Principles of Neuroimaging, 2/11/15

Optogenetics

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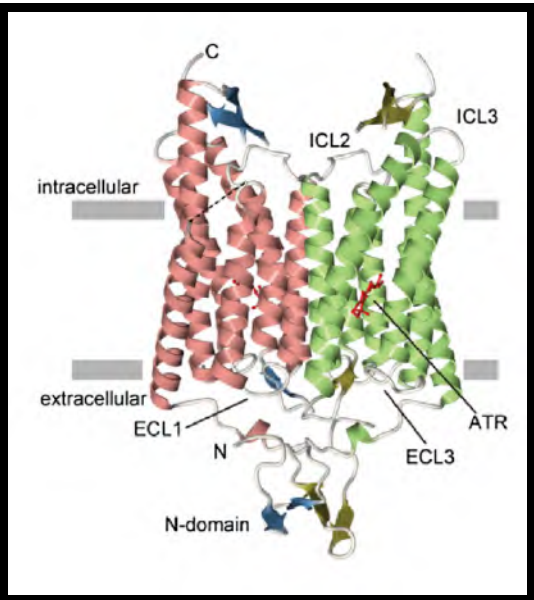
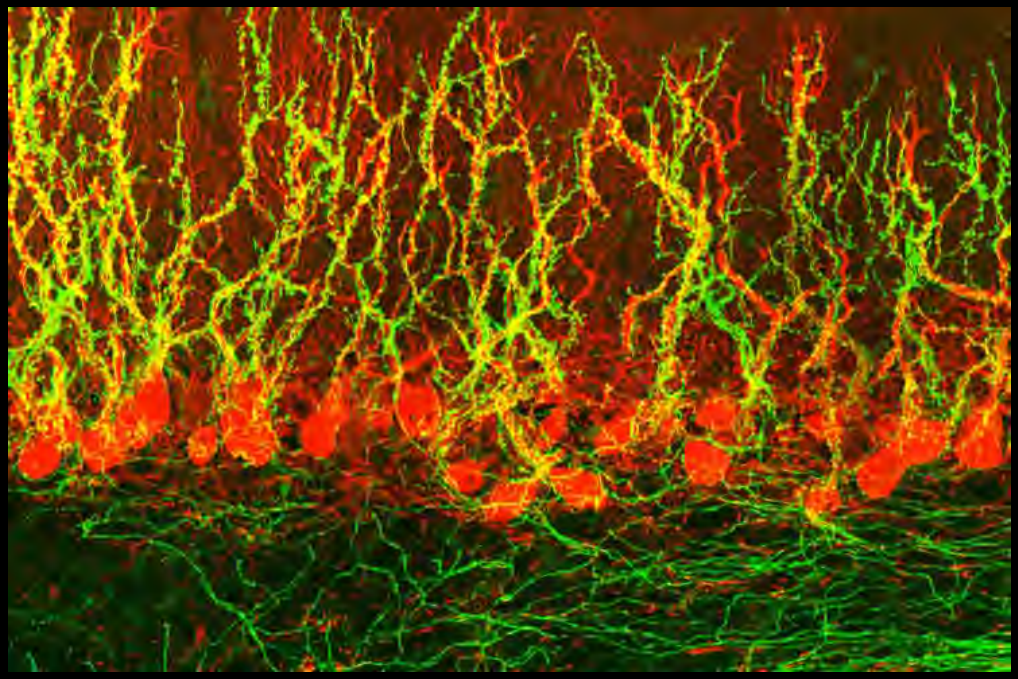
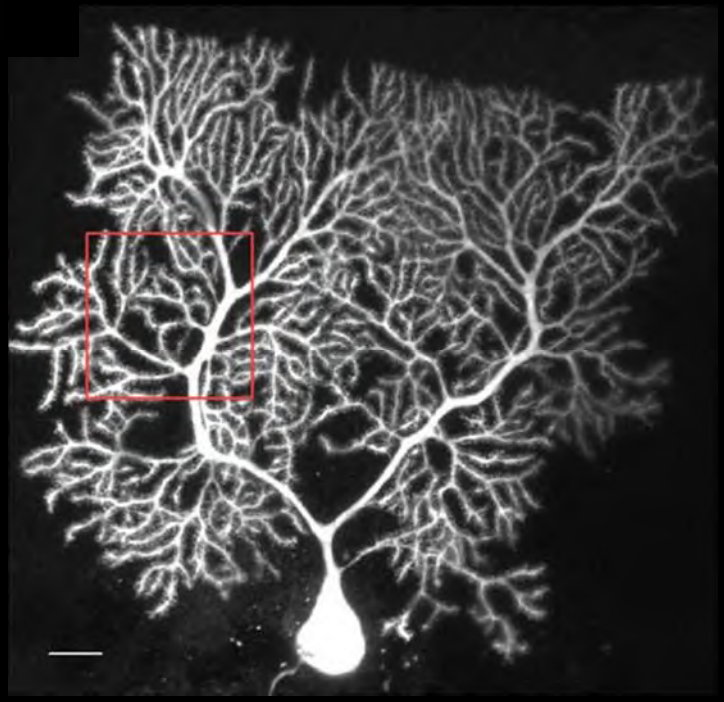


from Deisseroth & Schnitzer, 2013





GRETE LUNDBECK EUROPEAN BRAIN RESEARCH FOUNDATION

THE BRAIN PRIZE

'their invention and refinement of optogenetics. This revolutionary technique allows genetically specified populations of neurons to be turned on or off with light, offering not only the ability to elucidate the characteristics of normal and abnormal neural circuitry but also new approaches to treatment of brain disorders.'

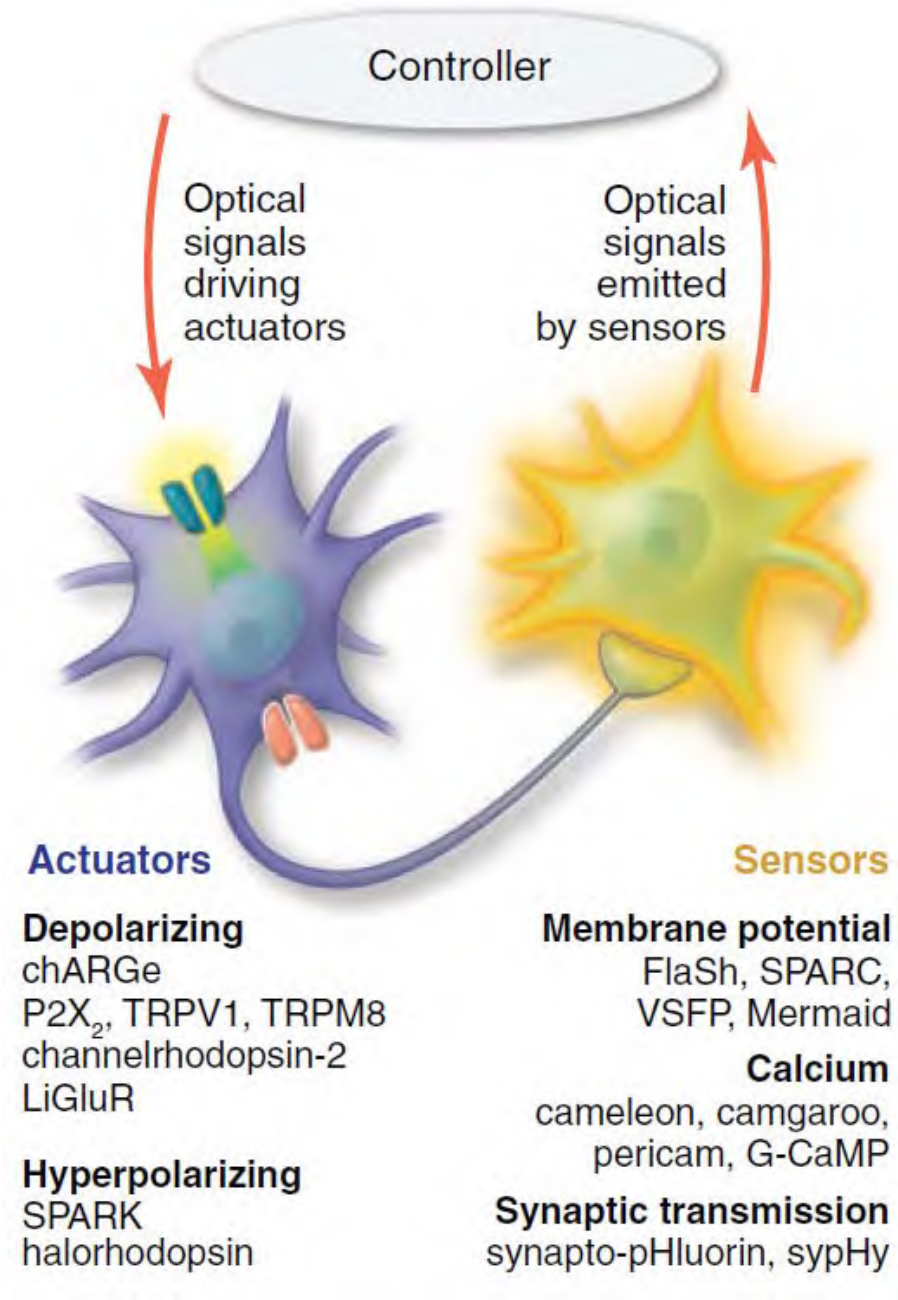
2013 winners for “optogenetics”, presented May 7, 2013 in Copenhagen
Bamberg, Boyden, Deisseroth, Hagemann, Meisenbock, Nagel

Optogenetic *sensors* and *actuators*

openoptogenetics.org

a terrific, up to date blog on the topics discussed today

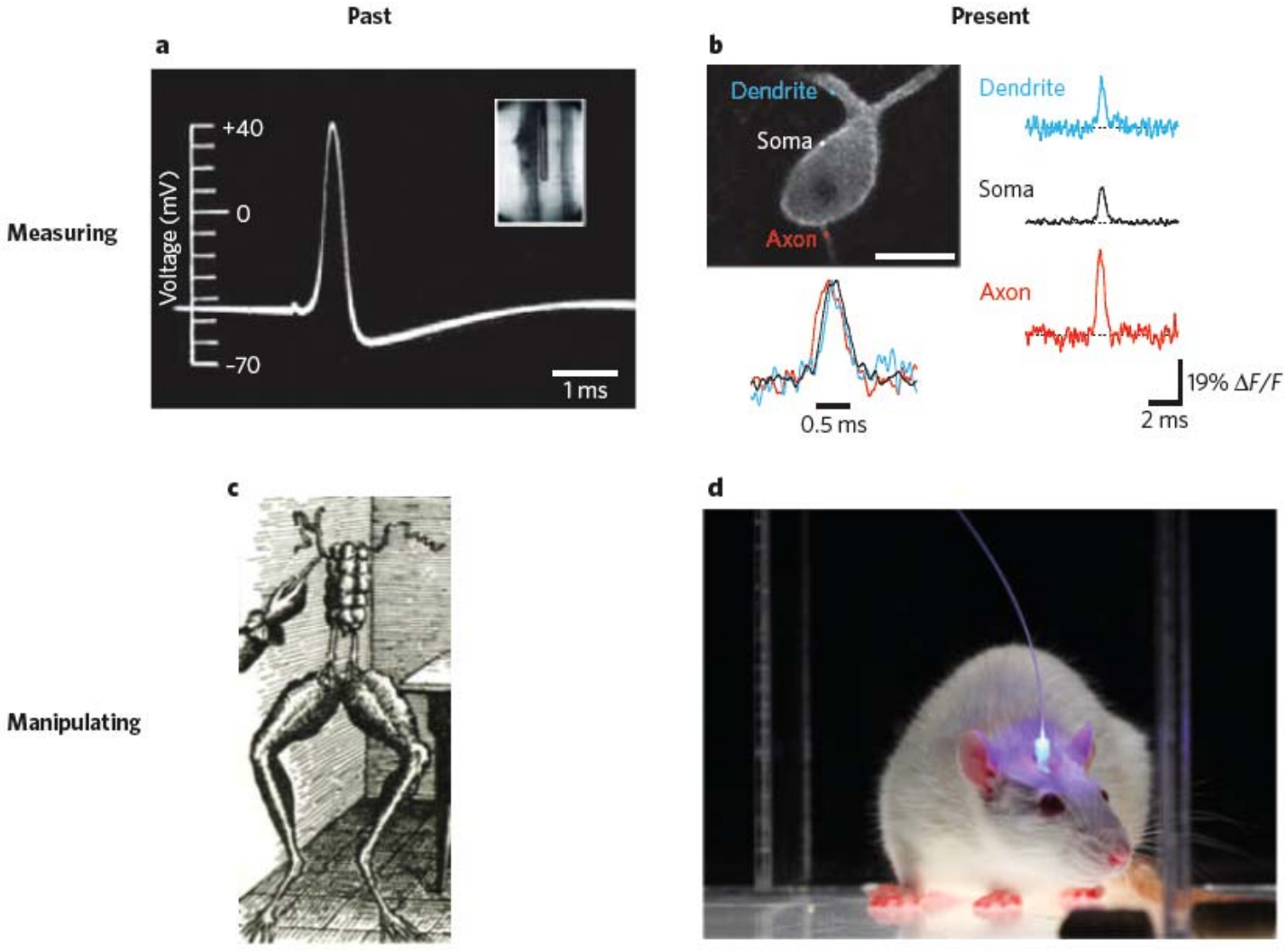
Miesenböck, 2009



NATURE|Vol 461|15 October 2009|doi:10.1038/nature08540

Electrophysiology in the age of light

Massimo Scanziani[1] & Michael Häusser[2]



Brain circuits are enormously complex

there are many neurons

~100 x 10^9 neurons in human brain

~80,000 neurons per mm^3 of cortex

~10,000 synapses per neuron

neurons are small

~5-25 µm diameter cell bodies

and signaling is fast

~ 1 millisecond nerve impulse

@ frequencies up to ~500 Hz

Tucker Nichols, *NY Times,* 6/24/13

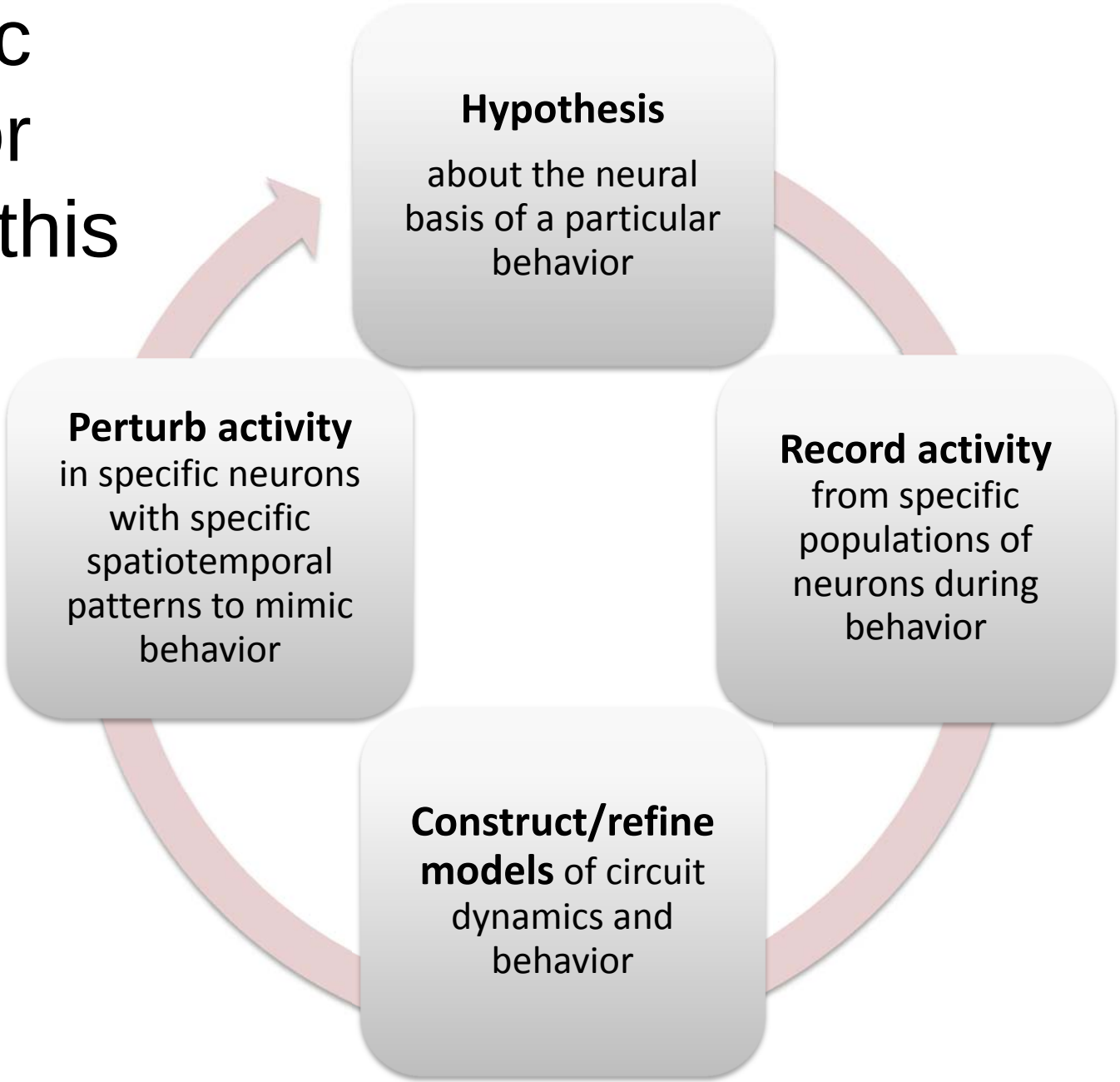
Why use light to probe neuronal function?

1) Various optical methods allow for precise control of light.

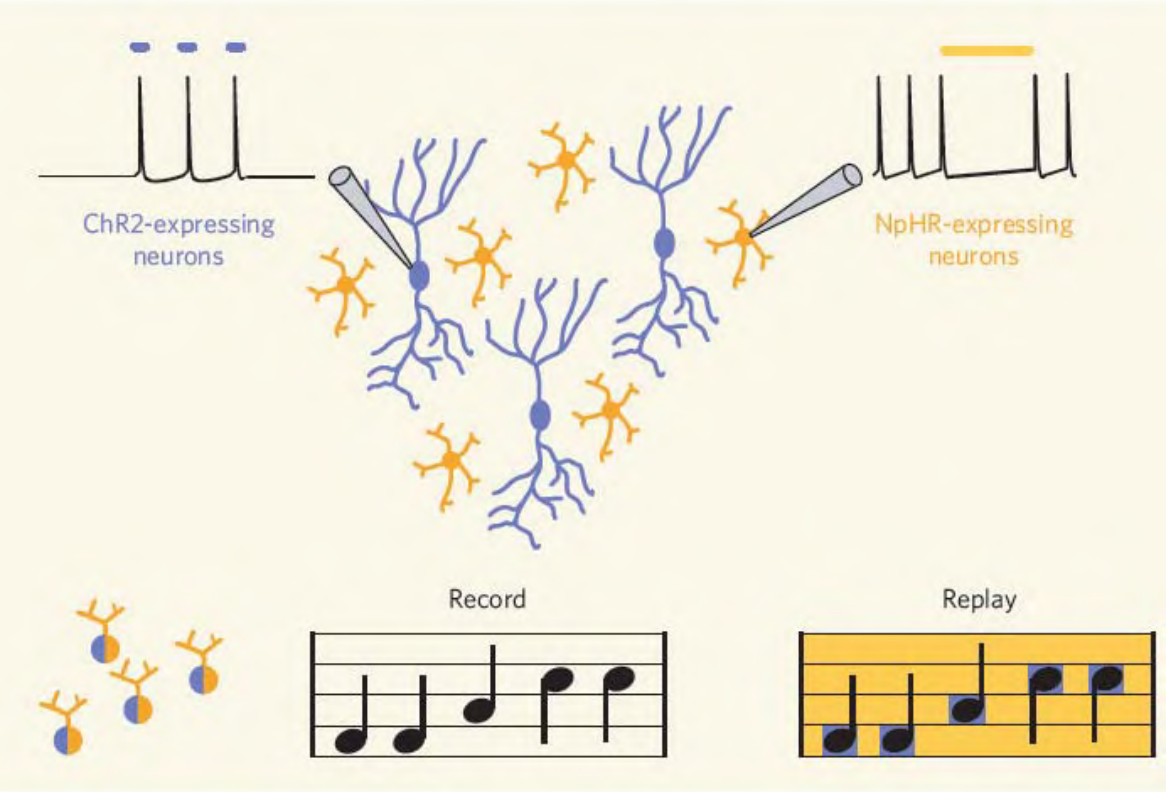
2) Light can be relatively non-invasive.

3) Light can be used to visualize neuronal structure. It can also to record, to stimulate, or to suppress neural activity (electrical and biochemical).

