Techniques in neuroscience

Prof. Pavan Ramdya

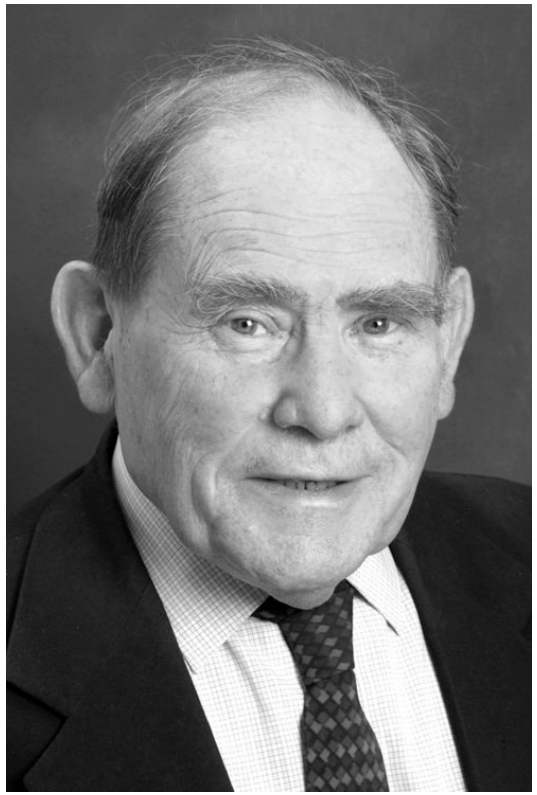
Reading: ***Purves* Chapter 1** pp. 10 – 28

BIO-311

Image credit:
https://neurosciencenews.com/files/2021/10/metacognition-look-neurosinces-public.jpg

Progress in science depends on new techniques,
new discoveries
and new ideas,
probably in that order.

-Sydney Brenner
Nobel Prize, 2002

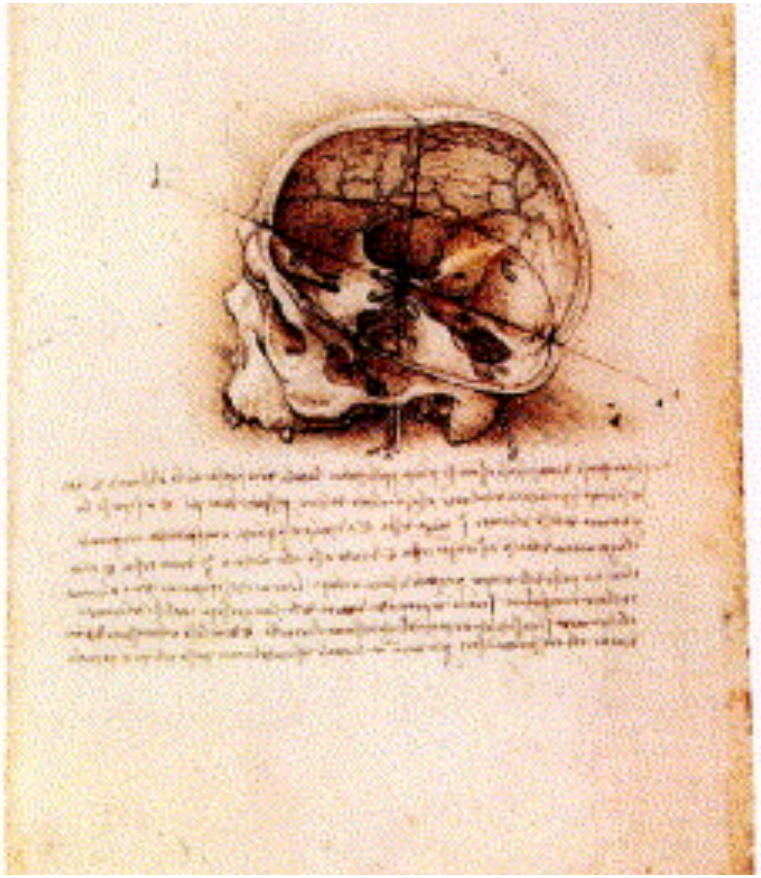


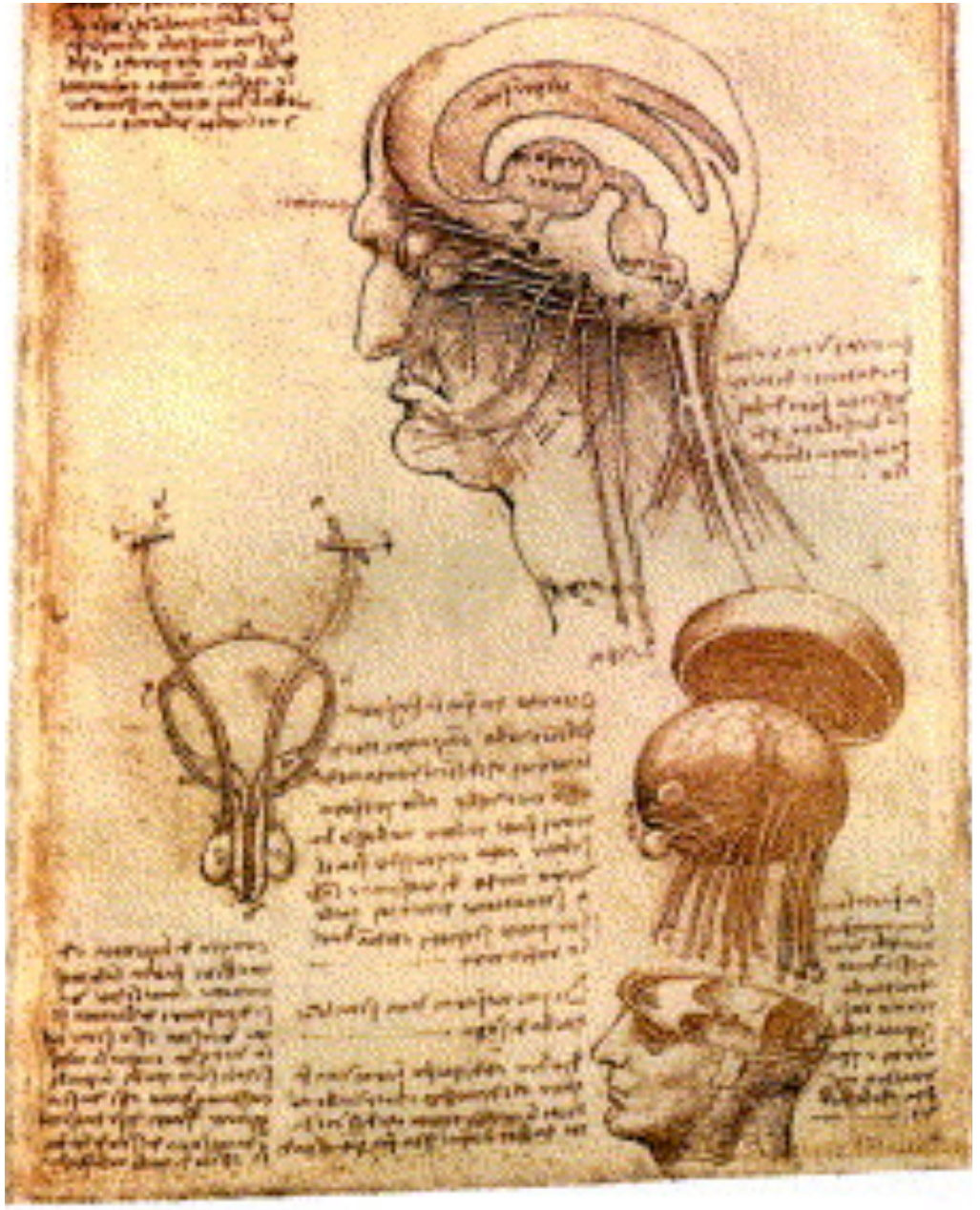
Common technical challenges in neuroscience

- Measuring brain **structure** / **connectivity**
- Measuring **neural activity**
- Measuring **neural activity during behavior**
- Quantifying **behavior**
- Perturbing **neural activity** (activation/silencing)

Measuring coarse brain structure / connectivity

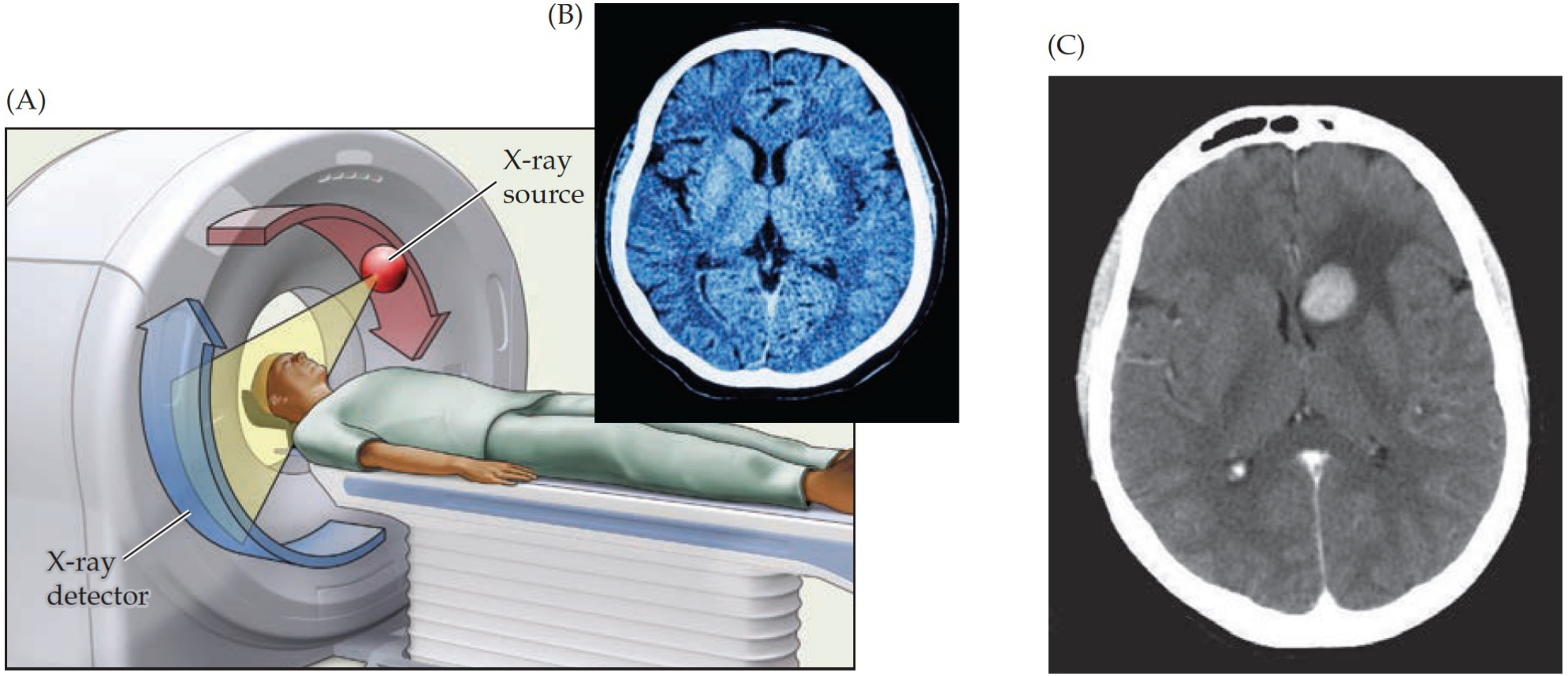
Dissections revealed coarse brain structure





Leonardo da Vinci (1452-1519)
Dissected and illustrated the brain

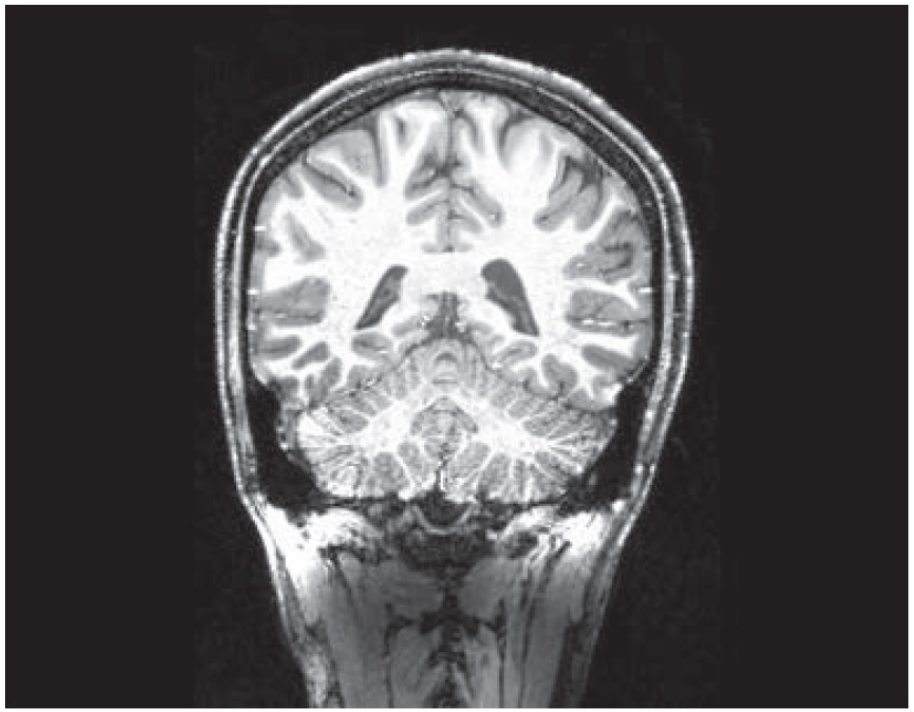
Modern CT scans reveal fine-grained brain structure



Computerized axial tomography.
- the X-ray source and detector are moved around the individual's head.
- Horizontal CT section of a typical adult brain.
- CT scan of an individual with multiple sites of a metastatic brain tumor (white spots throughout the cortical gray and white matter).

