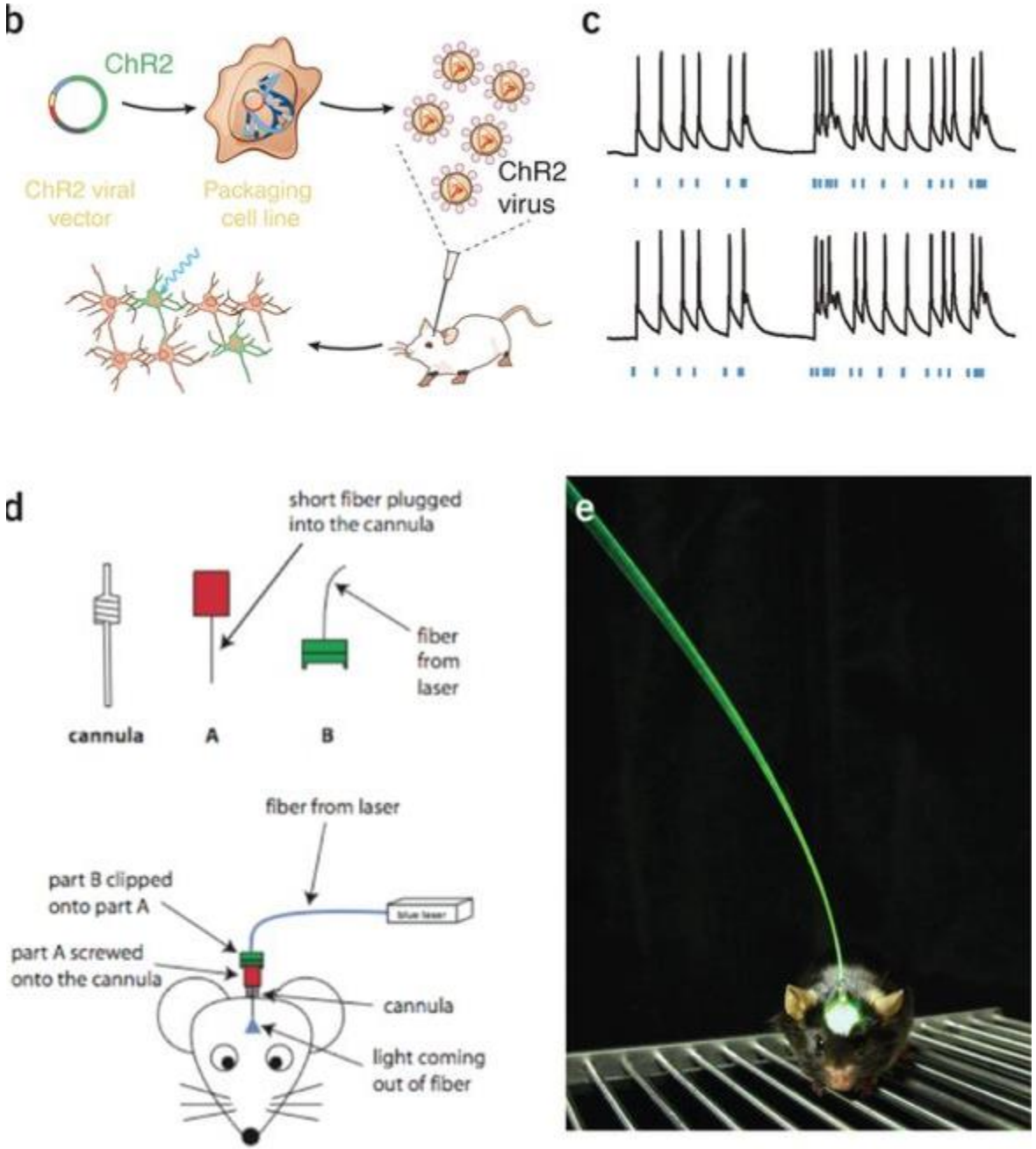
- ✓ **Expression** of specific opsin into well-defined cellular elements in the brain,

