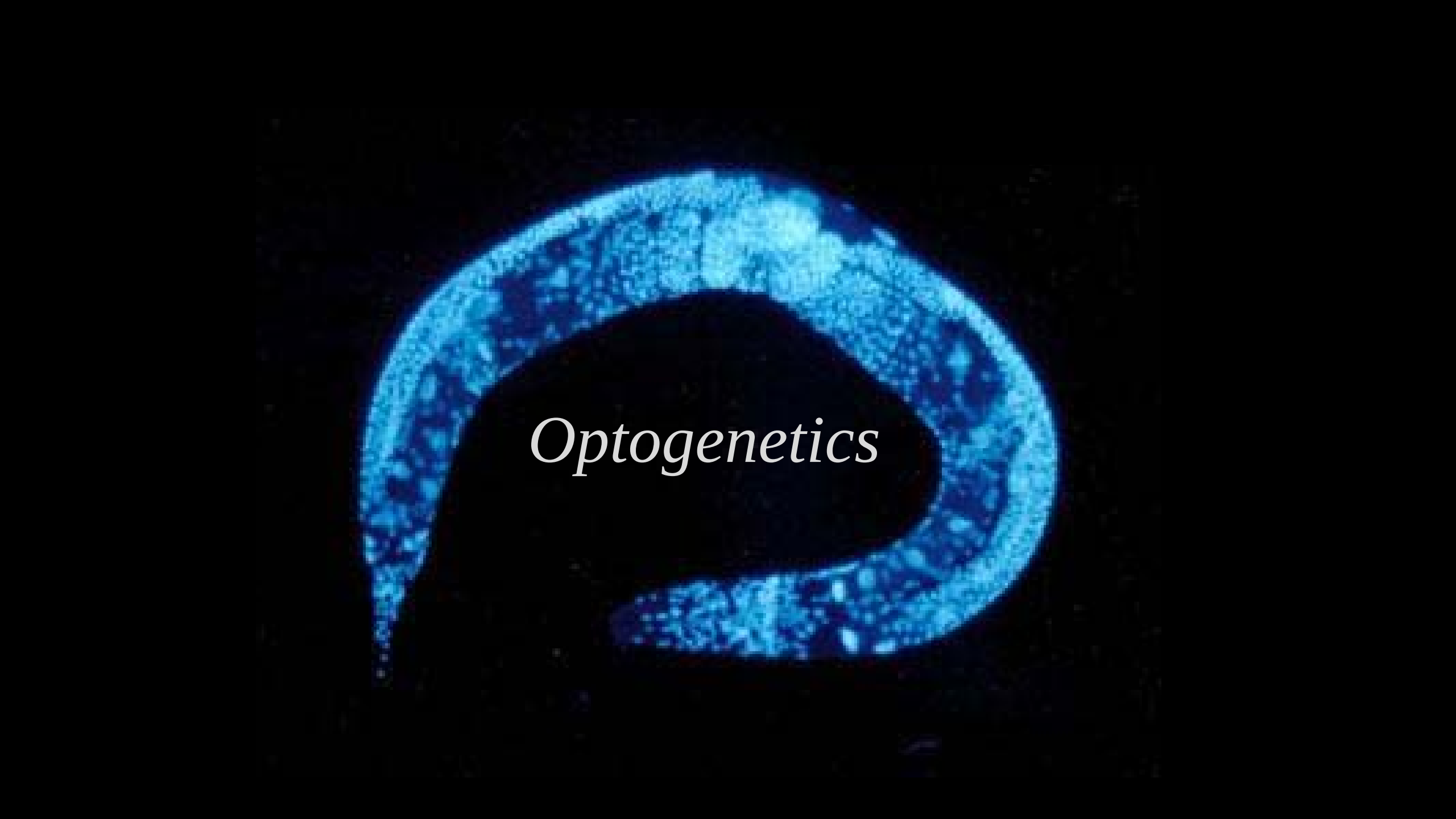
Shedding light on neural systems

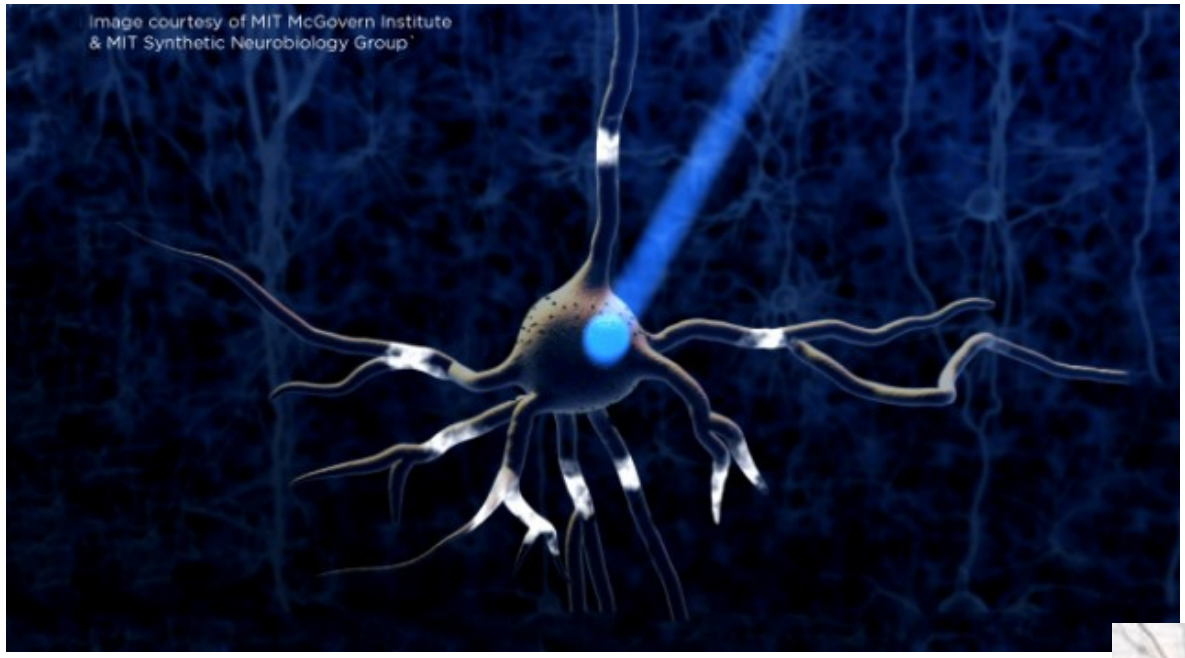
Journal Club

10.12.13

Despoina Goniotaki


Optogenetics

Optogenetics



✓ Drugs

✓ Electrical stimulation

integration of optics and genetics

→ to monitor well-defined events (e.g. action potentials)

→ within specified cells (e.g. targeted class of specific neurons)

→ in living tissues (e.g. the brains of freely behaving animals)

ILLUMINATING THE BRAIN

SIX STEPS TO OPTOGENETICS

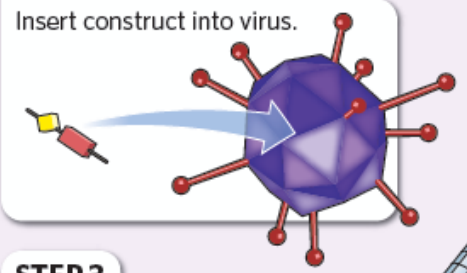
With optogenetic techniques, researchers can modulate the activity of targeted neurons using light.

STEP 1

Piece together genetic construct.

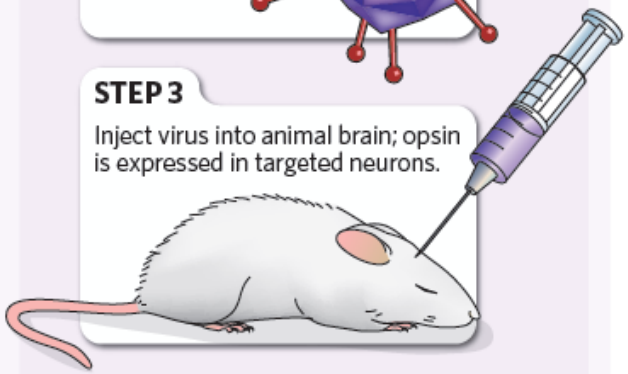
STEP 2

Insert construct into virus.



STEP 3

Inject virus into animal brain; opsin is expressed in targeted neurons.

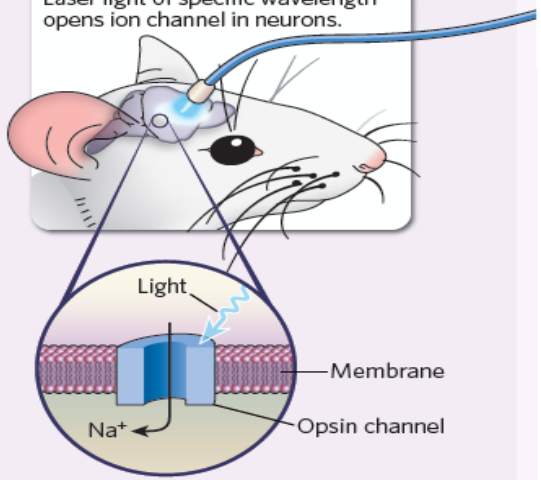


STEP 4

Insert 'optrode', fibre-optic cable plus electrode.

