«Здоровый и больной мозг: от молекулярной физиологии к патологии, клинике и лечению»

Наиль Бурнашев

III Современные методы исследования в нейронауках.

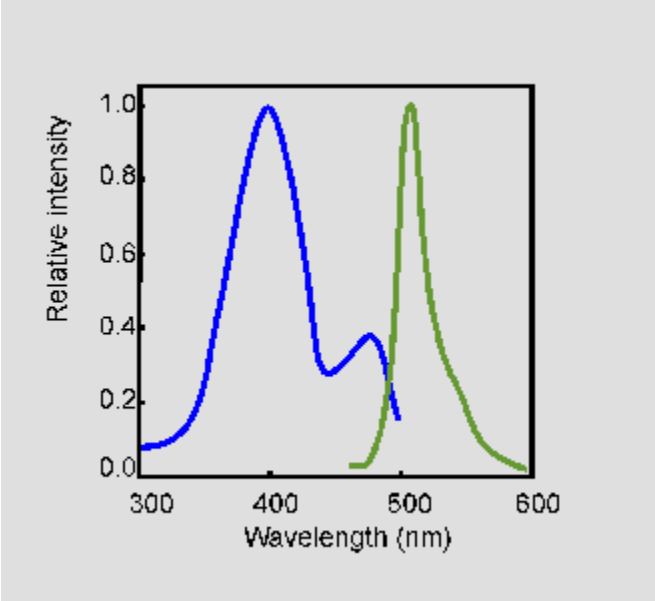
1. Электрофизиологические методы исследования мозга

2. Оптические методы исследования мозга

3. Животные модели патологий мозга

Флуоресценция

Феномен, в котором молекулярная абсорбция фотона вызывает эмиссию другого фотона с более длинной длиной волны



* Stimulate in the ultraviolet range, and the emitted light is in the visible range.*

Ca чувствительные красители

Low-affinity calcium indicators

Fura-FF
BTC
Fura-2
Fura-5,
Indo-1)

Excited by UV light

Intermediate-affinity calcium

Fura-4F
Fura-5F
Fura-6F

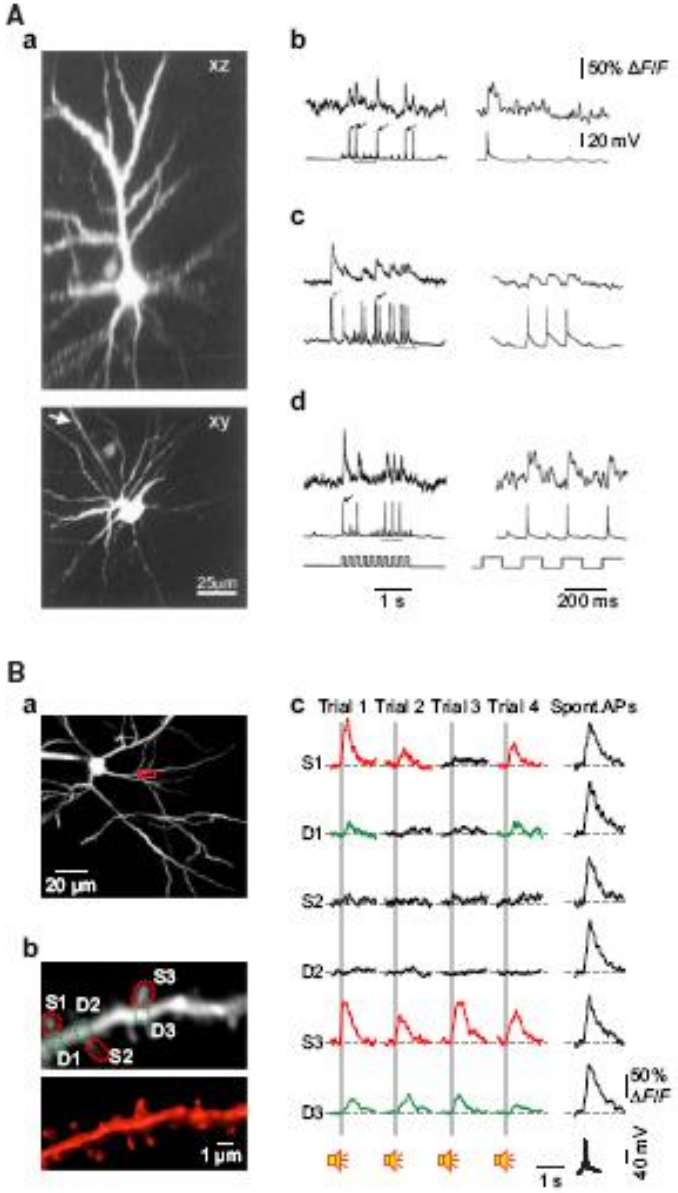
Excited by UV light

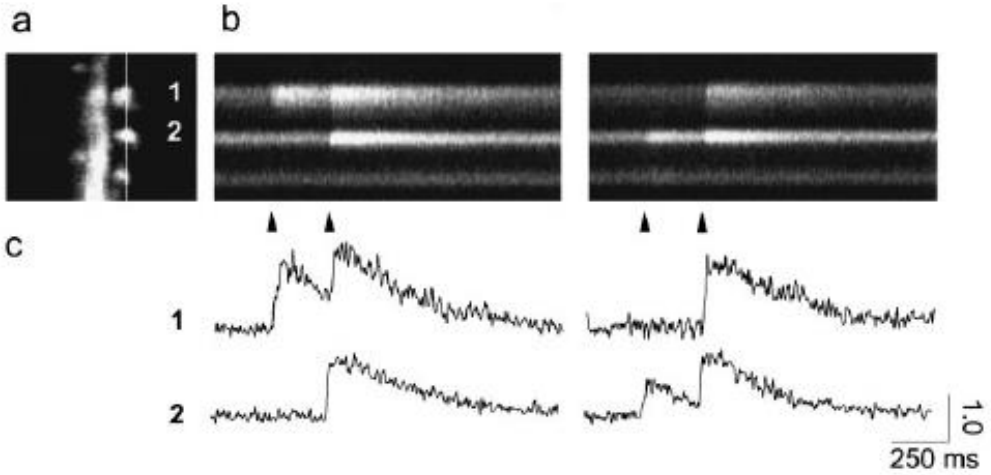
High-affinity and selectivity (BAPTA)

Excited by visible light under scanning laser confocal microscopy

Calcium Green, Calcium Orange – Tomchik et al 2007

Ca сигналы в дендритах и шипиках in vivo





Измерение мембранного Ca тока и входа ионов Ca

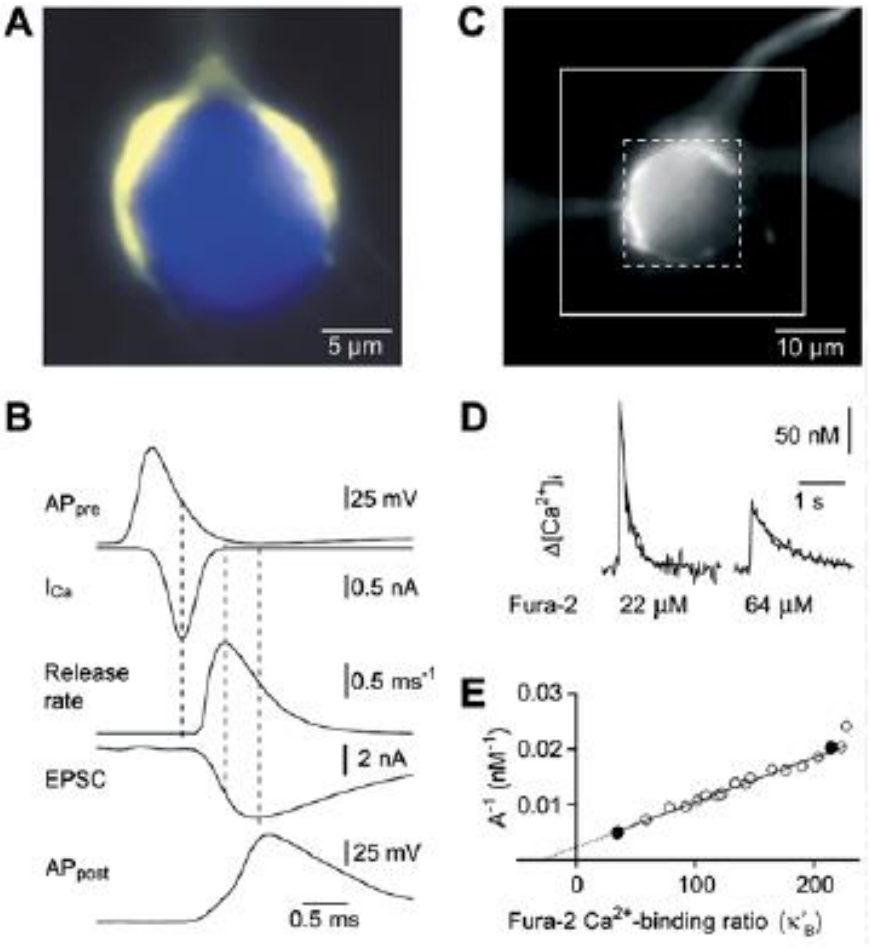
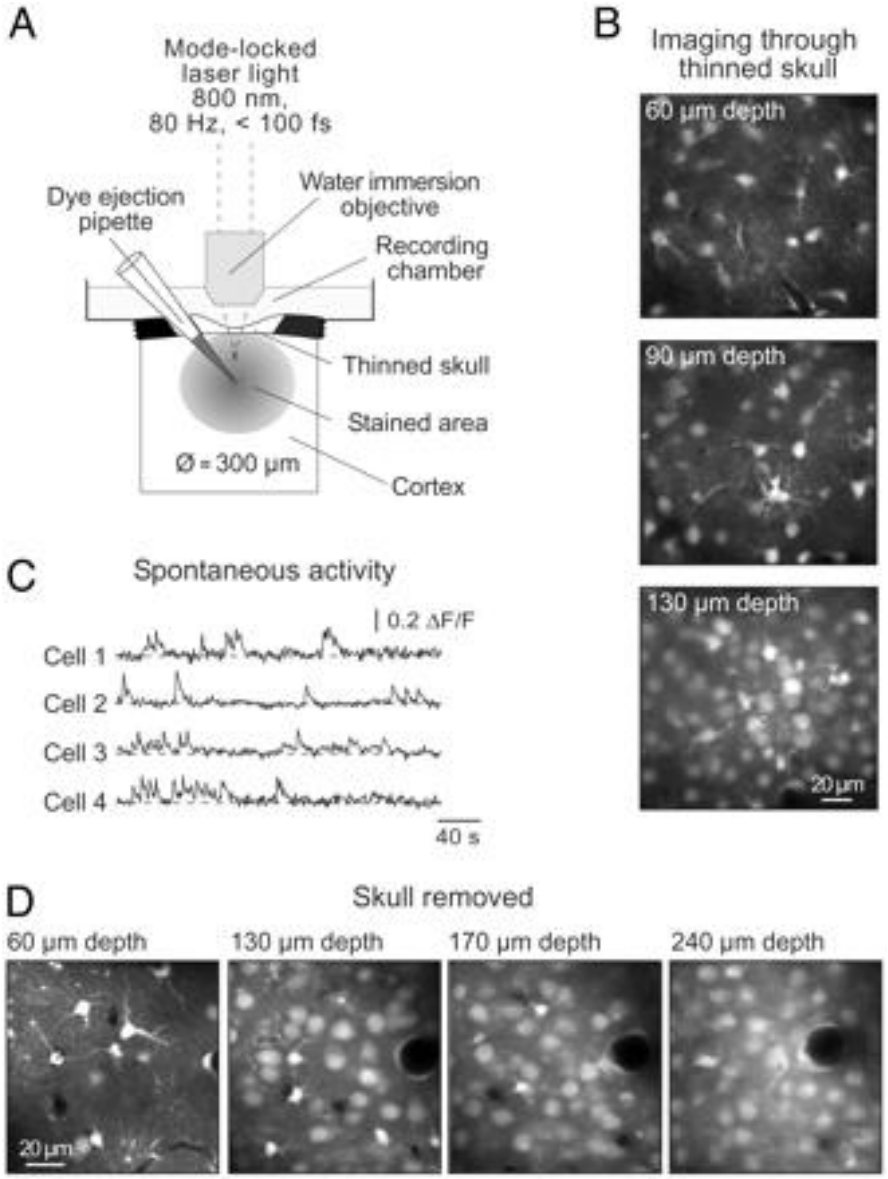