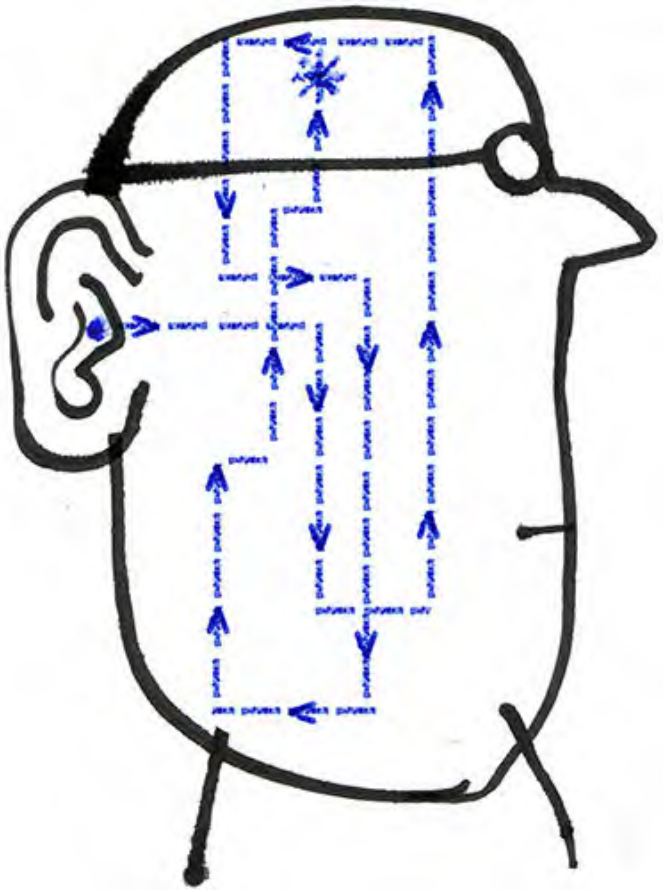
A systematic approach for addressing this problem



Some examples of what optogenetics can do…



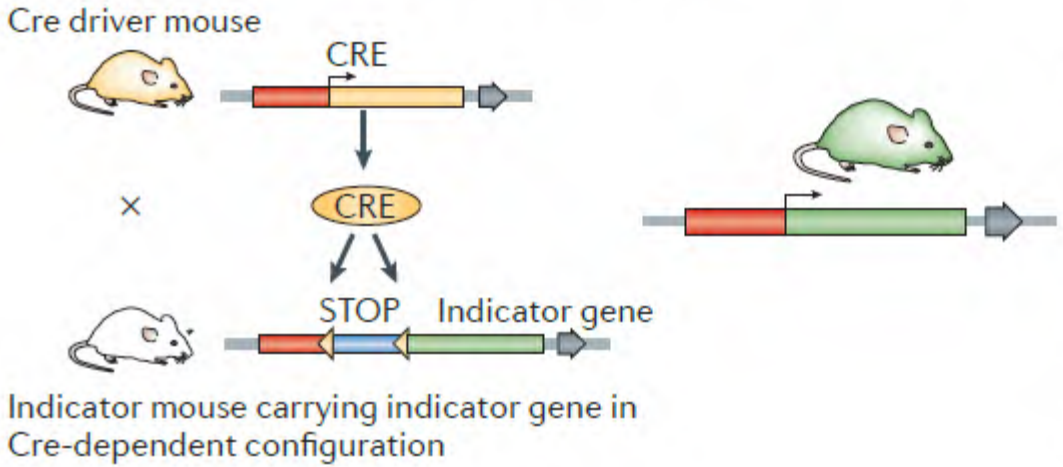
Smith & Häusser, *Nature*, 2007

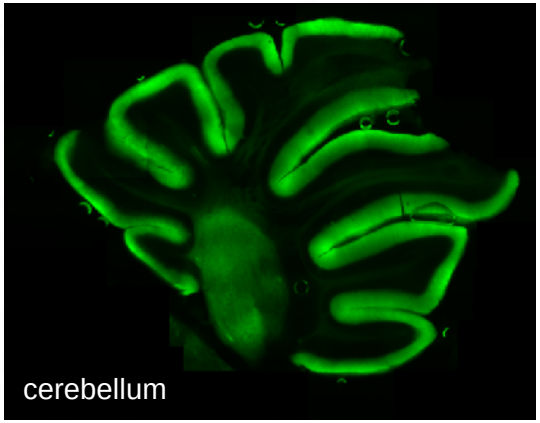


Serge Bloch, *NY Times*, 10/12/09

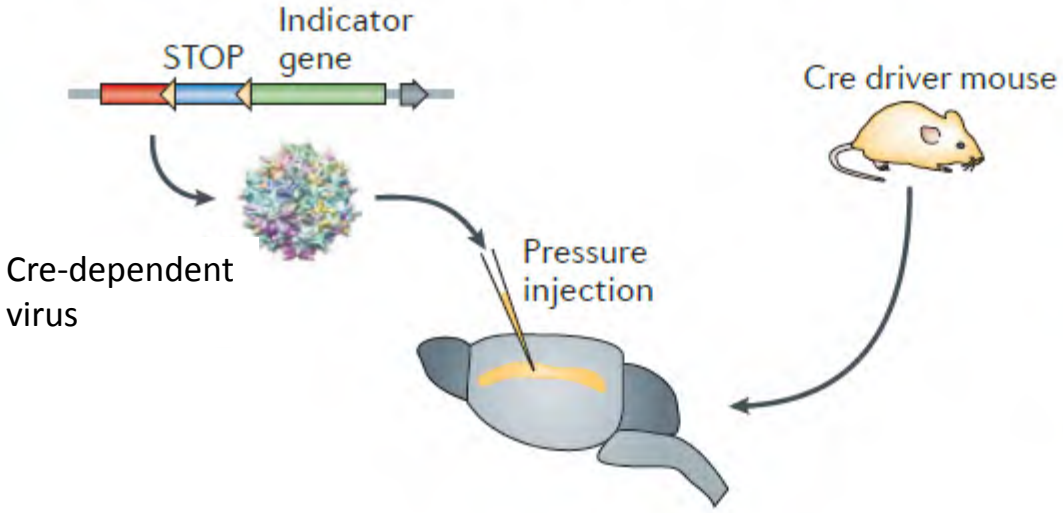
Conditional genetics and lab mice

Breeding strategy





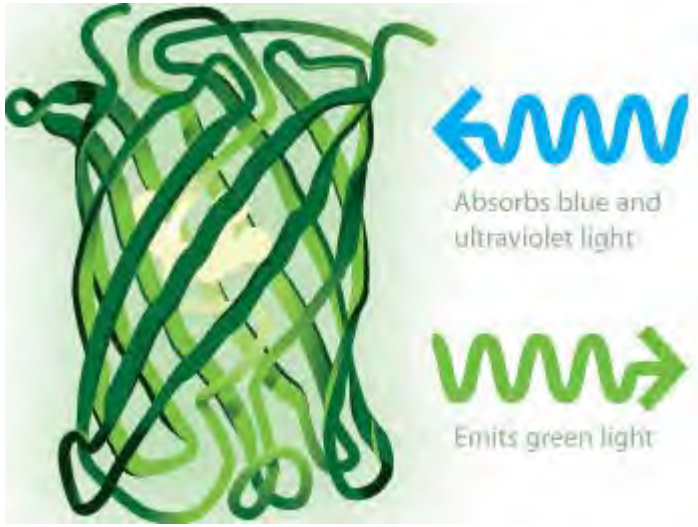
Viral strategy



from Knopfel,
Nat. Rev. Neurosci.
2012

A revolution in biotechnology caused by a protein from a jellyfish

Green fluorescent protein



2008 Nobel prize in Chemistry: Shimomura, Chalfie, & Tsien

Fundamentals of fluorescence

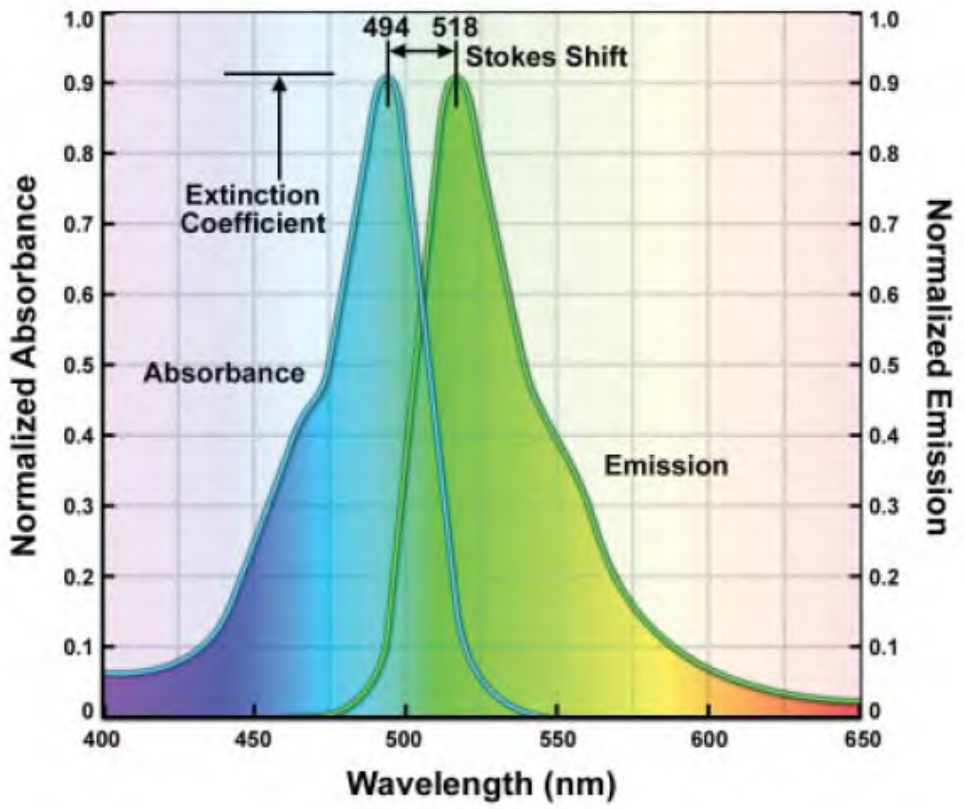


Figure 11.3

Normalized absorption and fluorescence emission spectra of fluorescein conjugated to IgG. Both spectra span a wide range of wavelengths. Fluorescein has an absorption/excitation peak at 494 nm and looks yellow-green to the eye, but actually fluoresces at wavelengths ranging from blue to red with a peak at 518 nm. The difference in nanometers between the excitation and emission maxima is called the Stokes shift. The molar extinction coefficient is measured at the peak of the absorbance spectrum as indicated in the figure.

From Ch.11, *Fundamentals of Light Microscopy and Electronic Imaging*, 2nd Ed., Murphy & Davidson

Multicolored fluorescent proteins

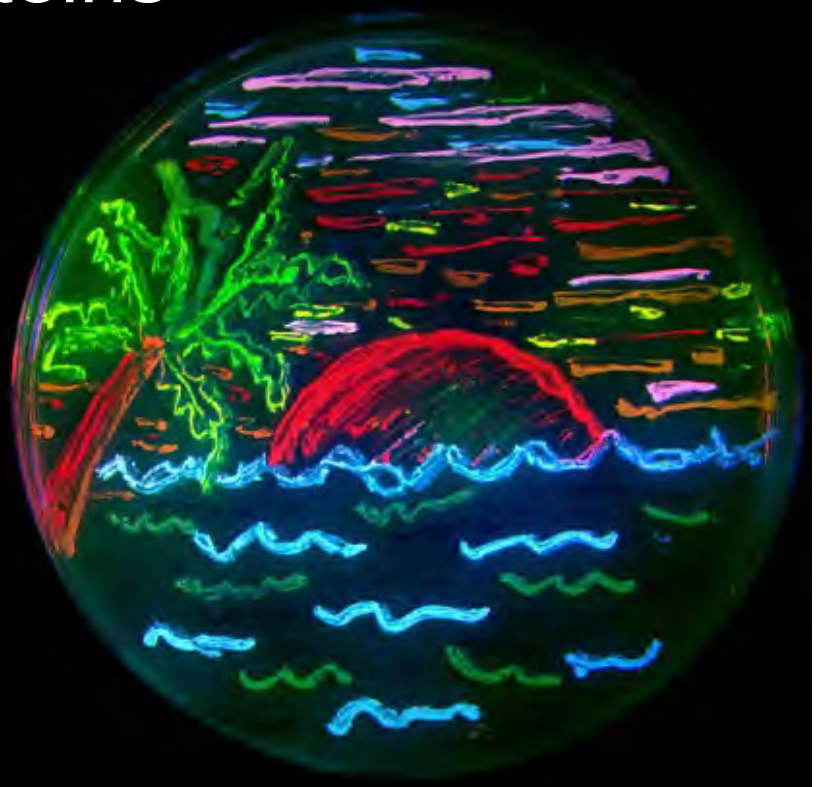


TABLE 11.2 Physical Properties of Useful Fluorescent Proteins

Protein[a]	Color[b]	Excitation (nm)	Emission (nm)	Brightness[c]	Photostability[d]	Filter Set[e]
EBFP2	Blue	383	448	18	++	DAPI
mCerulean	Cyan	433	475	17	++	CFP
mTurquoise	Cyan	433	474	25	+++	CFP
mTFP1	Teal	462	492	54	+++	CFP
mEGFP	Green	488	507	34	++++	FITC/GFP
mEmerald	Green	487	509	39	++++	FITC/GFP
mVenus	Yellow	515	528	53	++	FITC/YFP
mCitrine	Yellow	516	529	59	++	FITC/YFP
mKO2	Orange	551	565	40	+++	TRITC
tdTomato	Orange	554	581	95	+++	TRITC
TagRFP	Orange	555	584	48	++	TRITC
mApple	Orange	568	592	37	+++	TRITC
mCherry	Red	587	610	17	+++	TxRed
mKate2	Far-Red	588	633	25	++	TxRed
mPlum	Far-Red	590	649	3.2	+++	TxRed
mNeptune	Far-Red	600	650	13	++++	Cy5

[a] Common literature abbreviation.
[b] Spectral class.
[c] Product of the molar extinction coefficient and the quantum yield $(mM \times cm)^{-3}$.
[d] Relative to mEGFP (++++).
[e] Recommended filter set.

From Murphy and Davidson, Ch 11

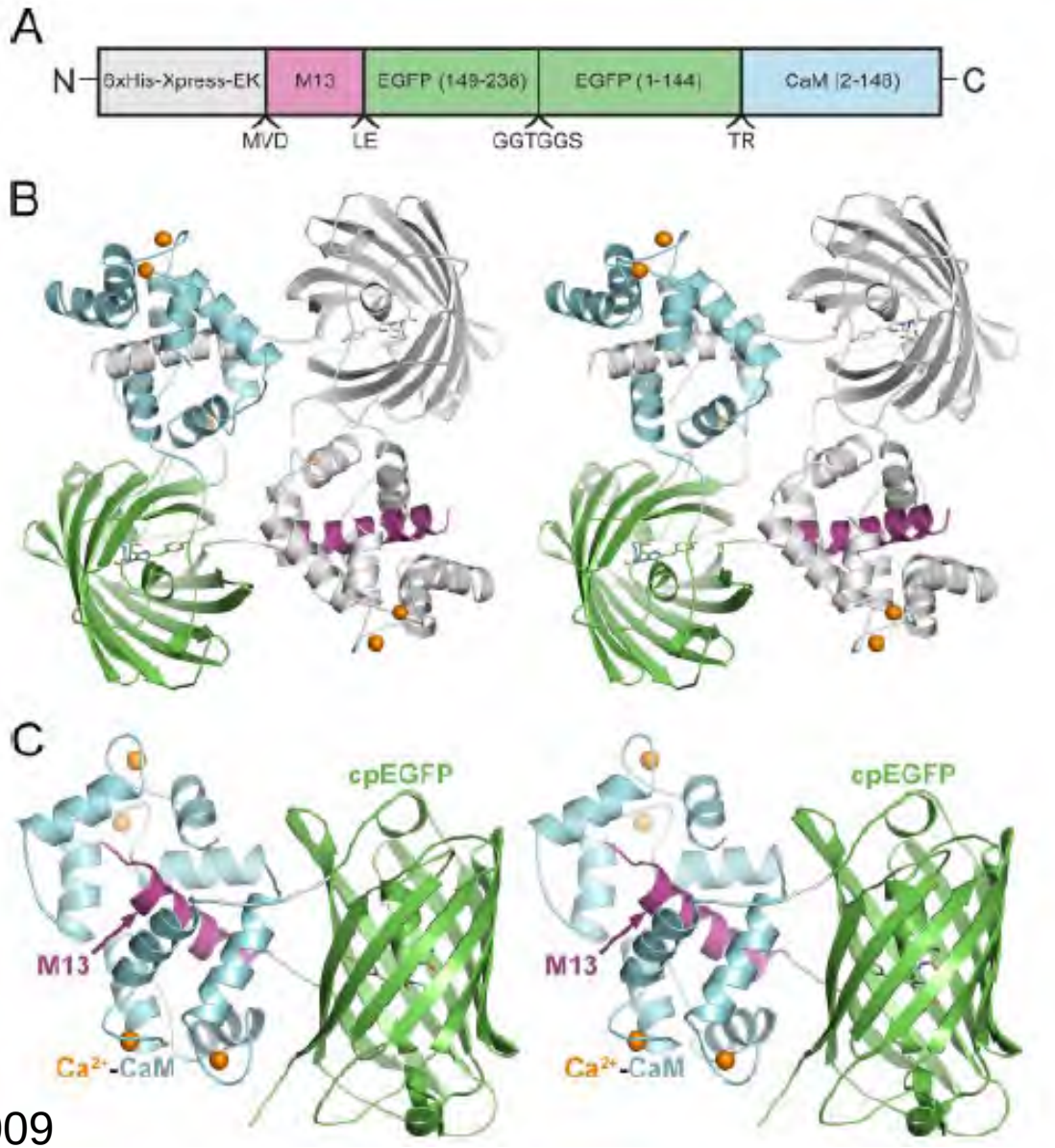
The GCaMP family of calcium sensors

GCaMP1 described in 2001:
Nakai et al.,, *Nat. Biotech.* 19:137

GCaMP6:
Chen et al., 2013
Nature, 499:295

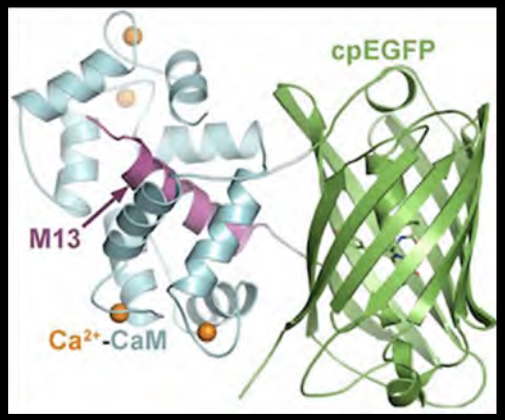
See also B-GECO and R-GECO

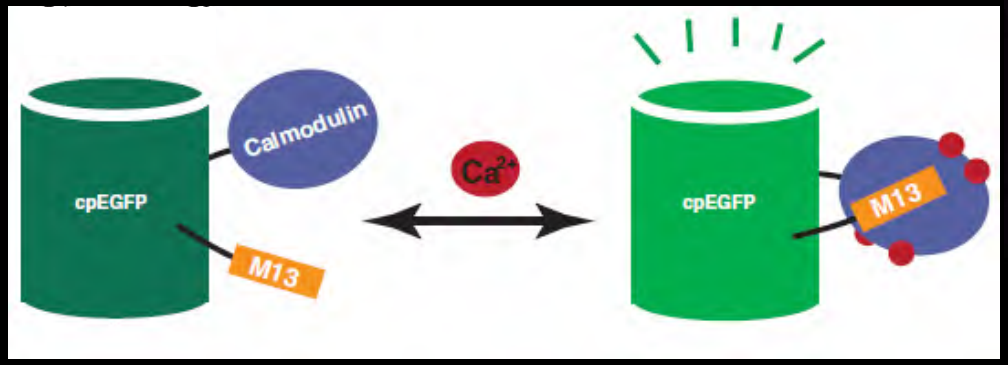
crystal structure of GCaMP2:
Akerboom et al., *JBC* 284:6455, 2009