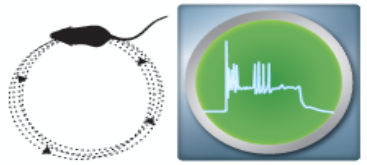
STEP 5

Laser light of specific wavelength opens ion channel in neurons.



STEP 6

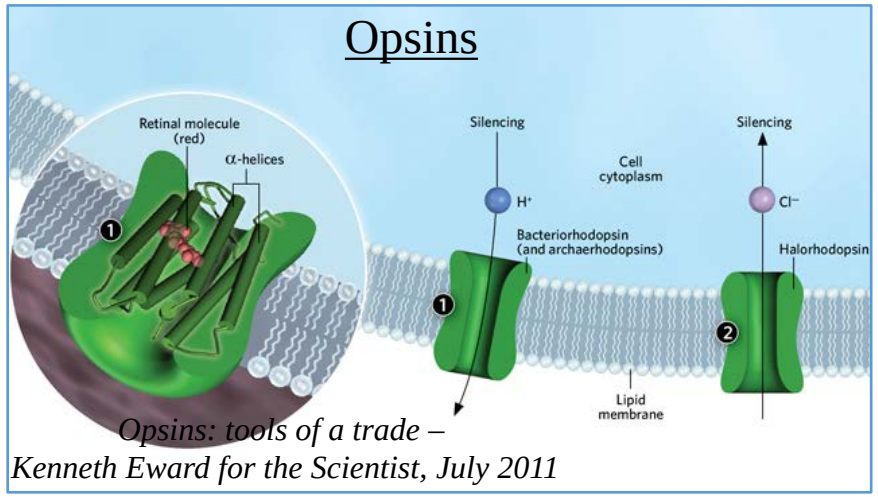
Record electrophysiological and behavioural results.



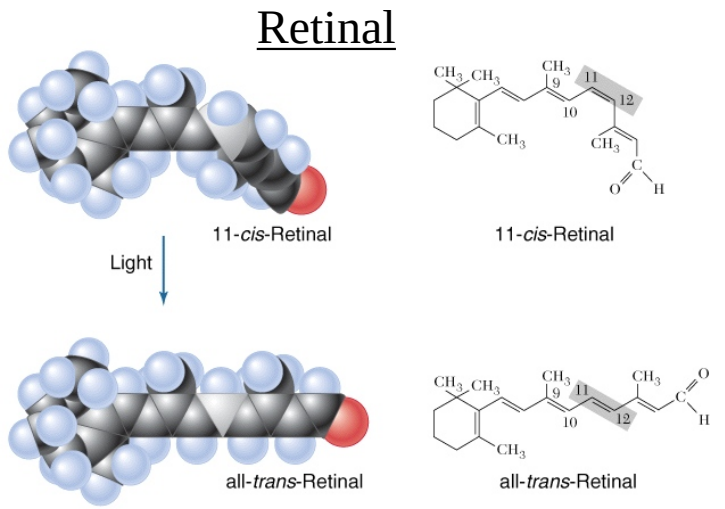
Grandinaru et al, Nature, 2010

Step I: Protein Engineering

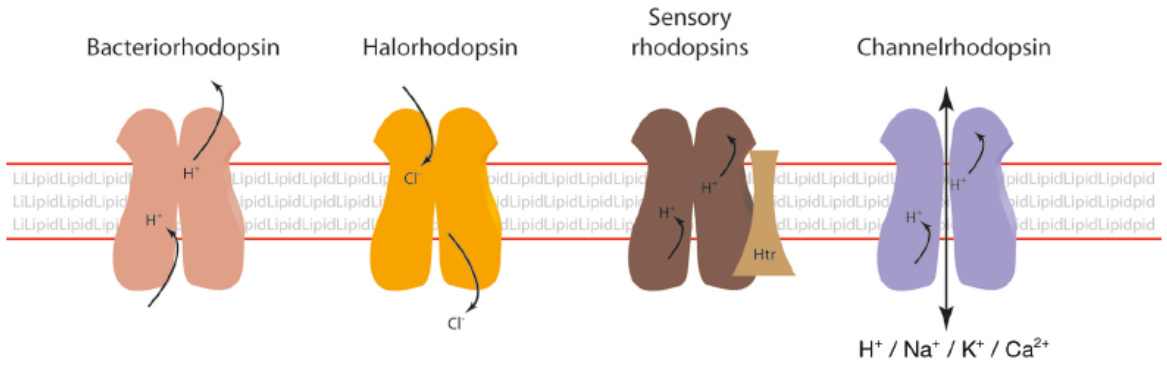
Opsins: light activated proteins that can be used to activate or deactivate neuronal activity



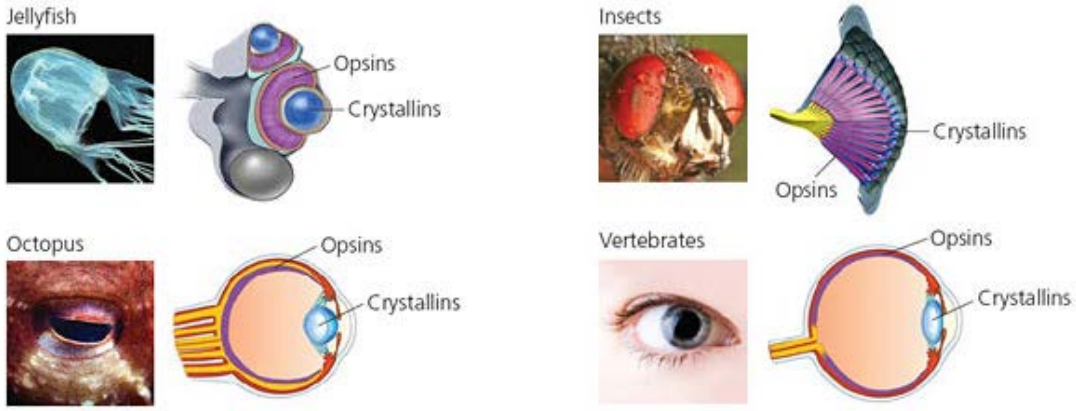
Opsins: tools of a trade –
Kenneth Eward for the Scientist, July 2011



microbial type opsins



animal type opsins

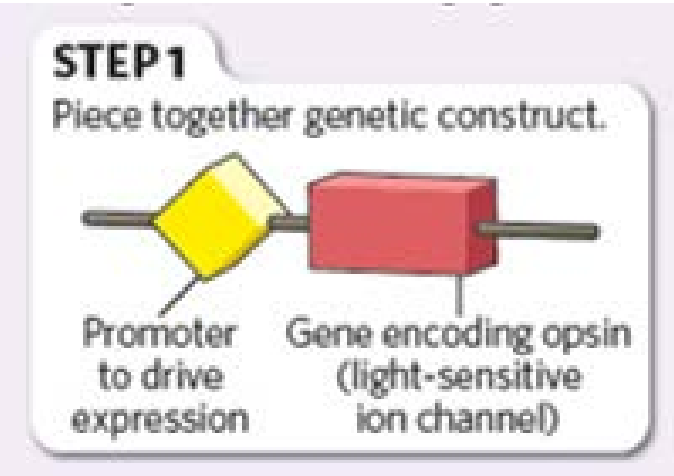


Step I: Protein Engineering

selecting an opsin

key factors to consider are:

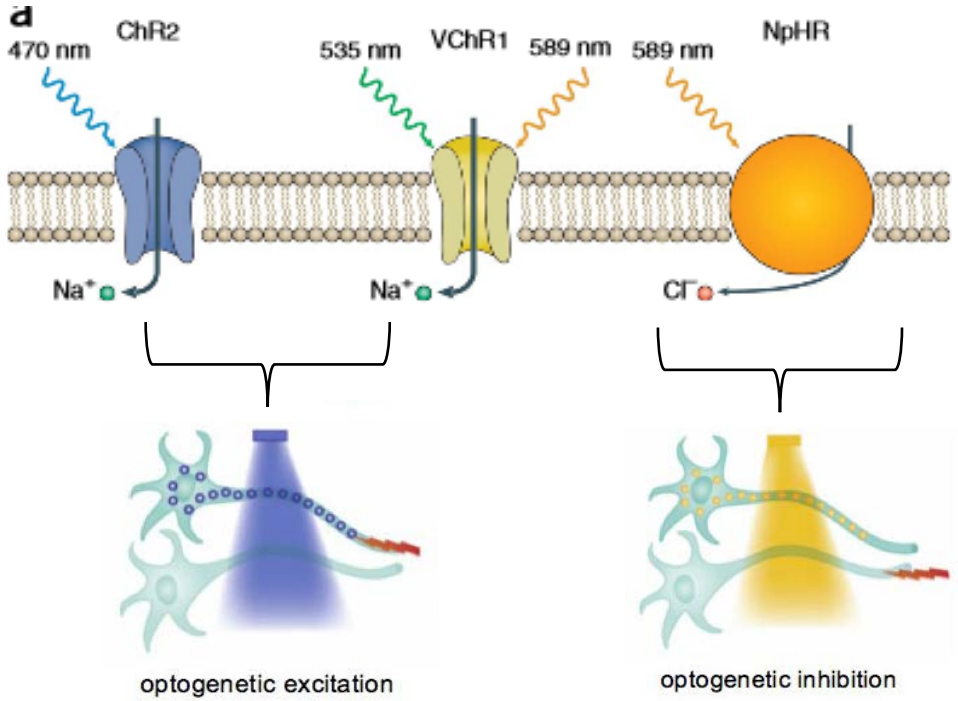
i. the experiment – polarity of manipulation (exciting, inhibiting or bidirectionally manipulating activity)



