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Kiev
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Оптогенетические методы исследования

26.05.2017

План

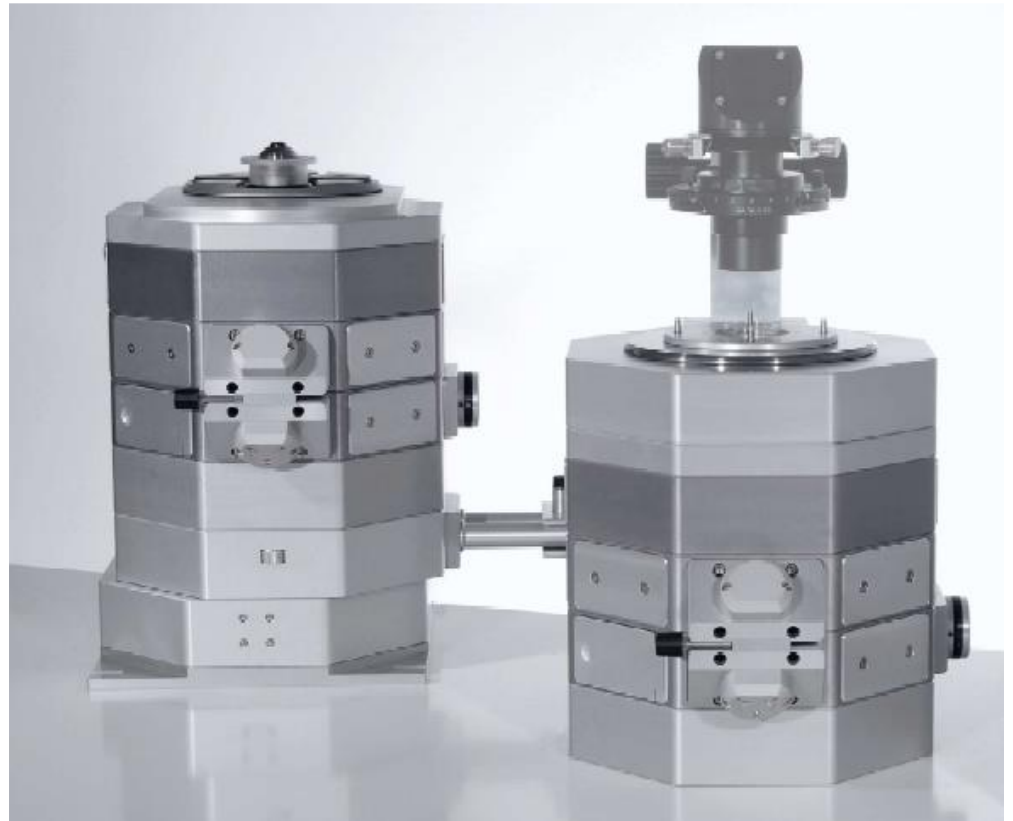
- *Флуоресцентная микроскопия основанная на применении флуоресцентных белков, Green Fluorescent Protein (GFP)*
- *Оптогенетика*

История развития оптических микроскопов

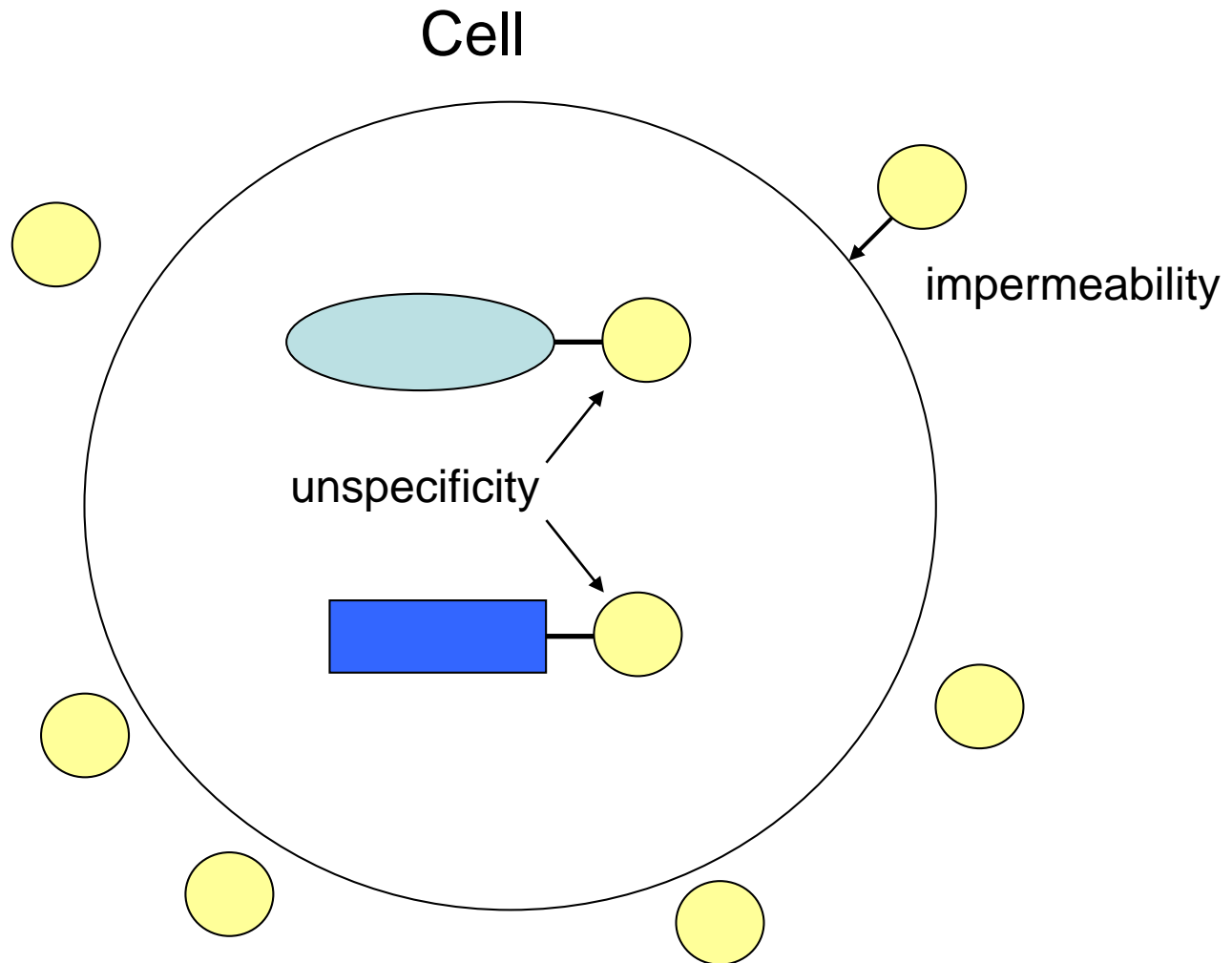
Compound microscope



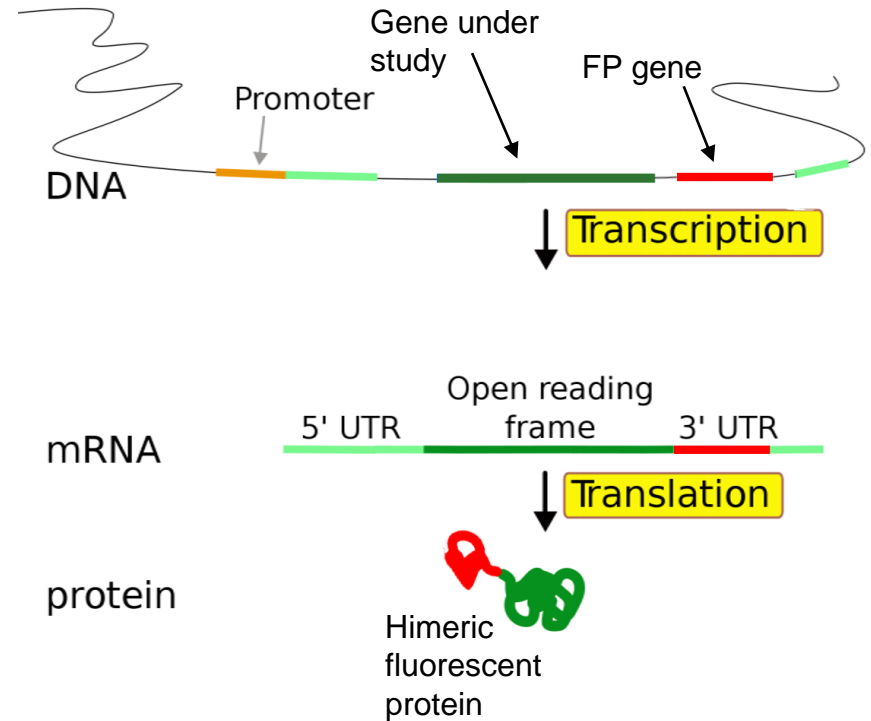
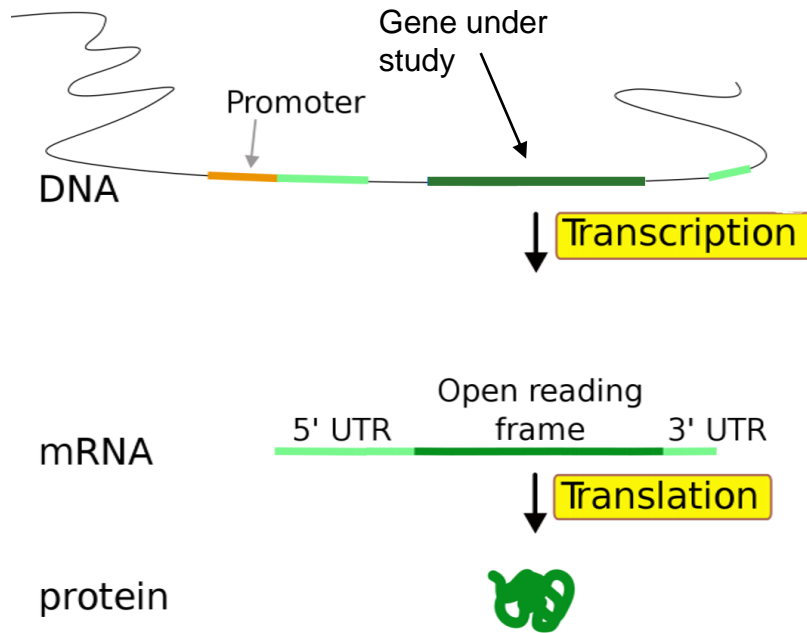
iMIC Digital Microscopes



