

- Measurements in general
- Extracellular electrophysiology
- Calcium imaging and analysis
- Voltage imaging

Measurement

the assignment of a number to a characteristic of an object or event, which can be compared with other objects or events

the correlation of numbers with entities that are not numbers

Requirements:

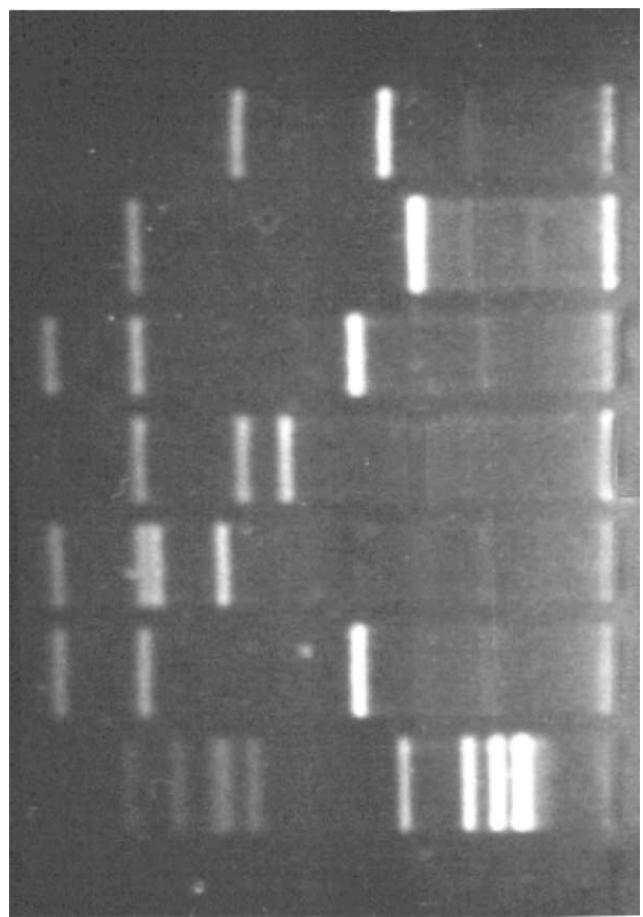
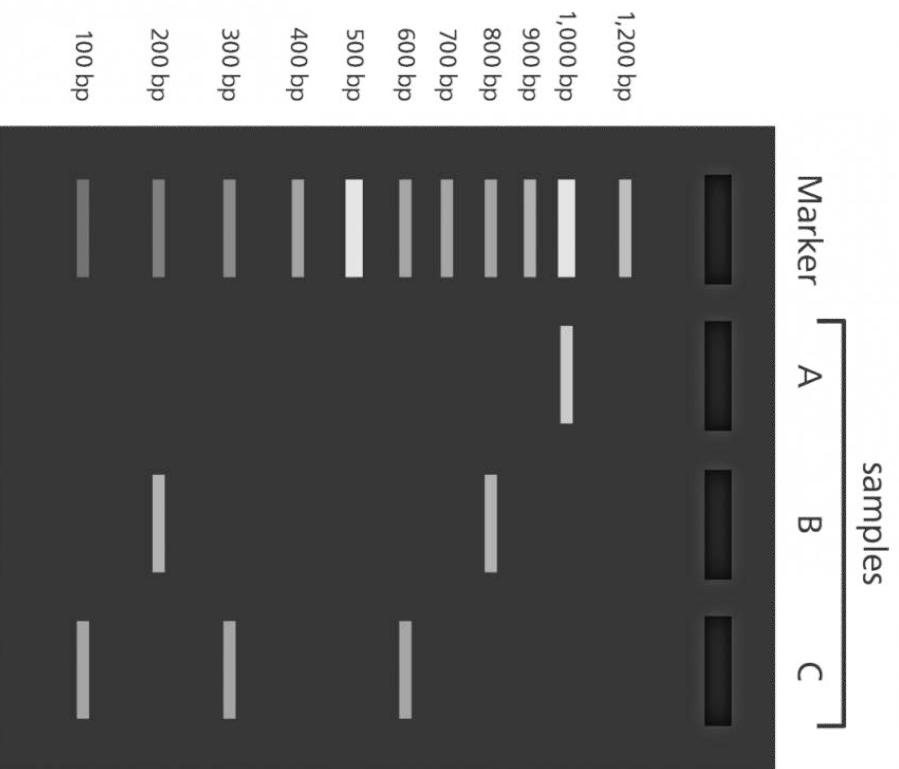
A well-defined, one-to-one (i.e. monotonic) mapping between the number and the characteristic of an object or event

A defined uncertainty or quality metric

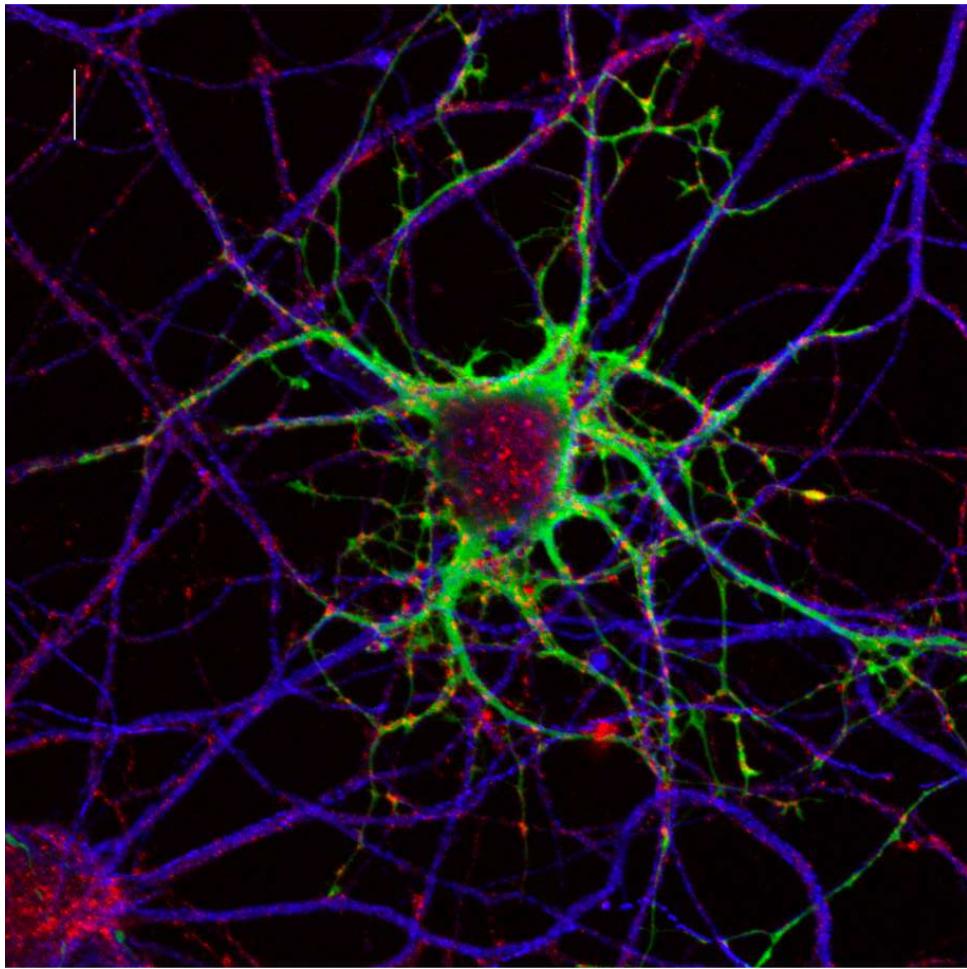
A physical model of the measurement procedure or assay

Reproducibility and provenance





Google images



SNR limited by shot-noise
 $(F \sim [\text{photon count}])$

$$\text{SNR} \sim (F-B)^{1/2}$$

$F(x) \sim g(x)$ - density of fluorophore

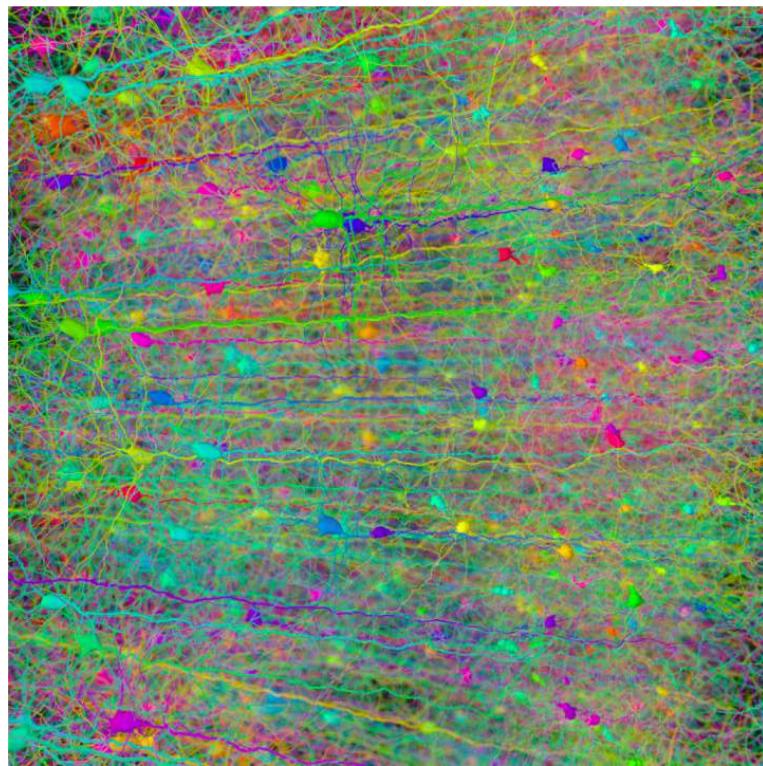
$$F(x) \sim (g * \phi)(x)$$

ϕ - 'point-spread function'

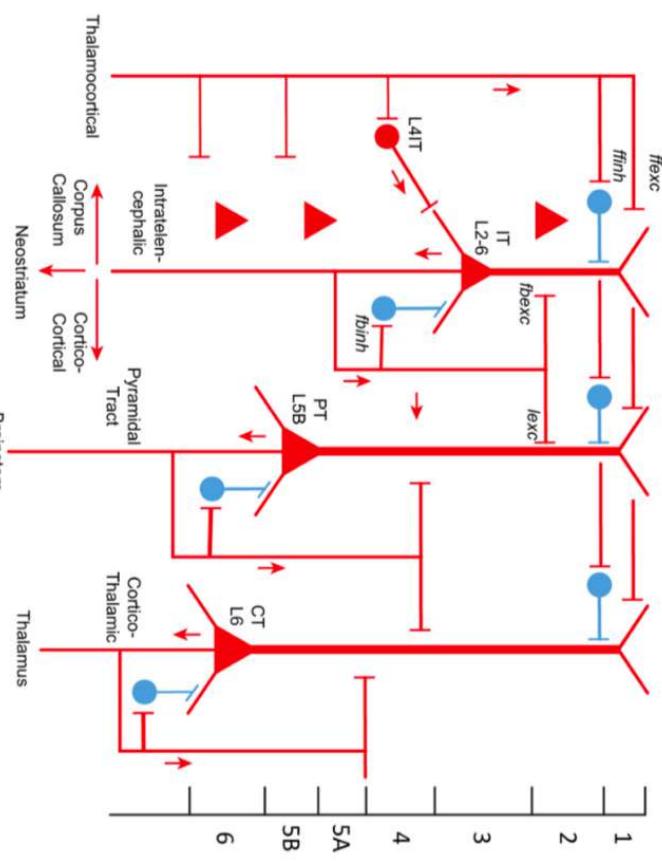
Google images

- Measurements in general
- **Extracellular electrophysiology**
- Calcium imaging and analysis
- Voltage imaging

Systems neuroscience



Google images



The 'shaft du jour'

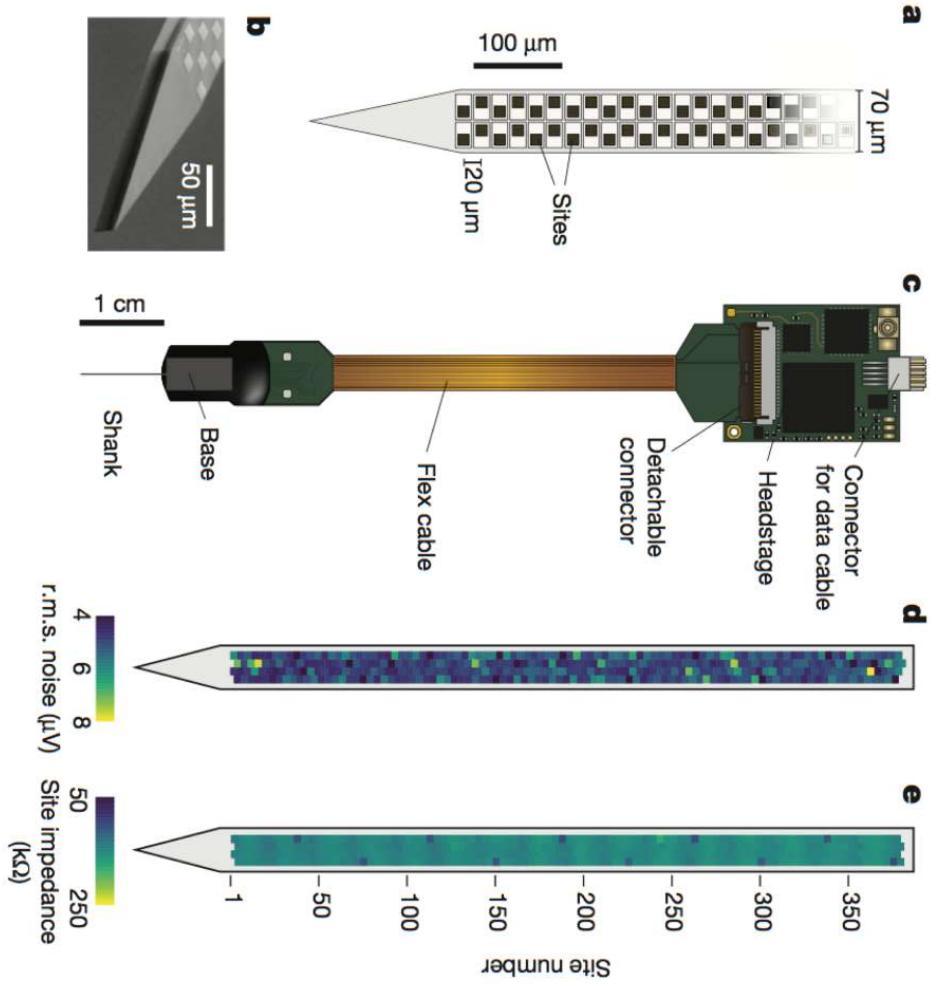
Extracellular neurophysiology

$\text{psd} = 4 \text{ kT R}$ - 'Johnson noise', white

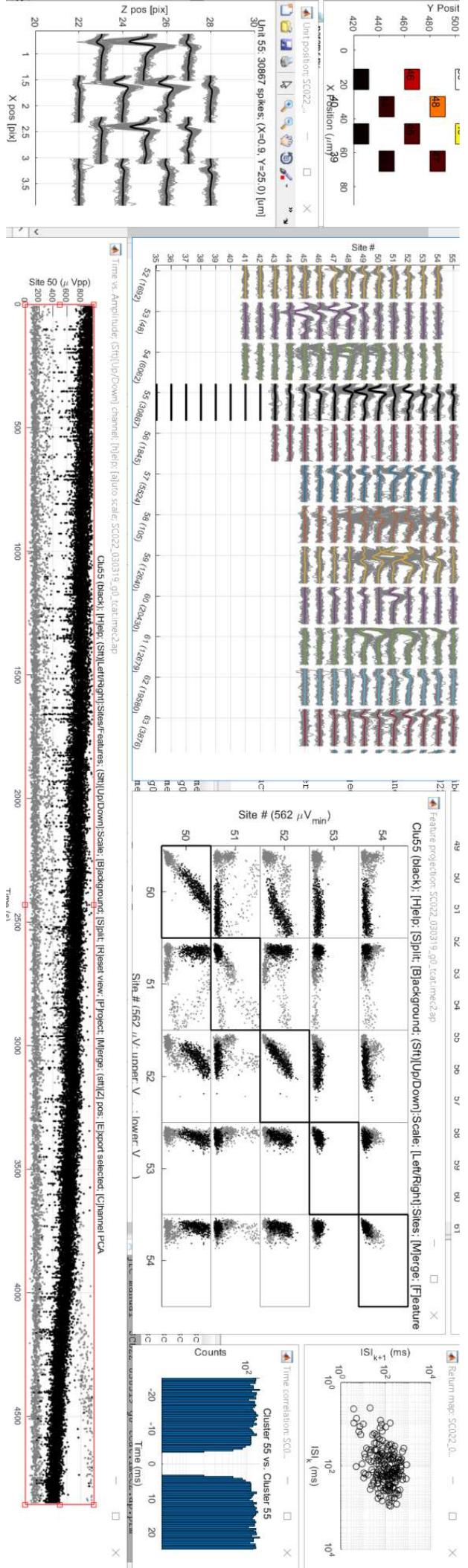
$$\langle V^2 \rangle = 4 \text{ kT R BW}$$

In the brain :

$\text{psd} = A f^{-2}$ - 'power law'; exponent varies



• Spike sorting



Susu Chen; Kilosort2

Metric	Icon	Description	Reference
Firing rate		Mean spike rate in an epoch	
Presence ratio		Fraction of epoch in which spikes are present	
ISI violations		Rate of refractory-period violations	
Amplitude cutoff		Estimate of miss rate based on amplitude histogram	
Isolation distance		Distance to nearest cluster in Mahalanobis space	Schmitzer-Torbert et al. (2005) <i>Neuroscience</i> 131, 1-11
L-ratio		"	Hill et al. (2011) <i>J Neurosci</i> 31, 8699-9705
d'		Classification accuracy based on LDA	Chung et al. (2017) <i>Neuron</i> 95, 1381-1394
Nearest-neighbors		Non-parametric estimate of unit contamination	
Silhouette score		Standard metric for cluster overlap	
Maximum drift		Maximum change in spike depth throughout recording	
Cumulative drift		Cumulative change in spike depth throughout recording	

Metrics for cluster isolation

https://github.com/AllenInstitute/ecephys_spike_sorting/blob/master/ecephys_spike_sorting/modules/quality_metrics/

Spike quality metrics are not robust

Q – proportion of spikes in refractory period

fp – false positive rate

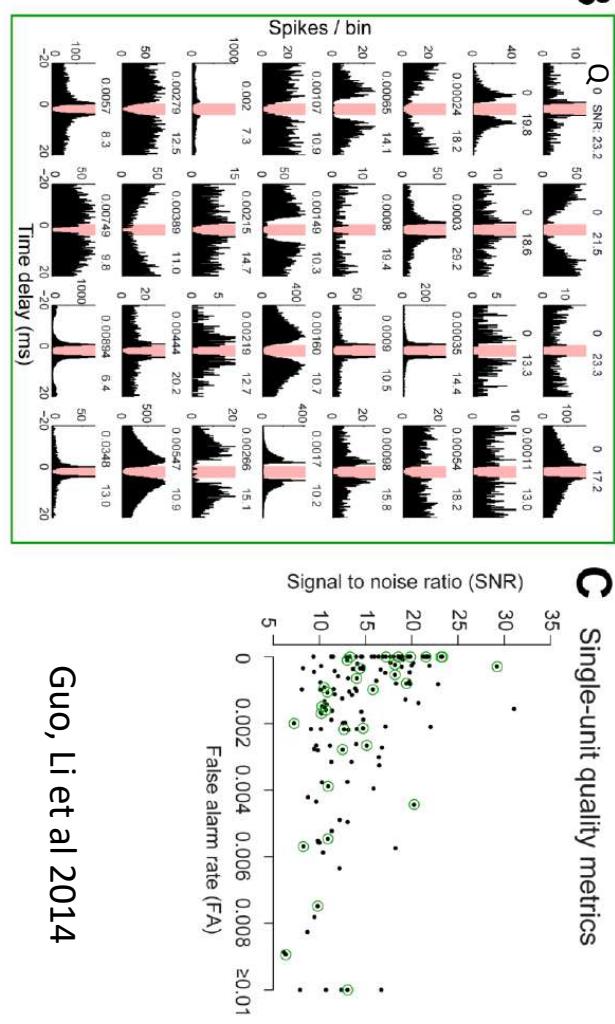
Tr – refractory period

Assuming constant Poisson spike rates r for the polluted neuron

$$fp = Q / (2 * Tr * r)$$

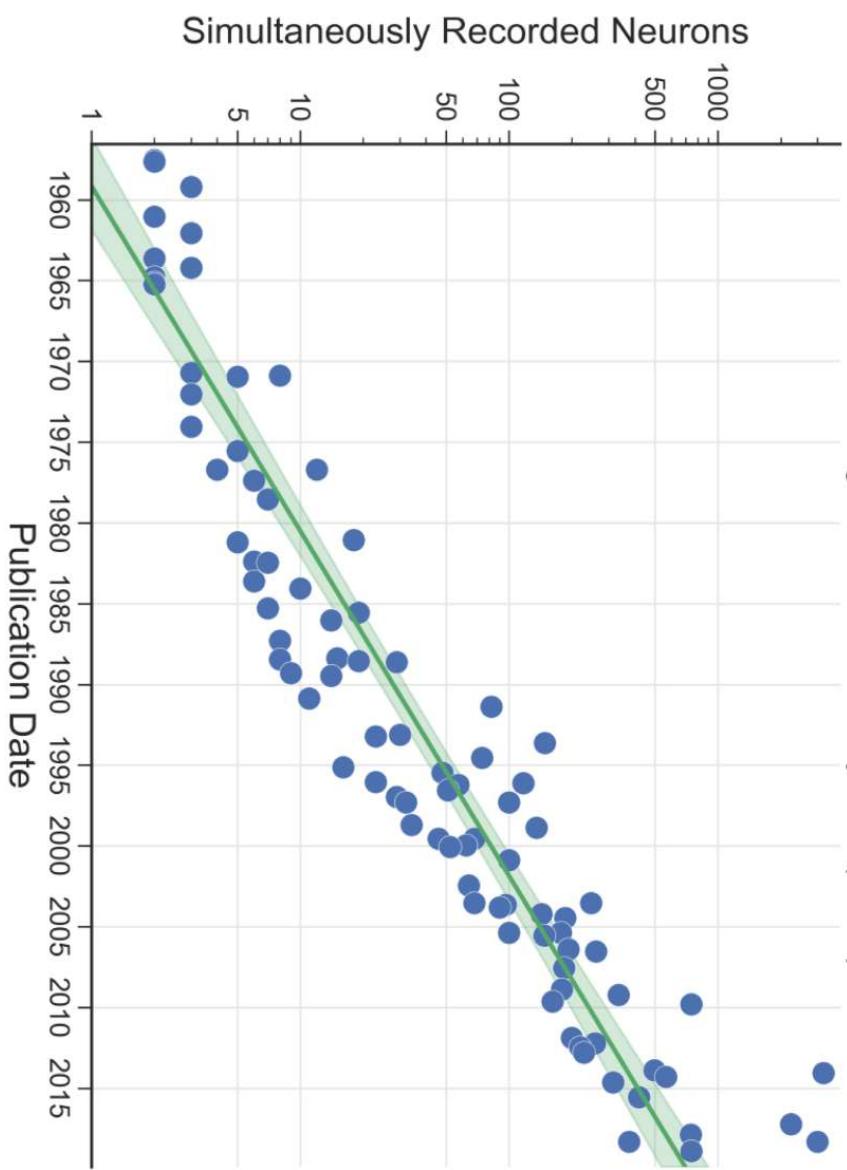
Assuming $Tr = 5$ ms; $r = 5$ Hz; $Q = 0.01$

$$fp = 0.2!$$



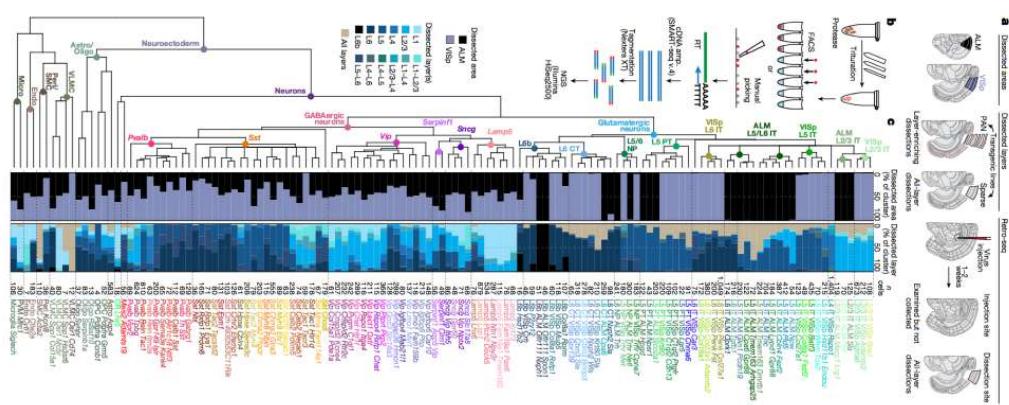
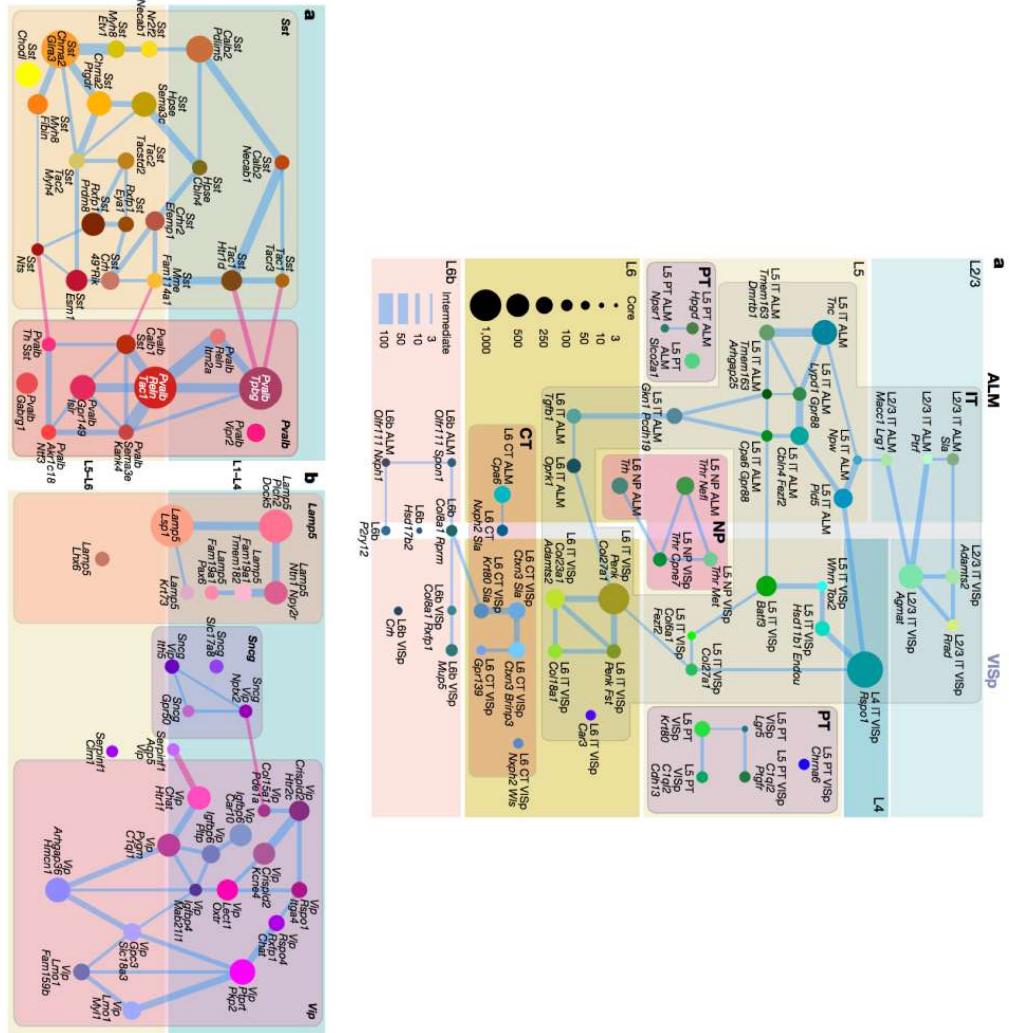
Potentially much worse correspondence between Q and fp for strongly modulated units!

