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Оптогенетические методы исследования

23.04.2015

План

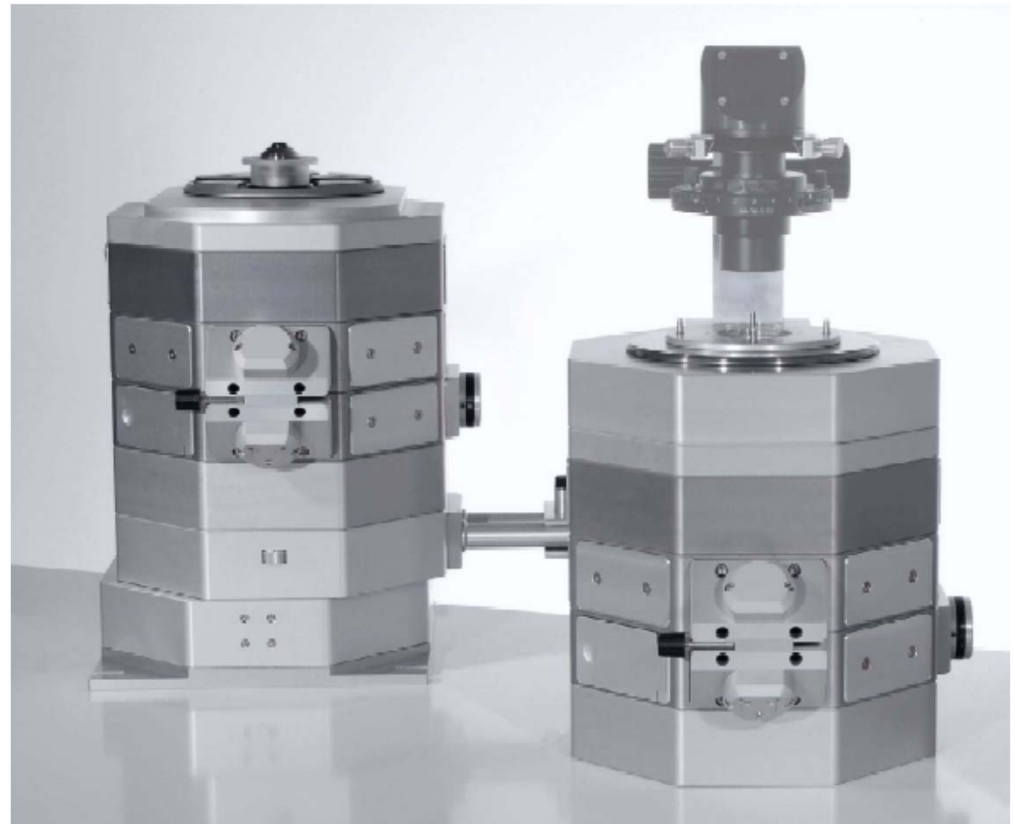
- *Флуоресцентная микроскопия основанная на применении флуоресцентных белков, Green Fluorescent Protein (GFP)*
- *Оптогенетика*

История развития оптических микроскопов

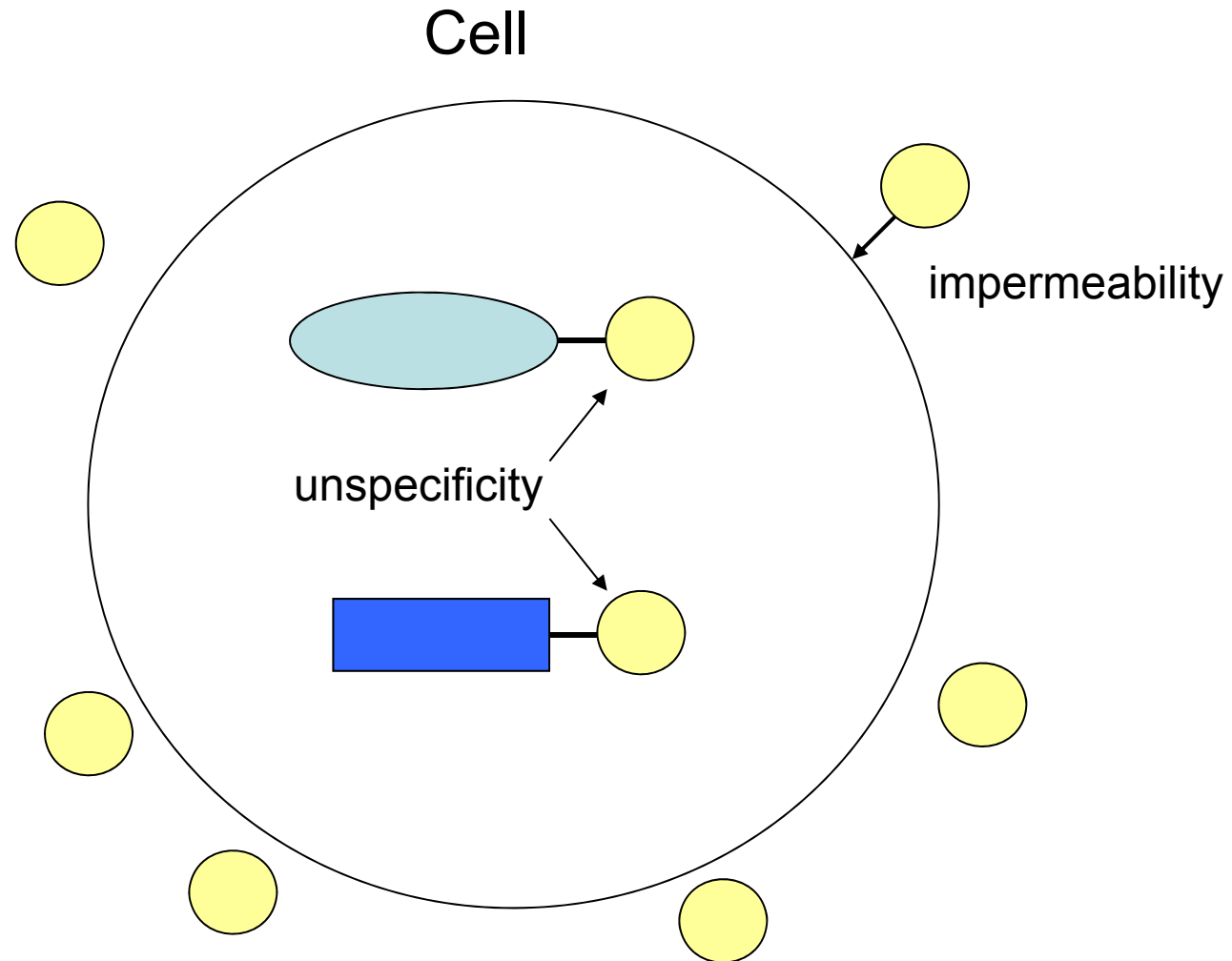
Compound microscope



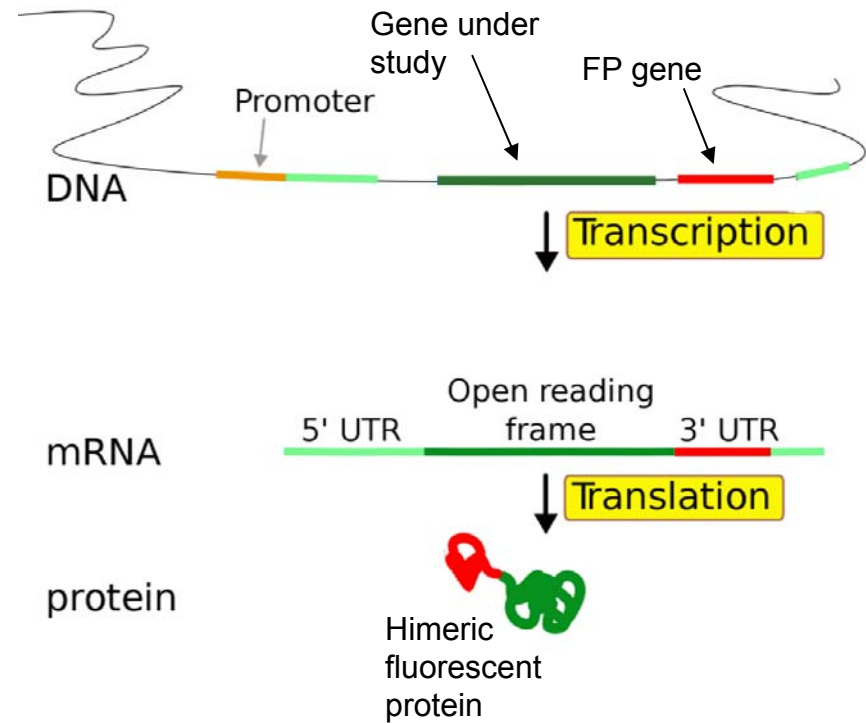
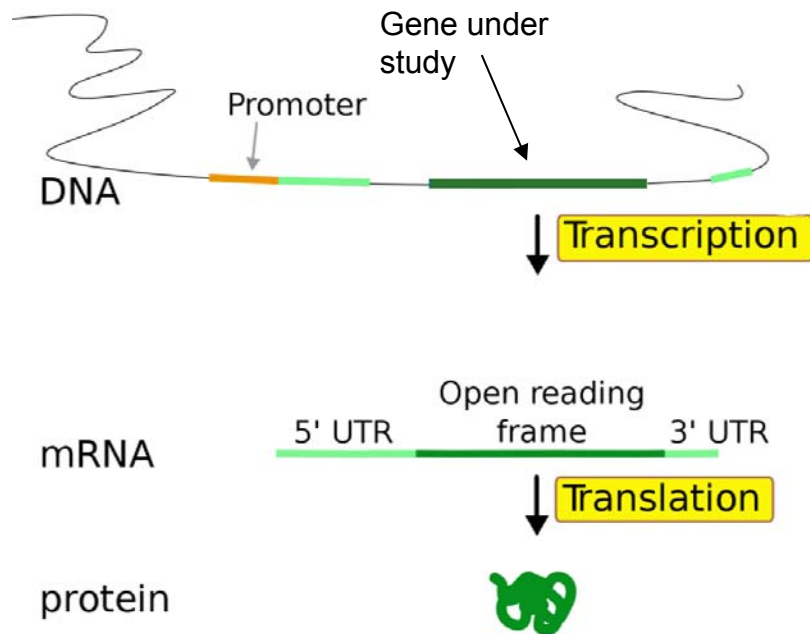
iMIC Digital Microscopes



