



Sainsbury Wellcome Centre

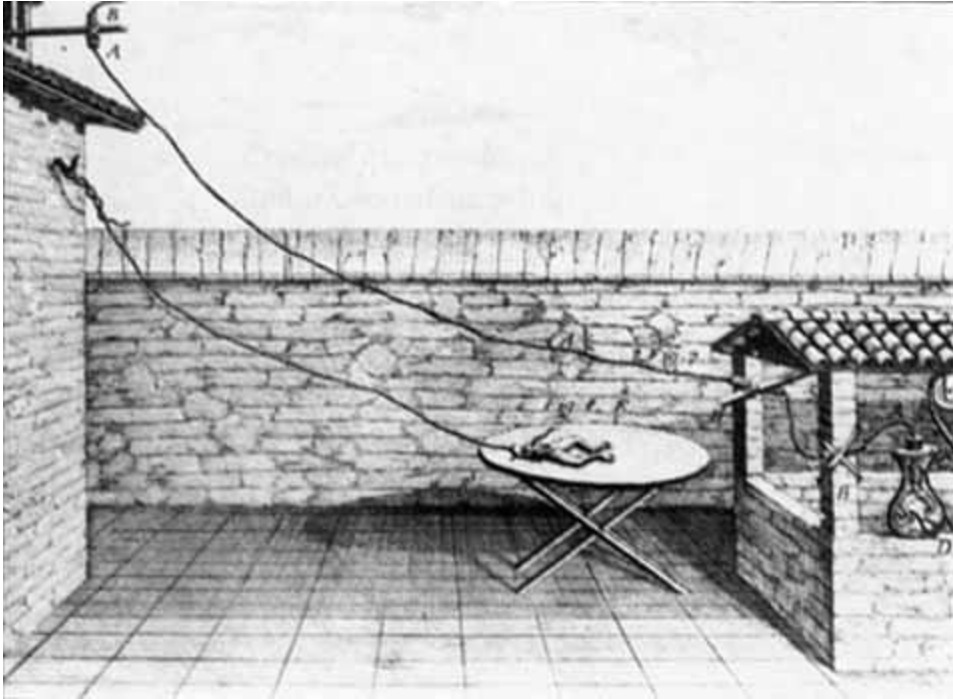
5 October 2020

# Methods for recording neuronal activity

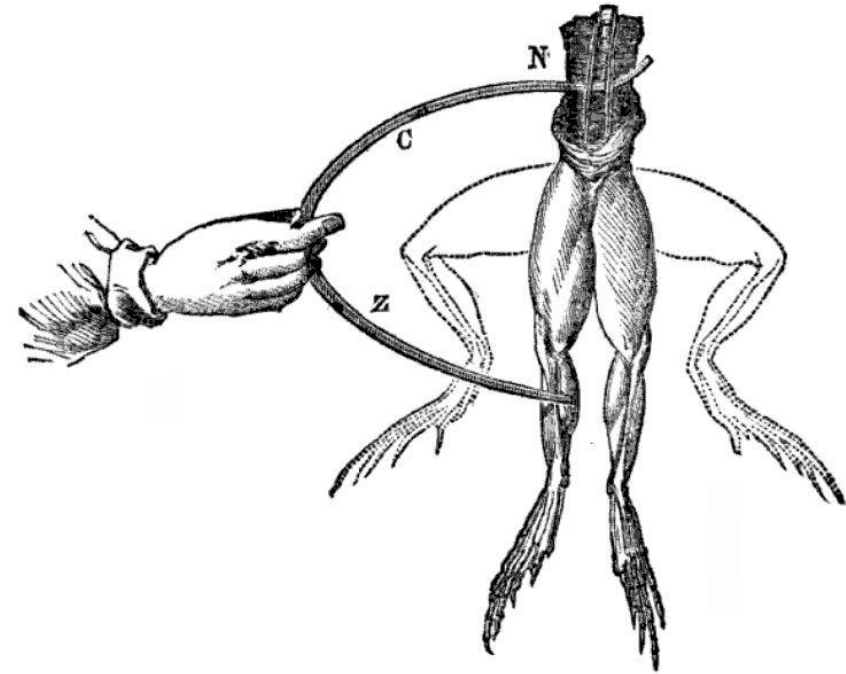
Prof. Tom Otis

t.otis@ucl.ac.uk

# From 'animal electricity'... to how nerves work



Galvani, 1780



Galvani, 1791

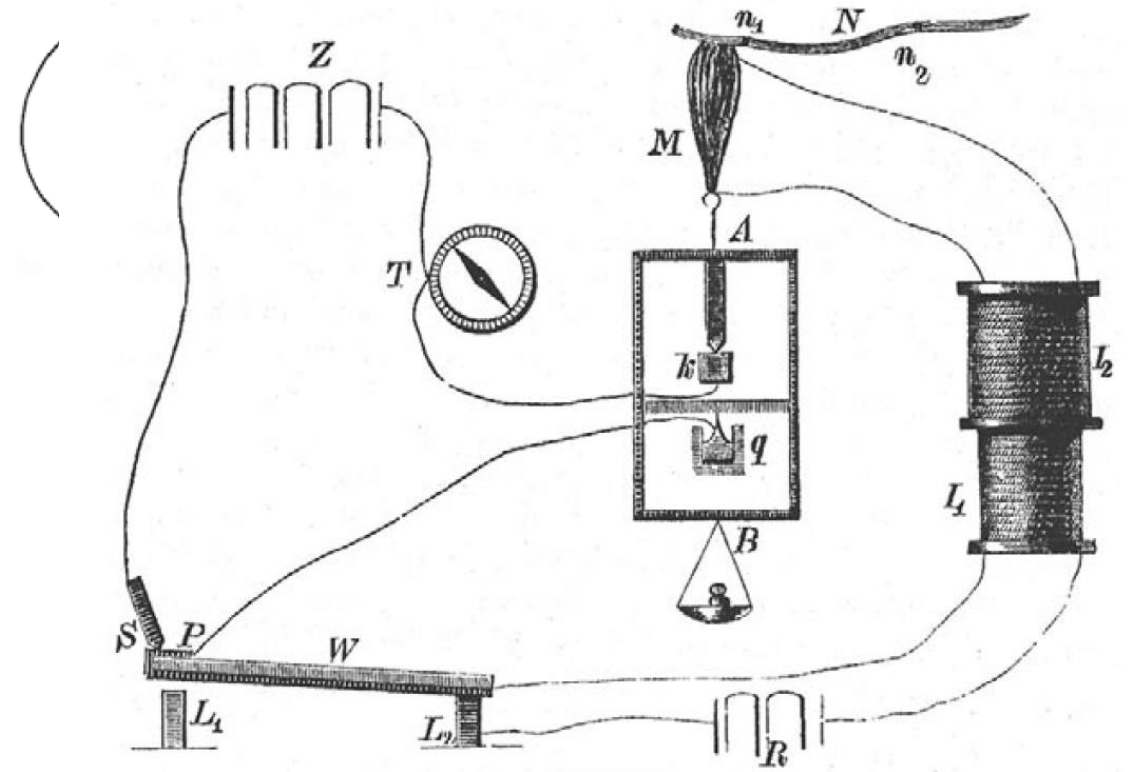
# Helmholtz's measurements of nerve conduction velocity

Devices to measure time intervals:

Charles Fizeau's sign for velocity device, 1844  
conduction velocity, c 1848



Hermann von Helmholtz

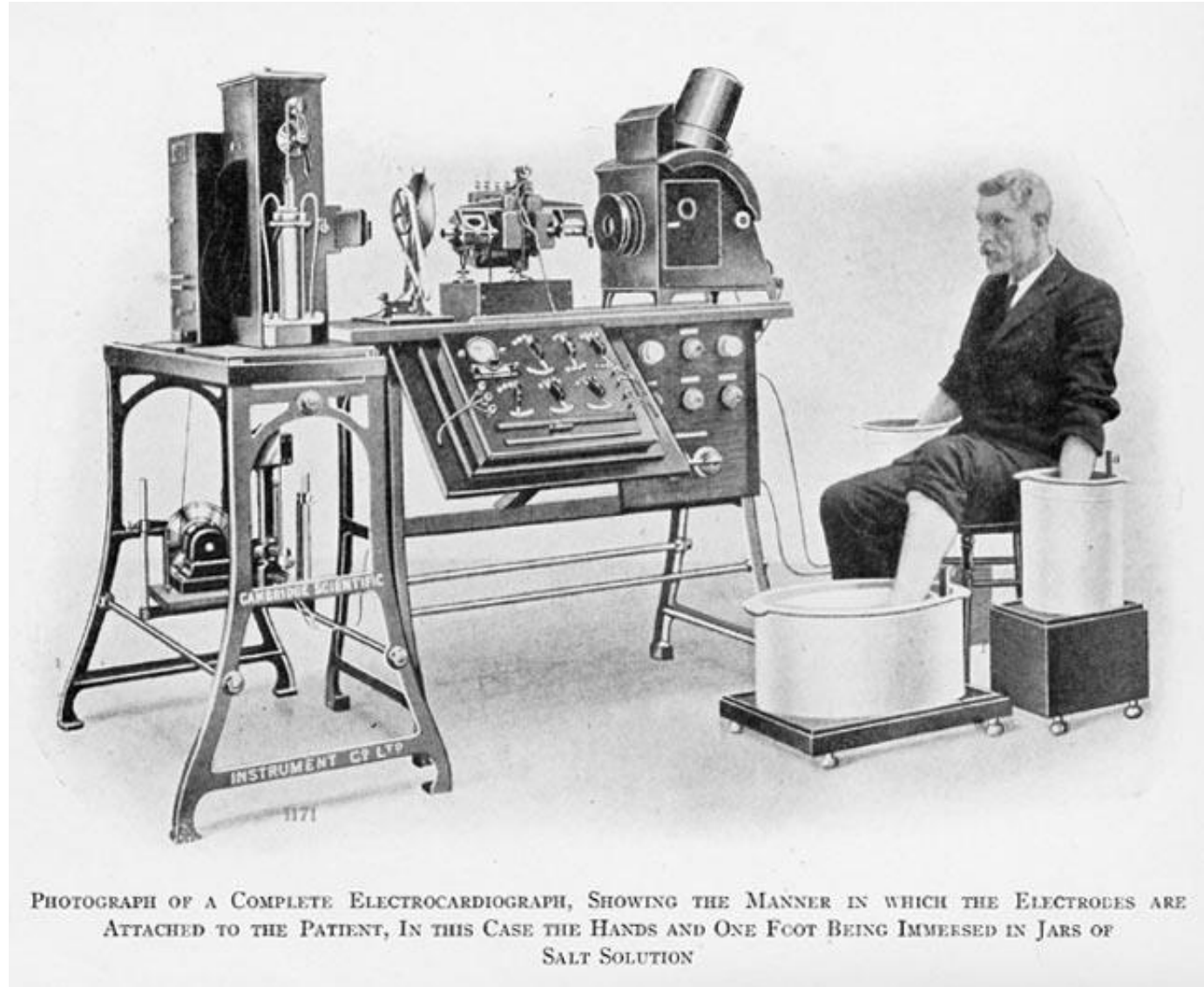


from Schnmidgen, *Endeavour*, 26:142 (2002)

# Willem Einthoven's string galvanometer



Willem Einthoven



# First electrical recordings of a nerve impulse

frog sciatic nerve

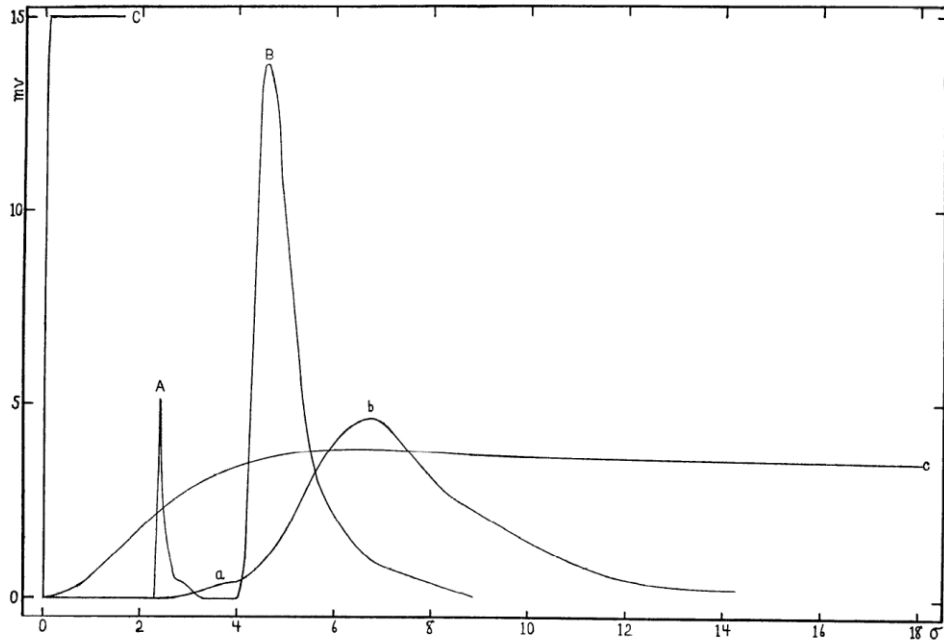


Fig. 3. The action currents of the bull frog sciatic, as recorded by the Braun tube and string galvanometer, plotted in rectangular linear coordinates. *A*, *B*, *C*, Braun tube records; *a*, *b*, *c*, string galvanometer records. *A*, *a*, shock; *B*, *b*, action current; *C*, calibration with a constant current of 15 mv.; *c*, with one of 3.75 mv.



Herbert Gasser

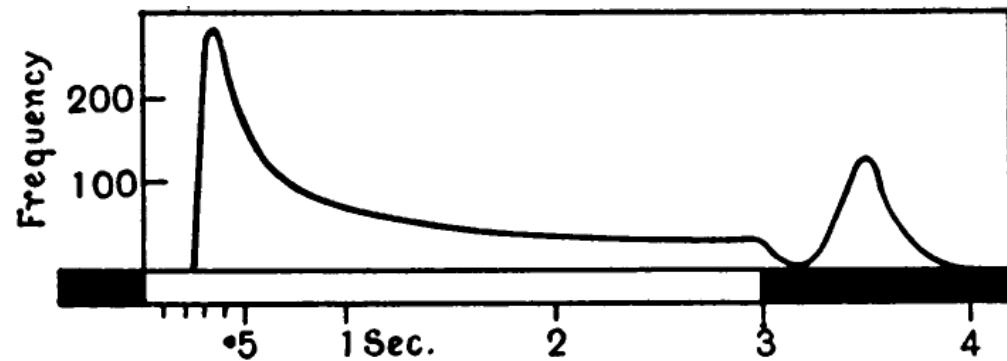
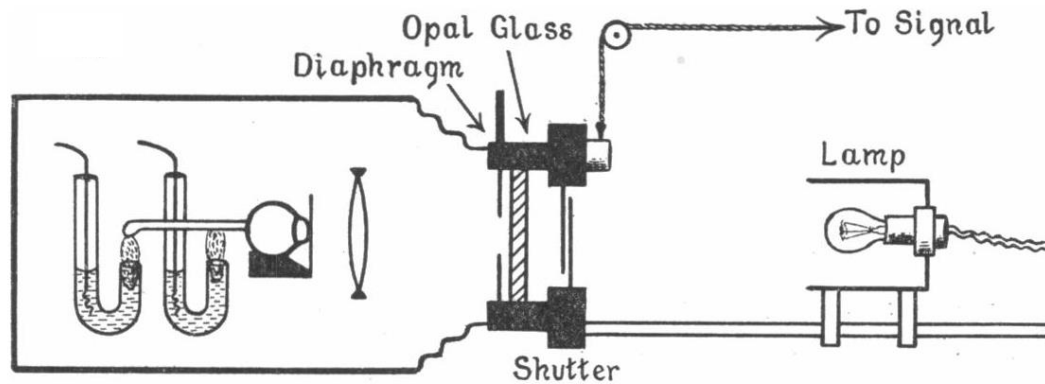


