

# Functional imaging from a data science perspective

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CSHL Neural Data Science

I will send you my slides

- And of course any reagents that you need as well

# Functional imaging

- Probe (exogenous, endogenous)
- Get light in
- Get light out
- Figure out where your signal was (if possible)
- Convert signal to [analyte] (if desired & if possible)

# Probes

- Small molecules
- Proteins
- Protein-small molecule hybrids

# Important probe properties

- Signal change magnitude
- Sensitivity
- Linearity / non-linearity
- Rise & decay kinetics
- Dynamic range
- Spectral properties (color, purity)
- Photostability
- Cell-to-cell comparability

# Important probe properties

- **Signal change magnitude**
- **Sensitivity**
- **Linearity / non-linearity**
- **Rise & decay kinetics**
- **Dynamic range**
- Spectral properties (color, purity)
- Photostability
- Cell-to-cell comparability

# Other important probe properties

- Doesn't mess up cells/sample
- Properties stable over time
- Not too big (fit in virus, fuse to things, etc.)
- Can express at good concentration
- Specificity for target molecule/state

# Important sample properties

- Light penetration
  - Background fluorescence
  - Movement artifacts
  - How well it expresses things
  - Buffering (competition with sensor)
  - Temperature
- 
- Virus, transgenic, electroporation, etc.

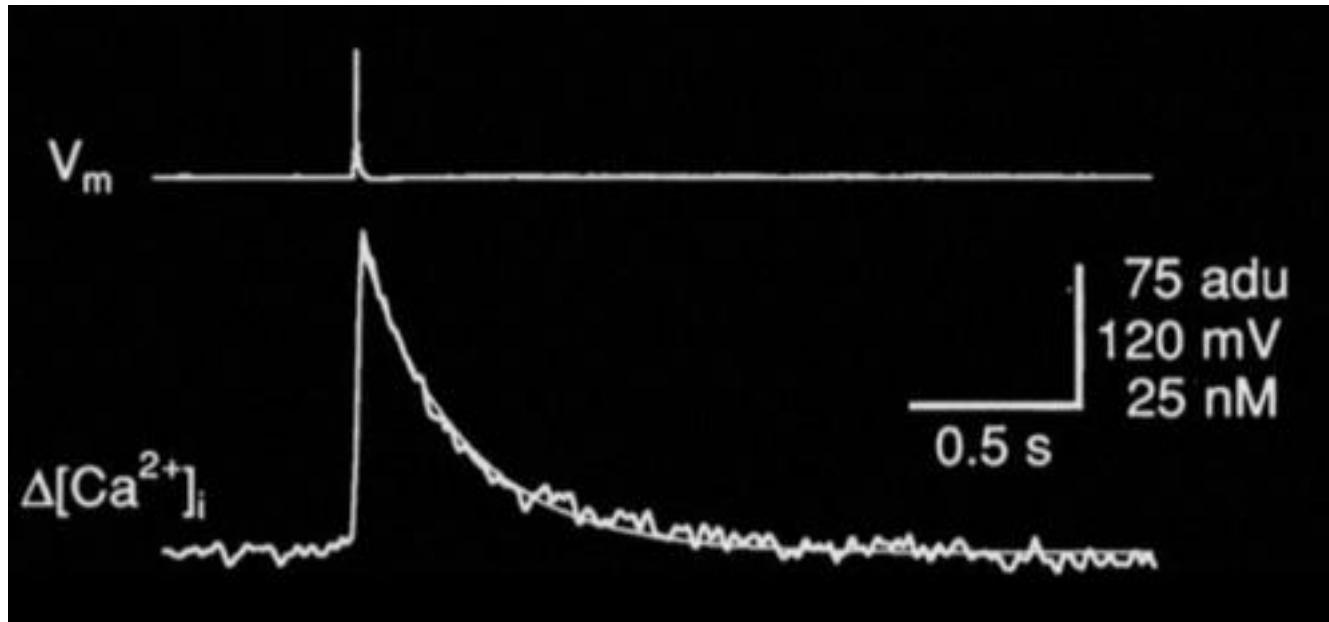
# Major classes of probes

- Calcium
- Voltage
- Neurotransmitter/neuromodulator
- Other signaling molecules

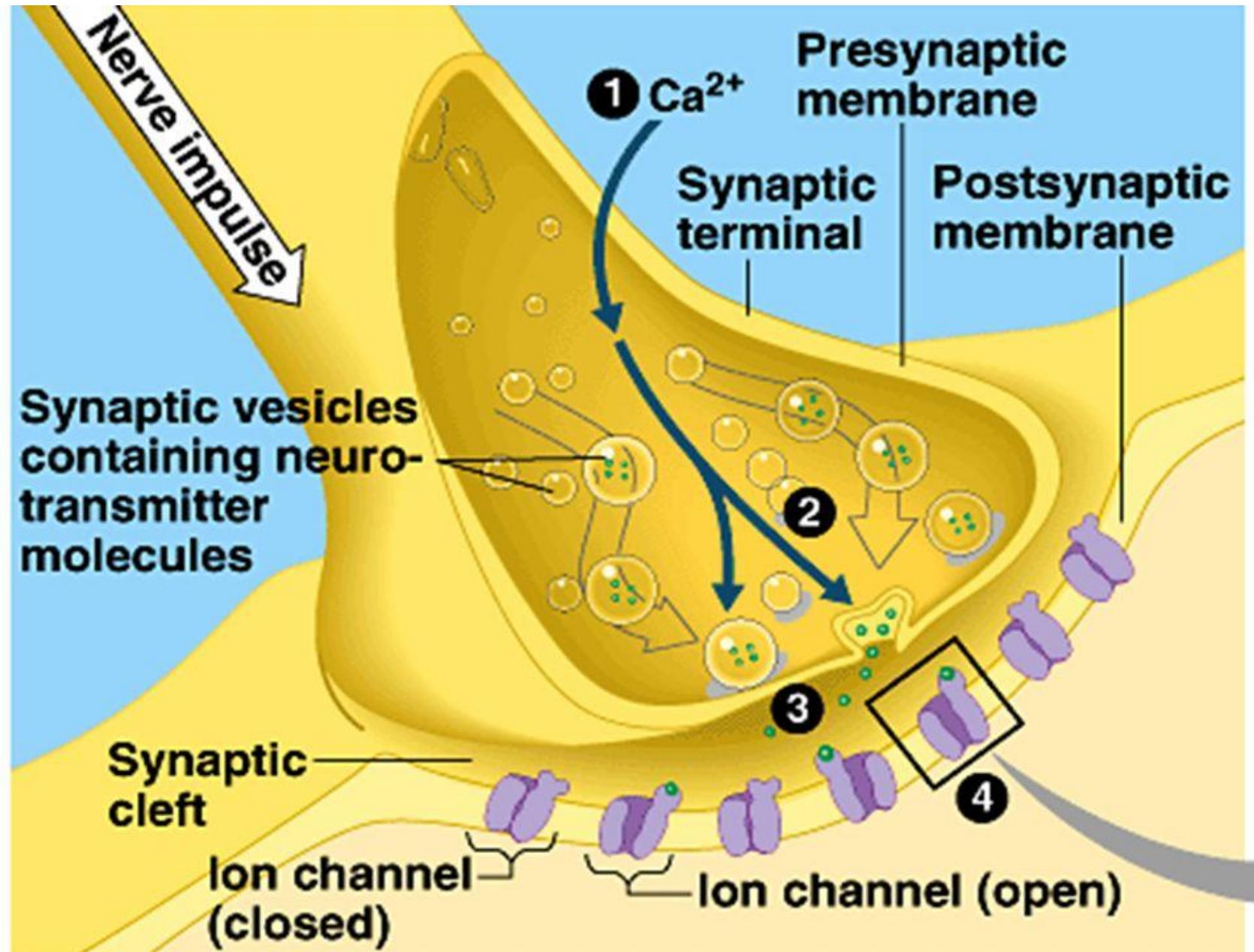
# Calcium



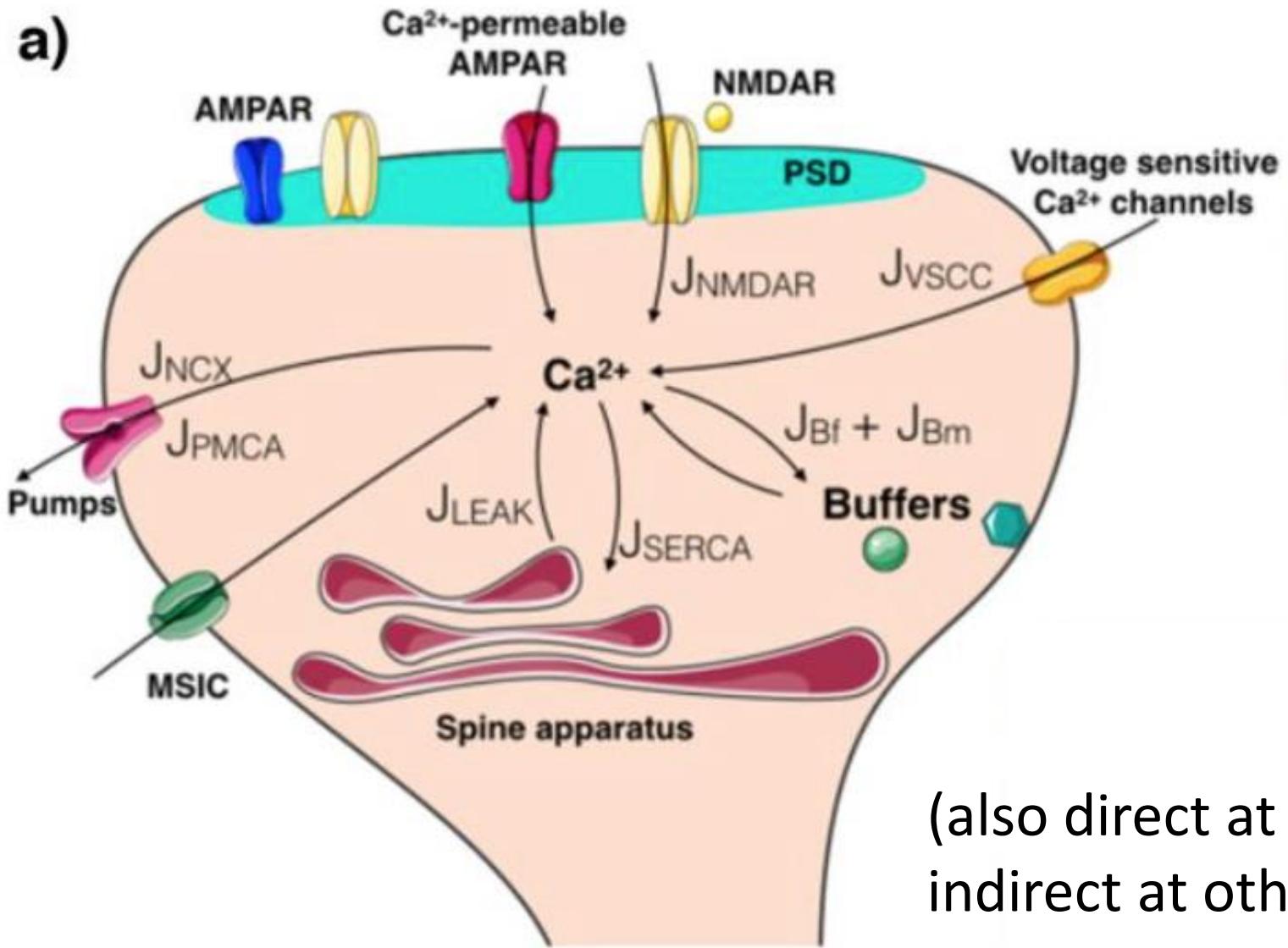
# Calcium



$[\text{Ca}^{2+}]$  is a proxy for neural activity



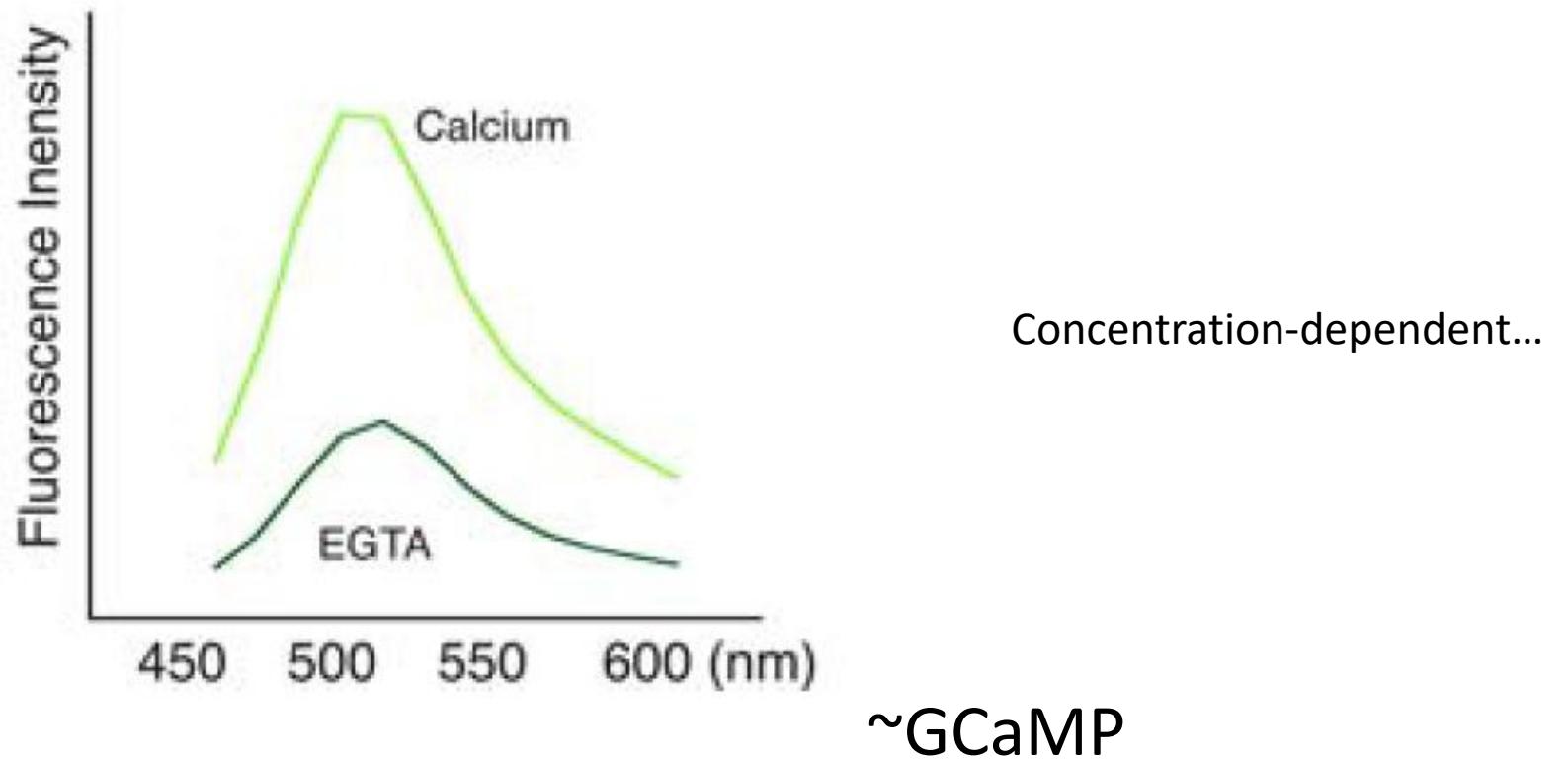
a)



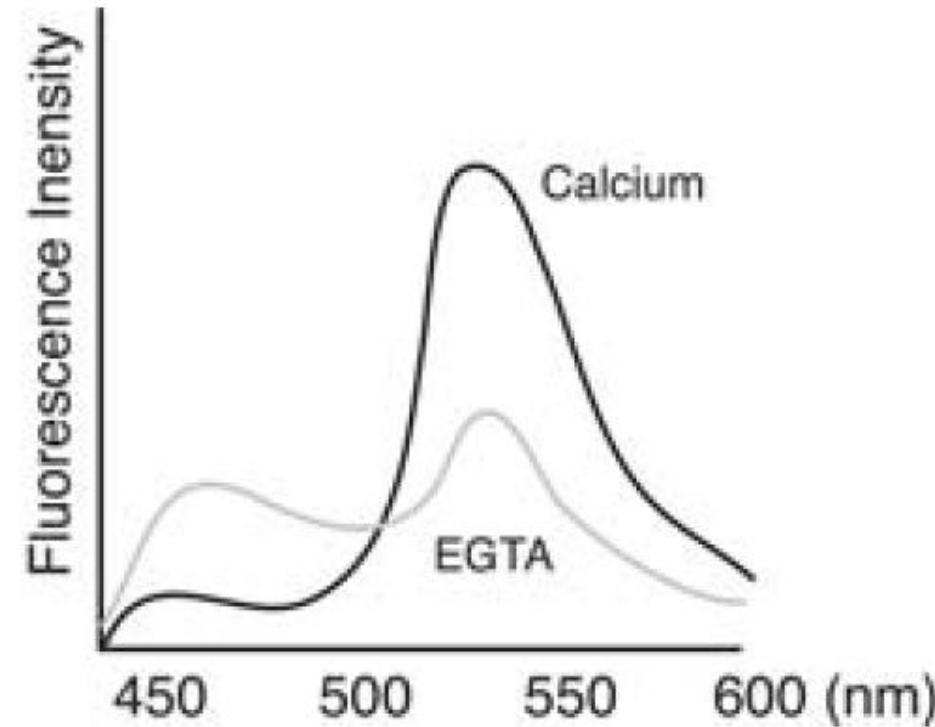
(also direct at ACh synapses,  
indirect at others)

# Fluorescence modalities

# Intensity



# FRET

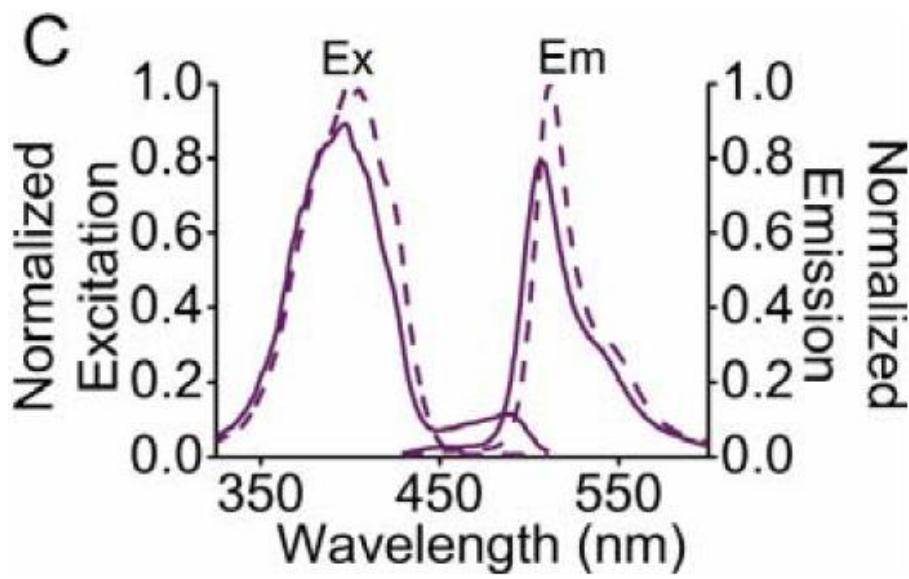


Some headaches...

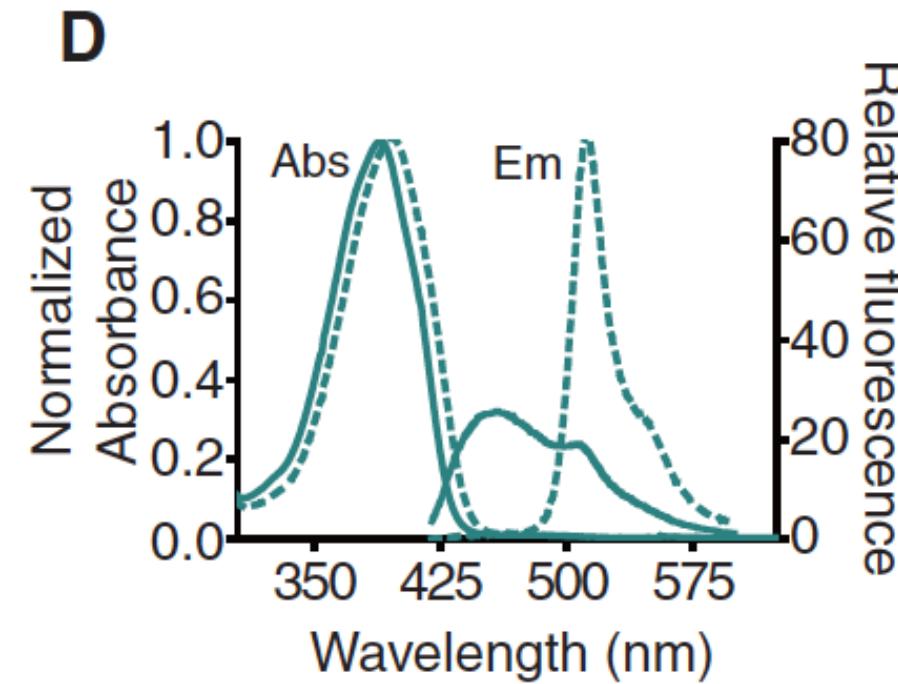
~Cameleon

# Spectral shift

~GEX-GECO



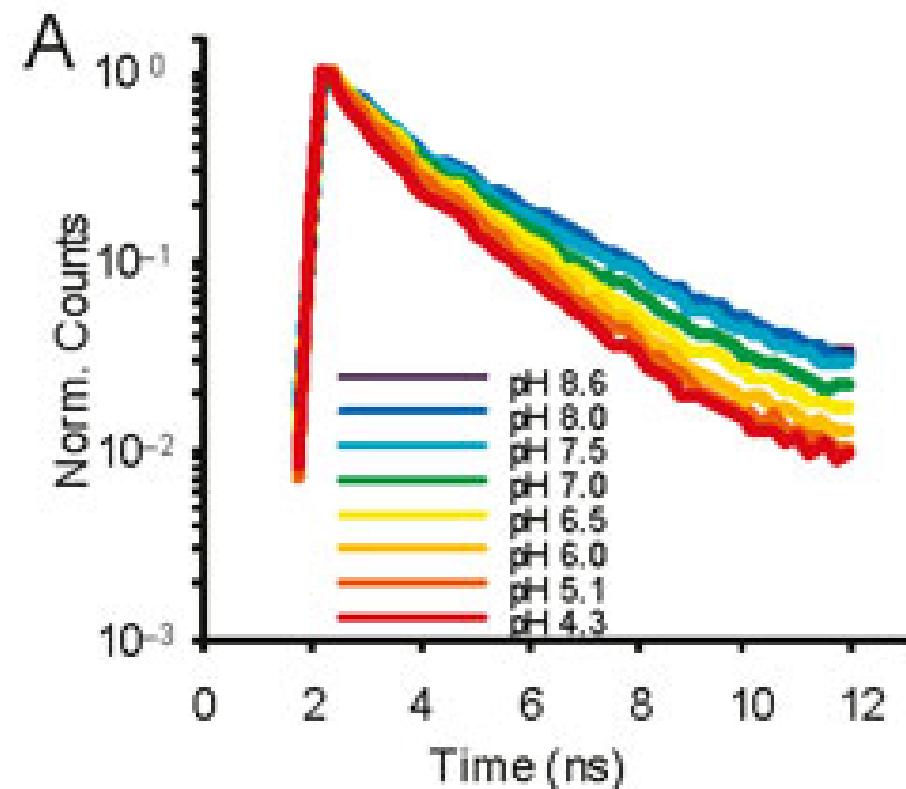
~GEM-GECO



# Fluorescence lifetime

**Table 1.** The calcium-dependent amplitudes and lifetimes of the fluorescence intensity decay components of CalciumGreen.

[Ca <sup>2+</sup> ] (μM)	$\alpha_1$	$\tau_1$ (ns)	$\alpha_2$	$\tau_2$ (ns)	$\chi^2$
<0.005	0.97	0.41	0.026	3.37	1.62
0.01	0.87	0.42	0.13	3.56	1.20
0.05	0.82	0.43	0.18	3.53	1.81
0.08	0.79	0.43	0.21	3.54	1.18
0.10	0.73	0.44	0.27	3.55	1.10
0.15	0.65	0.45	0.35	3.56	1.06
0.25	0.45	0.46	0.55	3.53	1.27
0.40	0.31	0.46	0.69	3.54	1.20
0.60	0.24	0.46	0.76	3.54	1.21
5.0	0.12	0.47	0.88	3.60	1.16



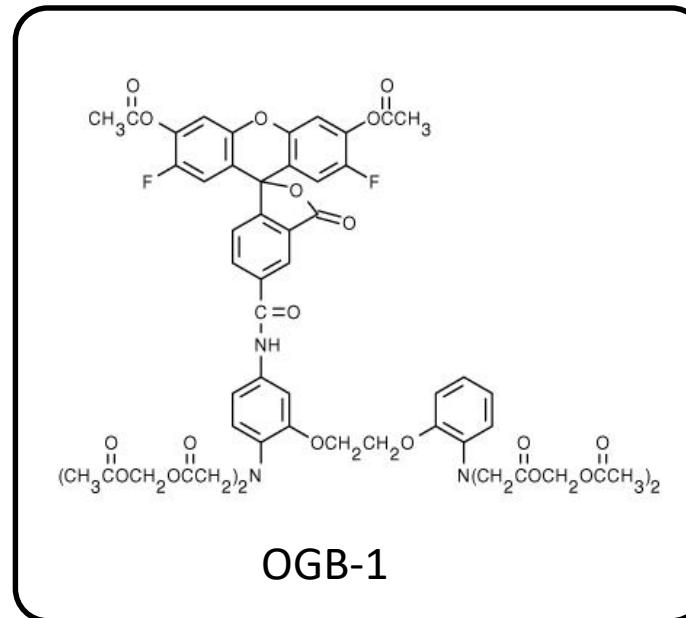
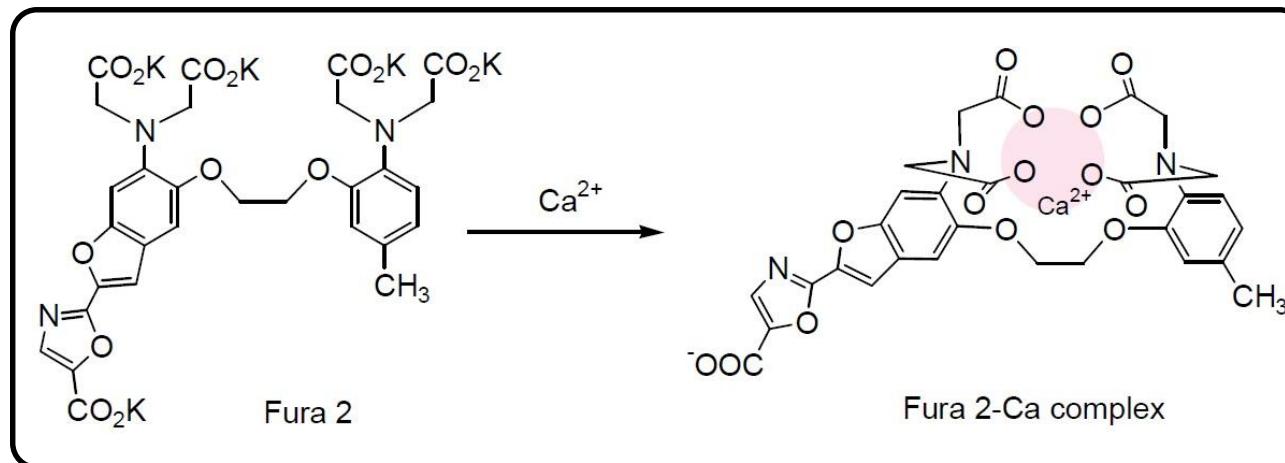
Fixes some things, headaches...

# Imaging modalities

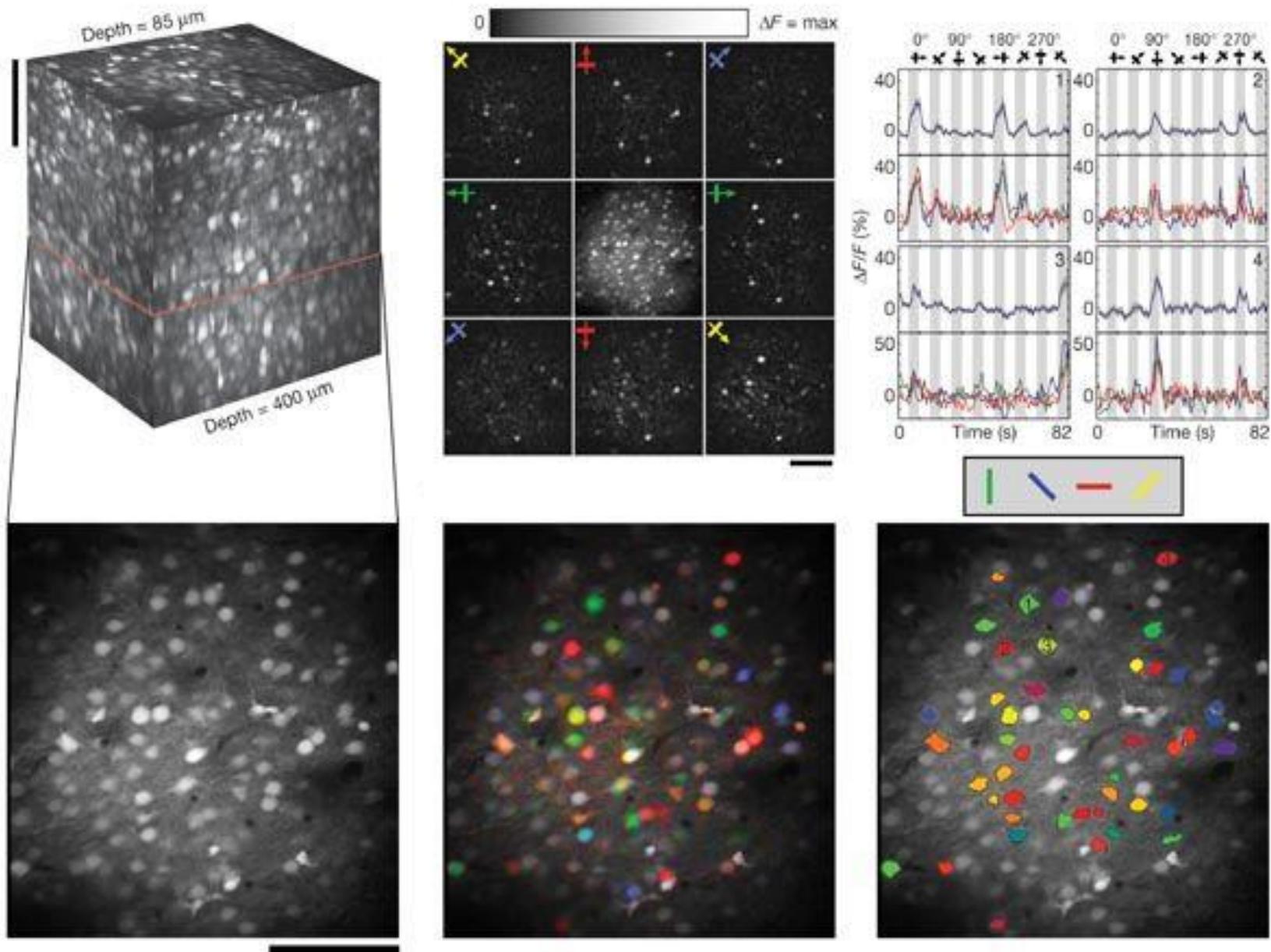
- 1-P
  - Widefield
  - Fiber photometry
- 2-P
  - Raster-scan
  - SLAP
- 3-P

# Types of sensors

# $\text{Ca}^{2+}$ dyes



Roger Tsien etc.



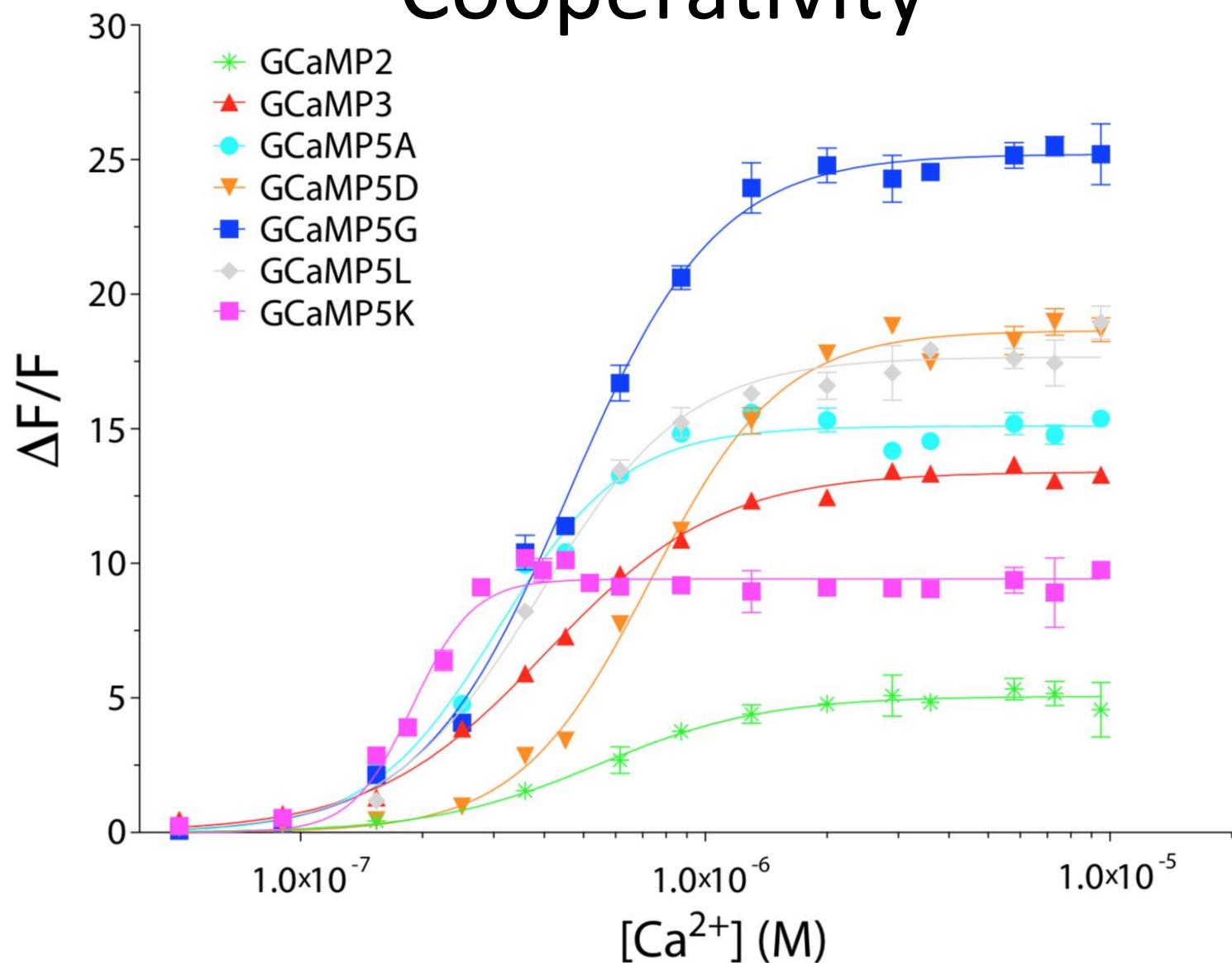
Ohki ... Reid (2005)

# Protein-based sensors

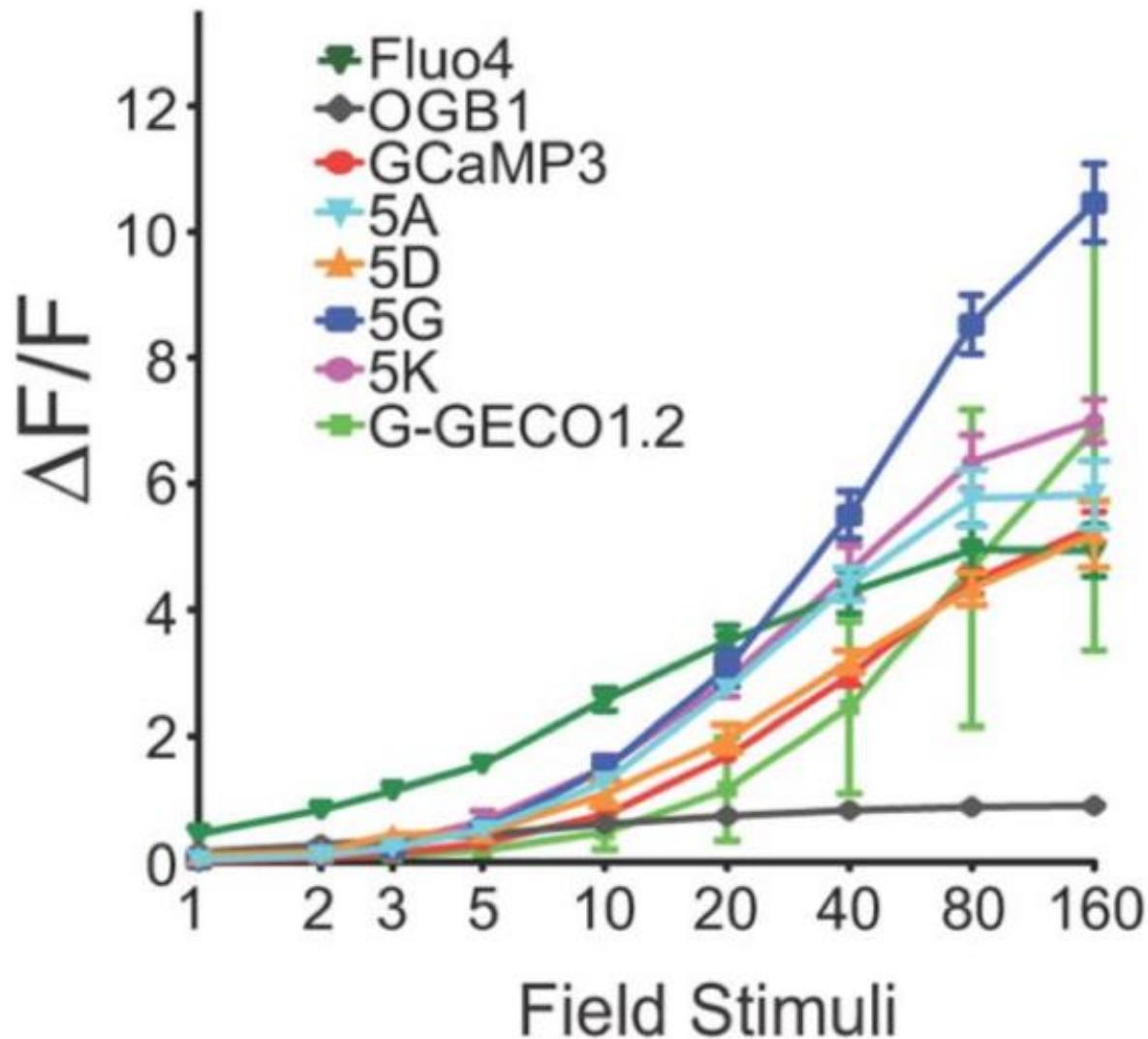
# Signal change

- Bigger is better
- SNR
- Resting fluorescence (ref. FP?)
- +/-
- Concentration-dependent?
- pH-dependent?

