

# **IN VIVO CA<sup>2+</sup> RECORDING**

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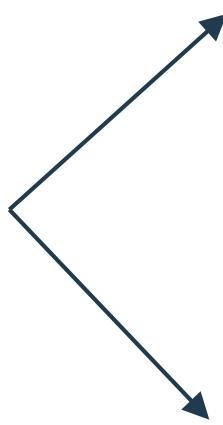
**PhD COURSE: ANIMAL MODELS OF DISEASE AND BEHAVIORAL ANALYSIS  
15.9.2022**

- 1. GENETICALLY ENCODED  $\text{Ca}^{2+}$  INDICATORS (GECIs)**
- 2.  $\text{Ca}^{2+}$  RECORDING IN FREELY MOVING MICE**
  - FIBER PHOTOMETRY**
  - ENDOSCOPIC  $\text{Ca}^{2+}$  IMAGING**
- 3. EXAMPLES**

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

*a proxy for neural activity*



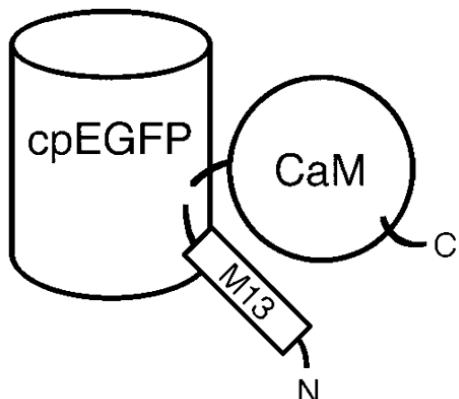
### Chemical $\text{Ca}^{2+}$ Indicators

- based on the EGTA homologue BAPTA ( $\text{Ca}^{2+}$  chelators)
- bathed onto tissue (transient)
- no cell specificity (neurons vs. astrocytes, neural subtypes)

### Genetically encoded calcium indicators (GECIs)

- based on cpEGFP
- genetically expressed (stable)
- option for cell type specificity

# GCaMP $\text{Ca}^{2+}$ SENSOR

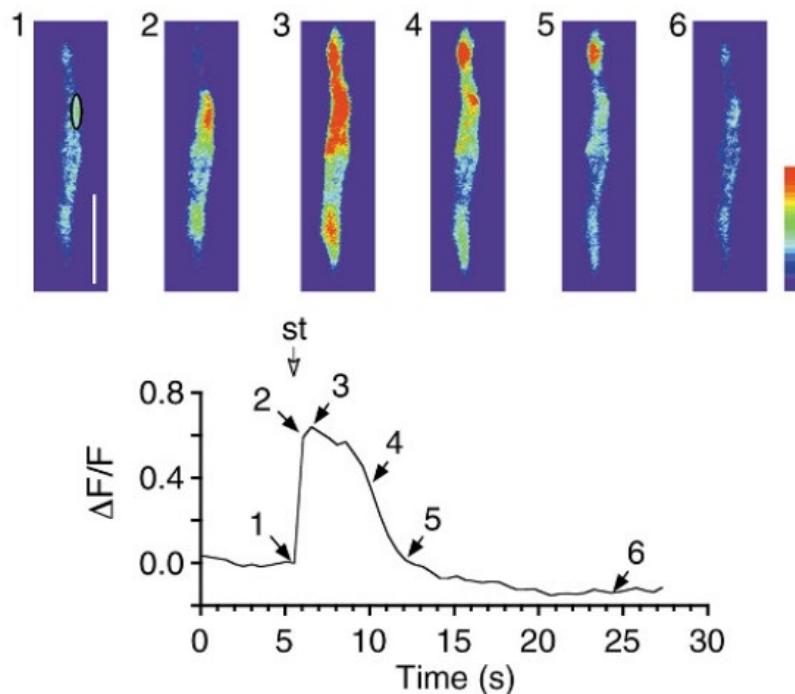


GCaMP consists of 3 parts:

- circularly permuted EGFP (cpEGFP)
- Calmodulin (CaM) domain
- M13 fragment of myosin light chain kinase

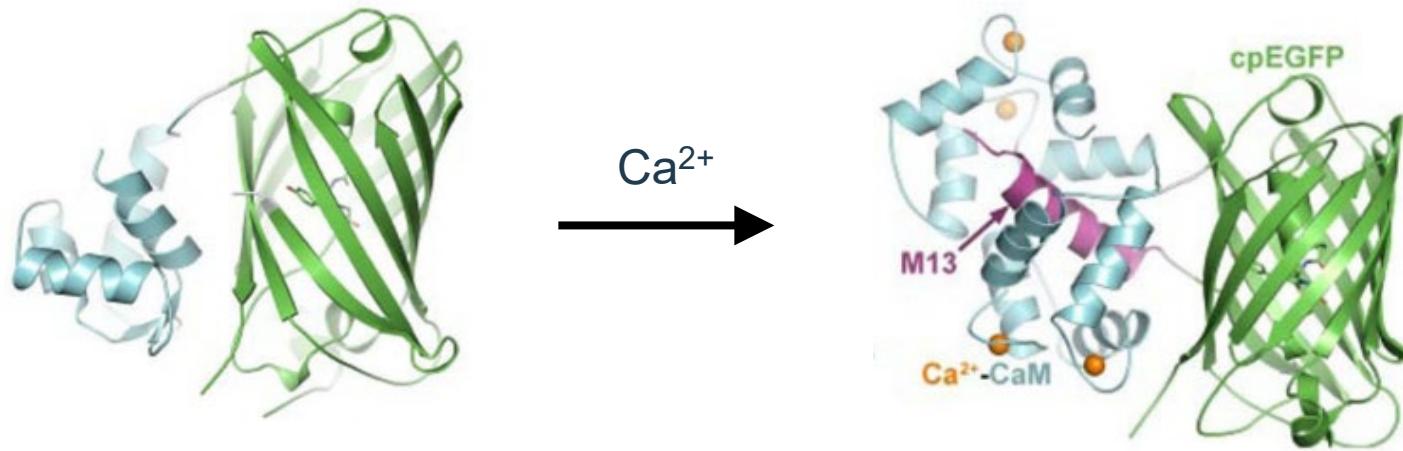
Ca<sup>2+</sup> sensing by GCaMP:

- cpEGFP does not fluoresce
- increases in  $[\text{Ca}^{2+}]_i$  cause CaM to bind  $\text{Ca}^{2+}$
- CaM associates with M13
- conformational change in cpEGFP induces GFP fluorescence
- decreases in  $[\text{Ca}^{2+}]_i$  reverse process



$$\Delta F/F = \frac{F - F_0}{F_0}$$

# CRYSTAL STRUCTURE OF GCAMP ± Ca<sup>2+</sup>



# IMPROVING GCAMP SIGNAL-TO-NOISE RATIO

GCaMP3 variants  
-site directed mutagenesis  
-4-6x SNR improvement

GCaMP5 variants  
-site directed mutagenesis  
+ high throughput screening  
-2-3x SNR improvement

GCaMP6 variants  
-imaging of dendritic spines

GCaMP7 variants  
-detection of individual spikes

ARTICLES  
**Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators**

Lin Tian<sup>1</sup>, S Andrew Hires<sup>1</sup>, Tianyi Mao<sup>1</sup>, Daniel Huber<sup>1</sup>, M Eugenia Chiappe<sup>1</sup>, Sreekanth H Chalasani<sup>2</sup>, Leopoldo Petreanu<sup>1</sup>, Jasper Akerboom<sup>1</sup>, Sean A McKinney<sup>1,4</sup>, Eric R Schreiter<sup>3</sup>, Cornelia I Bargmann<sup>2</sup>, Vivek Jayaraman<sup>1</sup>, Karel Svoboda<sup>1</sup> & Loren L Looger<sup>1</sup>