- ✓ **Target light** to specific brain regions

Bacterial opsin

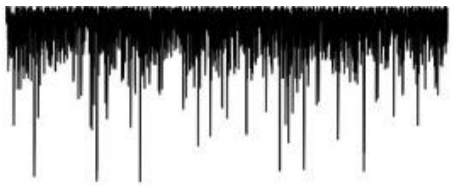


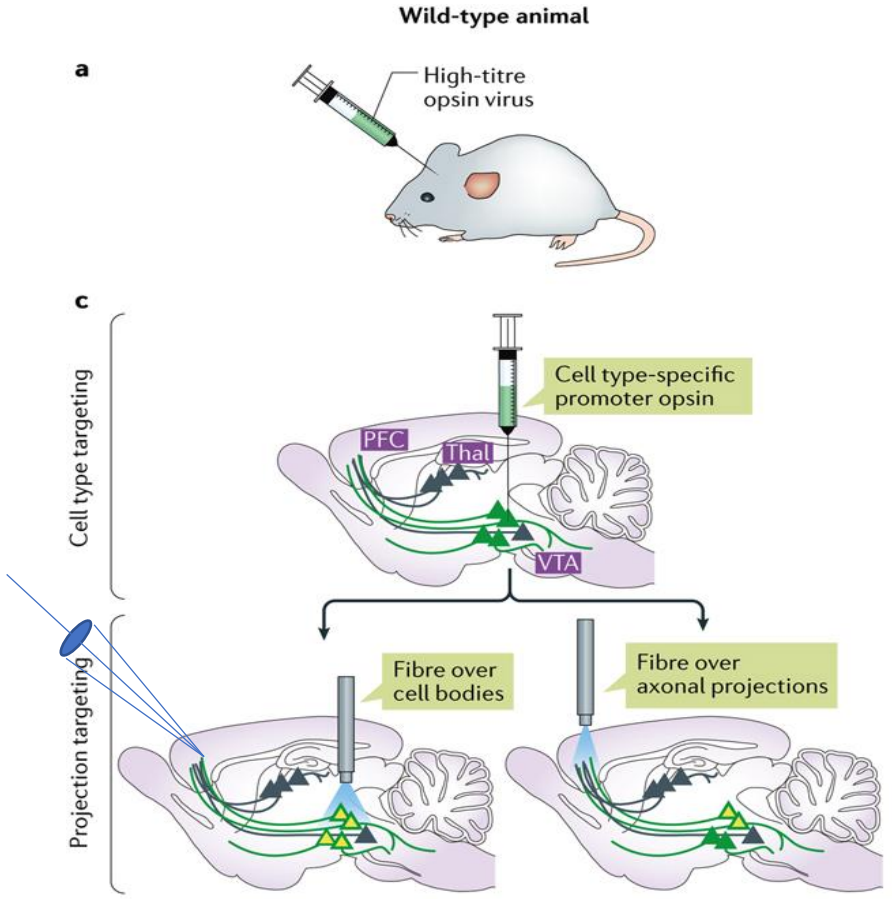
Deisseroth K, 2015



Deisseroth K, 2015

Stimulation and recording from different brain region





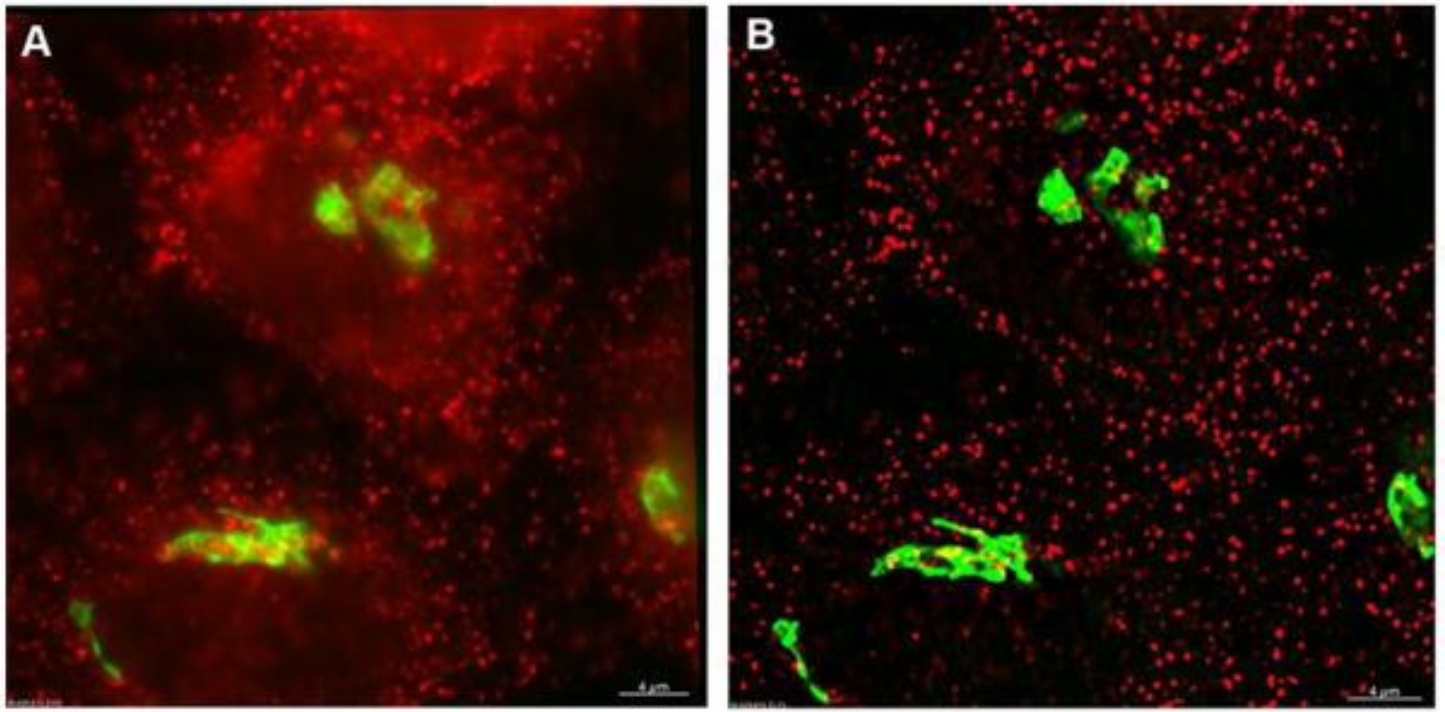
mod. from Deisseroth K, 2015

New imaging methods

- ✓ Conventional confocal microscopy has a limited resolution of ~ 250 nm
- ✓ Unfortunatly many protein complexes and cellular structures have a much smaller dimension
- ✓ In the mid 2000s scientists developed new techniques that allows a resolution of ~ 100 nm
- ✓ These techniques go under the general name of SUPER-RESOLUTION MICROSCOPY

- ✓ New imaging methods have enhanced our understanding of the neuronal molecular organization
- ✓ It is possible now to describe some neuronal mechanisms on a quantitative scale
- ✓ It is possible to look at cellular re-arrangemet *in vivo* in real time

