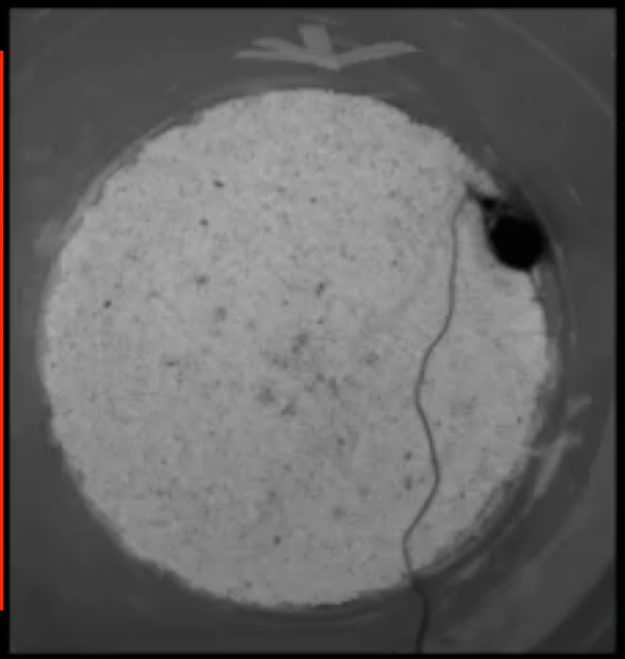
"Imaging large-scale ensemble neural codes underlying learning and long-term memory"

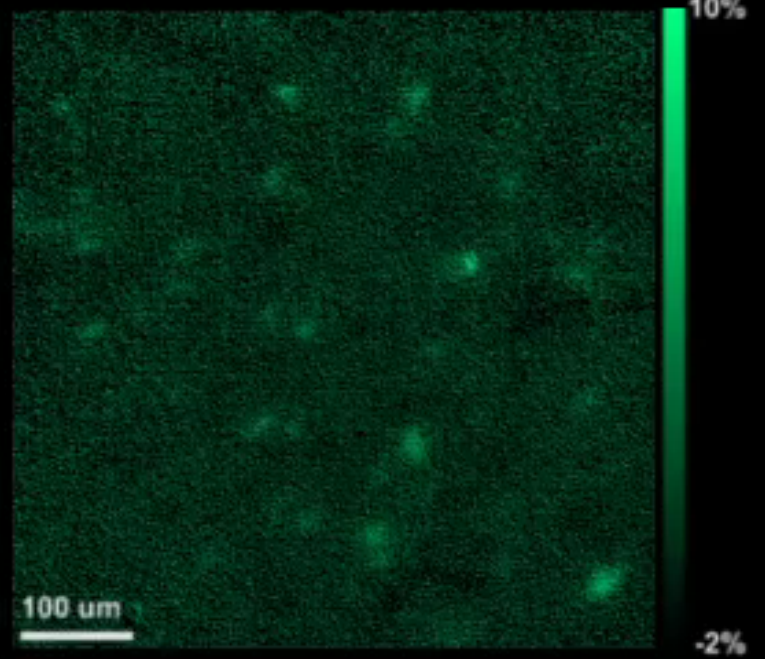
Brain Forum, presented by

Mark J. Schnitzer, HHMI / Stanford Univ.

Portable microscopy for mice

Imaging CA1, amygdala cell dynamics





What are biological substrates of long-term memories?

1. **Synaptic substrates**
   - *E.g.* Long-term changes in synaptic weight
2. **Cellular substrates**
   - *E.g.* Long-term changes in firing properties
3. **Network level substrates**
   - *Remains poorly explored due to lack of tools*

Need a combination of methods to monitor long-term network dynamics

1) Imaging of neurons lying deep in brain tissue

+

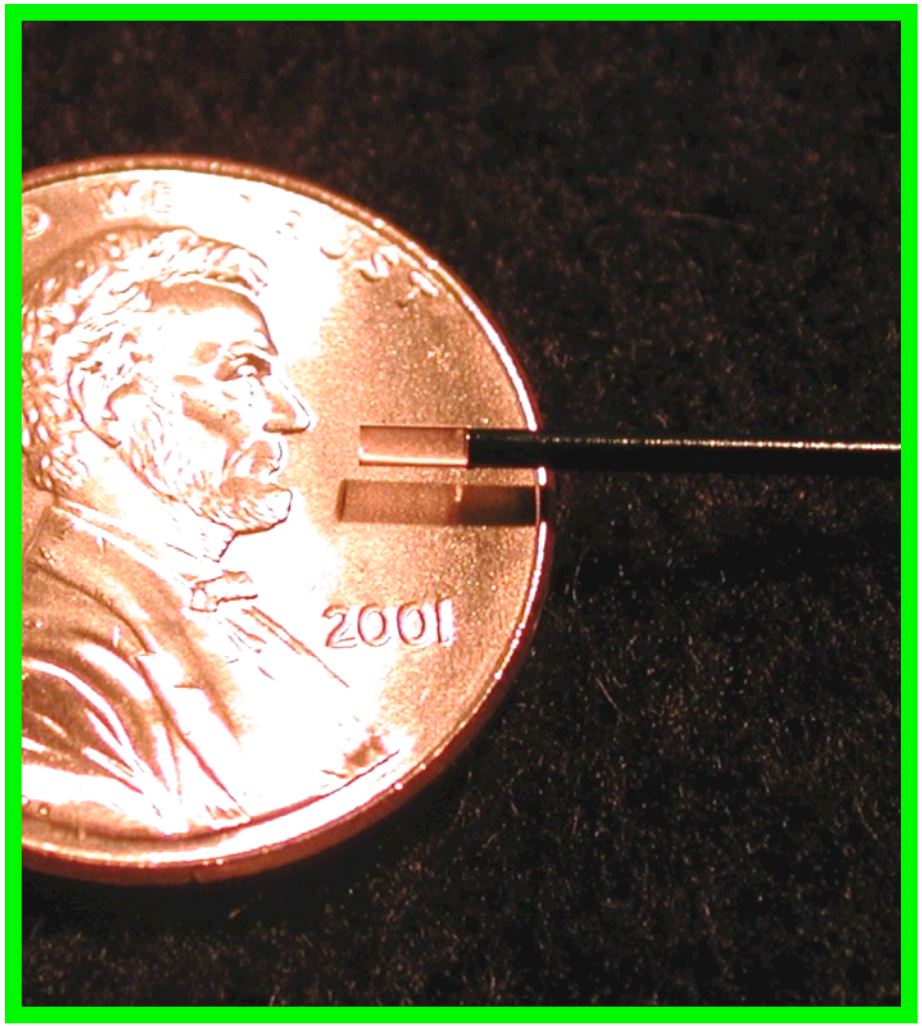
2) Time-lapse tracking of cells over multiple days

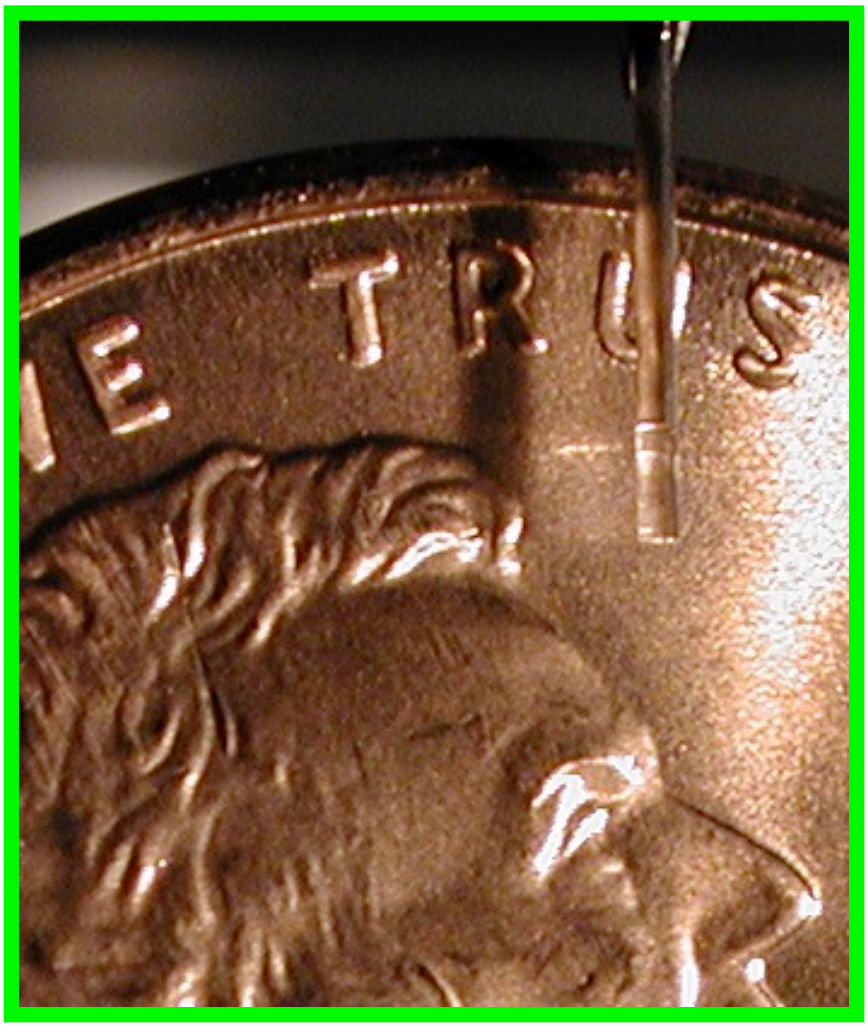
+

3) Ensemble neural Ca^{2+} imaging in freely behaving mice

= Long-term tracking of neural codes and dynamics

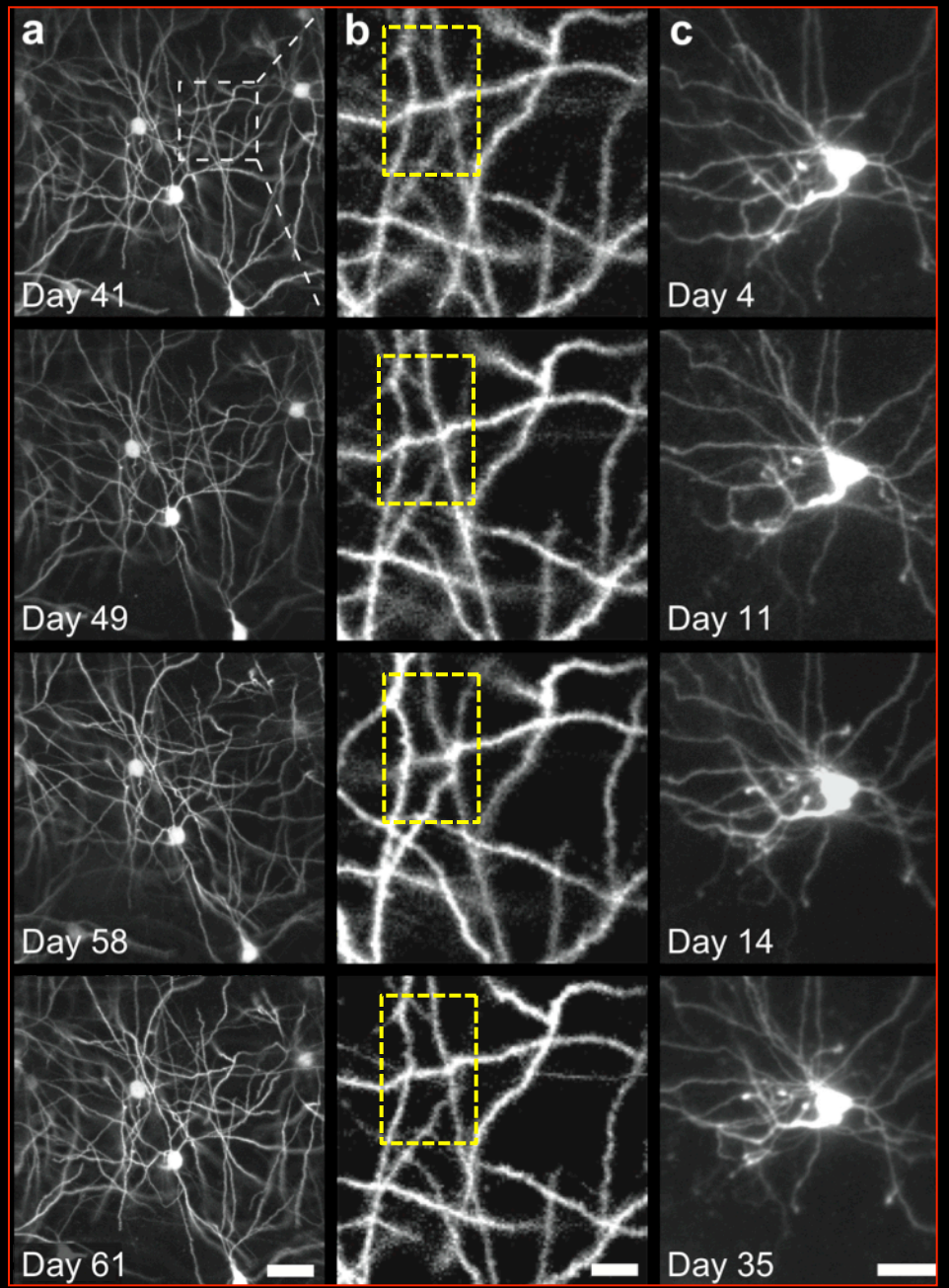
1) Implantable microendoscopes for imaging deep tissues at ~1 μm resolution



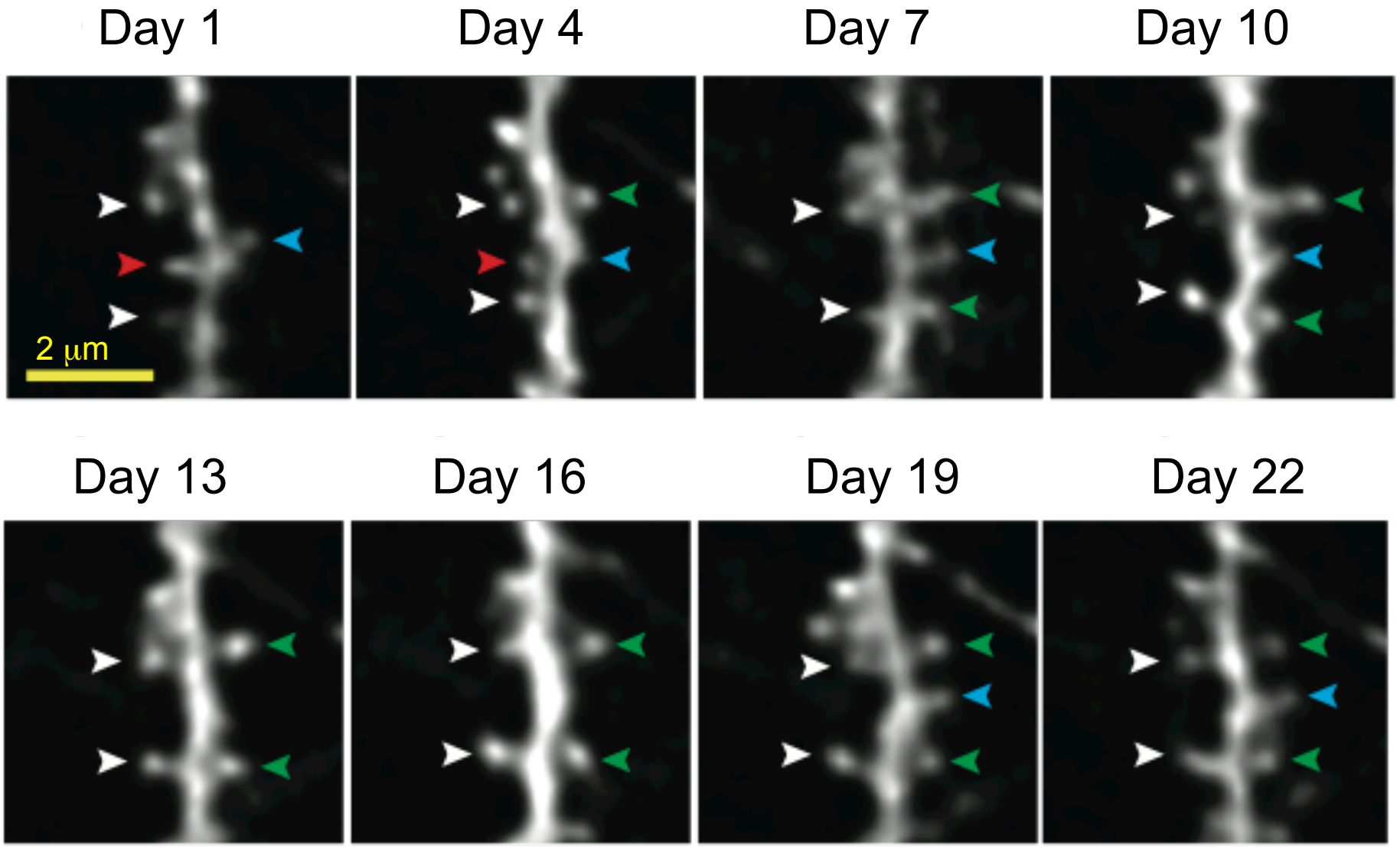


Jung & Schnitzer, Optics Lett. 2003; Jung et al., J. Neurophysiol., 2004

Time-lapse imaging deep in the live brain

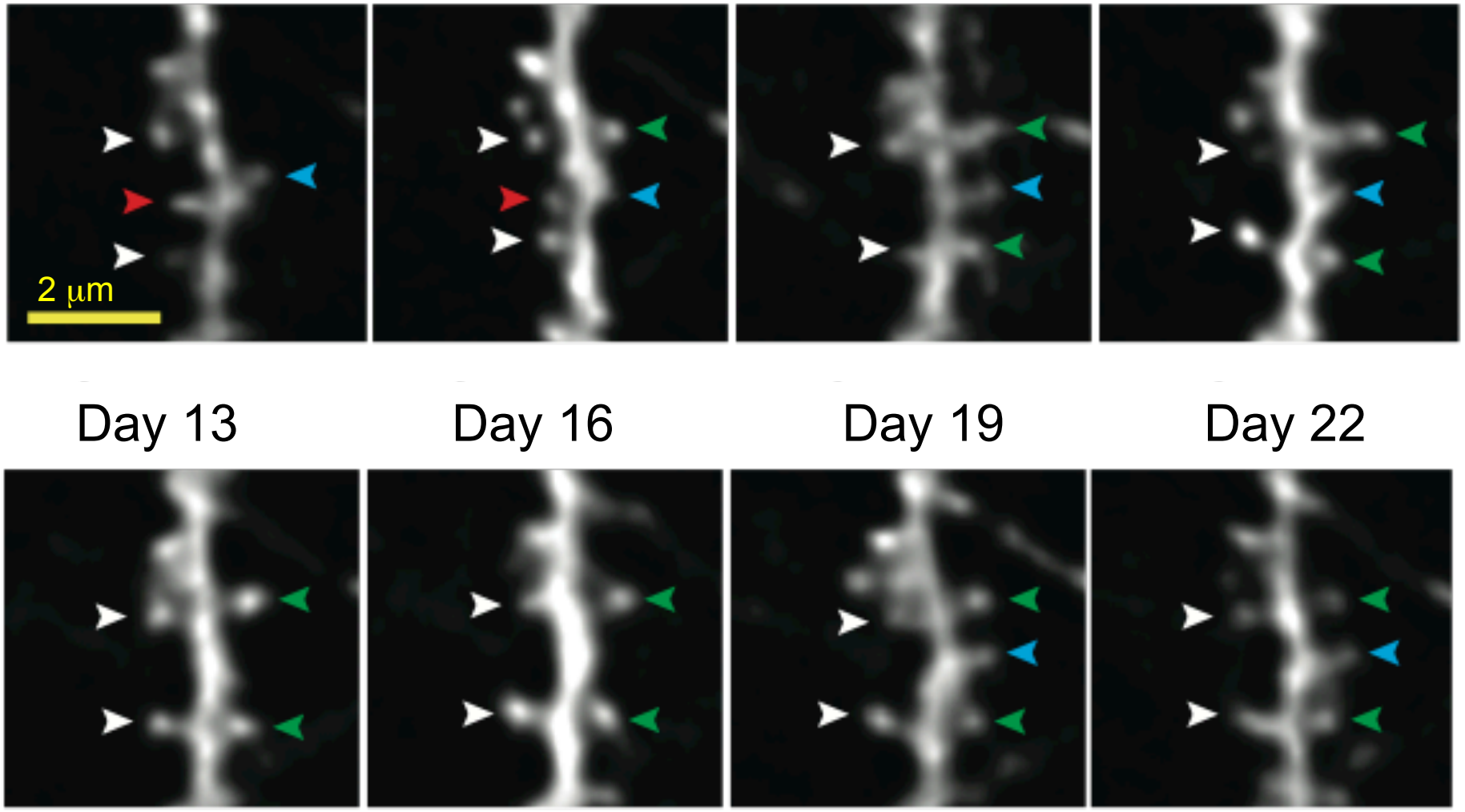


Turnover of CA1 dendritic spines in the live brain



Nature (2015)

Turnover of CA1 spines is consistent with ~1 month duration of hippocampal memory



Nature (2015)

How to track Ca^{2+} dynamics of CA1 neural ensembles in behaving mice?