Nat Methods, 2009

Cellular/Molecular

Optimization of a GCaMP Calcium Indicator for Neural Activity Imaging

Jasper Akerboom,<sup>1\*</sup> Tsai-Wen Chen,<sup>1,\*</sup> Trevor J. Wardill,<sup>1</sup> Lin Tian,<sup>1</sup> Jonathan S. Marvin,<sup>1</sup> Sevinç Mutlu,<sup>1,2</sup> Nicole Carreras Calderón,<sup>3,4</sup> Federico Esposti,<sup>3</sup> Bart G. Borghuis,<sup>1,5</sup> Xiaonan Richard Sun,<sup>6</sup> Andrew Gordus,<sup>7</sup> Michael B. Orger,<sup>2,8</sup> Ruben Portugues,<sup>8</sup> Florian Engert,<sup>8</sup> John J. Macklin,<sup>1</sup> Alessandro Filosa,<sup>9</sup> Aman Aggarwal,<sup>1,10</sup> Rex A. Kerr,<sup>1</sup> Ryousuke Takagi,<sup>11</sup> Sebastian Kracun,<sup>11</sup> Eiji Shigetomi,<sup>11</sup> Baljit S. Khakh,<sup>11</sup> Herwig Baier,<sup>9</sup> Leon Lagnado,<sup>3</sup> Samuel S.-H. Wang,<sup>8</sup> Cornelia I. Bargmann,<sup>2</sup> Bruce E. Kimmel,<sup>1</sup> Vivek Jayaraman,<sup>1</sup> Karel Svoboda,<sup>1</sup> Douglas S. Kim,<sup>1</sup> Eric R. Schreiter,<sup>1,4</sup> and Loren L. Looger<sup>1</sup>

J Neurosci, 2012

ARTICLE

doi:10.1038/nature12354

Ultrasensitive fluorescent proteins for imaging neuronal activity

Tsai-Wen Chen<sup>1</sup>, Trevor J. Wardill<sup>1,4</sup>, Yi Sun<sup>1</sup>, Stefan R. Pulver<sup>1</sup>, Sabine L. Renninger<sup>2</sup>, Amy Baohan<sup>1,3</sup>, Eric R. Schreiter<sup>1</sup>, Rex A. Kerr<sup>1</sup>, Michael B. Orger<sup>2</sup>, Vivek Jayaraman<sup>1</sup>, Loren L. Looger<sup>1</sup>, Karel Svoboda<sup>1</sup> & Douglas S. Kim<sup>1</sup>

Nature, 2013

nature|methods

ARTICLES

<https://doi.org/10.1038/s41592-019-0435-6>

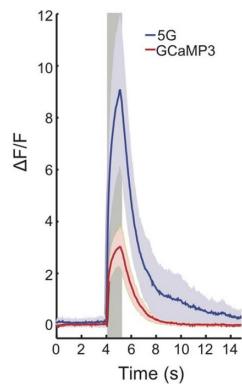
High-performance calcium sensors for imaging activity in neuronal populations and microcompartments

Hod Dana<sup>1,3,7</sup>, Yi Sun<sup>1,4,7</sup>, Boaz Mohar<sup>1,7</sup>, Brad K. Hulse<sup>1,7</sup>, Aaron M. Kerlin<sup>1,6</sup>, Jeremy P. Hasseman<sup>1</sup>, Getahun Tsegaye<sup>1</sup>, Arthur Tsang<sup>1</sup>, Allan Wong<sup>1</sup>, Ronak Patel<sup>1</sup>, John J. Macklin<sup>1</sup>, Yang Chen<sup>1</sup>, Arthur Konnerth<sup>2</sup>, Vivek Jayaraman<sup>1,8\*</sup>, Loren L. Looger<sup>1,9\*</sup>, Eric R. Schreiter<sup>1</sup>, Karel Svoboda<sup>1,10\*</sup> and Douglas S. Kim<sup>1,6</sup>