Purves, Figure 1.19

Modern MRI scans reveal fine-grained brain structure

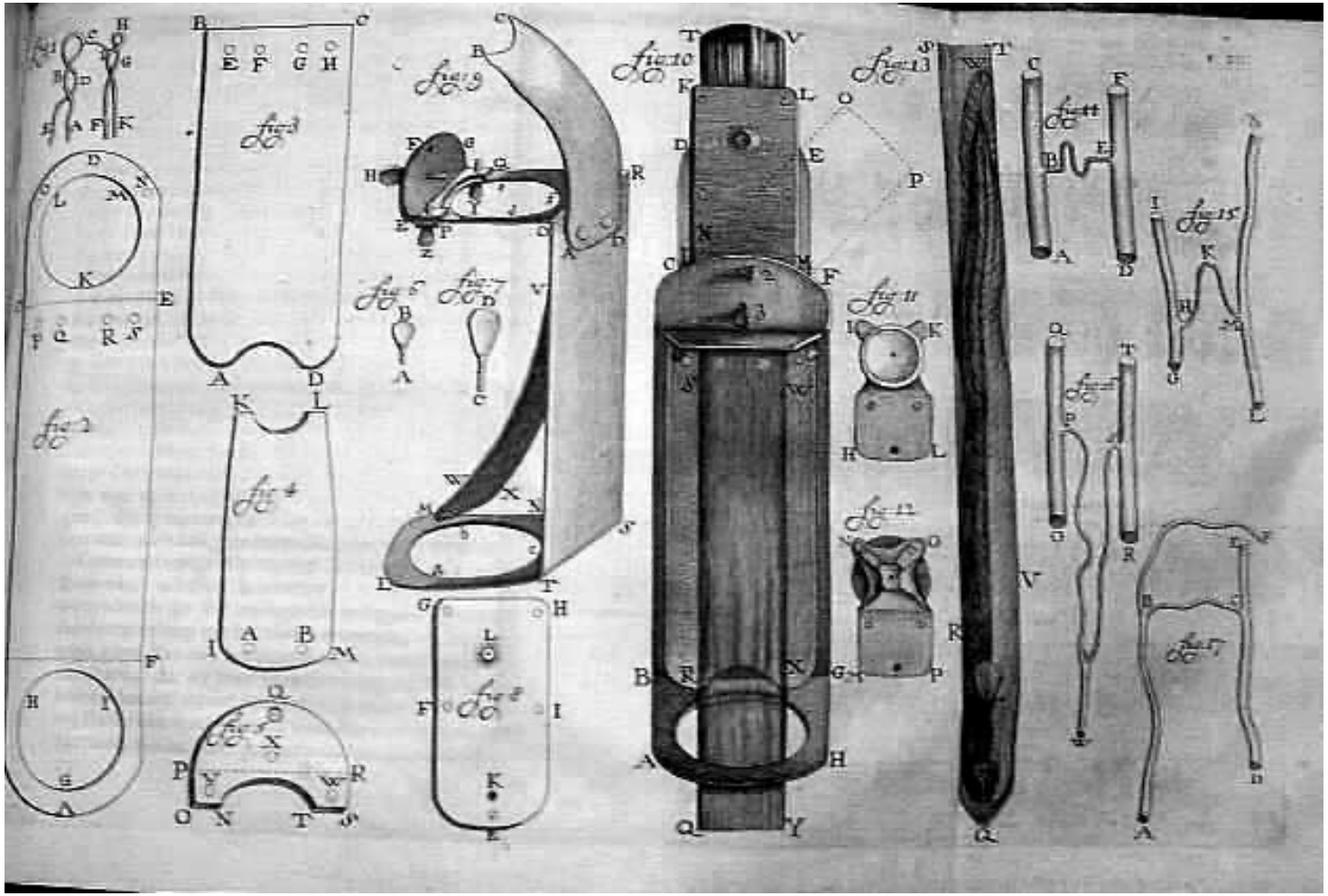


- **Magnetic Resonance Imaging** (MRI) scanner has a portal for the individual's head (or other region of the body to be imaged). A magnetic coil is placed around the head to activate and record magnetic resonance signal. Virtual reality goggles or earphones can be used to present visual or auditory stimuli.
-A pulse sequence yields data that record the white matter of the cerebral cortex as white and the gray matter as gray.

Purves, Figure 1.20

What about the underlying circuits?

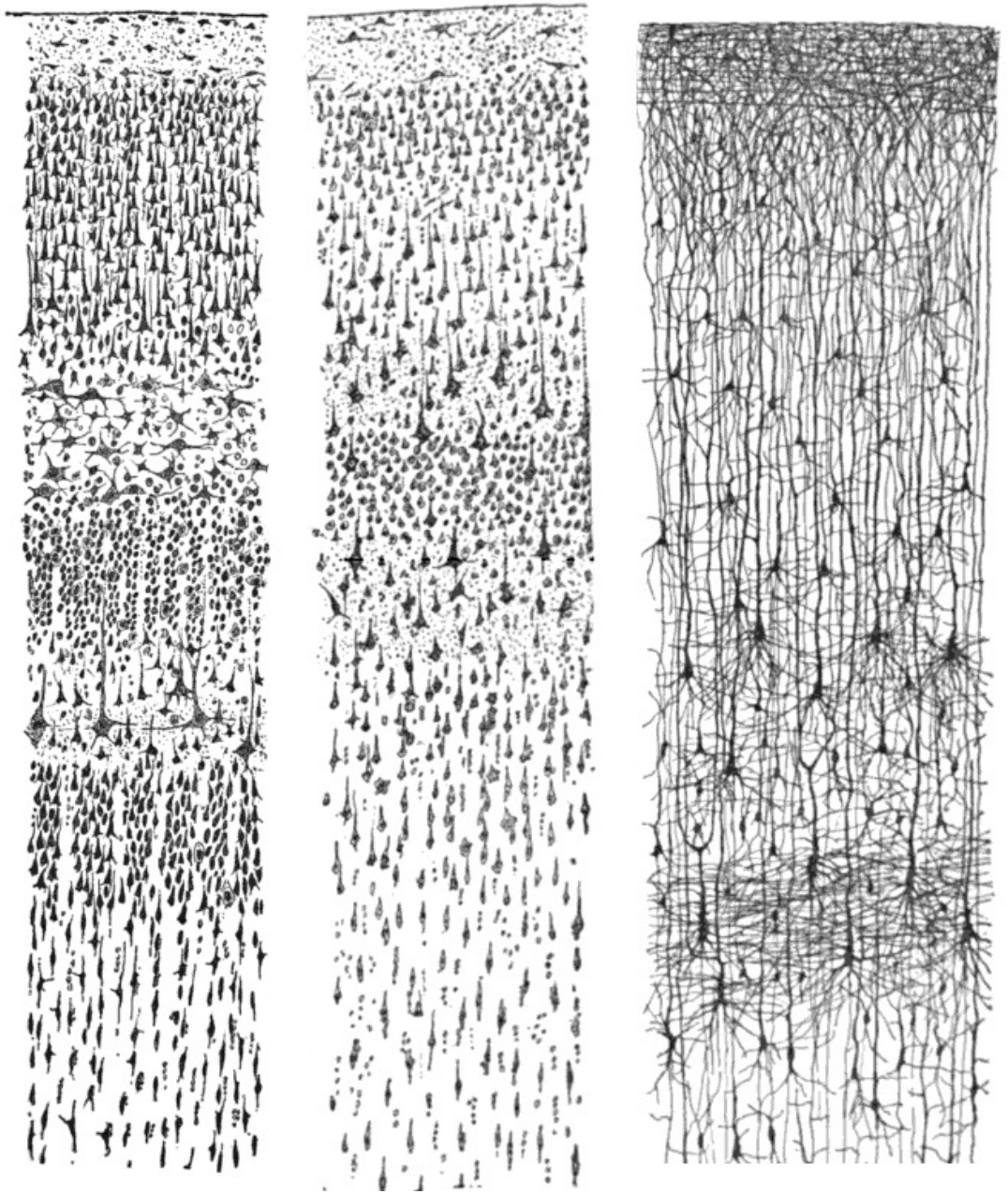
Microscopes reveal the structure of cells



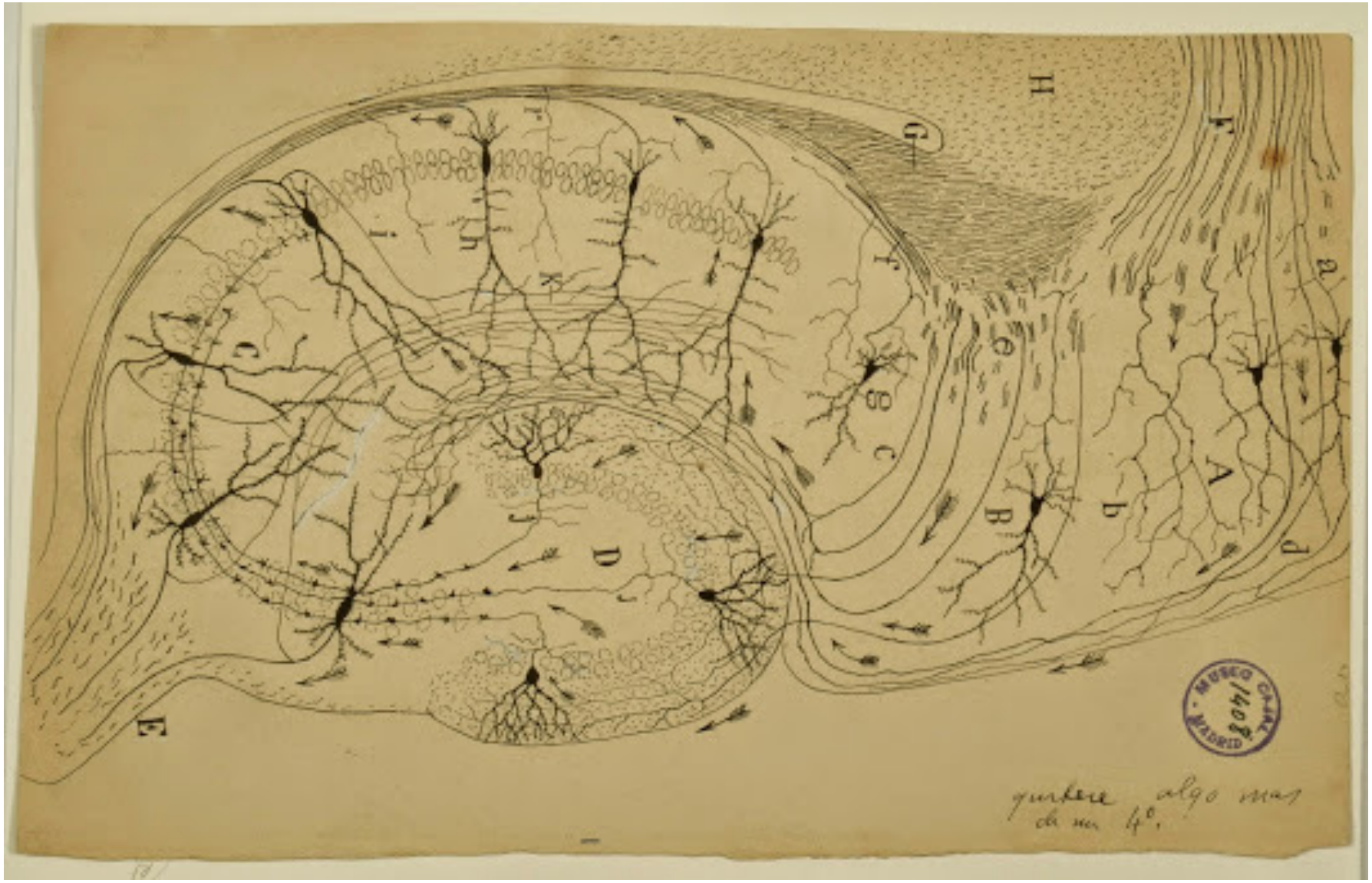
Antonie van Leeuwenhoek (1632-1723)
First microscopist (microbiologist)

Microscopes reveal the structure of neurons

Santiago Ramón y Cajal (1853-1934)
Nobel Prize (1906) for work on the structure of the nervous system



Microscopes reveal the structure of neurons



Santiago Ramón y Cajal; Drawing of the Hippocampus

What about living neurons/circuits?

Visualizing neurons using fluorescence microscopy

- Green fluorescent protein (‘GFP’)
- From the jellyfish, *Aequorea victoria*
- Activated by blue (higher energy) light, emits green (lower energy) light

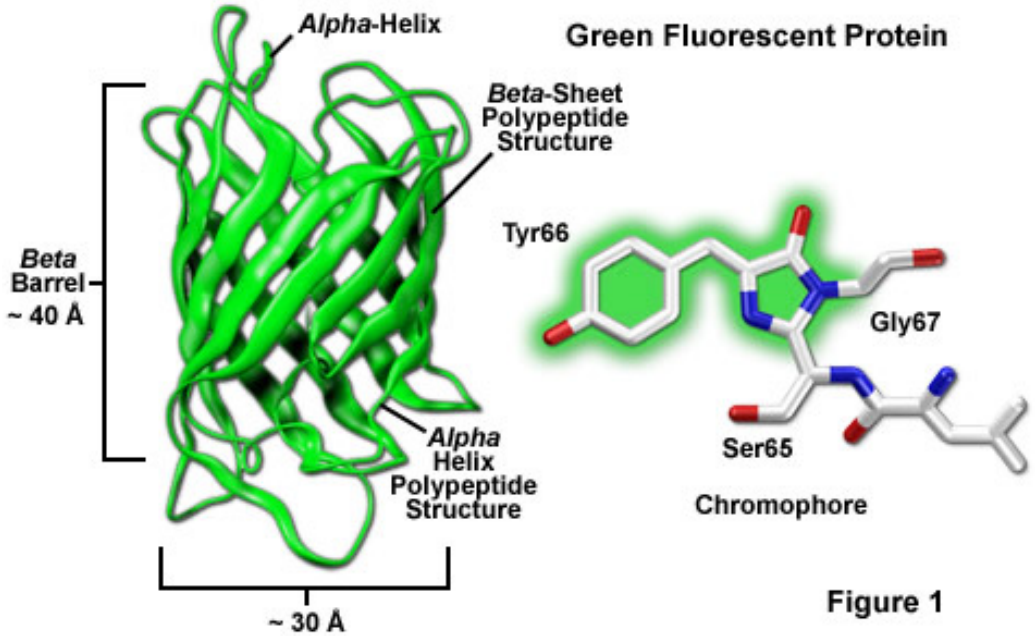
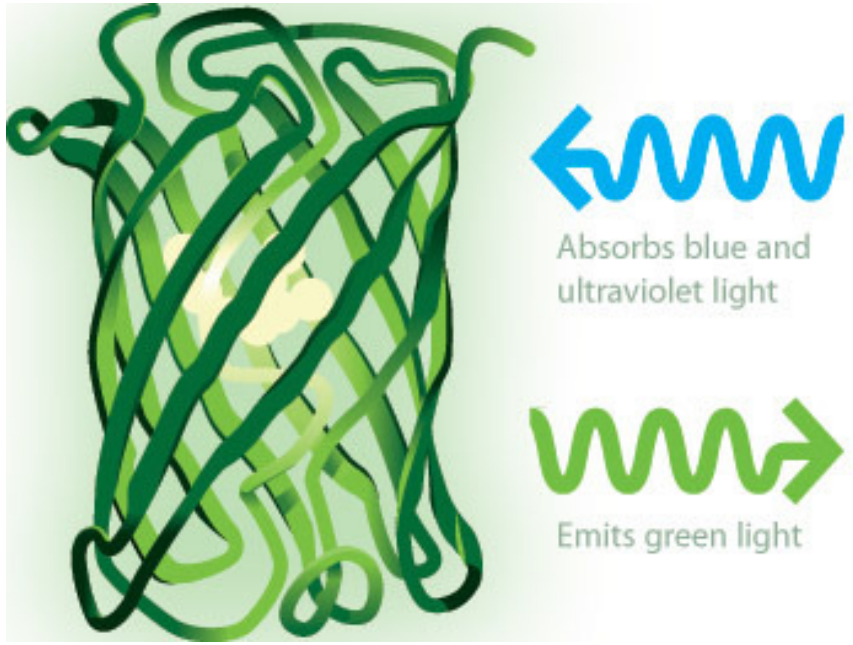
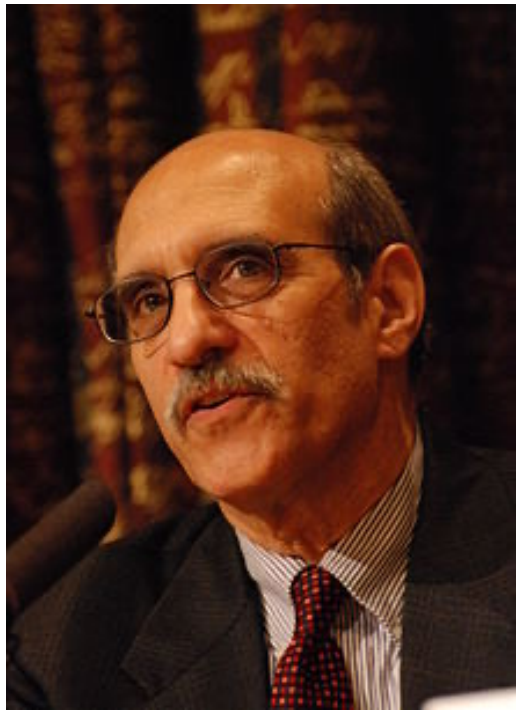