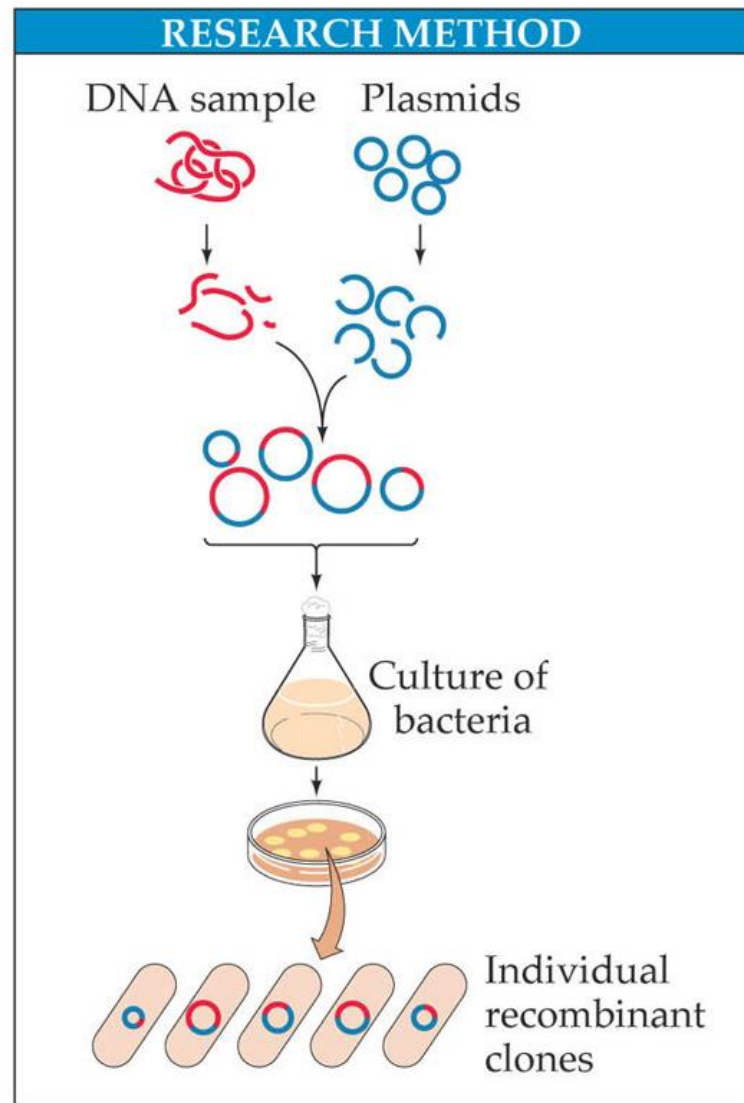
Проблемы введения флуоресцентных меток



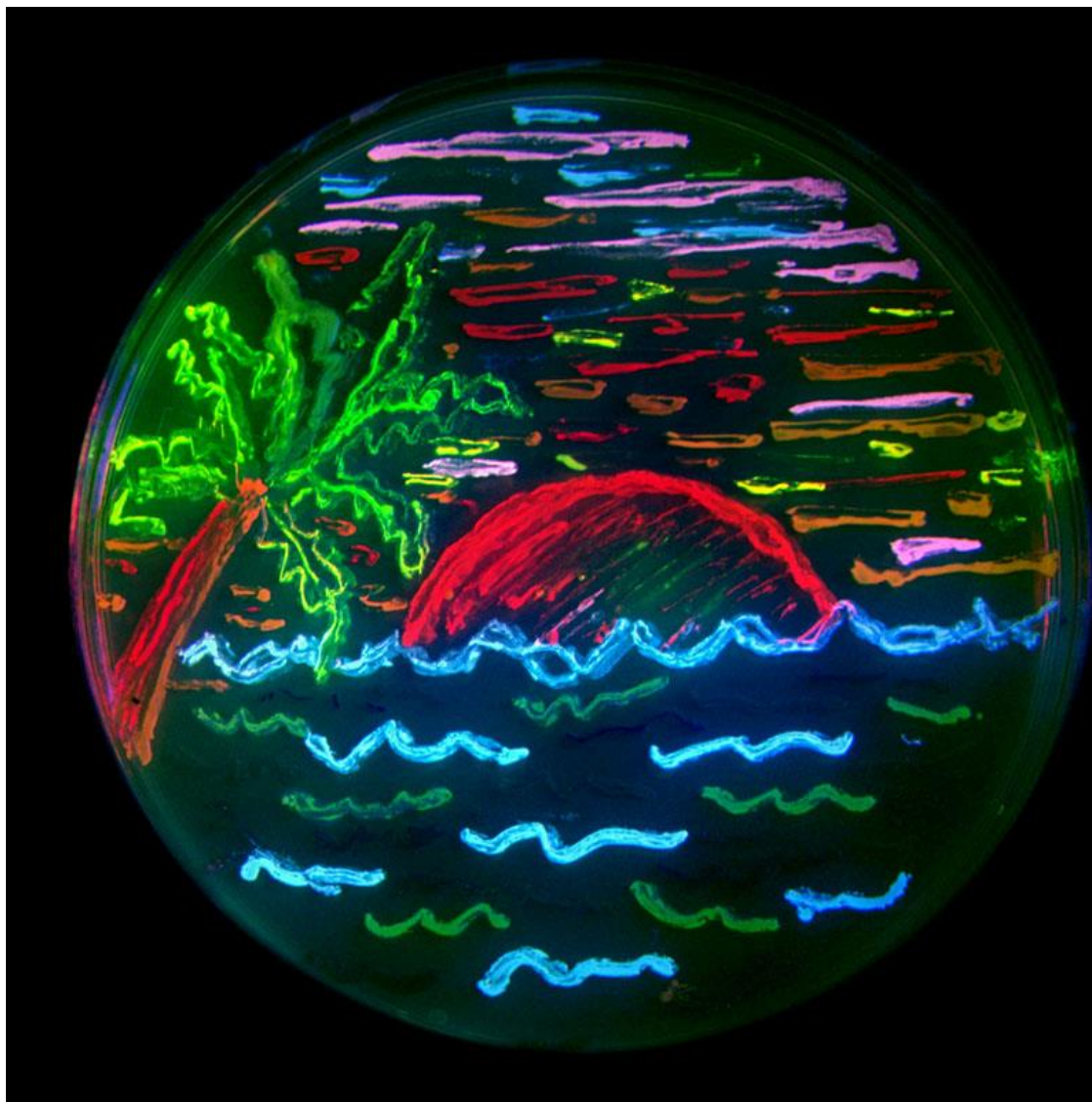
Химерная экспрессия генов



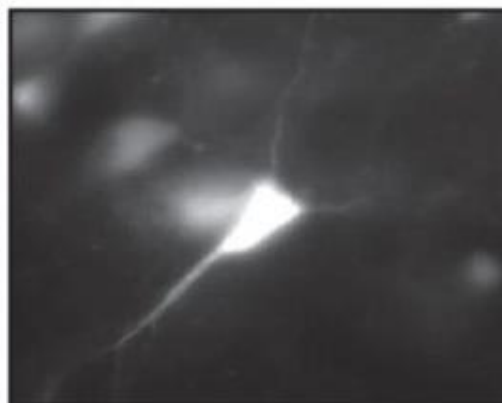
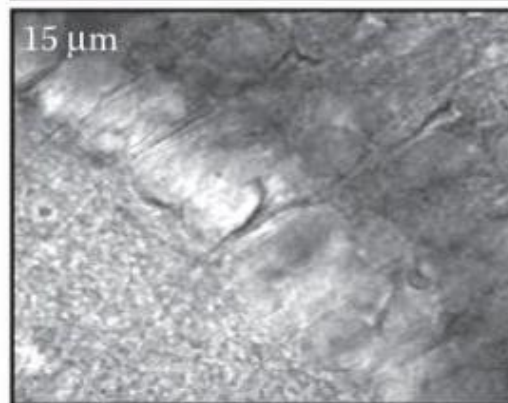
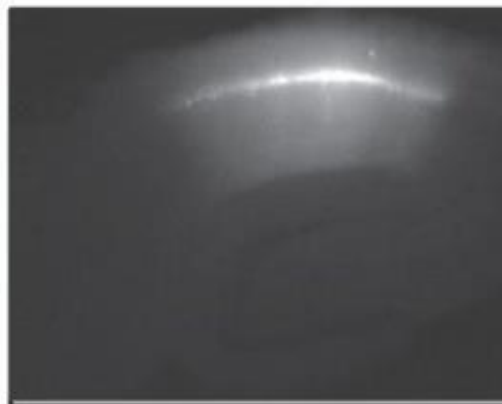
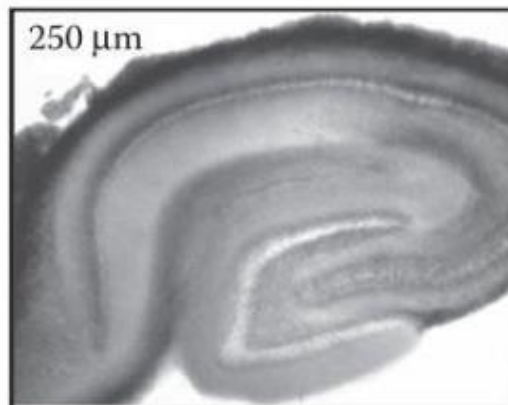
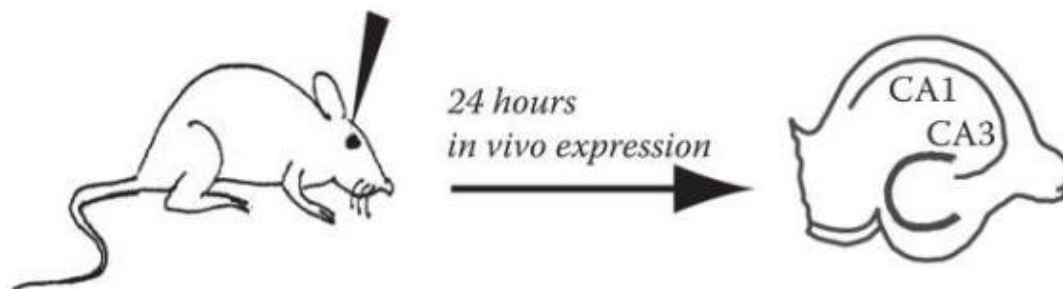
Конструирование плазмид



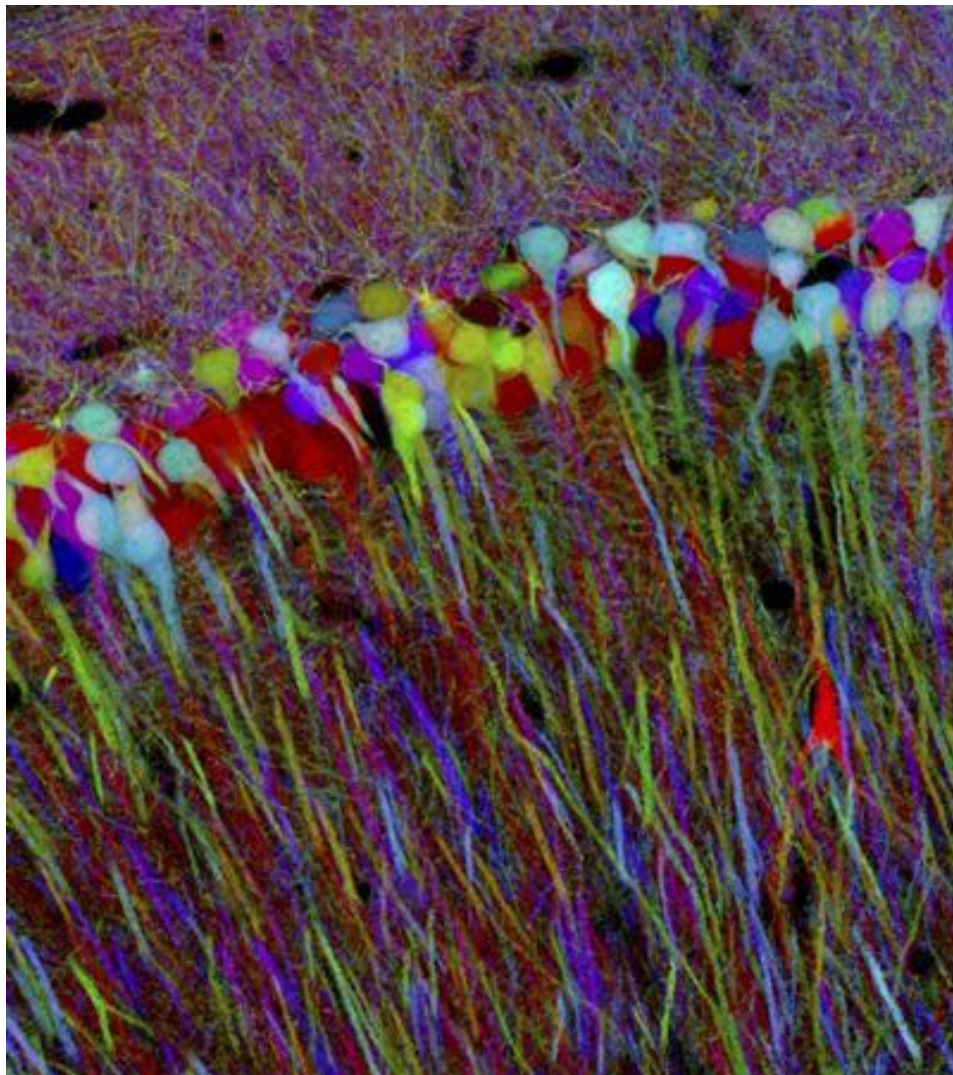
Зеленый флуоресцентный белок (GFP)



Методика введения вирусов



Пирамидные нейроны «радужных» крыс



- Флуоресцентные белки вместе с разнообразными способами доставки их генов внутрь клеток являются мощными инструментом исследований в области клеточной биологии, позволяющими исследовать динамику белков и клеточных органелл в живых клеточных системах.

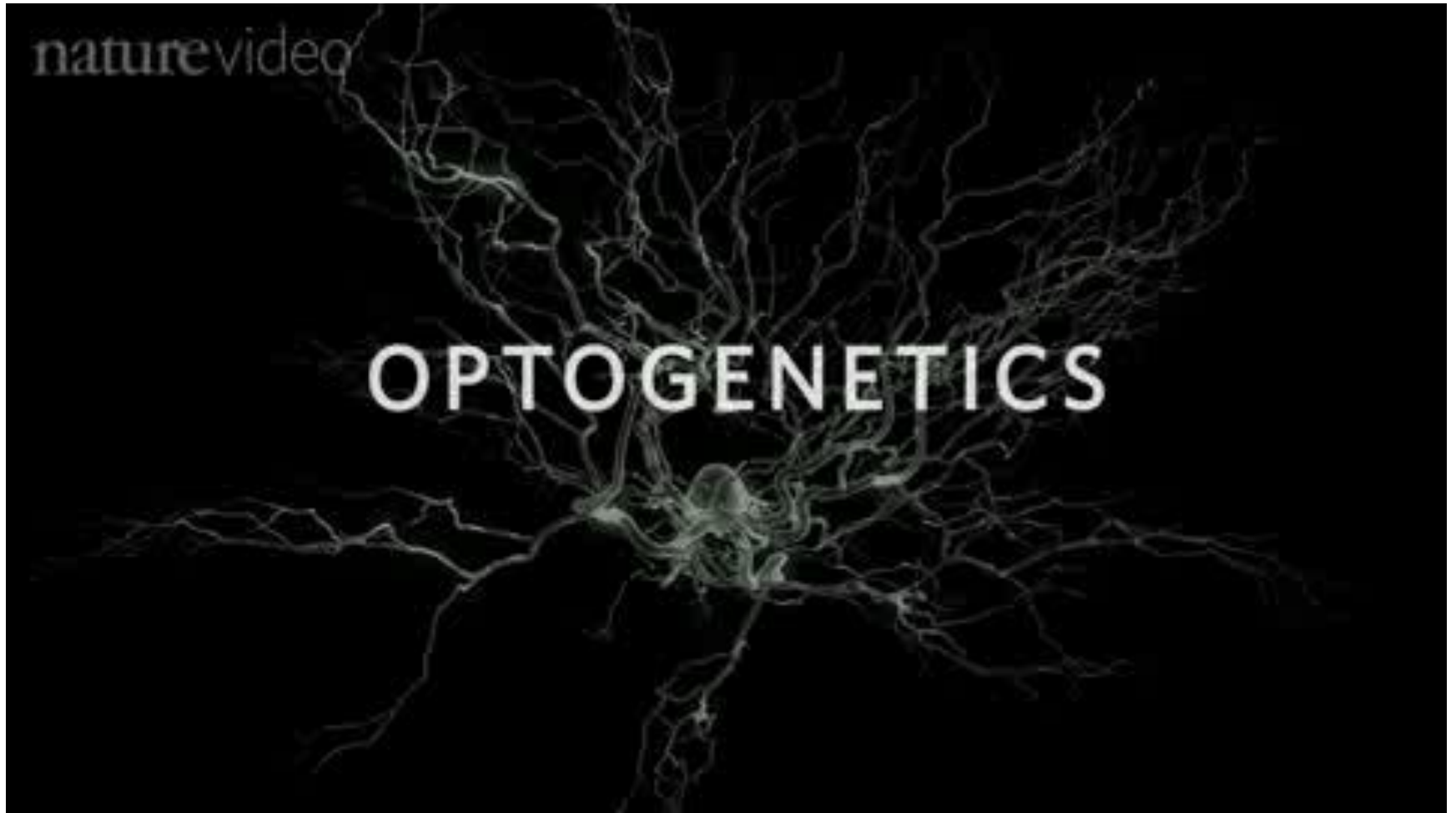
- Но можно ли не только исследовать клеточные процессы, но и управлять ими?