Confocal *vs* Super-resolution microscopy



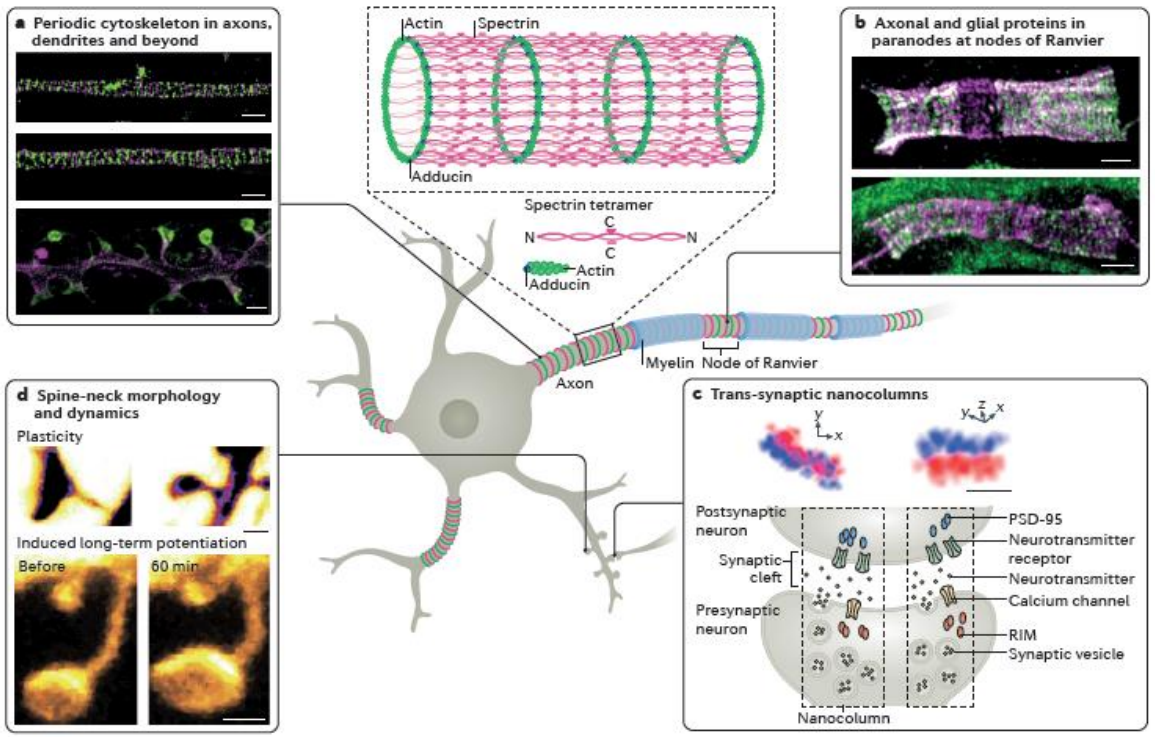
Clathrin (red) and the trans Golgi network (green) were imaged by confocal microscopy (A) and super-resolution (B)

McDonald et al.,2015

TECHNOLOGIES AND TECHNIQUES

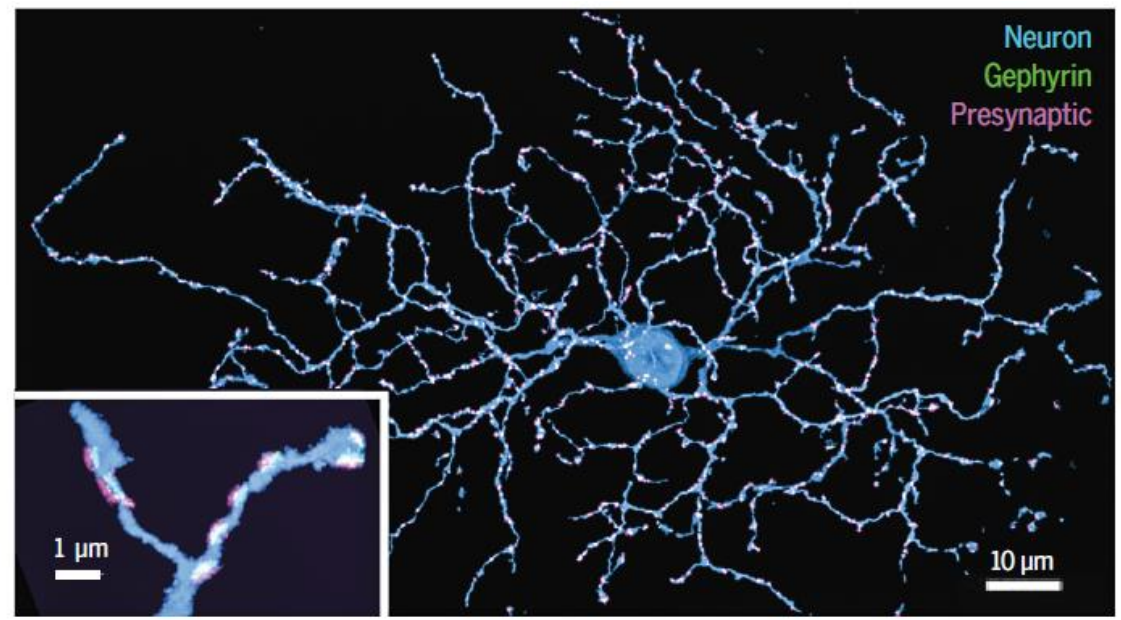
Fluorescence nanoscopy in cell biology

Steffen J. Sahl[1], Stefan W. Hell[1–3] and Stefan Jakobs[1,4]