# Cooperativity



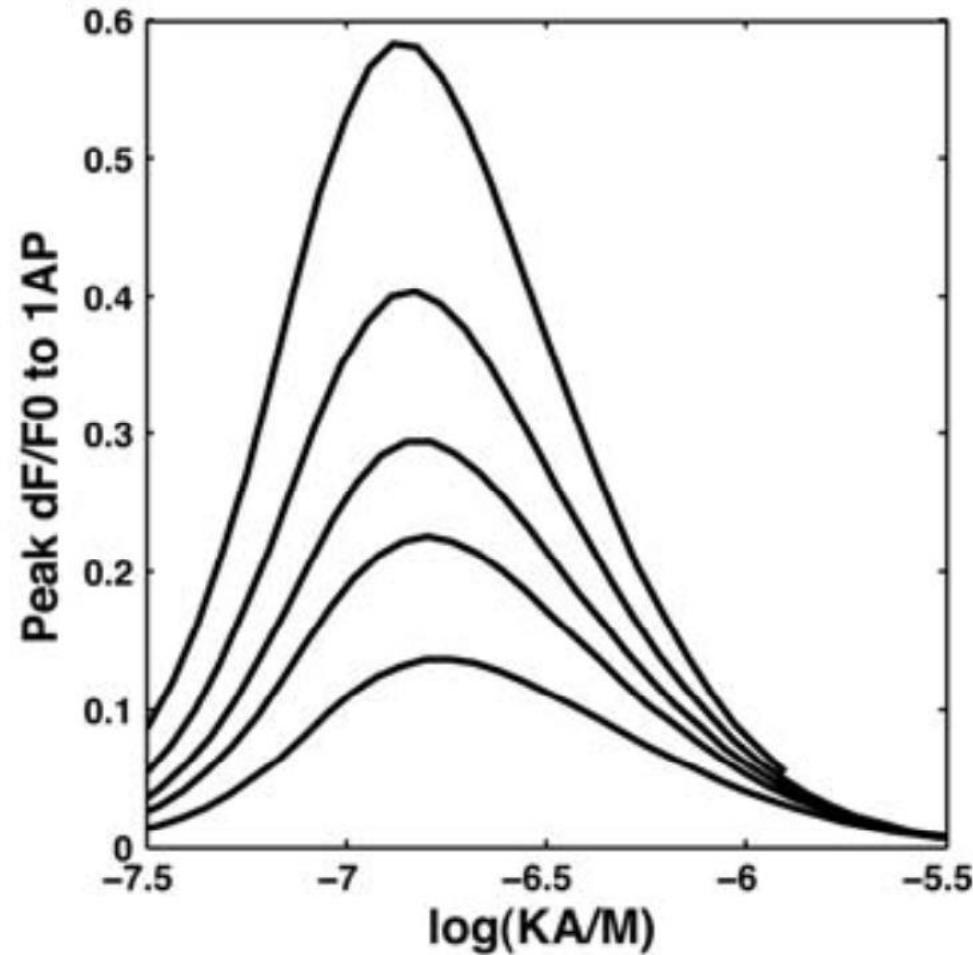
# Cooperativity



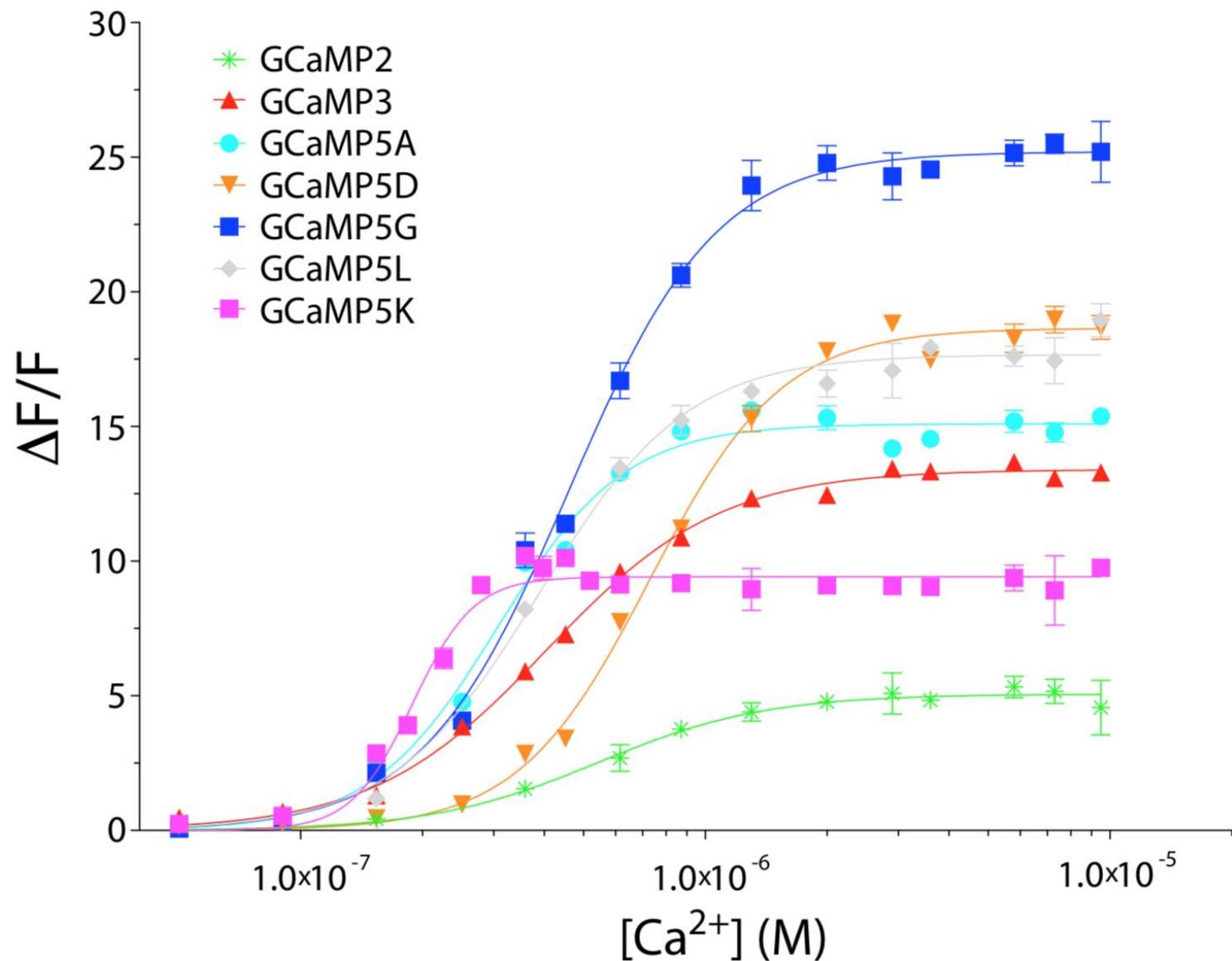
# Kinetics

- Resolve events
- Rise > decay

# Sensitivity from $k_{on}$



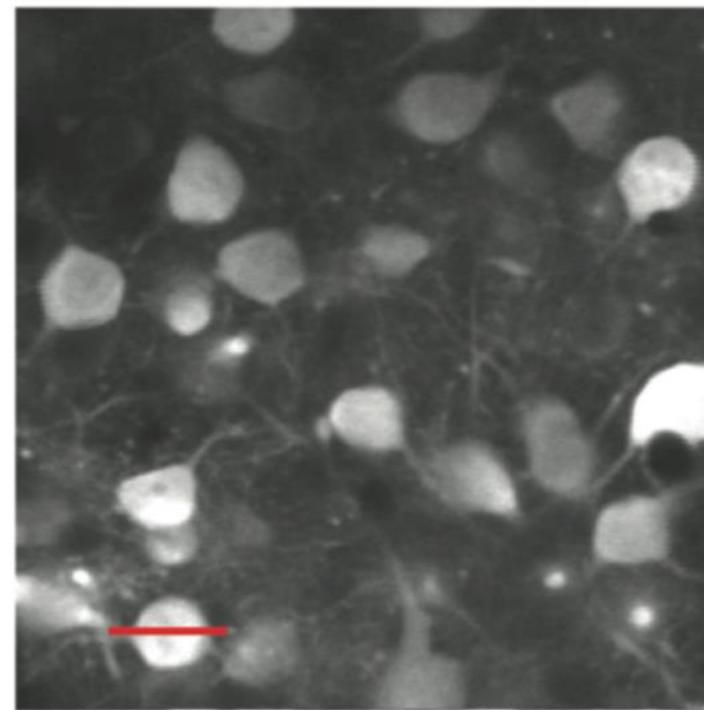
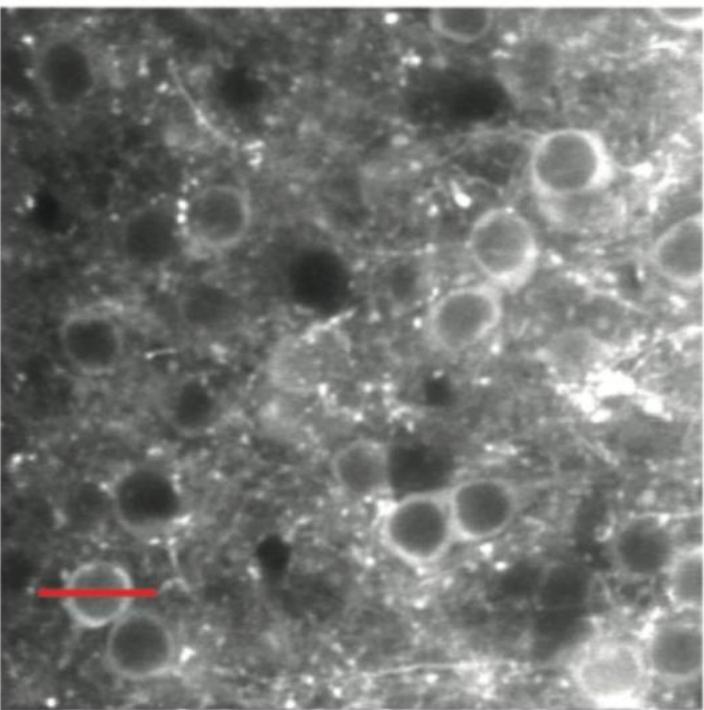
# Dynamic range



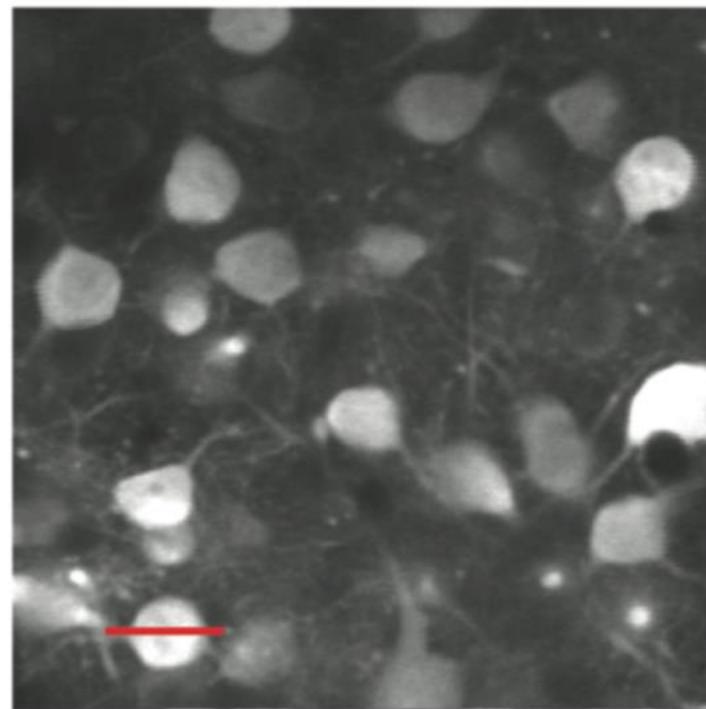
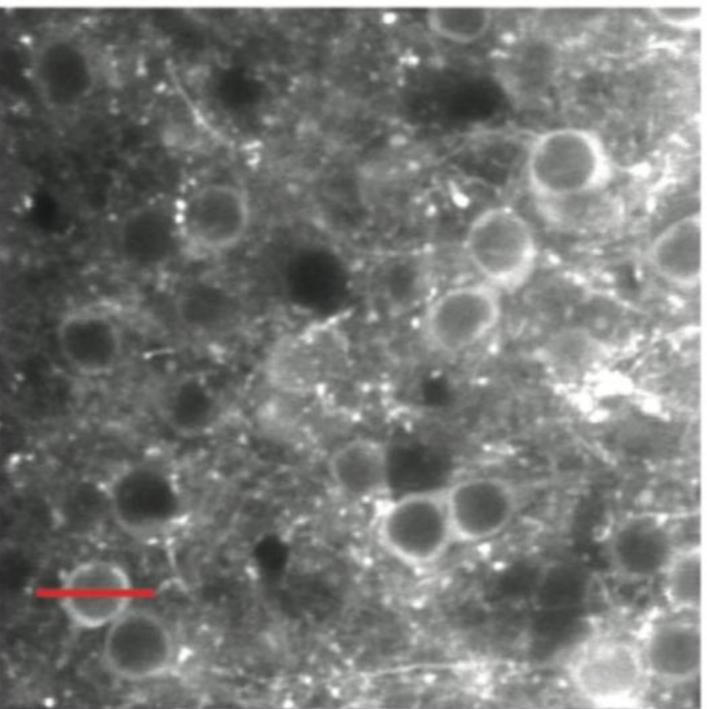
# Calcium buffering

- From sensor
- From sample

# Cytomorbidity



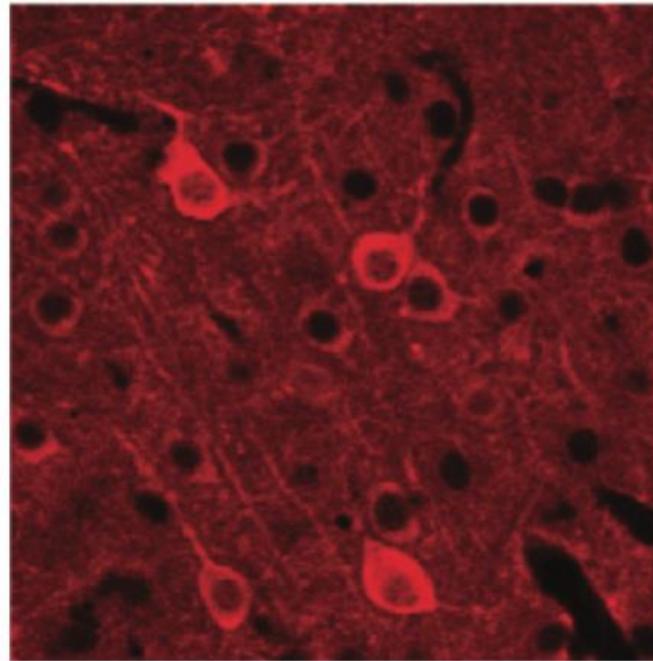
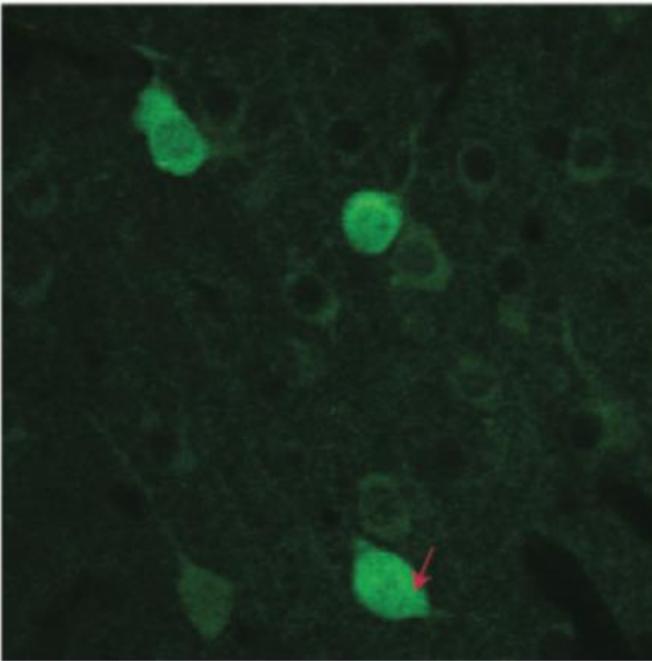
# Cytomorbidity



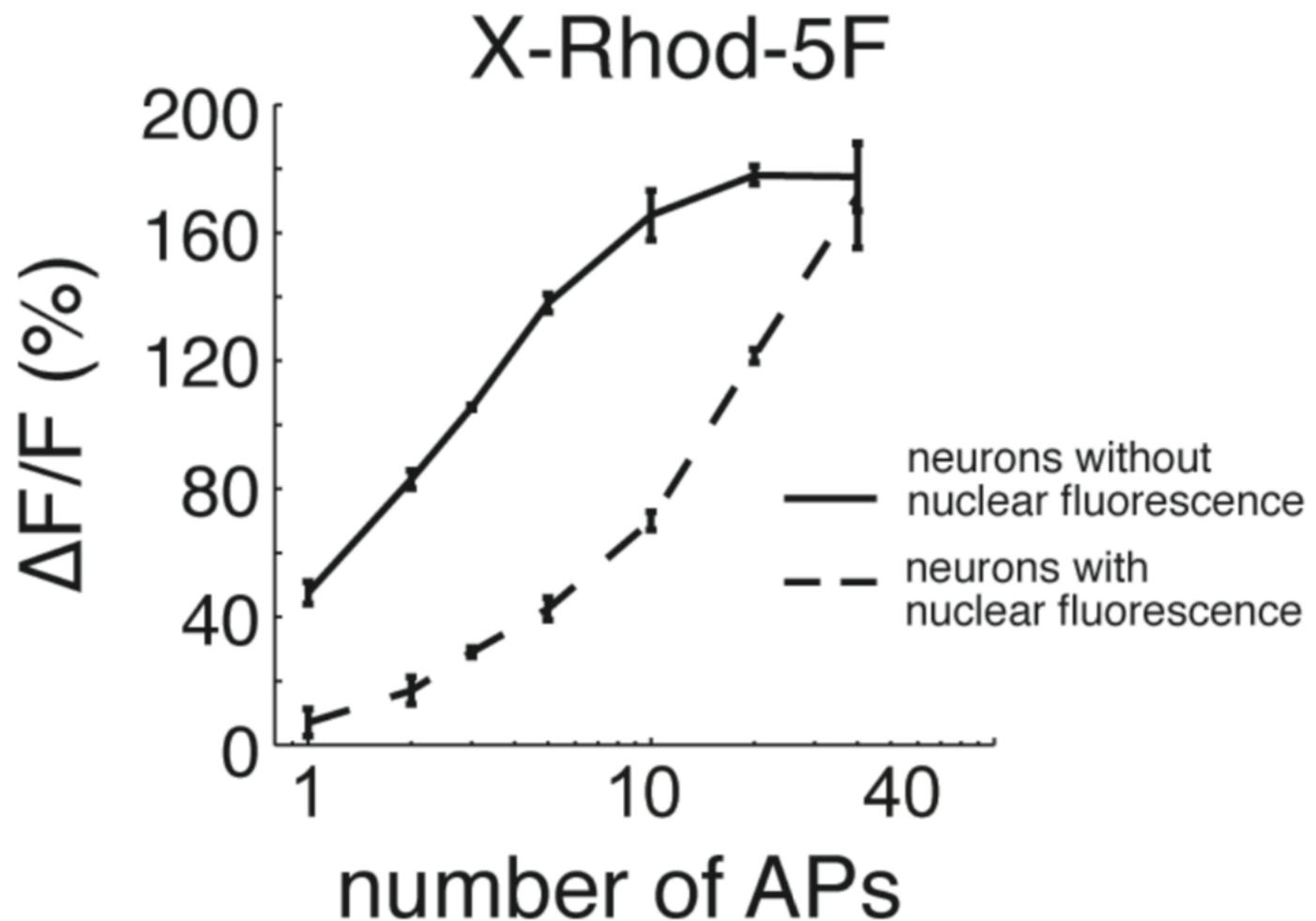
Cryptic NES sequence!

# Cytomorbidity

anti-HIS



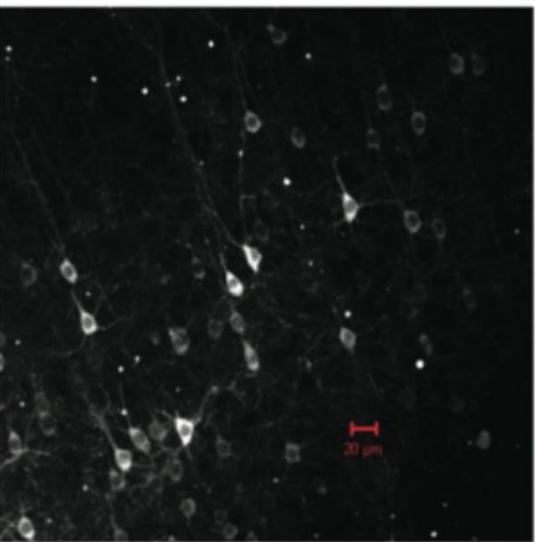
# Cytomorbidity



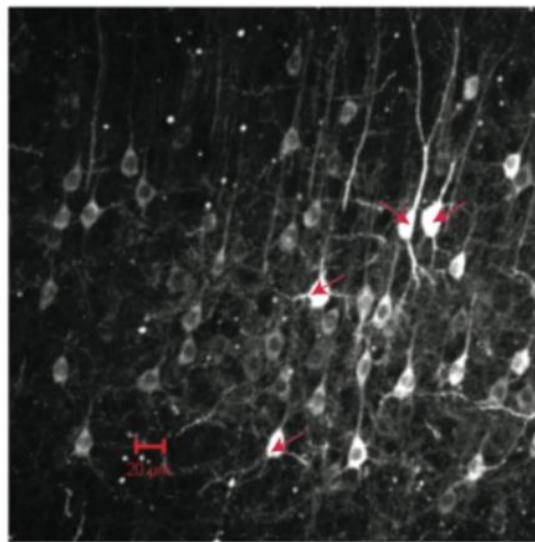
# Cytomorbidity

CAG-GCaMP3

P14

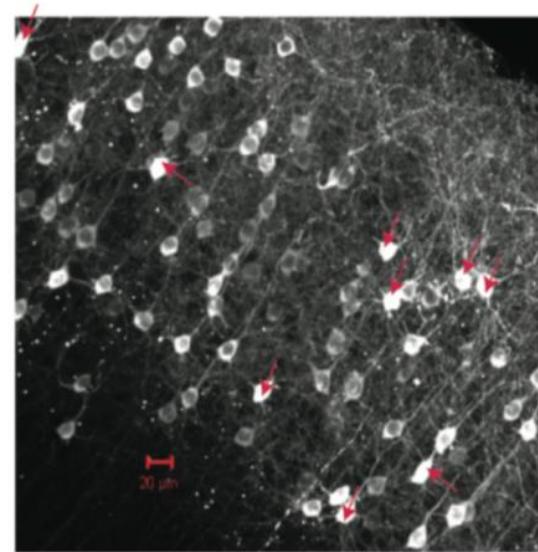


P28 (8.3%)



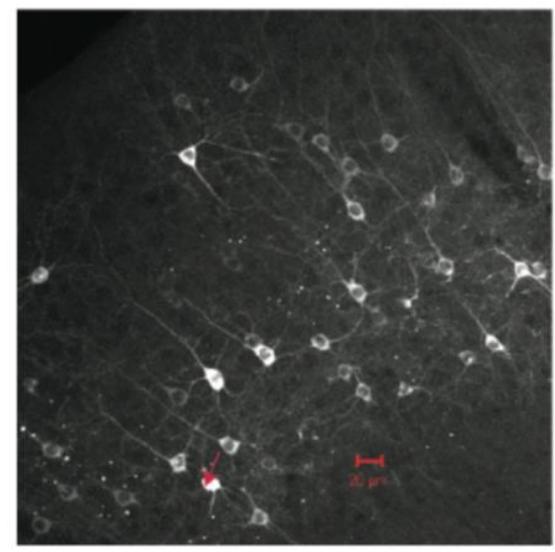
CAG-D3cpV

P25 (13%)



CAG-TN-XXL

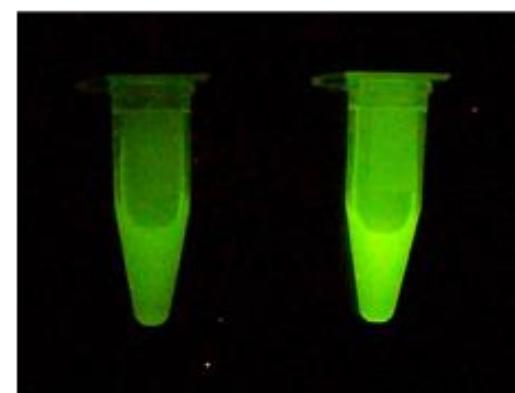
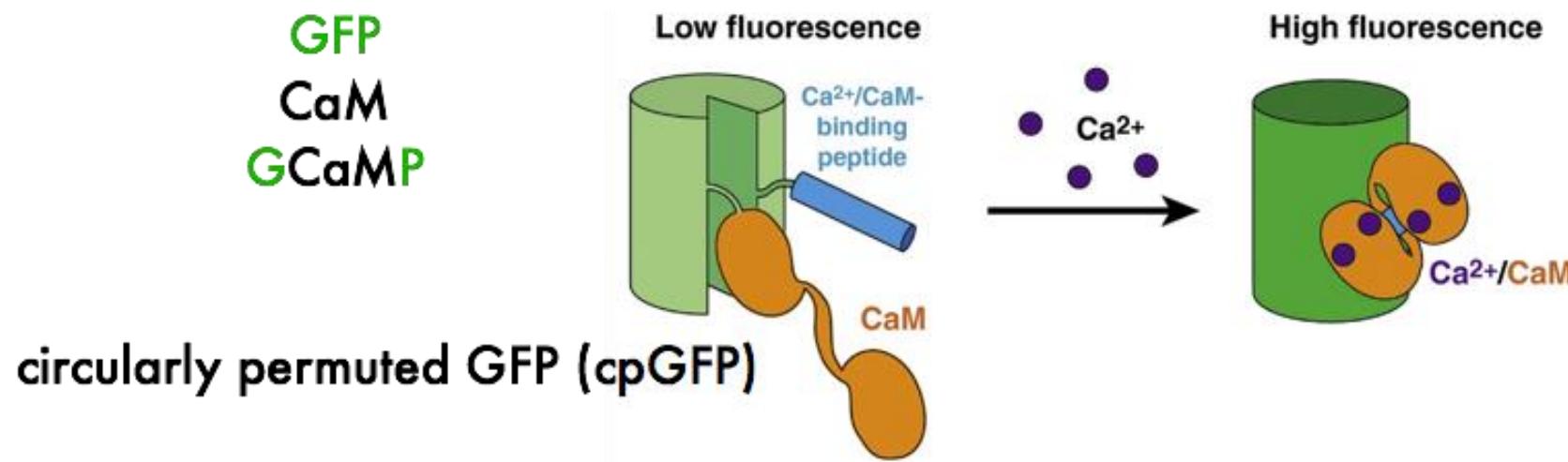
P25 (5%)



# Sensor concentration

- Too low bad
- Too high bad
- ~lowest level that gives good signals

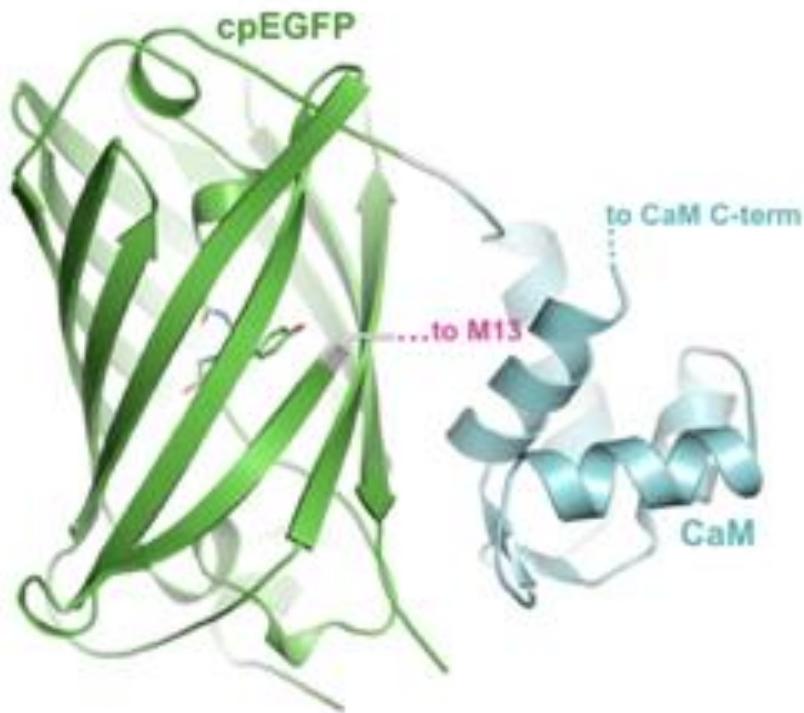
## Cartoon of proposed mechanism



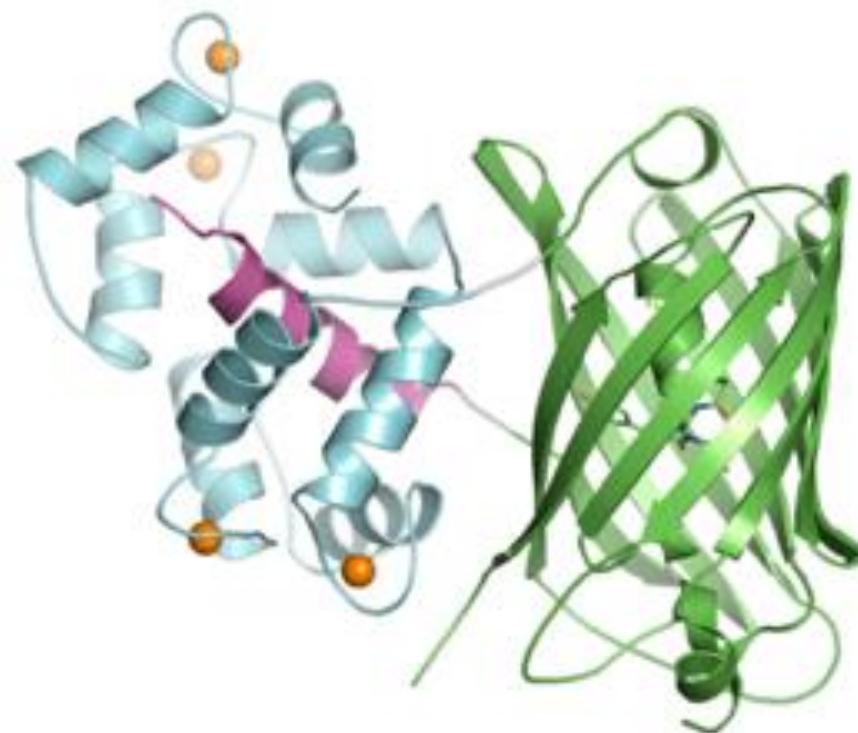
GCaMP2

Nakai & others from 2001 on

# GCaMP2 structure

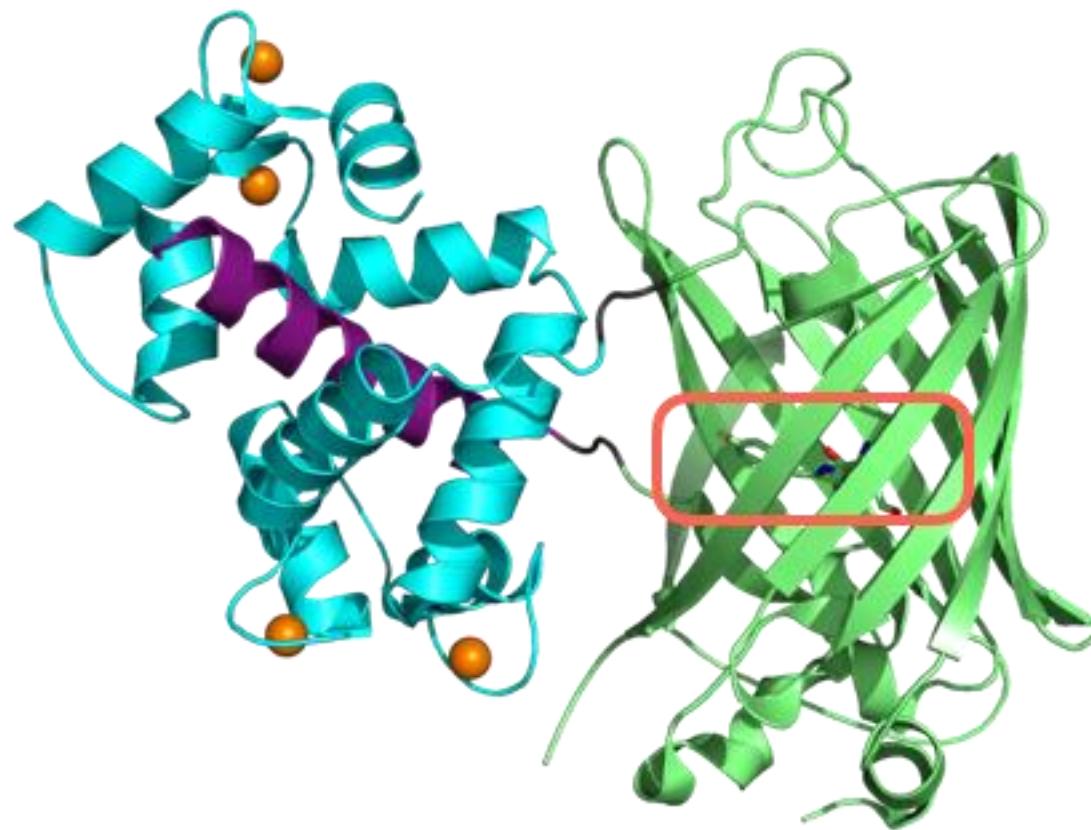


$\text{Ca}^{2+}$  free



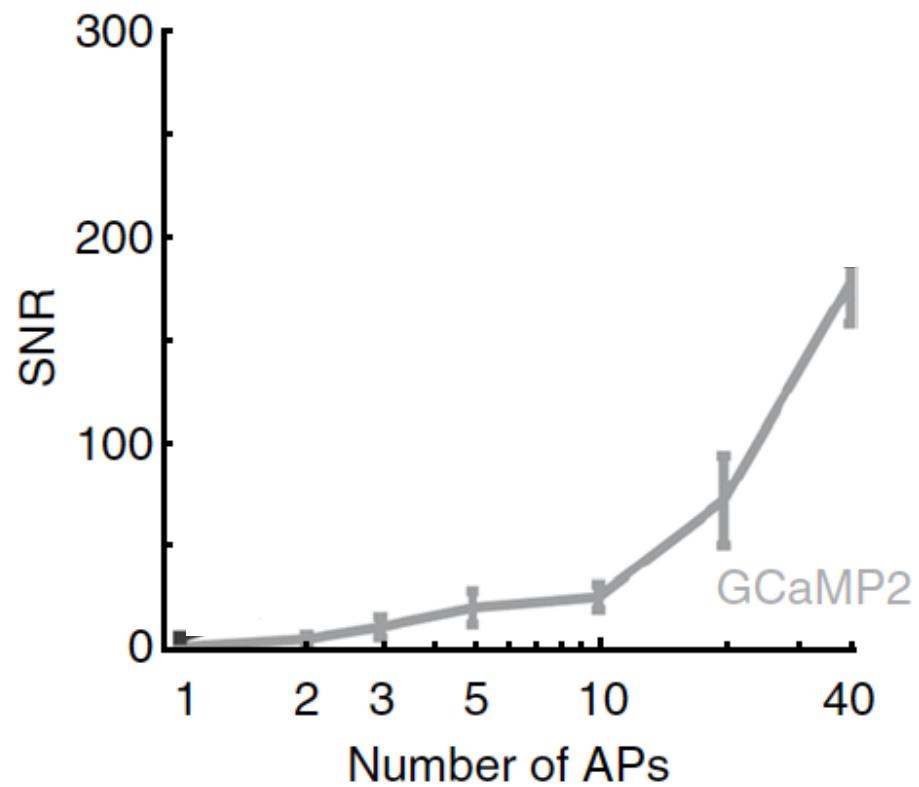
$\text{Ca}^{2+}$  bound

- Looger: GCaMP3
- Looger: GCaMP5
- GENIE: GCaMP6
- GENIE: jGCaMP7
- Looger: faster GCaMPs