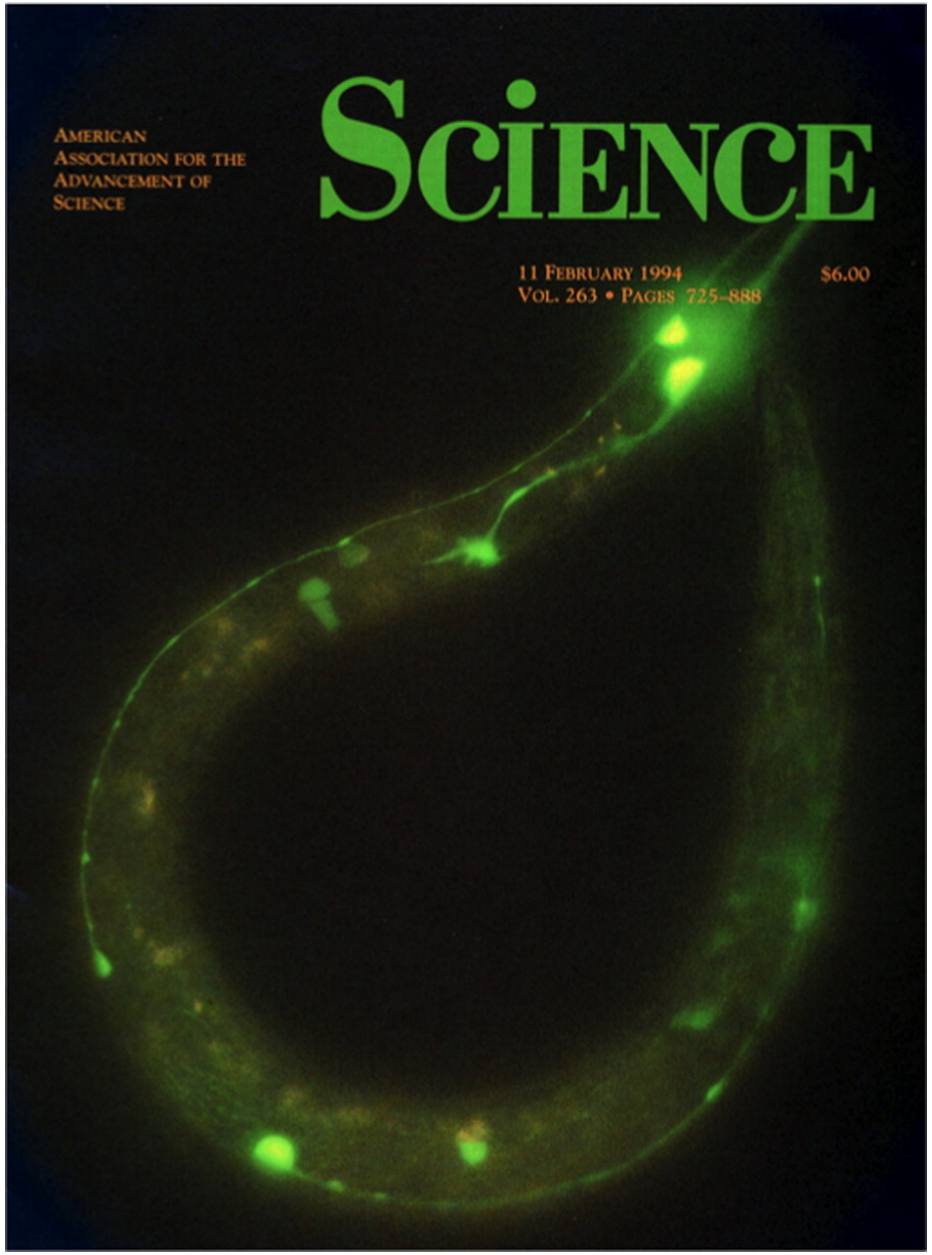
Figure 1



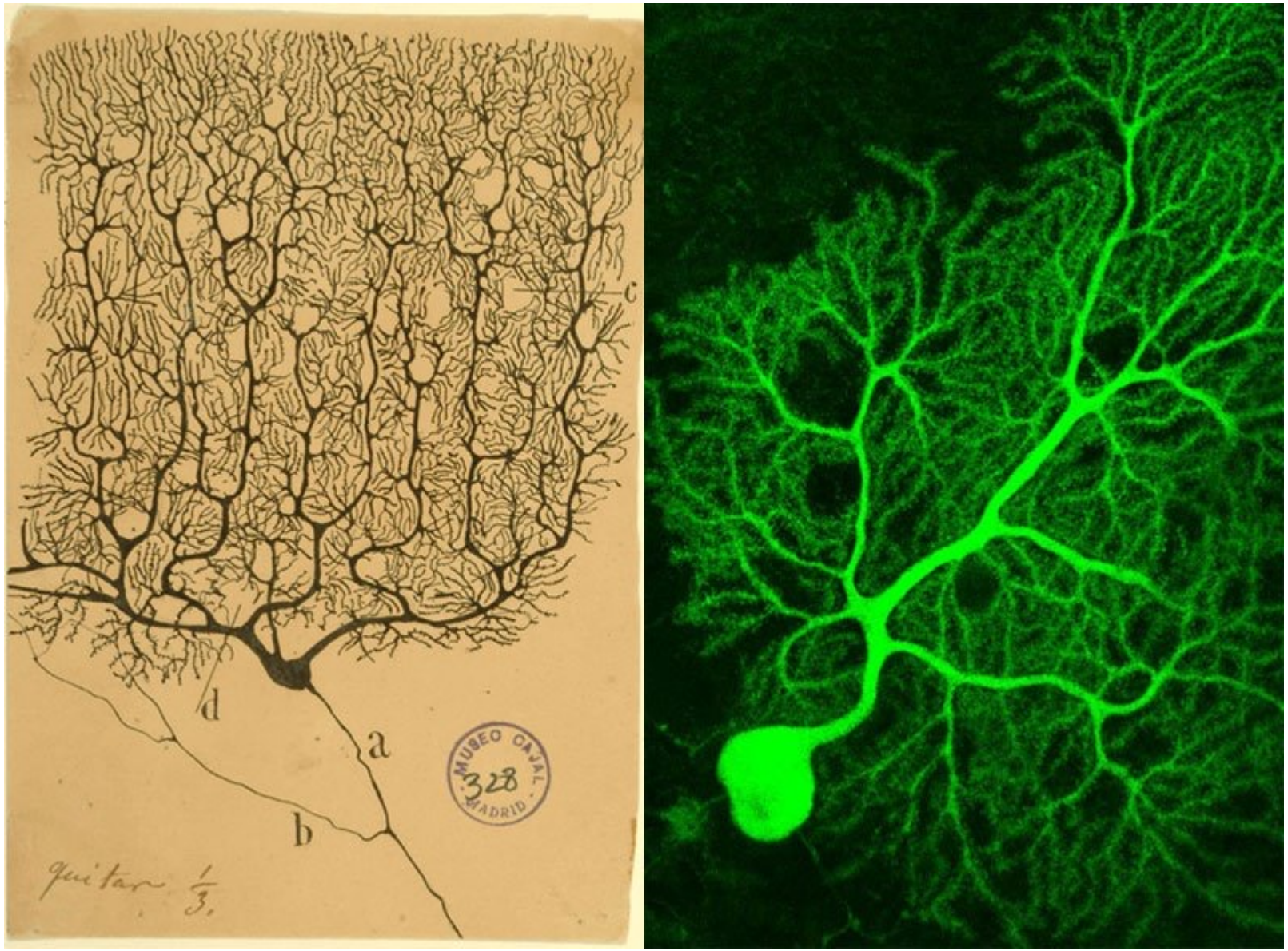
GFP reveals the structures of live neurons



Martin Chalfie (1947-)
First use of GFP to visualize cells
Nobel Prize 2008



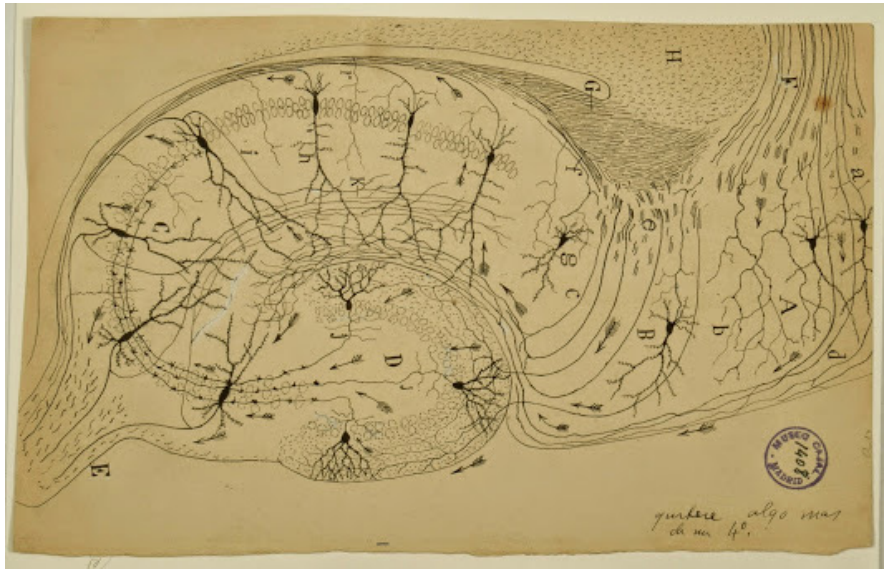
GFP reveals the structures of neurons

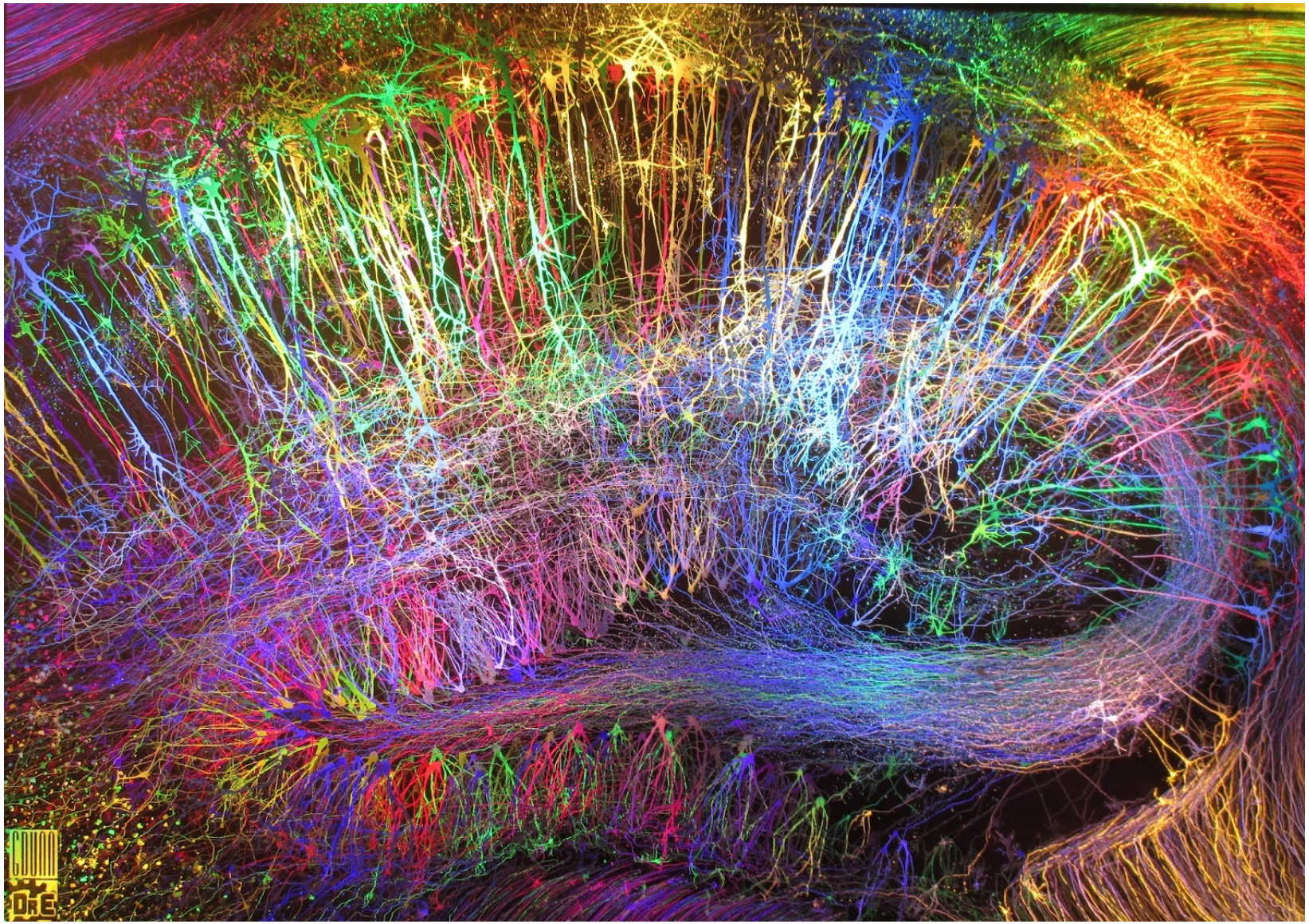


Santiago Ramón y Cajal; Drawing of the Purkinje cell

Fluorescence image of Purkinje cell

Multiple FP colors reveal the structures of many neurons

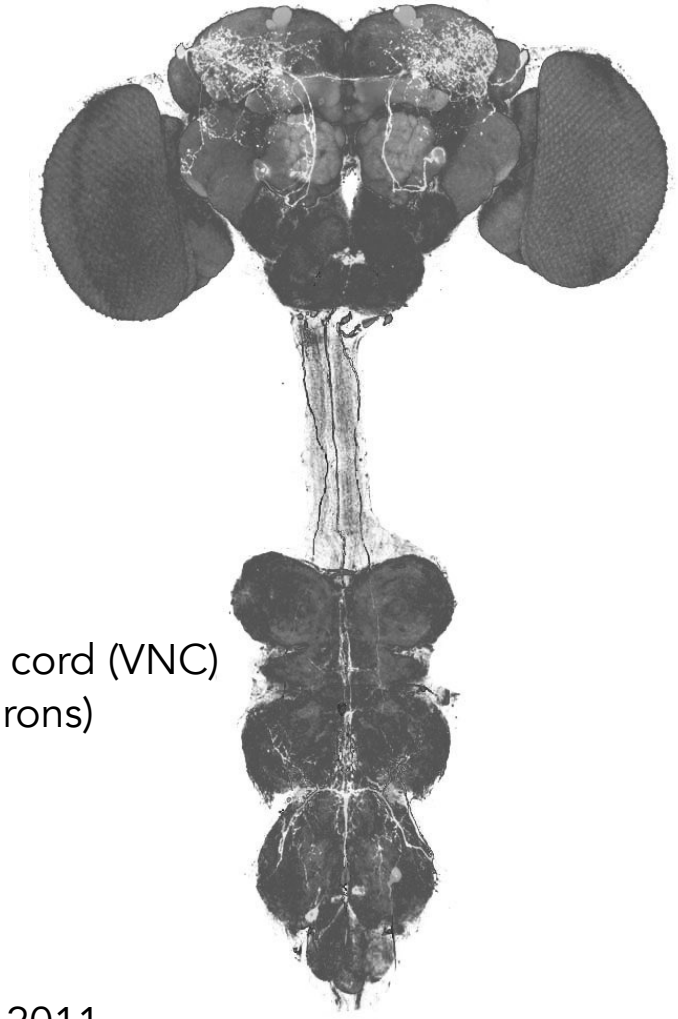




What about the connections between neurons?