Approach:

Miniature, digital fluorescence microscope

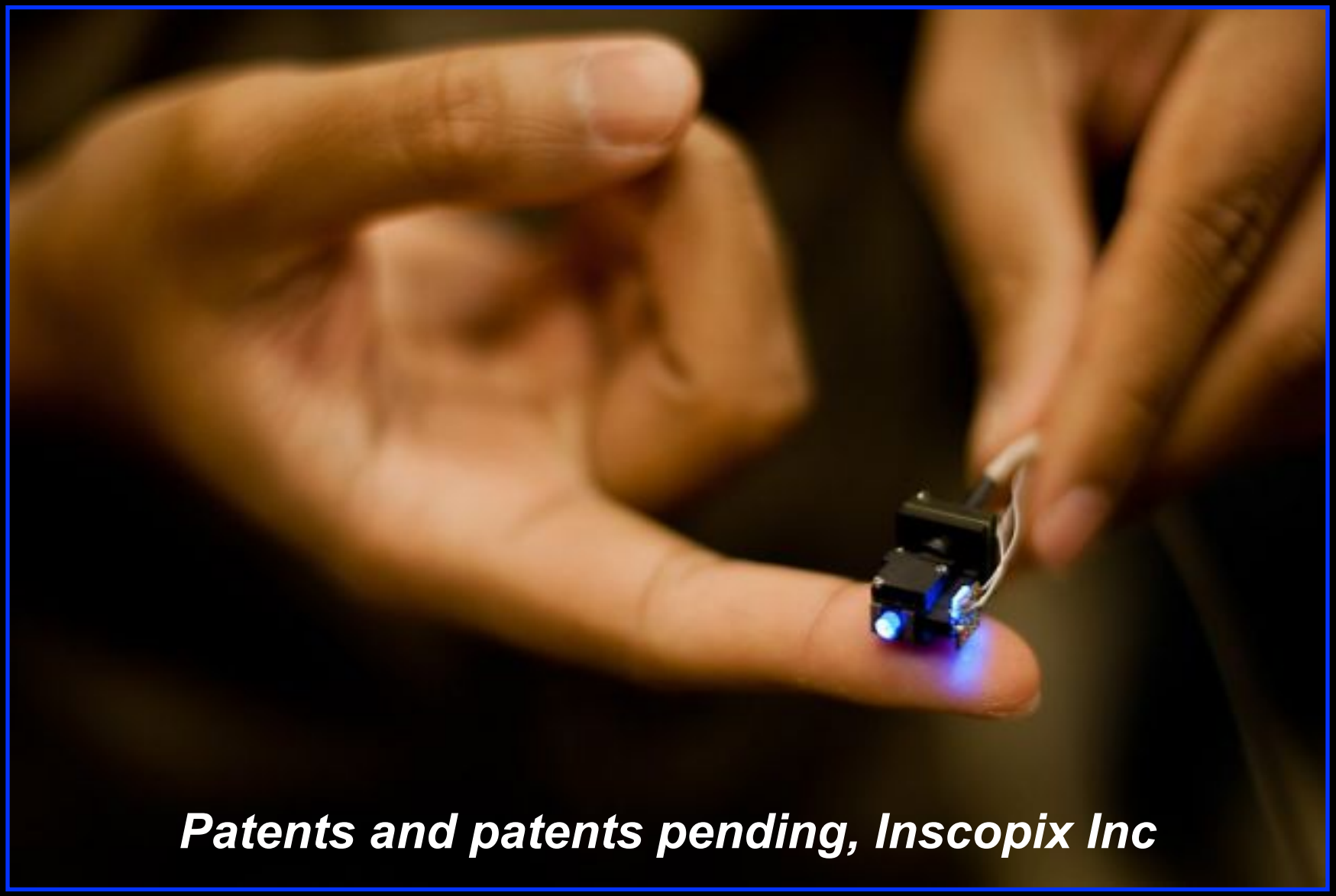
Has multiple advantages:

- **Mouse can behave freely**
- **Compatible with most behaviors**
- **Fast frame rates, up to ~100 Hz**
- **Broad field of view; recordings from ~1000 cells**

(20 to 50 times more cells than electrical recordings in mice)

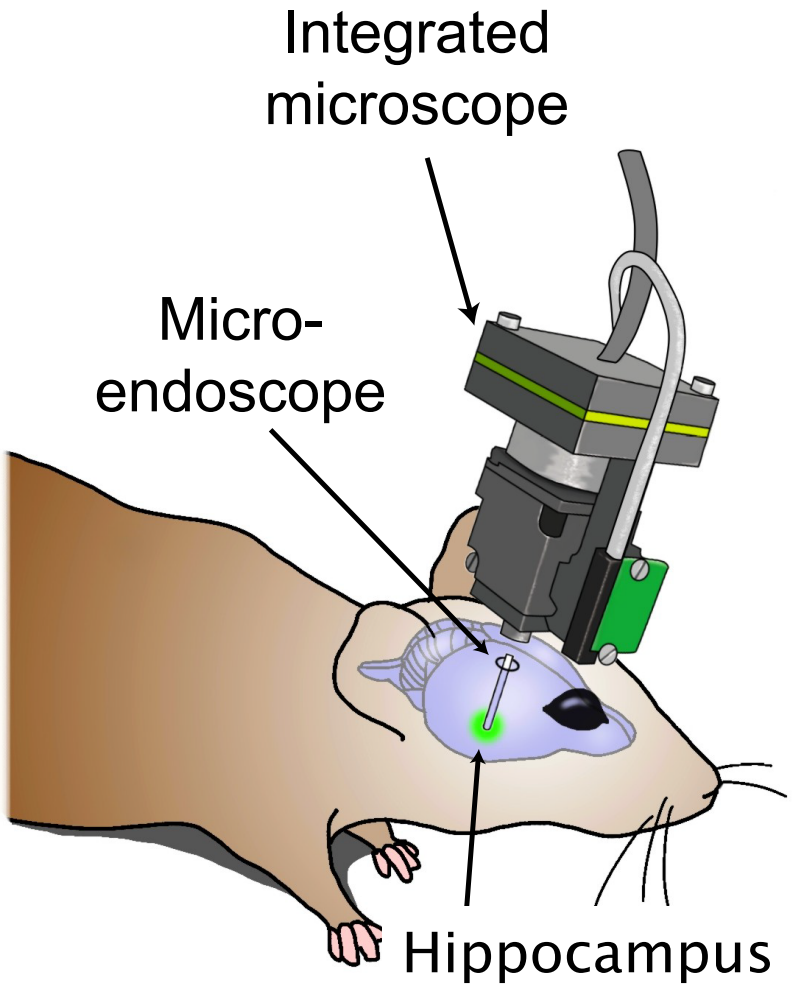
The integrated microscope is ~2 grams in mass and based on semiconductor optoelectronics

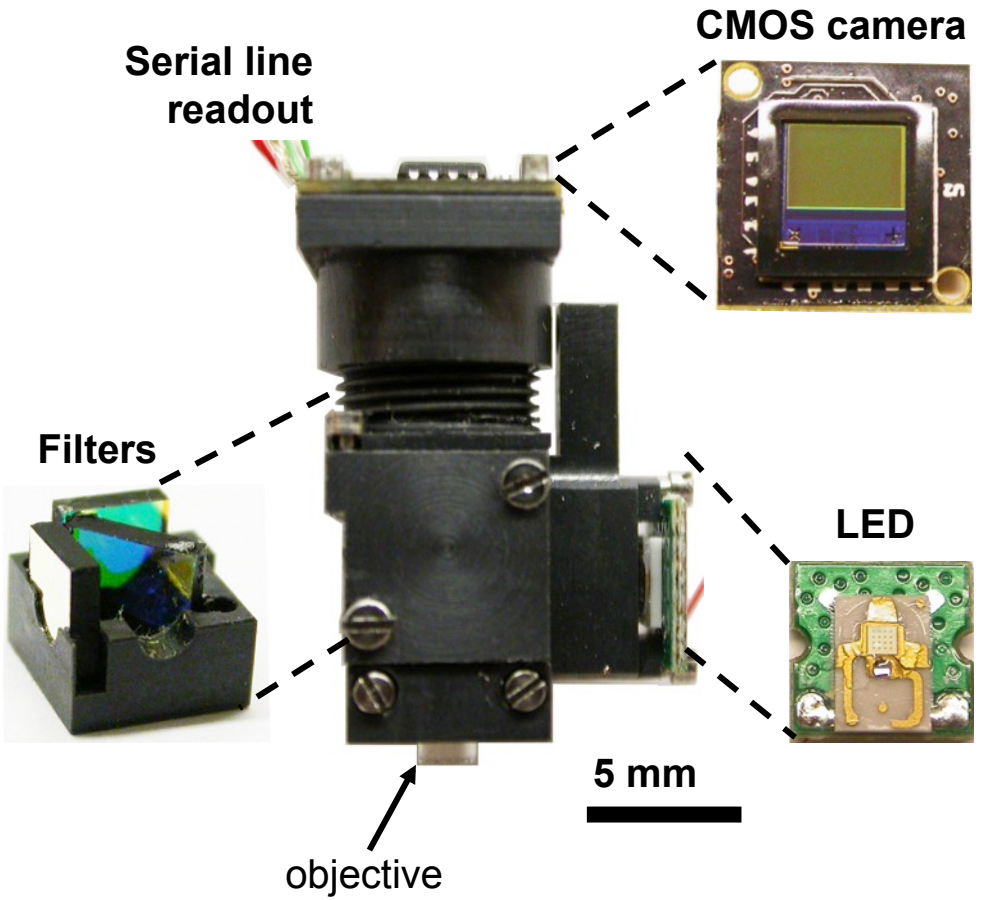
The integrated microscope is ~2 grams in mass and based on semiconductor optoelectronics



Patents and patents pending, Inscopix Inc

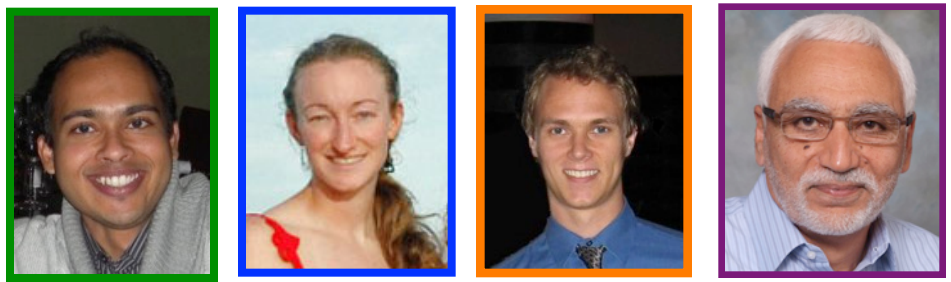
Time-lapse imaging in freely moving mice with a 2.0 g miniature microscope





Nature Methods (2008)
Nature Methods (2011)
Nature Neuroscience (2013)

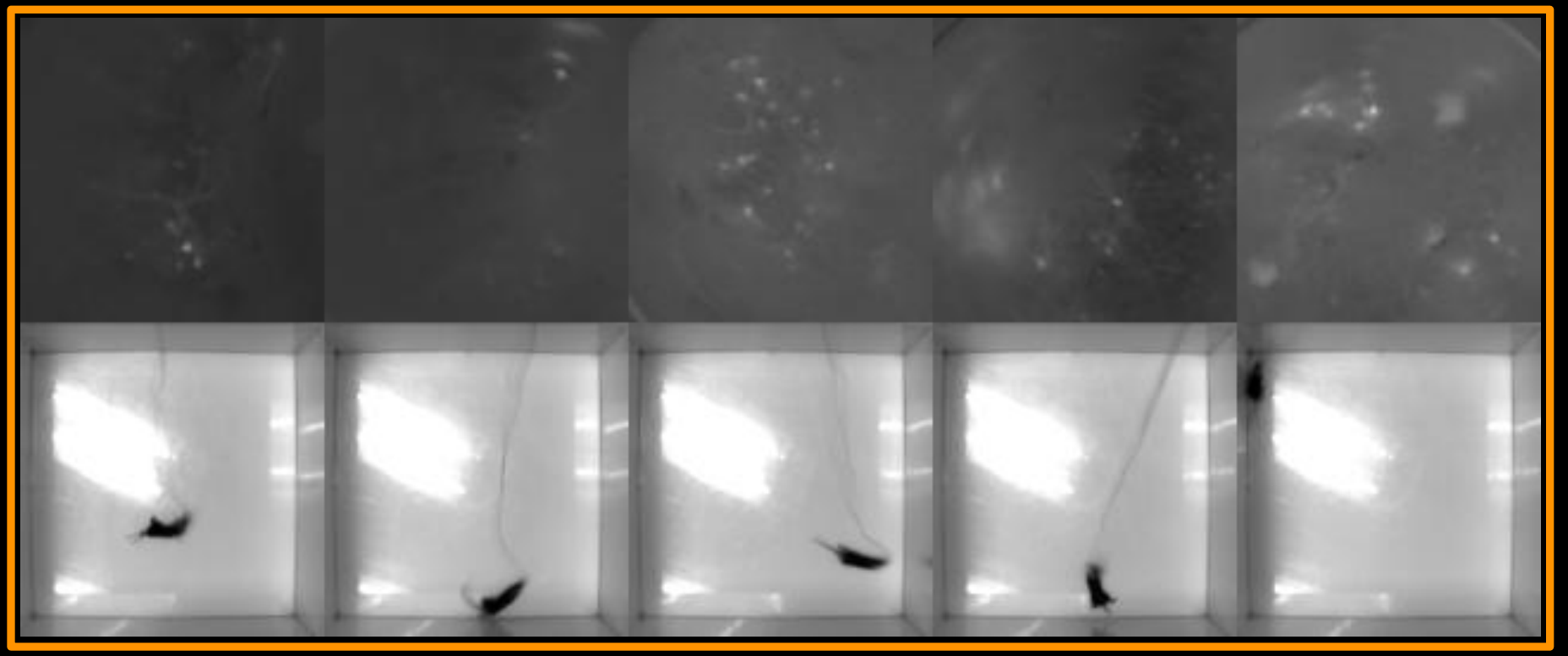
Kunal Ghosh Laurie Burns Eric Cocker Abbas El Gamal



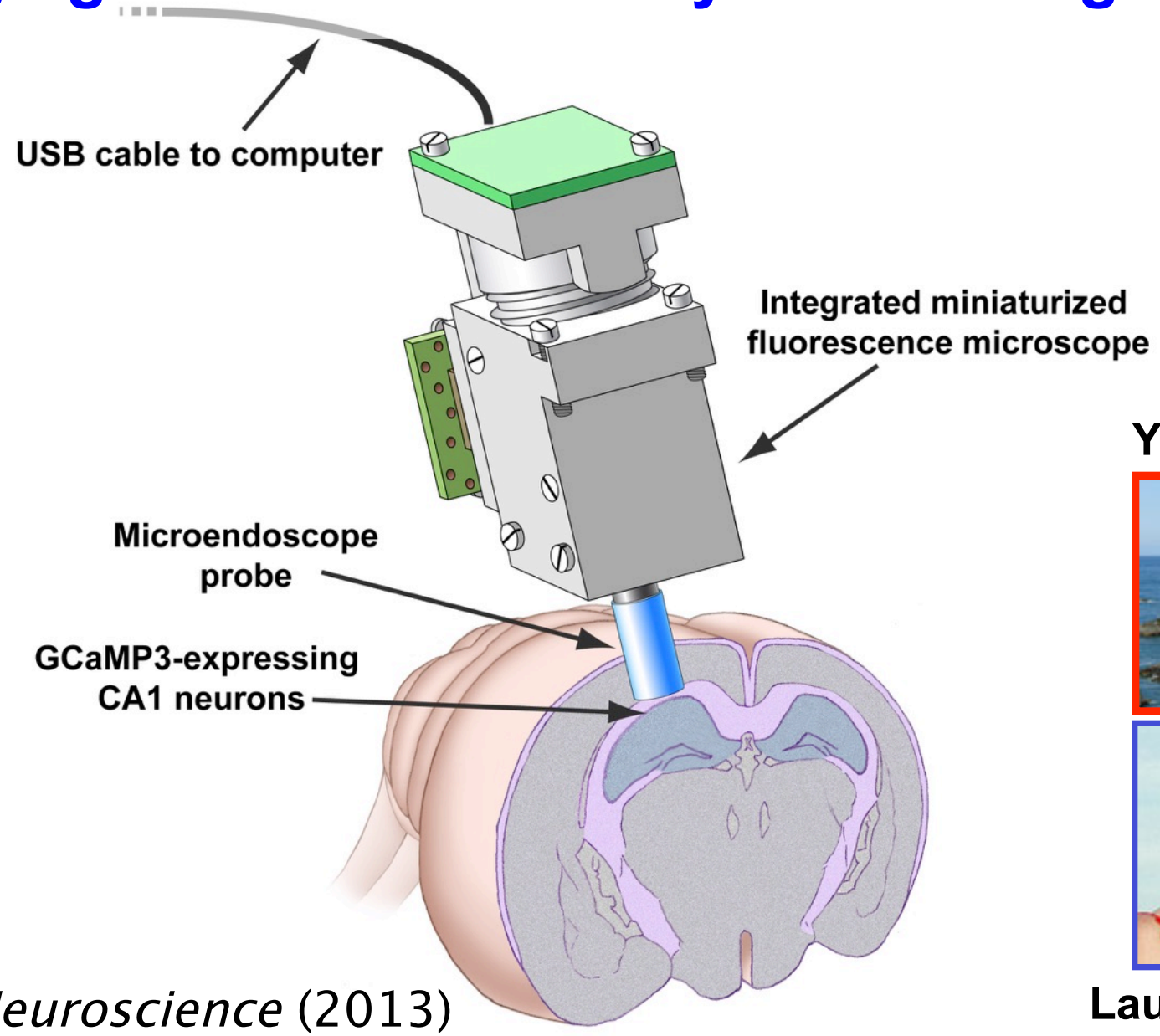
Mice behave naturally during digital imaging of activity in large neural ensembles

