Tools for Mapping the Circuits of Intelligence

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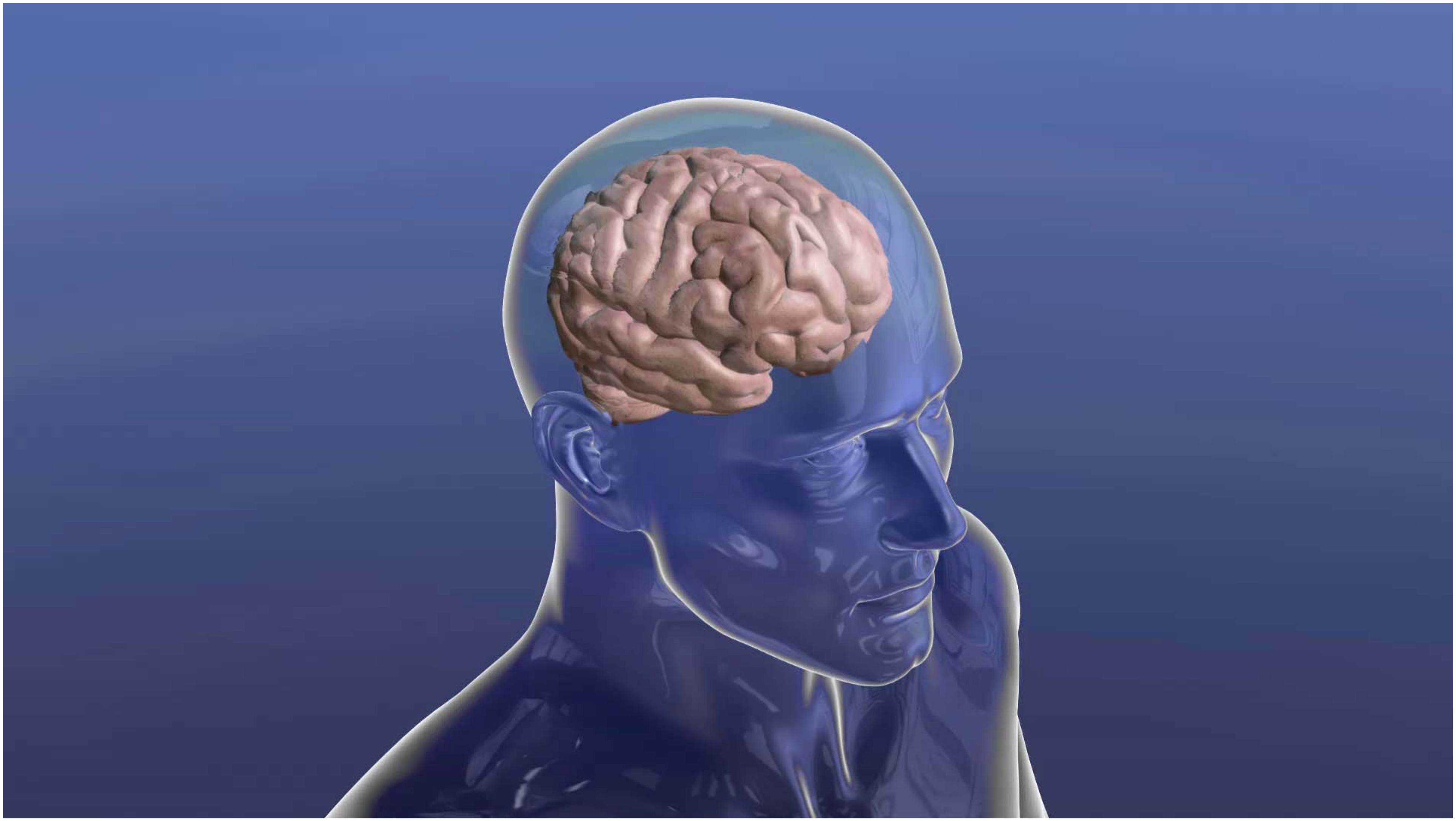
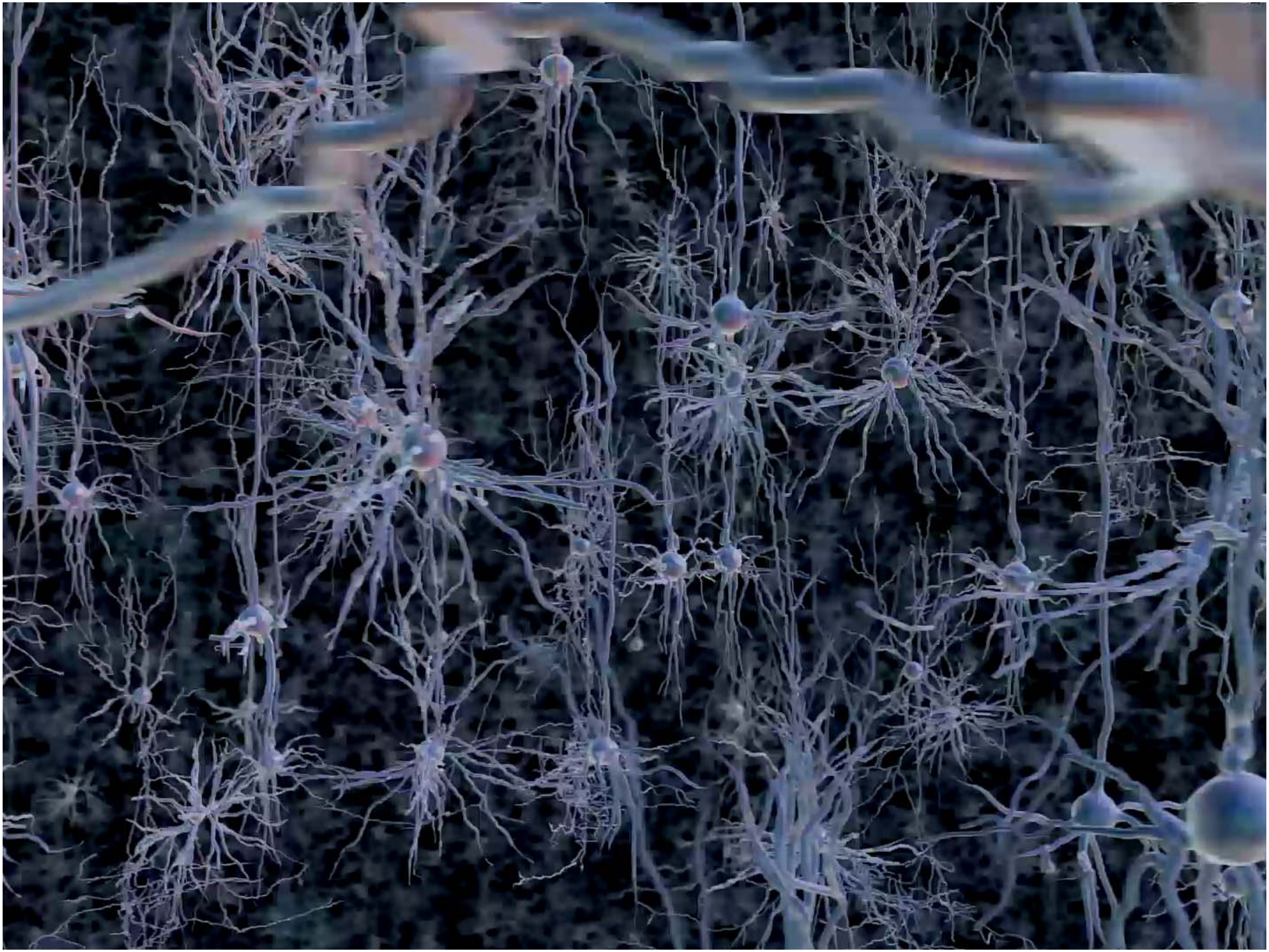
Understanding the brain is a problem of fundamental difficulty, but also of scale

Organized at nanoscale, but spanning centimeters (10^7 range)

Computing with millisecond timescale events, but spanning years (10^{12} range)





Principles of neuroengineering design:

Work **backwards** from properties of the brain,

Survey the entire scope of **engineering** possibility,

Invent technologies for analyzing and engineering the brain

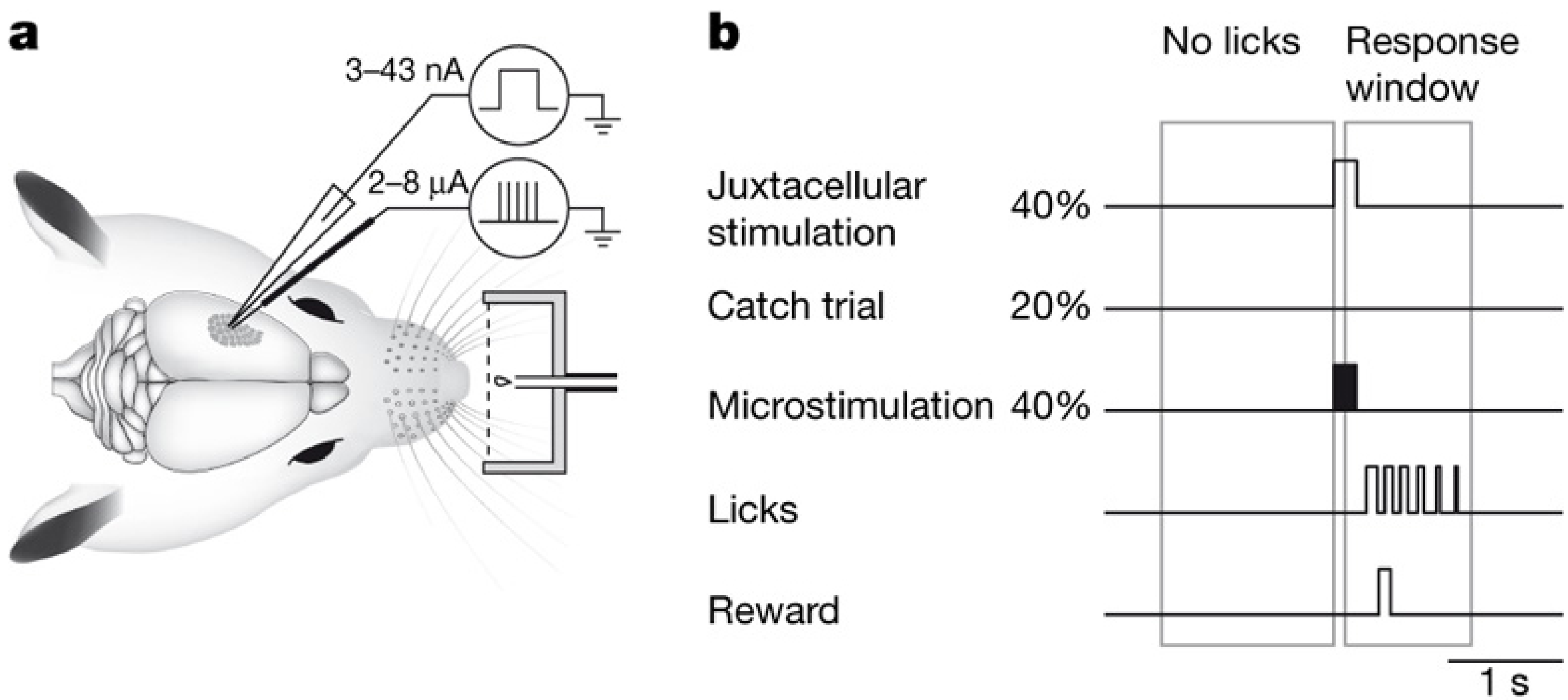
Map

Record

Control

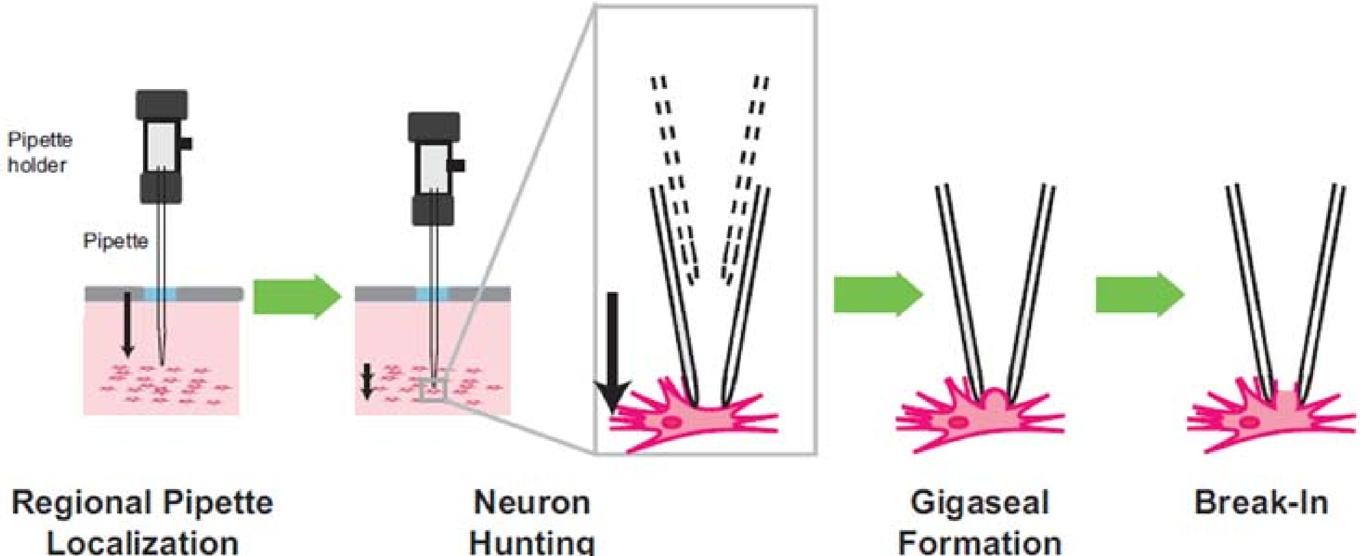
Build

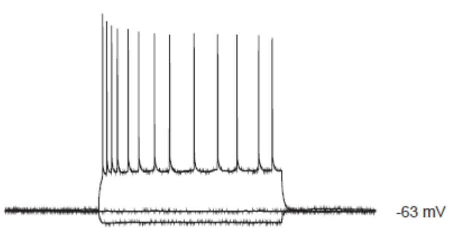
How does a single neuron compute?