Doubling Time: 6.4 ± 0.3 years (n=89)

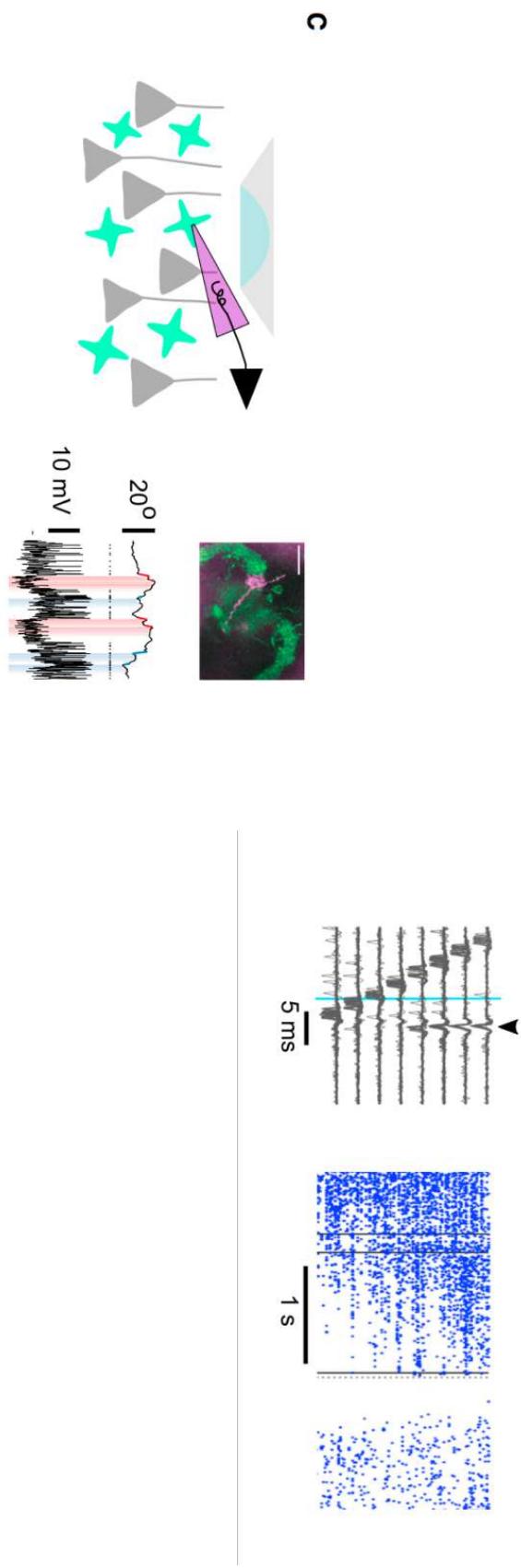
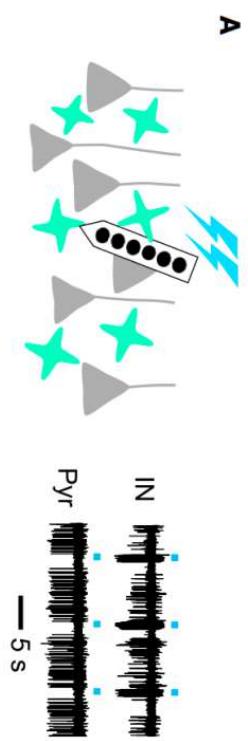


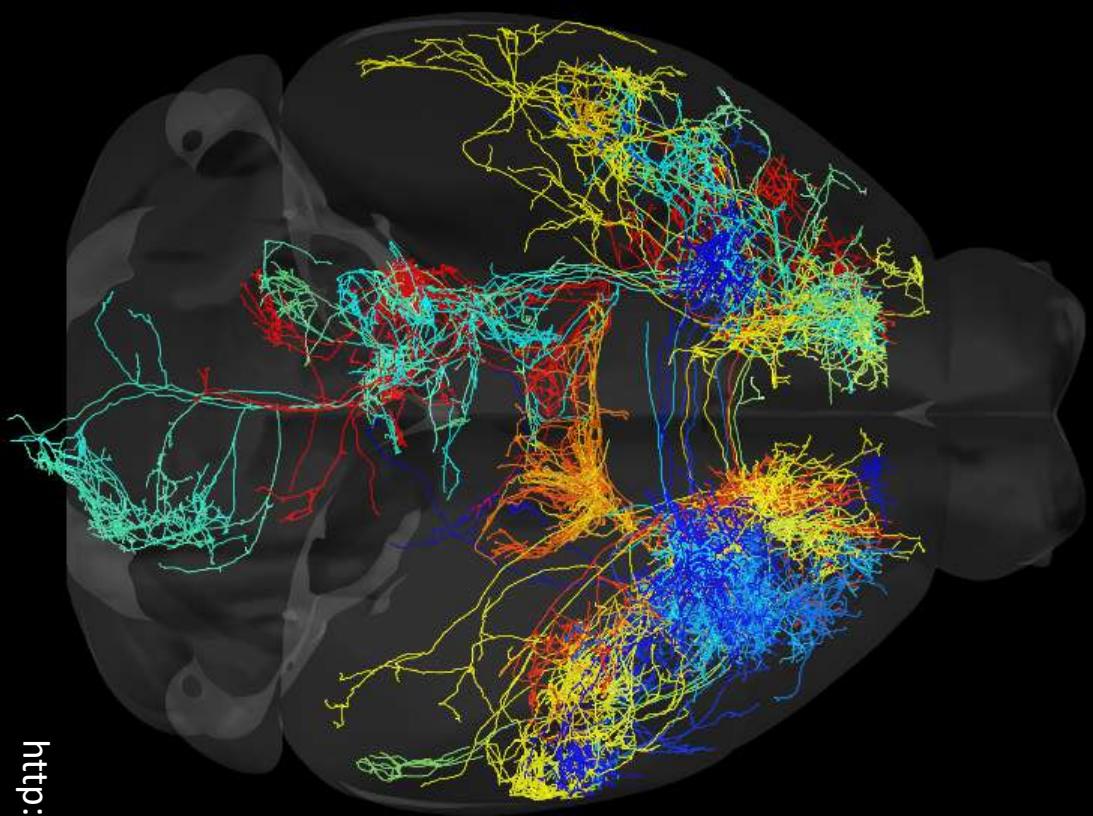
<https://stevenson.lab.uconn.edu/scaling>

See Steinmetz et al CONB 2018 or review



Cell type-specific ephys

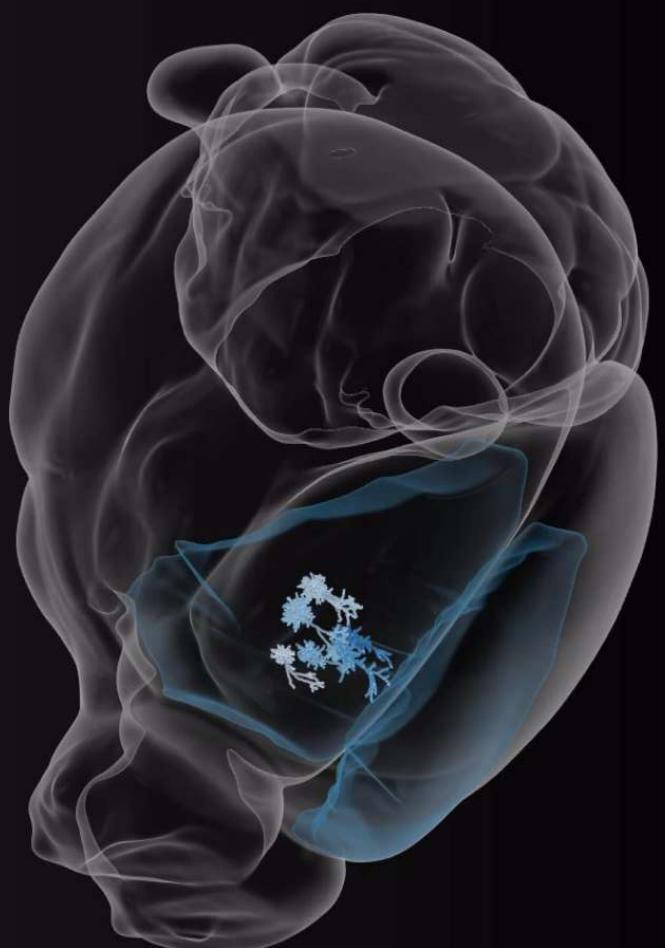




<http://mouselight.janelia.org/>



Motor Cortex Pyramidal Tract Neurons



3000 μm

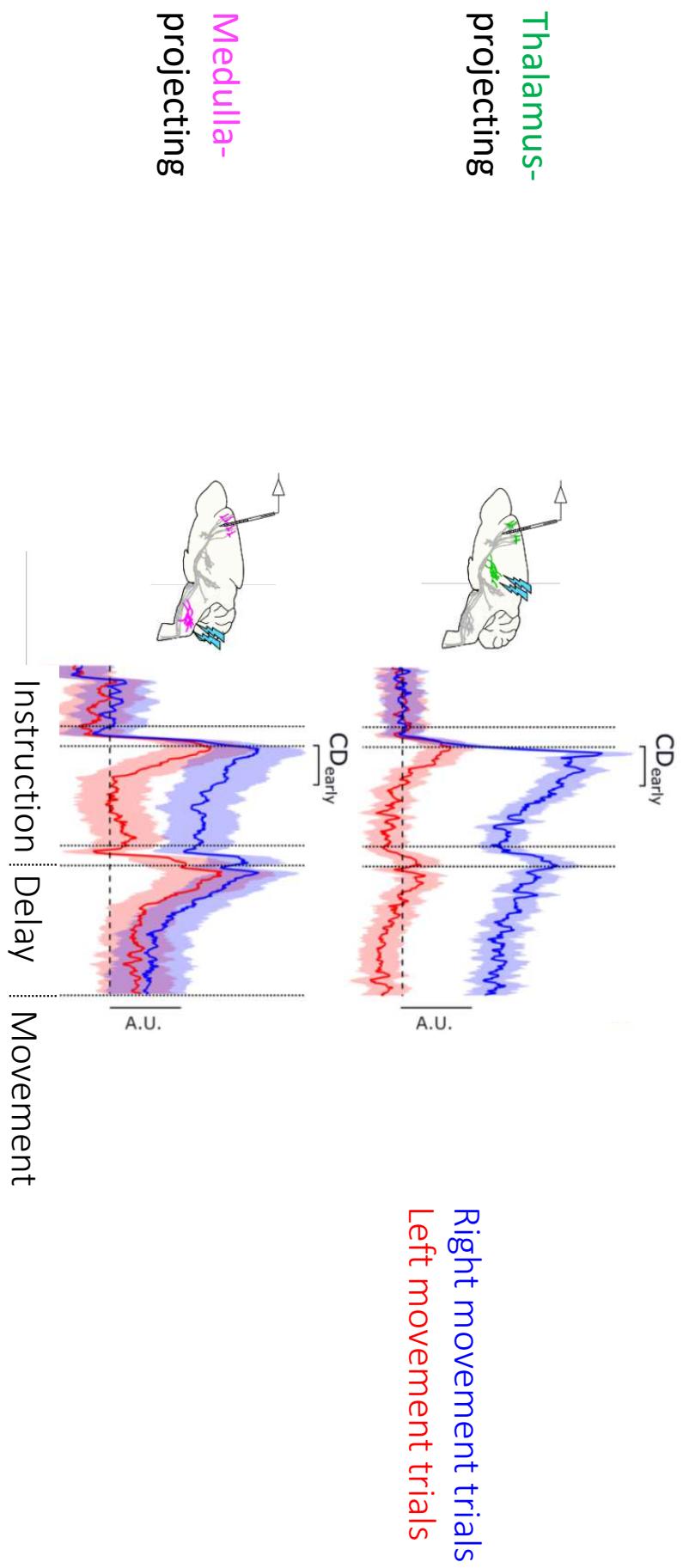
Johan Winnubst

<http://ml-neuronbrowser.janelia.org/>

Eonomo et al 2018



Motor planning in thalamus-projecting neurons

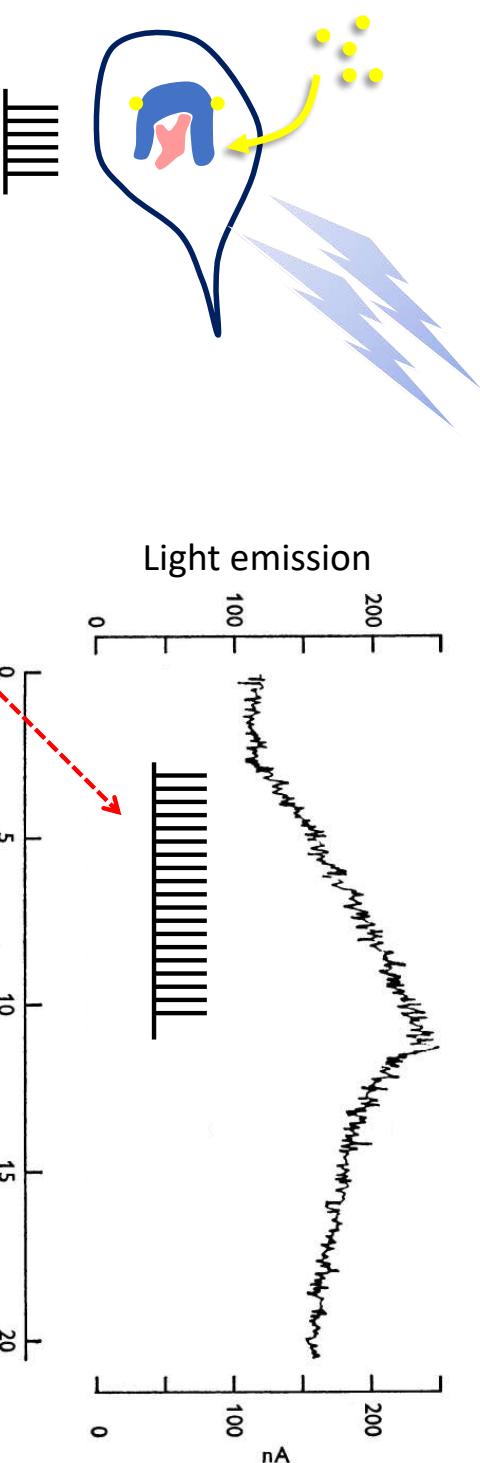


	Extracellular ephys
Yield	100's of neurons
Duration	Days to weeks
Signal-to-noise ratio	High
Time resolution	10 microseconds
Single unit isolation	Depends on SNR
Spatial reach	Entire brain
Access to cell types	Limited
Cell type biases	High
Dense sampling	No
Detect rare cell types	Limited
Longitudinal measurem.	Limited
Invasive?	Yes
Spatial localization	100 um

- Measurements in general
- Extracellular electrophysiology
- Calcium imaging and analysis
 - Voltage imaging

The first light-based measurement of neuronal activity

Blue light from aequorin



Baker, Hodkin, Ridgway, 1971

Calcium dynamics reflects neural activity

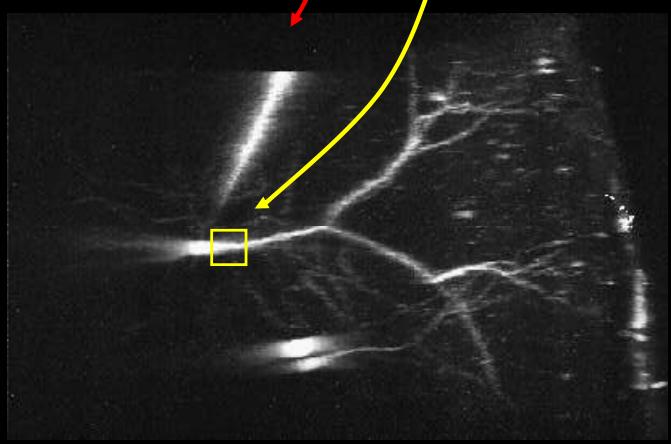
Top of the brain

Calcium-dependent fluorescence



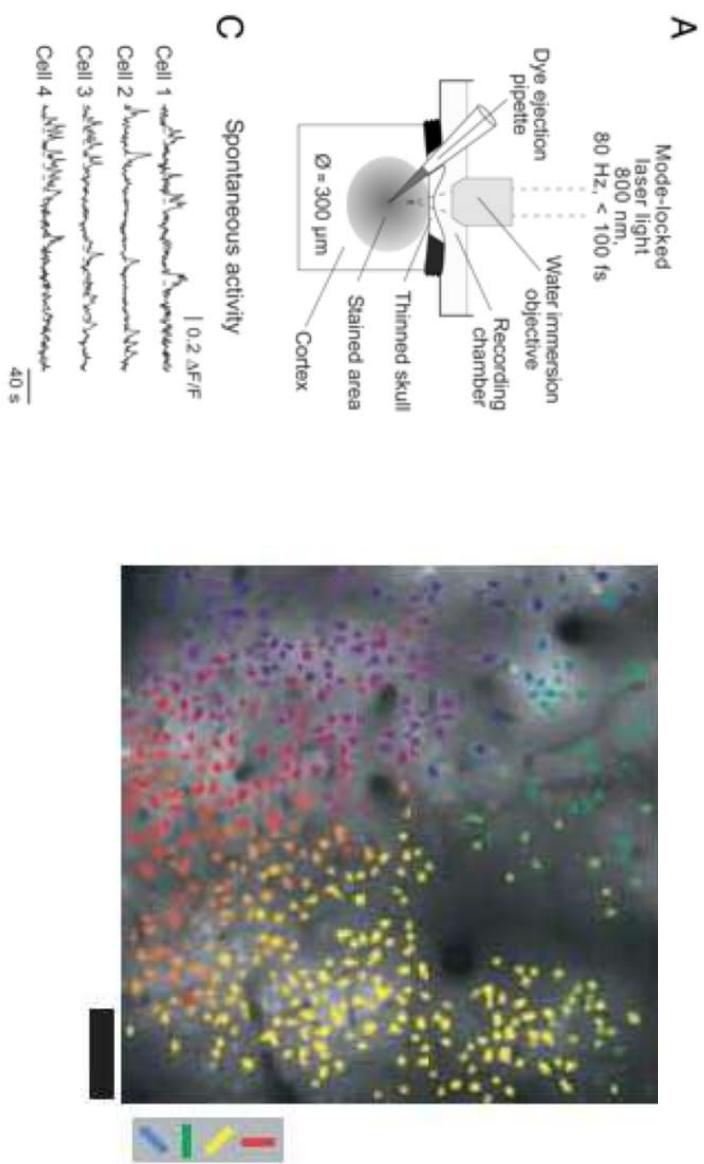
Spikes ('neural activity')

1 second



0.5
millimeter

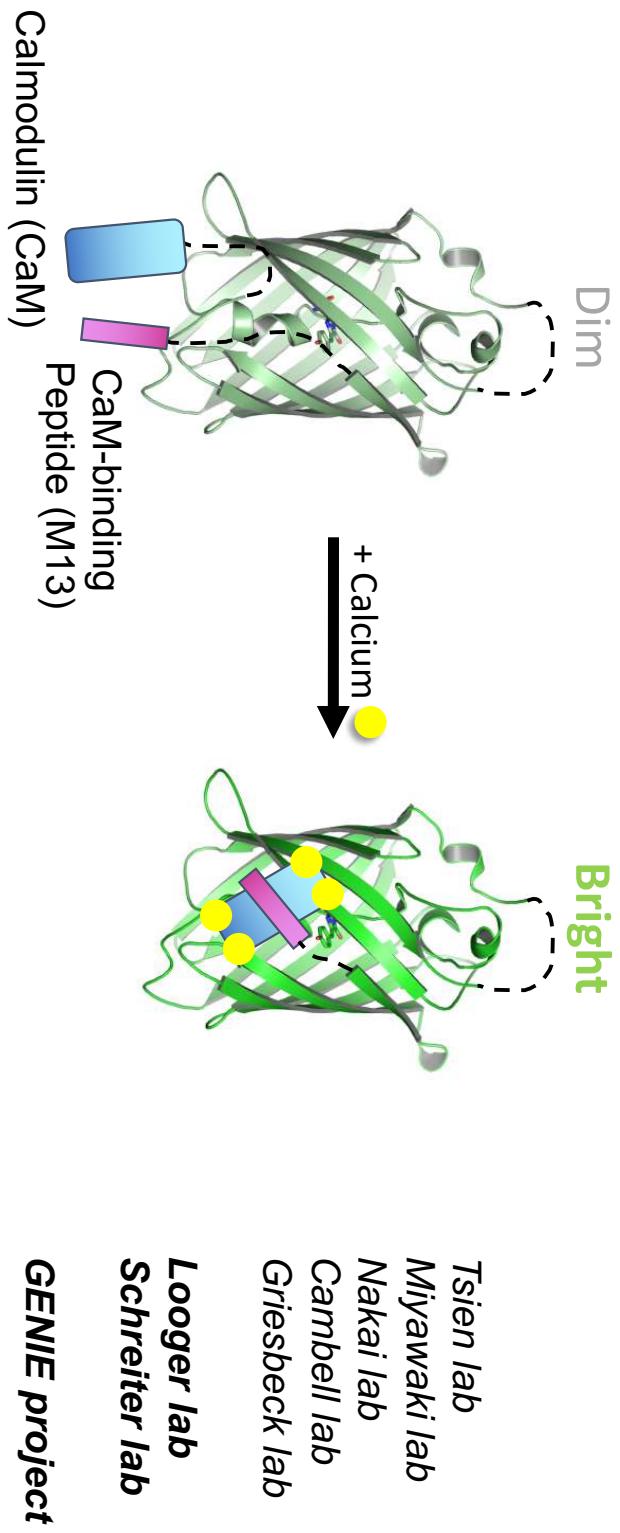
Imaging activity in neuronal populations



Stosiek et al 2003

Ohki et al 2006

Fluorescent proteins to spy on neuronal function



Nakai et al Nat. Biotech 2001; Tian et al Nat. Meth. 2009

GENIE team at Janelia

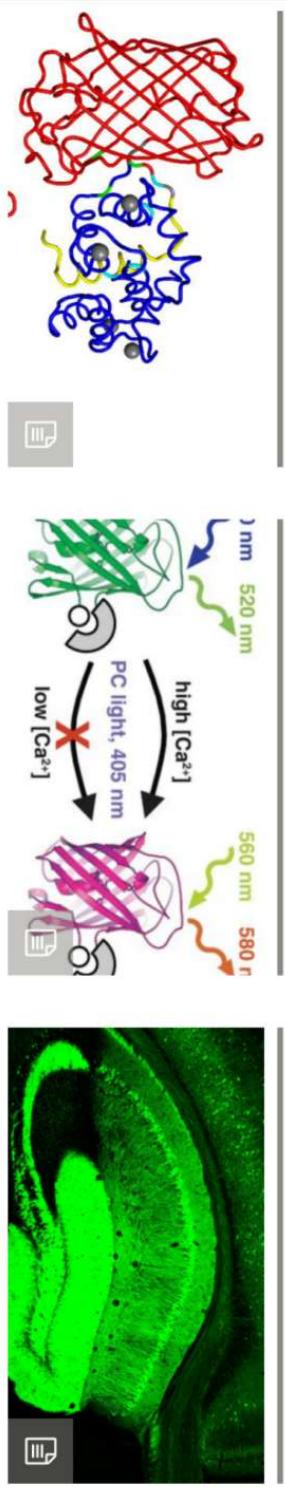


NEWS CAREERS CONFERENCES PEOPLE PUBLICATIONS RESEARCH GROUPS ▾ Our Research Support Teams Open Science You + Janelia About Us



GENIE

GENIE / A deep understanding of how neural circuits process information will require measurement of neural activity on many different scales, ranging from single synapses to large assemblies of neurons, in the intact brain. To advance this goal we engineer genetically encoded tools for measuring and controlling neuronal activity in the intact brain.