Joseph Erlanger

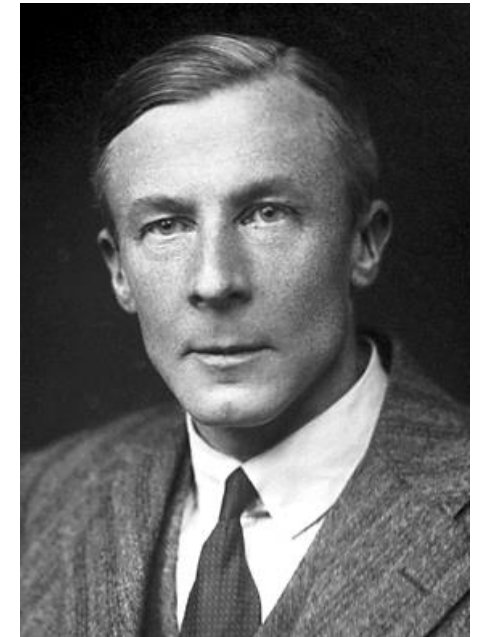
American J. Physiol., 1922

# First recordings of light-evoked activity in optic nerve

Conger eel optic nerve



J. Physiology, 1927

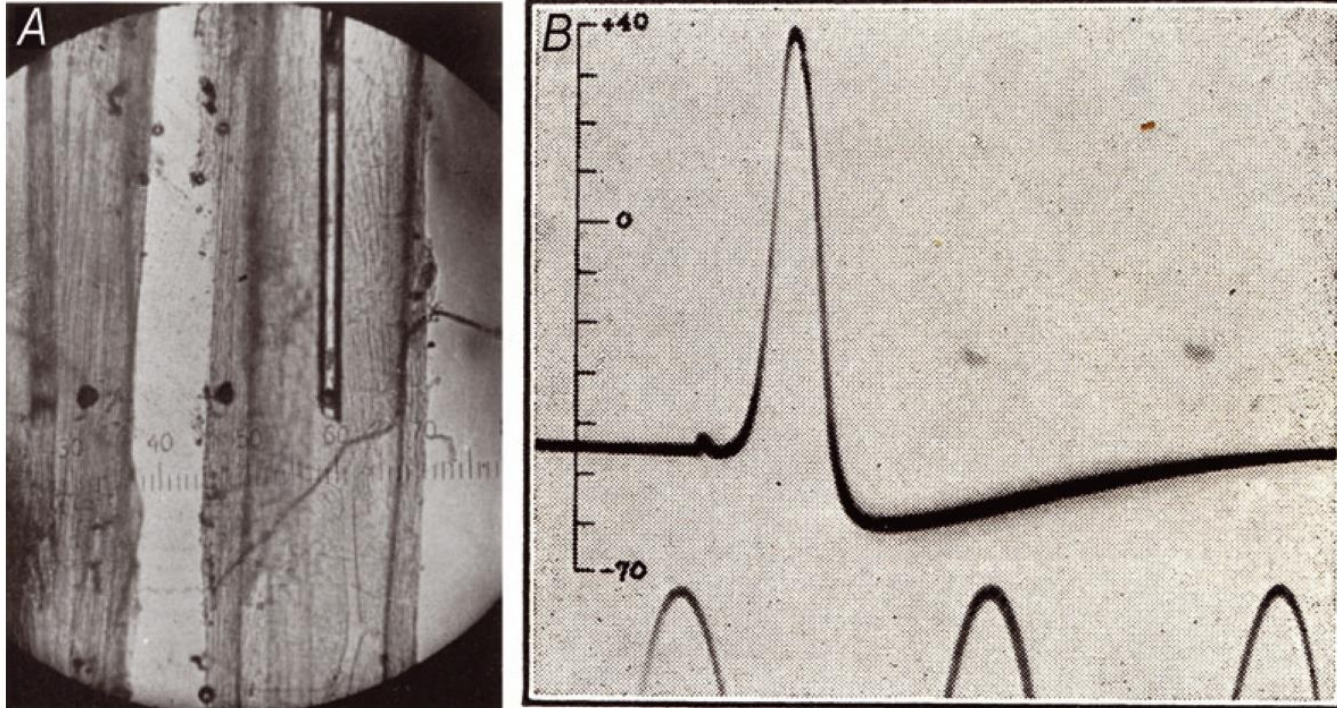


Lord Edgar Douglas  
Adrian

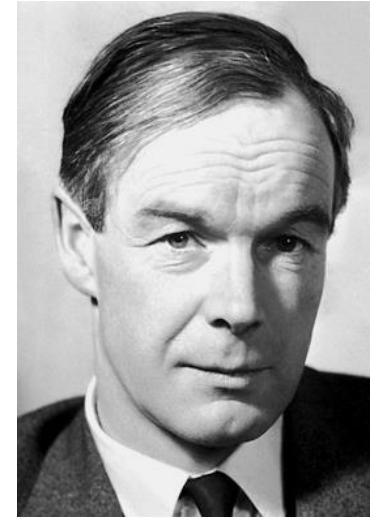
"I had arranged electrodes on the optic nerve of a toad in connection with some experiments on the retina. The room was nearly dark and I was puzzled to hear repeated noises in the loudspeaker attached to the amplifier, noises indicating that a great deal of impulse activity was going on. It was not until I compared the noises with my own movements around the room that I realised I was in the field of vision of the toad's eye and that it was signalling what I was doing."

# Mechanism of the nerve impulse

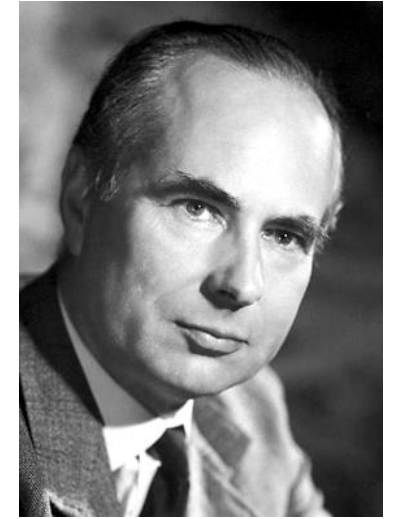
Squid giant axon



Nature, 1939



**Alan Hodgkin**



**Andrew Huxley**

# Hodgkin Huxley model of the action potential

<http://nerve.bsd.uchicago.edu/>

Fig.1

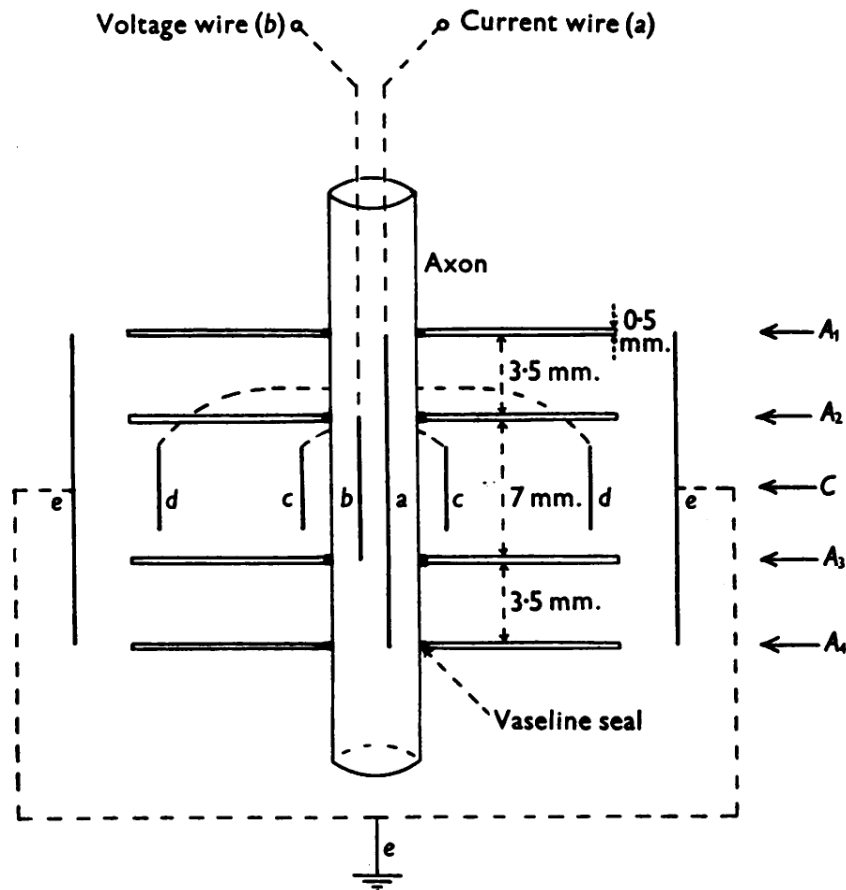
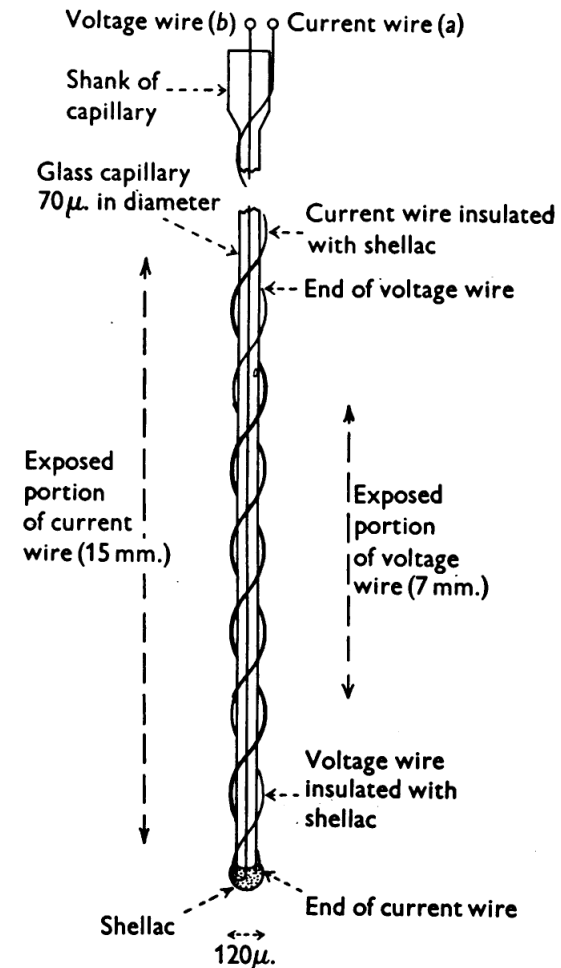


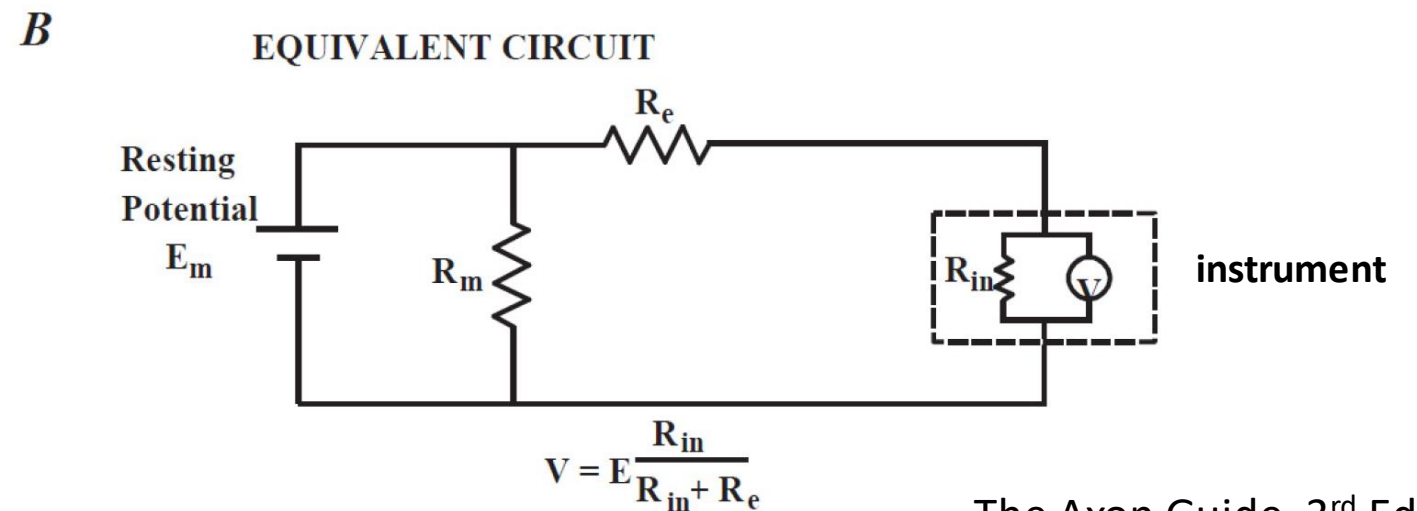
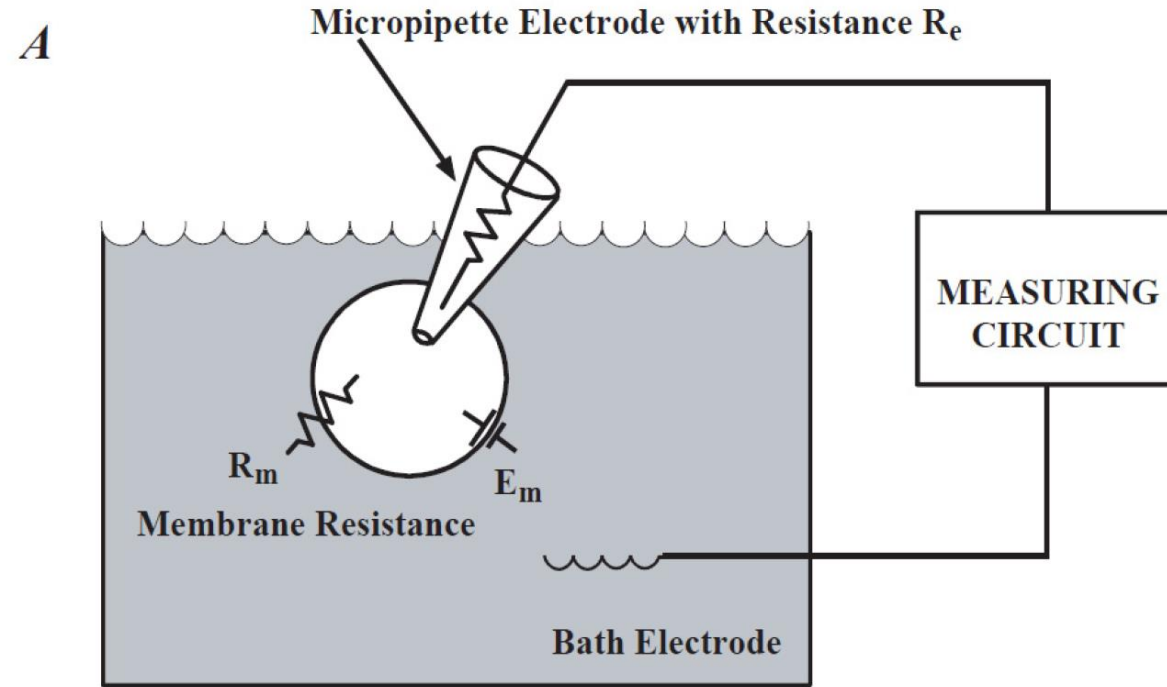
Fig.4





# Intracellular measurements with a microelectrode

Ag/AgCl wires are standard in physiological contexts due to their excellent bidirectional ionic mobility, stability



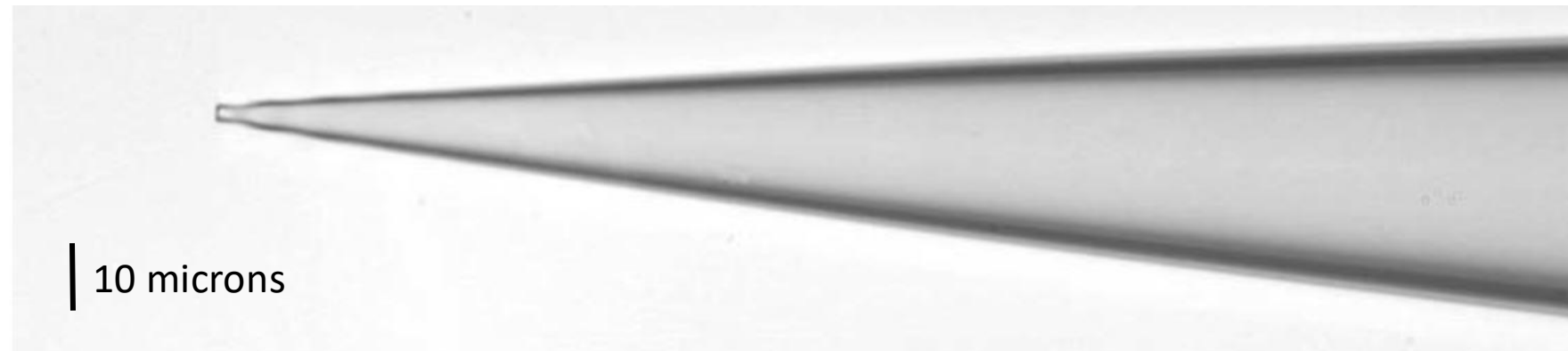
# Microelectrode methods for intracellular recording

