# Tools for Mapping and Engineering Brain Computations


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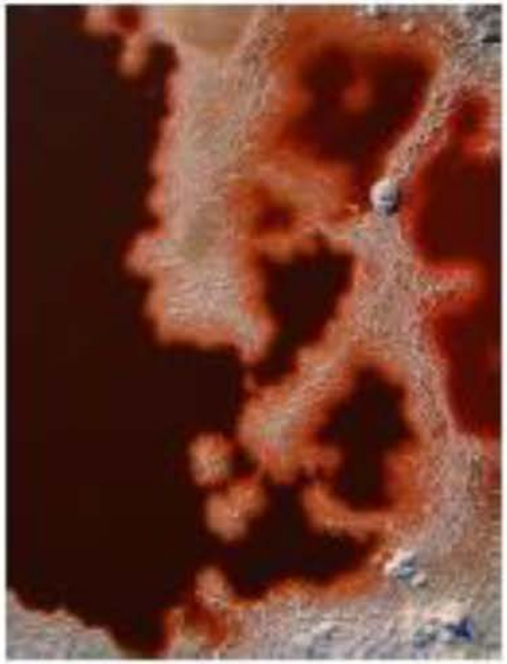
**Synthetic Neurobiology Group**
**MIT**


# Bacteriorhodopsins: Light-driven proton pumps





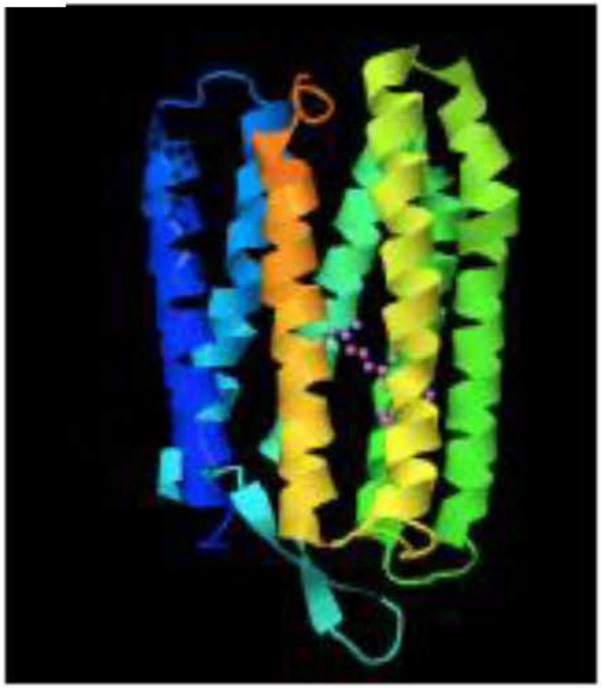


Image from the RCSB PDB of PDB ID 1DZE (Takeda, Kazuki, Yasuhiro Matsui, et al. "Crystal Structure of the M Intermediate of Bacteriorhodopsin: Allosteric Structural Changes Mediated by Sliding Movement of a Transmembrane Helix." *Journal of Molecular Biology* 341, no. 4 (2004): 1023–37.).

Oesterhelt, Dieter, and Walther Stoeckenius. "Rhodopsin-like Protein from the Purple Membrane of *Halobacterium Halobium*." *Nature* 233, no. 39 (1971): 149-52.

# Halorhodopsins: Light-driven chloride pumps

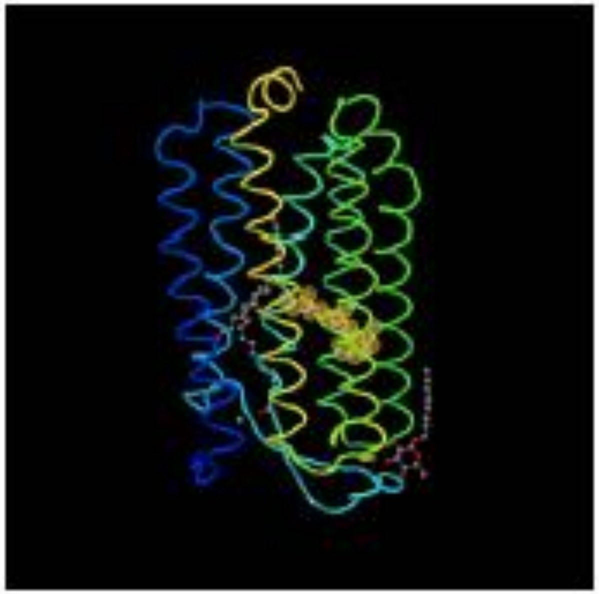


Image from the RCSB PDB of PDB ID 2JAF (Gmelin, Walter, Kornelius Zeth, et al. "The Crystal Structure of the L1 Intermediate of Halorhodopsin at 1.9 Å Resolution." *Photochemistry and Photobiology* 83, no. 2 (2007): 369–77.).

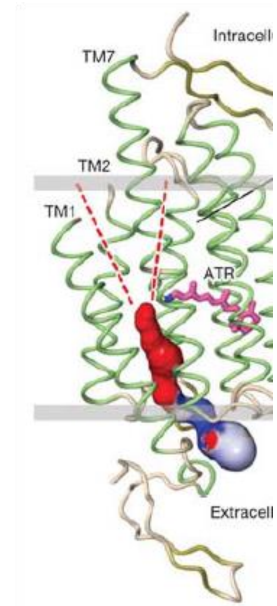
Matsuno-Yagi, Akemi, and Yasuo Mukohata. "Two Possible Roles of Bacteriorhodopsin; A Comparative Study of Strains of *Halobacterium Halobium* Differing in Pigmentation." *Biochemical and Biophysical Research Communications* 78, no. 1 (1977): 237-43.

Matsuno-Yagi, Akemi, and Yasuo Mukohata. "ATP Synthesis Linked to Light-dependent Proton Uptake in a Red Mutant Strain of *Halobacterium* Lacking Bacteriorhodopsin." *Archives of Biochemistry and Biophysics* 199, no. 1 (1980): 297-303.

Schobert, Brigitte, and Janos K. Lanyi. "Halorhodopsin is a Light-driven Chloride Pump." *Journal of Biological Chemistry* 257, no. 17 (1982): 10306-13.