Mini-microscope provides statistical power for computational studies of neural coding

dorsal striatum



Ensemble substrates for information storage: Imaging CA1 neural activity in behaving mice

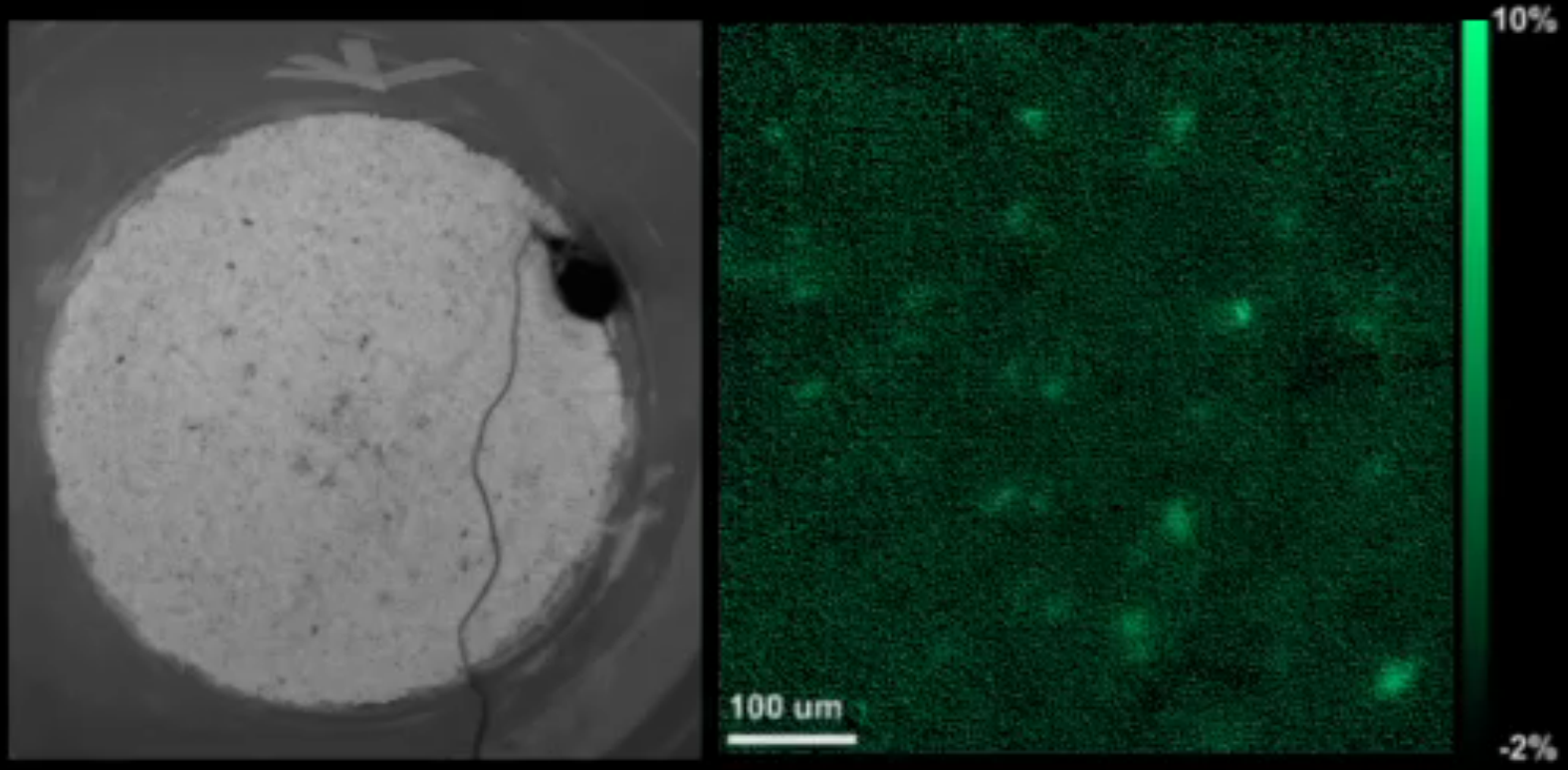


Yaniv Ziv

Laurie Burns

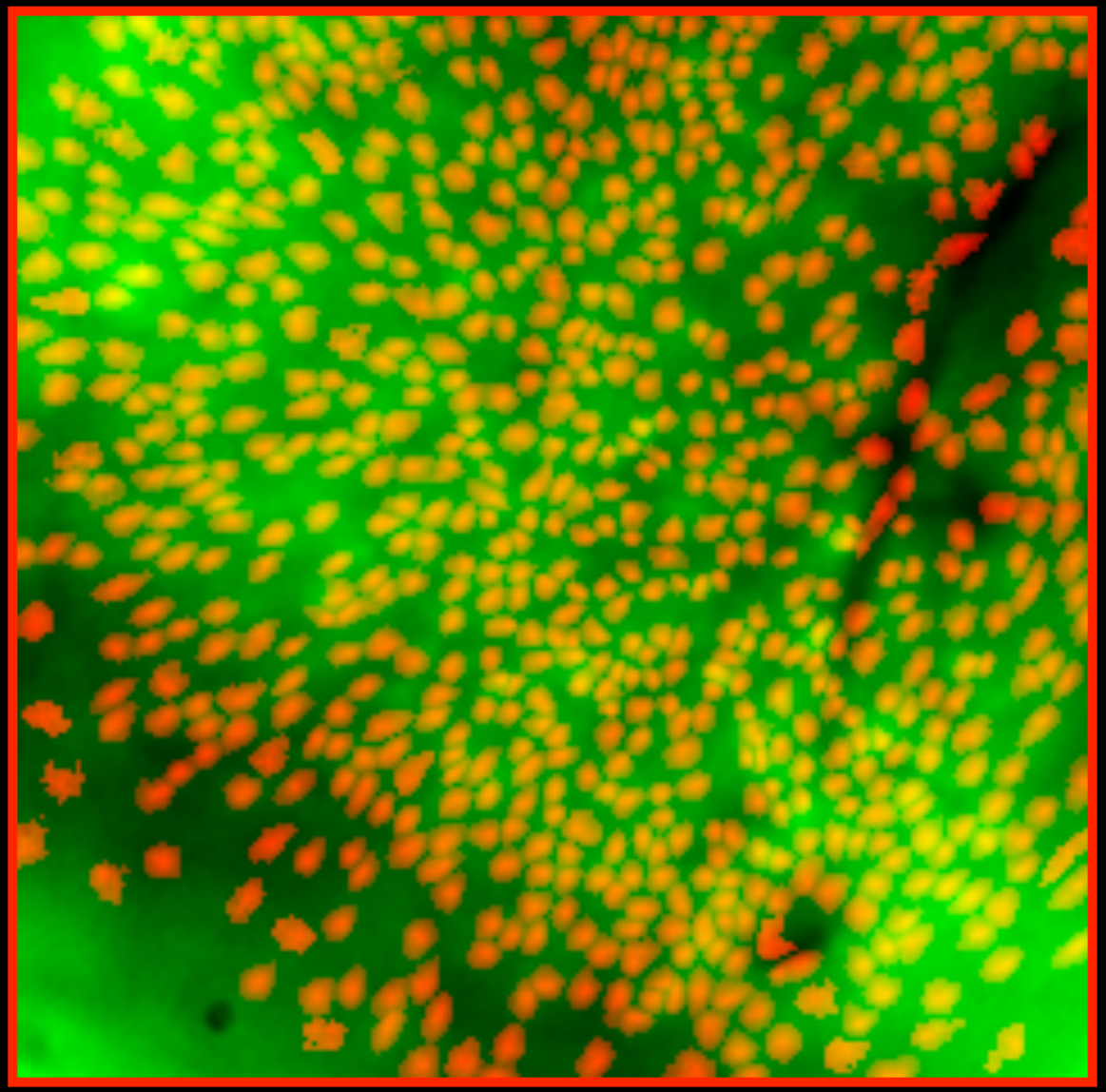
Nature Neuroscience (2013)

Visualizing Ca^{2+} dynamics in CA1 pyramidal neurons in behaving mice



Nature Neuroscience, 2013

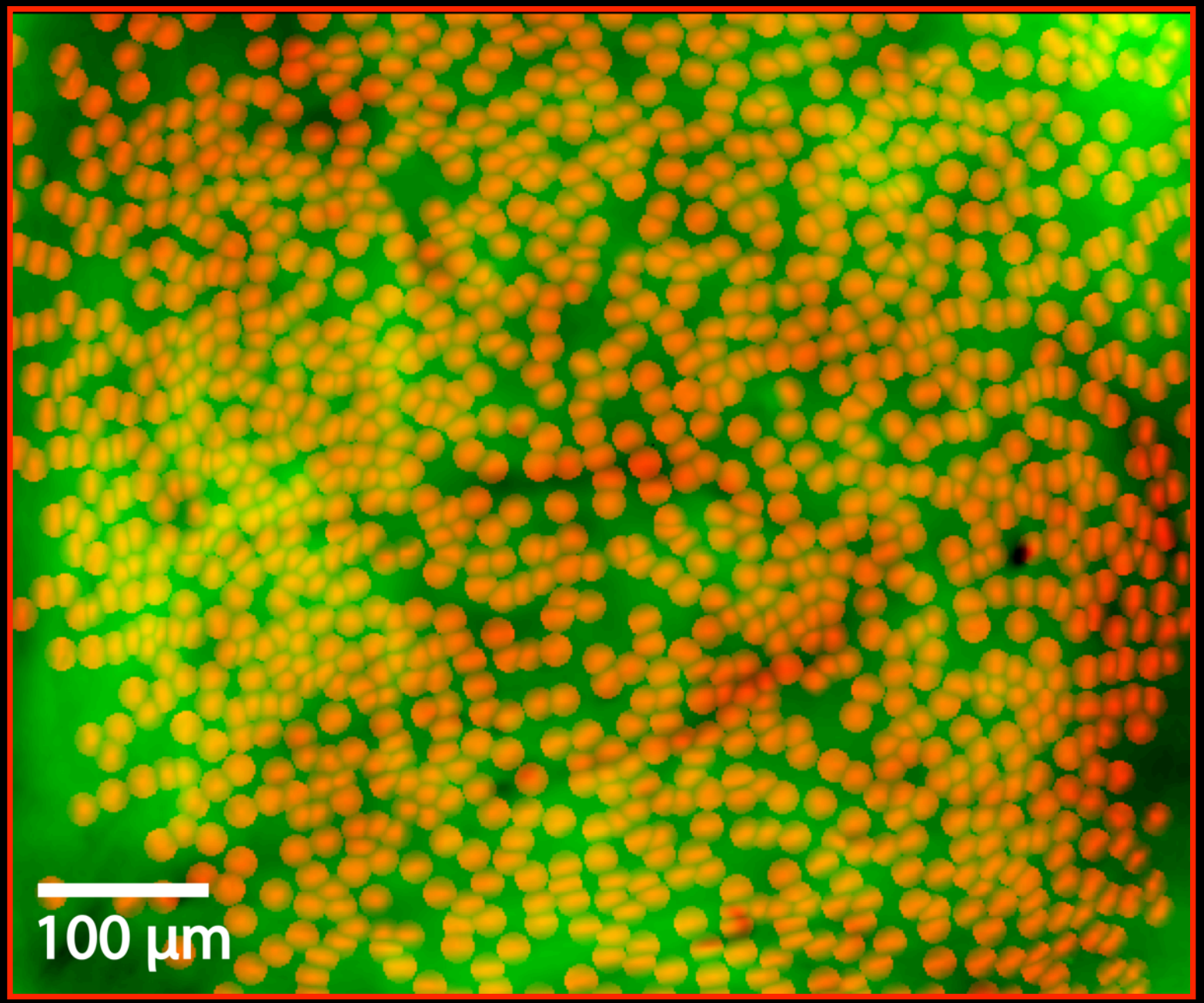
~700 CA1 neurons can be tracked concurrently



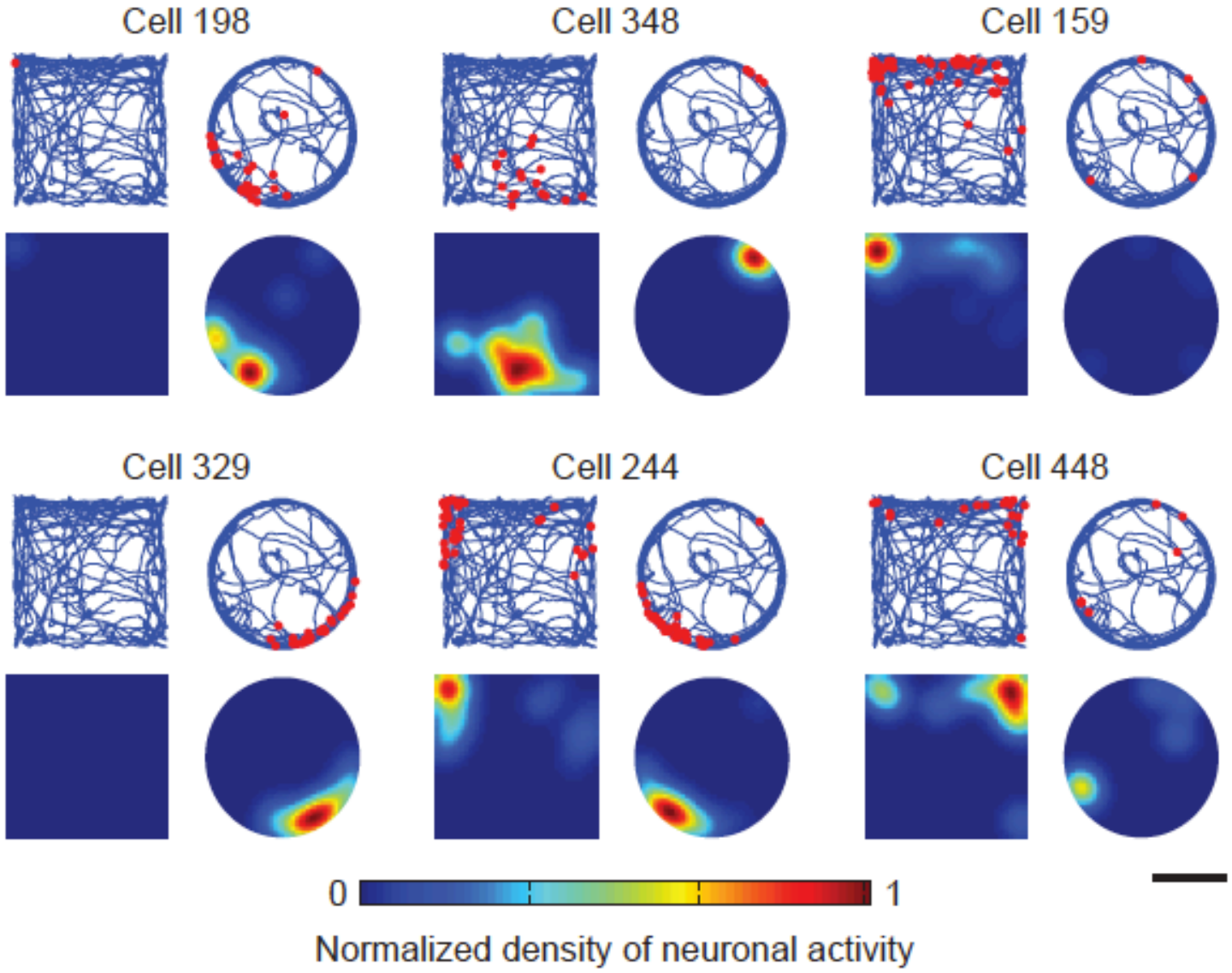
Nature Neuroscience (2013)