Channelrhodopsin-2 (ChR2)

cation-conducting channelrhodopsin (*VChR1*) from Volvox carteri

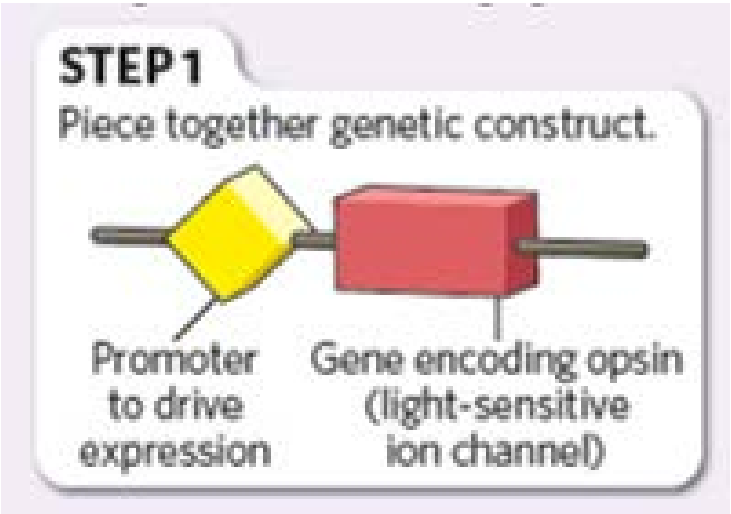
Halorhodopsin (NpHR)



Zhang et al, Nature Protocols, 2007

Step I: Protein Engineering

selecting an opsin



key factors to consider are:

i. the experiment – polarity of manipulation (exciting, inhibiting or bidirectionally manipulating activity)

ii. time course of manipulation (millisecond control of spiking, prolonged manipulation, subtle manipulation)

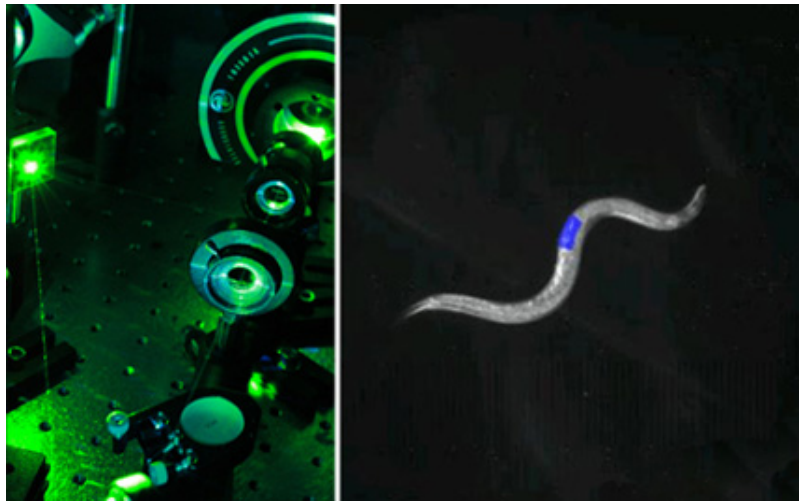
iii. selected wavelength of light (lower wavelengths for deeper penetration, differential wavelengths if 2 opsins are used simultaneously)

iv. the expression level of the chosen opsin (promoters & viruses can drive expression very high) (expression vs toxicity)

v. expression strategy (synchronous excitation, action potentials)

Step II: Selecting an animal model

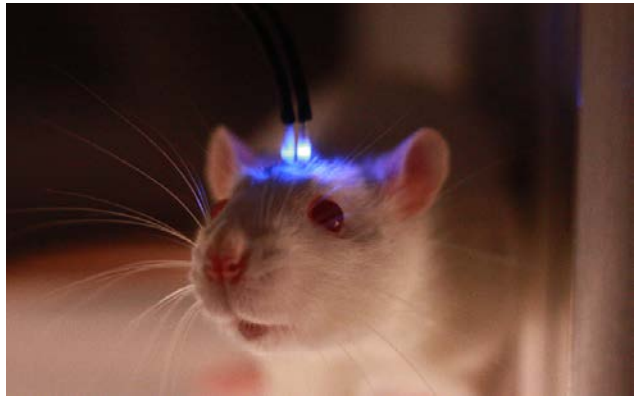
C. elegans



Zebrafish

Drosophila

Mouse



Step III: Selecting a light source

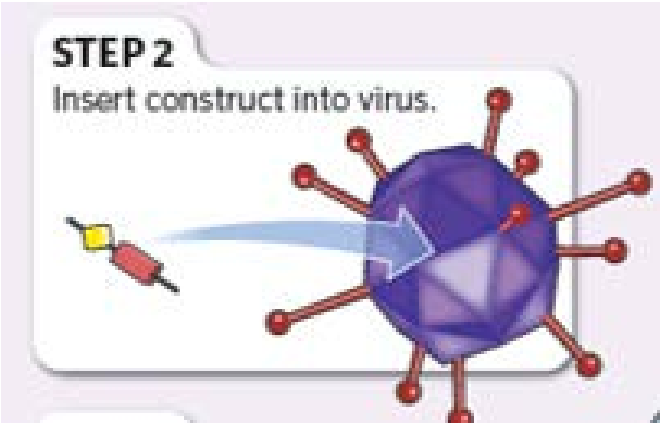
Mercury/Xenon bulb

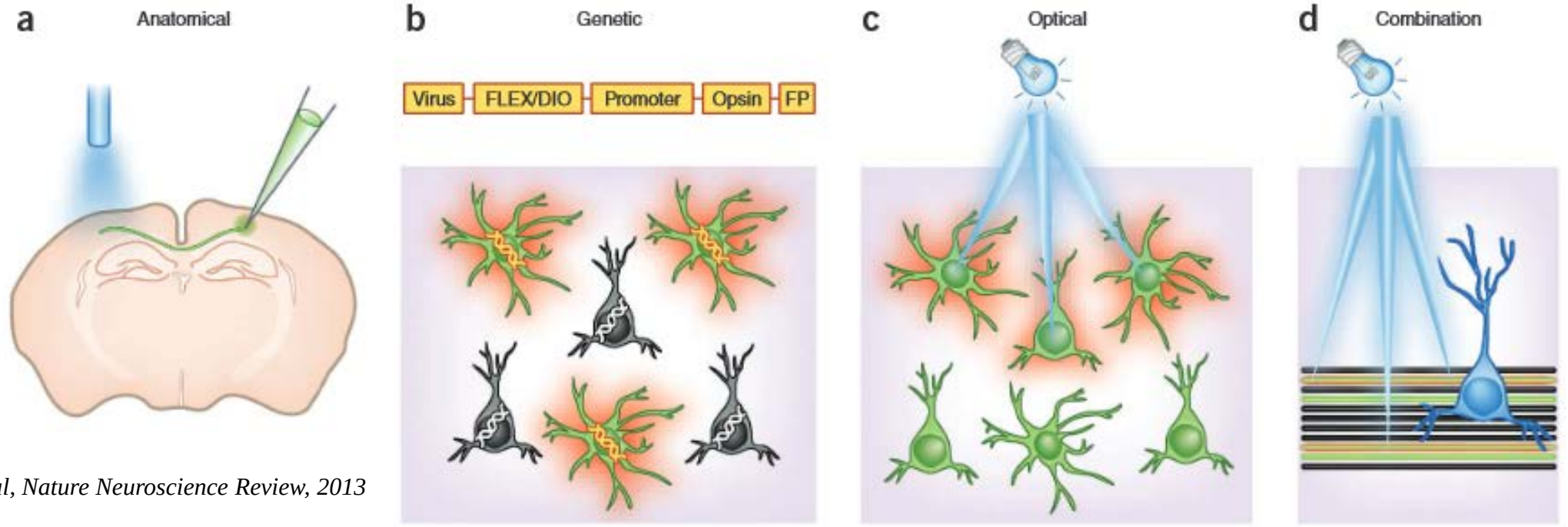
Light emitting diode (LED)

Continuous wave laser

Ultrafast laser

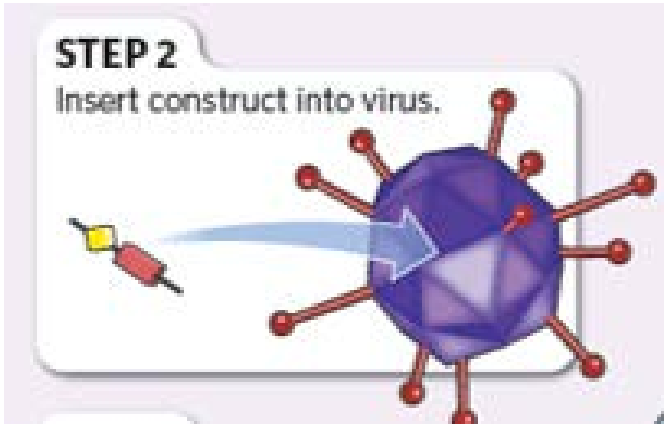
Step IV: Targeting optogenetic probes to the 'right' group of cells