Оптогенетика (Optogenetics)

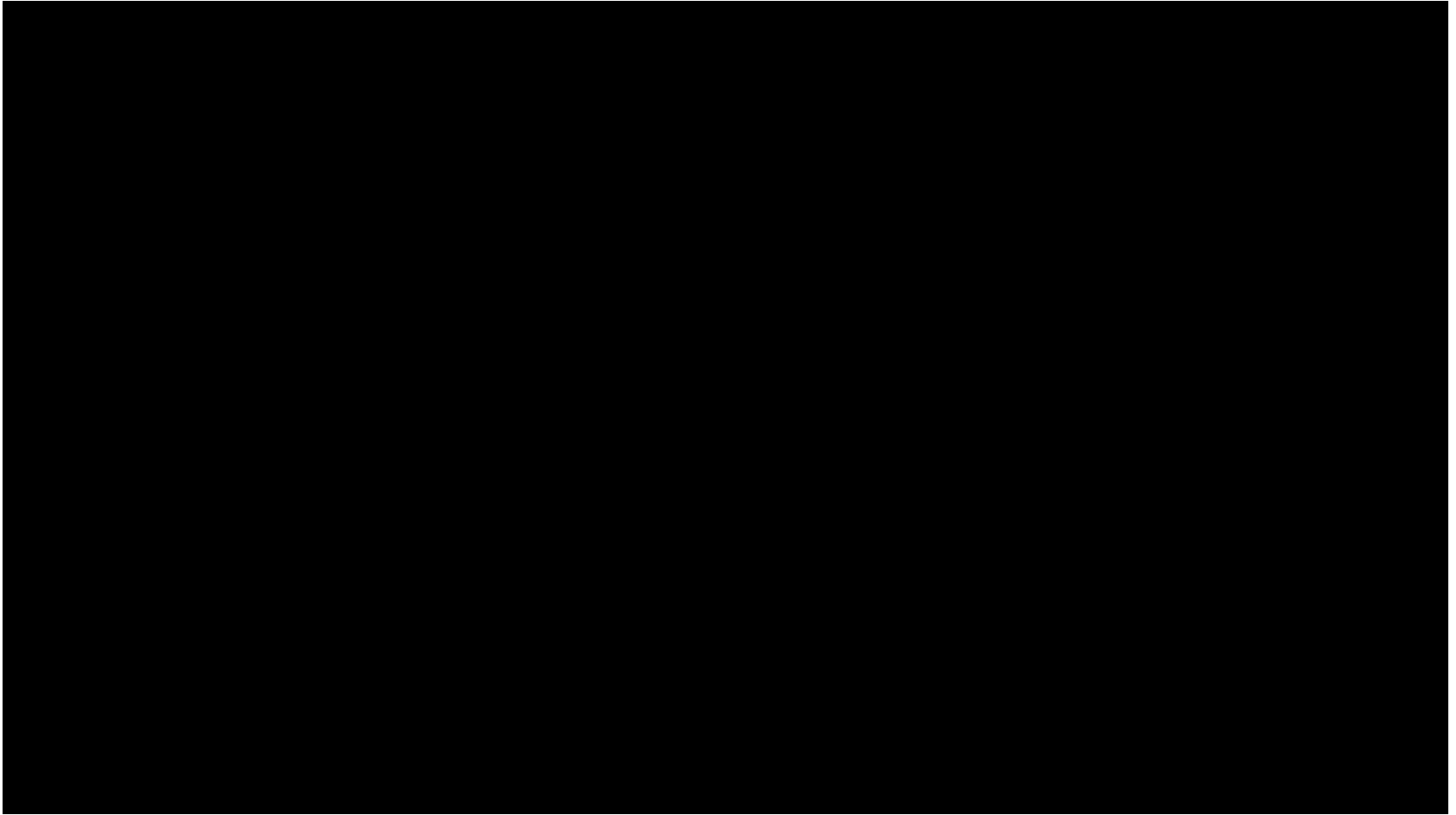
Оптогенетика это комбинация генетических и оптических методов с целью контролировать специфические события в определенных клетках живых тканей с миллисекундным временным разрешением.

Optogenetics is the combination of genetic and optical methods to control specific events in targeted cells of living tissue, even within freely moving [mammals](#) and other animals, with the temporal precision ([millisecond](#)-timescale) needed to keep pace with functioning intact biological systems.

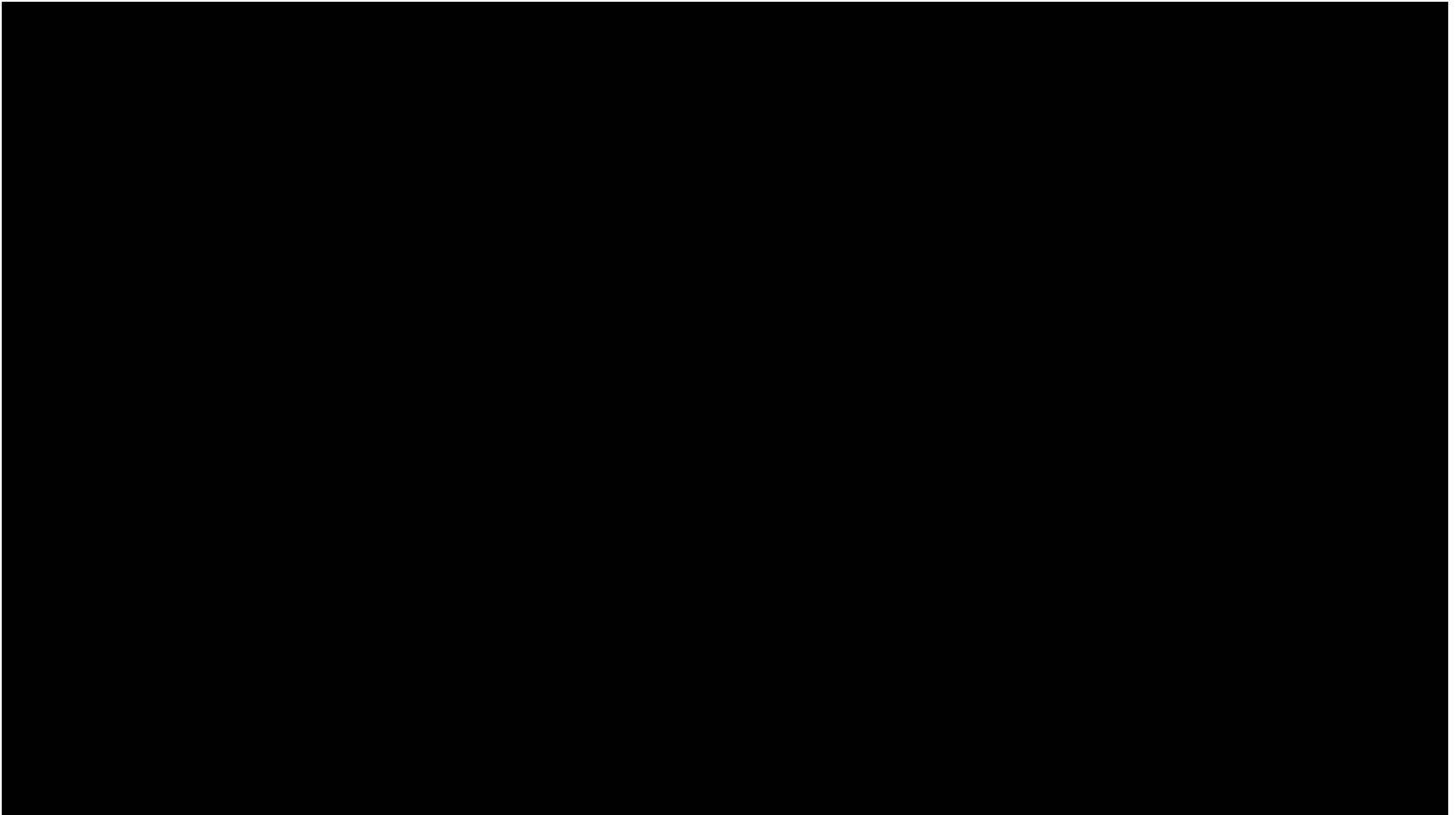
Фоточувствительные бактерии – основатели оптогенетики



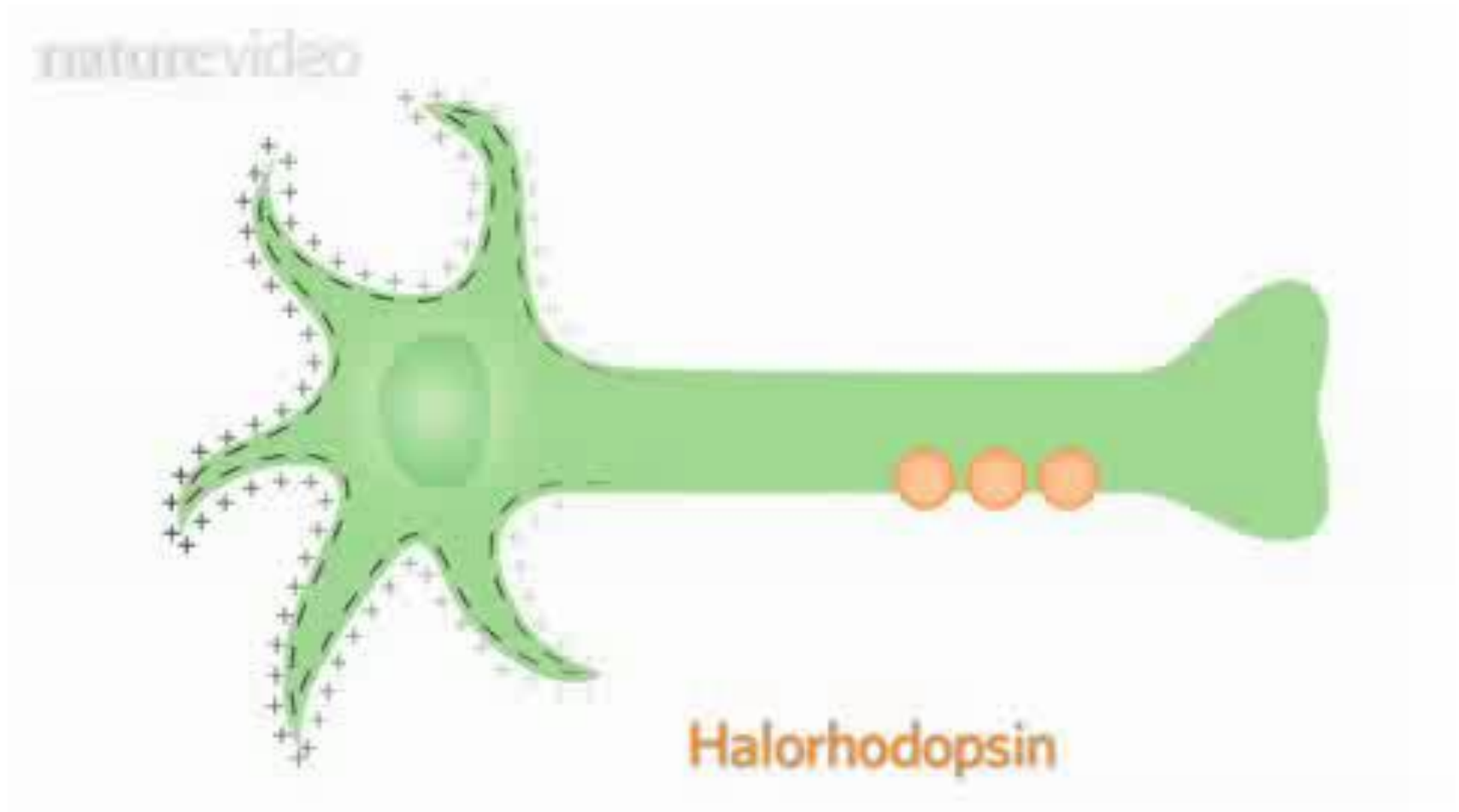
Каналородопсин Channelrhodopsin (ChR2) an electronic photoswitch exciting neurons