The latest integrated microscope can image >1000 cells in behaving mice

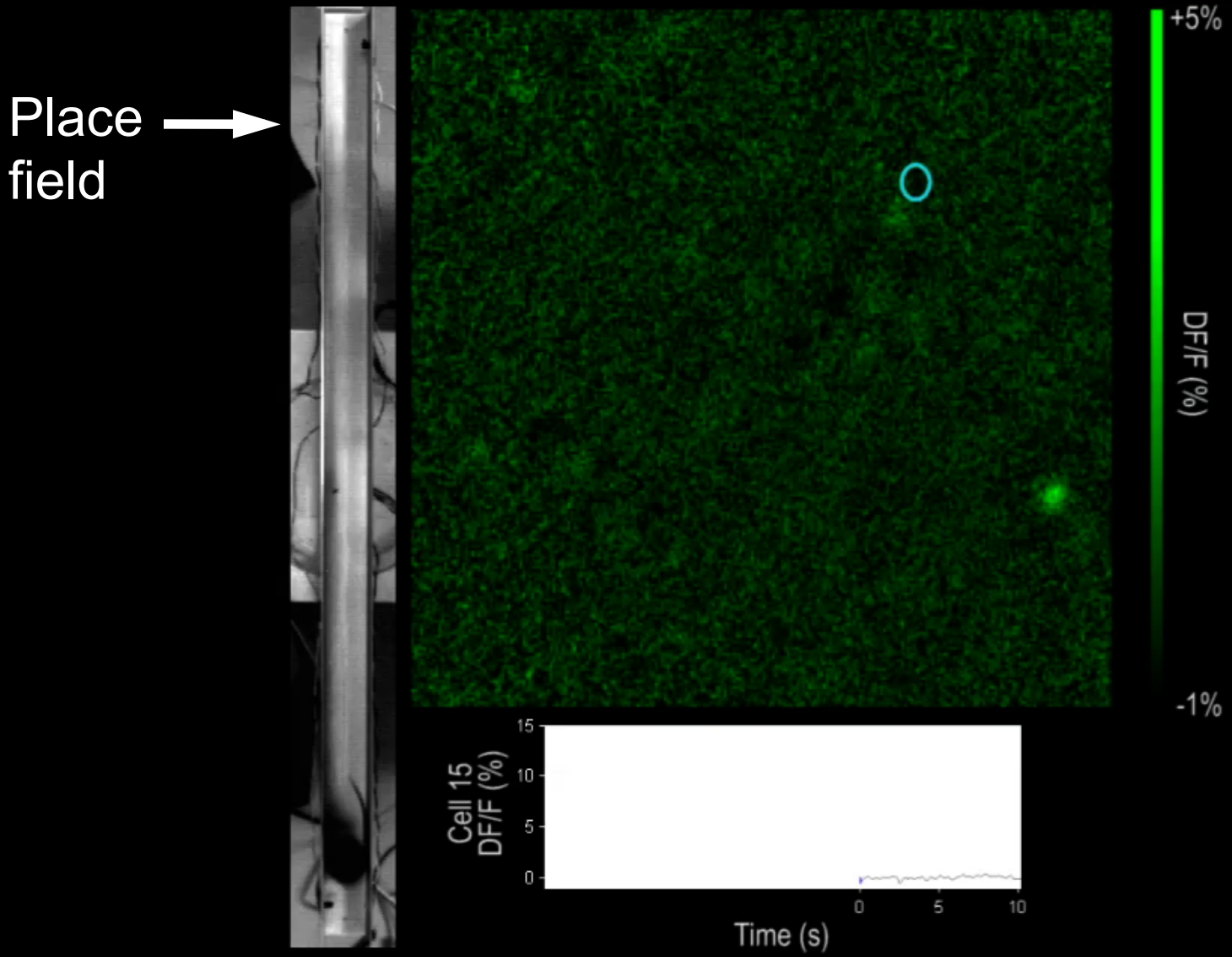
1202 CA1 neurons in one mouse



CA1 hippocampal place cells imaged optically in freely behaving mice



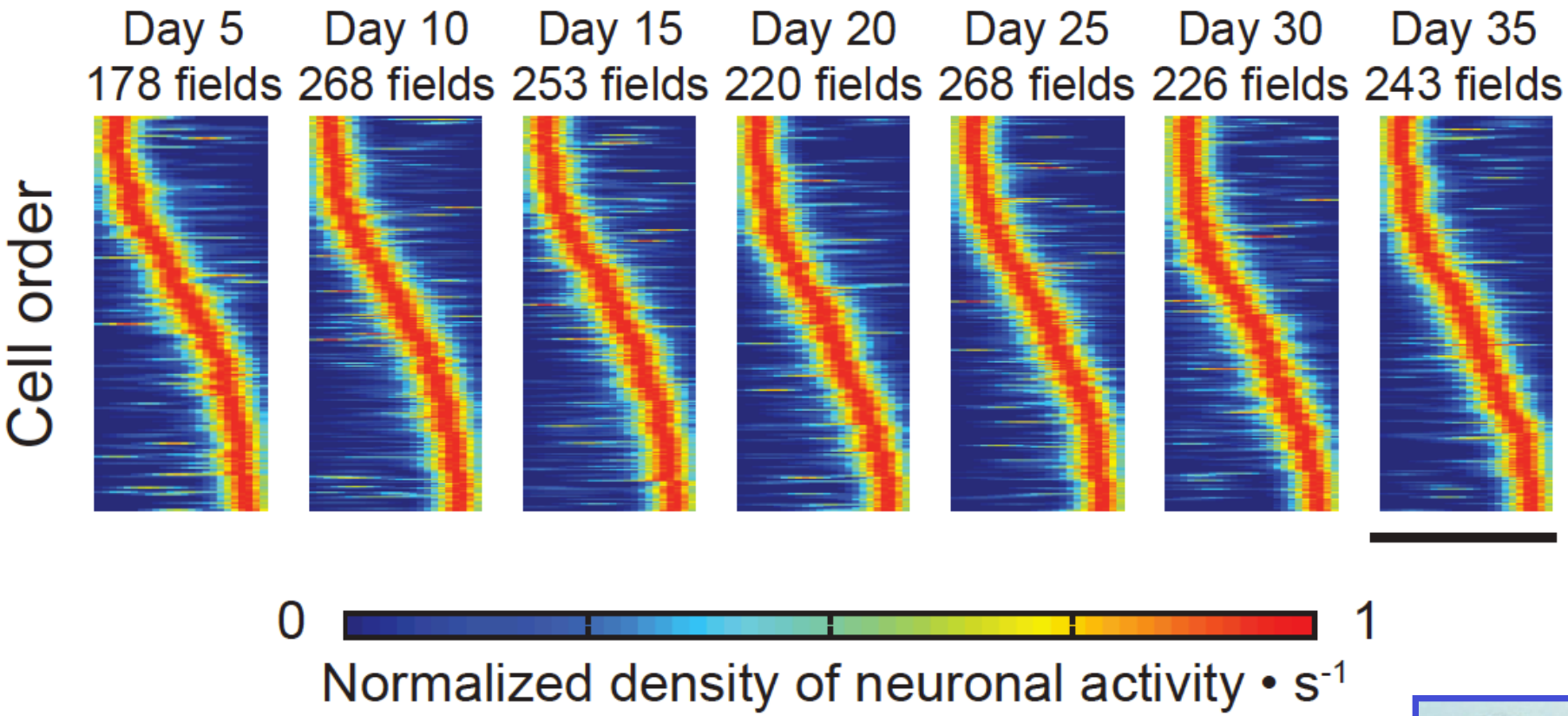
Probing the stability of the hippocampal representation of space over weeks



Yaniv Ziv

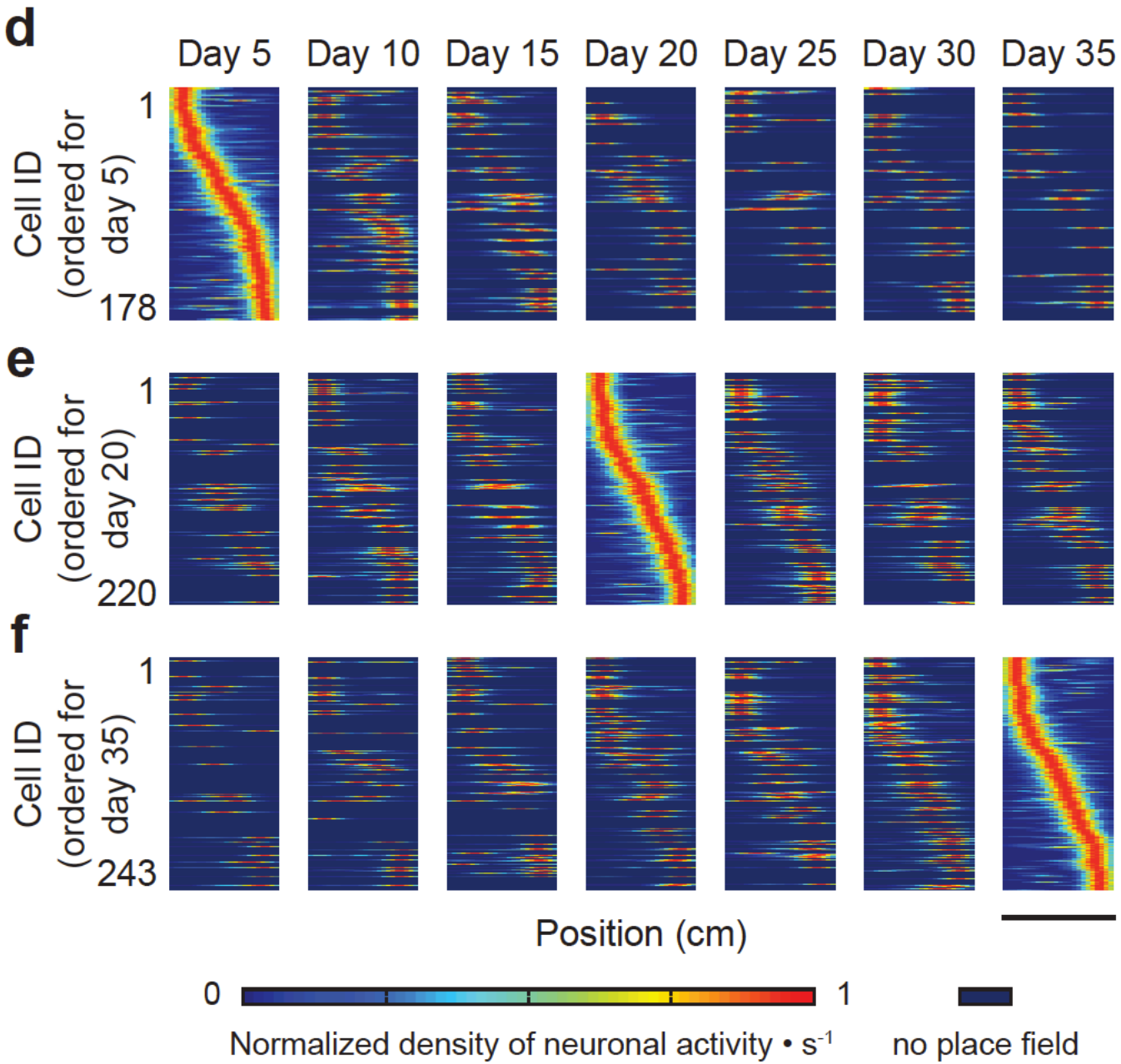
Laurie Burns

A complete spatial map on each day…



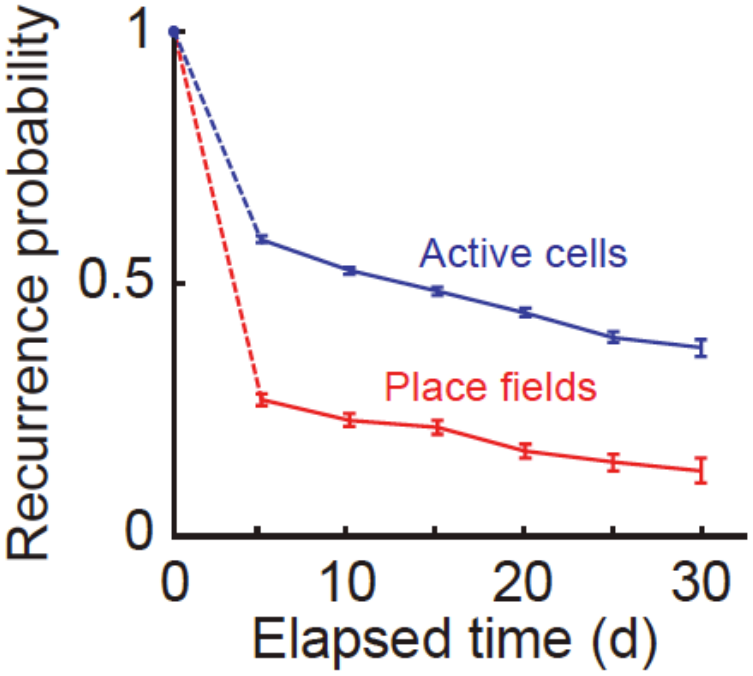
Laurie Burns

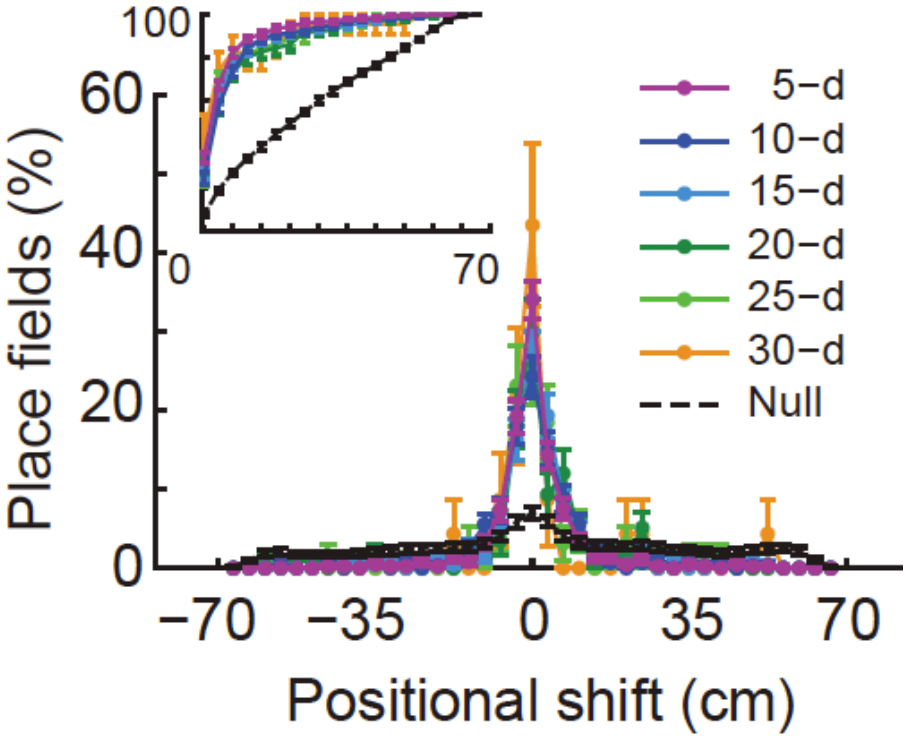
But the place cells involved slowly turnover



Laurie Burns

When they recur in the place-coding ensemble, cells retain same place fields

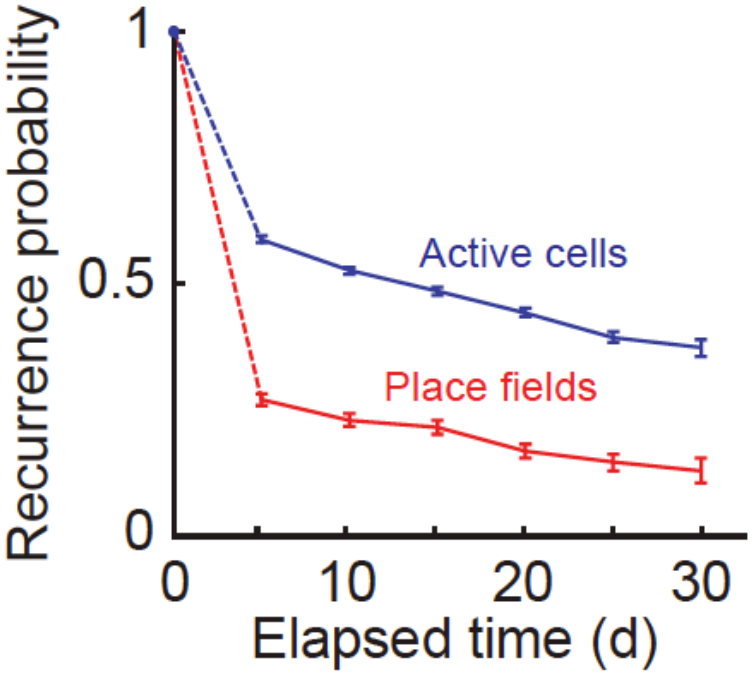


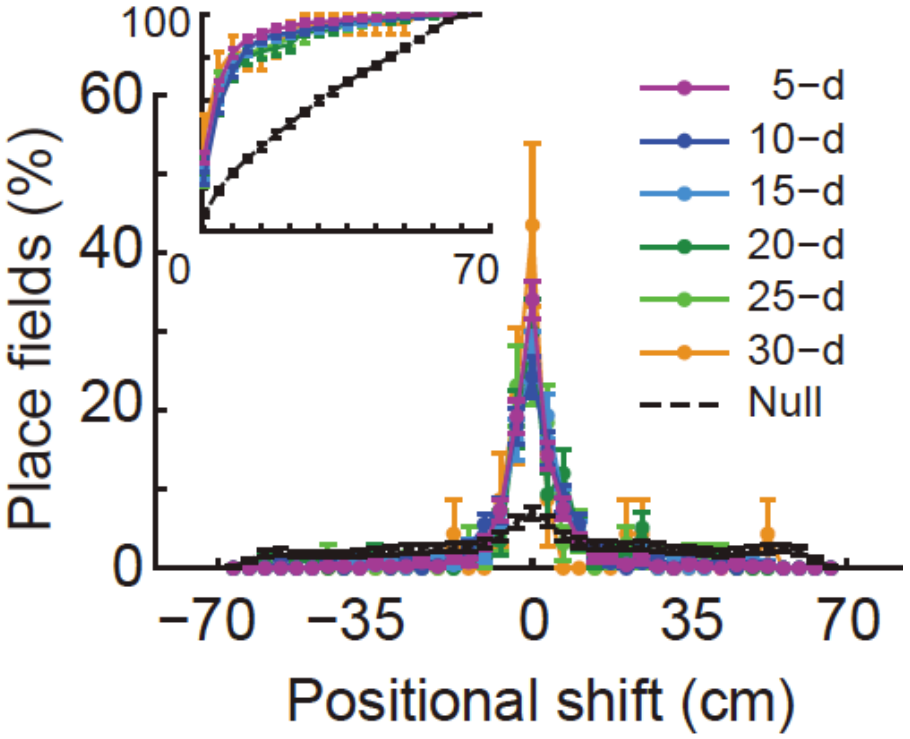


Laurie Burns

Nature Neuroscience (2013)

CA1 place fields are spatially invariant but temporally stochastic

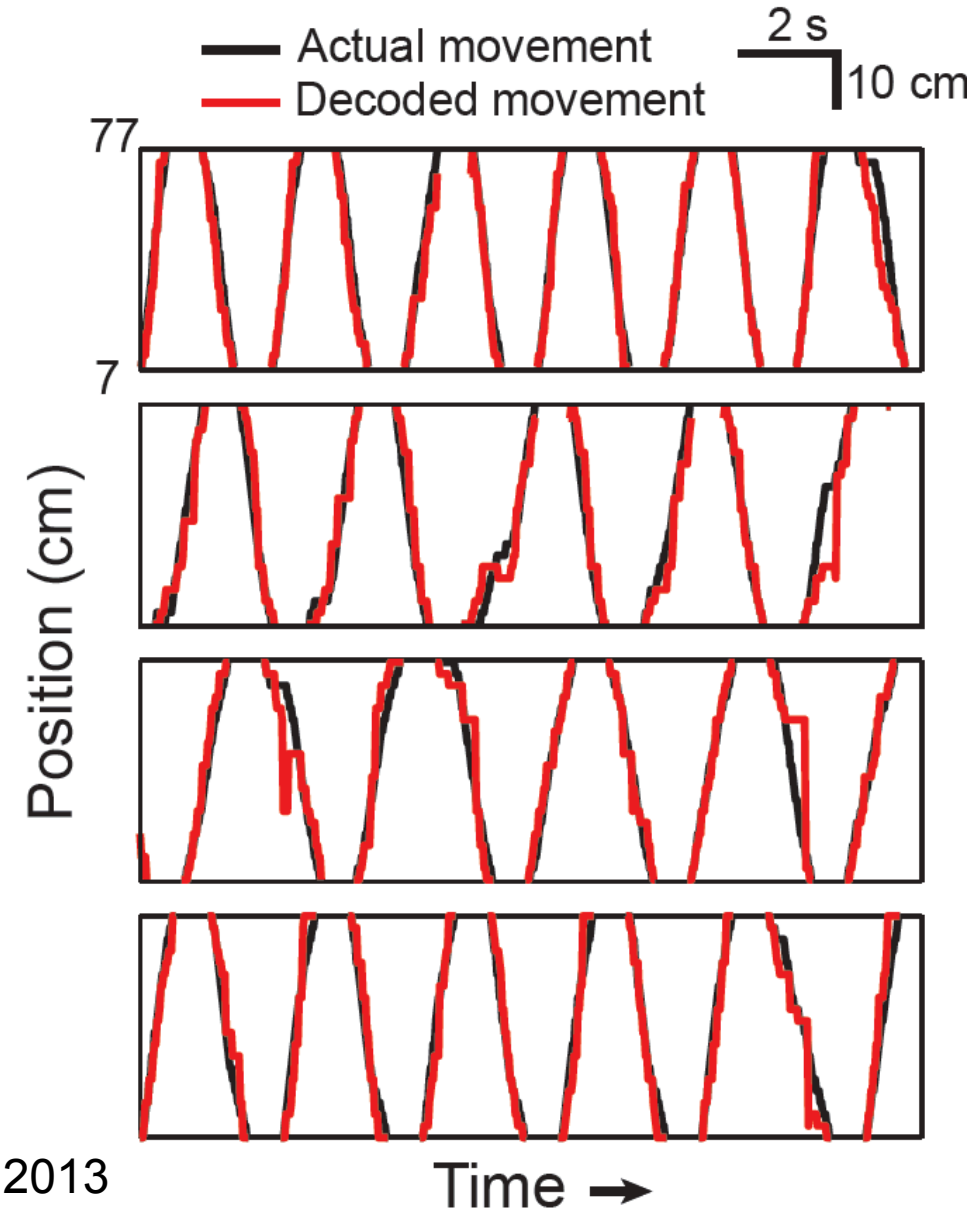




Laurie Burns

Nature Neuroscience (2013)