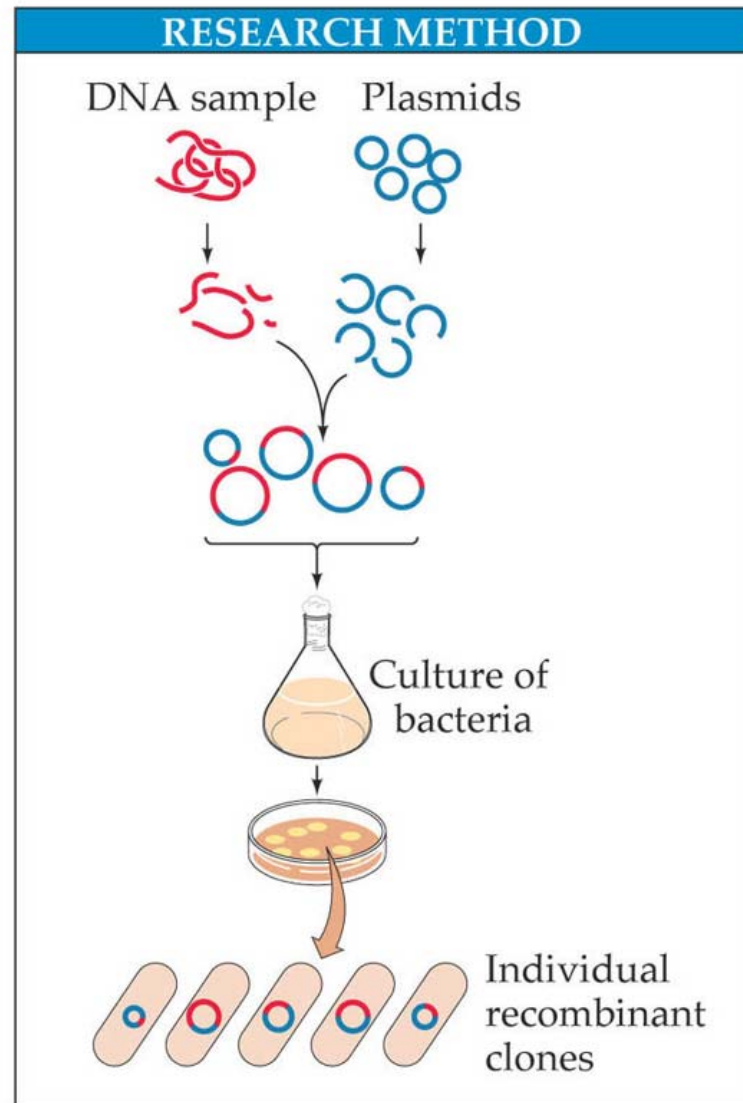
Проблемы введения флуоресцентных меток



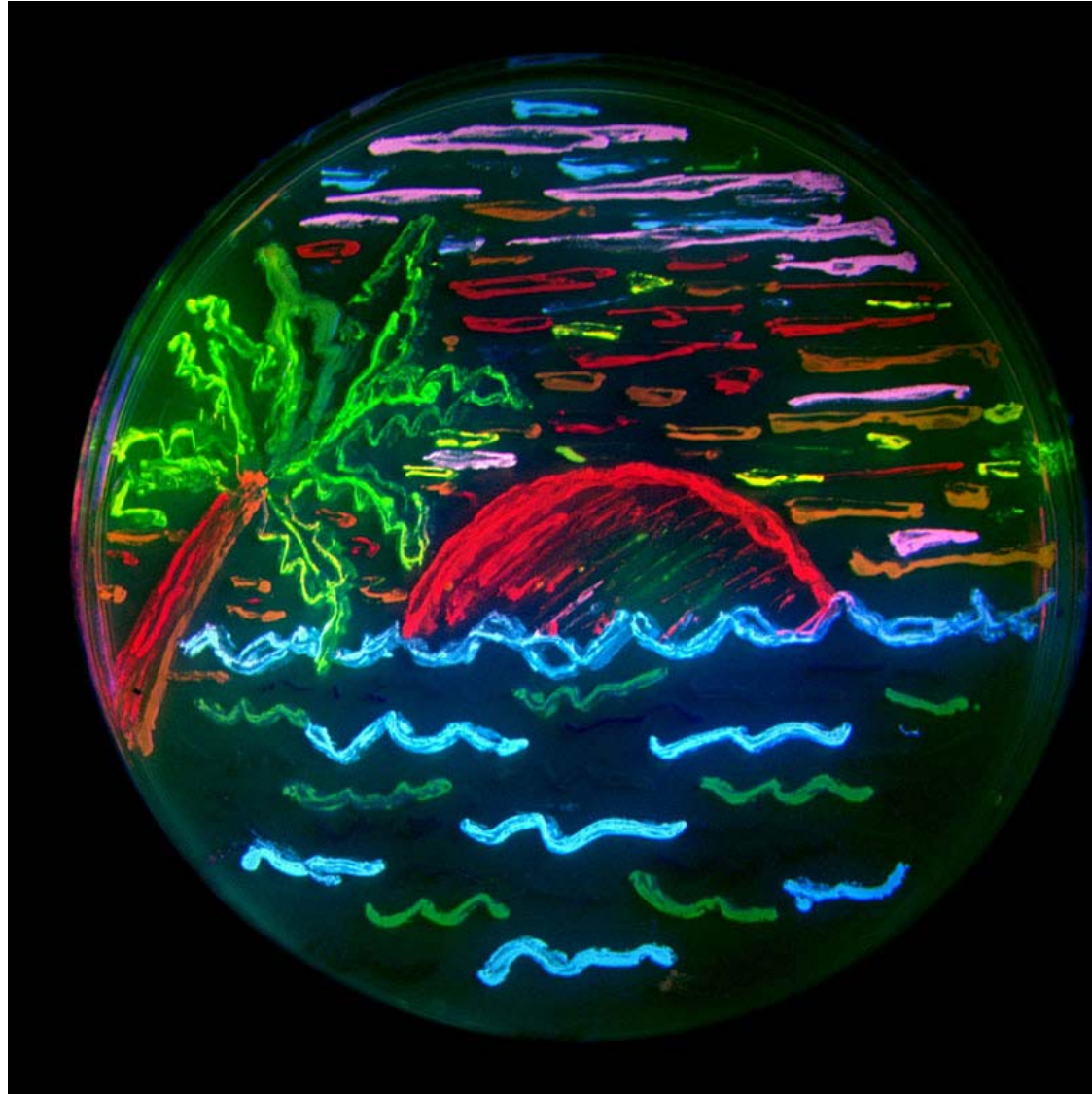
Химерная экспрессия генов



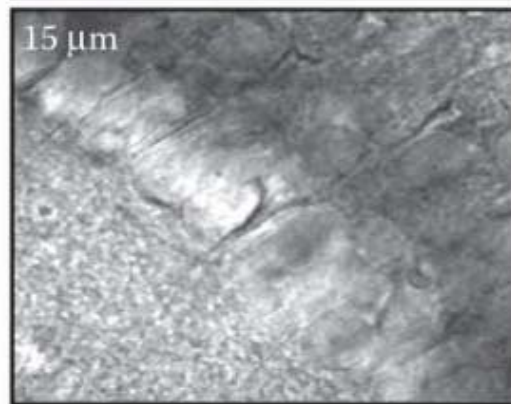
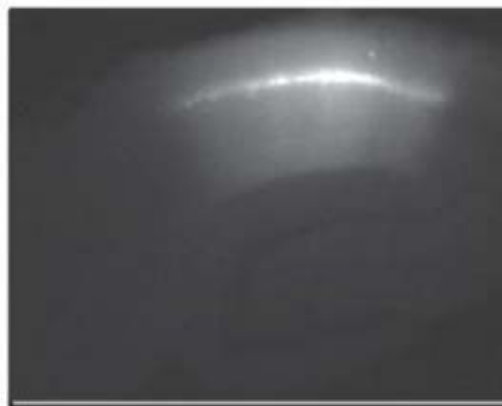
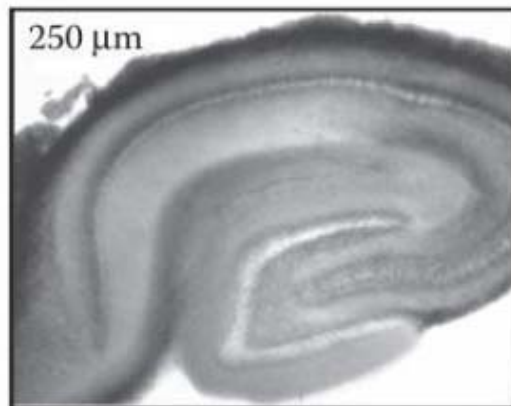
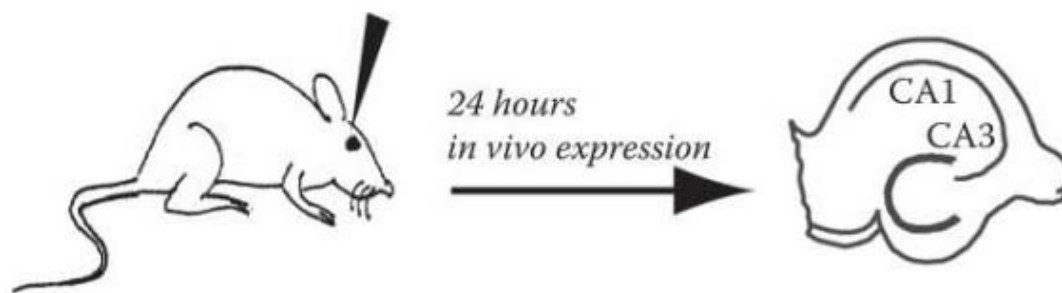
Конструирование плазмид