Packer et al, Nature Neuroscience Review, 2013

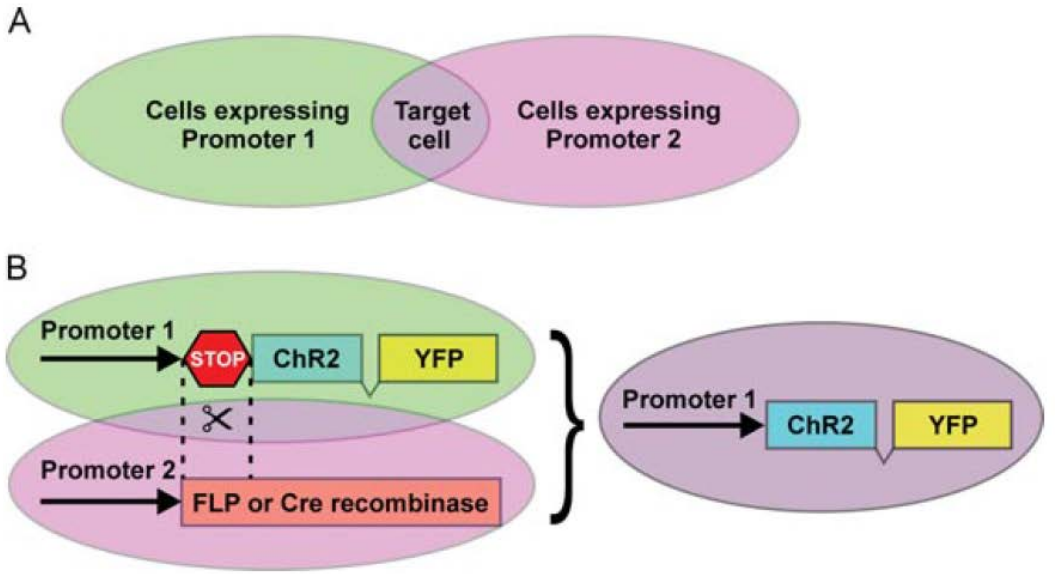
Step IV: Targeting optogenetic probes to the 'right' group of cells



Genetic Manipulation

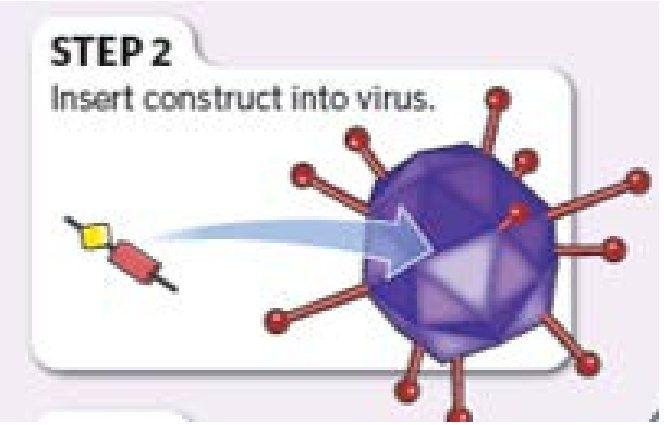
Combinations of partially overlapping promoters are used to restrict expression of opsins to a subpopulation of cells

Specificity of targeting may be increased using intersectional strategies



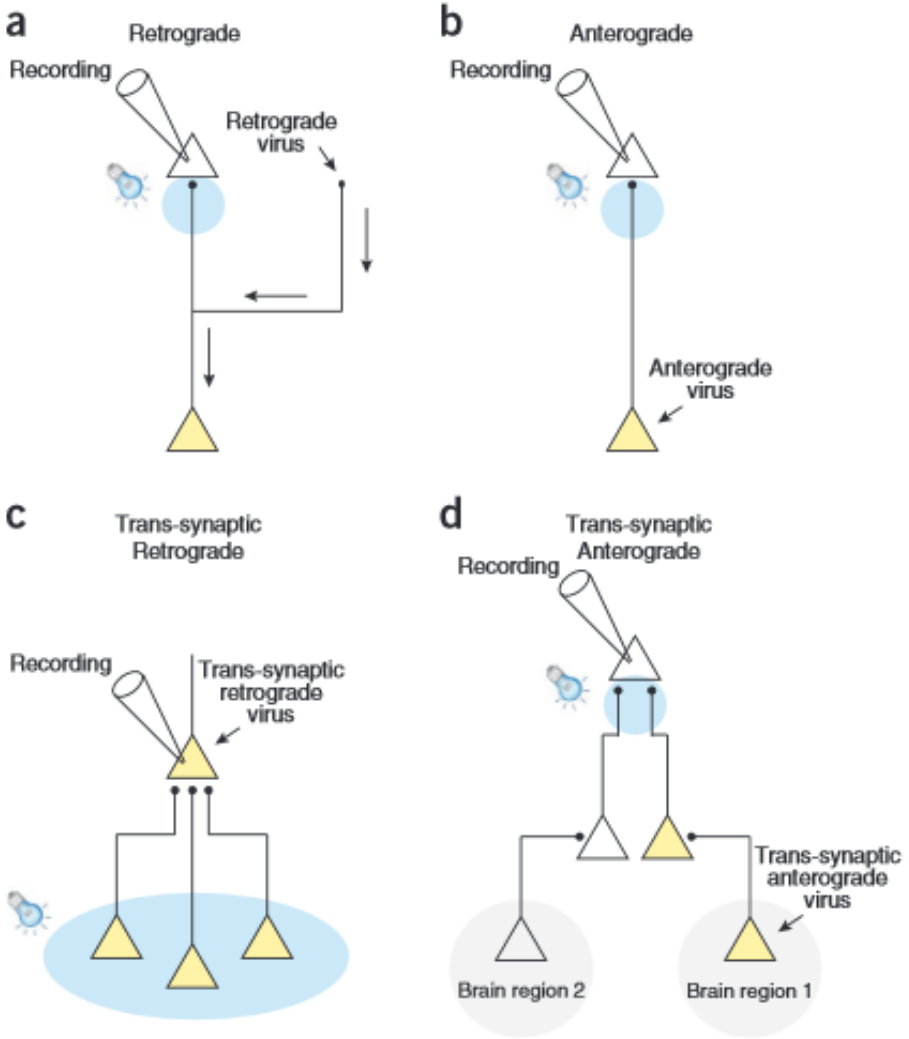
Husson et al, Biol Cell, 2013

Step IV: Targeting optogenetic probes to the 'right' group of cells



Specificity of targeting
may be increased
using intersectional strategies

Viral targeting of optogenetic tools
based on circuit connectivity.

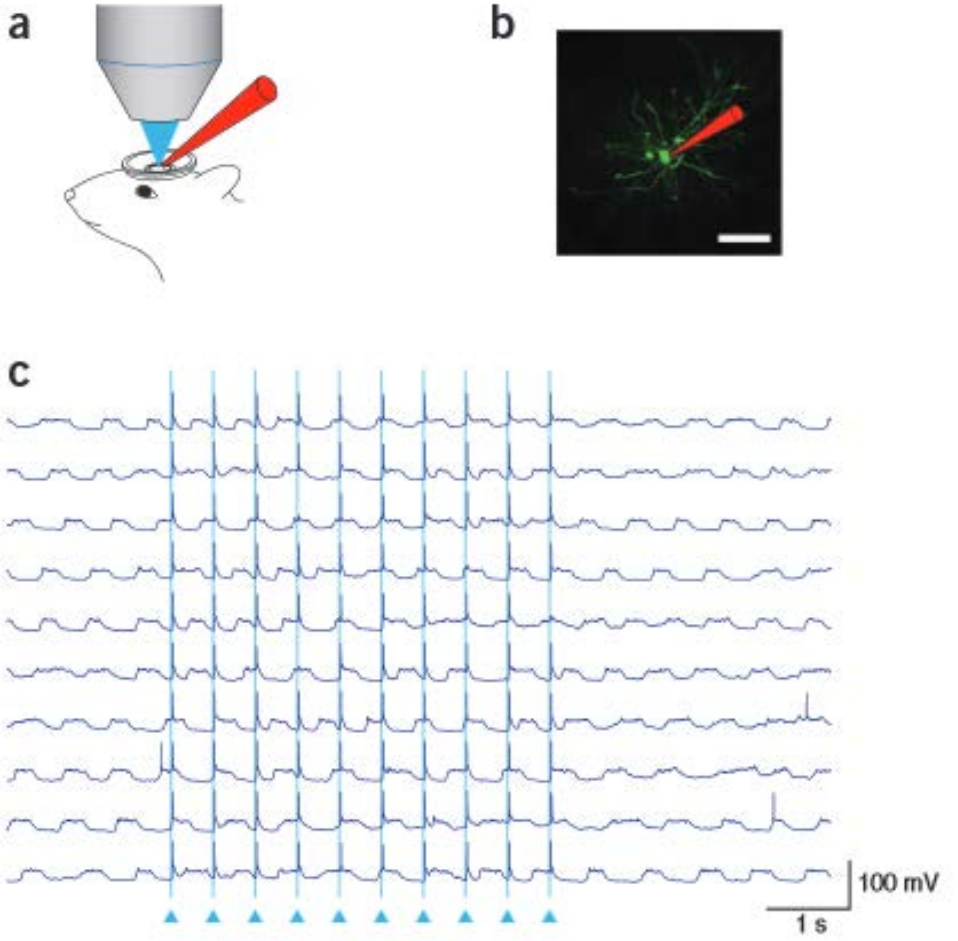


Packer et al, Nature Neuroscience Review, 2013

Step IV: Targeting optogenetic probes to the 'right' group of cells

Specificity of targeting
may be increased
using intersectional strategies

Single cell targeting of optogenetic tools
single cell electroporation and 2P microscopy.