# Channelrhodopsins: Light-driven cation pumps





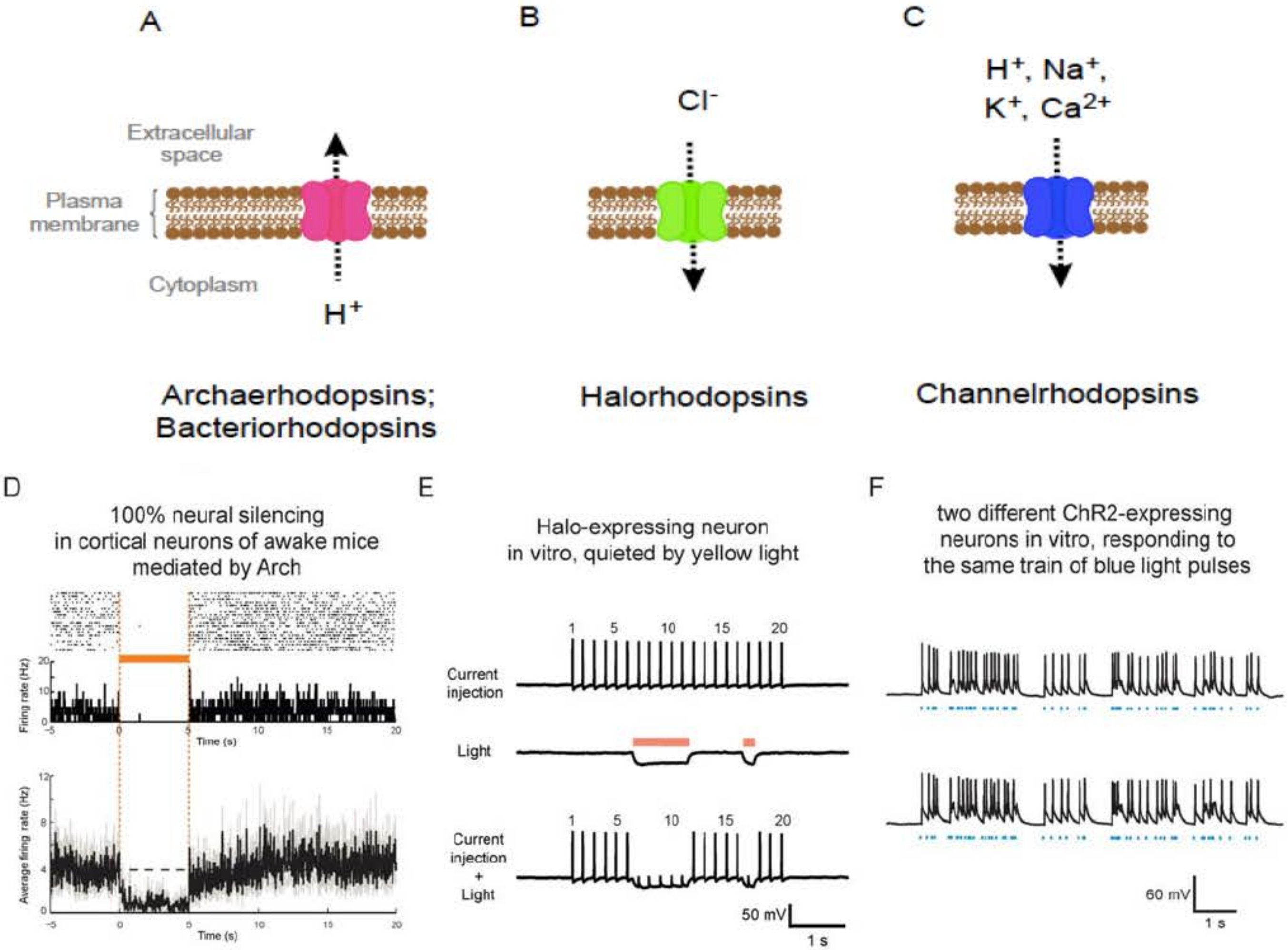



Source: Kato, Hideaki E., Feng Zhang, et al. "Crystal Structure of the Channel-rhodopsin Light-gated Cation Channel 482, no. 7385 (2012): 369–74.

Nagel, Georg, Doris Ollig, et al. "Channelrhodopsin-1: A Light-gated Proton Channel in Green Algae." *Science* 296, no. 5577 (2002): 2395-98.

Nagel, Georg, Tanjef Szellas et al. "Channelrhodopsin-2, a Directly Light-gated Cation-selective Membrane Channel." *Proceedings of the National Academy of Sciences* 100, no. 24 (2003): 13940-945.

# Three major optogenetic molecule classes: microbial opsins, seven-transmembrane proteins,

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Source: Chow, Brian Y., Xue Han, et al. "High-performance Genetically Targetable Optical Neural Silencing by Light-driven Proton Pumps." *Nature* 463, no. 7277 (2010): 98–102.

Courtesy of Han, Xue, and Edward S. Boyden. "Multiple-color Optical Activation, Silencing, and Desynchronization of Neural Activity, with Single-spike Temporal Resolution." *PloS One* 2, no. 3 (2007): e299. License CC BY.

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Source: Boyden, Edward S., Feng Zhang, et al. "Millisecond-timescale, Genetically Targeted Optical Control of Neural Activity." *Nature Neuroscience* 8, no. 9 (2005): 1263–68.

# Proto-optogenetic experiments: heterologous expression of opsins in different cell types

## Yeast

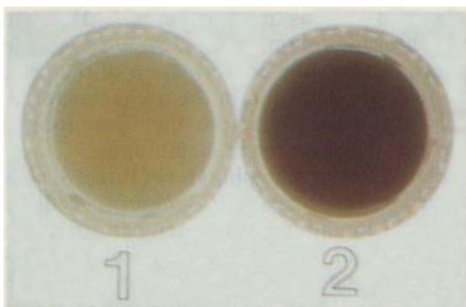



FIG. 1. Pelleted *S. pombe* cells. Pellet 1, cells transformed by the vector pEVP11, which lacks the *bop* gene. The yellow results from free retinal added to the culture medium. Pellet 2, cells transformed by the vector pEVBOp (pEVP11 containing the *bop* gene). The reddish color is a mixture of the purple of expressed bR and the yellow of free retinal.

Source: Hildebrandt, V., K. Fendler, et al. "Bacteriorhodopsin Expressed in Schizosaccharomyces Pombe Pumps Protons through the Plasma Membrane." *Proceedings of the National Academy of Sciences* 90, no. 8 (1993): 3578–82.

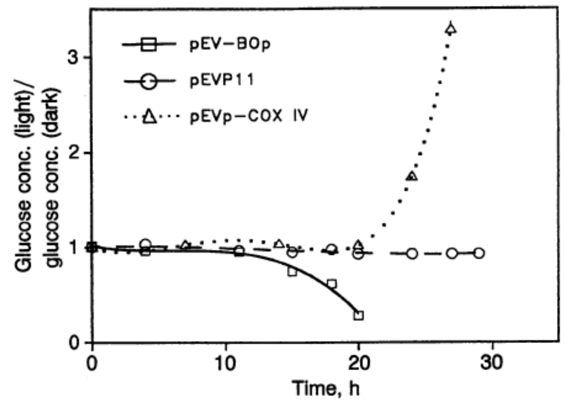




FIG. 5. Ratio of glucose concentrations in the growth medium for anaerobically growing *S. pombe* cultures with and without illumination [glucose conc. (light)/glucose conc. (dark)]. This ratio is plotted for three clones (pEV-BOp, pEVP11, and pEVp-COX IV). The absolute values of glucose concentrations are listed in Table 2.