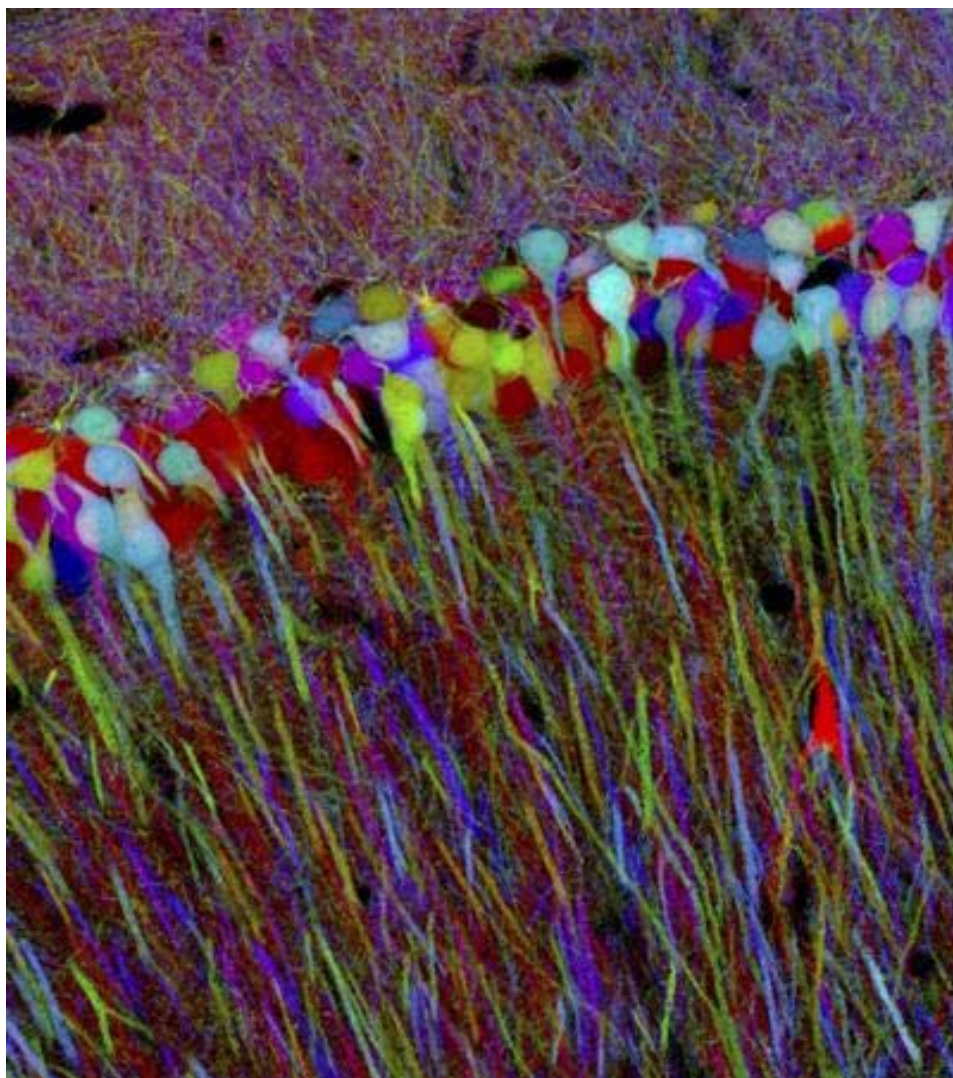
Зеленый флуоресцентный белок (GFP)



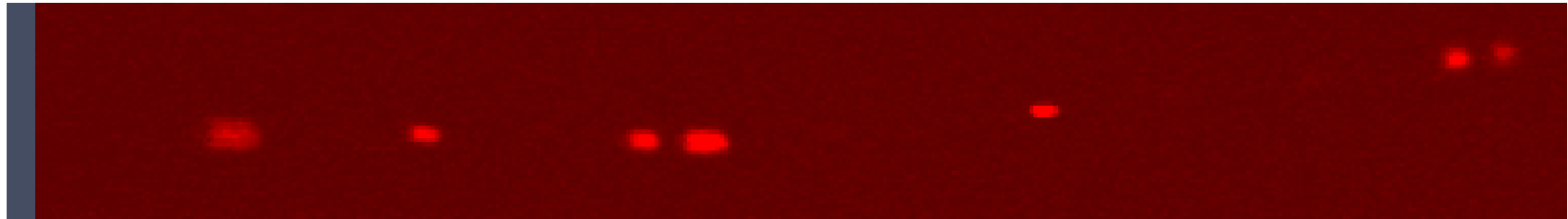
Методика введения вирусов



Пирамидные нейроны «радужных» крыс



Движение митохондрий в аксонах пирамидных нейронов гиппокампа



- 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.

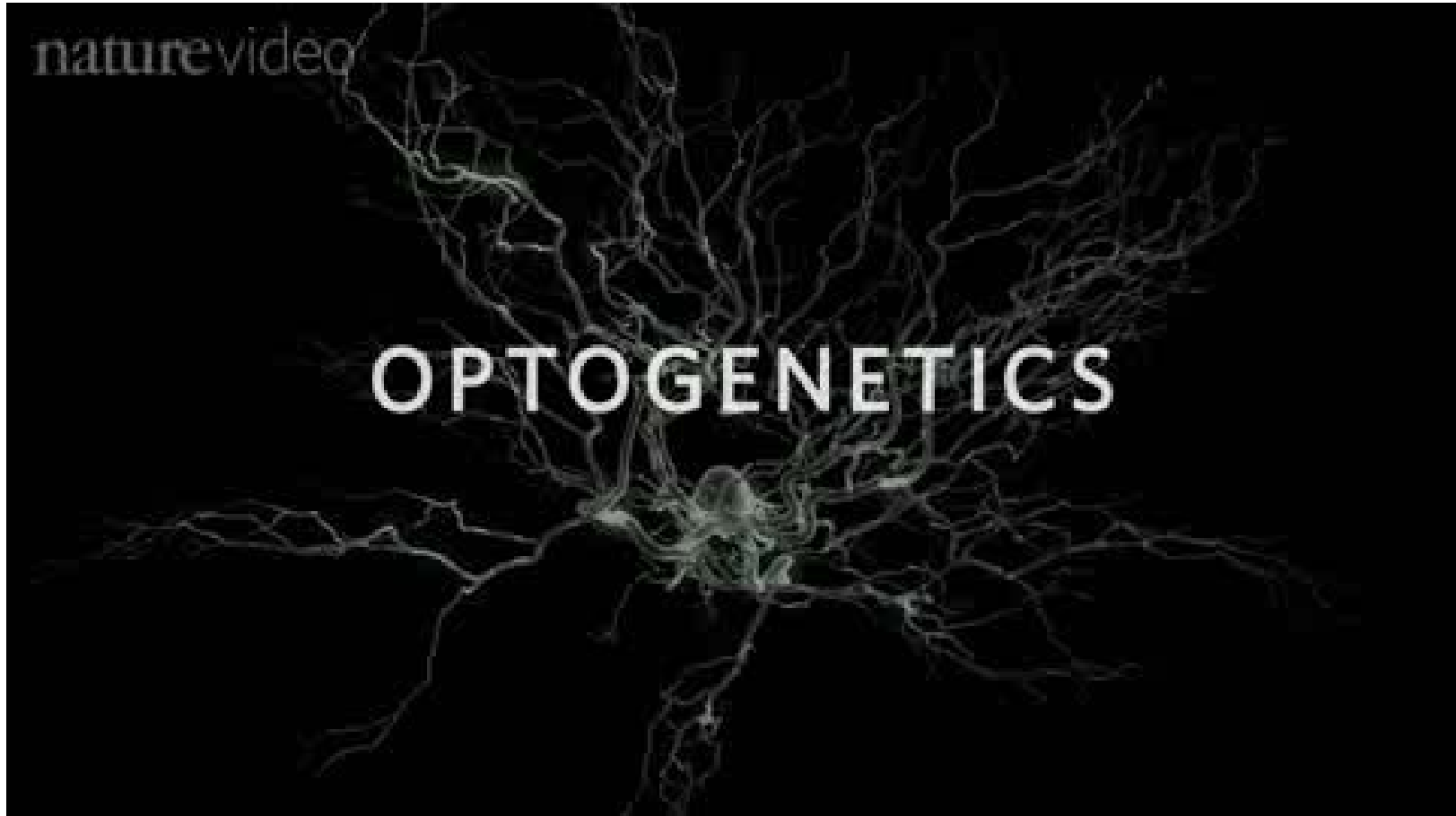
- Но можно ли не только исследовать клеточные процессы, но и управлять ими?

Оптогенетика (Optogenetics)

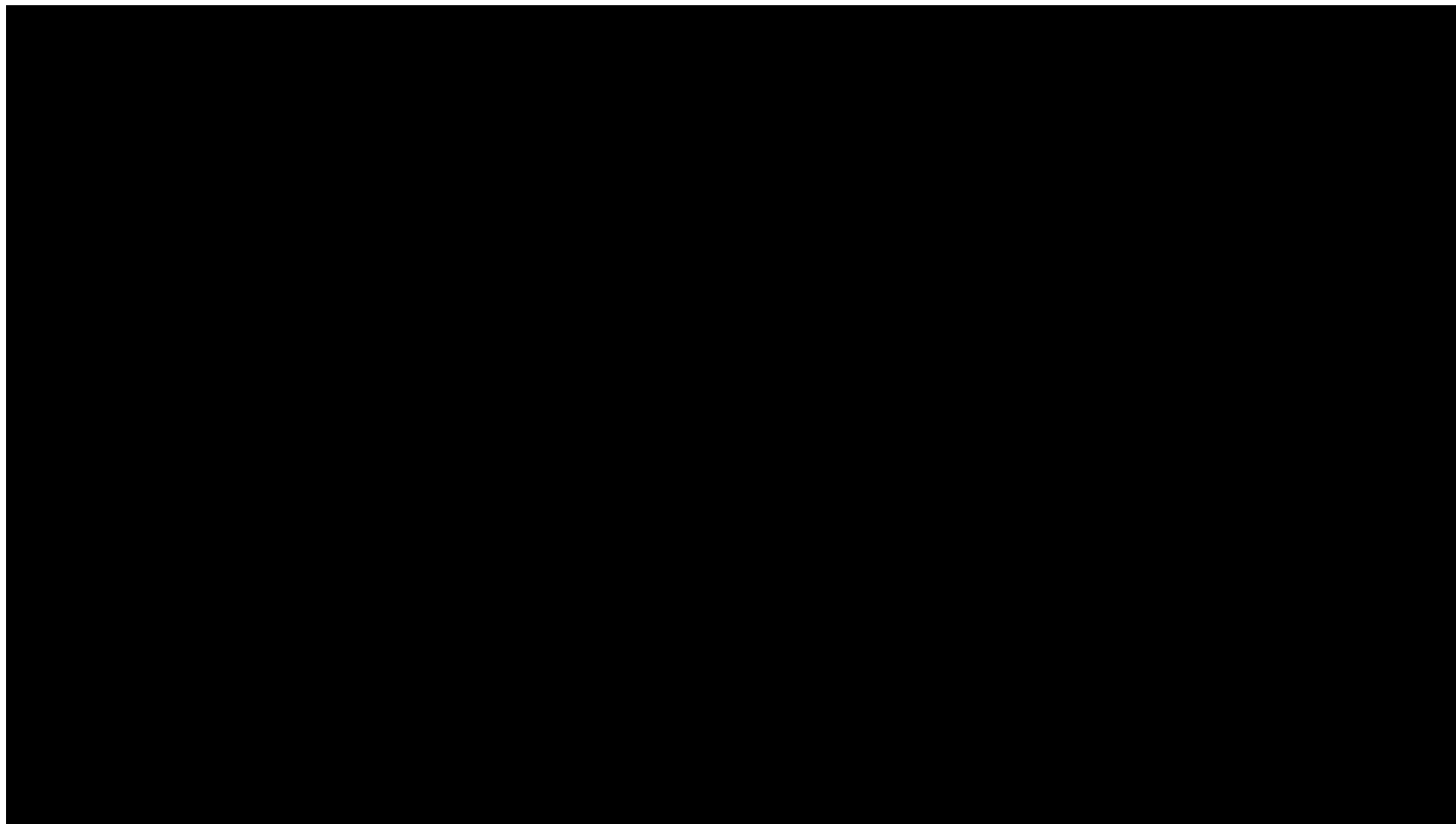
Оптогенетика это комбинация генетических и оптических методов с целью контролировать специфические события в определенных клетках живых тканей с миллисекундным временным разрешением.