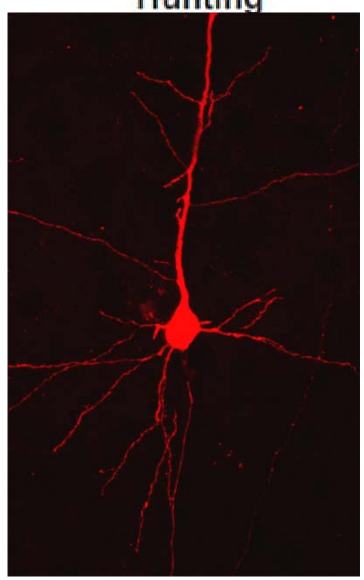


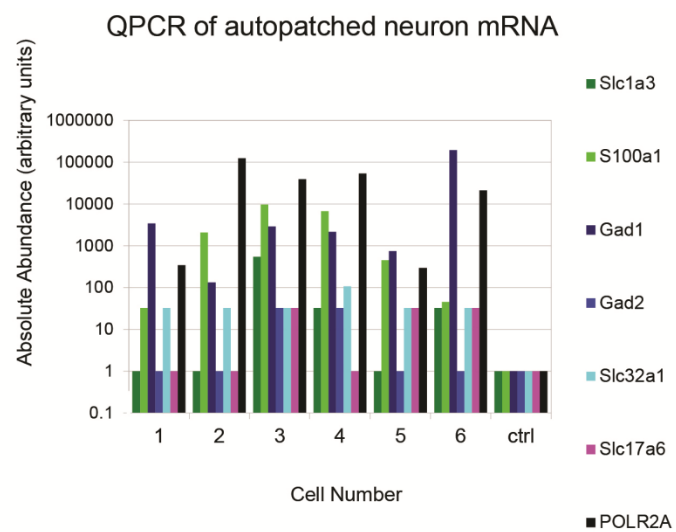
http://www.nature.com/nature/journal/v451/n7174/full/nature06447.html

Whole cell patch clamp: enables simultaneous measurement of electrophysiology, morphology, and gene expression in single cells in living brain

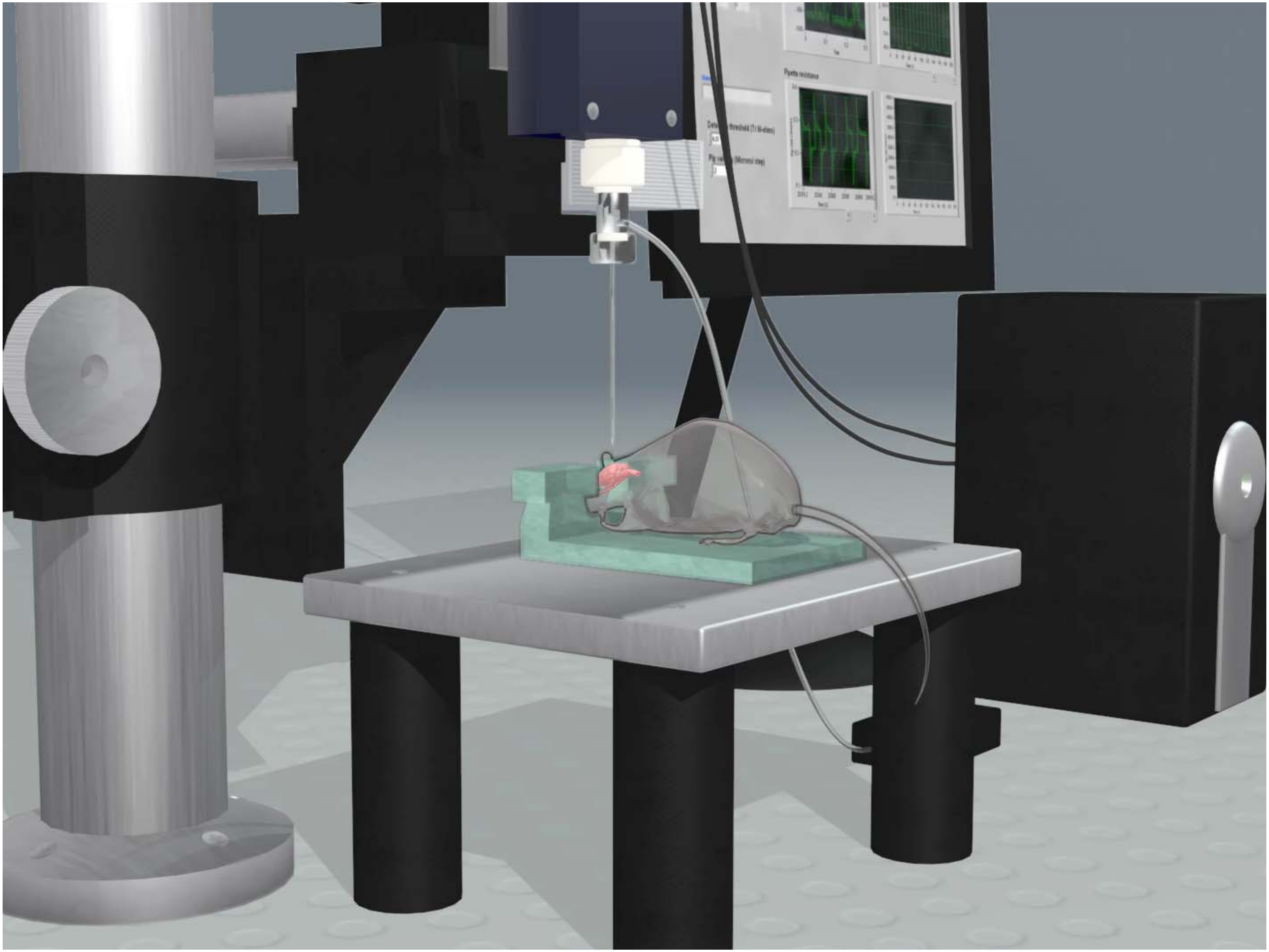




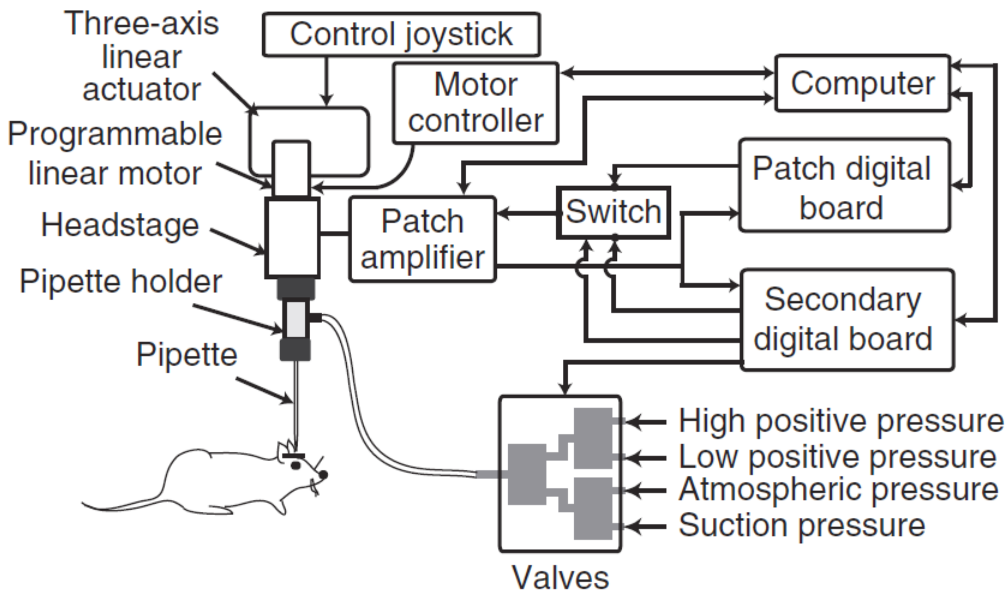


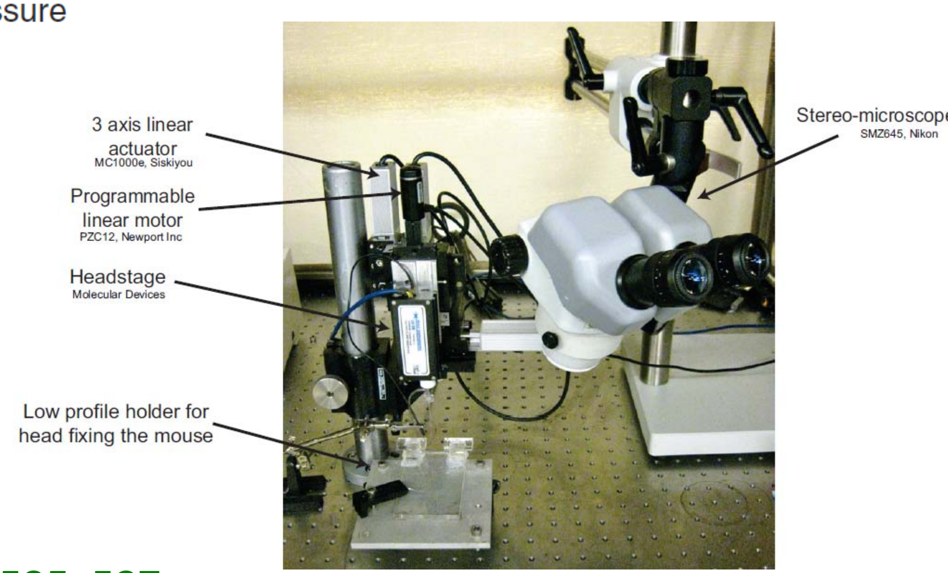


Kodandaramaiah et al. (2012) *Nature Methods* 9:585–587.



A robot that can automatically patch clamp neurons in living brain

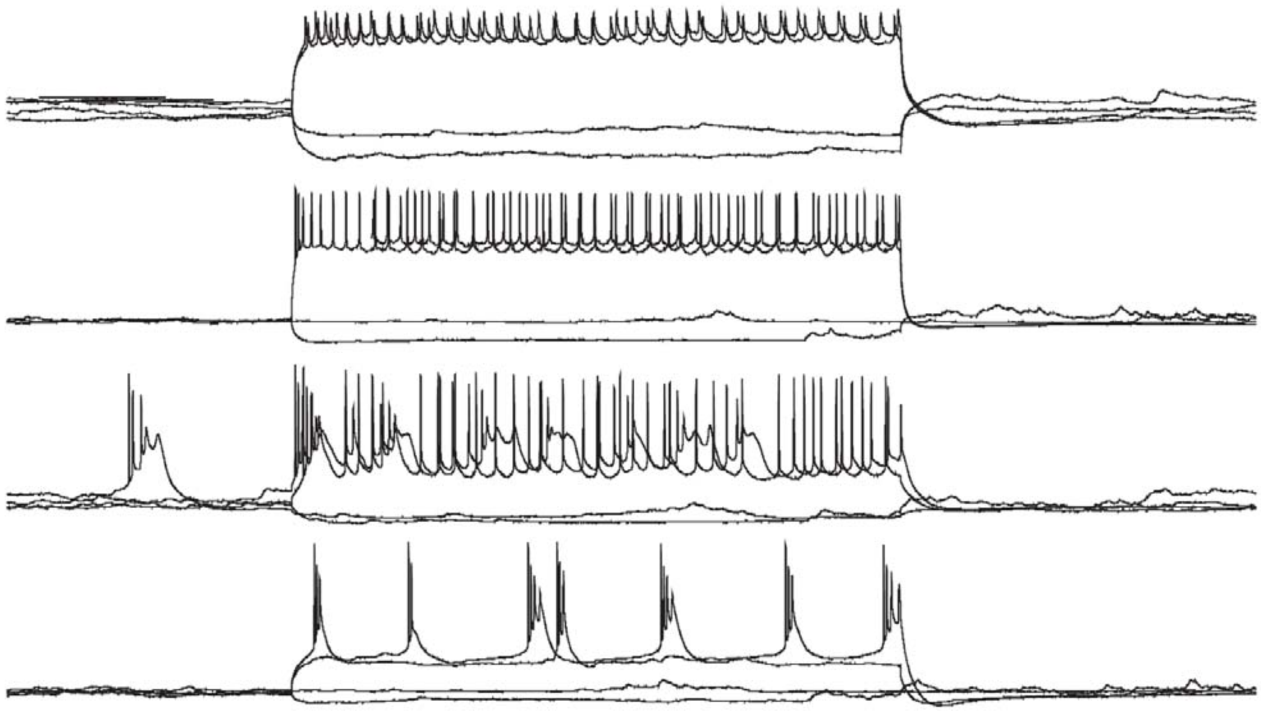




Kodandaramaiah et al. (2012) *Nature Methods* 9:585–587.

Commercialized by Neuromatic Devices, Inc. (ESB has no financial affiliation)

Robotic quad patching in living mouse brain



Suhasa Kodandaramaiah, Francisco Flores, Emery Brown, Craig Forest

How do neurons work together in entire circuits?

C. elegans

(Sulston and Horvitz, 1977)

"There are **302** neurons in the nervous system of *C. elegans*; this number is invariant between animals. Each neuron has a **unique combination of properties, such as morphology, connectivity and position, so that every neuron may be given a unique label**.

Groups of neurons that differ from each other only in position have been assigned to classes. There are **118 classes that have been made using these criteria, the class sizes ranging from 1 to 13**.

Thus *C. elegans* has a rich variety of neuron types in spite of having only a small total complement of neurons. This is in marked contrast to structures such as the mammalian cerebellum, which contains more than 10^{10} neurons (Braitenberg & Atwood 1958) and yet has only five classes of component neuron (Eccles *et al.* 1967)."