Visualizing and discovering cellular structures with super-resolution microscopy

Yaron M. Sigal, Ruobo Zhou, Xiaowei Zhuang*



TECHNOLOGIES AND TECHNIQUES

Fluorescence nanoscopy in cell biology

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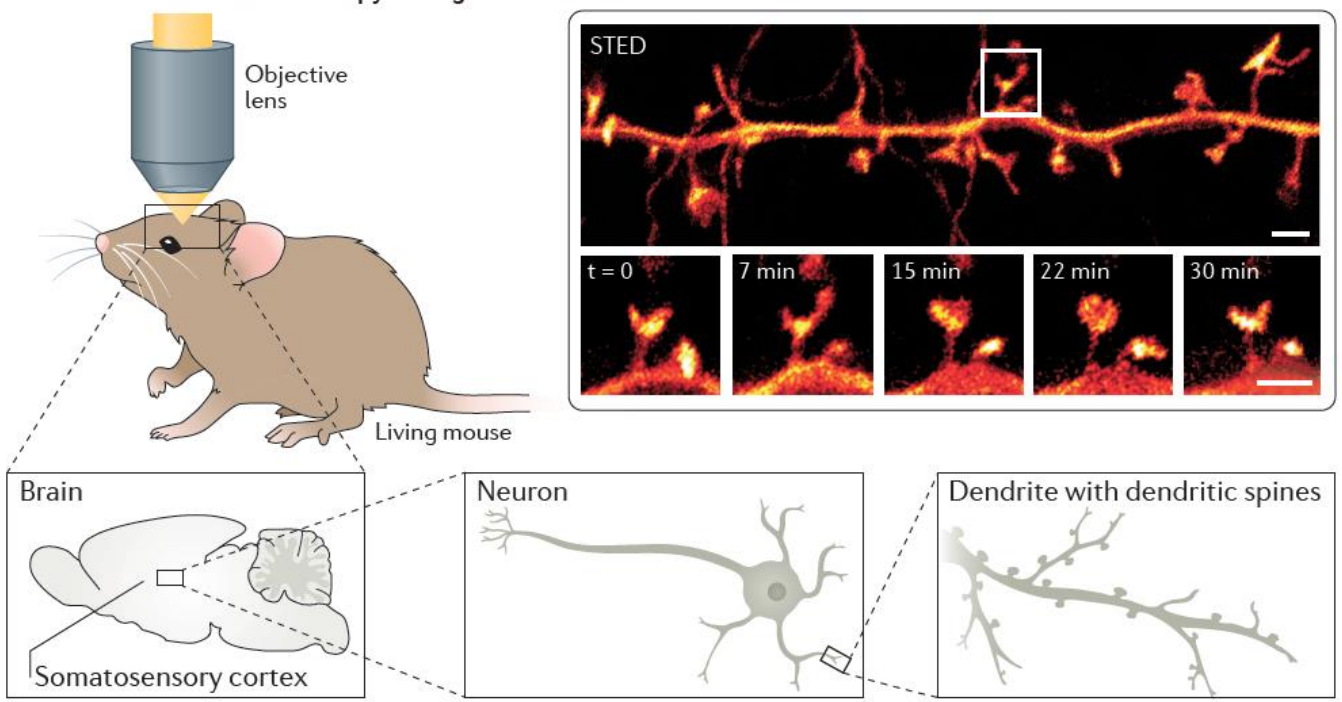
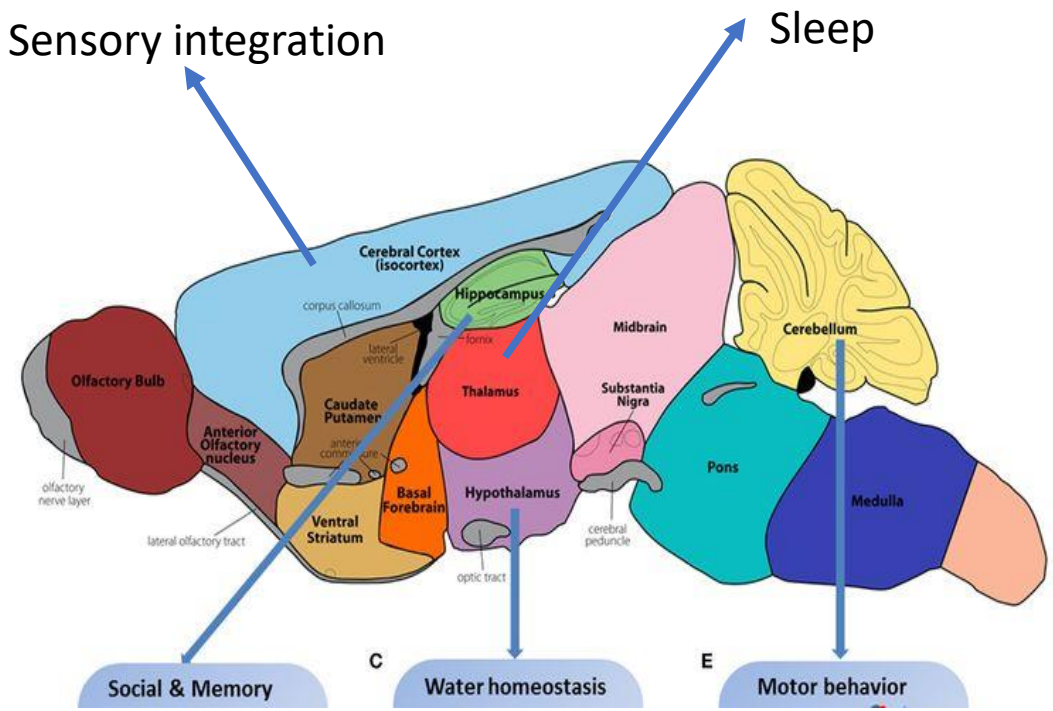
a ***In vivo*** **fluorescence nanoscopy through a cranial window in the mouse**



Brain areas and behavior



The synergistic combinations of the techniques described allow to investigate and correlate synaptic transmission with neuronal circuits and last behavior

In this way we can find a causal relationship between a given behavior and a brain area/region and thus investigate its cellular basis