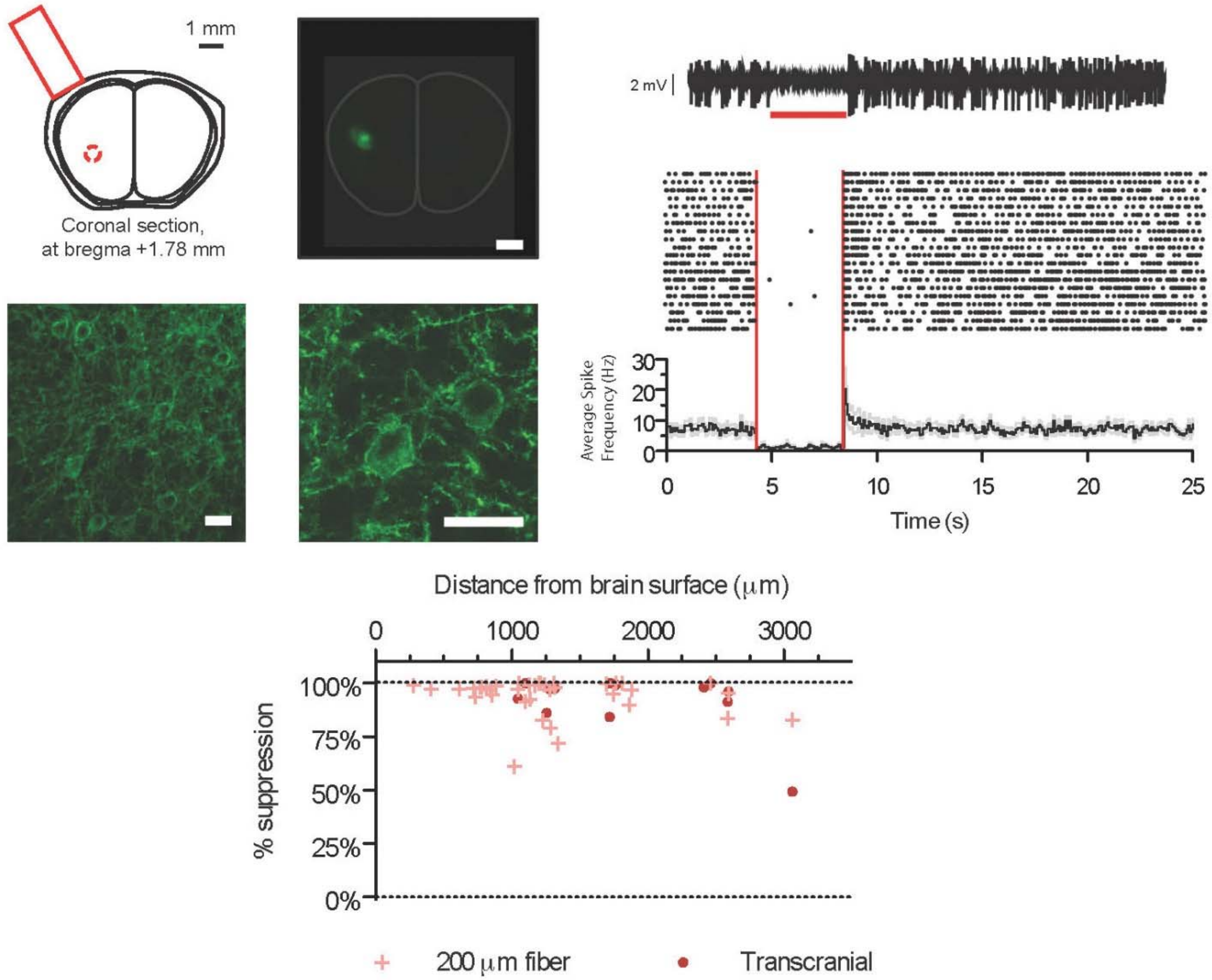
Source: Hoffmann, Astrid, Volker Hildebrandt, et al. "Photoactive Mitochondria: In Vivo Transfer of a Light-driven Proton Pump into the Inner Mitochondrial Membrane of Schizosaccharomyces Pombe." *Proceedings of the National Academy of Sciences* 91, no. 20 (1994): 9367–71.


Hildebrandt V, Ramezani-Rad M, Swida U, Wrede P, Grzesiek S, Primke M, Büldt G. (1989) Genetic transfer of the pigment bacteriorhodopsin into the eukaryote Schizosaccharomyces pombe. FEBS Lett. 243(2):137-40.

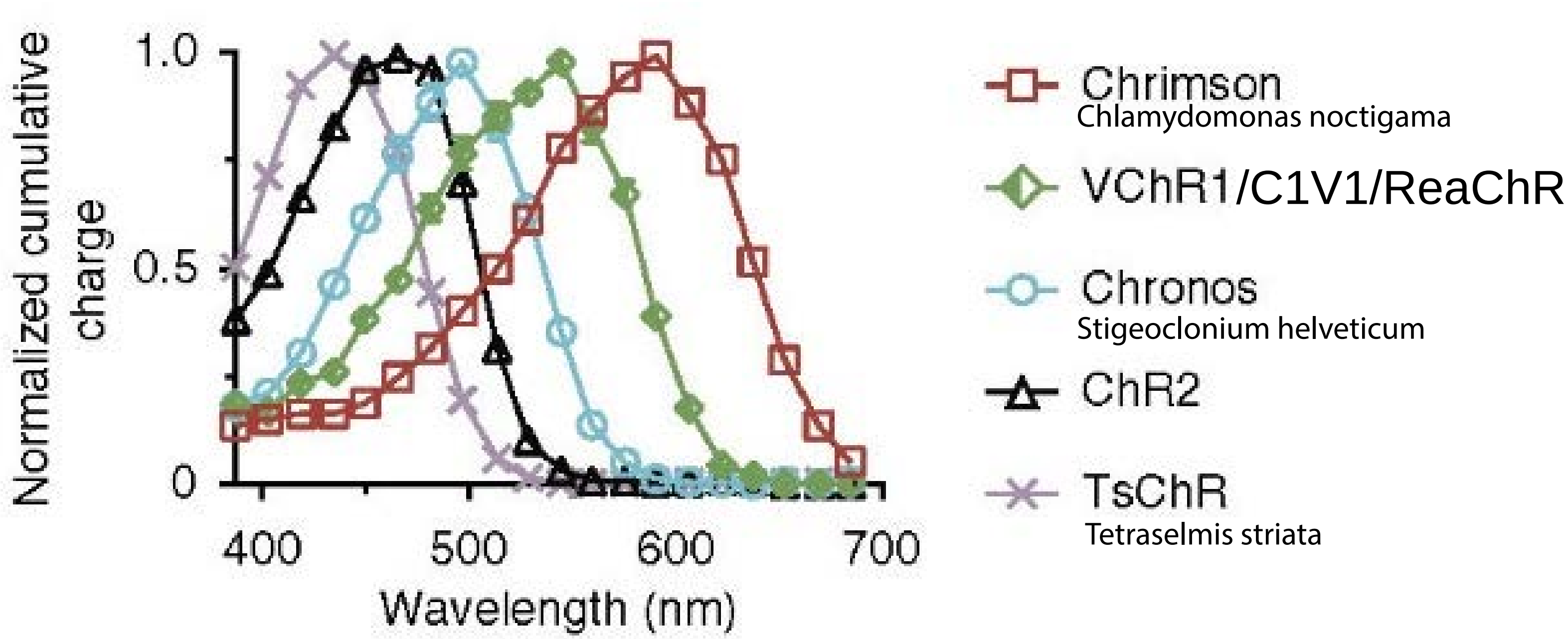
Hildebrandt V, Fendler K, Heberle J, Hoffmann A, Bamberg E, Buldt G (1993) Bacteriorhodopsin expressed in Schizosaccharomyces pombe pumps protons through the plasma membrane. Proc Natl Acad Sci U S A, 90:3578-82.

Hoffmann A, Hildebrandt V, Heberle J, Büldt G (1994) Photoactive mitochondria: in vivo transfer of a light-driven proton pump into the inner mitochondrial membrane of Schizosaccharomyces pombe. Proc Natl Acad Sci U S A. 91(20):93.