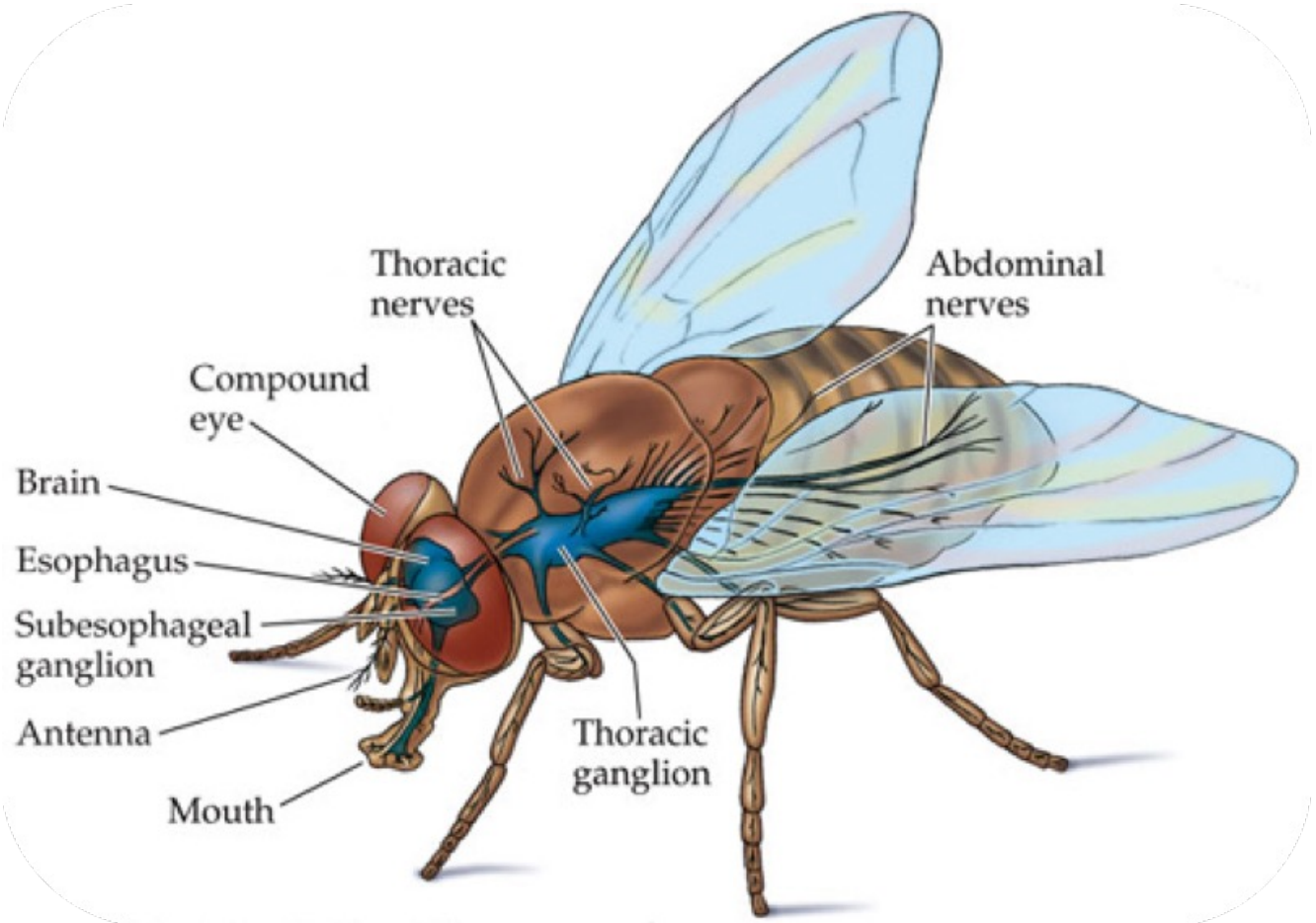
Connectomics measures neurons and their connectivity (The fruit fly, *Drosophila*, is an exemplar)

Brain (~10^5 neurons)



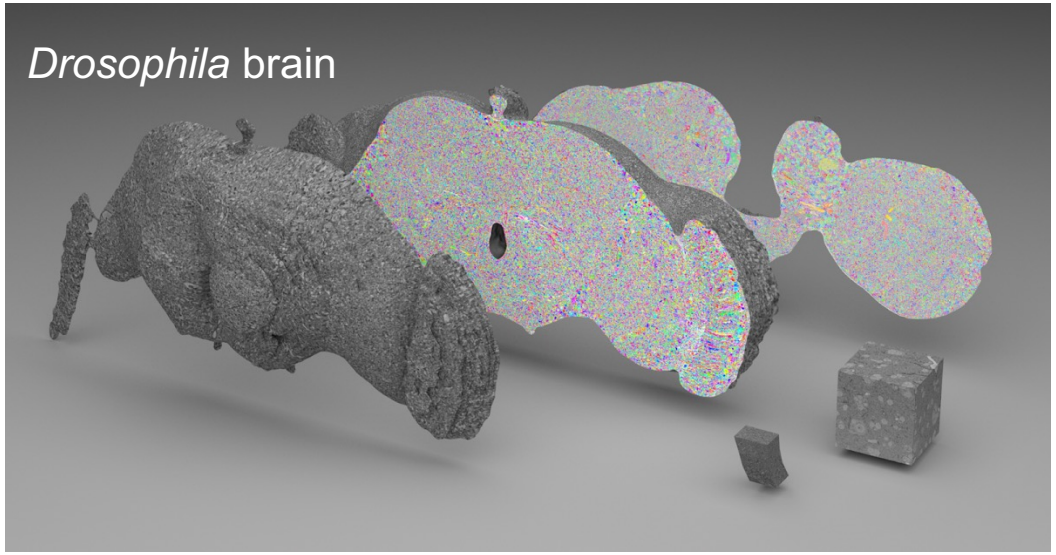
Ventral nerve cord (VNC)
(~16,000 neurons)

Chang & Shih, 2011

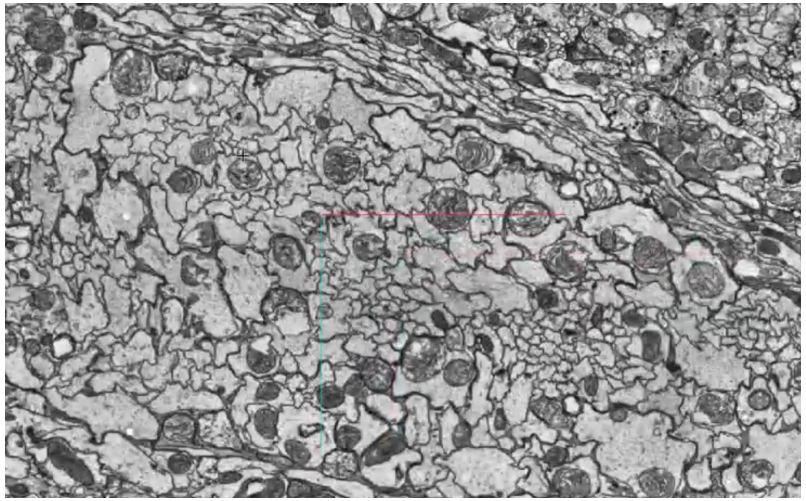


Breedlove & Watson, 2013

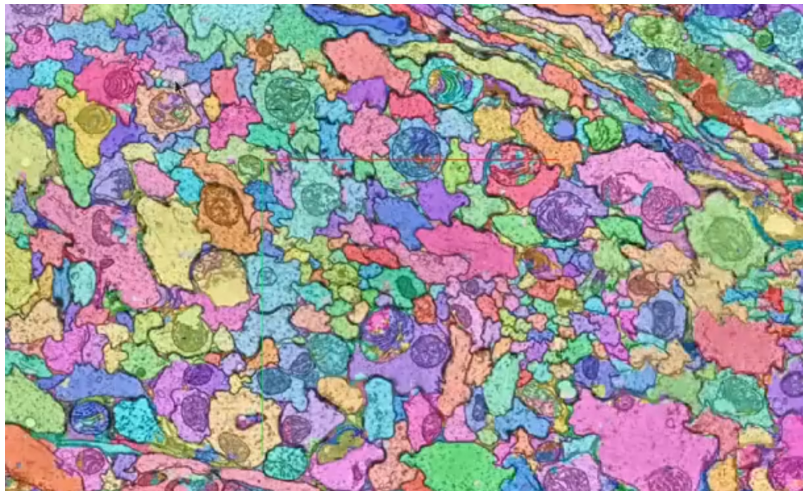
Obtaining a fly brain connectome



- High-throughput electron microscopy
- Automated neural tracing using human annotations and trained deep networks

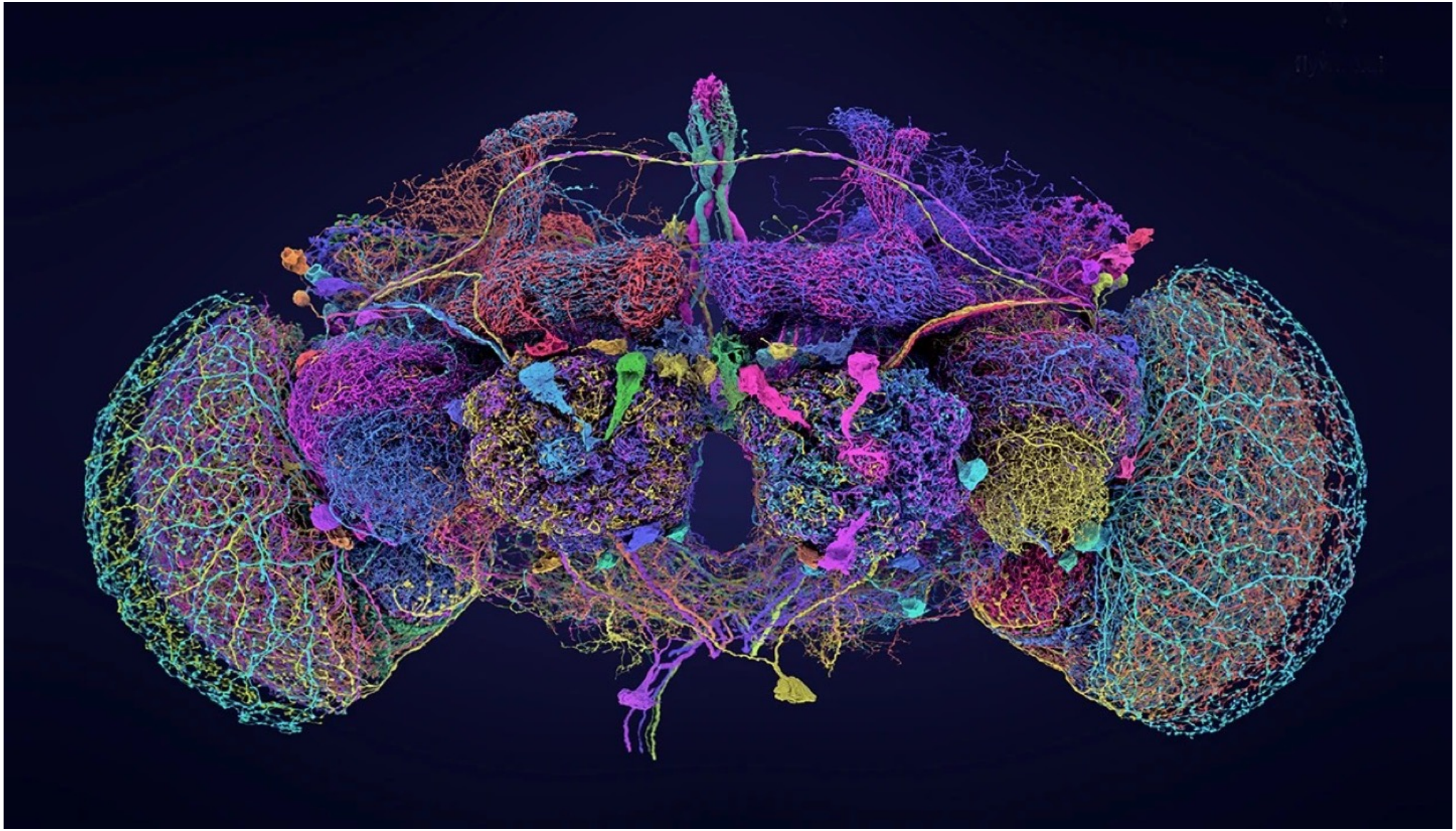


Deep network



Google Deep Mind

A complete connectome of the fly brain



Dorkenwald et al., *Nature* 2024; Schlegel et al., *Nature* 2024

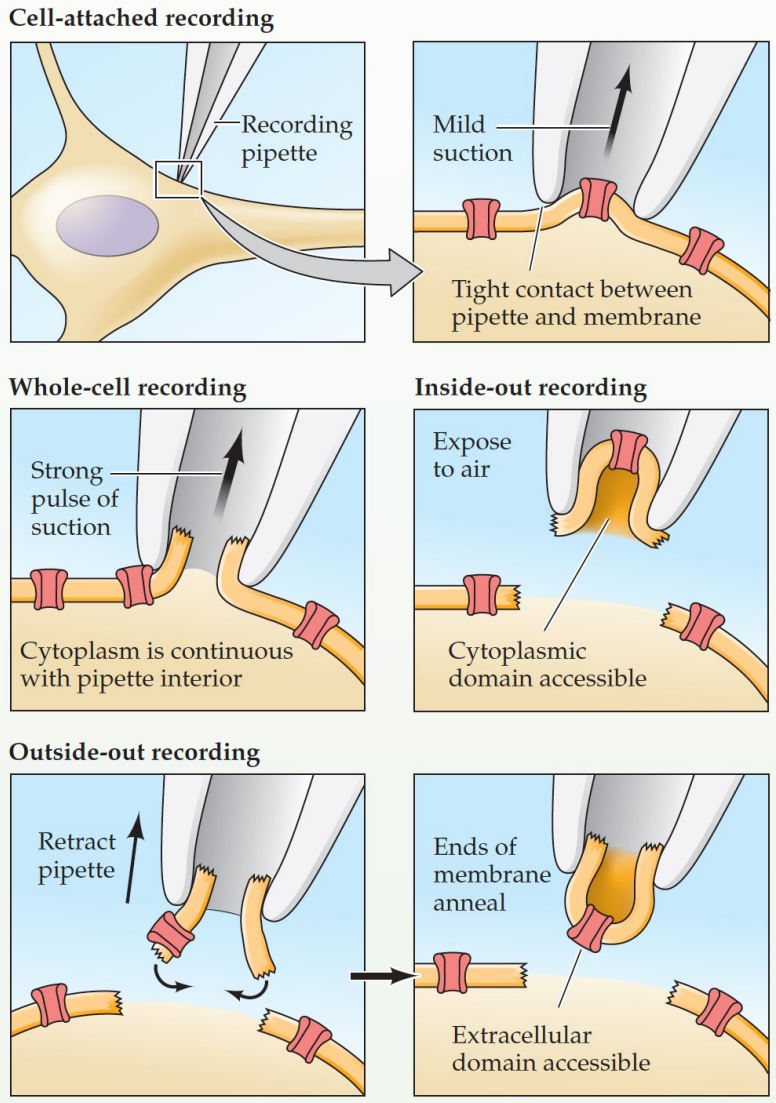
Recording neural activity

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

Lord Edgar Adrian (1889-1977) From: 1928 “The basis of sensation”
Some of the first recordings from neurons
Nobel Prize 1932