'sharp' microelectrode



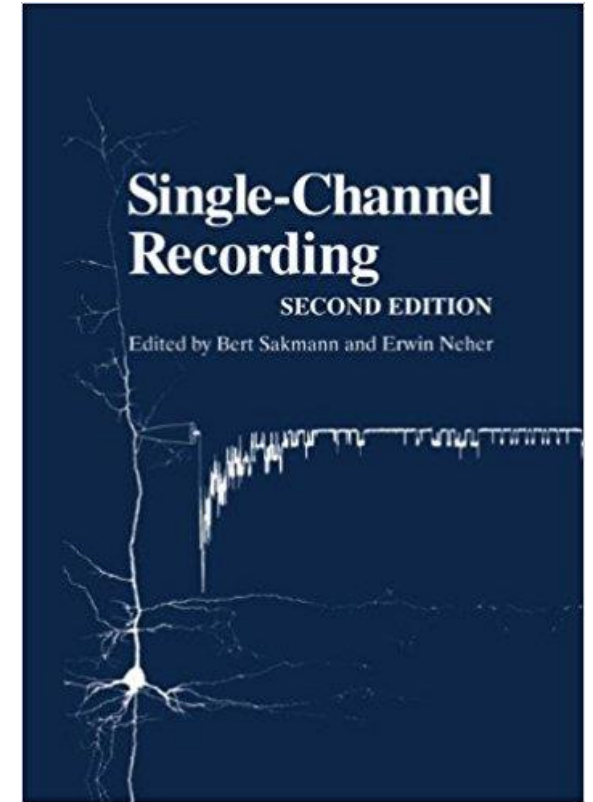
3 M KCl, 3 M K Acetate  
80-100 M $\Omega$

whole-cell patch pipette



physiological internal  
e.g. 130 K MeSO<sub>4</sub>  
2-5 M $\Omega$

# Patch clamping



<https://youtu.be/M3xN4lhmt7U>

from Purves et al, Neuroscience 5<sup>th</sup> Ed. 2012

# Microelectrode methods for intracellular recording

Rat dentate gyrus granule cells

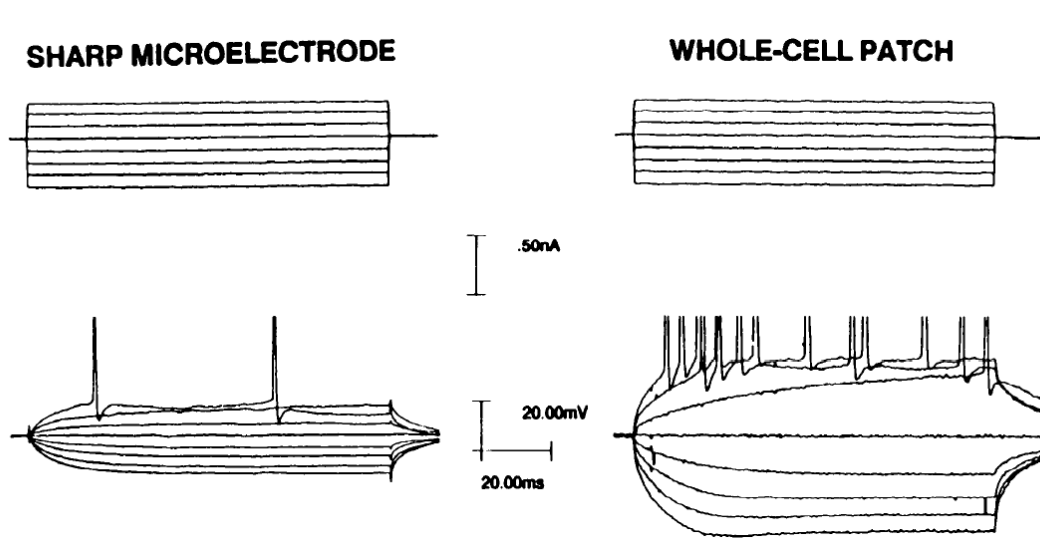


FIG. 4. Comparison of membrane voltage responses to current steps in GCs recorded with sharp electrode vs. whole-cell patch electrode. Sharp electrode filled with 3 M potassium acetate. The differences in  $R_N$  and  $\tau_m$  can be appreciated by visual inspection of the records. Spike threshold is similar for the 2 recording methods.

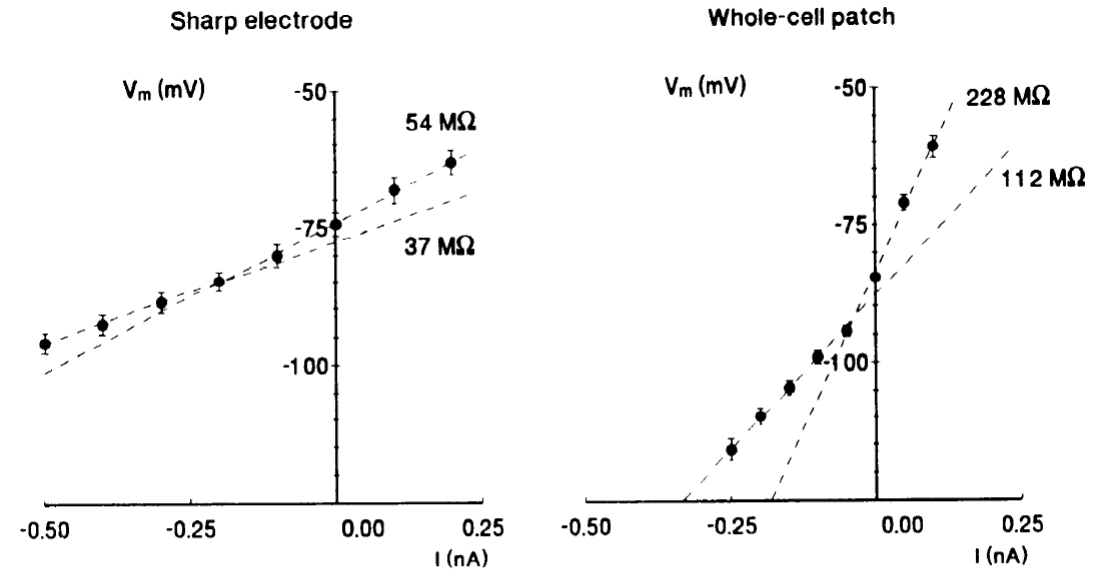
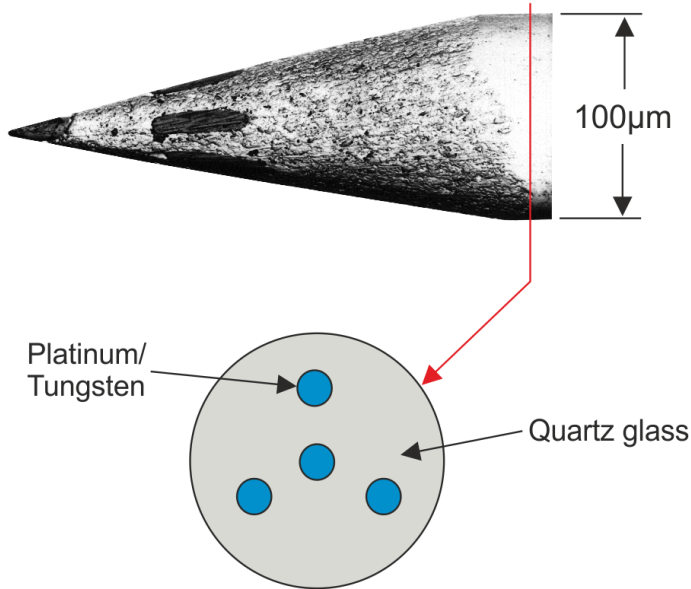


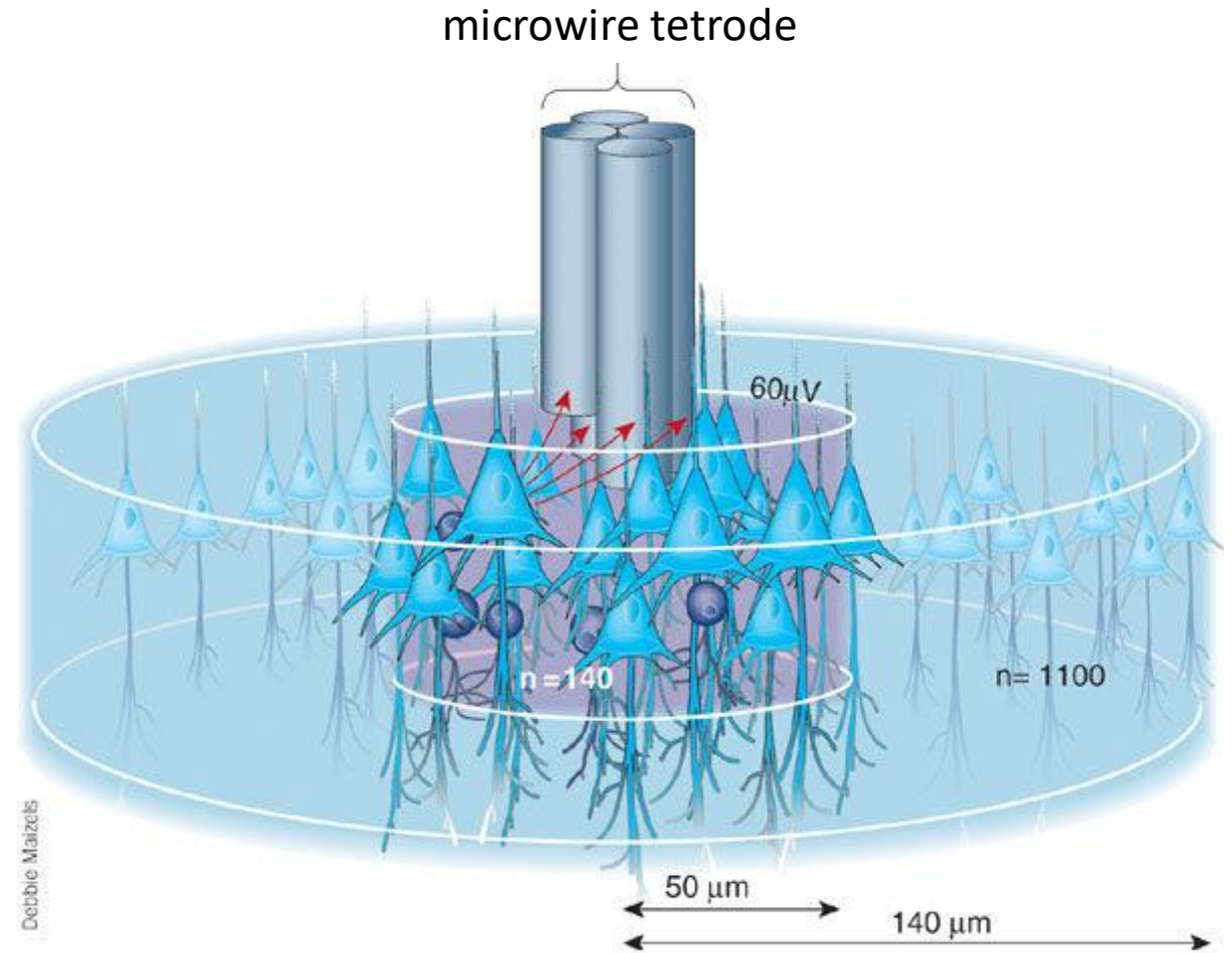
FIG. 5. Membrane voltage vs. injected current for 31 GCs recorded in whole-cell method and 10 GCs recorded with sharp electrodes; points are means  $\pm$  SE. Voltage was determined 180 ms after start of current pulse. Error bars indicate SE. Lines are fitted by least-squares method. Regions of linearity determined by eye.

# Recording from populations of single neurons: tetrodes

Thomas Recording tetrode

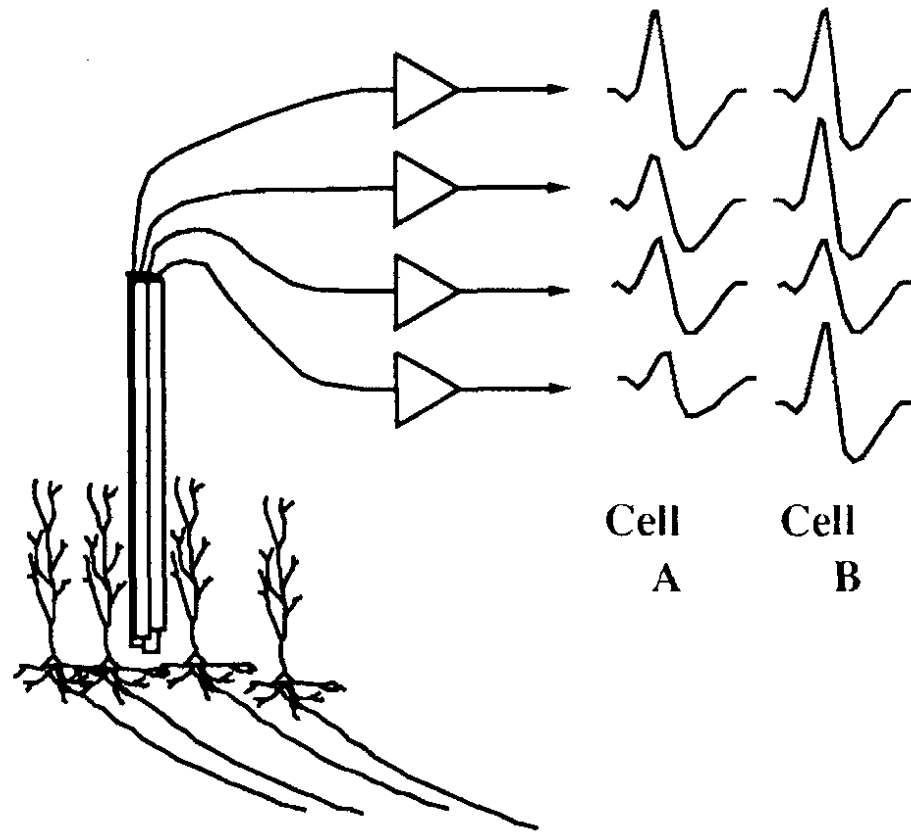


see Recce & O'Keefe, 1989

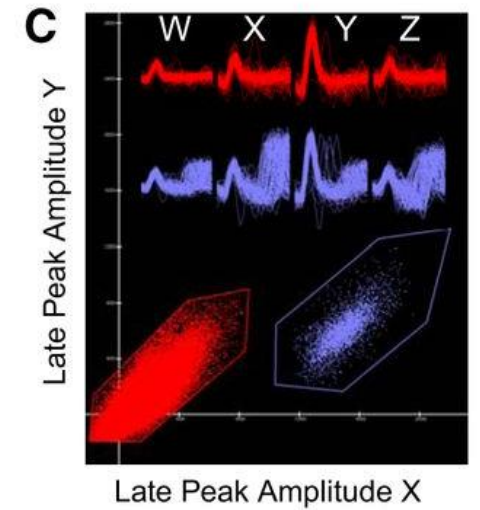
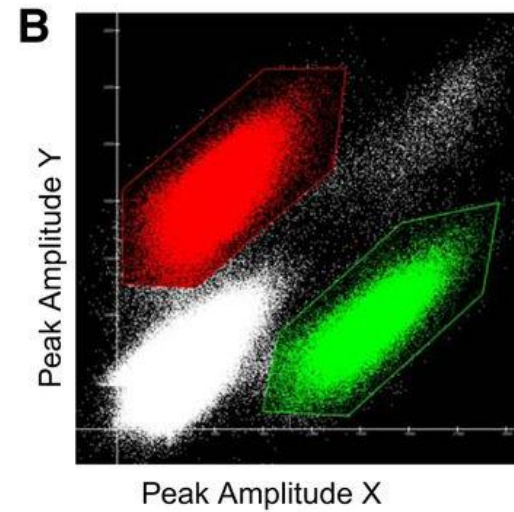