Examples of how Channelrhodopsins can control cells and animal behavior



Halorhodopsin (NpHR) - an electronic photoswitch inhibiting neurons

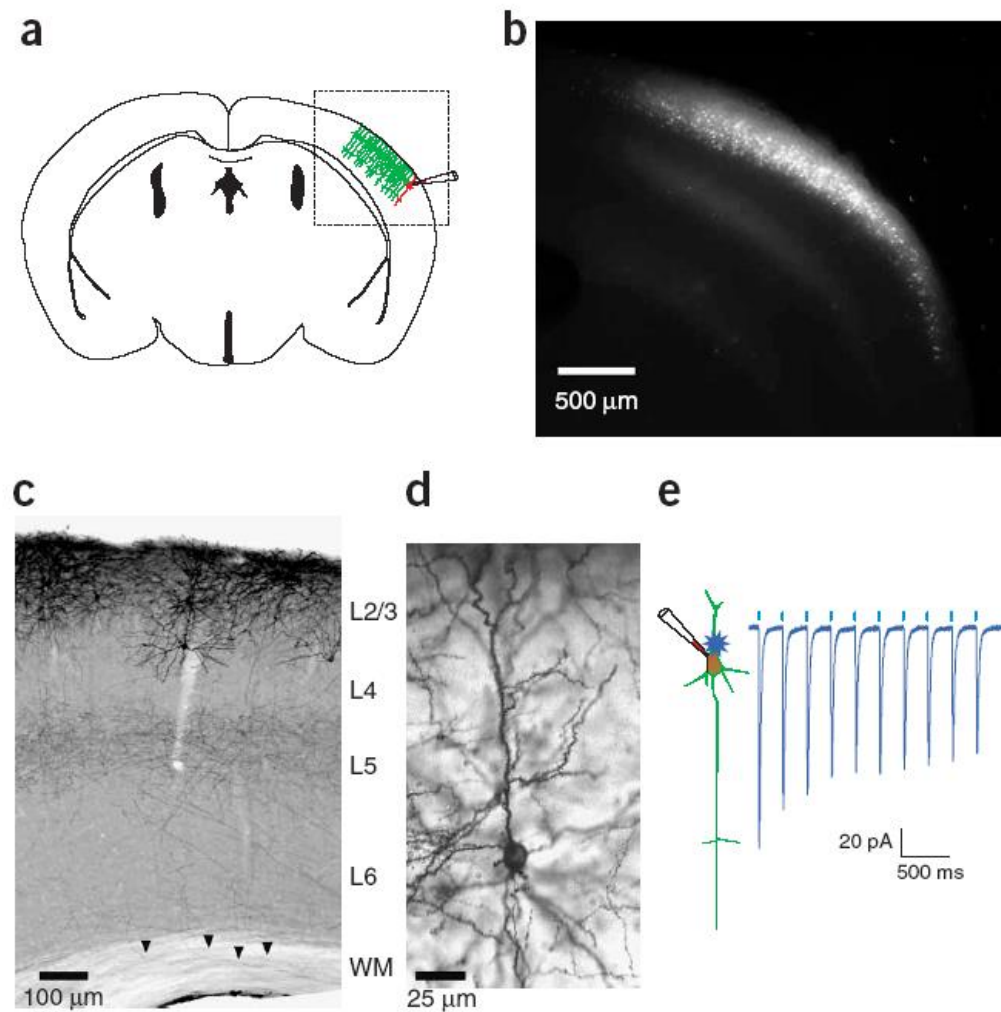


Molecular-genetic approach to introduce optogenetic constructs

naturevideo

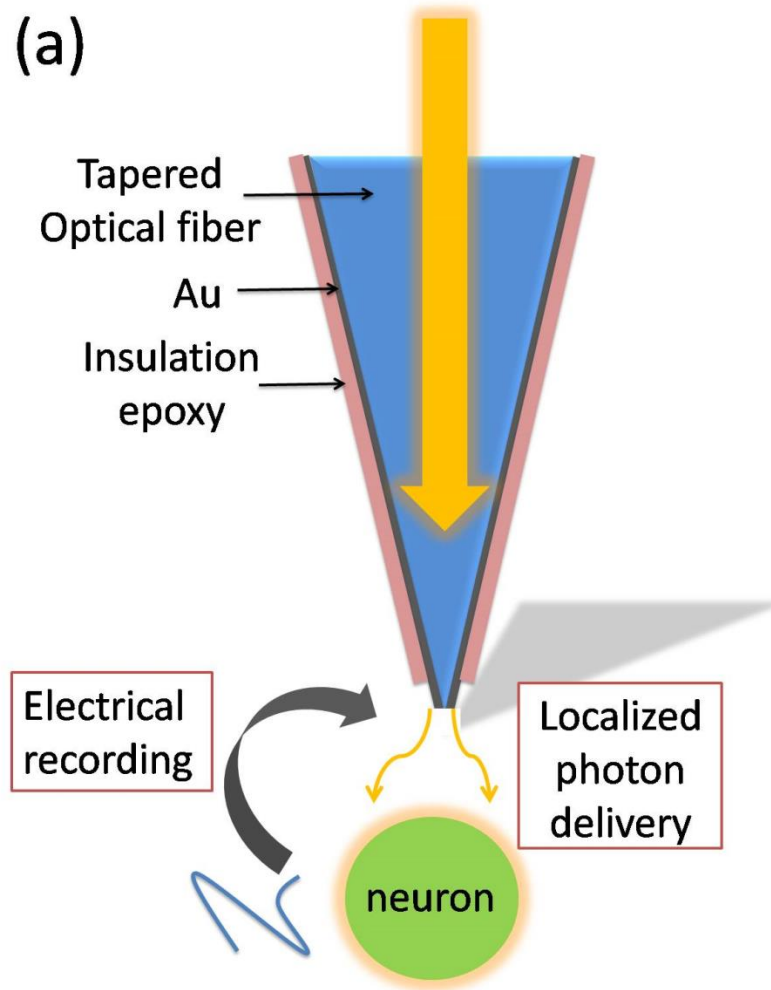


Photostimulation of ChR2-positive neocortical L2/3 pyramidal neurons

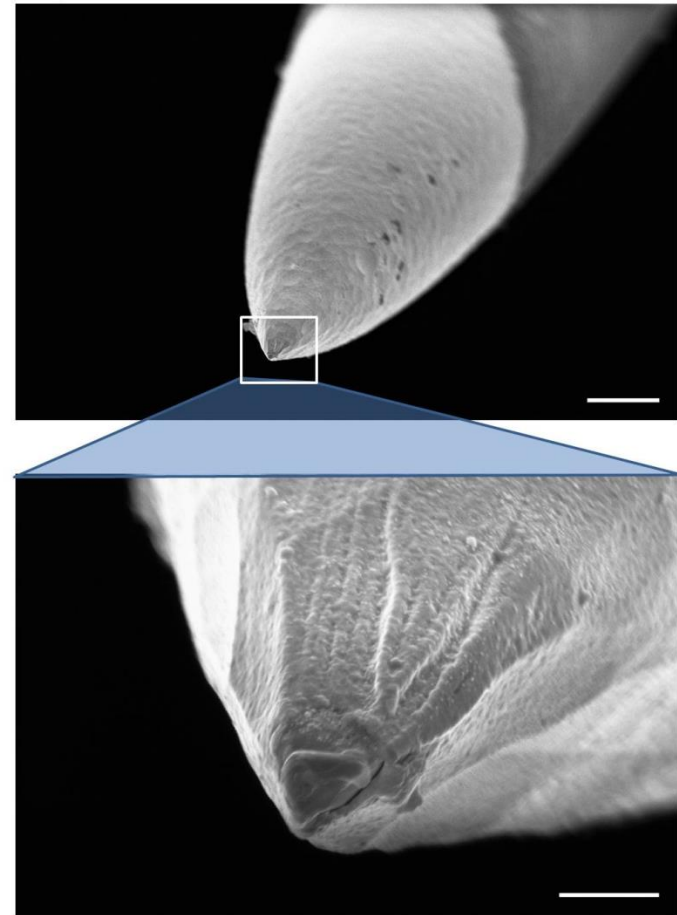


Schematics and SEM images of optrode

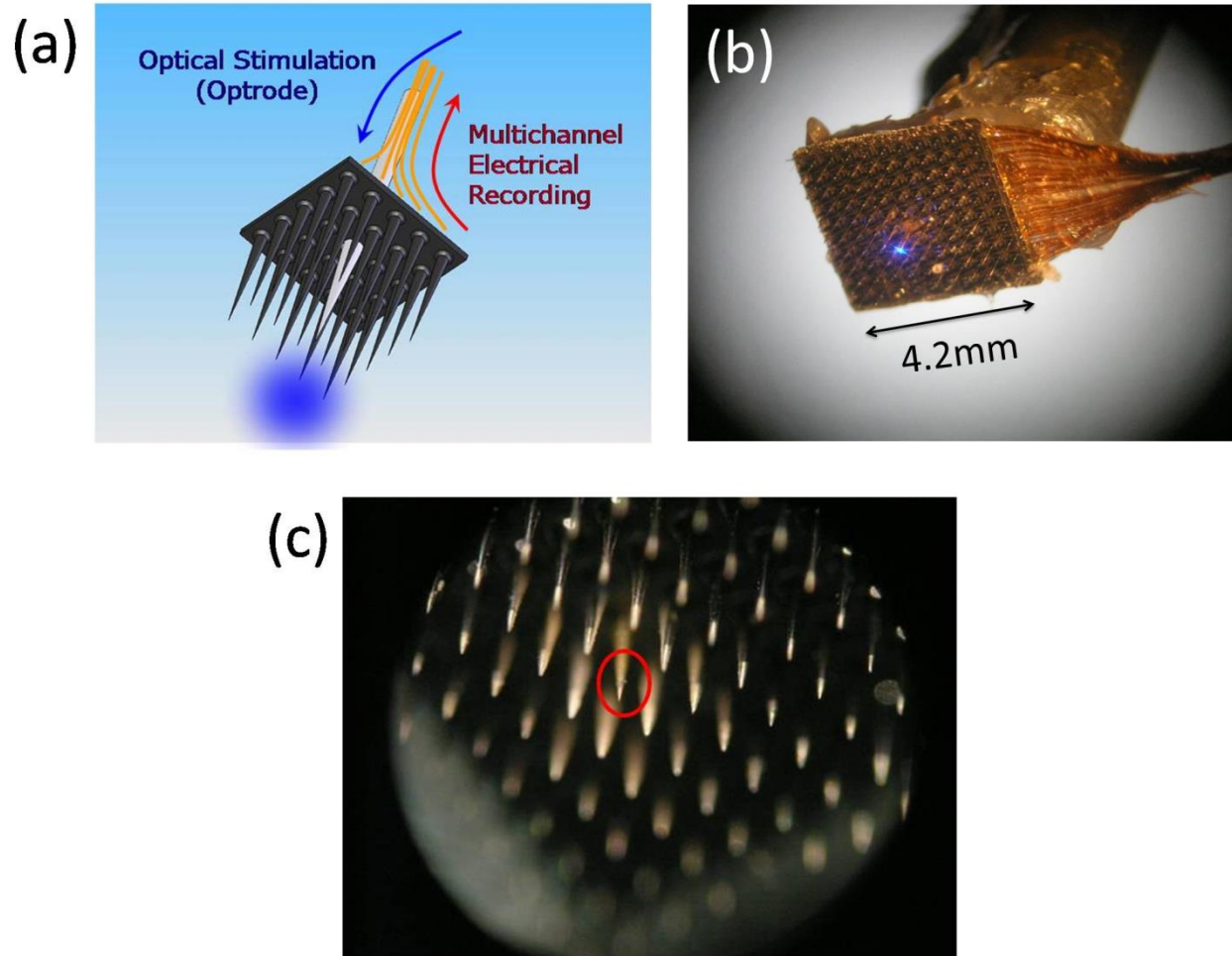
(a)



(b)