Figure 1. Membrane currents and volume-averaged $[Ca^{2+}]$

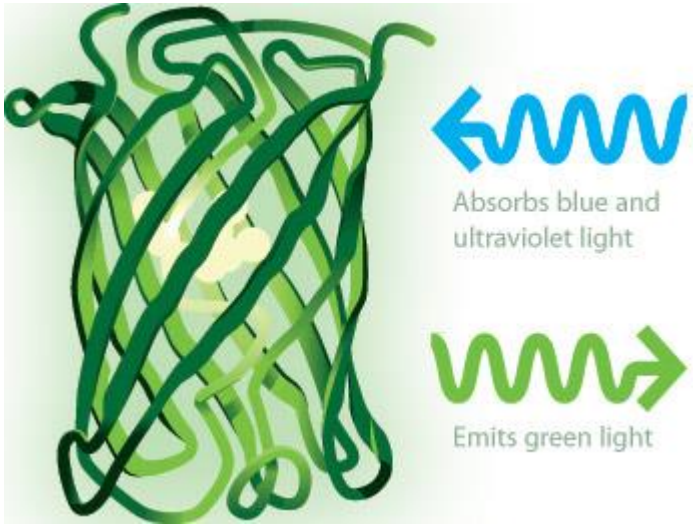
A, pseudocolour image of a calyx of Held (yellow) and the medial nucleus of the trapezoid body (MNTB) principal cell (blue) in a brain slice. The calyx was filled with Lucifer Yellow, the principal cell with Cascade Blue (after Borst *et al.* 1995). *B*, time course of the signalling cascade, showing (top to bottom) the presynaptic AP waveform and resulting Ca^{2+} current, (inferred) release rate, postsynaptic EPSC and postsynaptic AP (after Borst *et al.* 1995; Borst & Sakmann, 1998). *C*, fluorescence image of a calyx in a brain slice. The calyx was filled with 1 mM Fura-2. *D*, fluorescence of Fura-2 in calyx (at two different concentrations) to measure volume-averaged $[Ca^{2+}]$. Note that the measured decay of the fluorescent signal is much slower than the decay of calculated, local $[Ca^{2+}]$ transients (see Fig. 4). *E*, inverted whole-cell $[Ca^{2+}]$ amplitude (A^{-1}) in calyx as a function of exogenous buffer Ca^{2+}-binding ratio (κ_B). Regression line crosses *x*-axis at implied endogenous binding ratio ~40 (*C–E* reprinted after Helmchen *et al.* 1997).

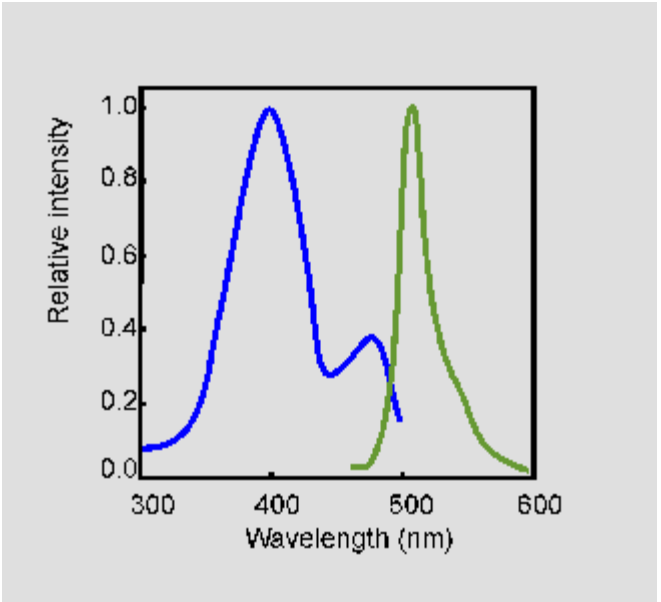
Ca сигналы на популяции нейронов in vivo



Флуоресцентные белки

GFP





Флуоресцентные белки

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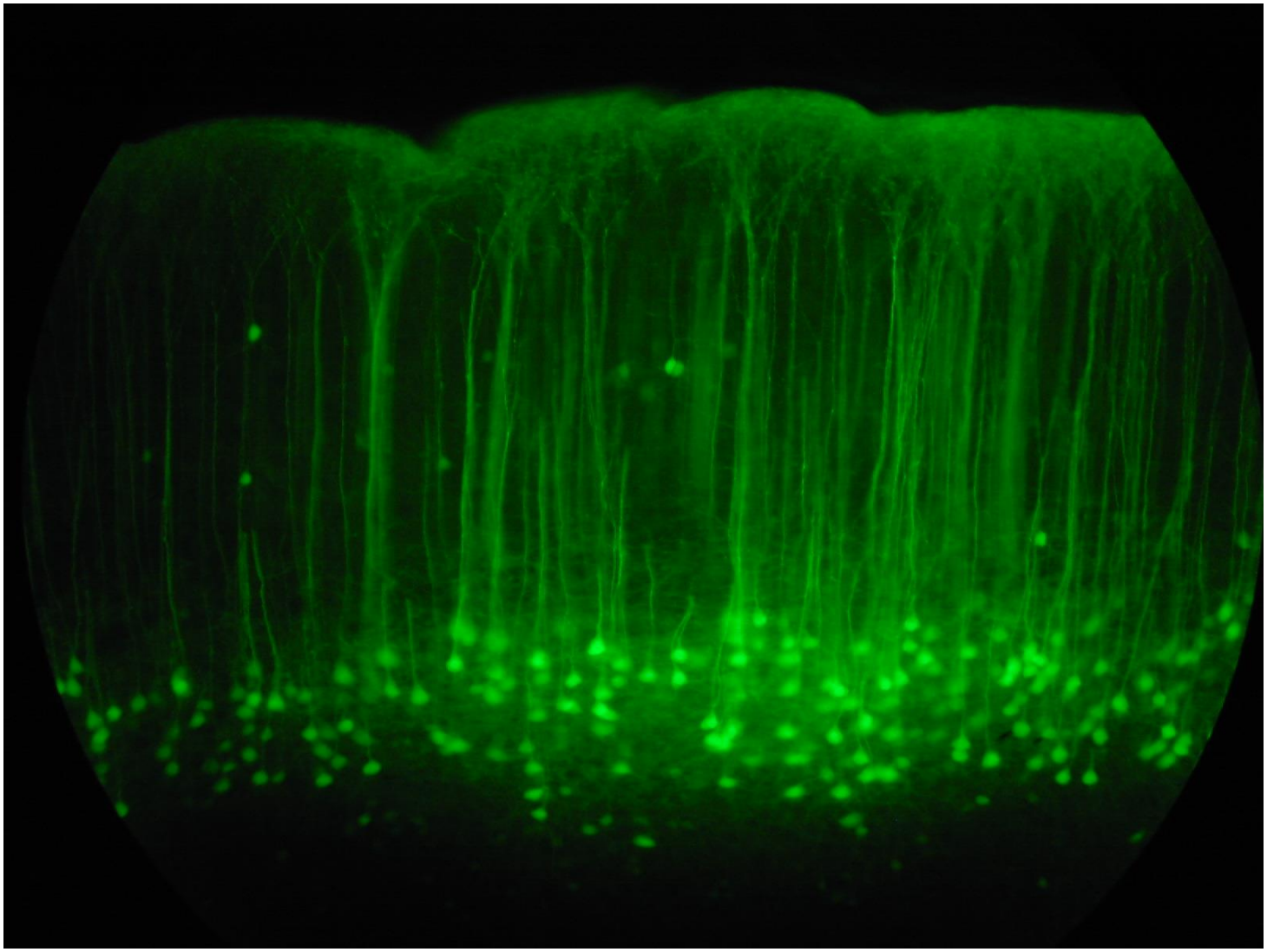
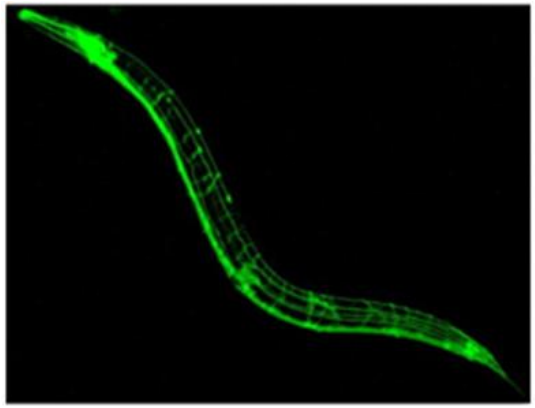
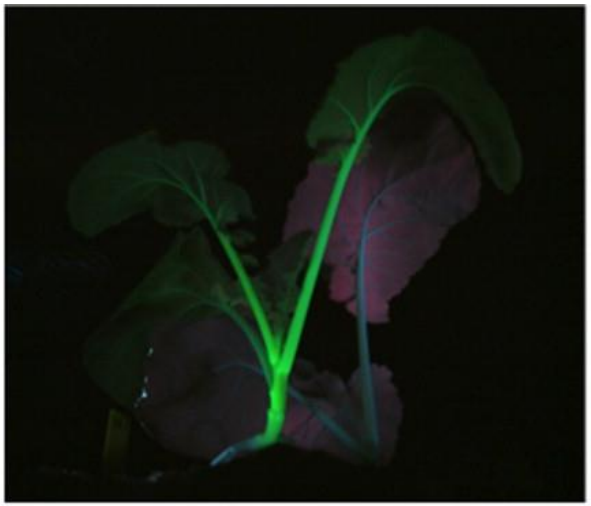
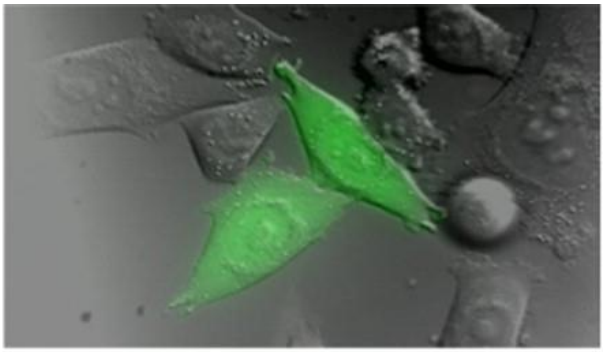
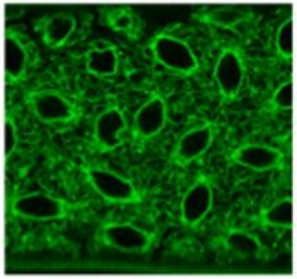
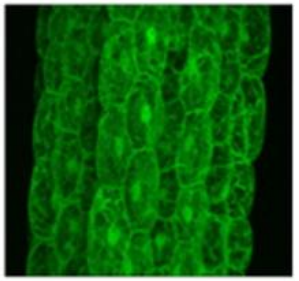
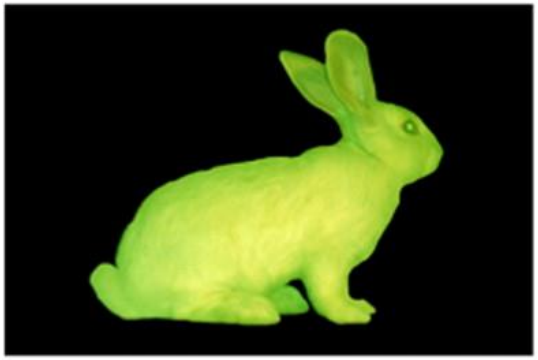
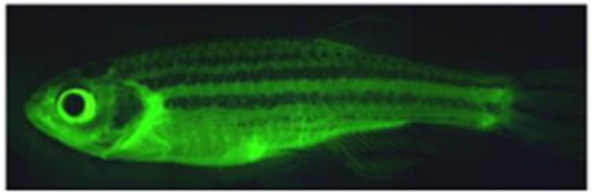
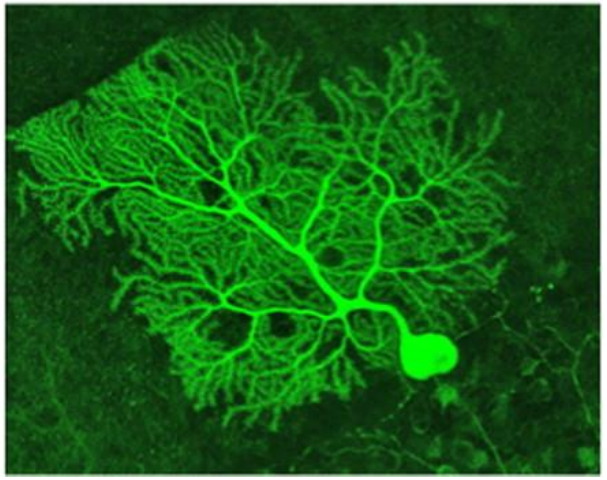