Genetically-Encoded Neuronal Indicator and Effector (GENIE) Project



Vivek Jayaraman
Allan Wong
Luke Lavis
Loren Looger
Eric Schreiter
Karel Svoboda



Jeremy Hasseman
Alison Howard
Ilya Kolb
Derek Merryweather
Amy Choung
Arthur Tsang
Getahun Tsegaye

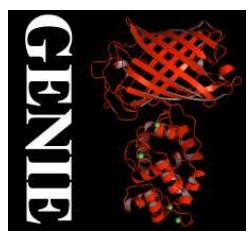
Collaborators

Misha Ahrens
Ben Arthur
Caiying Guo
Thomas Hughes (Montana State)
Takashi Kawashima
John Macklin
Jonathan Marvin
Boaz Mohar
Ondrej Novak
Amrita Singh
Yan Zhang

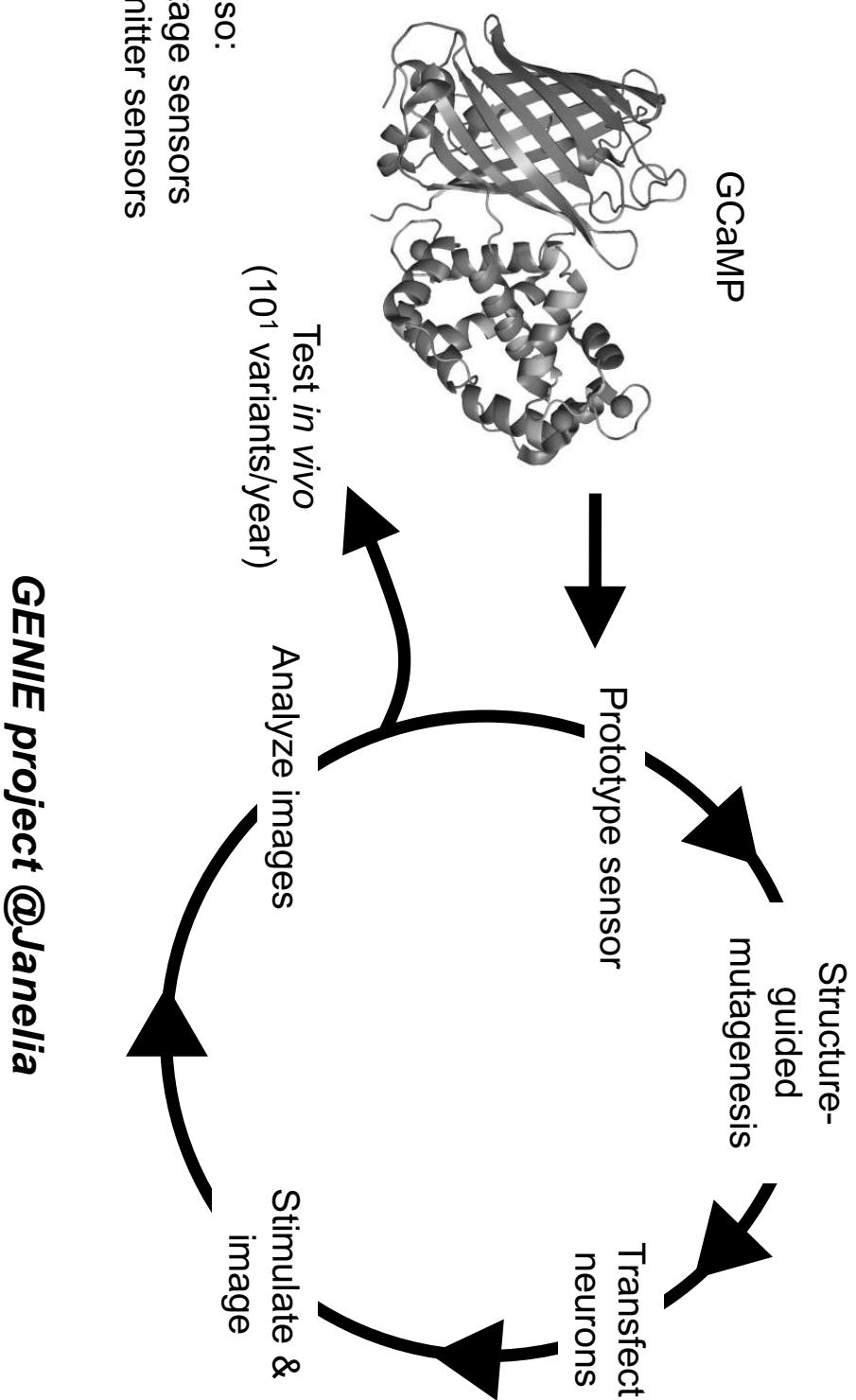
Alumni

Lauren Barnett (Scintillon Institute)
Stephan Brenowitz (Maryland)
Tsai-Wen Chen (National Yang-Ming University)
Hod Dana (Cleveland Clinic)
Benjamin Fosque (Chicago)
Graham Holt (Duke)
Rex Kerr (Calico Life Sciences)
Bei-Jung Lin (National Yang-Ming University)
Rajeev Rikhye (NYU)
Trevor Wardill (Cambridge)
Doug Kim (NIH)

Yi Sun (Westlake University)



Protein calcium sensor screening pipeline
 10^3 variants/year



GENIE project @Janelia

Spikes ↔ GCaMP6 fluorescence change

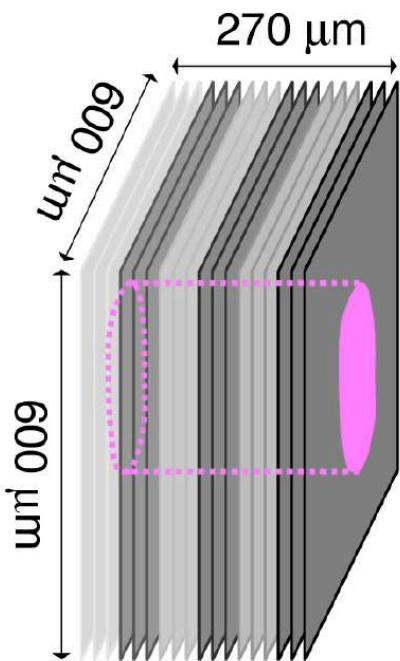
1s
20%
0.5mV

10 μm

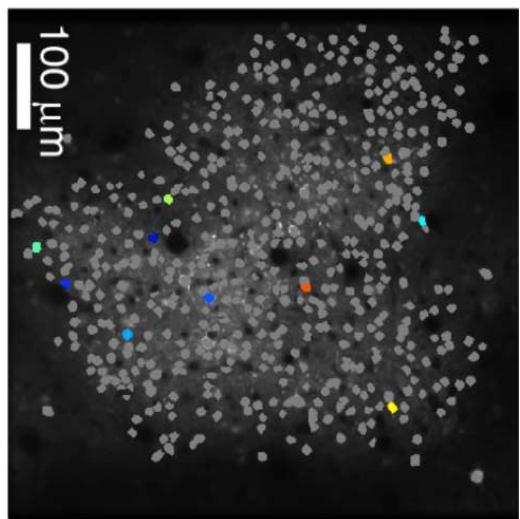
— | —

Comprehensive mapping of neural activity

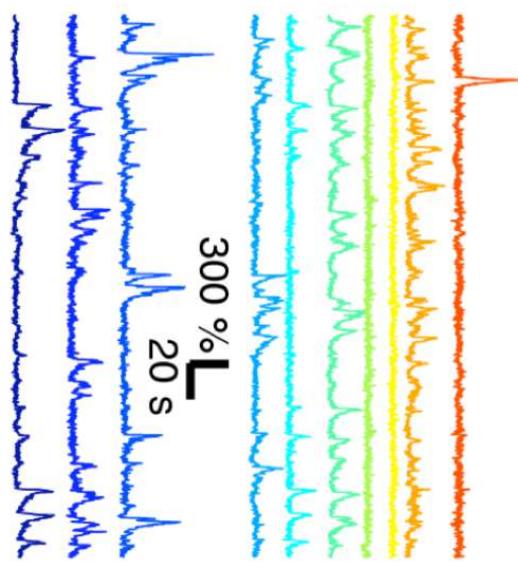
Cortical 'barrel' column



Imaging plane

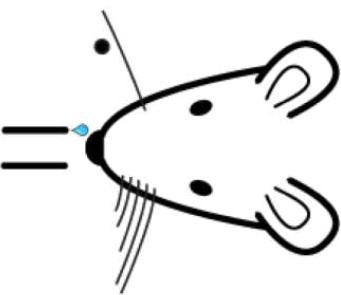


Example neuron fluorescence



Neural coding

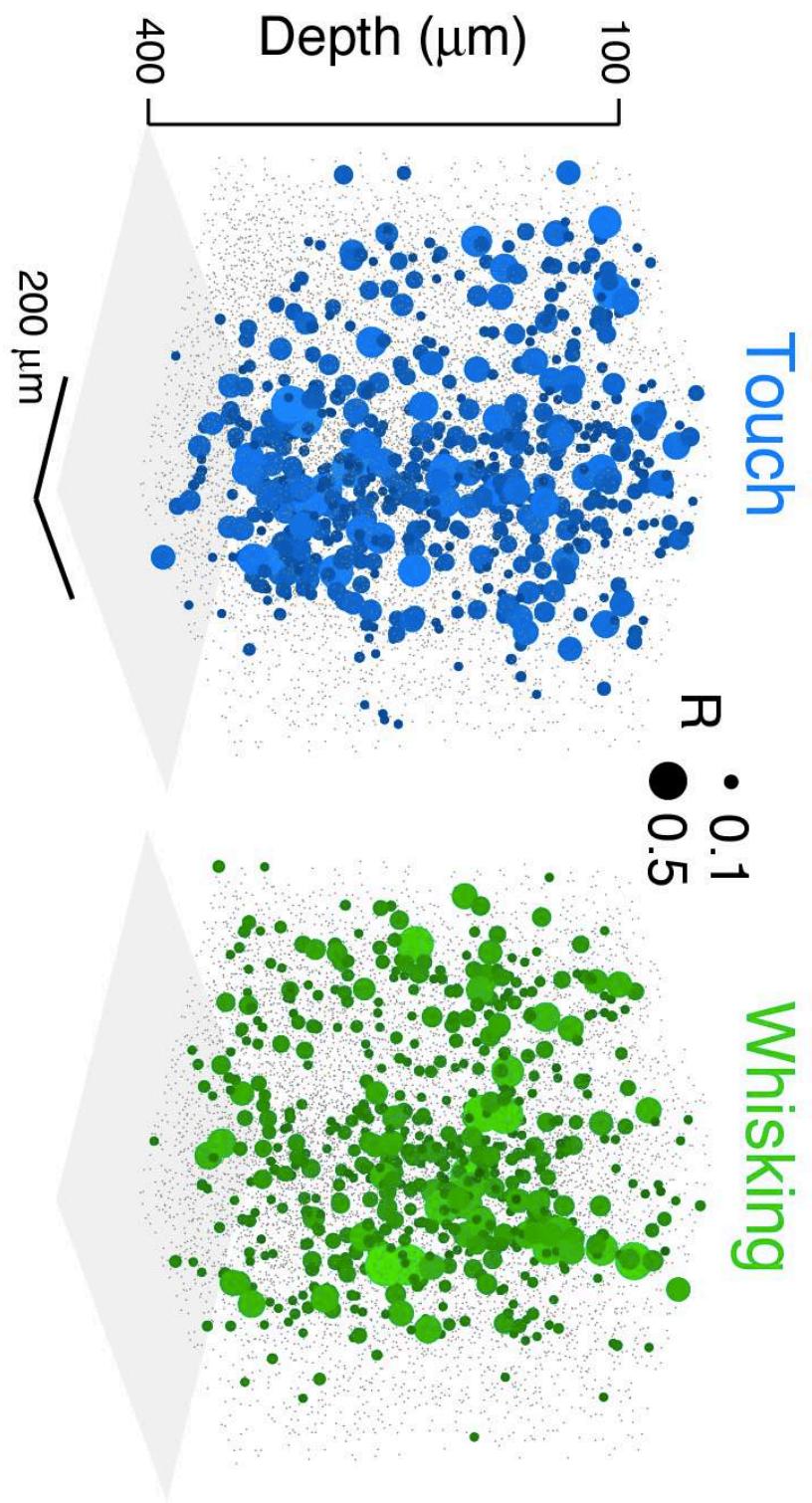
touch cell:



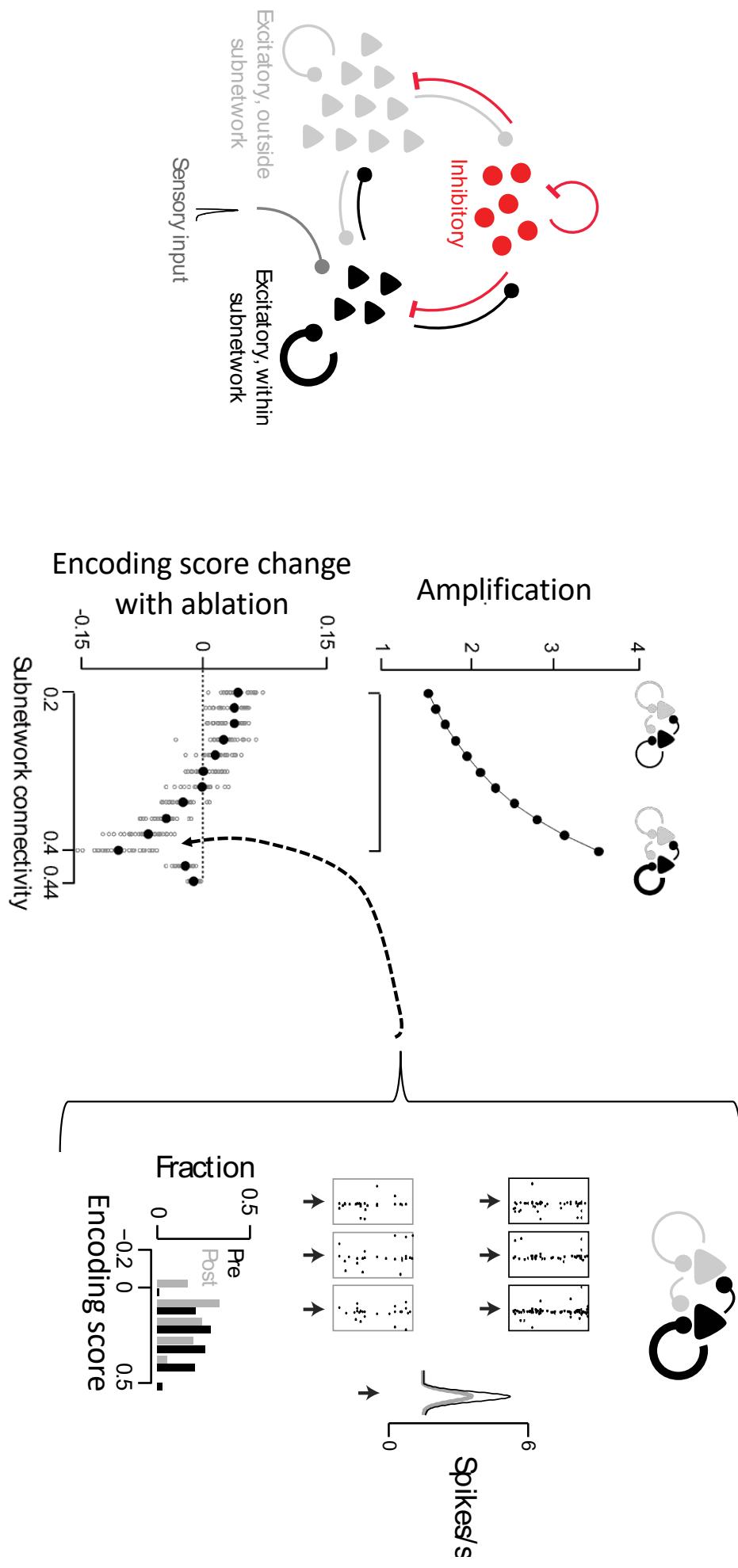
whisking cell:



Intermingled neural representations



Models of cortical circuits



Localization by microscopy → targeted manipulations

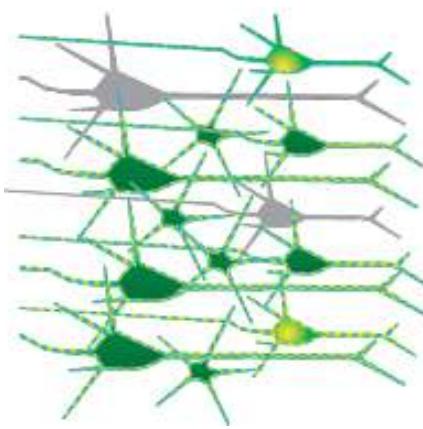
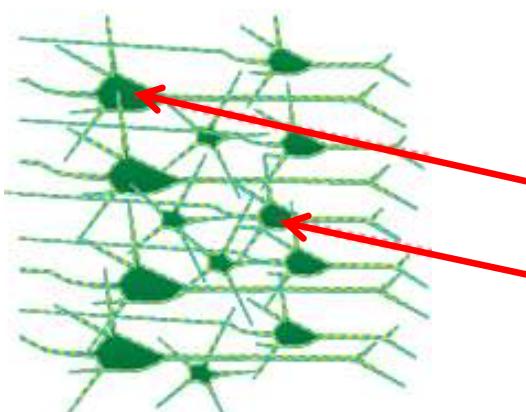
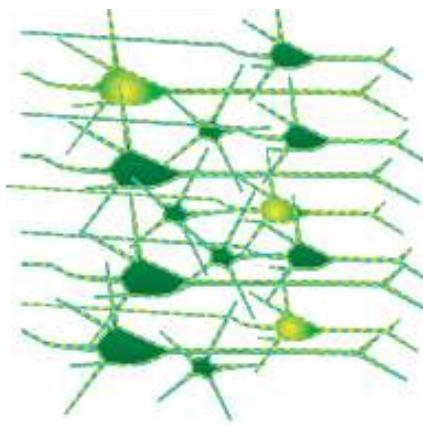


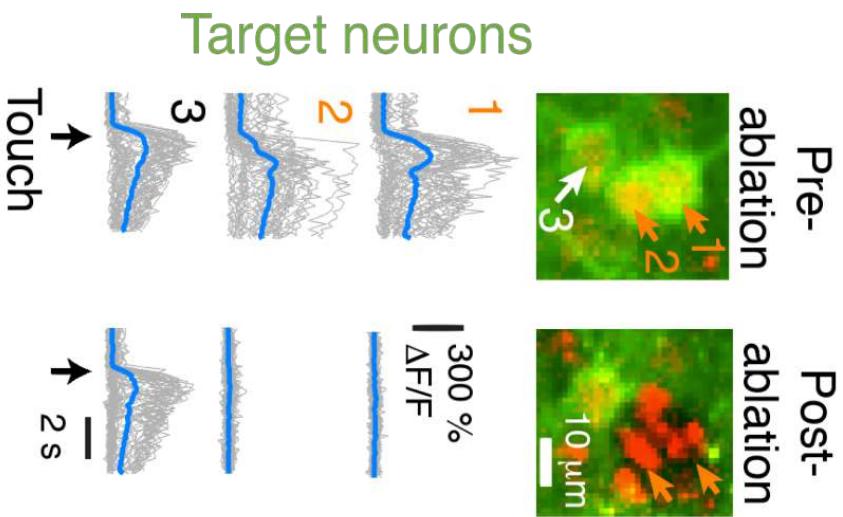
Image activity &
classify neurons

Ablate 10-20 % of
one representation

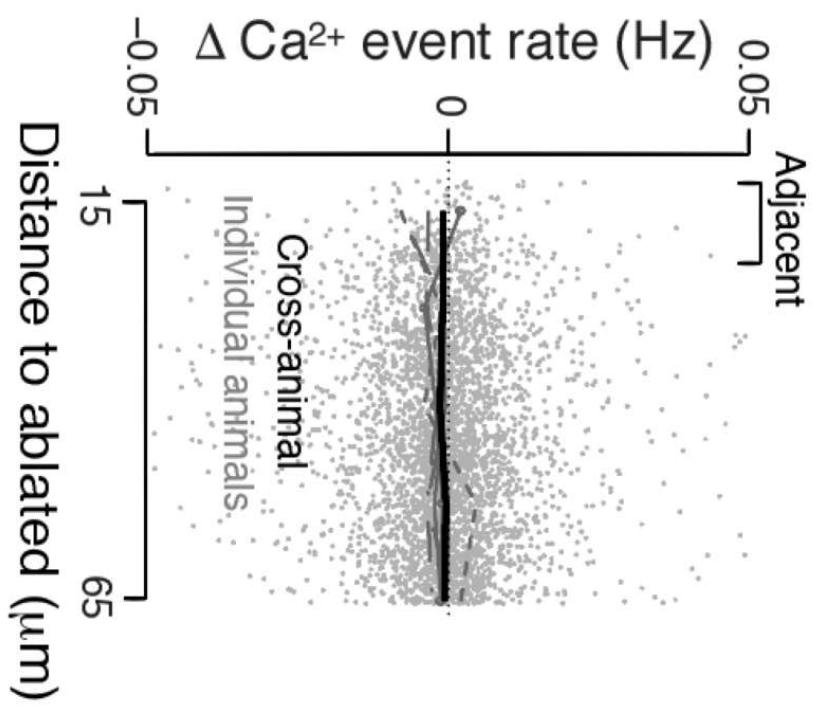
Image activity &
classify neurons

Targeted multi-photon ablations

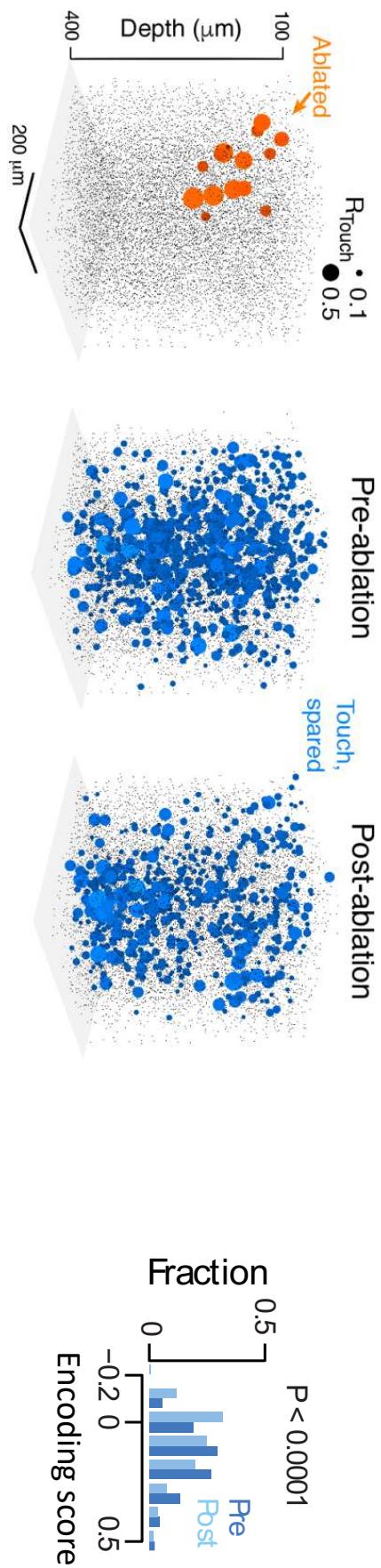
Target touch neurons:



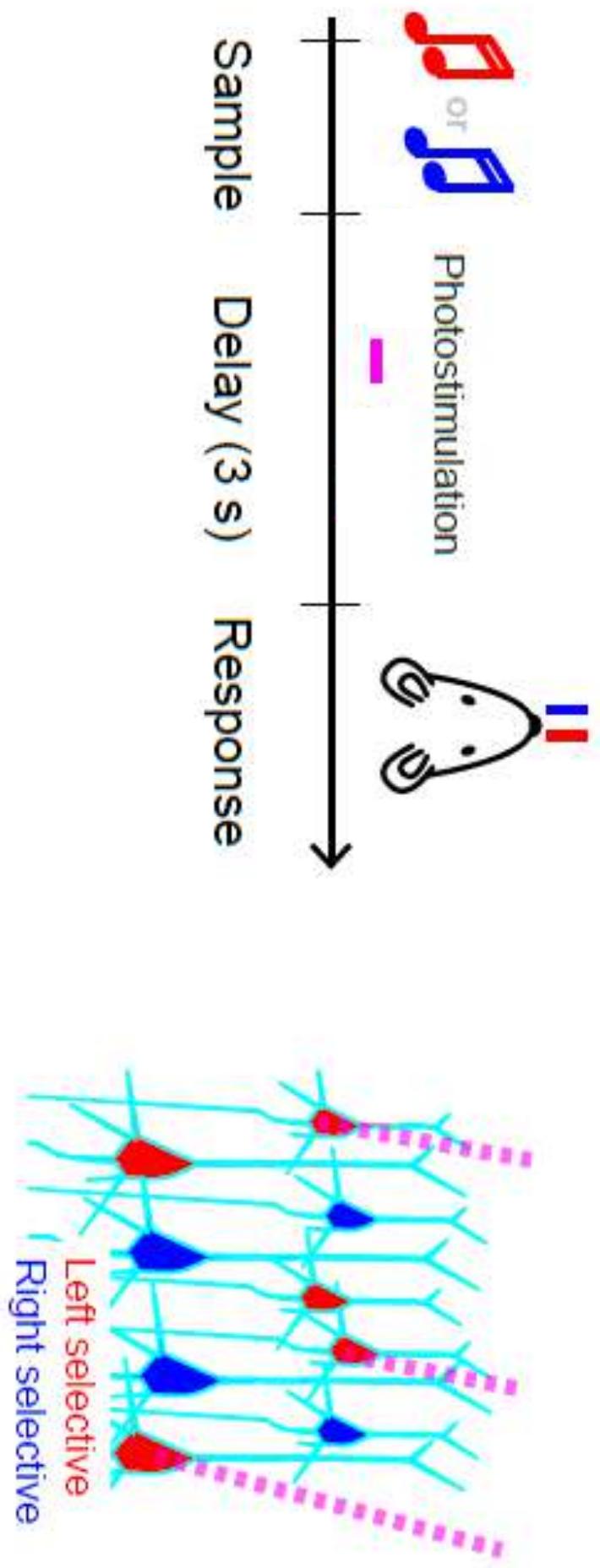
Target silent neurons:



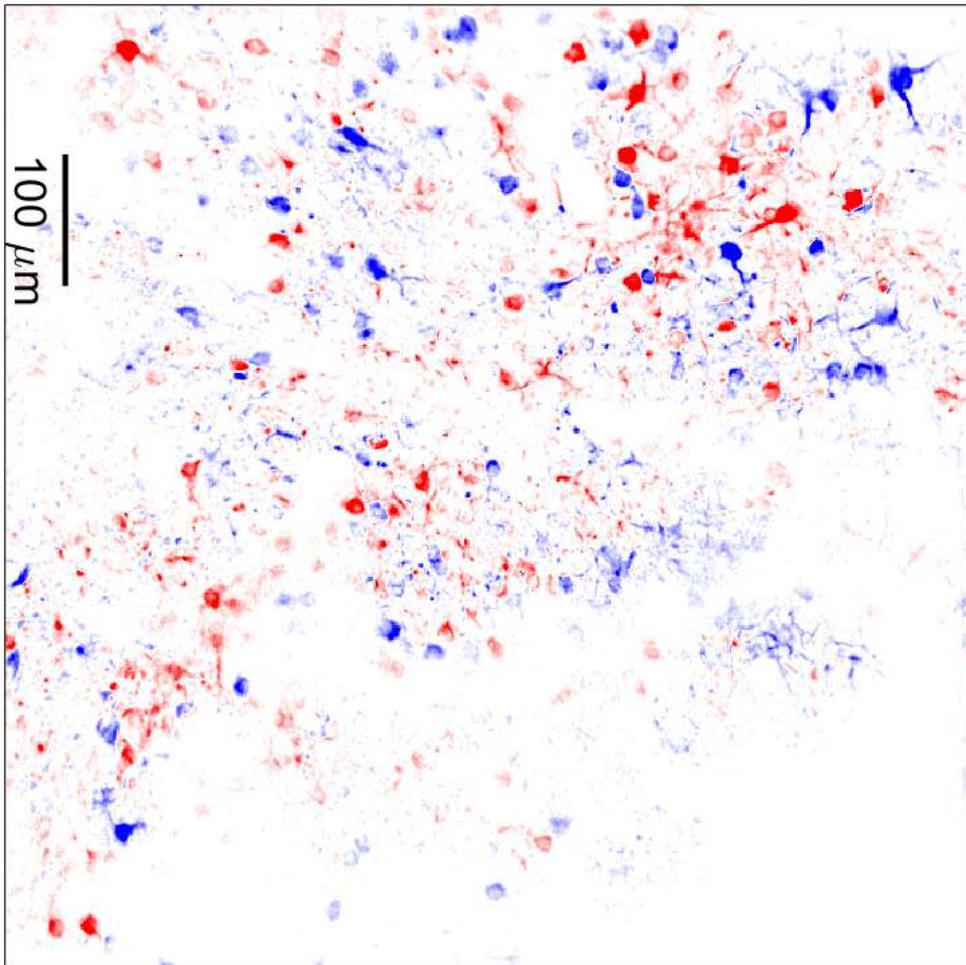
Strong amplification in somatosensory cortex