Packer et al, Nature Neuroscience Review, 2013

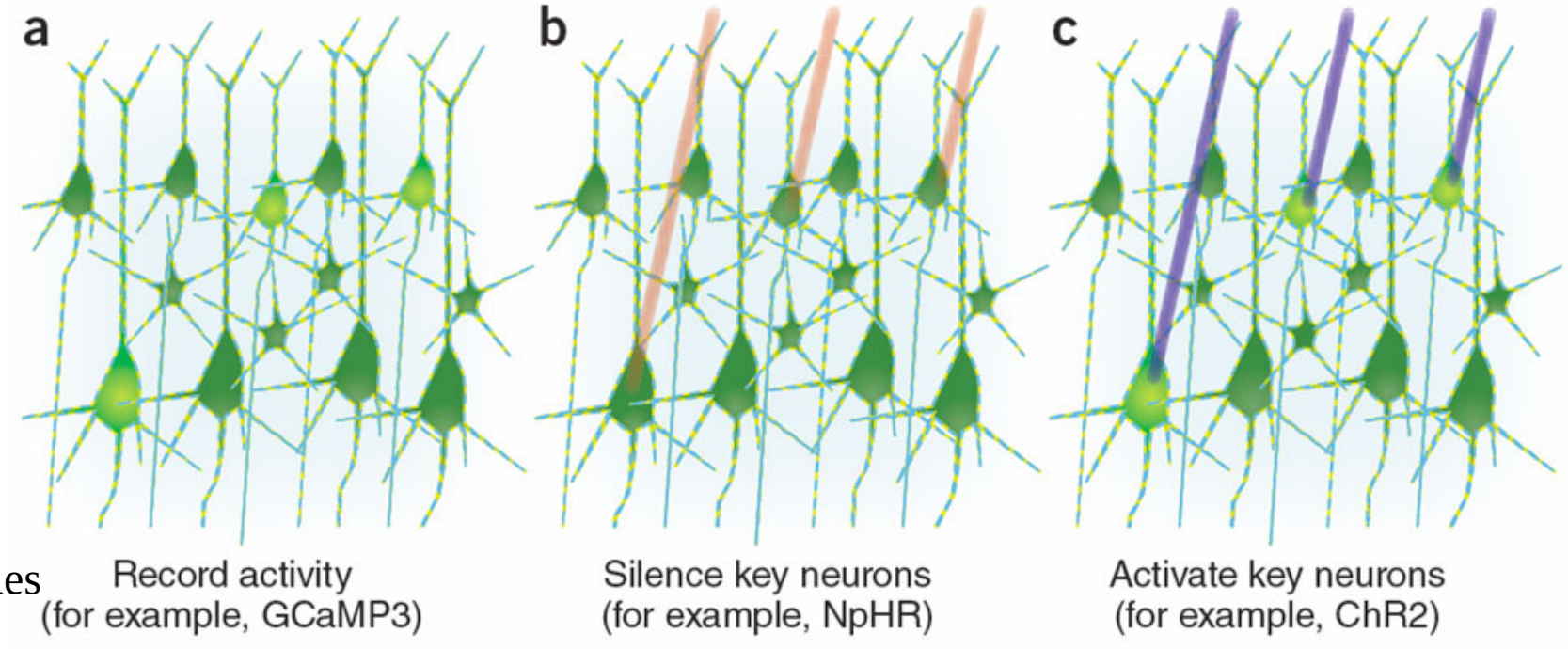
Step IV: Targeting optogenetic probes to the 'right' group of cells

DREAM scenario

Activity-dependent expression of optogenes.

spatially distributed and sparse neural assemblies can be precisely manipulated in behaving animals

Specificity of targeting may be increased using intersectional strategies

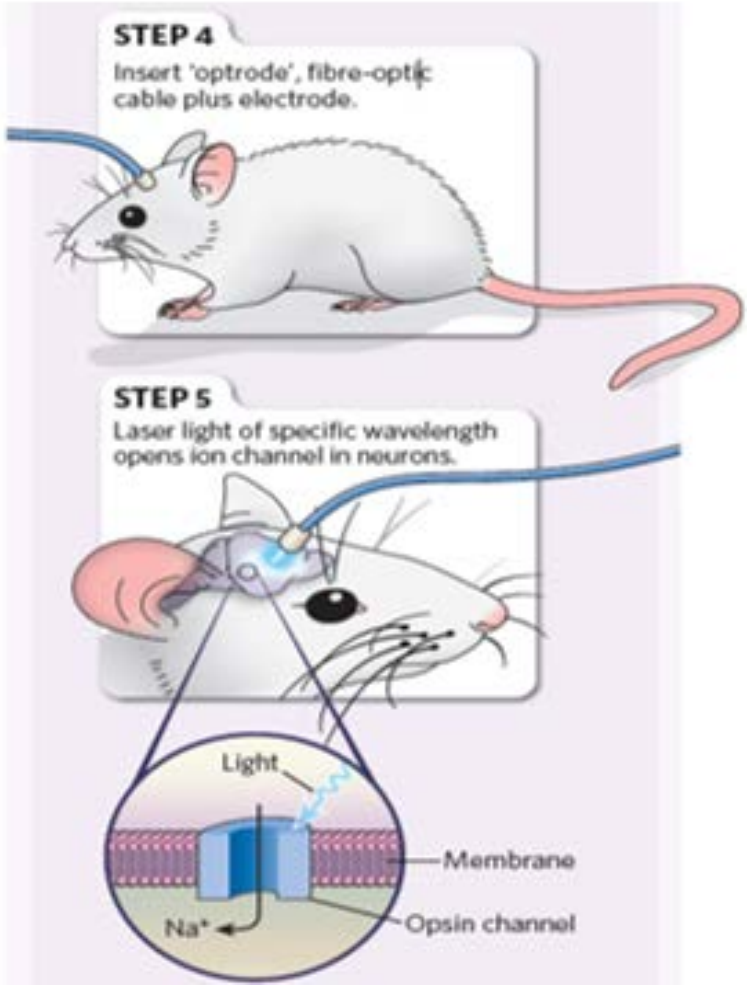


Functionally defined neuronal assemblies

e.g. reactivation of only the subset of neurons that had been active during a recent behavioral episode

Peron and Svoboda, Nature Methods, 2011

Step V: Targeted light delivery



Light targeting should be:
i. sufficiently precise and ii. rapid
to allow selective activation of individual cells

key factors to consider are:

i. the wavelength
ii. the intensity
iii. scattering of the light in the model system being used
iv. the optical delivery system

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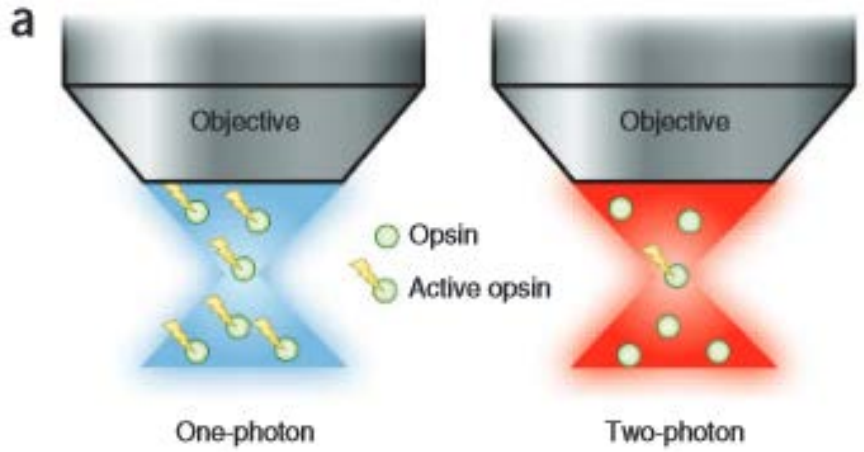
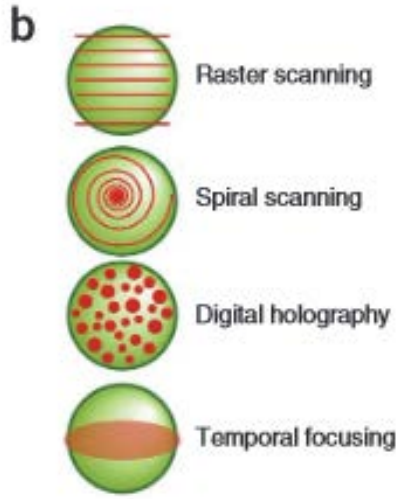
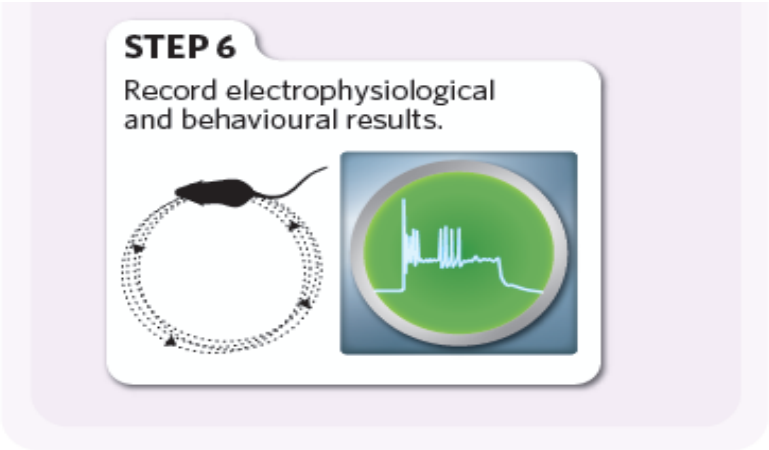
1P vs 2P microscopy