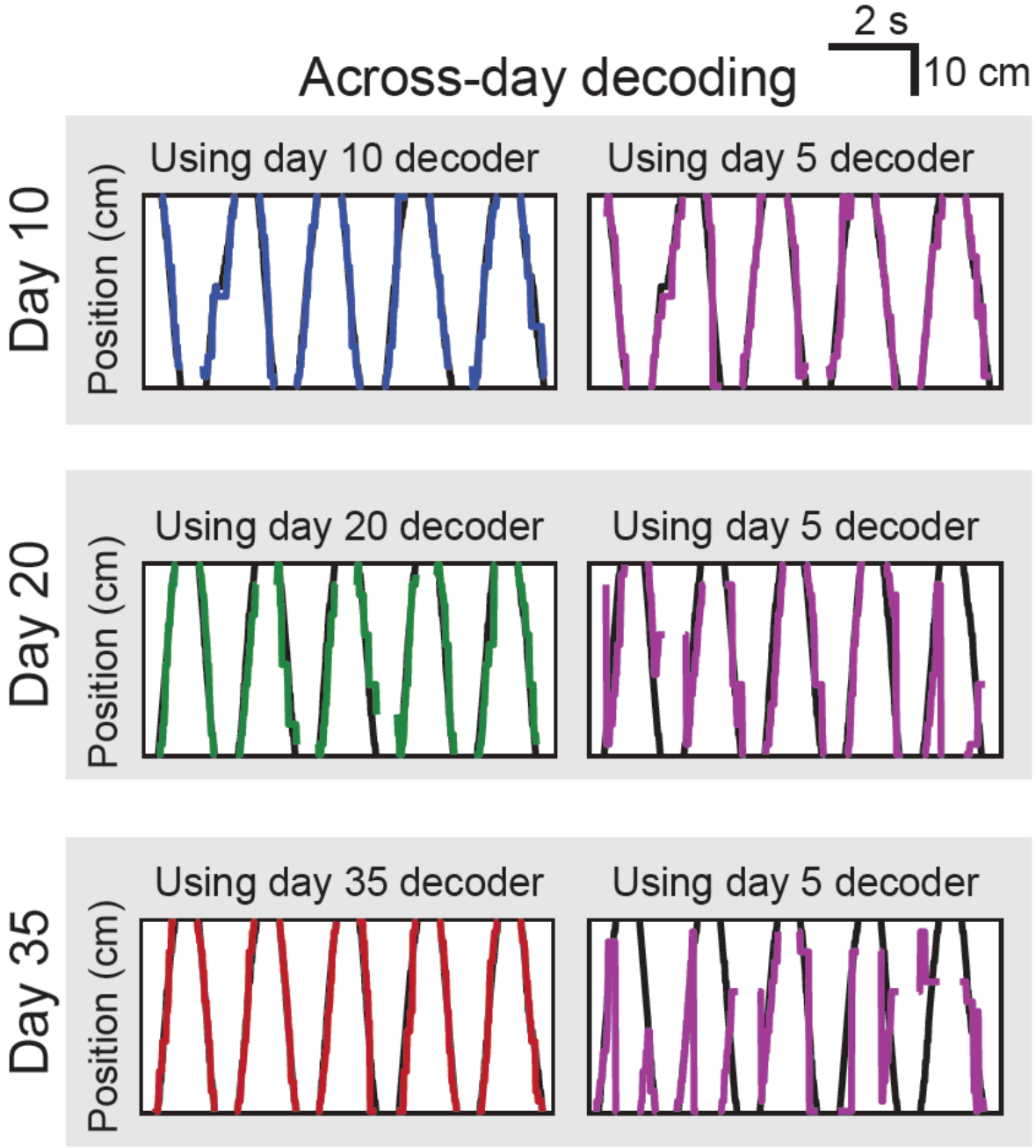
Decoding the mouse's position using CA1 ensemble activity….



Nature Neuroscience, 2013

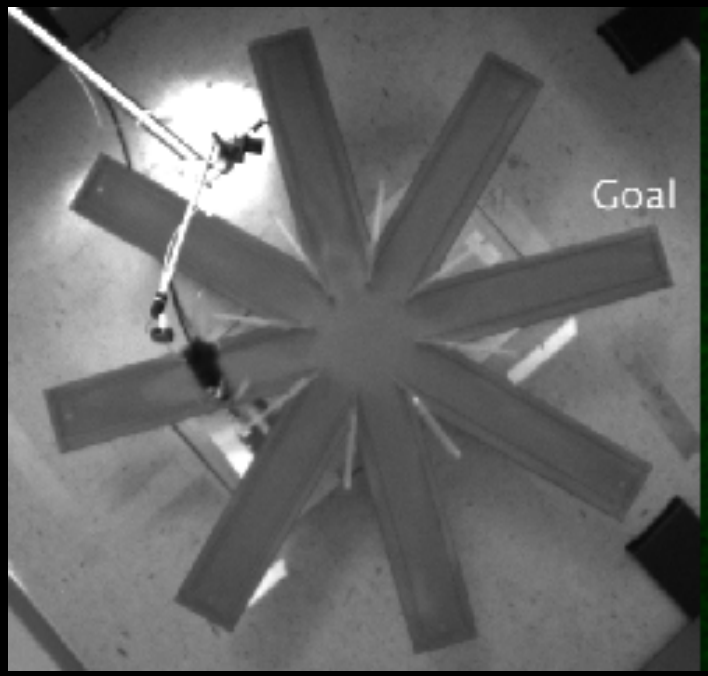
Lacey Kitch

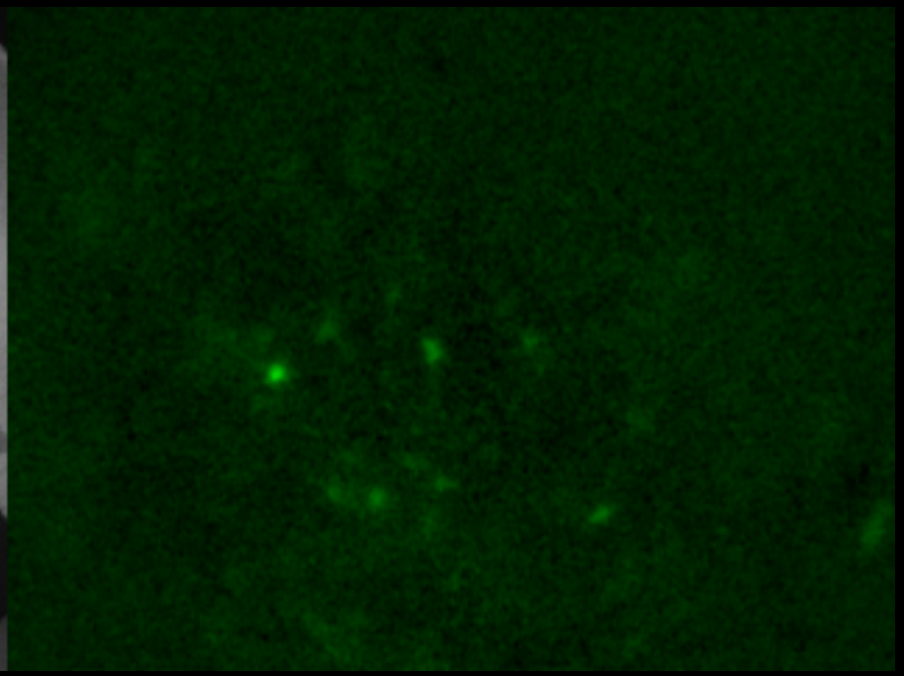
…yields an Ensemble substrate of memory



Lacey Kitch

We are now decoding the neural basis for correct and incorrect memory recall



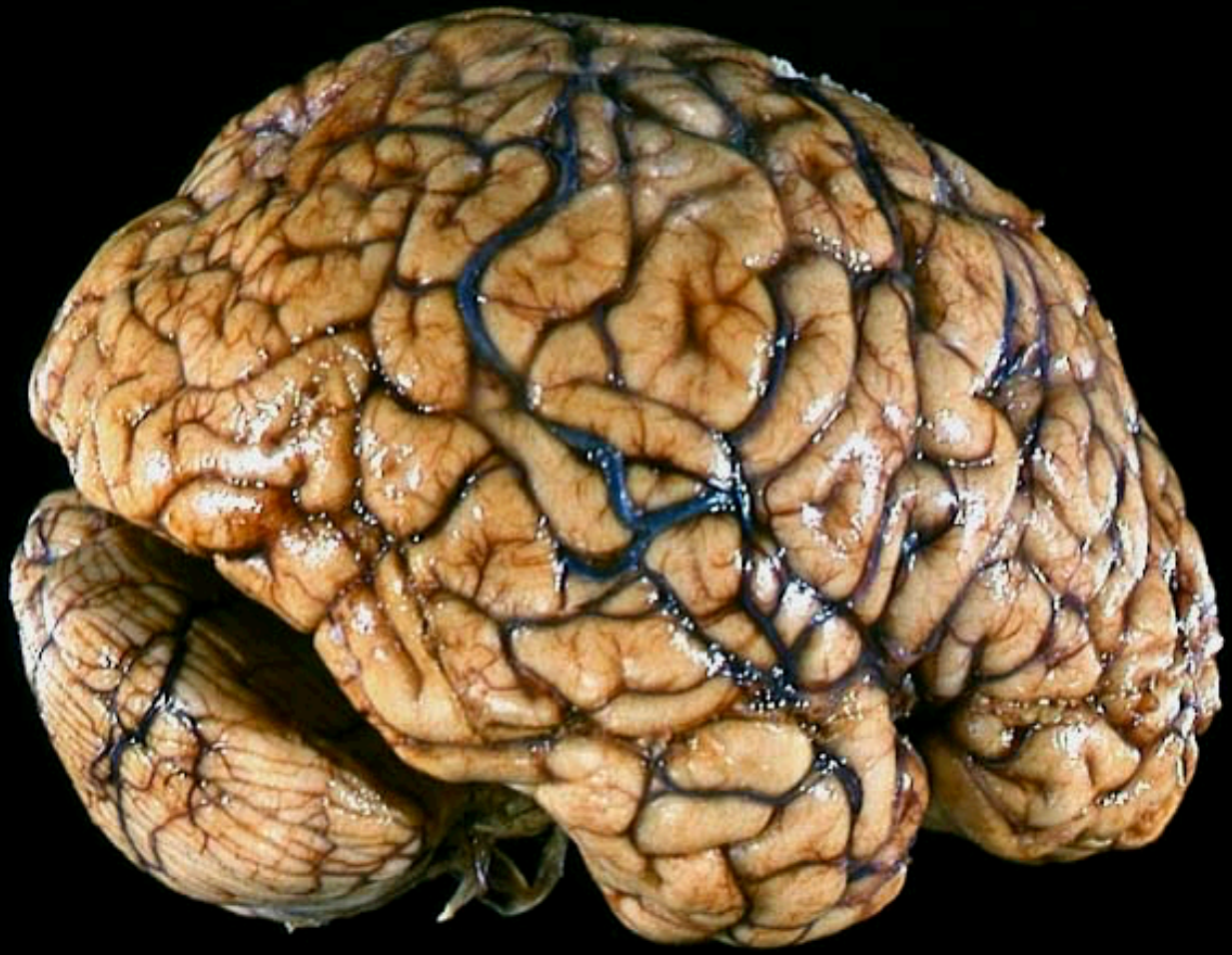


Yaniv Ziv

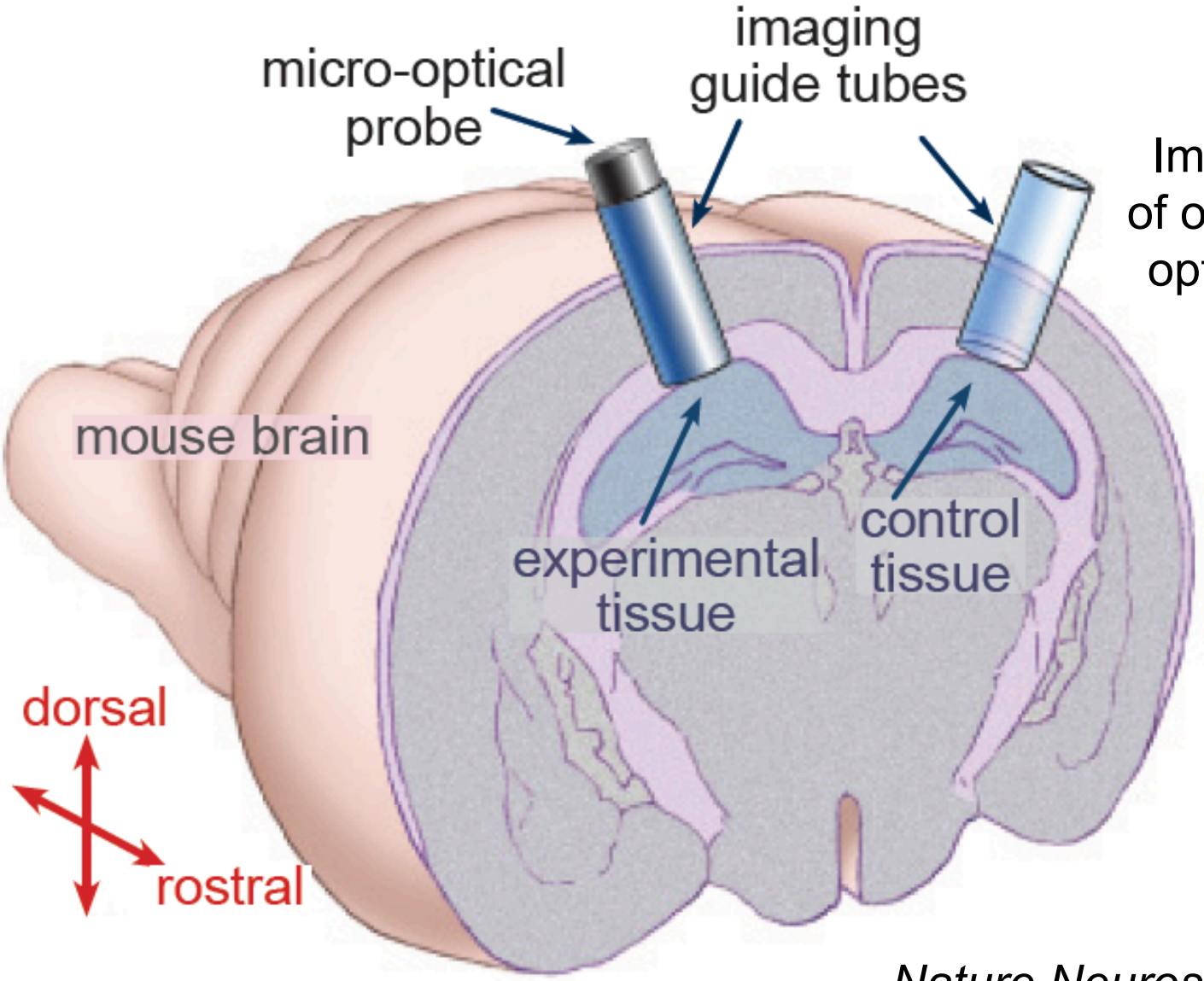
Lacey Kitch

"Next-generation technologies for the study of brain circuit dynamics"

Mark J. Schnitzer, HHMI / Stanford University

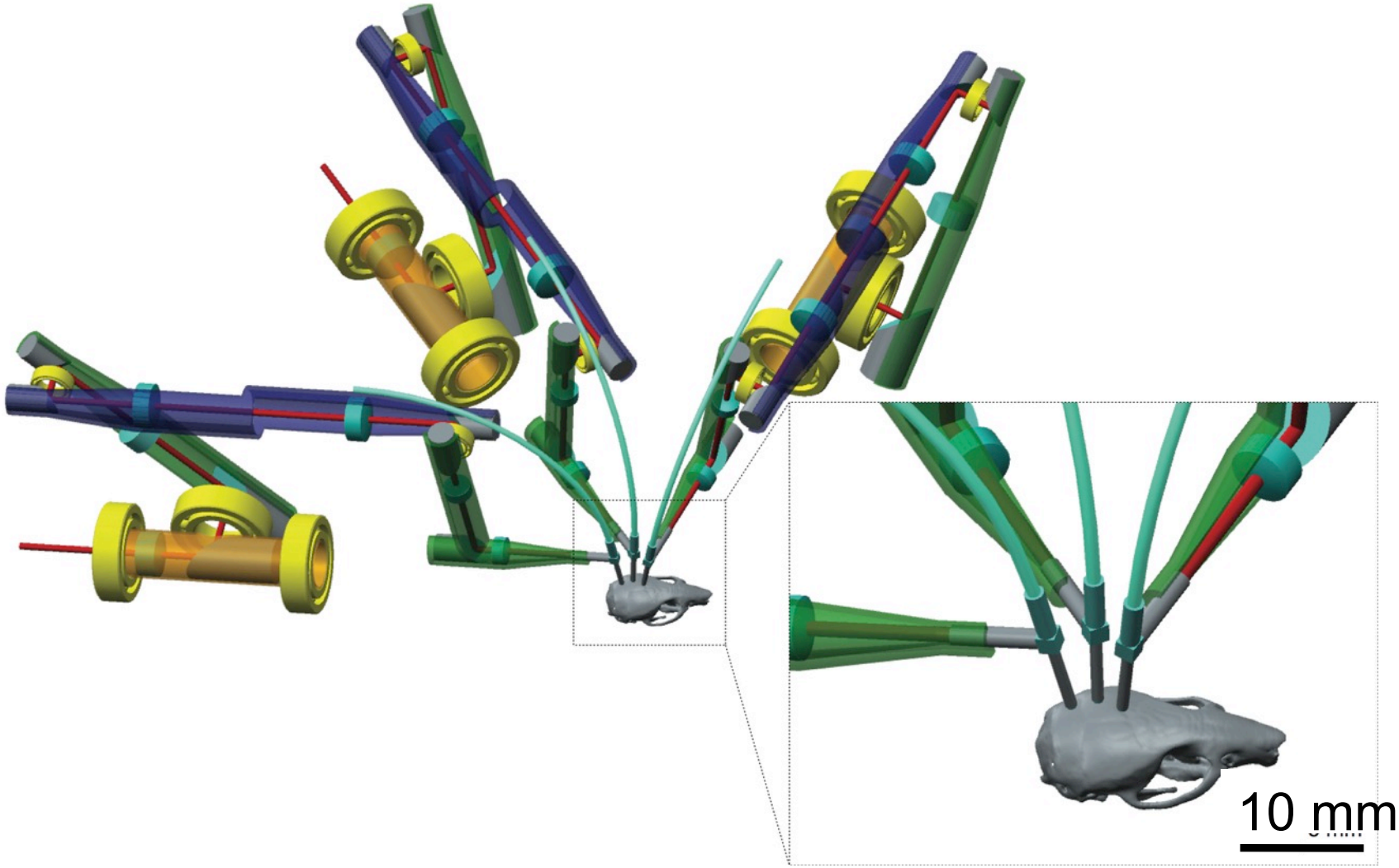


Imaging via two probes concurrently to examine brain area interactions?