Targeted 2-photon photostimulation to probe
the mechanisms of short-term memory



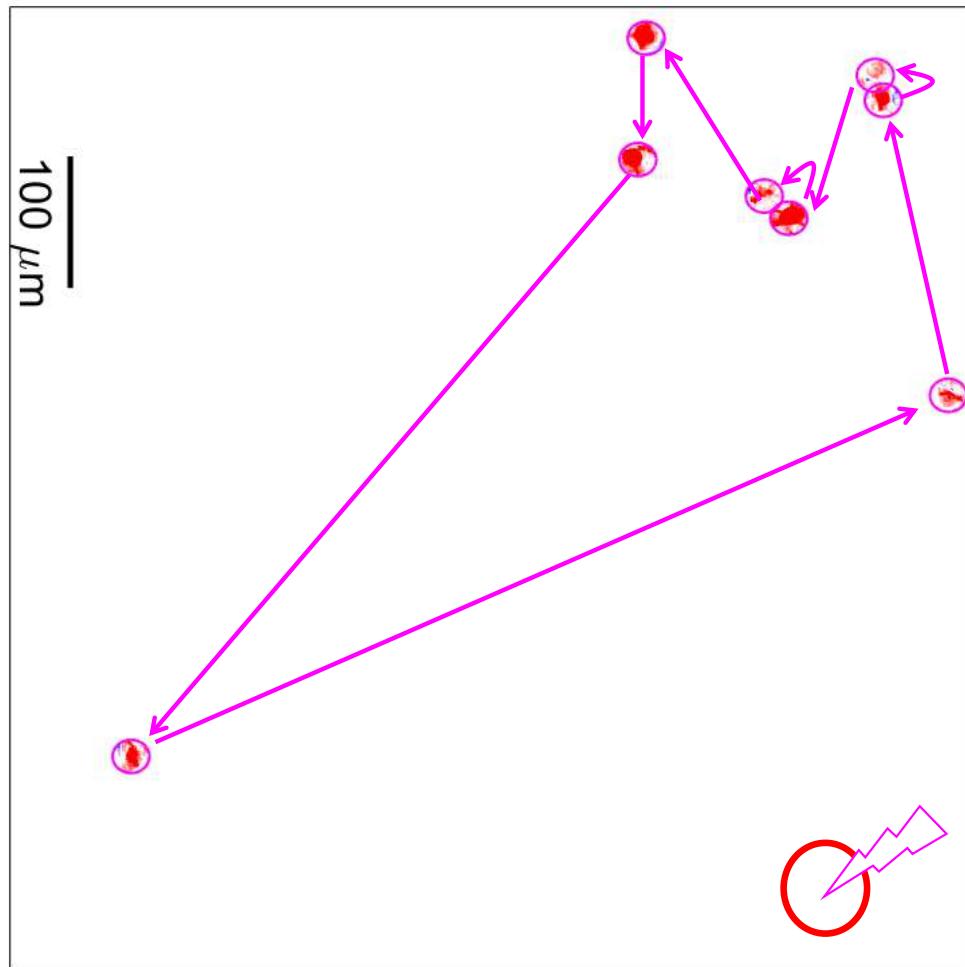
Selectivity map



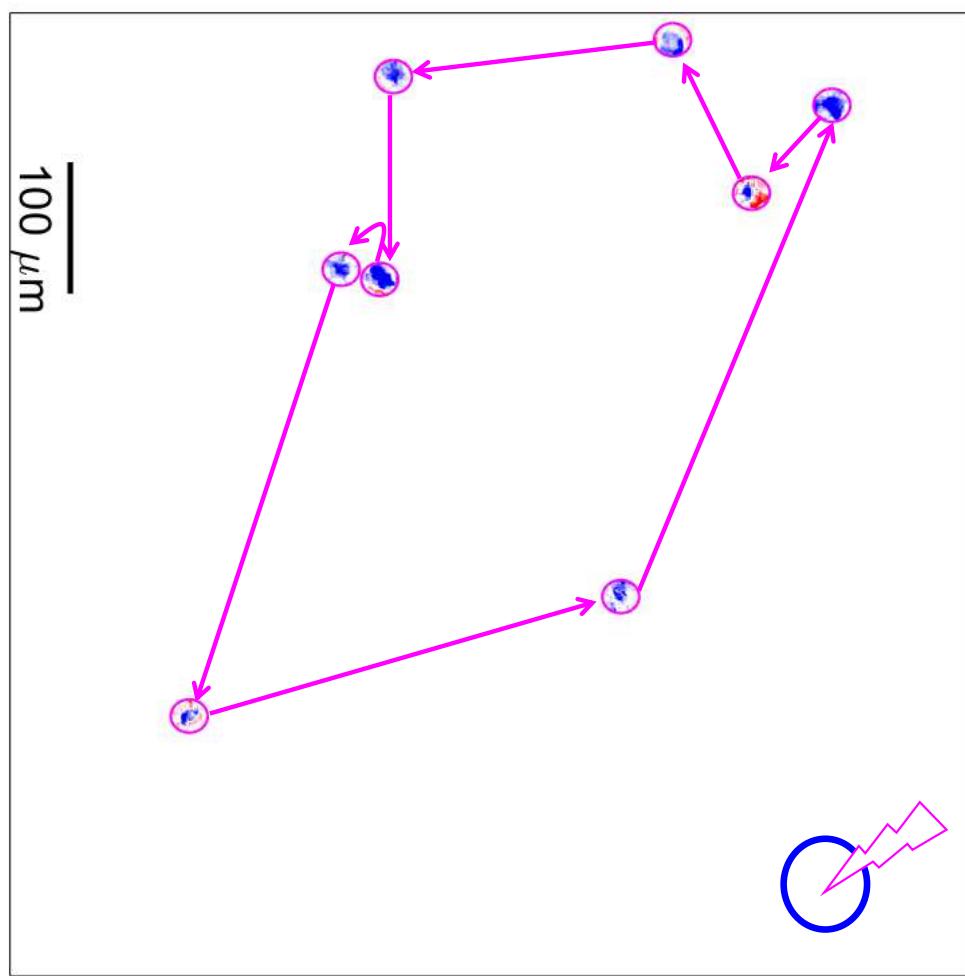
GCaMP6s & ChrimsonR

Kayvon Daie

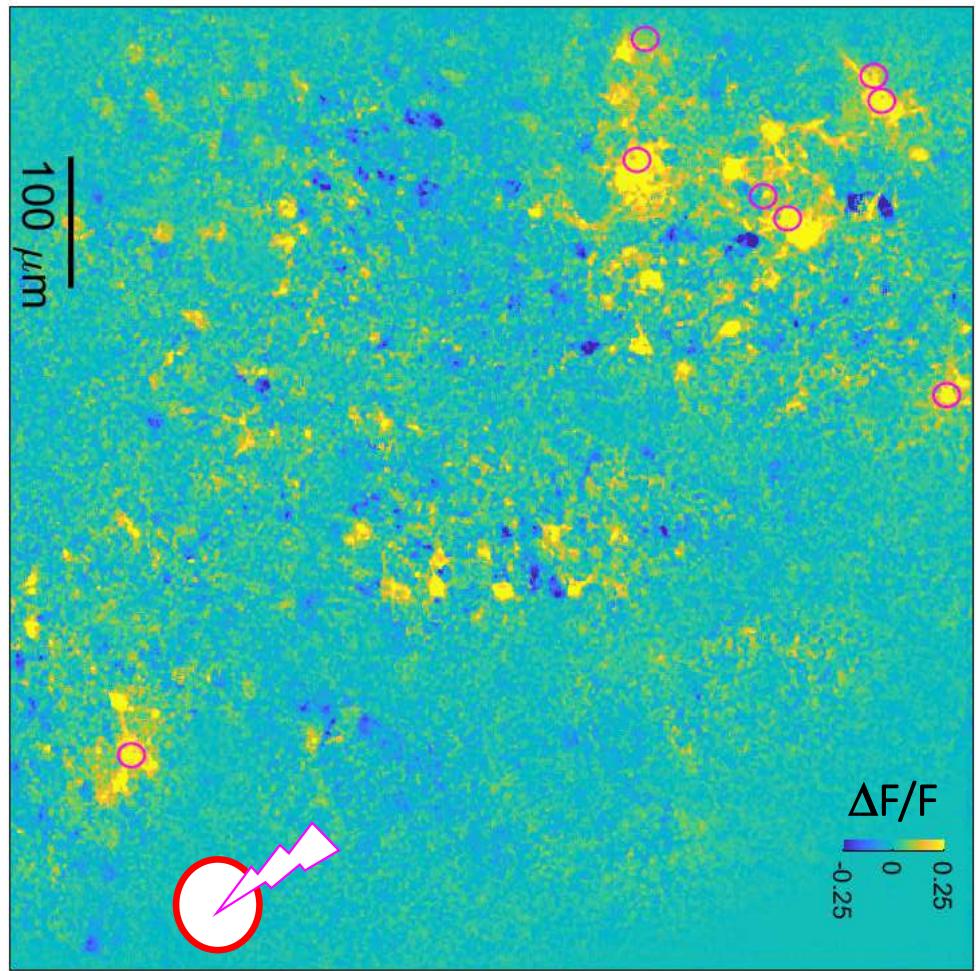
L group



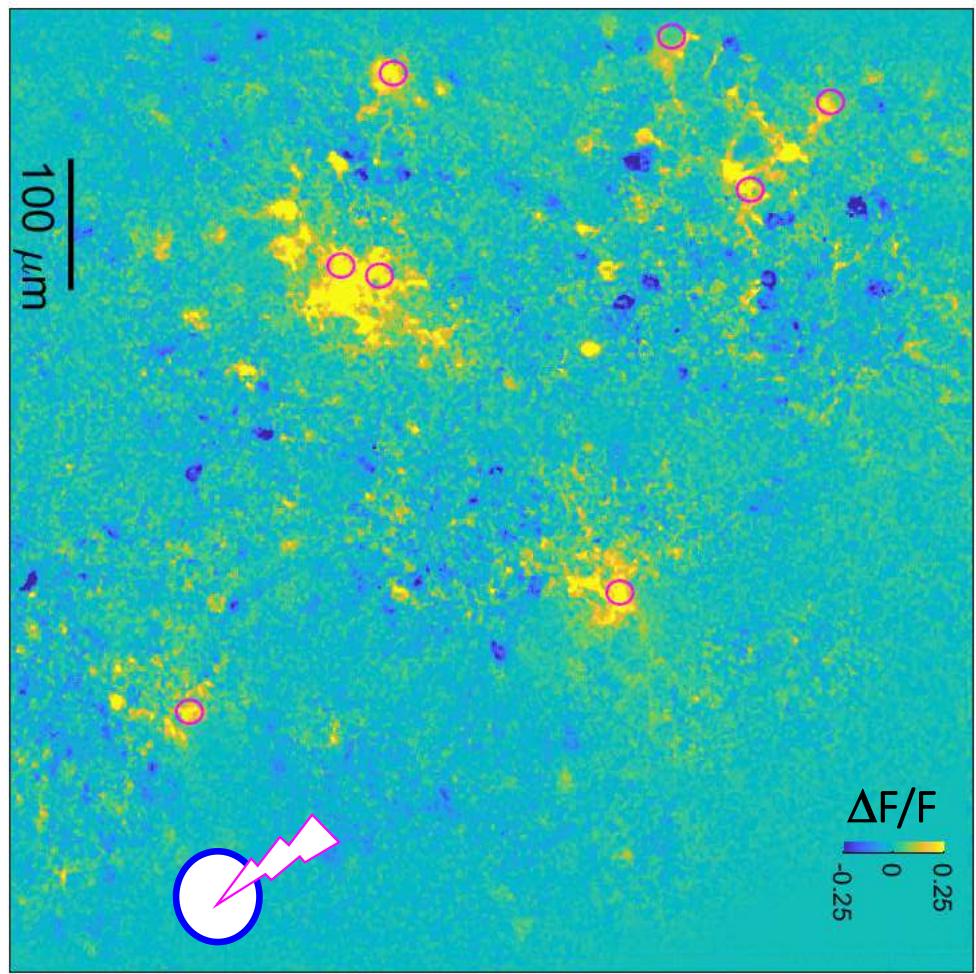
R group



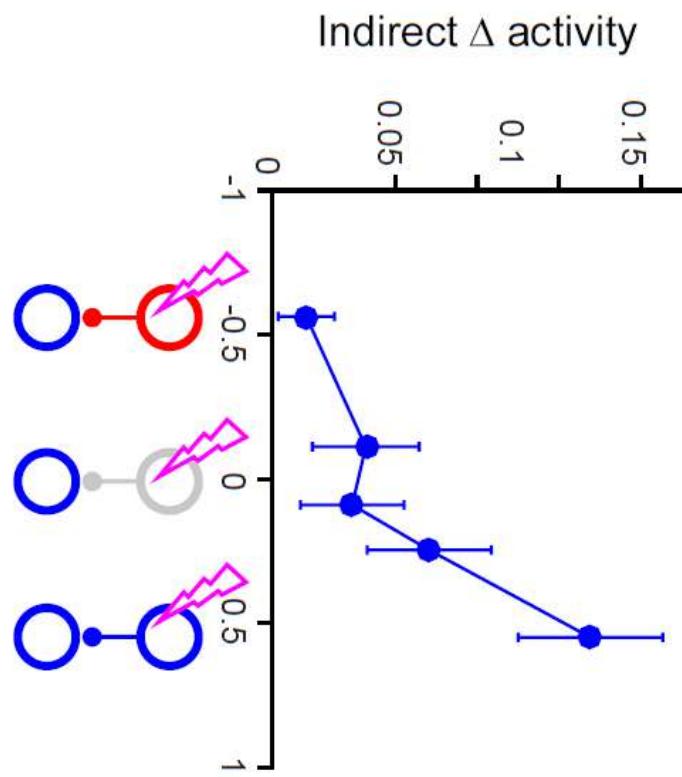
Δ Activity, L group



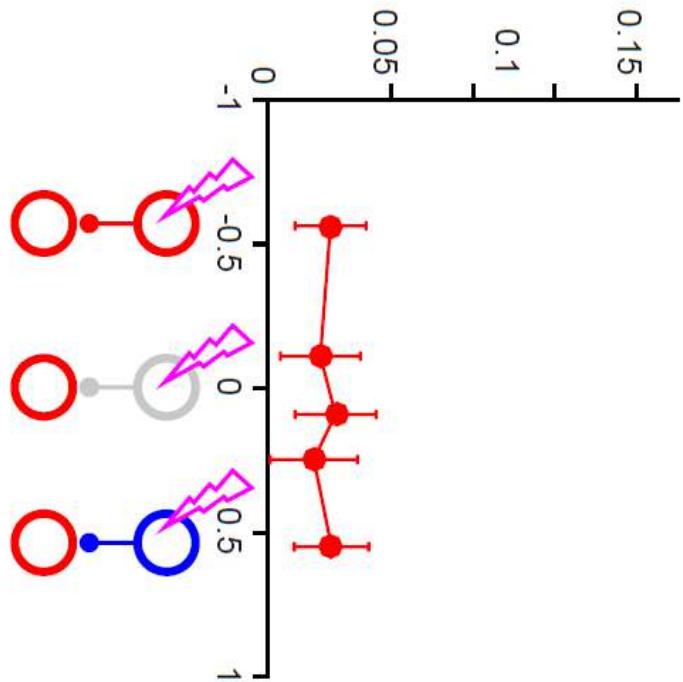
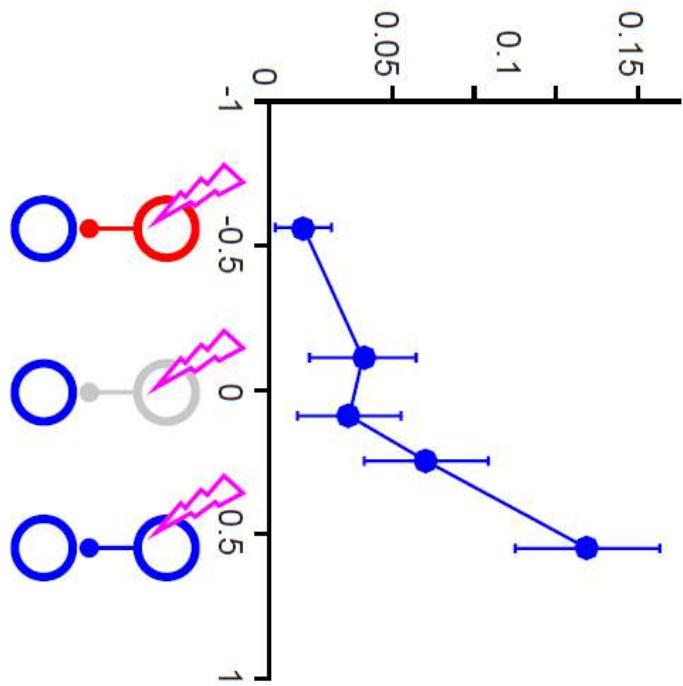
Δ Activity, R group

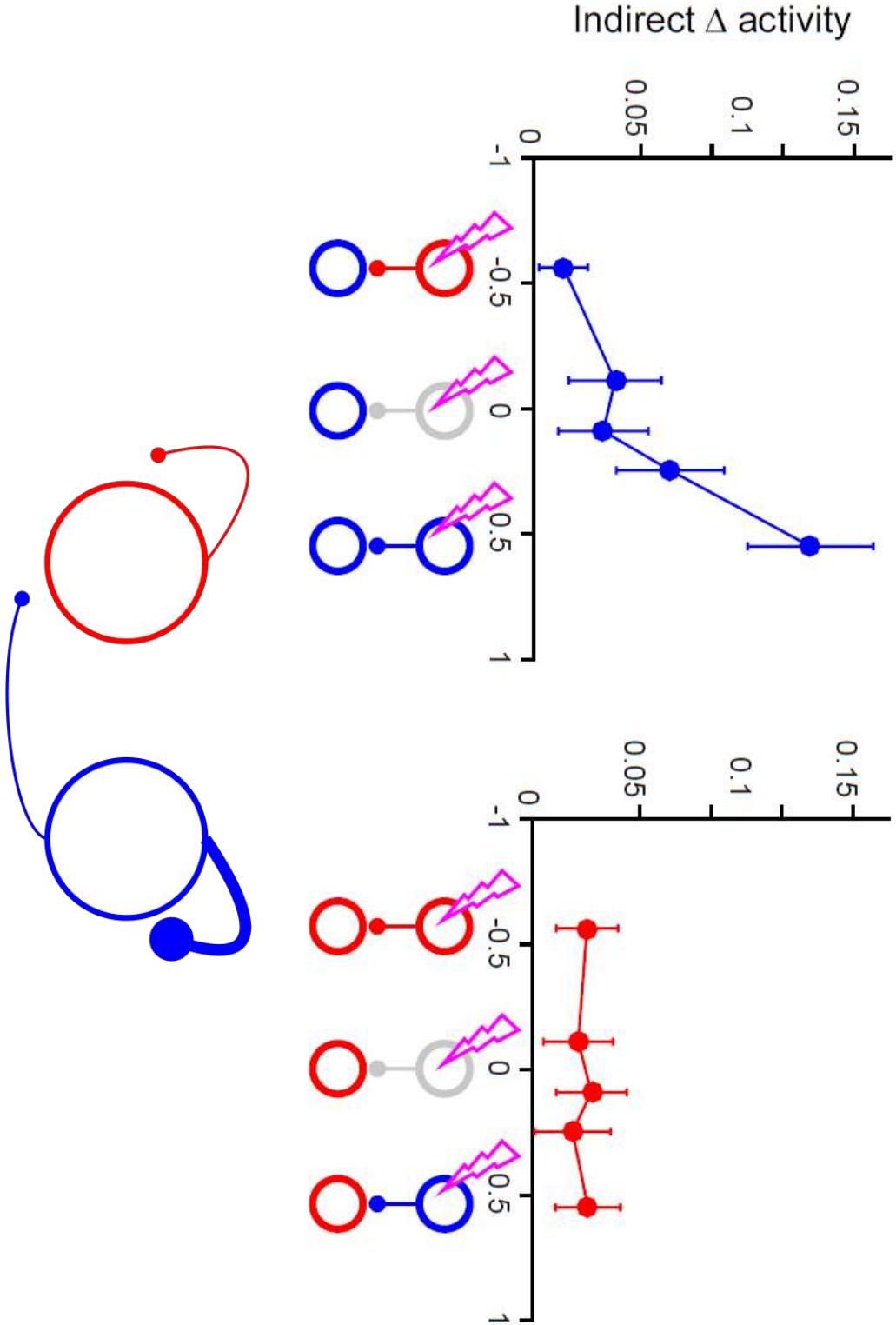


Non-targeted right neurons

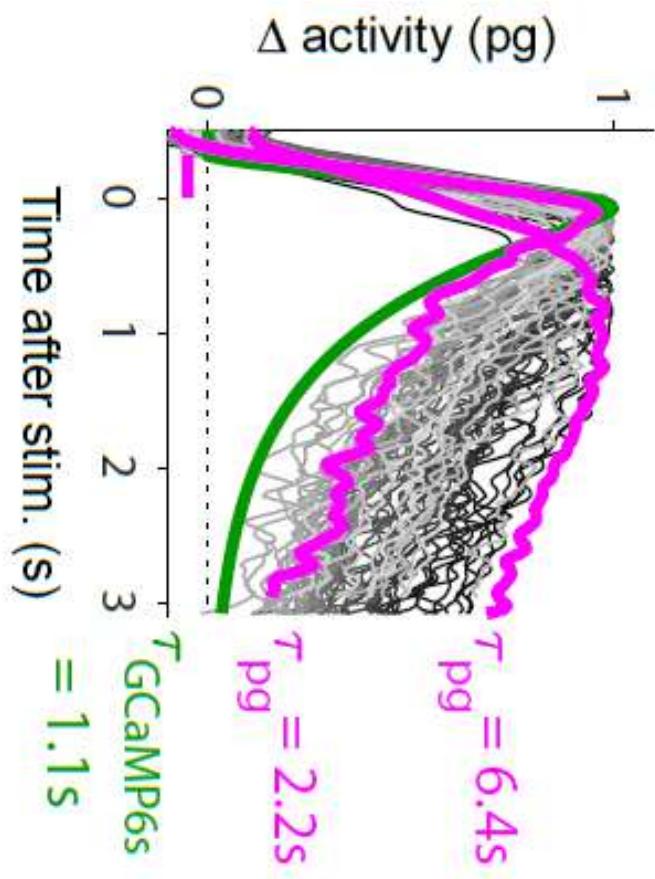


Indirect Δ activity

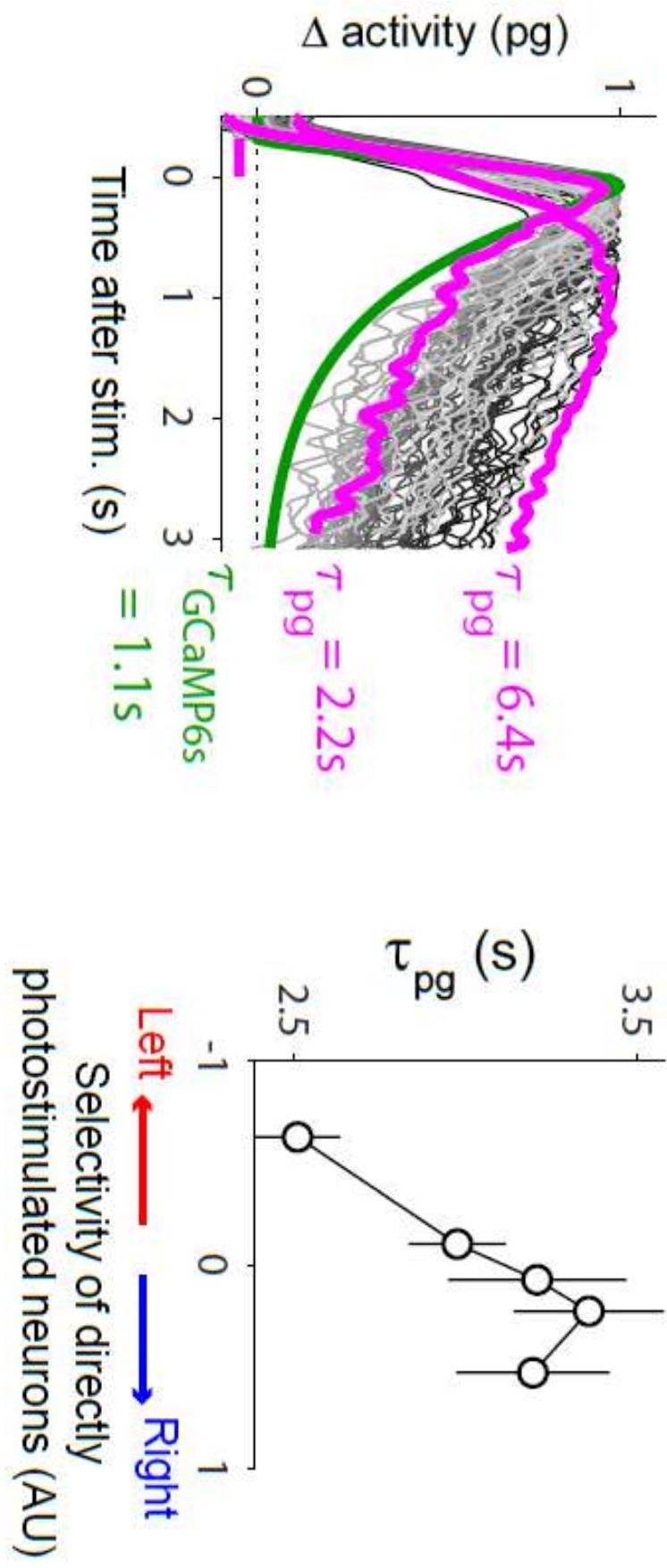




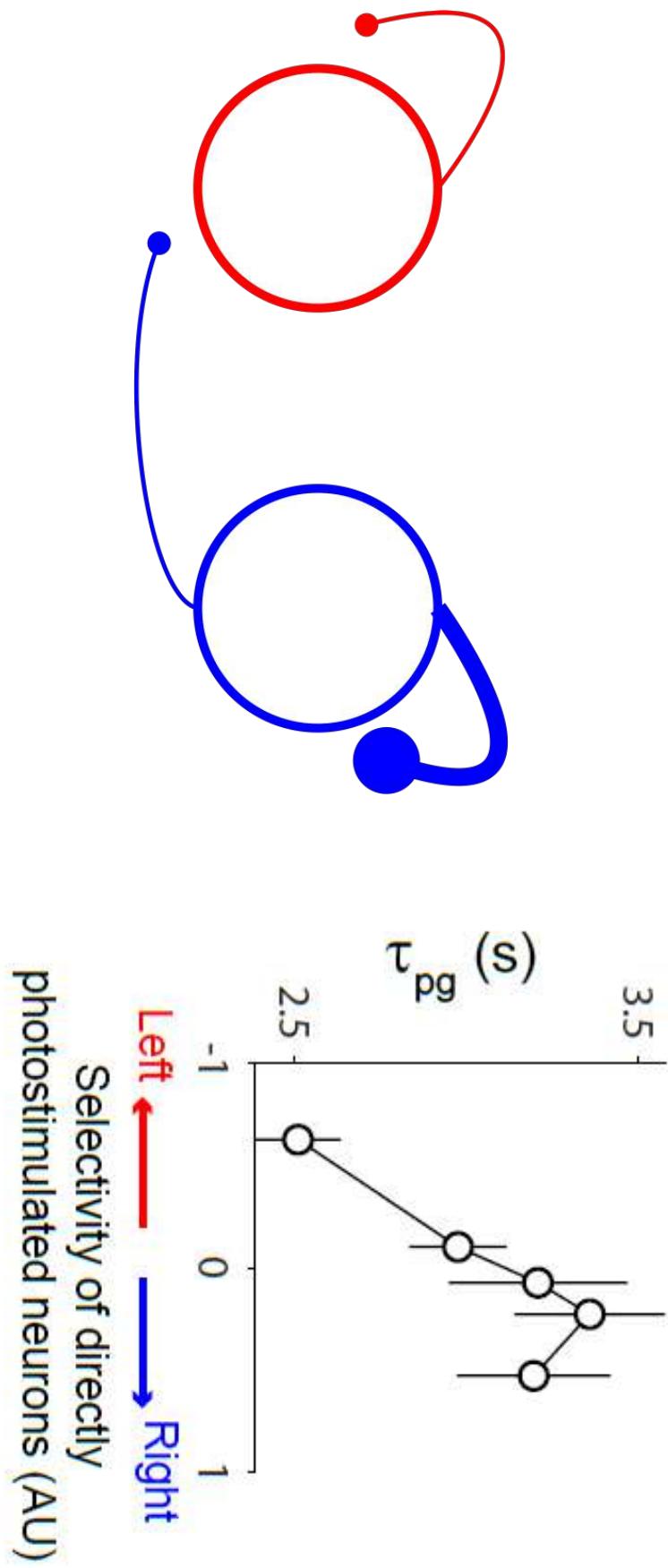
Persistence of photostimulated responses