Conclusions

- ✓ New techniques in Neurosciences allow a better understanding of electrical phenomena that are responsible for behaviour
- ✓ Every techniques has advantages but also limitations
- ✓ An integrated approach including the combination of more tehniques seems to be the best way to tackle scientific problems

Chloride homeostasis: basic mechanisms in physiological and pathological conditions

Rocco Pizzarelli
20-05-2019

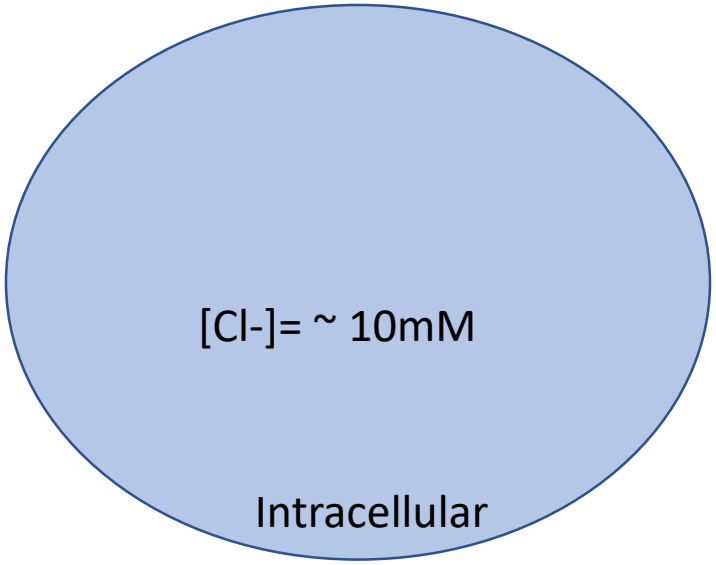
- ✓ Chloride is the main physiological anion, serving as the principal compensatory ion for the movement of major cations such as Na^+, K^+ and Ca^{2+}

- ✓ A fine regulation of chloride homeostasis is necessary in order to maintain a proper cellular functions.

- ✓ Functions attributed to chloride channels include the control of membrane potential, cell volume homeostasis and regulation of cell proliferation and apoptosis

Extracellular

$[Cl^-]$= ~ 110mM



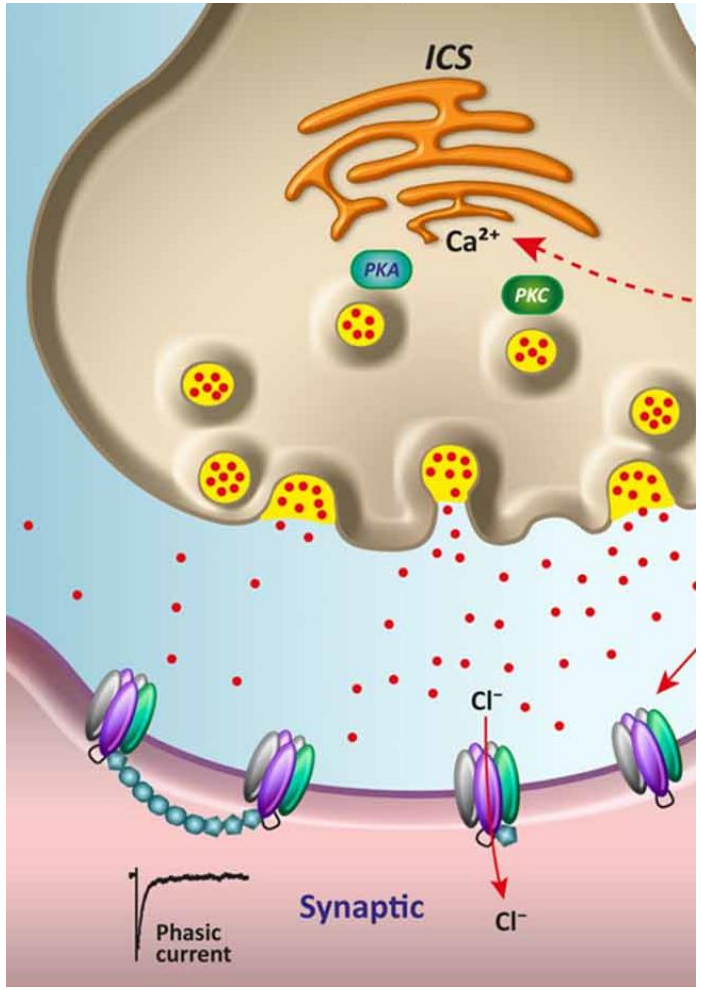
$$V_{Cl} = \frac{RT}{(-1)F} \ln \frac{[Cl^-]_o}{[Cl^-]_i}$$ =-64 mV

But let’s see what happen in neurons…

- During the first postnatal week [CL^-] is higher than what is regularly find in neurons (~25 mM)
- This has a very peculiar effect on neuron physiology
- Starting from the second postnatal week [CL^-] reach the value of ~ 5mM

$GABA_A$ and glycine receptor/channels are permeable to Cl^-

Pre-synaptic GABAergic/Glycinergic neuron



Post-synaptic

Forstera et al.,2016

- ✓ The binding of GABA or Glycine to the receptor opens a central pore, thus enabling Cl^- to move through the inner channel