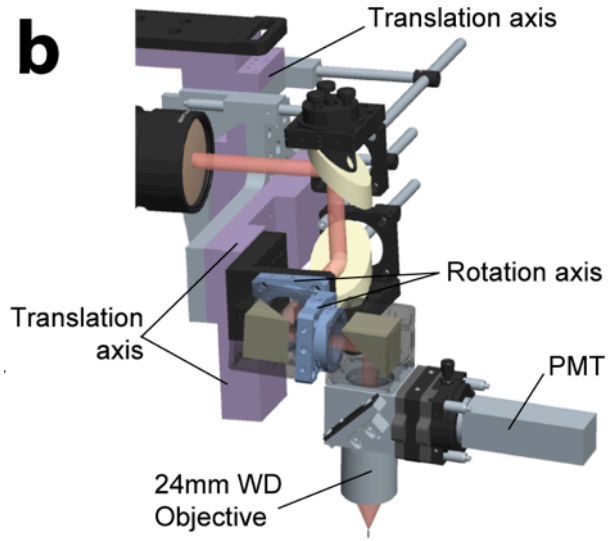
Nature Neuroscience, 2014

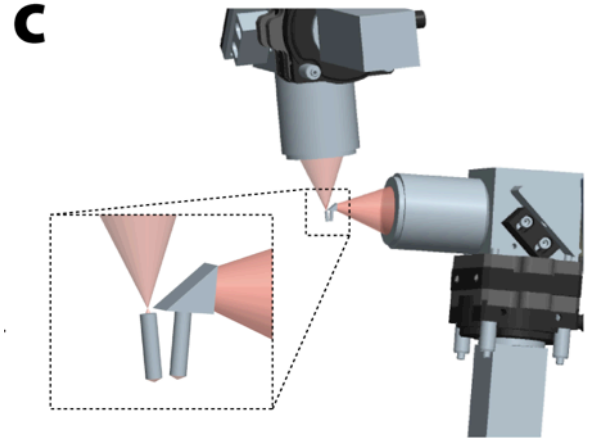
Optical needles can be densely packed to image multiple brain areas



The Duopus is a two-armed microscope that can image two areas concurrently

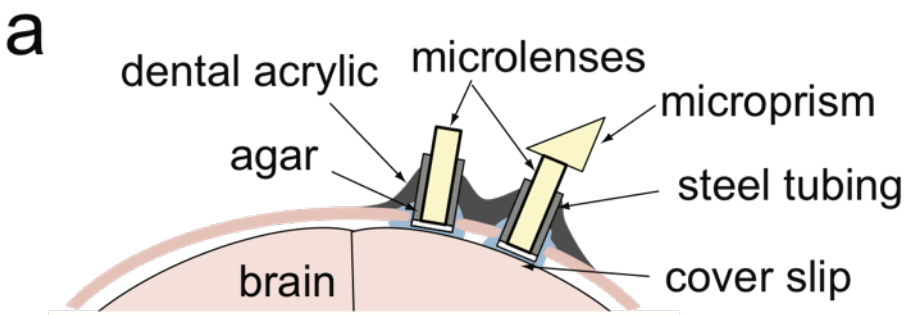
Jerome Lecoq

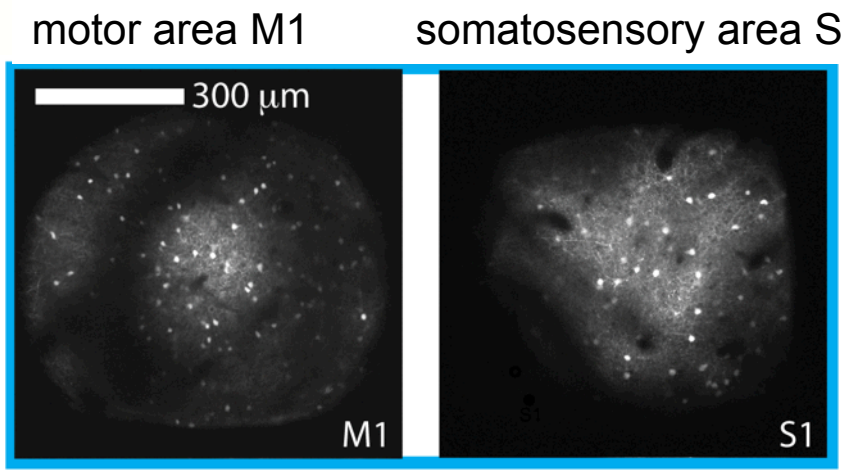


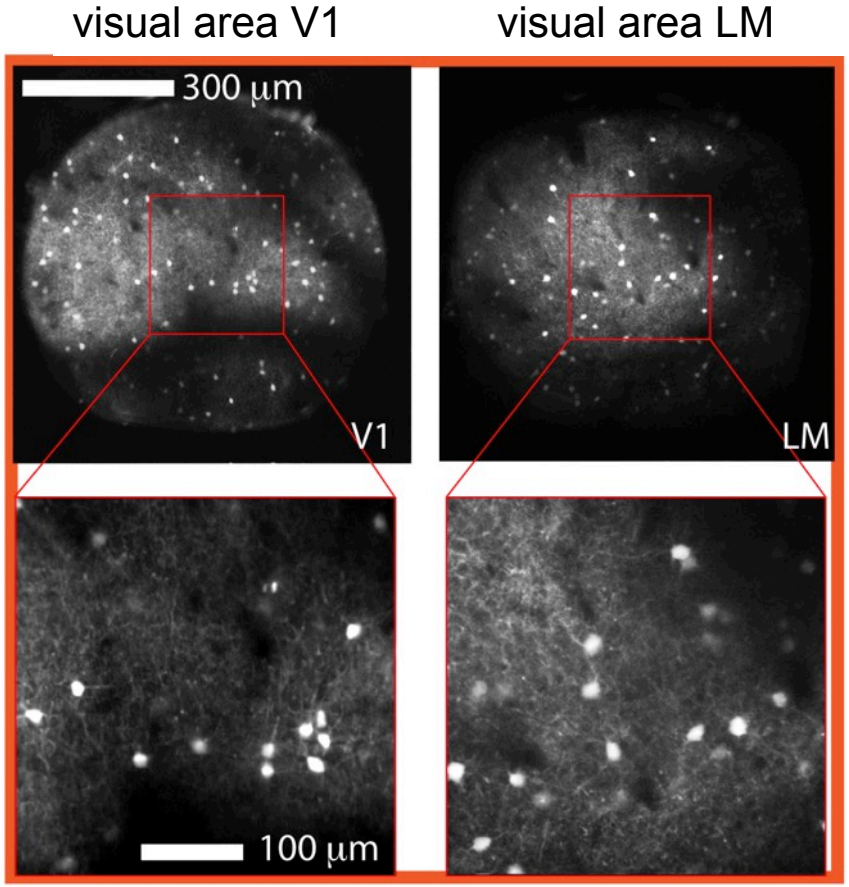


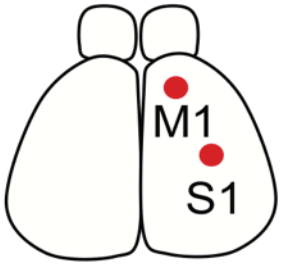
Nature Neuroscience, 2014

The Duopus can inspect either nearby or distal pairs of brain areas

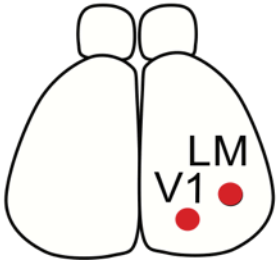




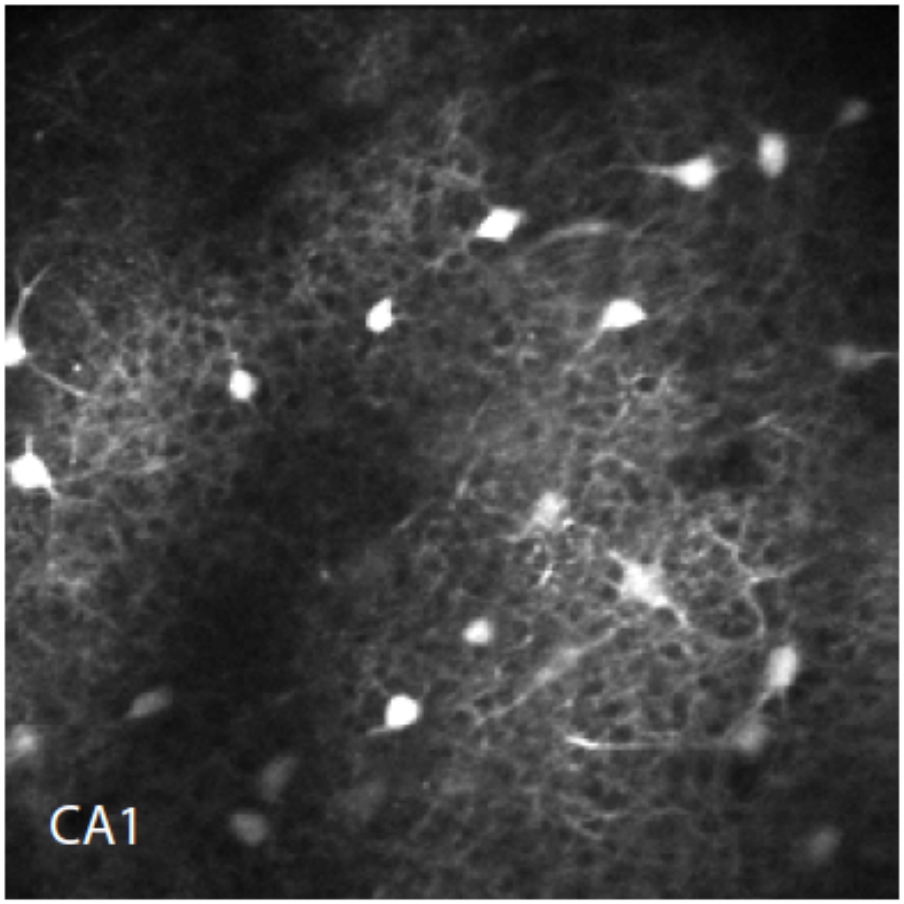


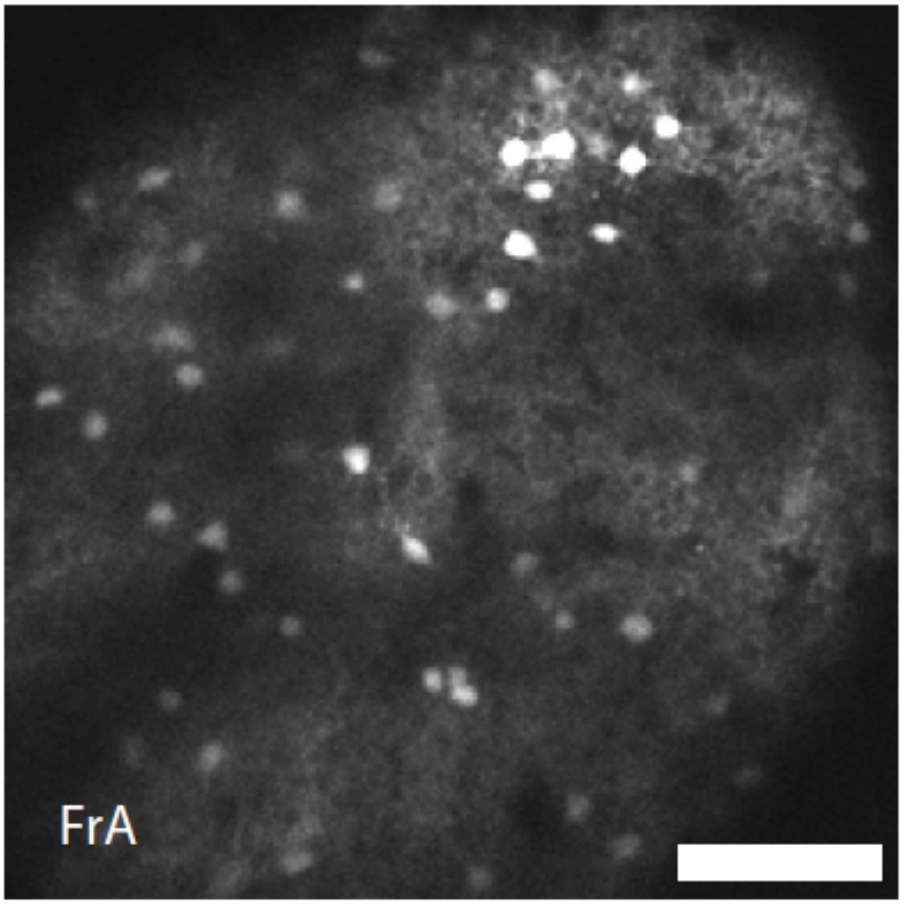


Parvalbumin interneurons labeled red with tdTomato



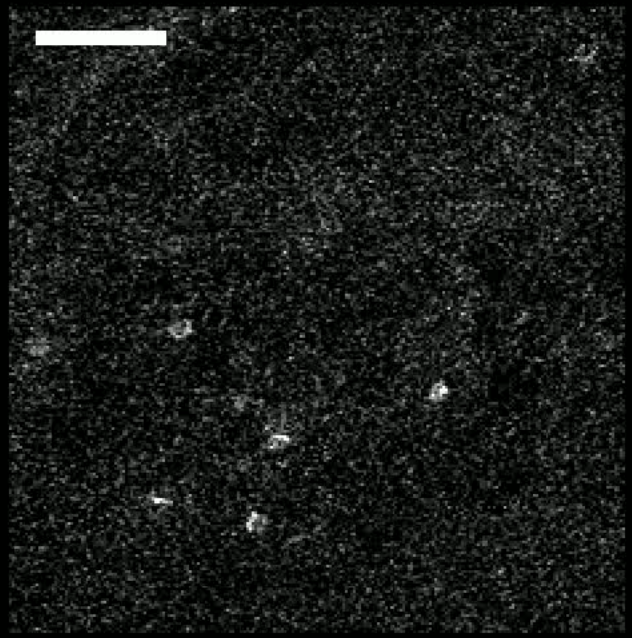
Example: CA1 hippocampus and frontal cortex imaged in tandem