White..Brenner, Phil. Trans. Royal Soc. London. Series B, Biol Scien. Vol.314, Issue 1165 (Nov 12, 1986), 1-340

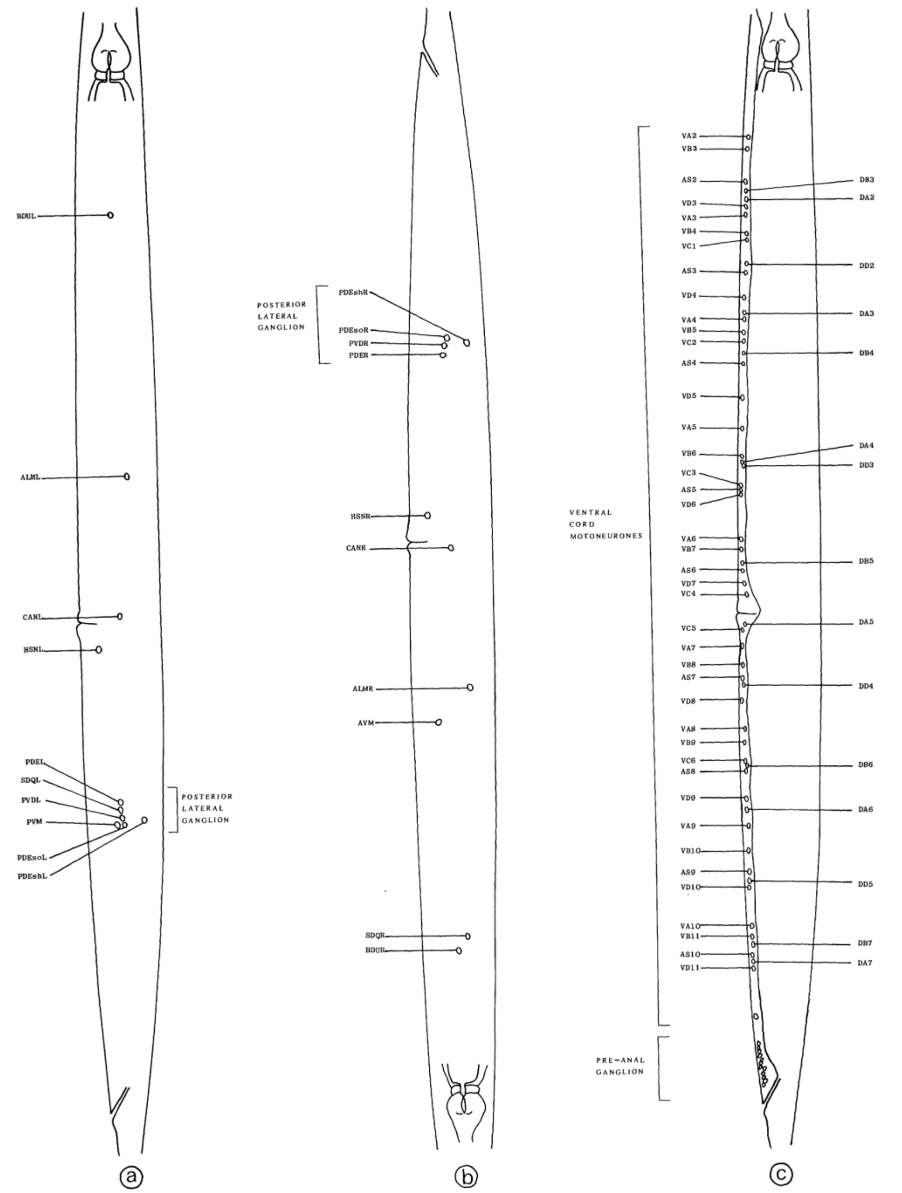


FIGURE 4. For description see opposite.

C. elegans

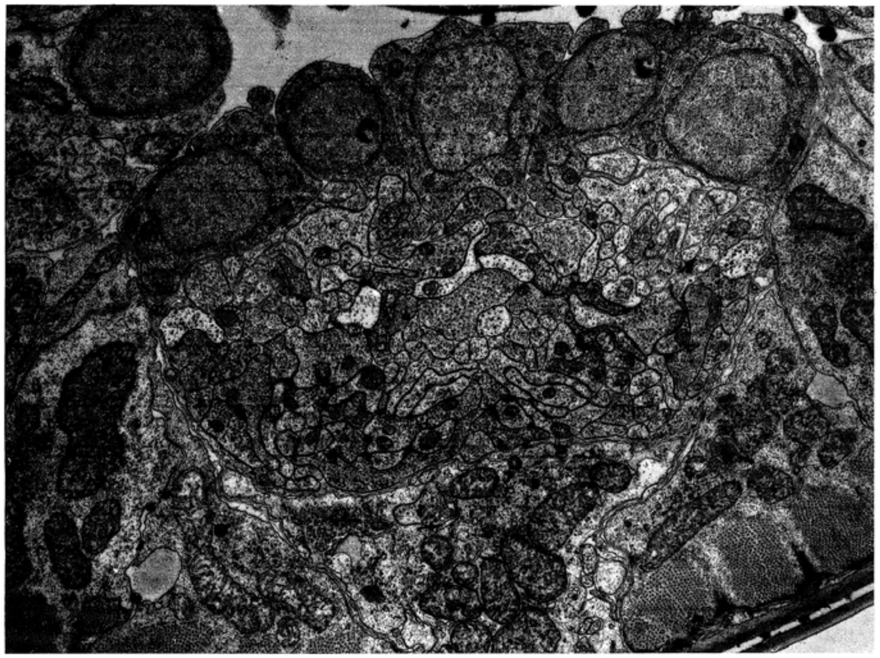
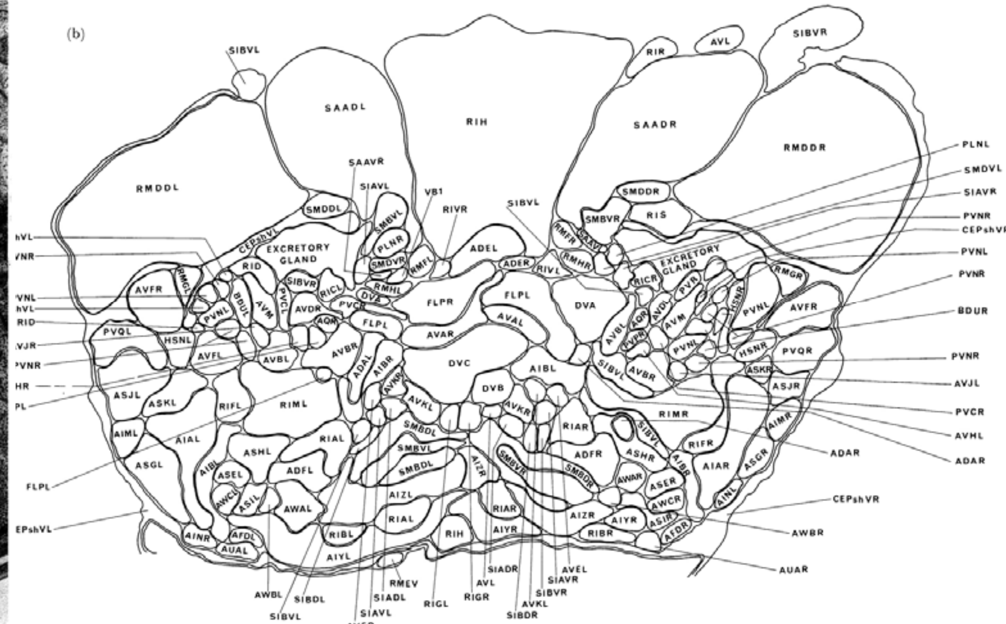


FIGURE 16(a). For description see opposite.



http://www.wormatlas.org/MoW_built0.92/nervous_system.html

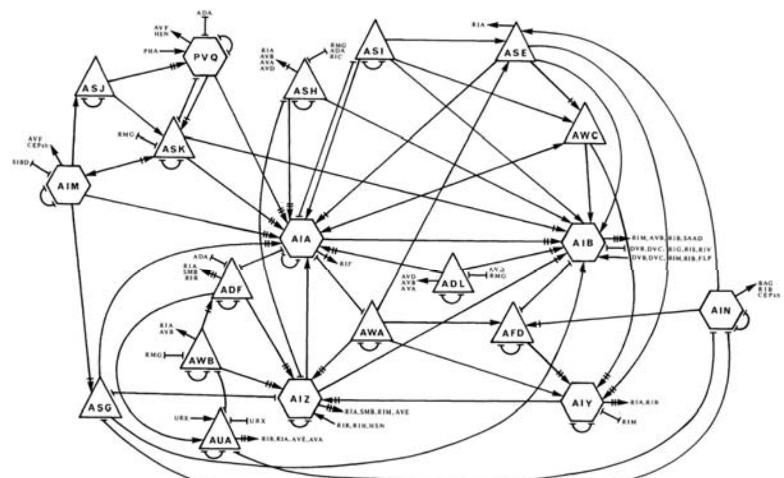
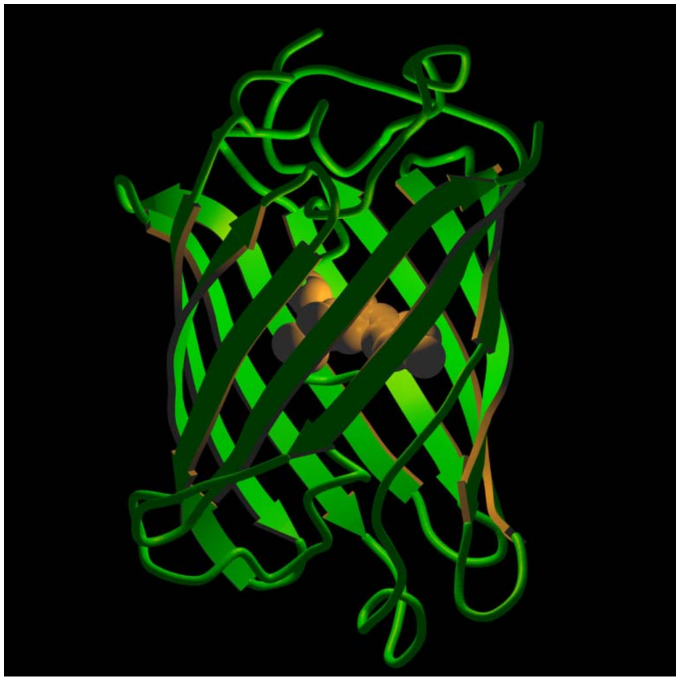
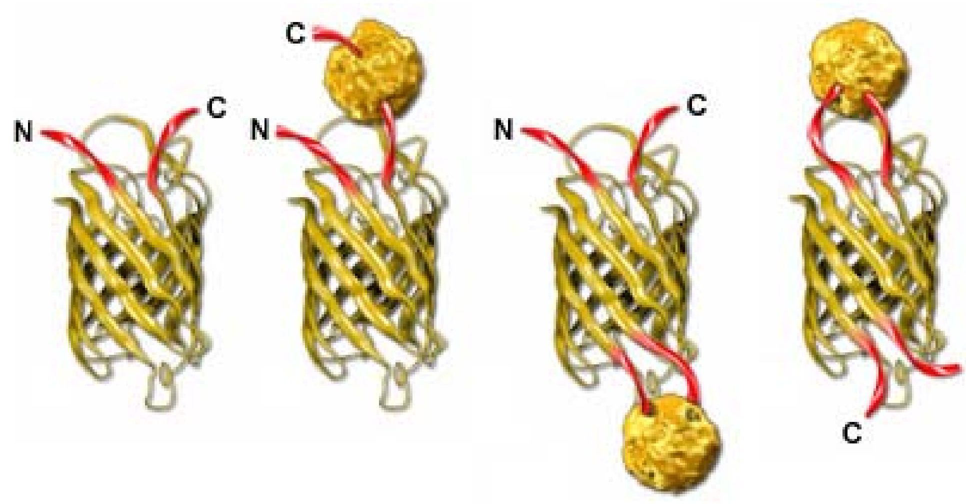


FIGURE 21. (a) Circuitry associated with amphids.