2P microscopy allows different spatiotemporal patterns



Step VI: Recordings



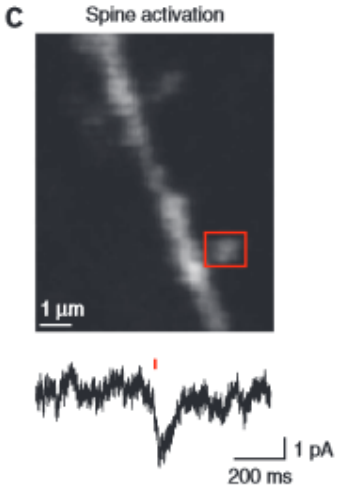
BRIEF COMMUNICATIONS

Two-photon optogenetics of dendritic spines and neural circuits

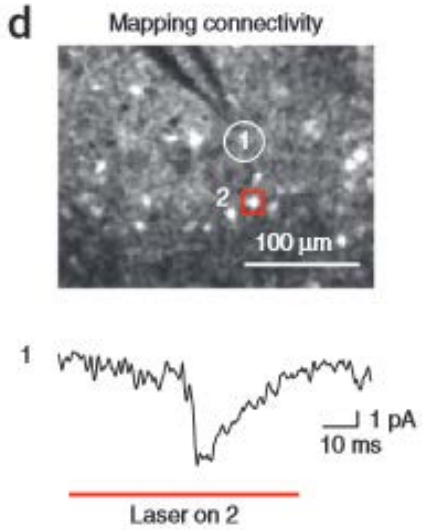
Adam M Packer[1,2,5,6], Darcy S Peterka[1,2,6], Jan J Hirtz[1], Rohit Prakash[3,4], Karl Deisseroth[3,4] & Rafael Yuste[1,2]

Two-photon optogenetics:

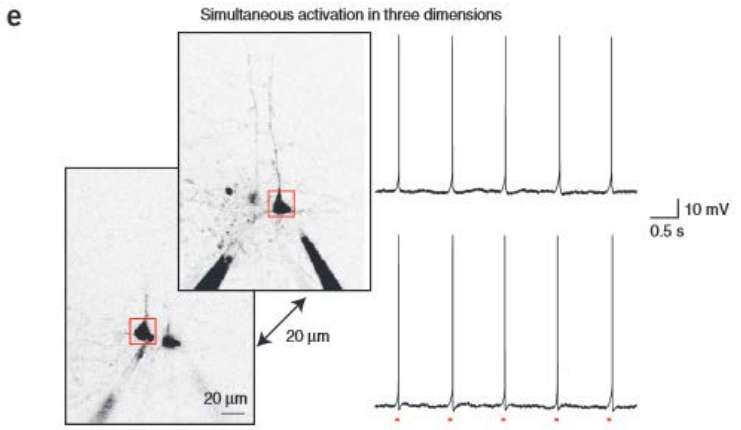
to generate action potentials in neurons with single-cell precision



2P point stimulation of a dendritic spine on a neuron expressing the red-shifted opsin $C1V1_T$ generates current – detectable at the soma.



Two-photon raster-scanning of neuron 2 during electrophysiological recording from neuron 1 indicates that neuron 2 is monosynaptically connected to neuron



Simultaneous action potential generation in two neurons in three dimensions using a spatial light modulator to generate separate laser beamlets over each neuron.

Packer et al, Nature Methods, 2012

C. elegans serves as a model organism for behavioral experiments

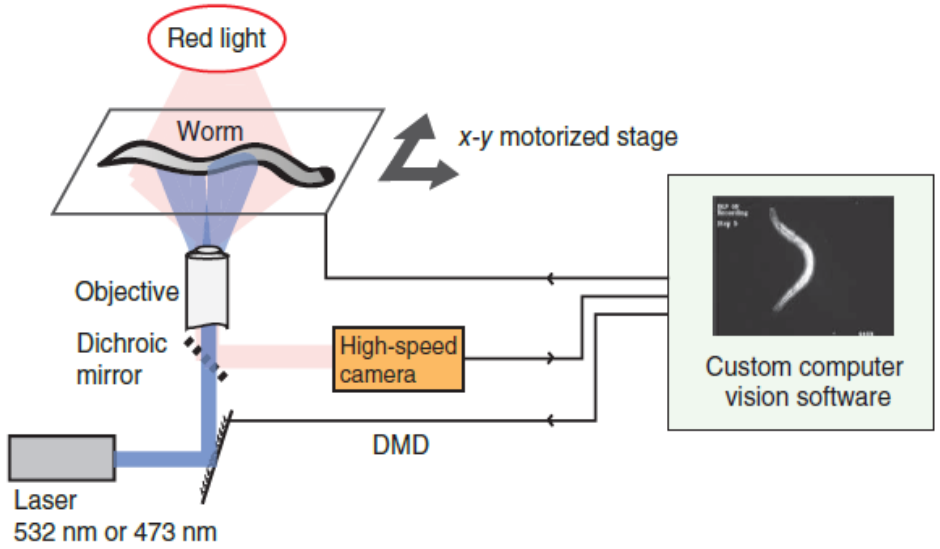
C. elegans was the first multicellular organisms to have its behavior manipulated by channelrhodopsin (*Nagel et al 2005*)

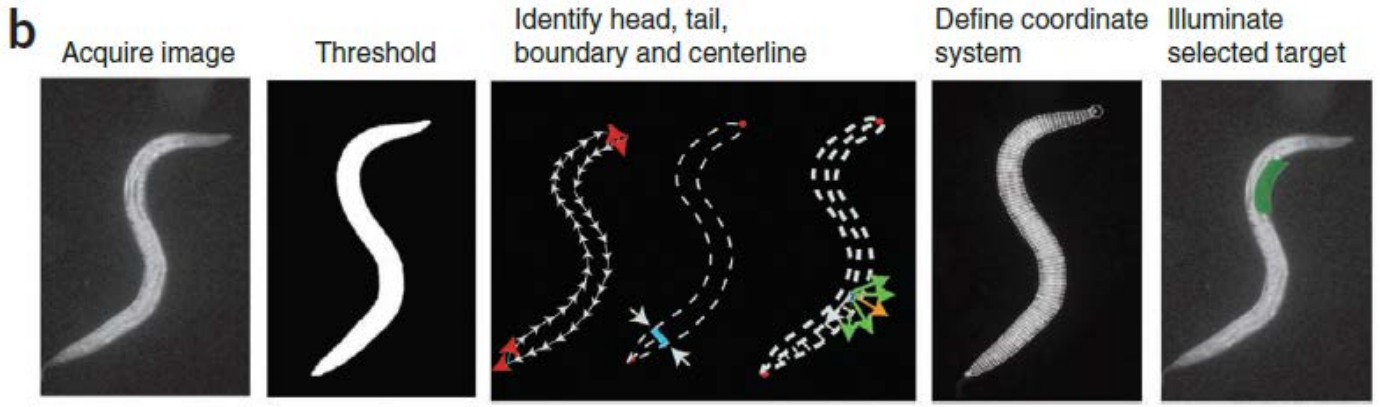
Optogenetic manipulation of neural activity in freely moving *Caenorhabditis elegans*

Andrew M Leifer[1,4], Christopher Fang-Yen[1,2,4], Marc Gershow[1], Mark J Alkema[3] & Aravinthan D T Samuel[1]

Imaging setup (Colbert)

Imaging experiments take advantage of the worm's stereotyped morphology to automatically identify targetted cells based on real-time images of the worm's body as it moves.





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Optogenetic Inactivation of muscle cells