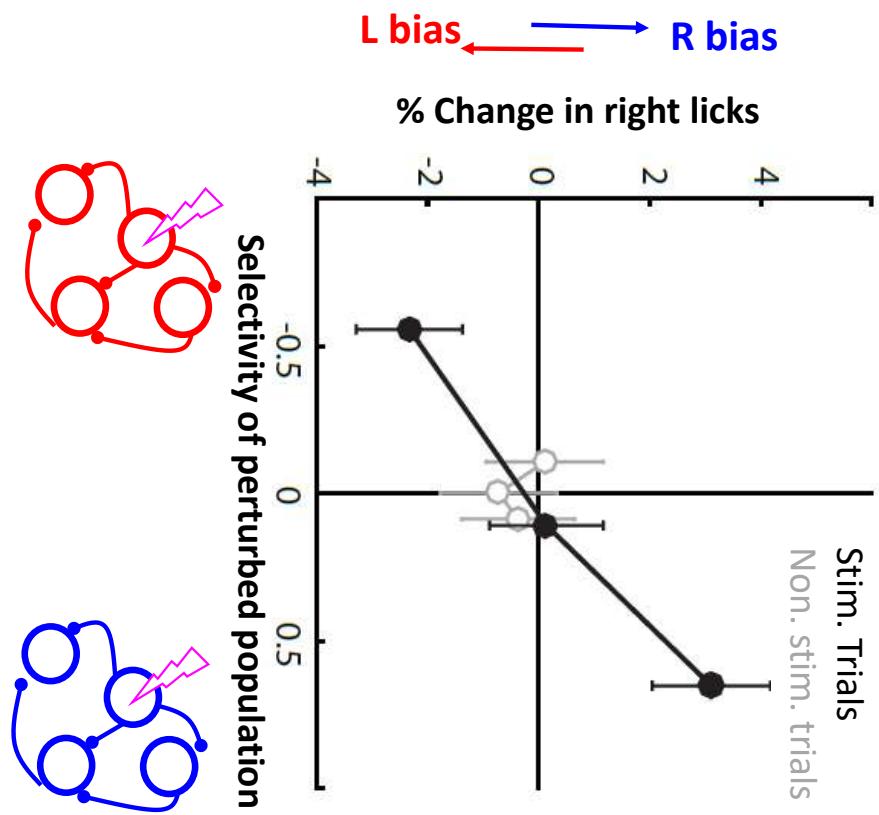
Persistence of photostimulated responses



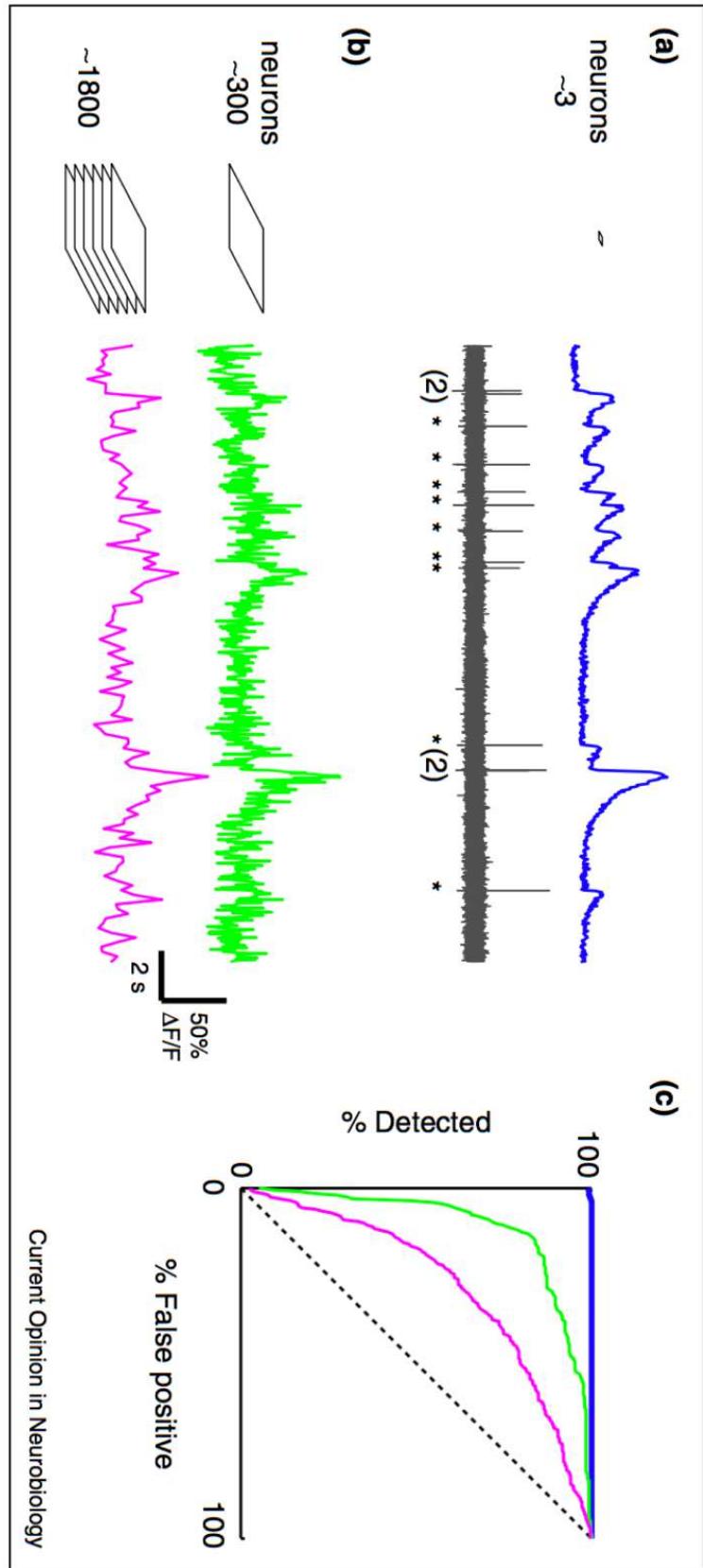
Persistence of photostimulated responses



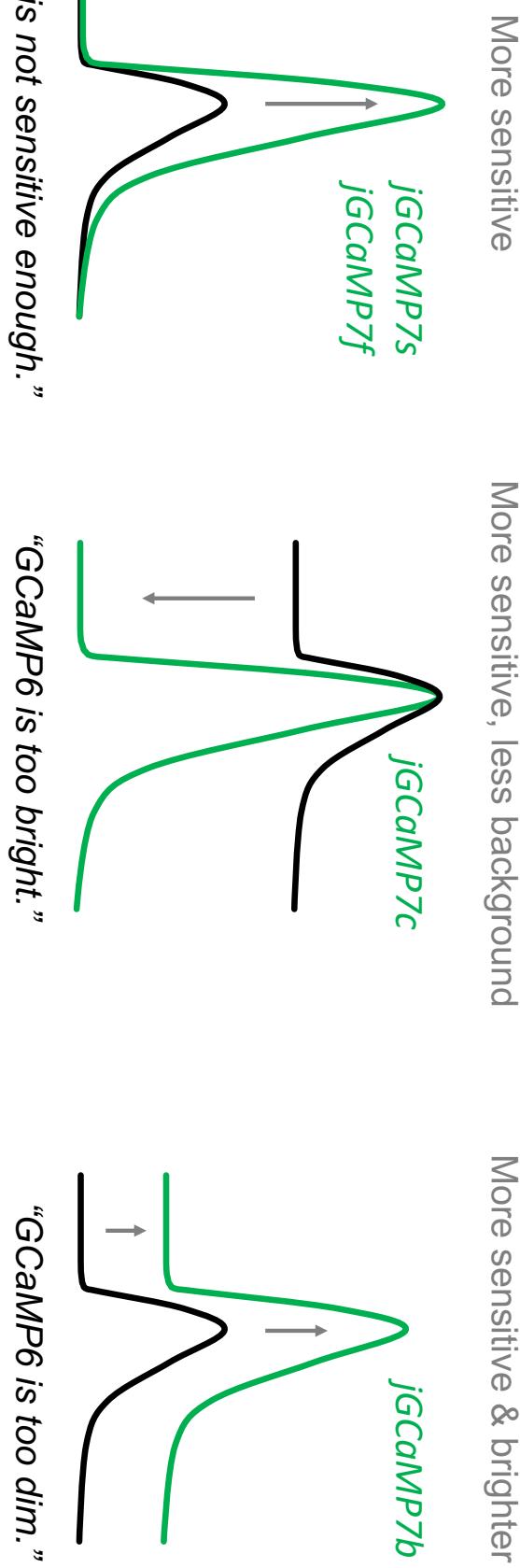
Perturbations cause predictable changes in behavior



Number of imaged neurons $\sim (1 / \text{SNR})^{1/2}$



jGCaMP7 calcium indicators



"*GCaMP6 is not sensitive enough.*"

"*GCaMP6 is too bright.*"

"*GCaMP6 is too dim.*"

Reagents freely available at Addgene, JAX, Upenn Vector core

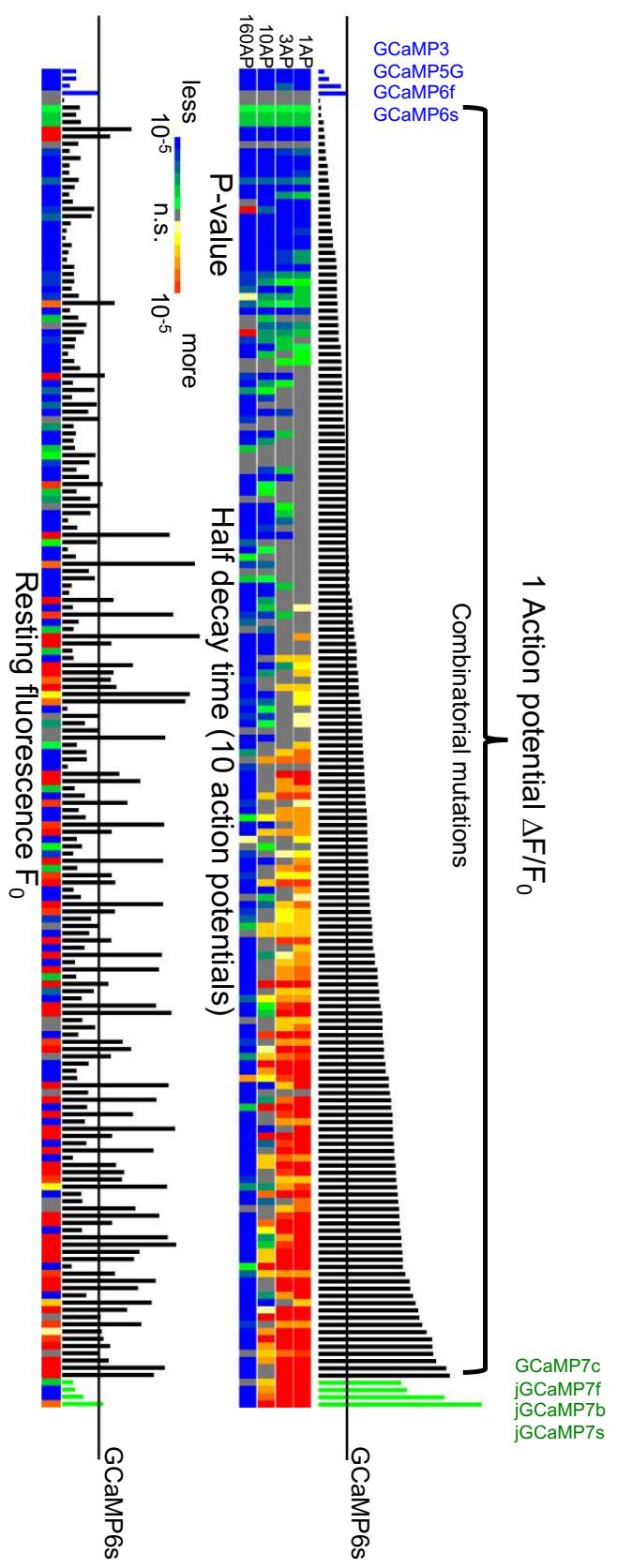
bioRxiv 434589; Dana et al 2019 *Nat Meth*

<https://www.janelia.org/project-team/genie>

[@janelia_genie](https://twitter.com/janelia_genie)



jGCaMP7 screening results



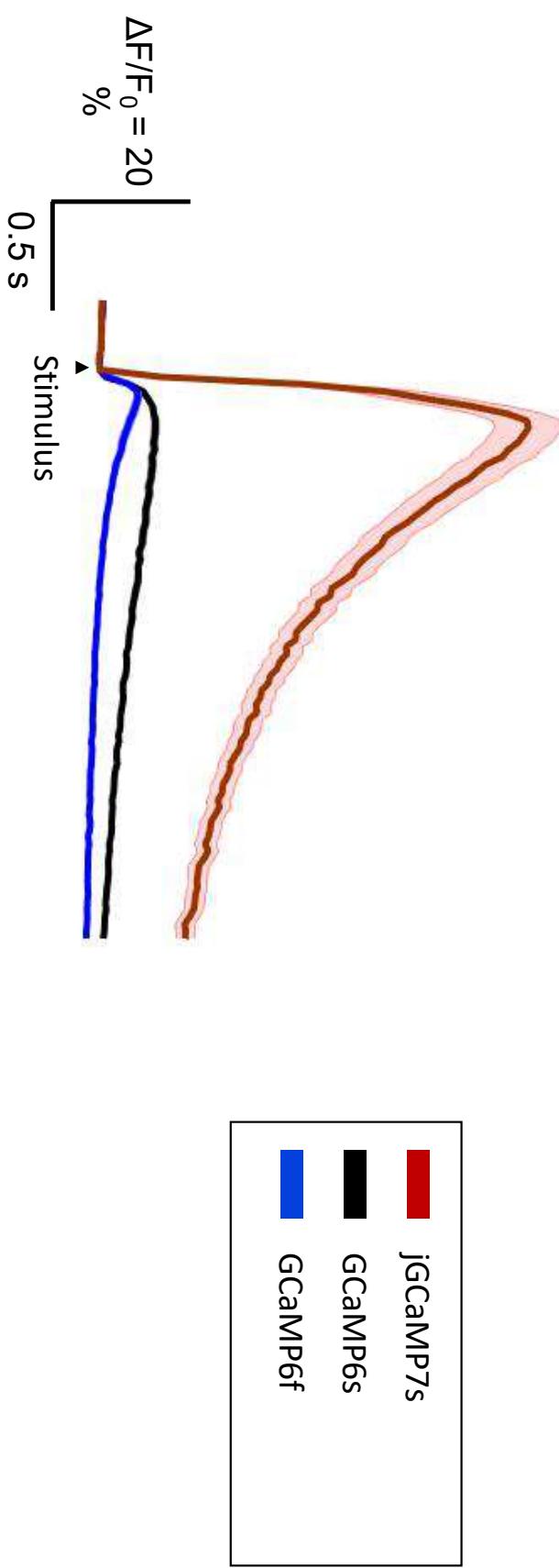
Reagents freely available at Addgene, JAX, Upenn Vector core

GENIE project @Janelia

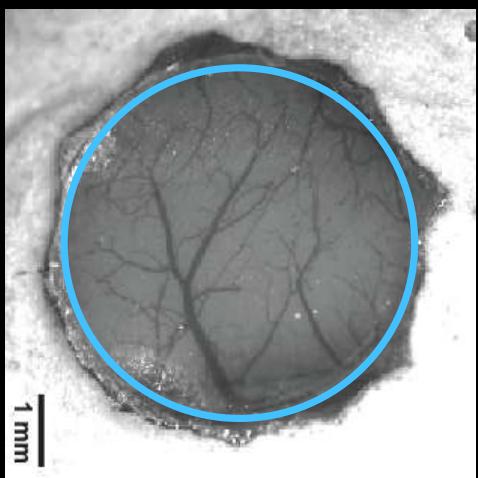
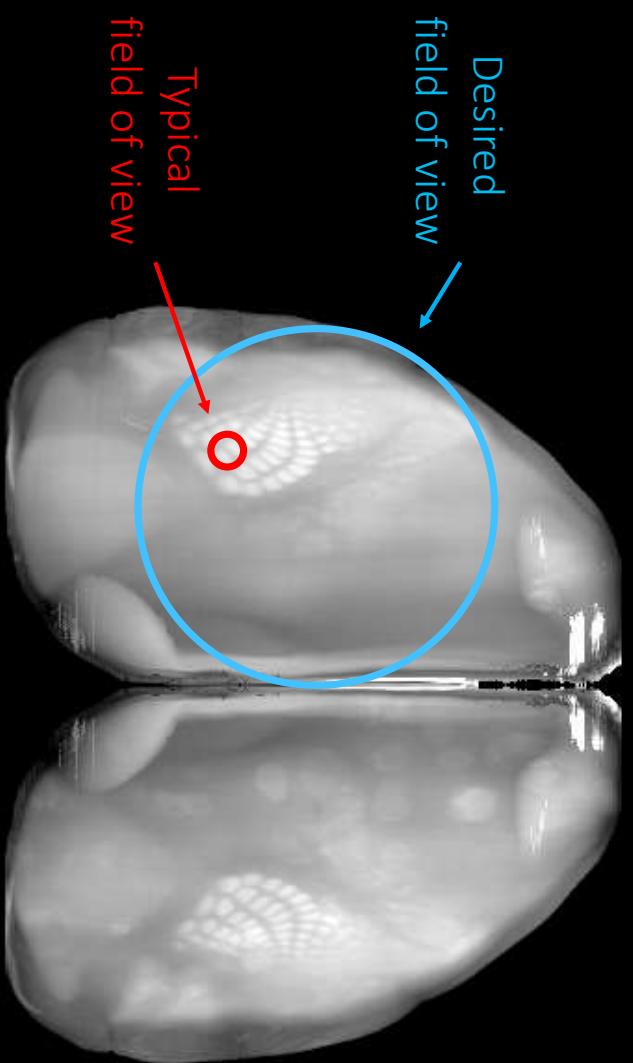
bioRxiv 434589

jGCaMP7s - improved sensitivity compared to GCaMP6s

Response to one action potential

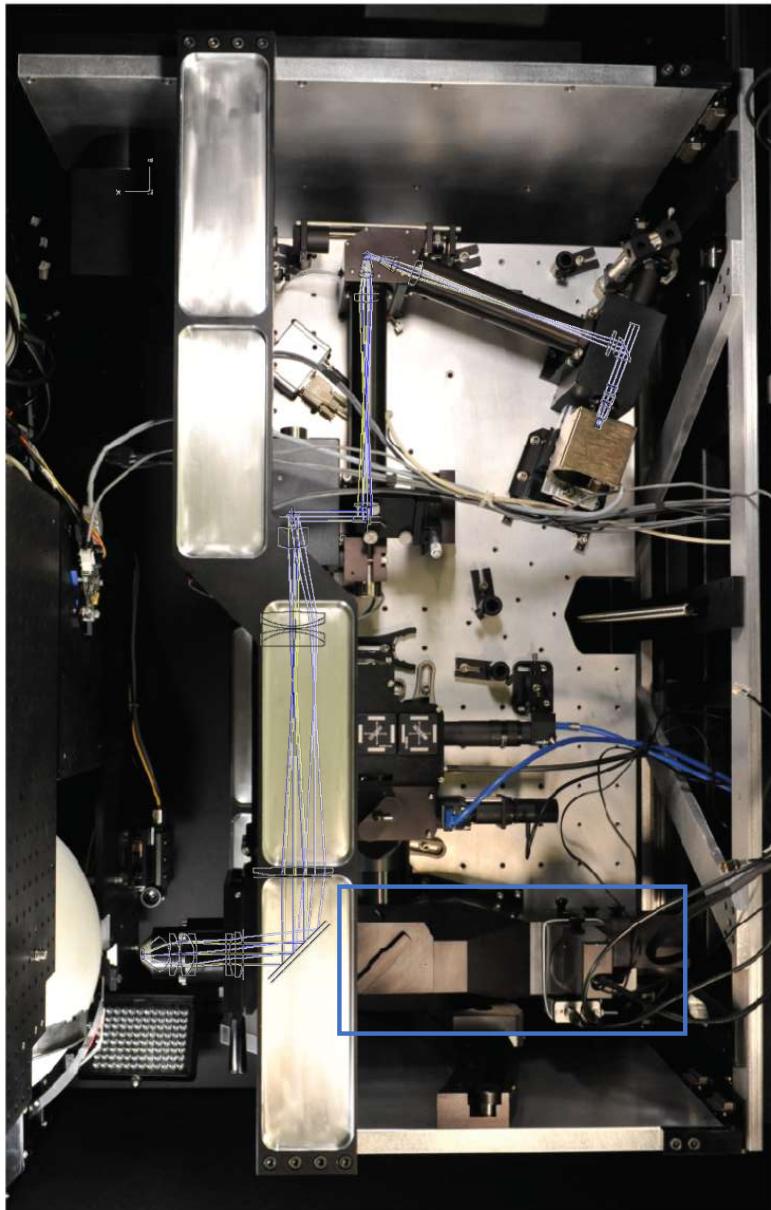


Mesoscale 2-photon microscopy



Sofroniew, Flickinger et al 2016

2-photon random access mesoscope



Key design specifications:

Field of view: 5.0 mm

Diffraction-limited excitation NA: 0.6

Collection NA: 1.0



Conventional
microscope





2016 Janelia 2p-RAM workshop



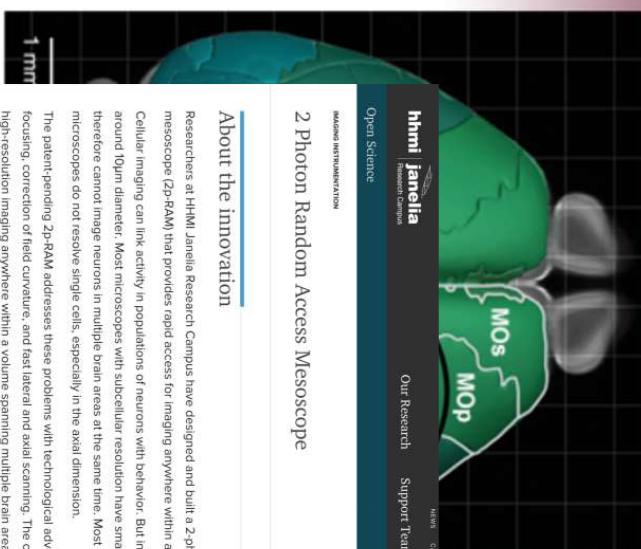
▲>>[Imaging Systems & Components](#)>>[Multiphoton Mesoscope](#)

Multiphoton Mesoscope

- ▼ Subcellular-Level Resolution over a Ø5 mm Field of View
- ▼ High-Speed Functional Imaging Across Several Brain Regions
- Operating in Concert



Mesoscope
Shown at -20° Rotation



2 Photon Random Access Mesoscope

About the Innovation

Researchers at HHMI Janelia Research Campus have designed and built a 2-photon random access mesoscope (2p-RAM) that provides rapid access for imaging anywhere within a large tissue volume. Cellular imaging can link activity in populations of neurons with behavior. But individual neurons are small, around 10µm diameter. Most microscopes with subcellular resolution have small fields-of-view and therefore cannot image neurons in multiple brain areas at the same time. Most large field-of-view microscopes do not resolve single cells, especially in the axial dimension.

The patent-pending 2p-RAM addresses these problems with technological advancements in remote focusing, correction of field curvature, and fast lateral and axial scanning. The complete 2p-RAM allows high-resolution imaging anywhere within a volume spanning multiple brain areas (Ø 5 mm x 1 mm cylinder) and resolution is near diffraction limited (axial, 0.66 µm; axial 4.09 µm at the center; excitation wavelength = 970 nm; numerical aperture = 0.6) over a large range of excitation wavelengths. A fast three-dimensional scanning system allows efficient sampling of neural activity in arbitrary regions of interest across the entire imaging volume.