Nat Methods, 2019

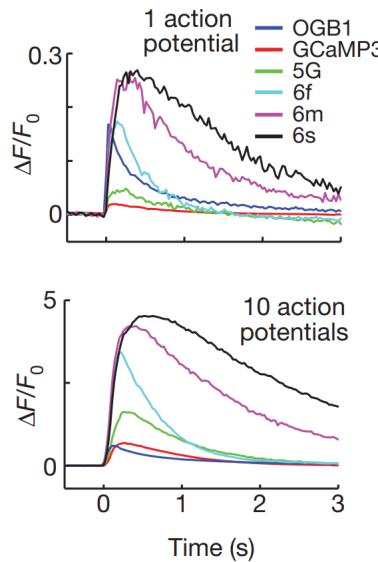
# IMPROVING GCAMP SIGNAL-TO-NOISE RATIO

GCaMP 5 vs. 3



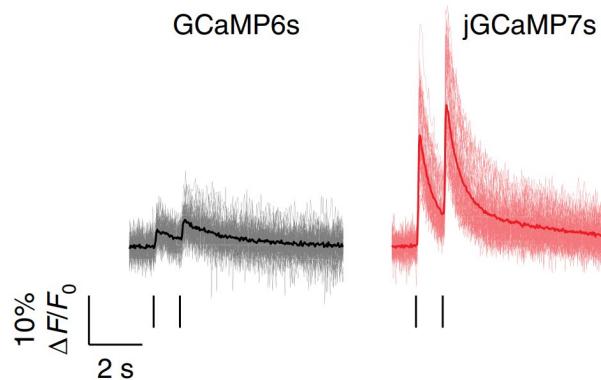
Akerboom, et al. *J Neurosci*, 2012

GCaMP 6 vs. 5



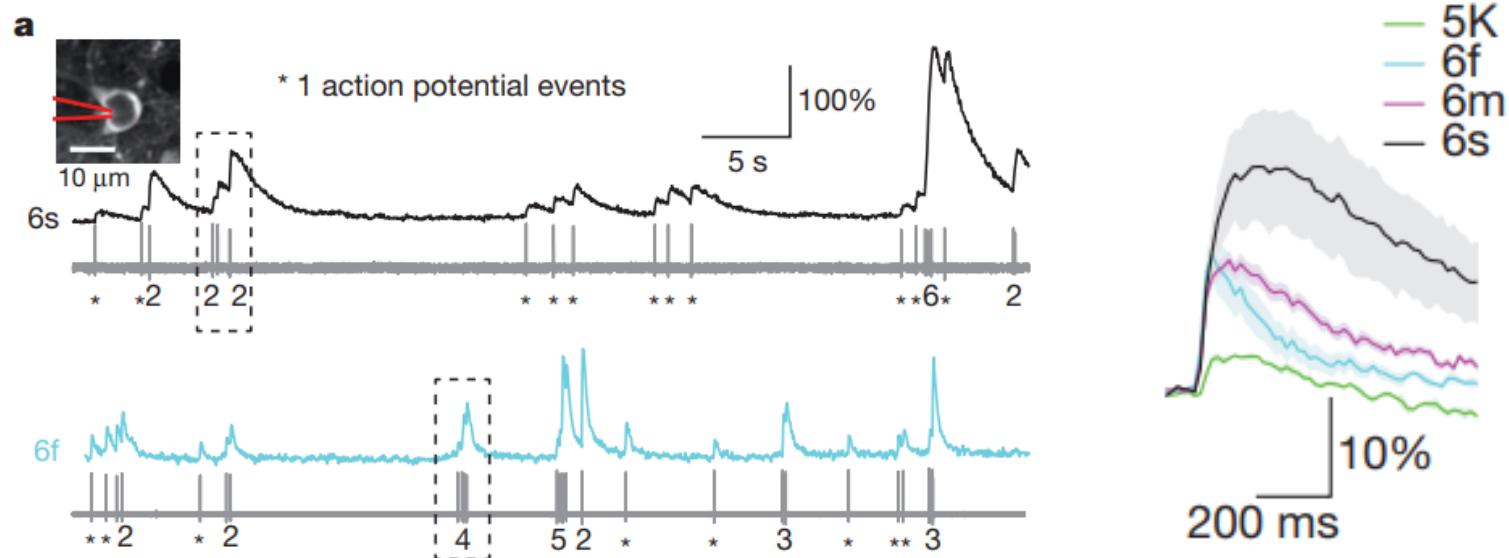
Chen TW, et al.  
*Nature*, 2013

GCaMP 7 vs. 6



Dona H, et al. *Nat Methods*, 2019

# GCAMP VARIANTS: SLOW AND FAST



Chen TW, et al. *Nature*, 2013

-Slow variants have higher signal-to-noise ratio, but have extended decay

-Fast variants have lower signal-to-noise ratio, but decay faster

# IMPROVING GCAMP TEMPORAL SENSITIVITY

GCaMP8 variants  
-rise time of 2 ms –  
exquisite time sensitivity



bioRxiv  
THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

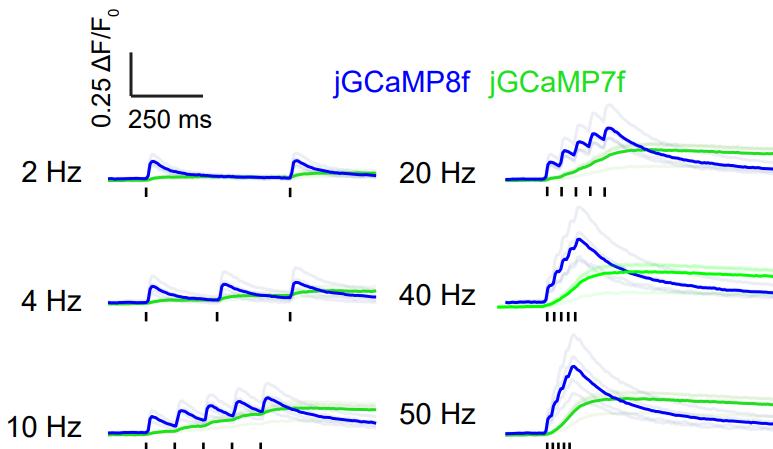
New Results

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## Fast and sensitive GCaMP calcium indicators for imaging neural populations

Yan Zhang, Márton Rózsa, Yajie Liang, Daniel Bushey, Ziqiang Wei, Jihong Zheng, Daniel Reep, Gerard Joey Broussard, Arthur Tsang, Getahun Tsegaye, Sujatha Narayan, Christopher J. Obara, Jing-Xuan Lim, Ronak Patel, Rongwei Zhang, Misha B. Ahrens, Glenn C. Turner, Samuel S.-H. Wang, Wyatt L. Korff, Eric R. Schreiter, Karel Svoboda, Jeremy P. Hasseman, Ilya Kolb, Loren L. Looger

BioRxiv, 2021



Example of temporal sensitivity (Zhang et al, 2021)

# RED SHIFTED $\text{Ca}^{2+}$ GECIs

eLife

NEWSLETTER ABOUT

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Tools and Resources  
Neuroscience

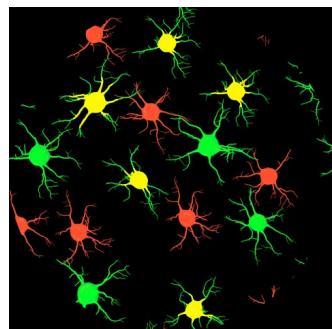
## Sensitive red protein calcium indicators for imaging neural activity

Hod Dana , Boaz Mohar, Yi Sun, Sujatha Narayan, Andrew Gordus, Jeremy P Hasseman, Getahun Tsegaye, Graham T Holt, Amy Hu, Deepika Walpita, Ronak Patel, John J Macklin, Cornelia I Bargmann, Misha B Ahrens, Eric R Schreiter, Vivek Jayaraman, Loren L Looger, Karel Svoboda, Douglas S Kim  « see less



V1 axons (GCaMP)  
LM dendrites (JRGECHO1a)

Dona H, et al. eLife, 2016



Green/Red GECIs allow simultaneous  $\text{Ca}^{2+}$  recording from 2-3 specific neuronal populations

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  - ENDOSCOPIC  $\text{Ca}^{2+}$  IMAGING**
- 3. EXAMPLES**

# DEEP BRAIN RECORDING OF $\text{Ca}^{2+}$ ACTIVITY

- $\text{Ca}^{2+}$  imaging has historically been limited to cortical surfaces (cortex and cerebellum) in head-fixed mice
- How does activity differ when animals can move freely? More complex behavior?
- How about  $\text{Ca}^{2+}$  activity in deep brain regions?

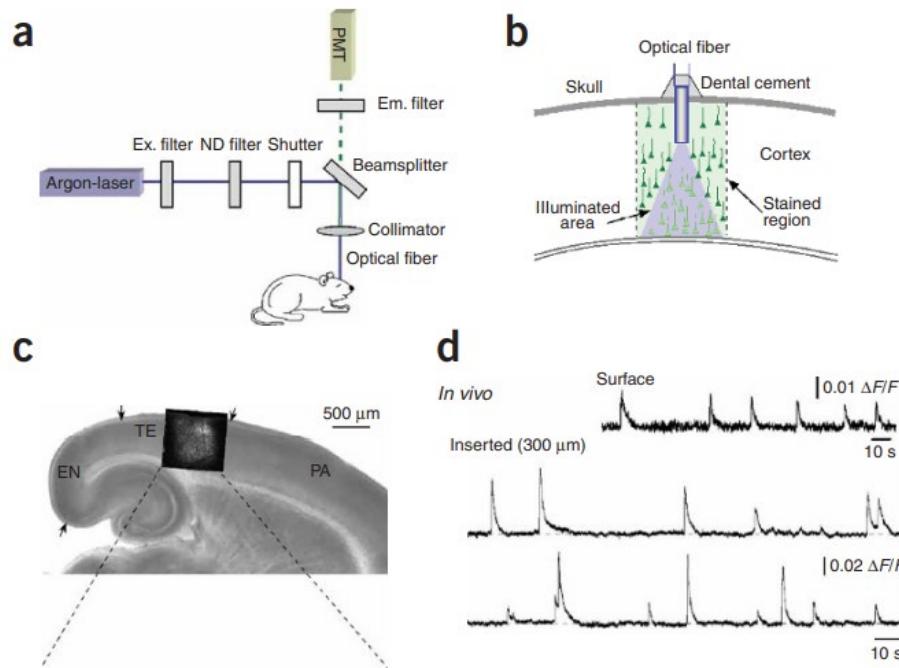
# FIBER PHOTOMETRY OF $\text{Ca}^{2+}$ ACTIVITY