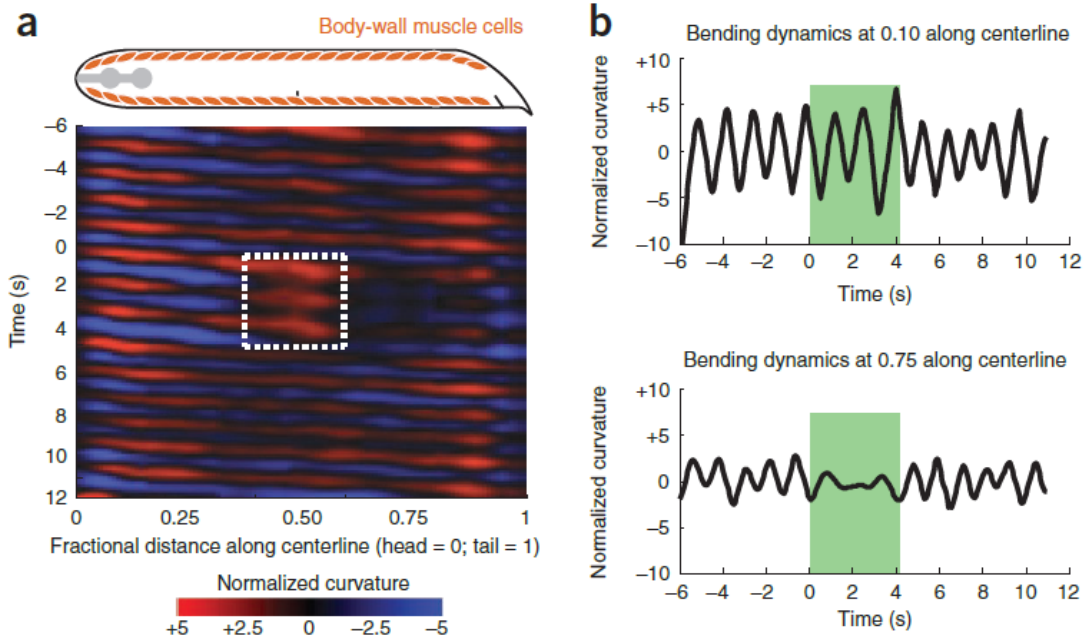
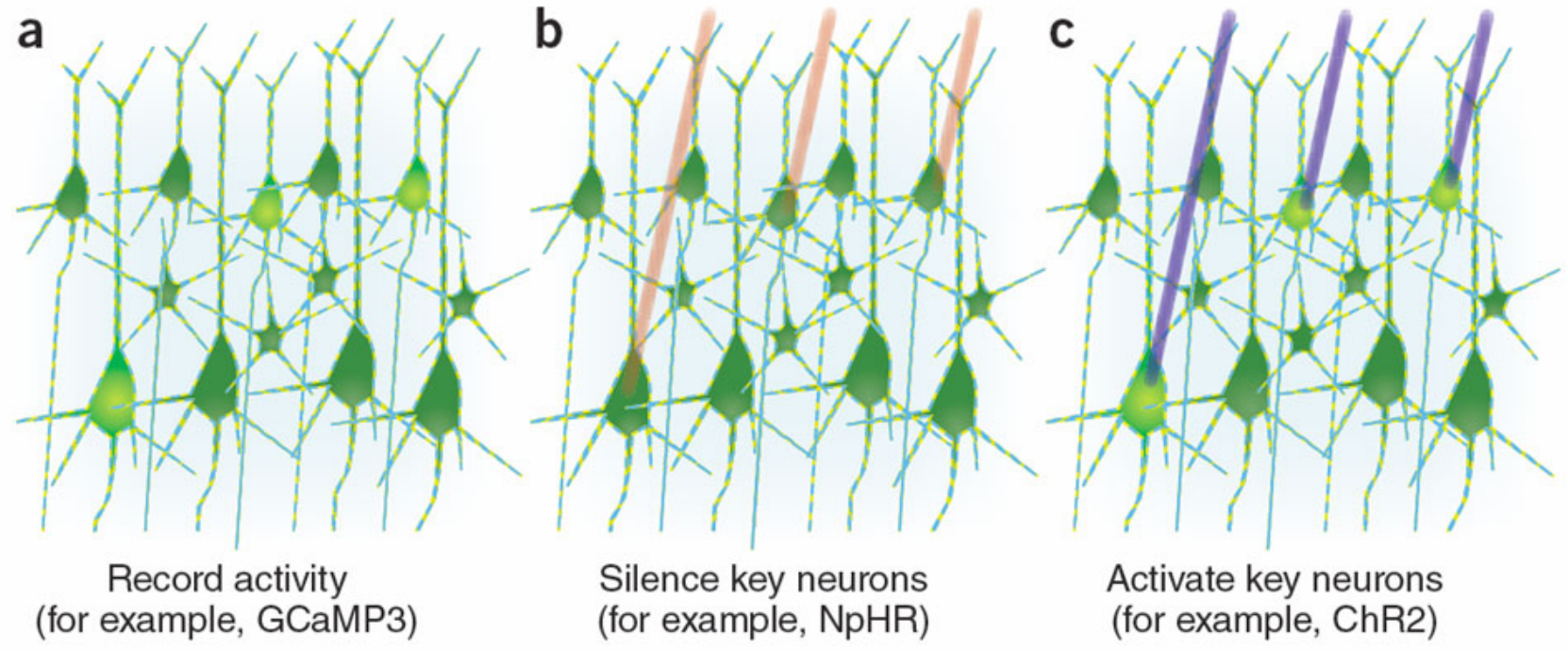


Figure 2 | Optogenetic inactivation of muscle cells. (**a**) Kymograph of time-varying body curvature along the centerline of a P*myo3::Halo/NpHR::CFP* transgenic worm. Between 0 s and 4 s, the worm was stimulated with green light (10 mW mm^{-2}) in a region spanning the worm diameter and between 0.38 and 0.6 of the fractional distance along the centerline. (**b**) For the kymograph in **a**, time-varying curvature at two points along the worm centerline, both anterior (top) and posterior (bottom) to the illuminated region.

DREAM experiment

Activity-dependent expression of optogenes.

e.g. reactivation of only the subset
of neurons that had been active
during a recent behavioral episode



Peron and Svoboda, Nature Methods, 2011

DREAM experiment

Reactivation of only the subset of neurons that had been active during a recent behavioral episode

Neuronal activity measurements

Ca^{2+} imaging

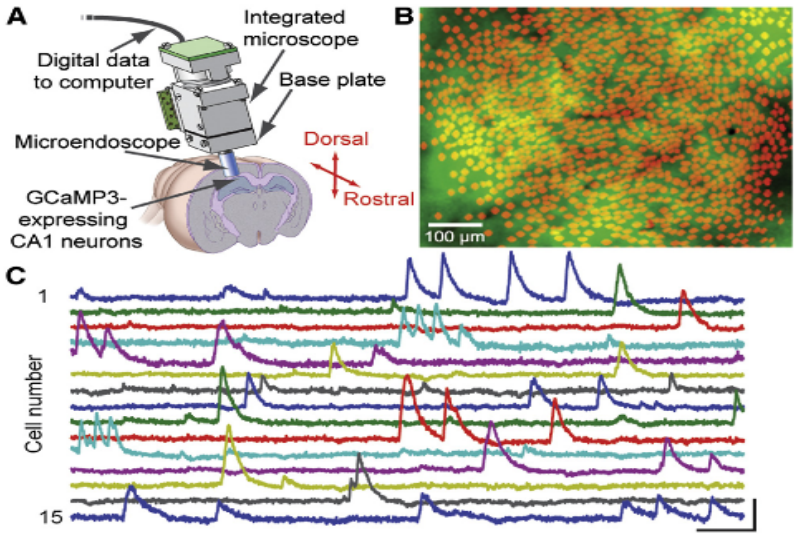


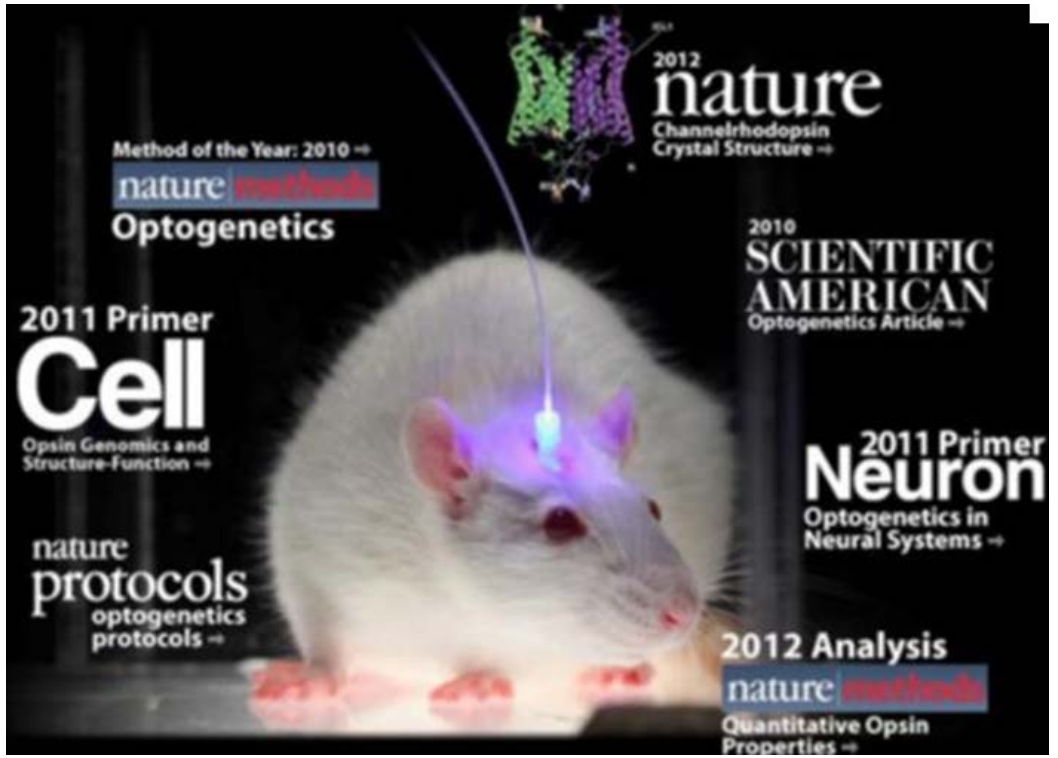
Figure 1. Optics and Protein Engineering Converge for Ca^{2+} Imaging in >1,200 CA1 Pyramidal Cells in Freely Moving Mice

(A) An integrated microscope is equipped with a microendoscope to image CA1 neurons expressing the engineered Ca^{2+} indicator GCaMP3 under control of the *Camk2a* promoter. The base plate and microendoscope are fixed to the cranium for repeated access to the same field of view. Republished from Ziv et al., 2013.