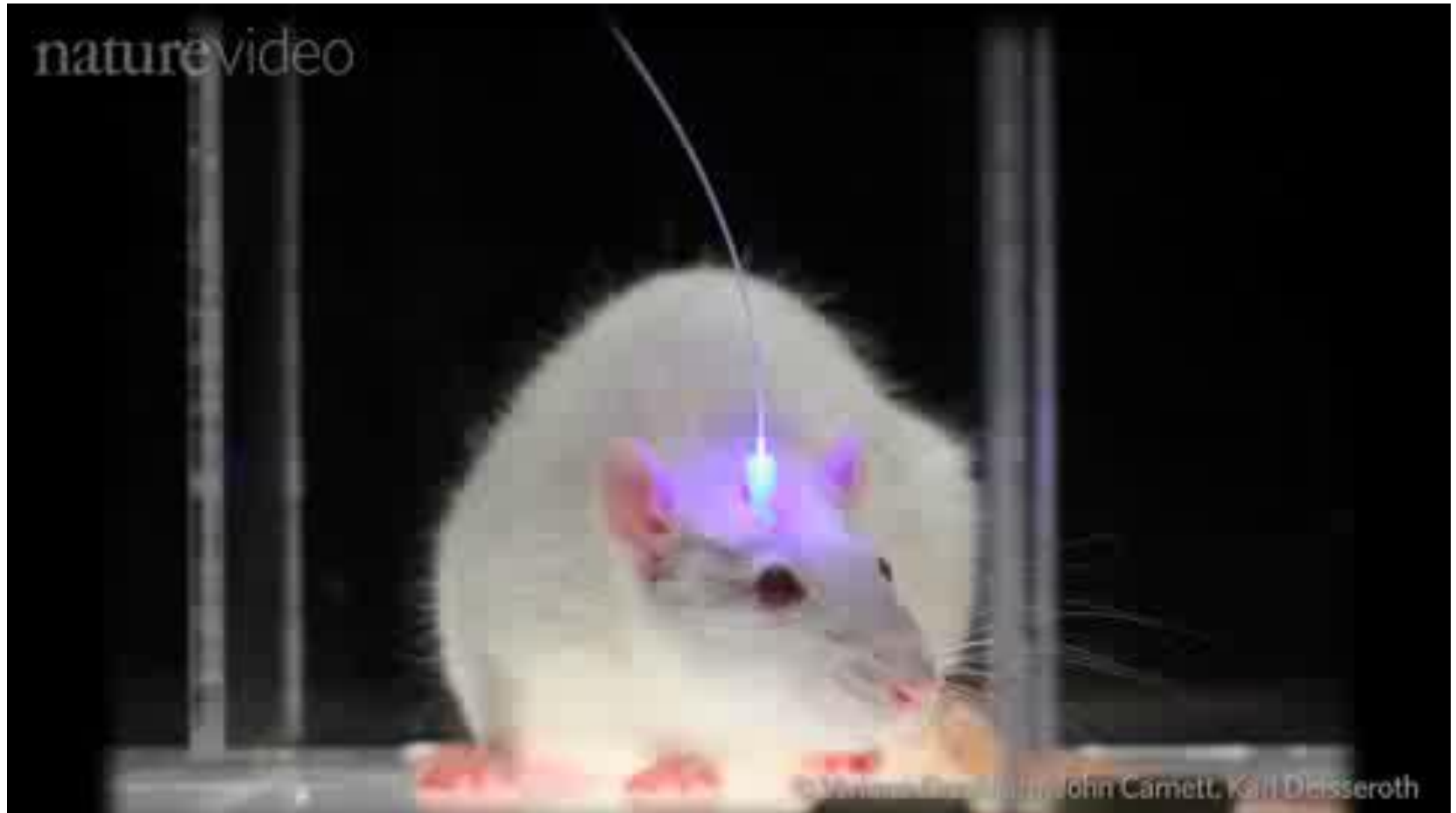
Optrode array assembly



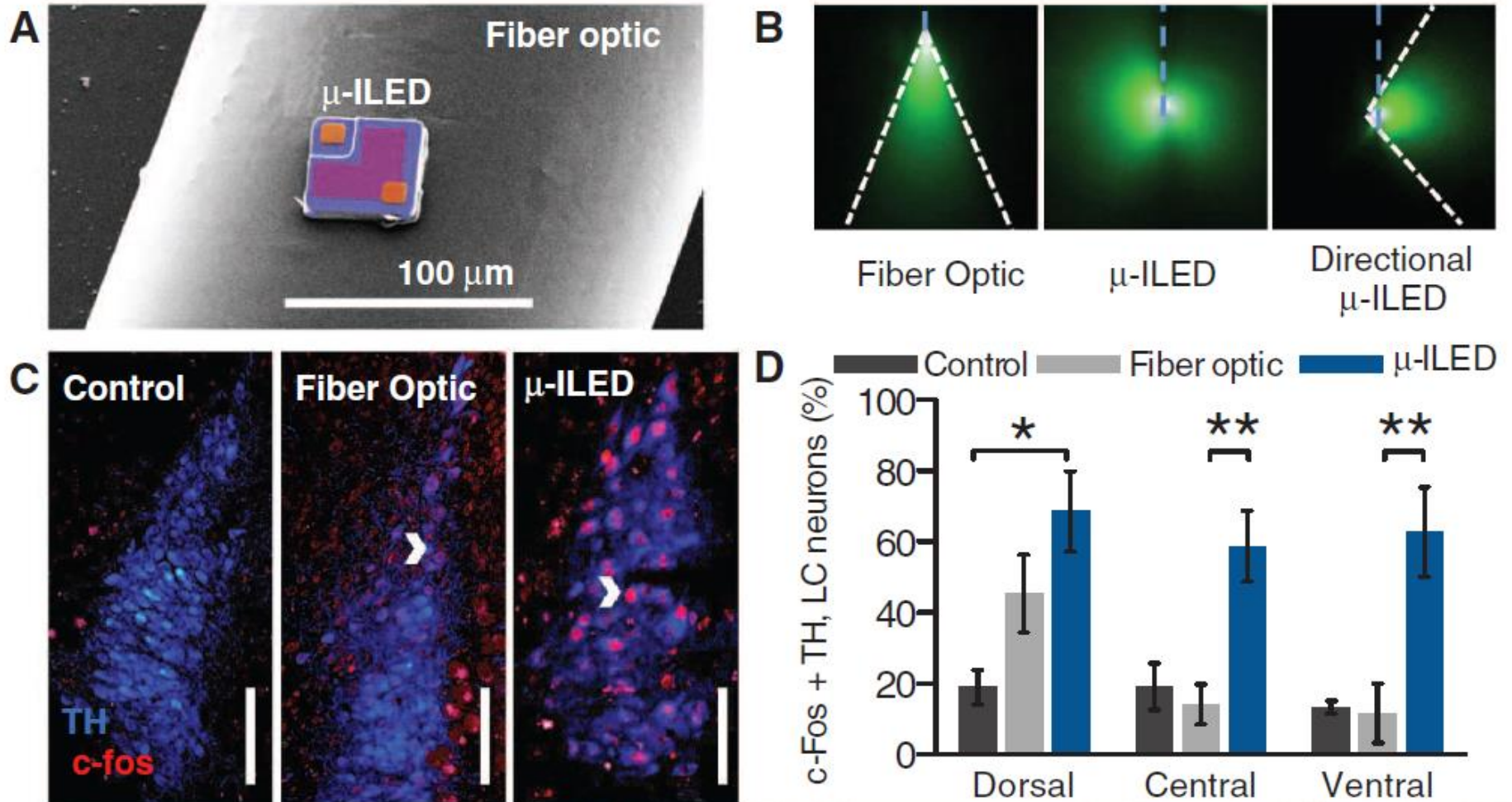
ChR2 mouse

Fiberoptic Control of Locomotion in ChR2 Mouse

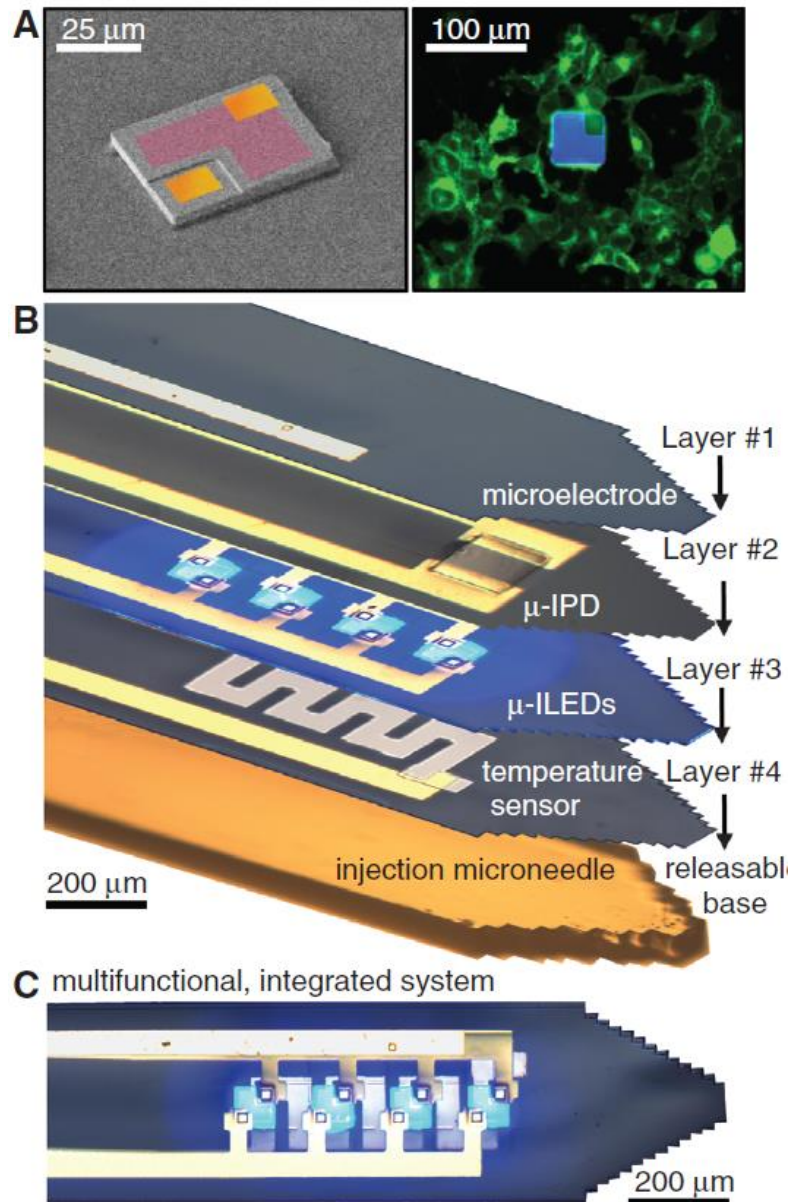
Parkinson disease treatment by optogenetic approaches



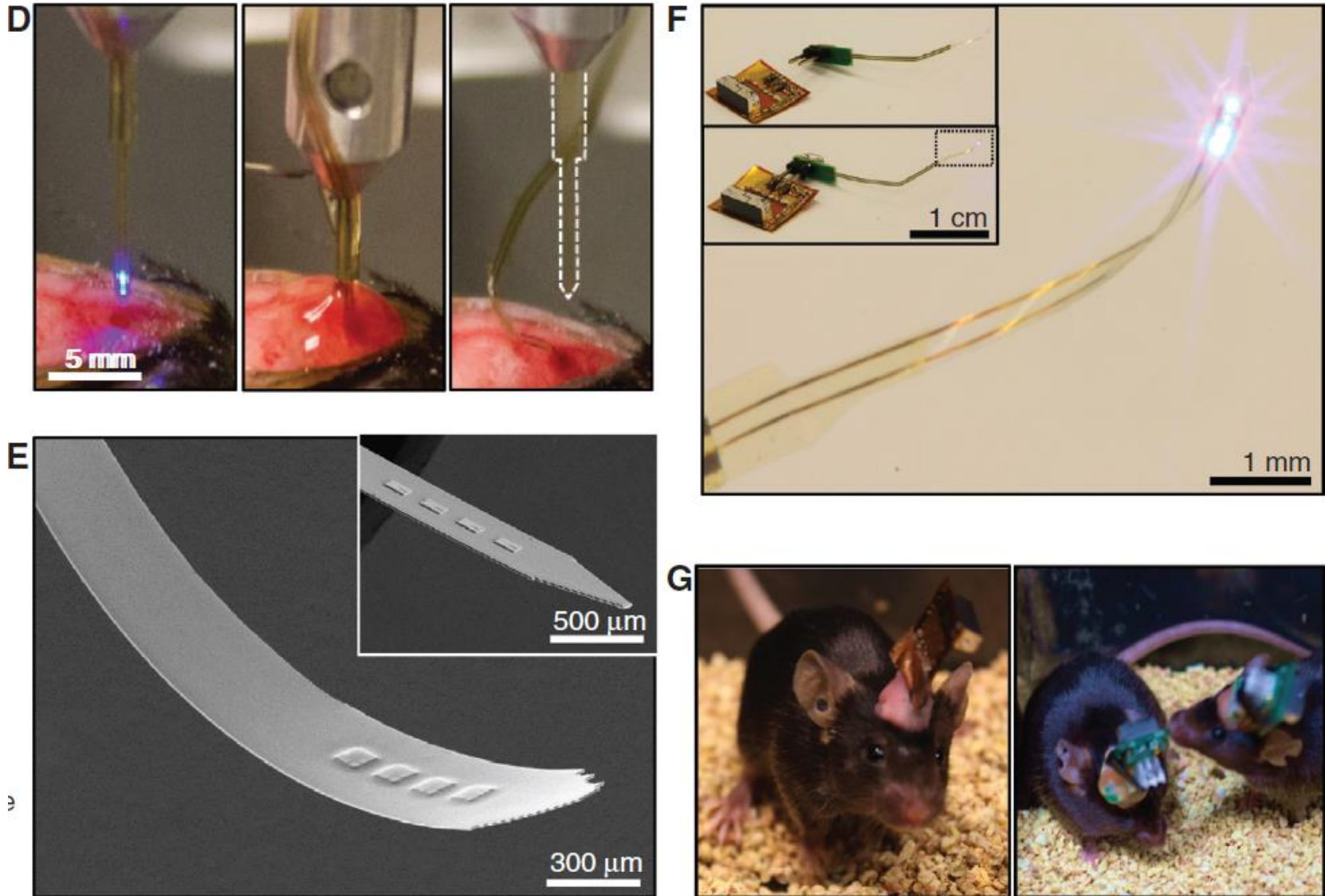
μ -ILED devices improve spatial targeting



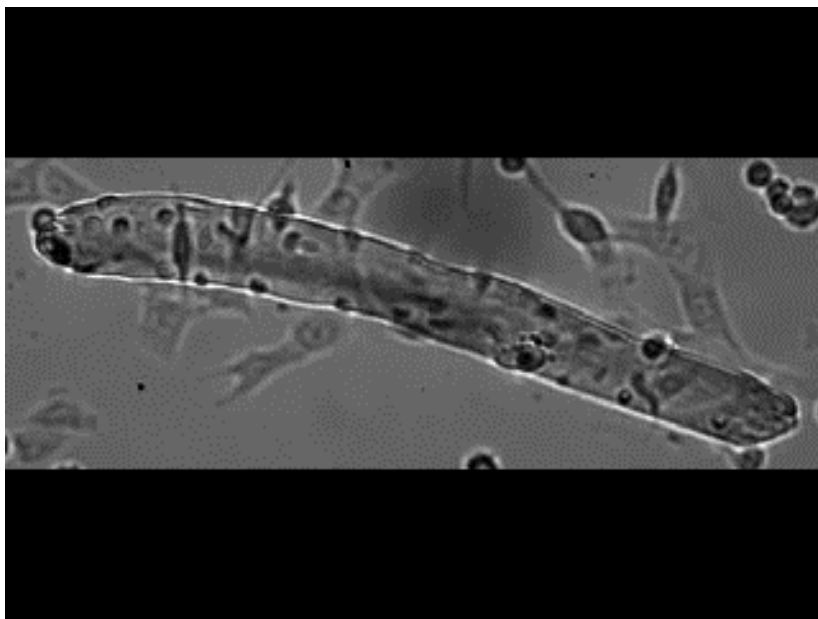
Injectable, cellular-scale semiconductor devices