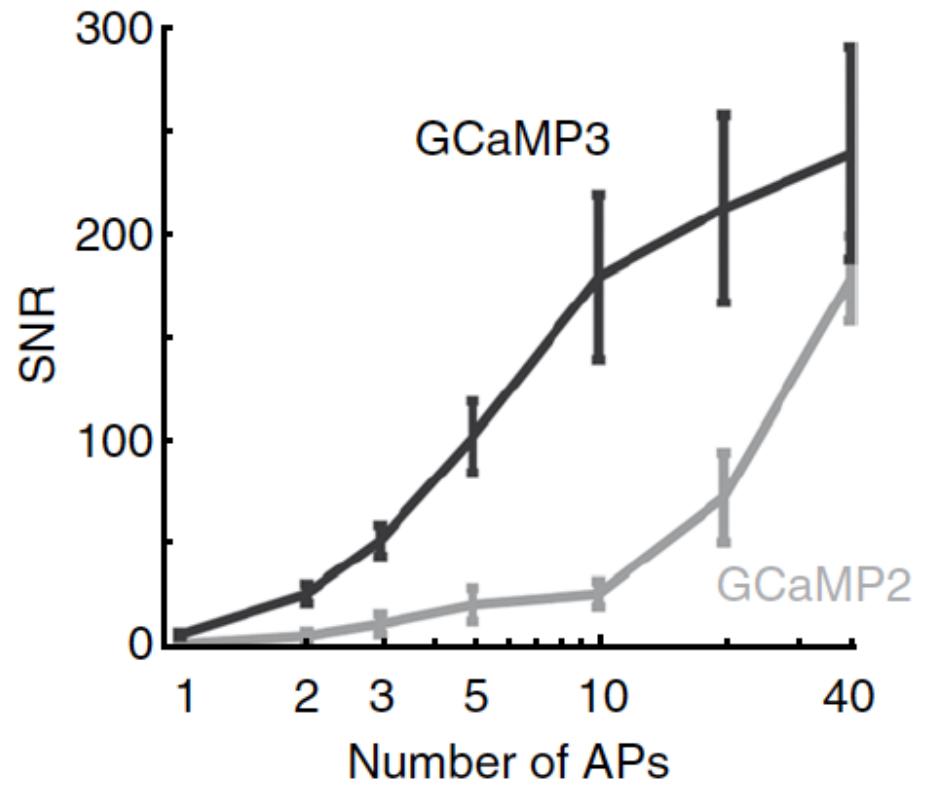


GCaMP3  
PDB 3EK8

# Systematic mutagenesis



Brain slice

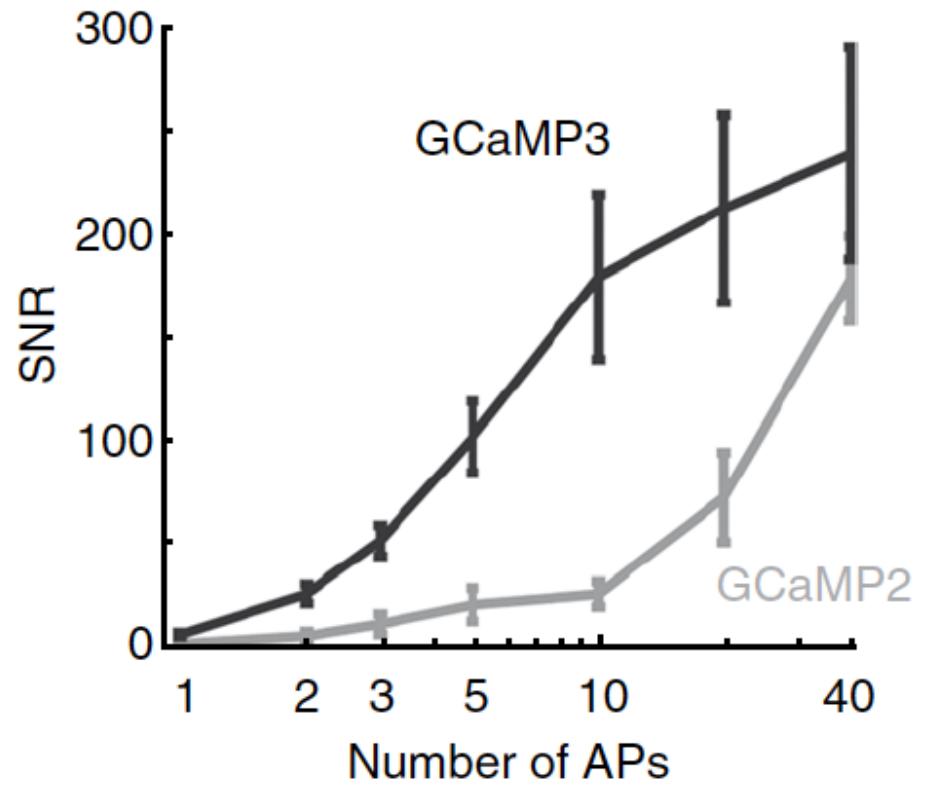


Brain slice

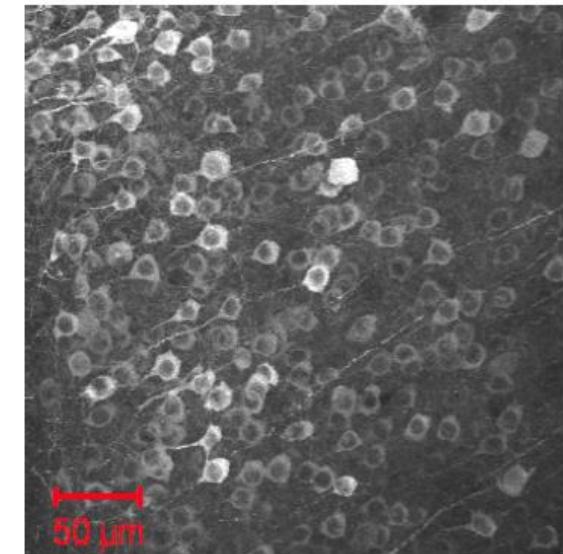
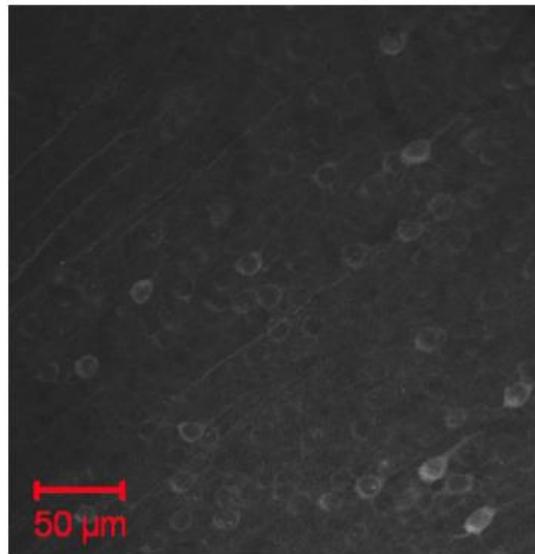


Lin Tian

Tian et al. (2009)

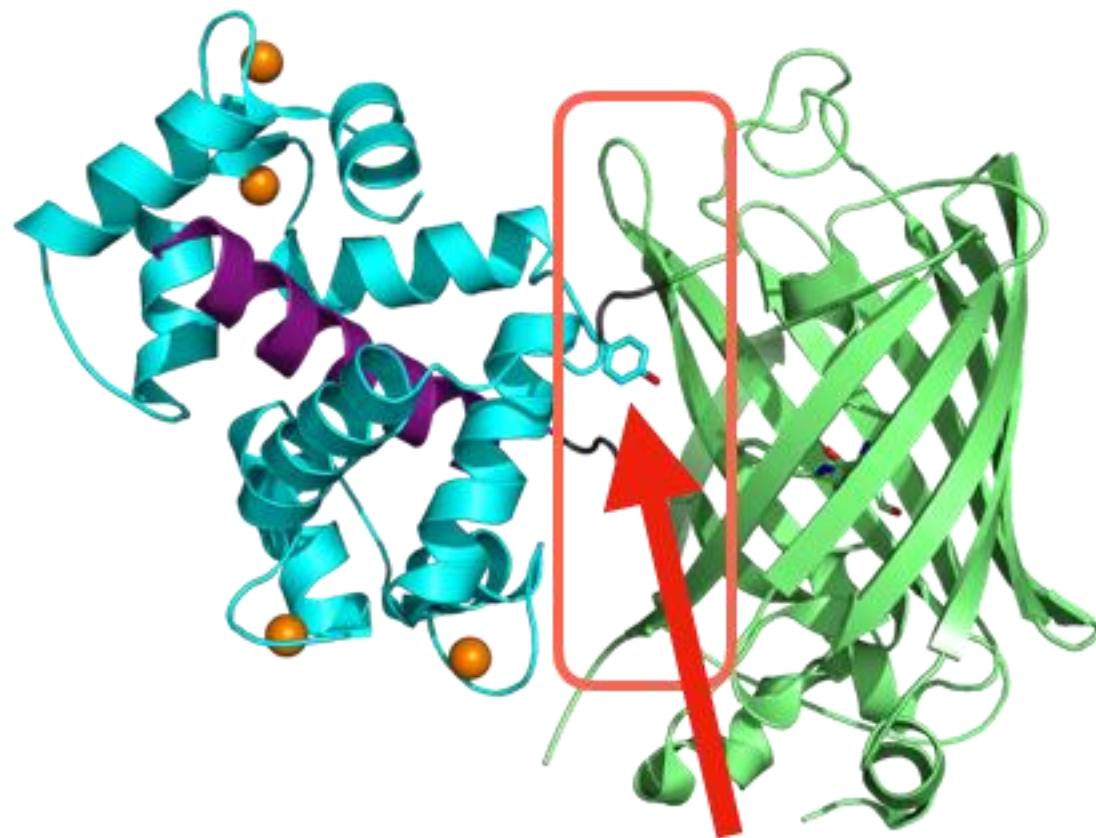


Brain slice



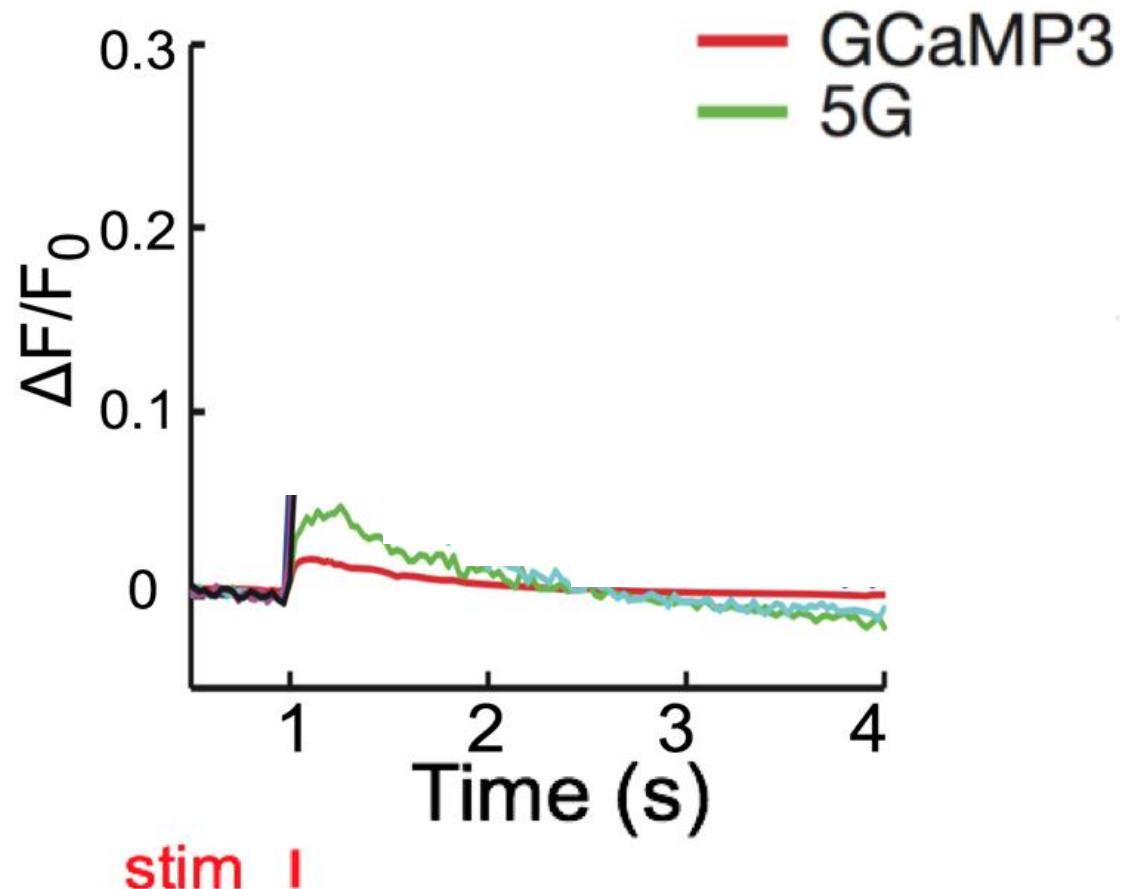
Tian et al. (2009)

- Looger: GCaMP3
- **Looger: GCaMP5**
- GENIE: GCaMP6
- GENIE: jGCaMP7
- Looger: faster GCaMPs



GCaMP5: Asp381Tyr  
PDB 3SG4

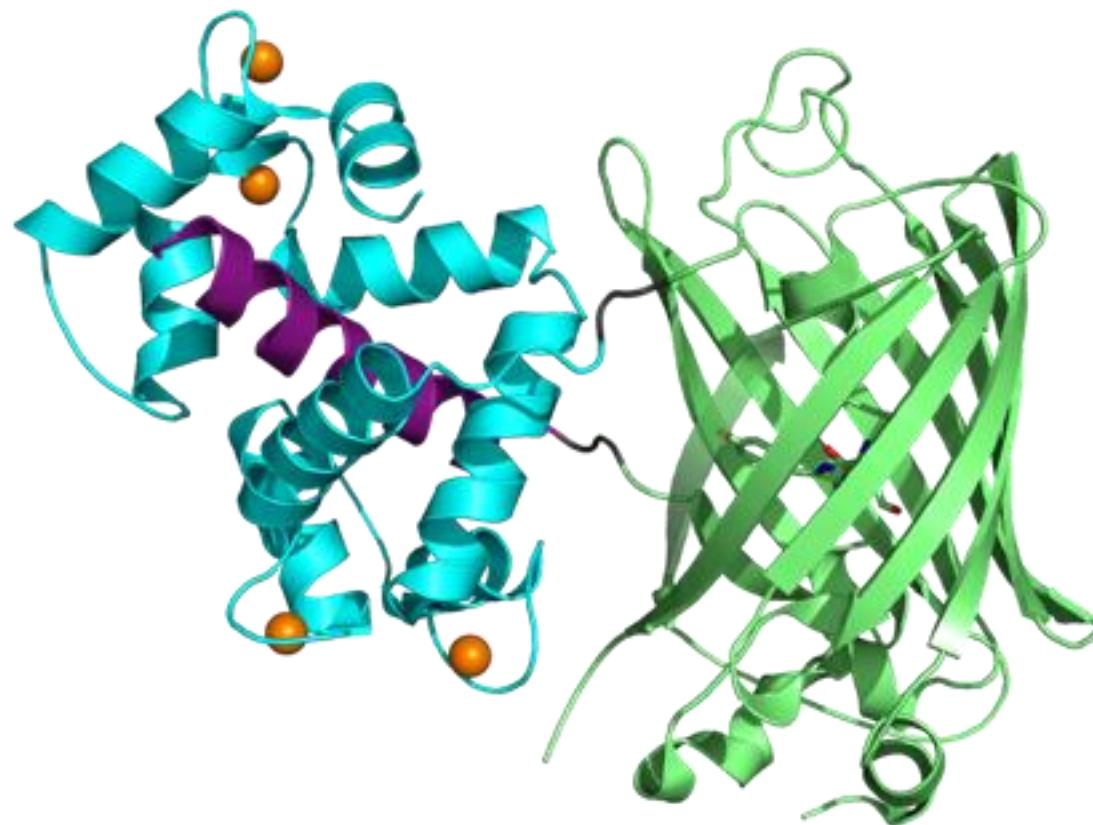
# More mutagenesis



Jasper Akerboom

Akerboom et al. (2012)

- Looger: GCaMP3
- Looger: GCaMP5
- GENIE: GCaMP6
- GENIE: jGCaMP7
- Looger: faster GCaMPs

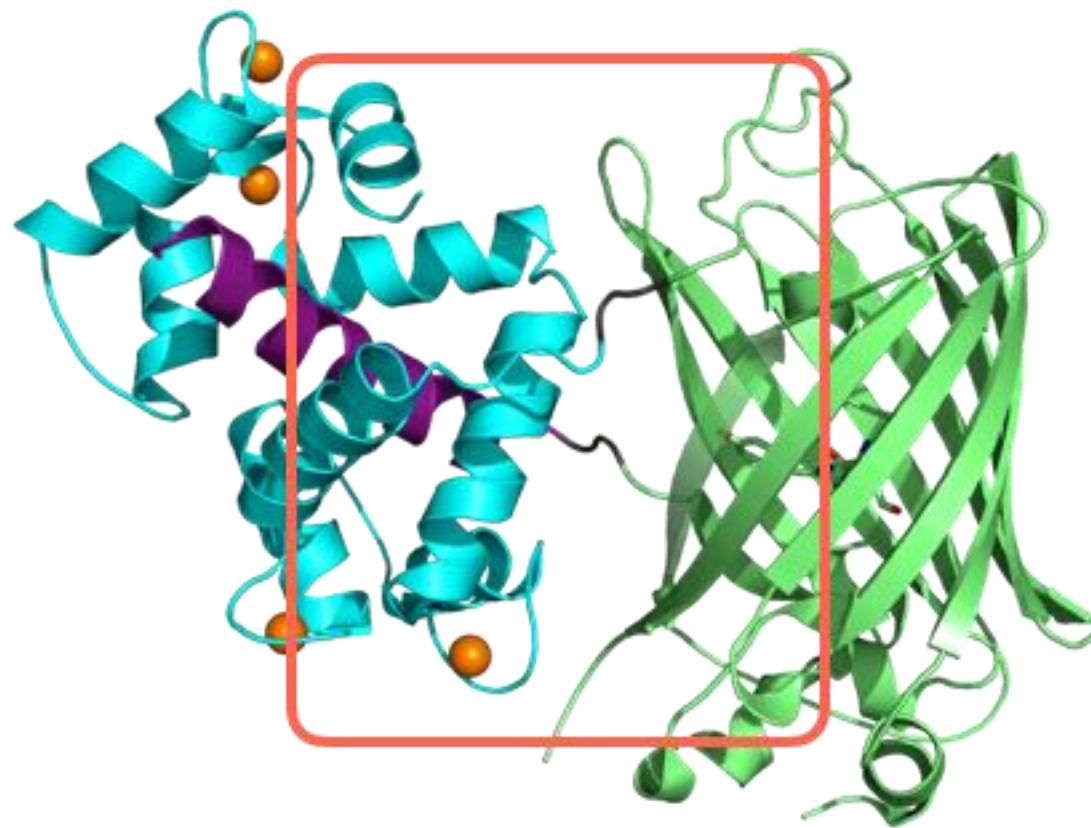


# GENIE

Looger, Svoboda, Jayaraman, Kerr, Kim et al.

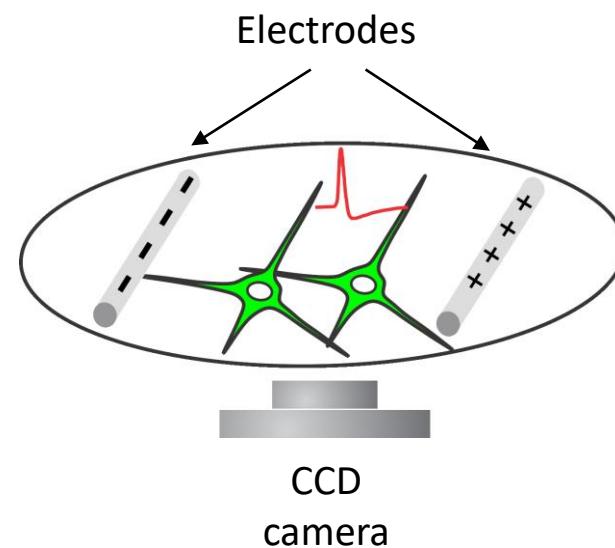
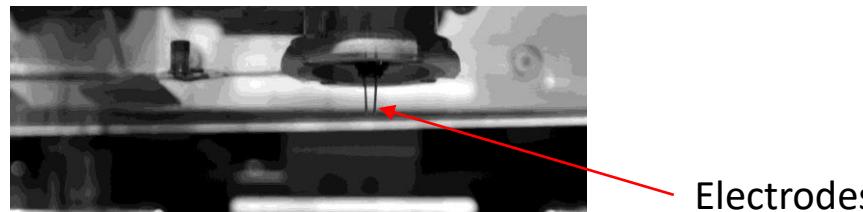
Looger, Svoboda, Jayaraman, Schreiter, Lavis, Spruston, Wong et al.

- Looger: GCaMP3
- Looger: GCaMP5
- **GENIE: GCaMP6**
- GENIE: jGCaMP7
- Looger: faster GCaMPs



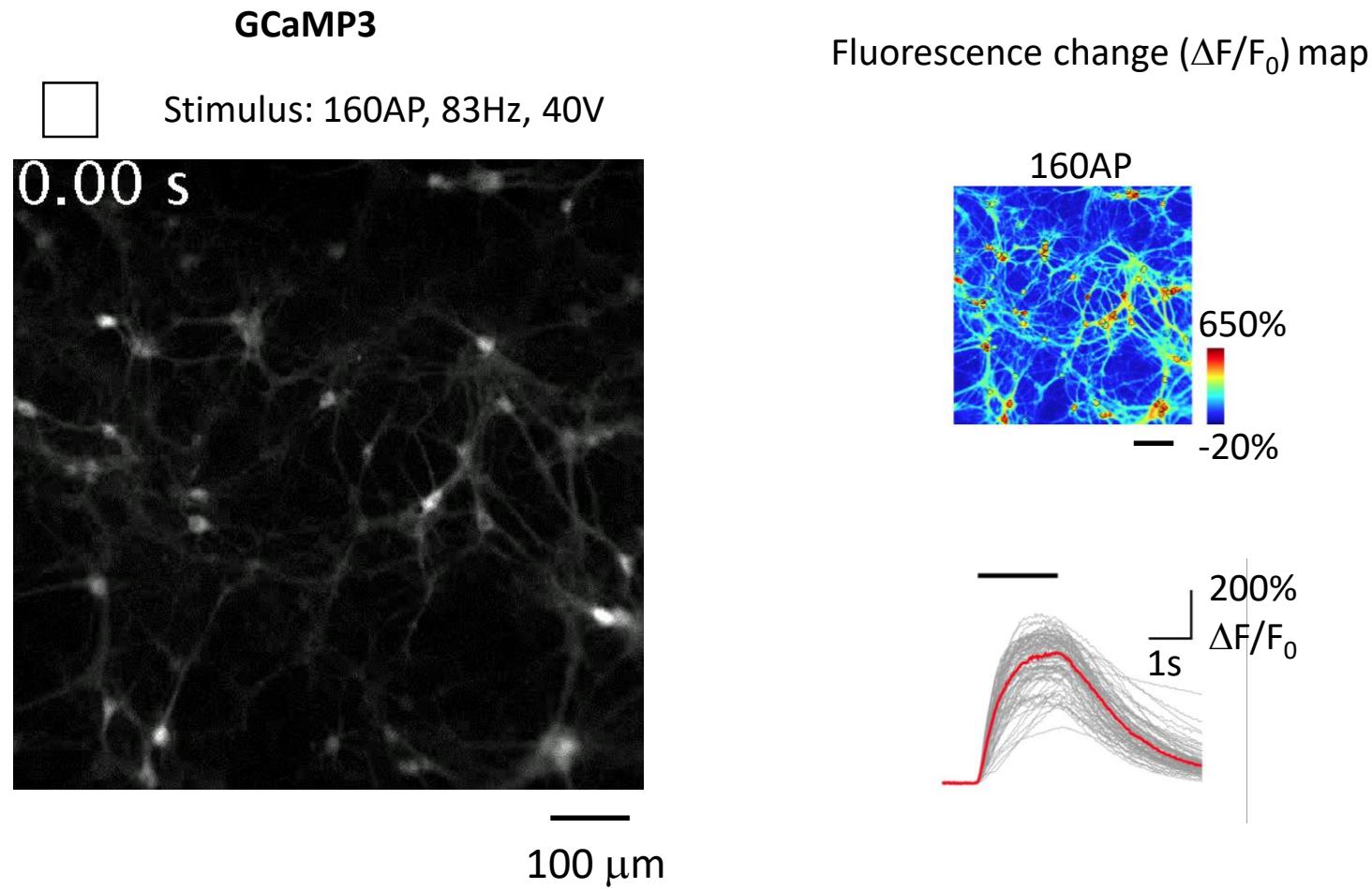
GCaMP6  
PDB 3WLD

# *Mammalian neuronal culture screening platform*

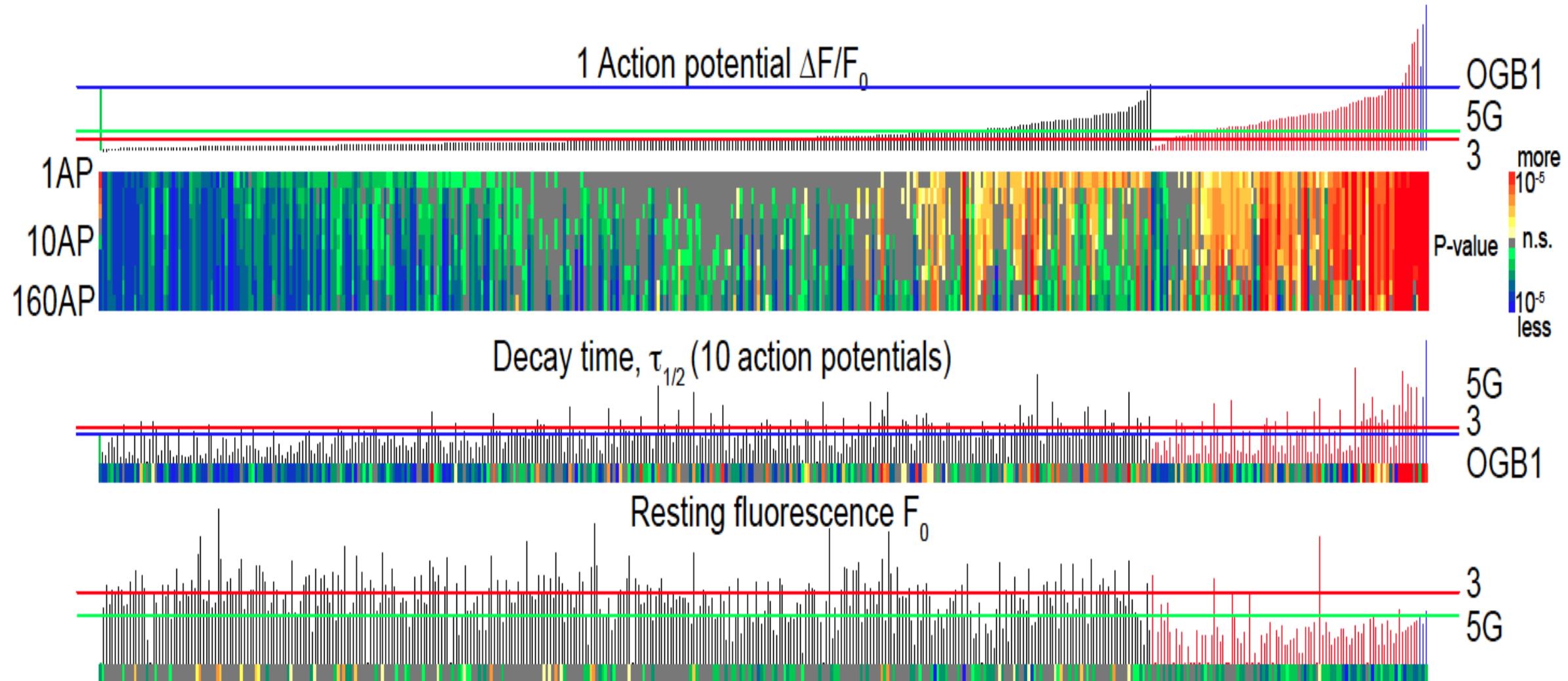


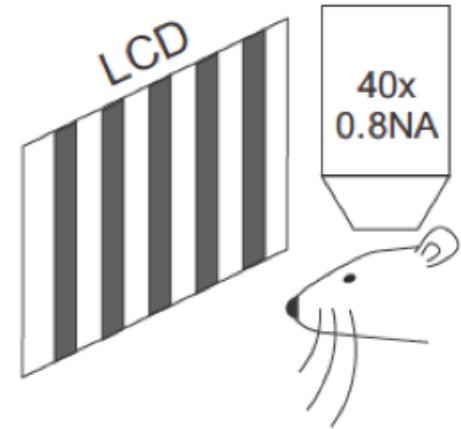
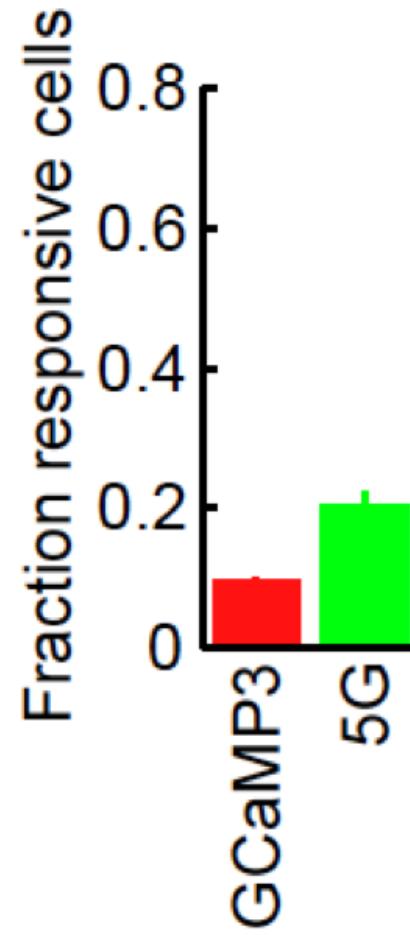
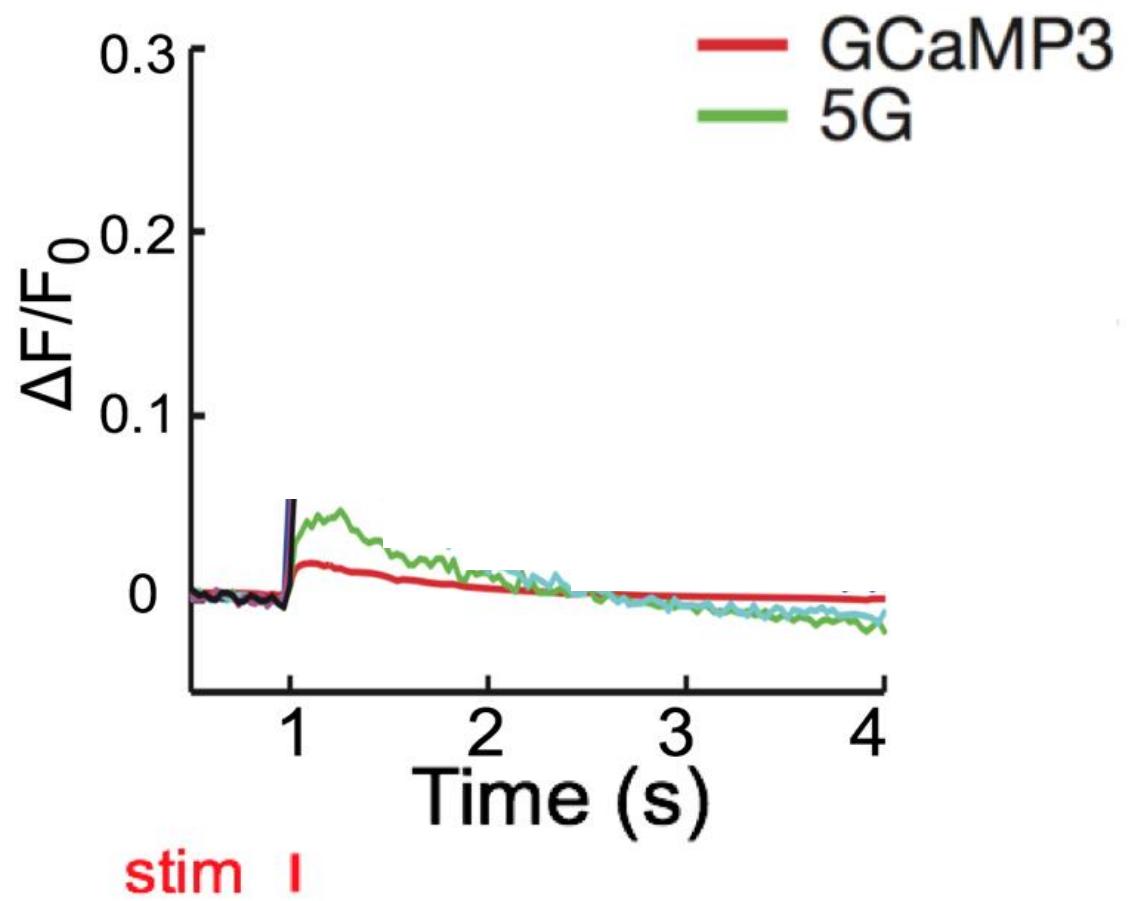
Wardill et al (2013)

# *Screening in mammalian neuronal culture*

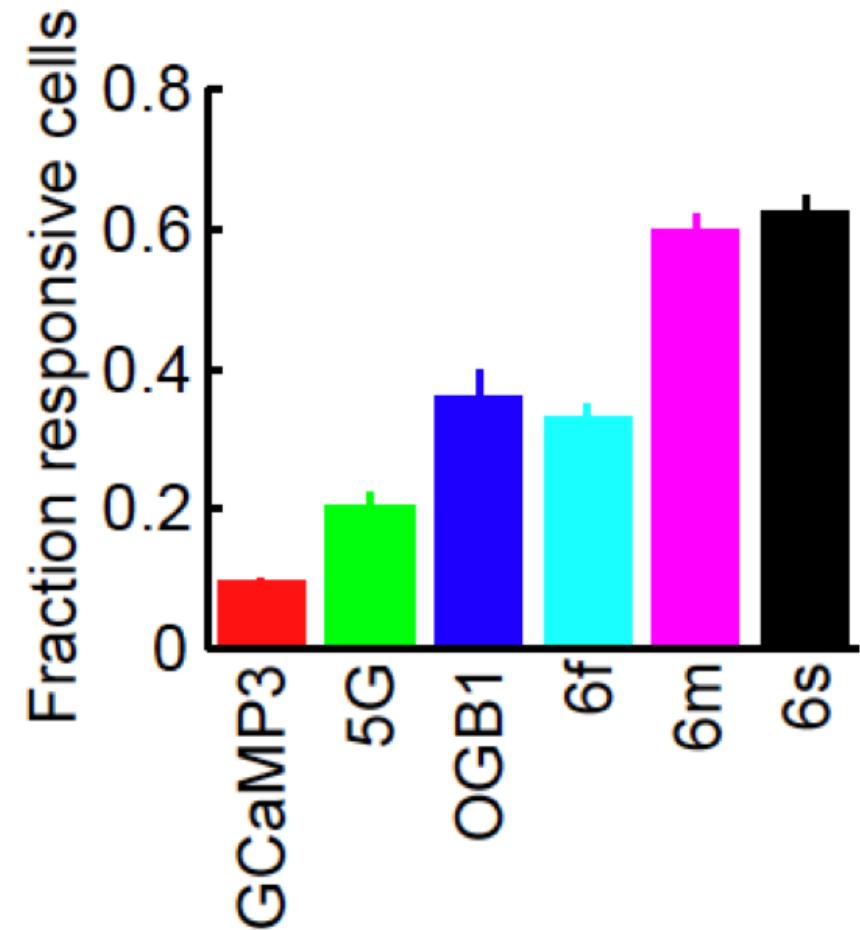
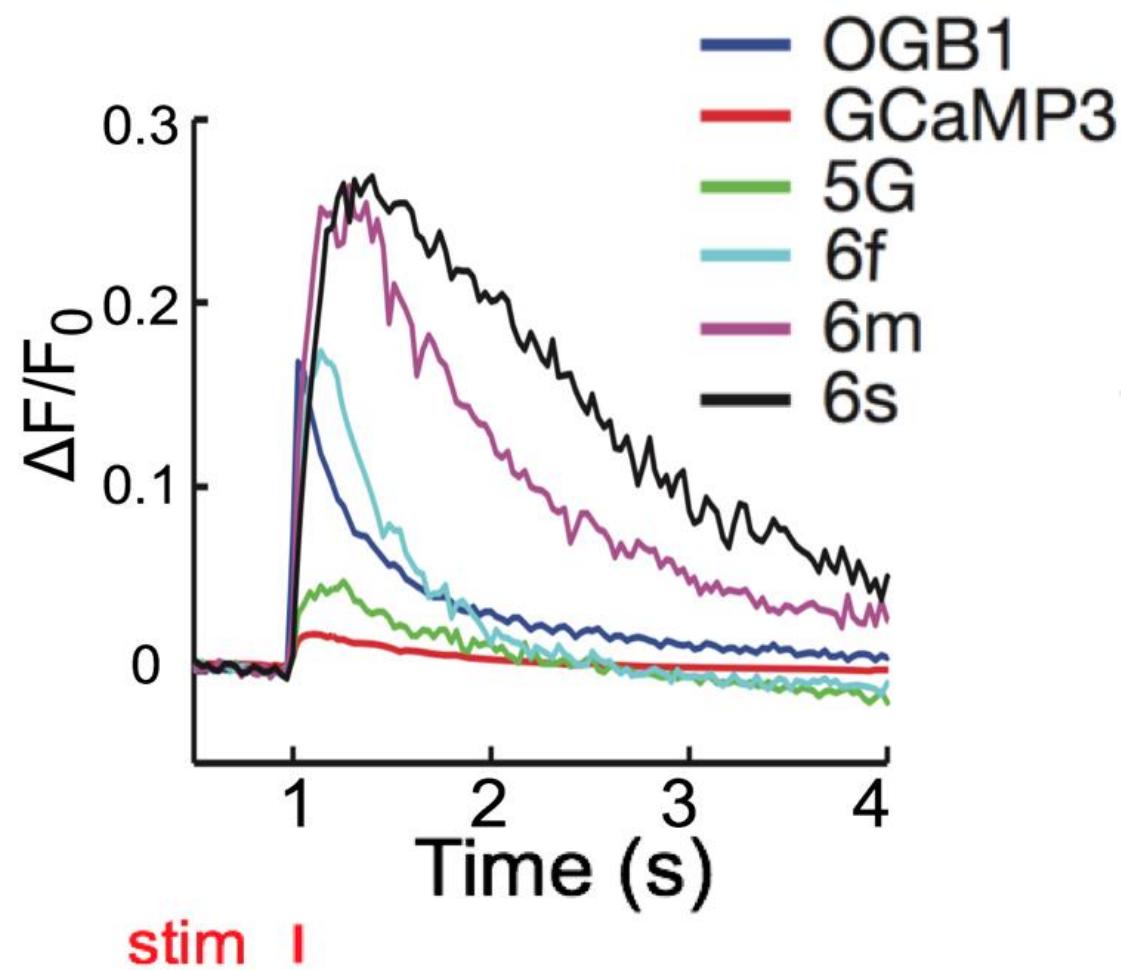


Wardill et al (2013)

**e**



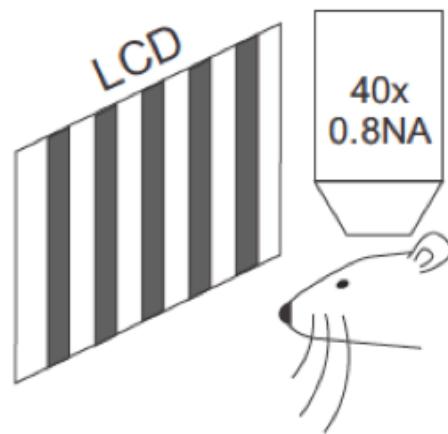
# GENIE



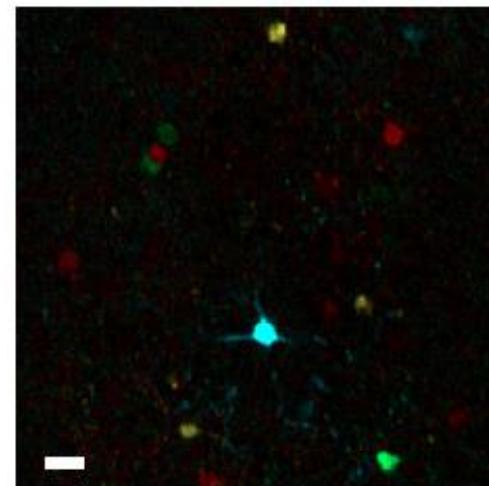
# GCaMP6s

20%  
0.5mV  
1s

10  $\mu\text{m}$

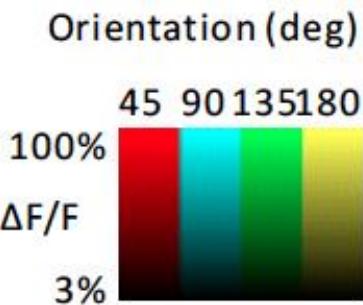
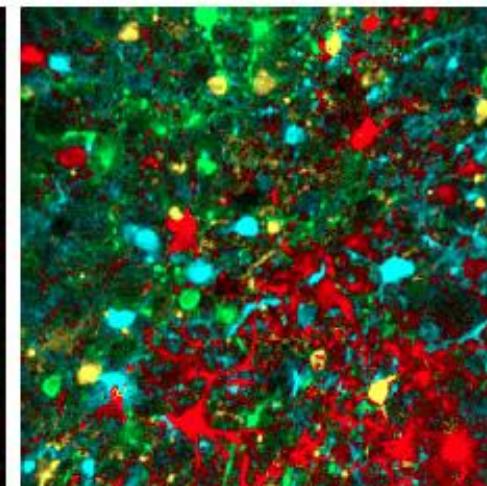