Buzsáki, *Nat. Neurosci.* 2004

# Recording from populations of single neurons: tetrodes

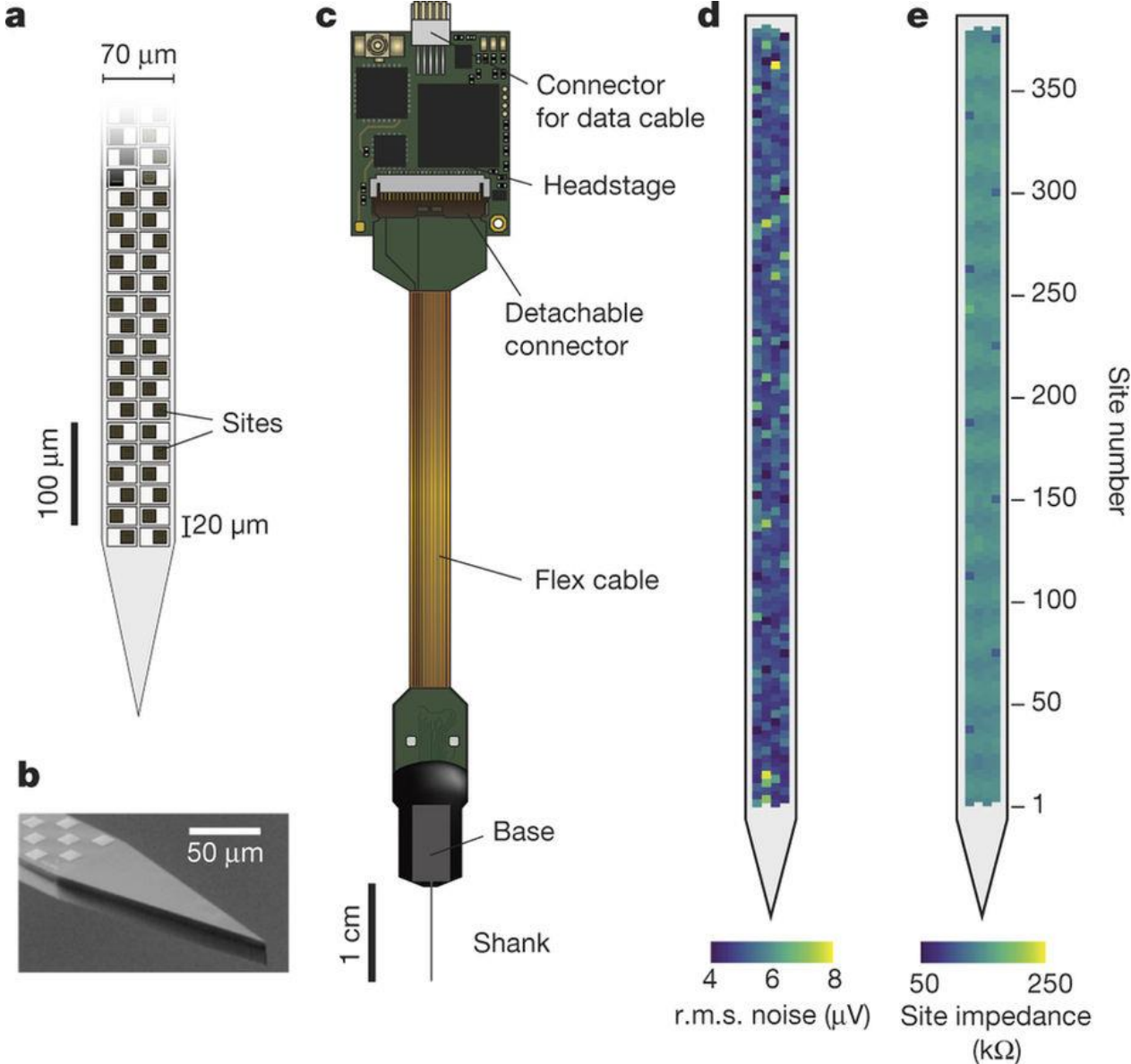


O'Keefe & Recce, 1993



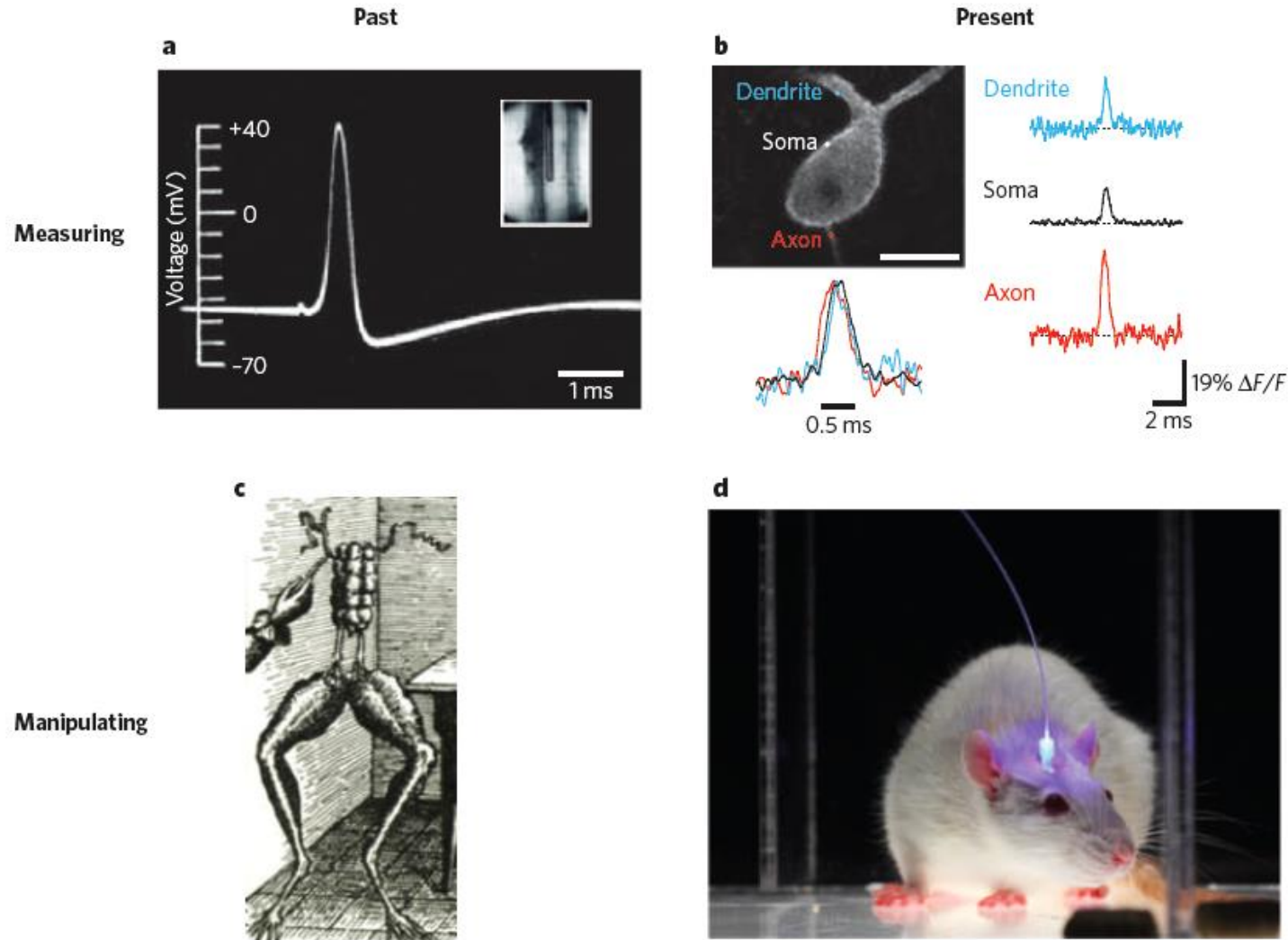
Halverson et al., J. Neurosci. 35:7182-32, 2015

# Neuropixel probes



# Electrophysiology in the age of light

Massimo Scanziani<sup>1</sup> & Michael Häusser<sup>2</sup>

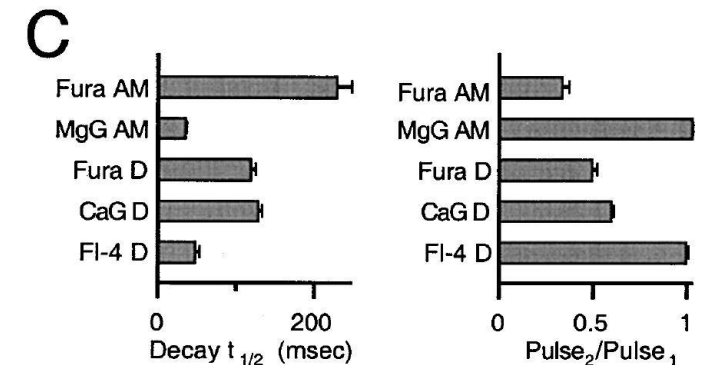
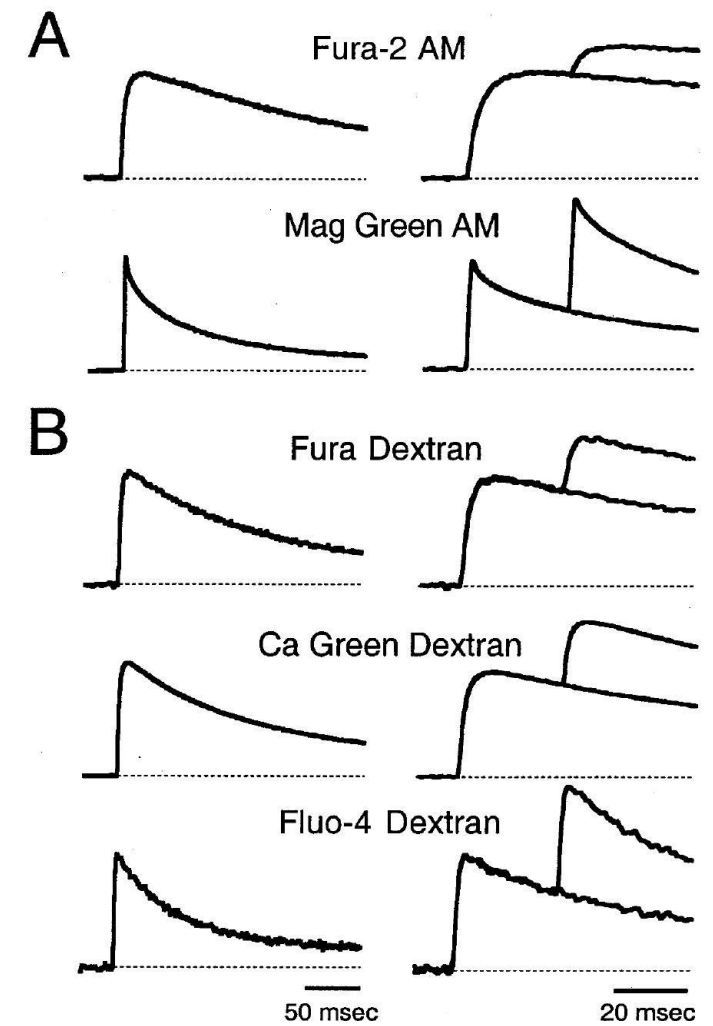


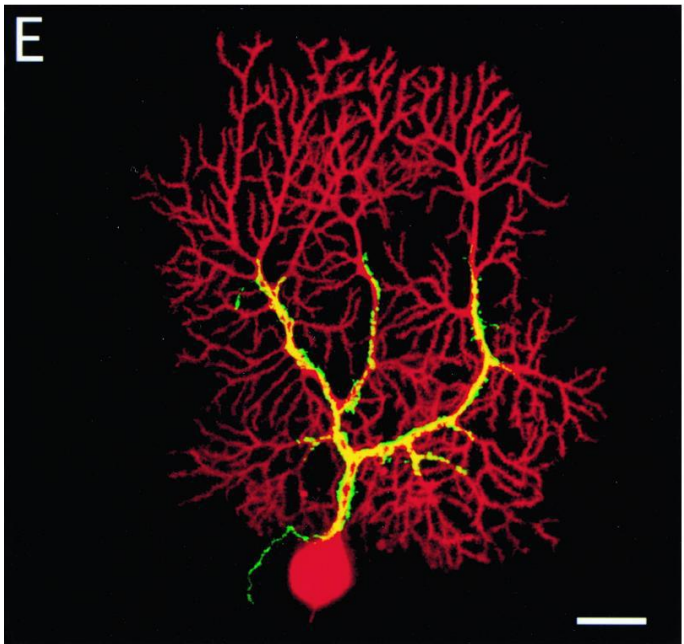
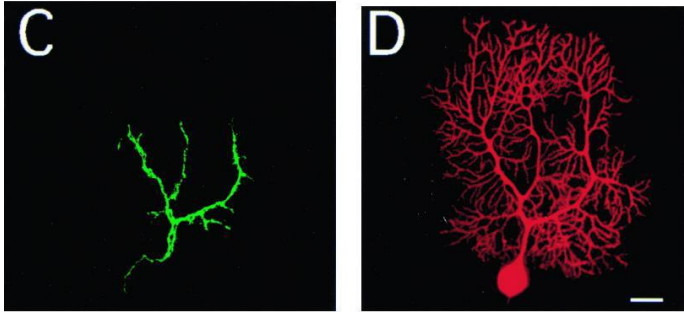
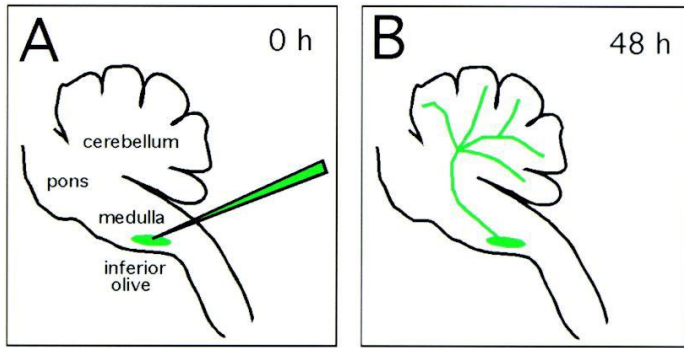


# A range of affinities and kinetics

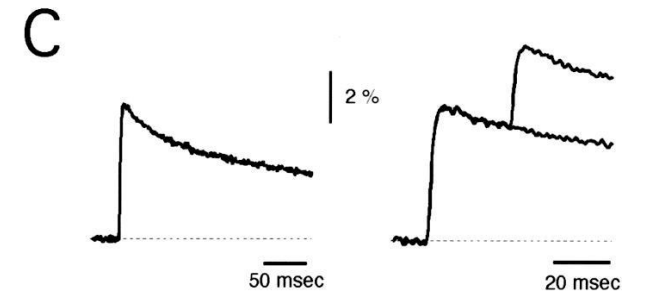
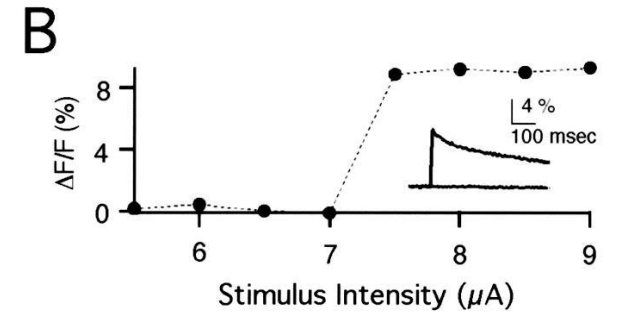
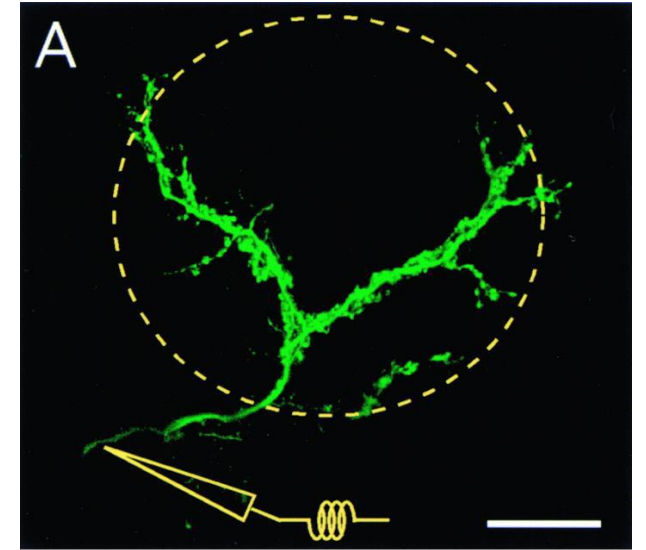
Table 1. Indicator Dissociation Constants for Calcium

Indicator	$K_D$ ( $\mu\text{M}$ )	Reference
Fura-2	0.16	( <a href="#">Kao and Tsien 1988</a> )
Magnesium green	7	( <a href="#">Zhao et al. 1996</a> )
Fura dextran (10,000 MW)	0.52	( <a href="#">Konishi and Watanabe 1995</a> )
Calcium green dextran (3,000 MW)	0.54	( <a href="#">Haugland 1996</a> )
Fluo-4 dextran (10,000 MW)	3.1	