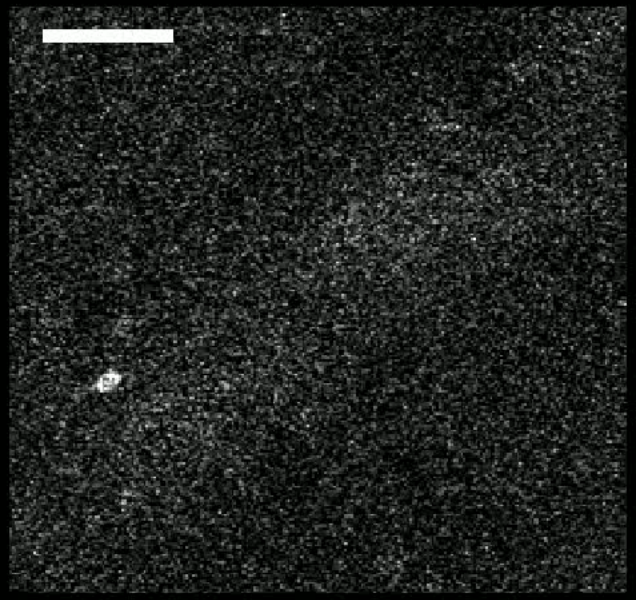


Duopus Ca^{2+}-imaging of visual areas V1 and LM in behaving mice

Behavior



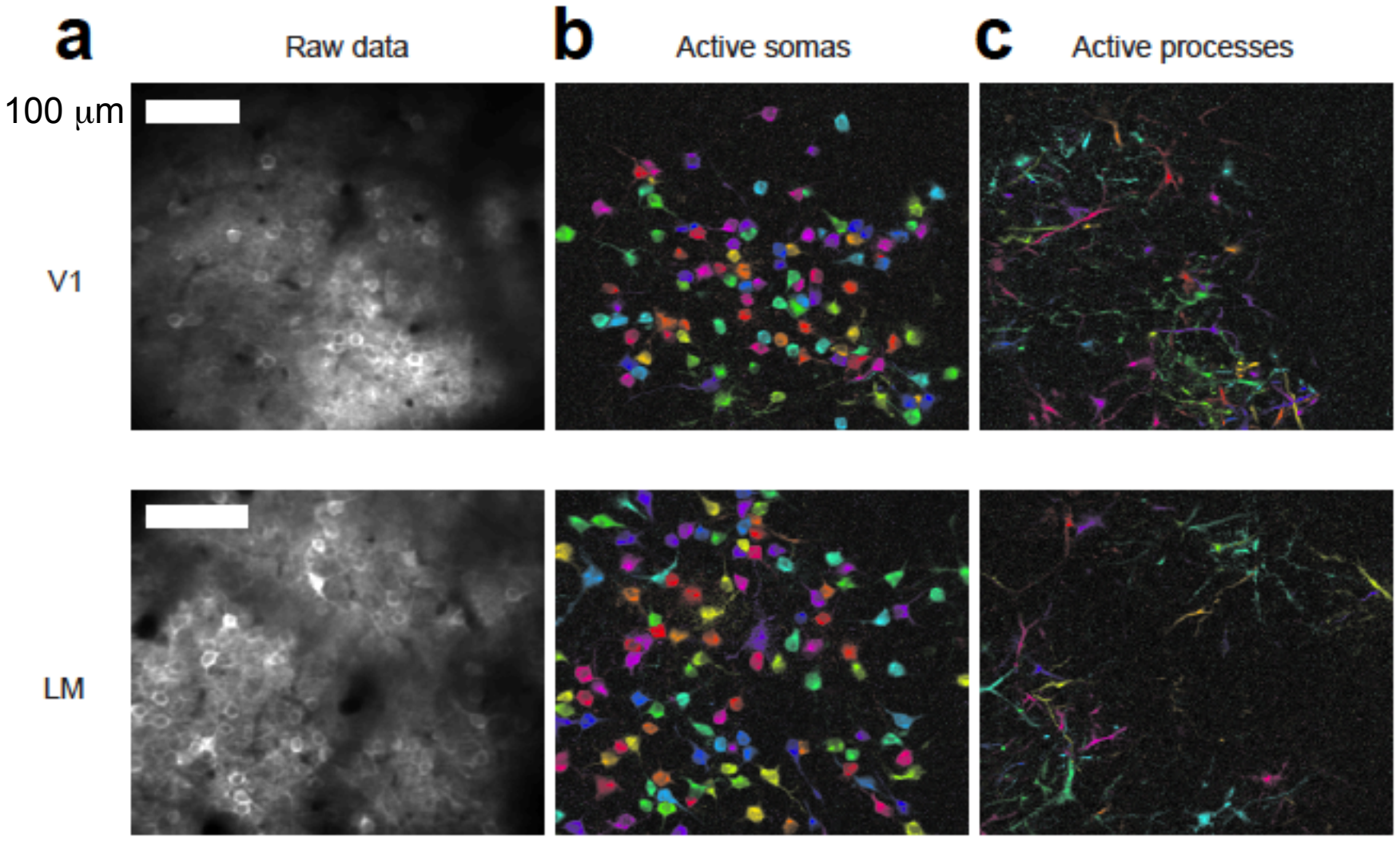
V1



LM

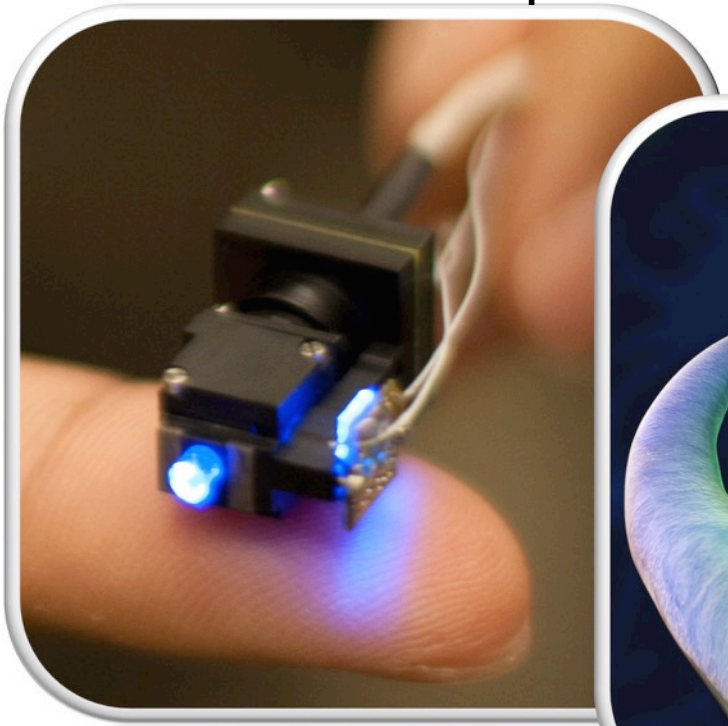
Jerome Lecoq

The Duopus allows Ca^{2+}-imaging in two brain areas in active mice

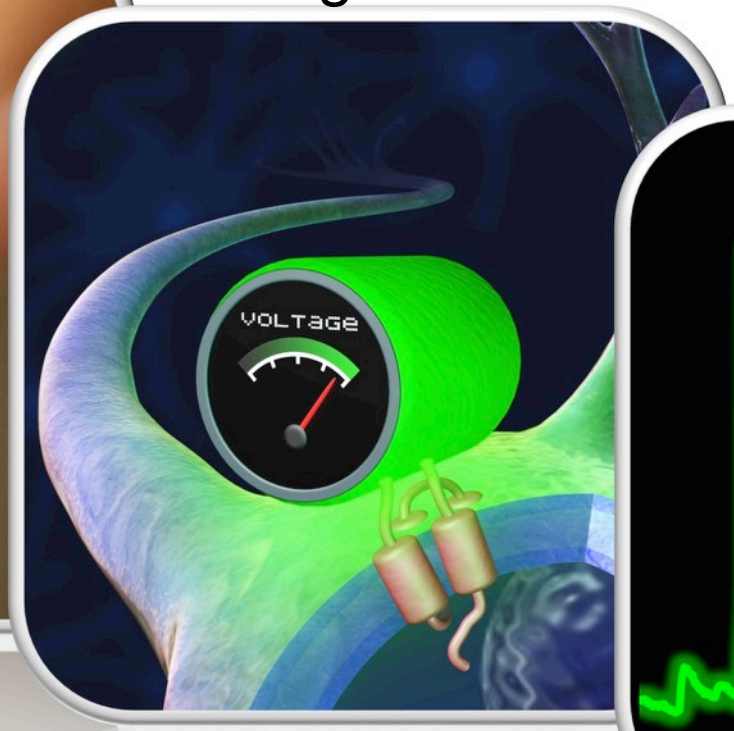


Are we on the brink of voltage-imaging in freely behaving animals?

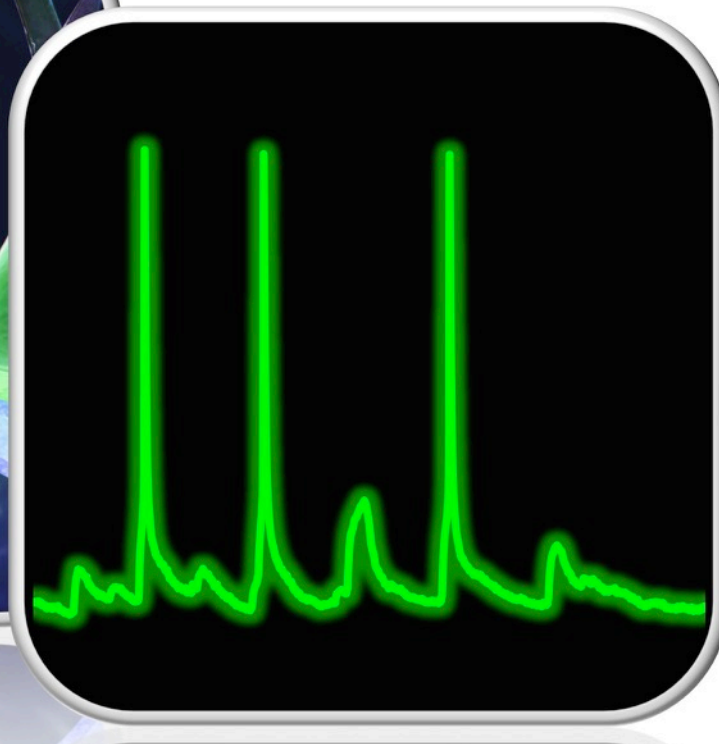
Mini-microscope



Voltage sensor



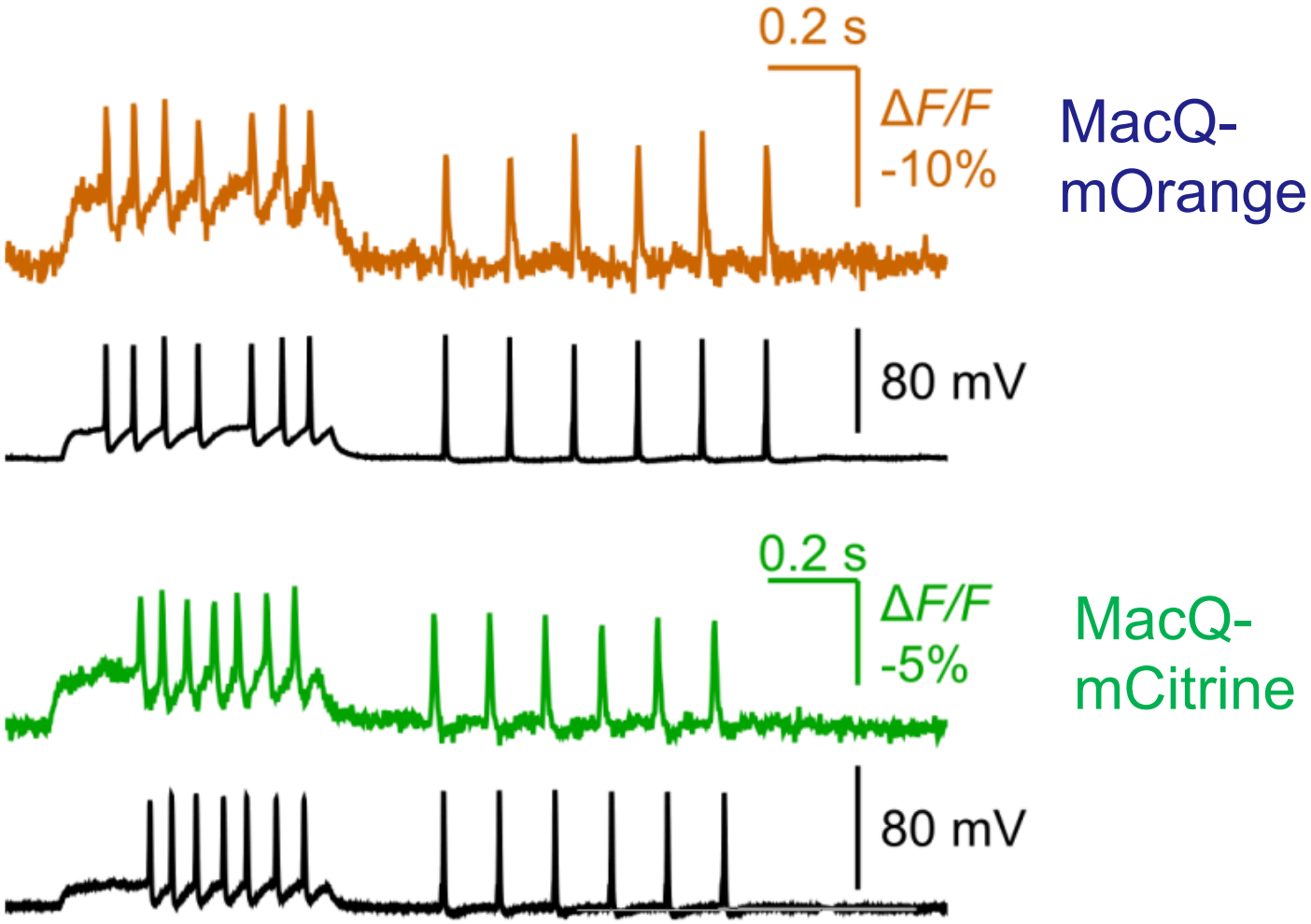
Fluorescence trace



NIH BRAIN Initiative grant

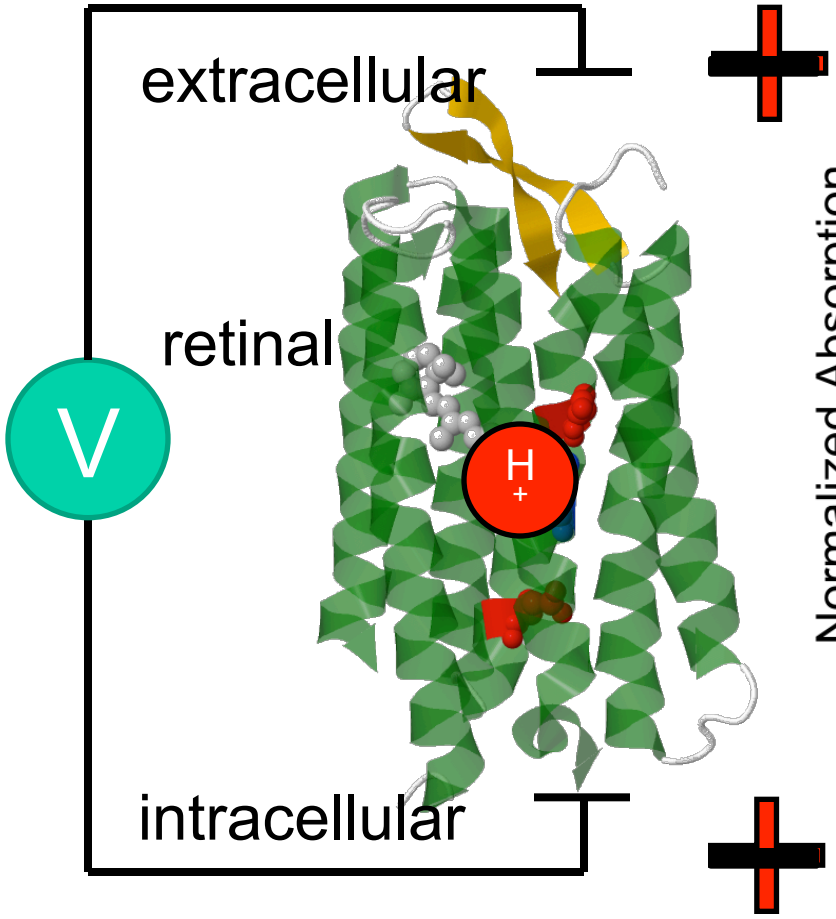
FRET-opsin voltage sensors detect spikes with high SNR using milliwatts of light

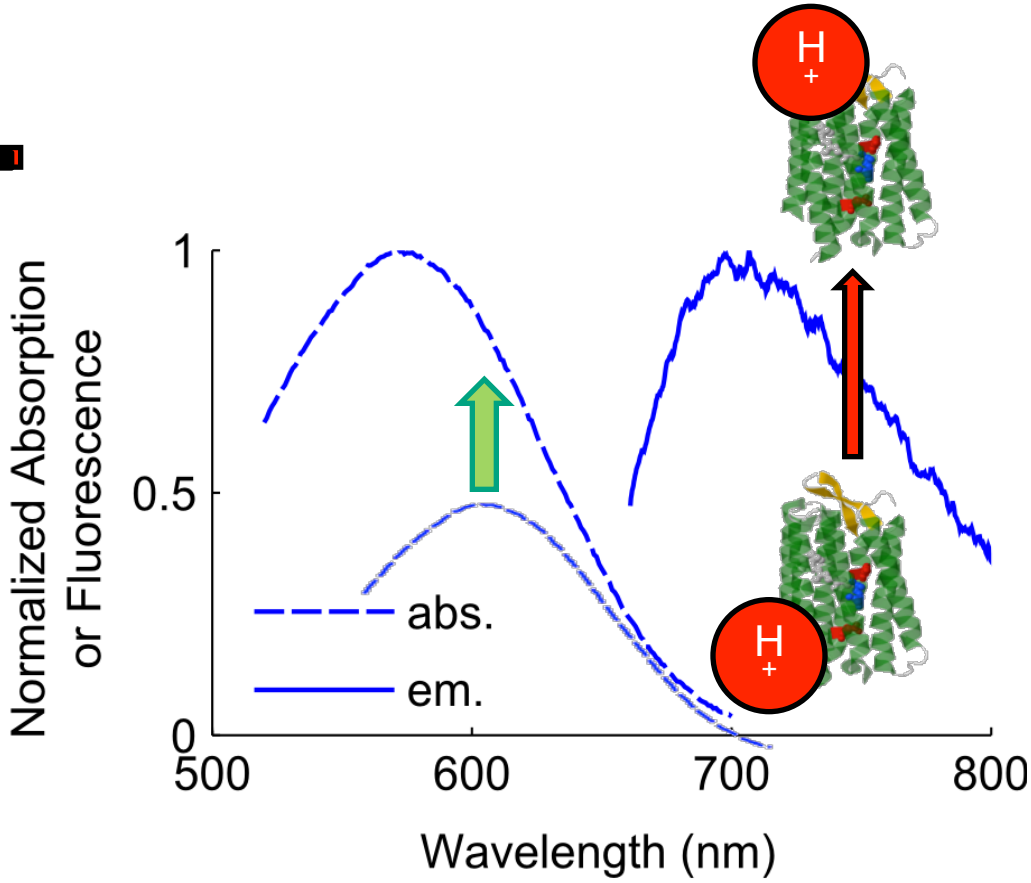
Yiyang Gong



Y. Gong *et. al*. *Nature Communications* (2014).

Archaerhodopsin acts as a voltage sensor by altering its light absorption

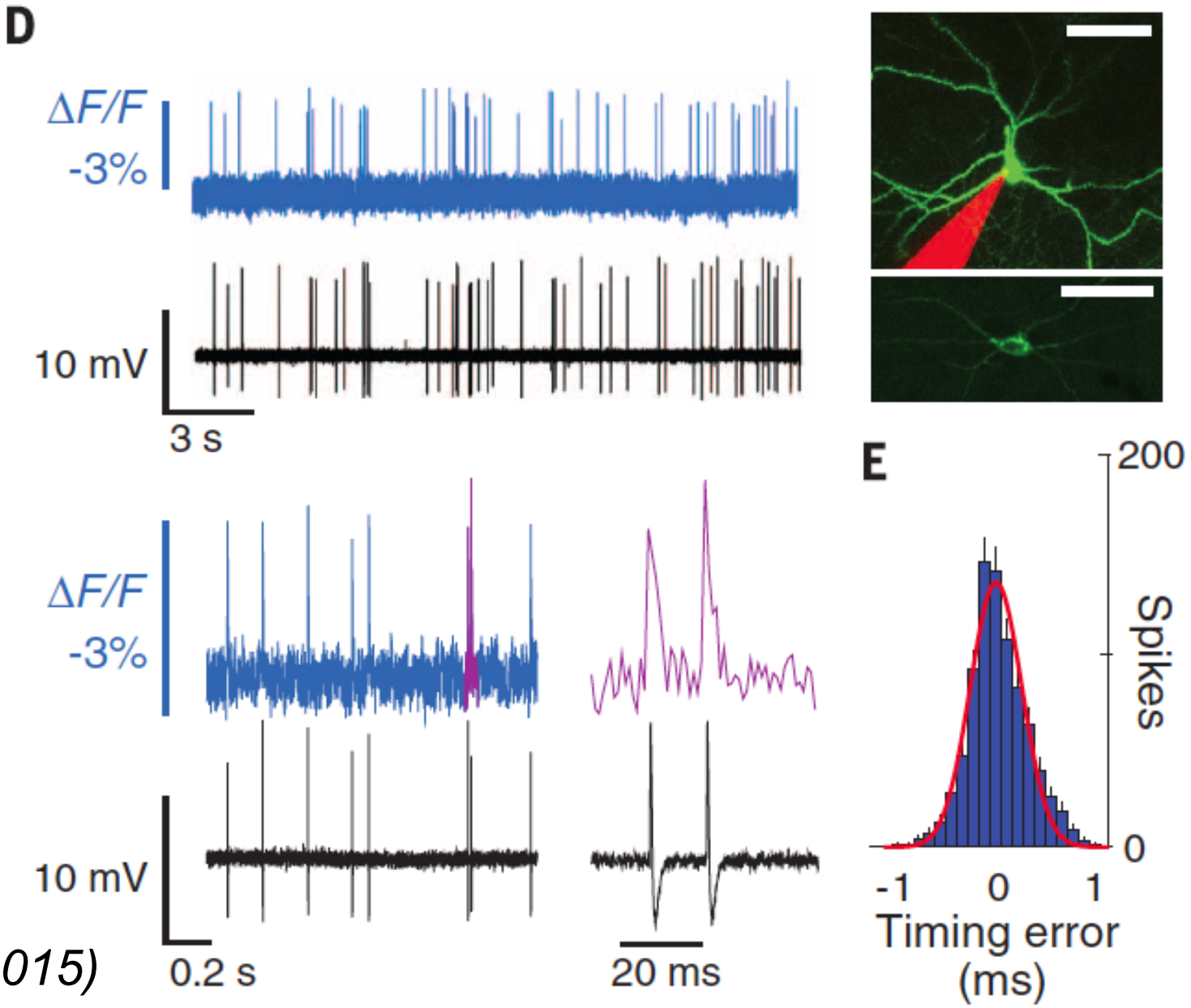




Bacteriorhodopsin, Alberts. *Molecular Biology of the Cell*

Kralj et. al. Nature Methods 9, 90-95 (2012).
Maclaurin et. al. PNAS 110, 5939-44 (2013).