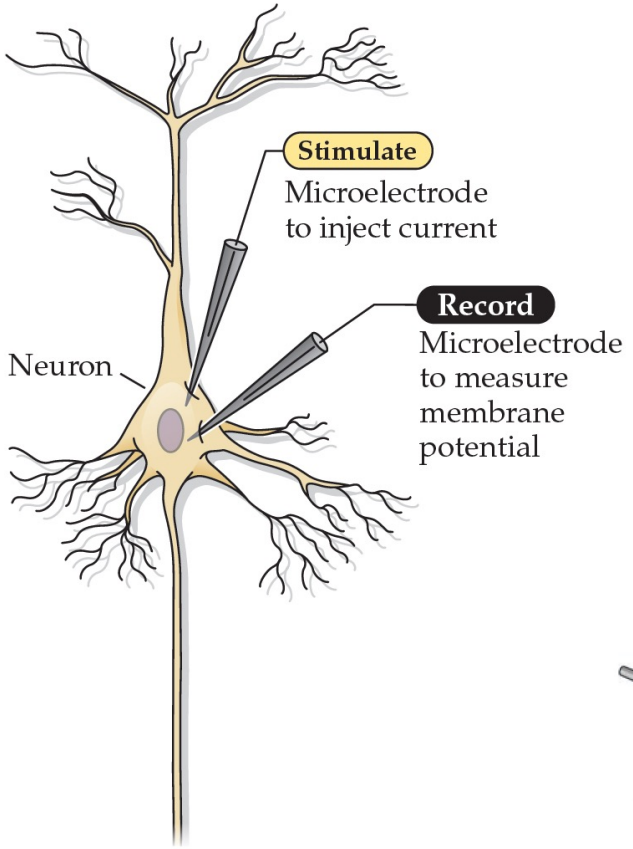
Electrophysiology (a review)

Synaptic potentials & ionic currents



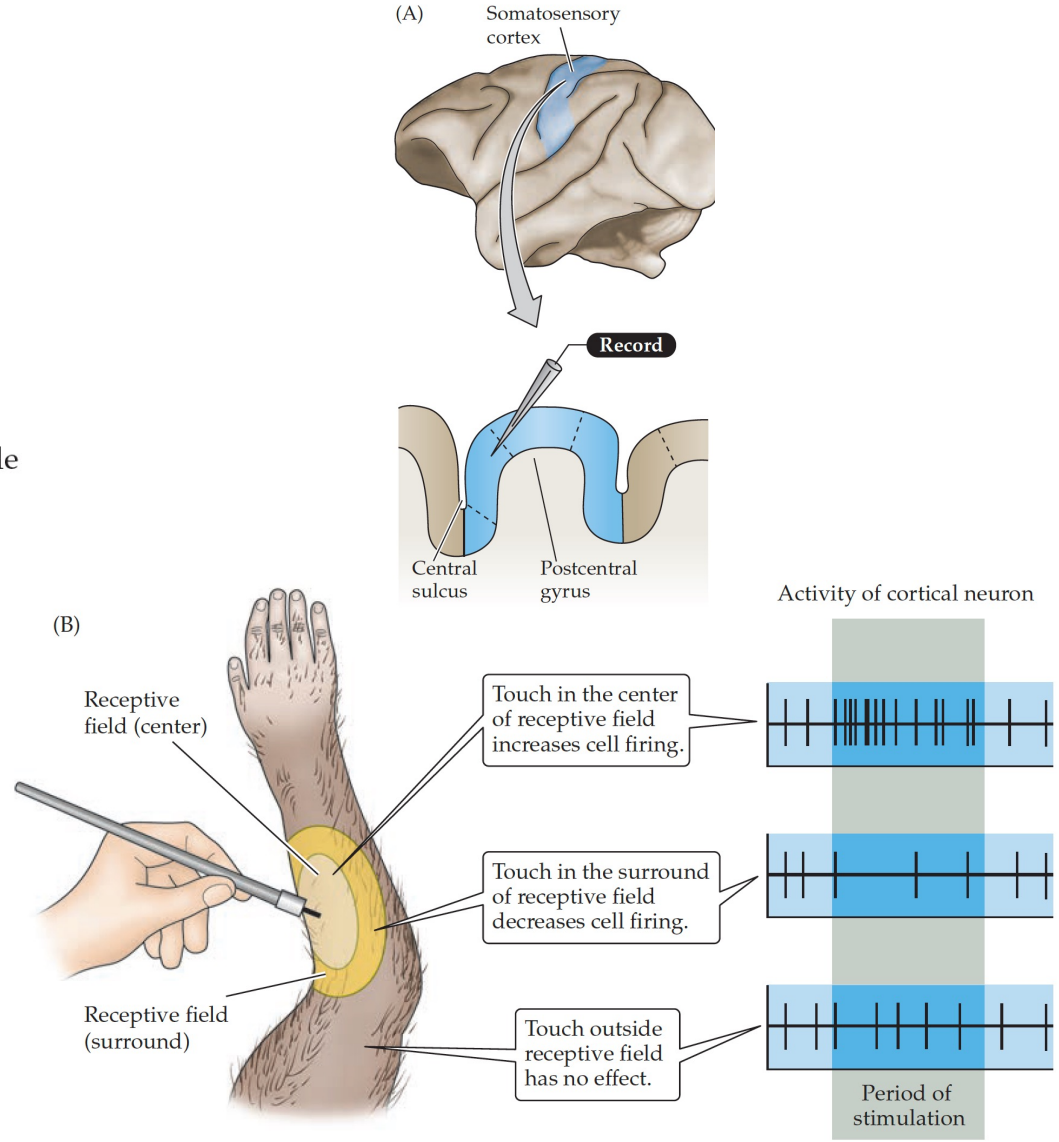
Purves, Box 4A

Voltage changes



Purves, Figure 2.2

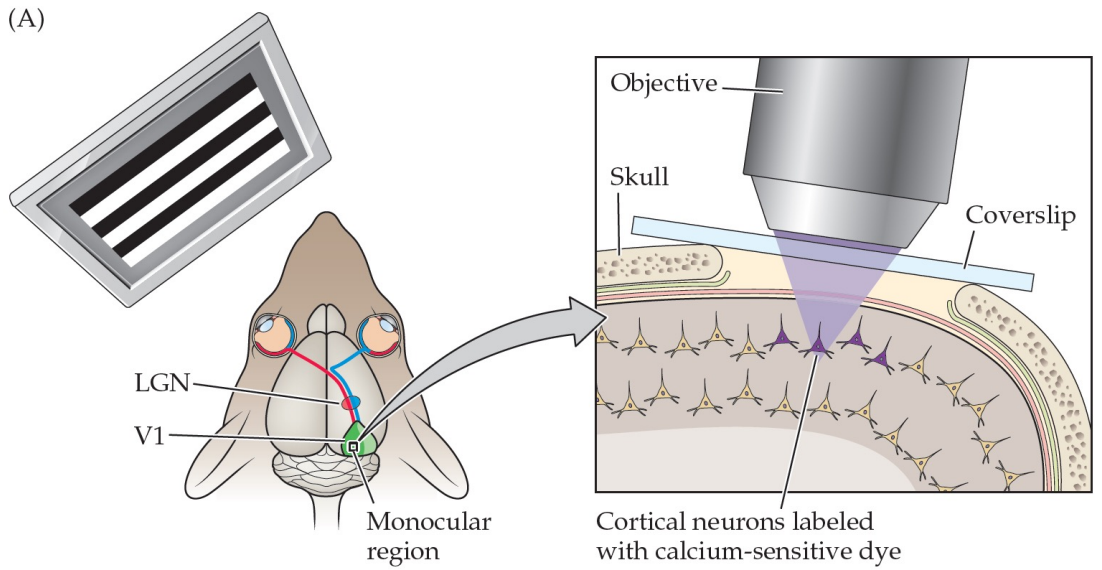
Trains of action potentials (spikes)

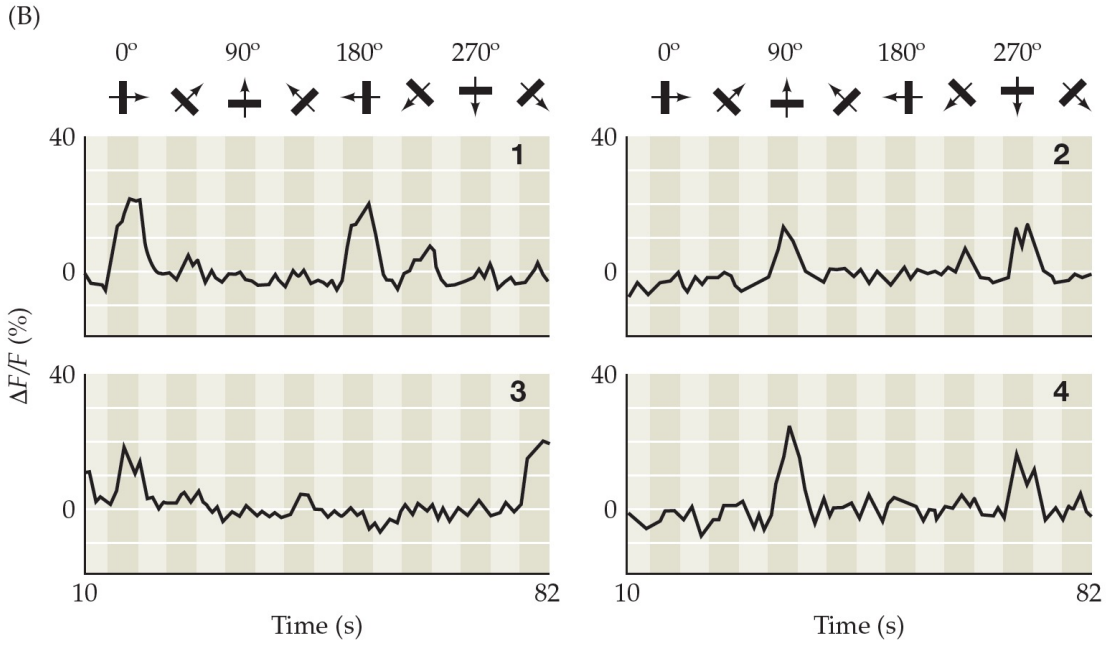


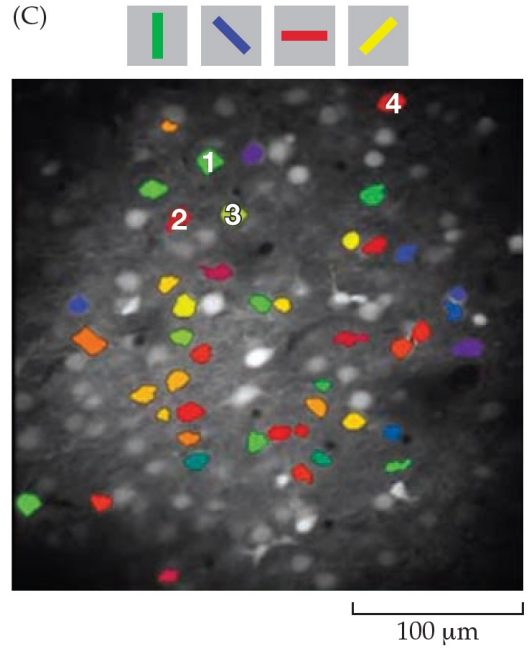
Purves, Figure 1.16

What are the downsides of electrophysiology that optical recordings can solve?