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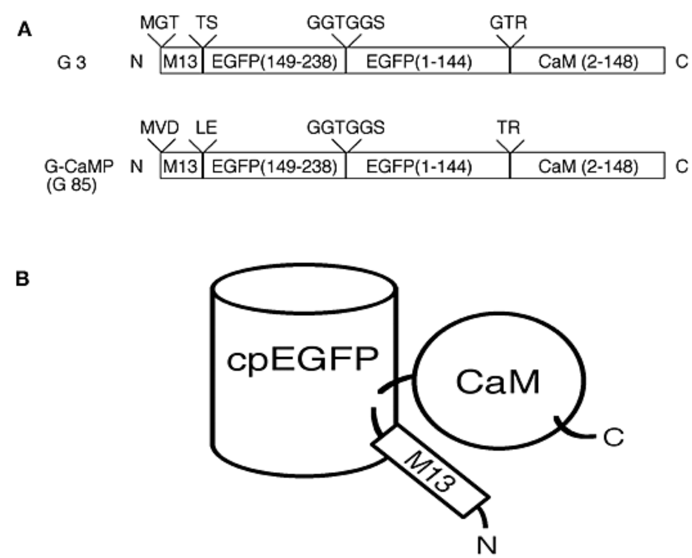
A
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TS
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EGFP(149-238)
EGFP(1-144)
CaM (2-148)
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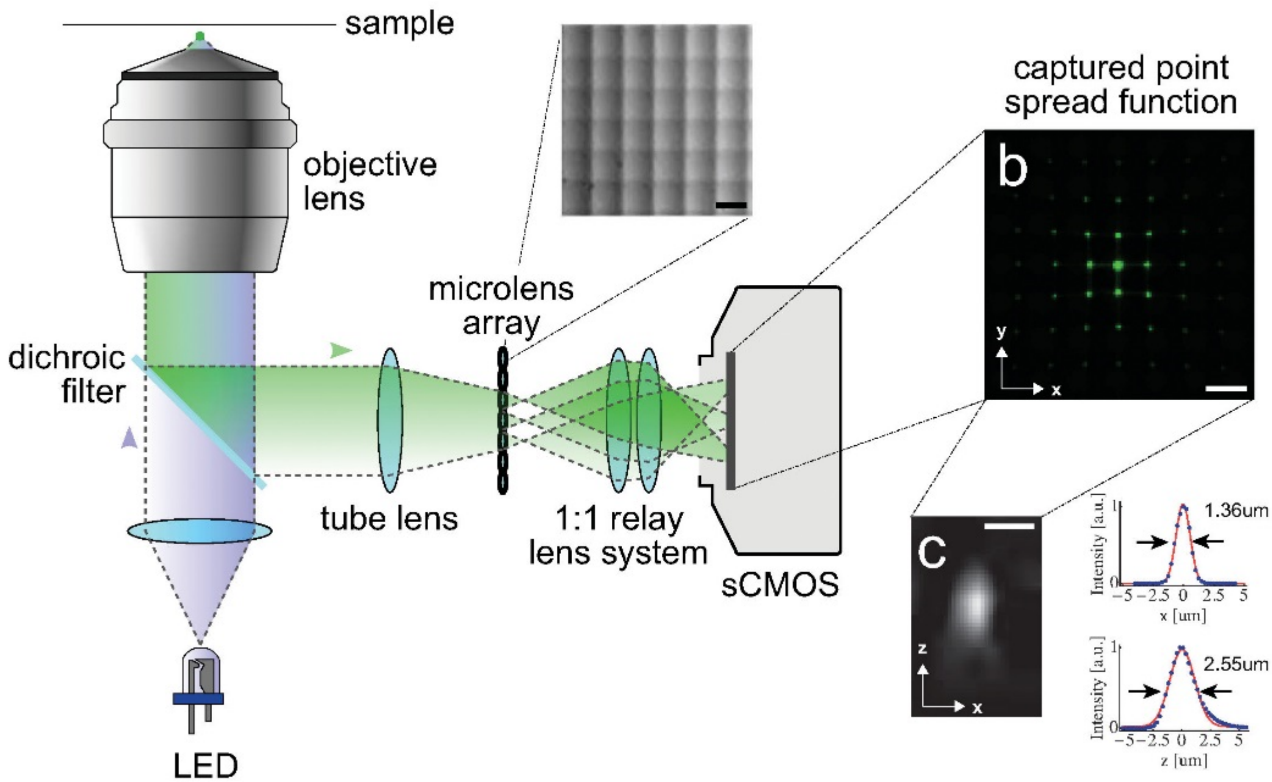
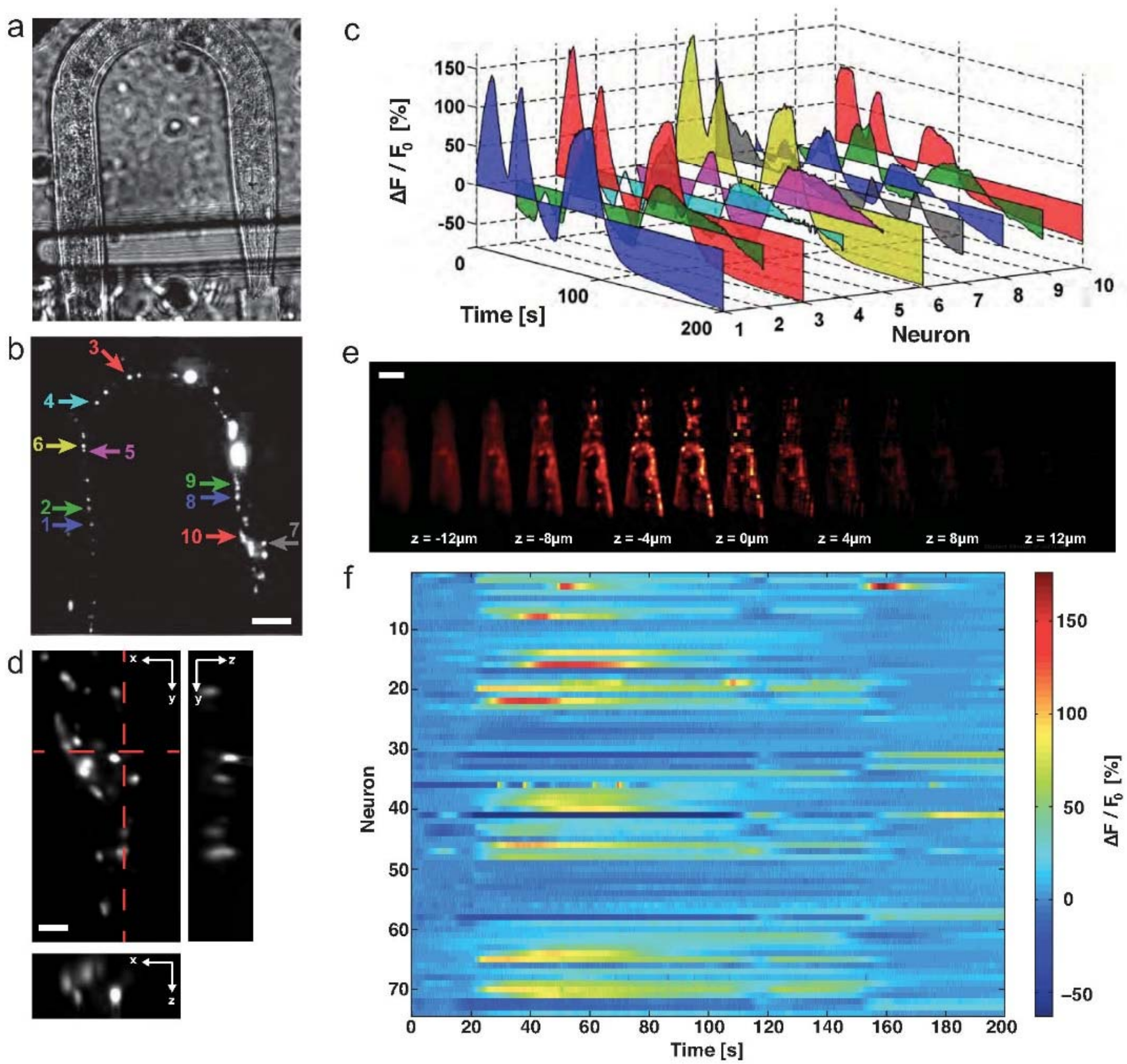
MVD
LE
GGTGGS
TR
G-CaMP
(G 85)
N
M13
EGFP(149-238)
EGFP(1-144)
CaM (2-148)
C
B
cpEGFP
CaM
M13
C
N

Simultaneous, whole-animal, 3-D microscopy: light-field imaging



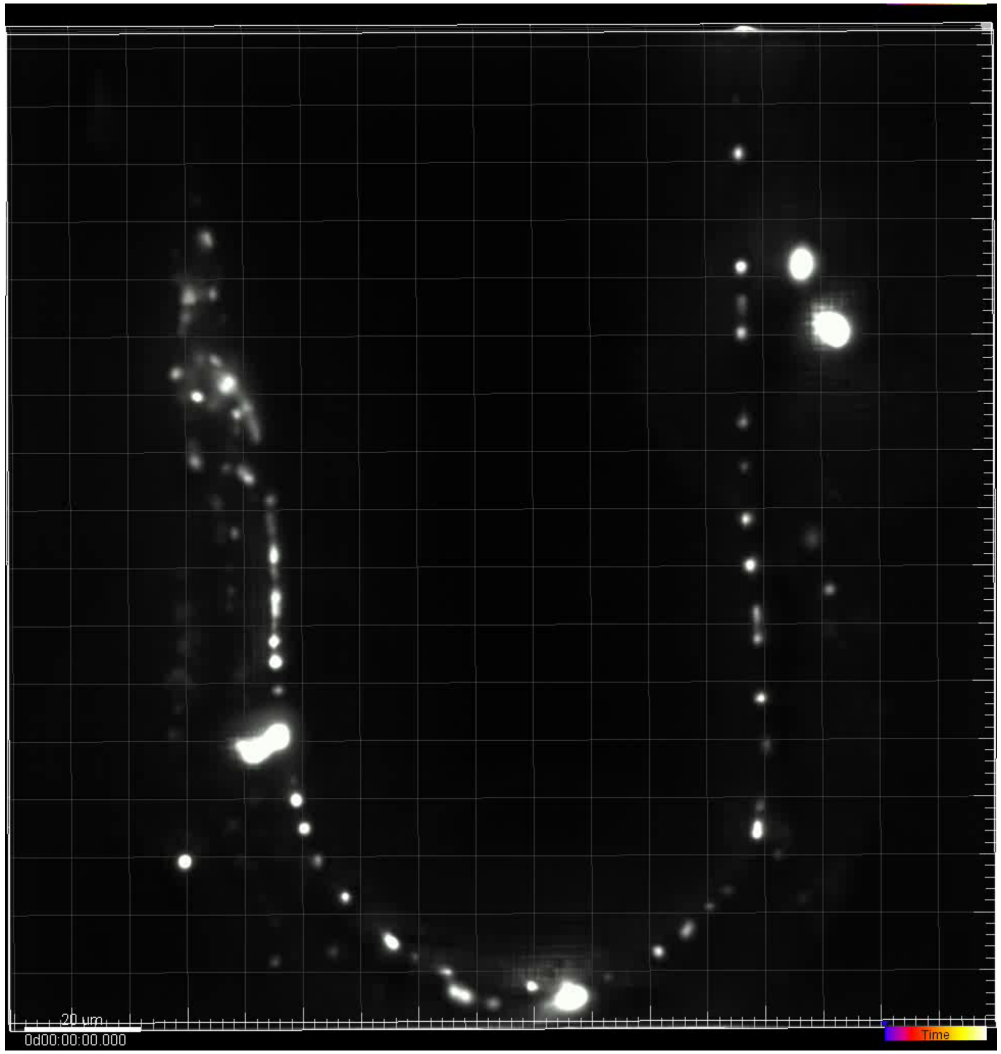
Prevedel*, Yoon*, et al. (2014) *Nature Methods,* advance online publication doi:10.1038/nmeth.2964.

Simultaneous, whole-animal, 3-D imaging of neural activity (at 5-50 Hz)



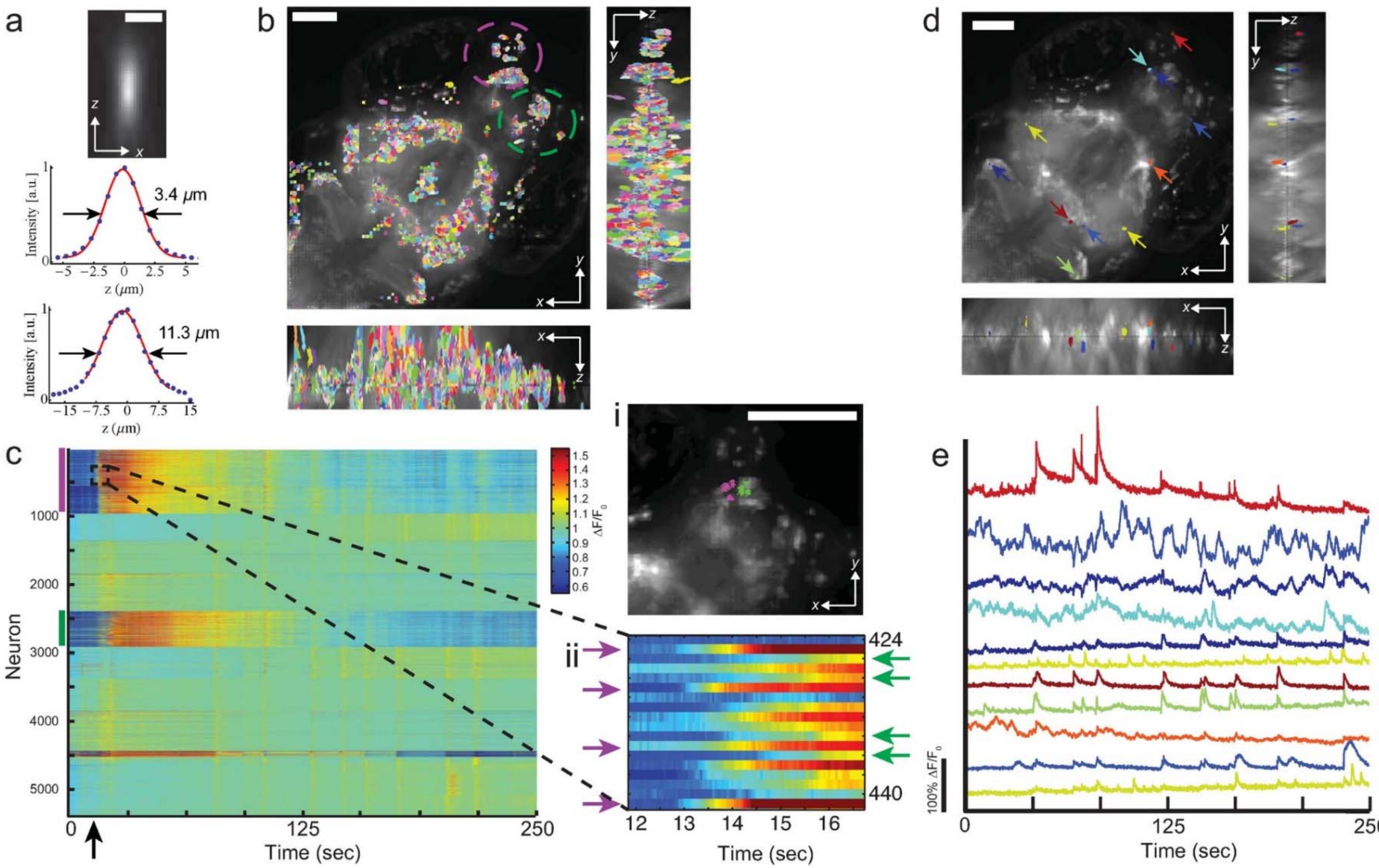
Prevedel*, Yoon*, et al. (2014) *Nature Methods,* advance online publication doi:10.1038/nmeth.2964.

Imaging neural activity throughout organism with known connectome



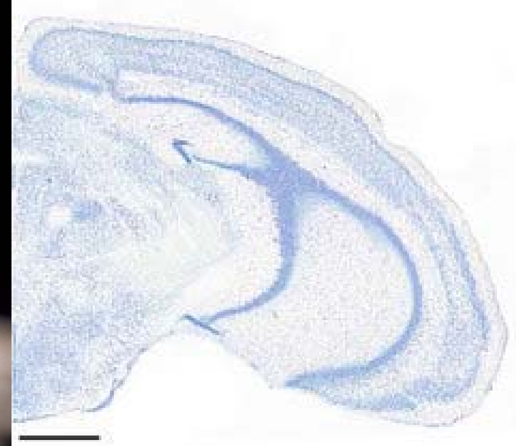
Prevedel*, Yoon*, et al. (2014) *Nature Methods,* advance online publication doi:10.1038/nmeth.2964.