- ✓ Cl^- electrochemical gradient determines the direction of its flux

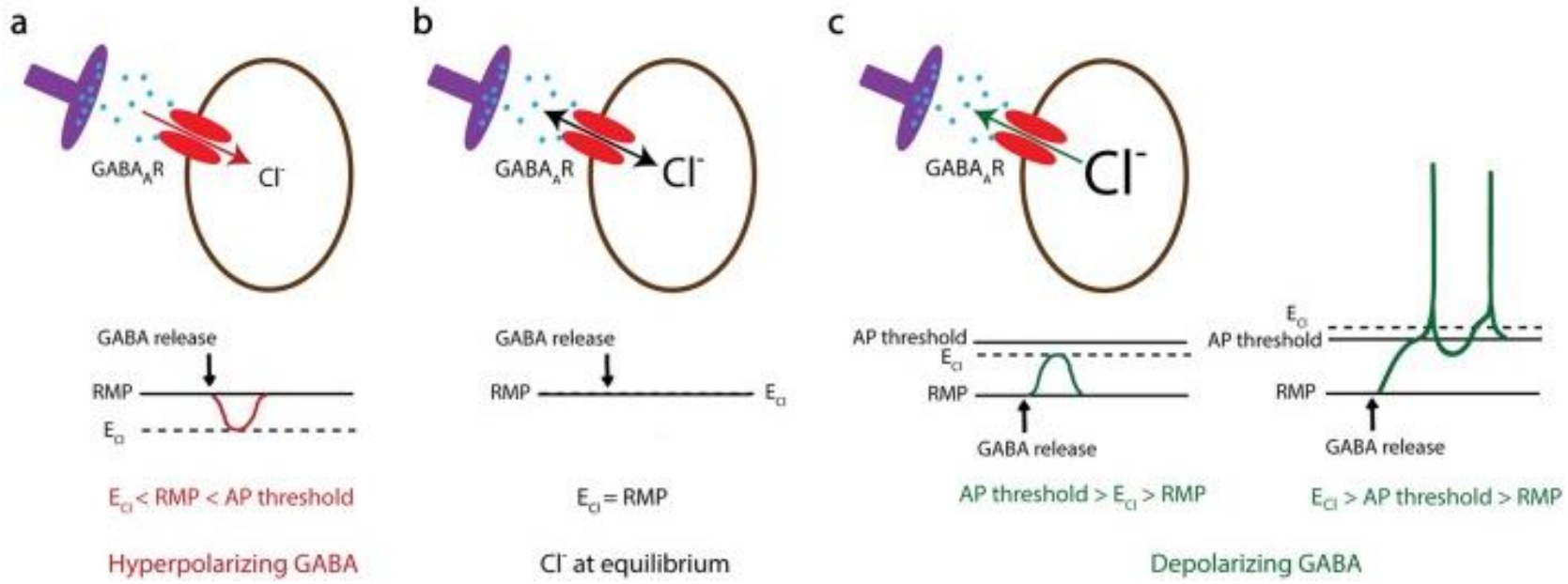
$$V_{DF} = V_m - V_{eq}$$

V_{DF}=electrochemical driving force

Vm= membrane potential

Veq= equilibrium potential for the ion of interest

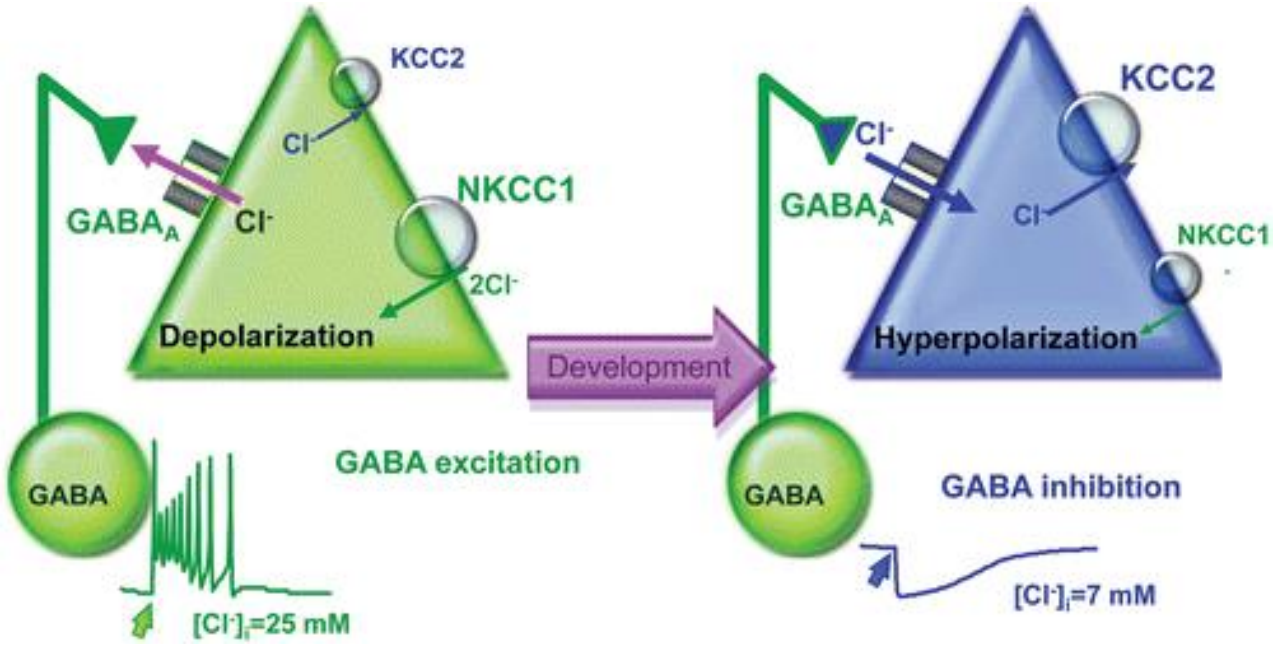
The $[Cl^-]_i$ dictates the polarity of the current through $GABA_A$ receptors



Modified from Rahmati et al.,2018

How is [Cl^-] regulated into neurons?