## BRIEF COMMUNICATIONS

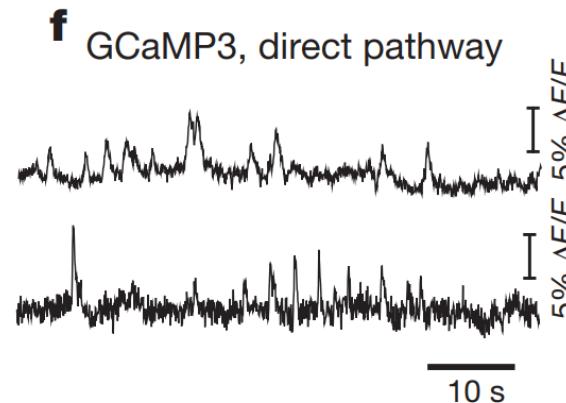
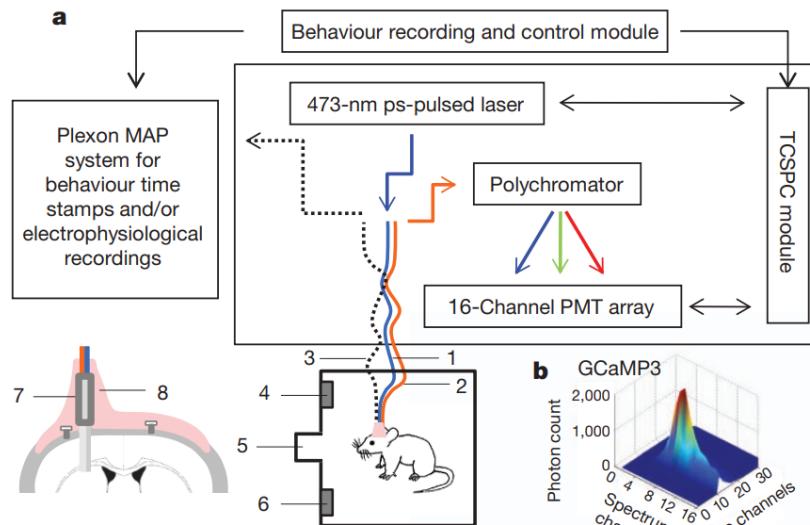
Cortical calcium waves in resting newborn mice

Helmut Adelsberger<sup>1,2</sup>, Olga Garaschuk<sup>1,2</sup> & Arthur Konnerth<sup>1</sup>

*Nat Neurosci*, 2005



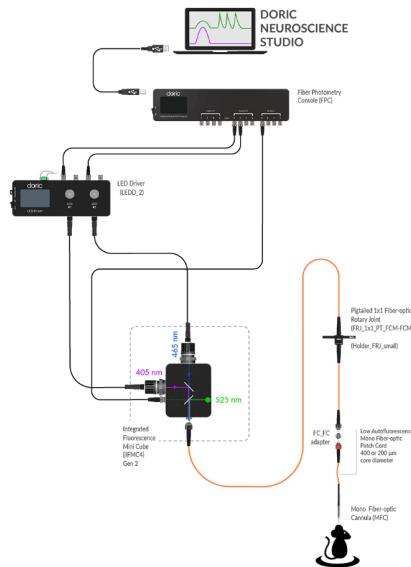
# FIBER PHOTOMETRY OF $\text{Ca}^{2+}$ ACTIVITY IN FREELY MOVING MICE



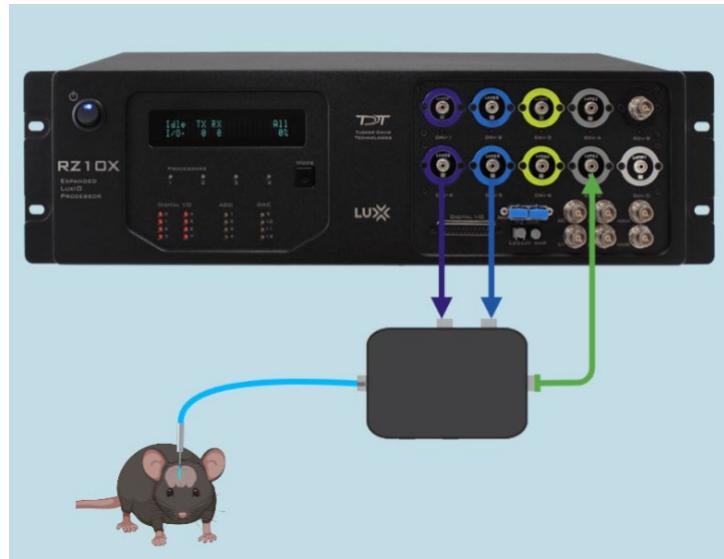
Cui G, et al. *Nature*, 2013  
Cui G, et al. *Nat Protoc*, 2014