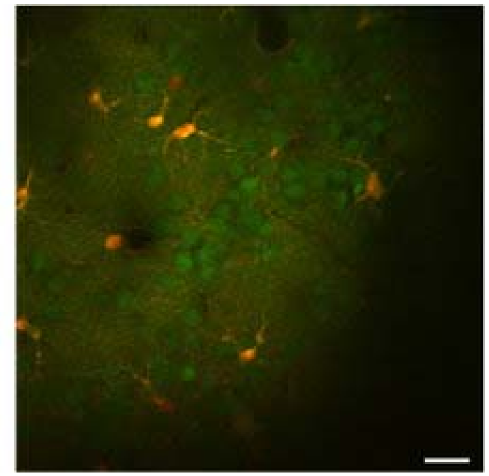
Imaging zebrafish neural activity in 3-D (at 20 Hz, below)

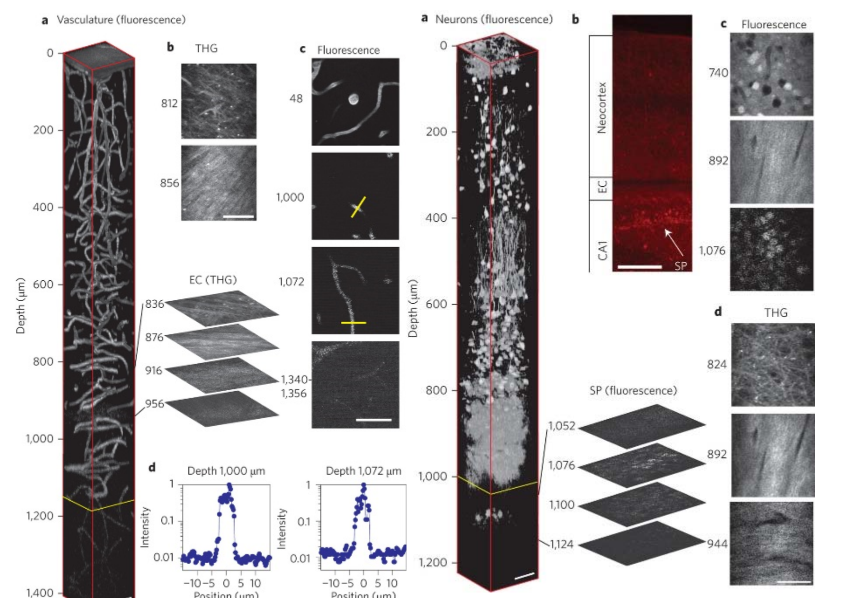


Prevedel*, Yoon*, et al. (2014) *Nature Methods,* advance online publication doi:10.1038/nmeth.2964.

The world's smallest mammal: towards whole-organism functional imaging



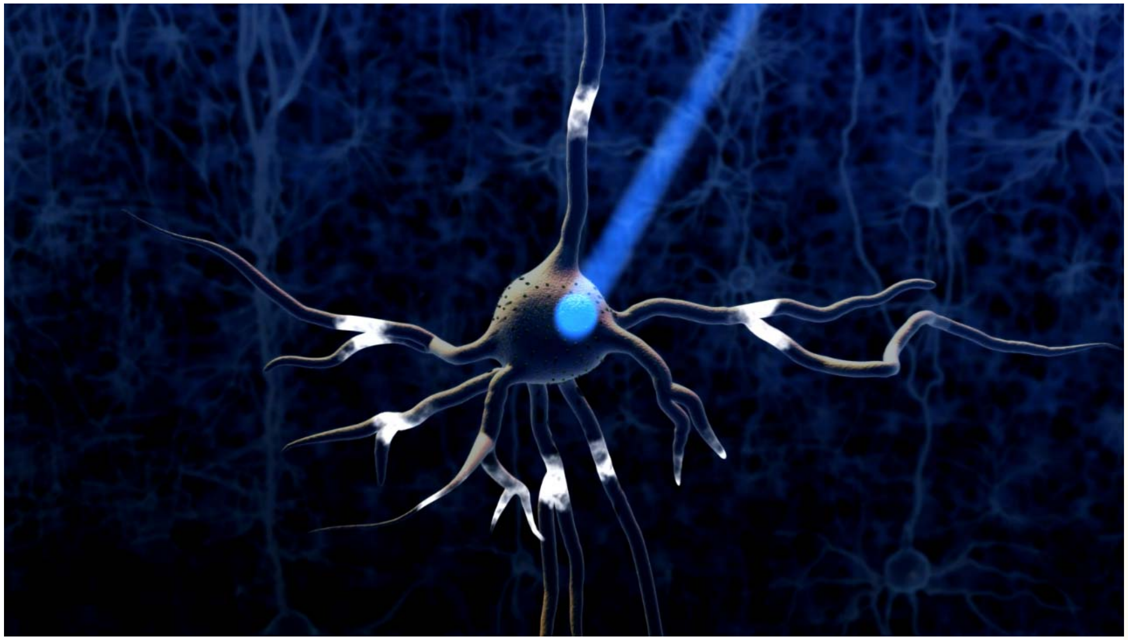
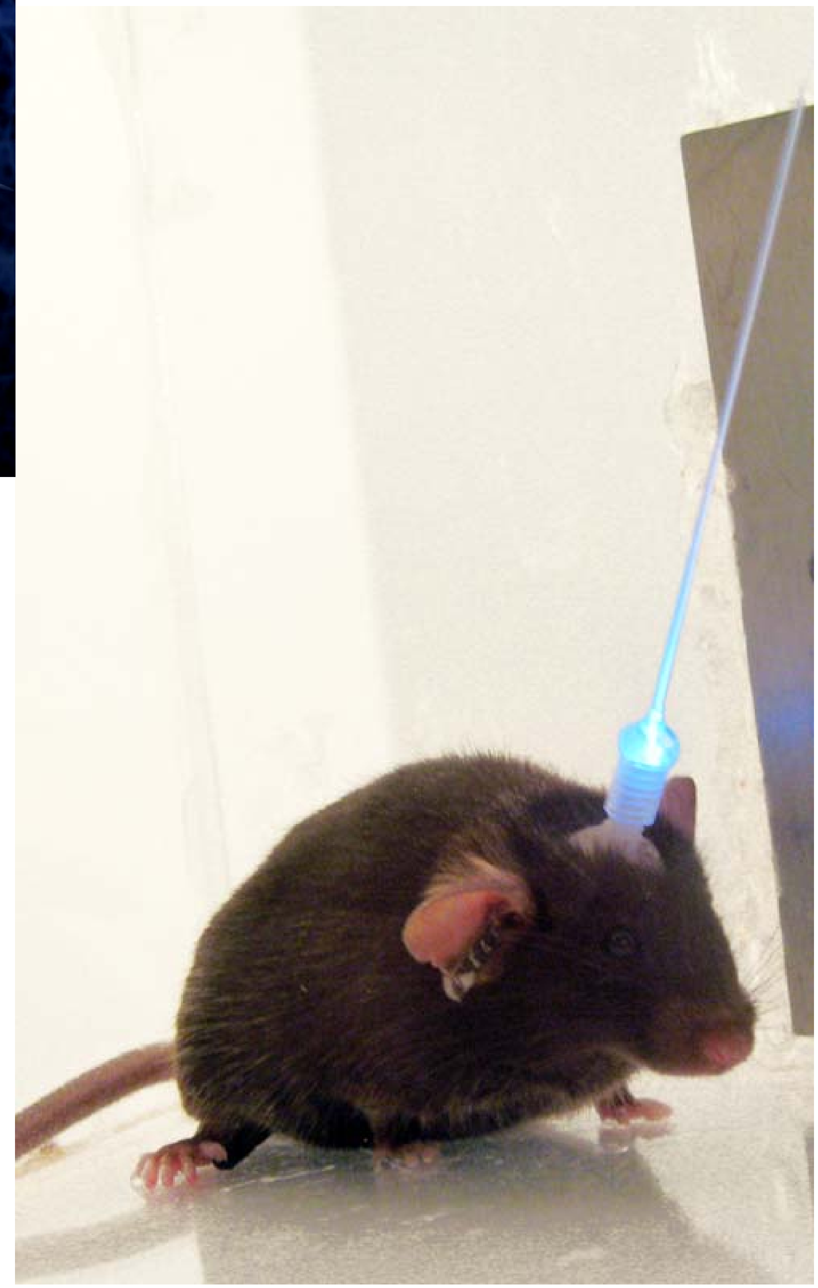




Michael Brecht, Ian Wickersham, Susan Erdman

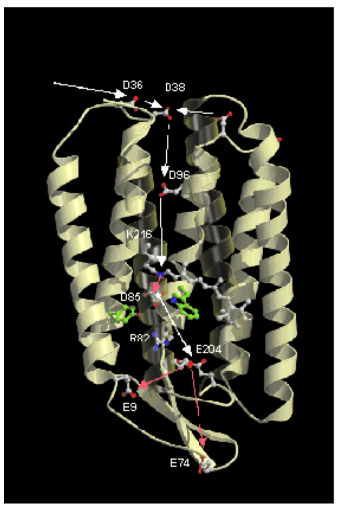
http://www.nature.com/nphoton/journal/v7/n3/abs/nphoton.2012.336.html

Can we understand causally how neurons function in circuits?

Bacteriorhodopsins:
Light-driven proton pumps

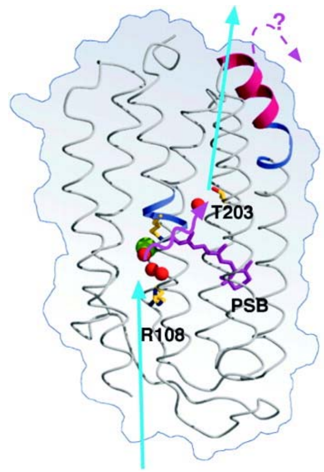
http://www.genome.duke.edu/genomelife/2011/03/systems-under-stress/



http://www.biochem.mpg.de/523002/Protein_BR

D. Oesterhelt and W. Stoeckenius (1971) Rhodopsin-like Protein from the Purple Membrane of Halobacterium halobium. Nature New Biology 233:149-152.

Halorhodopsins:
Light-driven chloride pumps



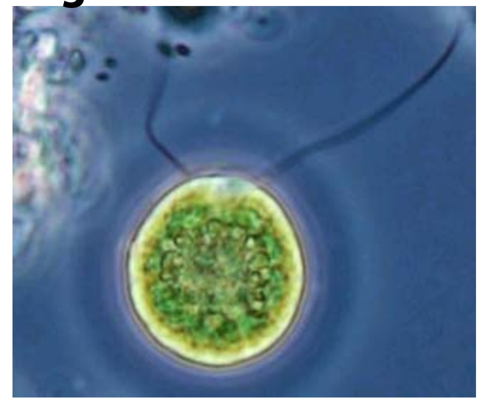
http://www.sciencemag.org/content/288/5470/1390.full

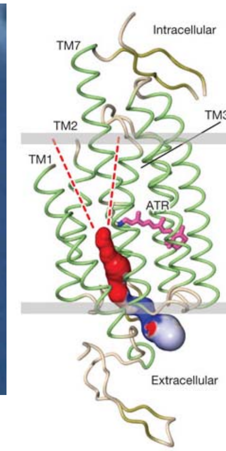
Matsuno-Yagi A, Mukohata Y (1977) Two possible roles of bacteriorhodopsin; a comparative study of strains of Halobacterium halobium differing in pigmentation. Biochem Biophys Res Commun 78:237-43.

Matsuno-Yagi A, Mukohata Y (1980) ATP synthesis linked to lightdependent proton uptake in a rad mutant strain of Halobacterium lacking bacteriorhodopsin. Arch Biochem Biophys, 199:297-303.

Schobert B, Lanyi JK (1982) Halorhodopsin is a light-driven chloride pump. J Biol Chem, 257:10306-13.

Channelrhodopsins:
Light-driven cation channels





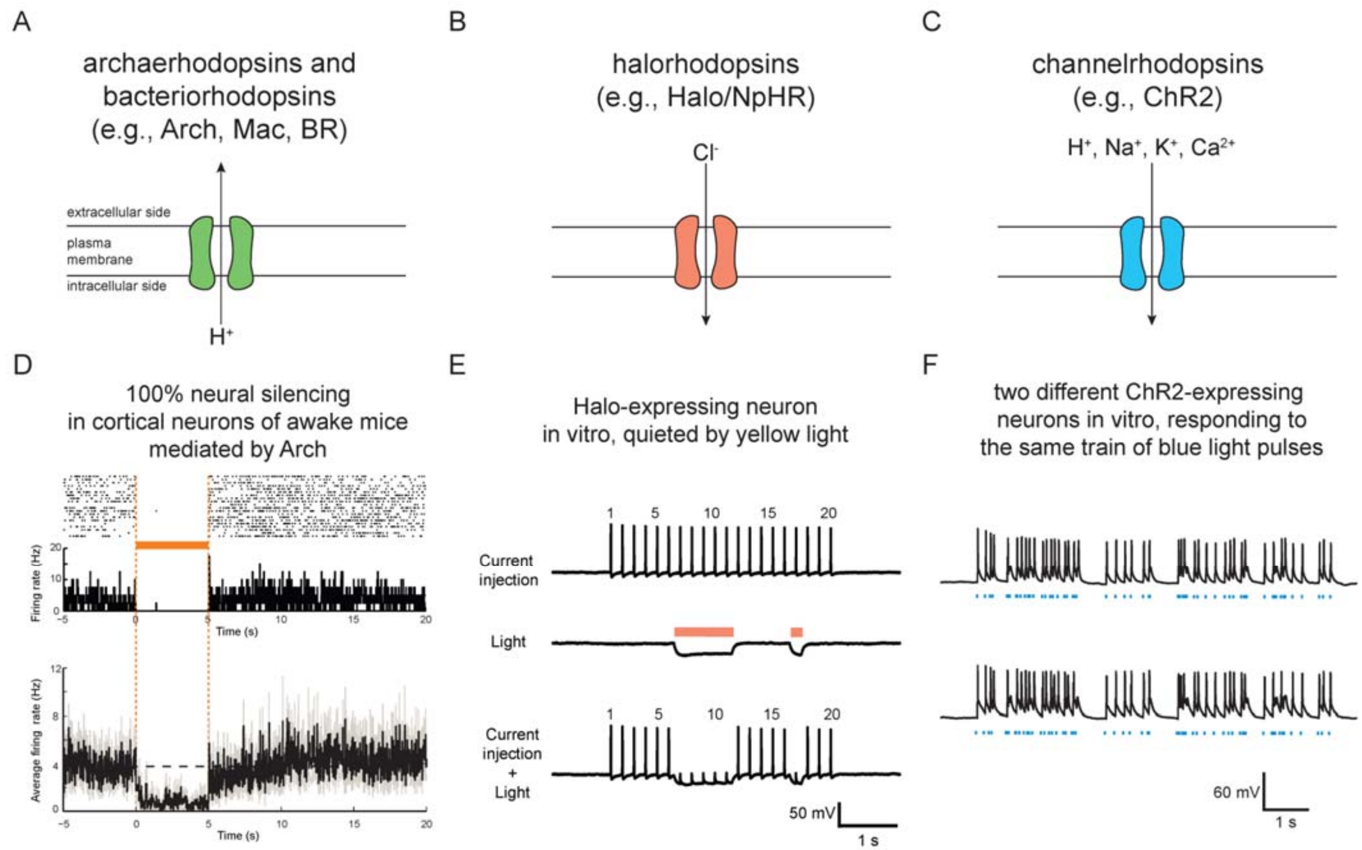
http://starcentral.mbl.edu/microscope/portal.php?pagetitle=assetfactsheet&imageid=3245

http://www.nature.com/nature/journal/v482/n7385/full/nature10870.html

Nagel G, Ollig D, Fuhrmann M, Kateriya S, Musti AM, Bamberg E, Hegemann P (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. Science, 296:2395-8.