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.

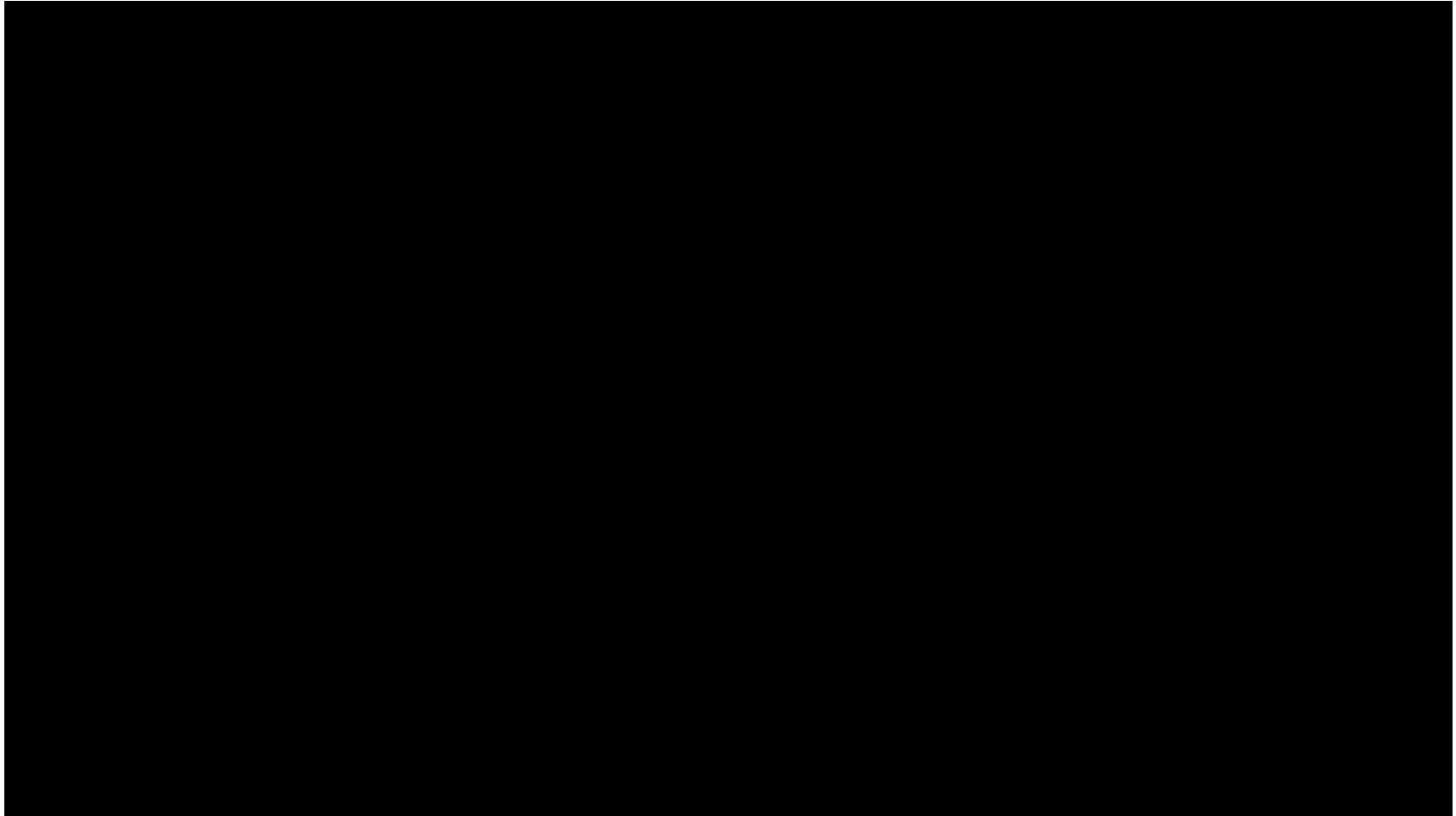
Фоточувствительные бактерии – основатели оптогенетики



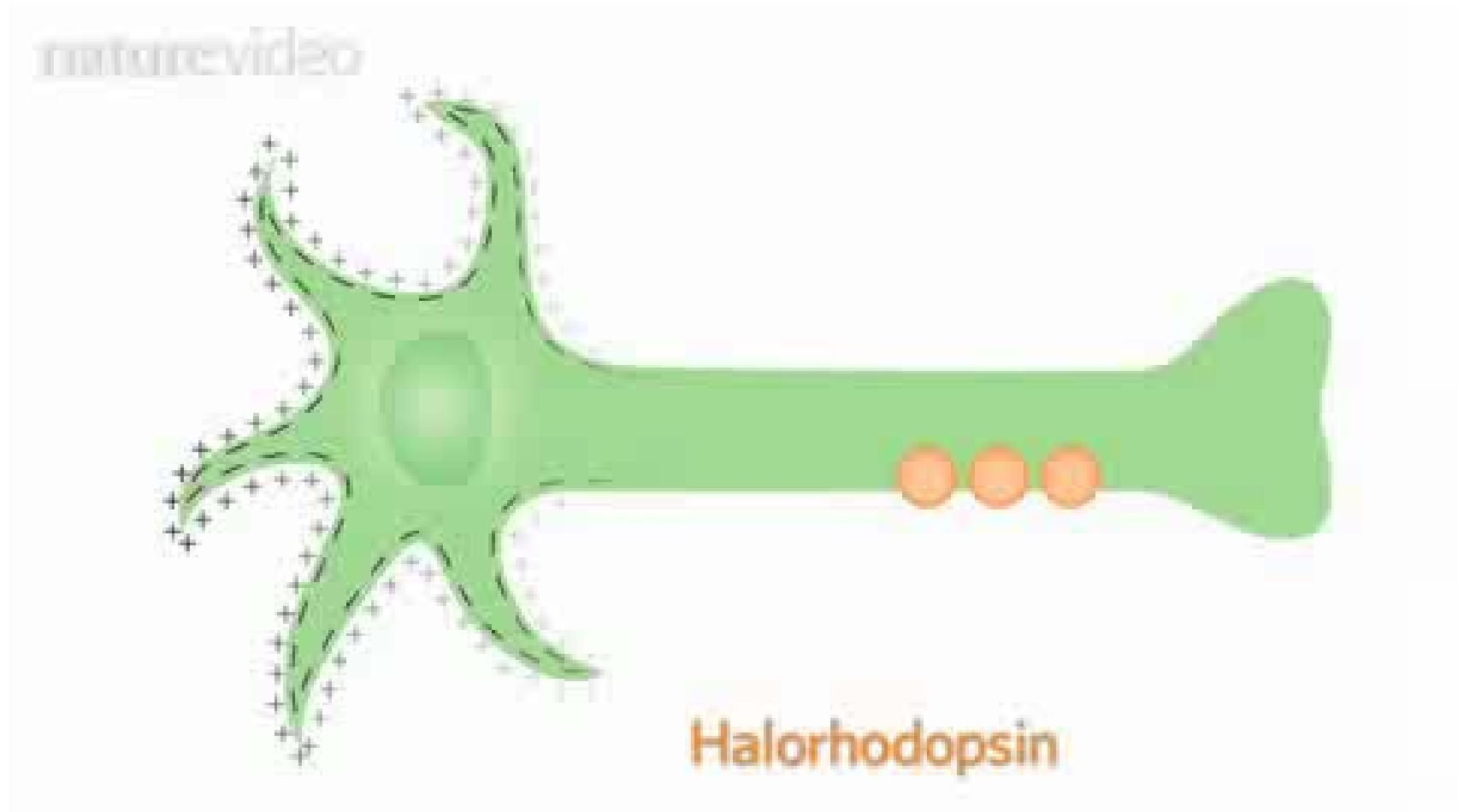
Каналородопсин Channelrhodopsin (ChR2) an electronic photoswitch exciting neurons