# Noninvasive optogenetic neural silencing: Jaws




**Chuong et al. (2014) *Nature Neuroscience*, doi:10.1038/nn.3752.**

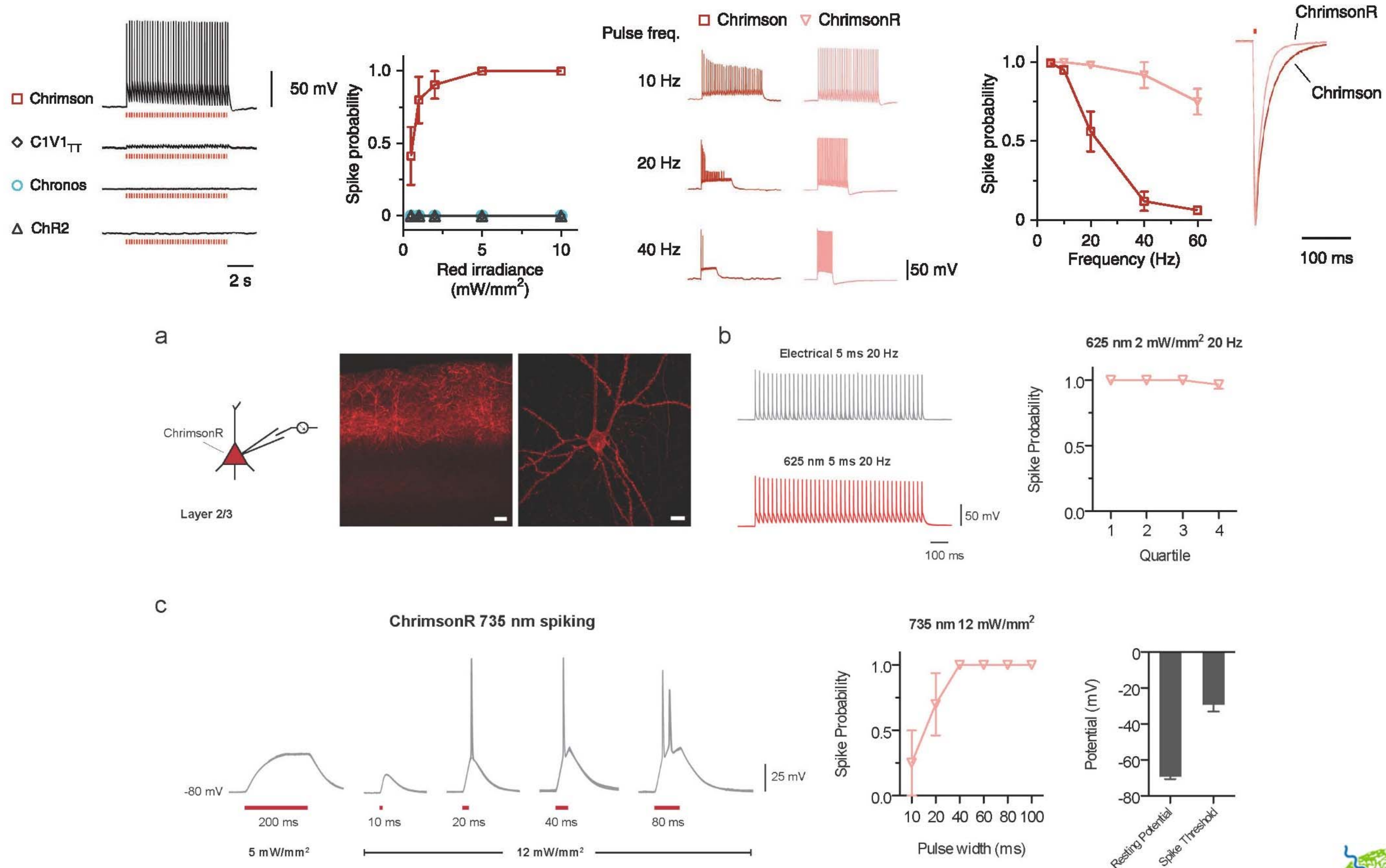




Source: Klapoetke, Nathan C., Yasunobu Murata, et al. "Independent Optical Excitation of Distinct Neural Populations." *Nature Methods* 11, no. 3 (2014): 338–46.

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

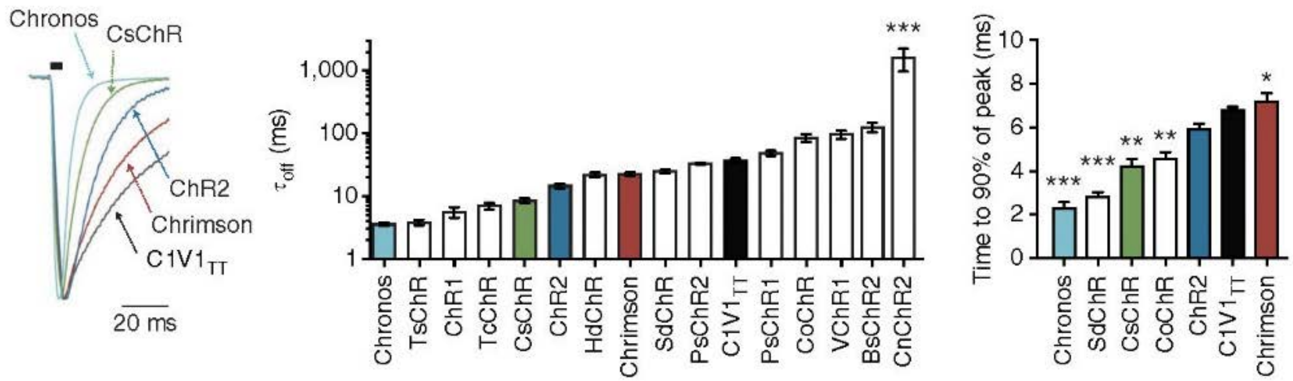
# Chrimson




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


Source: Klapoetke, Nathan C., Yasunobu Murata, et al. "Independent Optical Excitation of Distinct Neural Populations." *Nature Methods* 11, no. 3 (2014): 338–46.

# Chronos: a very fast channelrhodopsin…





Source: Klapoetke, Nathan C., Yasunobu Murata, et al. "Independent Optical Excitation of Distinct Neural Populations." *Nature Methods* 11, no. 3 (2014): 338–46.


Please see supplemental figure 3 and figure 2A, B from Klapoetke, Nathan C., Yasunobu Murata, et al. "Independent Optical Excitation of Distinct Neural Populations." *Nature Methods* 11, no. 3 (2014): 338–46.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3943671/