Флуоресцентные белки

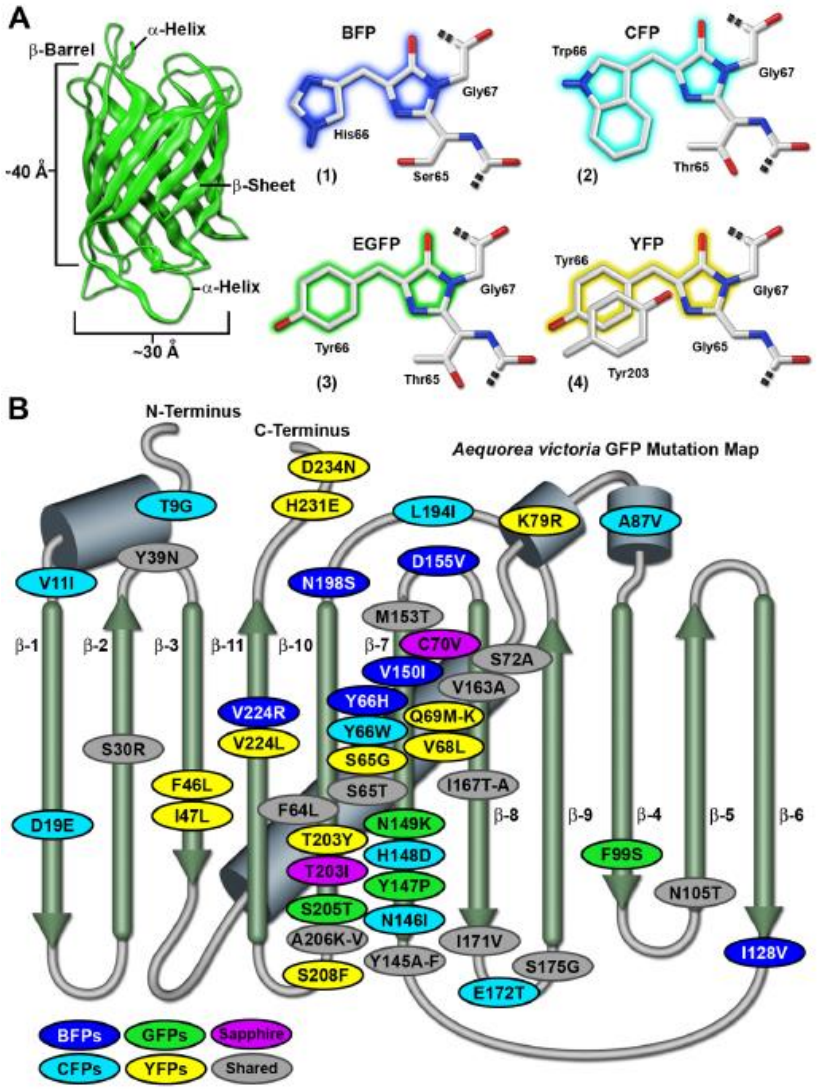
GFP

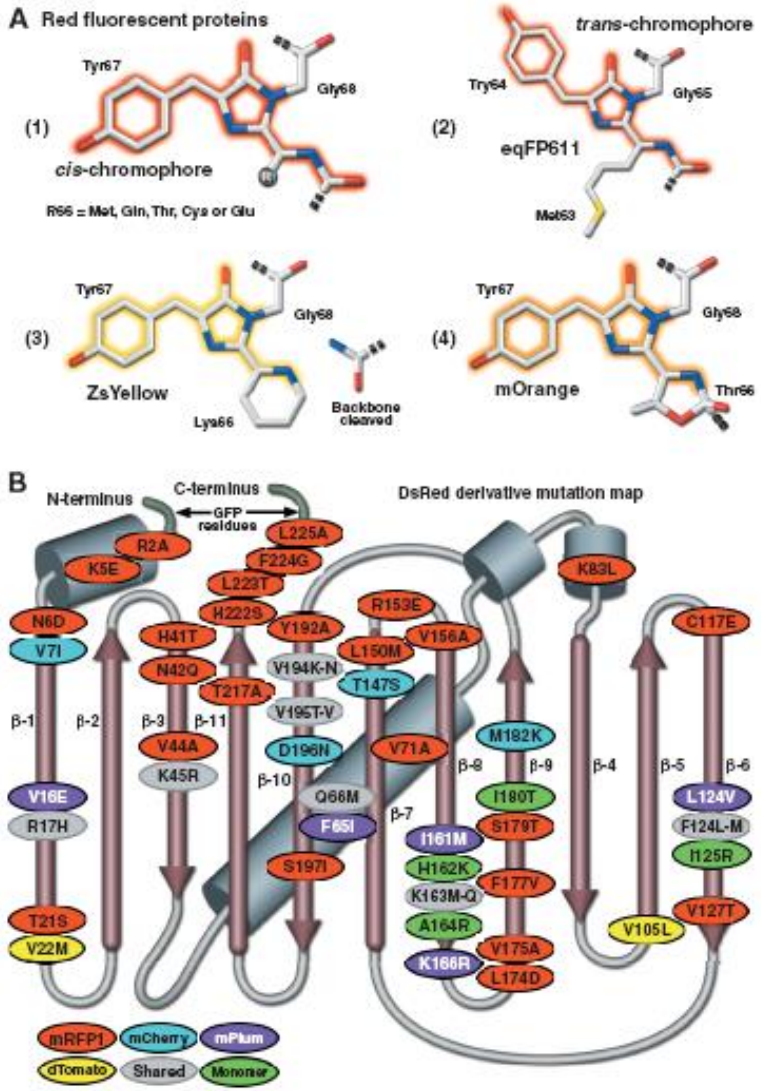







Флуоресцентные белки

GFP



RFP



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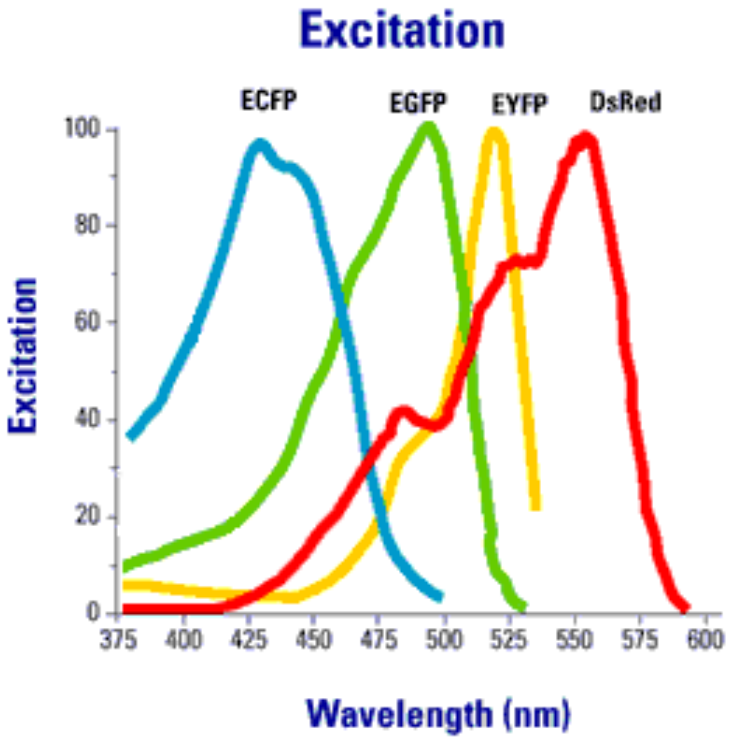
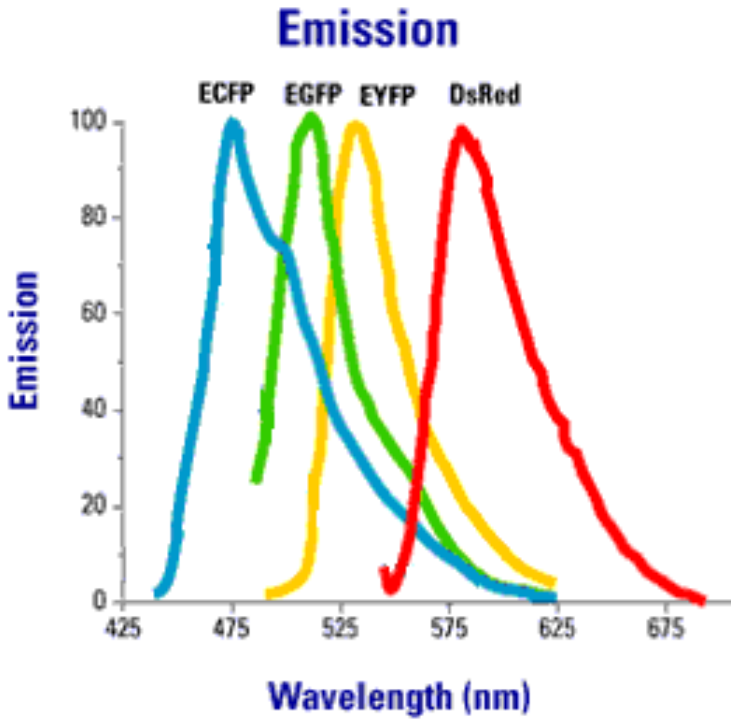
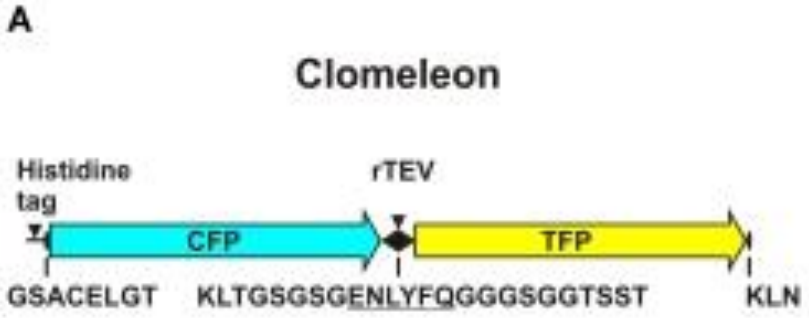
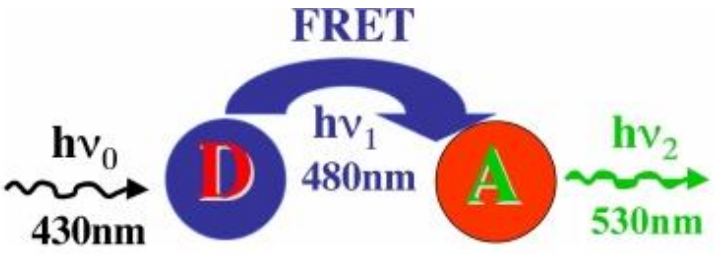
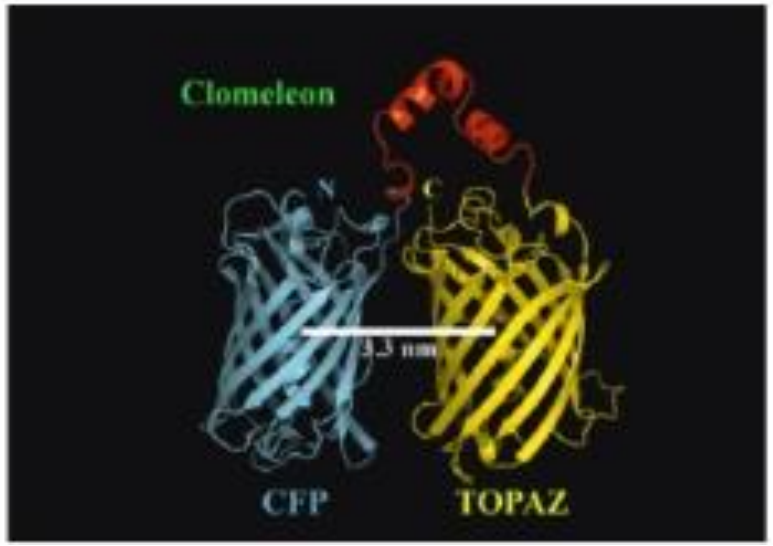