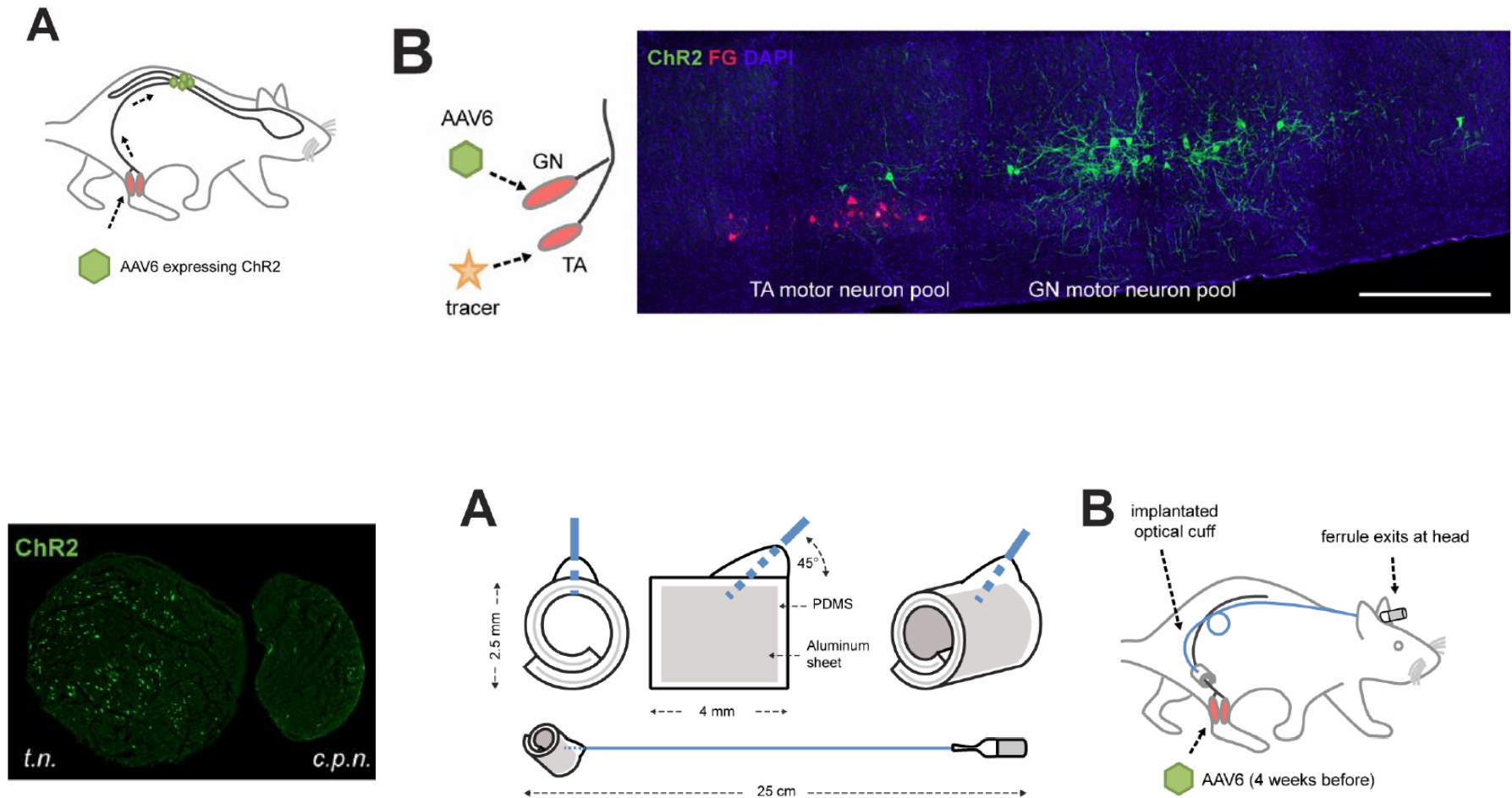
Injectable, cellular-scale semiconductor devices



Оптогенетический контроль сокращений мышц горла трансгенной мыши

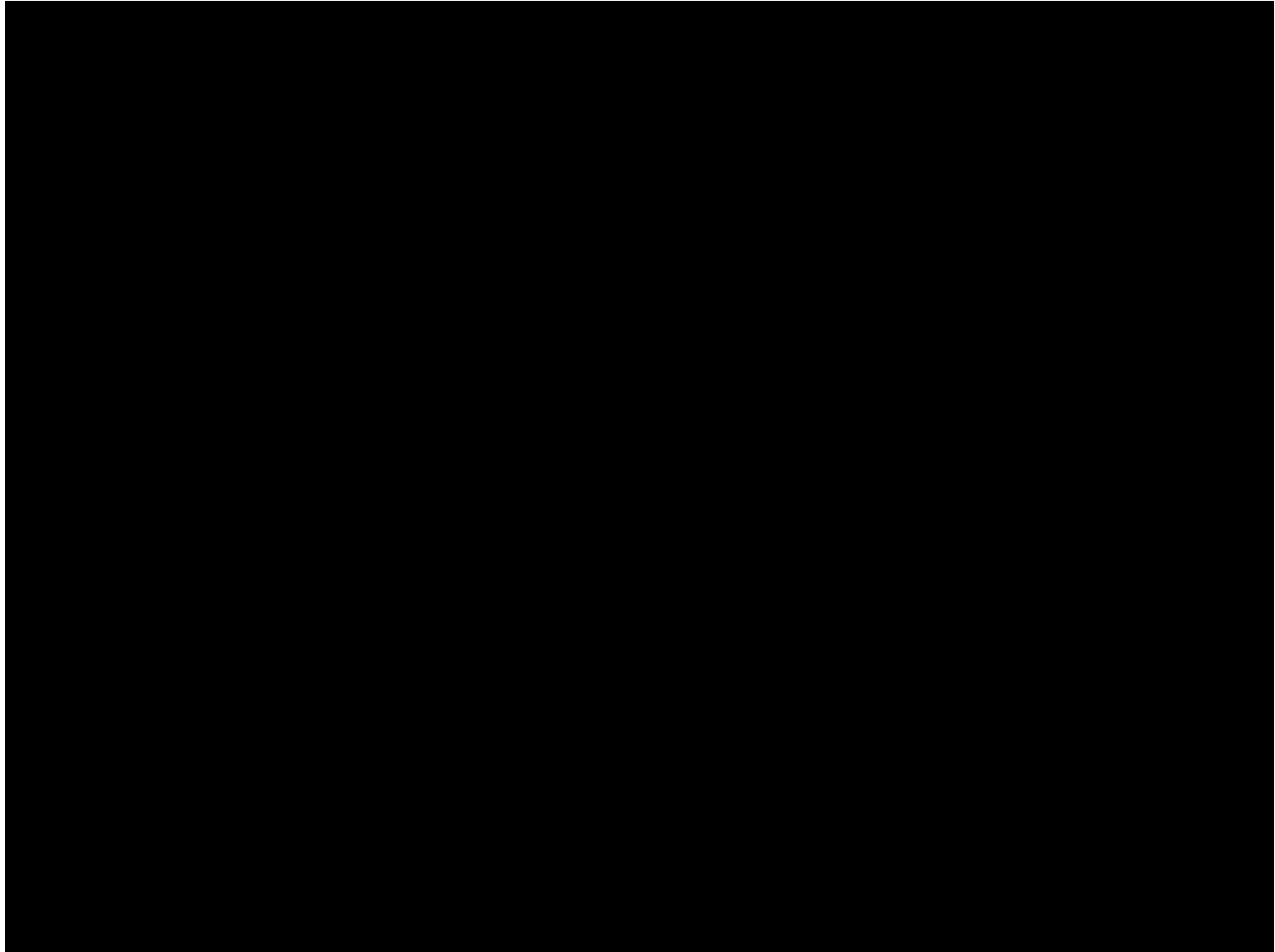


Имплантируемый «оптический» нерв

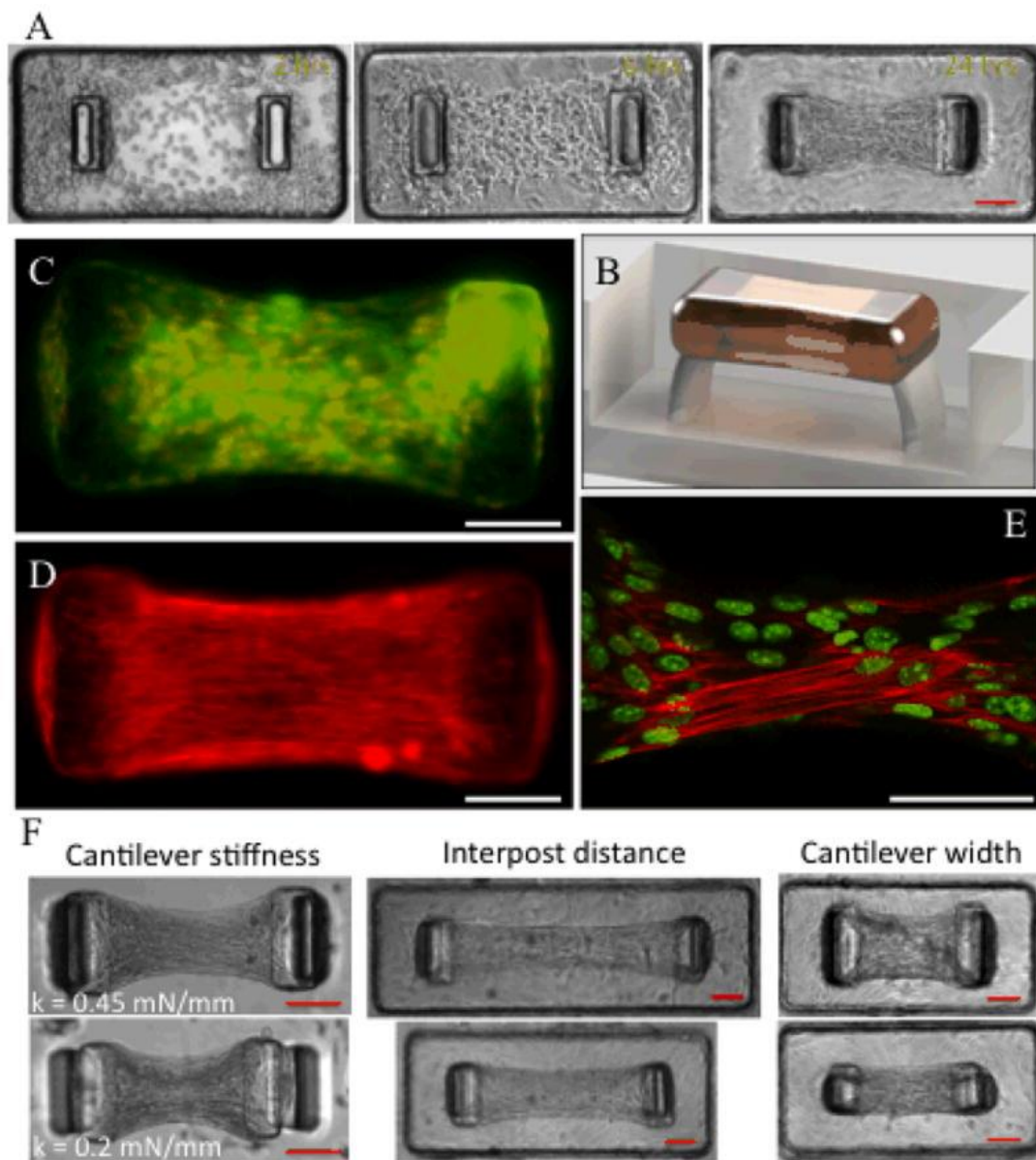


Towne C, Montgomery KL, Iyer SM, Deisseroth K, et al. (2013) Optogenetic Control of Targeted Peripheral Axons in Freely Moving Animals. PLoS ONE 8(8): e72691. doi:10.1371/journal.pone.0072691
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0072691>