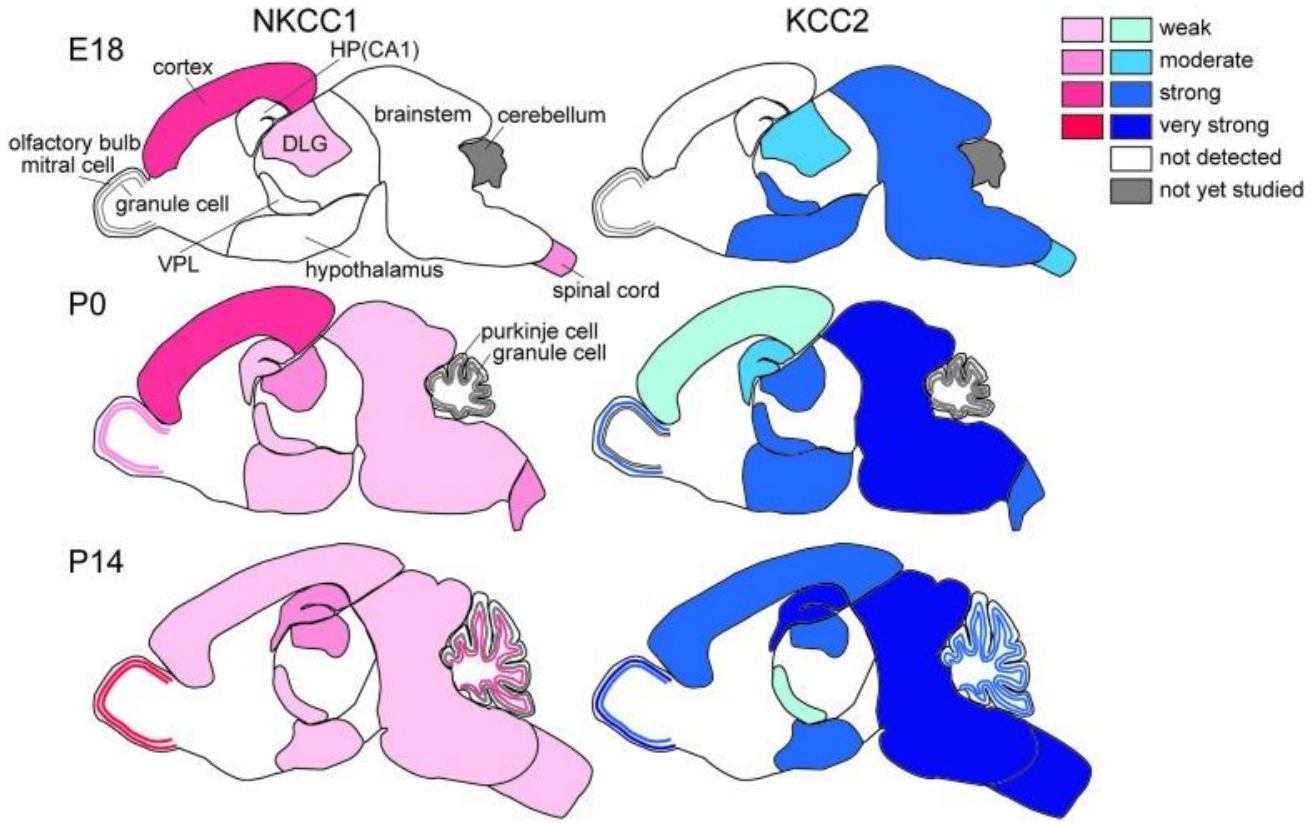
The cotransportes NKCC1 and KCC2 developmentally regulate Cl-



Ben-Ari et al.,2012

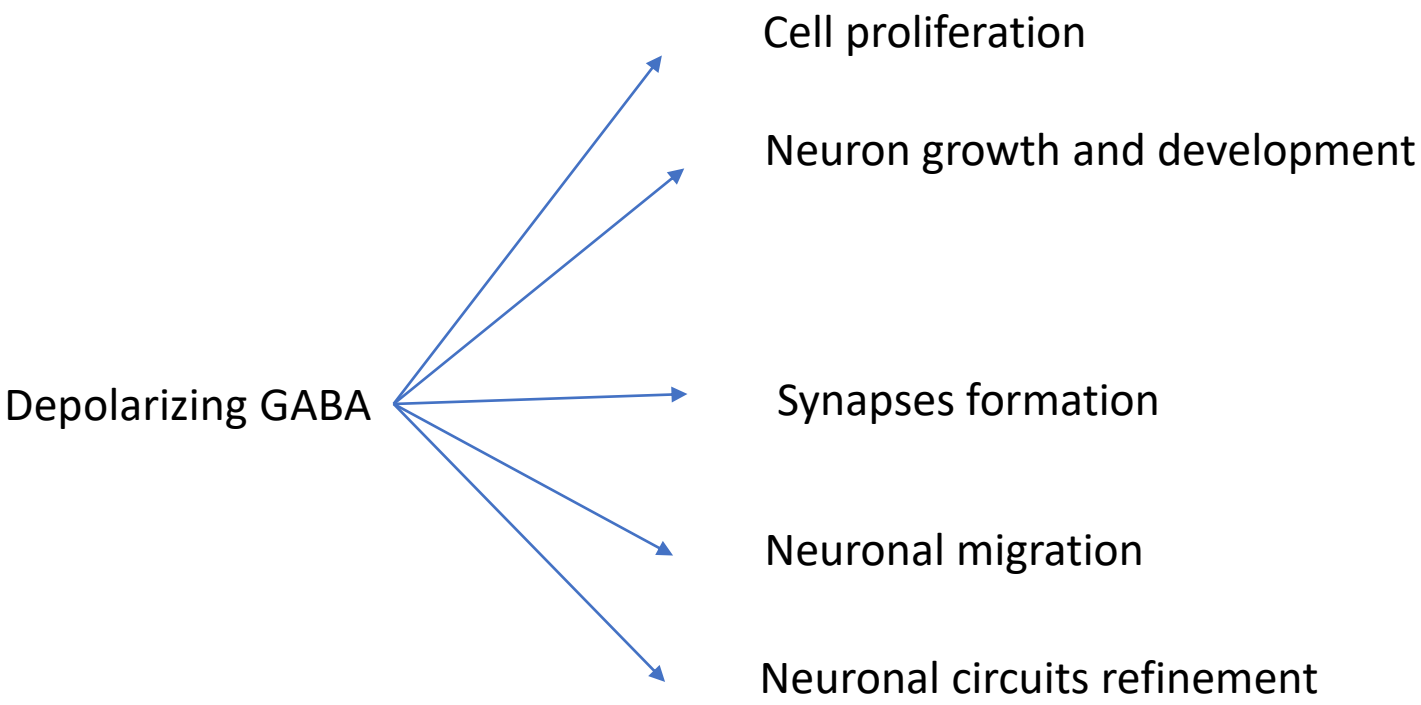
Differential development of NKCC1 & KCC2 expression in the brain