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

# …that is also very light sensitive!



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

# Chronos and Chrimson together

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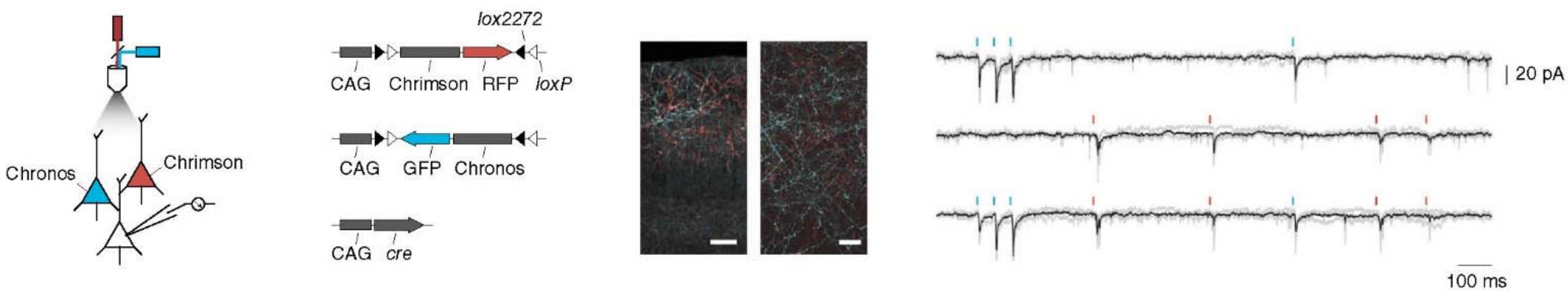
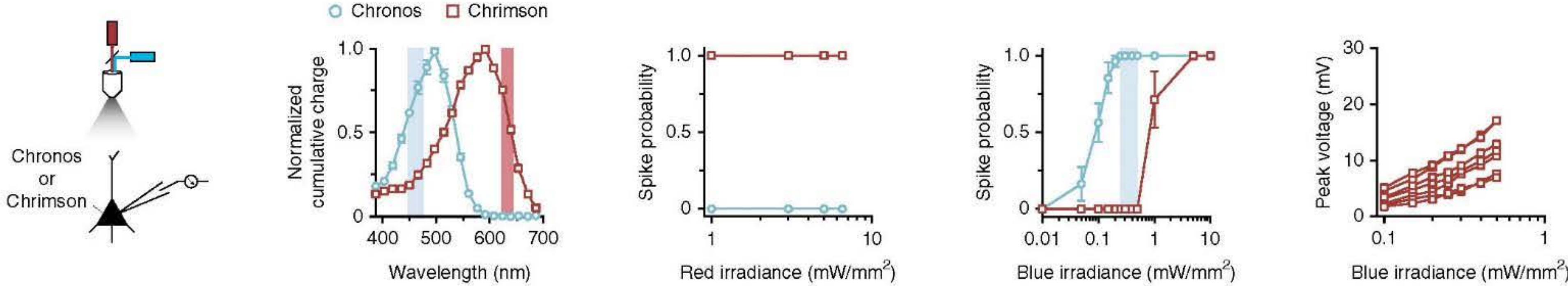
Please see supplemental figure 18 from Klapoetke, Nathan C., Yasunobu Murata, et al. "Independent Optical Excitation of Distinct Neural Populations." *Nature Methods* 11, no. 3 (2014): 338–46.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3943671/