Recording neural activity using a calcium sensitive dye



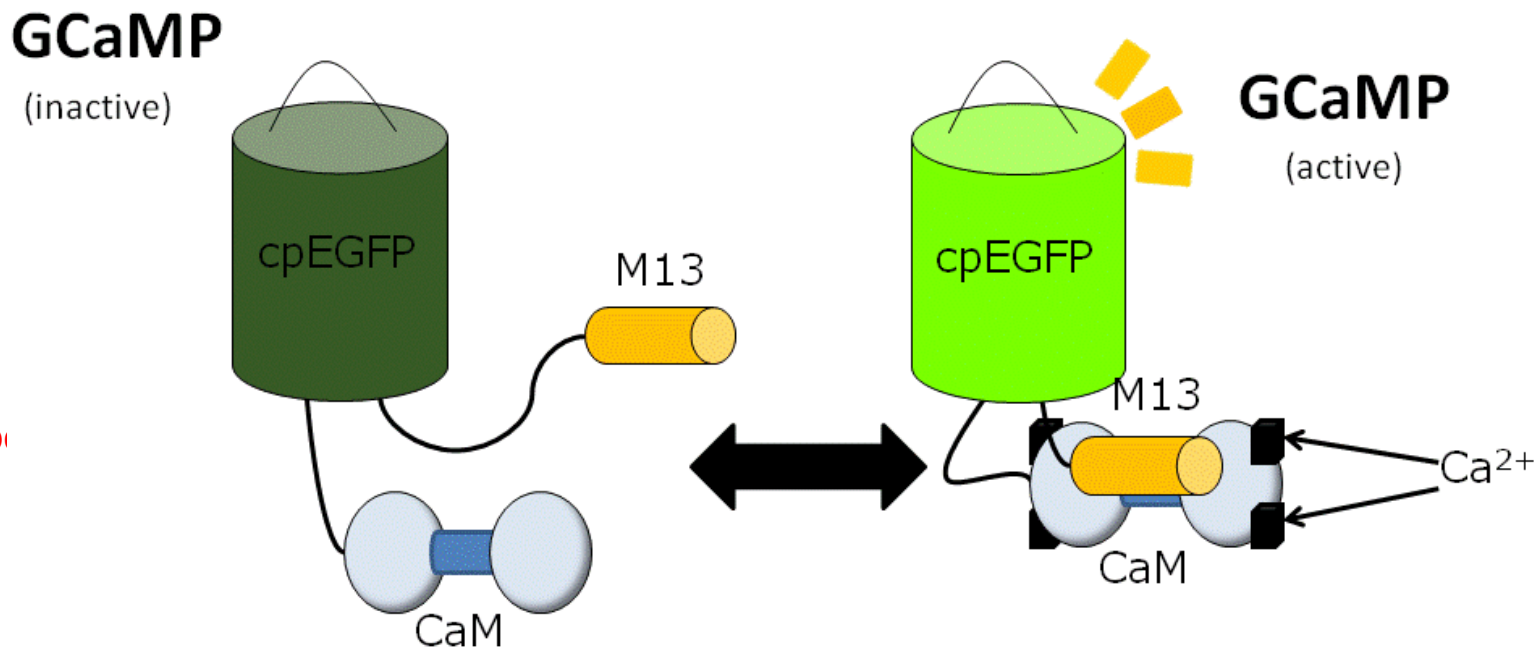




Purves, Figure 1.10

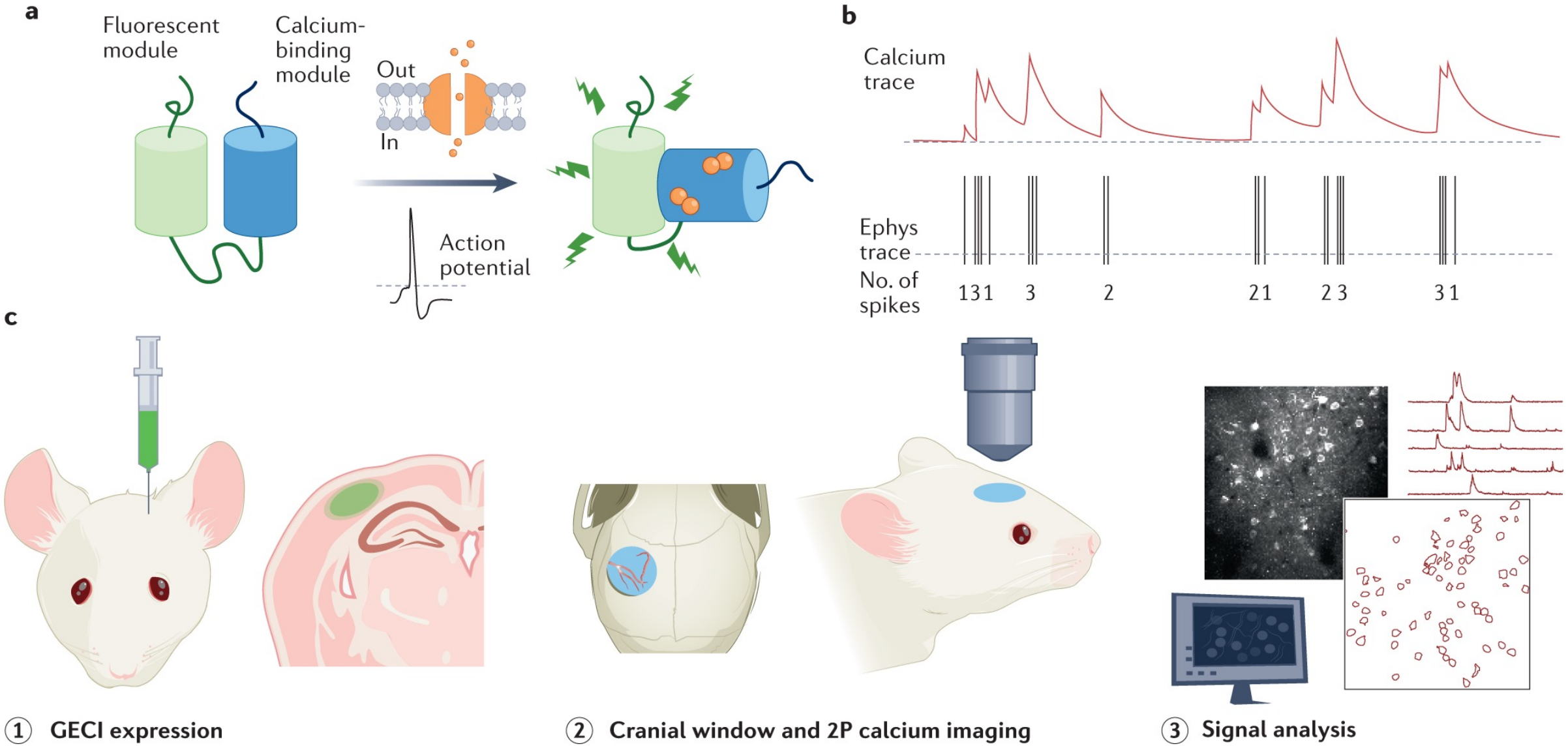
A calcium sensitive GFP called GCaMP

- GCaMP derived from GFP
- Neural activity leads to Ca^{2+} entry
- Calcium binding causes conformational change that increases fluorescence
- Visualized using a fluorescenc microscop



M13 – peptide from myosin light chain kinase
CaM – calmodulin
cpEGFP – circularly permutated GFP

Recording neural activity using GCaMP



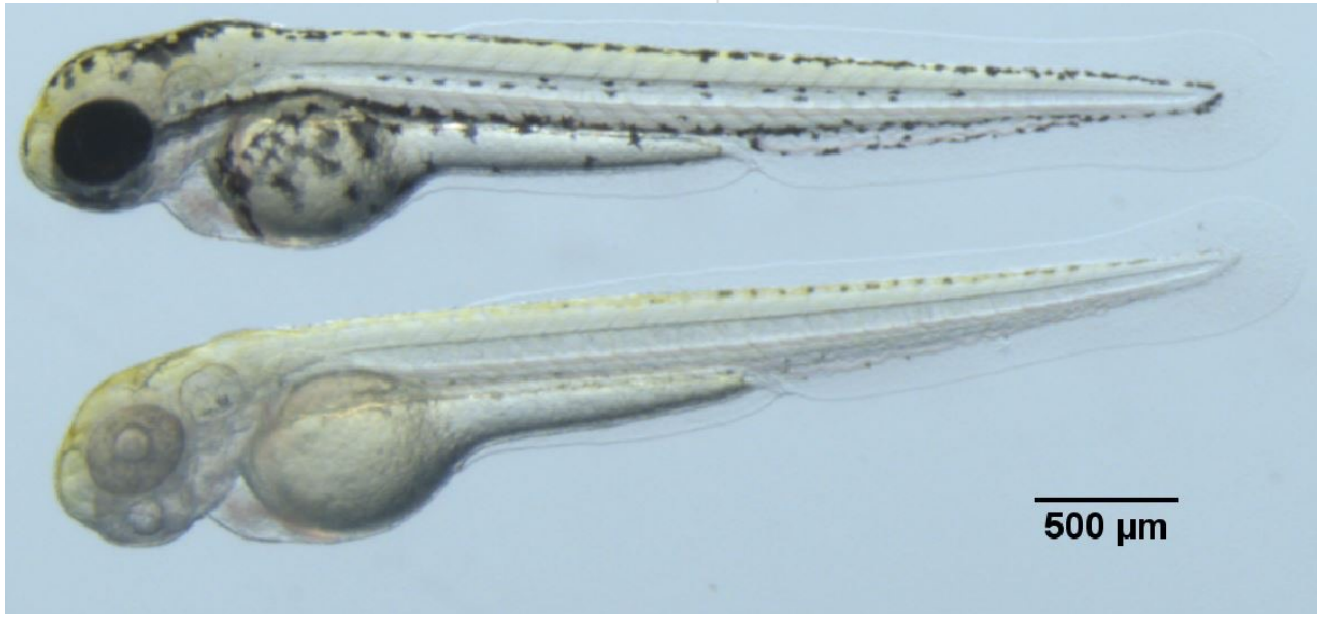
Grienberger et al., *Nature Reviews Methods Primer* 2022

Recording whole-brain activity using GCaMP and a light-sheet microscope

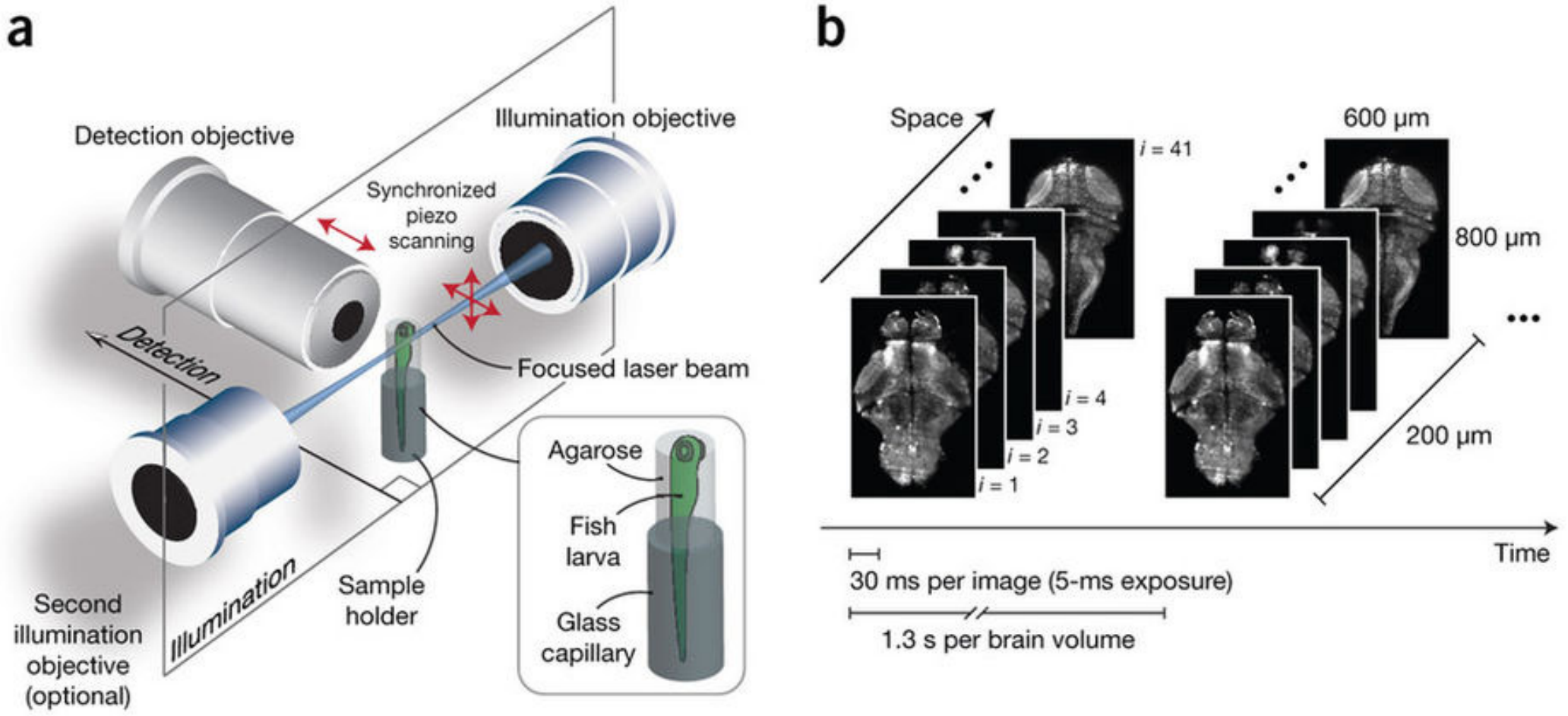
Whole-brain functional imaging at cellular resolution using light-sheet microscopy

Misha B Ahrens ✉, Michael B Orger, Drew N Robson, Jennifer M Li & Philipp J Keller ✉

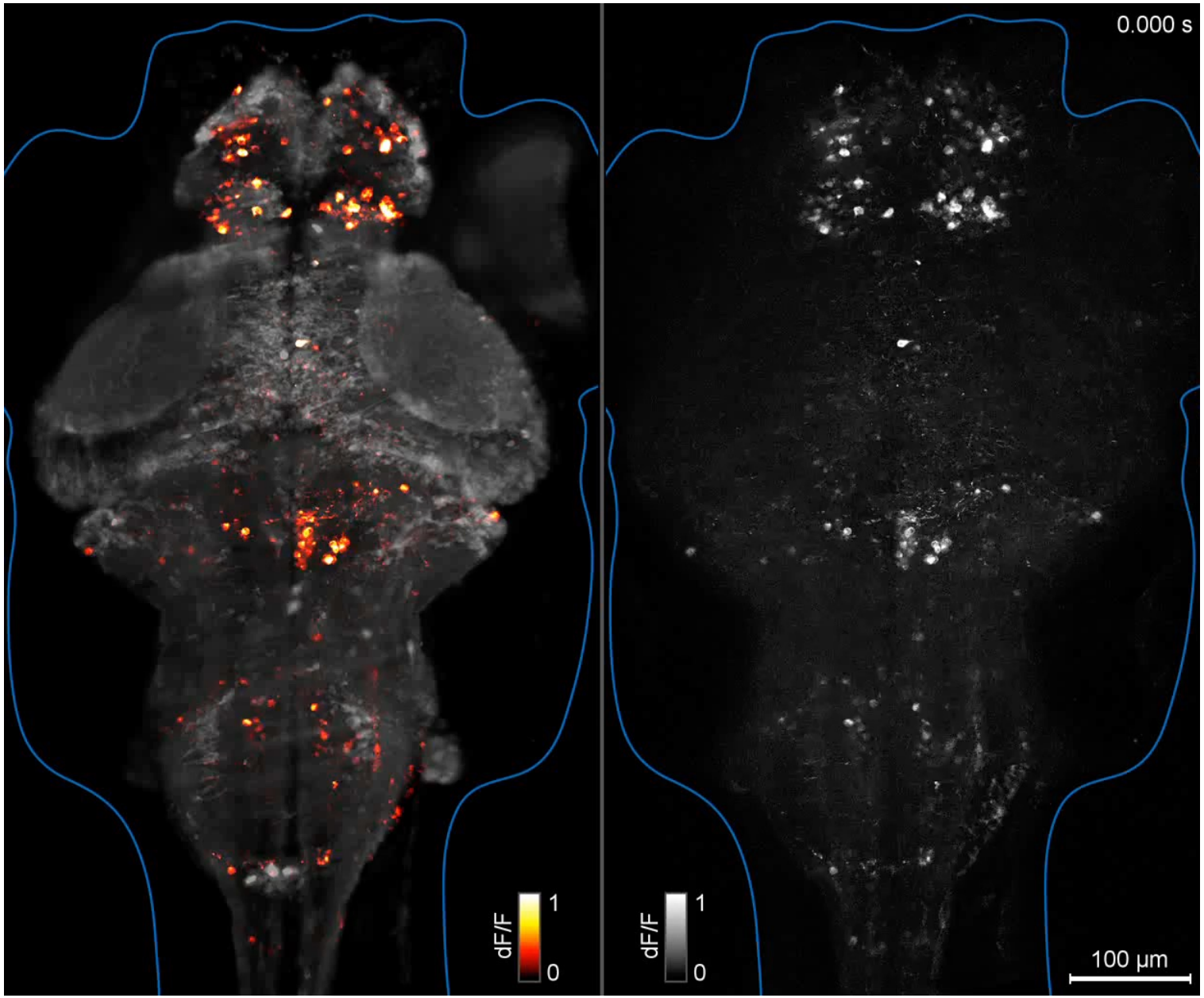
Nature Methods **10**, 413–420 (2013) | Received: 09 January 2013