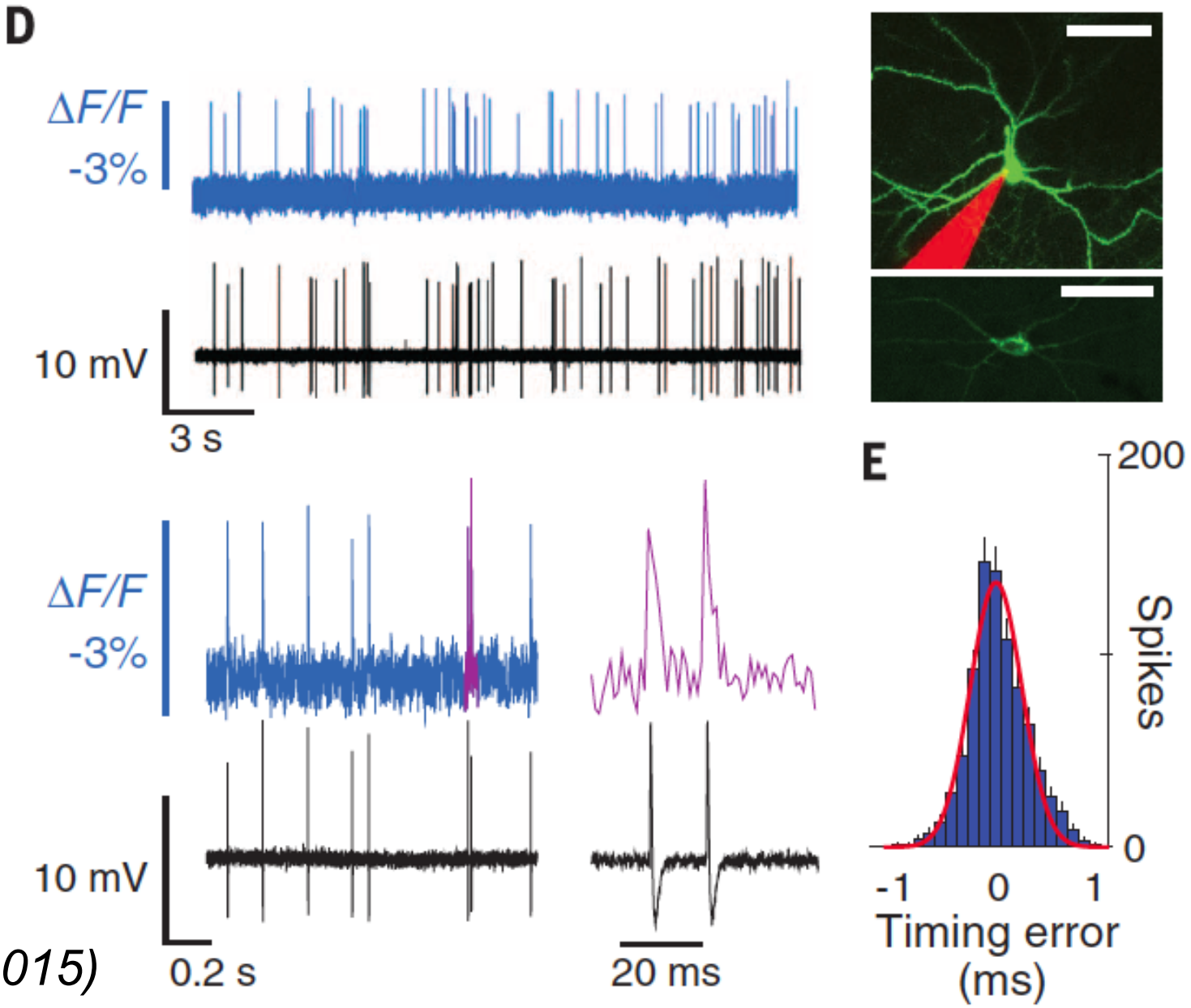
Single spike sensitivity in awake mice



Yiyang Gong

Science (2015)

200 μs spike timing precision



Yiyang Gong

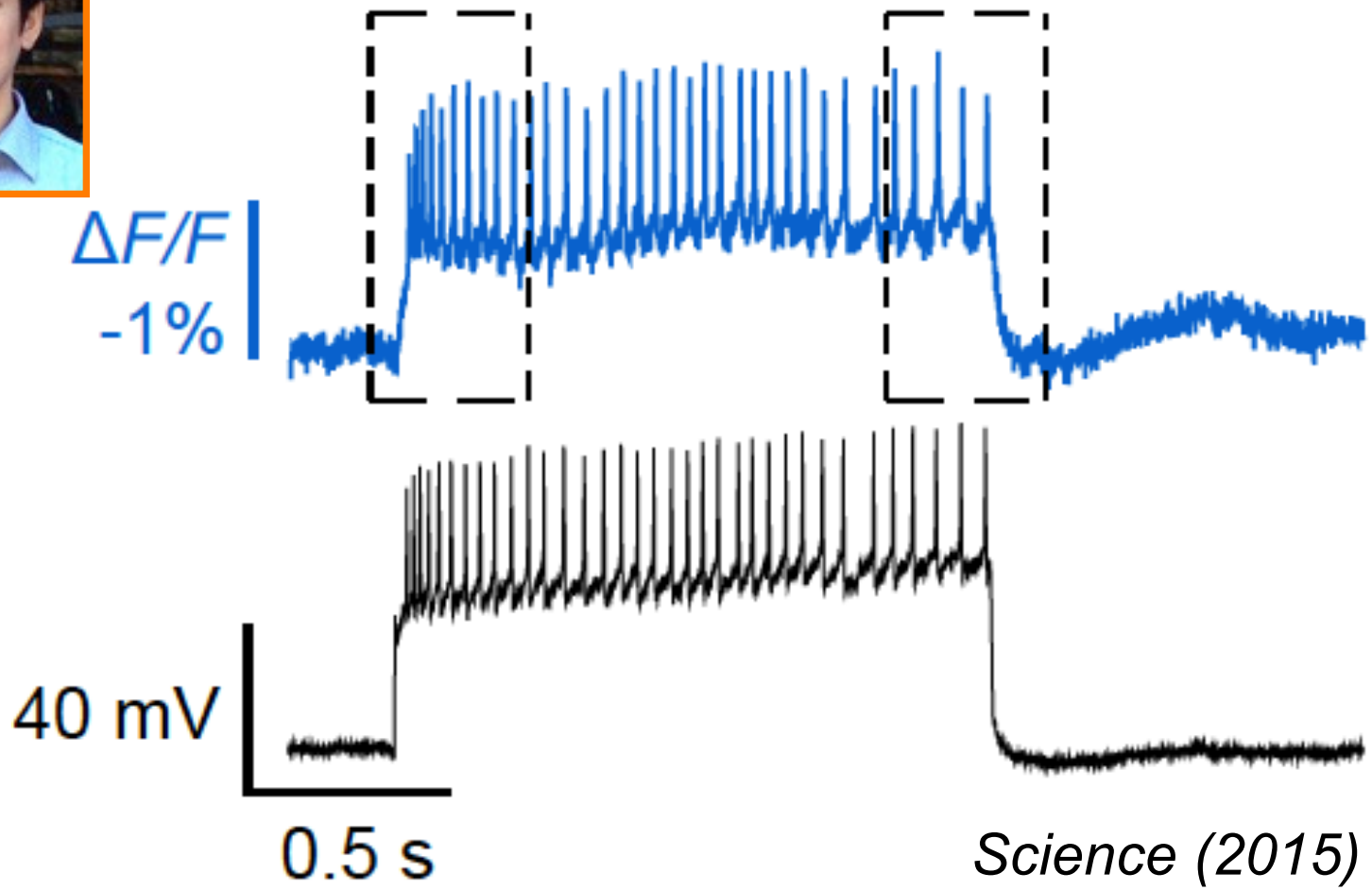
Science (2015)

Dual FRET-opsin and electrical recordings of neural spiking in the intact fly brain

Cheng Huang

Illumination:

20 mW · mm^{-2}



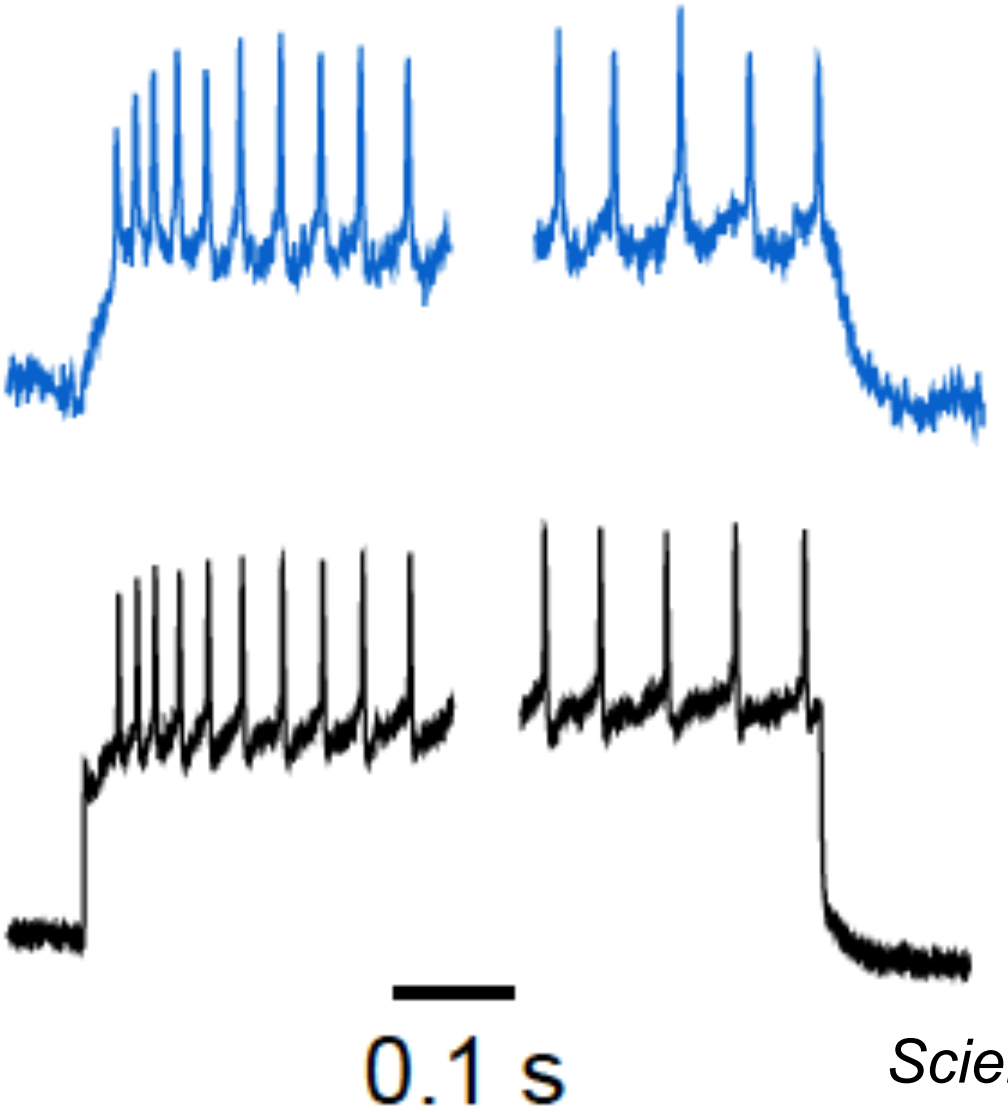
Science (2015)

FRET-opsin voltage imaging reveals sub-threshold voltage dynamics in fly brain

Cheng Huang

Illumination:

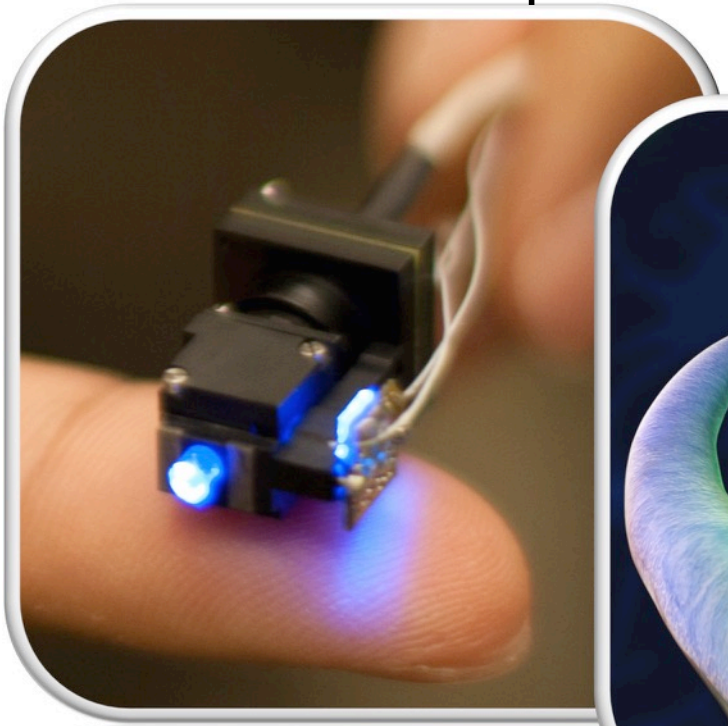
20 mW · mm^{-2}



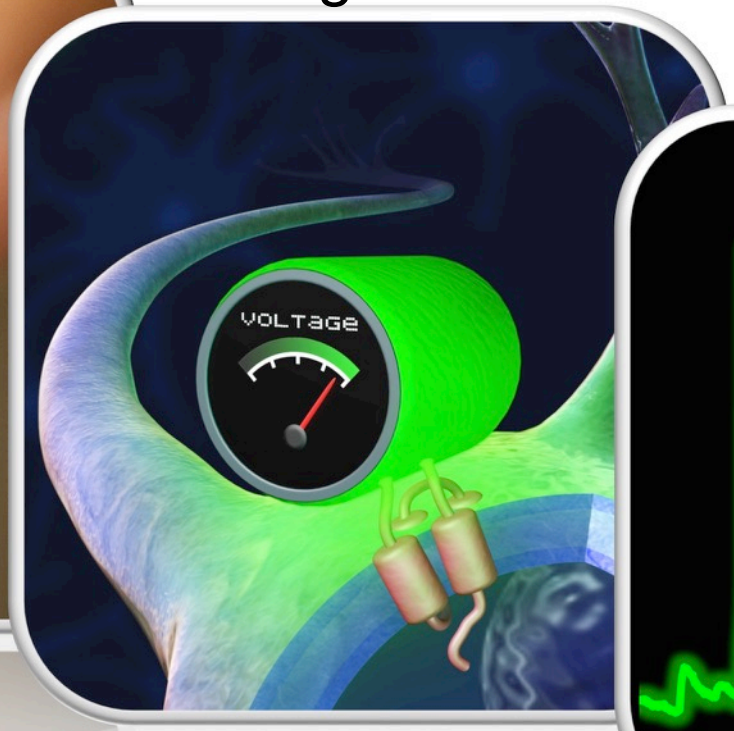
Science (2015)

Are we on the brink of voltage-imaging in freely behaving animals?

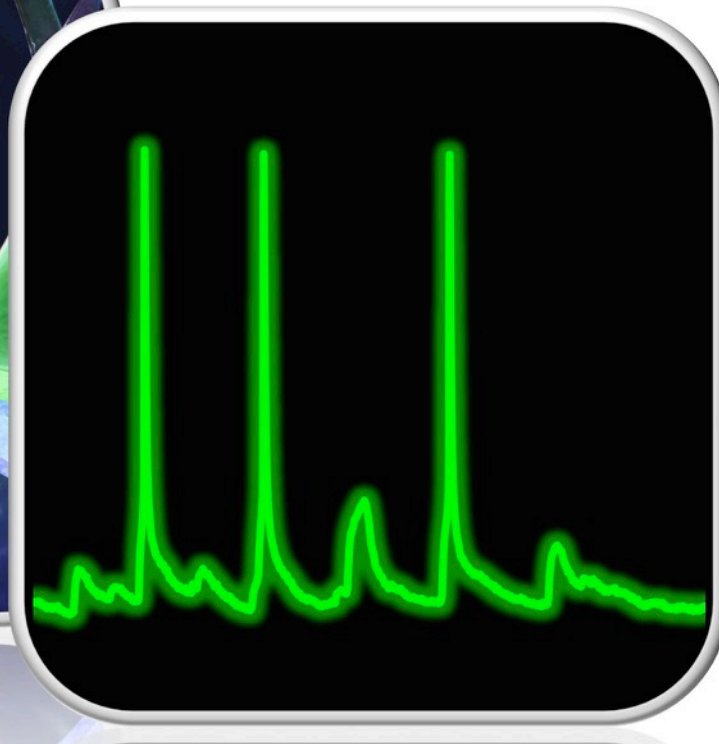
Mini-microscope



Voltage sensor



Fluorescence trace



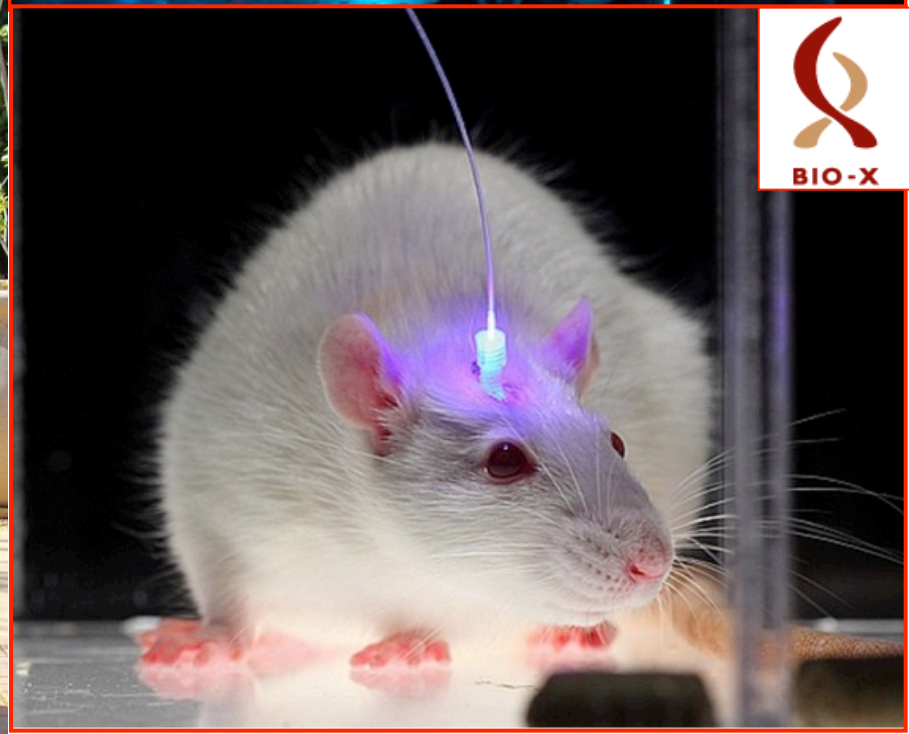
NIH BRAIN Initiative grant

HHMI

Thank you !

Pablo Jercog
Jerome Lecoq
Jesse Marshall
Jones Parker
Jin Zhong Li
Maggie Carr Larkin
Francois Grenier
Lacey Kitch

HHMI
NINDS, NIMH, NIH BRAIN
DARPA



Thank you!