# COMMERCIAL UNITS FOR FIBER PHOTOMETRY

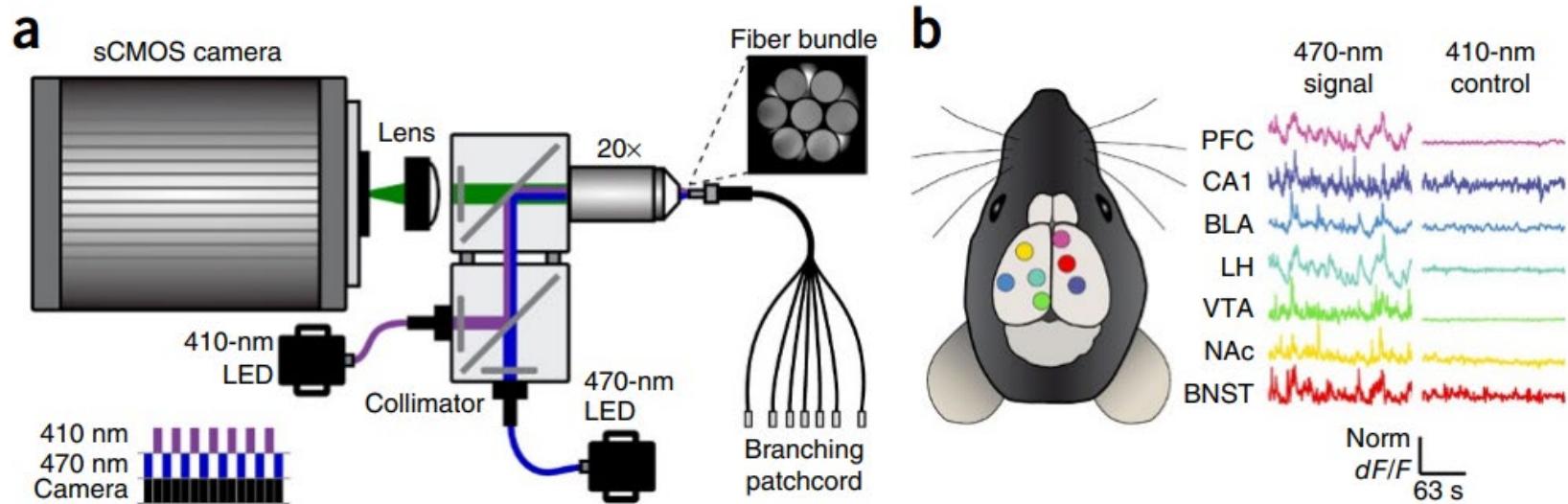
Doric Lenses



Tucker-Davis Technologies

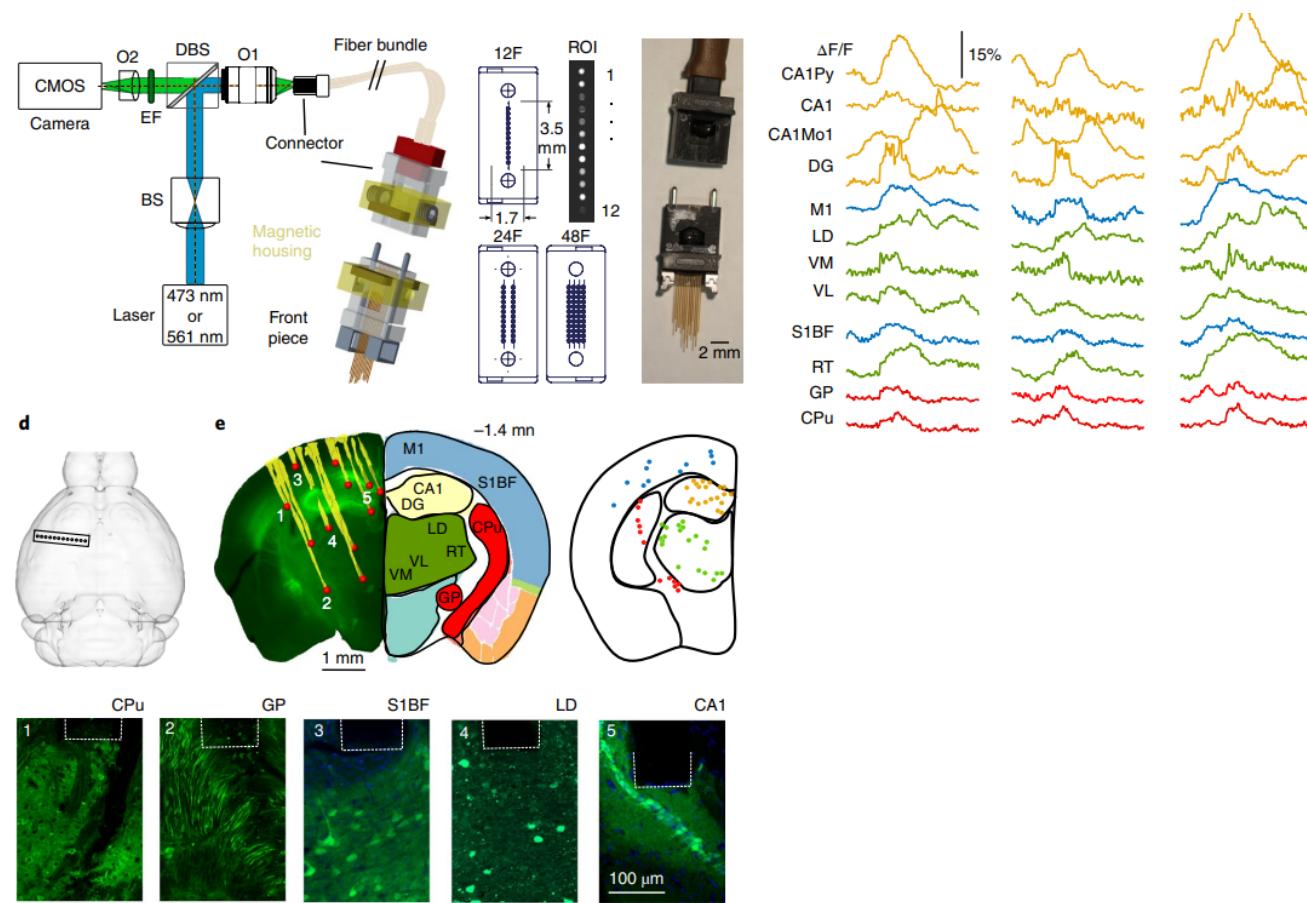


# MULTI-SITE FIBER PHOTOMETRY



Kim CK, et al. *Nat Methods*, 2016

# MULTI-SITE FIBER PHOTOMETRY (CONTINUED)



Sych Y, et al. *Nat Methods*, 2019

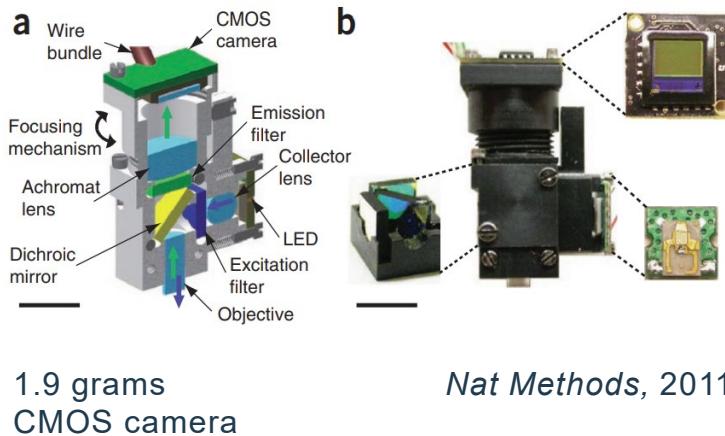
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# MINIATURE FLUORESCENCE MICROSCOPES

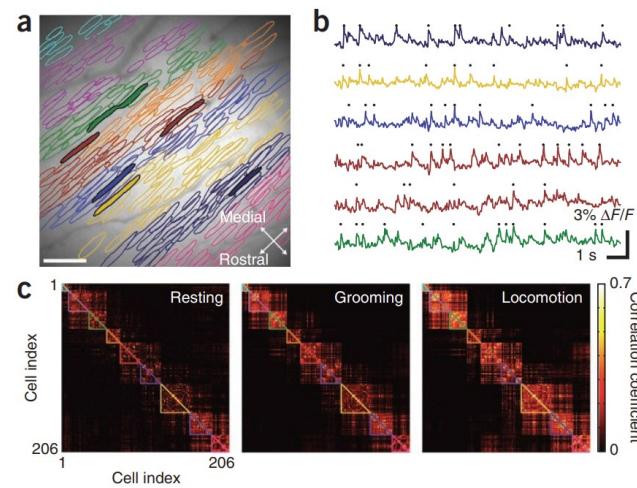
ARTICLES

## Miniaturized integration of a fluorescence microscope

Kunal K Ghosh<sup>1,2,5</sup>, Laurie D Burns<sup>2,5</sup>, Eric D Cocker<sup>2,5</sup>, Axel Nimmerjahn<sup>2</sup>, Yaniv Ziv<sup>2</sup>, Abbas El Gamal<sup>1</sup> & Mark J Schnitzer<sup>2-4</sup>

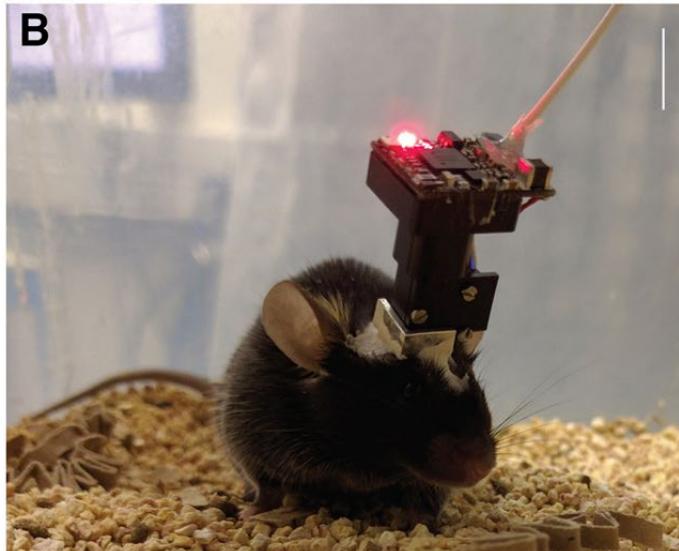
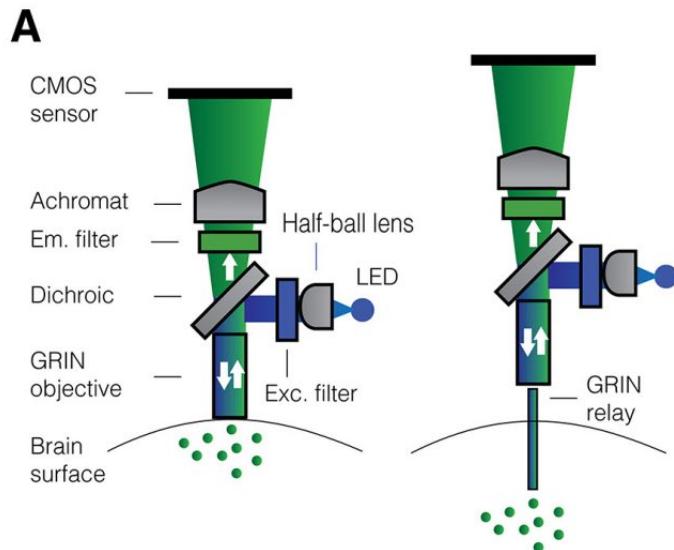