Examples of how Channelrhodopsins can control cells and animal behavior



Halorhodopsin (NpHR) - an electronic photoswitch inhibiting neurons



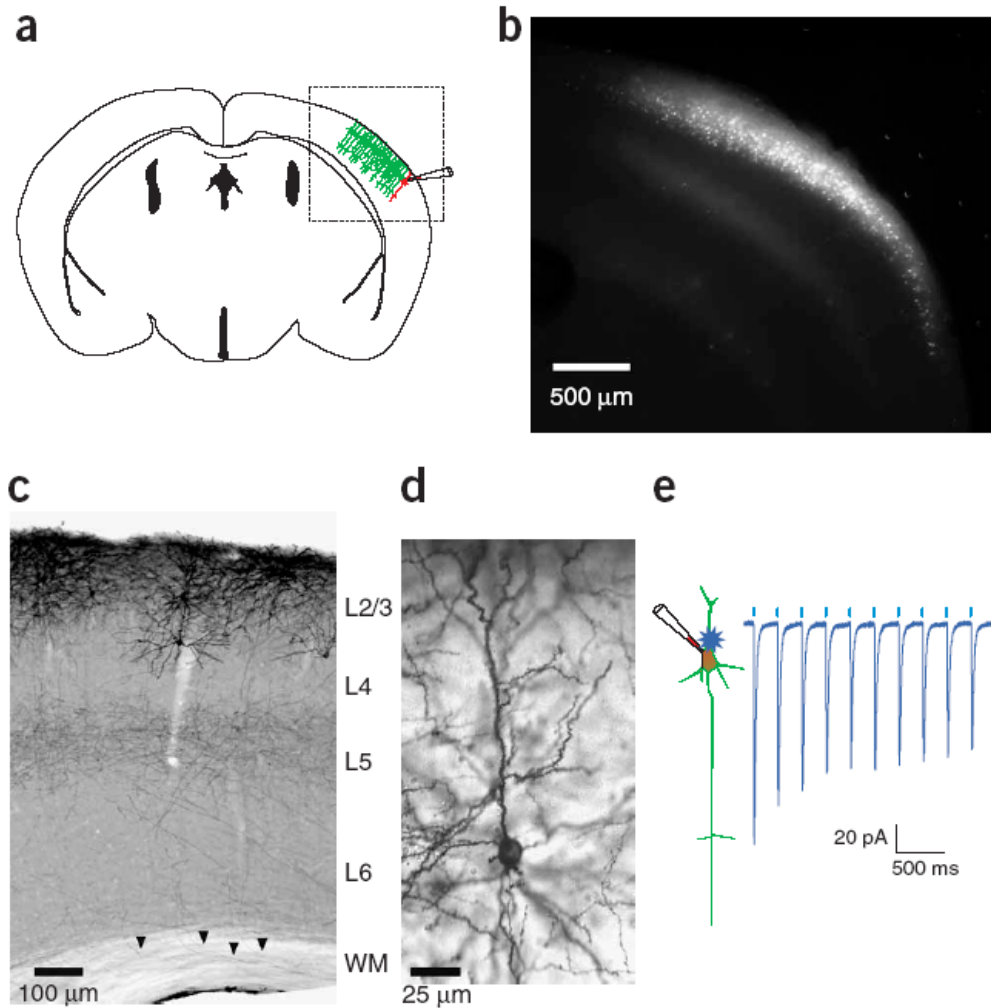
Molecular-genetic approach to introduce optogenetic constructs

nature video



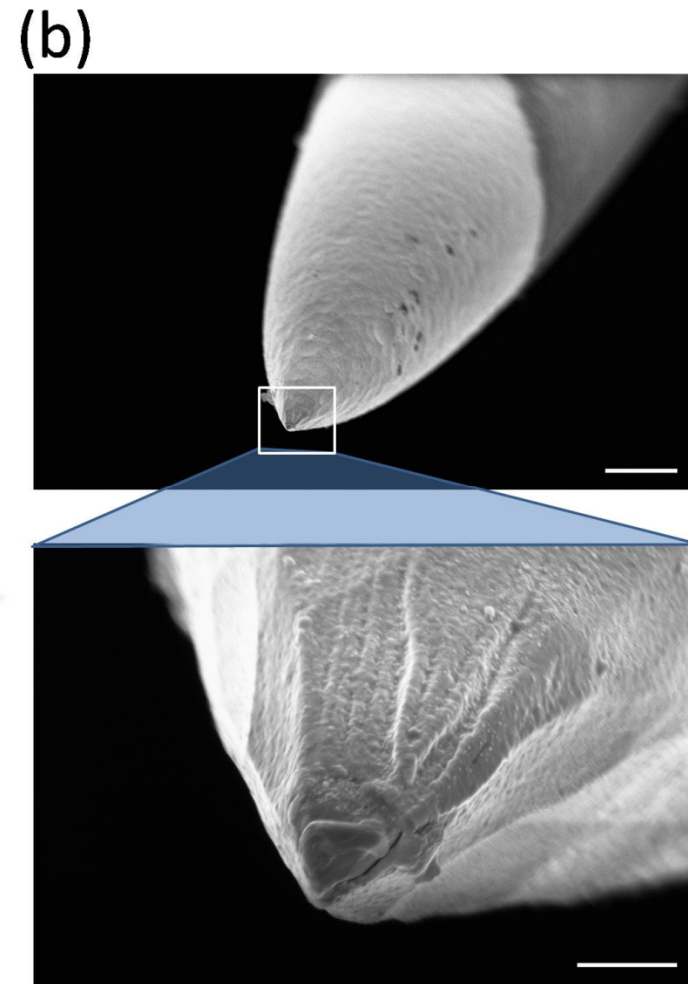
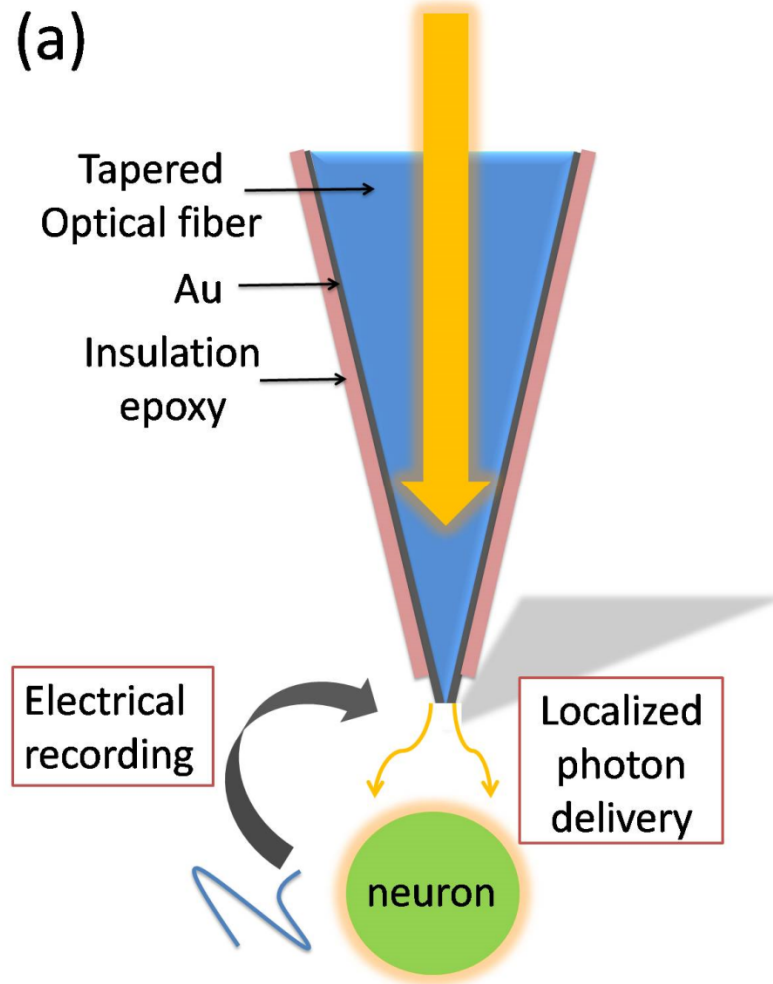
Nature web site

Photostimulation of ChR2-positive neocortical L2/3 pyramidal neurons

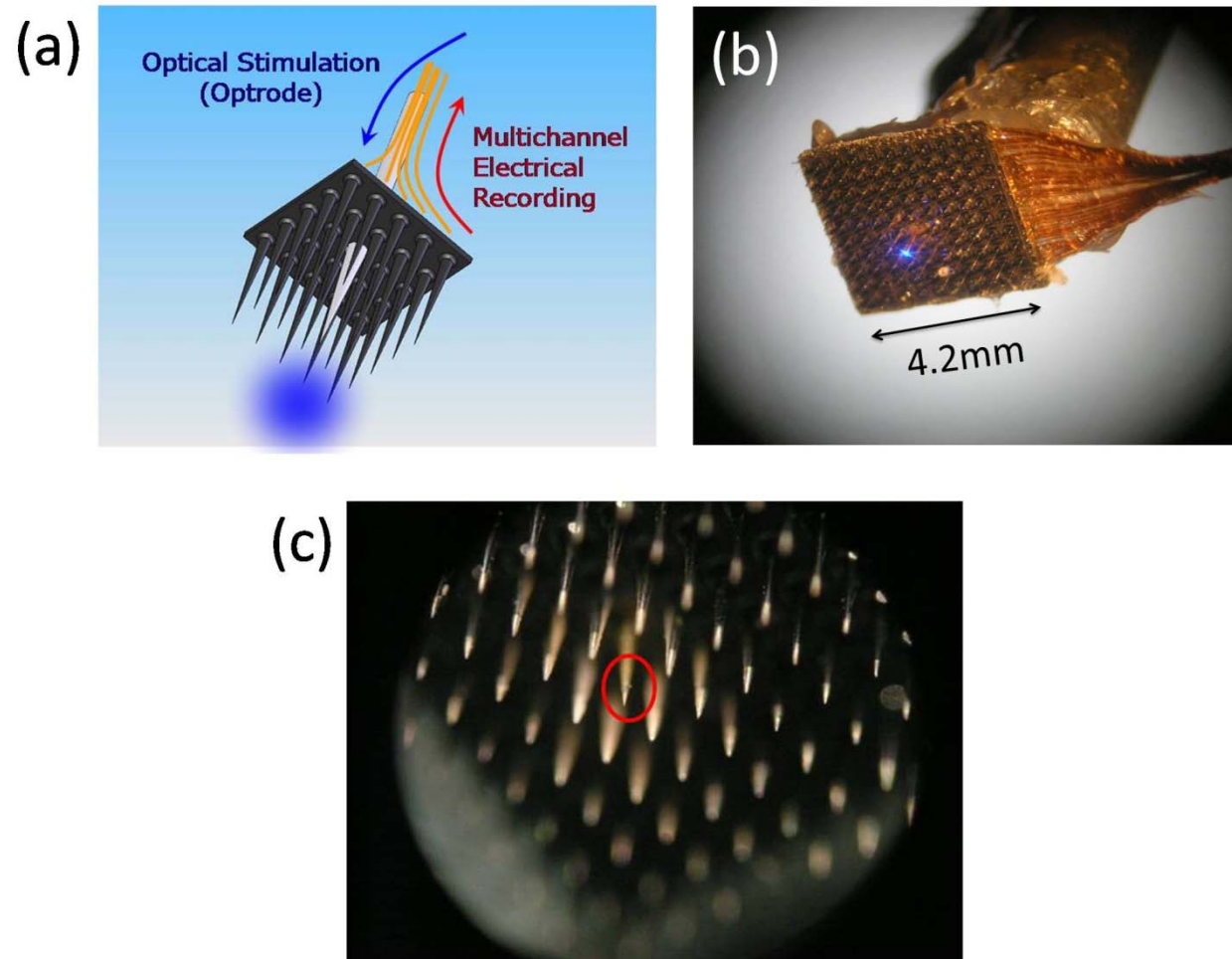


NATURE NEUROSCIENCE 10:663 2007.