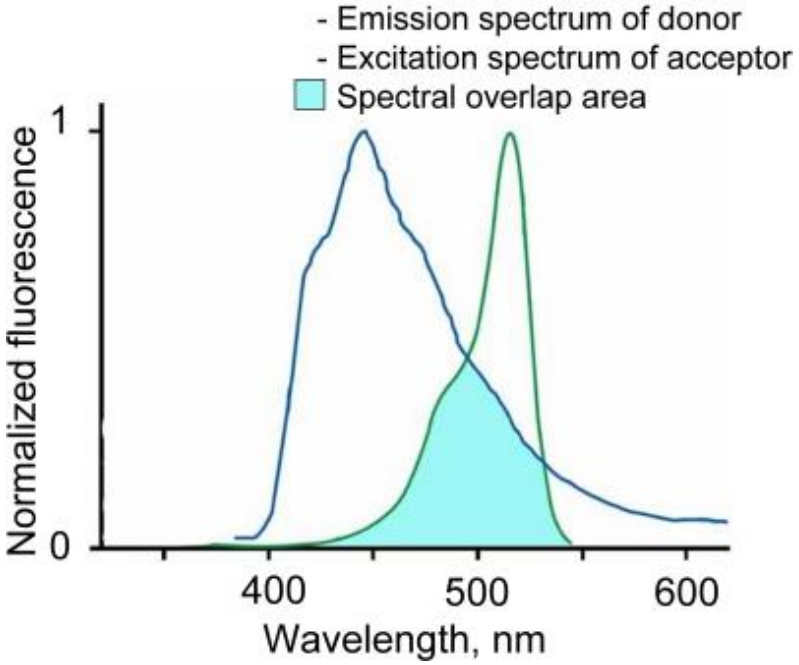


Хлор чувствительные белки

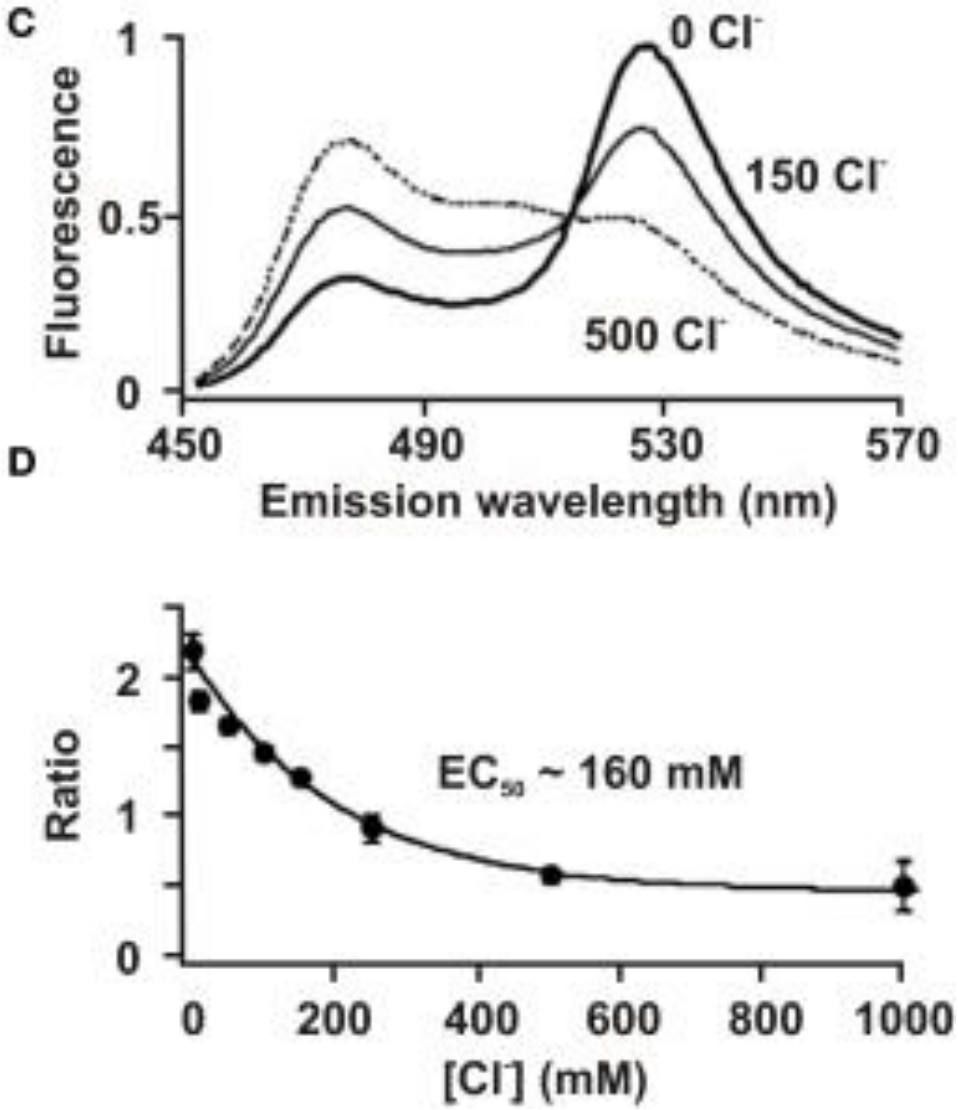








Измерение концентрации хлора



Потенциал-чувствительные красители

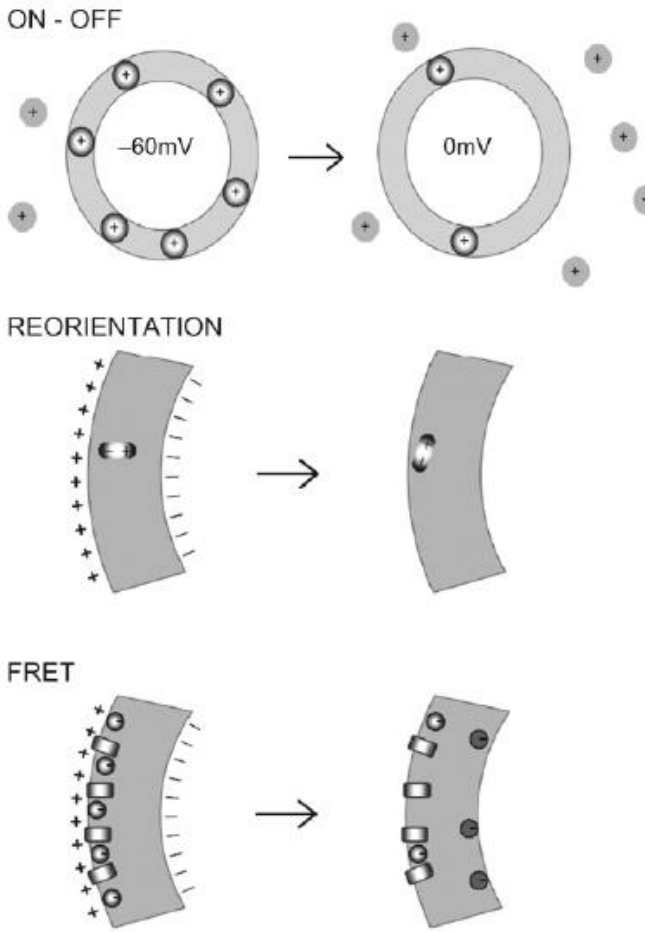


FIGURE 2.1. Mechanisms used by VSDs that can change their location in response to membrane depolarization.

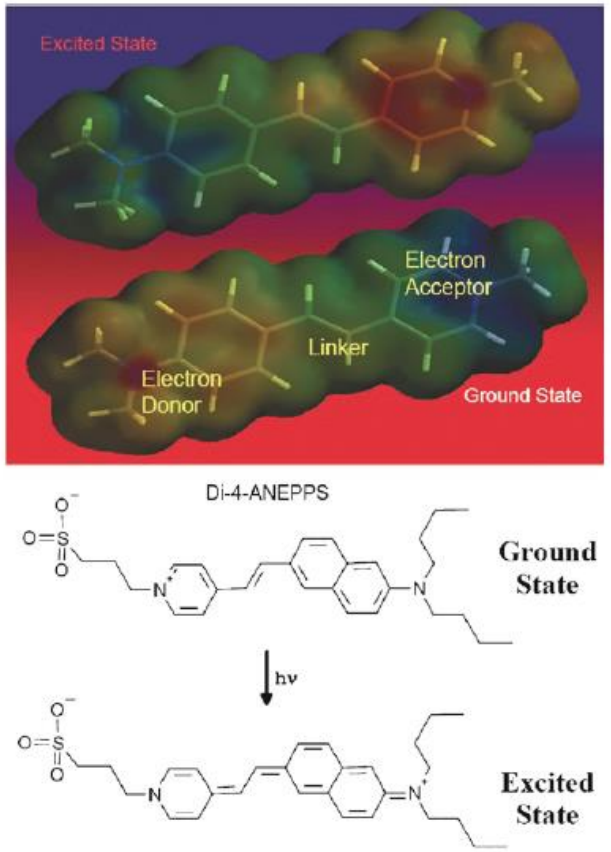


FIGURE 2.2. Electrochromic mechanism of voltage sensitivity. The *top* shows how the electrons, and therefore the charge distribution, shifts upon excitation of a typical electrochromic dye. These images were generated from molecular orbital calculations where low electron density (i.e., regions of positive charge) are represented by *bluer shades* and high electron density (i.e., negative charge) is represented by *redder colors*. The *lower portion* of the figure shows resonance structures for the ground and excited states of one of the most widely used VSDs, di-4-ANEPPS. In this chromophore, the donor moiety is an aminonaphthyl group, the linker is a simple double bond and the acceptor is a pyridinium moiety.

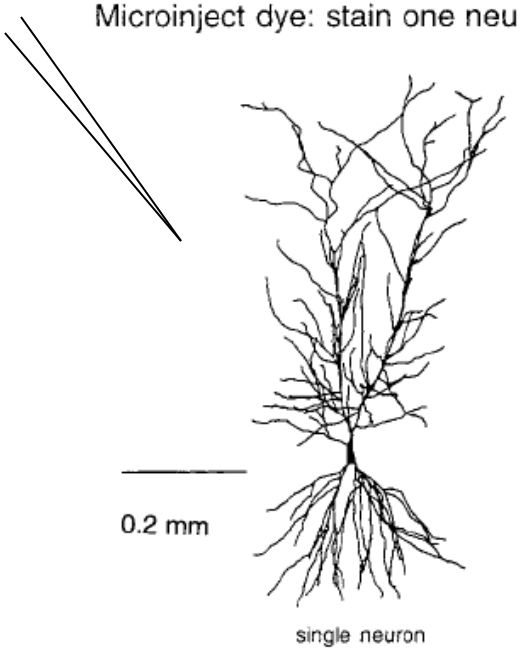
Потенциал-чувствительные красители

Измеряют активную проводимость – Na, K, Ca2+ каналы

PARTS OF A NEURON

Many Detectors - One neuron

Potential changes in dendrites.
Microinject dye: stain one neuron.

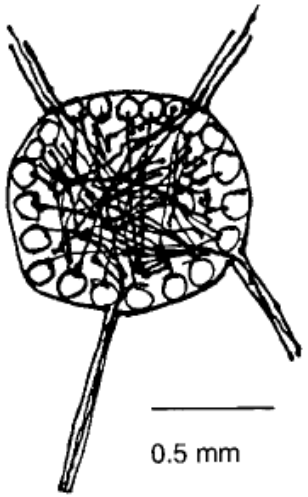


single neuron

INDIVIDUAL NEURONS

One Detector - One Neuron

Follow spike activity of many individual neurons.
Bath applied dye.