*Nat Methods*, 2011



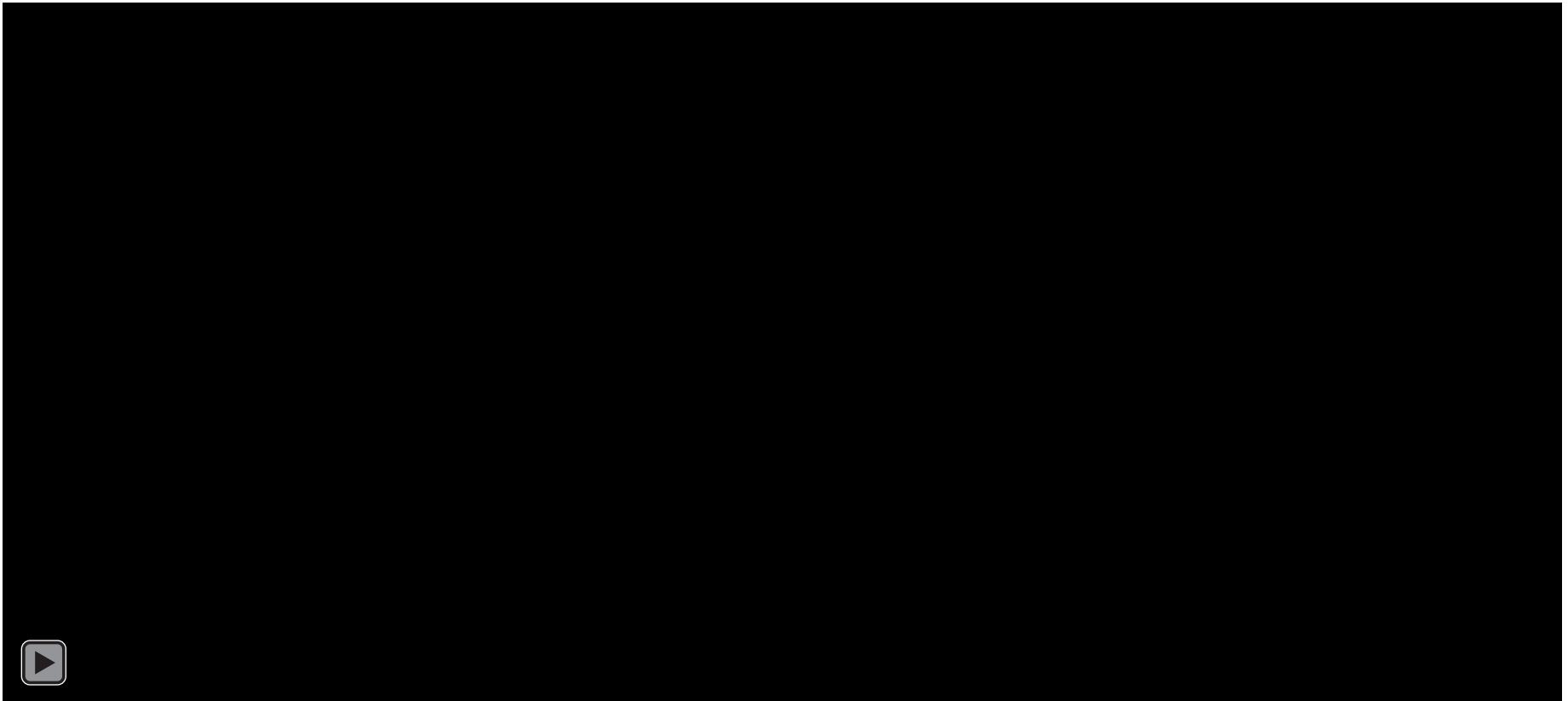
**iNSCOPIX**  
Palo Alto, CA

# SURFACE IMAGING VS. DEEP BRAIN IMAGING



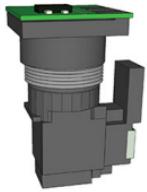
Aharoni and Hoogland, *Frontiers Cell Neurosci*, 2019

# EXAMPLE FOV - STRIATUM



Parker JG et al, *Nature*, 2018

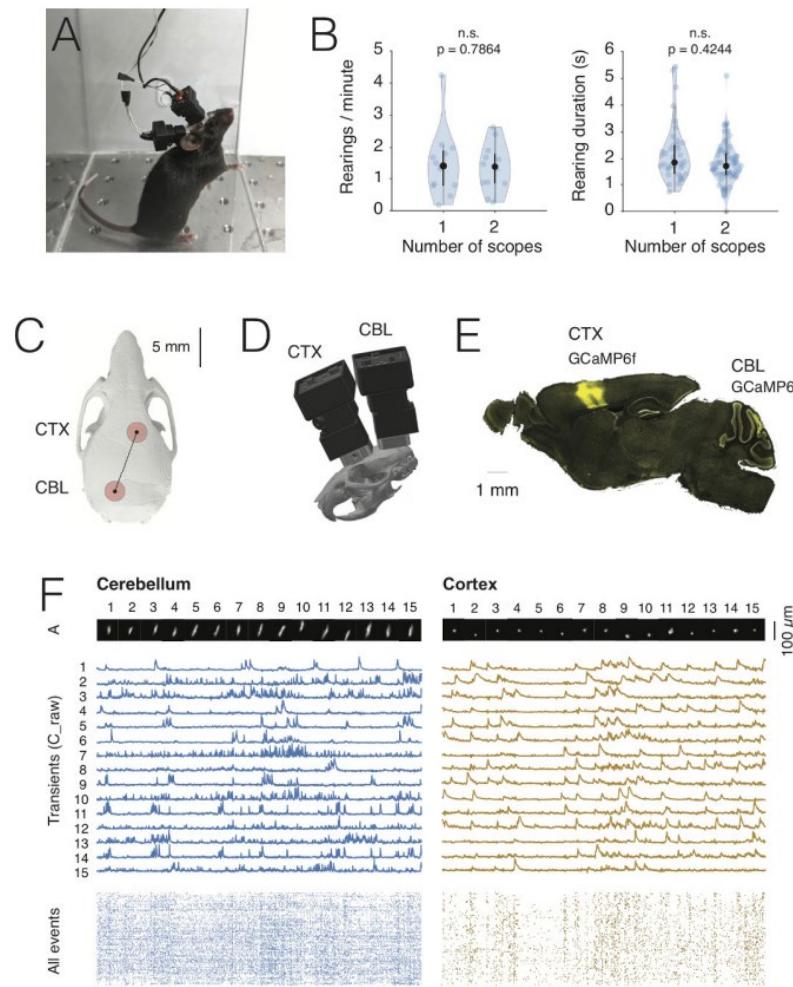
# OPEN-SOURCE MINISCOPES

A	B	C	D
			
FinchScope	miniScope	UCLA Miniscope	CHEndoscope
Dim: 10 x 6 x 21 mm Wired: 1.8 gram Wireless: ~ 4 gram FOV: 880 x 600 $\mu\text{m}$ Frame Rate: 30 Hz Focus: turret DAQ: Arduino Software: MacOS	Dim: 12 x 12 x 20 mm Wired: 2.4 gram FOV: 1.1 x 1.1 mm Frame Rate: 10 Hz Focus: turret DAQ: Opal Kelly Software: Win & Mac	Dim: 16.5 x 13 x 22.5 mm Wired: ~ 3 gram Wire-free: 4.5 gram FOV: 700 x 450 $\mu\text{m}$ Frame Rate: 60 Hz Focus: linear slider DAQ: custom PCB Software: Win	Dim: 15.9 x 17 x 32.5 mm Wired: 4.5 gram FOV: ~ 500 $\mu\text{m}$ across Frame Rate: 20 Hz Focus: turret DAQ: direct to PC Software: Win & Linux

**FIGURE 2 |** Open-source miniscopes released in the public domain. **(A)** FinchScope (<https://github.com/gardner-lab/FinchScope>), image credit: W.A. Liberti III. **(B)** miniScope ([https://github.com/giovannibarbera/miniscope\\_v1.0](https://github.com/giovannibarbera/miniscope_v1.0)). **(C)** UCLA Miniscope (<http://www.miniscope.org>). **(D)** CHEndoscope (<https://github.com/jf-lab/chendoscope>), image credit: A. Jacob, Josselyn lab.