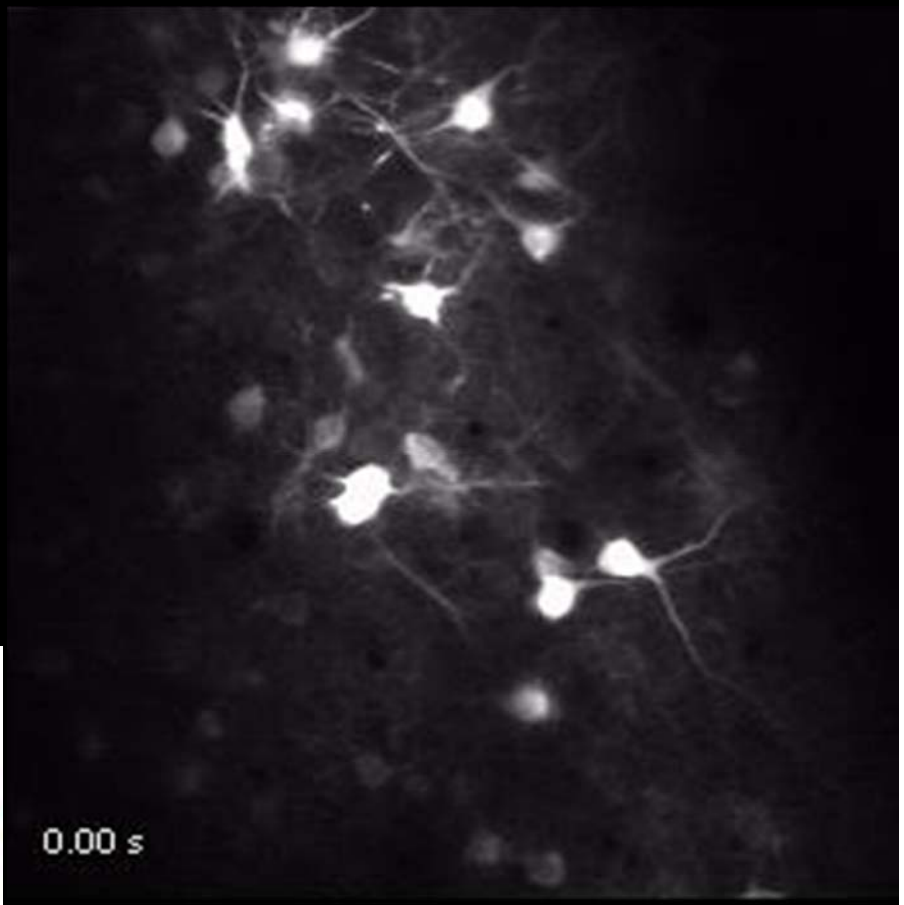


Prefrontal cortical activity recorded in a living, behaving mouse

GCAMP6



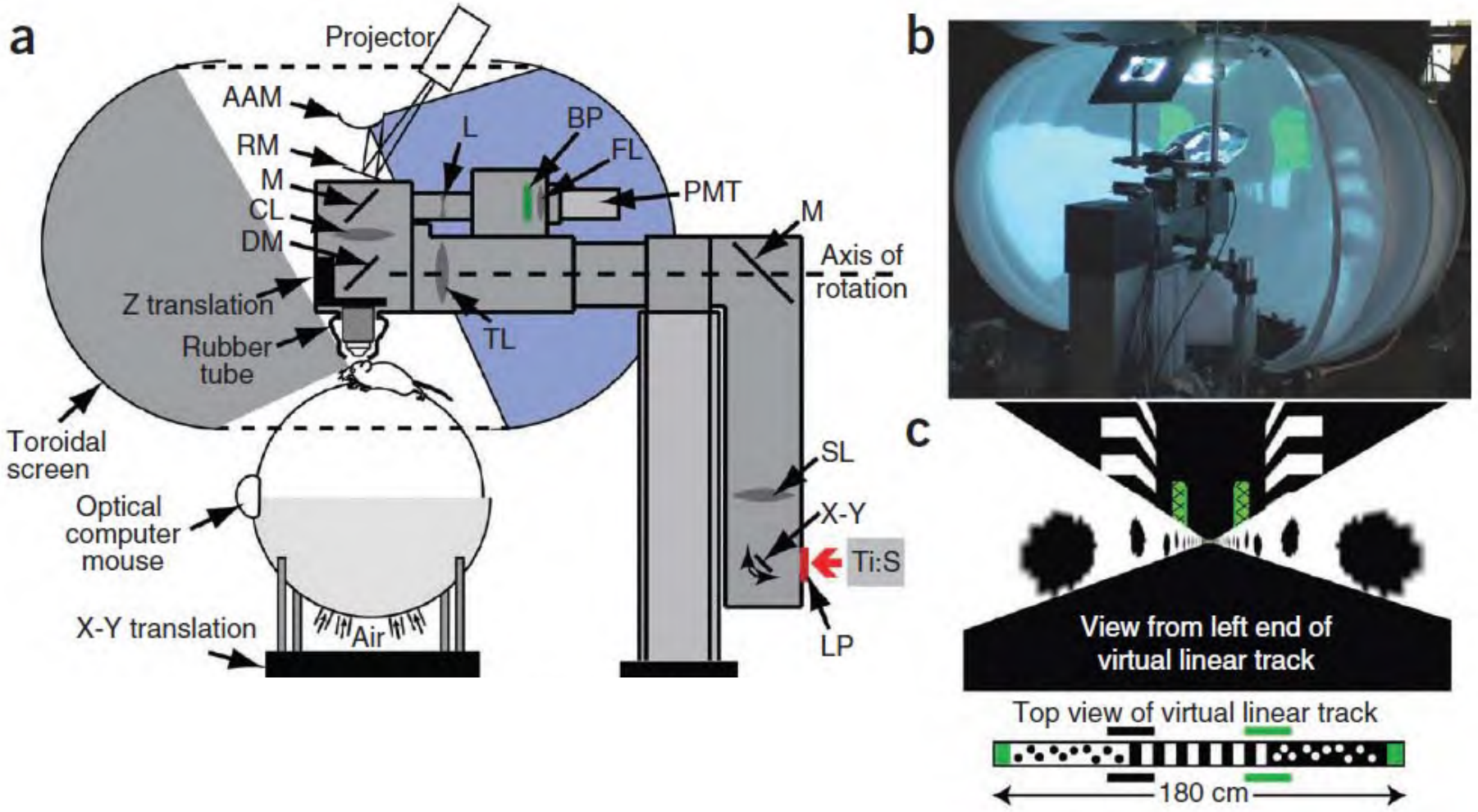




L. Looger, Janelia Farm HHMI

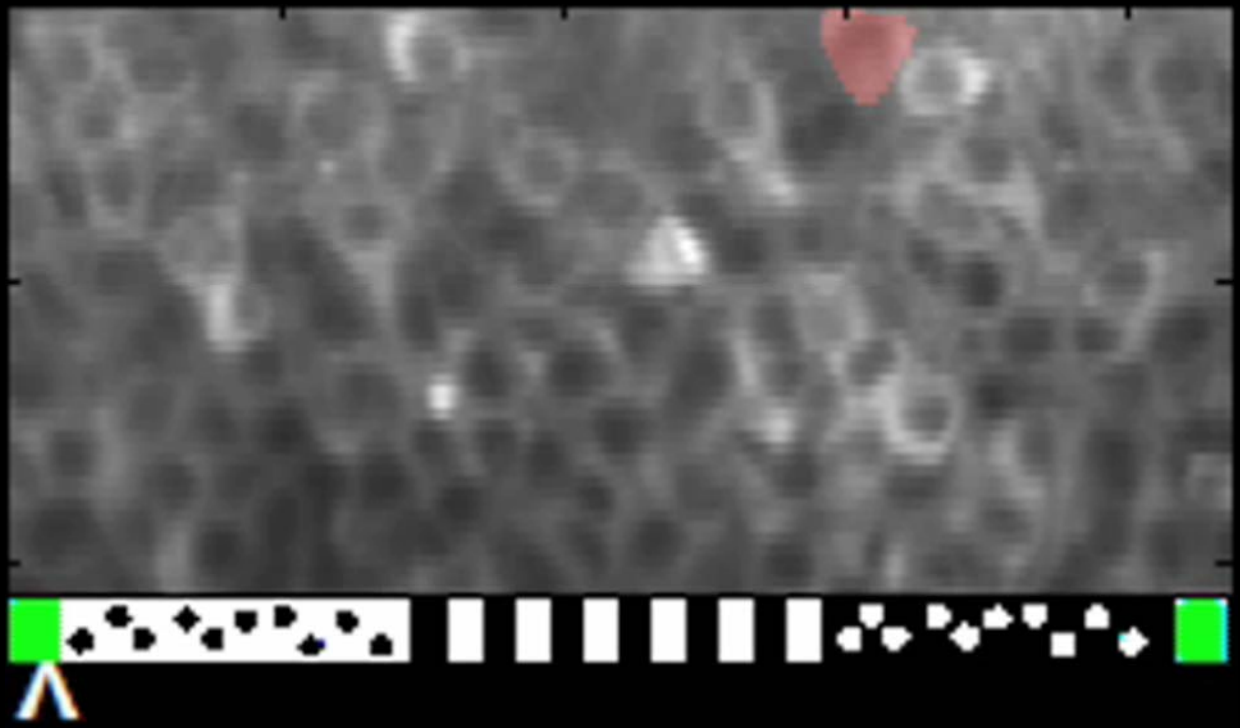
Pablo Garcia Junco Clemente
& Josh Trachtenberg, UCLA

A virtual reality environment



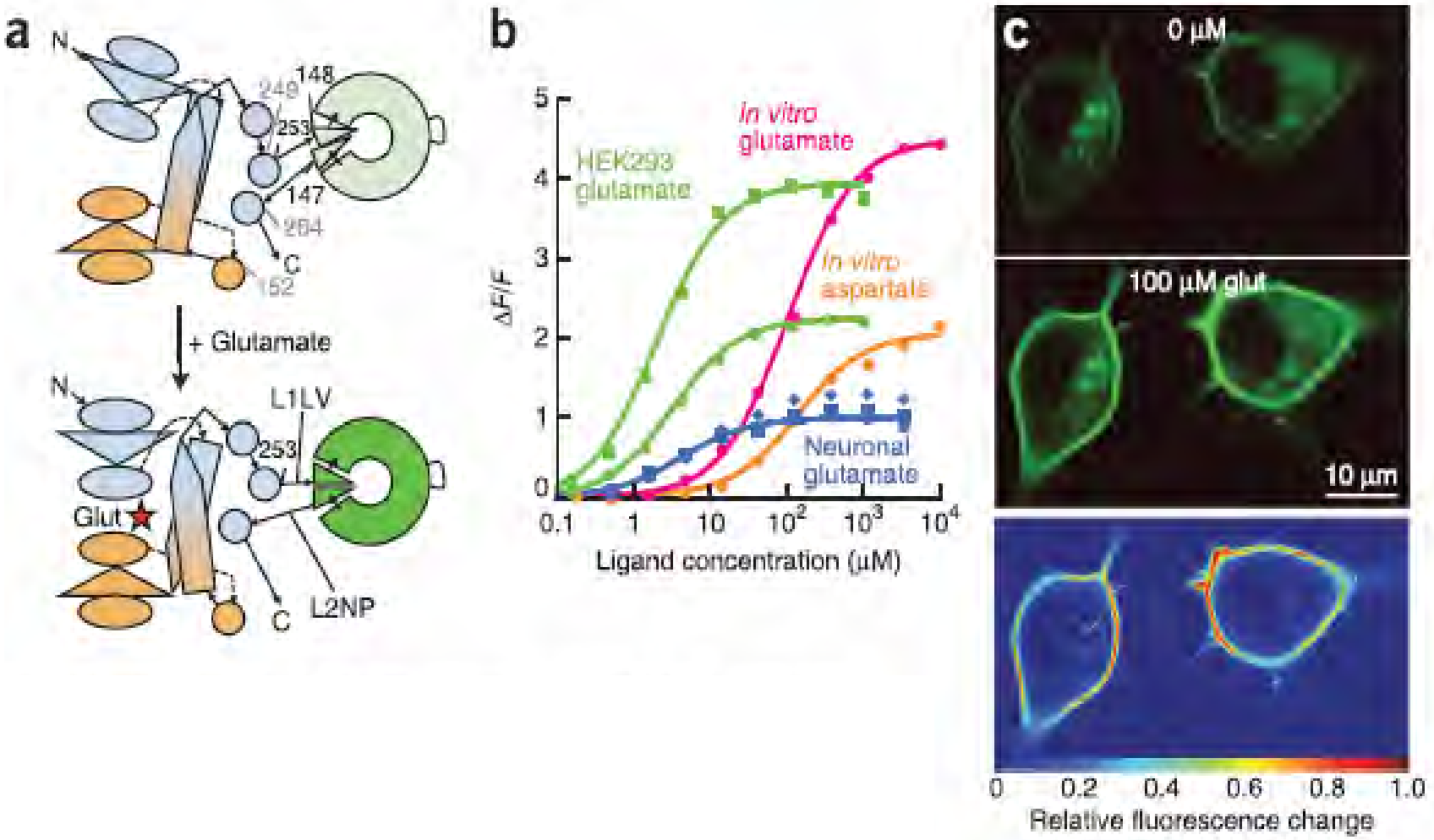
Dombeck et al., *Nat. Neurosci.* 2010

Imaging while the mouse navigates a virtual reality maze



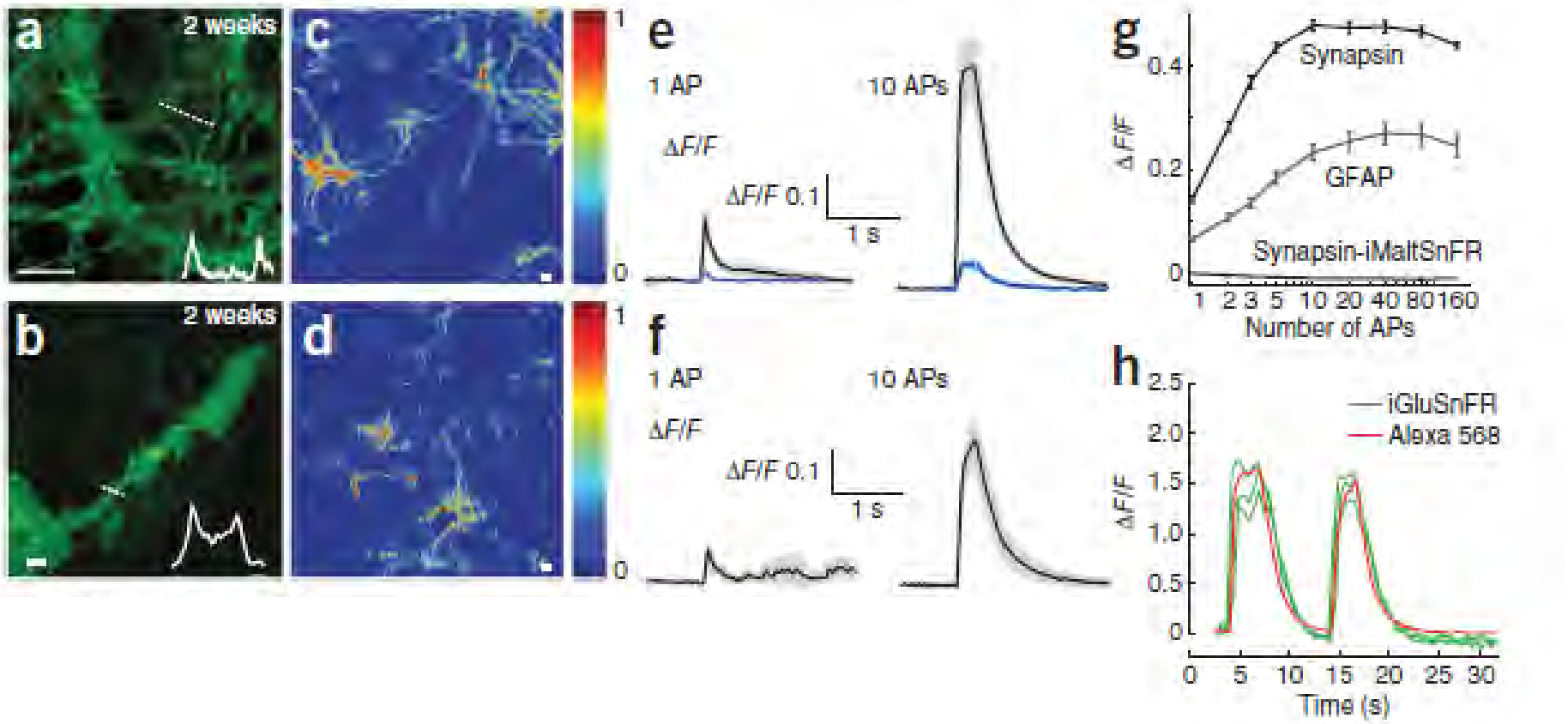
Dombeck et al., *Nature Neuroscience* 13:1433

iGluSnFR, a genetically encoded glutamate sensor



Marvin et al., 2013, *Nature Methods* 10: 162

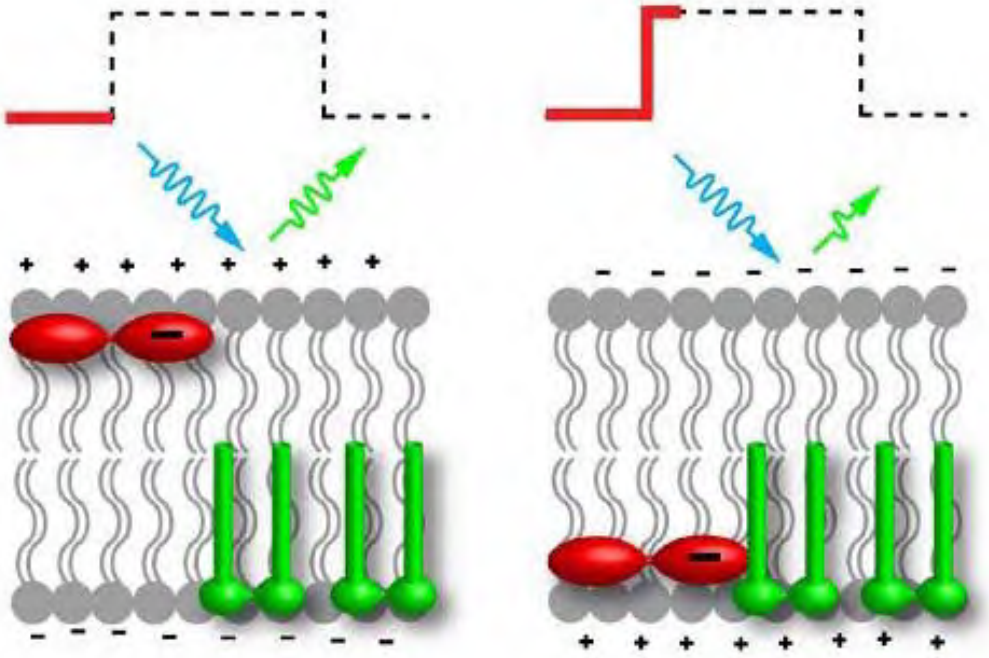
Signals from cultured neurons and astrocytes in response to neural activity

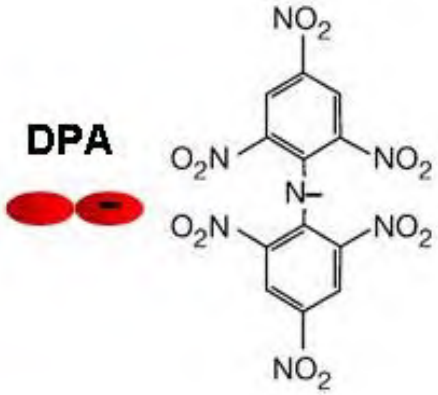


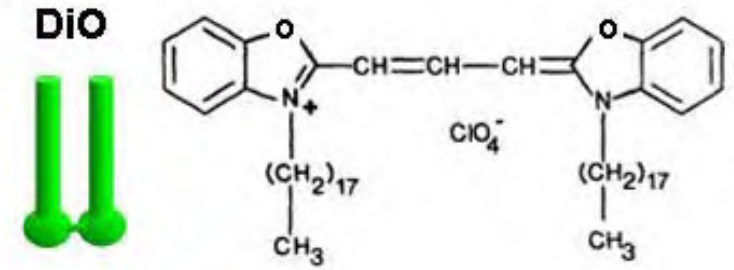
Marvin et al., 2013, *Nature Methods* 10: 162