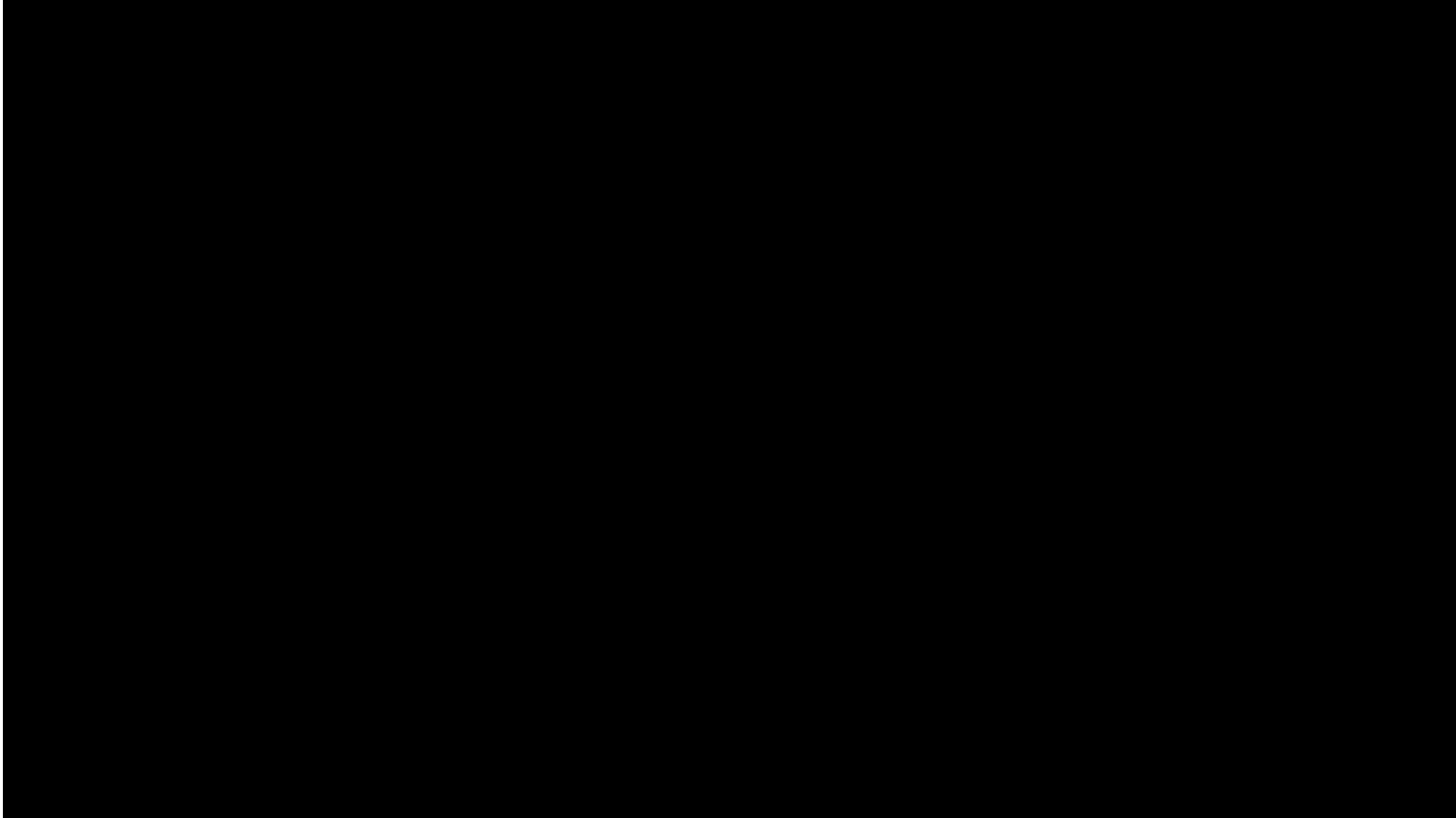
Schematics and SEM images of optrode



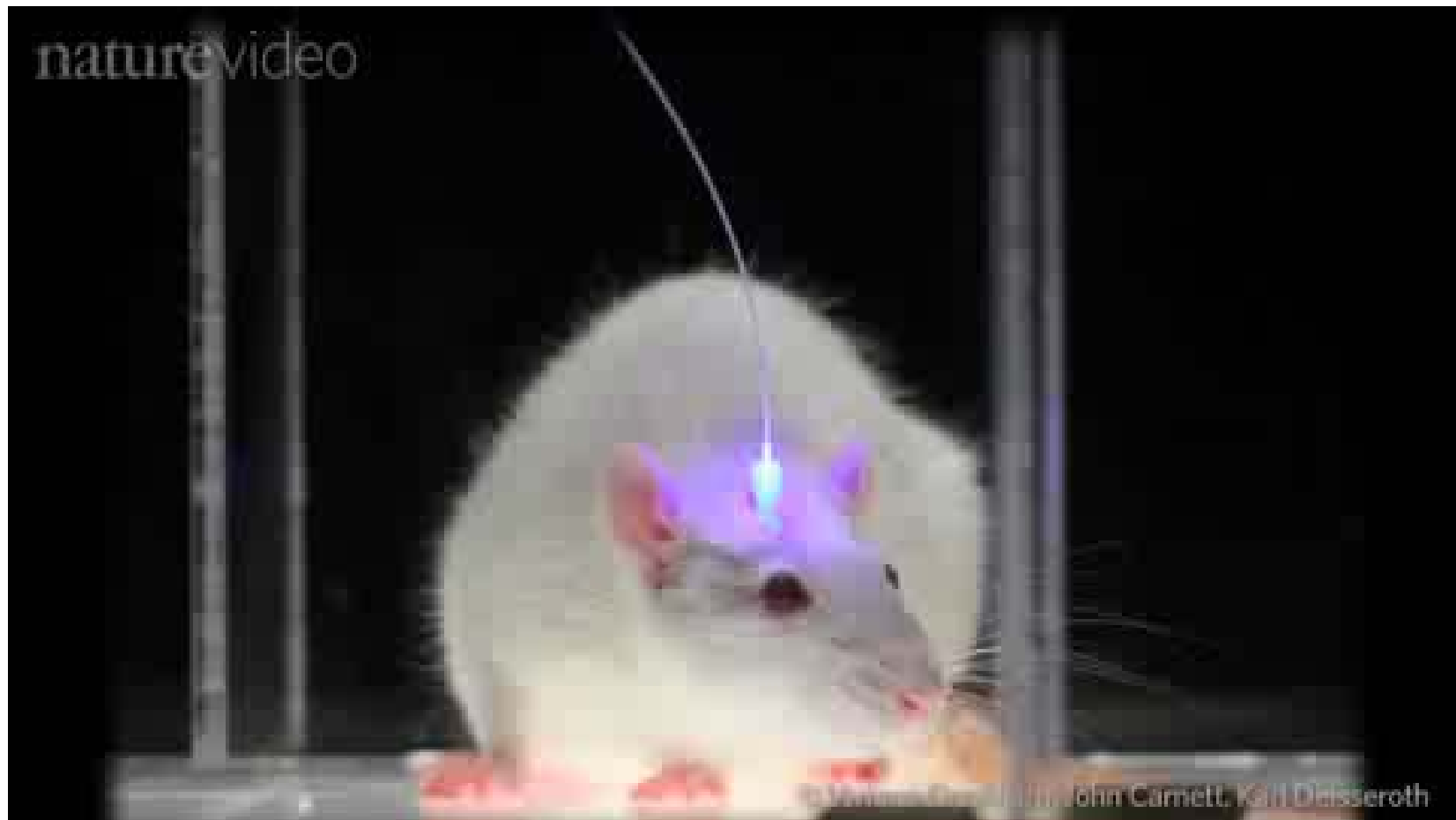
Optrode array assembly



ChR2 mouse

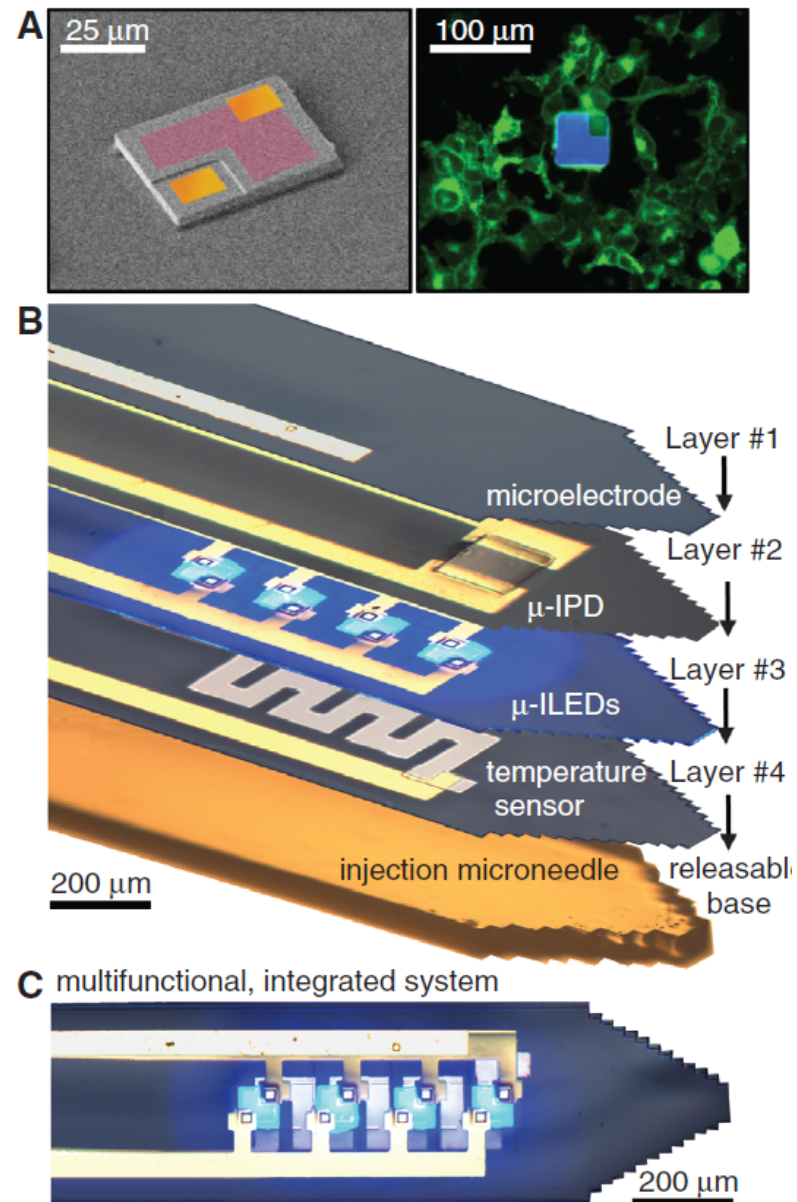