NKCC1 and KCC2 levels during development

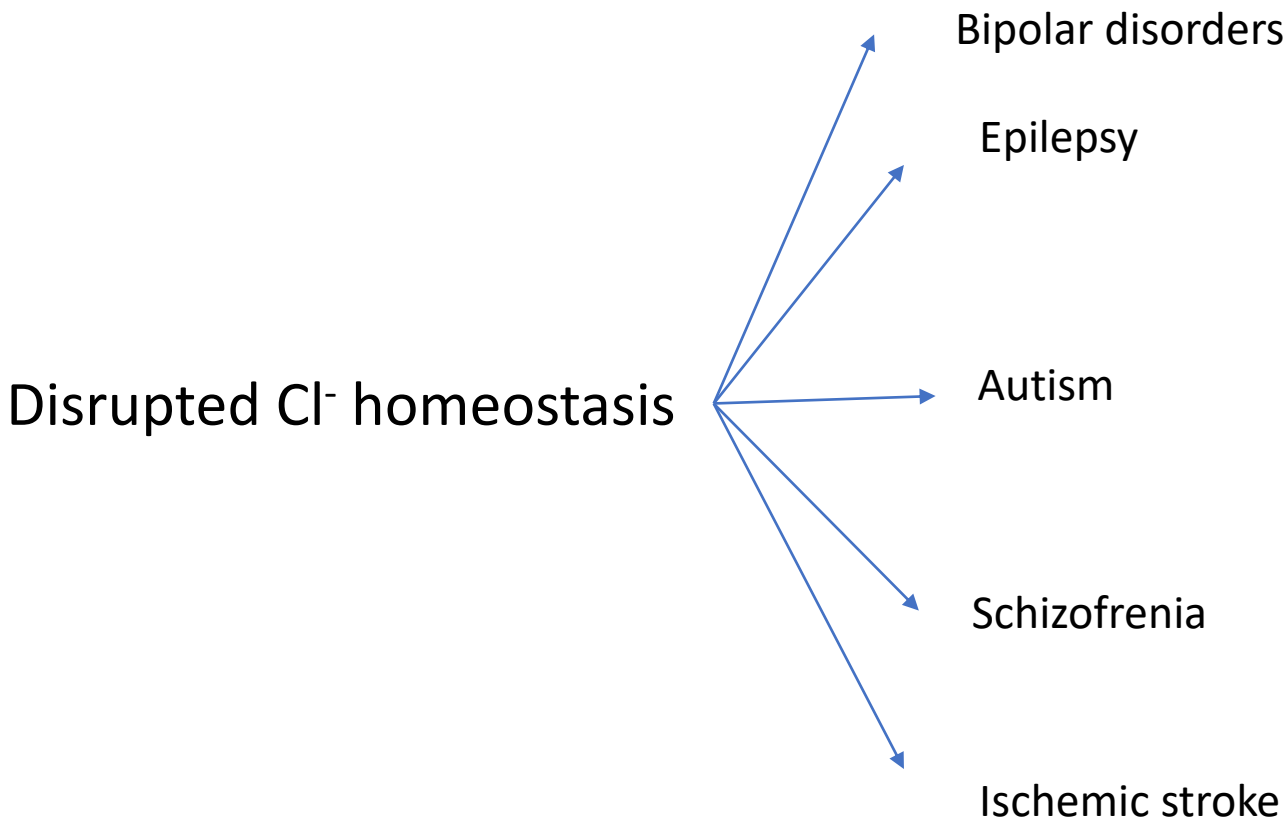


Watanabe & Fukuda,2015

What are the main function of depolarizing GABA ?



Alterations in Cl^- homeostasis during development or at later stages can affect neuronal functions.



Conclusions

- ✓Cl- ion is involved in many important cellular functions
- ✓In neurons intracellular [Cl-] is developmentally regulated
- ✓Alterations in Cl- homeostasis during development seems to be implicated in some neurological disorders

Suggested readings

- ✓ Sahl SJ, Hell SW, Jakobs S Fluorescence nanoscopy in cell biologyNat Rev Mol Cell Biol. 2017 Nov;18(11):685-701. doi: 10.1038/nrm.2017.71.

- ✓ Deisseroth K, Hegemann P. The form and function of channelrhodopsin. Science. 2017 Sep 15;357(6356). pii: eaan5544. doi: 10.1126/science.aan5544

- ✓ Grienberger C, Konnerth A. Imaging calcium in neurons. Neuron. 2012 Mar 8;73(5):862-85. doi: 10.1016/j.neuron.2012.02.011

- ✓ Ben-Ari Y. The GABA excitatory/inhibitory developmental sequence: a personal journey. Neuroscience. 2014 Oct 24;279:187-219. doi: 10.1016/j.neuroscience.2014.08.001