Optical sensors of voltage

A non-genetic voltage sensor that relies on FRET-based quenching

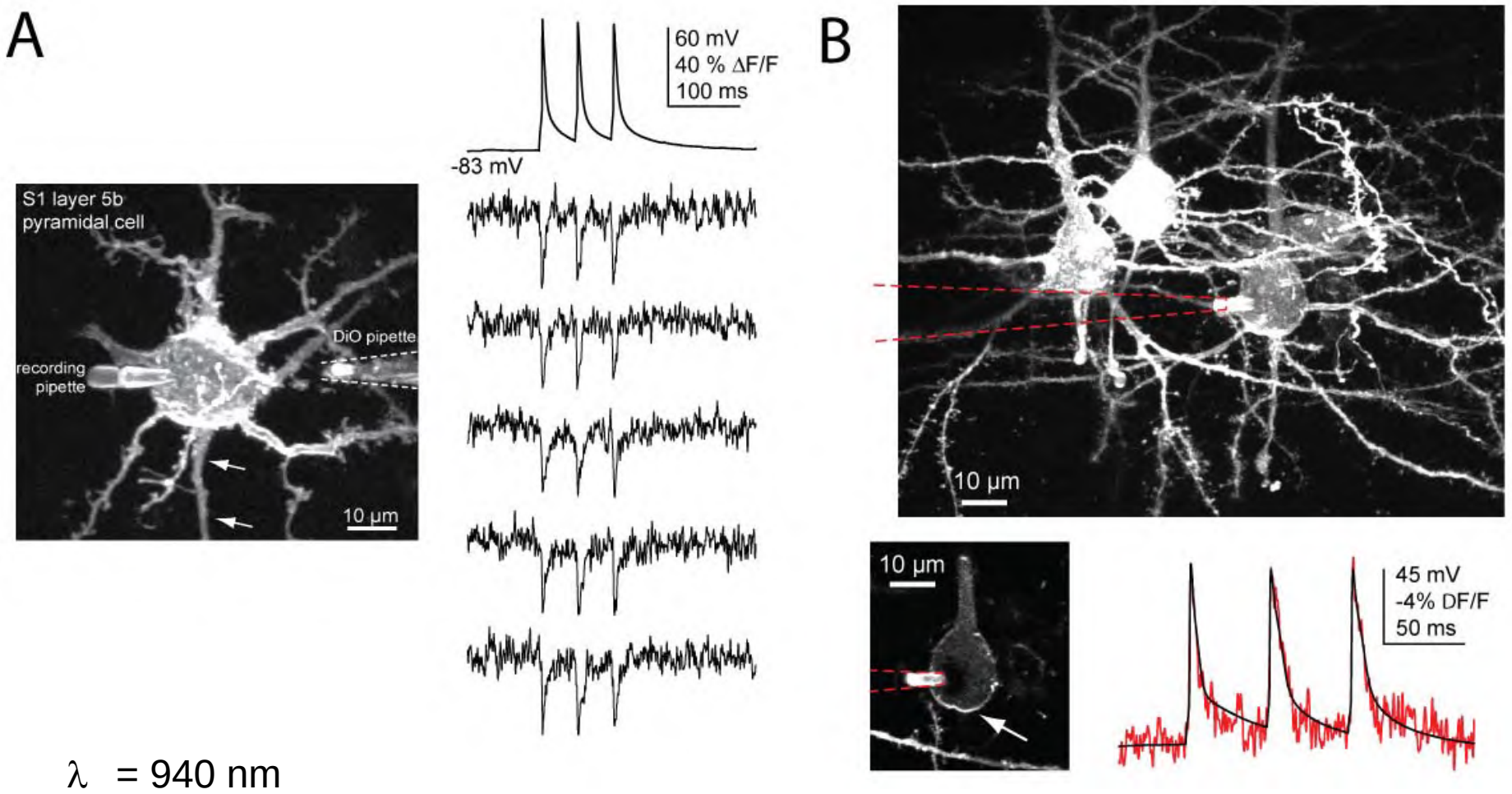






Bradley et al., *J. Neurosci.*, 2009

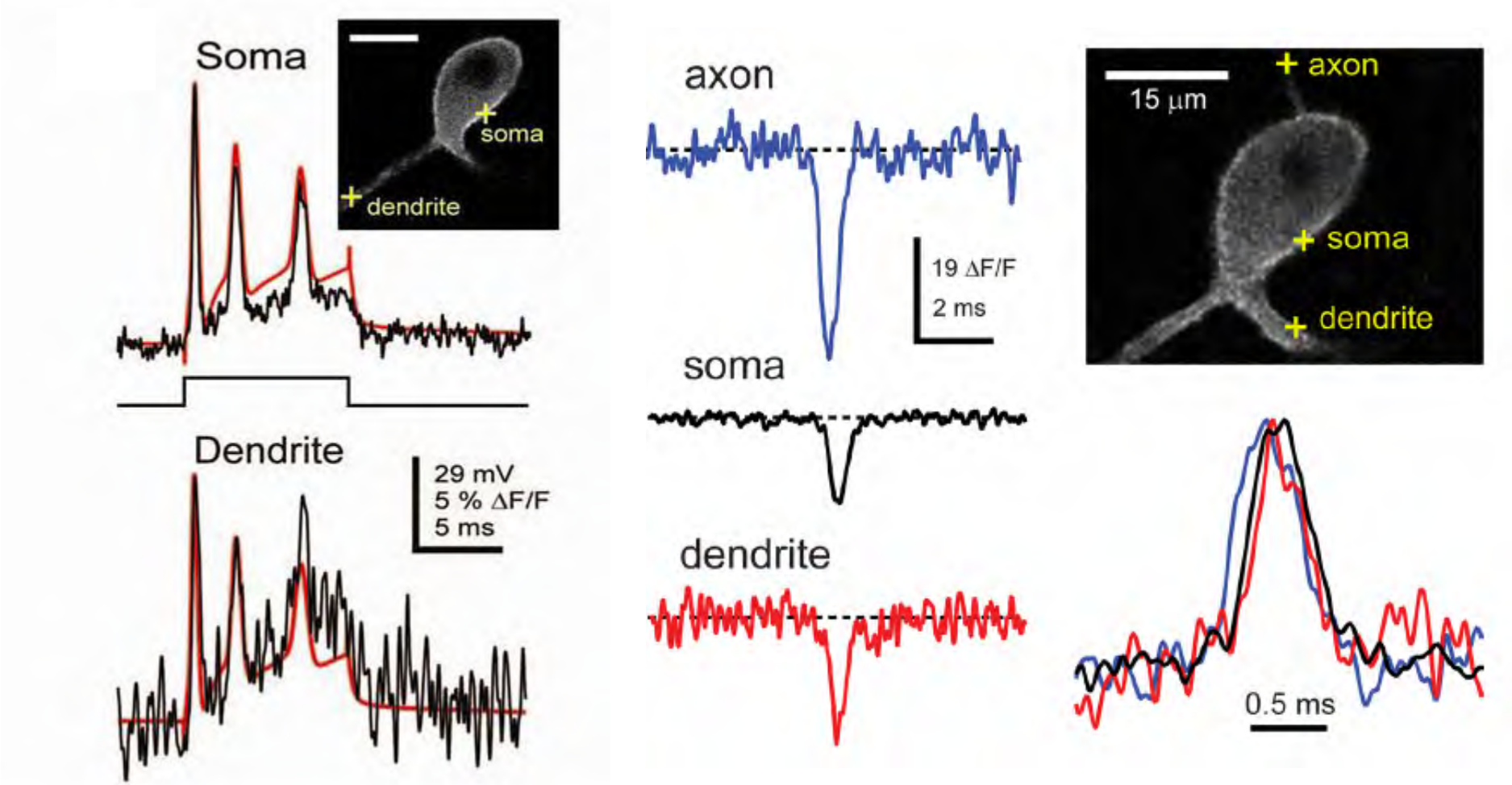
Two photon compatibility, high SNR



λ = 940 nm
3 µM DPA

Fink et al., PLOS One, 2012

Laser spot photometry from different regions of the same neuron



Bradley et al., *J. Neurosci.*, 2009

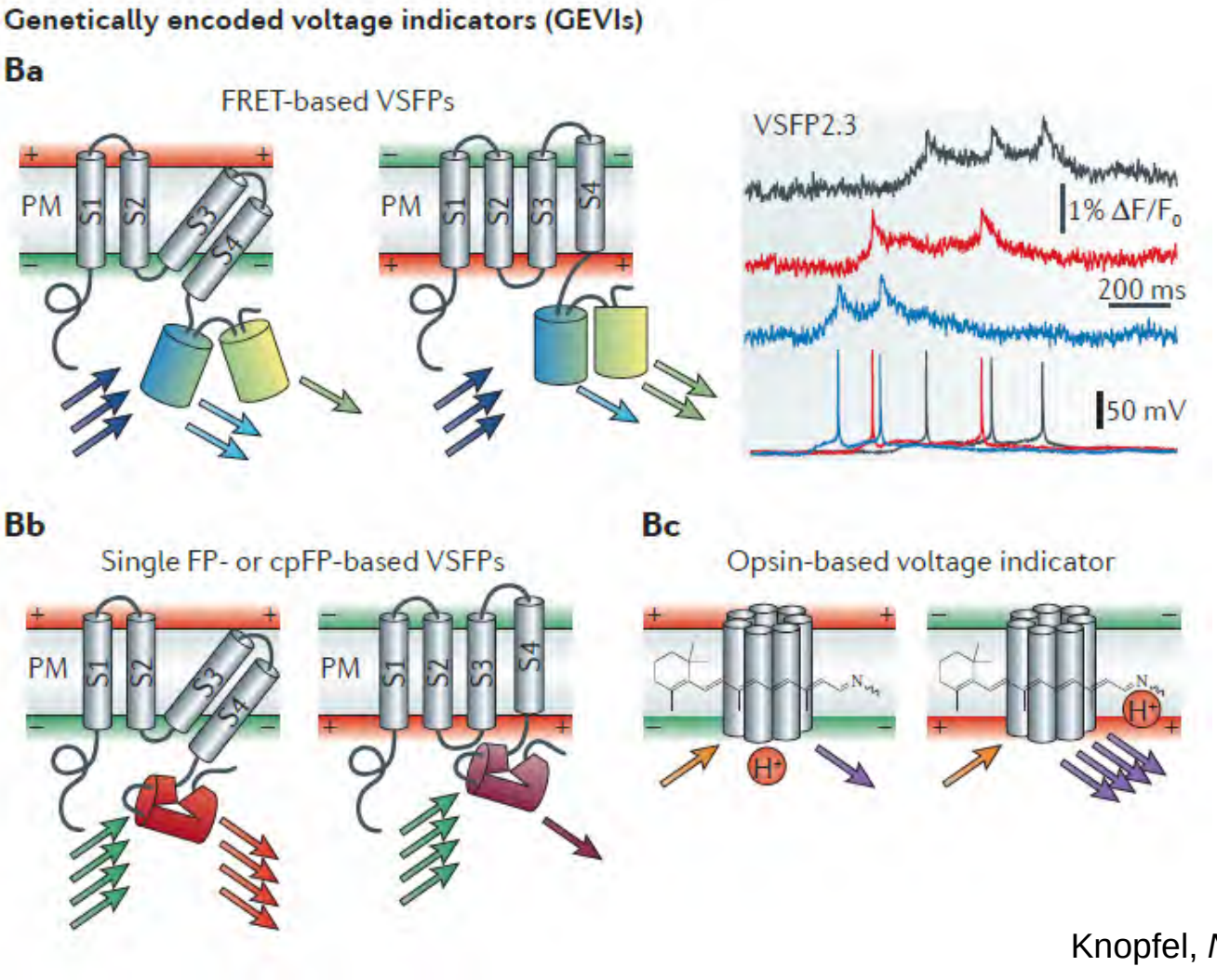
A comparison of genetic and non-genetic optical voltage sensors

Molecule	Approx ΔF/F per 100 mV	Approx response time	Comments
VSFP 2.3 [1]	9.5%	78 ms	Ratiometric (ΔR/R)
VSFP 2.4 [1]	8.9%	72 ms	Ratiometric (ΔR/R)
VSFP 3.1 [2]	3%	1-20 ms	Protein
Mermaid [3]	9.2%	76	Ratiometric (ΔR/R)
SPARC [4]	0.5%	0.8 ms	Protein
FlaSh [5]	5.1%	2.8 – 85 ms	Protein
Flare [6]	0.5%	10 – 100 ms	Protein
PROPS [7]	150%	5 ms	Protein
di-4-ANEPPS [8]	8%	< 1 ms	Dye
di-8-ANEPPS [9]	10%	< 1 ms	Dye
RH237 [10]	11%	< 1 ms	Dye
RH421 [11]	21%	< 1 ms	Dye
ANNINE-6plus [12]	30%	< 1 ms	Dye
hVOS [13]	34%	< 1 ms	hybrid
DiO/DPA [14]	56%	< 1 ms	hybrid

Supplementary Table 1 Approximate characteristics of fluorescent voltage indicating proteins. In some cases numbers were estimated from published plots. The table contains representative members of all families of fluorescent indicators but omits many.

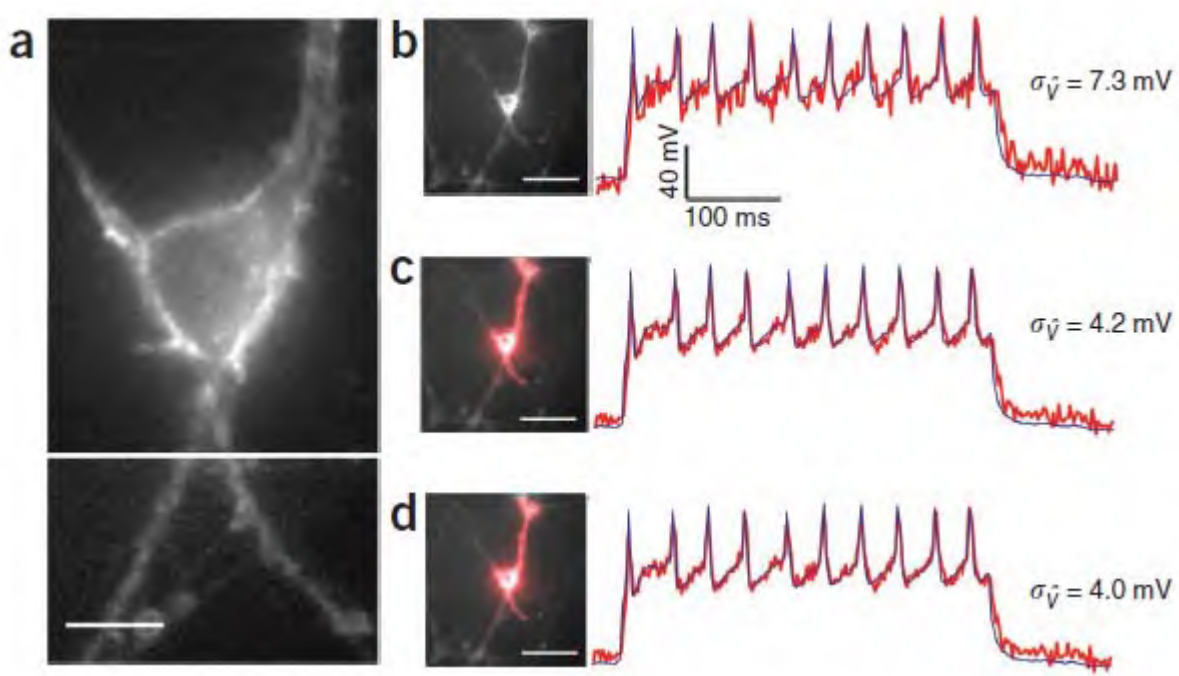
from Supplementary Material Kralj et al., *Nat. Methods*, 2011

Genetically encoded voltage sensing strategies



Knopfel, *Nat. Rev. Neurosci.*, 2012

Arch, the best genetically encoded voltage indicator (so far)

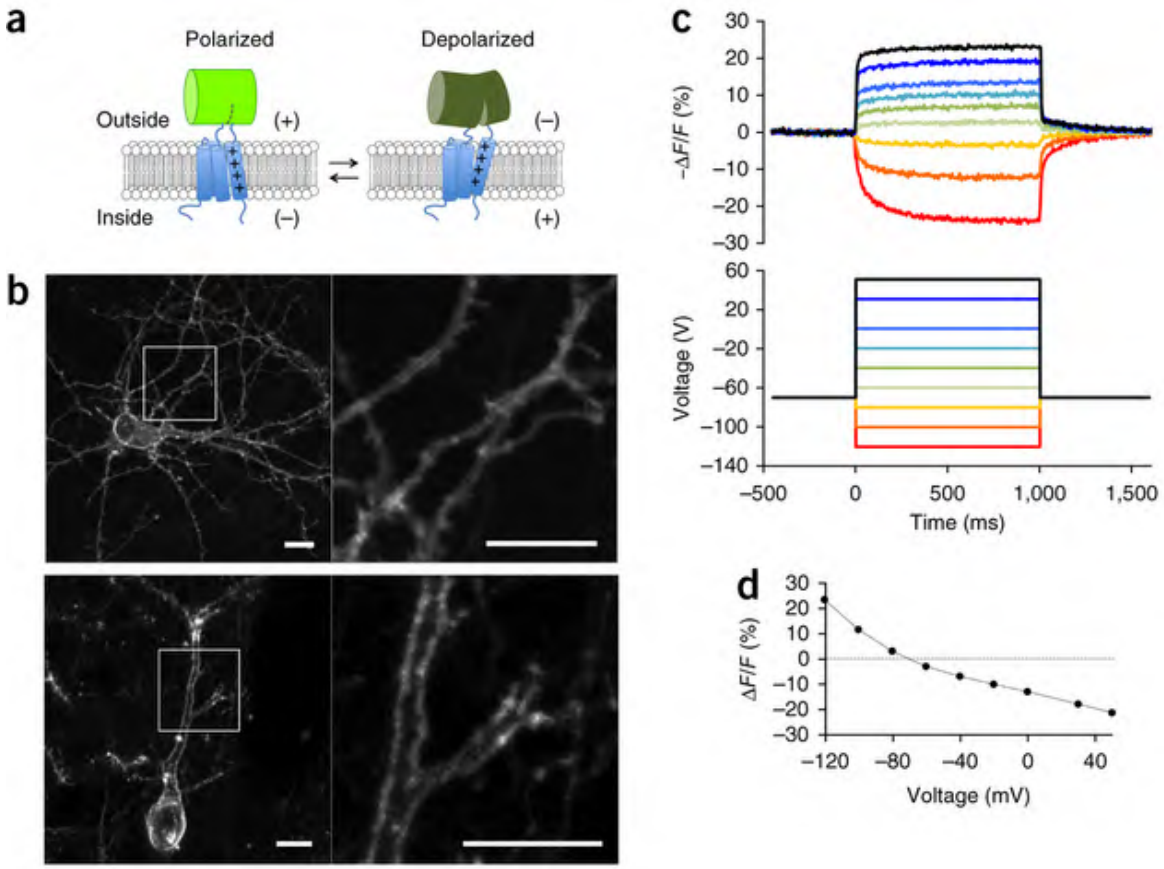


Kralj et al.,
Nat. Methods, 2011

Table 1 | Optical and electrical response of Arch and Arch(D95N)

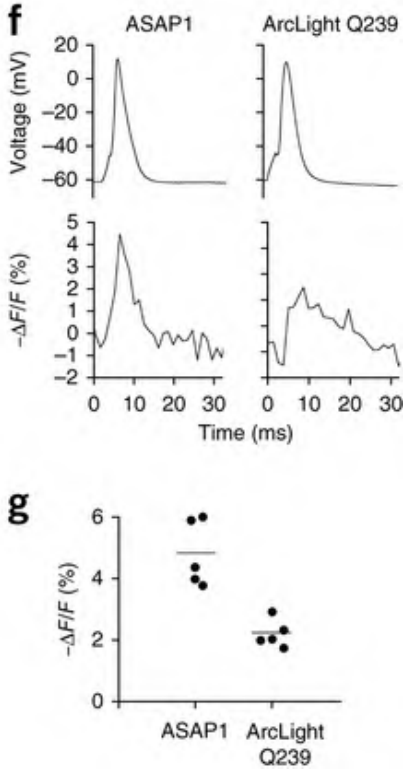
	λ_{max} absorbance (nm)	λ_{max} emission (nm)[a]	ε_{633} (M^{-1} cm^{-1})[b]	QY[c]	Photostability relative to eGFP[d]	pK_a of Schiff base[e]	$\tau_{response}$ (ms)[f]	Noise in $\hat{V}_{FL}$ (μV $Hz^{-0.5}$)[g]	Photo-current
Arch	558	687	6,300	9×10^{-4}	0.25	10.1	<0.5	625	Yes
Arch(D95N)	585	687	37,500	4×10^{-4}	0.1	8.9	41	260	No

The newest FRET-based VSP



e

		ASAP1	ArcLight Q239
Activation	τ_1 (ms)	2.1 ± 0.2	14.5 ± 1.7
	τ_2 (ms)	71.5 ± 1.6	122 ± 18
	Percentage fast	60.2 ± 1.2	50.3 ± 3.3
Inactivat.	τ_1 (ms)	2.0 ± 0.1	44.6 ± 7.2
	τ_2 (ms)	50.8 ± 1.2	273 ± 101
	Percentage fast	43.7 ± 0.6	63.7 ± 3.5



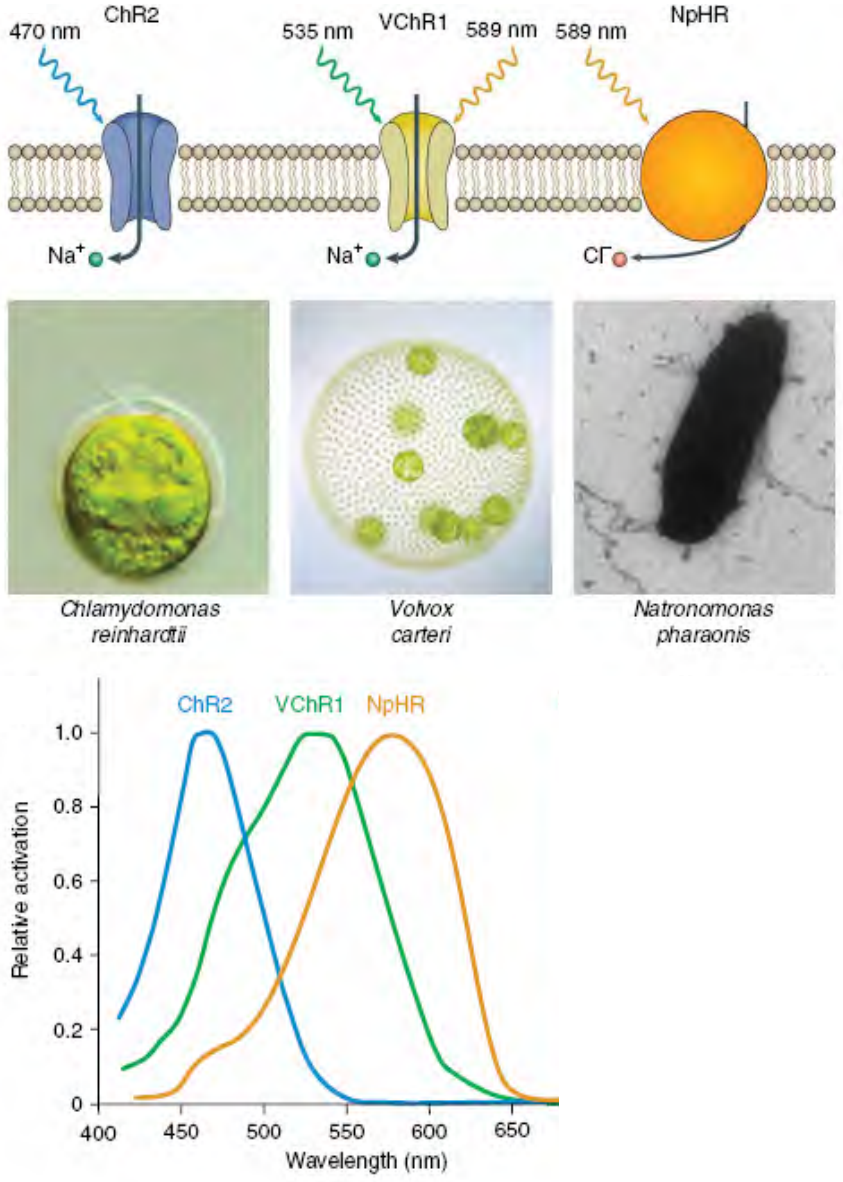
St-Pierre et al., *Nature Neuroscience* 17:884-89 (2014) doi:10.1038/nn.3709