Parkinson disease treatment by optogenetic approaches

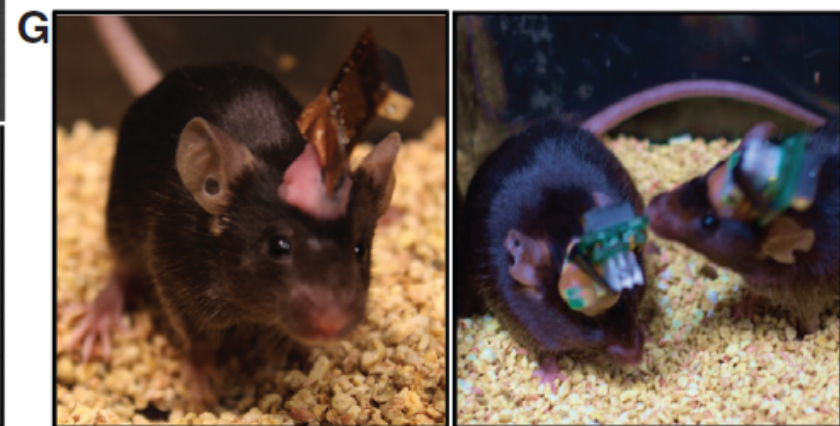
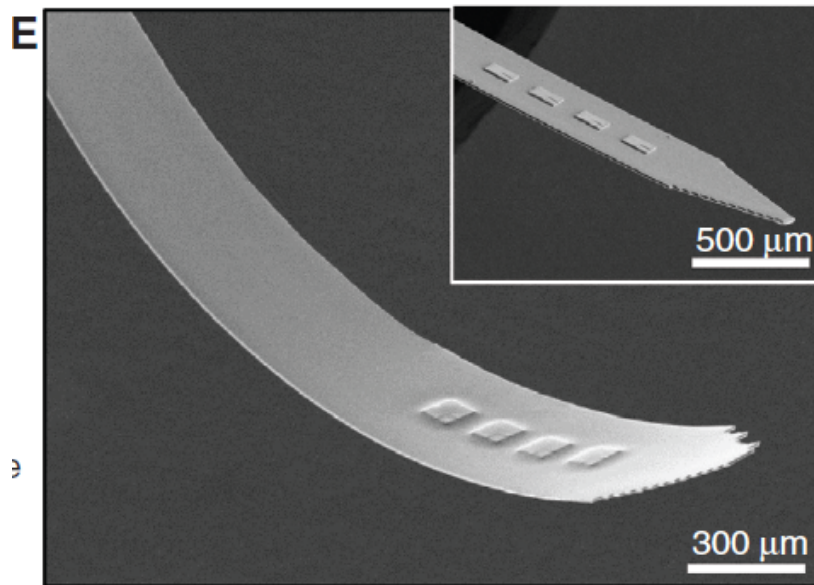
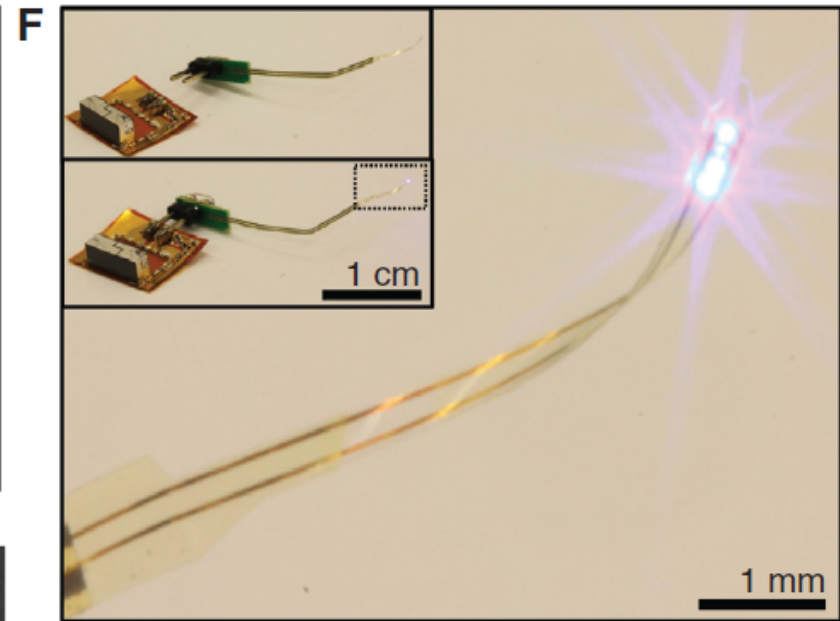
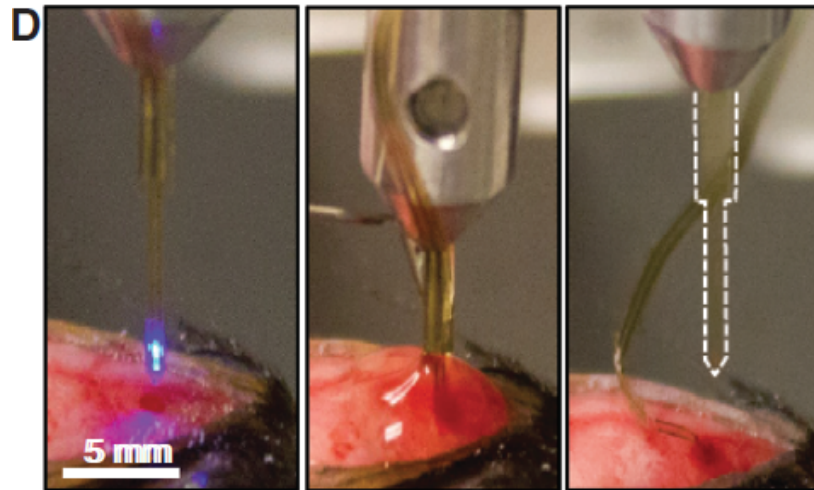