Свето-активируемые скелетные мышцы



Роботы на свето-активируемых скелетных мышцах

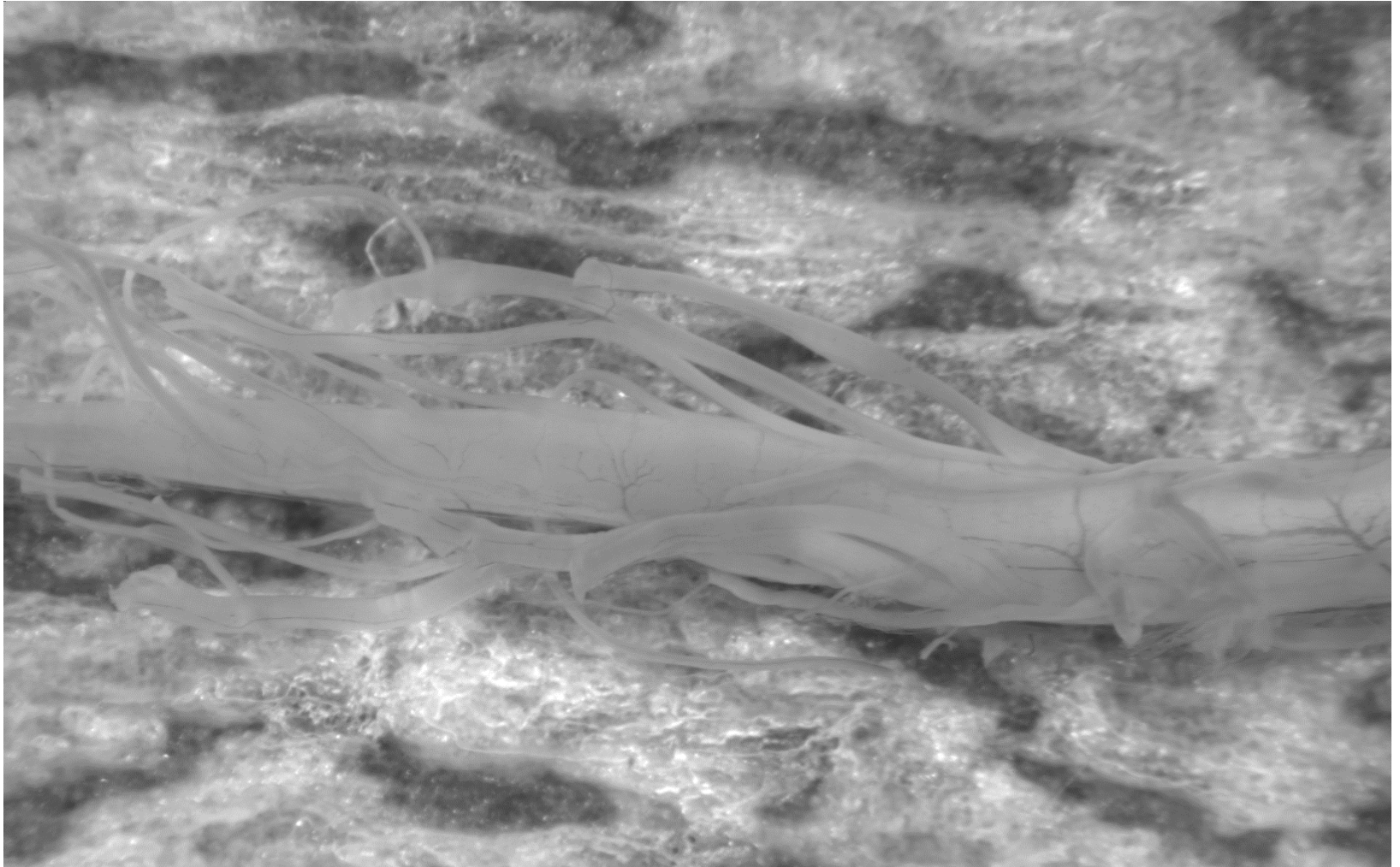


Specific optogenetic photostimulation of
nociceptive afferents

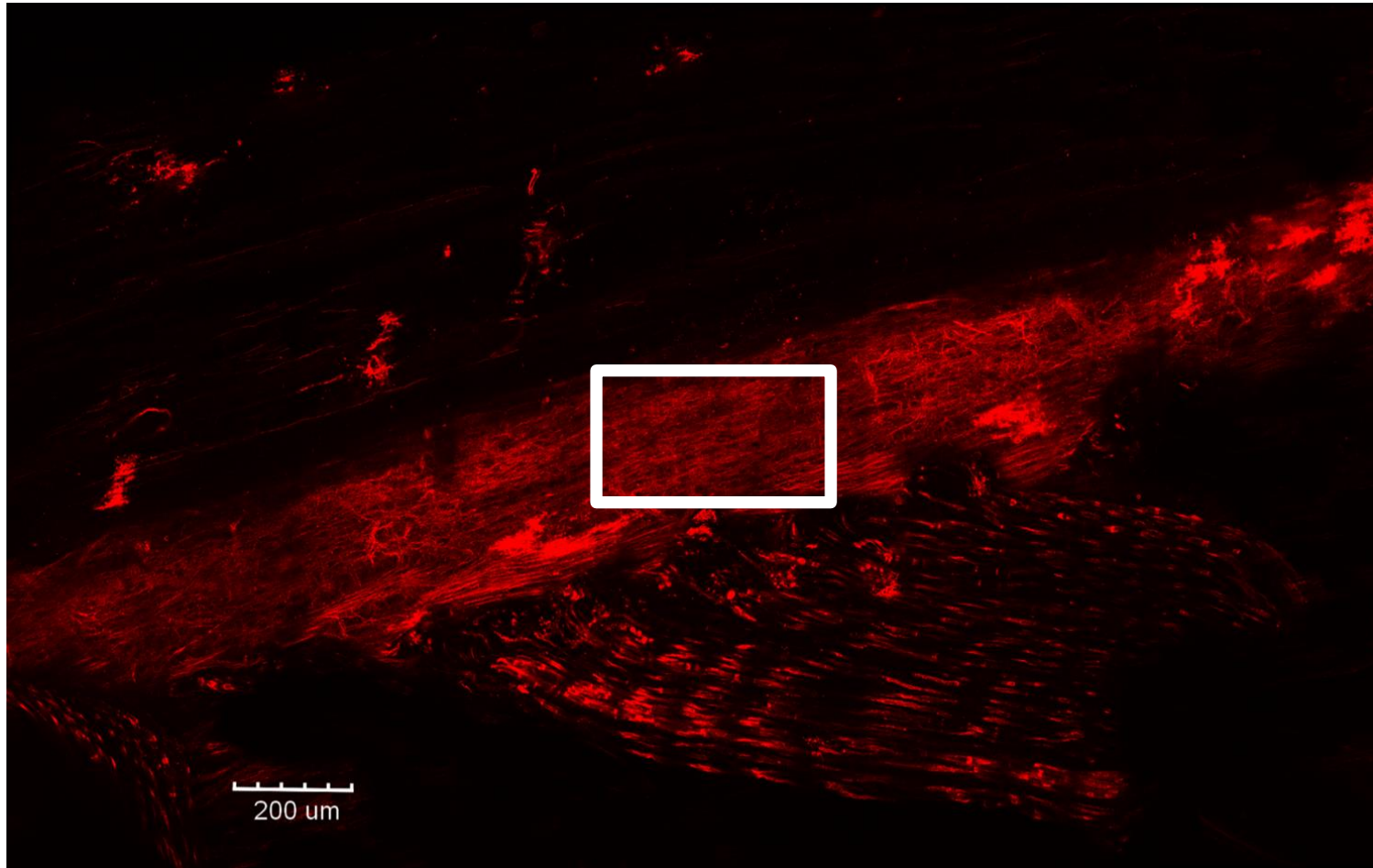
Isolated spinal cord and sciatic nerve preparation



Isolated spinal cord preparation

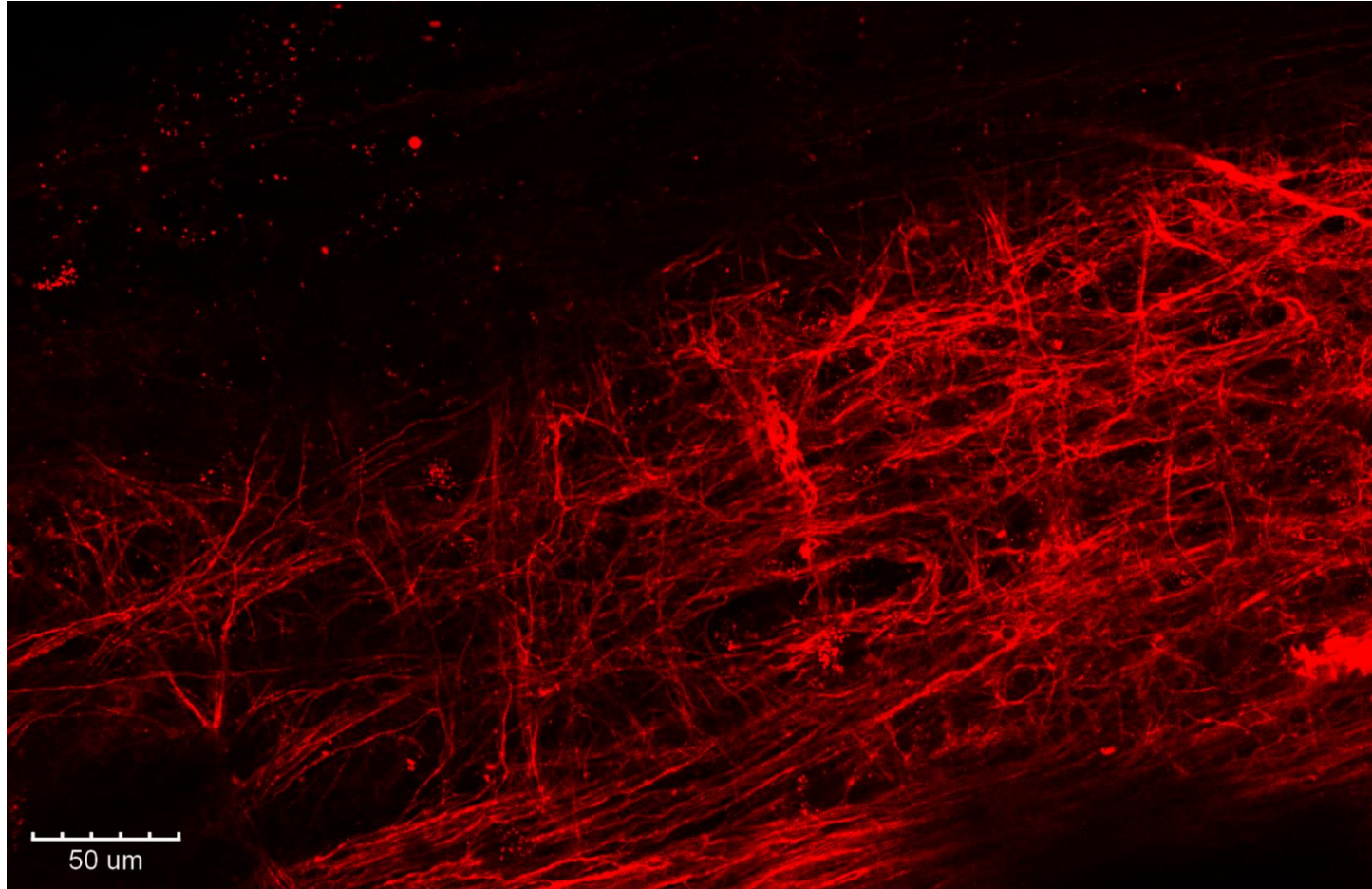


Thermal nociceptor afferent inputs to the spinal cord labeled by TRPV1-TdTomato-ChRh2

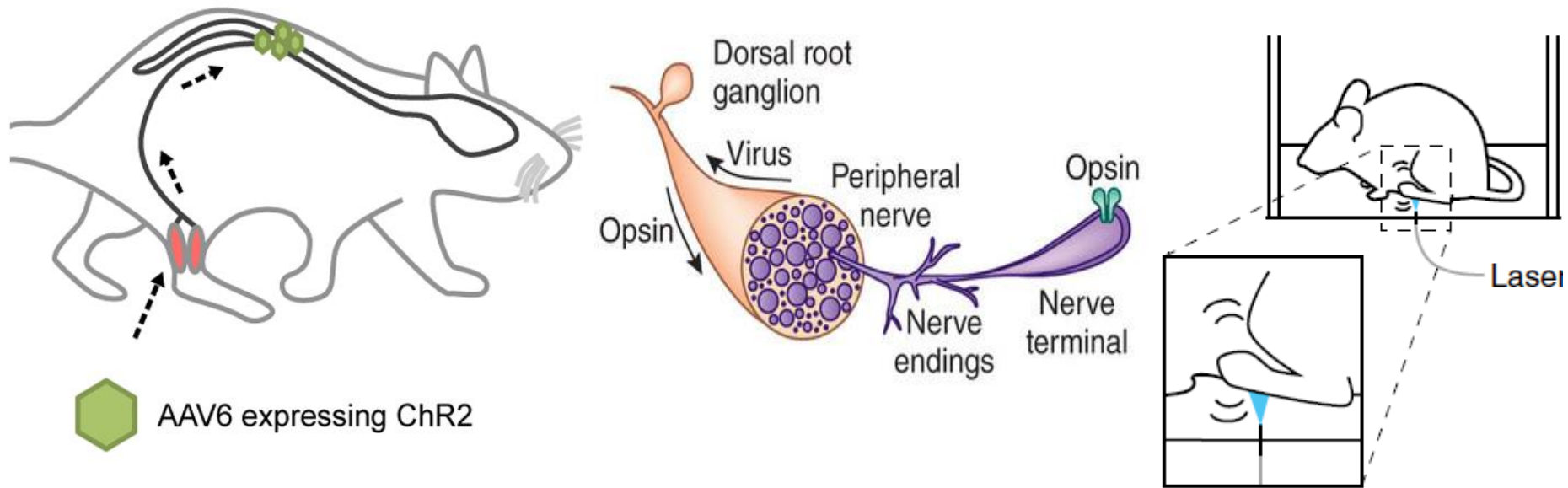


TdTomato-ChRh2 mice (floxed) and TRPV1-Cre (Cre expression is controlled by TRPV1 promoter) are from Jackson lab, stock# 012567 and 017769, respectively.

The pre-synaptic network is very dense and complex



Intrasciatic AAV6-hSyn-ChR2-eYFP injection ChR2 expression in nociceptive fibers



PLOS ONE 2013, Volume 8, Issue 8, e72691

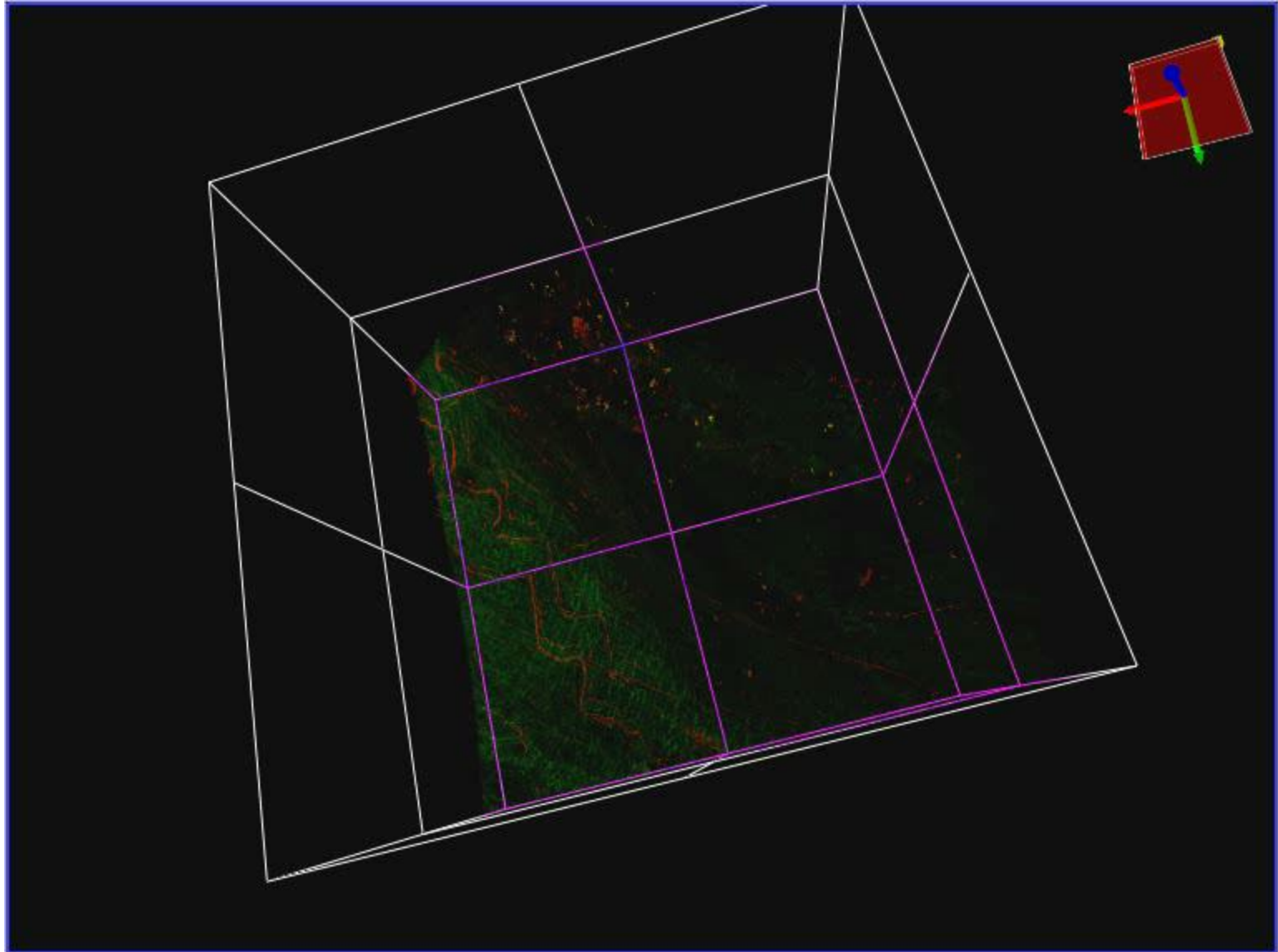
Iyer, S. M. et al.(2014). Nat. Biotechnol., 32: 274