# Dye imaging from a presynaptic terminal

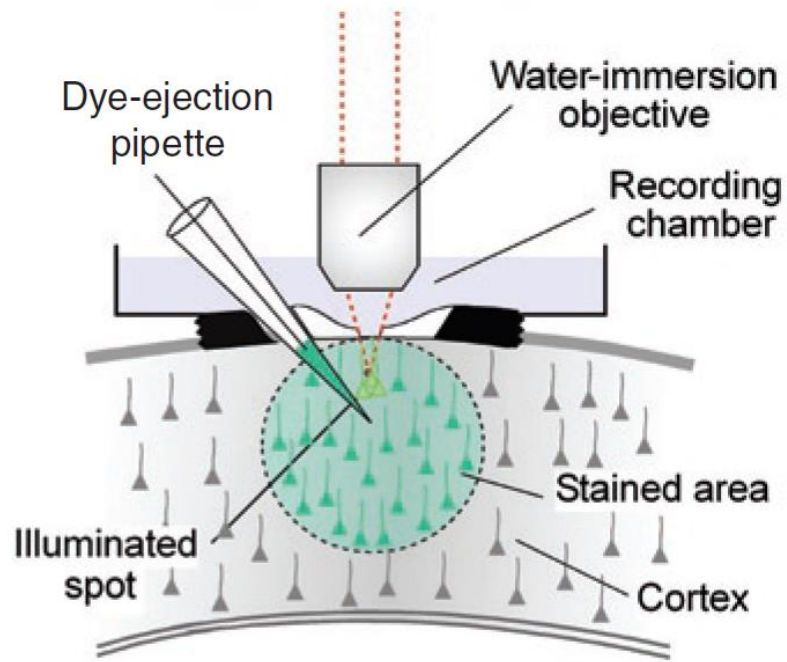


# Ca<sup>++</sup> dyes *in vivo*

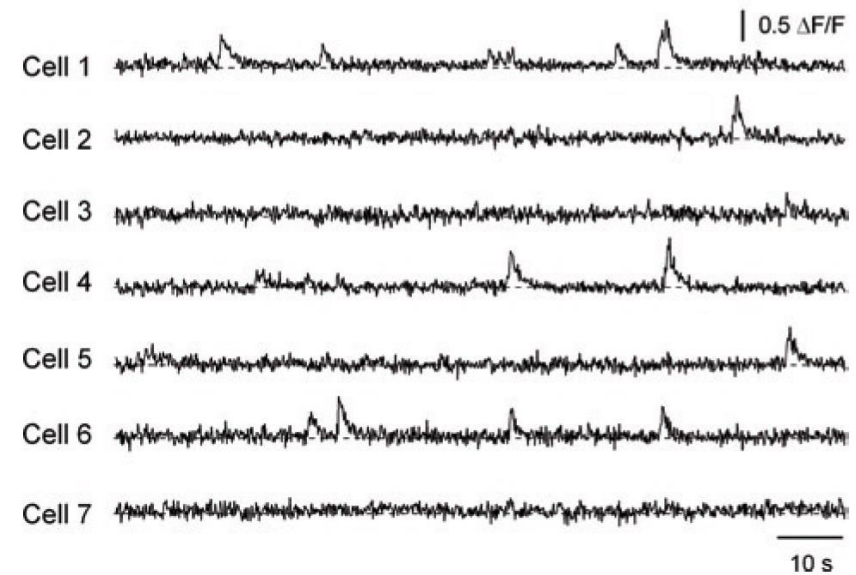
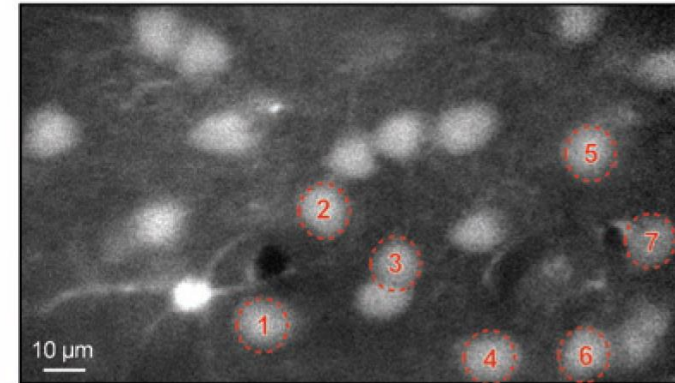
'Bulk loading'

a

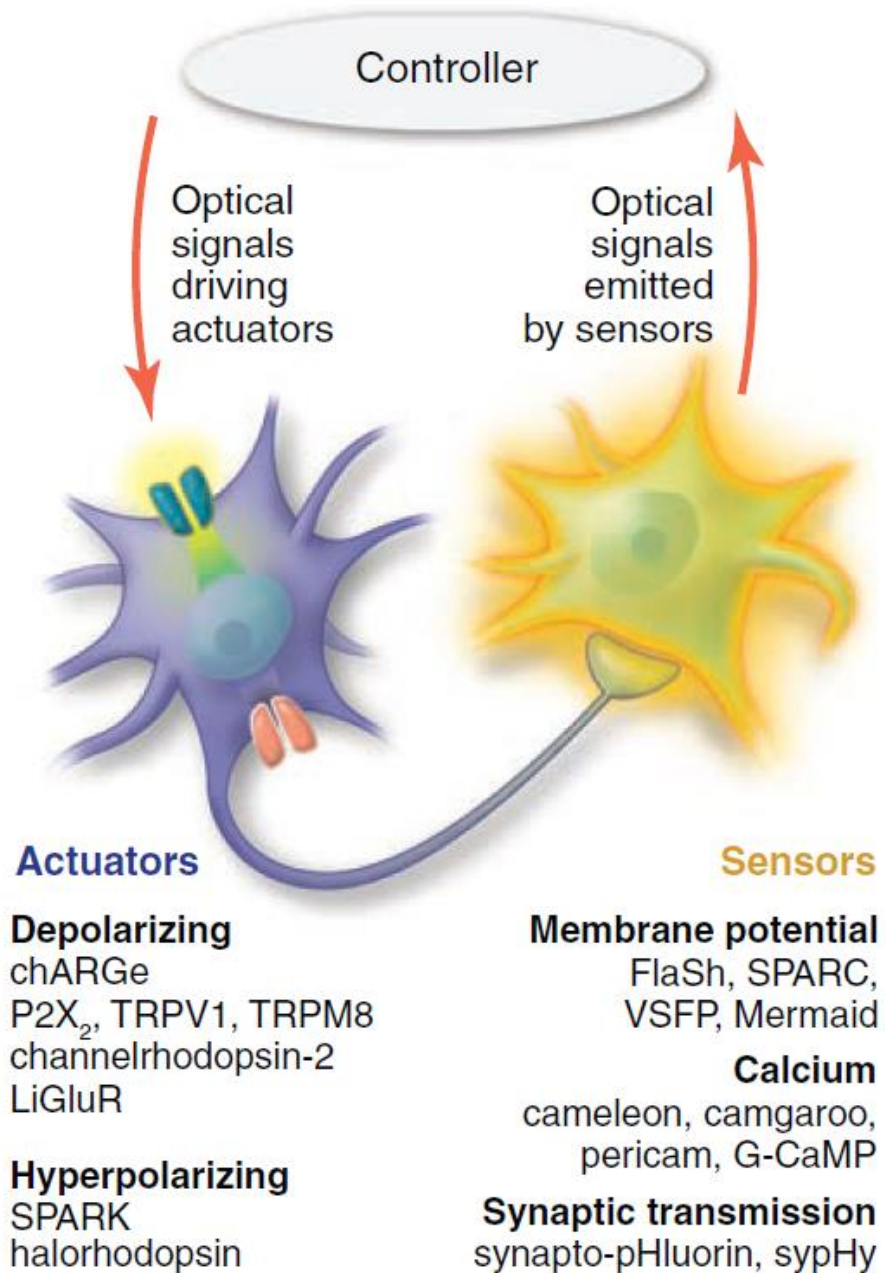
Mode-locked  
laser light  
800 nm,  
80 MHz, < 100 fs



Adult (6-month-old)



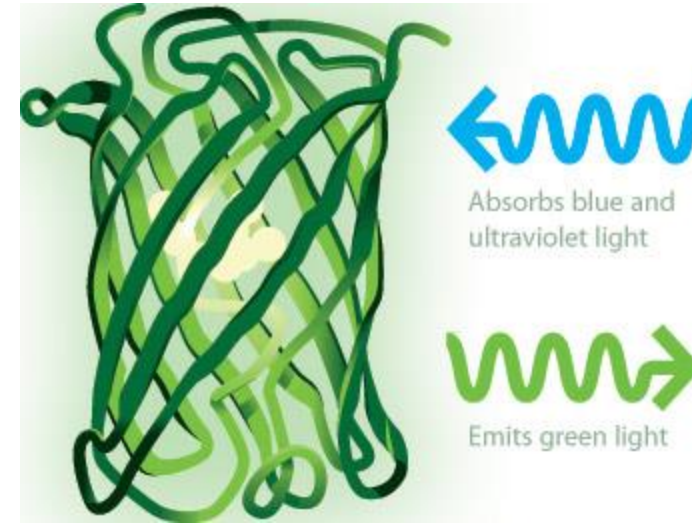
# Optogenetic *sensors* and *actuators*



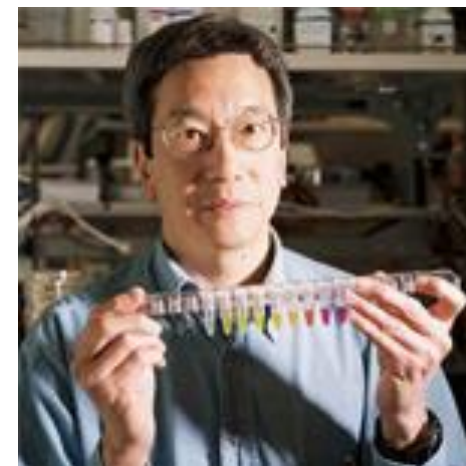
# A revolution in biotechnology caused by a protein from a jellyfish



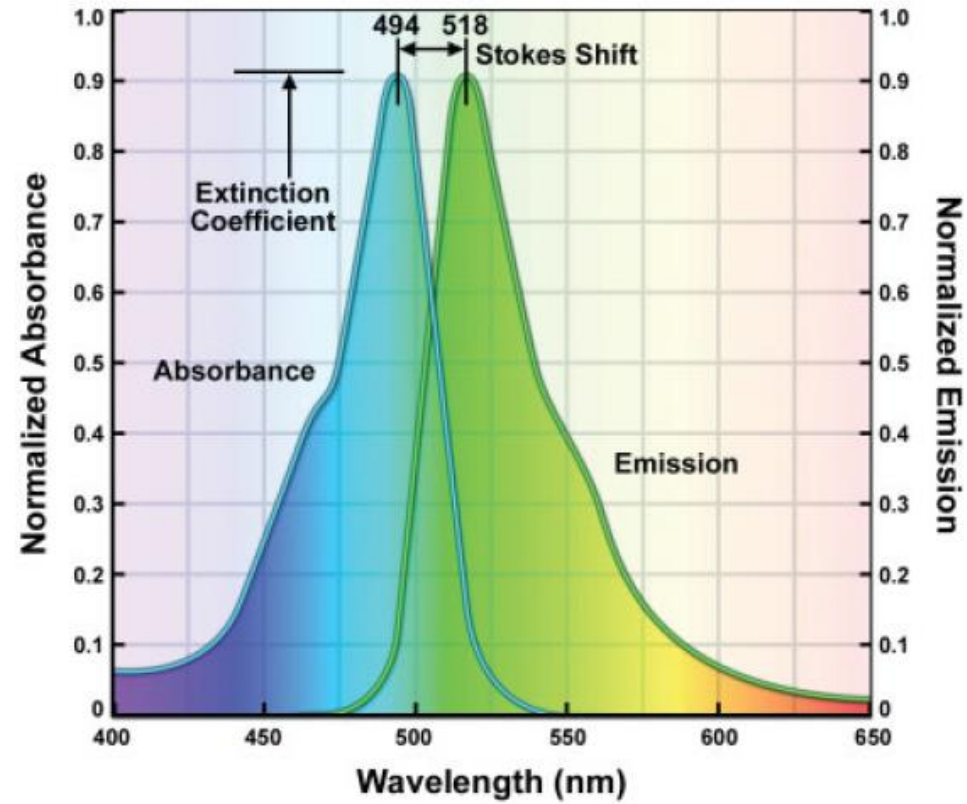
Green fluorescent protein



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



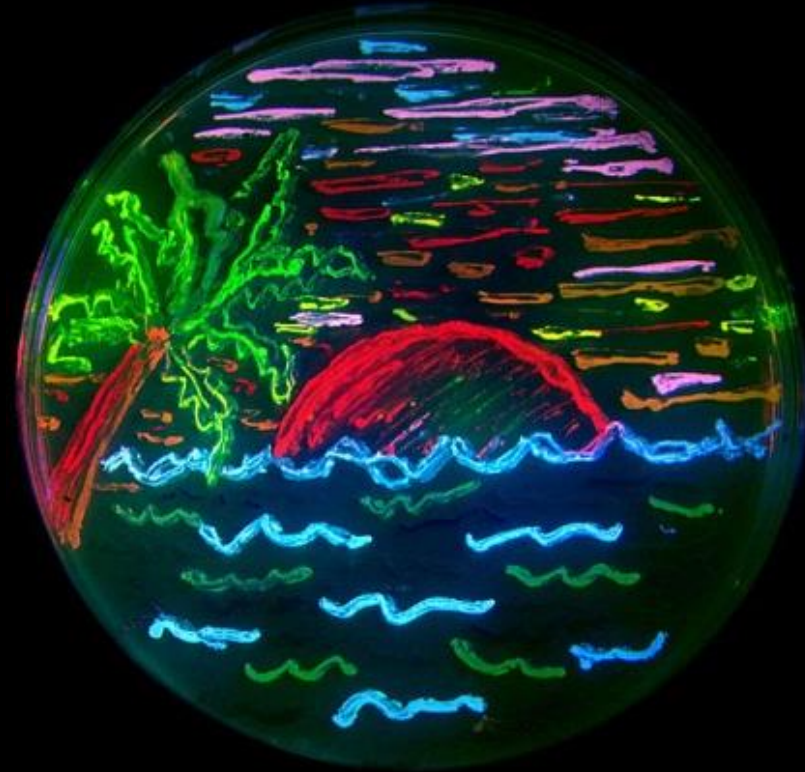
# Fundamentals of fluorescence



[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.

# Multicolored fluorescent proteins



**TABLE 11.2** Physical Properties of Useful Fluorescent Proteins

Protein <sup>a</sup>	Color <sup>b</sup>	Excitation (nm)	Emission (nm)	Brightness <sup>c</sup>	Photostability <sup>d</sup>	Filter Set <sup>e</sup>
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

<sup>a</sup> Common literature abbreviation.

<sup>b</sup> Spectral class.

<sup>c</sup> Product of the molar extinction coefficient and the quantum yield (mM × cm)<sup>-3</sup>.

<sup>d</sup> Relative to mEGFP (++++).

<sup>e</sup> Recommended filter set.

From Murphy and Davidson, Ch 11

# Circularly-permuted GFP and 'CAMgaroo'

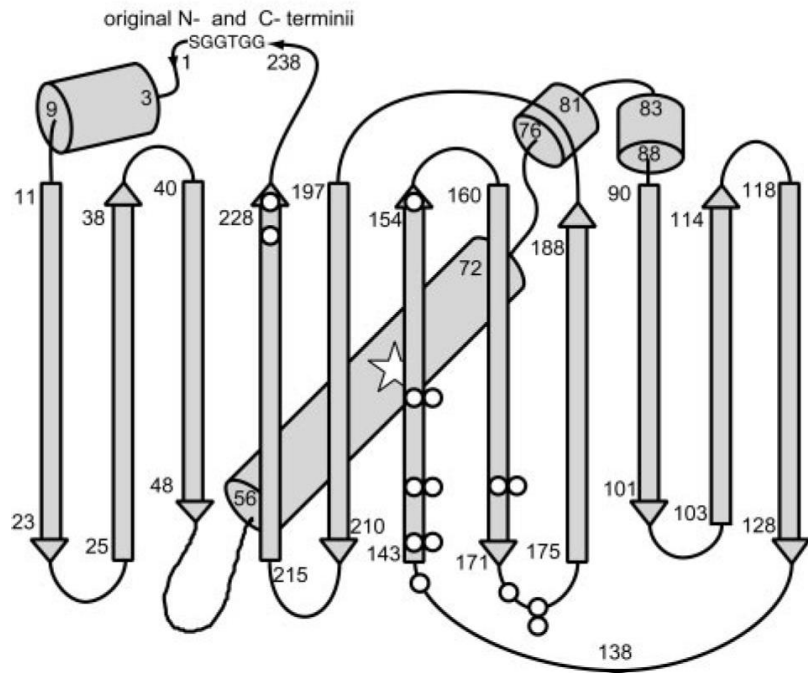


FIG. 2. Schematic drawing of the overall fold of GFP (12) modified to show starting points of fluorescent circular permutations (○), the linker (GGTGGG) connecting the original N and C termini, and the approximate location of the chromophore (open star, residues 65–67). Locations with two circles indicate where circular permutations with two different ending amino acids were isolated (Table 1).