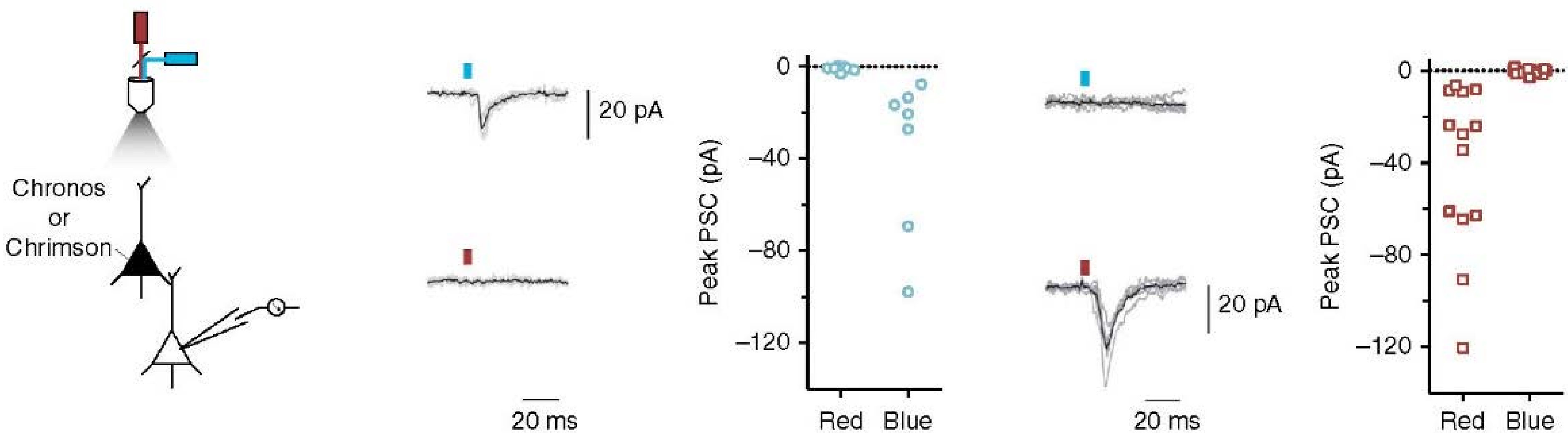



**Klapoetke et al. (2014) *Nature Methods* 11:338–346.**

# Chronos and Chrimson together: zero-crosstalk control of spikes…

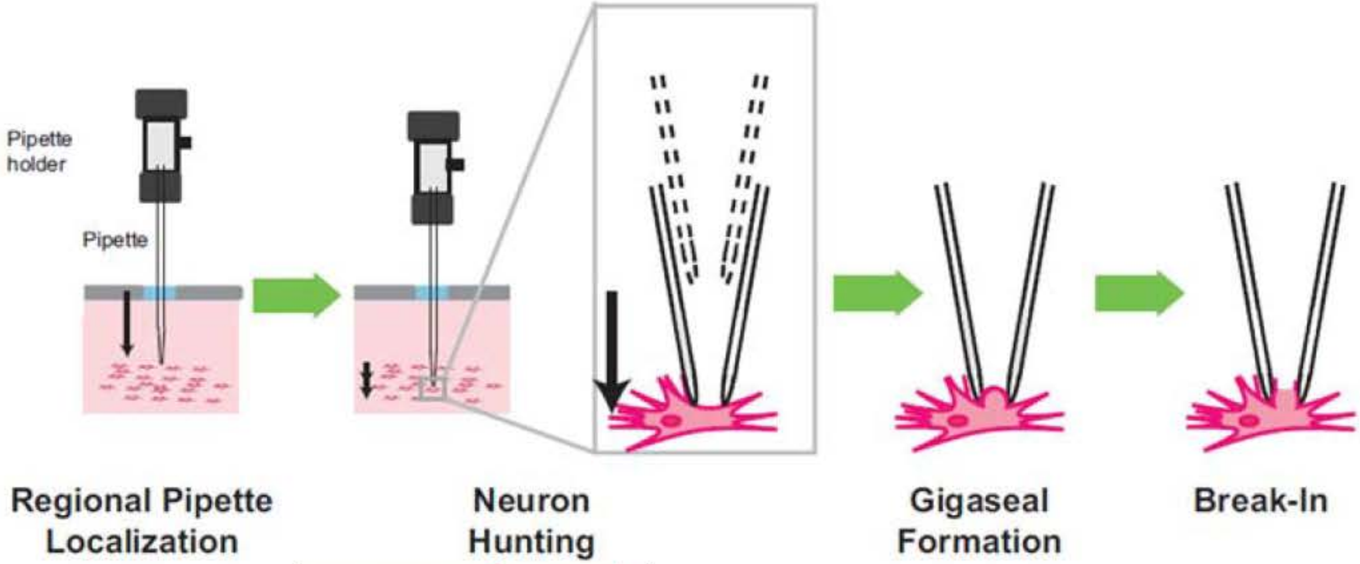


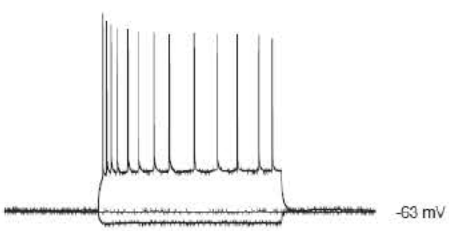

# …and synaptic release events

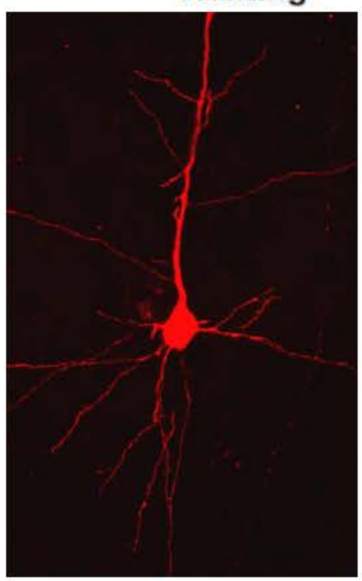



**Klapoetke et al. (2014) *Nature Methods* 11:338–346.**

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

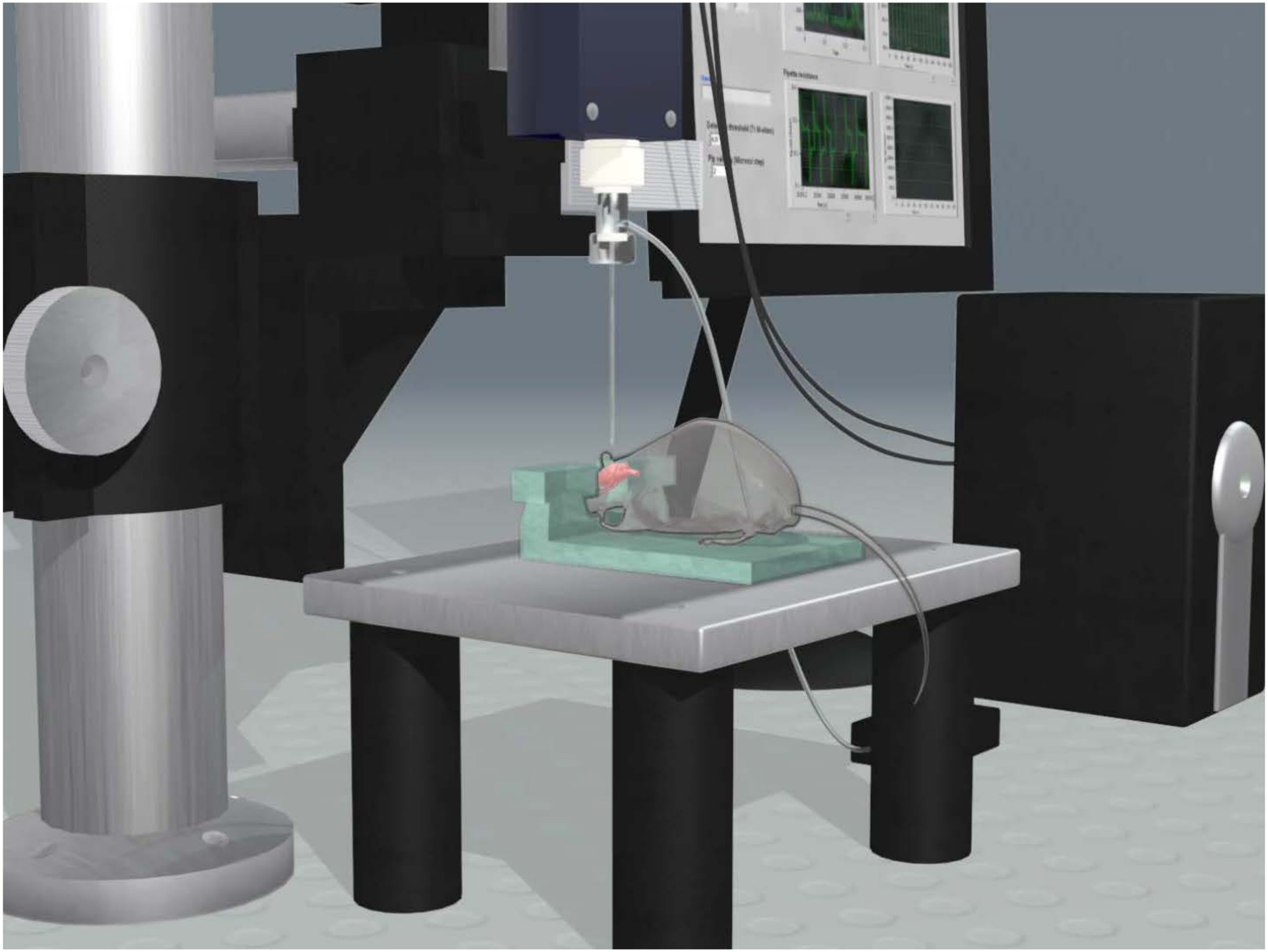


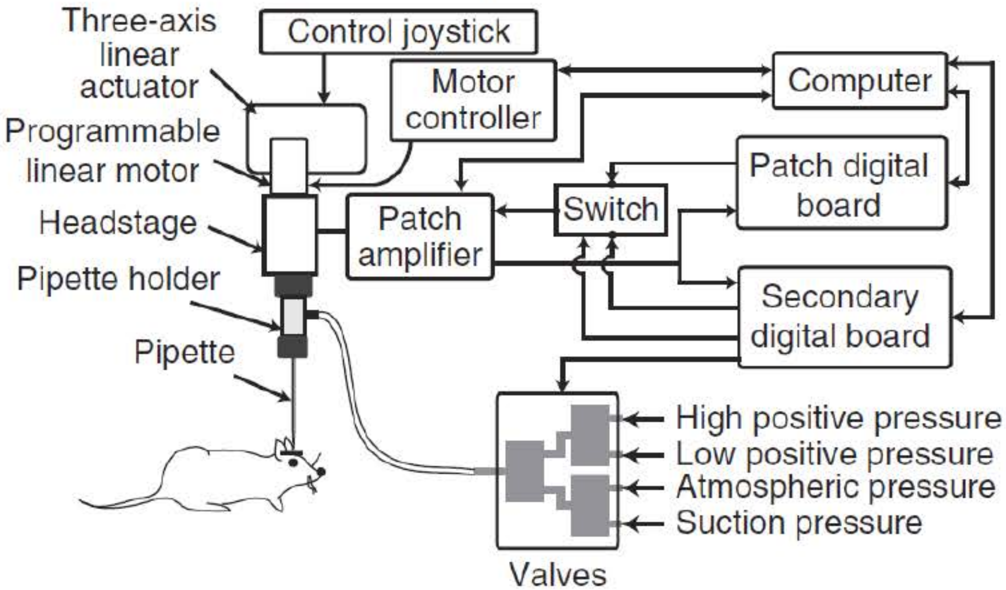



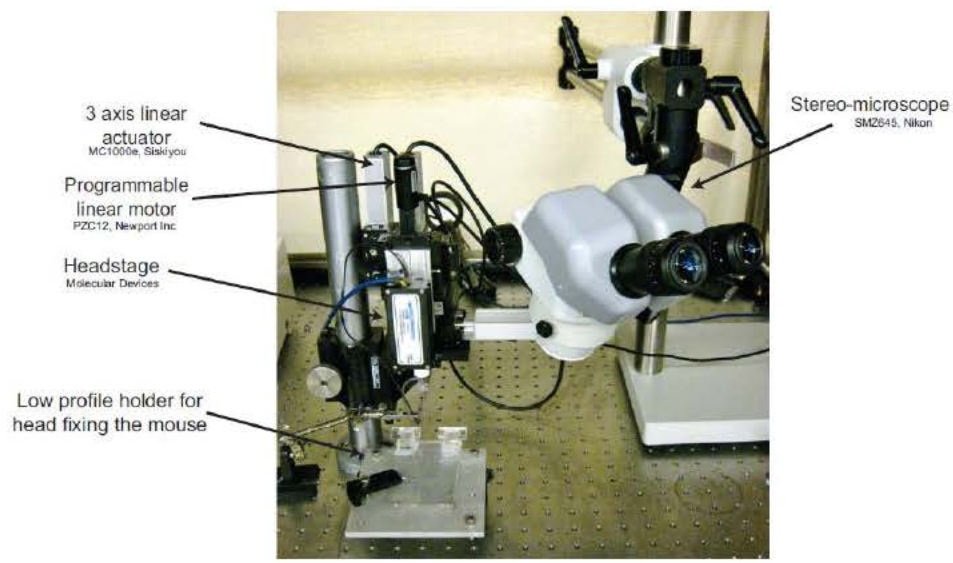