Recording whole-brain activity using GCaMP and a light-sheet microscope



Recording whole-brain activity using GCaMP and a light-sheet microscope

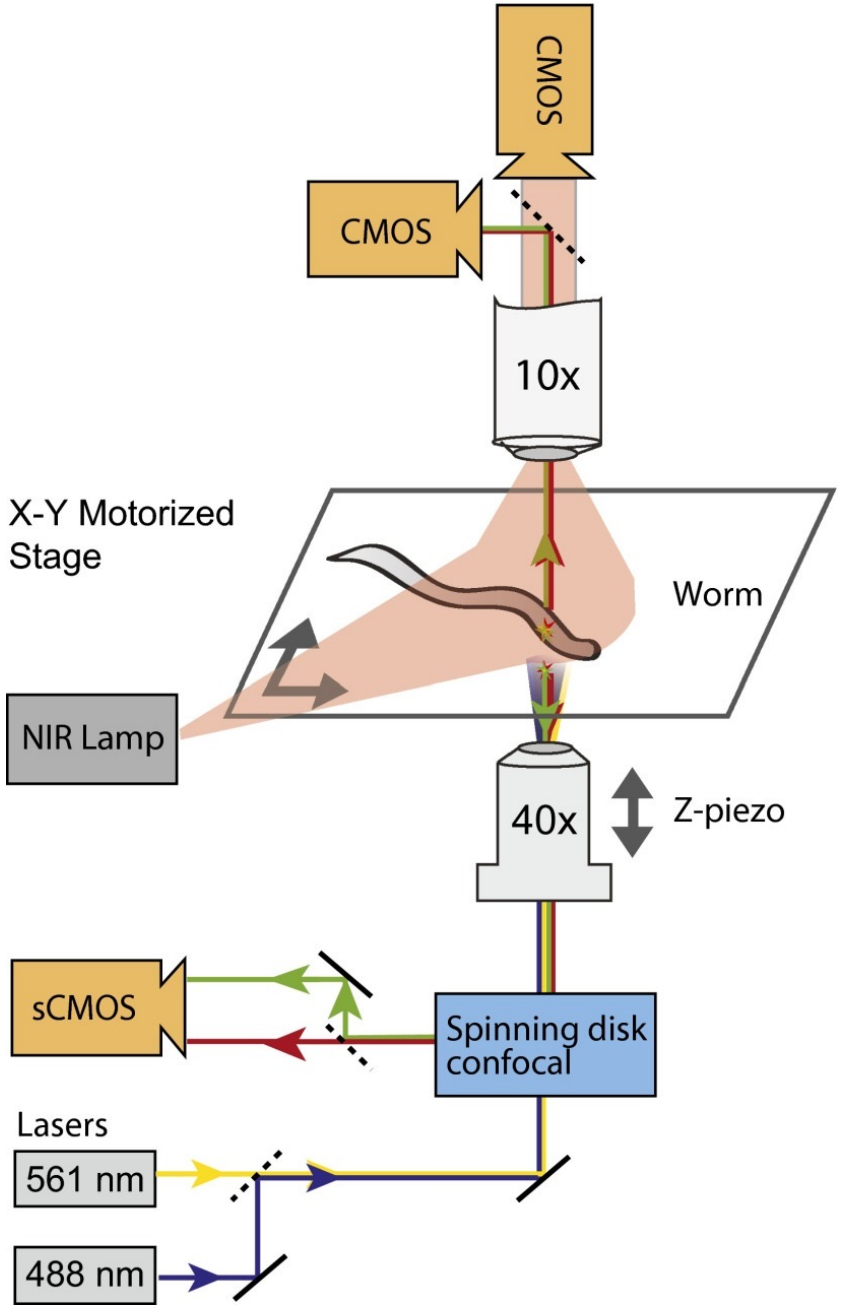


Recording neural activity **during behavior**

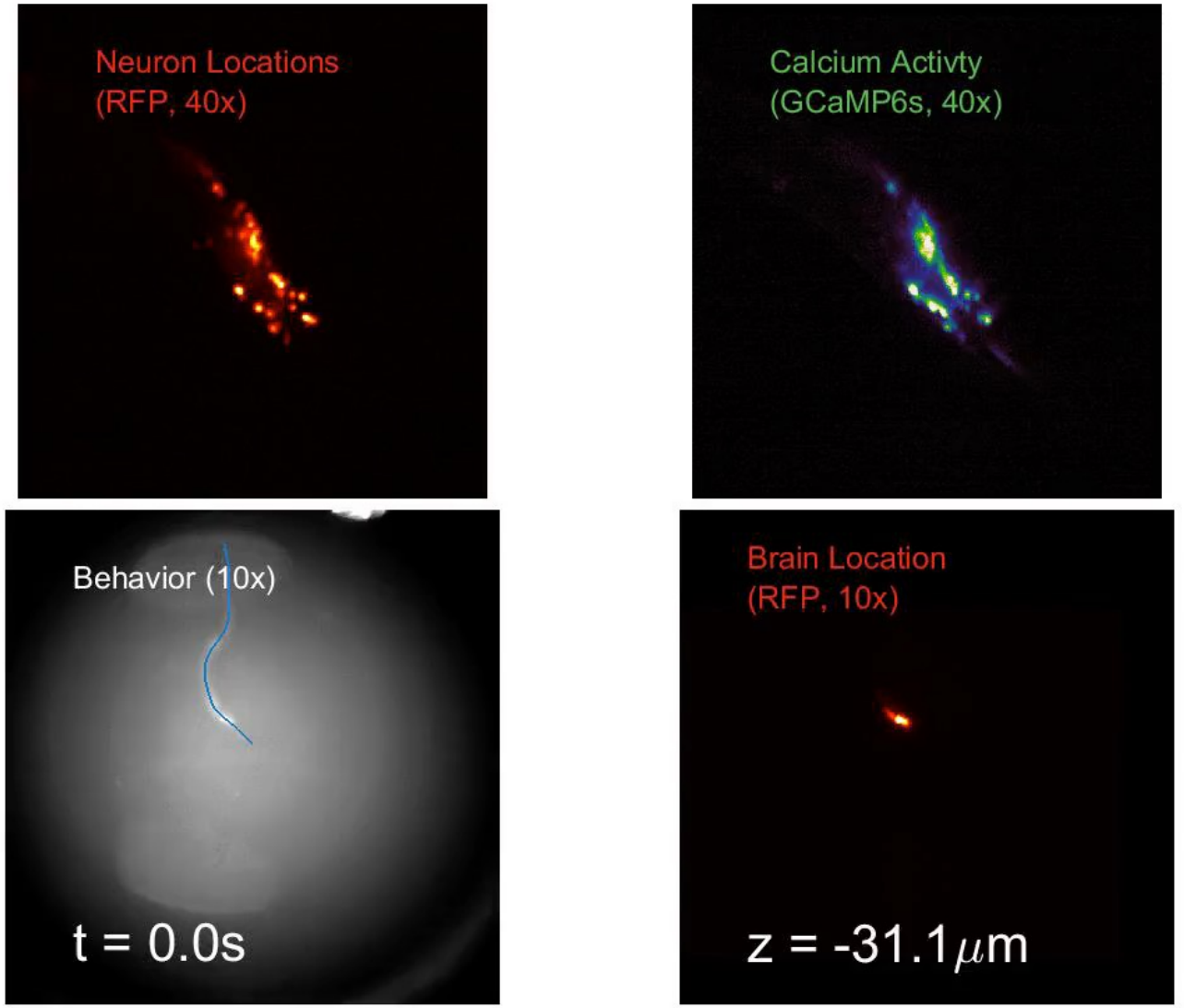
Recording neural activity in freely-behaving worms

Whole-brain calcium imaging with cellular resolution in freely behaving *Caenorhabditis elegans*

Jeffrey P. Nguyen[a,b,1], Frederick B. Shipley[a,1], Ashley N. Linder[c], George S. Plummer[a], Mochi Liu[a], Sagar U. Setru[a], Joshua W. Shaevitz[a,b], and Andrew M. Leifer[a,2]



Recording neural activity in freely-behaving worms

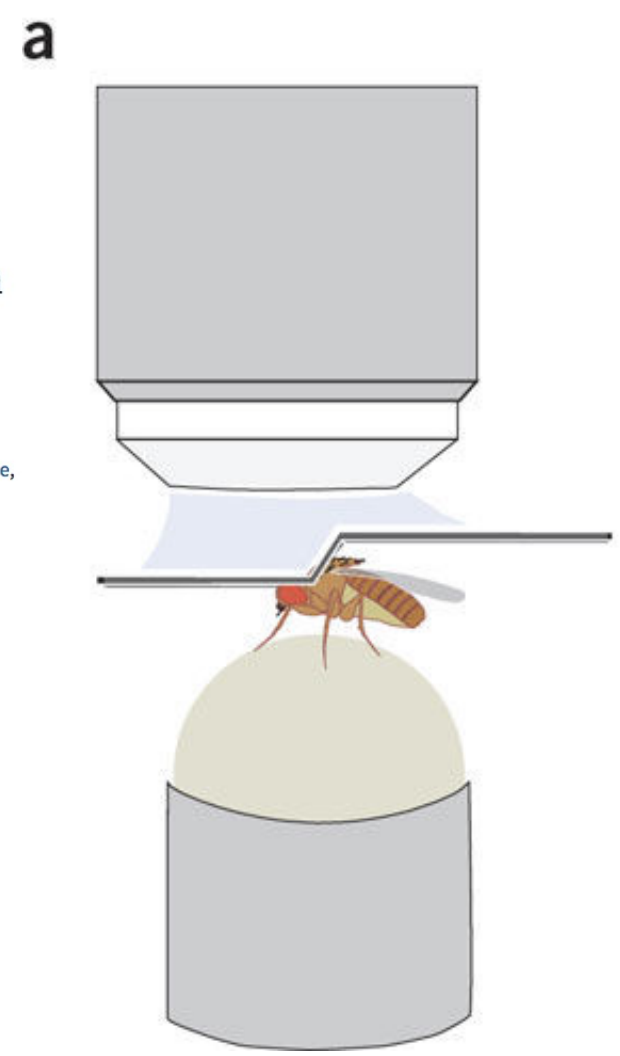


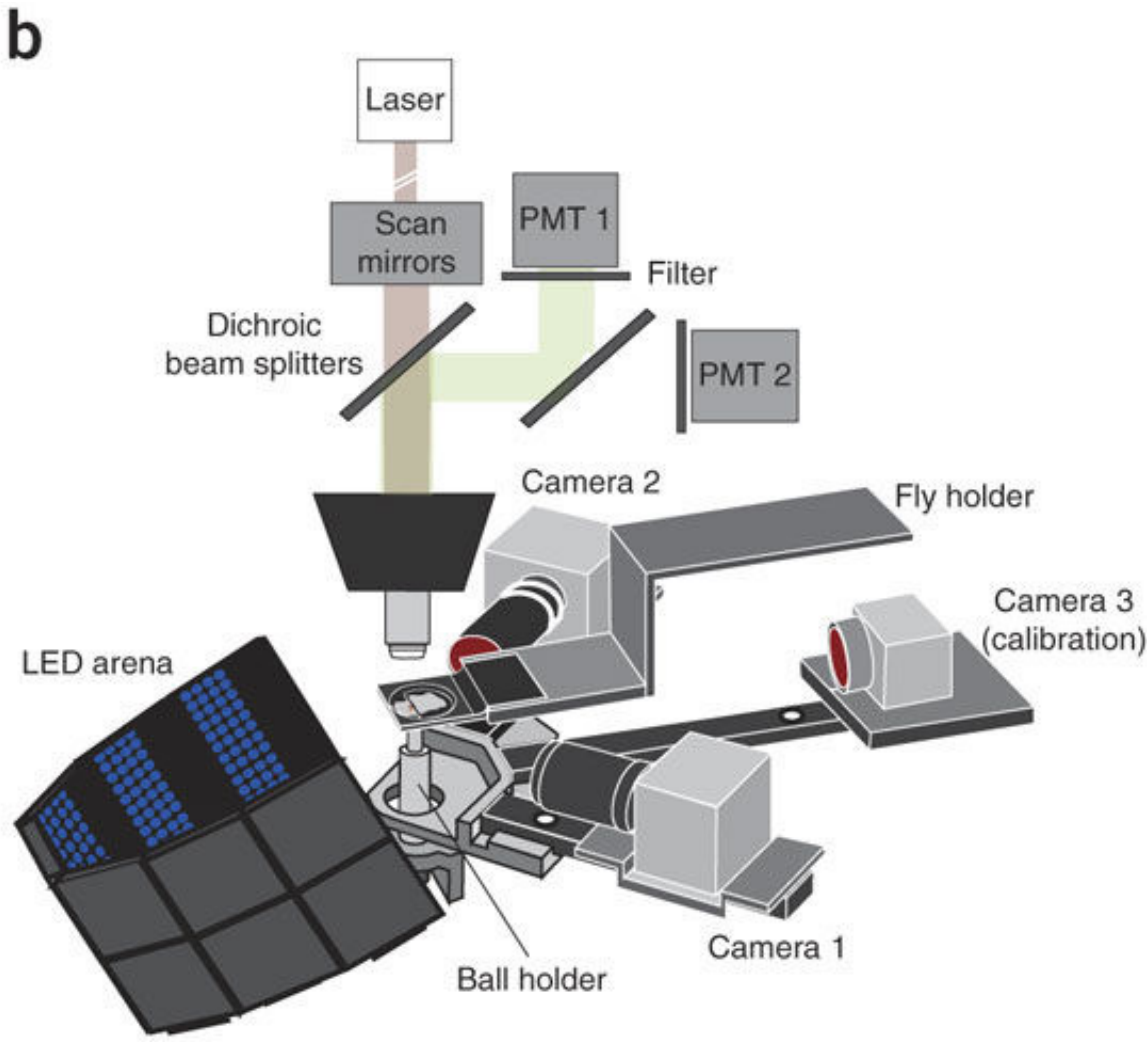
Recording neural activity in tethered flies

Two-photon calcium imaging from head-fixed *Drosophila* during optomotor walking behavior

Johannes D Seelig, M Eugenia Chiappe, Gus K Lott, Anirban Dutta, Jason E Osborne, Michael B Reiser & Vivek Jayaraman