Optogenetic actuators

NEUROSCIENCE

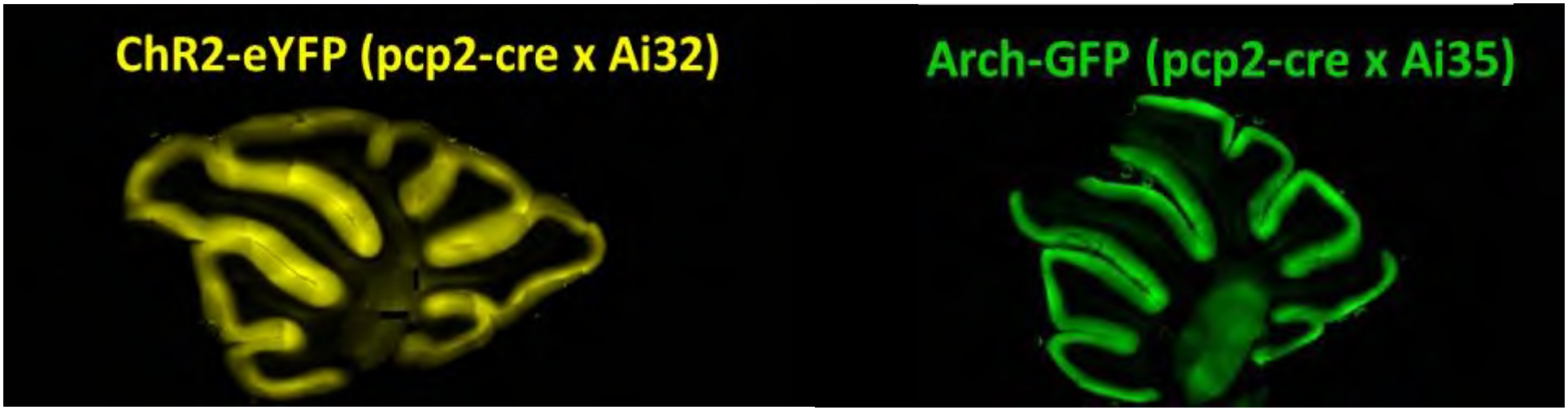
Controlling the Brain with Light

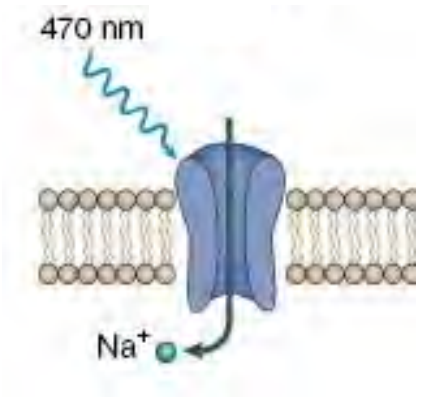
By Karl Deisseroth
Scientific American, Nov. 2010

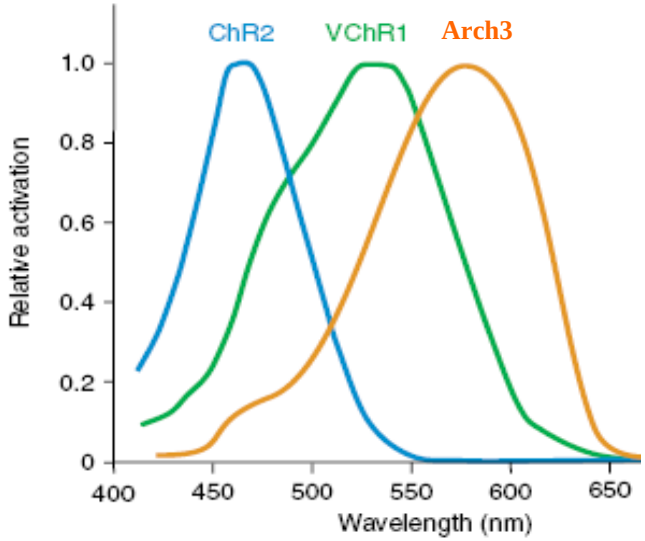


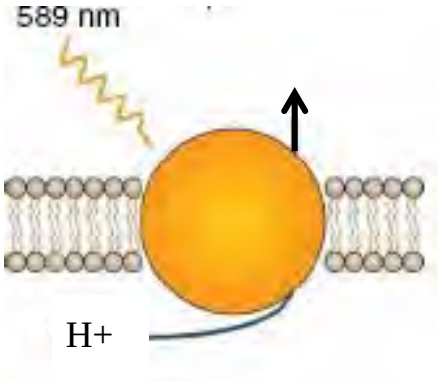
Zhang et al., *Nature Protocols,* 5:449 (2010)

Optogenetic control of Purkinje cell excitability





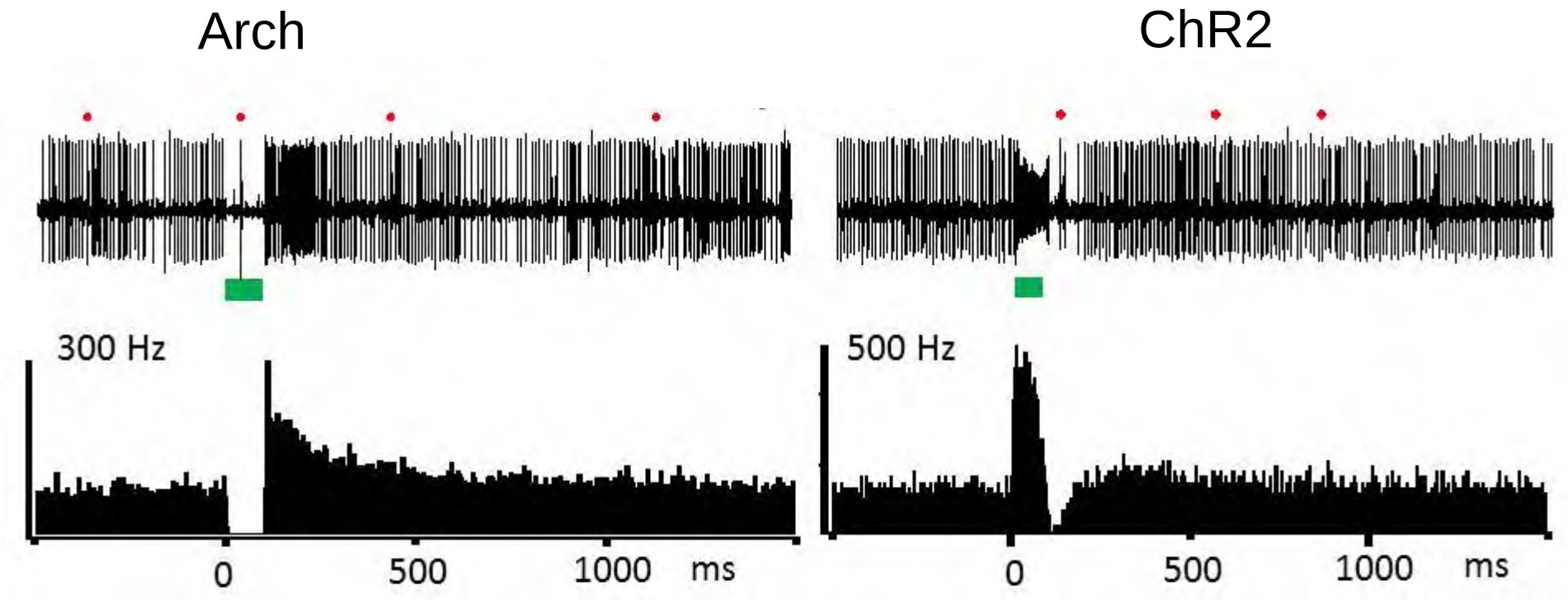




Lee, Mathews, Reeves, Jamil, Serrano, and Otis unpublished

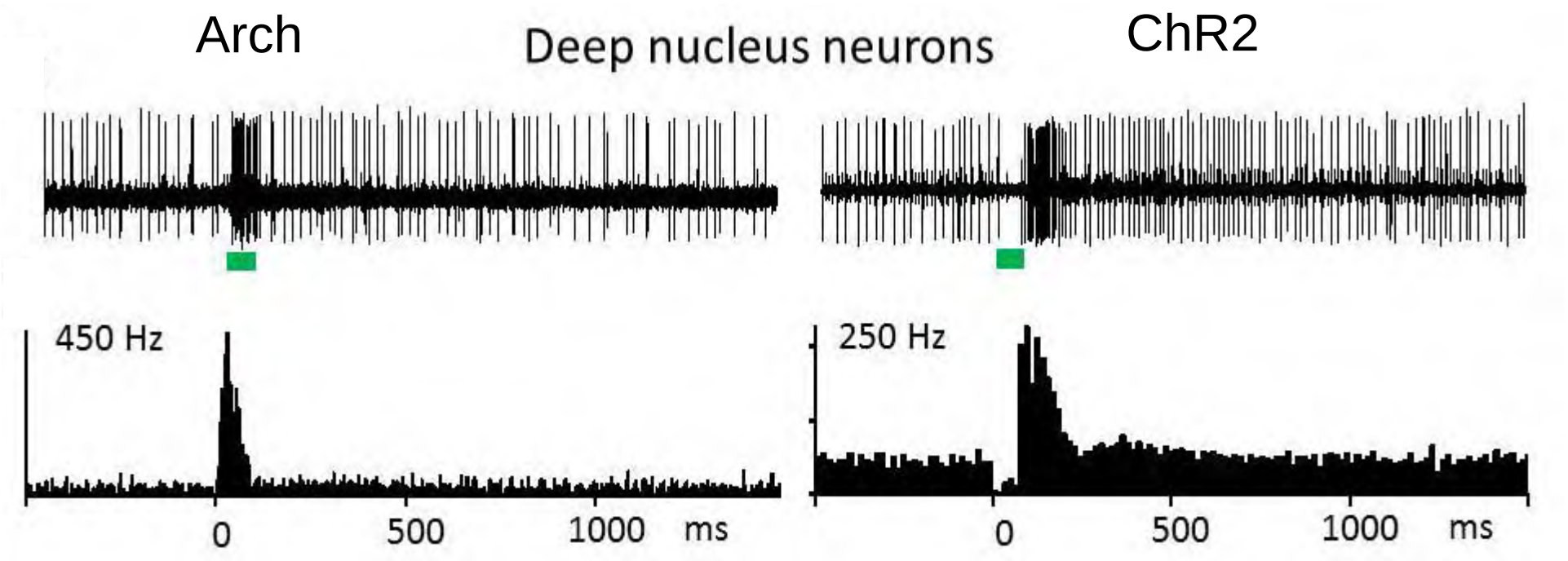
Zhang et al., *Nature Protocols,* 5:449 (2010)

In vivo recordings from PNs in awake mice



Lee, Mathews, Reeves, Jamil, Serrano,
and Otis unpublished

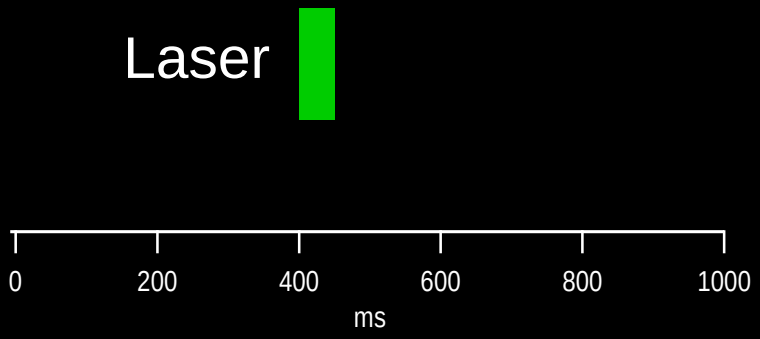
In vivo recordings from deep nucleus neurons

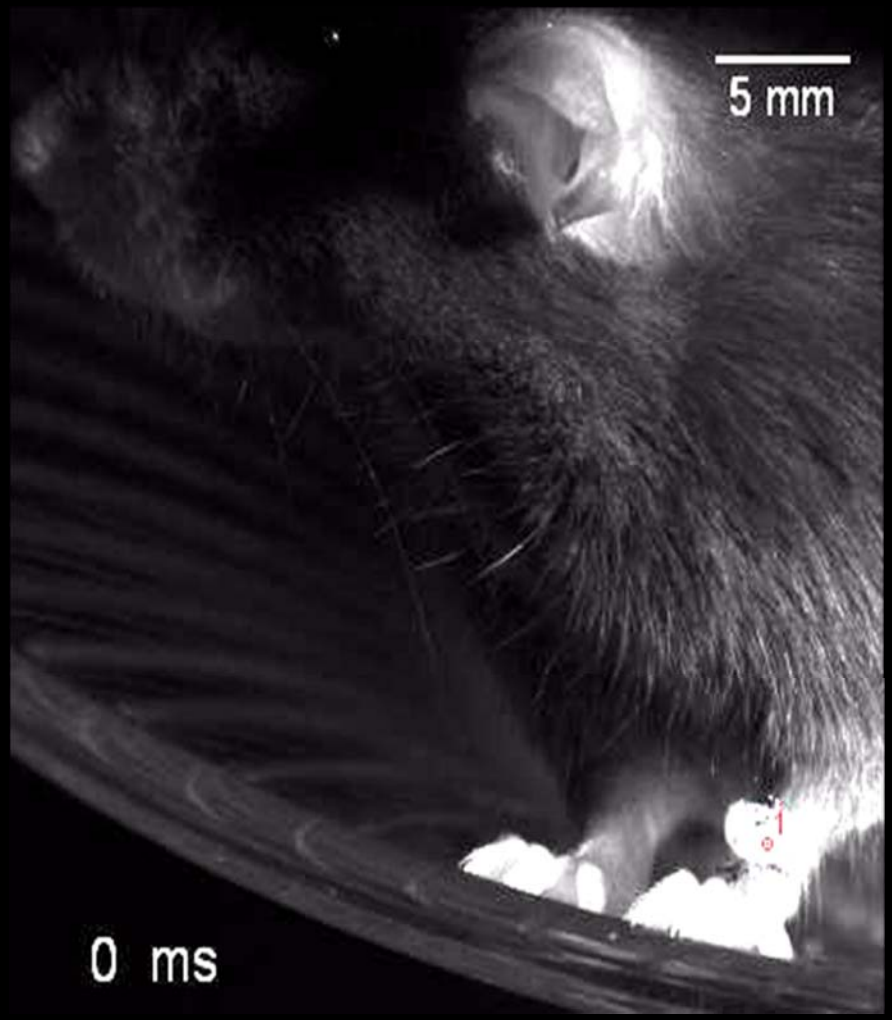


Maximal burst frequency during 100 ms Arch pulse illumination was 291 ± 34 Hz (n=6), representing a 1700 ± 330 % increase above baseline

Lee, Mathews, Reeves, Jamil, Serrano, and Otis unpublished

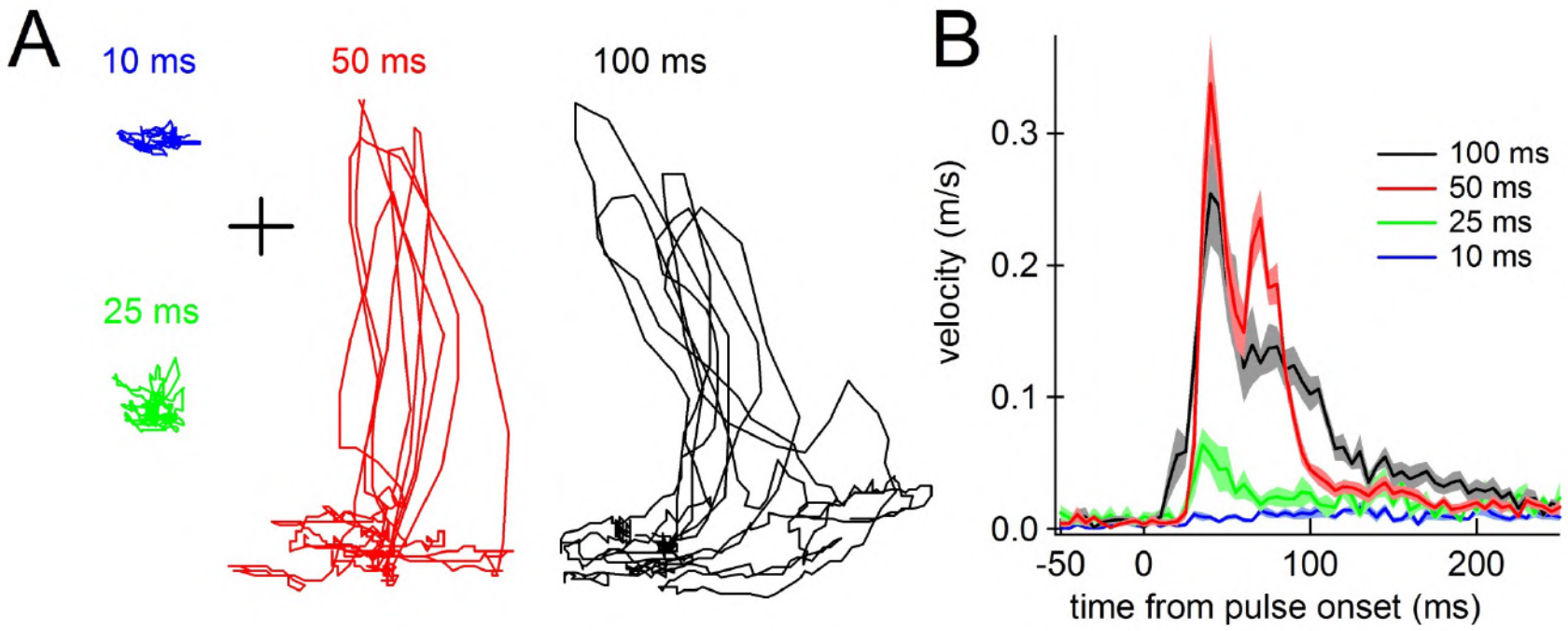
Movement trigged by inhibiting PNs





Lee, Mathews, Reeves, Choe, Jamil, Serrano, and Otis unpublished

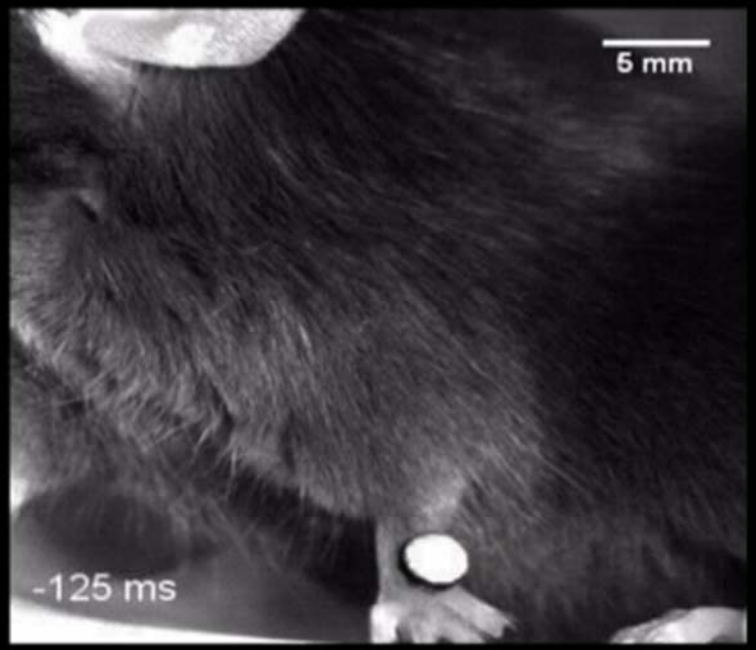
Kinematics of Arch-induced movement as a function of pulse duration



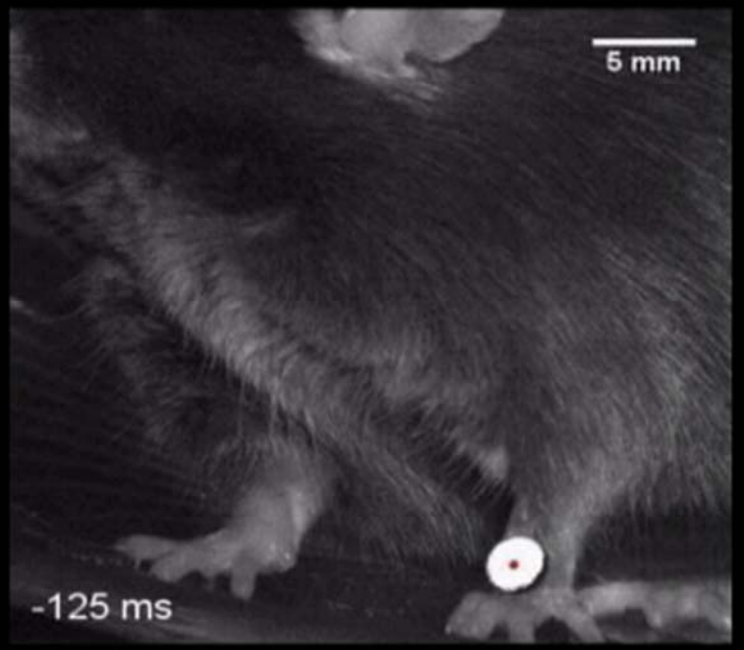
Lee, Mathews, Reeves, Jamil, Serrano, and Otis unpublished