Aharoni and Hoogland, *Frontiers Cell Neurosci*, 2019

# IMAGING WITH 2 MINISCOPES



De Groot A et al, *eLife*, 2020

## Fiber Photometry

Record population  $\text{Ca}^{2+}$  activity

Small footprint ( $0.2 \text{ mm } \varnothing$ )

Multicolor

Multiple sites (1-30)

Dendritic  $\text{Ca}^{2+}$  vs. Soma?

Lightweight (<0.1 gram)

Small surgery time

Less time-intensive data analysis

High success rate (>50%)

## Endoscopic $\text{Ca}^{2+}$ Imaging

Record single neuron  $\text{Ca}^{2+}$  activity

Large footprint ( $>0.5 \text{ mm } \varnothing$ )

Multicolor

1-2 sites (usually 1 site)

Resolution of neuronal compartments

Heavy (>1.5 g) + cement

Long surgery time

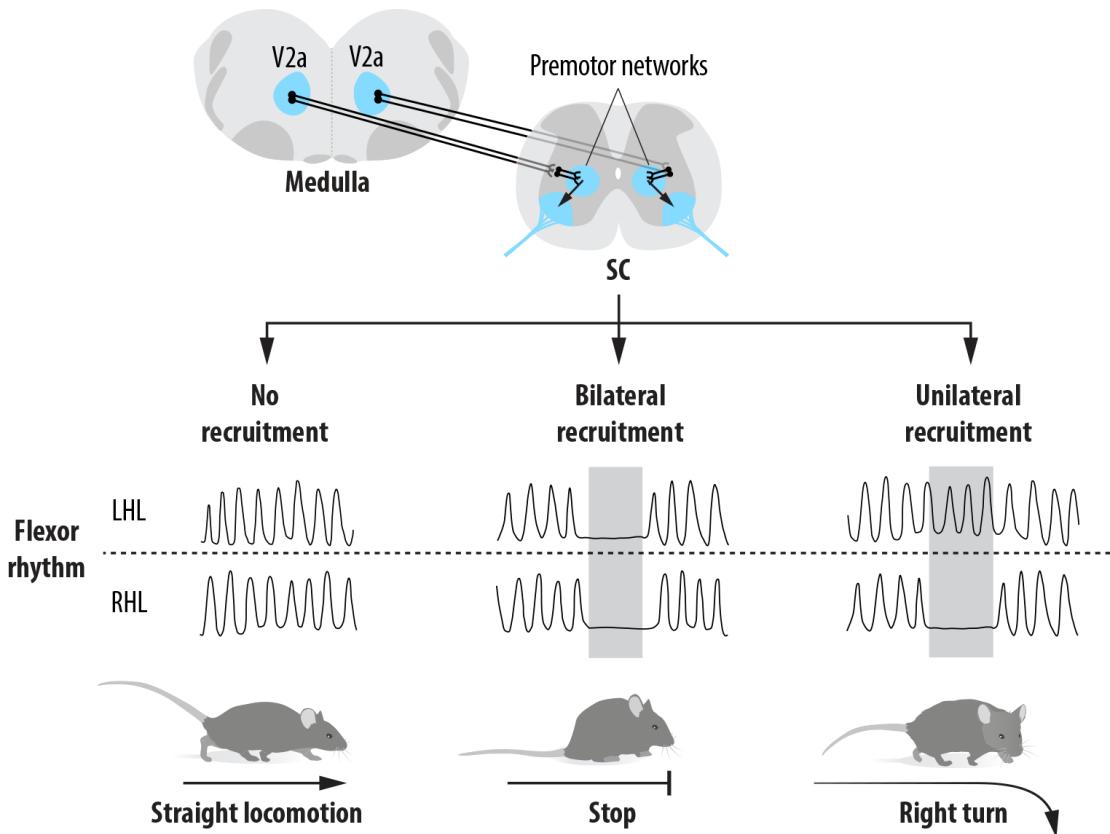
Time-intensive data analysis

Low success rate (<10%)

complementary approaches for understanding cell-type specific activity

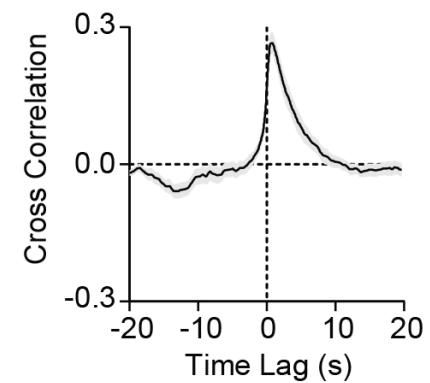
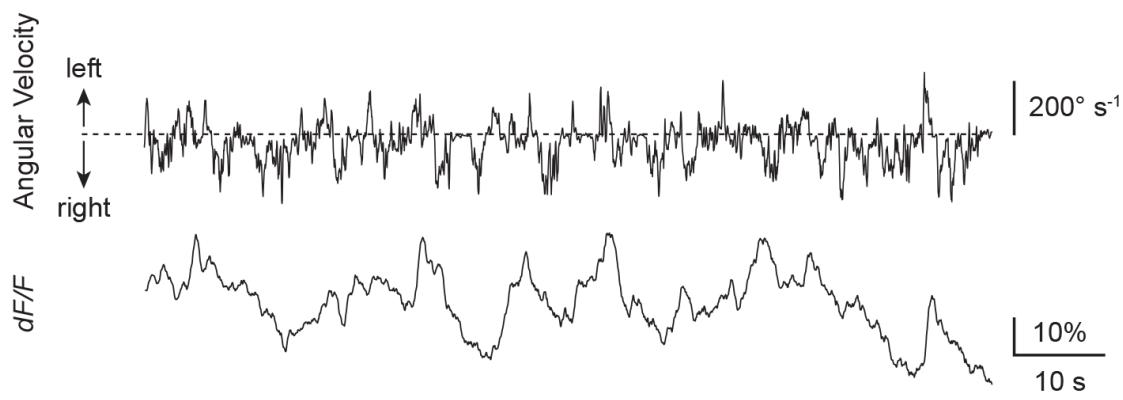
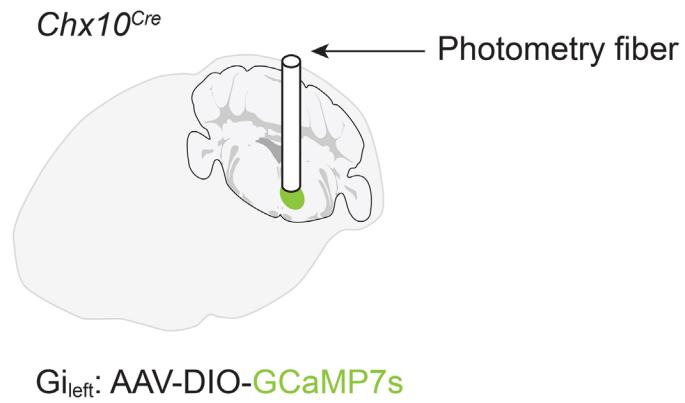
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# A BRAINSTEM COMMAND FOR LOCOMOTOR STOP & TURN