**Kodandaramaiah et al. (2012) *Nature Methods* 9:585–587.**


Source: Kodandaramaiah, Suhasa B., Giovanni Talei Franzesi, et al. "Automated Whole-cell Patch-clamp Electrophysiology of Neurons in Vivo." *Nature Methods* 9, no. 6 (2012): 585–87.



# A robot that can automatically patch clamp neurons in living brain





Source: Kodandaramaiah, Suhasa B., Giovanni Talei Franzesi, et al. "Automated Whole-cell Patch-clamp Electrophysiology of Neurons in Vivo." *Nature Methods* 9, no. 6 (2012): 585–87.

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

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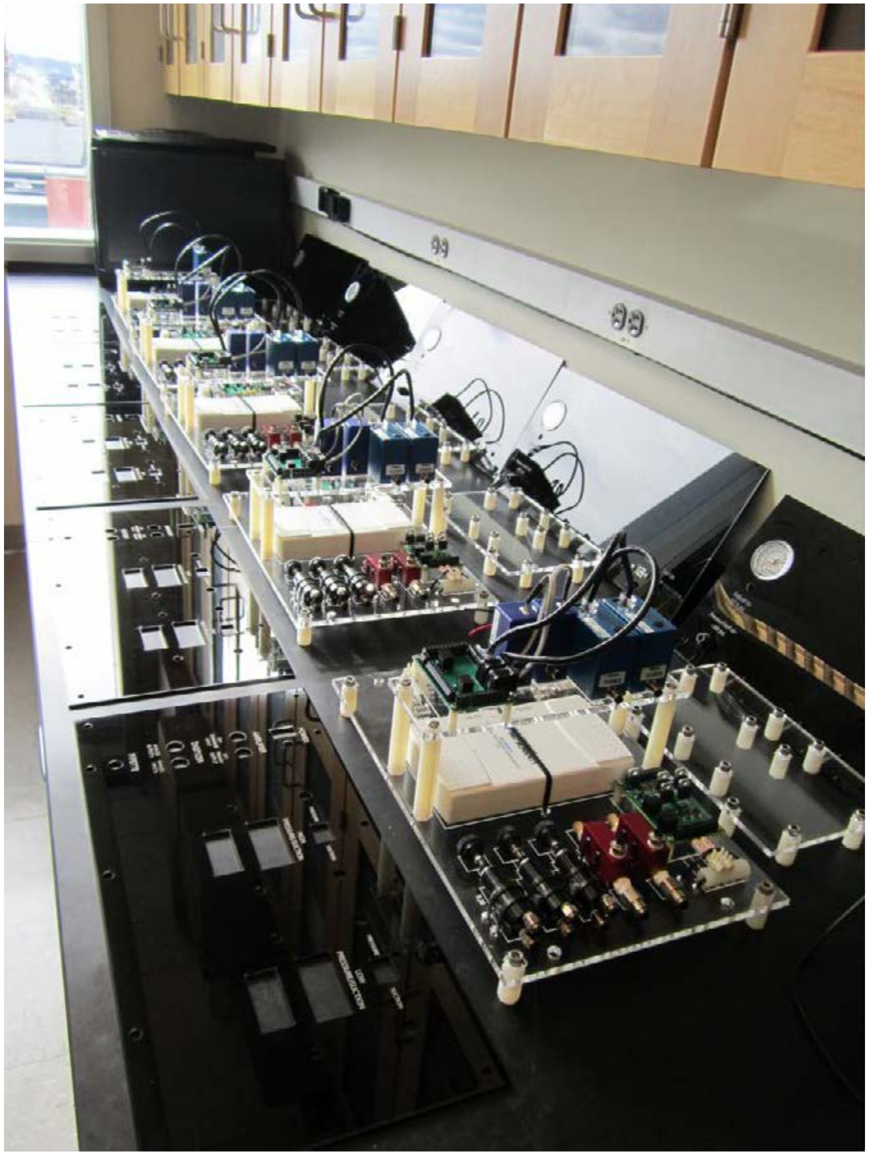
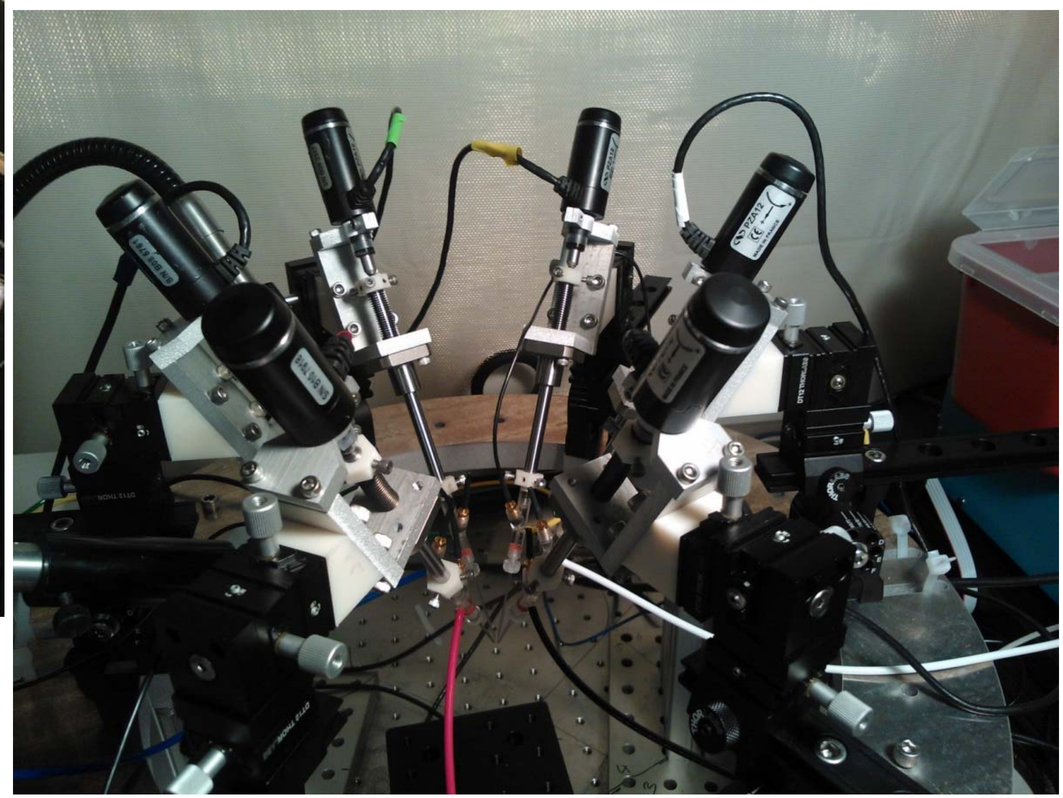
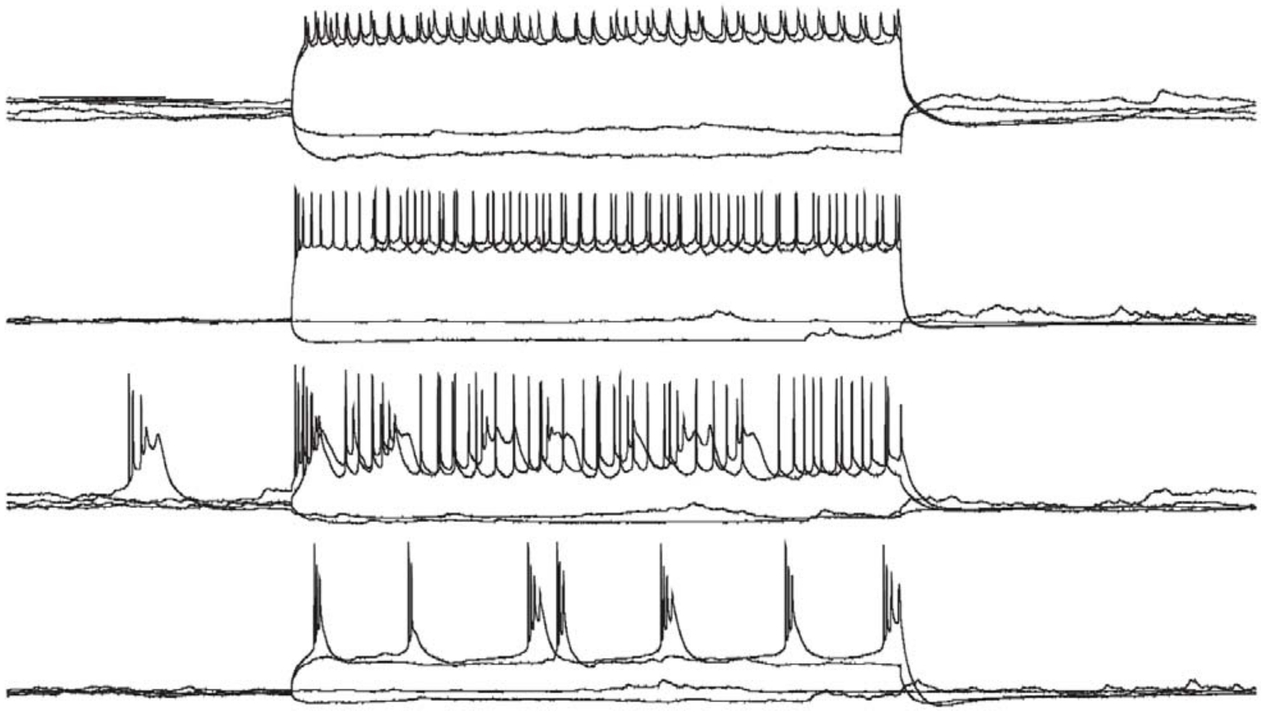