GCaMP5g

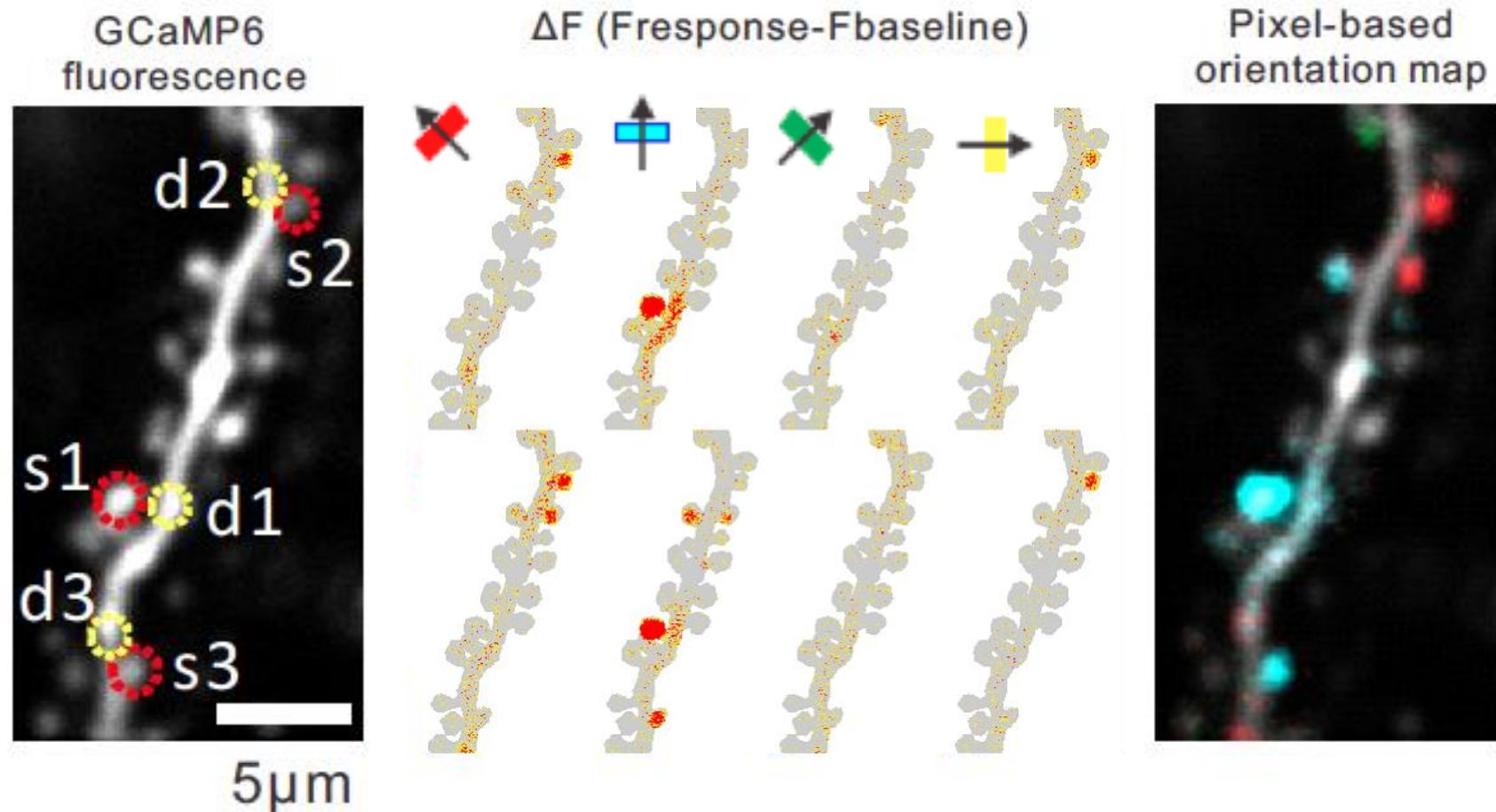


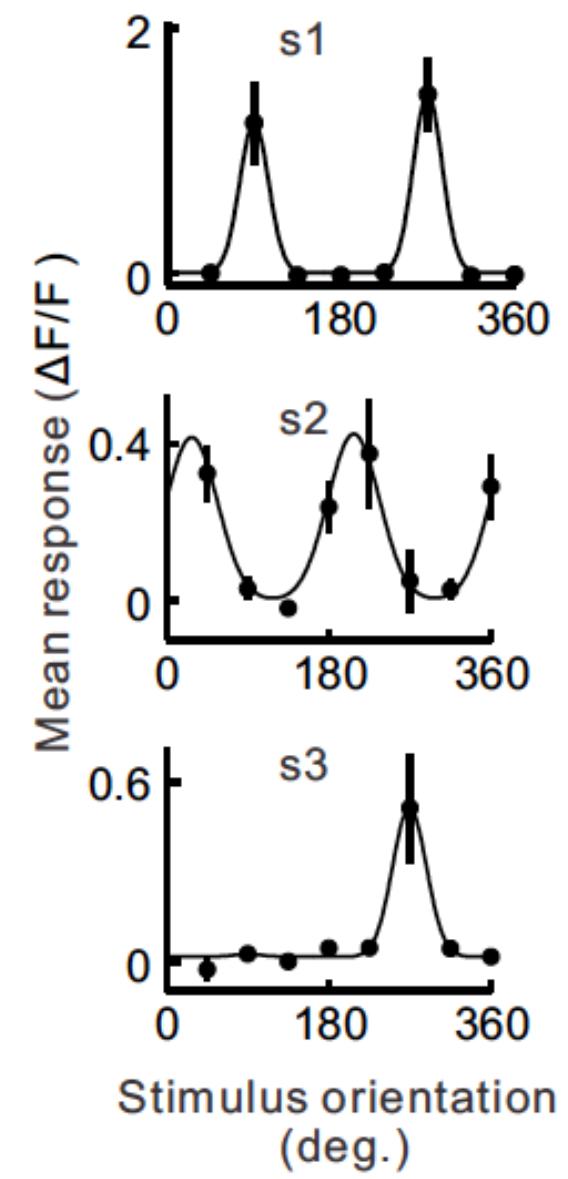
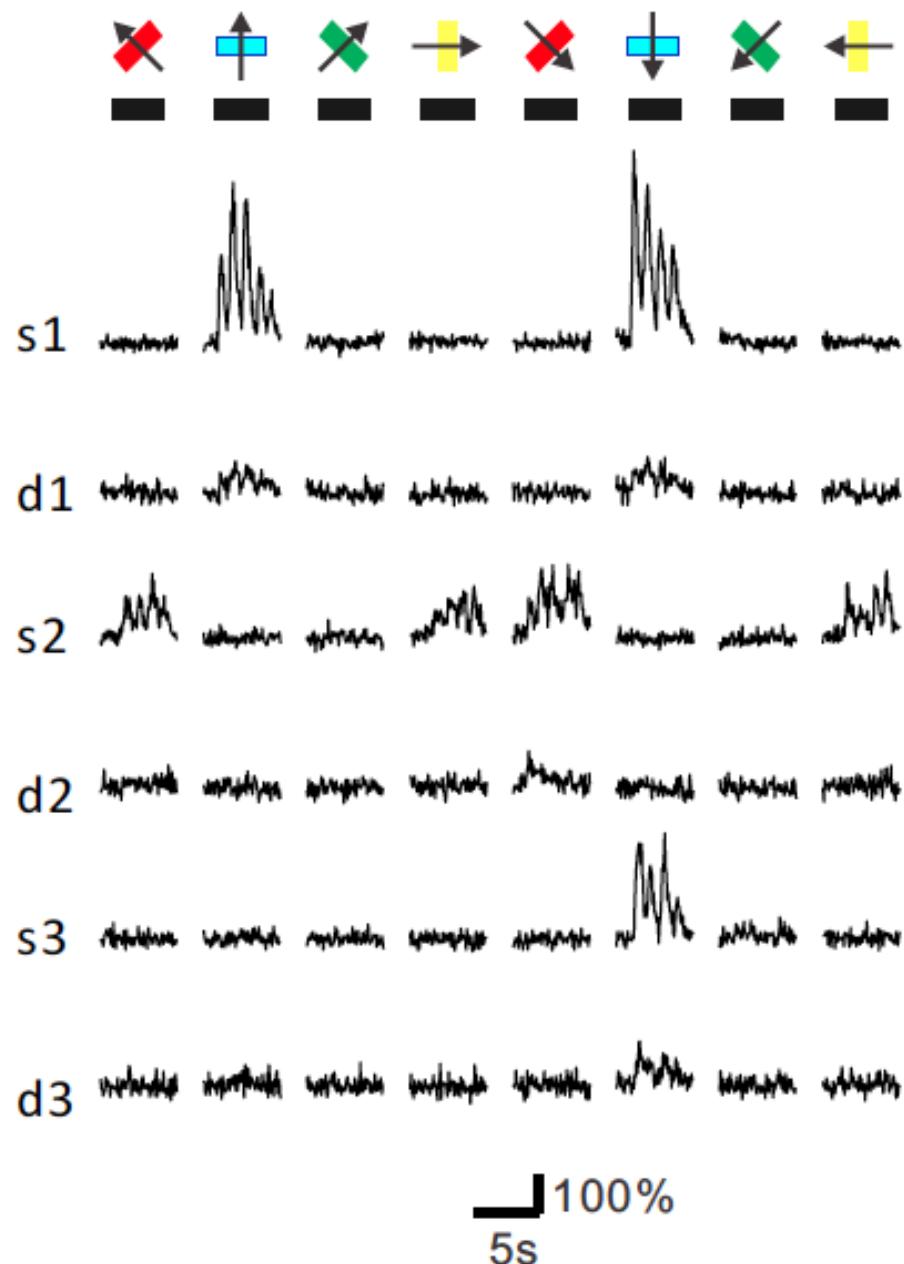
20μm

GCaMP6s

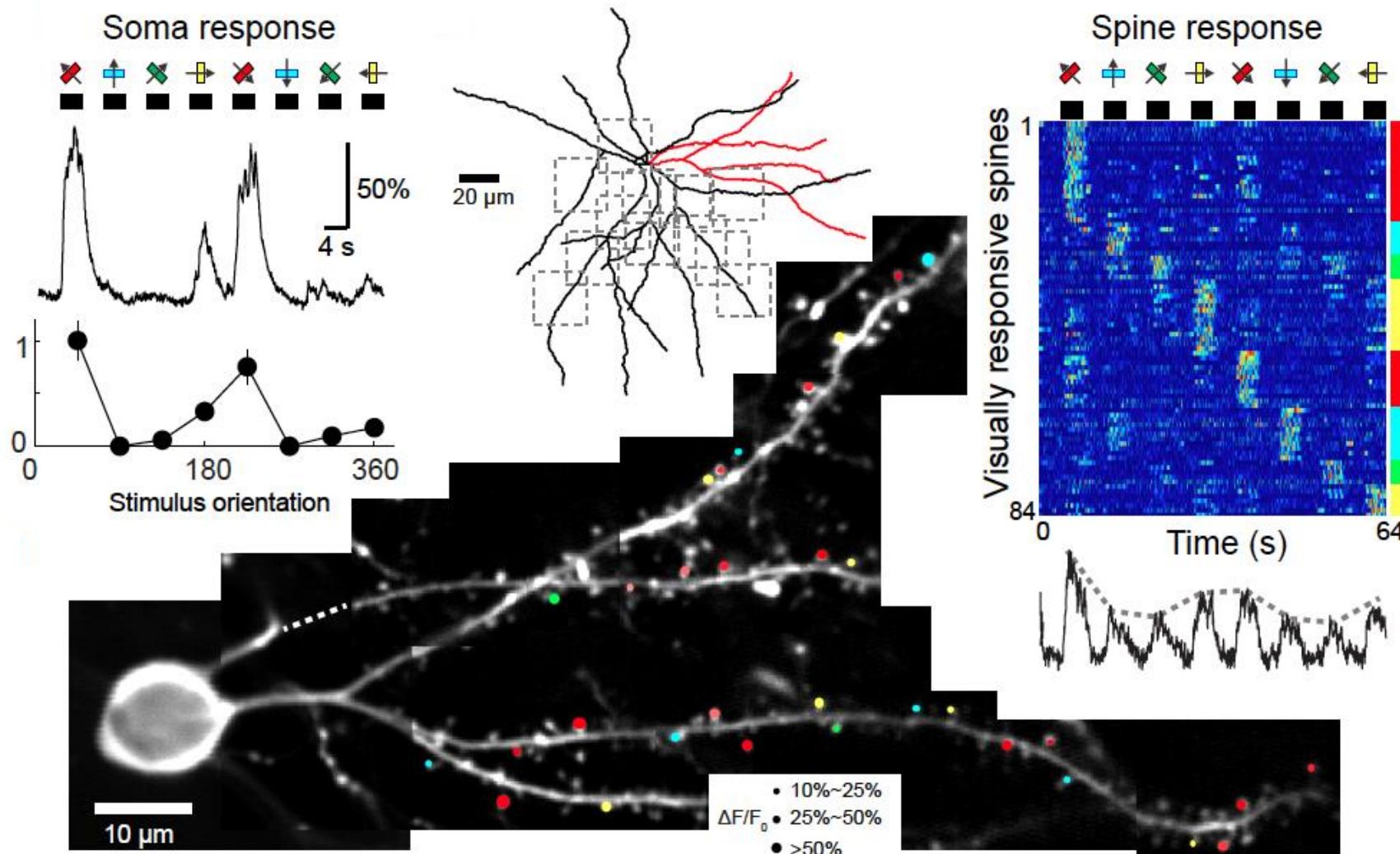


# Single-spine responses

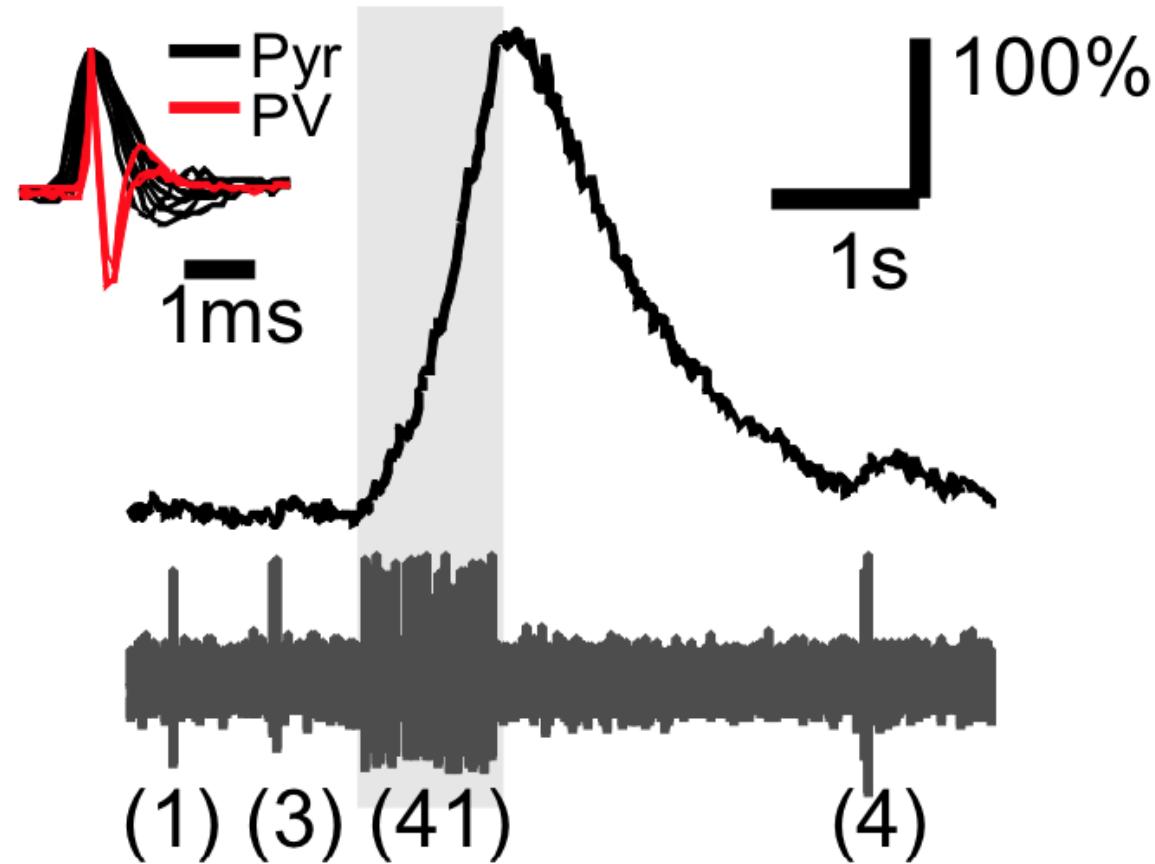




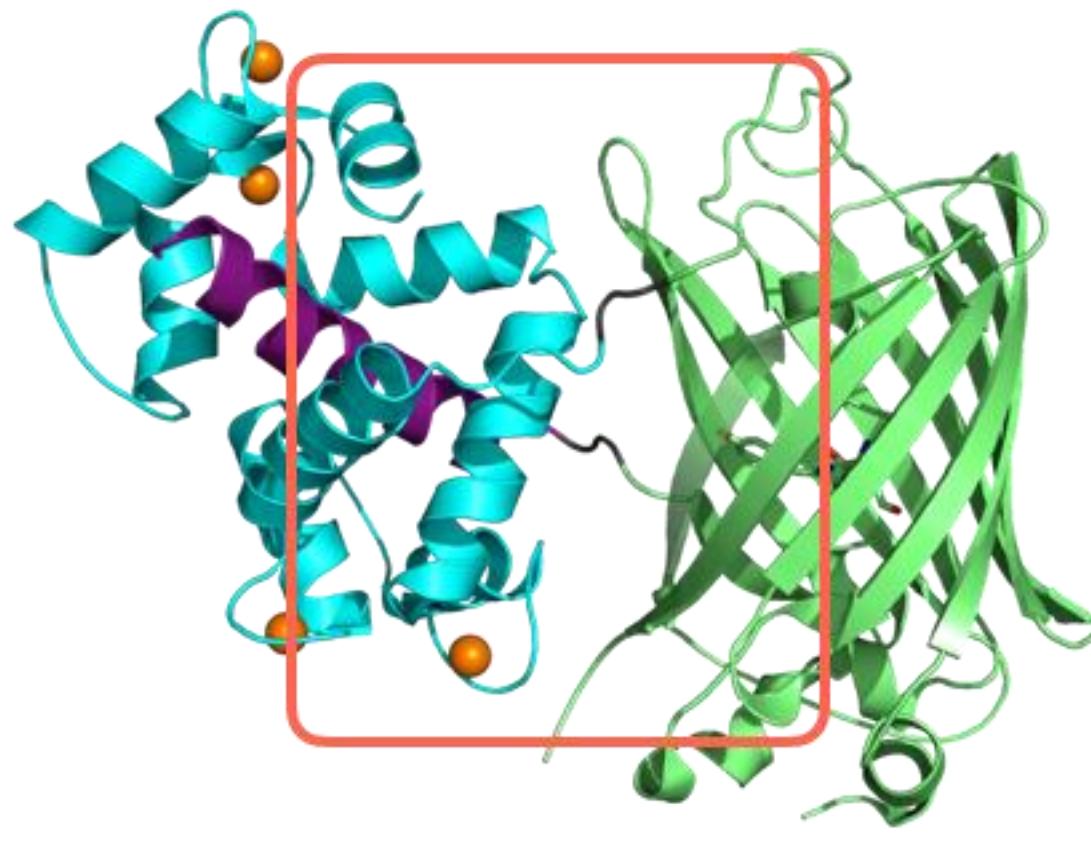
# Whole-neuron spine responses



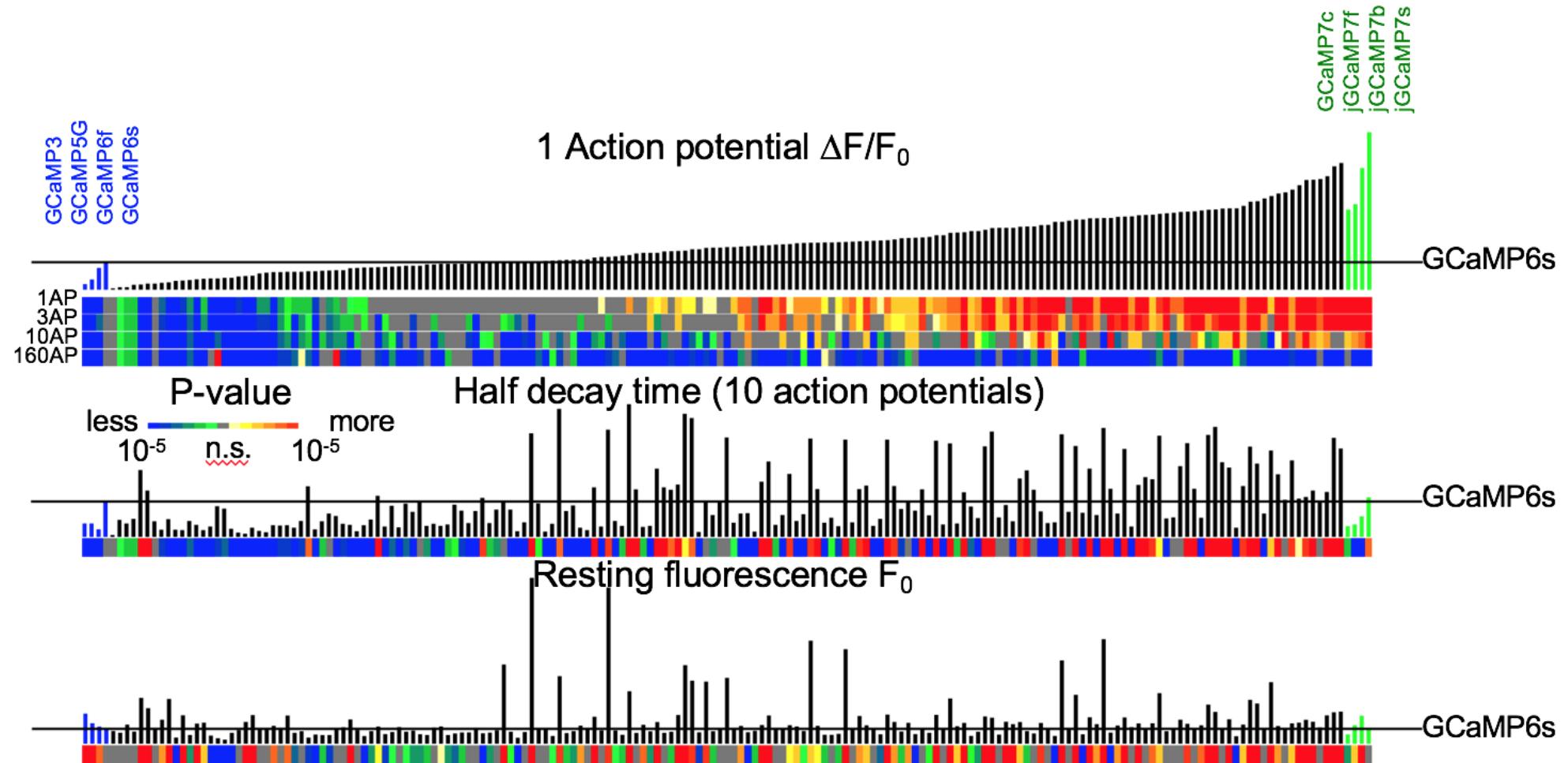
# Highly-buffered cells



- Looger: GCaMP3
- Looger: GCaMP5
- GENIE: GCaMP6
- **GENIE: jGCaMP7**
- Looger: faster GCaMPs



# GENIE

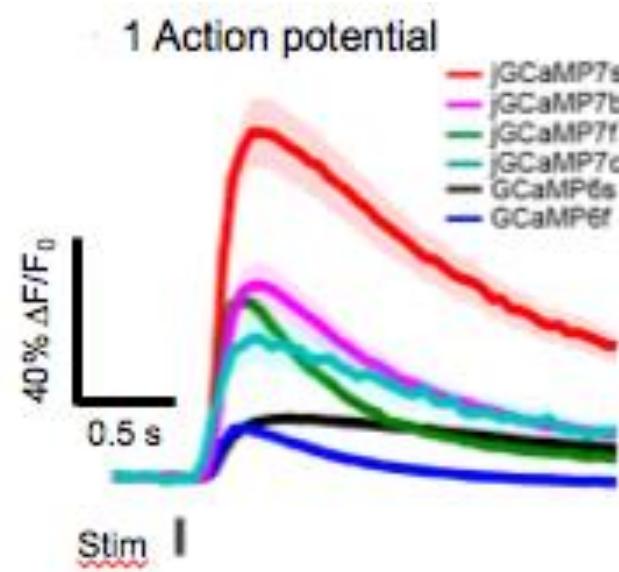


Hod Dana

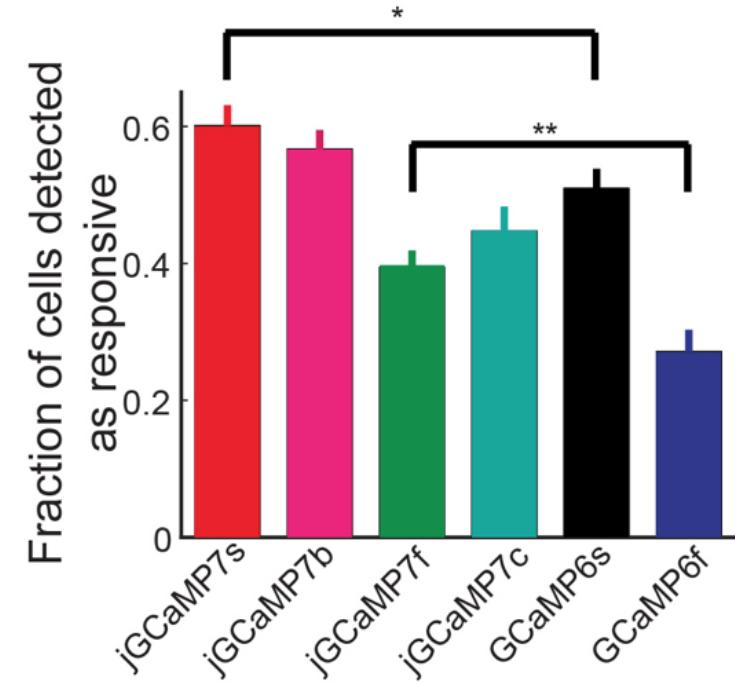


Yi Sun

# GENIE



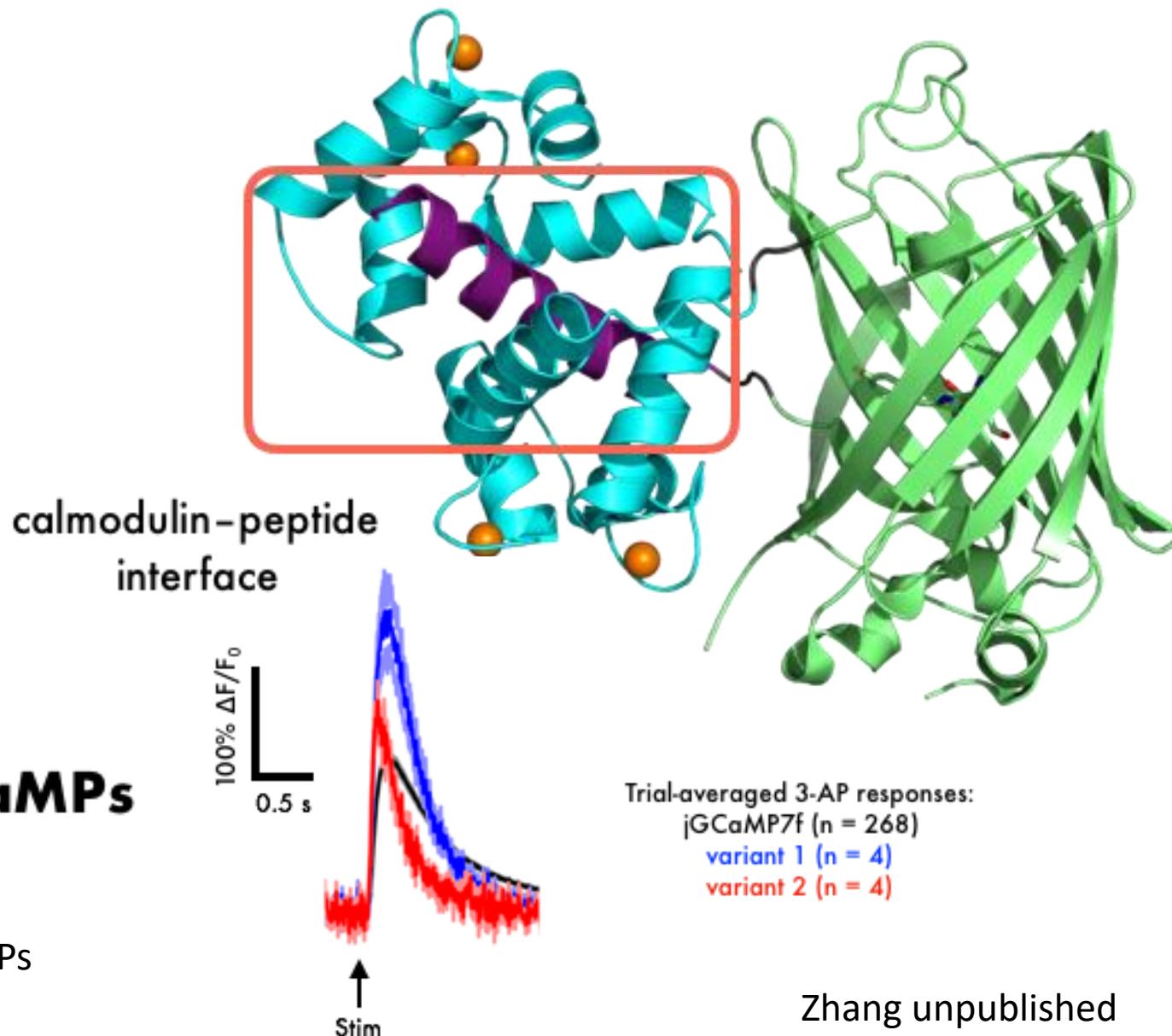
**s: sensitive**  
**b: high baseline**



**f: fast**  
**c: low baseline**

- Looger: GCaMP3
- Looger: GCaMP5
- GENIE: GCaMP6
- GENIE: jGCaMP7
- **Looger: faster GCaMPs**

Also GCaMP-uf, XCaMPs



Zhang unpublished

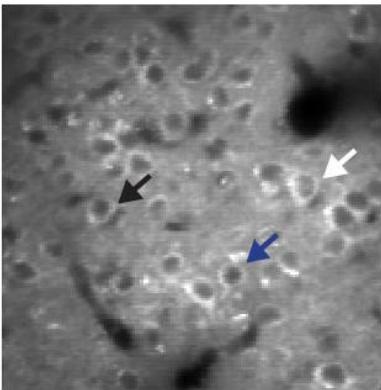
# Non-CaM GECl's

- Fungal GCaMP
- TN-XXL (based on troponin-C)
- NTnC / YTnC / new stuff?
- Jenny Yang's

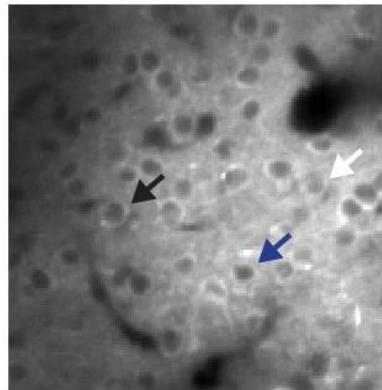
# GCaMP6 thy1 transgenic mice

C

GP4.3, Imaging day 1

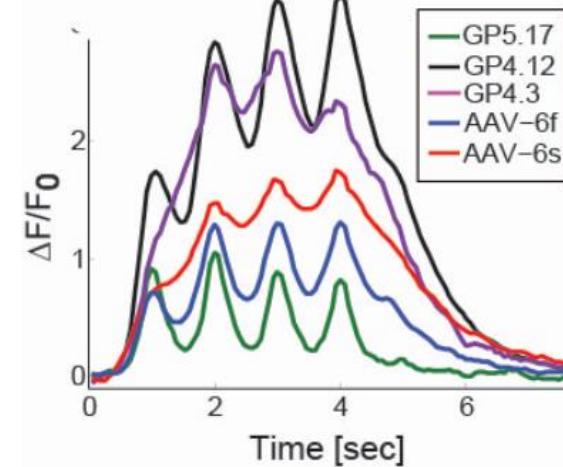


GP4.3, Imaging day 42

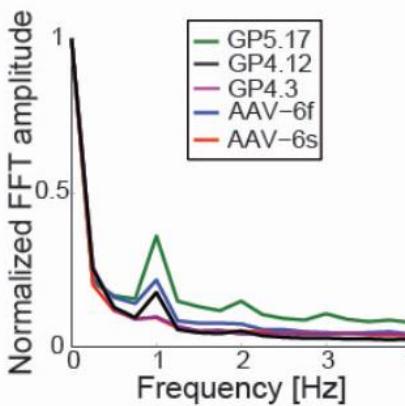


20  $\mu$ m

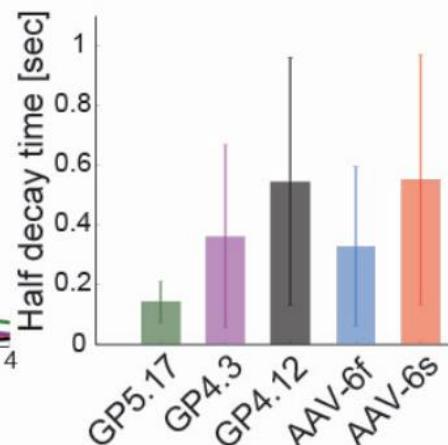
d



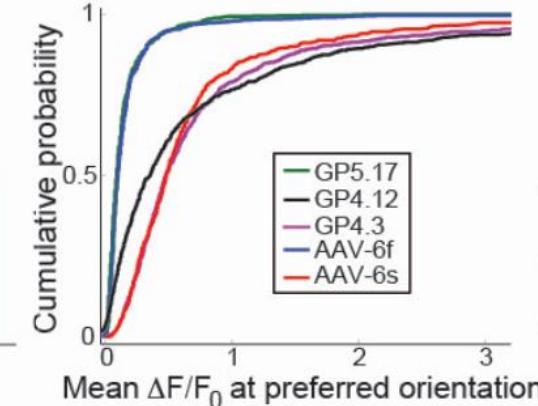
e



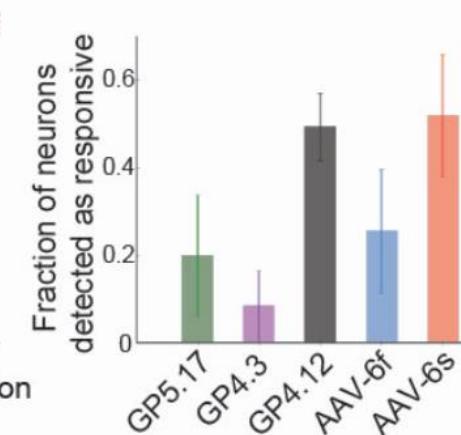
f



g



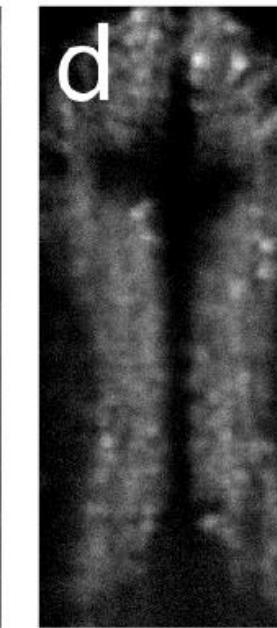
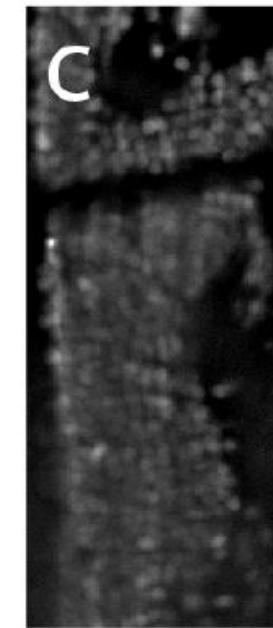
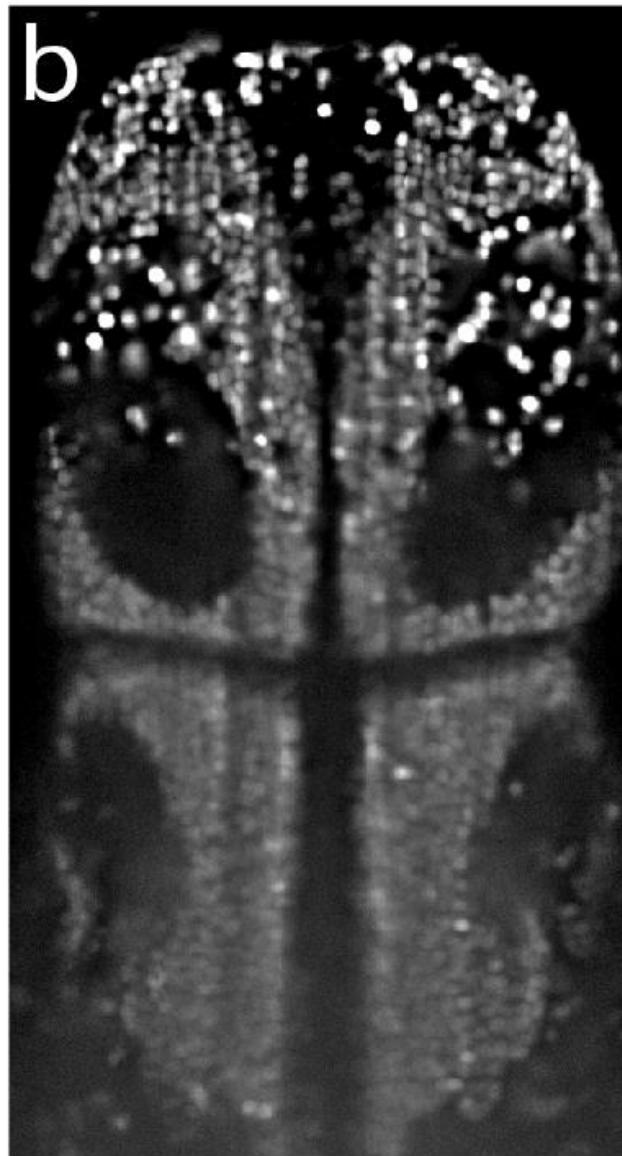
h