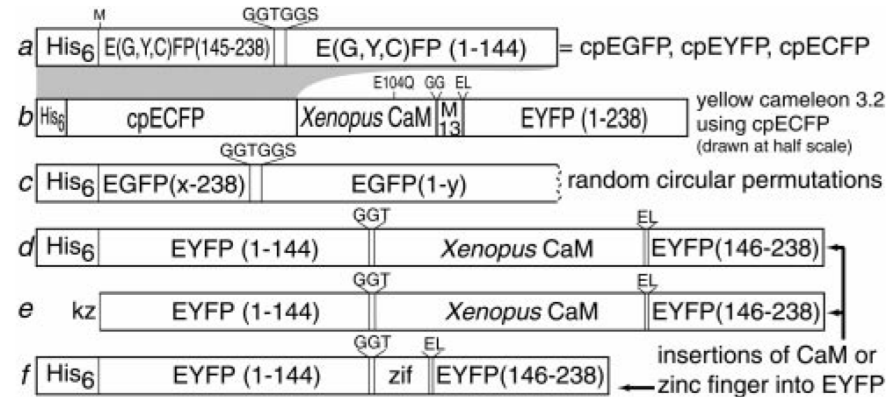
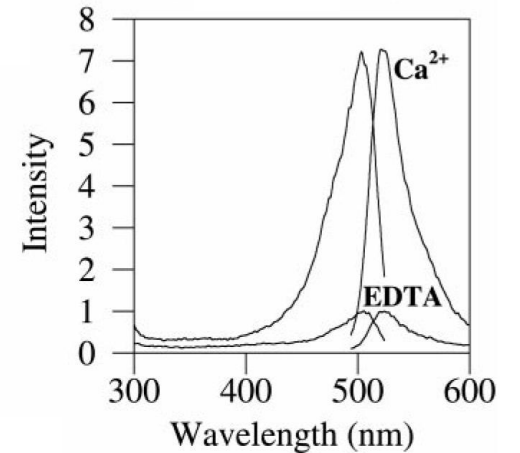


FIG. 1. Schematic structures of major new constructs. (a) Designed circular permutations of EGFP, EYFP, and ECFP starting at Y145M. His<sub>6</sub> indicates the polyhistidine tag MRGSHHHHHGMASMTG-GQQMGRDLYDDDDDKDP. Linkers and substitutions are shown above the main sequence. (b) Yellow cameleon 3.2 (YC3.2) incorporating cpECFP instead of ECFP. This sequence is drawn at half the scale of all the other constructs. M13 is the CaM-binding peptide derived from skeletal muscle myosin light chain kinase (7). (c) Random circular permutations of EGFP. The successful values of *x* and *y* are shown in Table 1. (d and e) Insertions of CaM in place of Y145 of EYFP as expressed in bacteria (d) for *in vitro* purification or in HeLa cells (e) for *in situ* monitoring of cytosolic Ca<sup>2+</sup>. kz, Kozak sequence (10) for optimal translation initiation. (f) Insertion of a zinc finger (zif), residues 334–362 of zif268 (8), in place of Y145 of EYFP.



An apt nickname for this construct is “camgaroo1,” because it is yellowish, carries a smaller companion (calmodulin) inserted in its “pouch,” can bounce high in signal, and may spawn improved progeny.



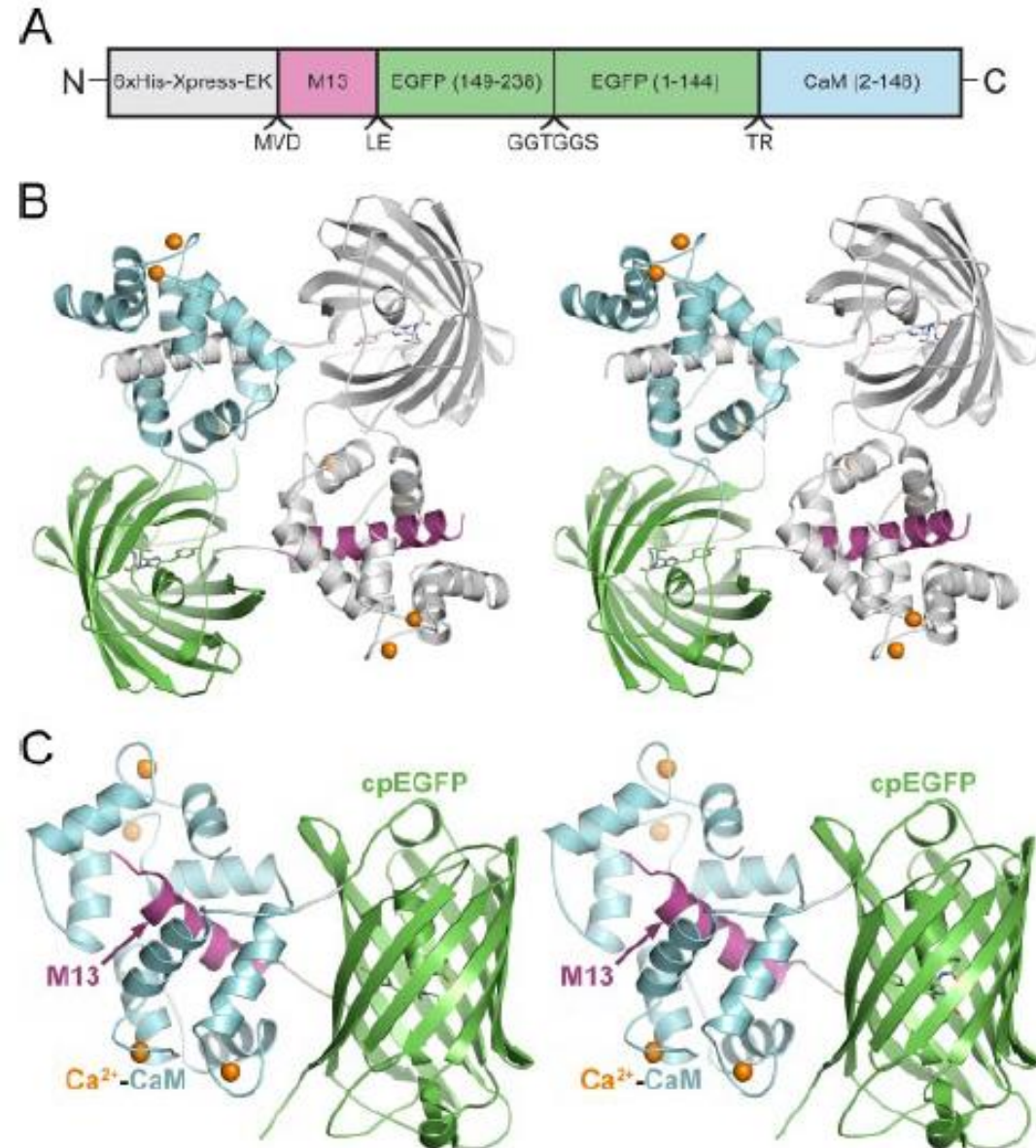
# The GCaMP family of calcium sensors

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

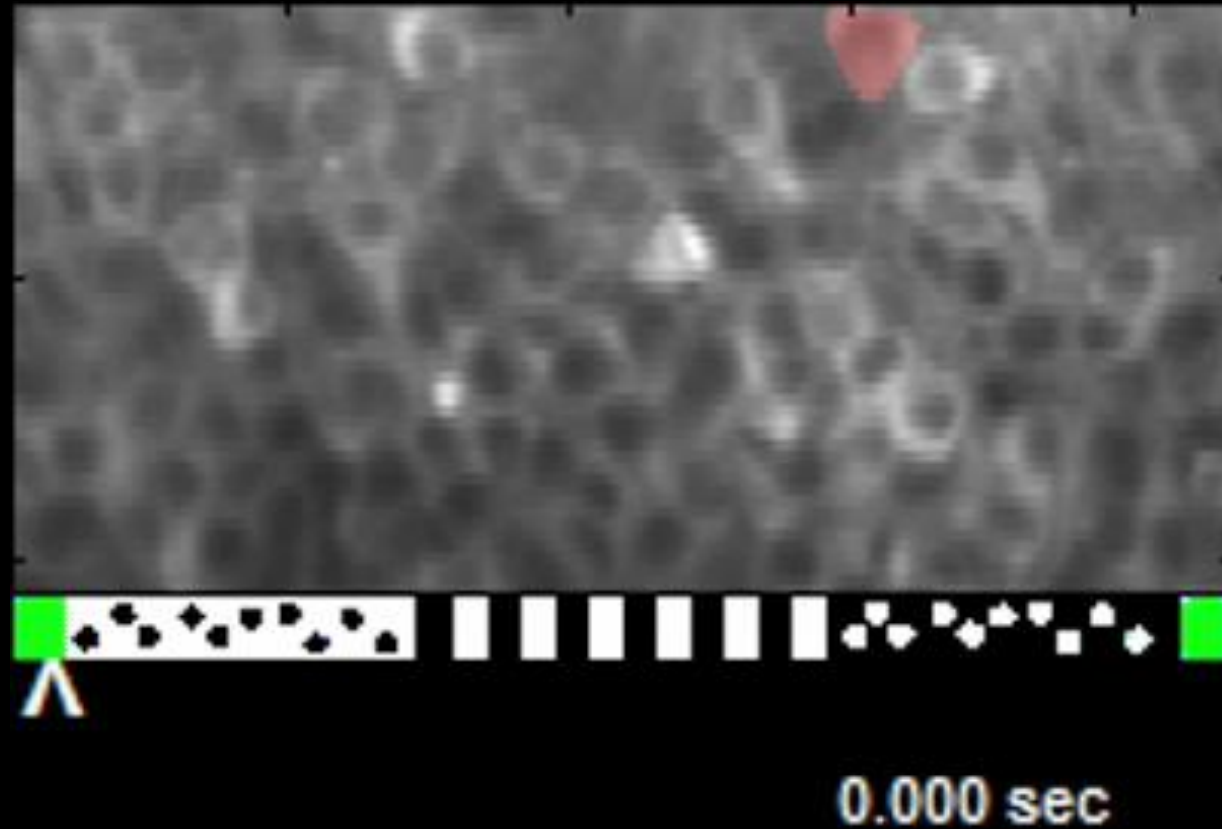
GCaMP6:  
Chen et al., 2013  
*Nature*, 499:295

See also **B-GECO** and  
**R-GECO**

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



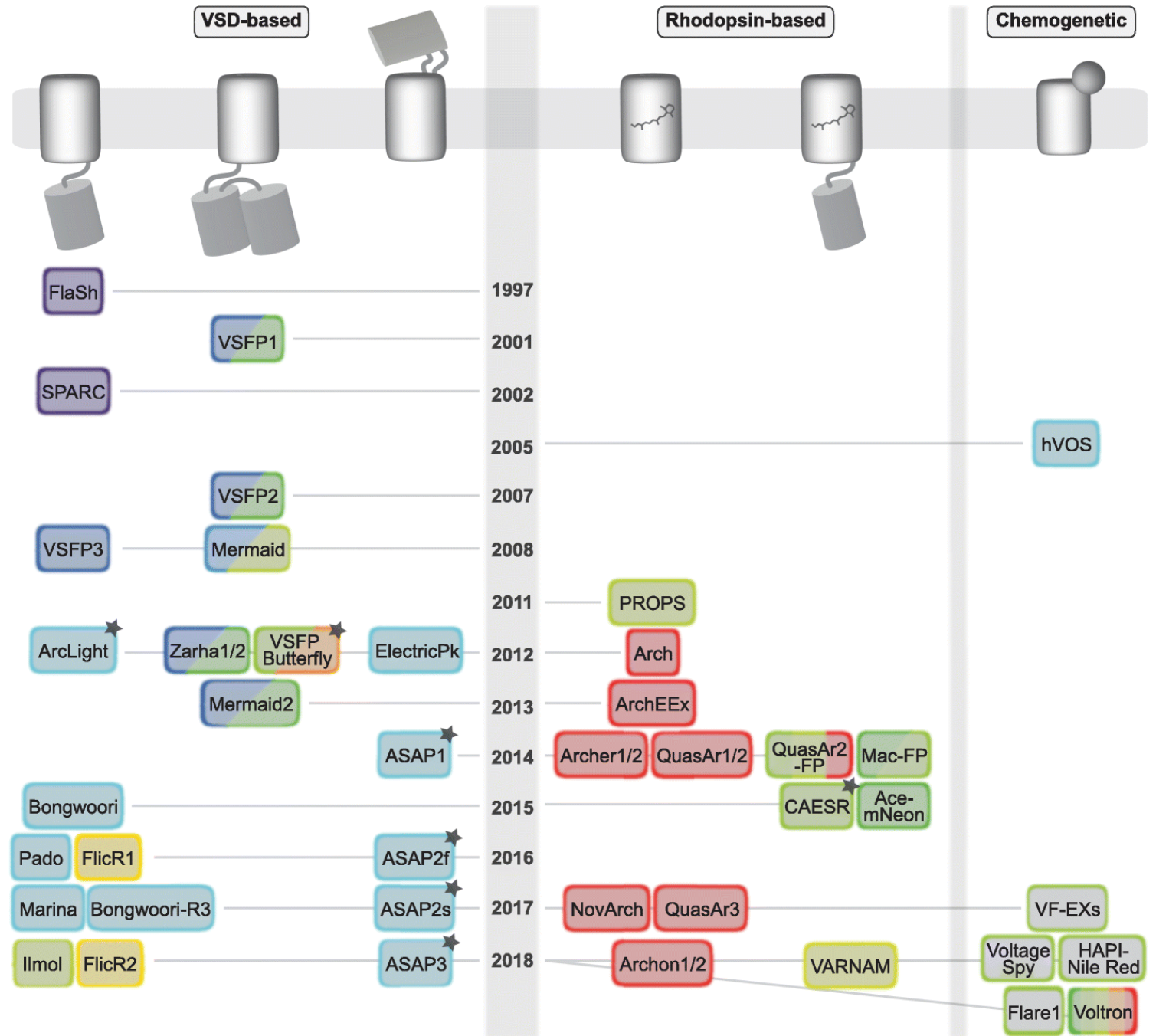
# Imaging place cells while the mouse navigates a virtual reality maze



- GCaMP3
- CA1 region

# Optical sensors of voltage

# Genetically encoded voltage sensing strategies



# All of the genetically-encoded voltage sensors compared...

**Table 1** Comparative performance of genetically targeted voltage indicators. Values extracted from the literature. *NR* not reported, *RT* room temperature