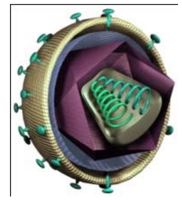
Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A, 100:13940-5.

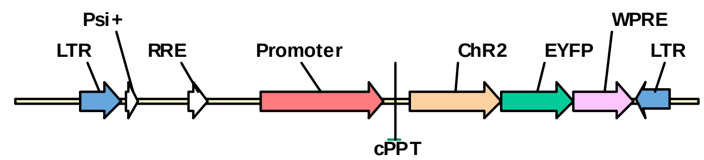
Three major optogenetic molecule classes: microbial opsins, seven-transmembrane proteins, binding endogenous all-trans-retinal



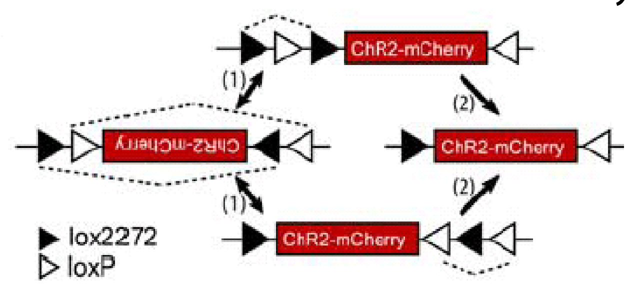
Boyden (2011) *Faculty of 1000 Biology Reports* 3:11.

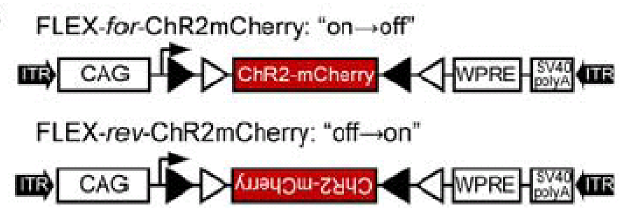
Targeting different neurons of the brain in genetic model organisms, and beyond

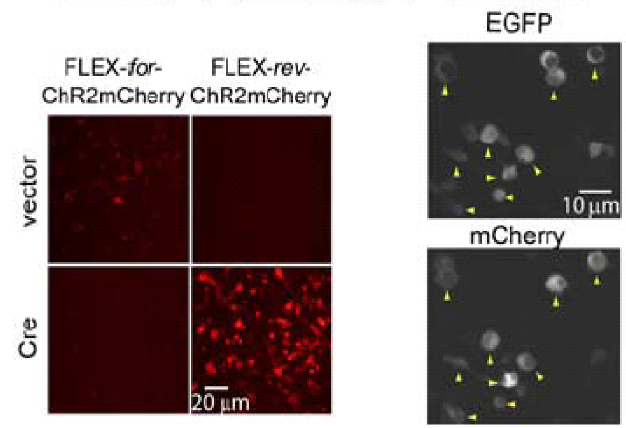




Atasoy et al., 2008







- **Lentiviruses and adeno-associated viruses**
  - Have **intrinsic** tropism for certain cell types (e.g., lenti – excitatory neurons of the cortex)
  - Can tune the **promoter**: synapsin pan neuronal, CaMKII excitatory, TH/dopamine, GAD, SOM, CCK, …
  - AAV serotypes – AAV8, 5, 2, 9, …
- Lots of **Cre recombinase expressing** mice (e.,g dopamine, serotonin, parvalbumin, etc.)
  - Administer a **floxed and reversed** opsin AAV into such a mouse, and the opsin will be flipped around into the correct direction
- Takes **2-3 weeks** to express after injection; electroporation and other methods may be of use

Rules of thumb for blue/green/yellow light

200 mW/mm^2 is good **irradiance** to shoot for (higher okay for brief neural activations; lower should be considered for long duration silencings; molecules are sensitive to 0.1-10 mW/mm^2)

50 micron **fibers** = can easily go into tetrode drives

100-200 micron fibers = stiff enough to go into brain

400-800 micron fibers = for specialized uses

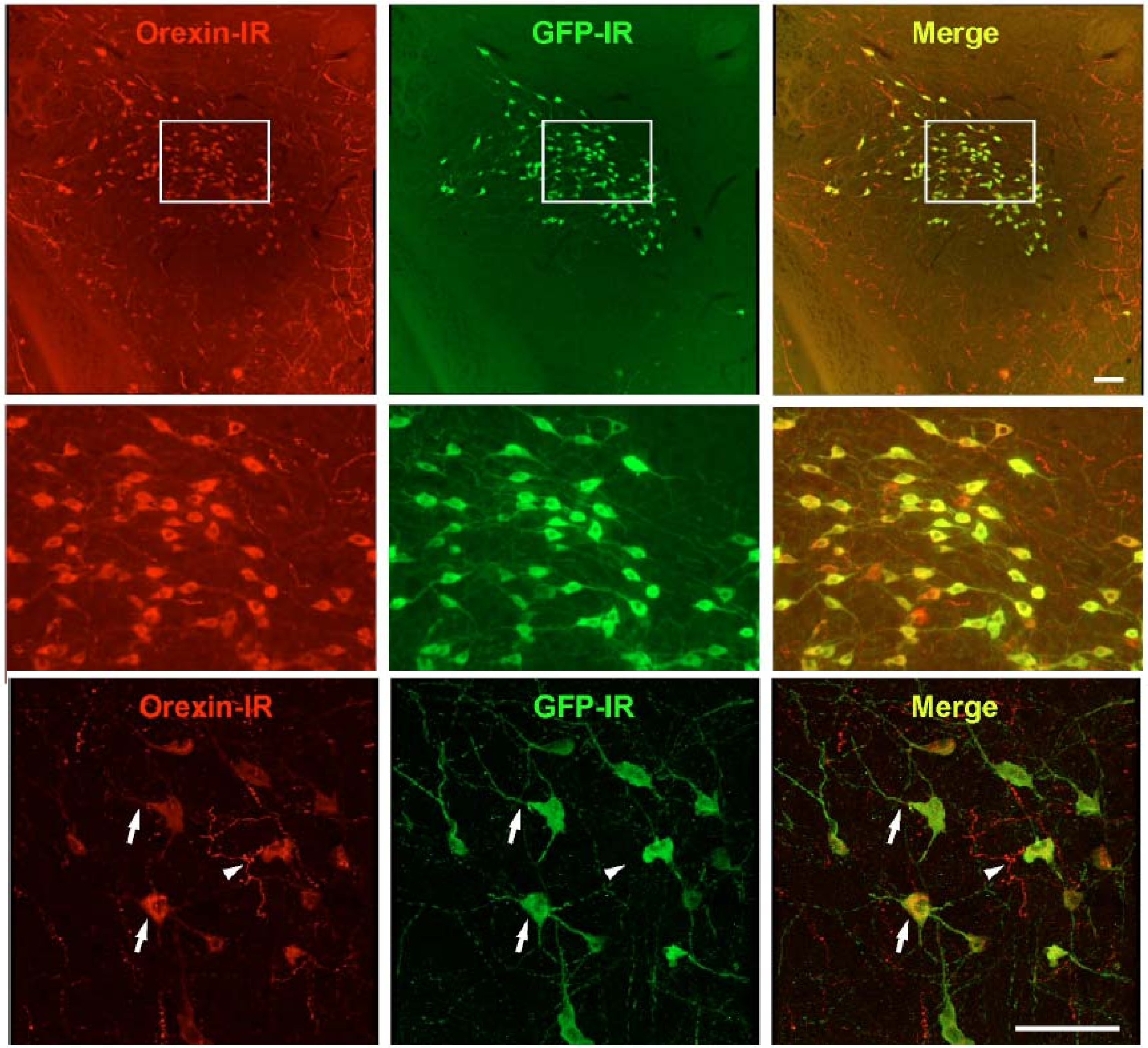
200 micron fiber, 200 mW/mm^2 affects **~1 mm^3** of tissue

Close to fiber tip: light goes forward

Beyond a **scattering** length (~50-100 microns), starts to look spherical (power falls off as $1/r^2$)

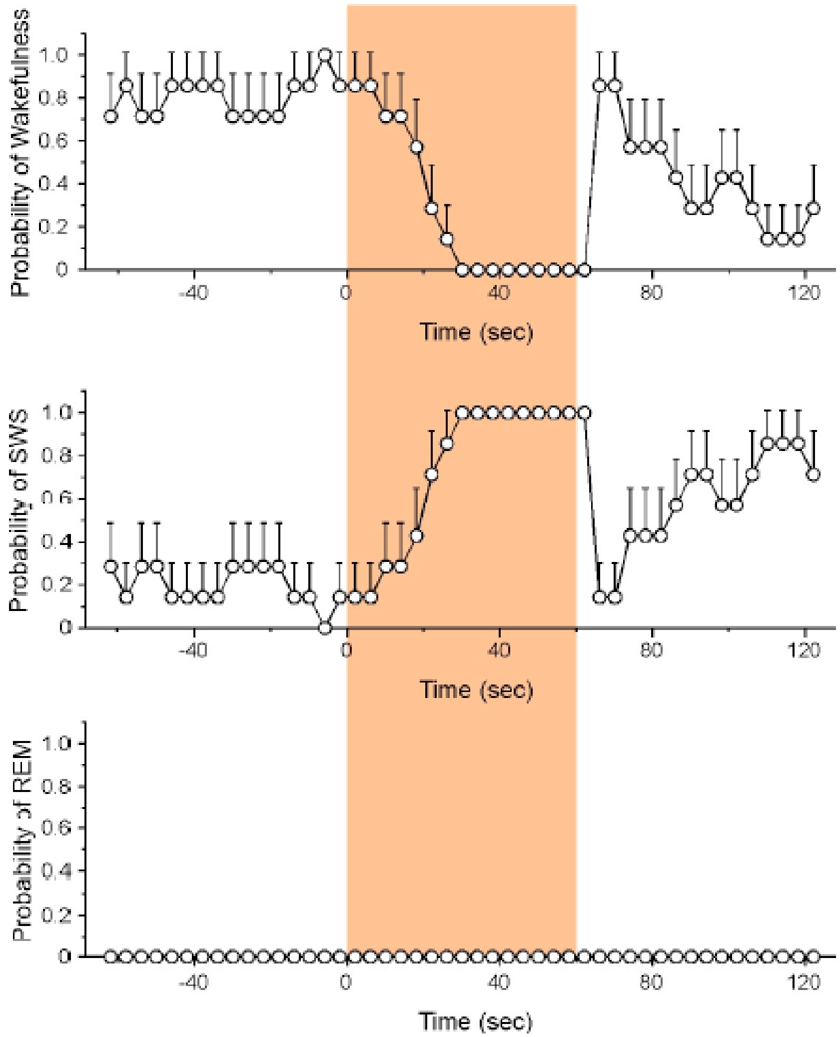
Beyond the **absorbance** length (~500-1000 microns), falls off exponentially

Transgenic mice expressing original-N. pharaonis halorhodopsin, tagged with GFP, in hypocretin neurons



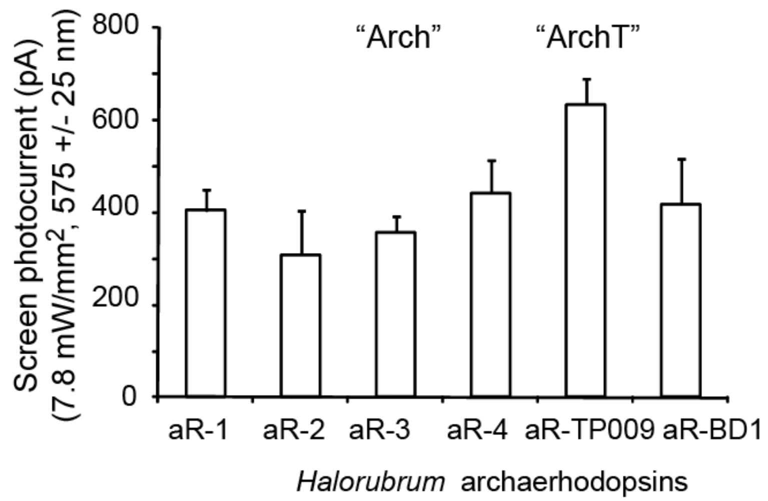
Tsunematsu et al. (2011) *Journal of Neuroscience* 31(29): 10529-10539.

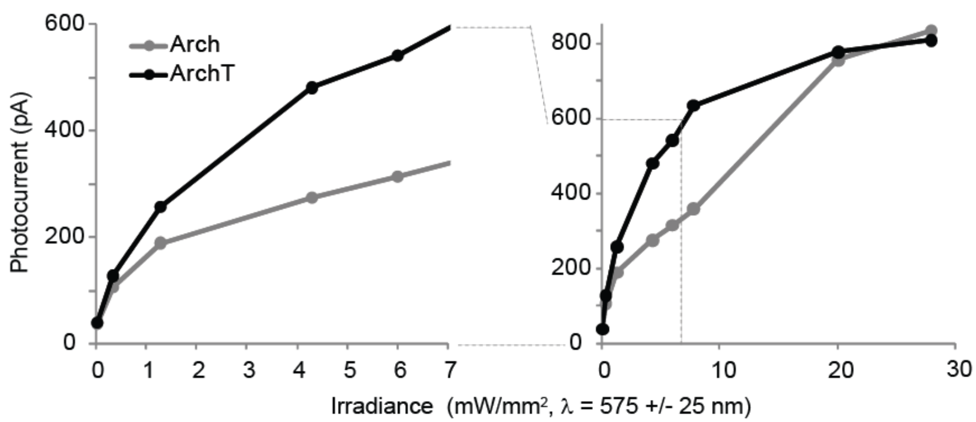
Light silences the neurons, resulting in slow-wave sleep



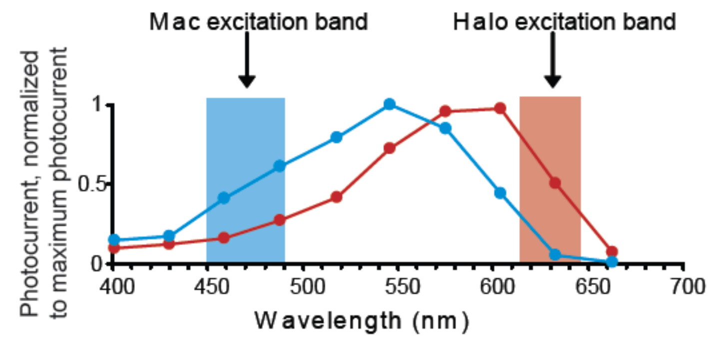
Tsunematsu et al. (2011) *Journal of Neuroscience* 31(29): 10529-10539.