Delayed movement trigged by exciting PNs

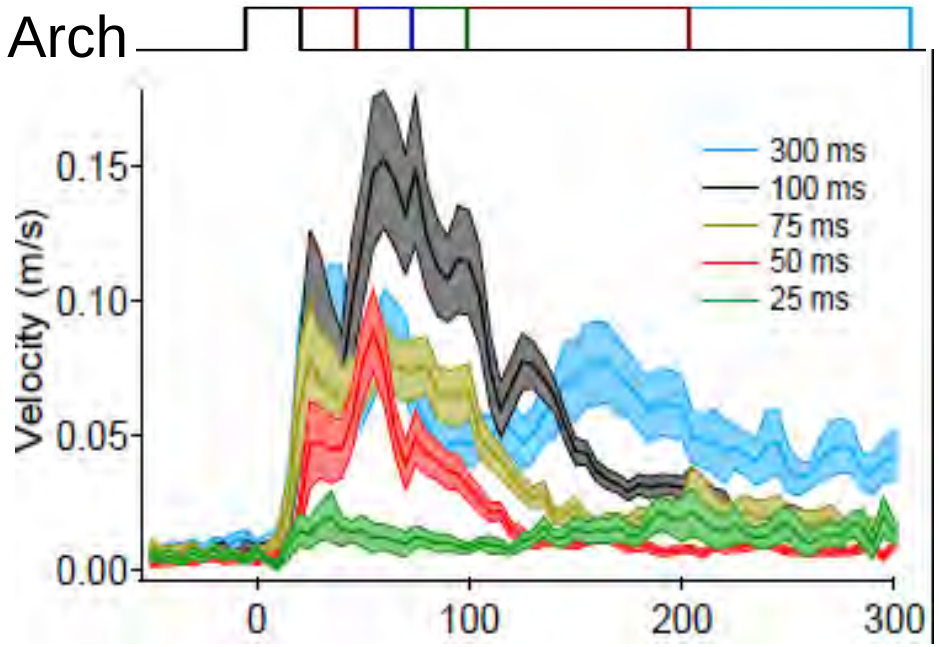
ChR2



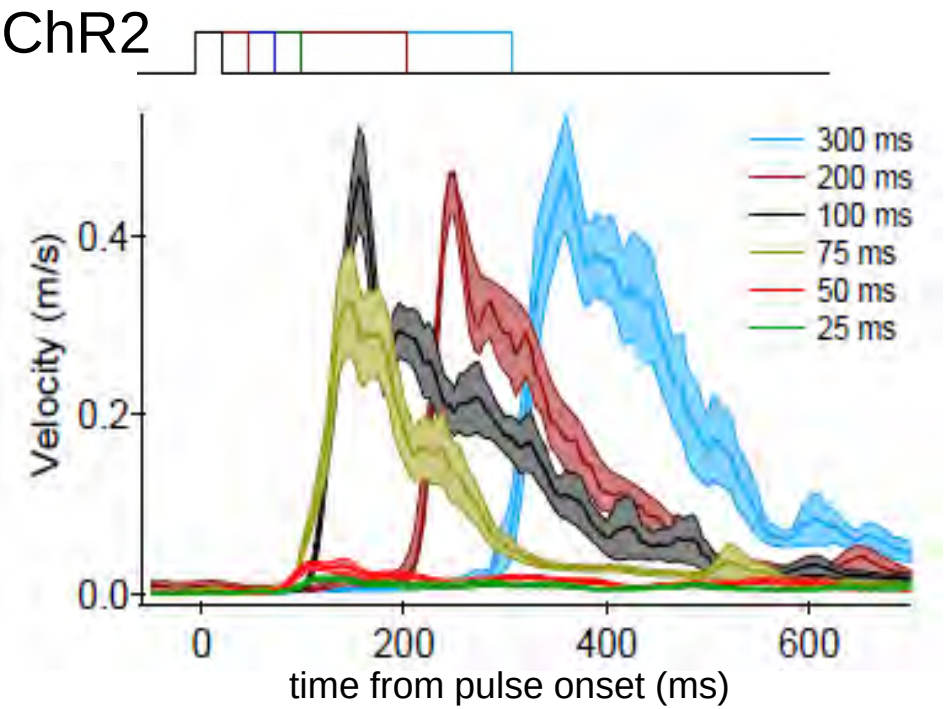
Arch



Lee, Mathews, Reeves, Choe, Jamil, Serrano,
and Otis unpublished



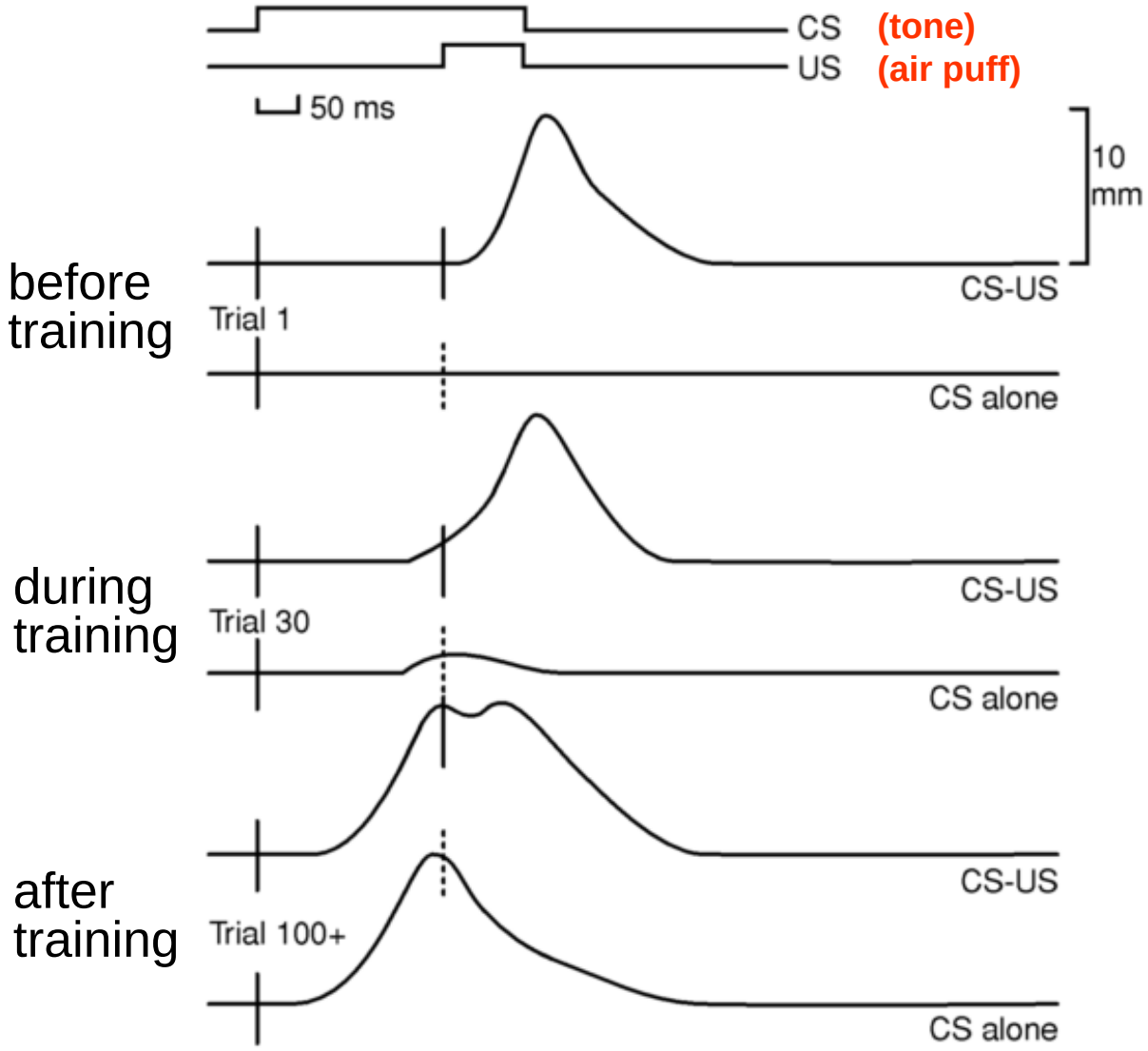
Inhibiting PNs causes movement during laser pulse



Exciting PNs causes movement following a short delay at end of laser pulse

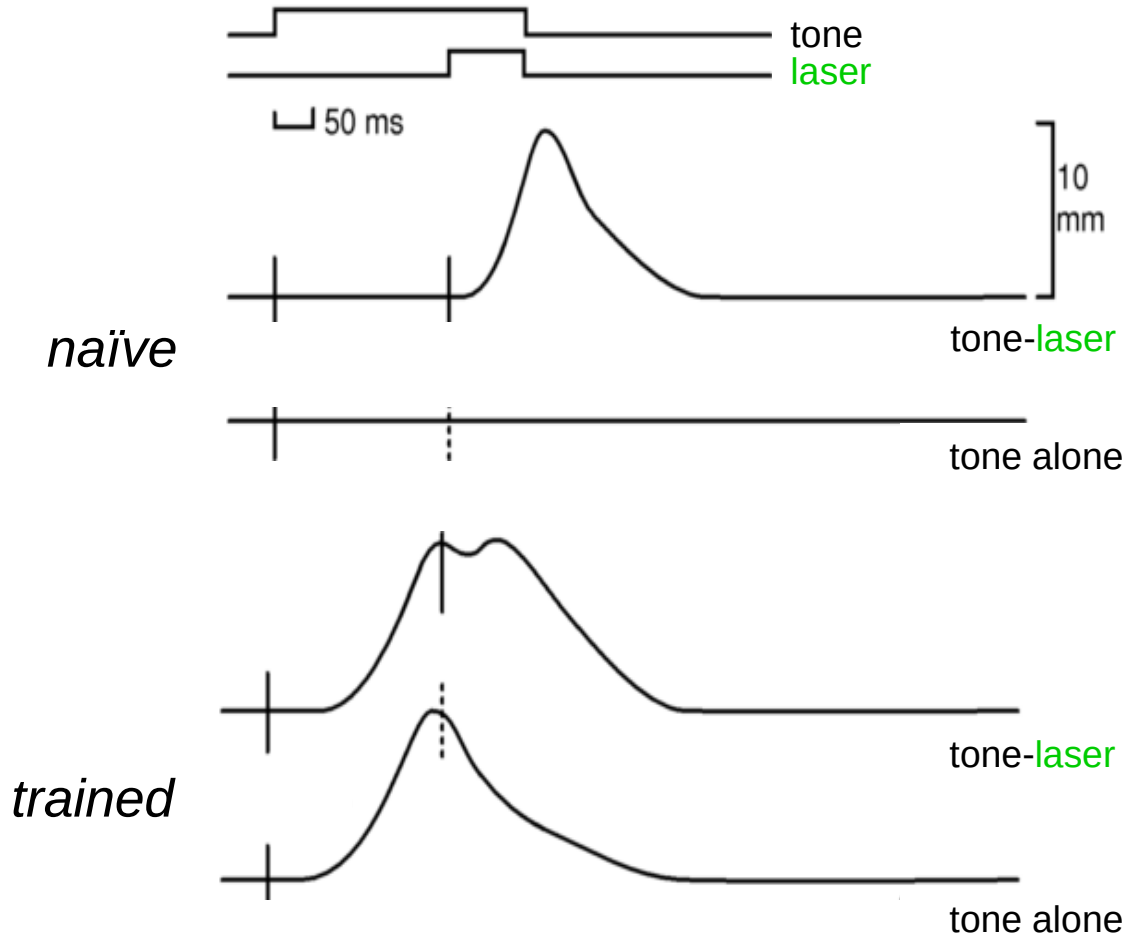
Motor learning is *associative* and *predictive*

eye blink conditioning



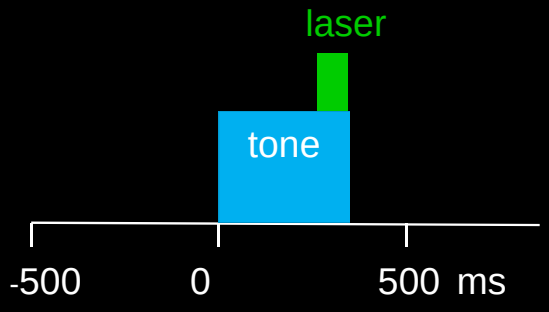
Zigmond et al., 1999

An associative conditioning experiment to test whether pauses are instructive

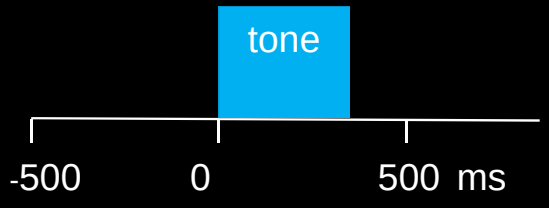


Pairing PC excitation with a tone leads to robust learned movements

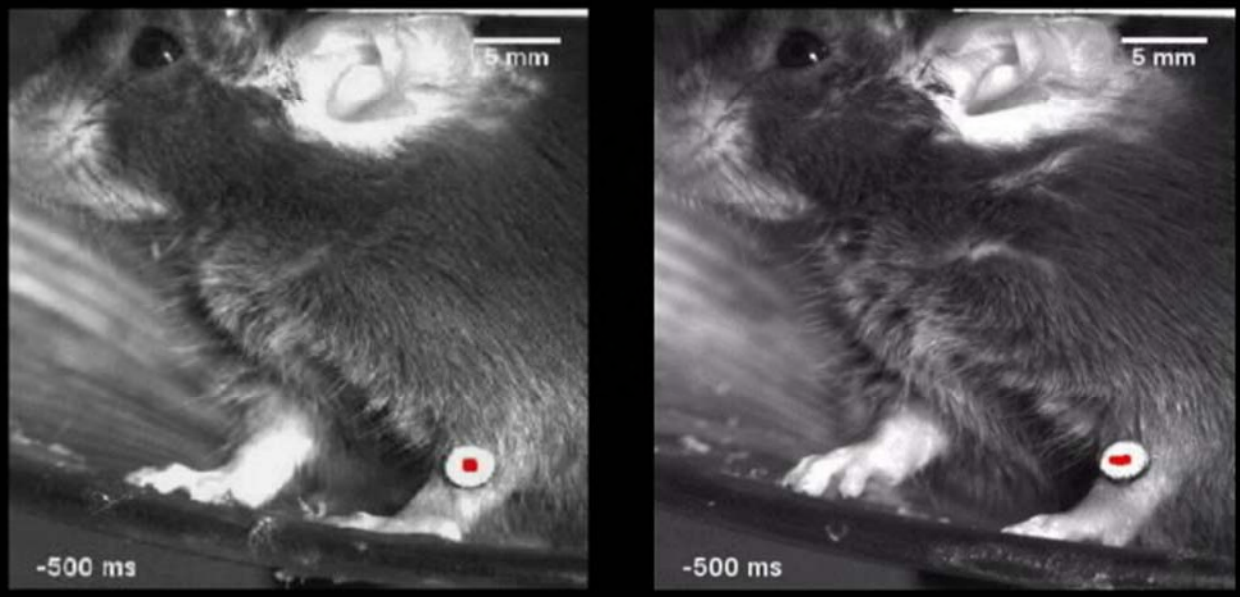
Training: 90 trials/day



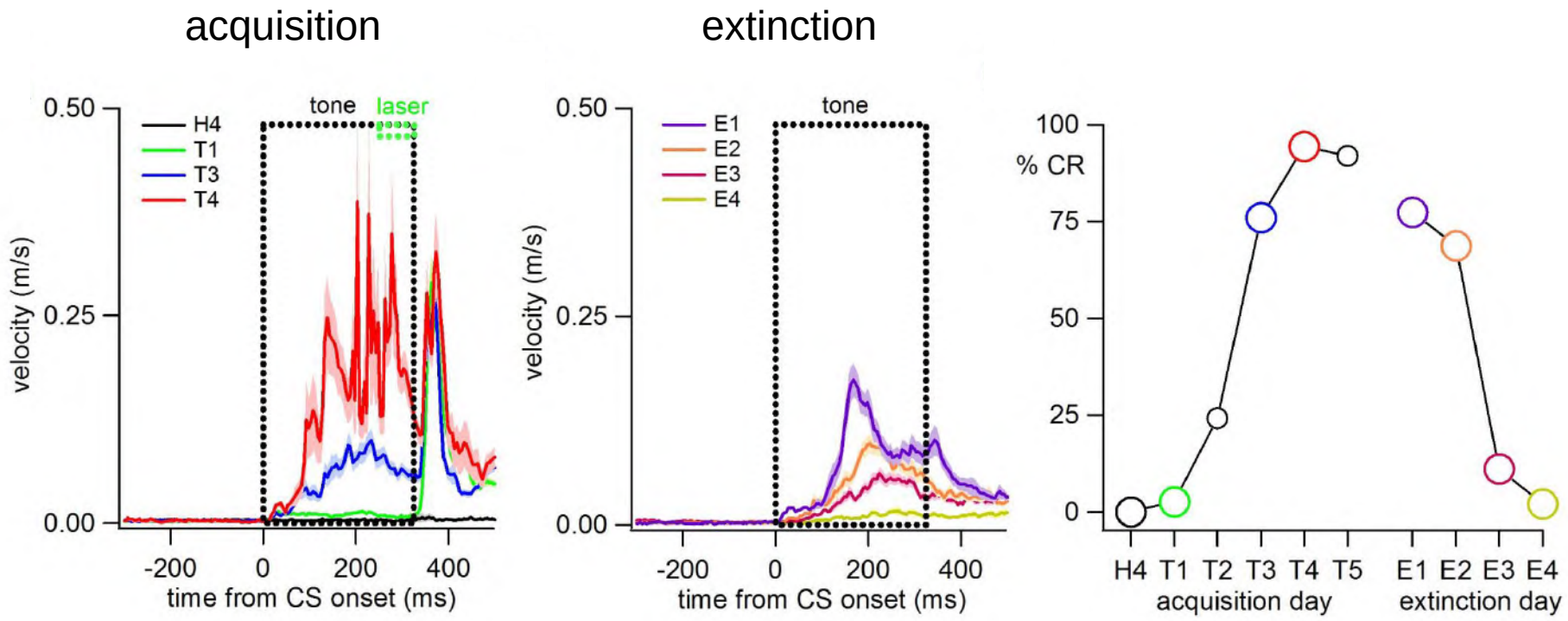
Testing:



ChR2-induced learning



ChR2 excitation of PNs drives associative learning

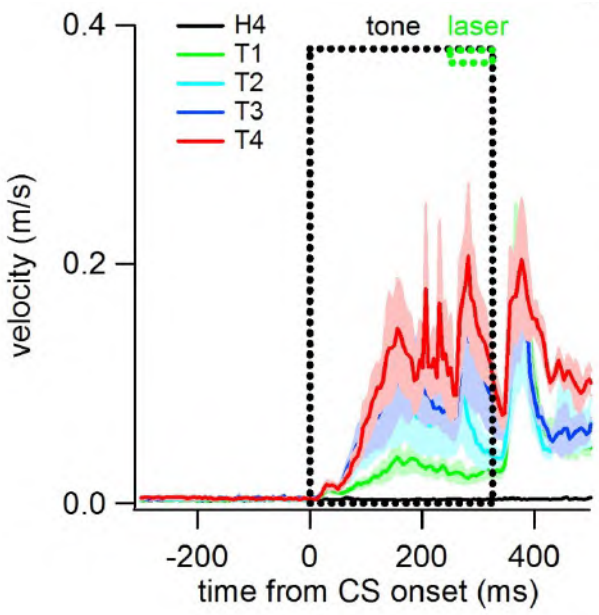


Lee, Mathews, Reeves, Choe, Jami, Serrano, and Otis unpublished

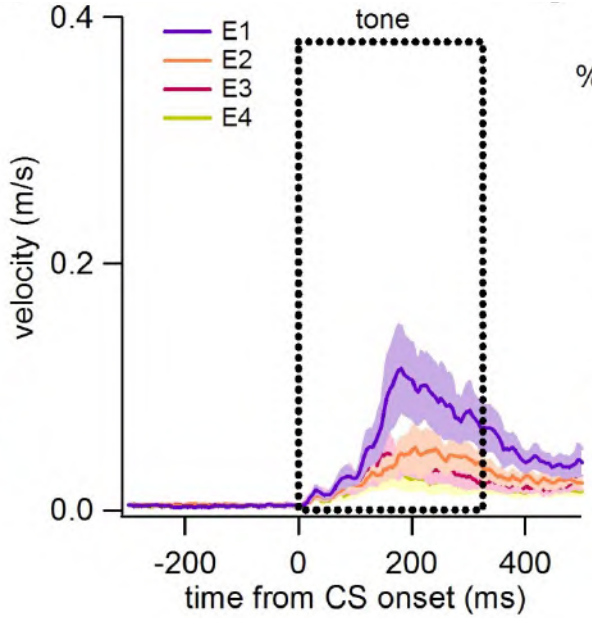
ChR2 excitation of PNs drives associative learning