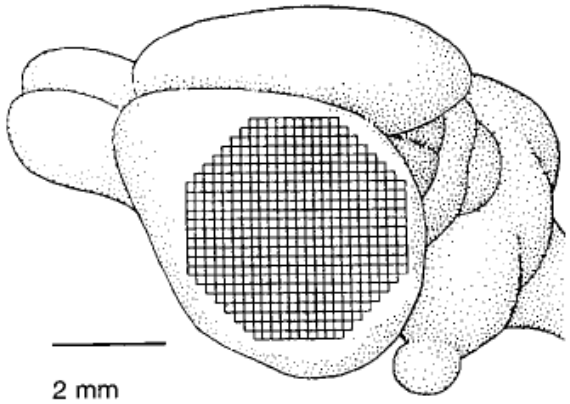


invertebrate ganglion

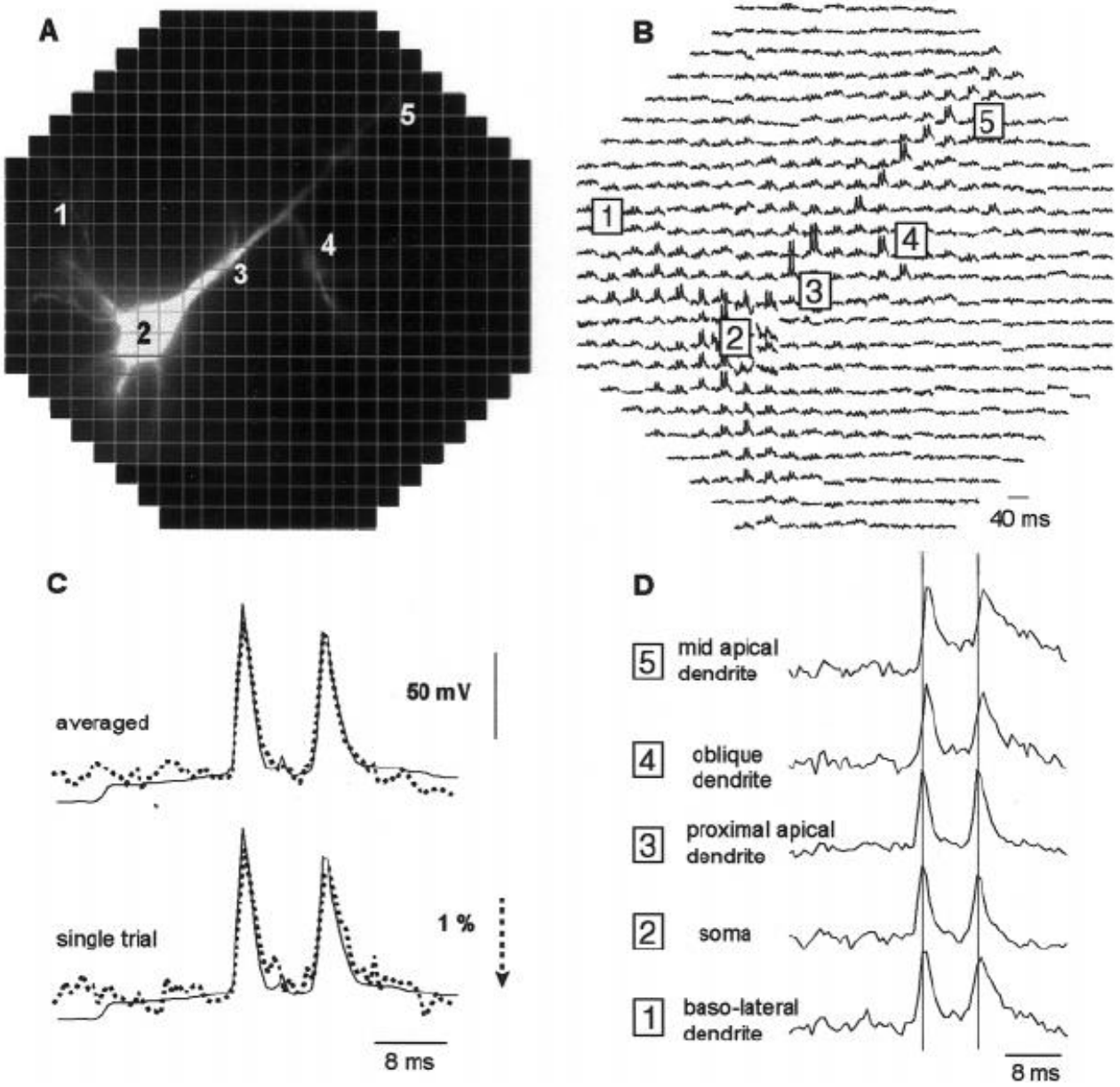
POPULATION SIGNALS

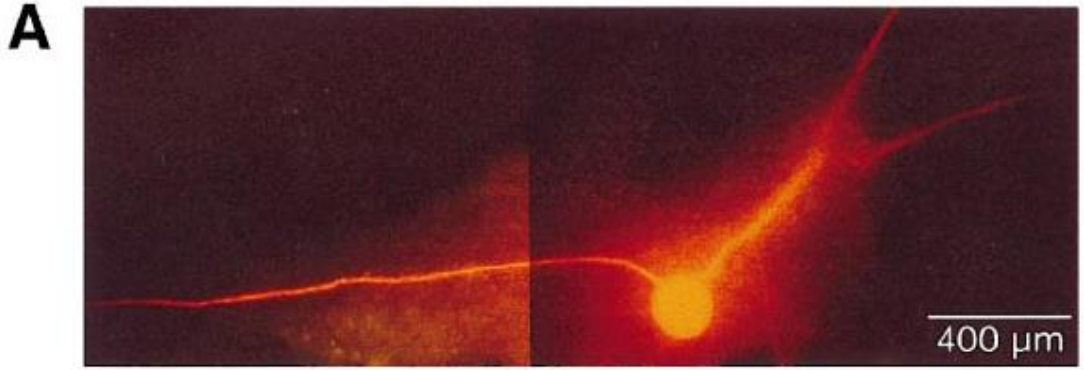
One Detector - Many Neurons

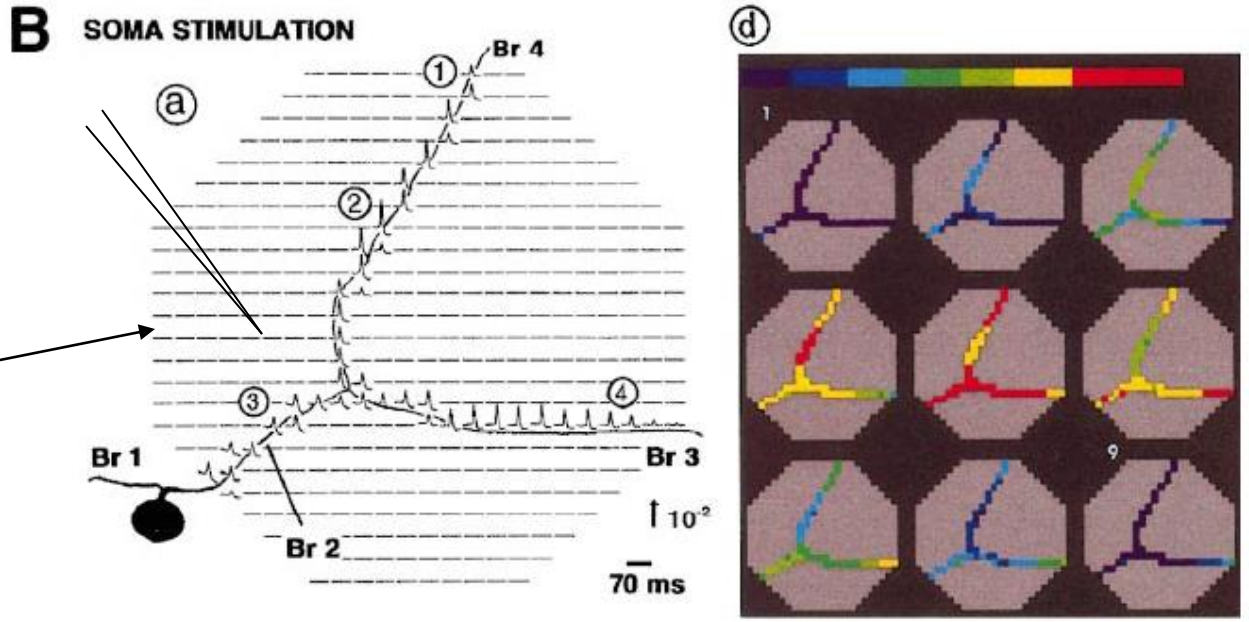
Signals are population average.
Vertebrate brain.
Bath applied dye.