Suhasa Kodandaramaiah, Xue Han, Craig Forest

# Robotic quad patching in living mouse brain



Suhasa Kodandaramaiah, Francisco Flores, Emery Brown, Craig Forest

# Can we automate the rest of in vivo neuroscience?

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Please see Figures 1A, B and Figure 3B, C from Pak, Nikita, Joshua H. Siegle, et al. "Closed-loop, Ultraprecise, Automated Craniotomies." *Journal of Neurophysiology* (2015).

**Nikita Pak, Justin Kinney**

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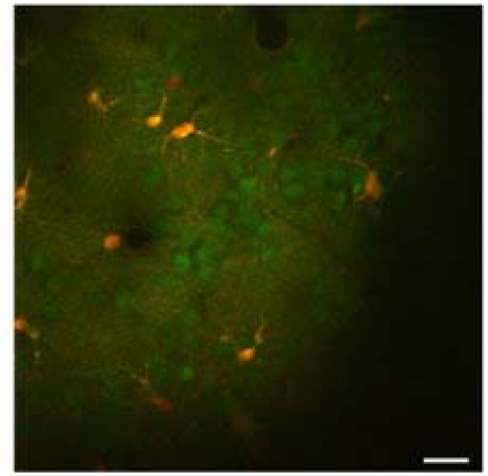
Please see Figures 1A, B and Figure 3B, C from Pak, Nikita, Joshua H. Siegle, et al. "Closed-loop, Ultraprecise, Automated Craniotomies." *Journal of Neurophysiology* (2015).

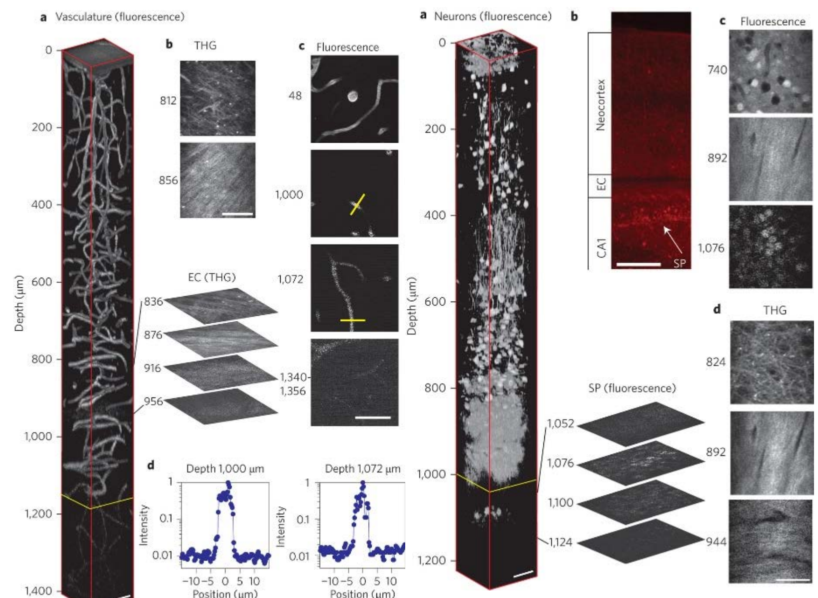
**Nikita Pak, Justin Kinney**

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











Source: Horton, Nicholas G., Ke Wang, et al. "In Vivo Three-photon Microscopy of Subcortical Structures within an Intact Mouse Brain." *Nature Photonics* 7, no. 3 (2013): 205–9.

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# http://syntheticneurobiology.org/

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***Primate work:*** Ann Graybiel, Bob Desimone, Bob Wurtz, Roderick Bronson, Wim Vanduffel
***Transgenics:*** Hongkui Zeng

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