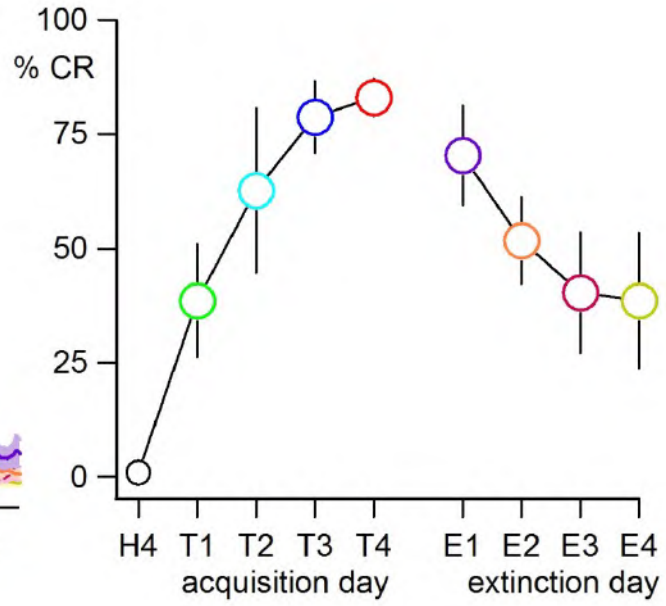
group average, n=4 mice

acquisition



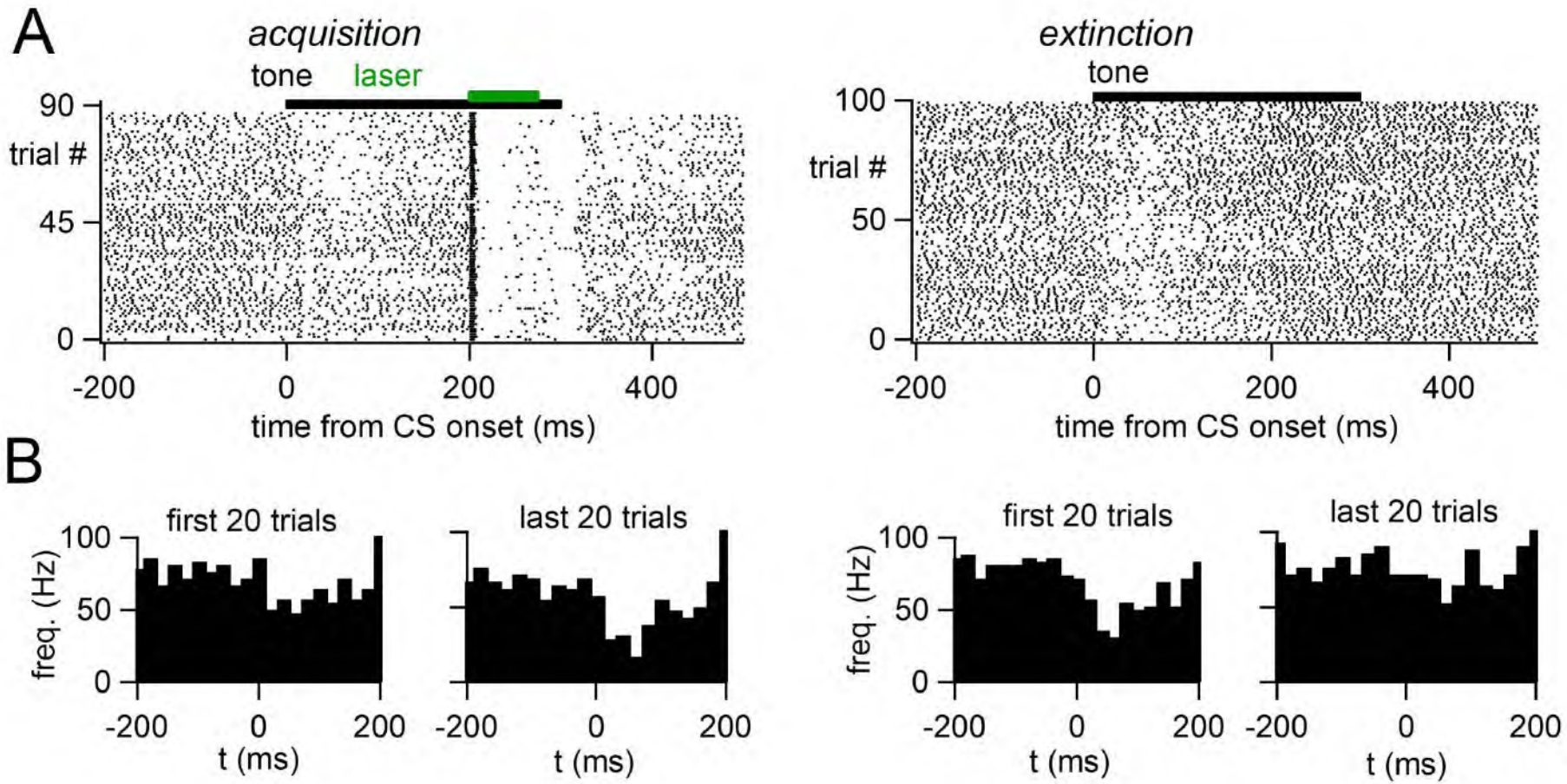
extinction





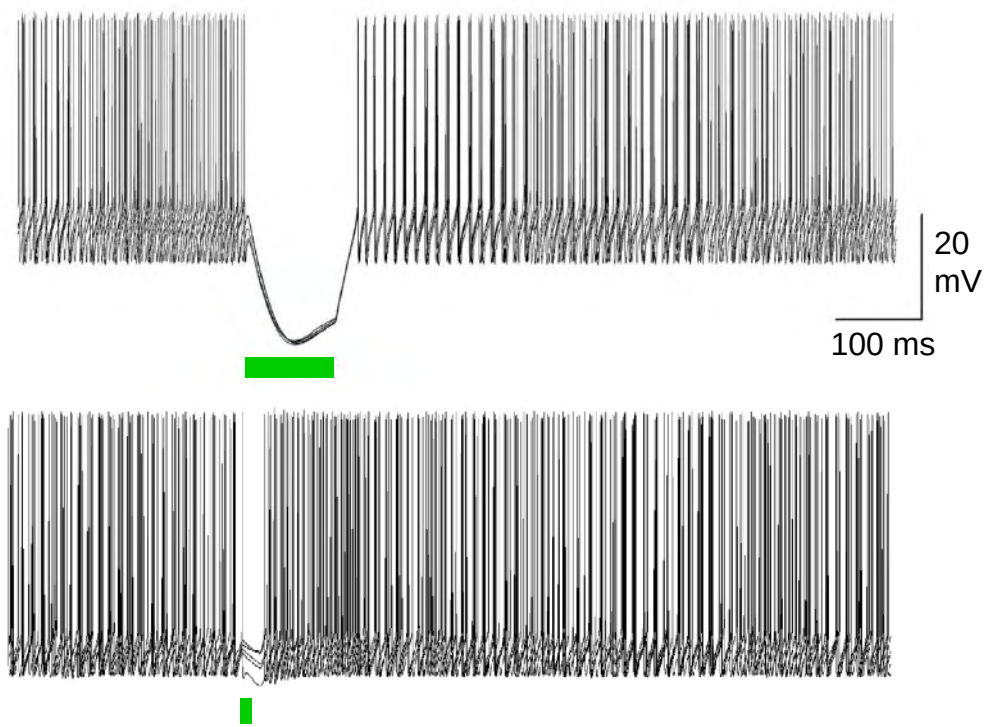
Lee, Mathews, Reeves, Choe, Jami, Serrano, and Otis unpublished

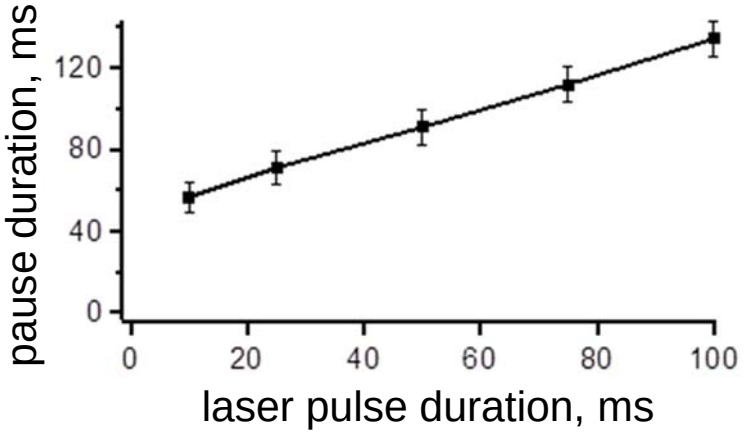
Single PNs can be trained with ChR2



Lee, Mathews, Reeves, Jamil, Serrano, and Otis unpublished

Arch activation silences PNs

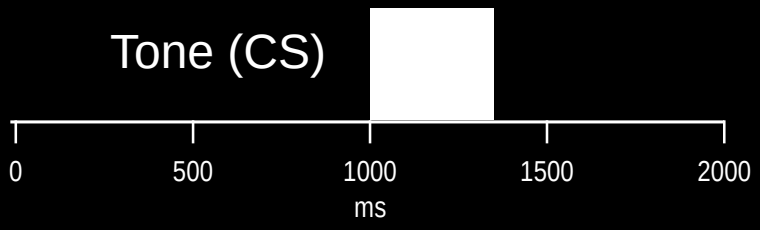




Lee, Mathews & Otis, unpublished

Tone alone, same mouse

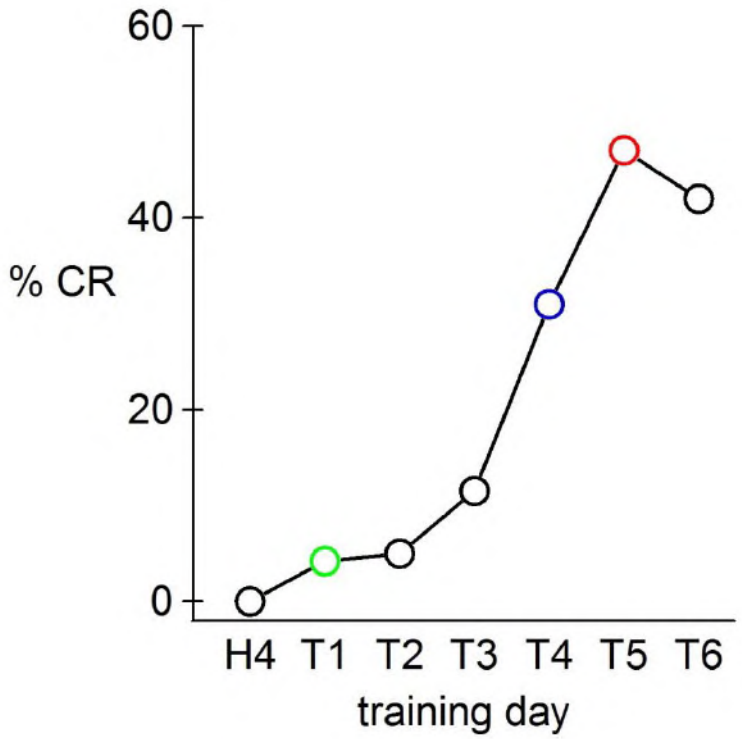
no US
CS: 1,000 – 1,350 ms

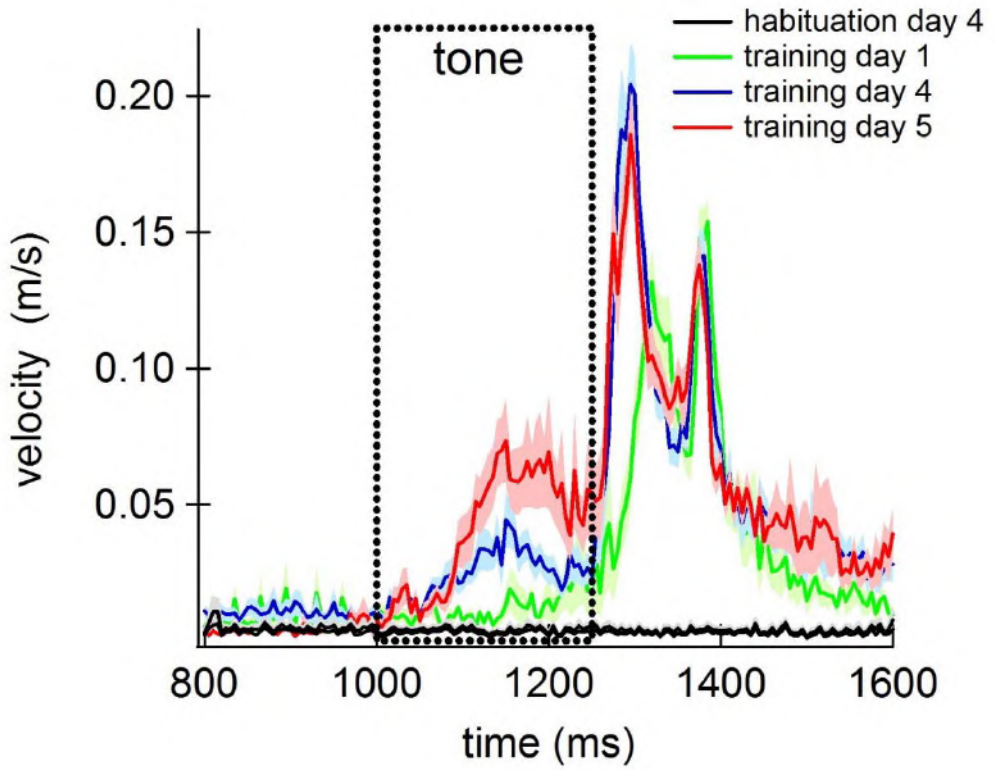


mouse 2, training day 4, trial 20, 9/14/12

Lee, Mathews and Otis unpublished

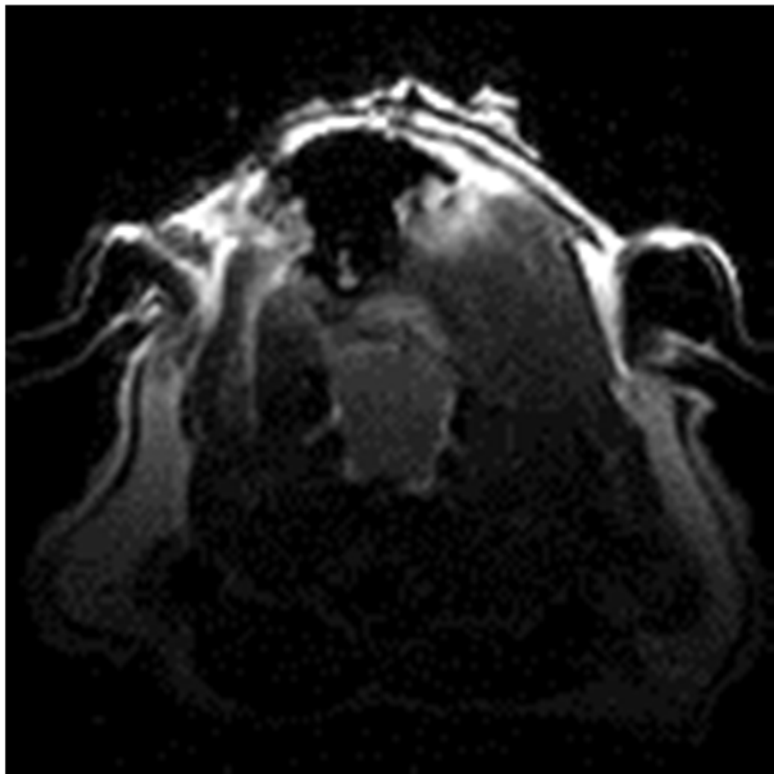
Arch-induced pauses in PN firing also drive associative learning

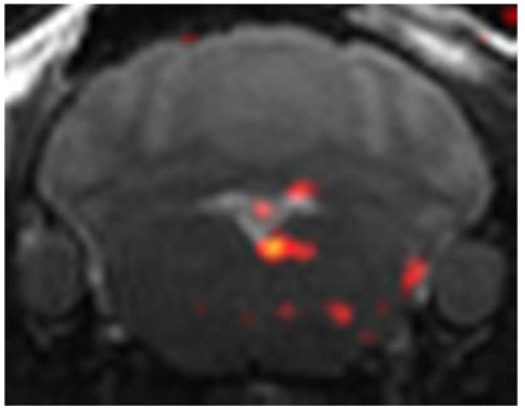




Lee, Mathews, and Otis unpublished

Scan Nov. 12, 2014
Mouse no. 2





Pseudocolor indicates negative BOLD signal
(5 cycles, 30s ON/OFF)
Laser ON = 100 ms pulses at 5 Hz

ARTICLE 10 FEBRUARY 2011 | VOL 470 | NATURE | 221

doi:10.1038/nature09736

Functional identification of an aggression locus in the mouse hypothalamus

Dayu Lin[1,2], Maureen P. Boyle[3], Piotr Dollar[4], Hyosang Lee[1], E. S. Lein[3], Pietro Perona[4] & David J. Anderson[1,2]

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7 JUNE 2013 VOL 340 SCIENCE www.sciencemag.org

Repeated Cortico-Striatal Stimulation Generates Persistent OCD-Like Behavior

Susanne E. Ahmari,[1,2,3,4]* Timothy Spellman,[5] Neria L. Douglass,[1,2] Mazen A. Kheirbek,[1,2] H. Blair Simpson,[1,3,4] Karl Deisseroth,[6] Joshua A. Gordon,[1,2] René Hen[1,2]

7 JUNE 2013 VOL 340 SCIENCE www.sciencemag.org

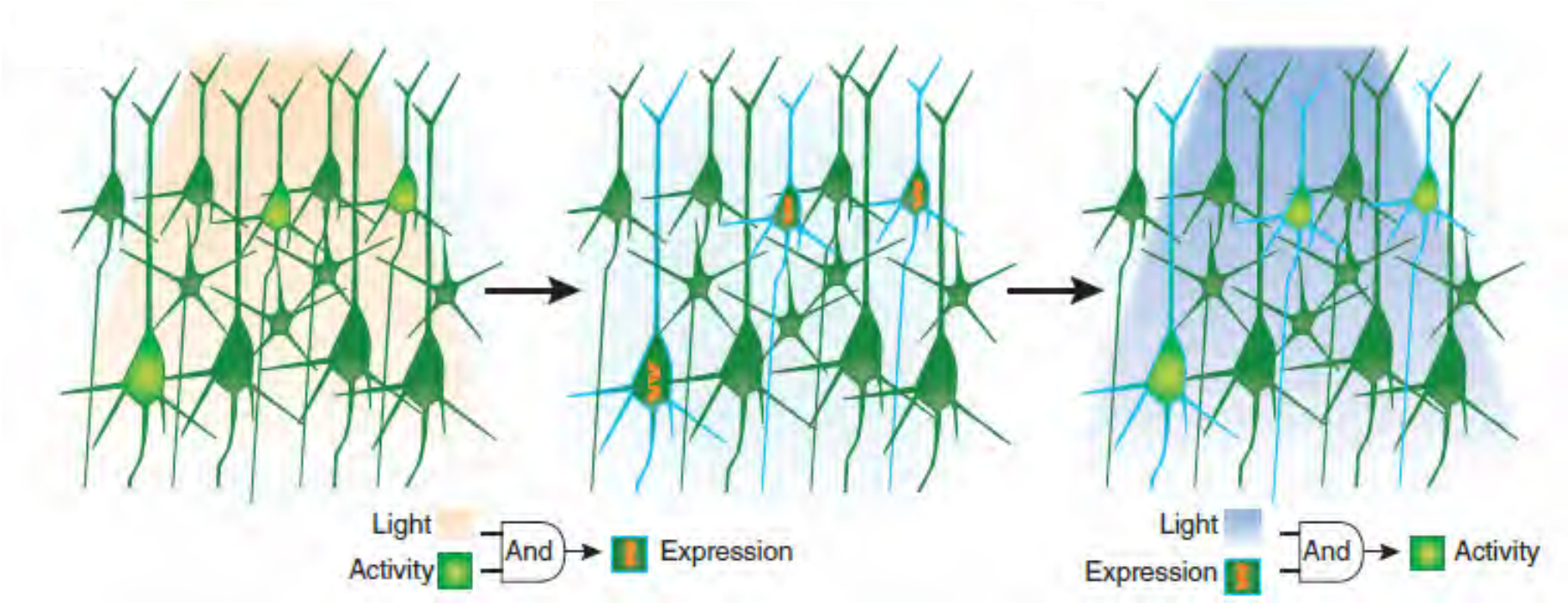
Optogenetic Stimulation of Lateral Orbitofronto-Striatal Pathway Suppresses Compulsive Behaviors

Eric Burguière,[1] Patrícia Monteiro,[1] Guoping Feng,[1] Ann M. Graybiel[1]*

Future tools that combine light and neuronal activity

red light enables gene expression but only in active neurons

if ChR2 is expressed, those neurons can be activated later by blue light



Peron and Svoboda, *Nat. Methods*, 2011

Perturb activity in specific neurons with specific spatiotemporal patterns to mimic behavior

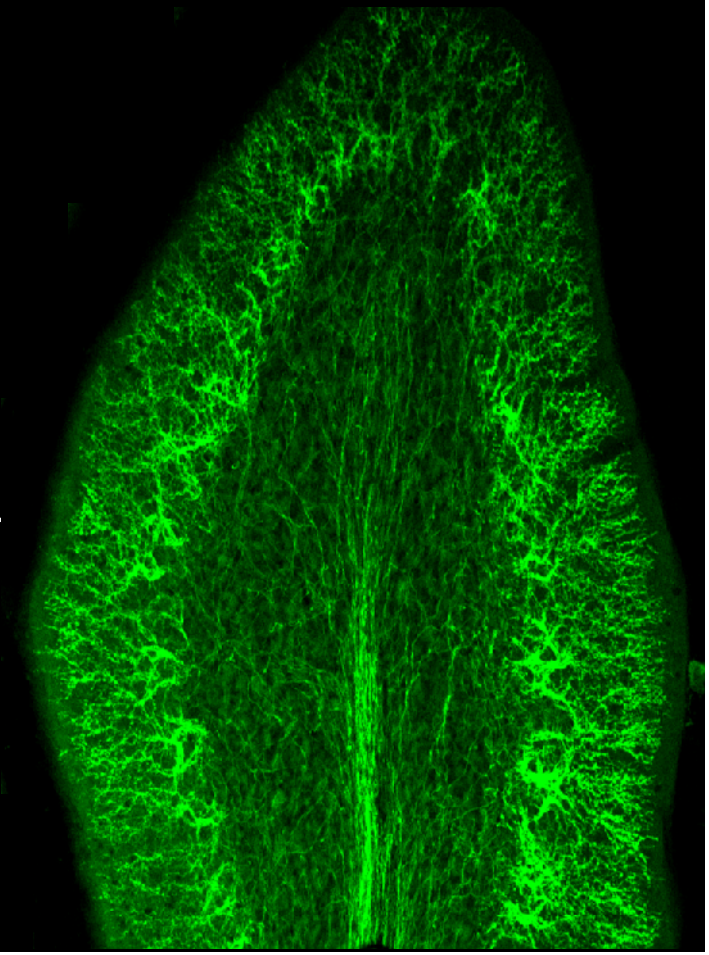
1. What is “optogenetics”?
2. *Create* activity in circuits to study normal brain processes: movement, motivation, memory formation
3. *Impose* aberrant activity to mimic neurological or psychiatric disease
4. *Restore* normal activity patterns to treat disease