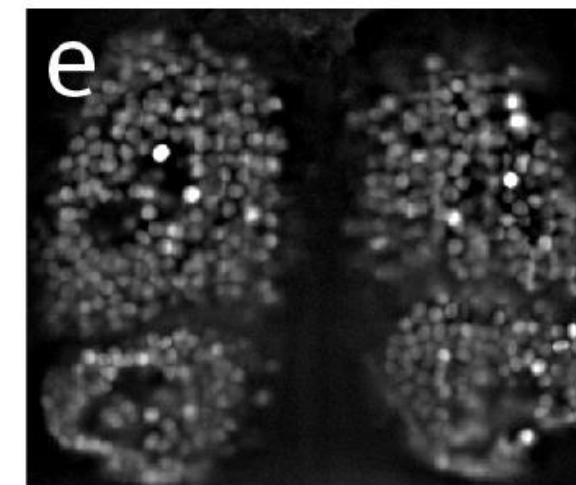
# Cre-dependent mice from Allen

GECI doesn't have to be cytosolic

# H2B-GCaMP

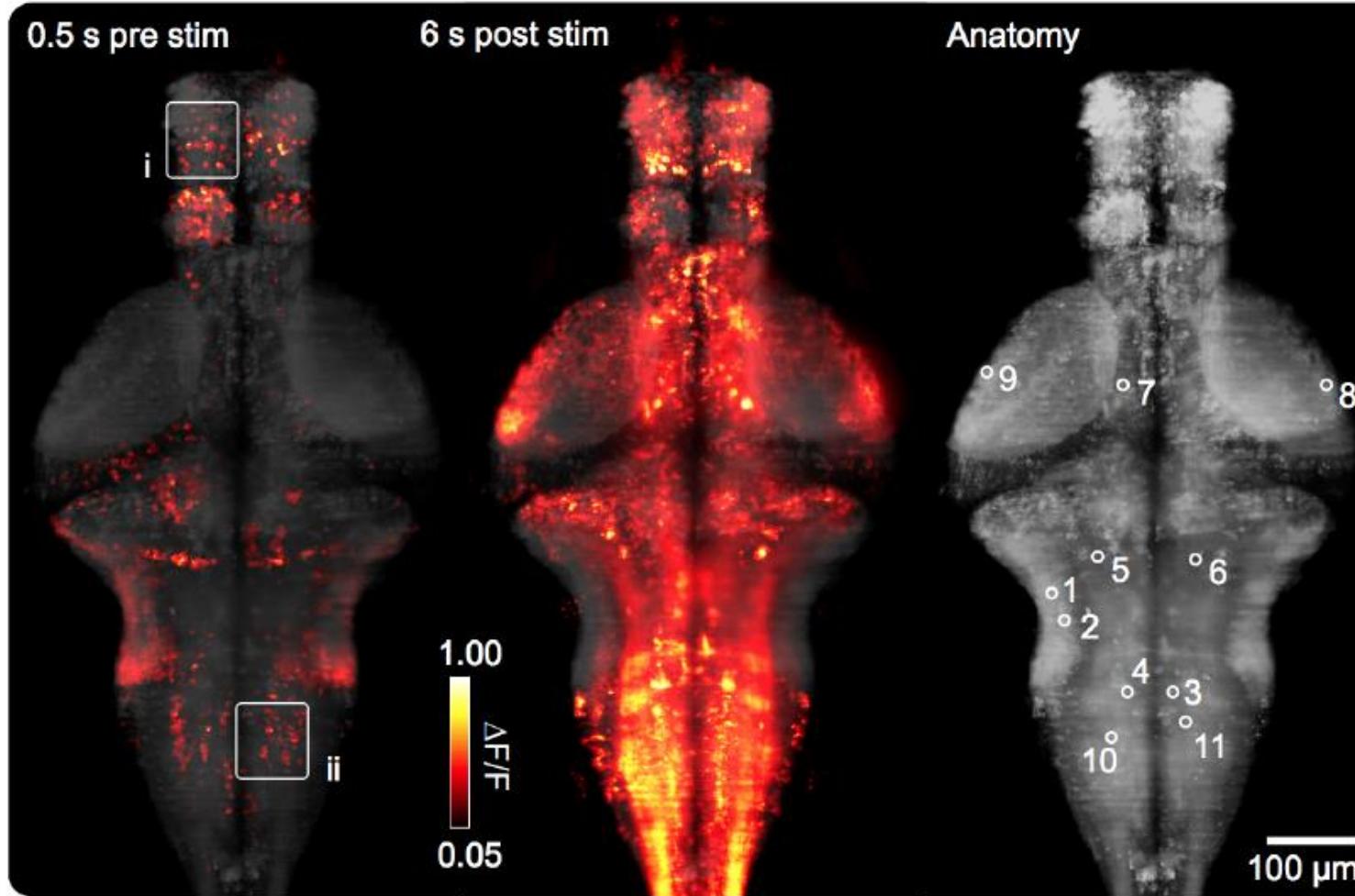
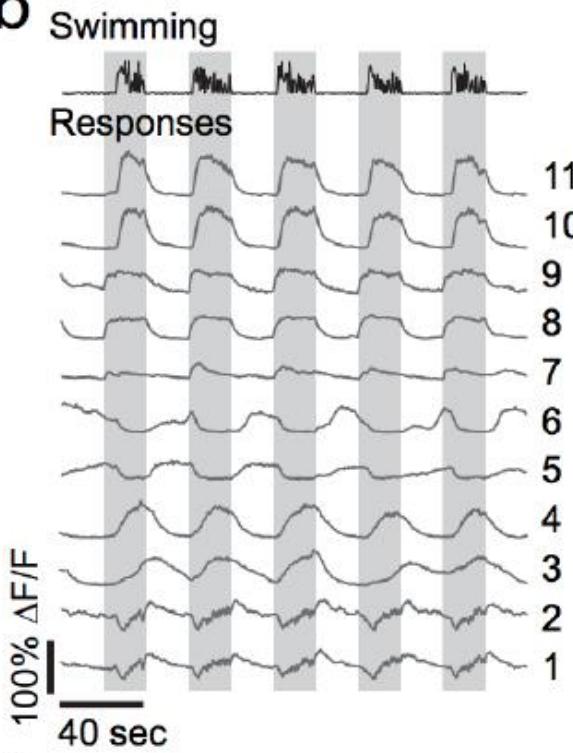
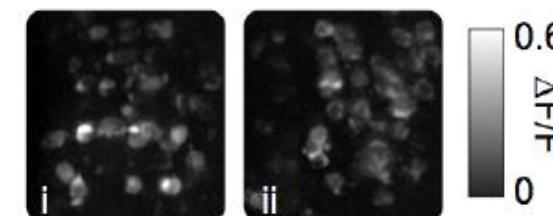


(better than NLS)



## H2B-GCaMP

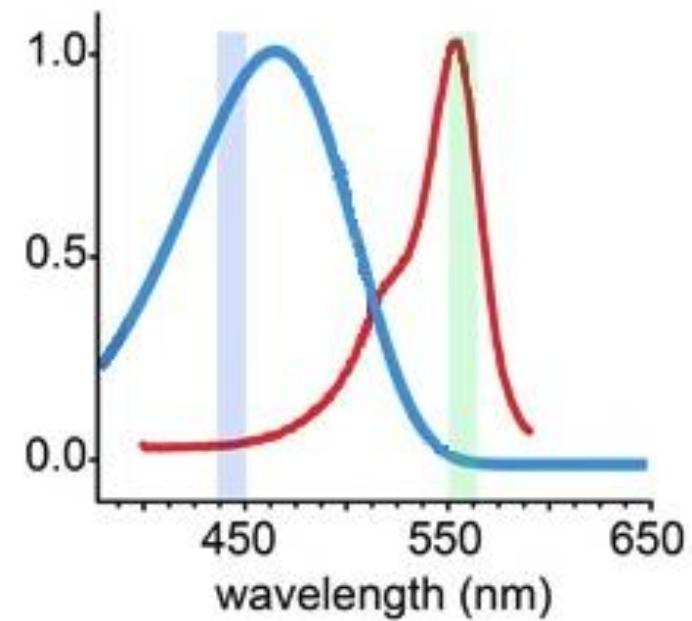
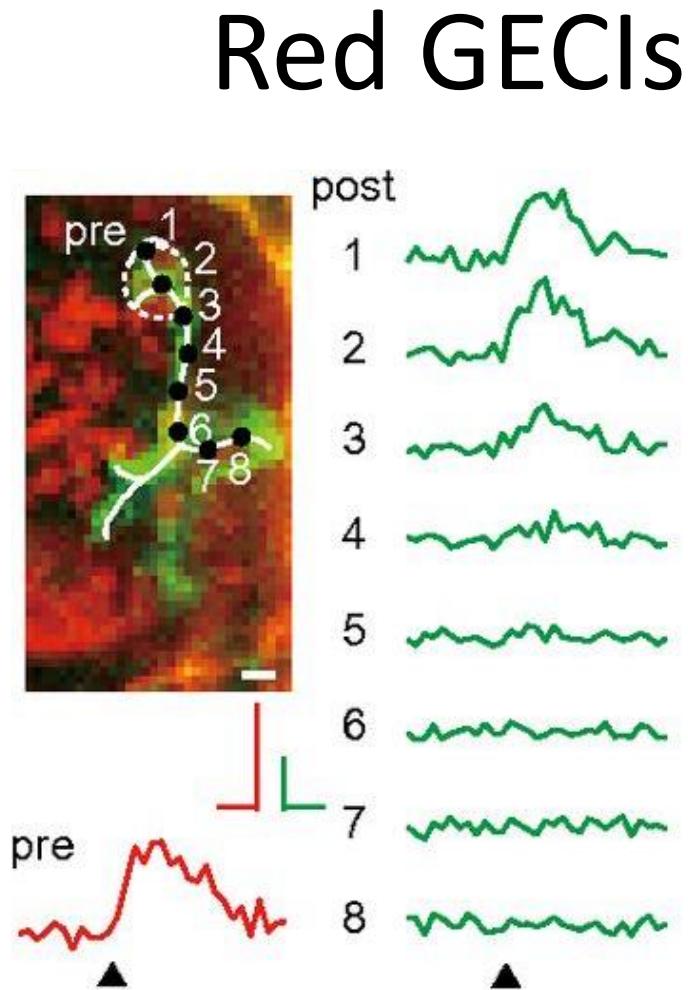
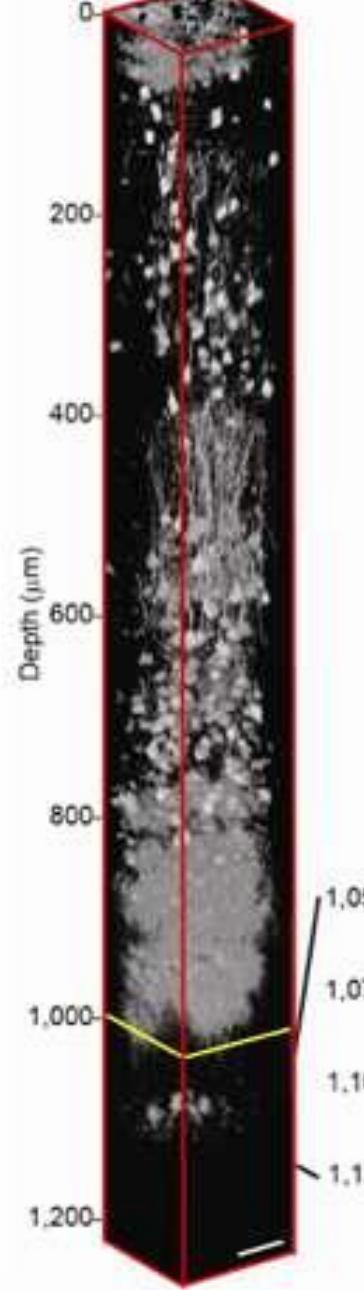
Available as AAVs as well

**a****b****c**

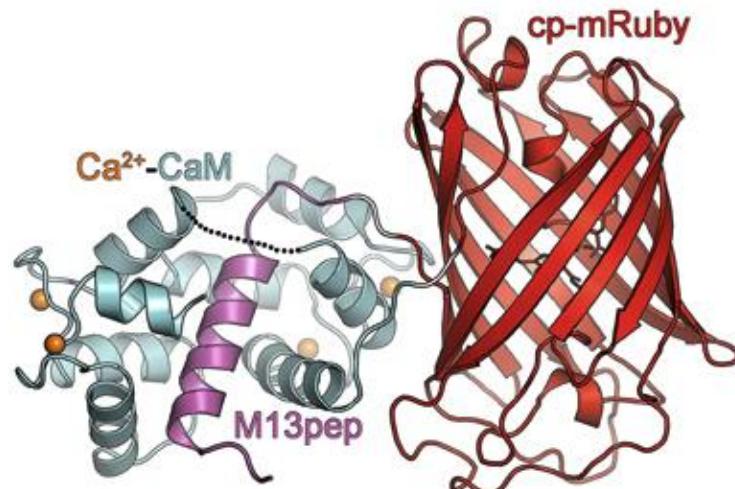
- Terminal (Synaptophysin)
- Membrane (Lck)
- ER
- Mito
- Extracellular
- Etc...

■ neurons (fluorescence)

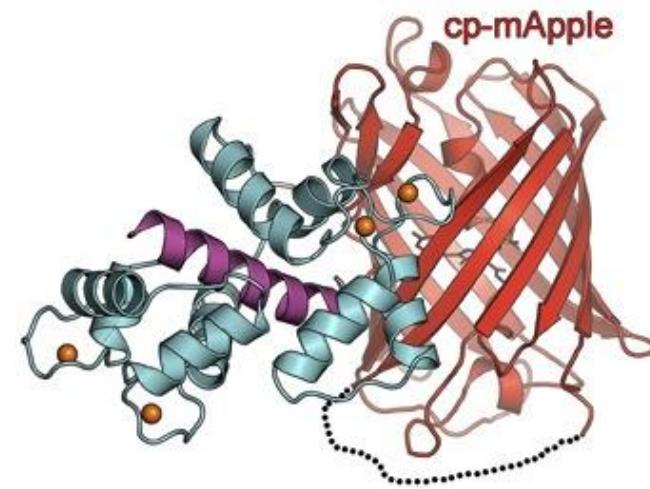
■ GECIs (fluorescence)



# Red GECLs



**RCaMP1**  
Akerboom et al., 2013

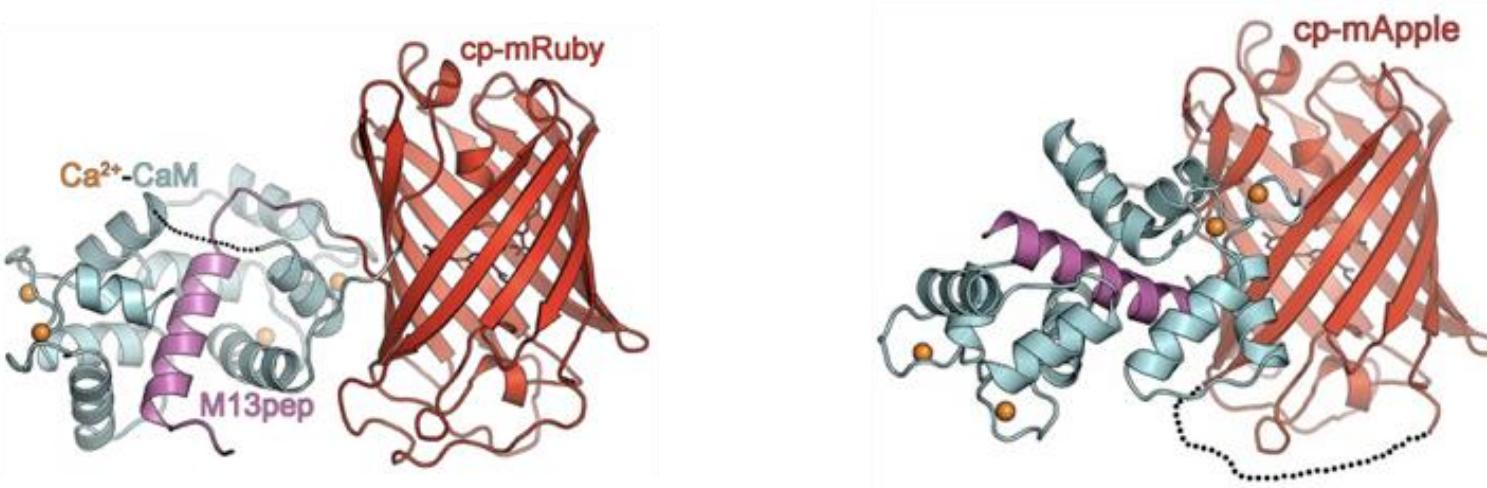


**R-GECO1.0**  
Zhao et al., 2011



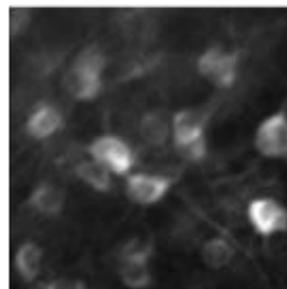
Robert Campbell

# GENIE

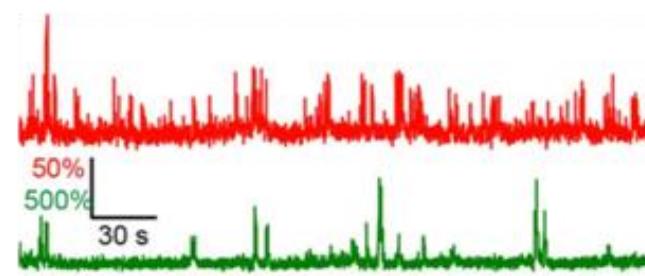
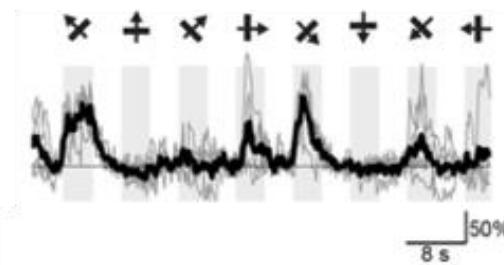


Akerboom et al. 2013

RCaMP (Looger)  
↓  
jRCaMP1



L6, 850  $\mu$ m



Dana 2018

# Issues with reds

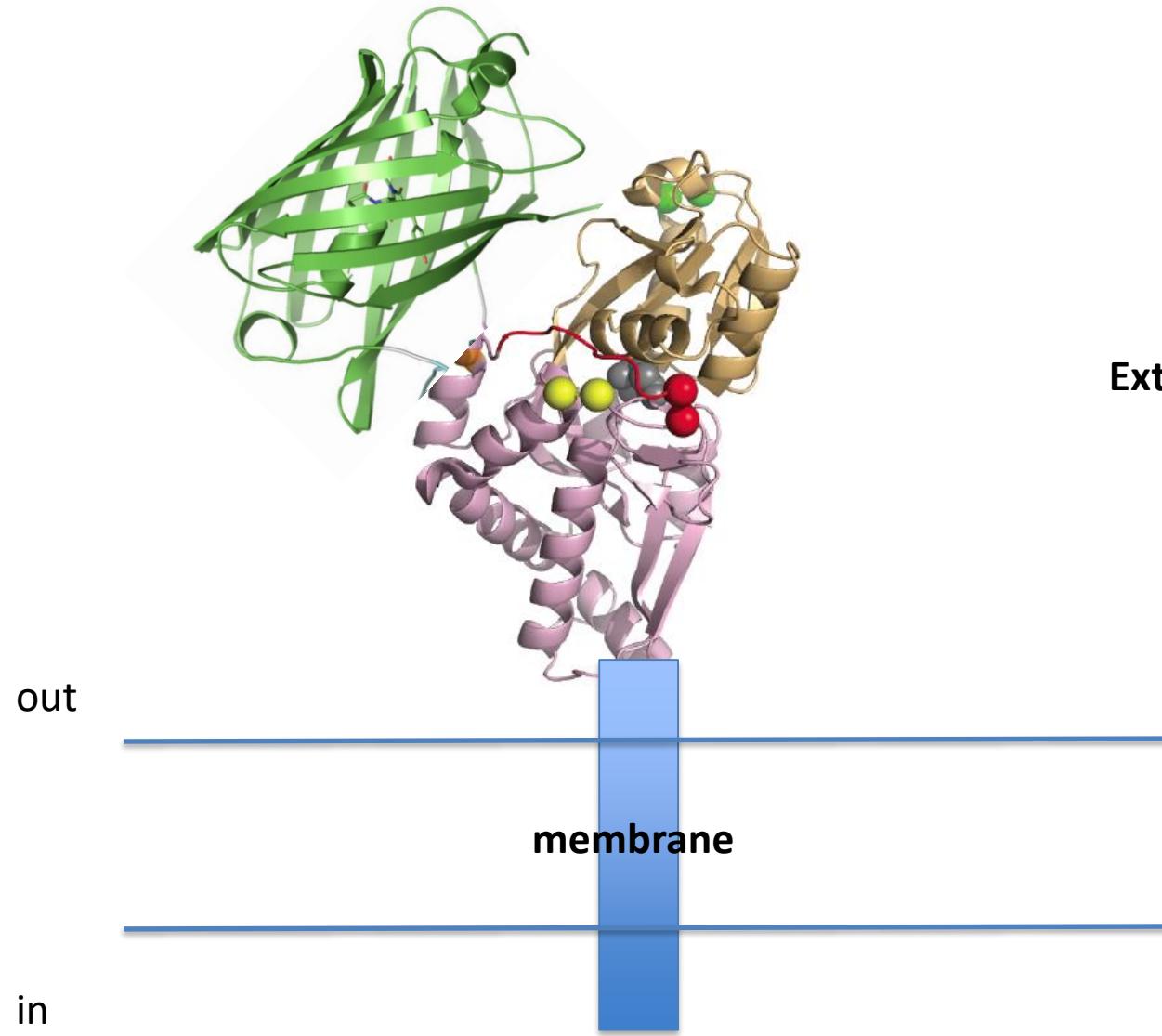
- Didn't have NES, had to add! (Impt for >=2 reasons)
- Bleach much faster
- Can pollute the green channel
- Can photoswitch from light regardless of [Ca<sup>2+</sup>]
- Form non-productive “blobs” in endosomes/lysosomes
  - Reds less monomeric than greens
  - Only partially degraded by cathepsins?

Also....

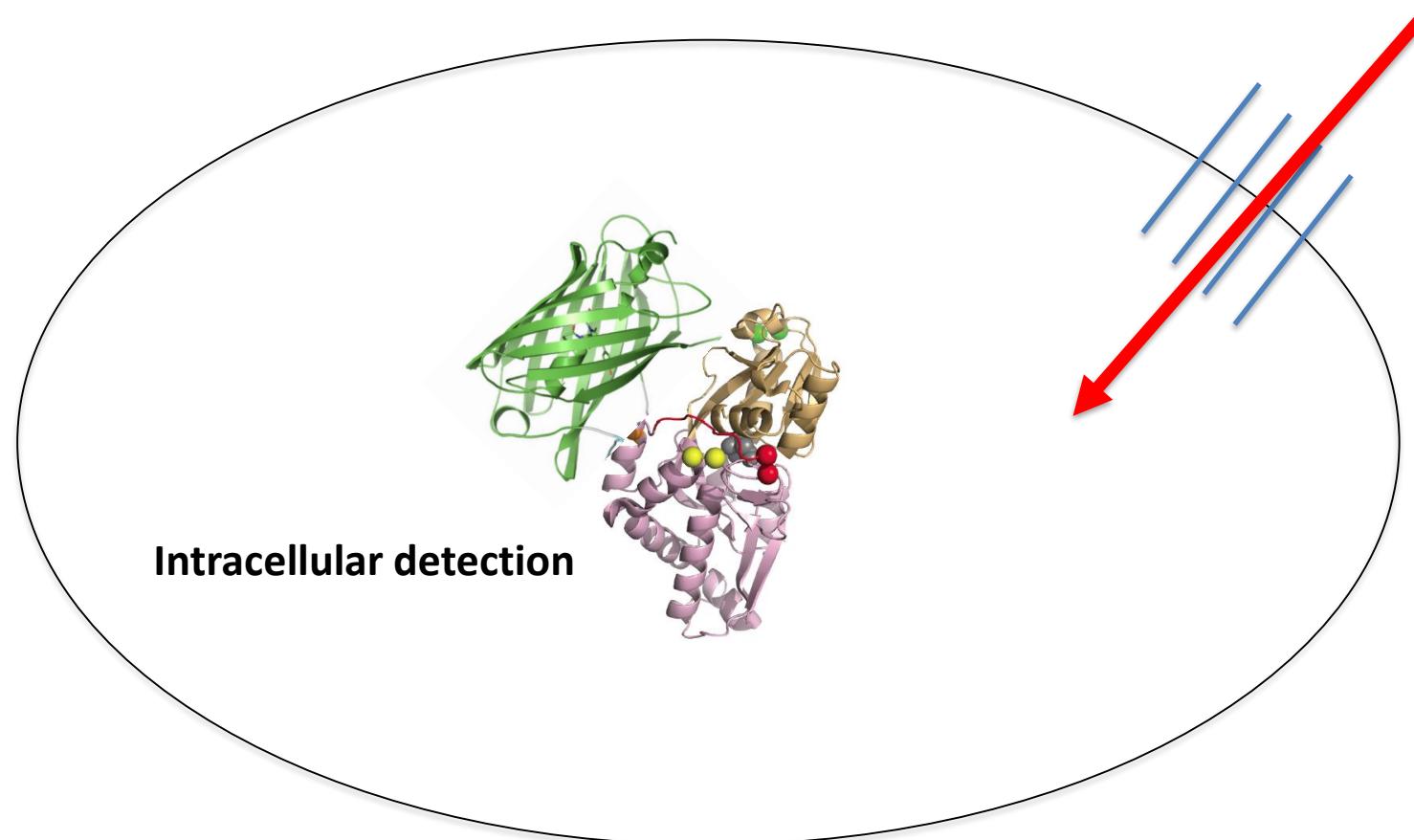
- Yellow, cyan, blue, infrared/far-red



# Neurotransmitter sensors



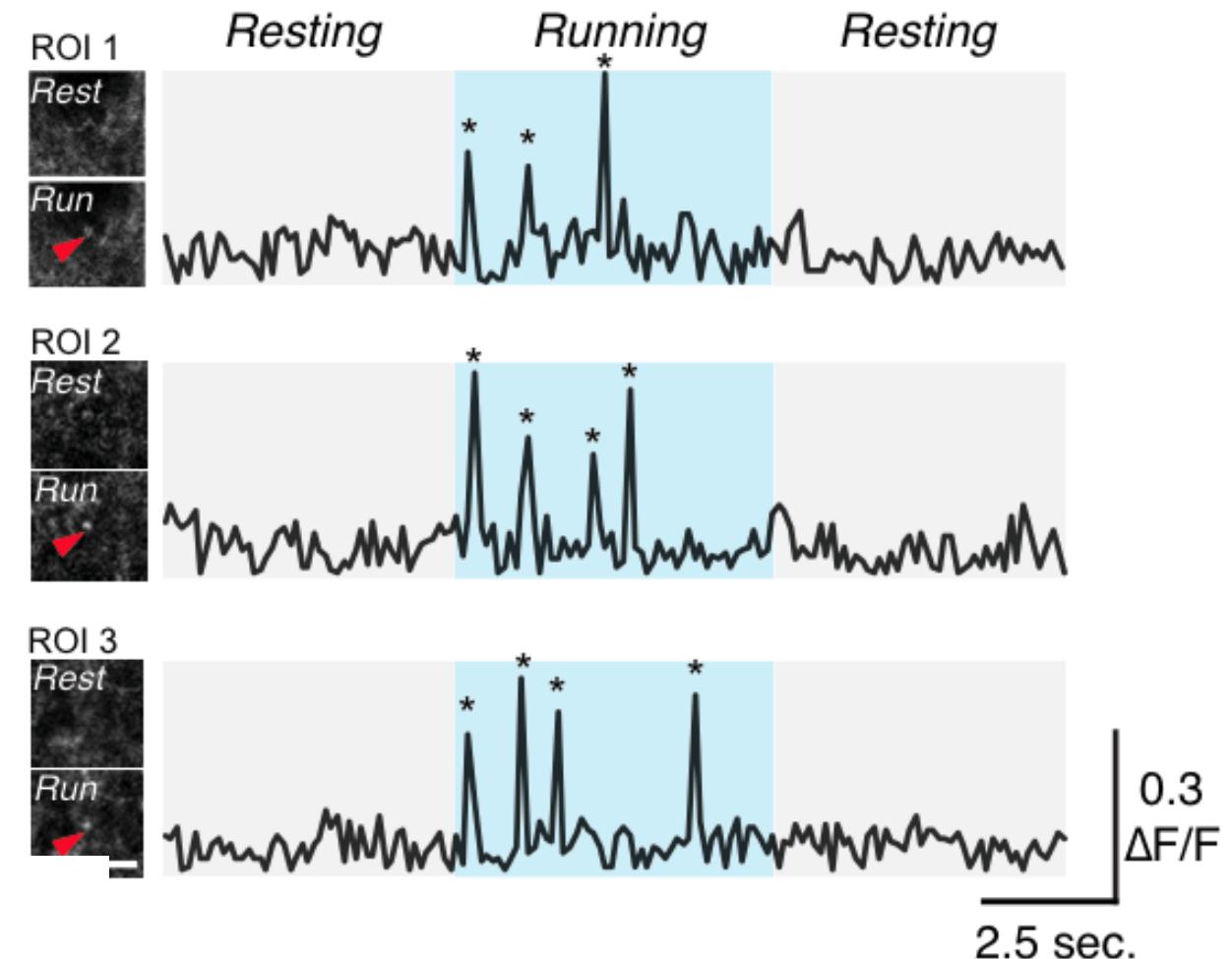
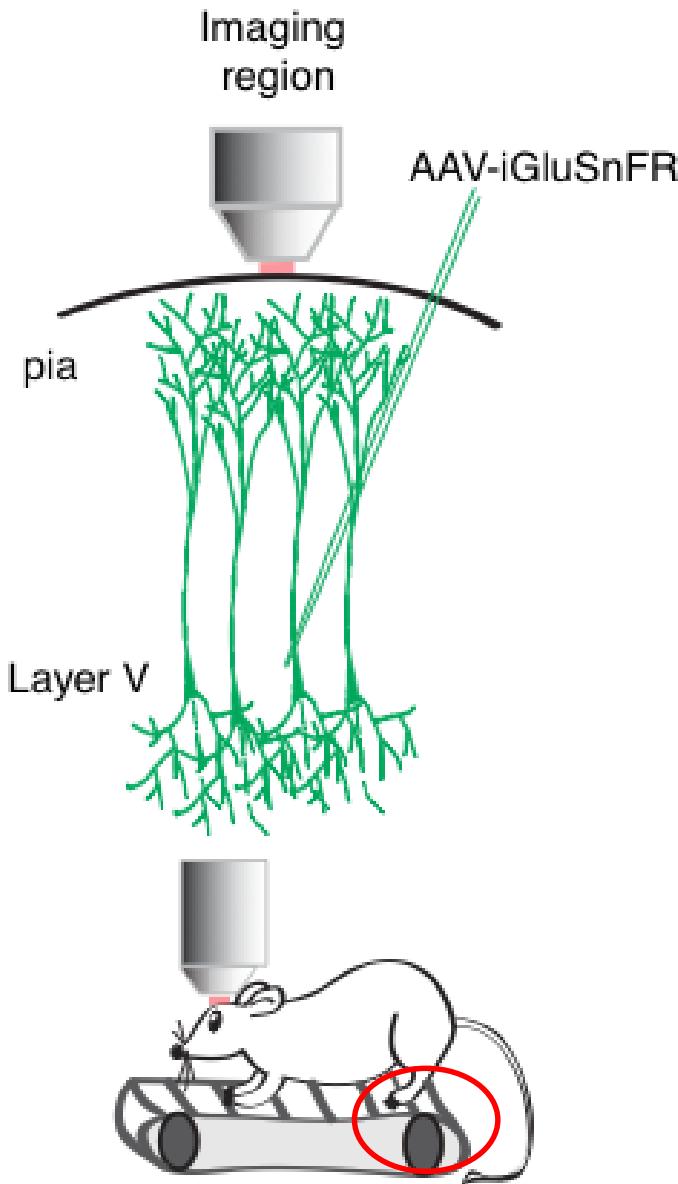
**Extracellular detection**



# Needs

- Genetically encoded, targetable, bright, photostable, high SNR, sensitive, fast, specific, long-term expression, bio-orthogonal (microbial proteins)

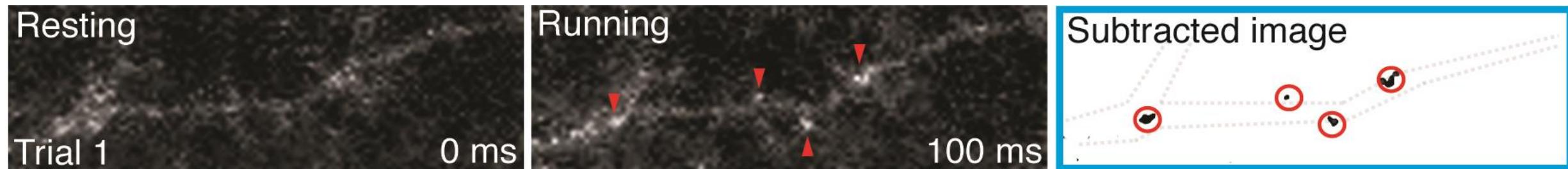
# Glutamate



Joe Cichon

Marvin et al. 2013

# Signal at spines



# Activity-dependent

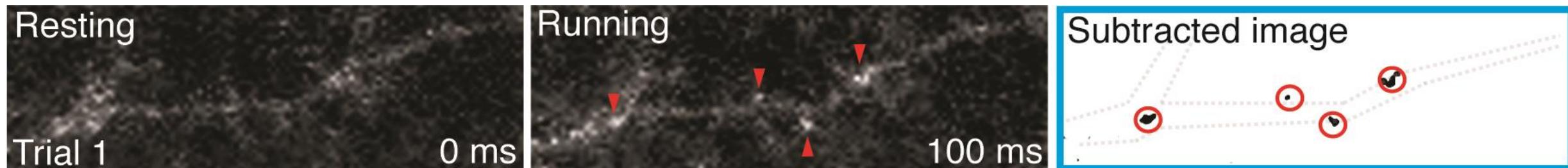


# Glu-specific



**Not affected by any other molecules or drugs**

# Measures Glu



TTX

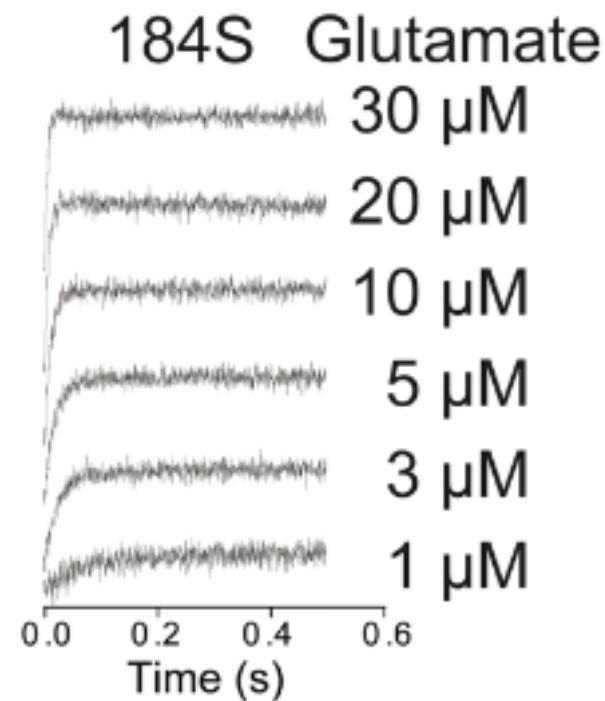
-> no iGluSnFR signals

Not affected by any other molecules or drugs

Pilocarpine, ketamine -> 2x iGluSnFR signals

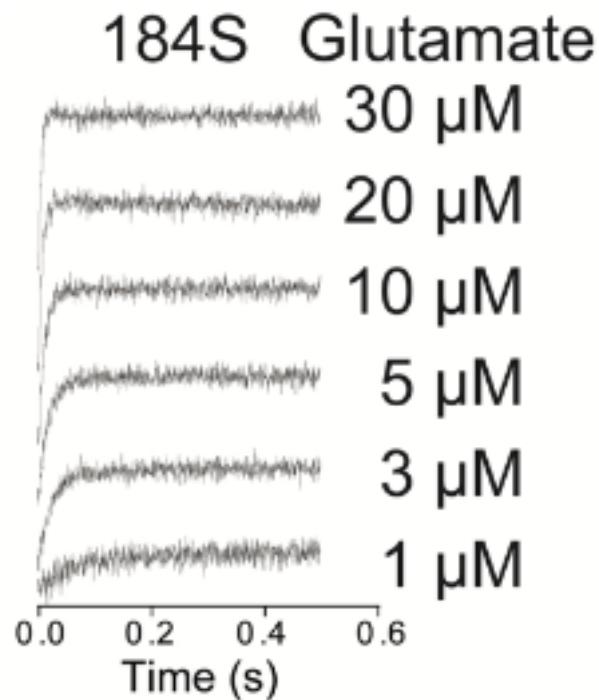
# iGluSnFR is extremely fast

Stopped-flow fluorescence



# iGluSnFR is extremely fast

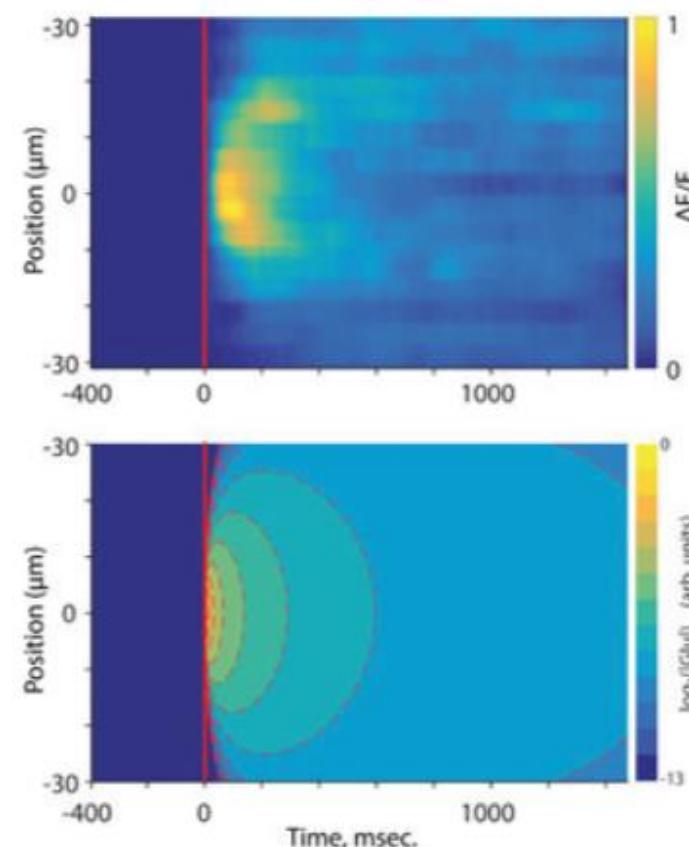
Stopped-flow fluorescence



$$k_{\text{on}} \sim 1 \times 10^{-7} \text{ M}^{-1}\text{s}^{-1}$$

1 kHz frame-rate (1.5 Mpixel) imaging of Glu uncaging & diffusion

Cool imaging trick!