(B) Shown are 1,202 CA1 pyramidal cells (red somata) identified by Ca^{2+} imaging in a freely moving mouse atop a mean fluorescence image (green) of CA1. Vessels appear as dark shadows. Image courtesy of Yaniv Ziv and Lacey Kitch, Stanford University.

(C) Example traces of $[Ca^{2+}]_i$ dynamics from 15 cells. Scale bars denote 5% ΔF/F (vertical) and 10 s (horizontal).

Optogenetics



Deisseroth and Schneitzer, Neuron Perspective, 2013

Suggested Title: **Simultaneous optogenetic manipulation and calcium imaging in freely moving *C. elegans***

Frederick B. Shipley[1], Christopher M. Clark[2], Mark J. Alkema[2], Andrew M. Leifer[1*]
[1] Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA
[2] Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA, USA
* To whom correspondence should be addressed: leifer@princeton.edu

They induced neural activity using Channelrhodopsin (ChR2) and simultaneously monitored calcium dynamics
(by parallel measuring the fluorescence of an optical calcium indicator (GCaMP3)and a calcium insensitive reference mCherry).

Suggested Title: **Simultaneous optogenetic manipulation and calcium imaging in freely moving *C. elegans***

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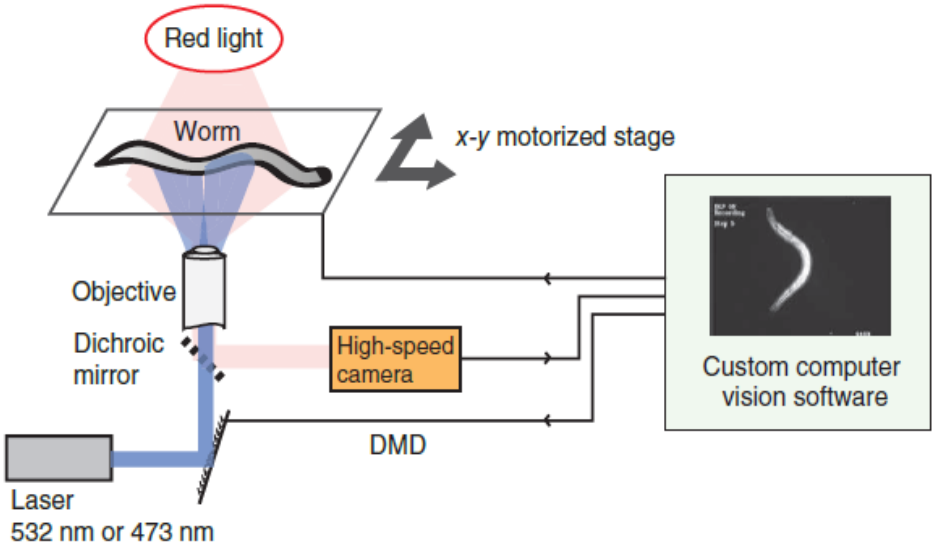
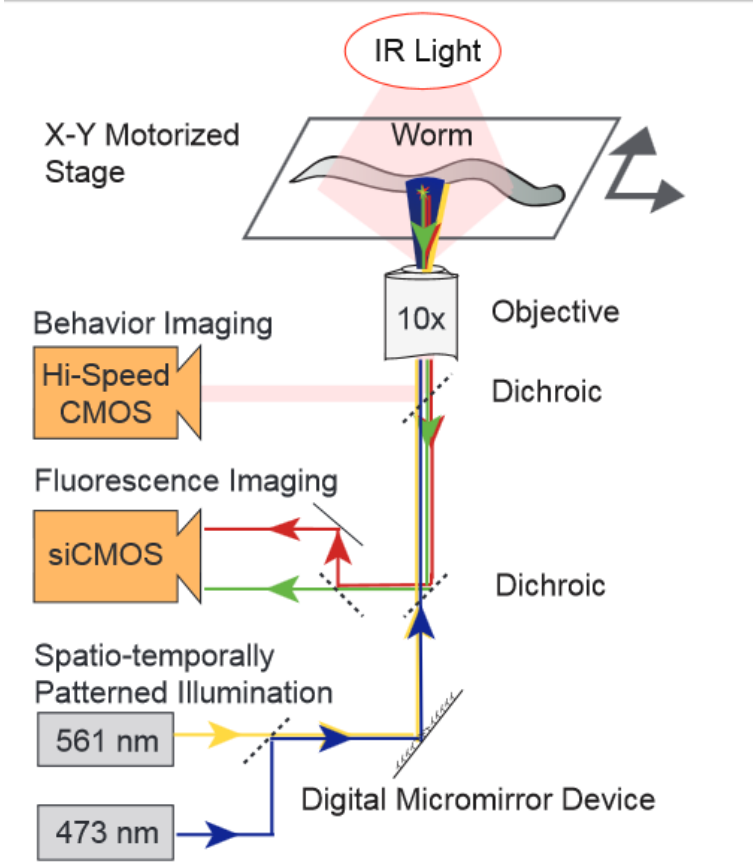
Imaging setup (Colbert)





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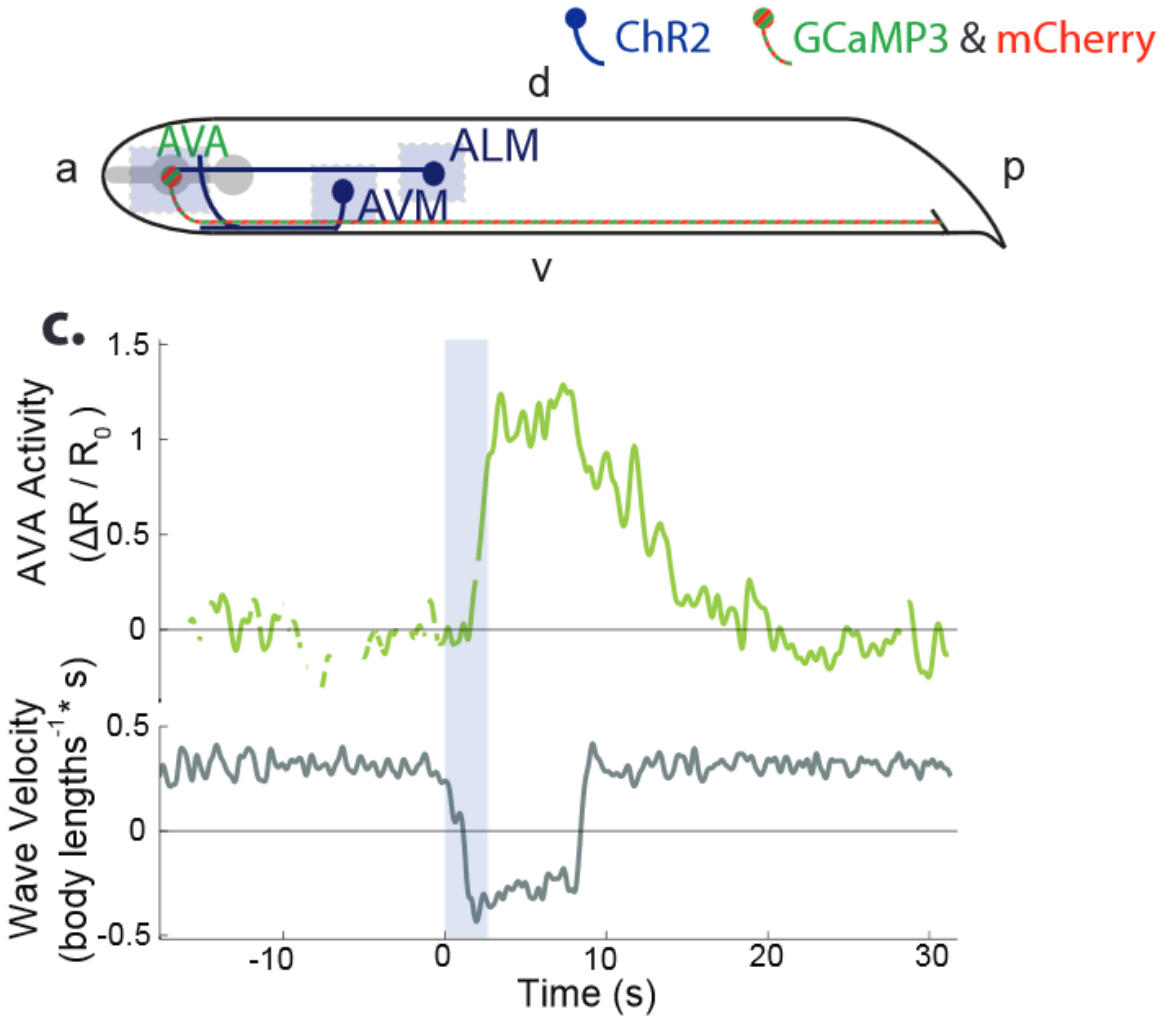
[1] Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA
[2] Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA, USA
* To whom correspondence should be addressed: leifer@princeton.edu

<u>System</u>: calcium measurements in the backward locomotion command interneurons AVA,
in response to activation of the anterior mechanosensory neurons ALM, AVM or both



→ continuously observe calcium dynamics from one neuron while transiently activating others.

Optogenetics

- Powerful technique for manipulating neurons

- Coupled with calcium imaging:

  ✓ better understanding of neuronal circuits

  ✓ precise neural control

Thank you!