Summary

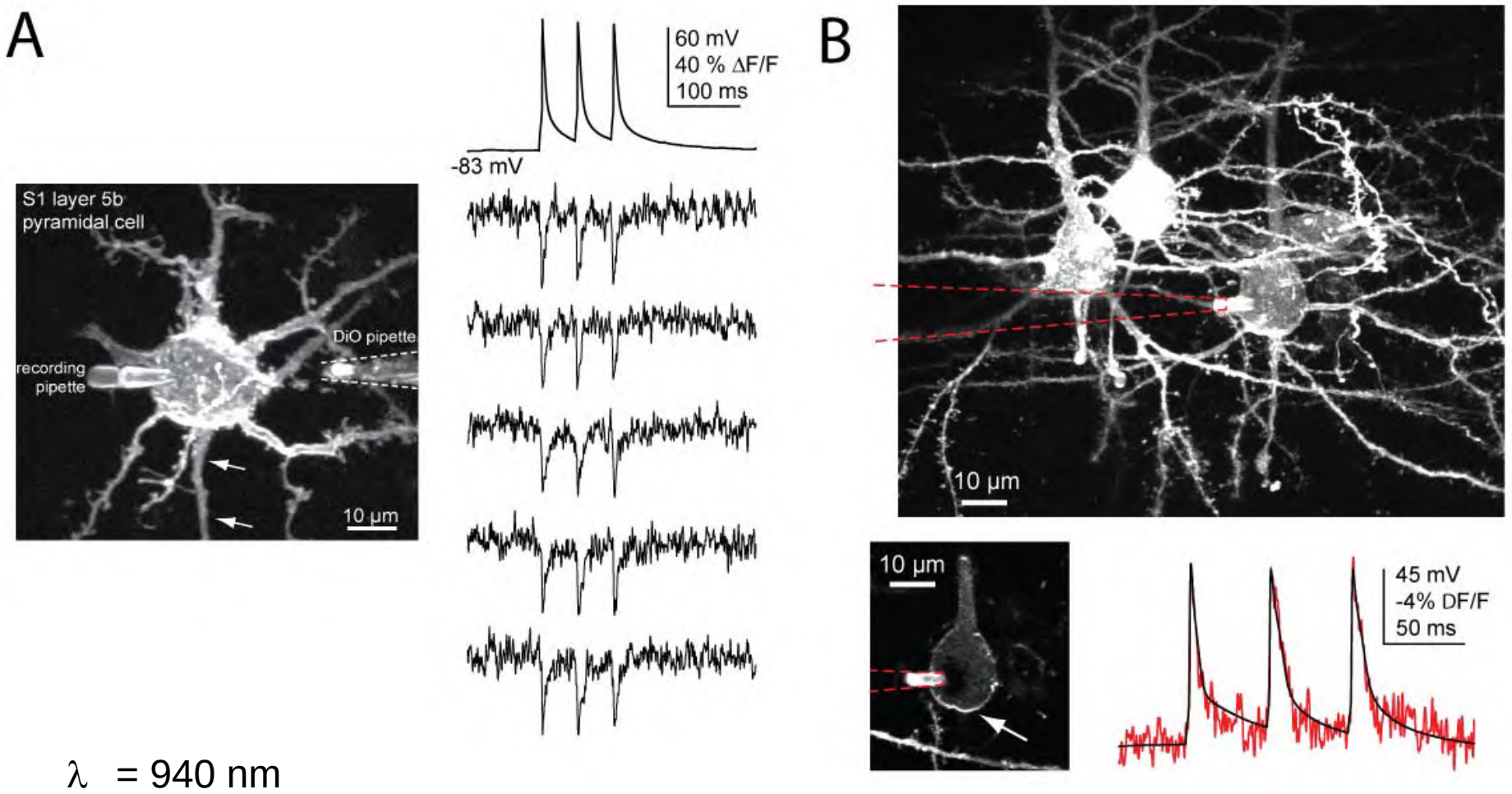
The BRAIN initiative will likely focus on three essential areas for understanding how the brain works at a circuit level: recording neuronal activity, data analysis/circuit modeling, and manipulating neuronal activity.

Technological revolutions in genetics and neuroscience allow us to record from specific circuits of neurons, stimulate them, or prevent their activity during behavior.

New approaches such as these will be critical for elucidating the inner workings of brain and mind. They should also prove useful in defining how circuits fail in a range of neurological and psychiatric diseases.



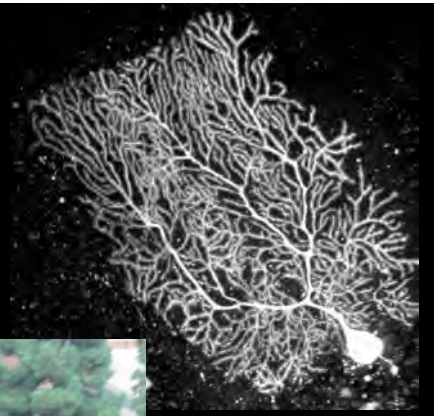
Labeling neurons so that electrical signals can be converted into light



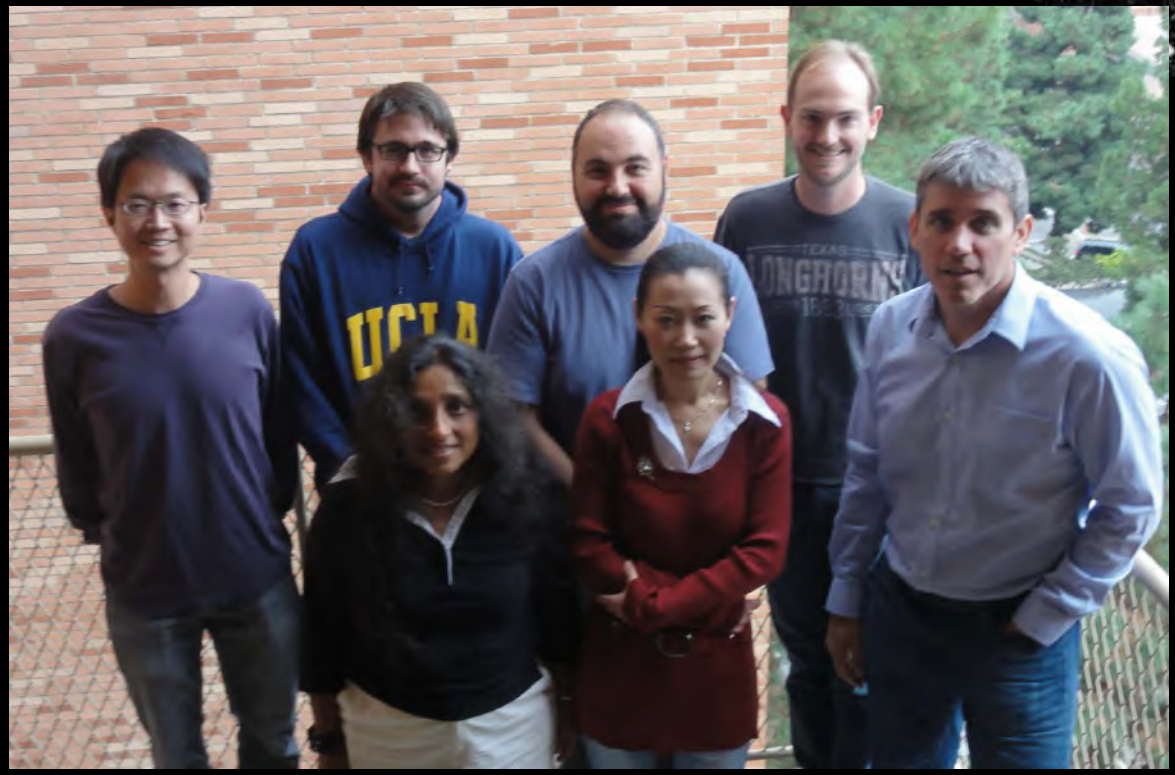
λ = 940 nm
3 µM DPA

Fink, Bender, Trussell, Otis, & DiGregorio, PLOS One, 2012

Otis lab members & collaborators



Current lab members:
Shekib Jamil (rotation)
Ka-Hung Lee
Paul Mathews
Meera Pratap
Alex Reeves
Raul Serrano
Matt Shtrahman
Xiaoping Tong



Collaborators

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Carolyn Houser
Zechun Peng
Pierre-Olivier Polack
Ben Novitch
Julio Vergara

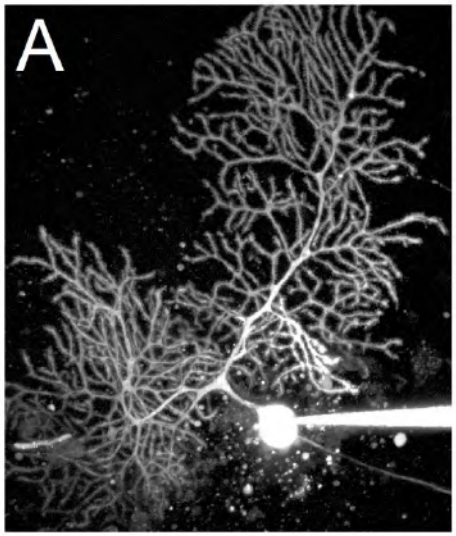
Cedars Sinai MC
Clive Svendsen
Bob Baloh

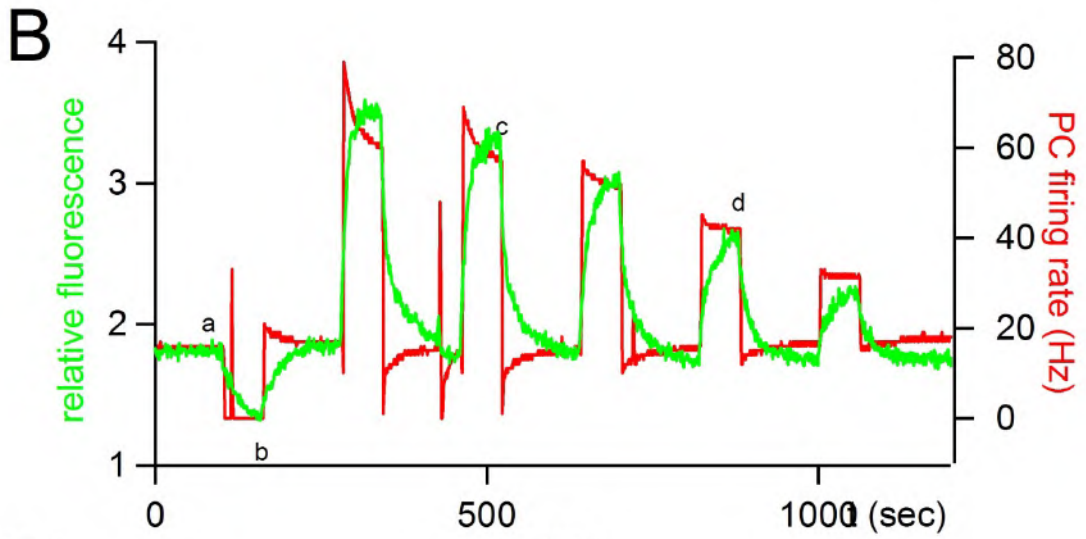
University of Utah
Stefan Pulst

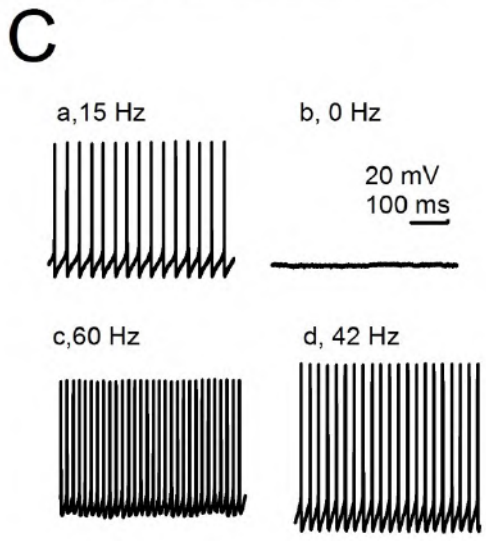
Pasteur Institute
David Digregorio

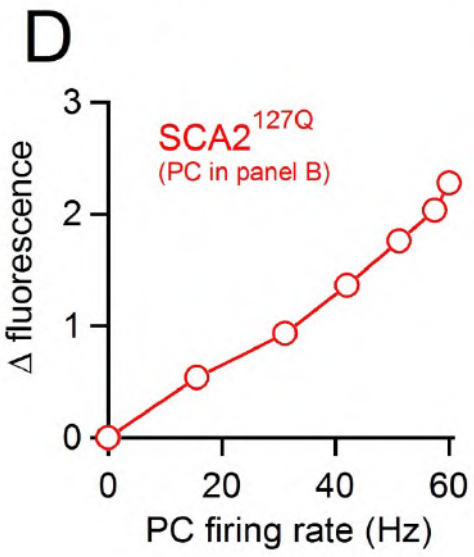
Funding: NINDS, NIAAA, NCRR, NSF, ALS, McKnight, & Whitehall Foundations

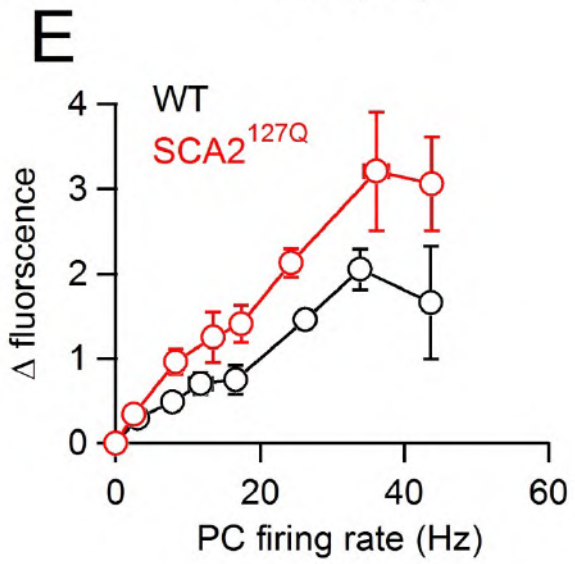
SCA2 PCs have elevated calcium across the physiological firing range





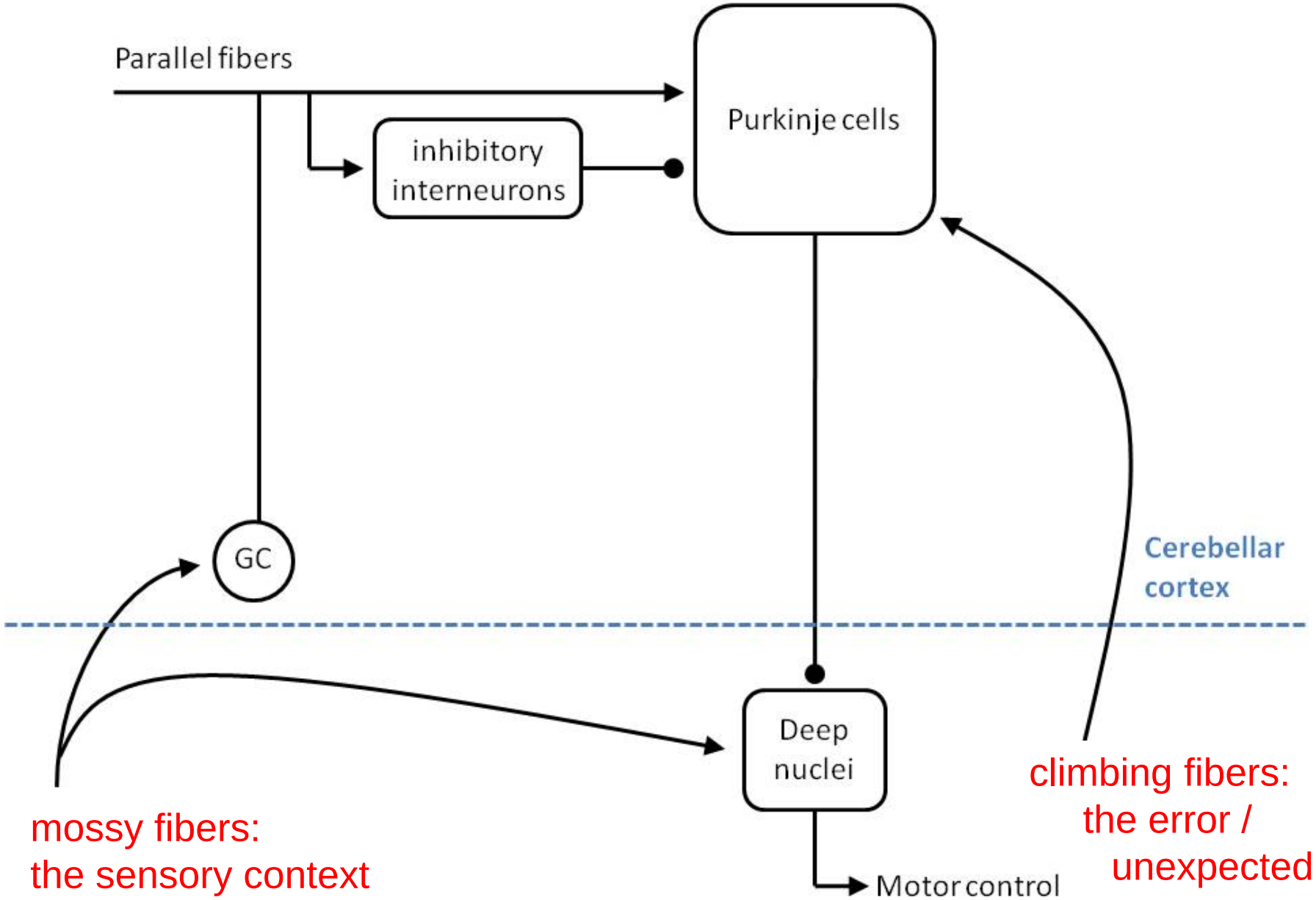




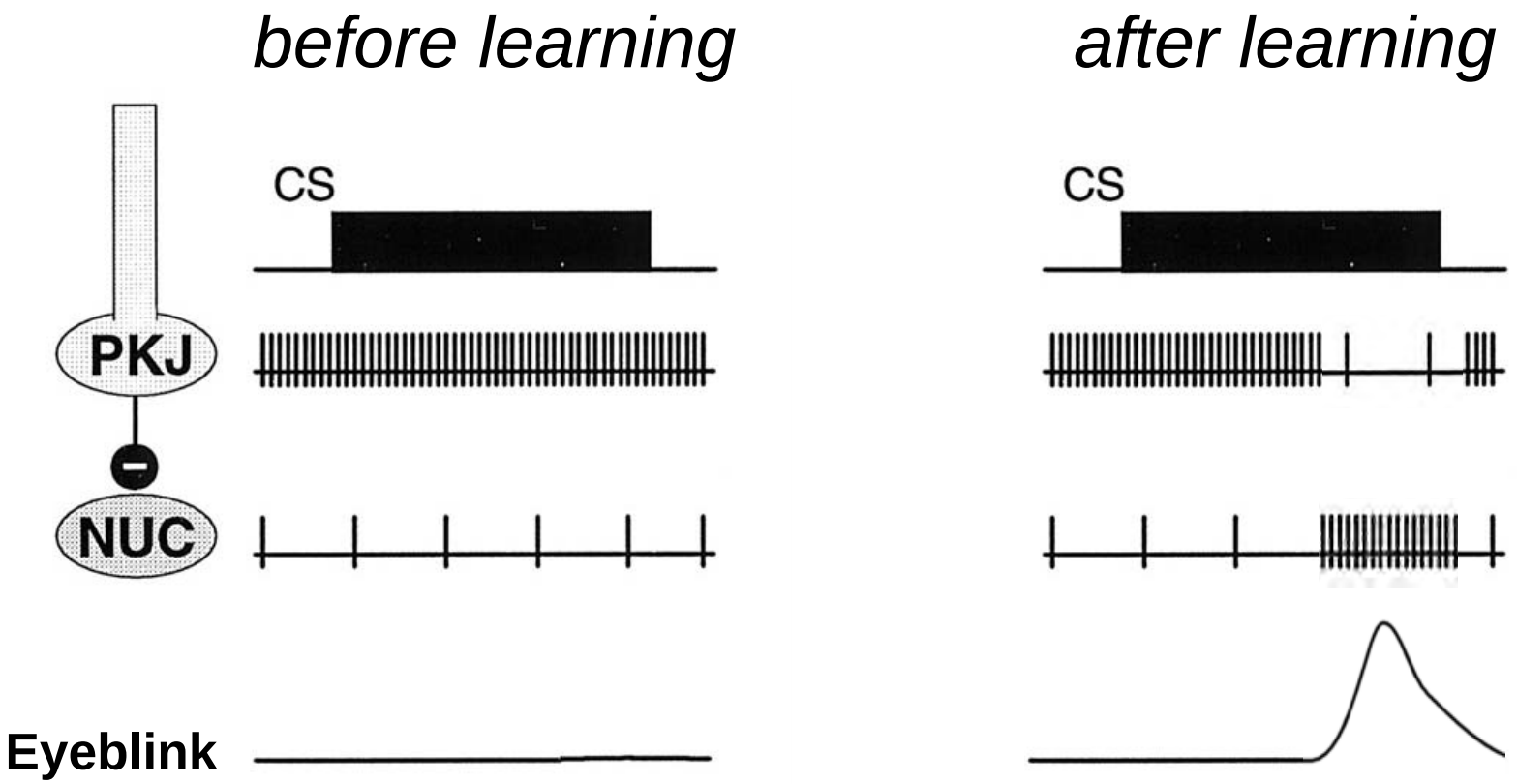


Meera & Otis, unpublished

The essential circuit:



How does learning change neuronal firing?



Purkinje cells "learn" when to pause and deep nuclear cells "learn" when to burst

from Garcia, Steele, and Mauk, *J. Neurosci.* 19:10940, 1999

Hypothesized sites of plasticity supporting associative learning