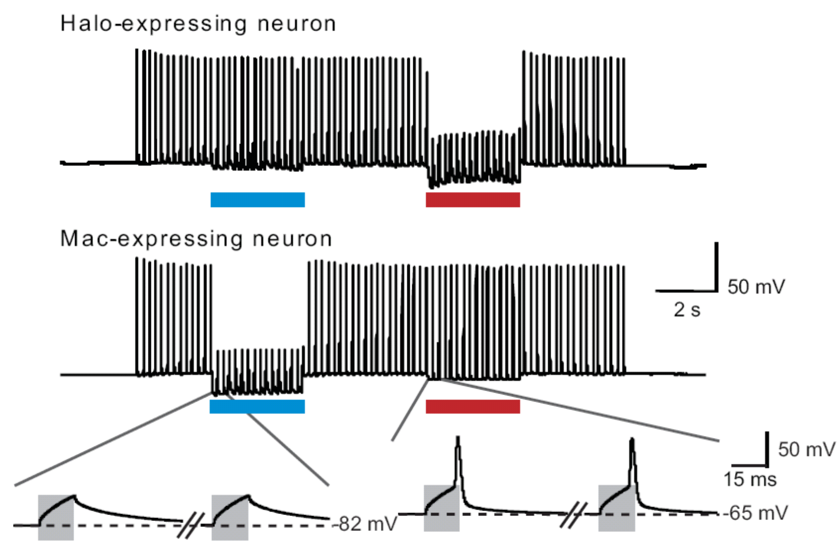
Search locally in genomic space: ArchT, higher light sensitivity relative of Arch





Search broadly in genomic space: Mac, blueshifted relative to all other silencers

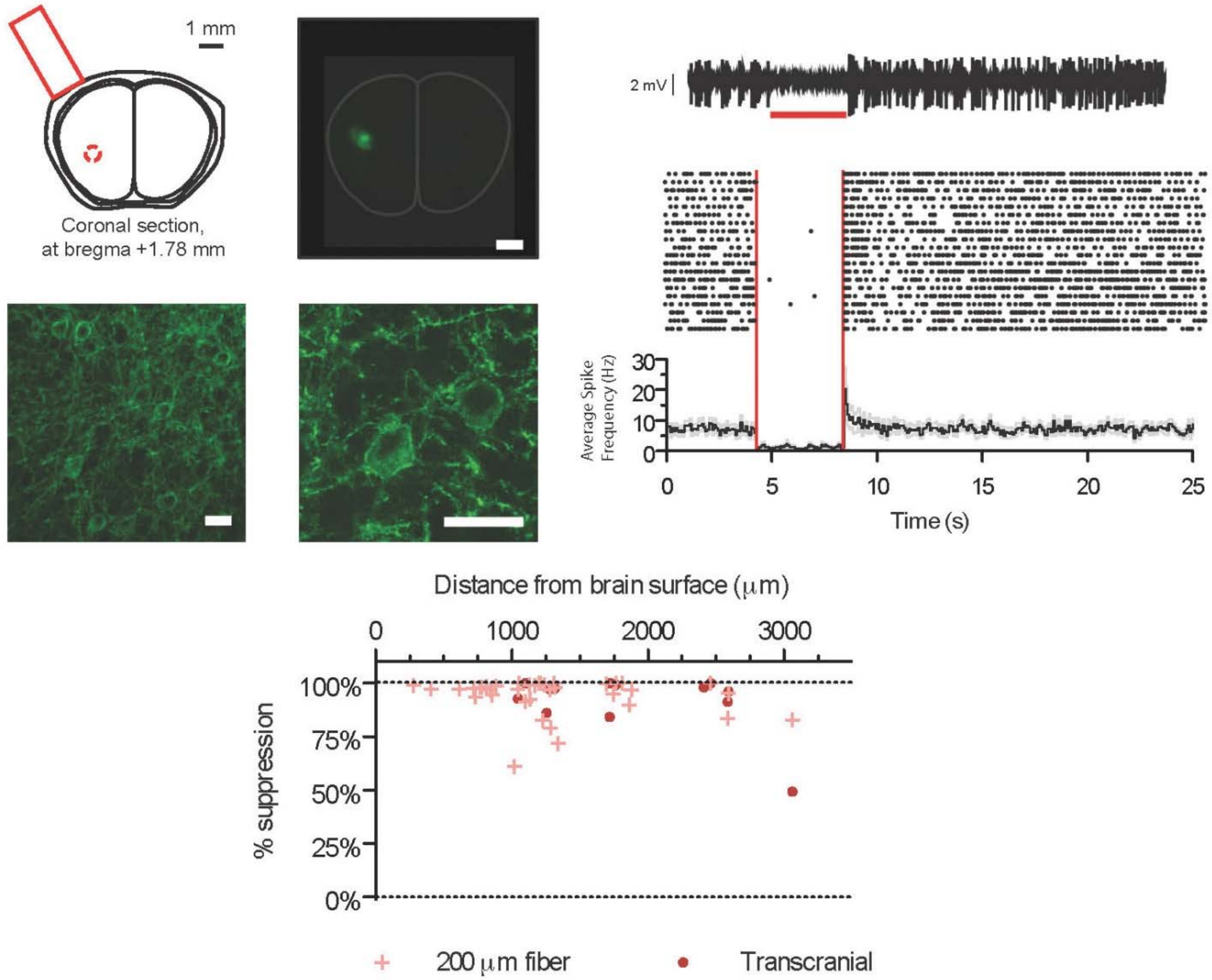




Han*, Chow*, et al. (2011) ***Frontiers in Systems Neuroscience*** **5:18.**

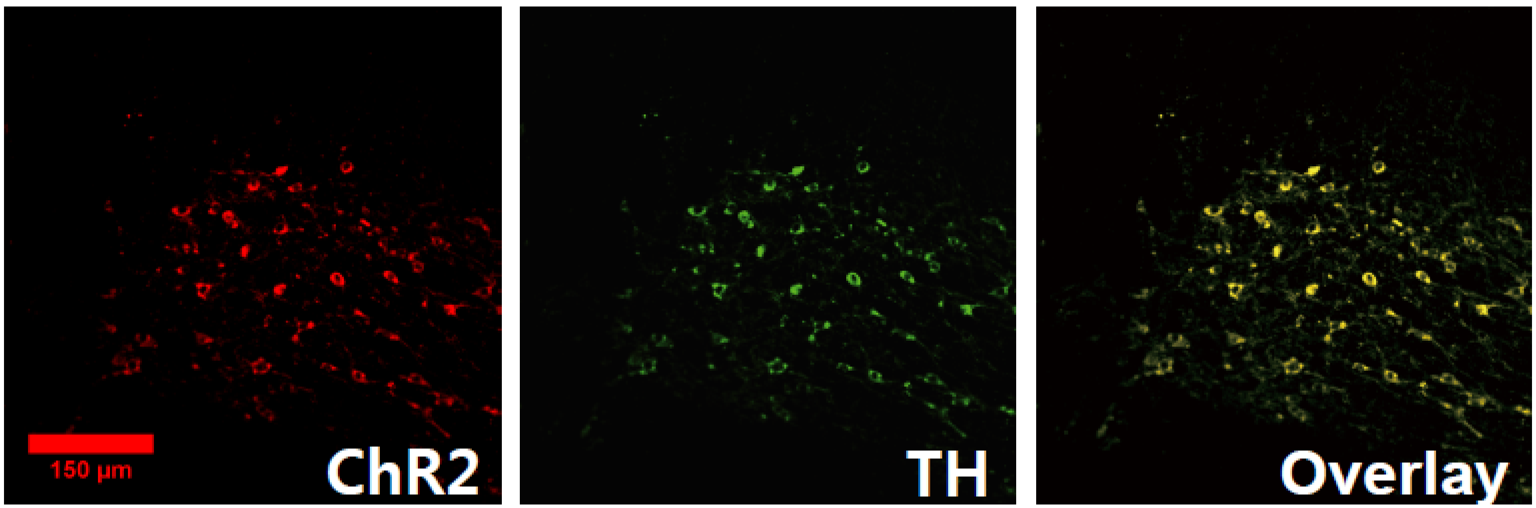
Chow*, Han*, et al. (2010) ***Nature*** **463:98-102.**

Noninvasive optogenetic neural silencing: Jaws



Chuong et al. (2014) ***Nature Neuroscience*****, accepted.**

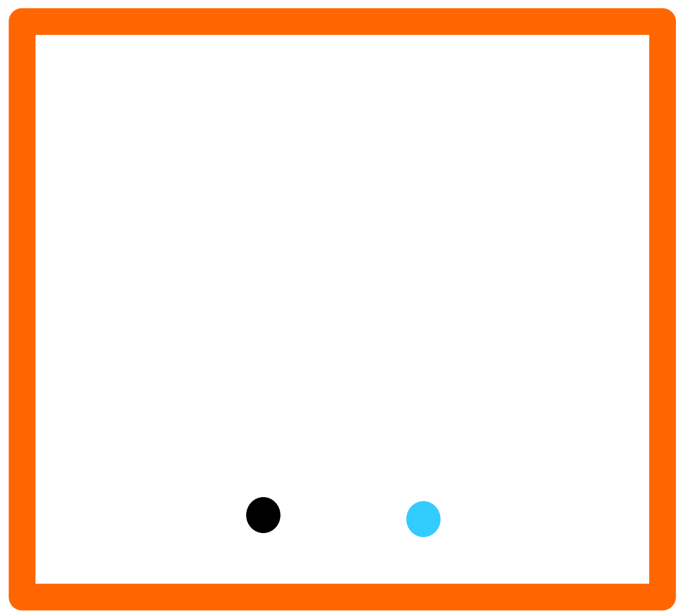
DAT-Cre + AAV-FLEX-ChR2-tdTomato



Kim et al. (2012) *PLoS One* 7(4):e33612

Finding circuits in the brain that can mediate reward

- **Dopamine neurons**: implicated in reward and addiction, but largely through pharmacological and electrical means
- Is a **brief activation** of them sufficient to drive reward?



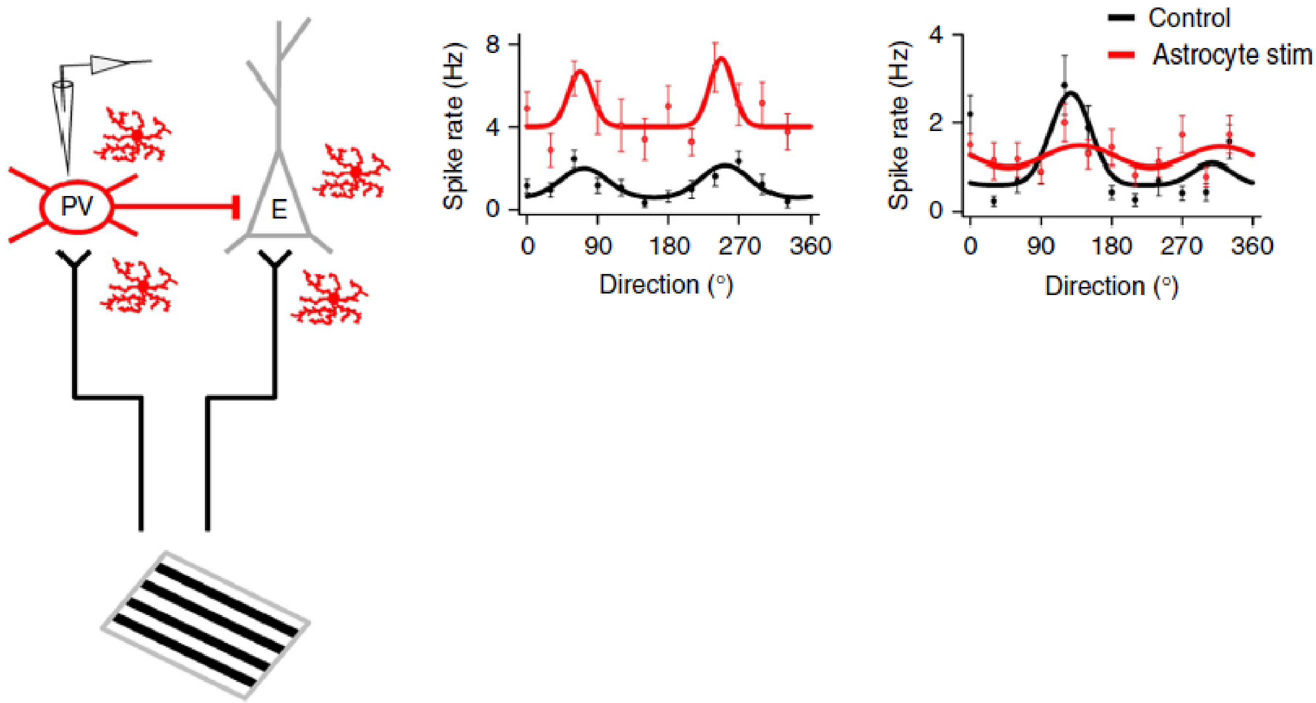
no light stimulation

light stimulation

Kim et al. (2012) *PLoS One* 7(4):e33612

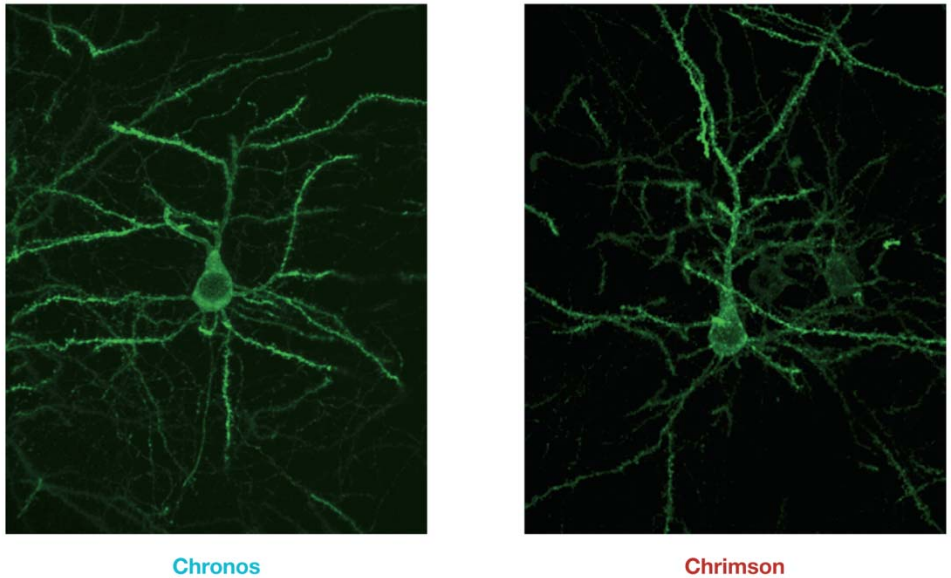
Kim et al. (2012) *PLoS One* 7(4):e33612

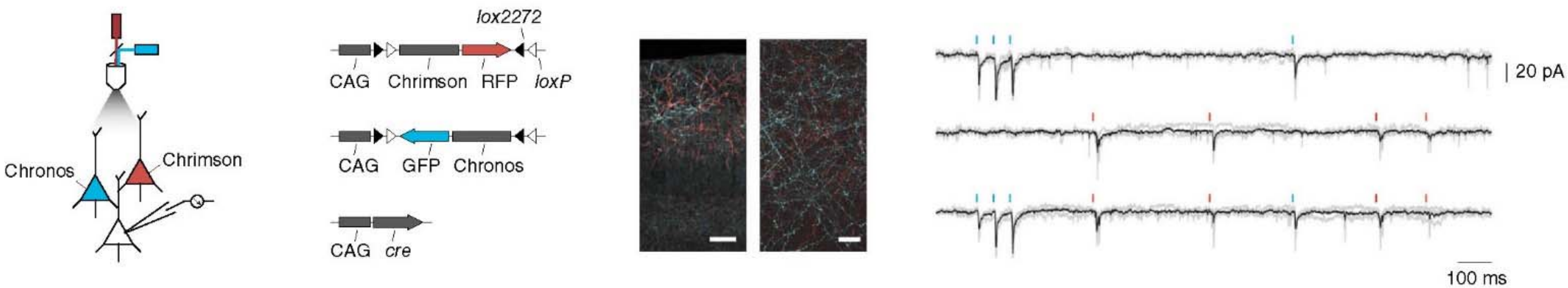
Optogenetic activation of glia can change neural codes



Perea et al. (2014) *Nature Communications* 5:3262.