Kaspar  
Podgorski  
(SLAP)

$\Delta F/F$

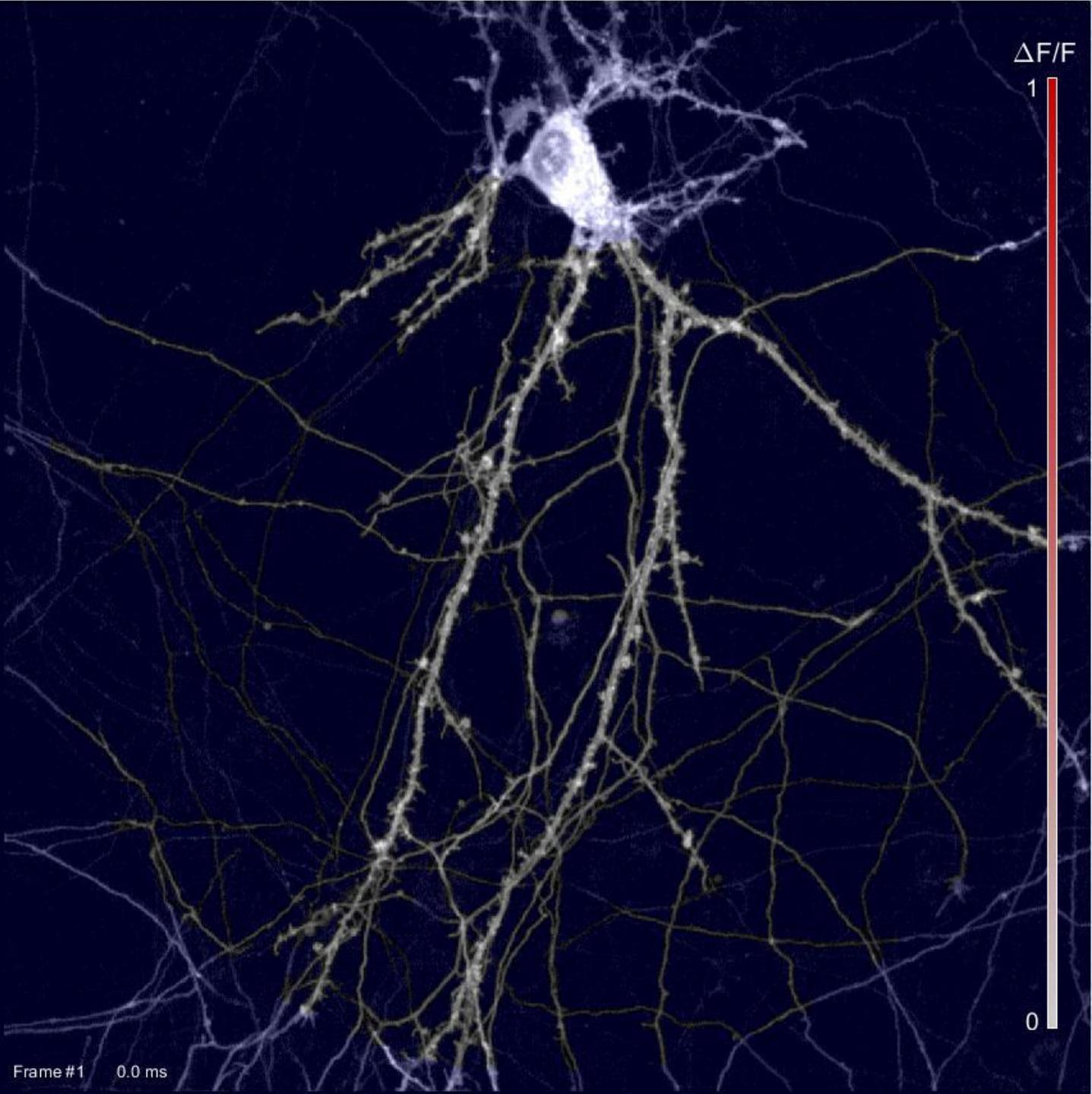
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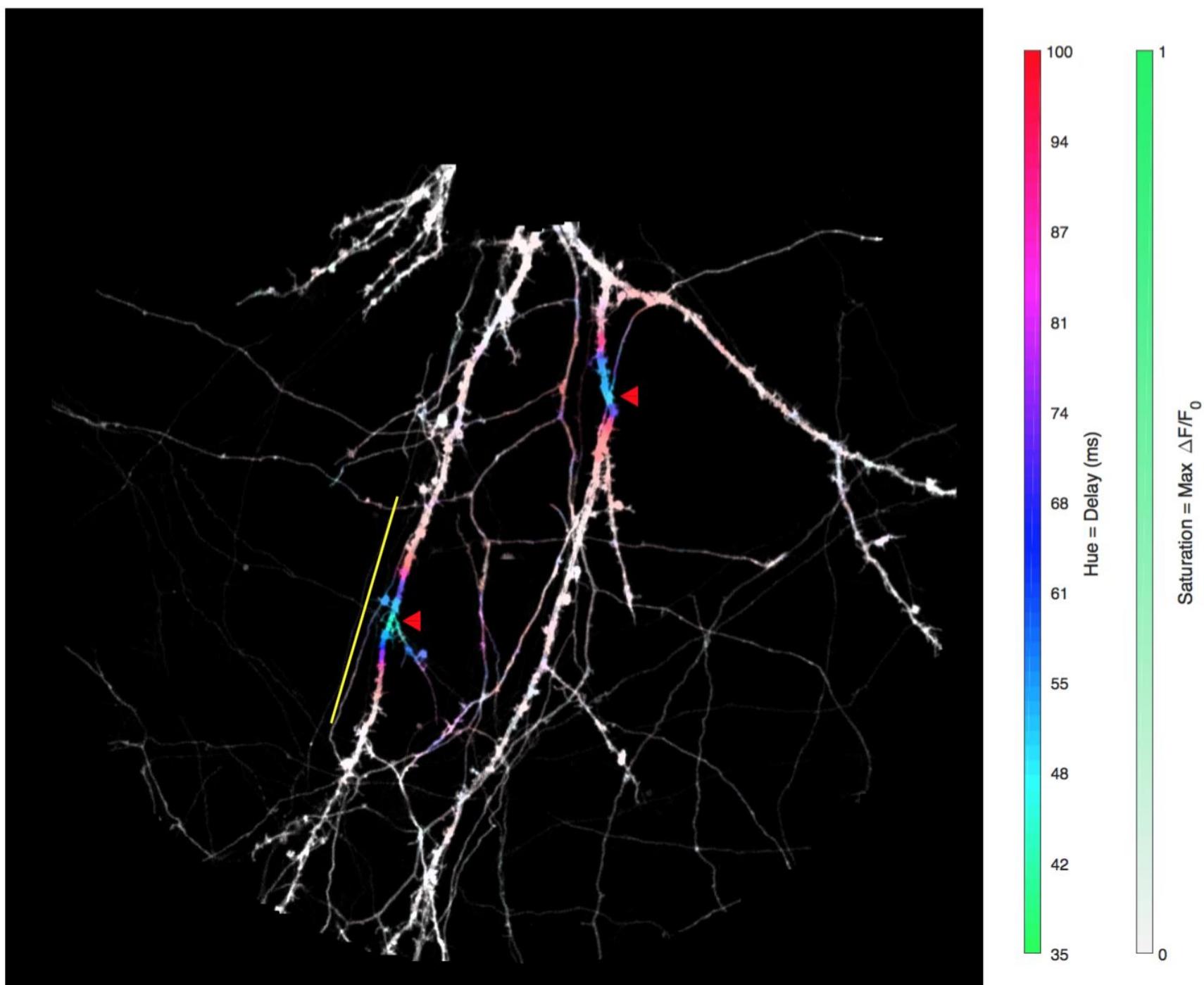
0

~1.5 Megapixels at 1016 Hz



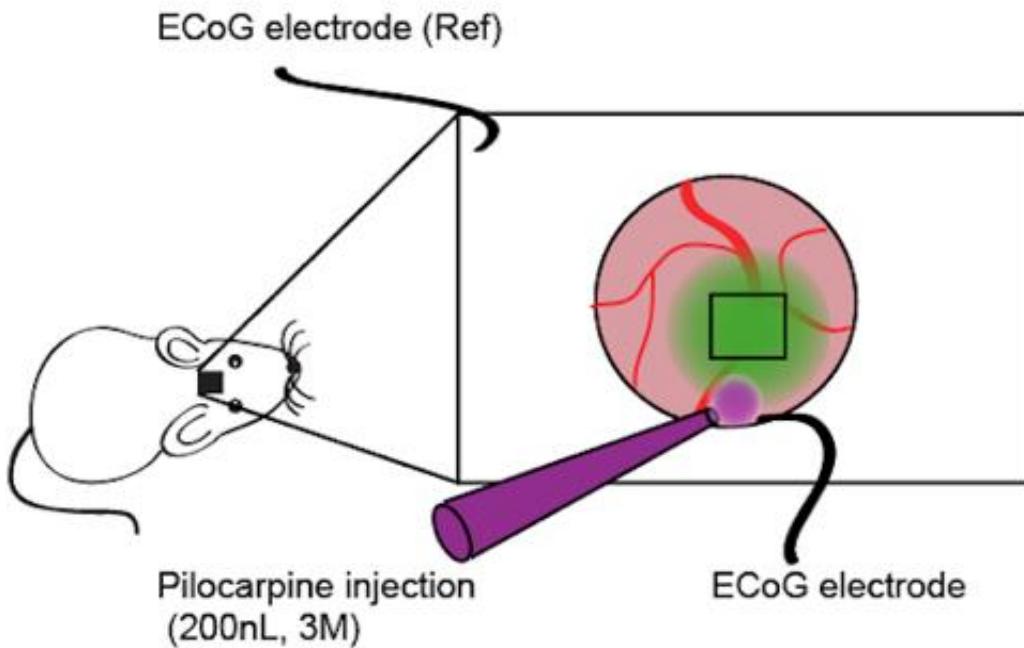
Kaspar Podgorski

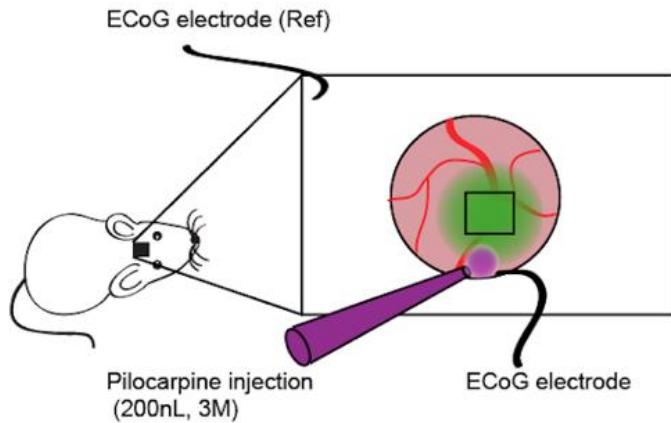




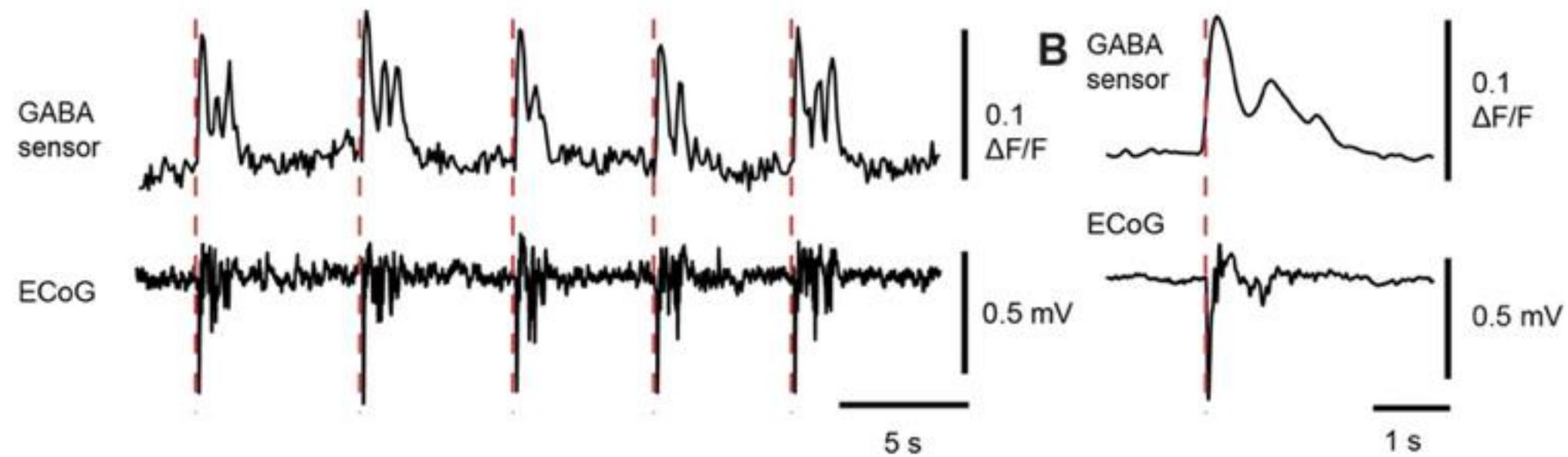
# GABA

# GABA



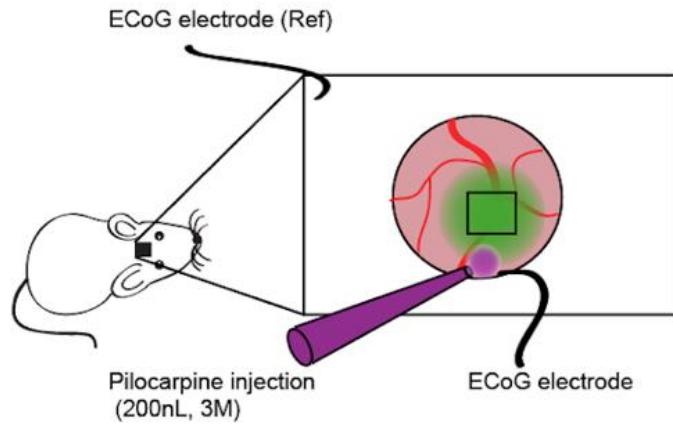


# GABA

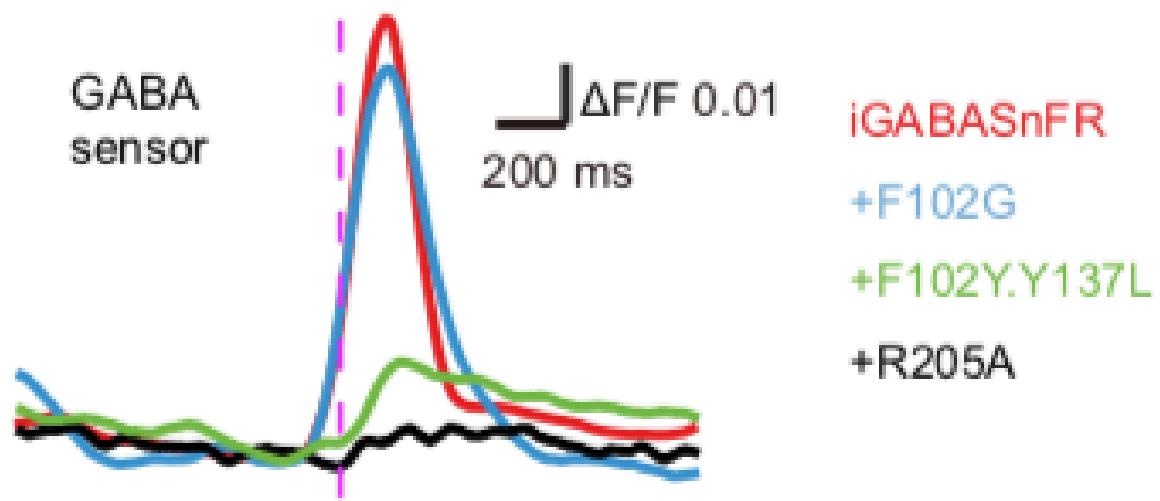


Dimitri Kullmann et al. (UCL)

Marvin et al. bioRxiv 2018, in press Nature Methods



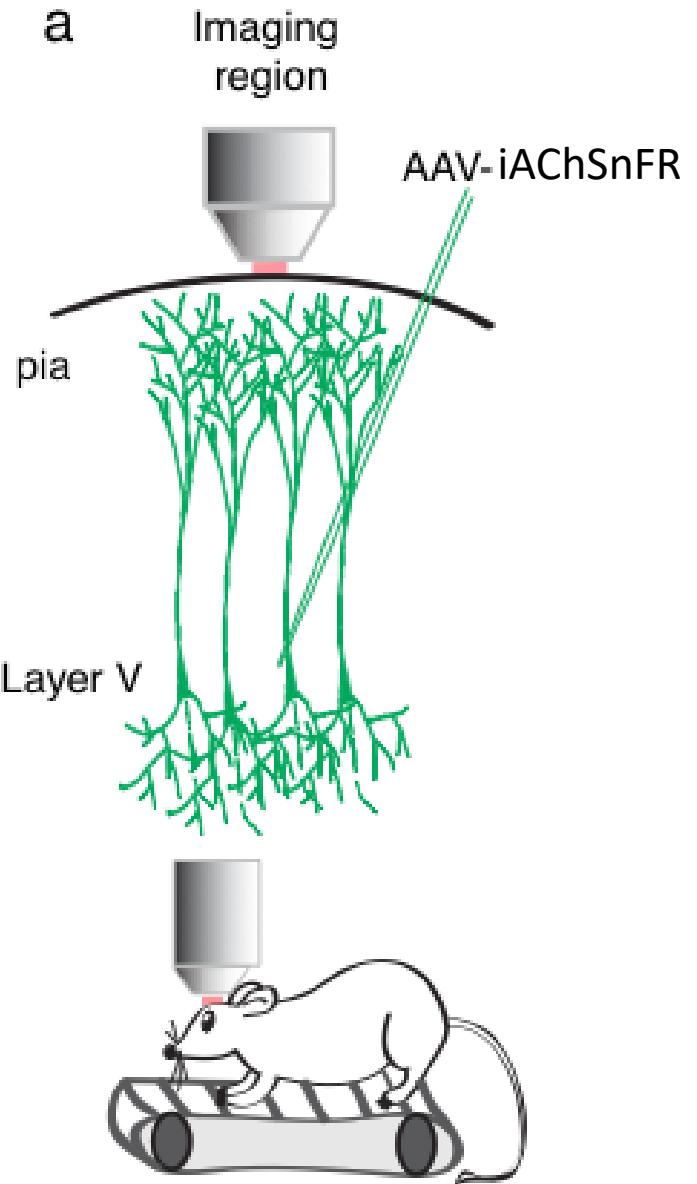
# GABA



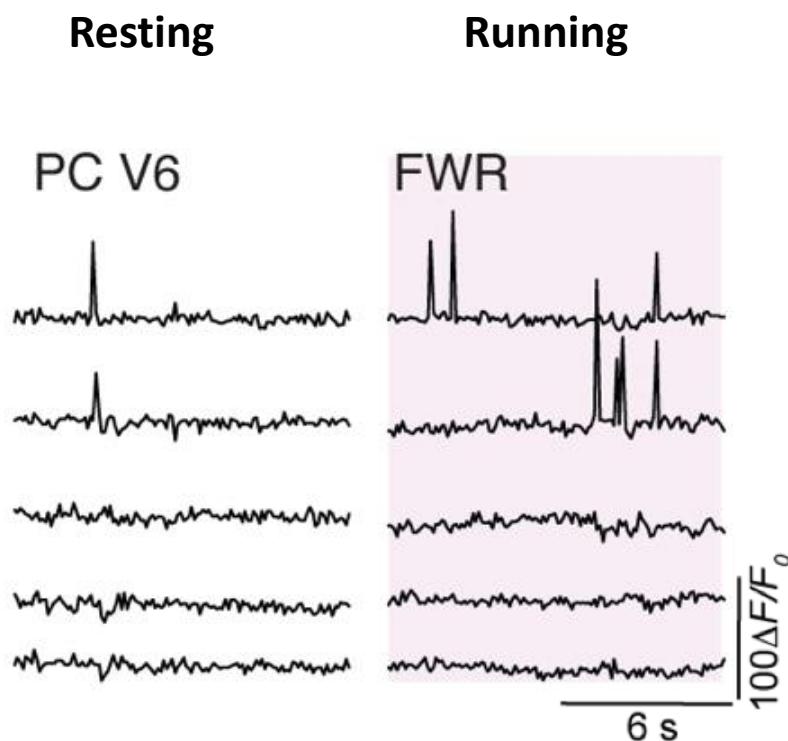
Dimitri Kullmann et al. (UCL)

Marvin et al. bioRxiv 2018, in press Nature Methods

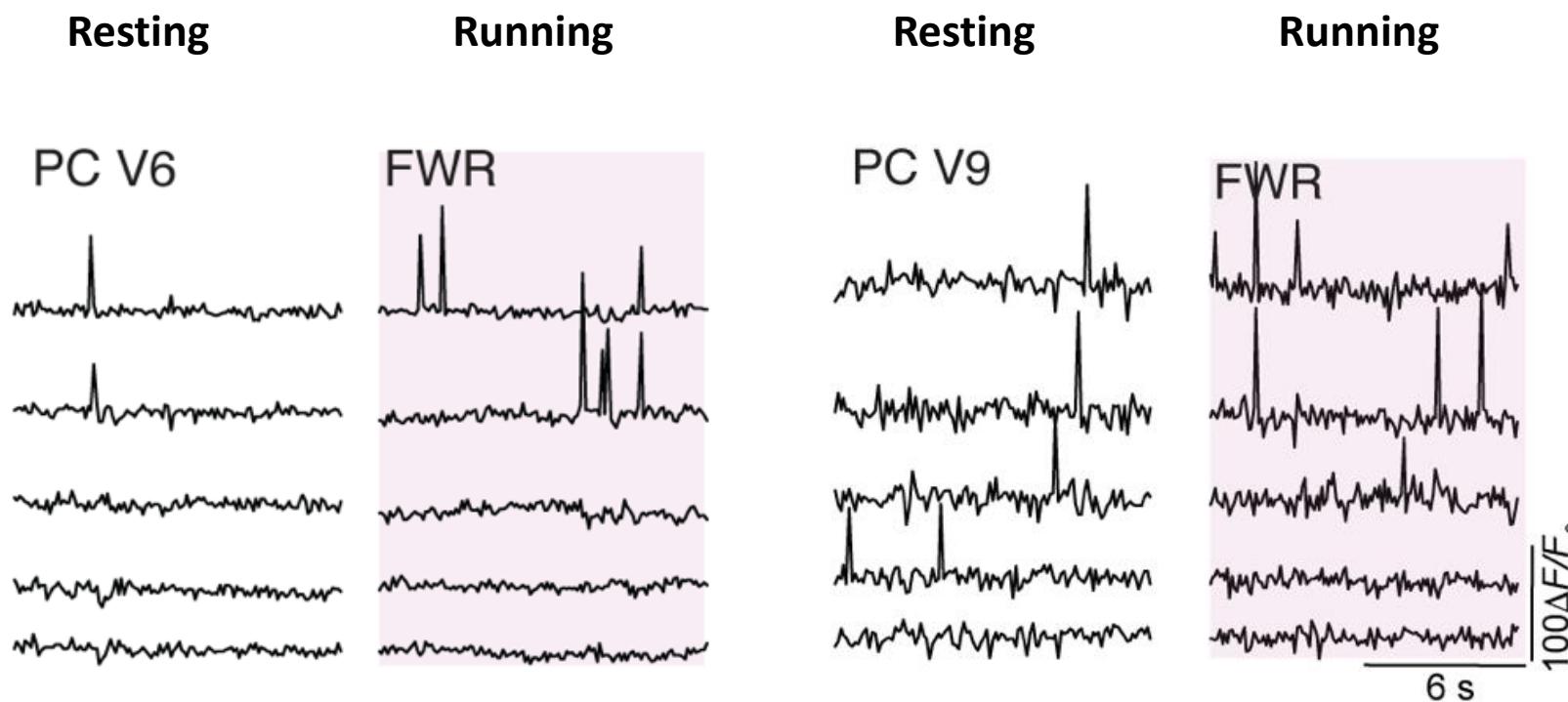
# Acetylcholine



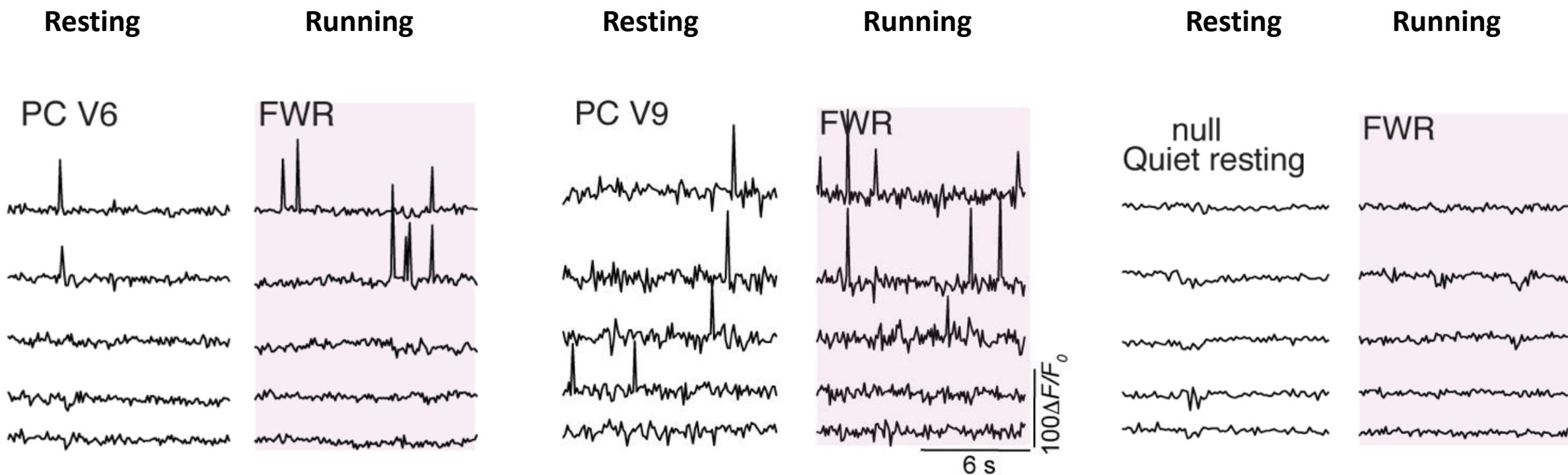
# iAChSnFR signal increases during running



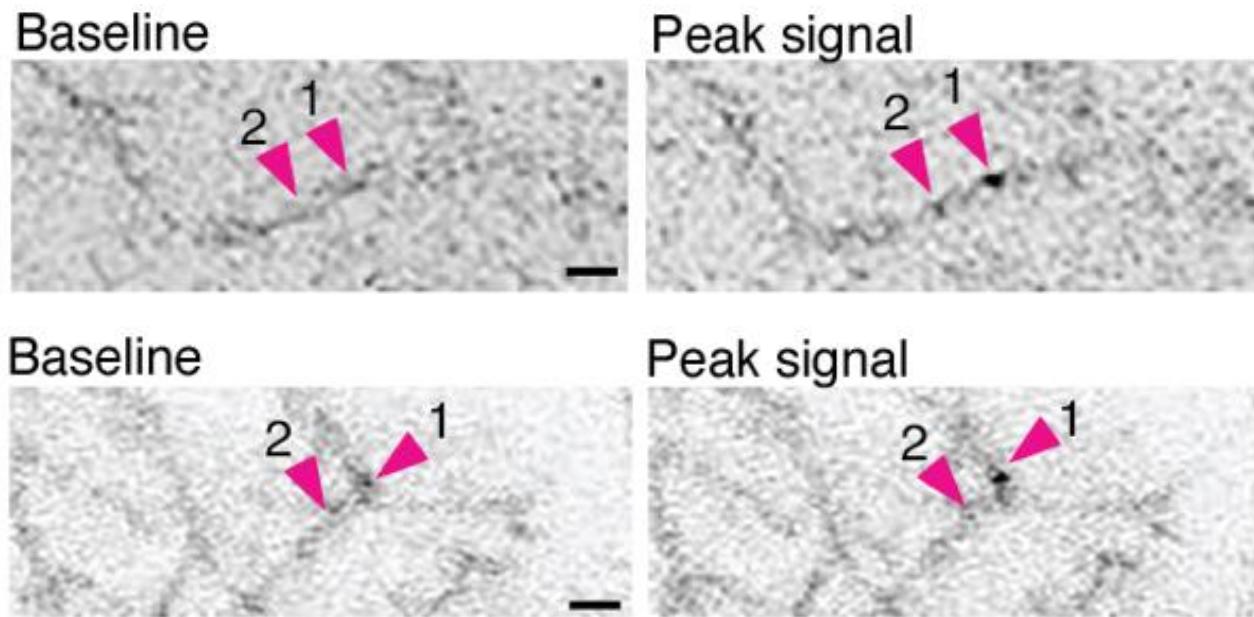
# iAChSnFR signal increases after AChE block



# iAChSnFR signal really measures ACh

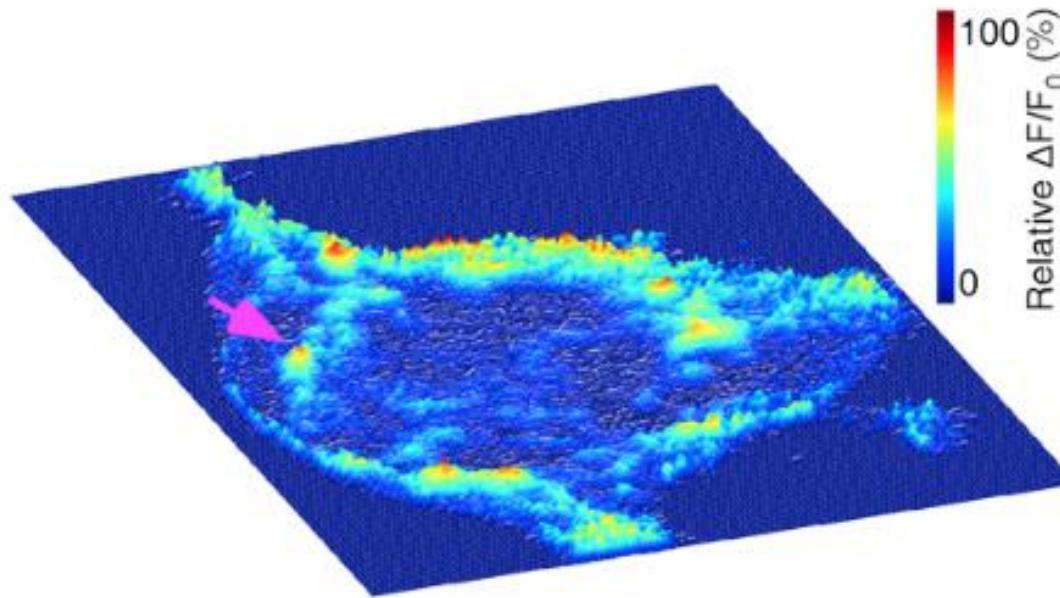


# ACh signal is localized on neuropil



# And at somatic release sites

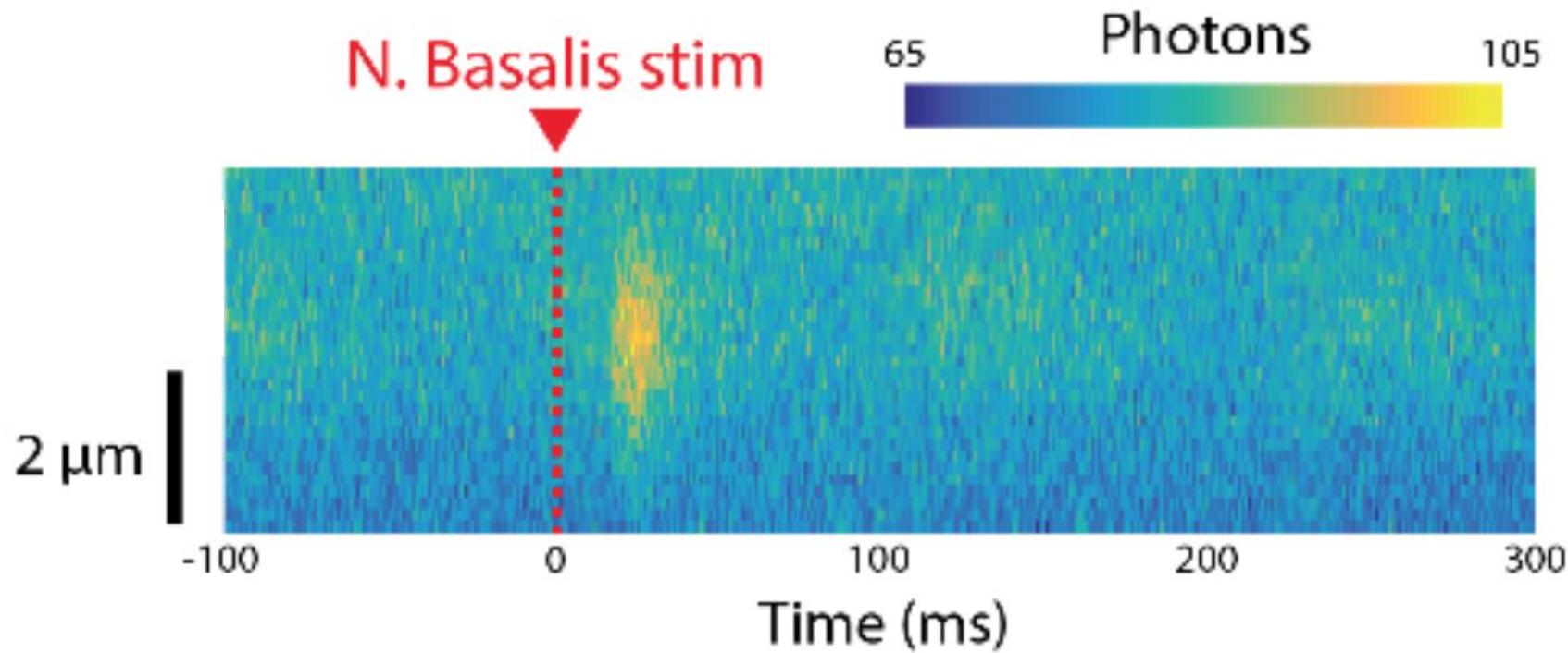
Cool imaging trick!