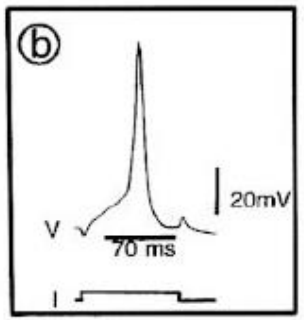
Мультидиодная матрица

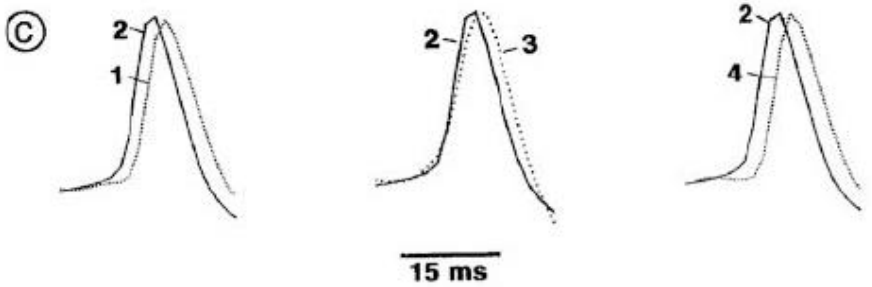



A
400 µm

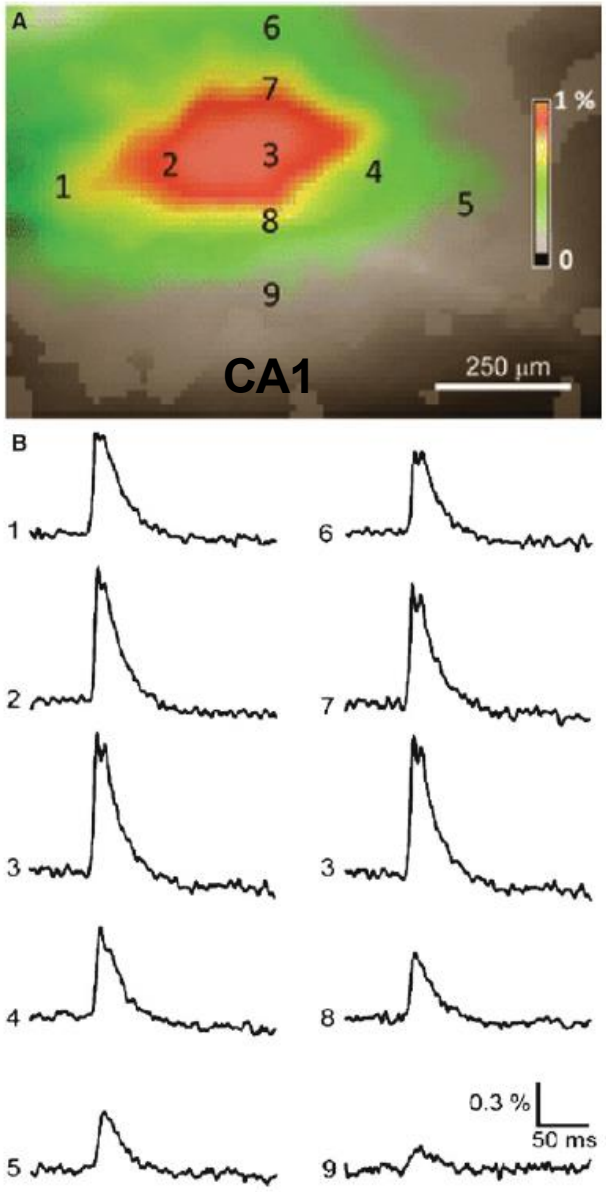

B
SOMA STIMULATION
Br 4
a
1
2
3
4
Br 1
Br 3
Br 2
10⁻²
70 ms
d
1
9

Photodiode

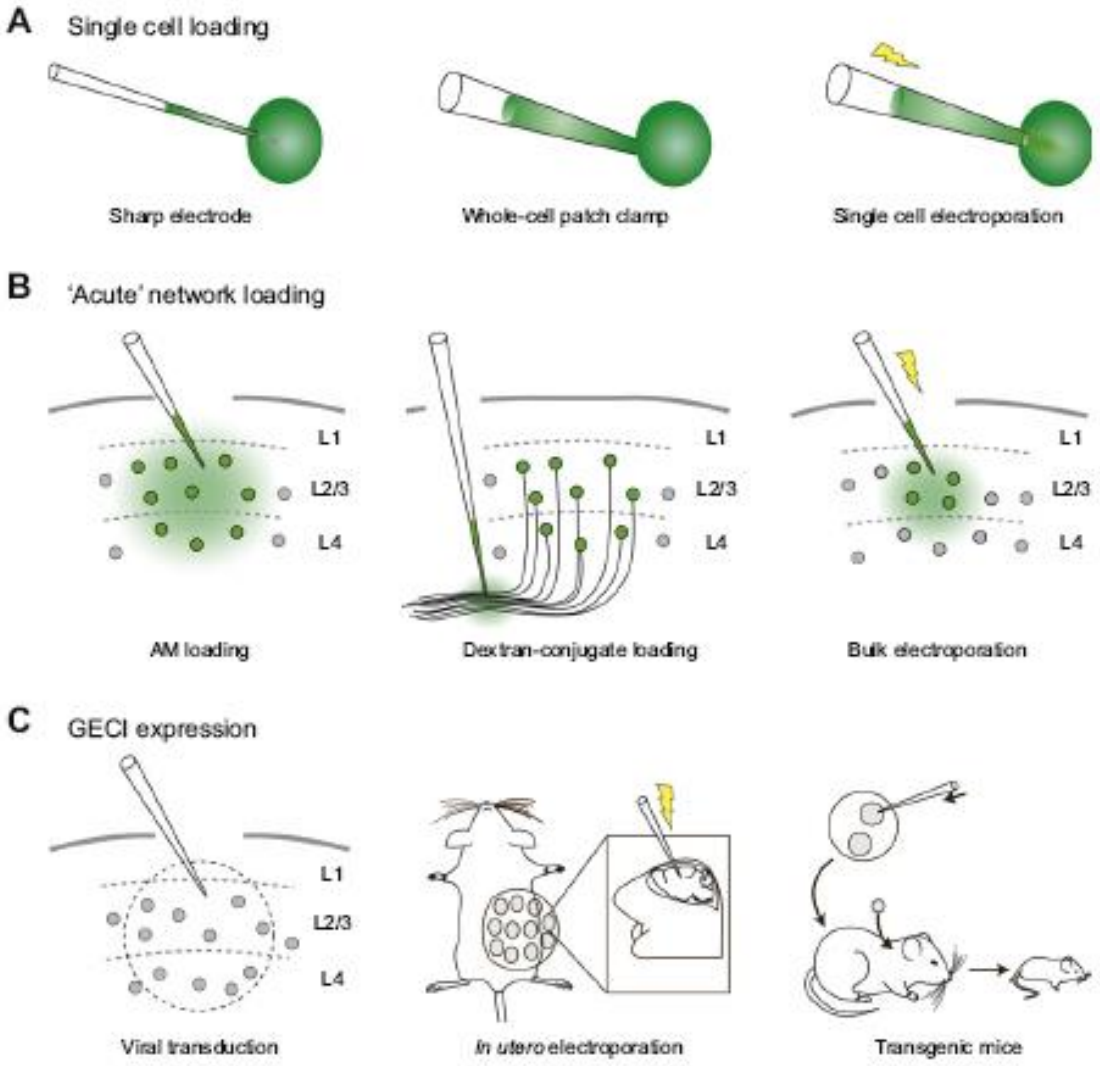

b
20mV
V
70 ms
I


c
2
1
2
3
2
4
15 ms

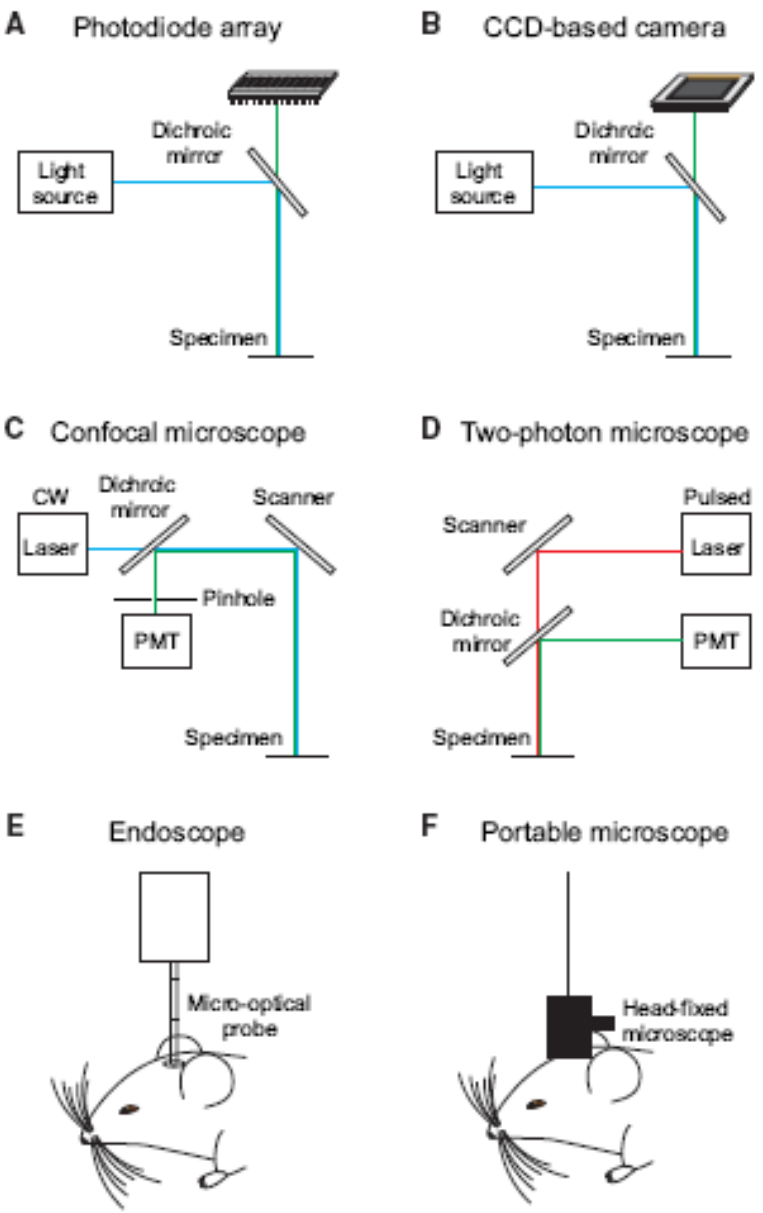
Потенциал-чувствительные красители



Методы загрузки краситилей

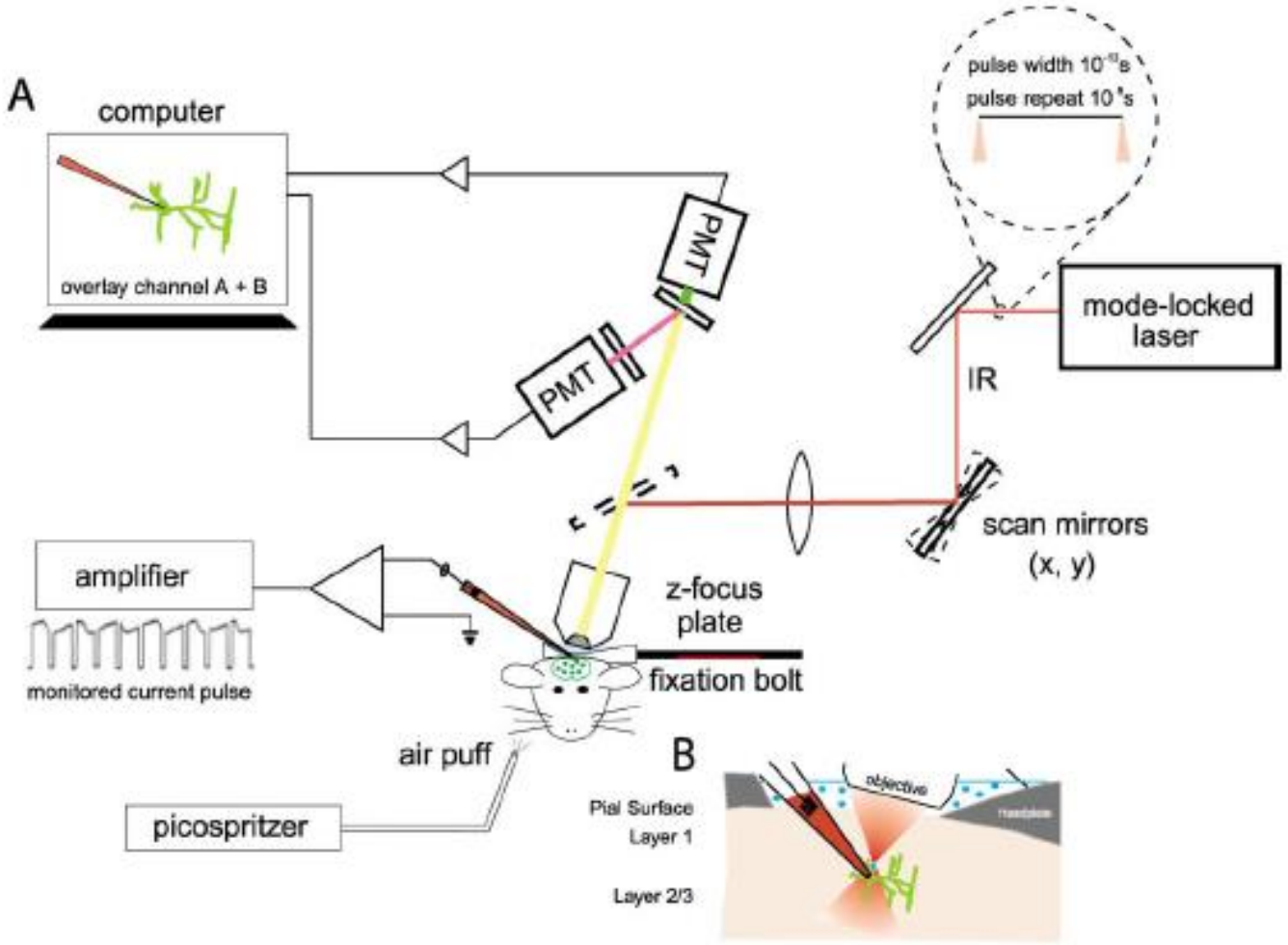


Методы визуальной регистрации

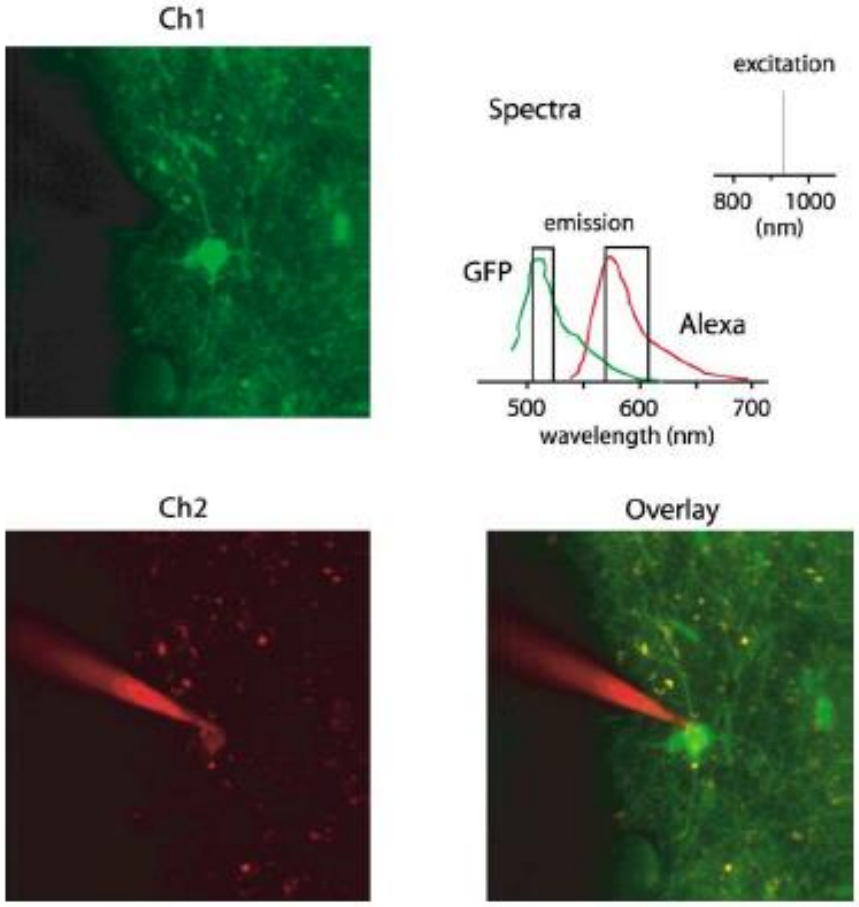


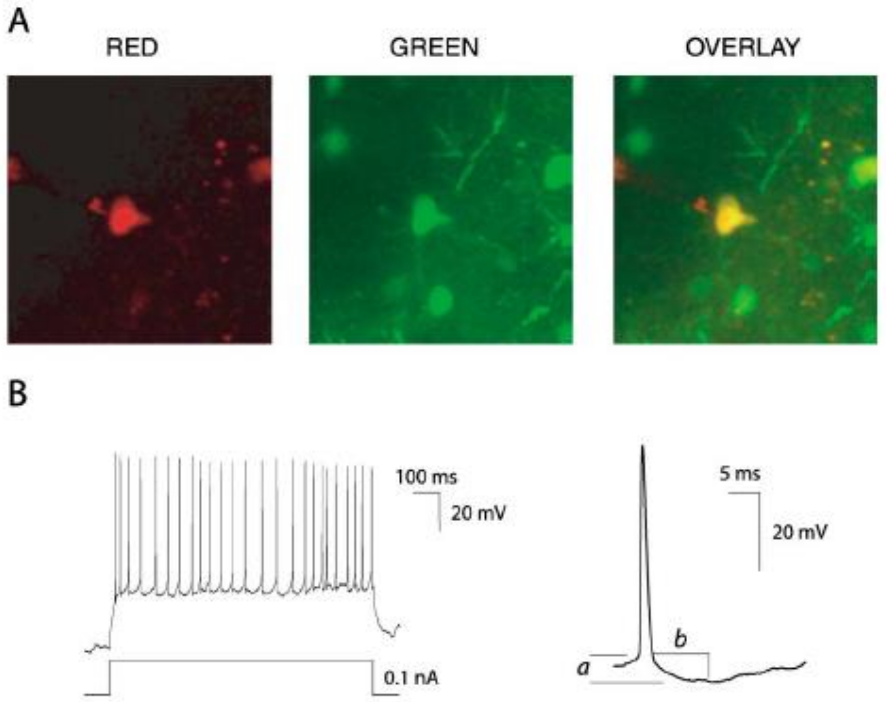
Точечная whole cell регистрация *in vivo*