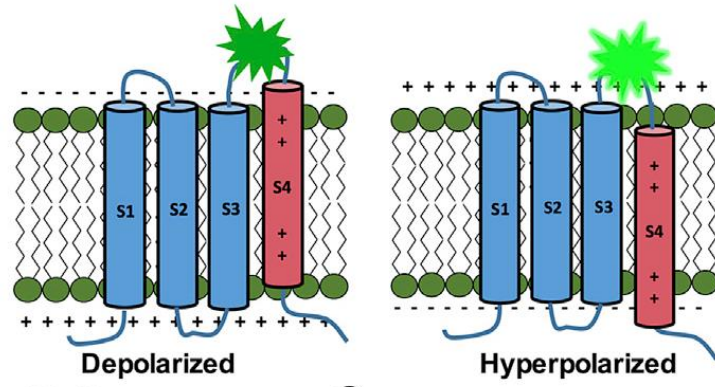
	$\lambda_{ex}$ [nm]	$\lambda_{em}$ [nm]	Rise time (depol.) [ms]	Decay time (hyperpol.) [ms]	Intensity $\sim$ [W/cm <sup>2</sup> ]	SNR	Res. spike rate [Hz]	$\Delta F/F$ for 100 mV	Bleaching	Reference
<i>VSD-based</i>										
ArLight Q239	480	520	$\tau_1 = 9$ (50%) $\tau_2 = 48$ (RT)	$\tau_1 = 17$ (79%) $\tau_2 = 63$ (RT)	Xenon arc lamp	3.7 per AP	10	-35%	$\tau = 244$ s	Jin 2012 [34]; Bando 2019 [49]
ArLight-MT	480	535	$\tau = 84.8$ (RT)	$\tau = 91.9$ (RT)	Mercury arc lamp	5.6 per AP	10	-20%	$\tau = 360$ s	Kwon 2017 [46]; Bando 2019 [49]
ASAP1	472	525	$\tau_1 = 2.1$ (60%) $\tau_2 = 71.5$ (RT)	$\tau_1 = 2.0$ (43.7%) $\tau_2 = 50.8$ (RT)	$10^{-1}$	14.6 per AP	200	-20%	$\tau = 35$ m	St-Pierre 2014 [38]
ASAP2f	470	525	$\tau_1 = 2.8$ (81%) $\tau_2 = 135$ (RT)	$\tau_1 = 2.4$ (71%) $\tau_2 = 155$ (RT)	$10^0$	5 per AP	100	-20%	$\tau = 404$ s	Yang 2016 [39]; Chamberland 2017 [41]
ASAP2s	480	525	$\tau_1 = 5.2$ (56%) $\tau_2 = 63$ (RT)	$\tau_1 = 24$ (49%) $\tau_2 = 106$ (RT)	$10^0$	8 per AP	100	-38%	$\tau_1 = 121$ s (69%) $\tau_2 = 1017$ s	Chamberland 2017 [41]
ASAP3	484	525	$\tau_1 = 3.7$ (81%) $\tau_2 = 48$ (RT)	$\tau_1 = 16$ (81%) $\tau_2 = 102$ (RT)	$10^{-1}$	2.6 per AP (2P)	100	-50%	19.6%/first 10 s 0.99%/m after that (2P)	Chavarha 2018 [40]
Bongwoori	472	496	$\tau_1 = 8$ (91%) $\tau_2 = 30$ (RT)	$\tau = 7$ (RT)	Xenon arc lamp	19 per AP	60	-15%	> 450 s	Piao 2015 [37]; Lee 2017 [91]
Bongwoori-R3	472	497	$\tau_1 = 7$ (90%) $\tau_2 = 45$ (RT)	$\tau_1 = 6$ (91%) $\tau_2 = 46$ (RT)	Xenon arc lamp	52 per AP	65	-20%	> 450 s	Lee 2017 [91]
FlicR1	561	595	$\tau_1 = 3$ (90%) $\tau_2 = 42$ (RT)	$\tau_1 = 2.8$ (70%) $\tau_2 = 18$ (RT)	$10^1$	6 per AP	100	6.40%	$\tau = 150$ s	Abdelfattah 2016 [42]; Kannan 2018 [44]
FlicR2	561	630	$\tau_1 = 2.9$ $\tau_2 = 29.5$ (RT)	$\tau_1 = 3.1$ $\tau_2 = 28.5$ (RT)	$10^0$	NR	NR	12.90%	NR	Kannan 2018 [44]
Marina	488	520	$\tau = 29.2$ (RT)	$\tau_1 = 15.6$ (61%) $\tau_2 = 59.4$ (RT)	$10^0$	4.5 per AP	NR	29.20%	$\tau = 206$ s	Platasa 2017 [43]
Mermaid	455	480 (donor) 575 (acceptor)	$\tau_1 \sim 12$ $\tau_2 \sim 200$ (RT)	$\tau \sim 128$ (RT)	$10^0$	NR	100	$\sim 30\%$ $\Delta R/R$	NR	Tsutsui 2008 [28]
VSFP Butterfly1.2	483	542 (donor) 594 (acceptor)	$\tau_1 \sim 1.5$ (35%) $\tau_2 \sim 15$ (RT)	NR	Xenon arc lamp	NR	40	5% $\Delta R/R$	NR	Akemann 2012 [92]
<i>Rhodopsin-based</i>										
Arch	640	687	< 1	< 1	$10^3$	NR	NR	40% at 640 nm	NR	Kralj 2012 [29]; Maclaurin 2013 [58]; Hochbaum 2014 [93]
Arch (D95N)	640	687	$\tau_1 = 0.5$ (20%) $\tau_2 = 41$ (RT)	NR	$10^3$	NR	NR	50% at 640 nm	NR	Kralj 2012 [29]; Maclaurin 2013 [58]
Archer1	655	NR	Like Arch	Like Arch	$10^2$	NR	40	85% at 655 nm	NR	Flytzanis 2014 [53]
QuasAr1	640	715	$\tau_1 = 0.05$ (94%) $\tau_2 = 3.2$ (RT)	Similar to rising	$10^2$	20-30 per AP	NR	32% at 640 nm	$\tau = 440$ s	Hochbaum 2014 [93]

**Table 1** Comparative performance of genetically targeted voltage indicators. Values extracted from the literature. *NR* not reported, *RT* room temperature (Continued)

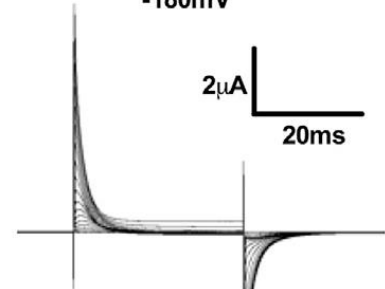
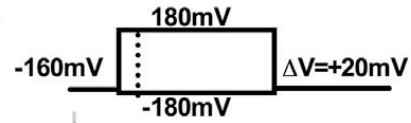
	$\lambda_{ex}$ [nm]	$\lambda_{em}$ [nm]	Rise time (depol.) [ms]	Decay time (hyperpol.) [ms]	Intensity $\sim$ [W/cm <sup>2</sup> ]	SNR	Res. spike rate [Hz]	$\Delta F/F$ for 100 mV	Bleaching	Reference
QuasAr2	640	715	$\tau_1 = 1.2$ (68%) $\tau_2 = 11.8$ (RT)	Similar to rising	$10^2$	40-70 per AP	NR	90% at 640 nm	$\tau = 1020$ s	Hochbaum 2014 [93]
QuasAr3	Like QuasAr2	Like QuasAr2	$\tau_1 = 1.2$ (77%) $\tau_2 = 10.0$ (34 °C)	$\tau_1 = 0.9$ (91%) $\tau_2 = 9.0$ (34 °C)	$10^2$	27 per AP	NR	54% at 640 nm	NR	Adam 2018 [55]
Archon1	637	NR	$\tau_1 = 0.6$ (88%) $\tau_2 = 8.1$ (34 °C)	$\tau_1 = 1.1$ (88%) $\tau_2 = 1.3$ (34 °C)	$10^1-10^2$	21 per AP	NR	43% at 637 nm	0.01%/s	Piatkevich 2018 [57]
Archon2	637	NR	$\tau_1 = 0.6$ (70%) $\tau_2 = 6.7$ (34 °C)	$\tau_1 = 0.17$ (92%) $\tau_2 = 7.0$ (34 °C)	$10^1-10^2$	16 per AP	200	19% at 637 nm	0.03%/s	Piatkevich 2018 [57]
QuasAr2-mOrange	549	565	$\tau_1 = 3.9$ (60%) $\tau_2 = 27$ (23 °C)	$\tau_1 = 4.3$ (45%) $\tau_2 = 26$ (23 °C)	$10^1$	9 per AP	NR	-10%	NR	Zou 2014 [61]
MacQ-mCitrine	515	530	$\tau_1 = 2.8$ (74%) $\tau_2 = 71$ (RT)	$\tau_1 = 5.4$ (77%) $\tau_2 = 67$ (RT)	$10^1$	NR	NR	-20%	1.3%/s	Gong 2014 [60]; Gong 2015 [52]
Acc2-4aa-mNeon	505	515	$\tau_1 = 0.37$ (58%) $\tau_2 = 5.5$ (RT)	$\tau_1 = 0.5$ (60%) $\tau_2 = 5.9$ (RT)	$10^1$	NR	NR	-12%	0.6%/s	Gong 2015 [52]
VARNAM	558	605	$\tau_1 = 0.88$ $\tau_2 = 5.2$ (RT)	$\tau_1 = 0.80$ $\tau_2 = 4.7$ (RT)	$10^1$	36 per AP	100	-14% for 120 mV	$\tau = 256$ s	Kannan 2018 [44]
<i>Chemogenetics based</i>										
HVOS	480	535	< 1	< 1	Mercury arc lamp	NR	667	34%	NR	Chanda et al. 2005 [70]
Flare1	488	570	$\tau_1 = 0.92$ (96%) $\tau_2 = 23.5$	$\tau_1 = 1.41$ (91%) $\tau_2 = 25.6$	$10^0$	53	20	35.9%	NR	Xu et al. 2018 [74]
Voltron 525	532	553	$\tau_1 = 0.64$ (61%) $\tau_2 = 4.1$	$\tau_1 = 0.78$ (55%) $\tau_2 = 3.9$	$10^0$	4.4	10	30%	$\tau = 206$ s	Abdelfattah 2016 [42]
VF-EX	525	540	< 1	< 1	NR	20	3	21%	NR	Liu et al. 2017 [77]
VoltageSpy	525	540	< 1	< 1	$10^{-1}-10^0$	7.7	NR	60%	NR	Grenier et al. 2019 [82]
HAPI-Nile	540-552	581	$\tau_1 = 1.9$ (85%)	$\tau_1 = 1.9$ (85%)	$10^1$	12.4	10	5.50%	NR	Sundukova et al. 2019 [83]

# ASAP1, a VSD-based indicator using a circularly permuted GFP

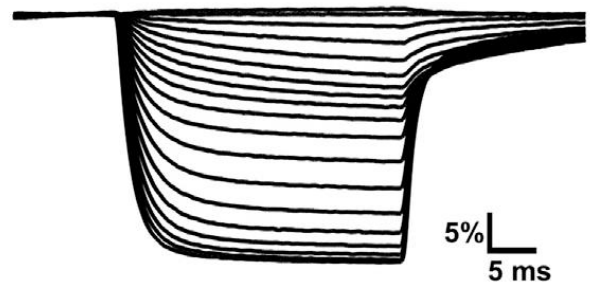
A



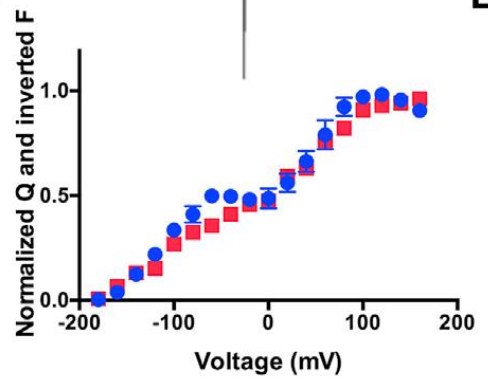
B



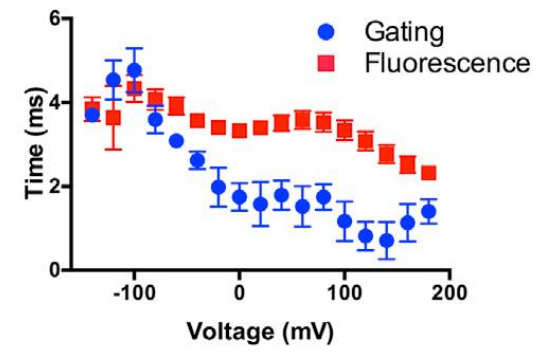
C



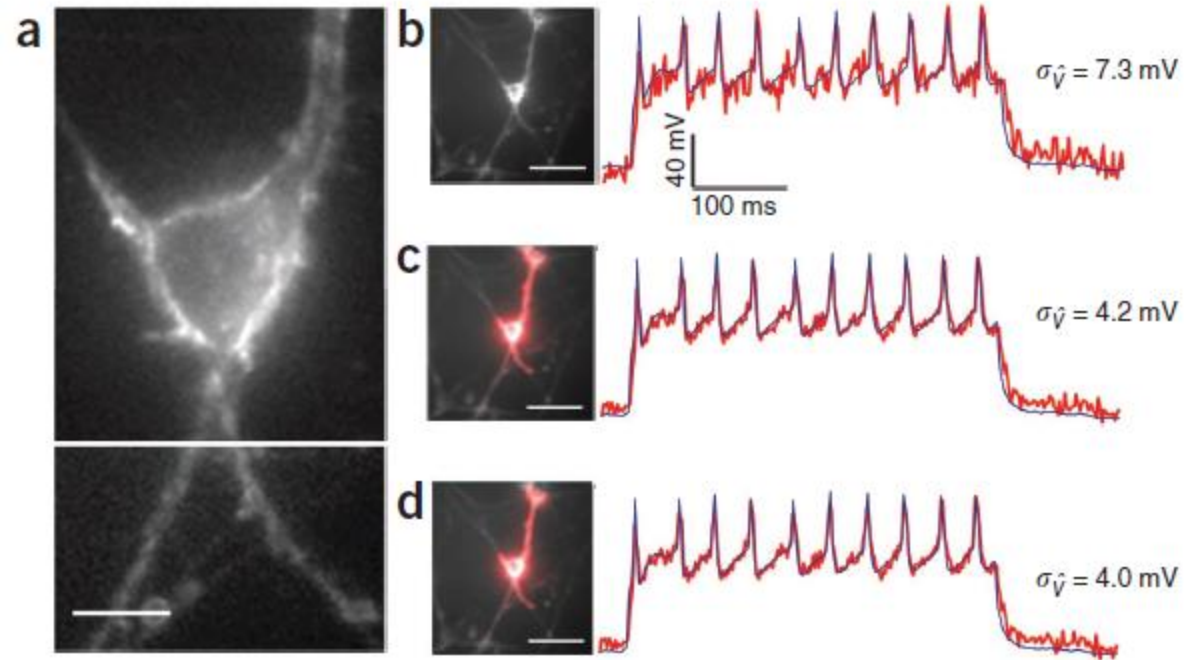
D



E



# Archaeorhodopsin 3, a rhodopsin-based voltage indicator - fast but low QY and super dim

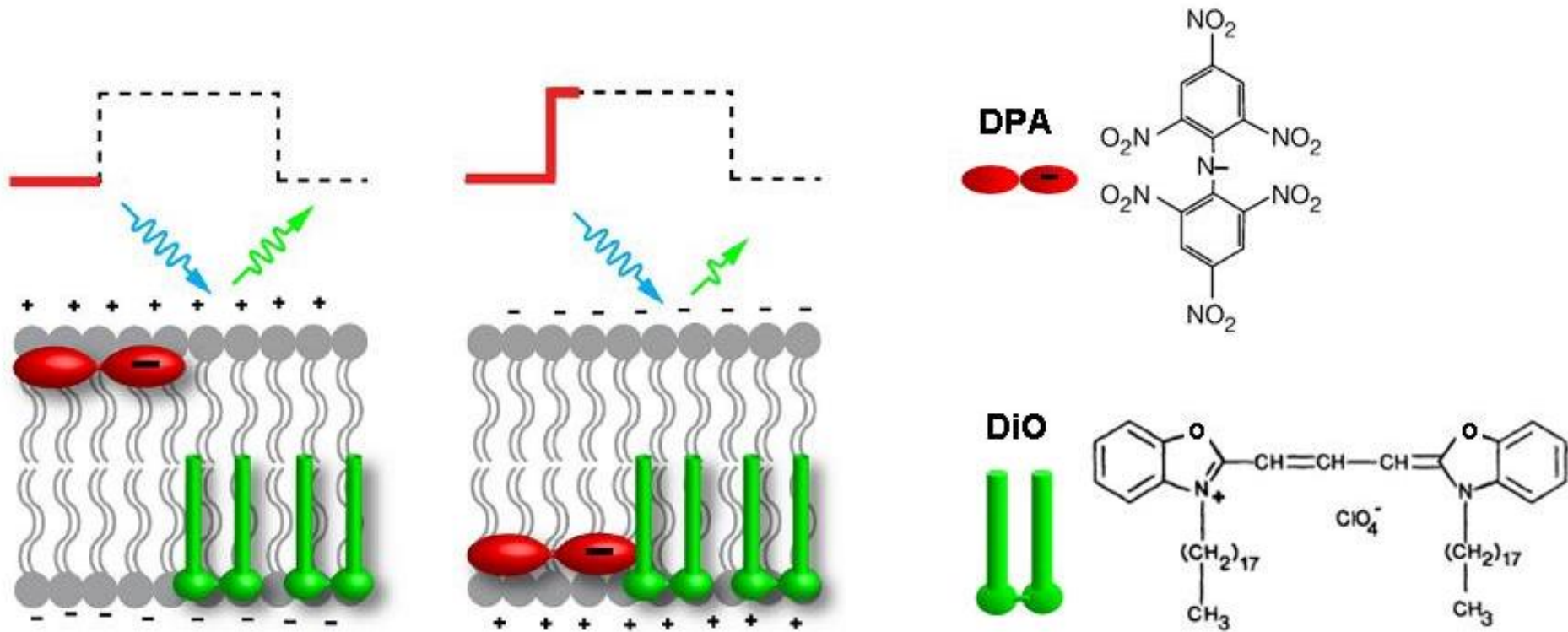


Kralj et al., *Nat. Methods*, 9:90, 2011

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

	$\lambda_{\max}$ absorbance (nm)	$\lambda_{\max}$ emission (nm) <sup>a</sup>	$\epsilon_{633}$ ( $M^{-1} \text{ cm}^{-1}$ ) <sup>b</sup>	QY <sup>c</sup>	Photostability relative to eGFP <sup>d</sup>	pK <sub>a</sub> of Schiff base <sup>e</sup>	$\tau_{\text{response}}$ (ms) <sup>f</sup>	Noise in $\hat{V}_{FL}$ ( $\mu\text{V Hz}^{-0.5}$ ) <sup>g</sup>	Photo-current
Arch	558	687	6,300	$9 \times 10^{-4}$	0.25	10.1	<0.5	625	Yes
Arch(D95N)	585	687	37,500	$4 \times 10^{-4}$	0.1	8.9	41	260	No