The 2p-RAM was made public and training offered to attendees at a Janelia 2p-RAM workshop in August 2016, where researchers learned to make and use the first 13 instruments outside of Janelia. The instrument is now available for purchase at Thorlabs, Inc. (see link to right), and the plans are available via

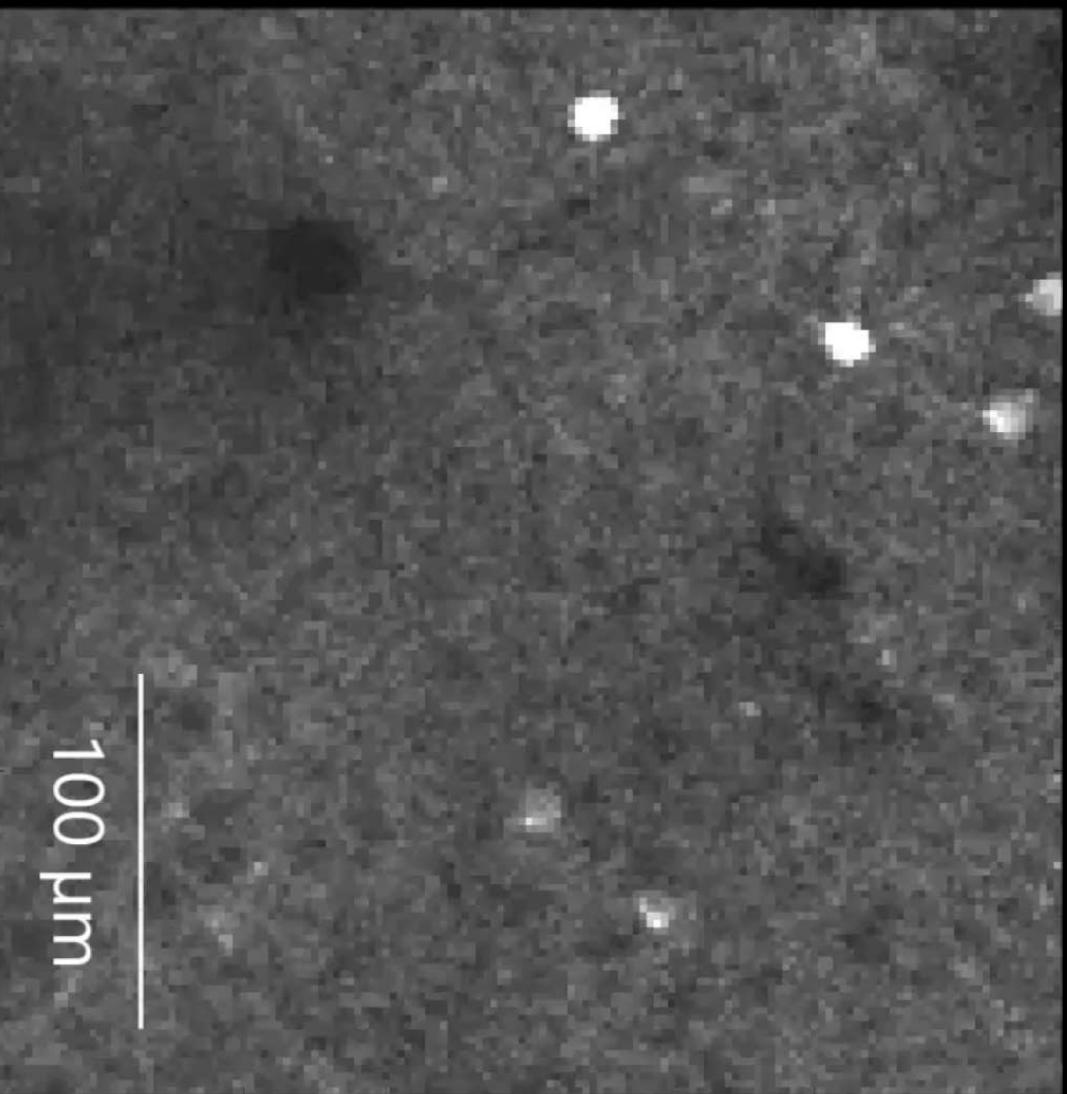


Related Items

Multiphoton Microscopes

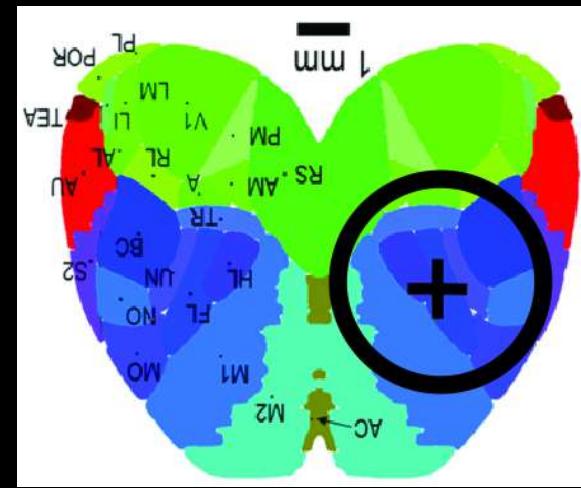


Tunable fs Ti:Sapphire Laser



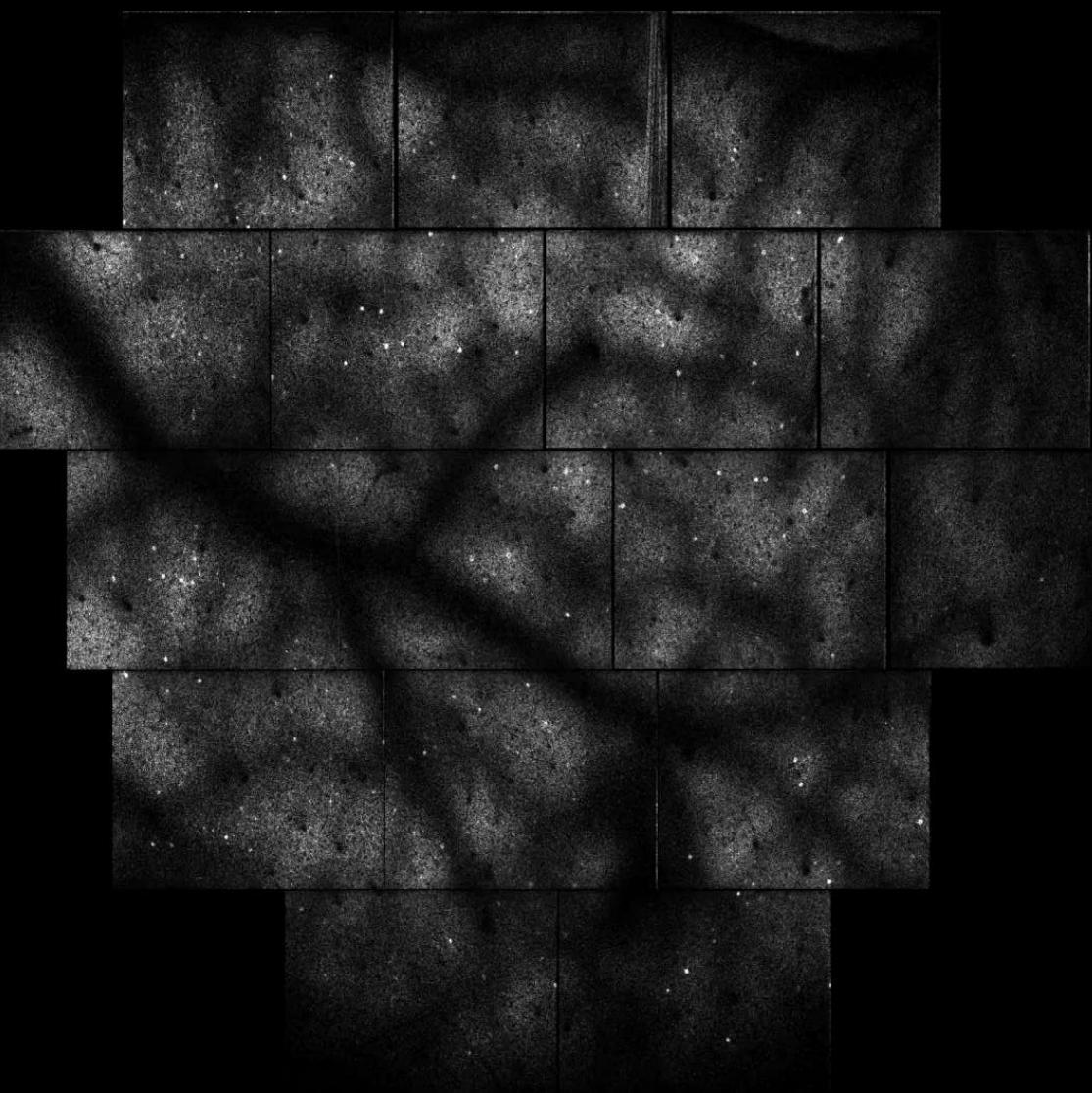
Reimer, Froudarakis, Tolias, Baylor College Medicine

Movie – registered and 10x speed



5mm window
Sensorimotor cortex

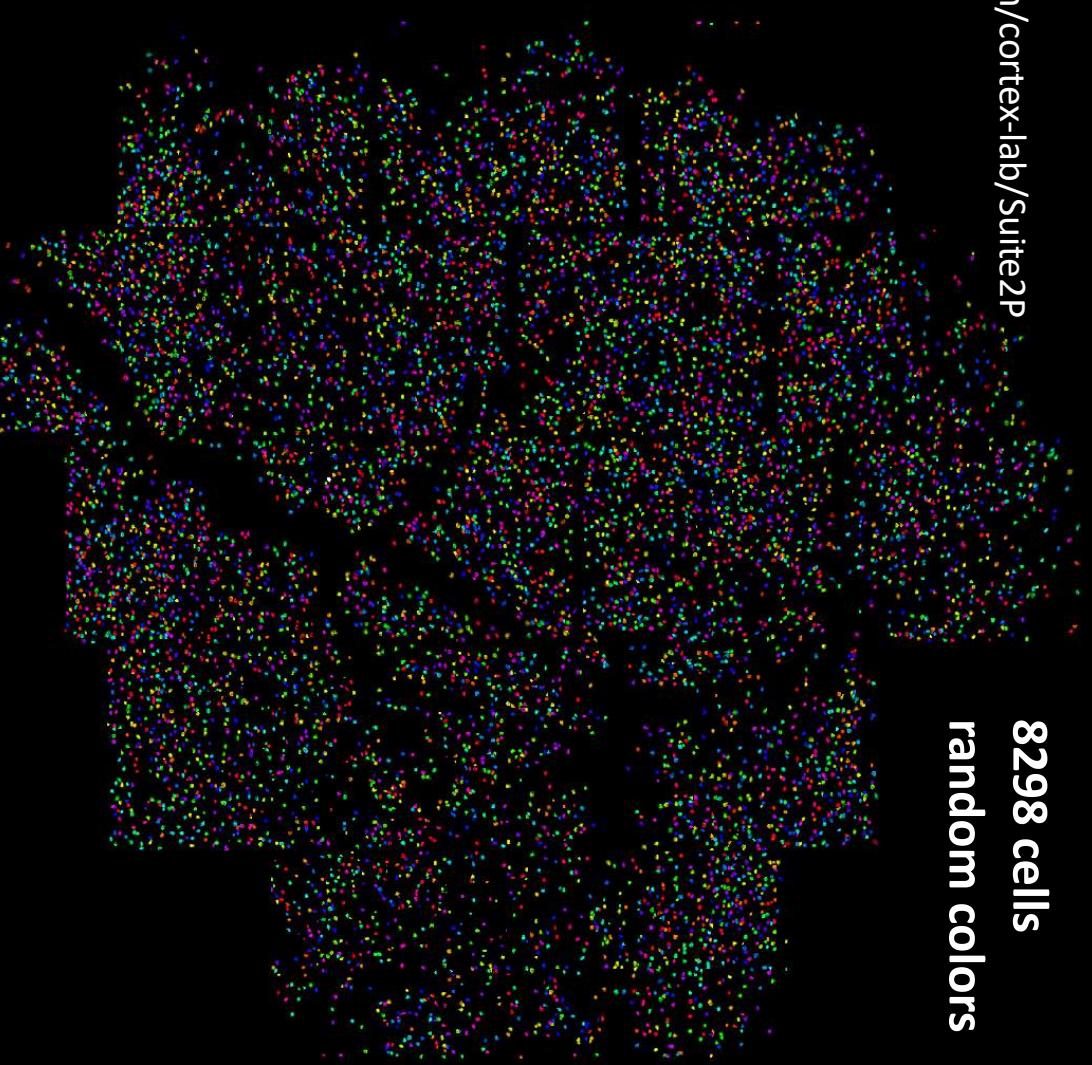
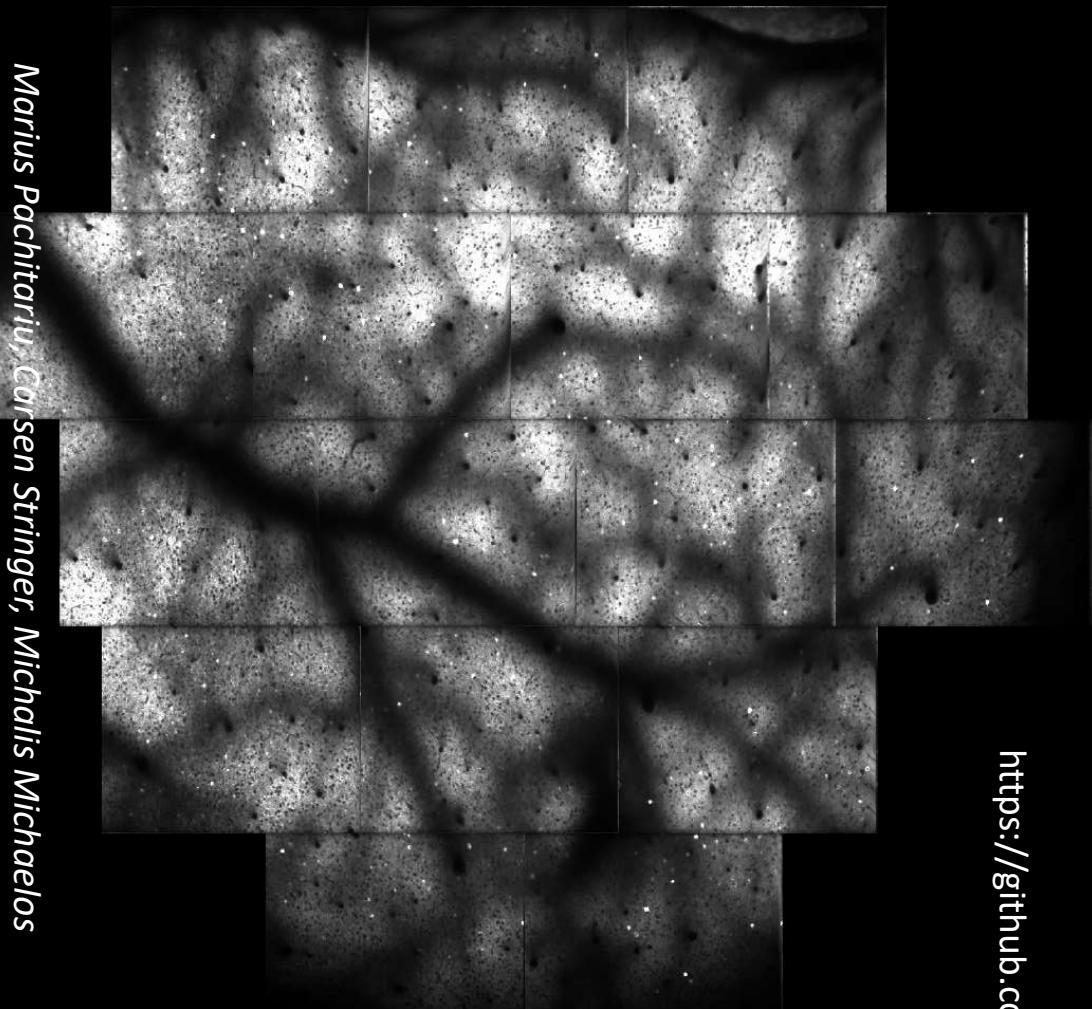
Marius Pachitariu, Carsen Stringer, Michalis Michaelos



Sensorimotor cortex

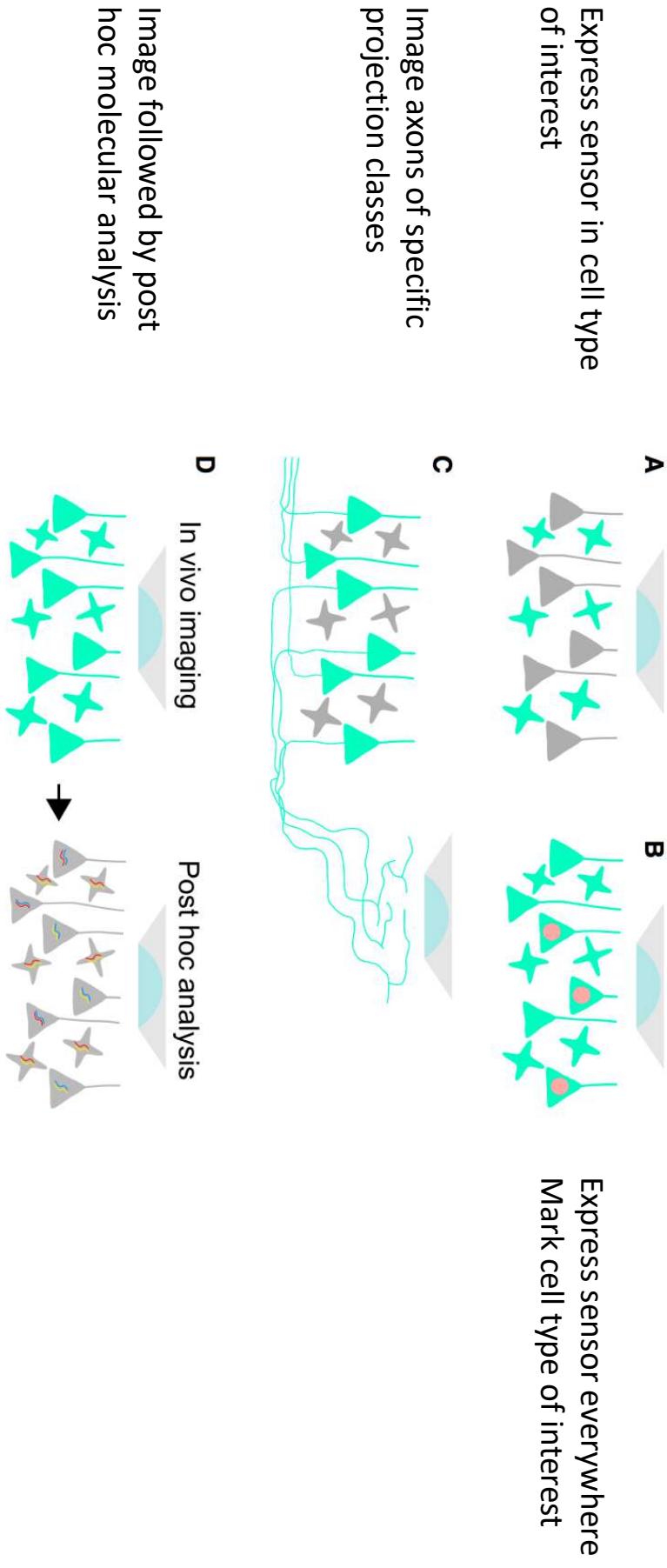
<https://github.com/cortex-lab/Suite2P>

8298 cells
random colors

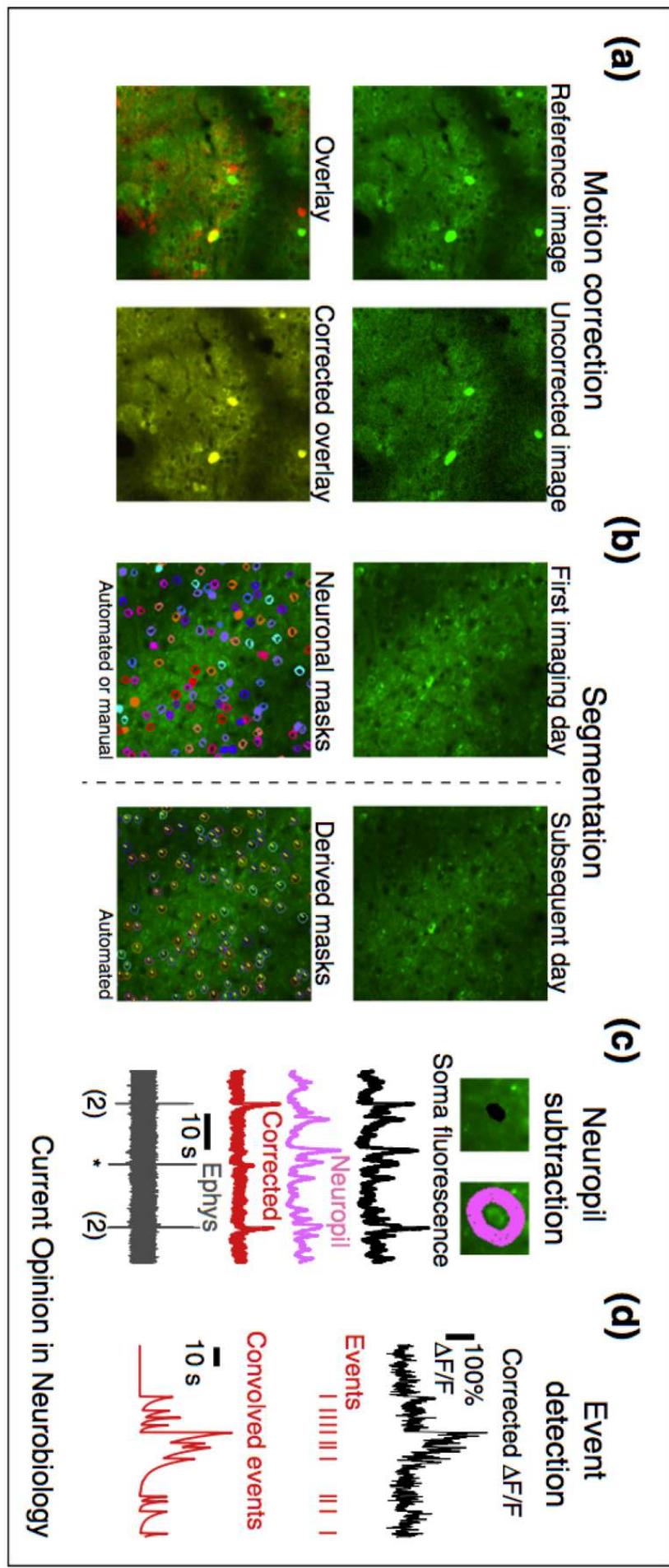


Marius Pachitariu, Carsen Stringer, Michalis Michaelos

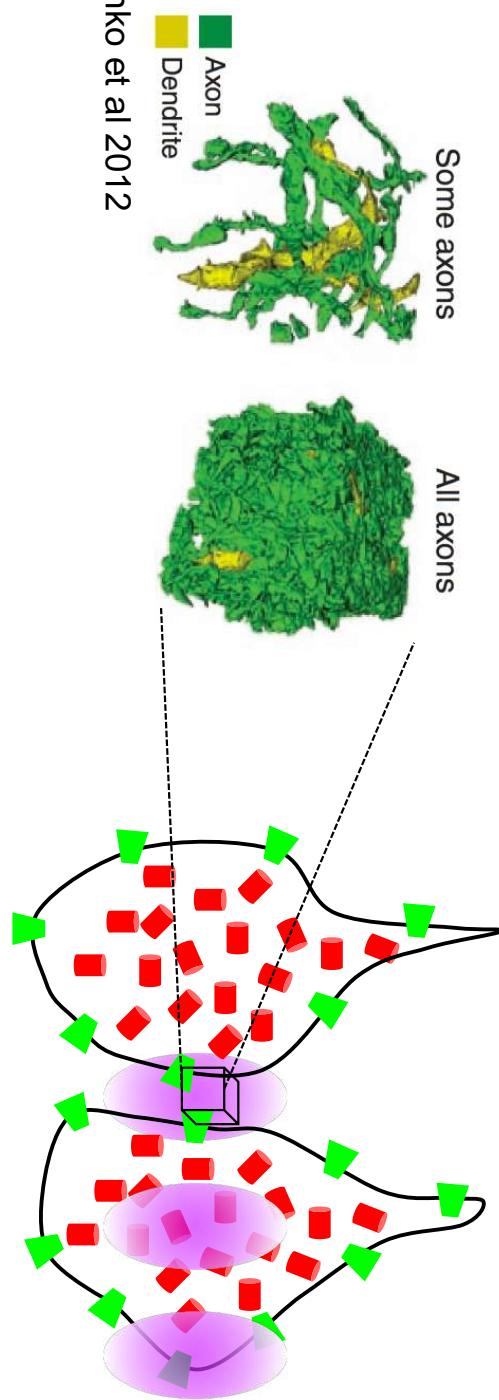
Cell type-specific imaging



'Cell / Spike sorting'



Origin of the neuropil signal



Mishchenko et al 2012

Probes in the

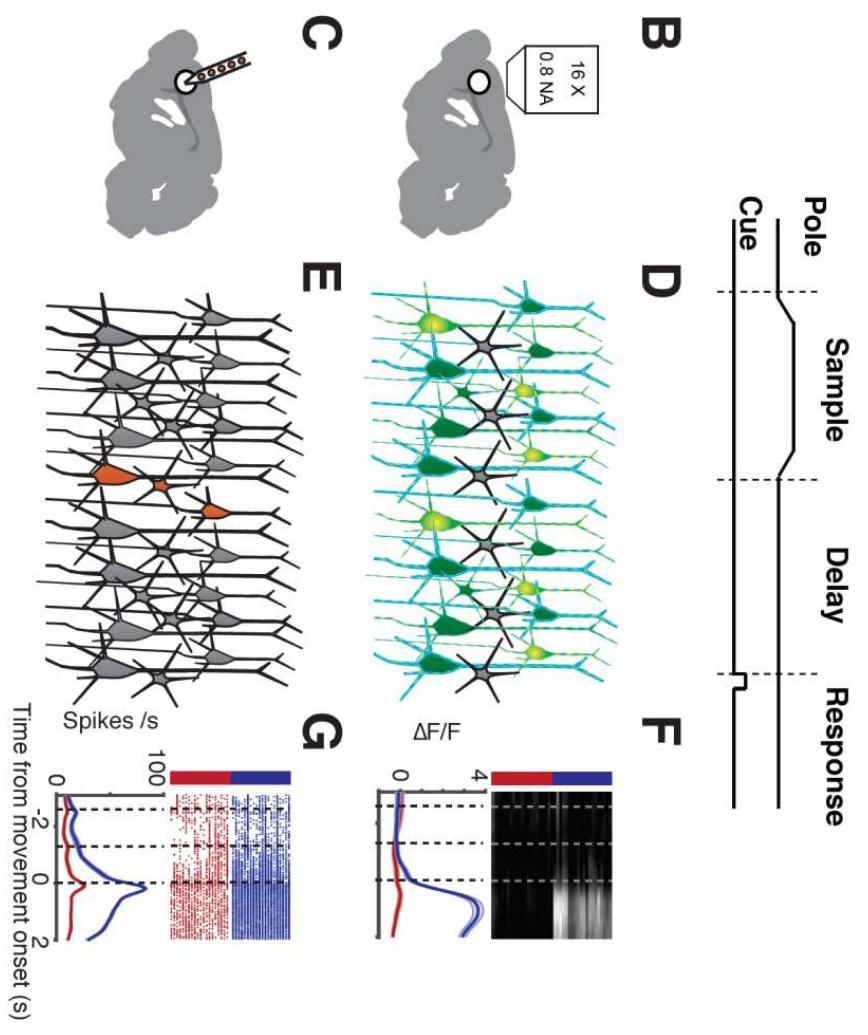
Cytoplasm

Membrane

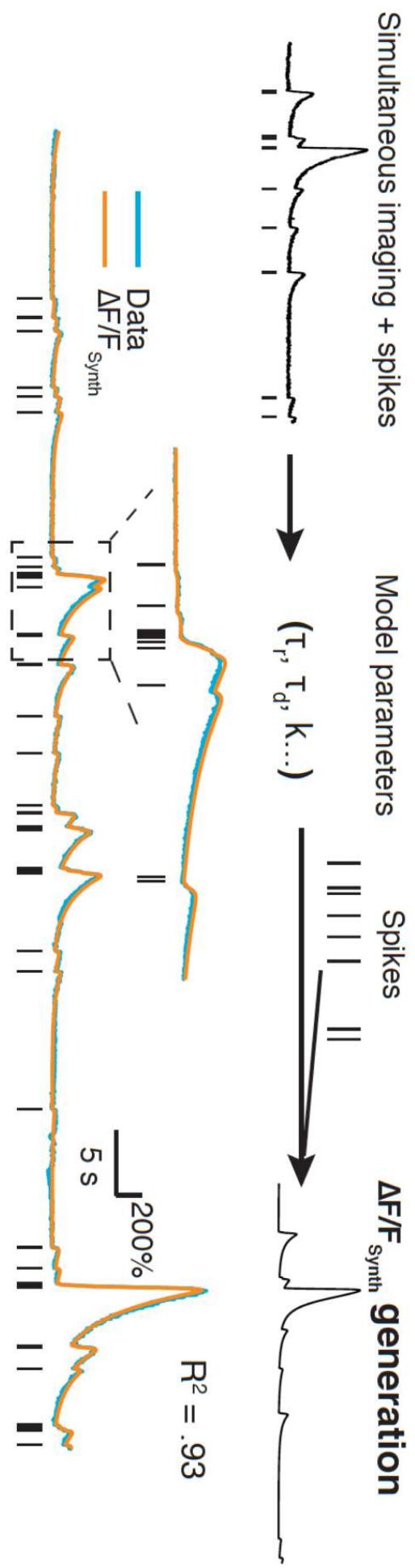
Shot-noise limited performance ($F \sim [\text{photon count}]$)