Targeted Whole-Cell Recordings in the Mammalian Brain In Vivo



Точечная whole cell регистрация *in vivo*

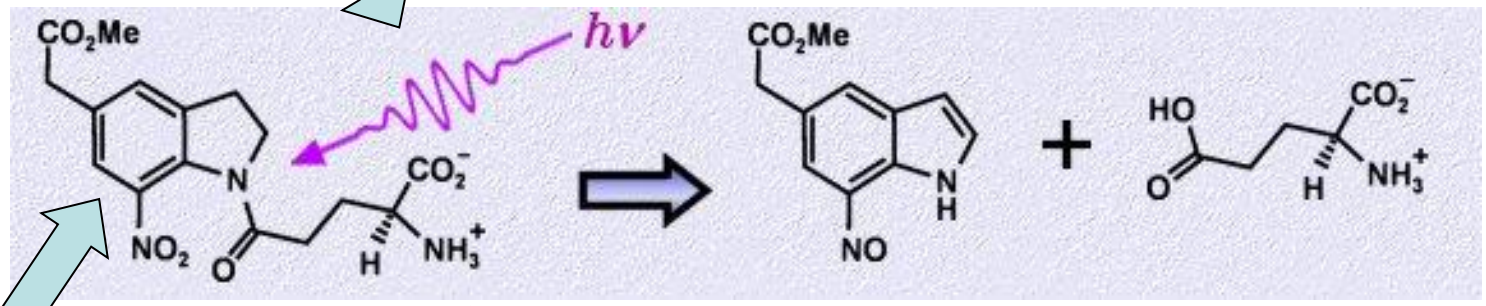




Визуальное и электрофизиологическое подтверждение успешного контакта

Фотолиз

UV photon



Caging compound

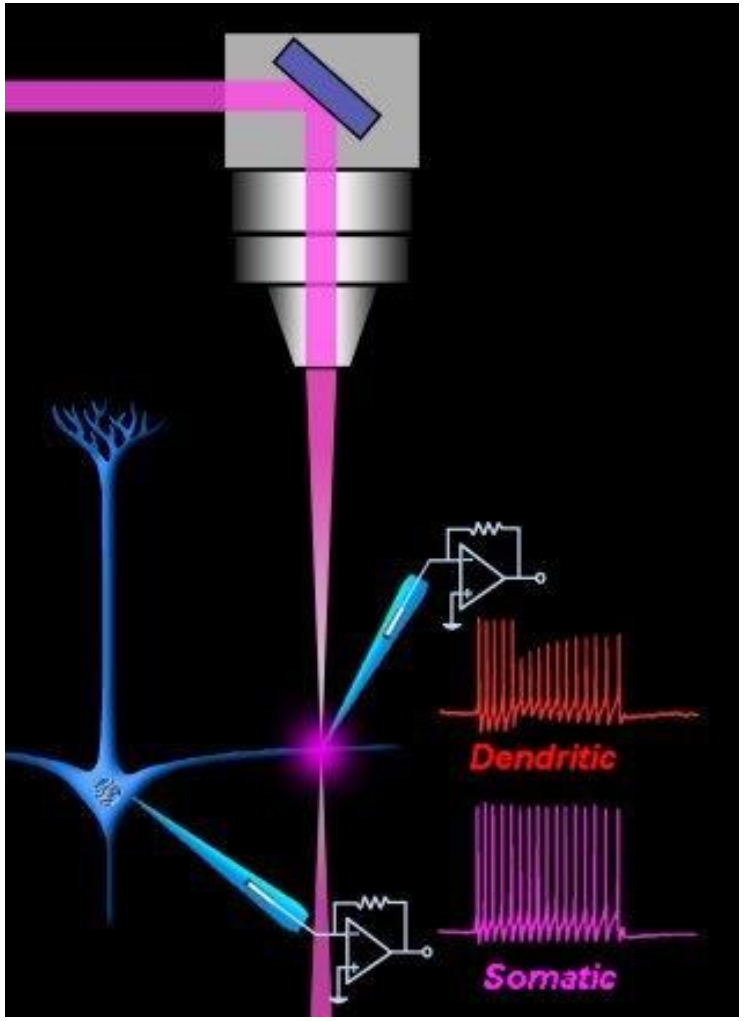
Glutamate

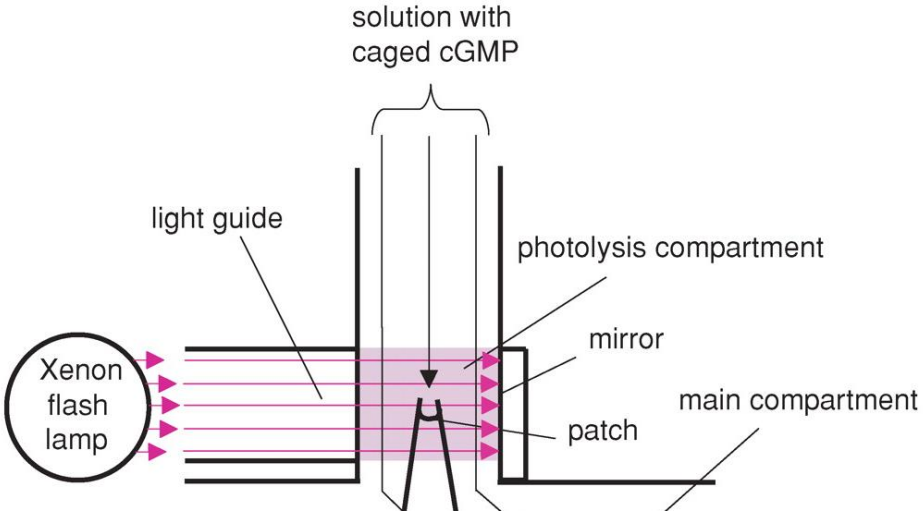
Requirements of caging compounds/ systems:

- Masks the biomolecule completely
- Releases the biomolecule completely
- No deleterious by-products
- No biomolecule breakdown