Mouse infected with ChR2-YFP by injection
into sciatic nerve



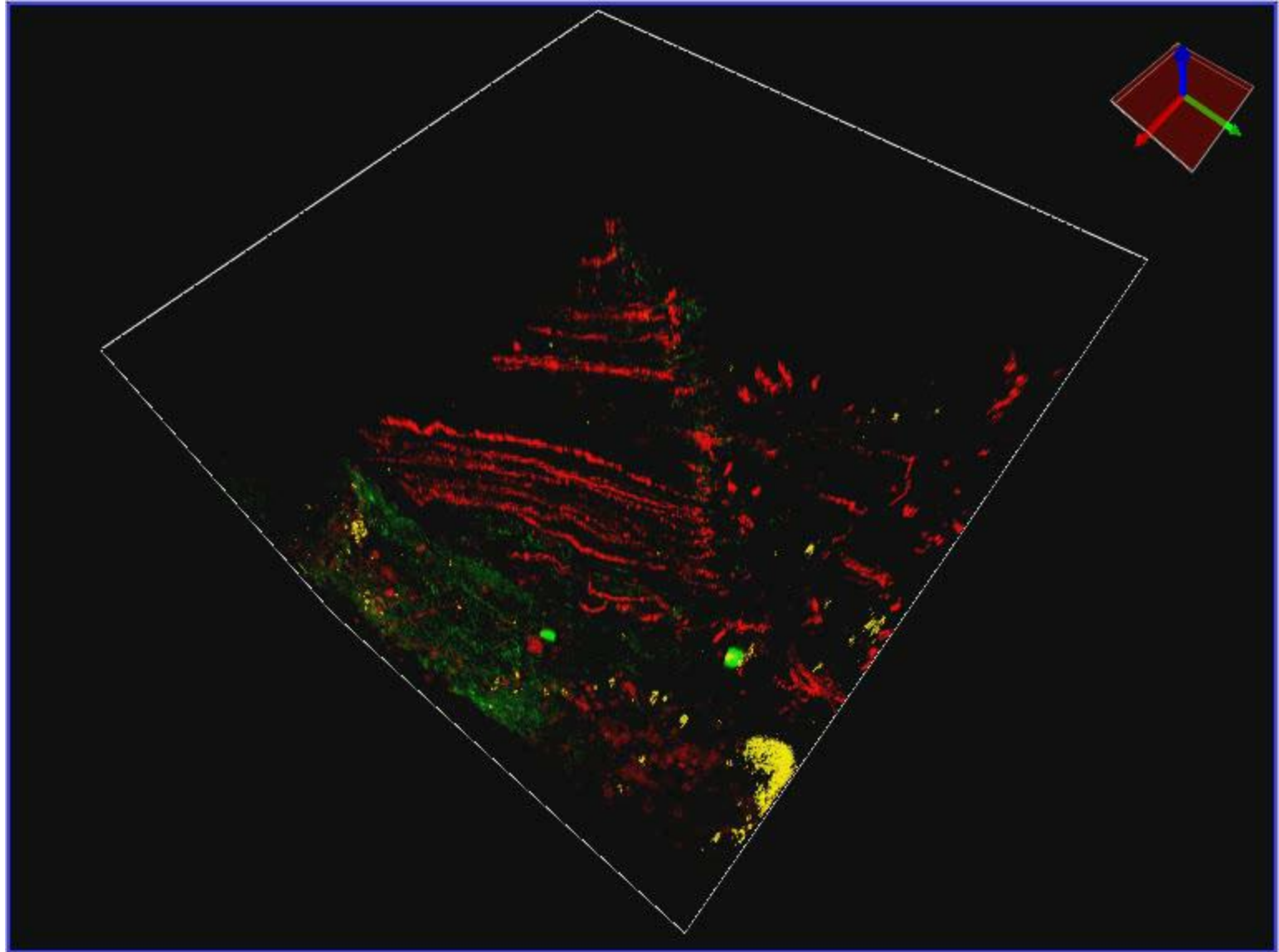
Sciatic nerve

fluorescence (red pseudocolor) indicates fibers infected with ChR2

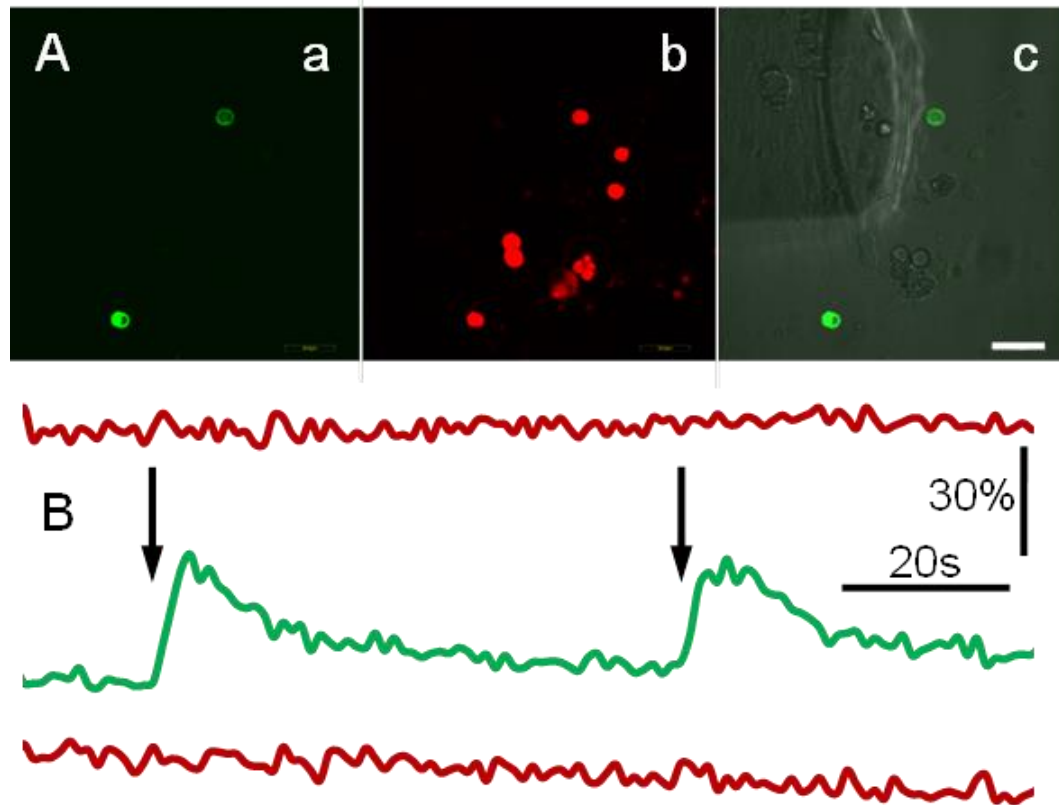


Dorsal roots

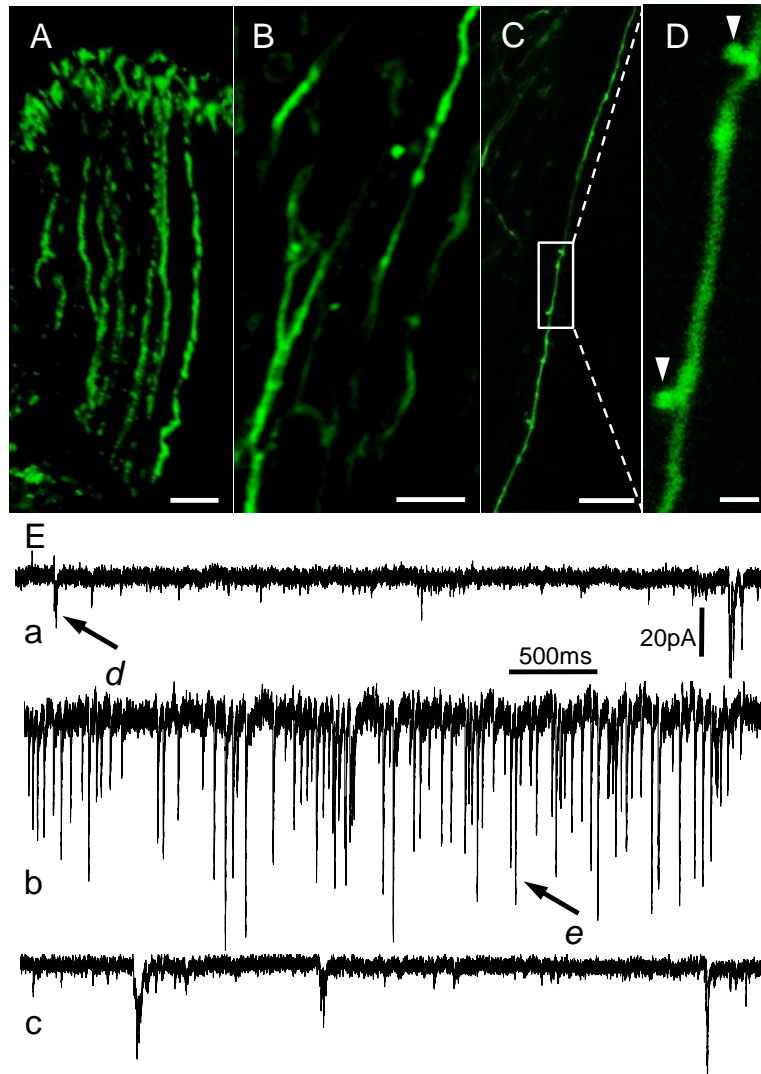
fluorescence (red pseudocolor) indicates iChR2 infected nociceptive fibers



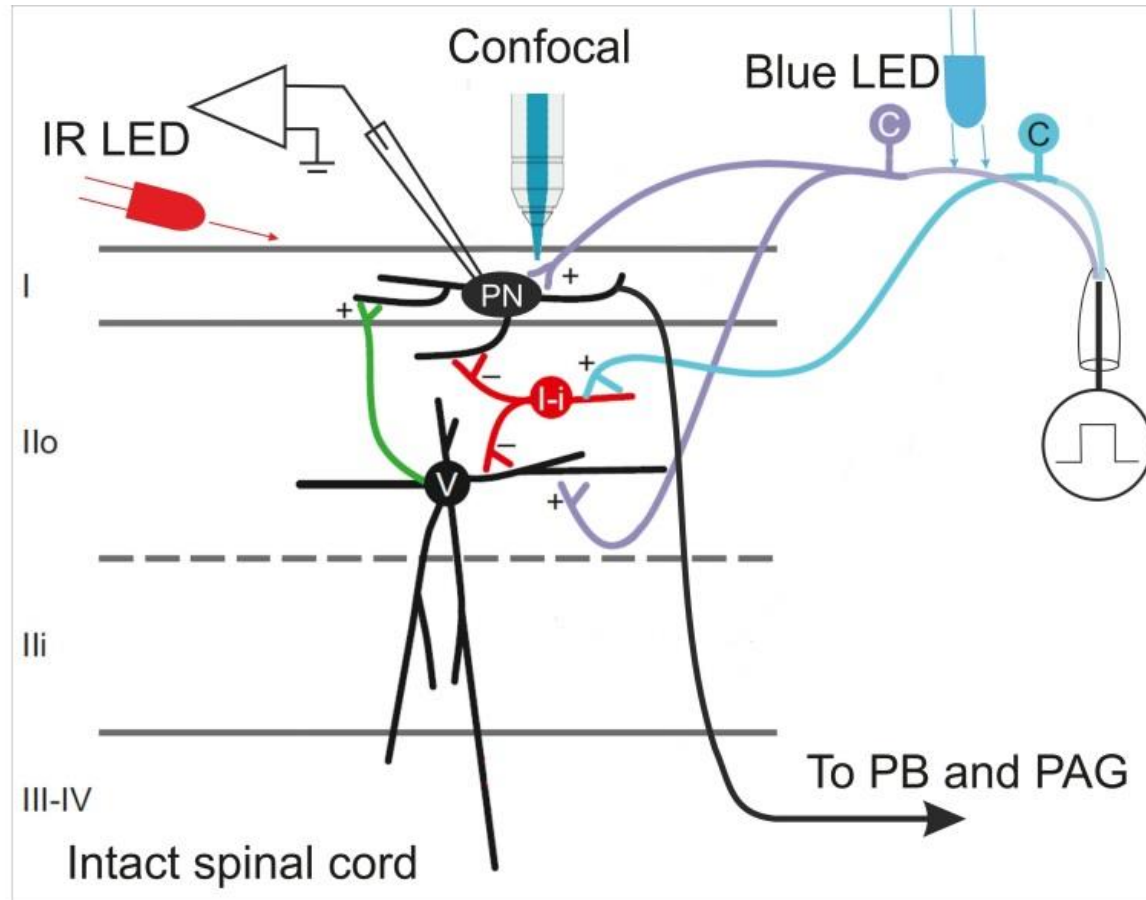
Small size DRG neurons of mice infected with AAV6-hSyn-ChR2-eYFP



Evoked EPSCs induced in dorsal horn neurons by photostimulation of ChR2 expressed in primary nociceptive afferents



Experimental design



Conclusions

- **Optogenetics** is a new experimental methodology based upon the combination of genetic and optical methods to control specific mainly electrical events in targeted cells of living tissue.
- **Optogenetics** allows to almost instantaneously switch on and off certain neuronal groups in order to study their functions and relationships with other cells of animal body.
- **Optogenetics** also allows to correct pathological changes of signaling processes being a prerequisite for treatment of many neurodegenerative disorders.

- Спасибо за внимание 😊