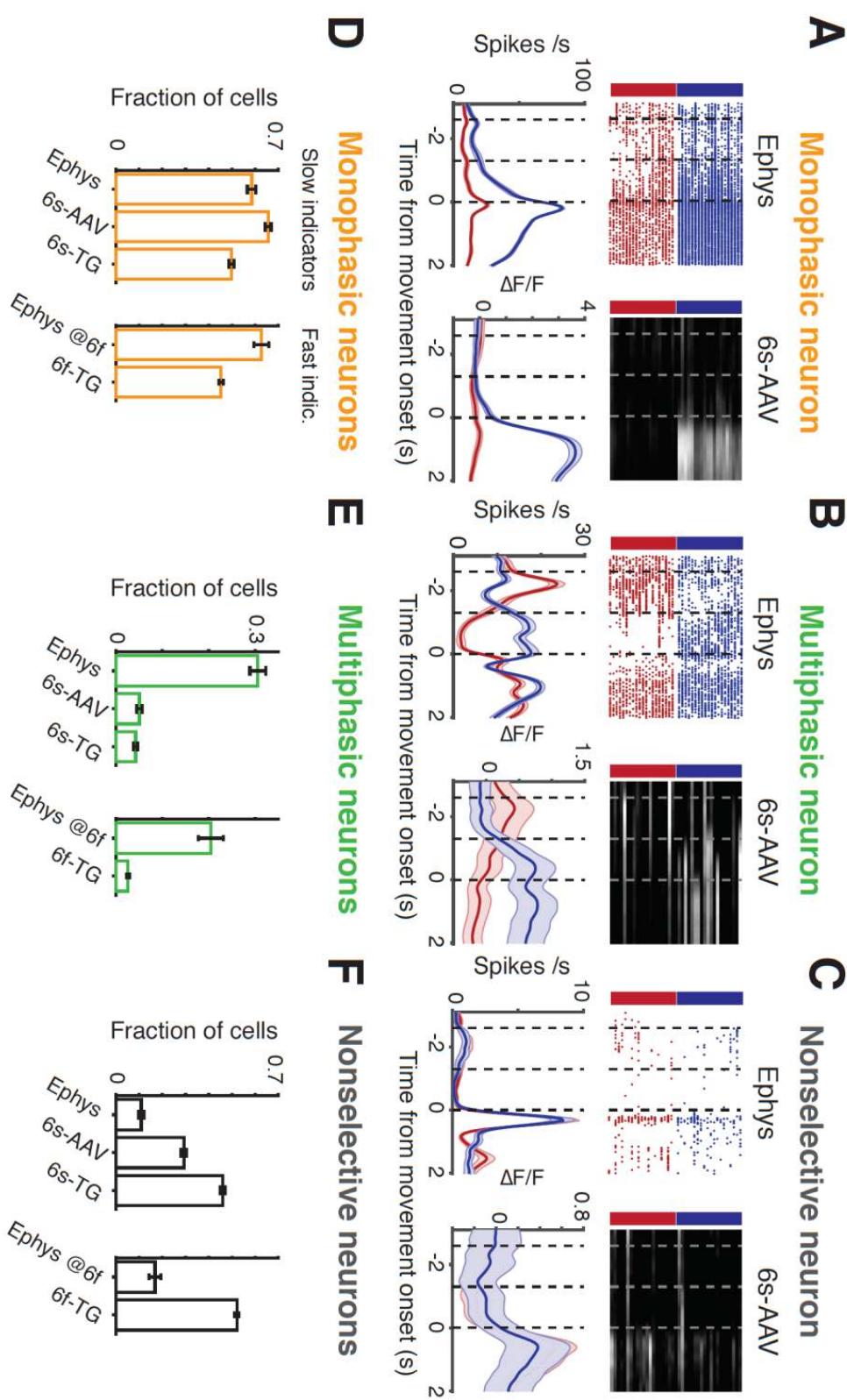
$$\text{SNR} = (F - F_0) / (F_0 + B)^{1/2}$$

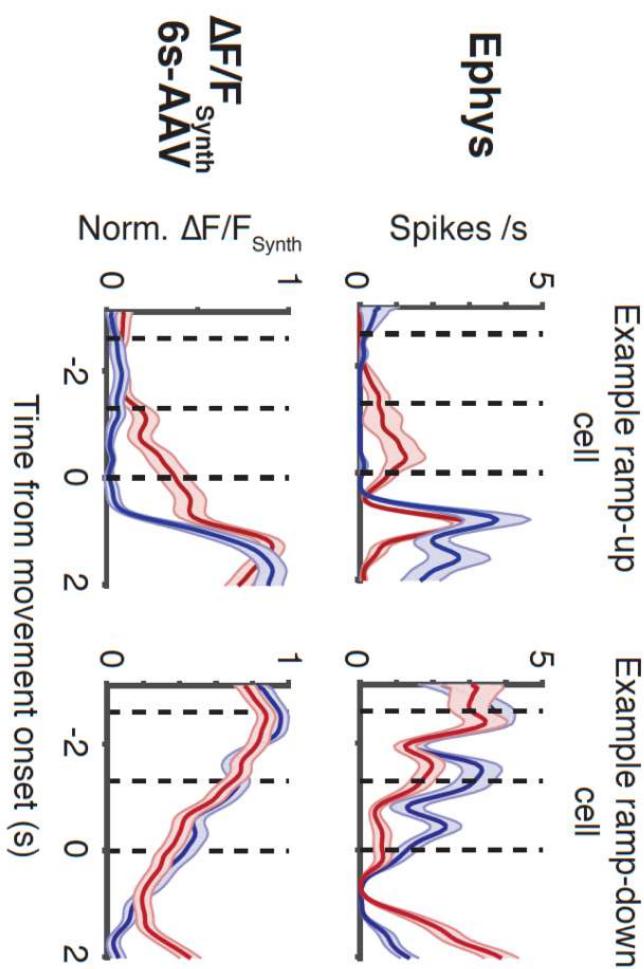
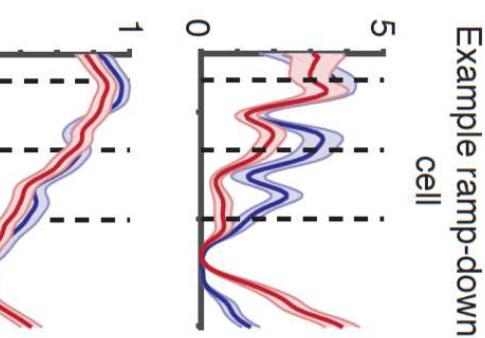
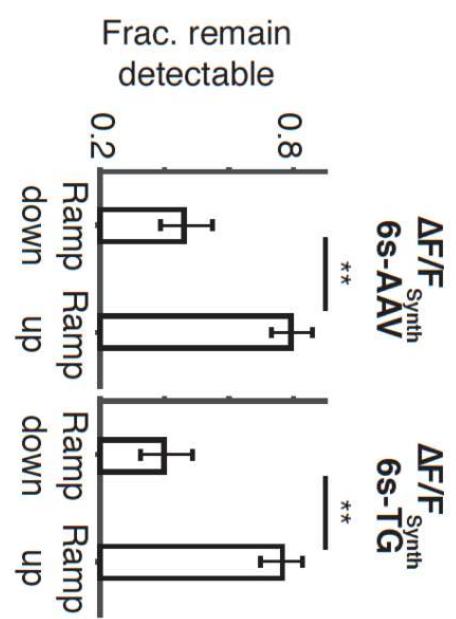
Compare imaging and ephys side-by-side

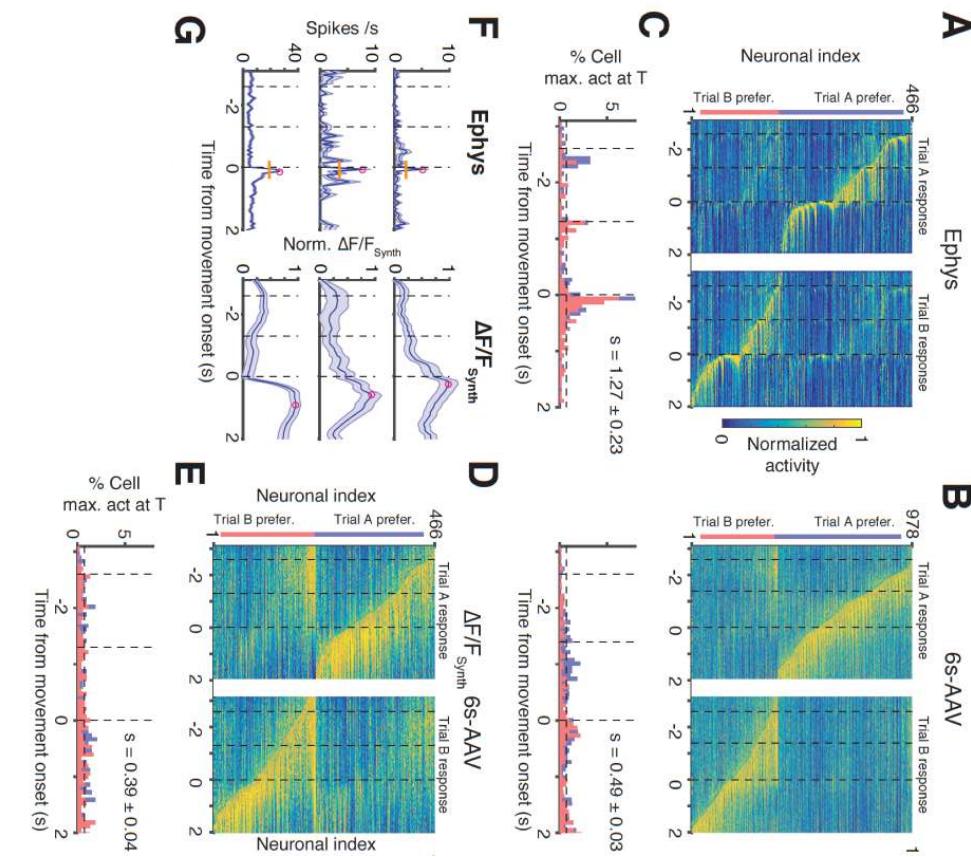


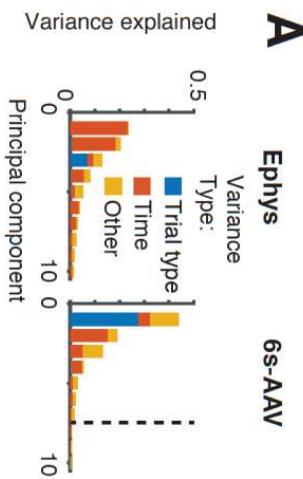
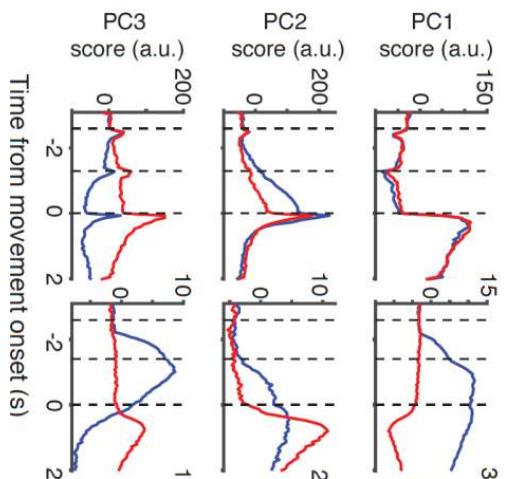
Forward (spike-to-synthetic-calcium) model





E**F****G**



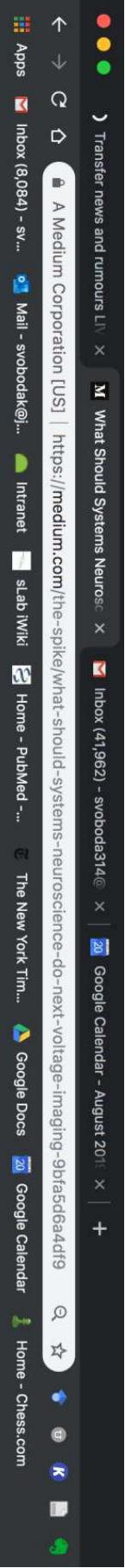
A**B**

Imaging produces a distorted version of dynamics and selectivity

- Low pass filtering
- Nonlinear and asymmetric transformation
- The coupling between spikes and F varies across individual cells of the same type and is not obviously invertible
- (Not shown – coupling between spikes and F differs across cell types)

	Extracellular ephys	Ca imaging
Yield	100's of neurons	✓
Duration	Days to weeks	✓
Signal-to-noise ratio	High	✓
Time resolution	10 microseconds	✓
Single unit isolation	Depends on SNR	✗
Spatial reach	Entire brain	✓
Access to cell types	Limited	✗
Cell type biases	High	✗
Dense sampling	No	✗
Detect rare cell types	Limited	✗
Longitudinal measurem.	Limited	✗
Invasive?	Yes	✗
Spatial localization	100 um	✗

- Measurements in general
- Extracellular electrophysiology
- Calcium imaging and analysis
- **Voltage imaging**



M | The Spike

What Should Systems Neuroscience Do Next? Voltage Imaging

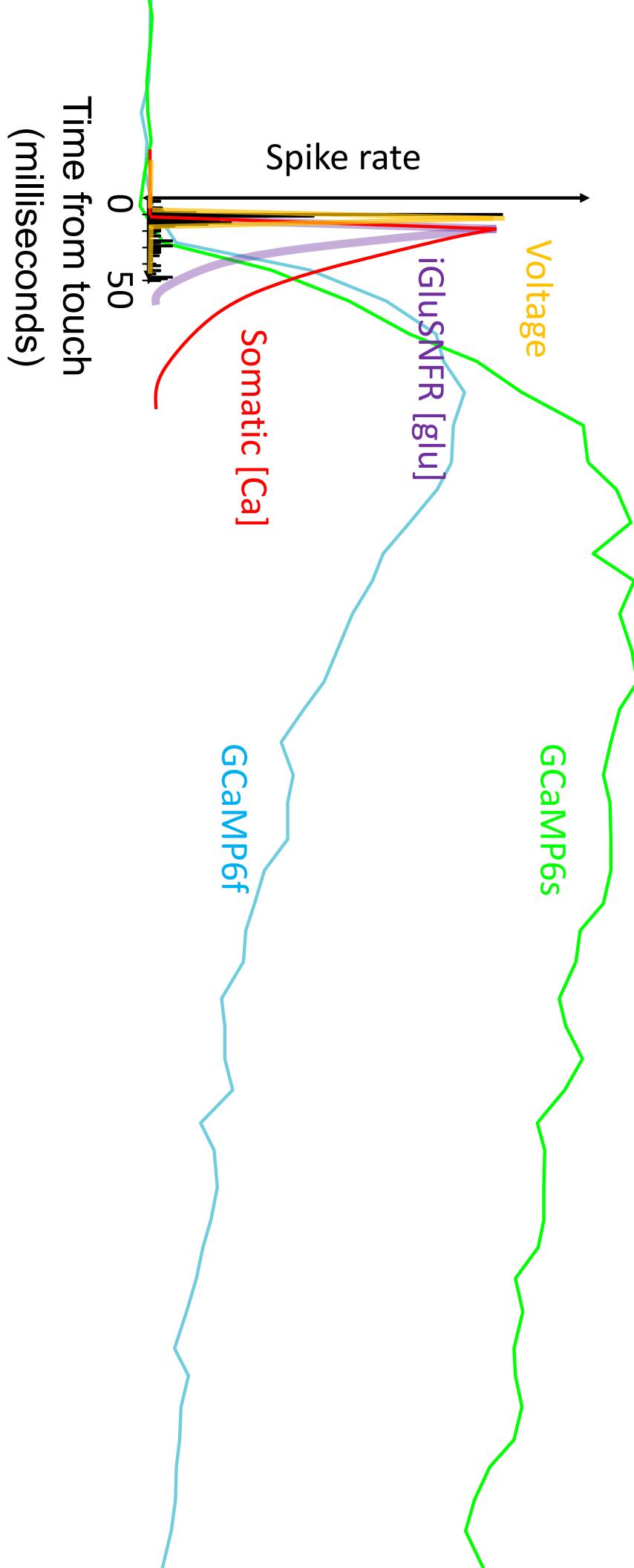
The firing and the wiring at the same time



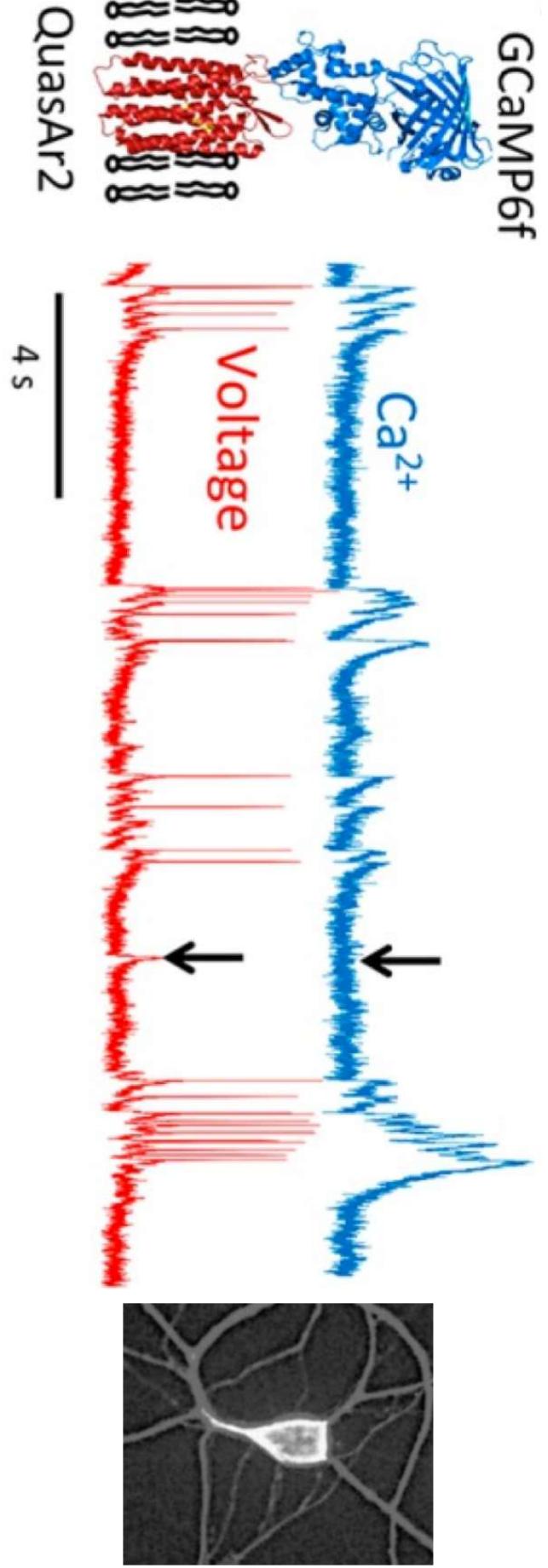
Mark Humphries
[Follow](#)
Jun 3 · 10 min read ★



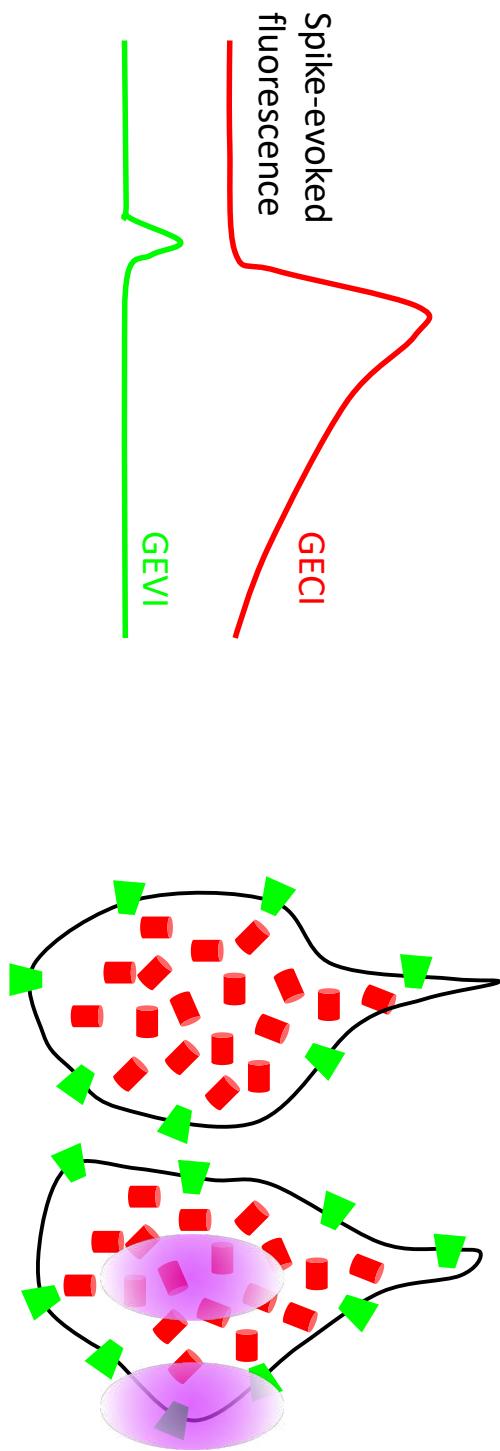
*Protein voltage indicators
(genetically encoded voltage indicators, GEViS):
millisecond time scale imaging*



GEVis: millisecond time scale imaging

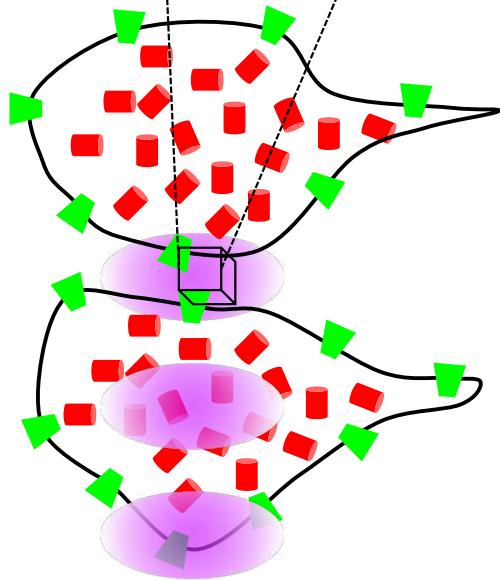
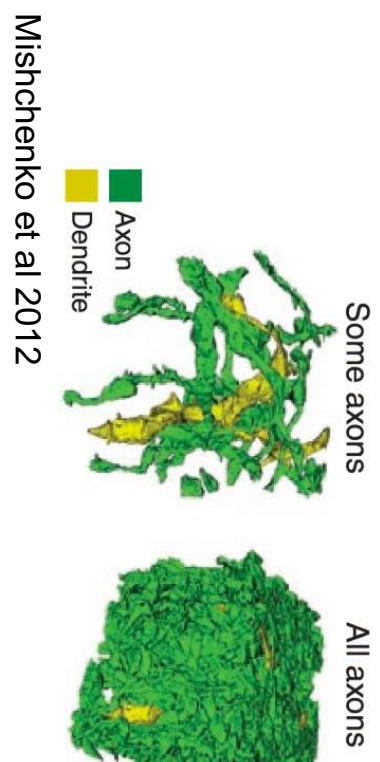


Challenges for imaging GEVIs in vivo



	GEVI - voltage	GECI - calcium	x10 ⁵
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**



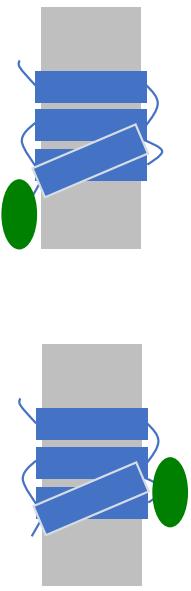
	GEVI - voltage	GECI - calcium	x10 ⁵
Fluorescence change per action potential	5 %	40 %	x10
Time per AP	4 ms	400 ms	x100
Sensor distribution	Membrane	Cytosol	
Molecules per resolution element	100	10,000	x100

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

**ArcLight,
MARINA**

ASAP

cIVSP-based

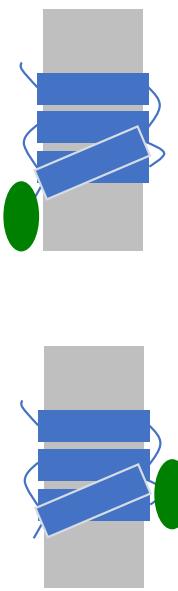


*Protein voltage indicators
(genetically encoded voltage indicators, GEVis)*

**ArcLight,
MARINA**

ASAP

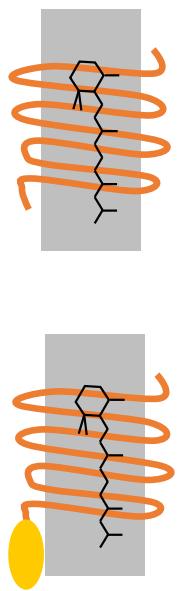
cIVSP-based



Arch

**Arch-FP,
MacQ-FP,
Ace2N-FP**

Voltron

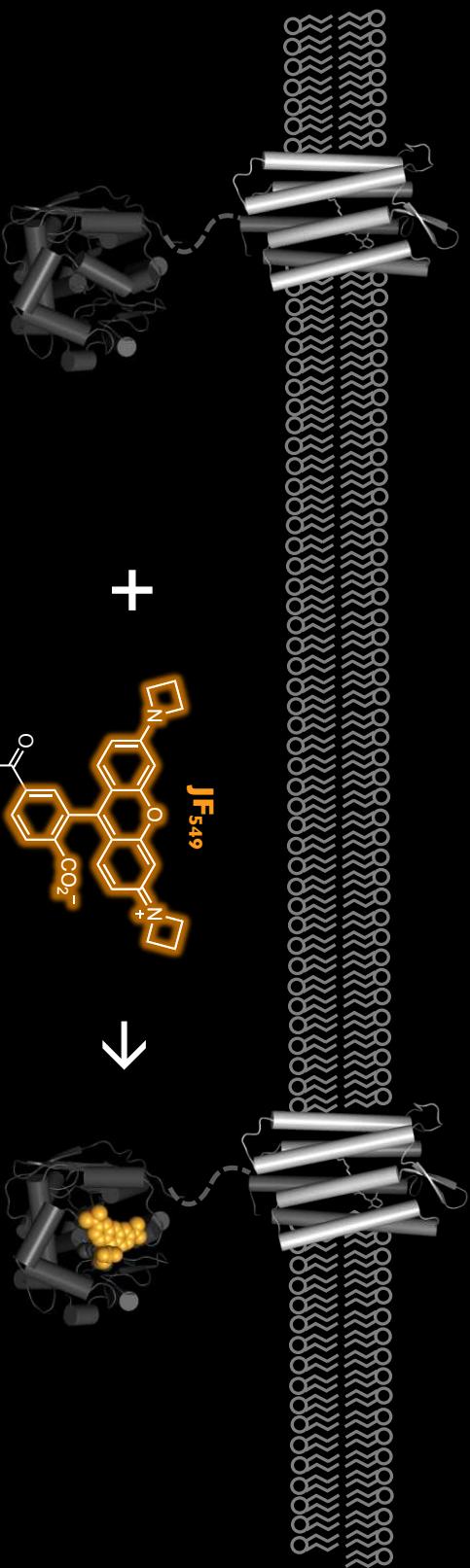


HaloTag

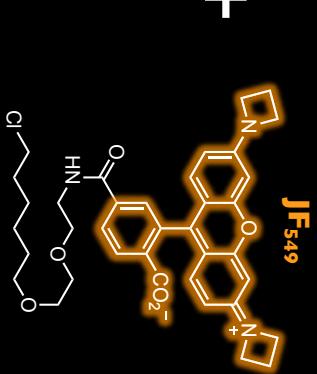
Rhodopsin-based

Voltron – a chemigenetic voltage sensor

rhodopsin



HaloTag



Ahmed Abdelfattah



Eric Schreiter

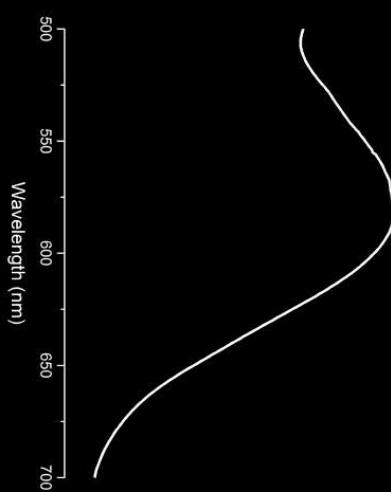
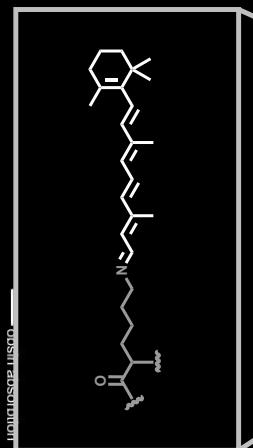
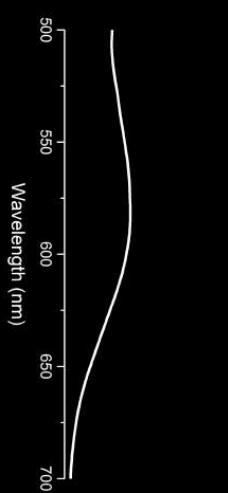
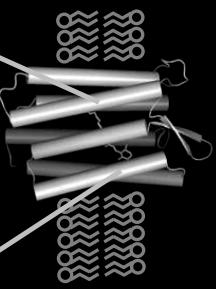