Nature web site

Injectable, cellular-scale semiconductor devices



Injectable, cellular-scale semiconductor devices



μ -ILED devices improve spatial targeting

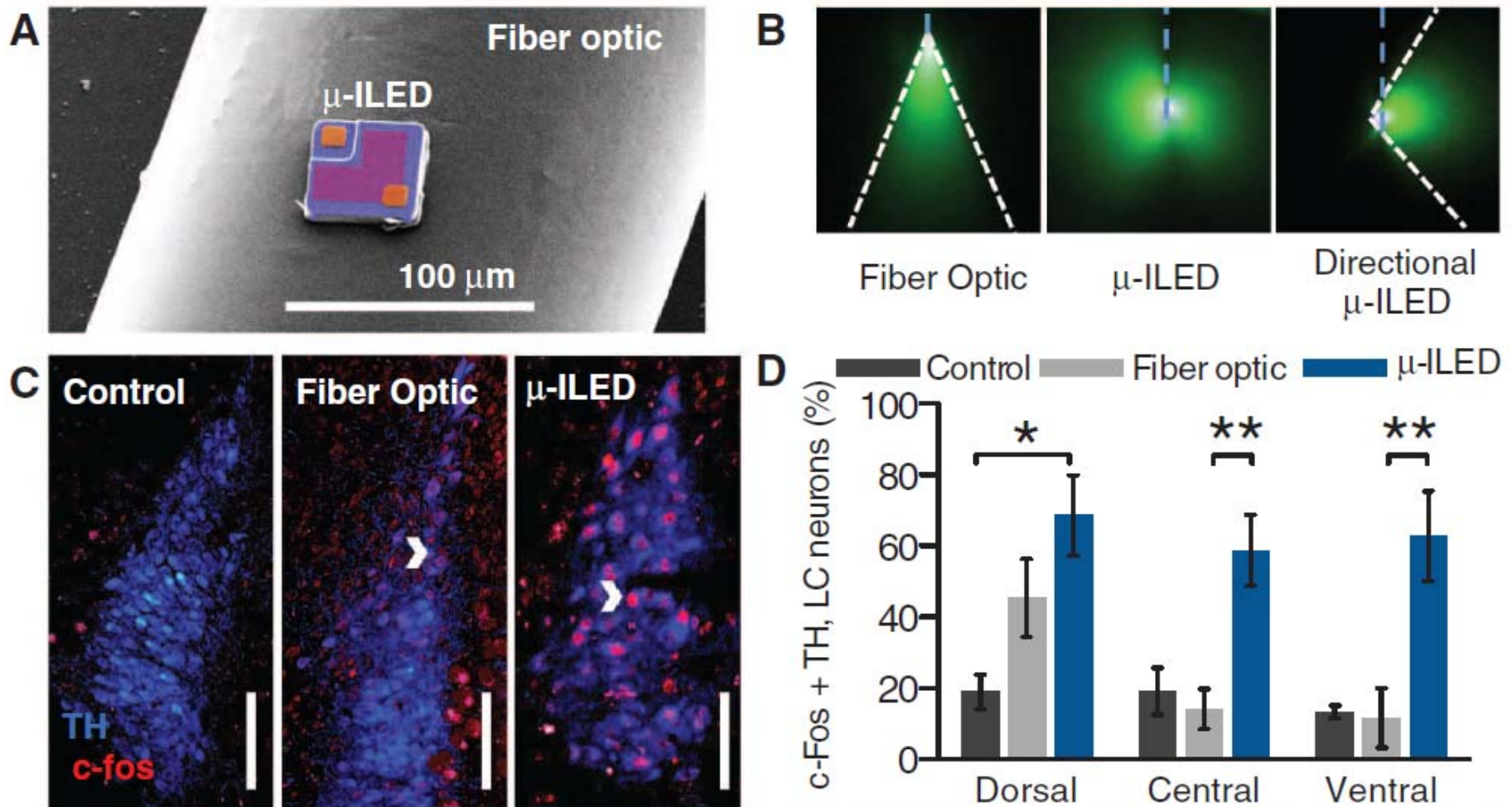
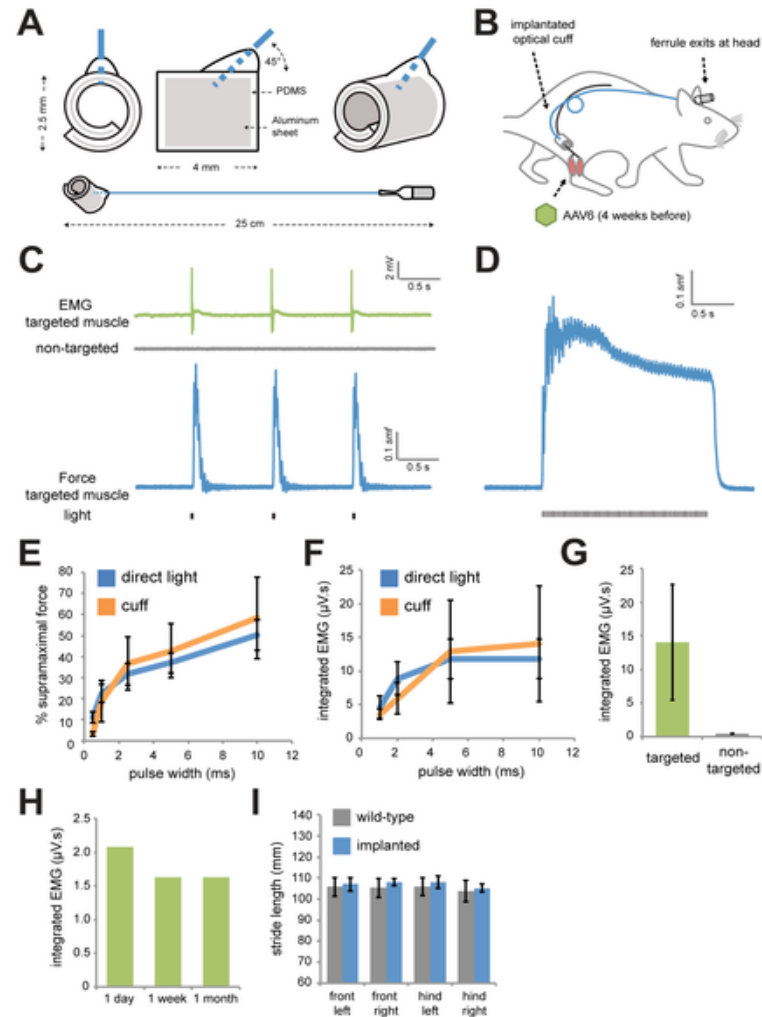
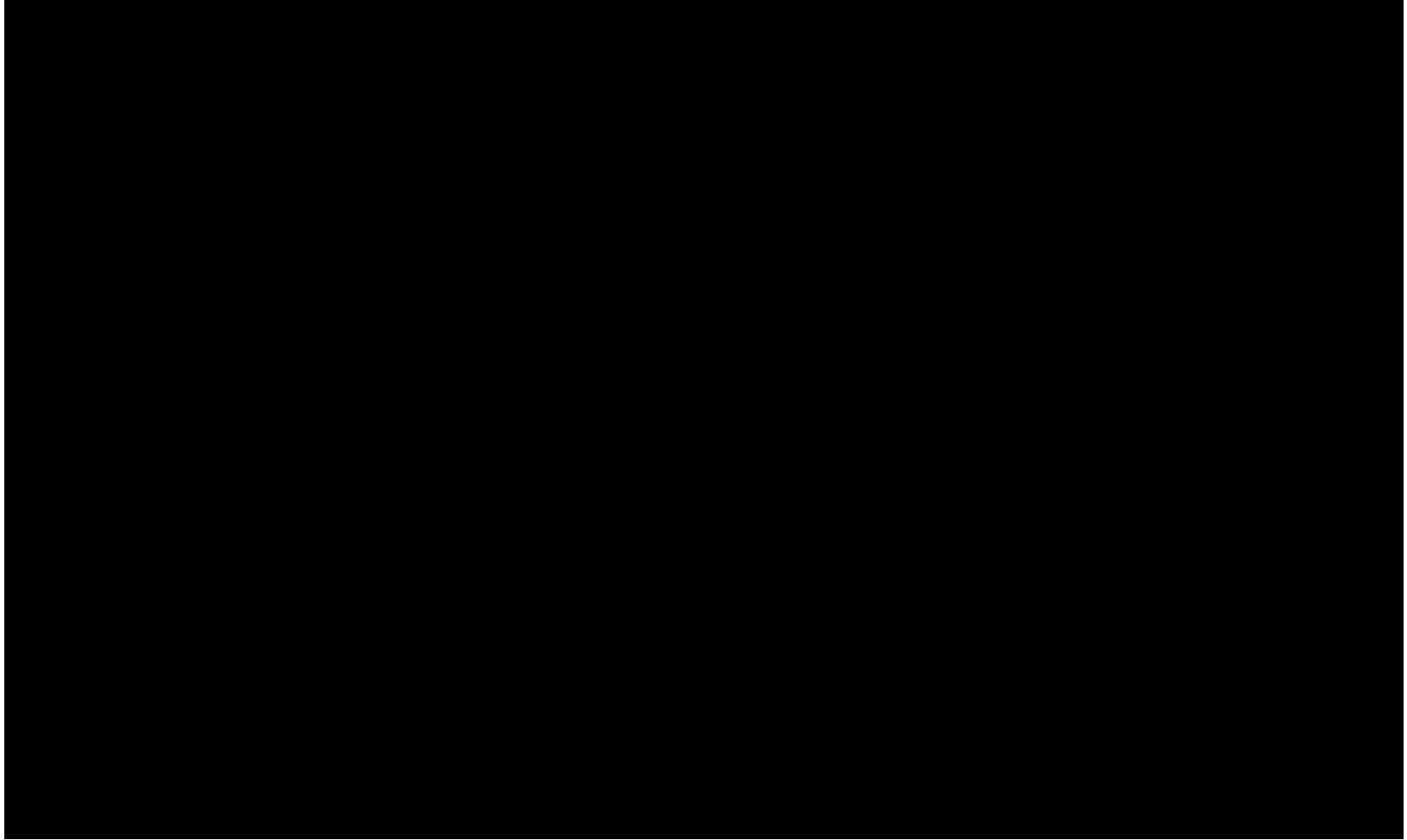


Figure 3. Implantable optical nerve cuffs are well tolerated and activate MNs in anesthetized rats.

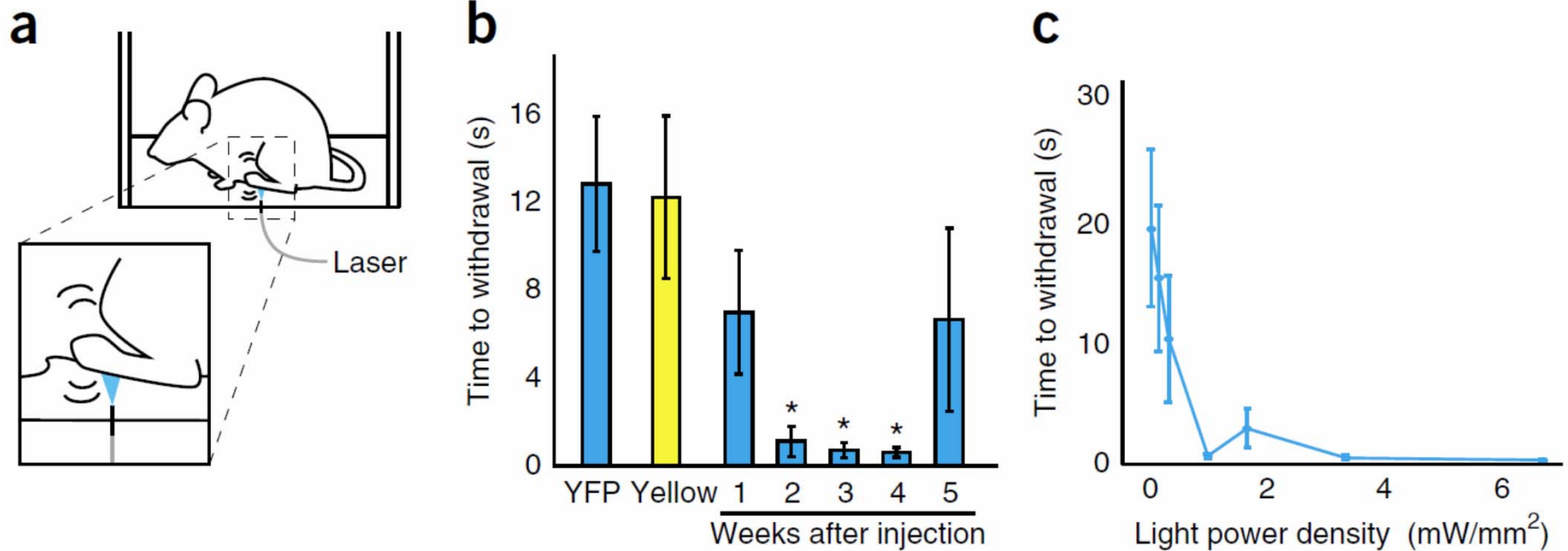


Towne C, Montgomery KL, Iyer SM, Deisseroth K, et al. (2013) Optogenetic Control of Targeted Peripheral Axons in Freely Moving Animals. *PLoS ONE* 8(8): e72691. doi:10.1371/journal.pone.0072691
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0072691>

Transdermal illumination of AAV6-hSyn-ChR2-eYFP–
injected mice results in tunable pain-like behavior



Transdermal illumination of AAV6-hSyn-ChR2-eYFP–injected mice results in tunable pain-like behavior



Nature biotechnology (2014) 32:274

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 😊