Super-resolution (~20 nm localization)

Julius Zhu, UVA

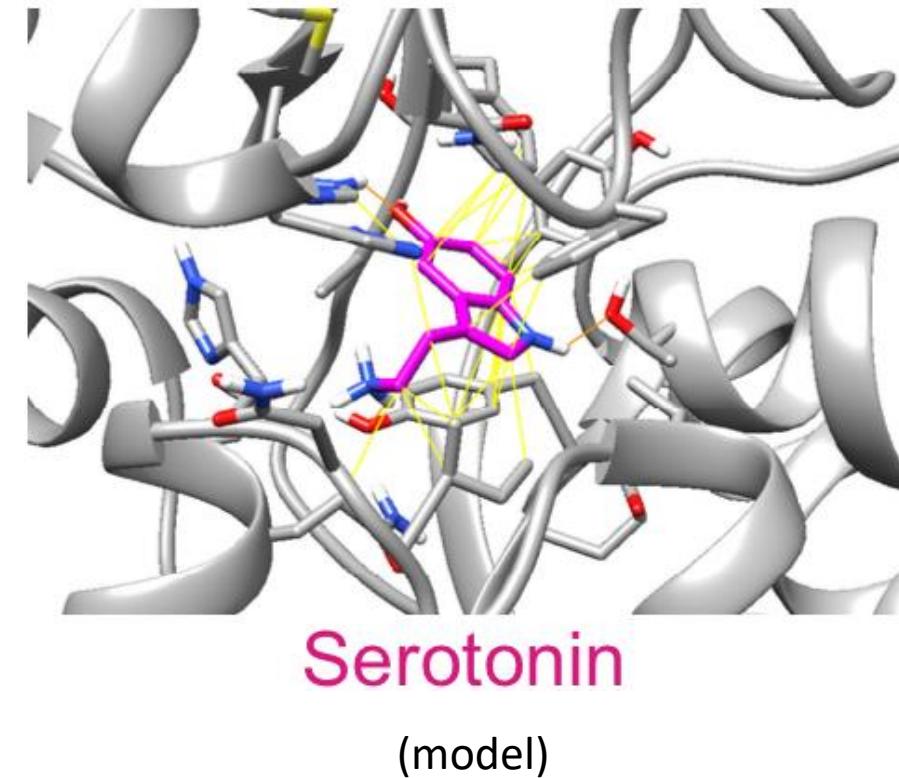
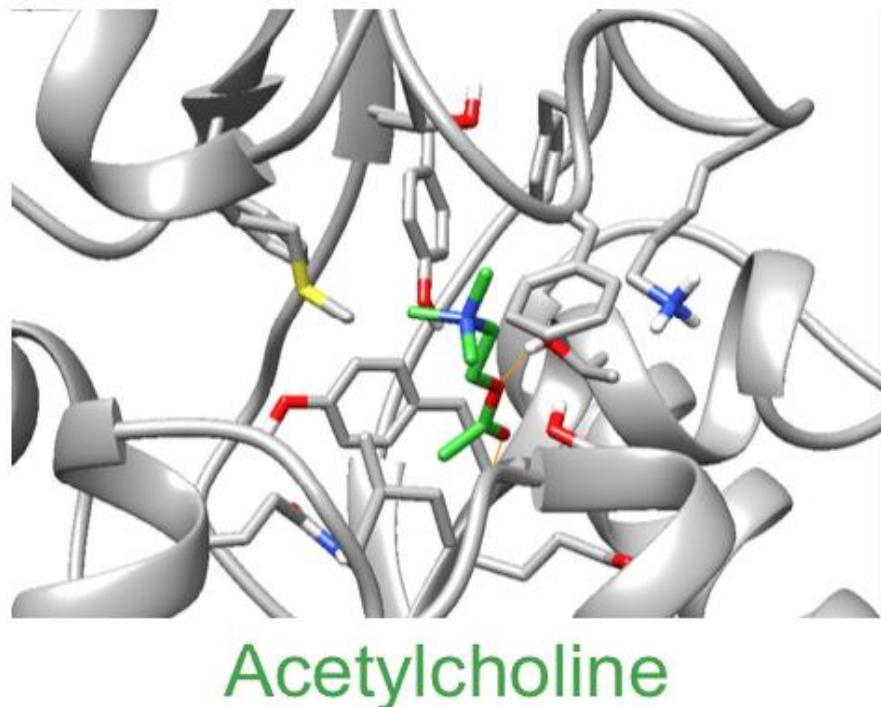
And is quite rapid (phasic ACh signaling)



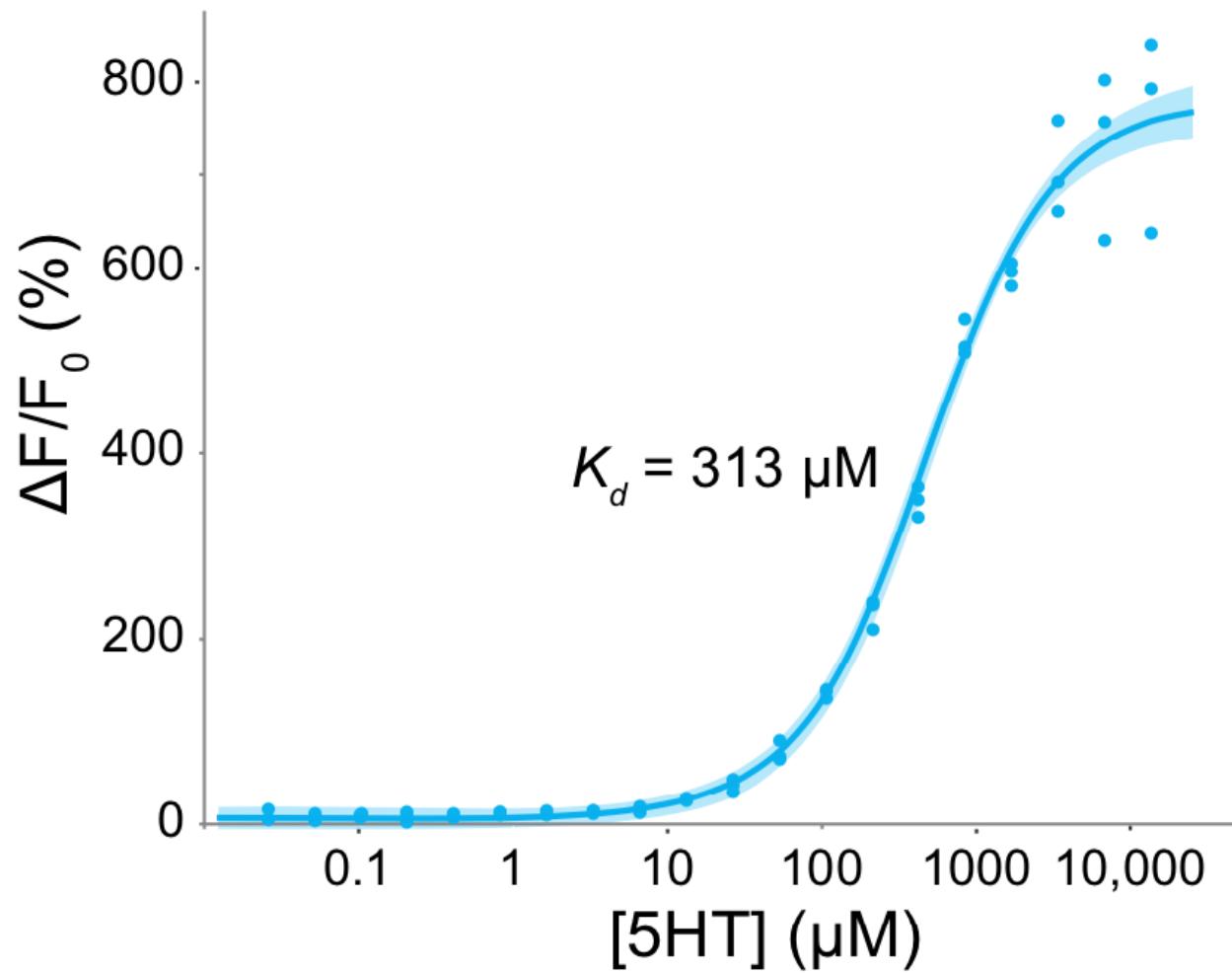
1 kHz 2-P frame-rate iAChSnFR imaging in cortex  
current injection in nucleus basalis

# Serotonin

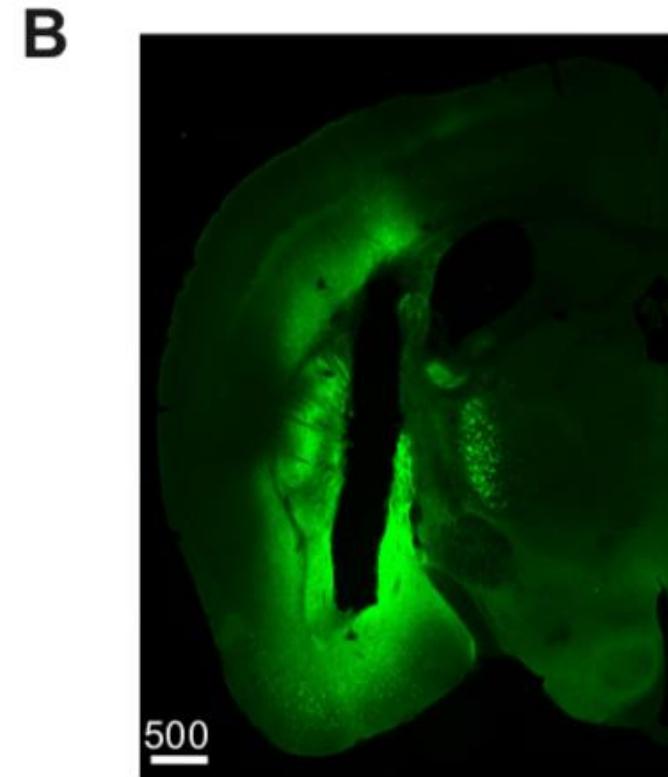
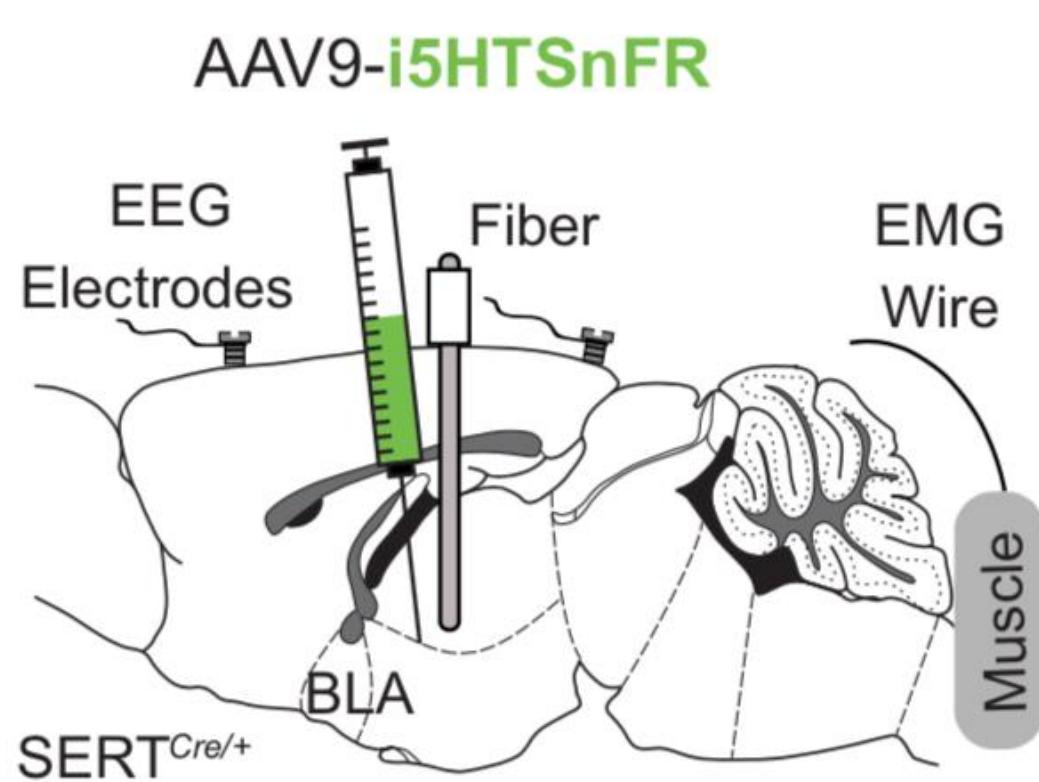
No existing serotonin BP, had to make one



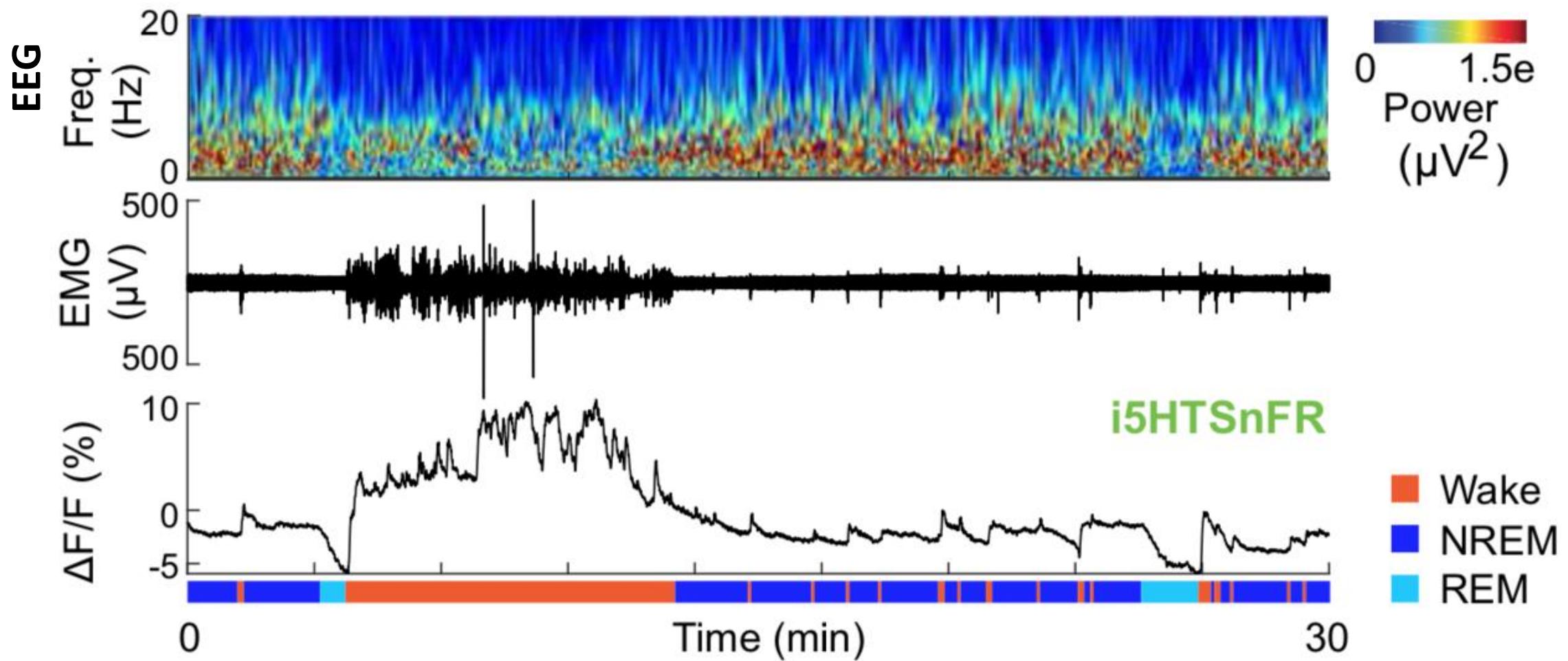
Lin Tian, Liz Unger, Jacob Keller

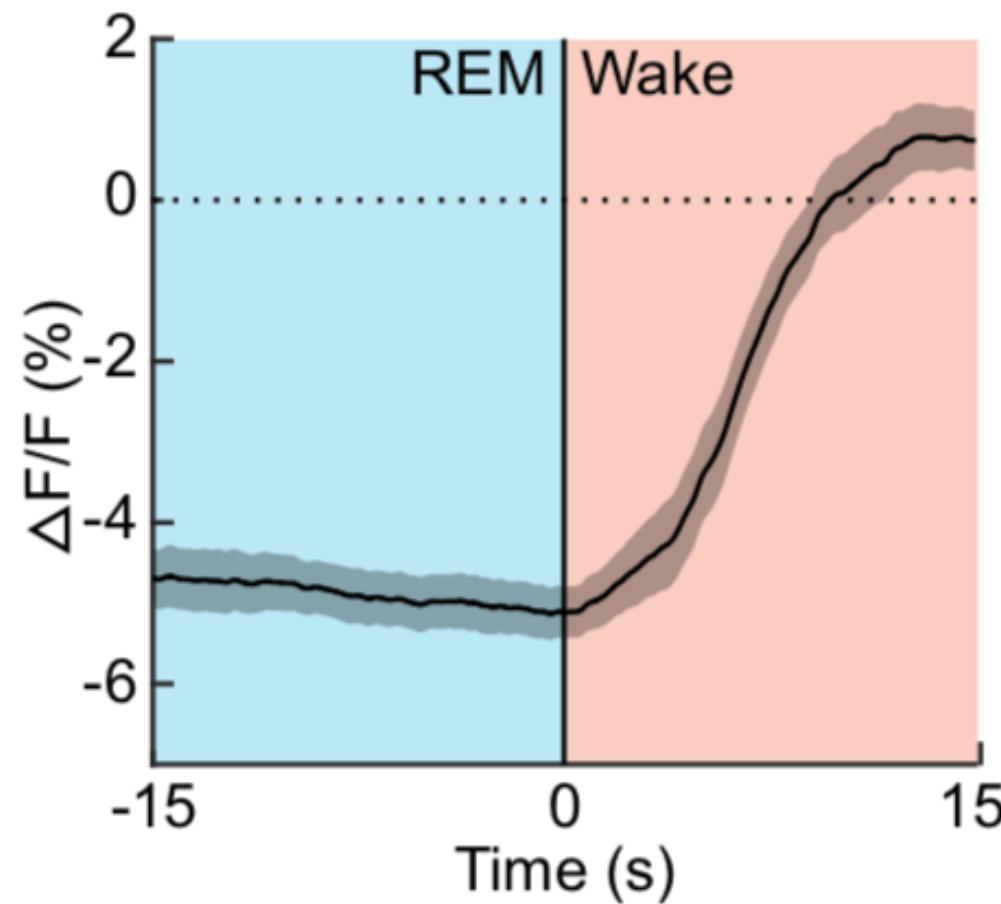


Lin Tian, Liz Unger, Jacob Keller

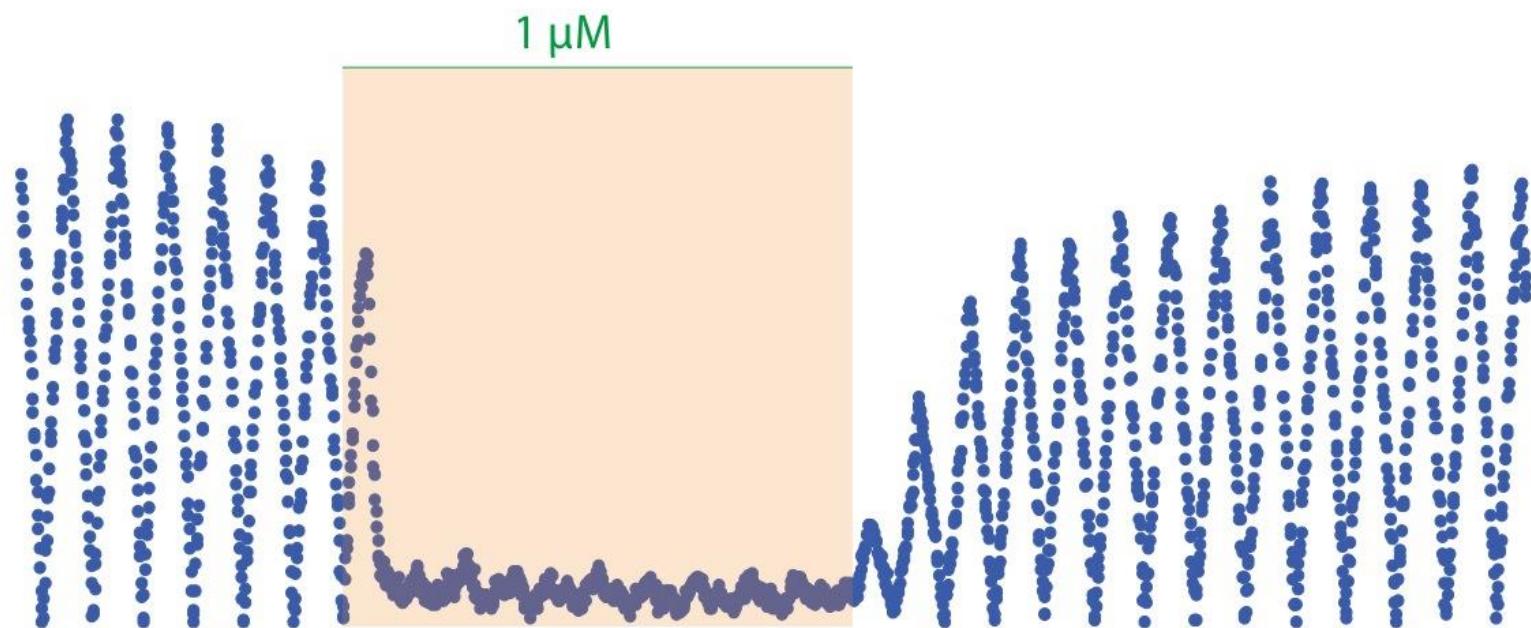
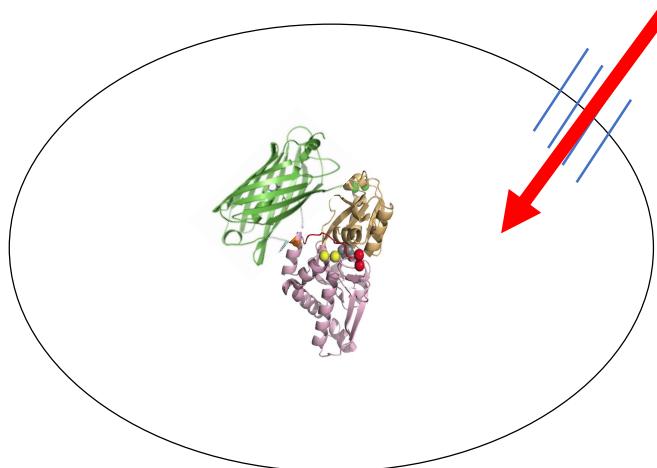


Viviana Gradinaru et al.





# Lexapro (SSRI) effect on SERT



Jacob Keller

# Other GETIs

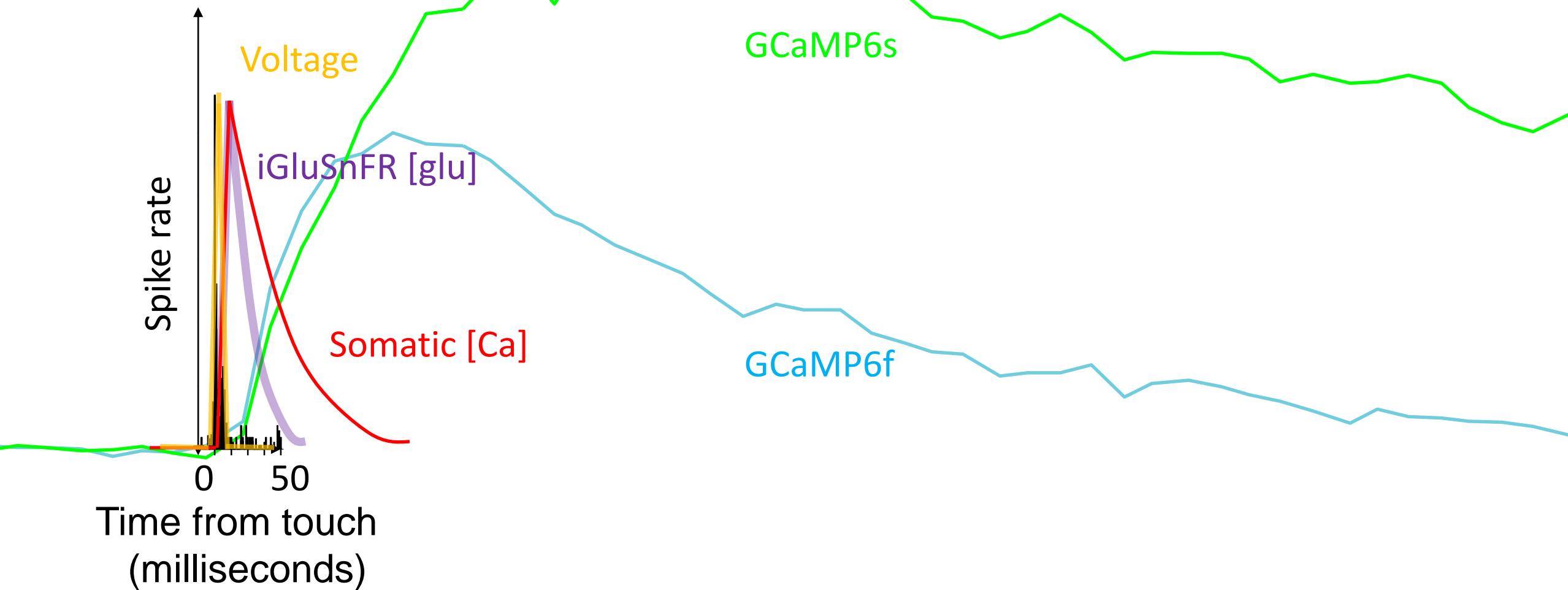
- GPCR-based:
  - dLight/GRAB-DA (dopamine) (also red-dLight)
  - Norepenephrine
  - ACh
- Others

# Key future goals for GETIs

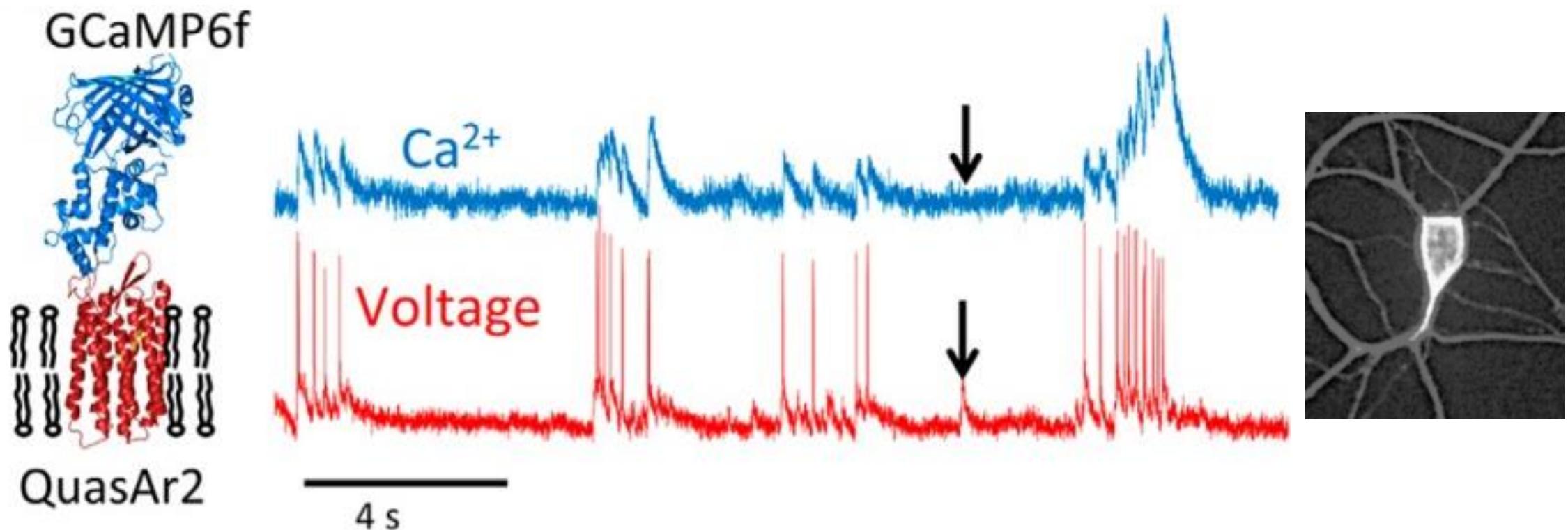
- Target to synapses!

# Voltage sensors

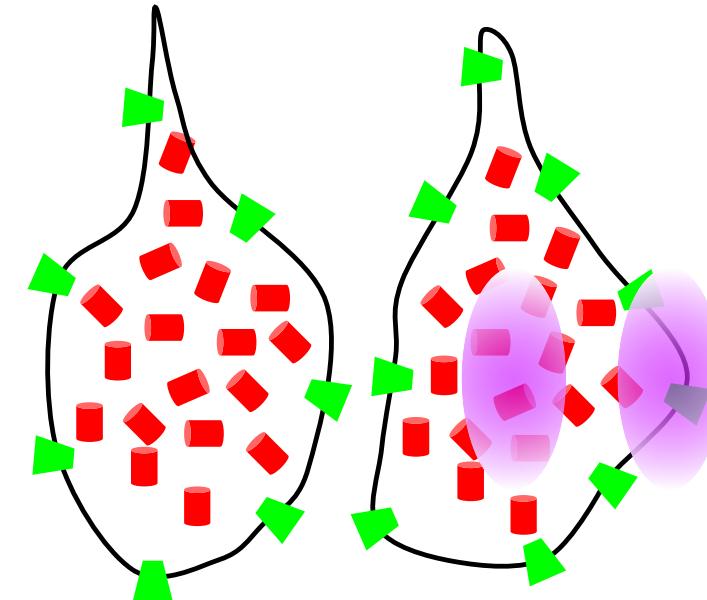
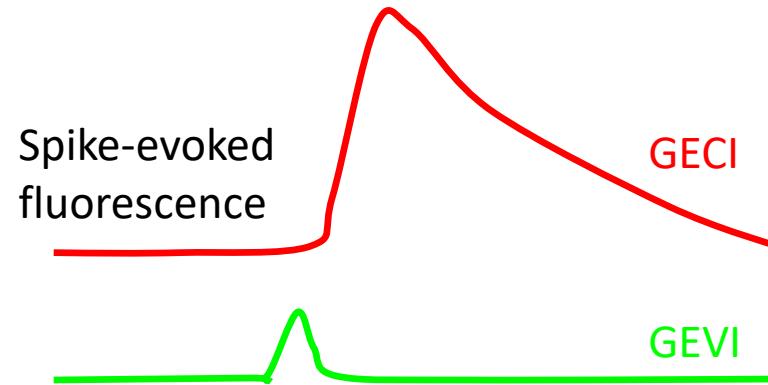
*Protein voltage indicators  
(genetically encoded voltage indicators, GEVIs):  
millisecond time scale imaging*



*GEVIs: millisecond time scale imaging*



## *Challenges for imaging GEVIs in vivo*



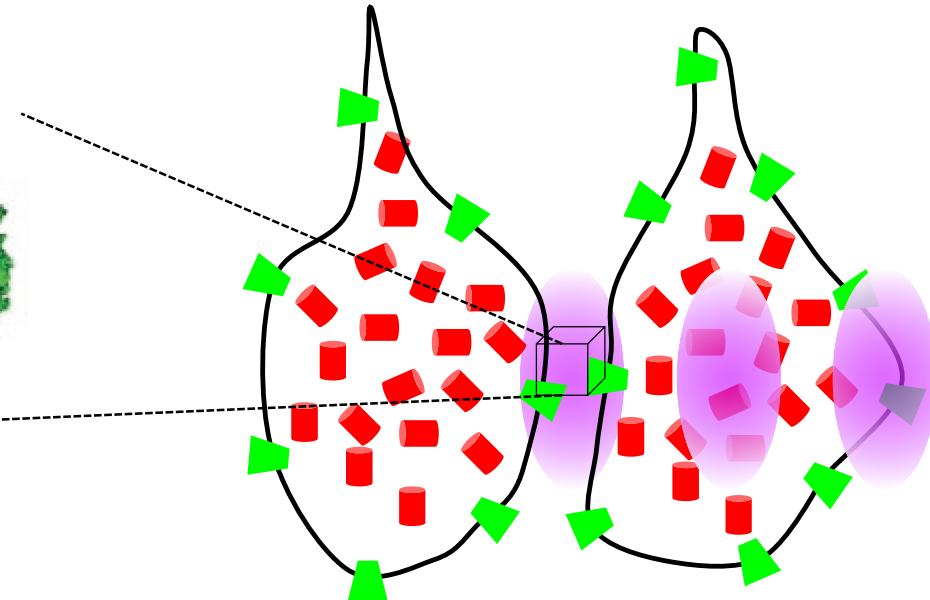
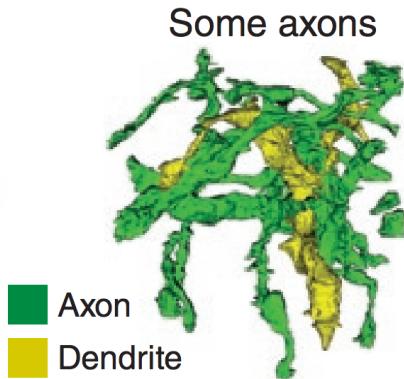
	GEVI - voltage	GECI - calcium	
Fluorescence change per action potential	5 %	40 %	x10
Time per AP	4 ms	400 ms	x100
Sensor distribution	Membrane	Cytosol	
Molecules per resolution element	100	10,000	x100

## *Challenges for imaging GEVIs in vivo*

GEVI - voltage

GECI - calcium

Mishchenko et al 2012



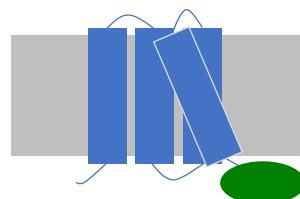
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*Protein voltage indicators  
(genetically encoded voltage indicators, GEVIs)*

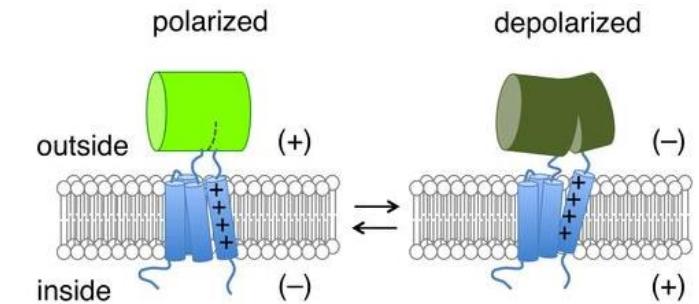
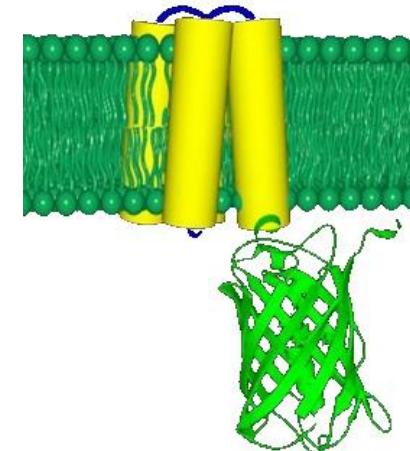
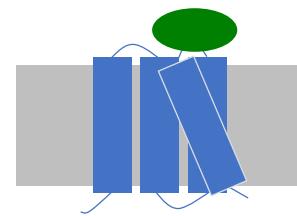
ArcLight

ASAP1

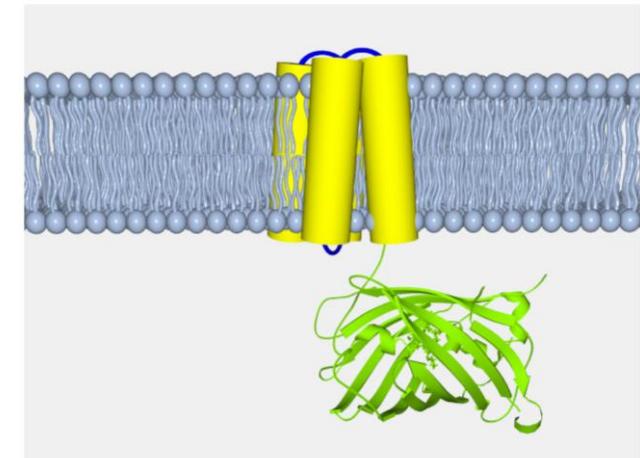
ArcLight,  
MARINA



ASAP



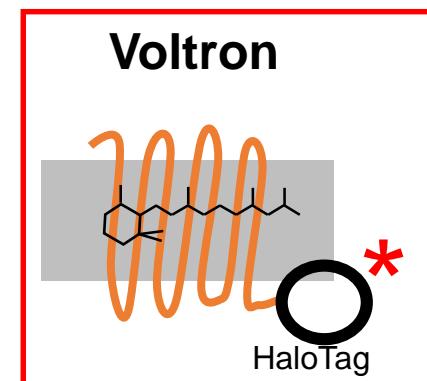
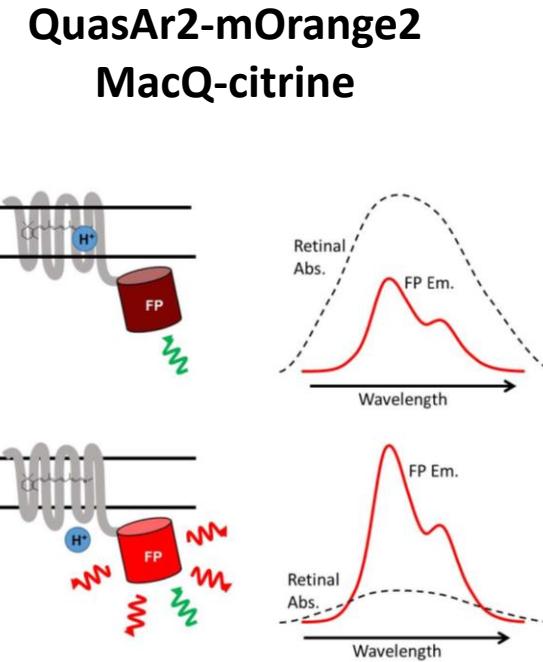
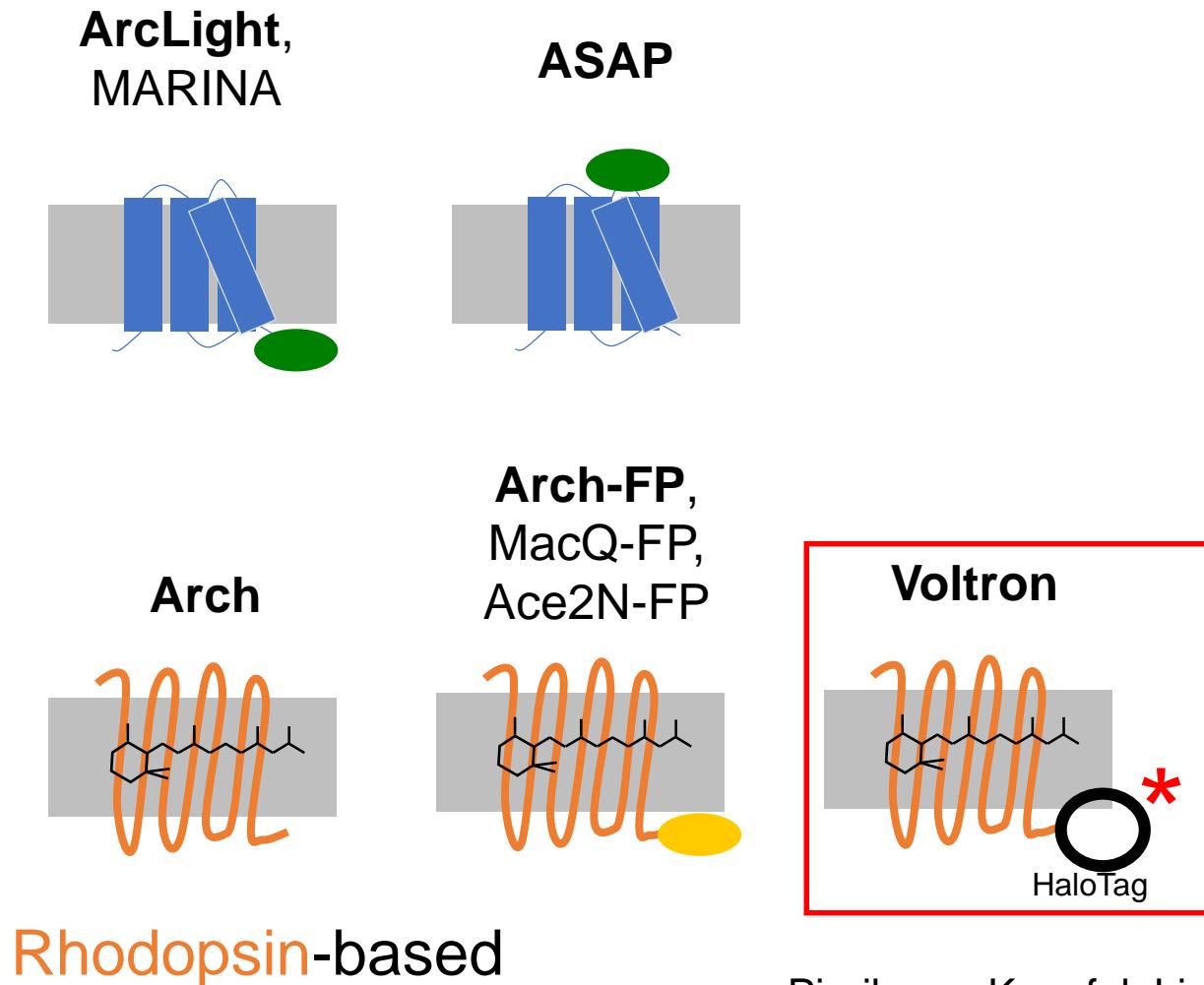
ElectricPk



ciVSP-based

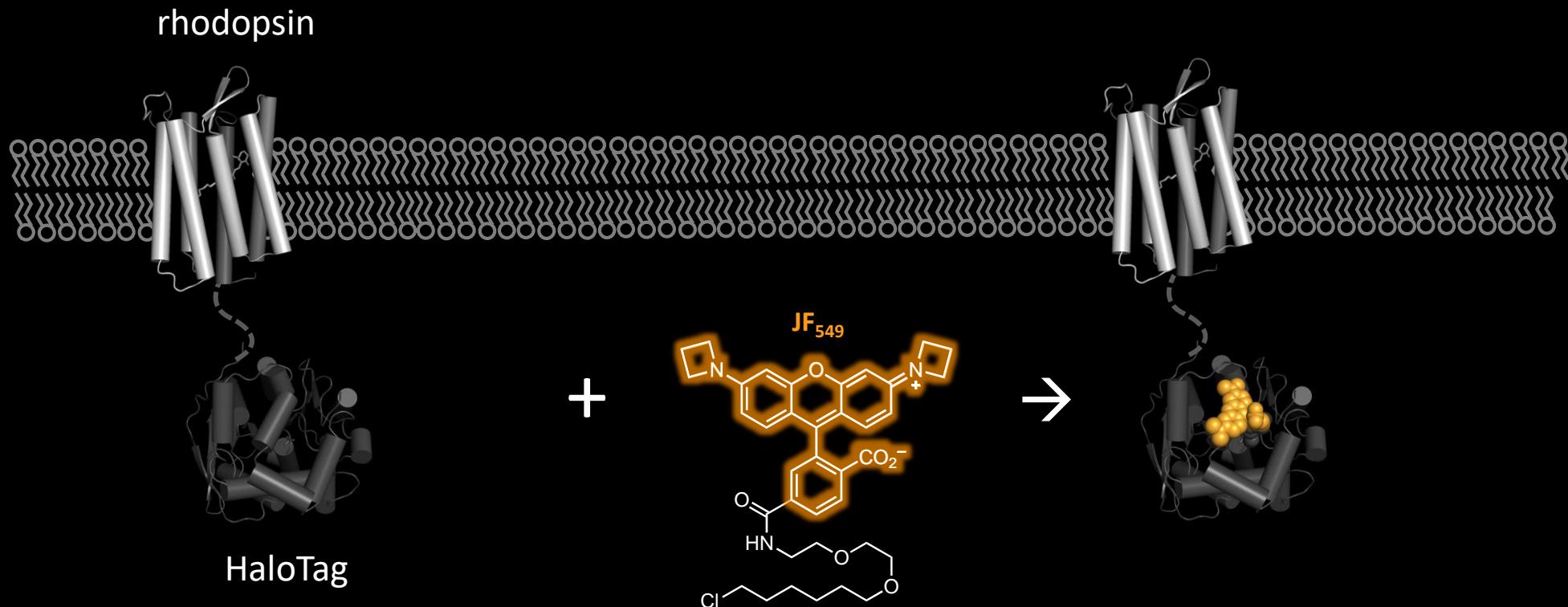
Pieribone, Knopfel, Lin, Campbell, Cohen, Schnitzer, Schreiter, Lavis