Chronos and Chrimson together





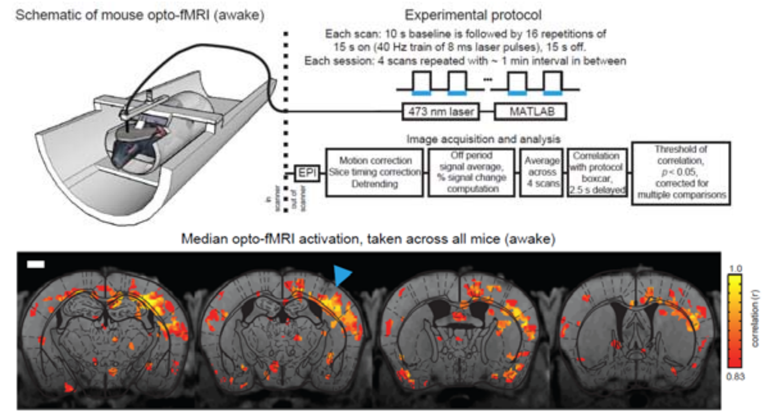
Klapoetke et al. (2014) *Nature Methods* 11:338–346.

Accessory strategies for a diversity of systems

Wireless, multisite optogenetics

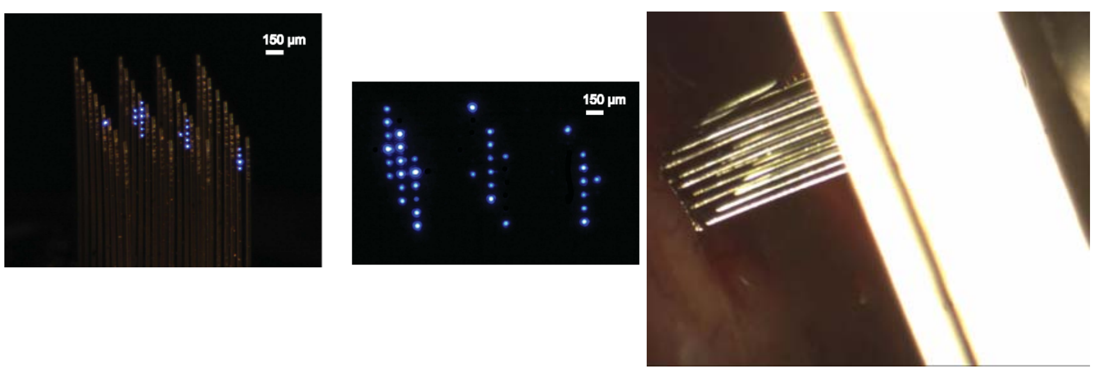
Wentz et al. (2011) *Journal of Neural Engineering* 8(4): 046021, commercialized by Kendall Research Systems, Inc. (ESB has no financial affiliation); Bernstein and Boyden (2011) *Trends in Cognitive Sciences* 15(12):592-600; Bernstein et al. (2011) *Current Opinion in Neurobiology* 22(1):61-71.

Opto-fMRI



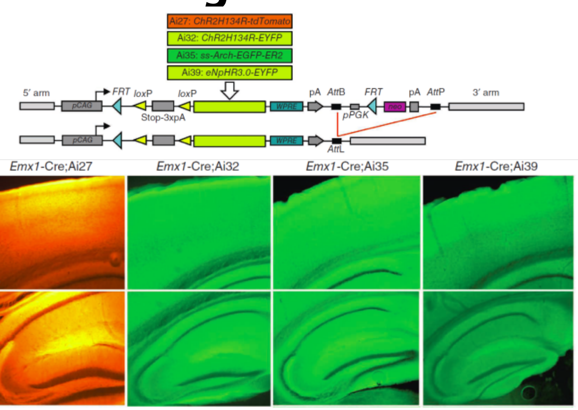
Desai et al. (2011) *Journal of Neurophysiology* 105(3):1393-405; Kahn et al. (2011) *Journal of Neuroscience* 31(42):15086-15091.

3-D optogenetic control



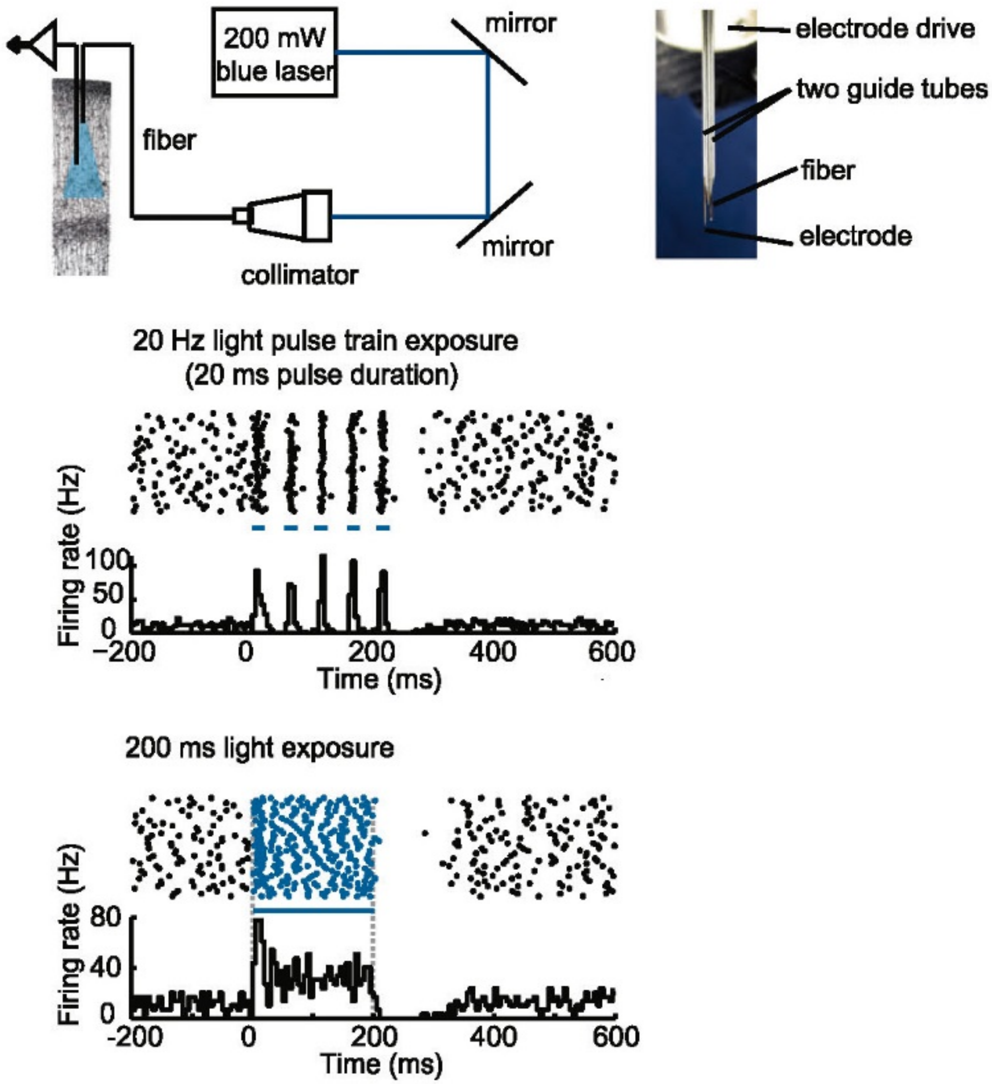
Zorzos et al. (2010) *Optics Letters* 35(24):4133-5; Zorzos et al. (2012) *Optics Letters* 37(23):4841-4843

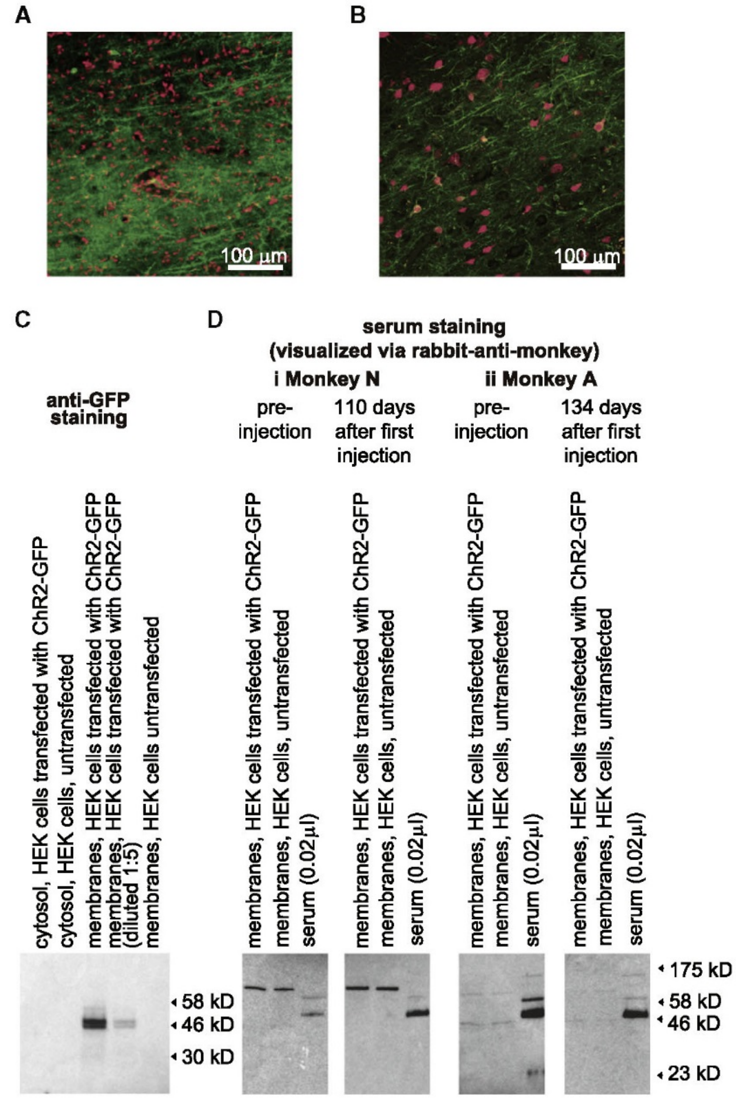
Transgenic mice



Madisen et al. (2012) *Nature Neuroscience* 15(5):793-802.

Primate optogenetics





Han et al. (2009) *Neuron* 62(2):191-198.

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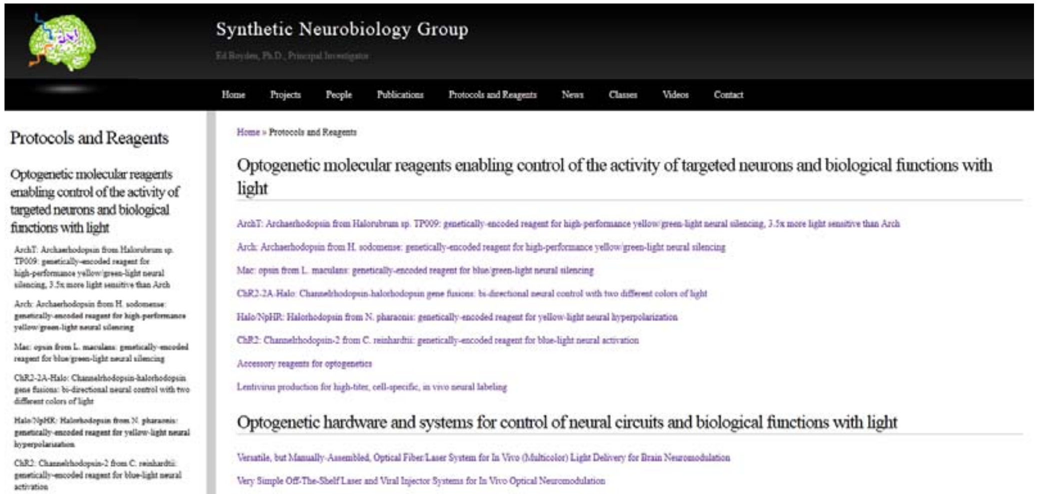

Tools distributed to >1000 labs worldwide

- Addgene, DNA
- UNC (Lori Nisi), viruses
- Allen Institute (Hongkui Zeng), floxed-stop transgenics
- Host visitors (1-2x/week) to teach procedures

Synthetic Neurobiology Group

http://syntheticneurobiology.org/

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Pre-MIT: Hertz Foundation, Helen Hay Whitney Foundation.

Collaborators

3-D Brain-building: Utkan Demirci
Blindness: Alan Horsager, Ben Matteo, Bill Hauswirth, Alapakkam Sampath
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Neural recording: Leaflabs, Konrad Kording, George Church
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Transgenics: Hongkui Zeng