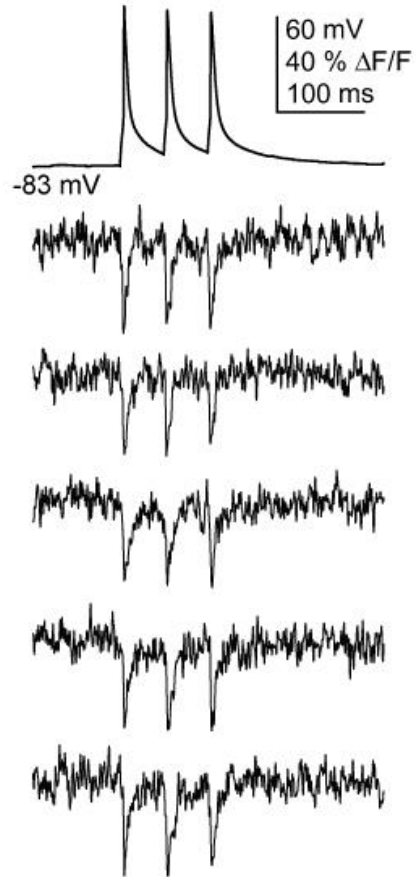
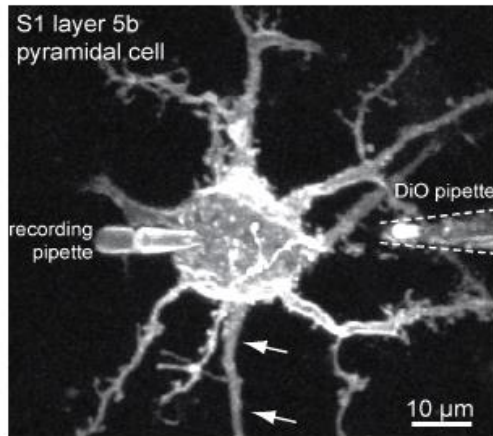
# A non-genetic voltage sensor that relies on FRET-based quenching



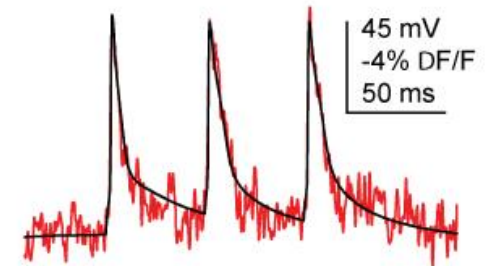
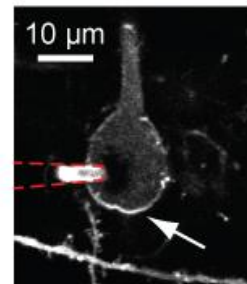
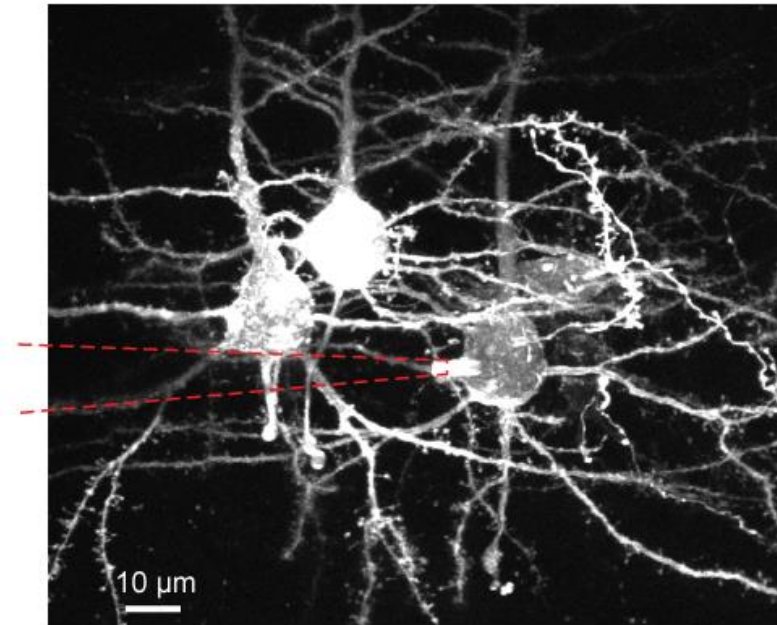


# Two photon compatibility, high SNR

A

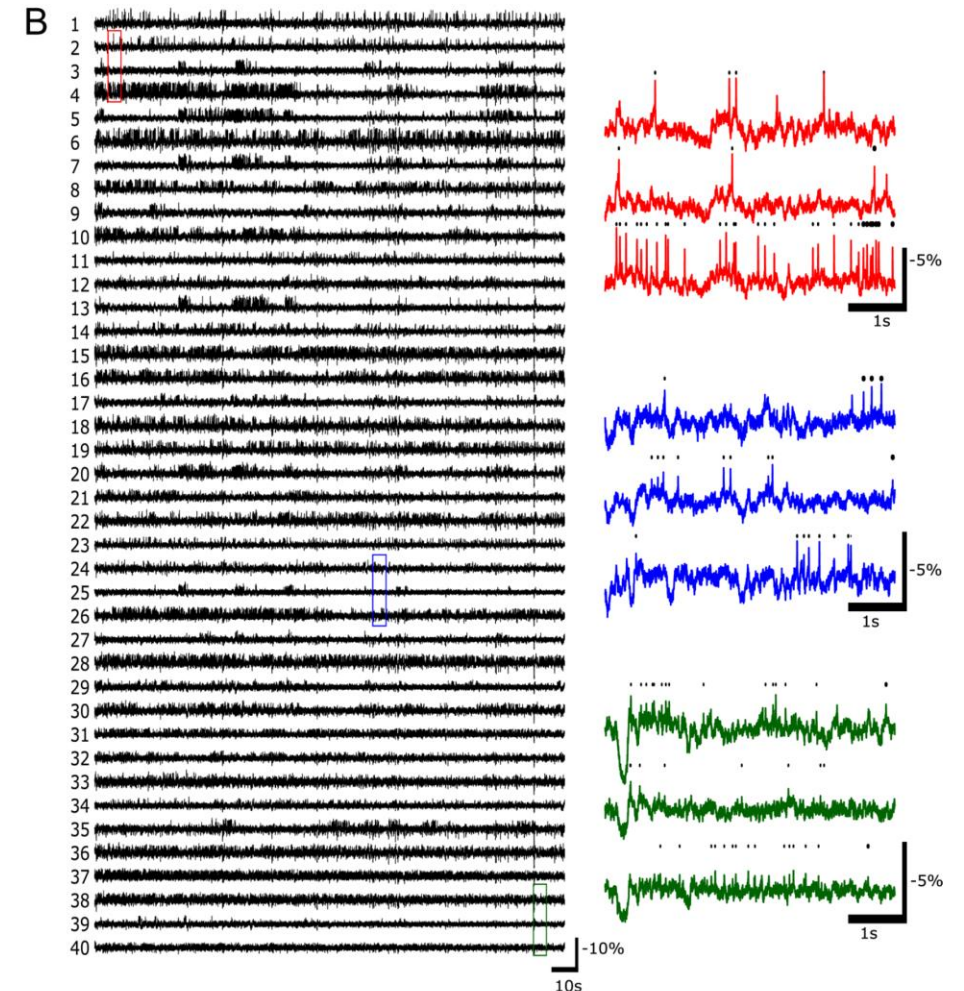
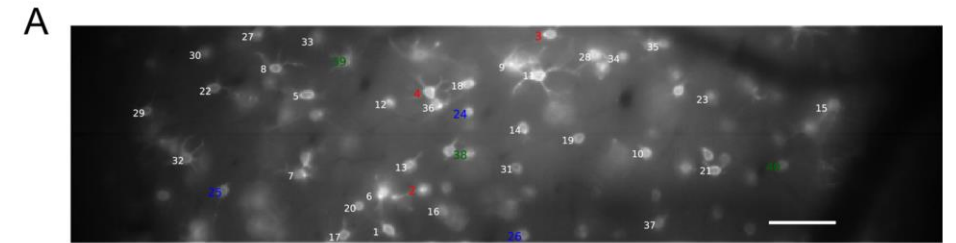
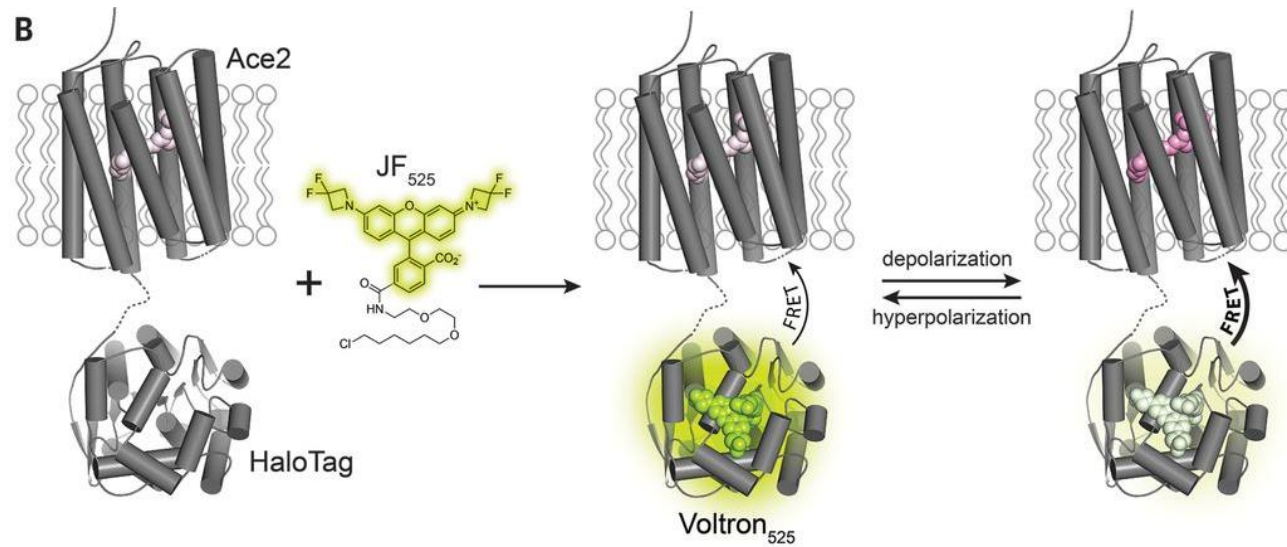


B



$\lambda = 940 \text{ nm}$   
3  $\mu\text{M}$  DPA

# Voltron, a 'modular' chemogenetic-based voltage sensor



# Essential things to consider in comparing GEVIs

## photophysical properties

- QY, brightness
- 2P compatibility
- kinetics
- SNR
- bleaching
- linearity

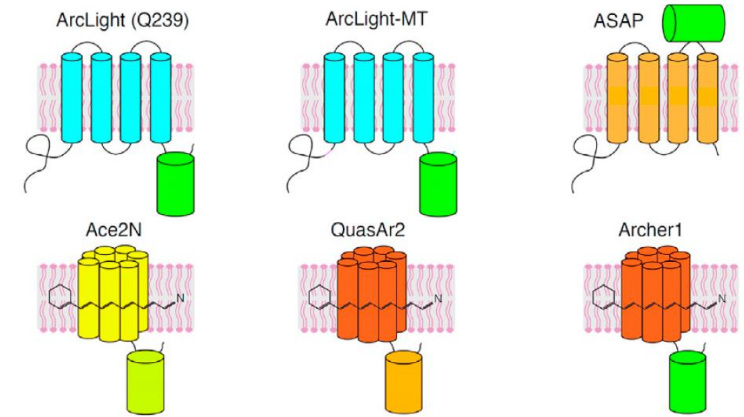
## ease of expression, protein trafficking

- if it's not in the PM its background

## instrumentation

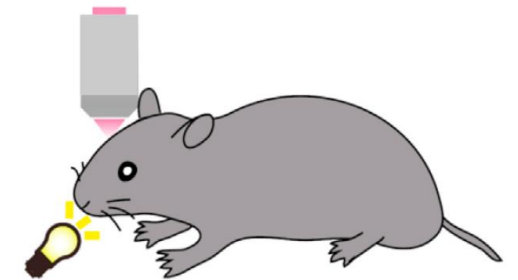
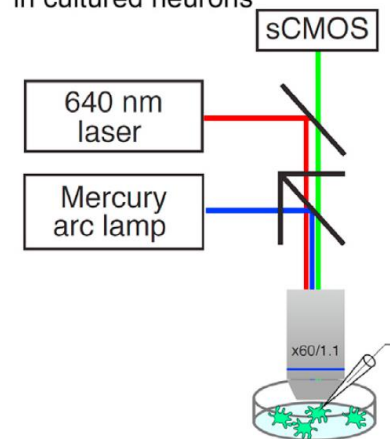
## other challenges

- tolerance/side effects for chemo-based
- capacitive load for VSD-based



1. One-photon imaging  
in cultured neurons

2. One-photon wide-field and two-photon  
imaging in vivo



Bando et al., *Cell Reports*, 2019

See also Box 1 from Bando et al, *BMC Biology*, 2019



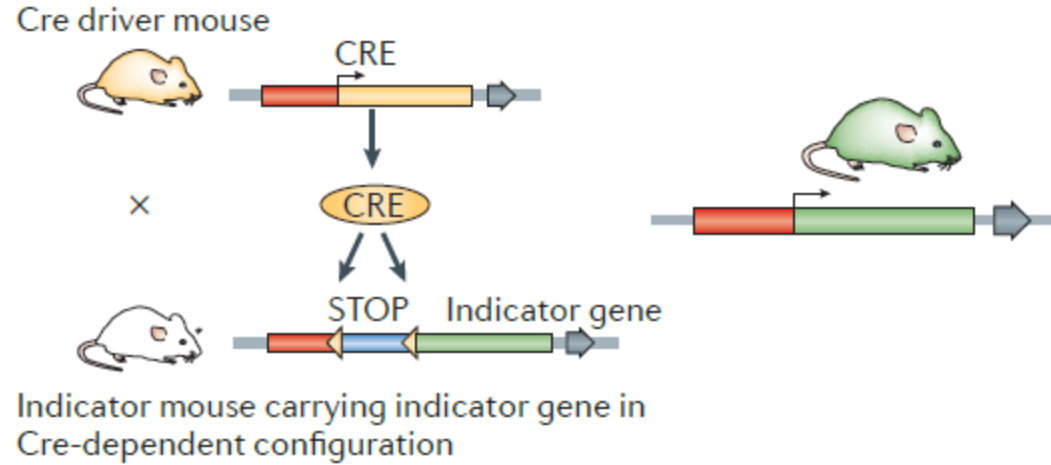
*Unfinished Marx-Engels sculpture at Ludwig Engelhardt's studio in Gummlin, Usedom, Sybille Bergemann, 1984*

**More from Adam Cohen @ SWC Virtual Tea Hour,  
29 May 2020**

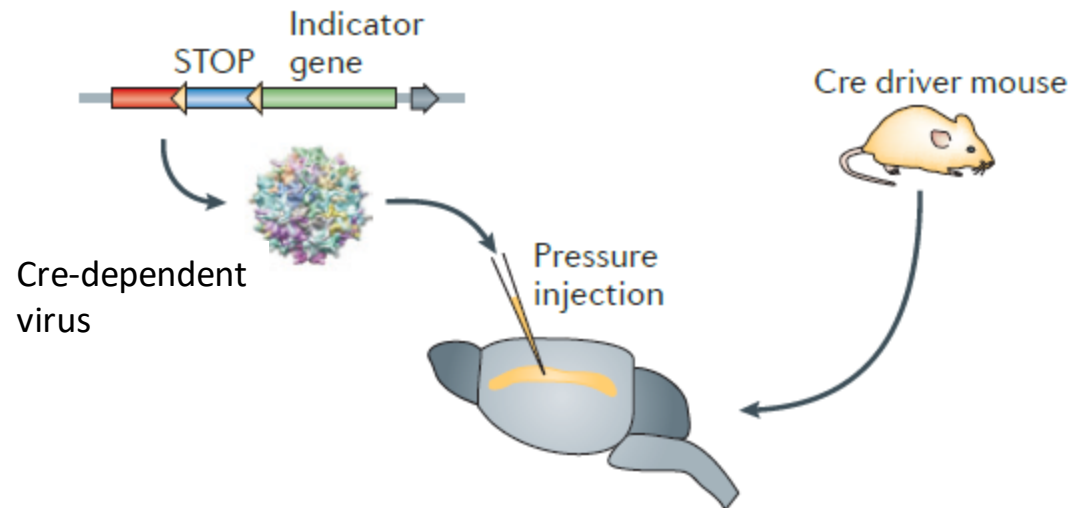
**Backup/extra**

# Conditional genetics and lab mice

## Breeding strategy



## Viral strategy



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

# CAMPARI, a conditional integrator of neural activity