# *Protein voltage indicators* (genetically encoded voltage indicators, GEVIs)



Pieribone, Knopfel, Lin, Campbell, Cohen, Schnitzer, **Schreiter, Lavis**

# *Voltron – a chemigenetic voltage sensor*



Ahmed Abdelfattah



Eric Schreiter

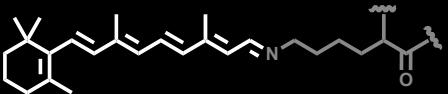
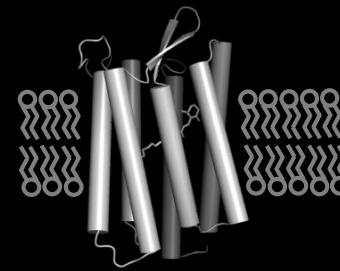
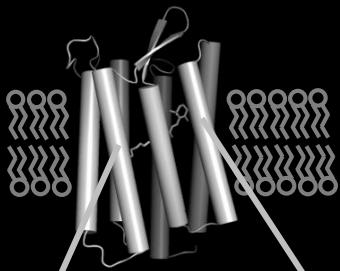


Luke Lavis

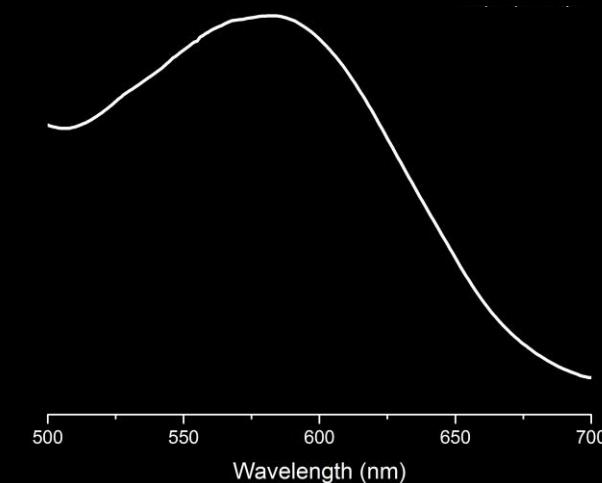
Biorxiv 436840

hyperpolarized

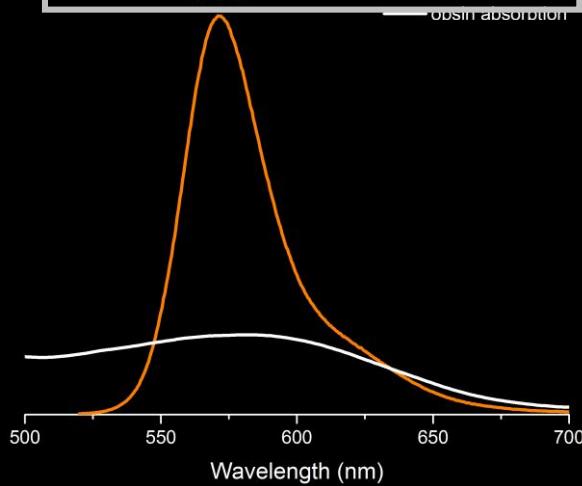
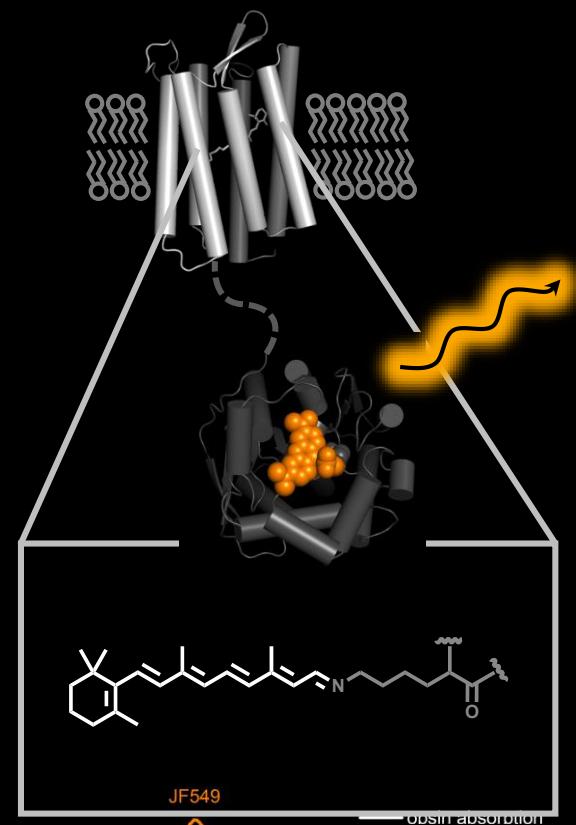
depolarized



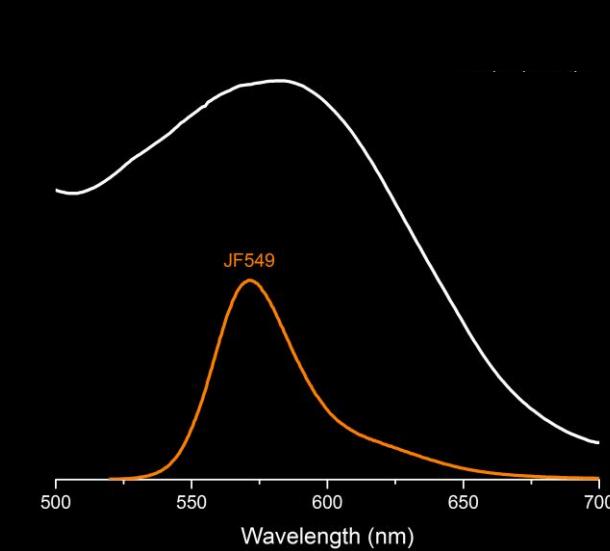
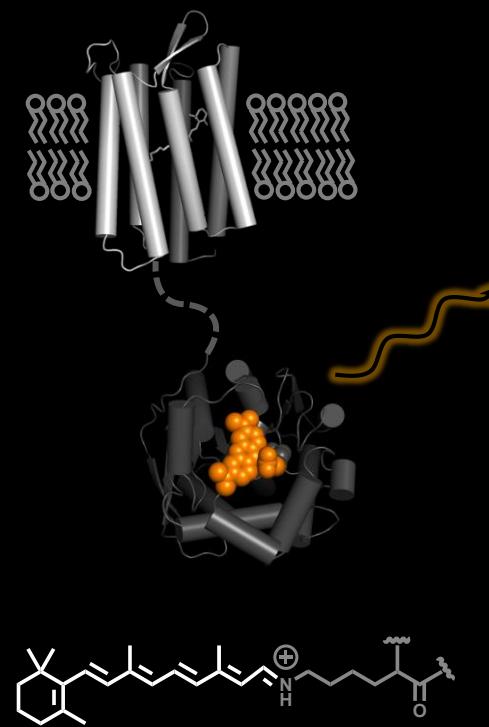
rhodopsin absorption



hyperpolarized

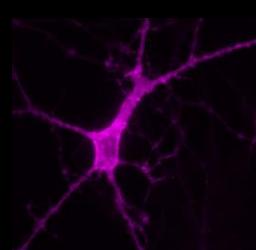
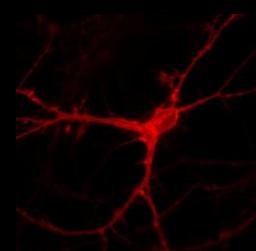
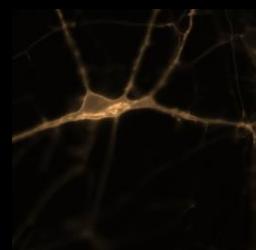
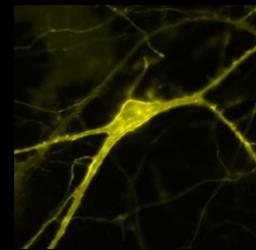
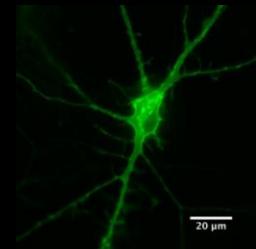
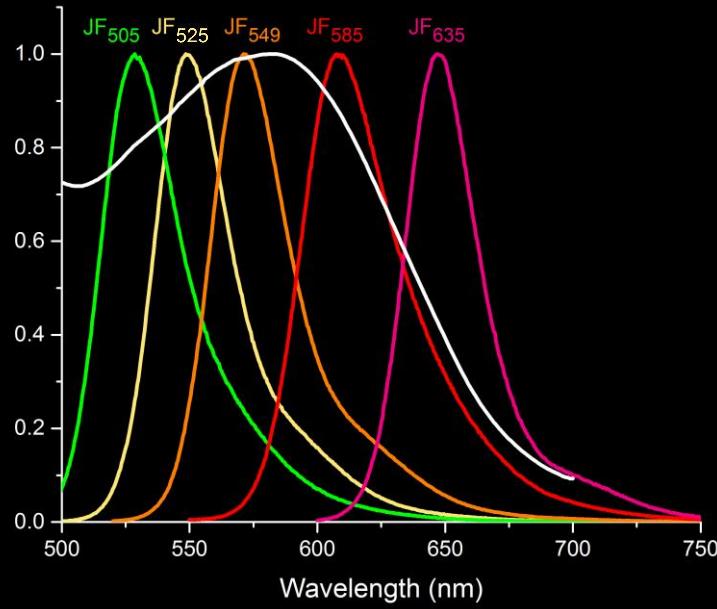


depolarized



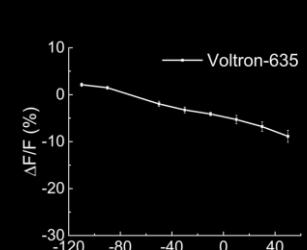
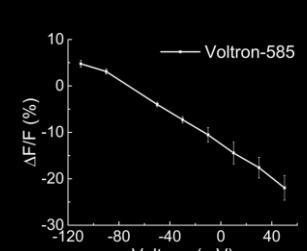
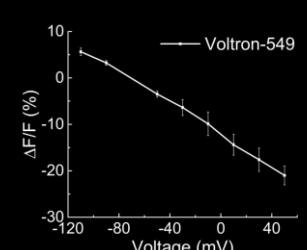
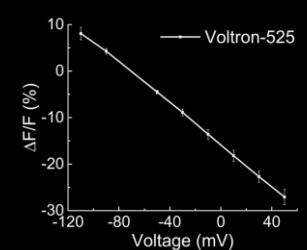
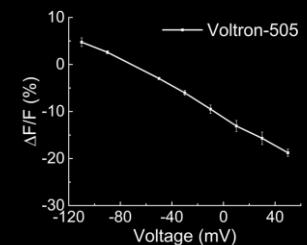
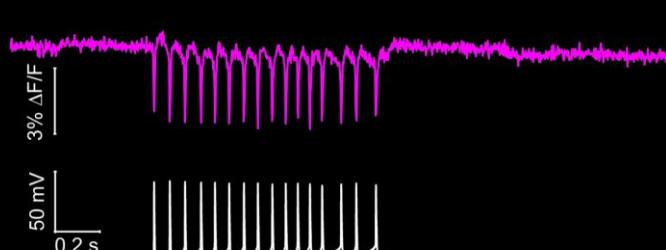
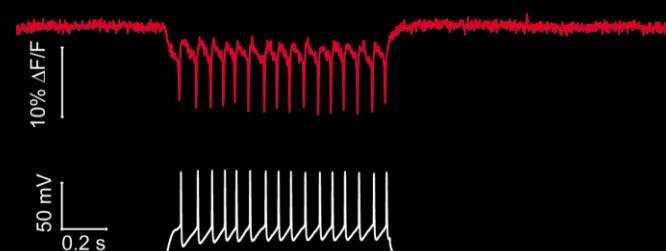
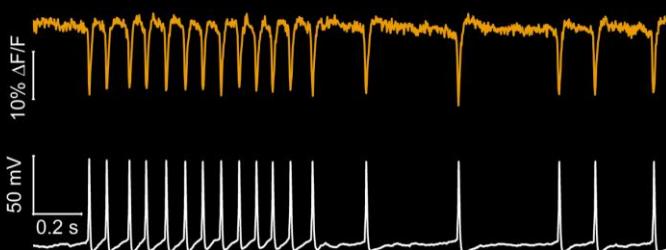
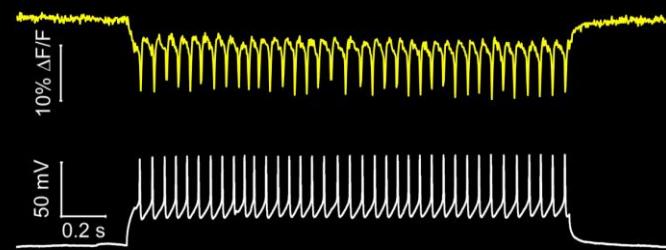
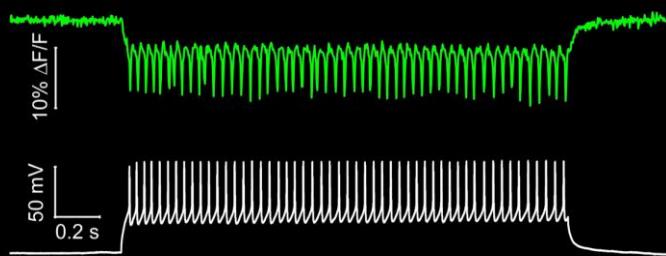
Ace2N

HaloTag



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Ahmed Abdelfattah



Mouse cortex, NDNF-Cre

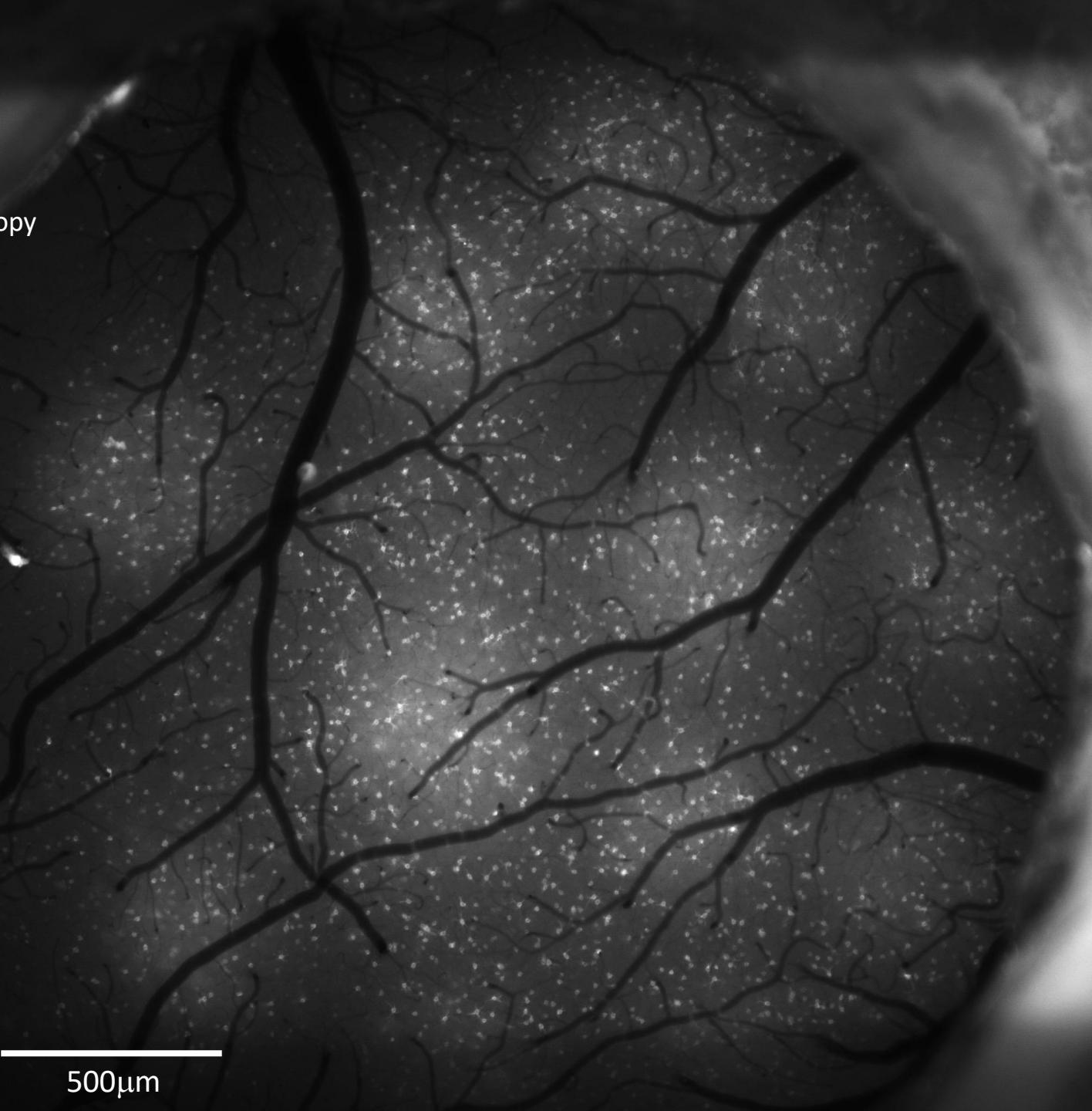
AAV-syn-FLEX-Voltron-ST; JF<sub>525</sub>

Widefield fluorescence microscopy

Soma-  
localized

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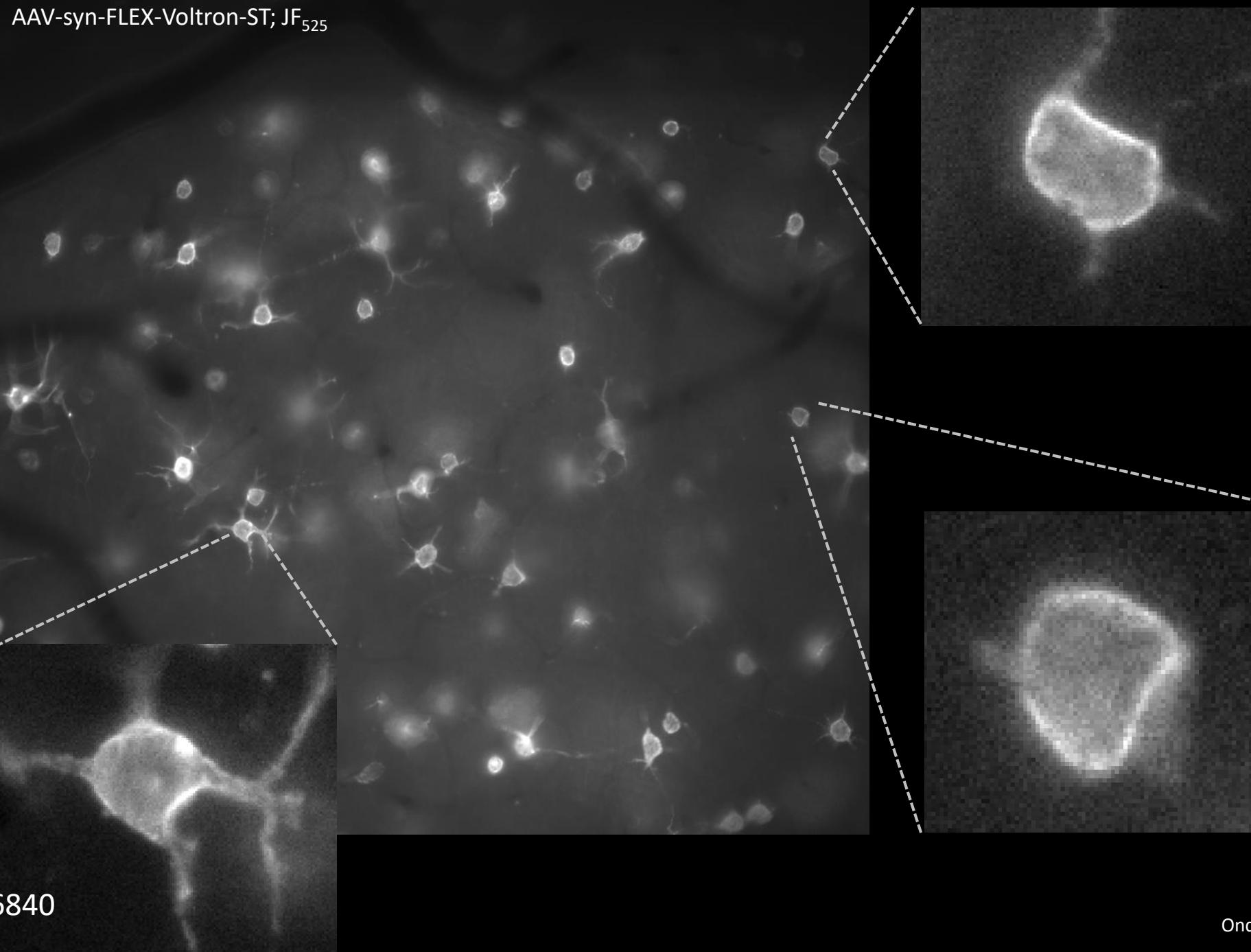
500μm



Ondřej Novák, Amrita Singh

Mouse cortex, NDNF-Cre  
AAV-syn-FLEX-Voltron-ST; JF<sub>525</sub>

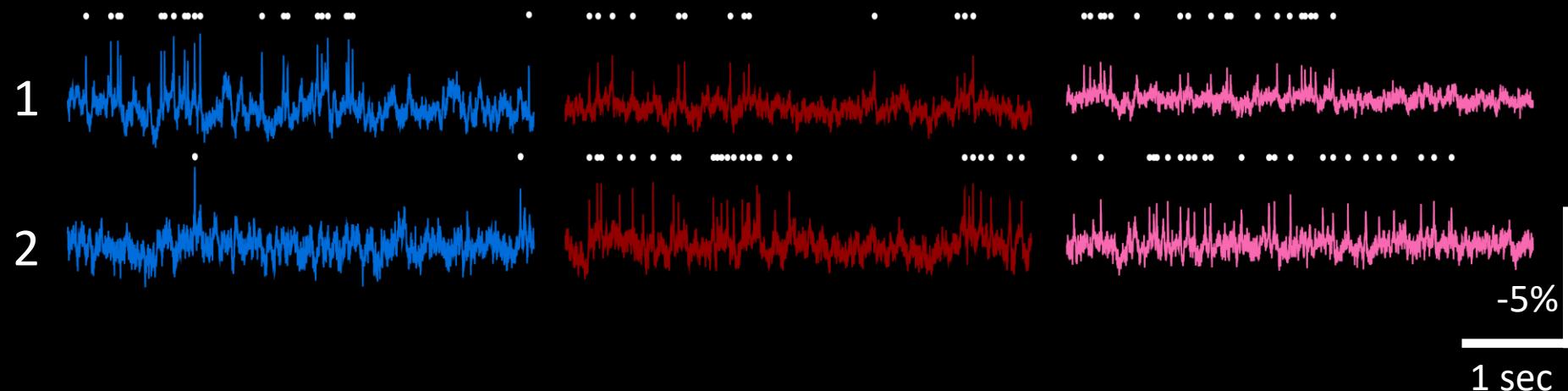
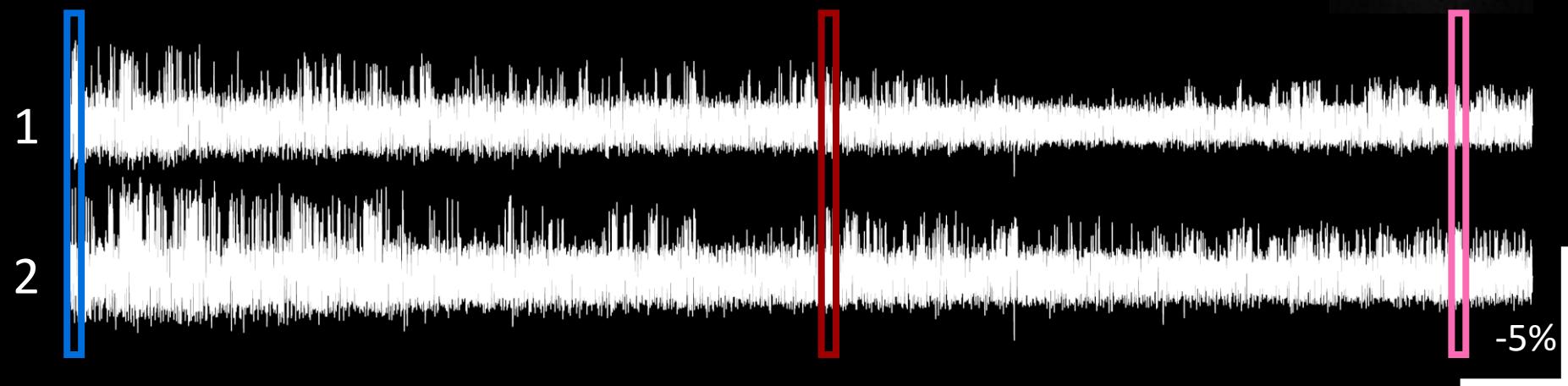
Soma-  
localized

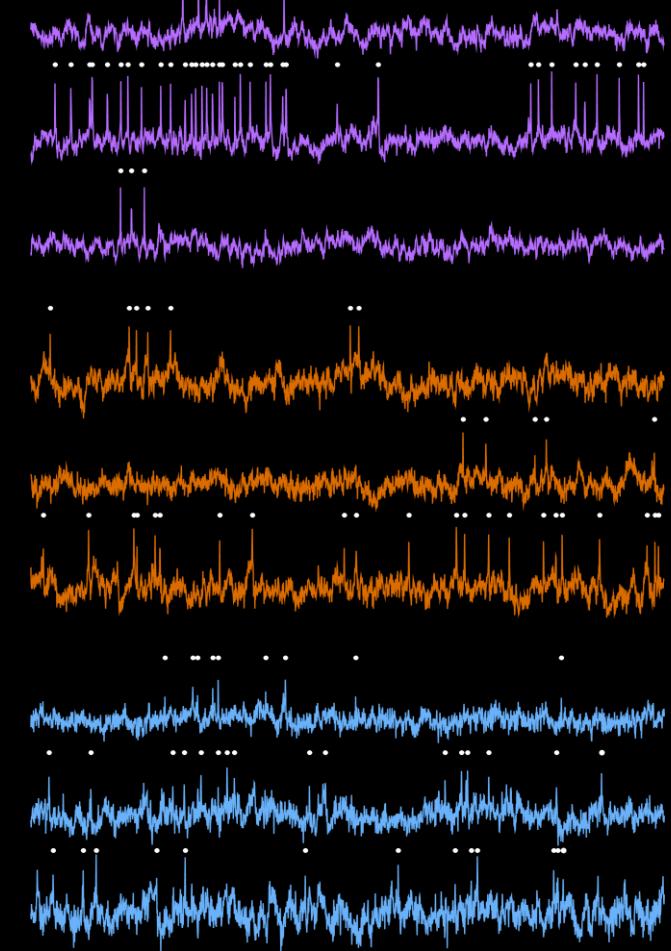
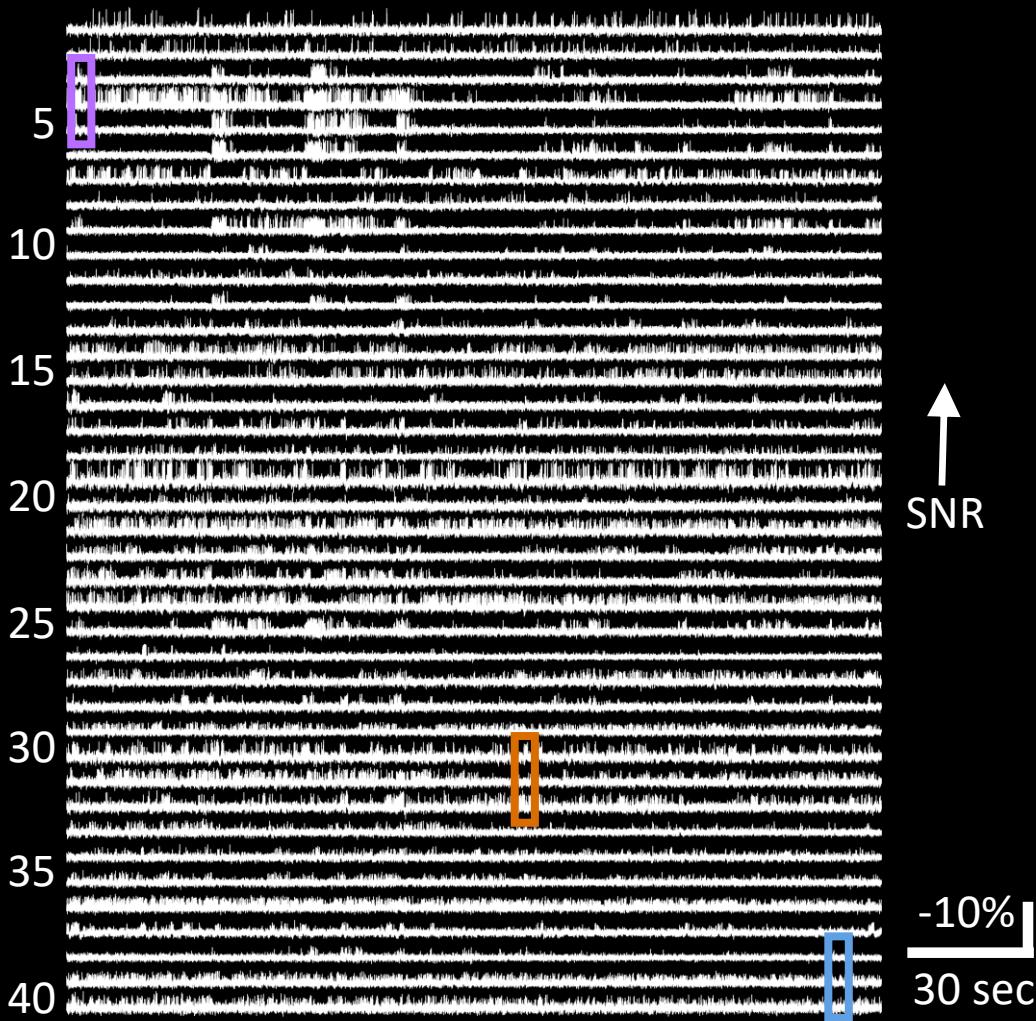
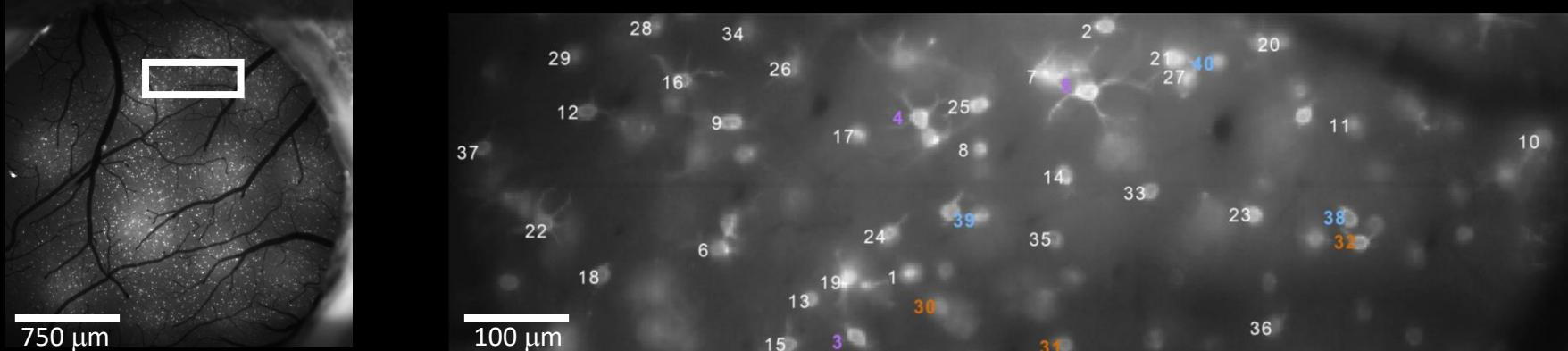


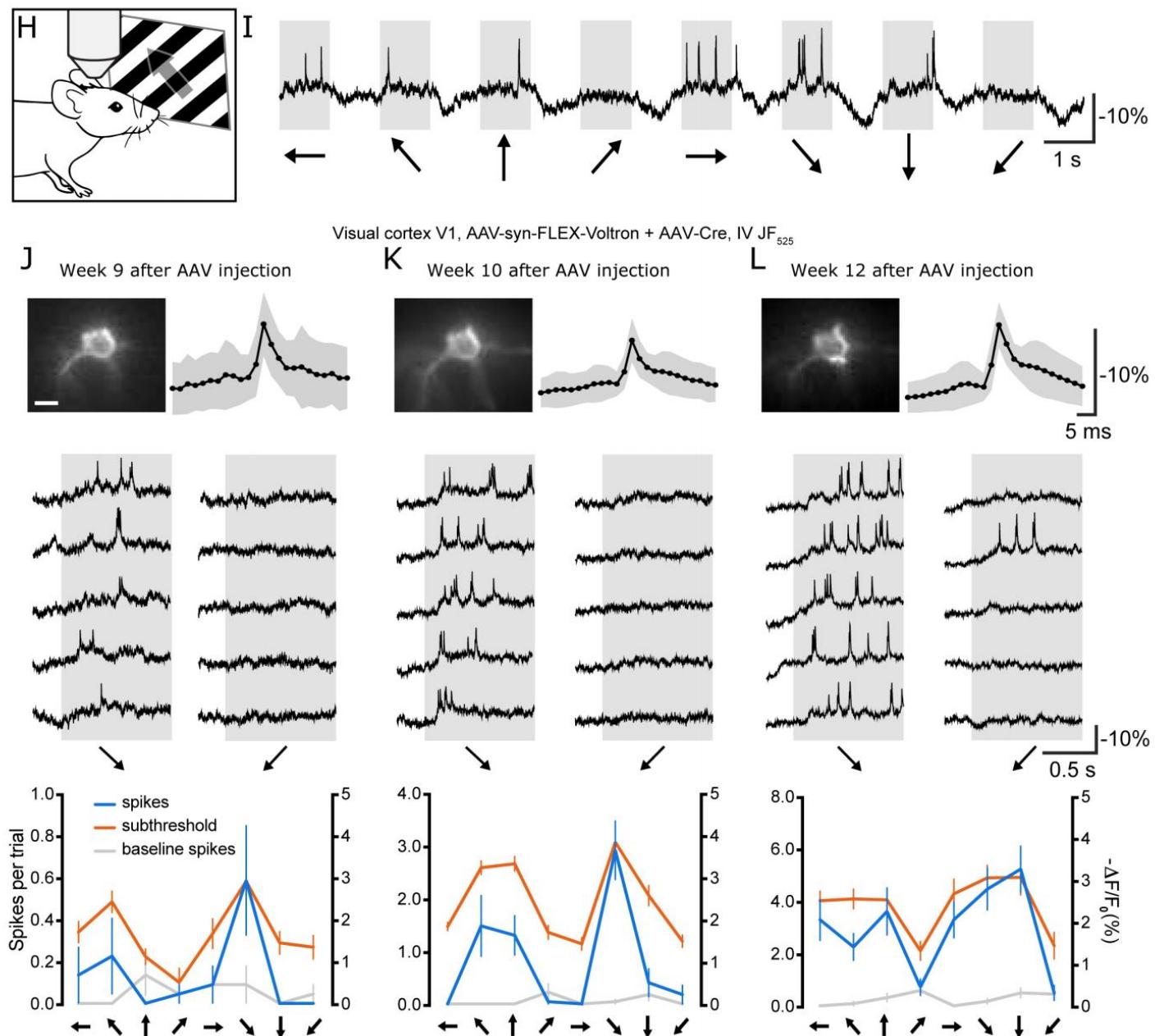
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Mouse cortex, NDNF-Cre  
AAV-syn-FLEX-Voltron-ST; JF<sub>525</sub>







	Extracellular ephys	Ca imaging	Voltage imaging			
<b>Yield</b>	100's of neurons	✓	100's of neurons	✓	10 neurons	✗
<b>Duration</b>	Days to weeks	✓	Days to weeks	✓	Tens of minutes	✗
<b>Signal-to-noise ratio</b>	High	✓	Modest & improving	✗	Low	✗
<b>Time resolution</b>	10 microseconds	✓	100 milliseconds	✗	1-5 milliseconds	✓
<b>Single unit isolation</b>	Depends on SNR	✗	Depends on SNR	✗	Depends on SNR	✗
<b>Spatial reach</b>	Entire brain	✓	Limited to ~ 0.5 mm or invasive	✗	Limited to 0.2 mm	✗
<b>Access to cell types</b>	Limited	✗	Good	✓	Good	✓
<b>Cell type biases</b>	High	✗	Low	✓	Low	✓
<b>Dense sampling</b>	No	✗	Yes	✓	No	✗
<b>Detect rare cell types</b>	Limited	✗	Yes	✓	Yes	✓
<b>Longitudinal measurem.</b>	Limited	✗	Yes	✓	Limited	✗
<b>Invasive?</b>	Yes	✗	Yes	✗	Yes	✗
<b>Spatial localization</b>	100 um	✗	1 um	✓	1 um	✓