Luke Lavis

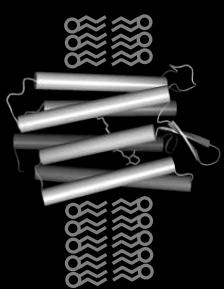


Biorxiv 436840

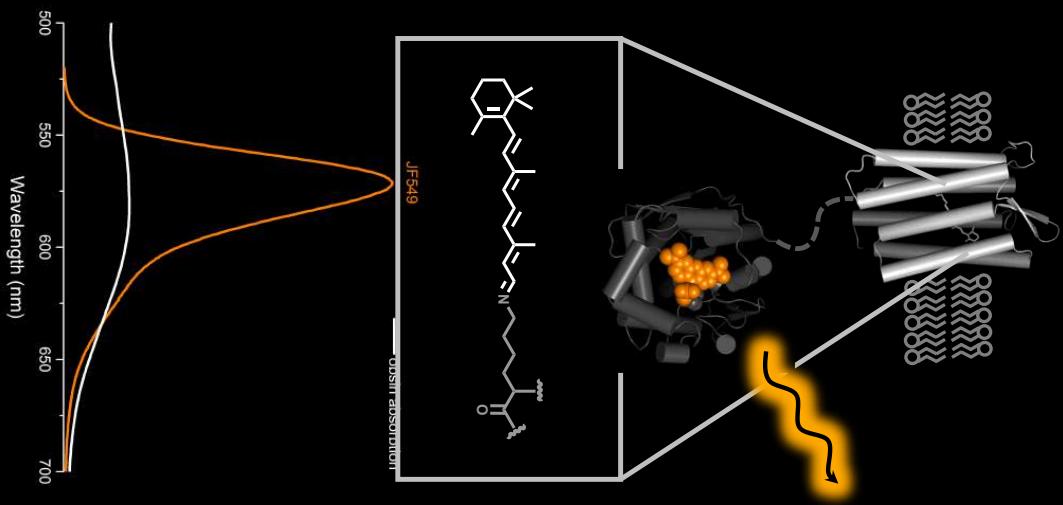
hyperpolarized



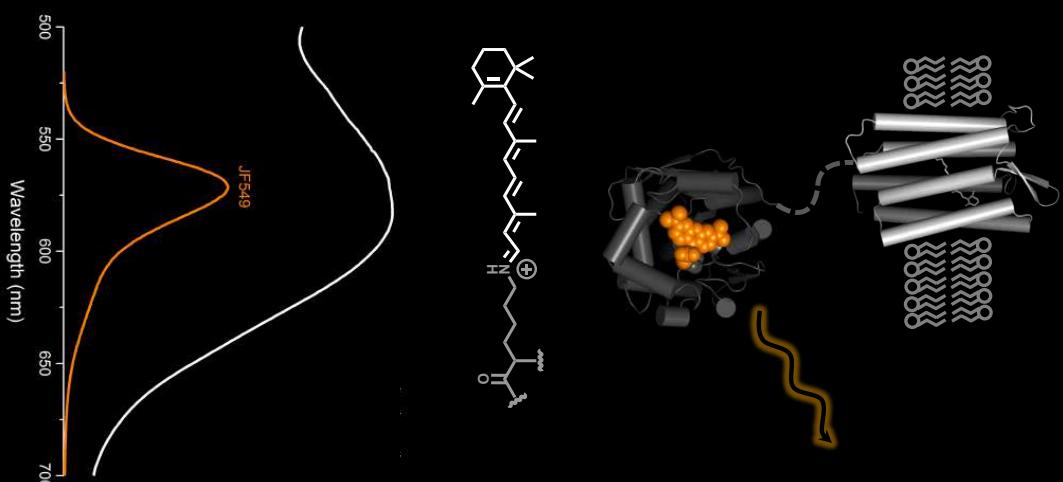
depolarized



hyperpolarized

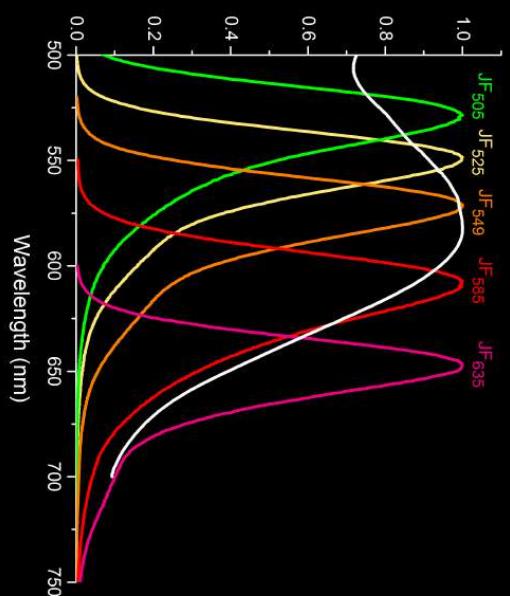


depolarized

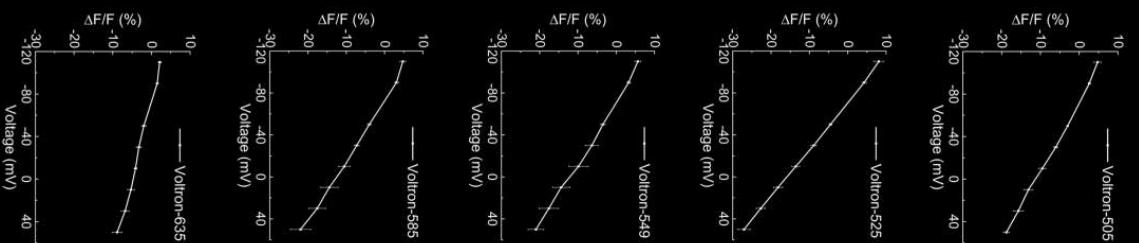
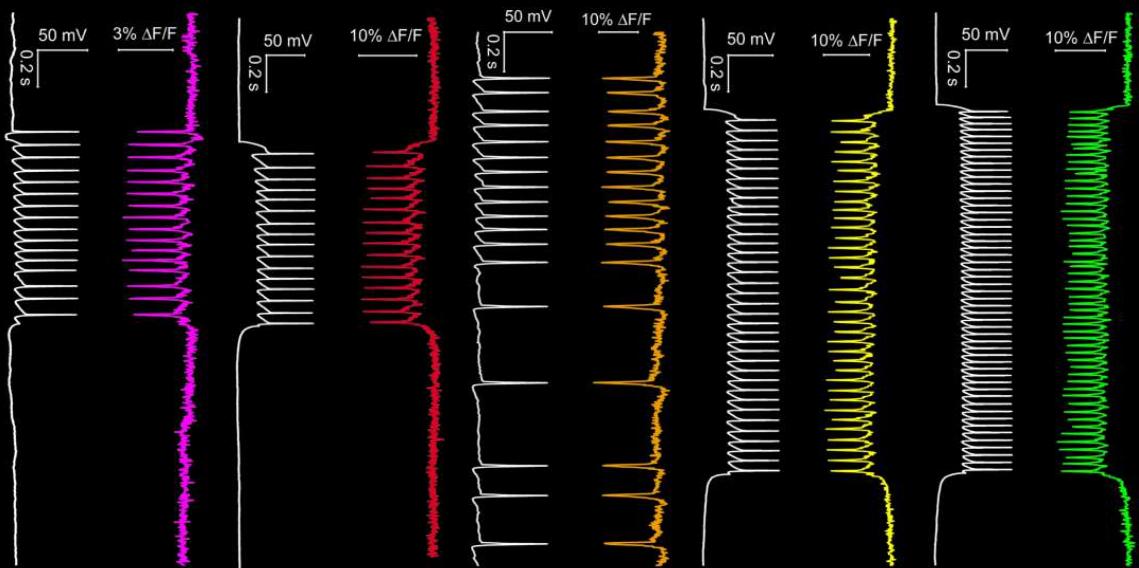
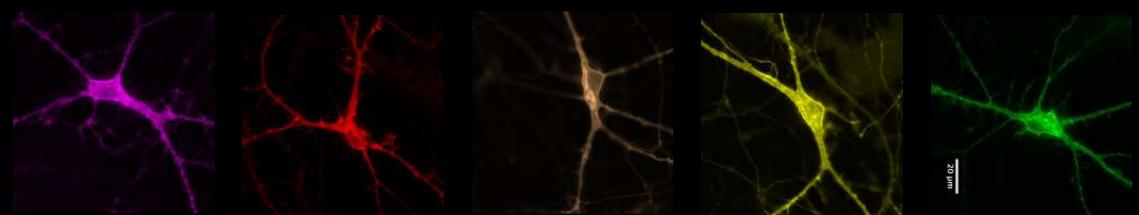


HaloTag

Ac₂N



Biorxiv 436840



Mouse cortex, NDNE-Cre
AAV-syn-FLEX-Voltron-ST;JF₅₂₅
Widefield fluorescence microscopy

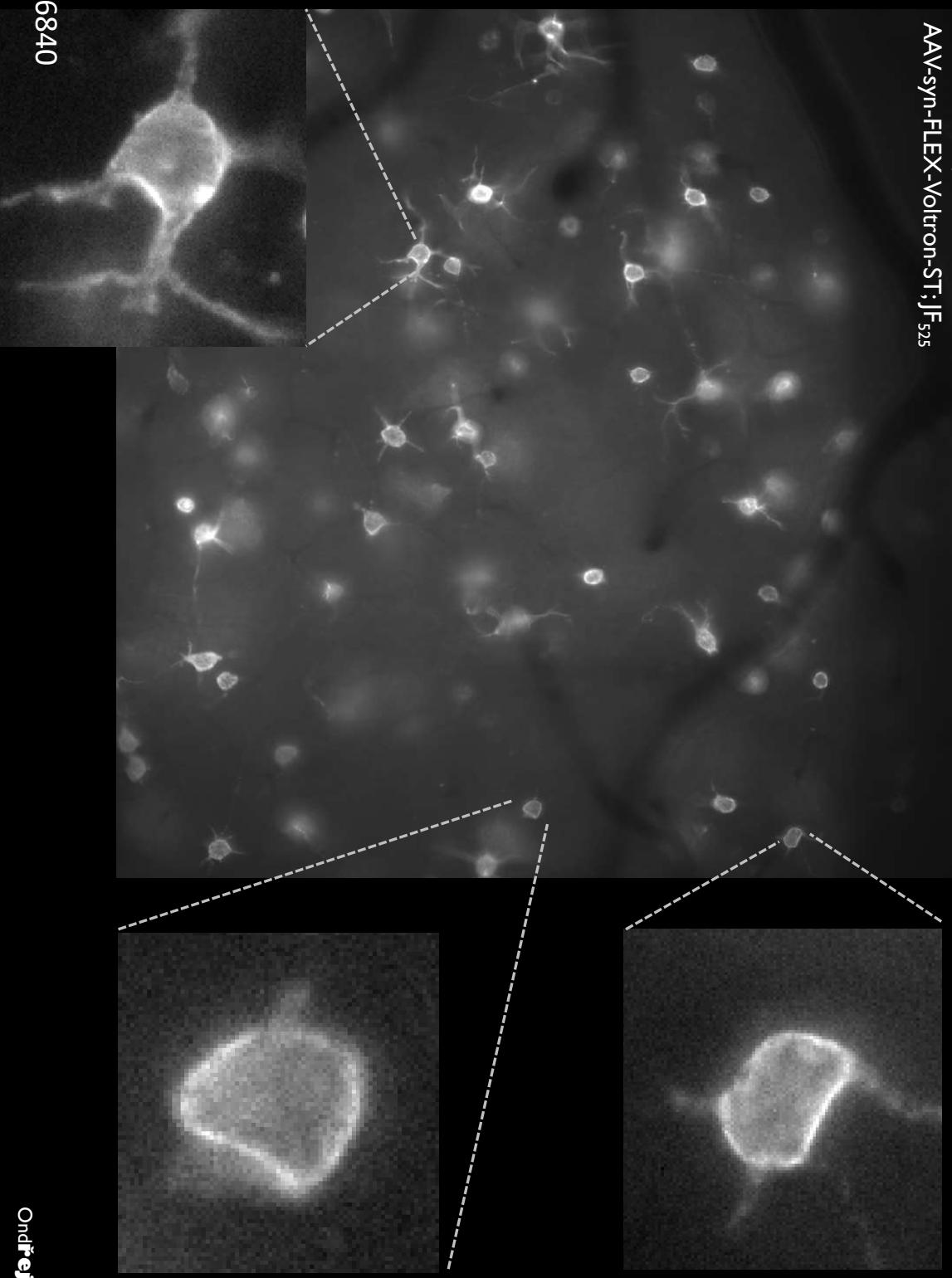


Biorxiv 436840

Ondřej Novák, Amrita Singh



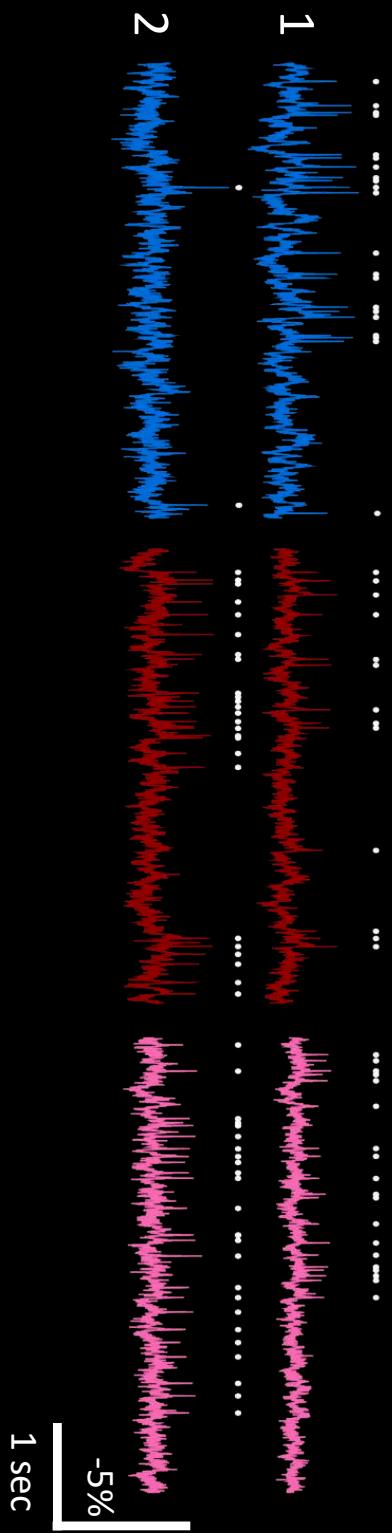
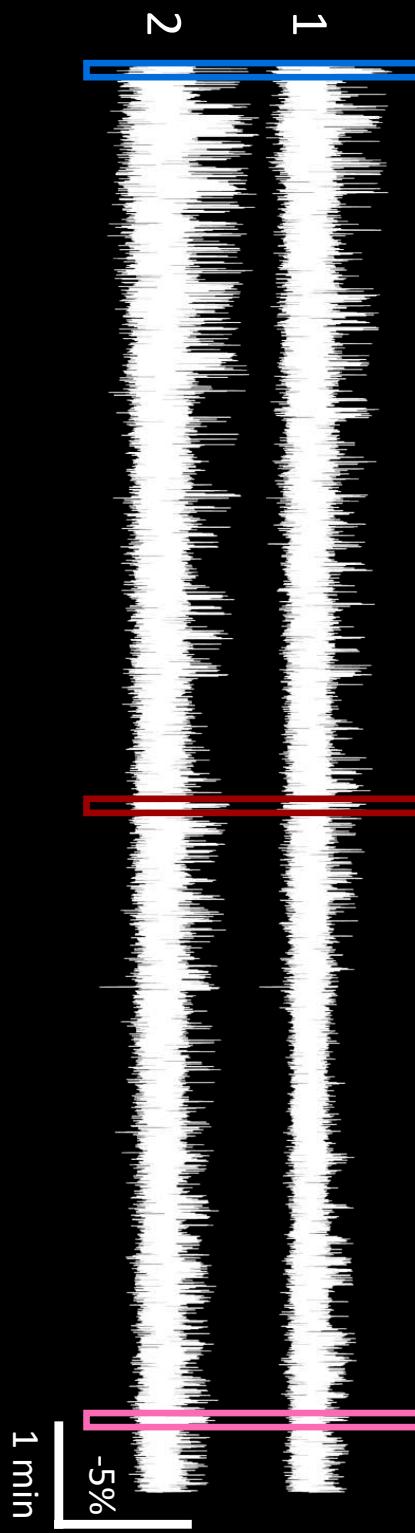
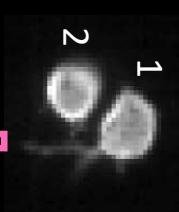
Mouse cortex, NDNF-Cre
AAV-syn-FLEX-Voltron-ST;JF₅₂₅

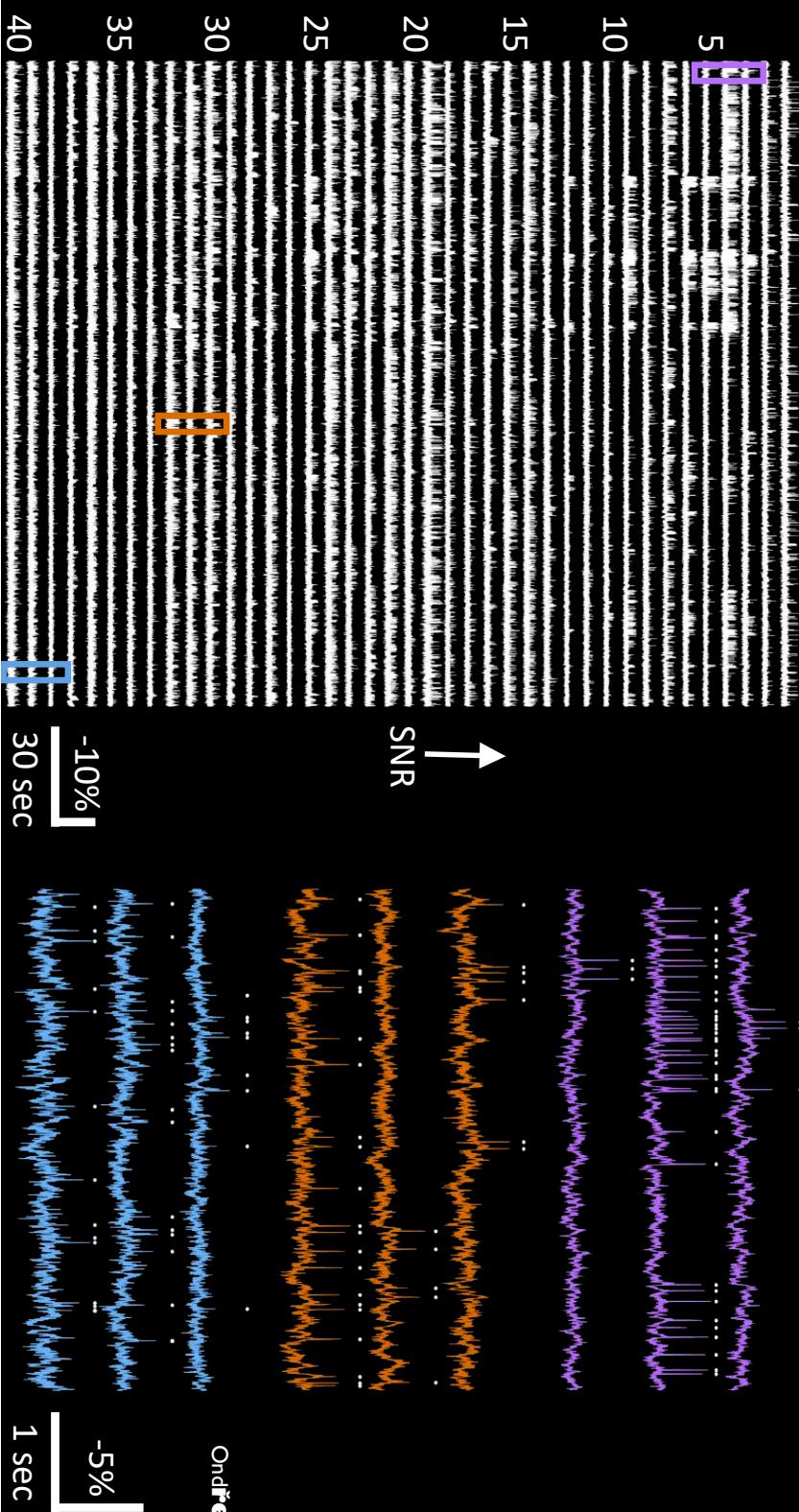
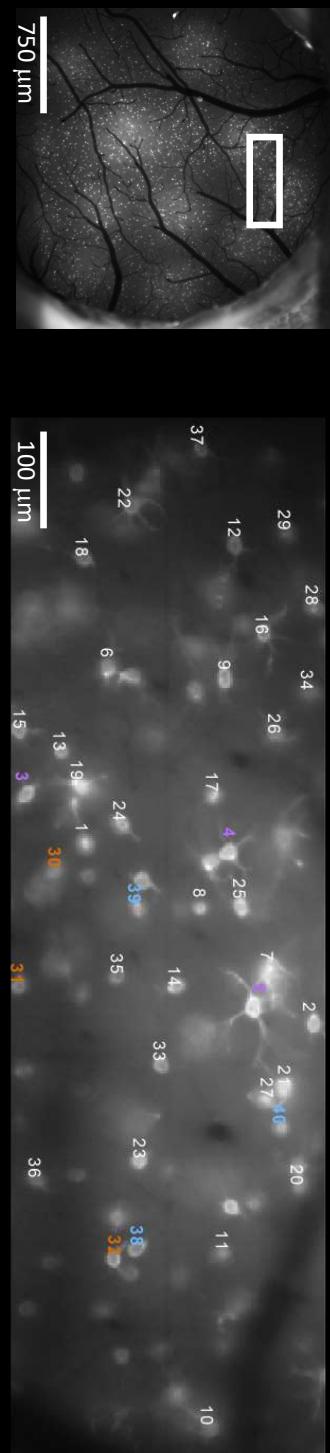


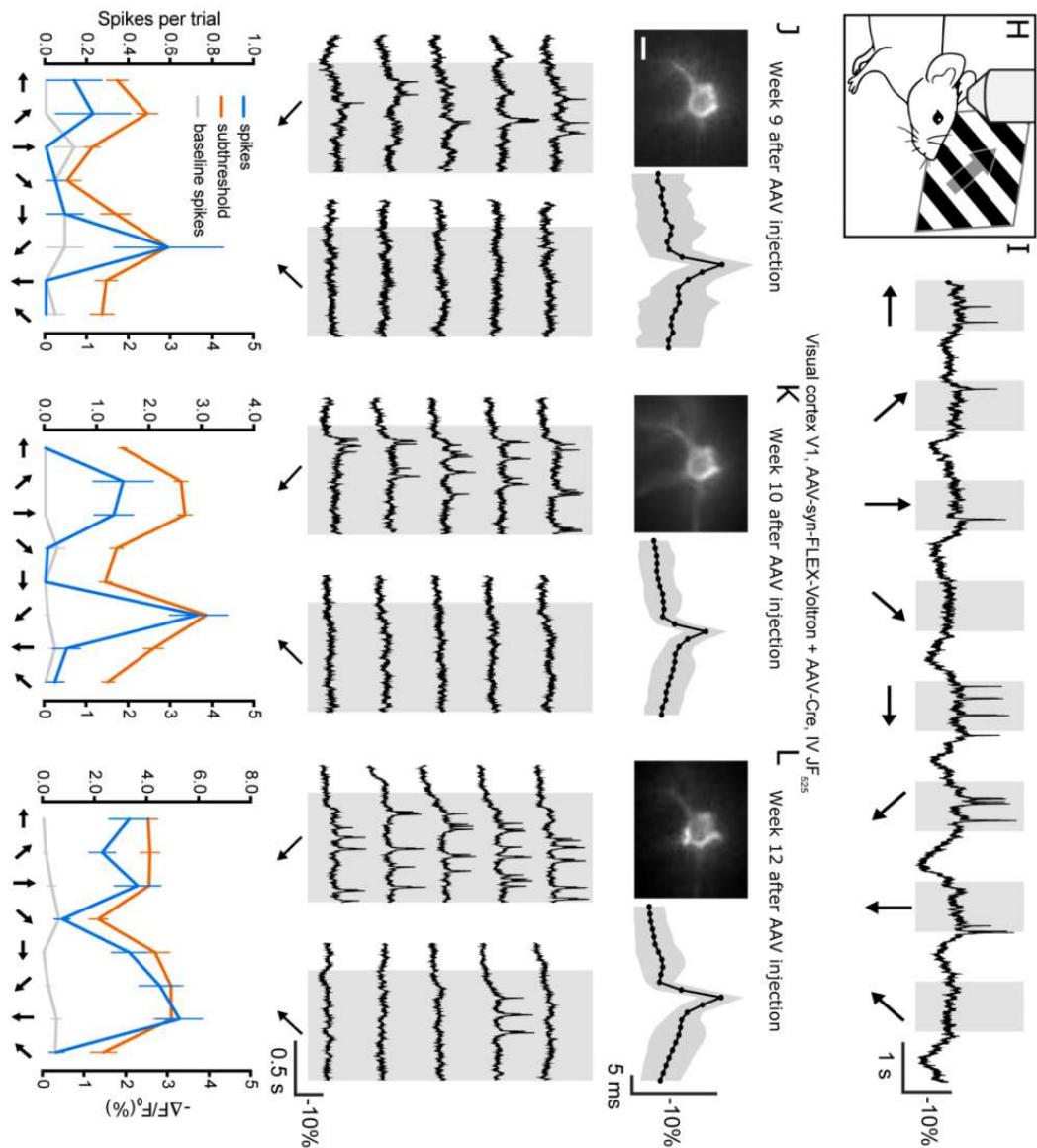
Biorxiv 436840

Ondřej Novák, Amrita Singh

Mouse cortex, NDNF-C^{re}
AAV-syn-FLEX-Voltron-ST;JF₅₂₅







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