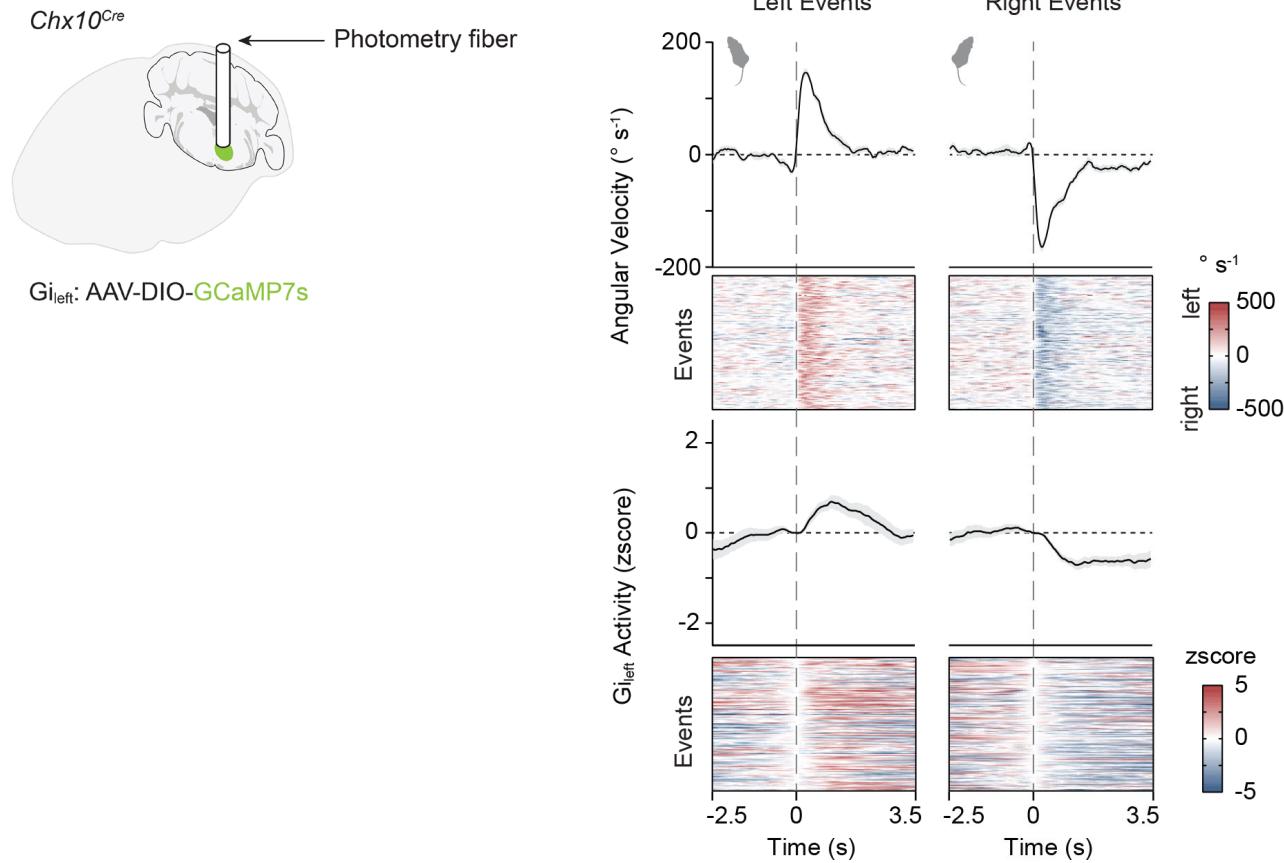


Bouvier et al, *Cell* 2015  
Cregg et al, *Nat Neurosci*, 2020  
Leiras, Cregg, Kiehn, *Ann Rev Neurosci*, 2022

# DEEP BRAINSTEM FIBER PHOTOMETRY OF *CHX10* GI $\text{Ca}^{2+}$ ACTIVITY

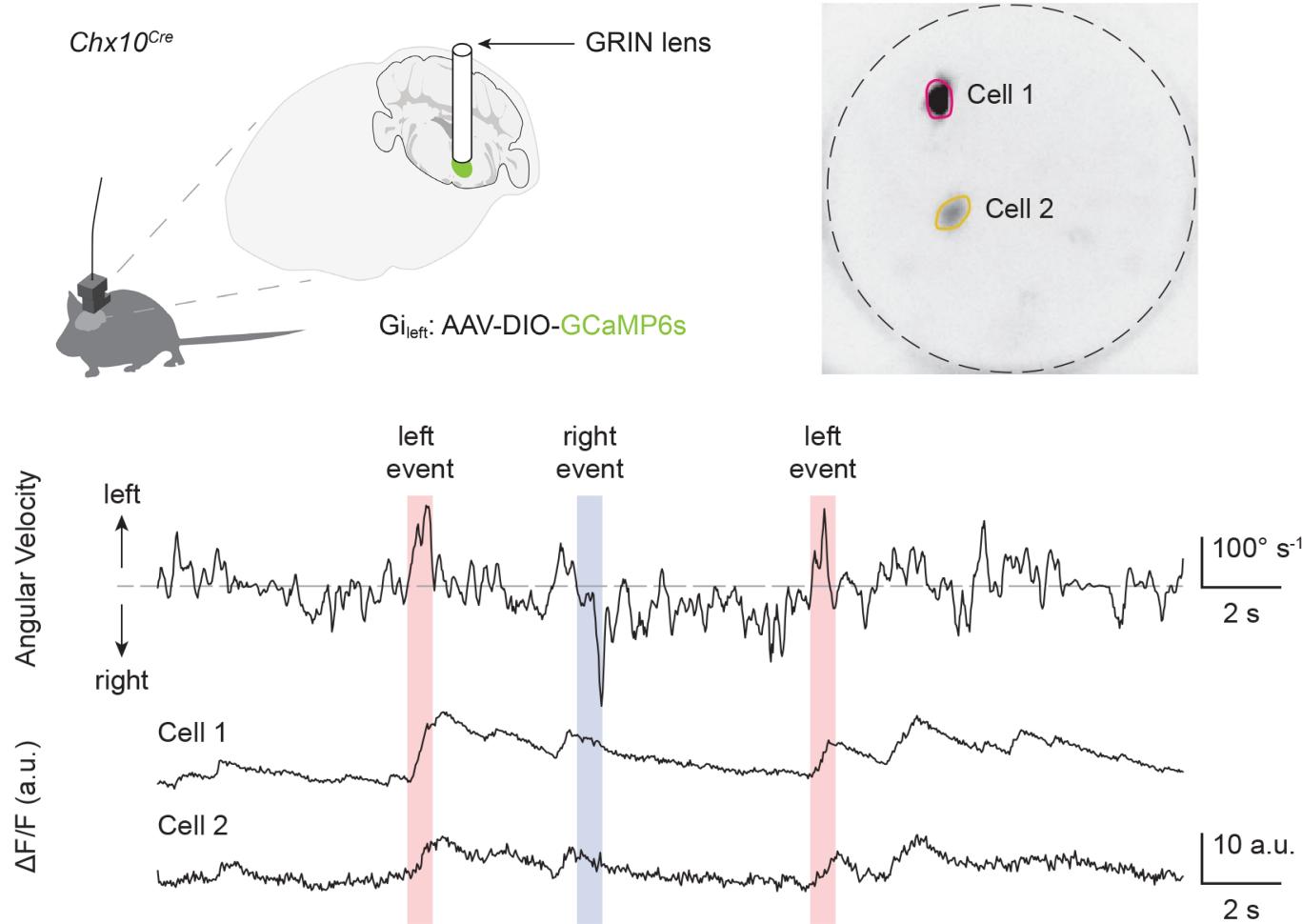


## *CHX10* GI ACTIVITY IN SPONTANEOUS TURNS – FIBER PHOTOMETRY



*Chx10* Gi population activity encodes angular velocity

# DEEP BRAINSTEM ENDOSCOPIC $\text{Ca}^{2+}$ IMAGING OF *Chx10* Gi ACTIVITY



Single *Chx10* Gi neurons encode angular velocity

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THANK YOU!

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