http://flavor.monell.org/%7Eloweg/Techniques.htm

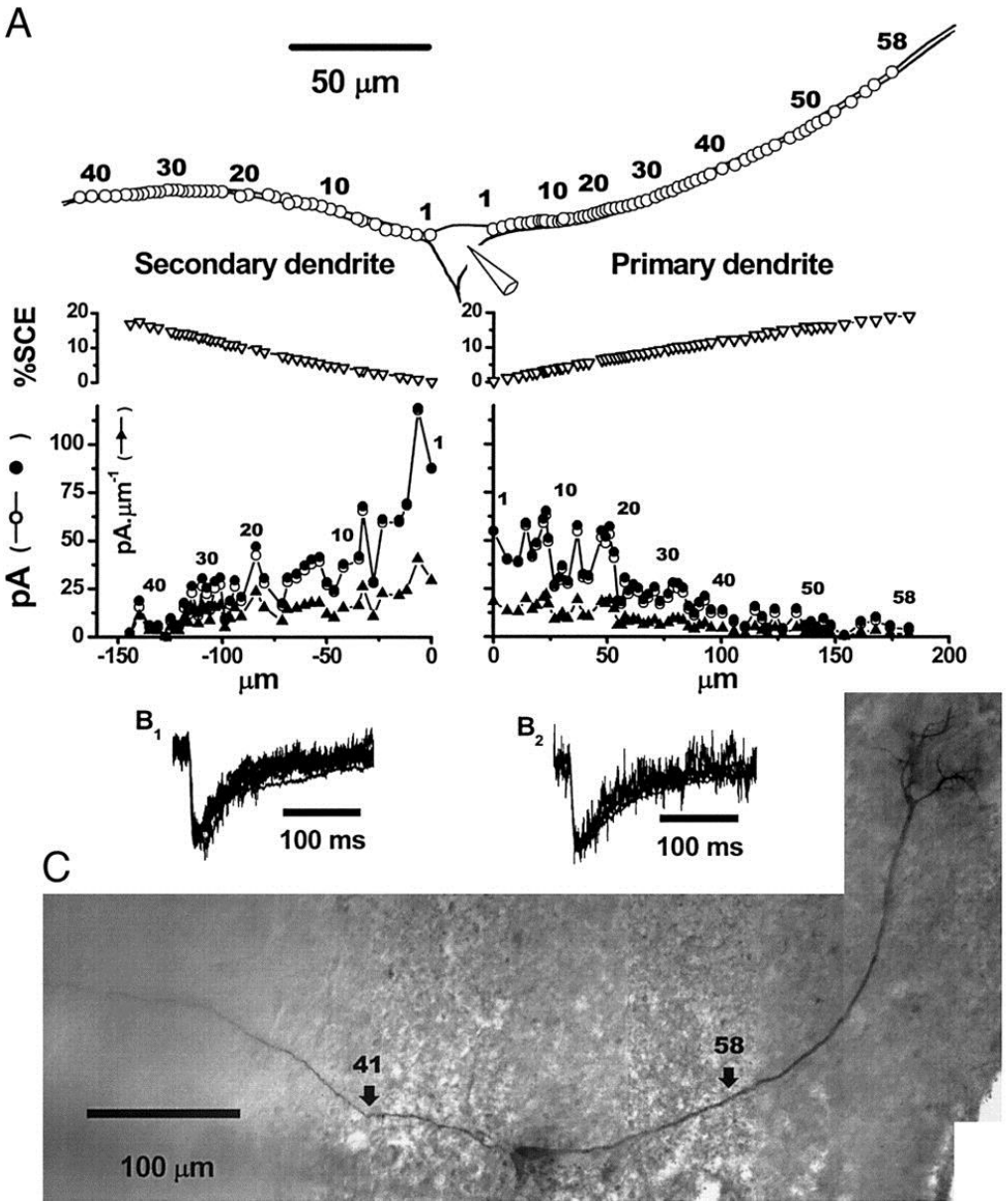
Установкка для регистрации фотолизом

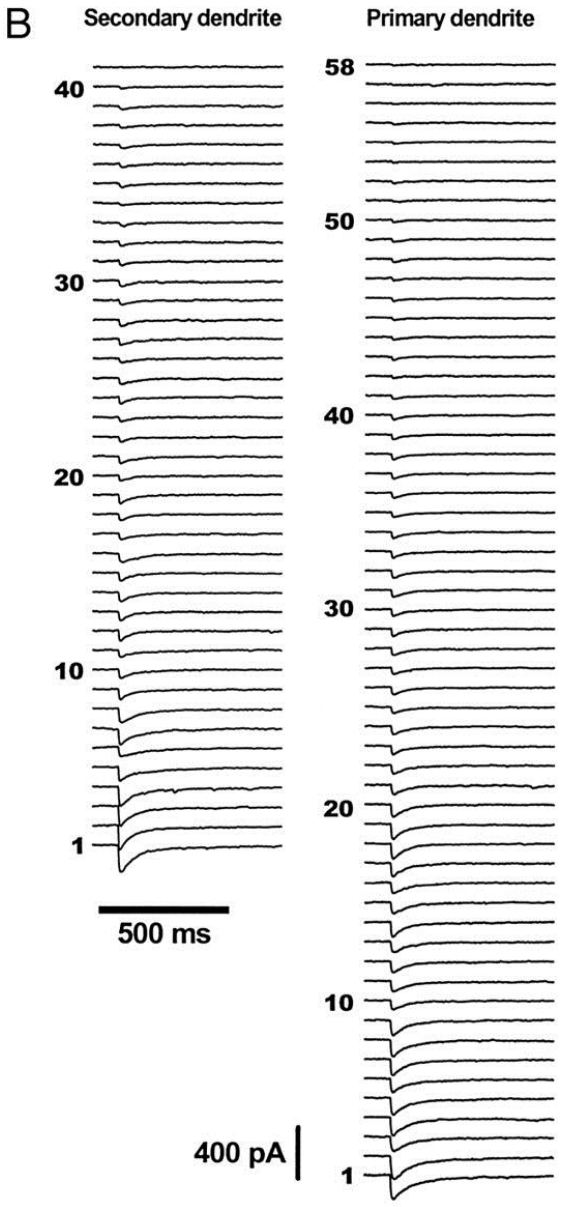




http://flavor.monell.org/%7Eloweg/Techniques.htm

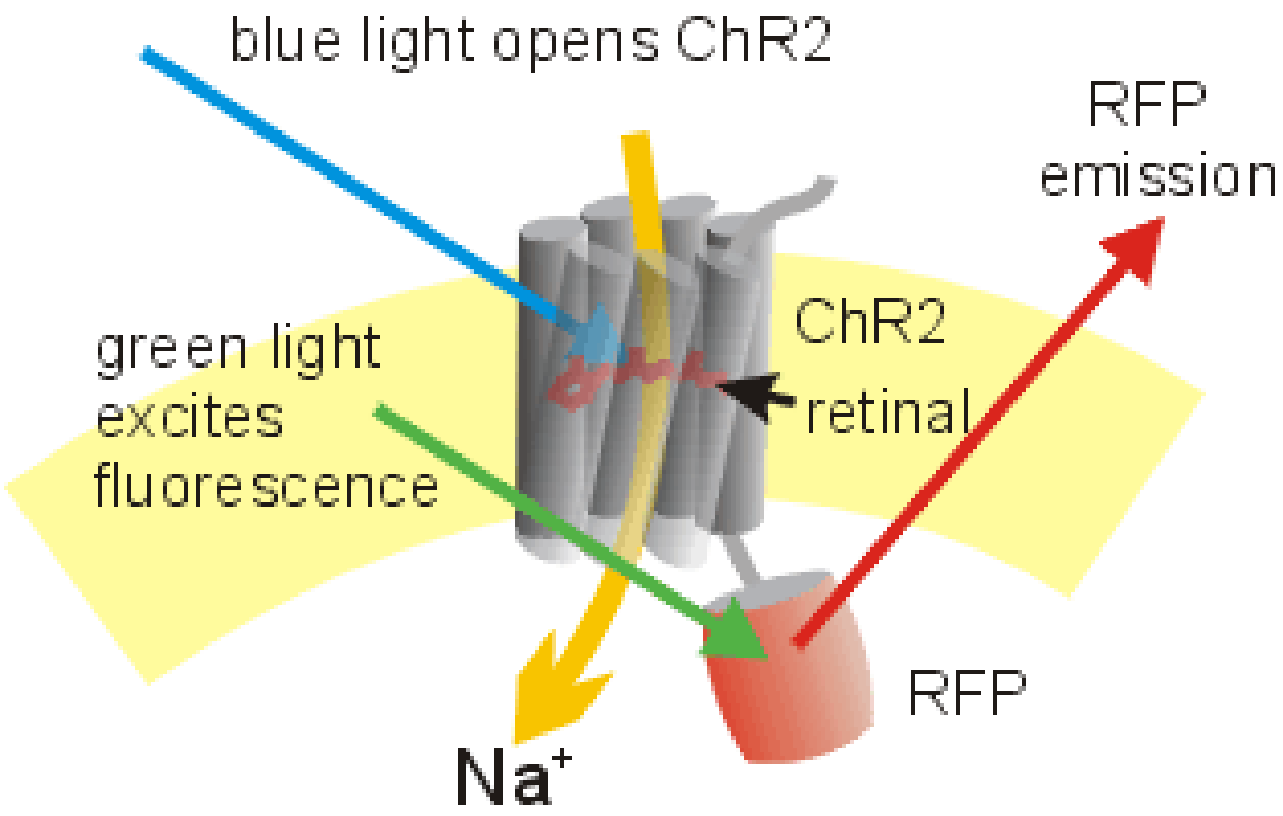
Регистрация фотолизом





Lowe, G. J Neurophysiol 88: 64-85 2002

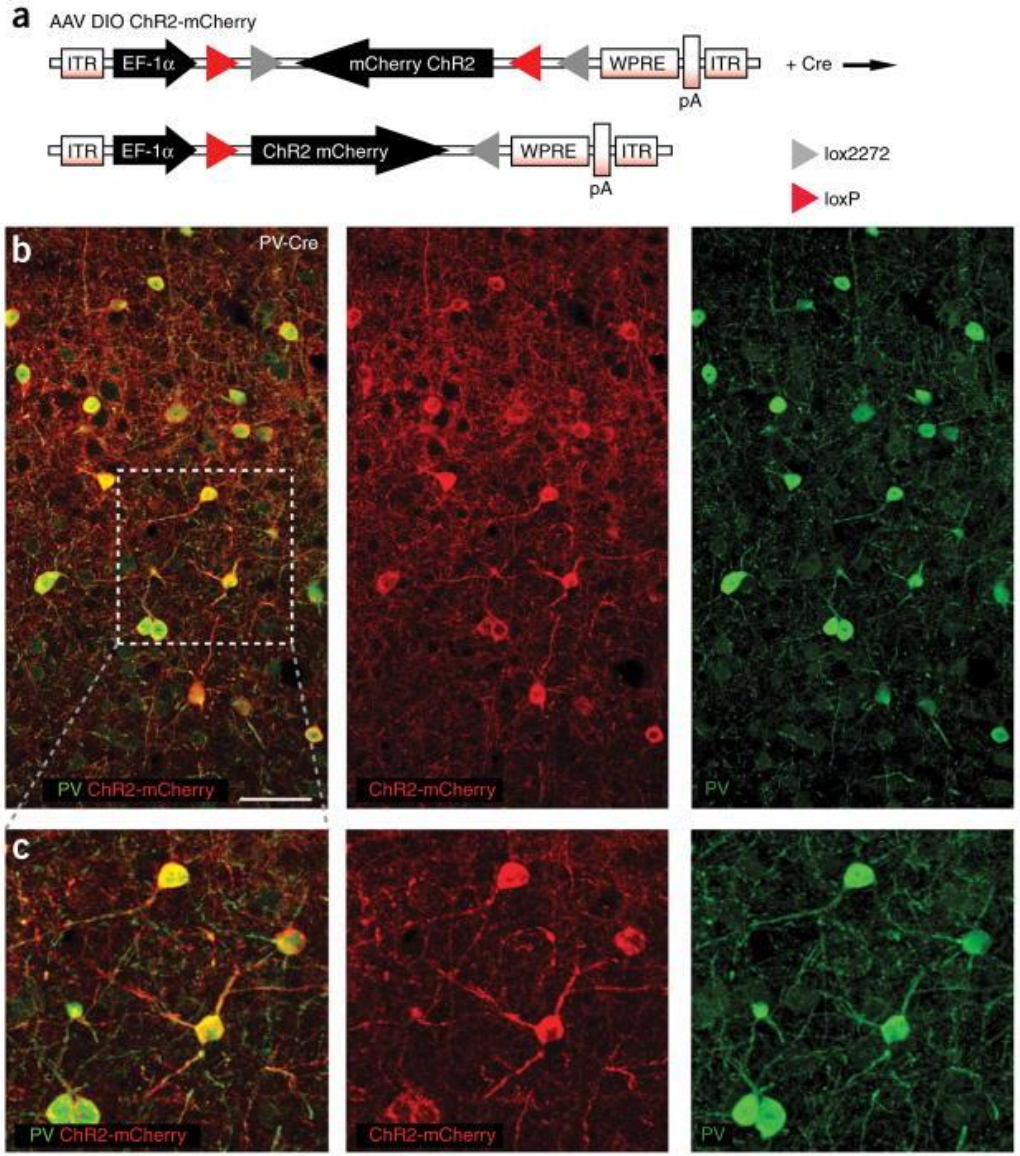
Оптогенетика



Channel Rhodopsin

Light-activated channels originally isolated from an algae. Non-selective cation channel, so opening induced by blue light can be used to depolarize neurons transfected to express ChR

Оптогенетика



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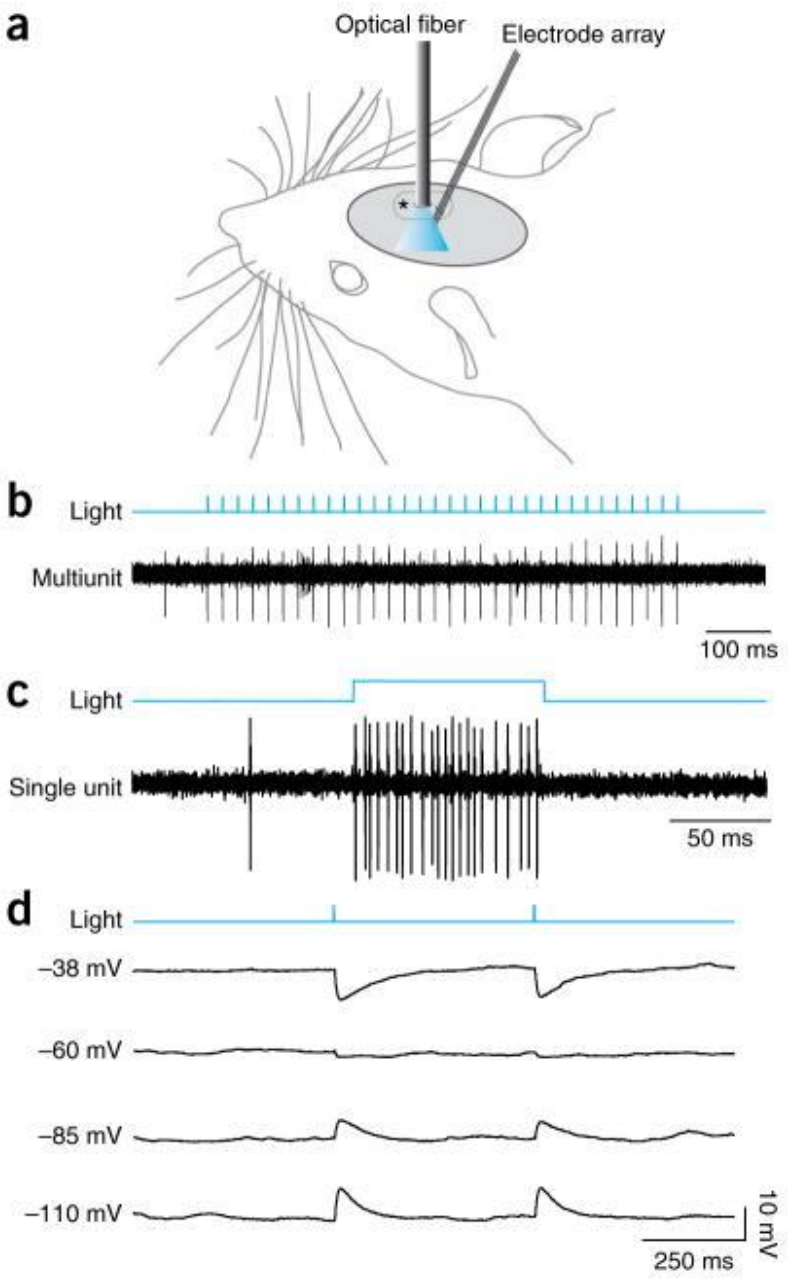
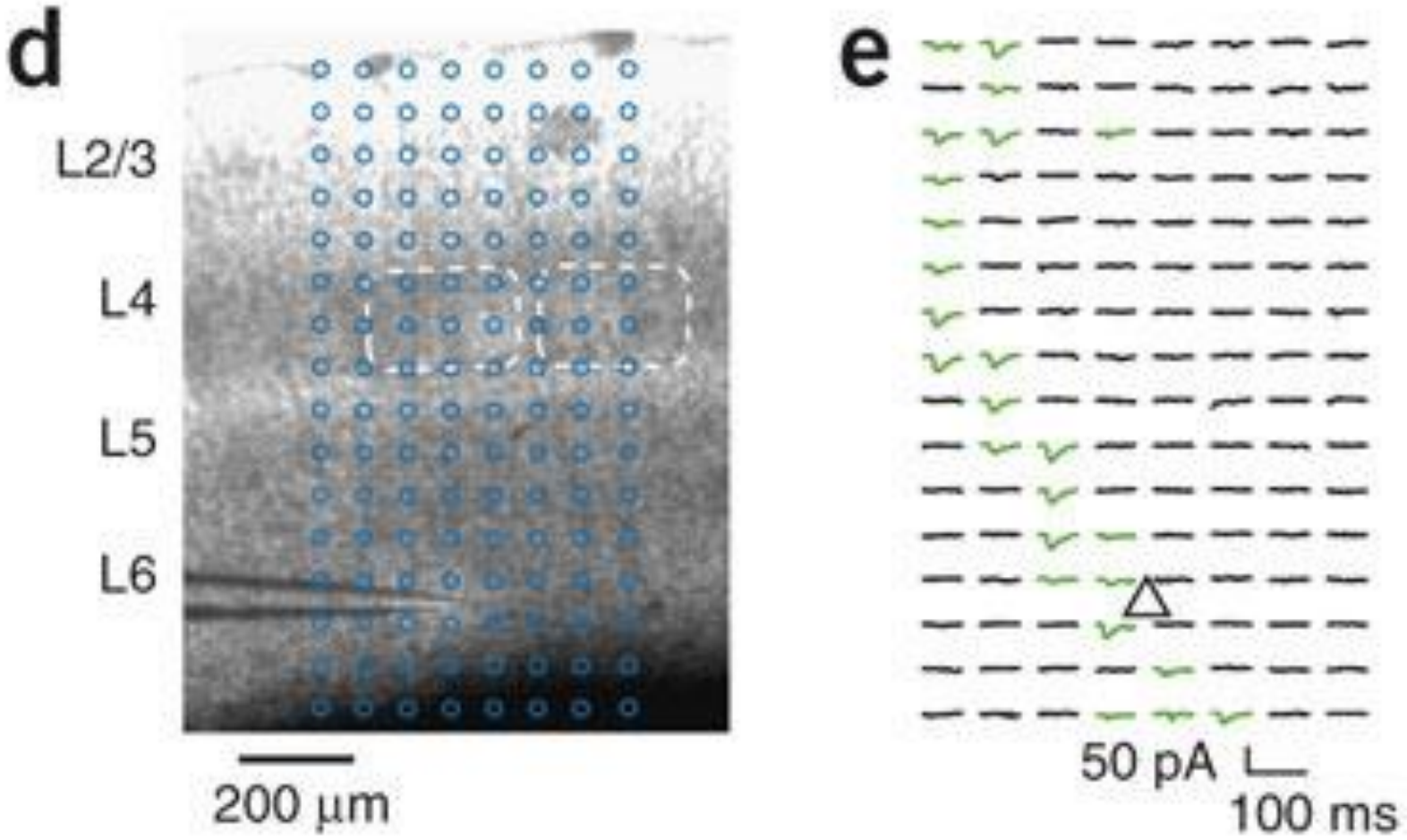


Оптогенетика

Картирование нейрональных порекций активацией ChR2



Leopoldo Petreanu, Daniel Huber, Aleksander Sobczyk & Karel Svoboda
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Intrinsic imaging