Nature Methods **7**, 535–540 (2010) Received: 10 February 2010



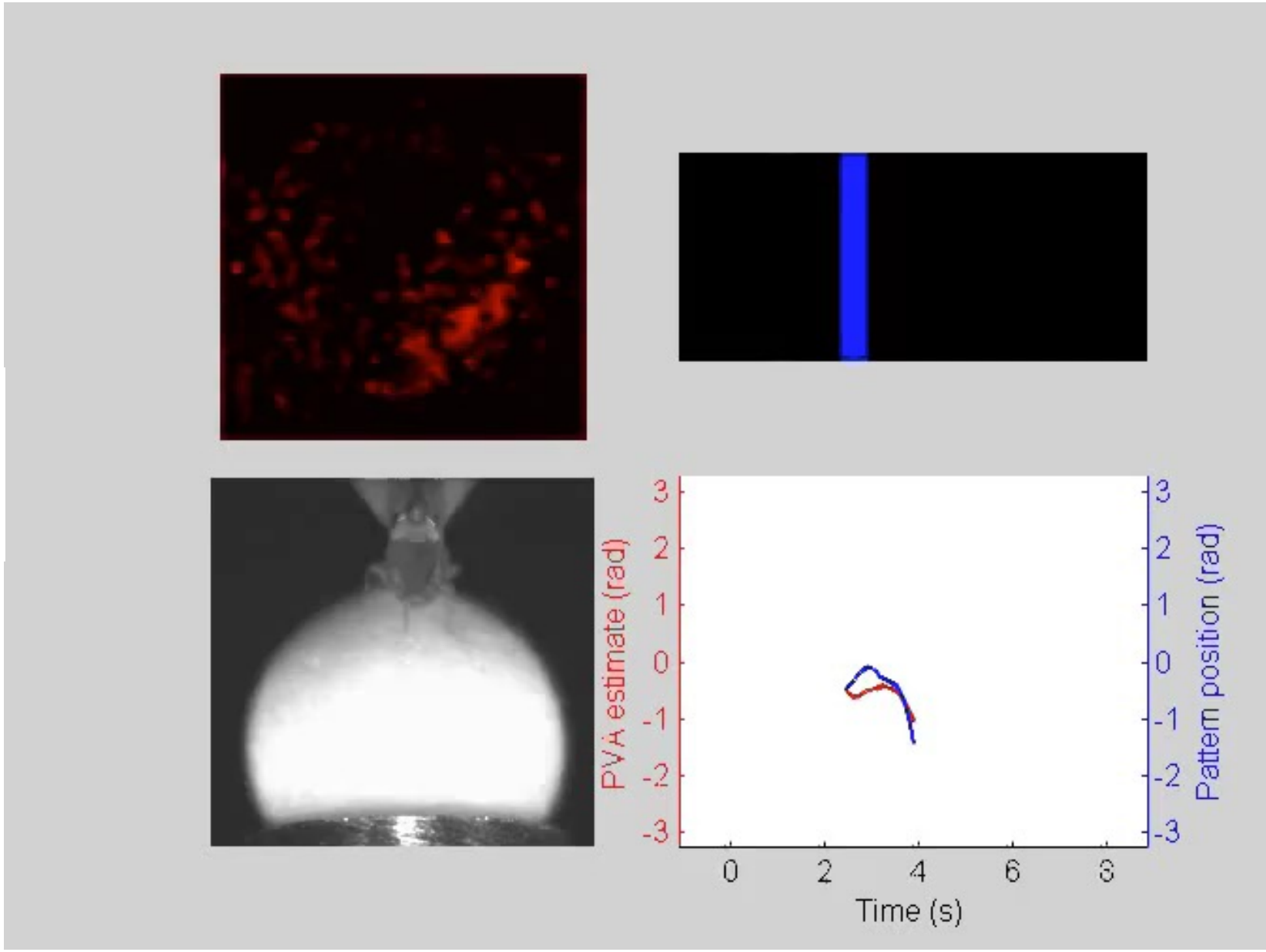


Recording neural activity in tethered flies

Neural dynamics for landmark orientation and angular path integration

Johannes D. Seelig & Vivek Jayaraman

Nature **521**, 186–191 (14 May 2015) Received: 01 December 2014



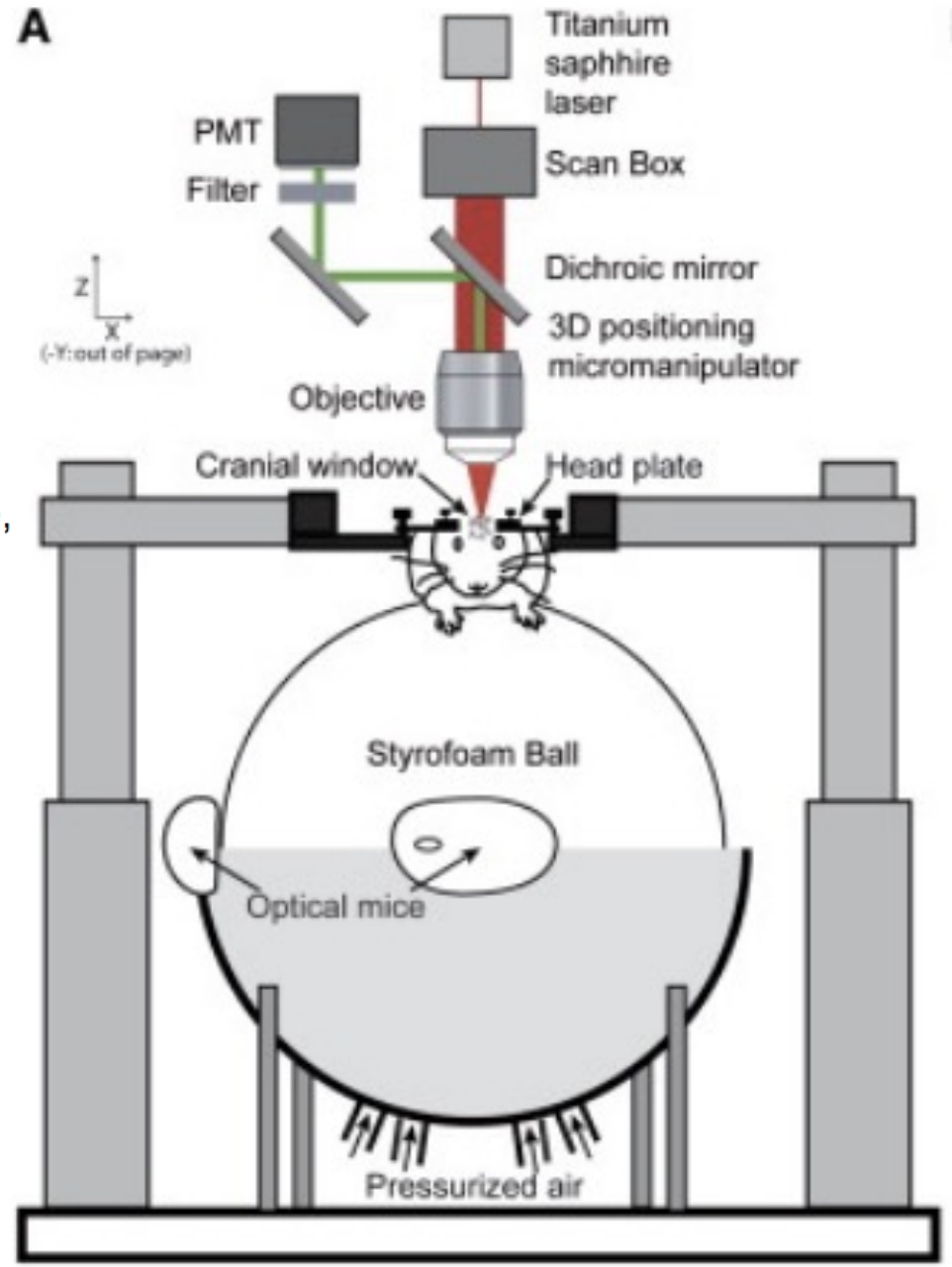
Recording neural activity in tethered mice

Imaging Large-Scale Neural Activity with Cellular Resolution in Awake, Mobile Mice

Daniel A. Dombeck[3], Anton N. Khabbaz[3], Forrest Collman, Thomas L. Adelman, David W. Tank

[3] These authors contributed equally to this work.

Volume 56, Issue 1, p43–57, 4 October 2007

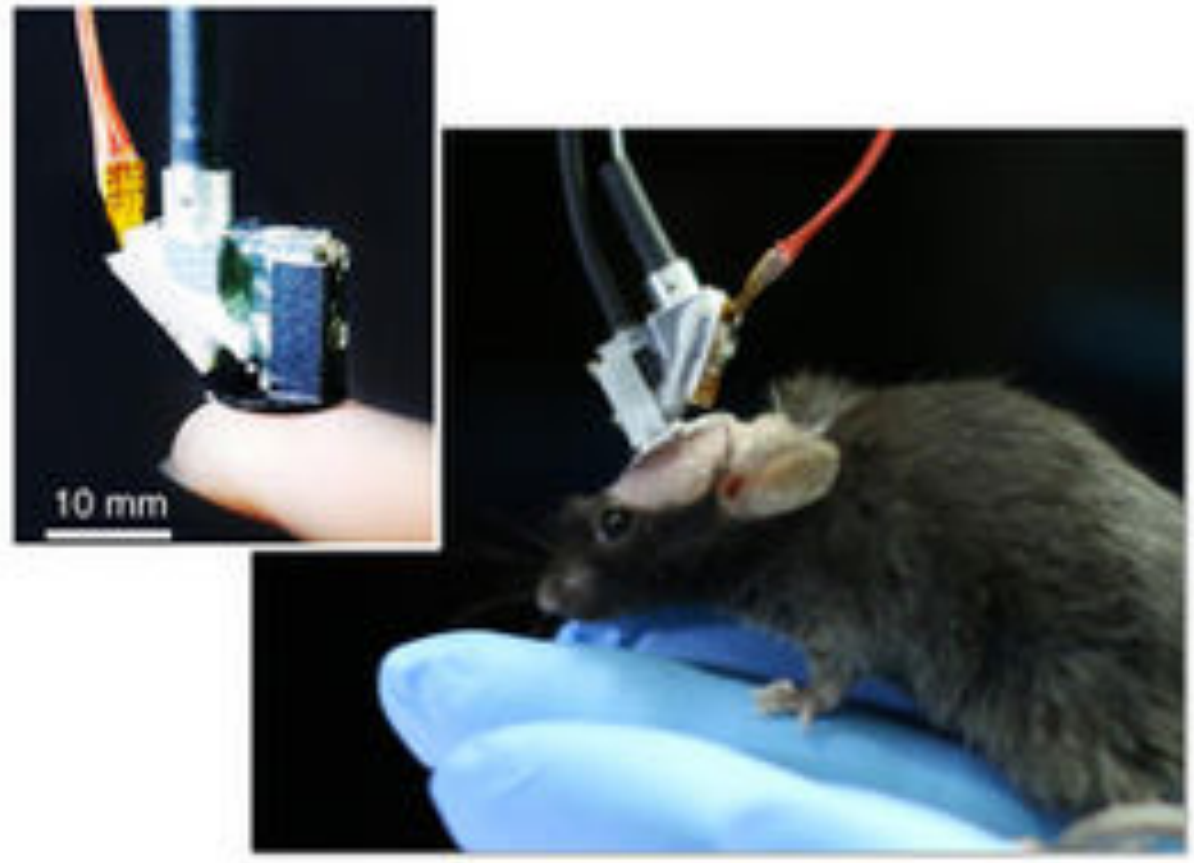


Recording neural activity in **freely-behaving** mice

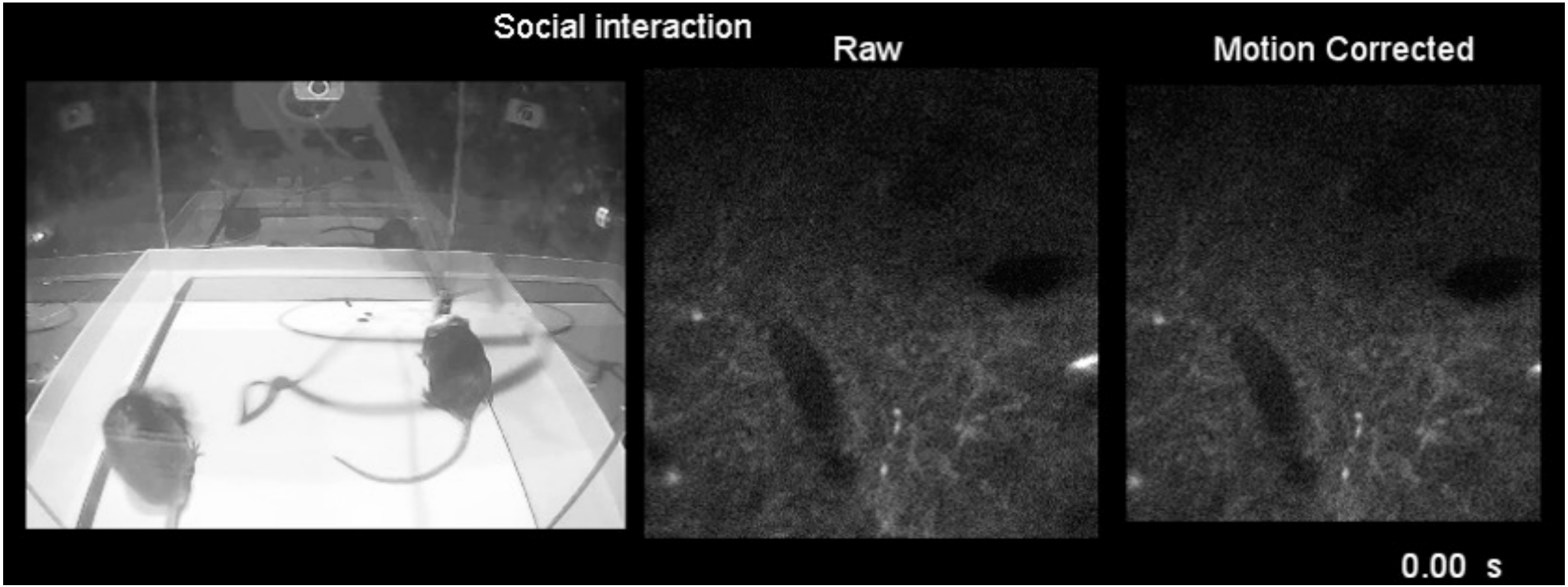
Fast high-resolution miniature two-photon microscopy for brain imaging in freely behaving mice

Weijian Zong, Runlong Wu, Mingli Li, Yanhui Hu, Yijun Li, Jinghang Li, Hao Rong, Haitao Wu, Yangyang Xu, Yang Lu, Hongbo Jia, Ming Fan, Zhuan Zhou, Yunfeng Zhang ✉, Aimin Wang ✉, Liangyi Chen ✉ & Heping Cheng

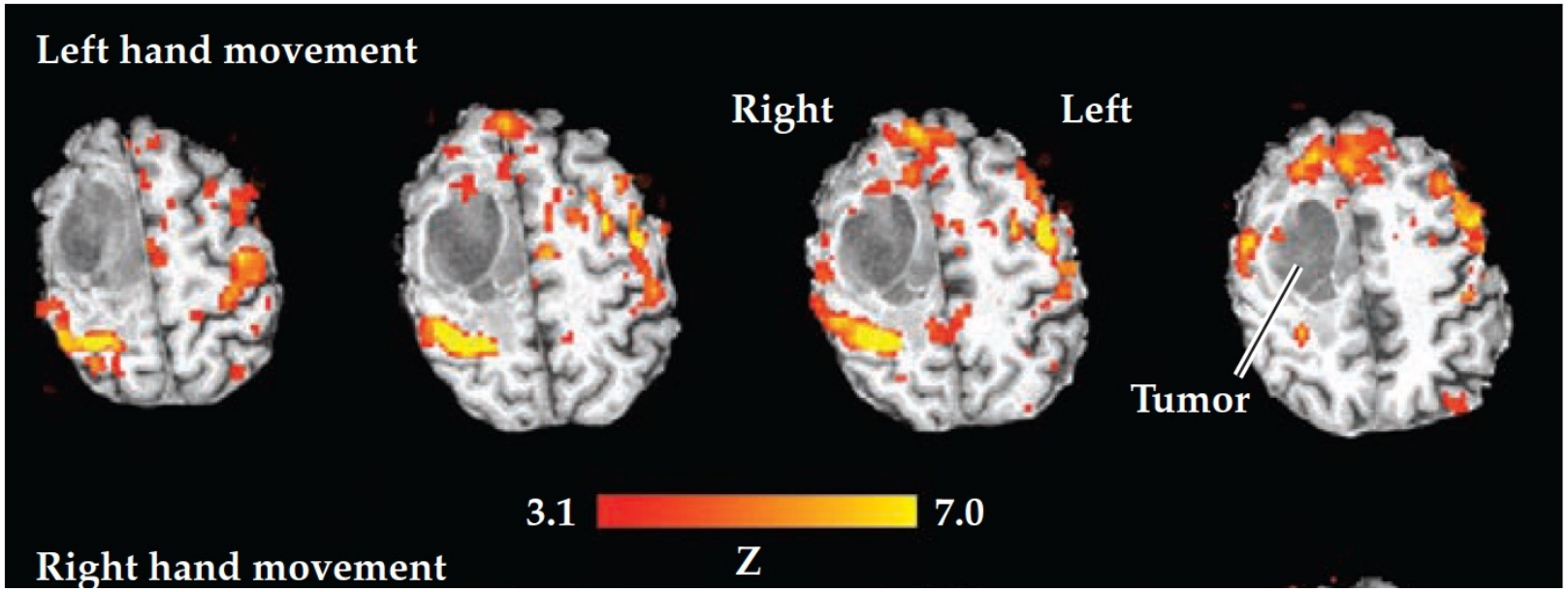
Nature Methods **14**, 713–719 (2017) | Received: 12 December 2016



Recording neural activity in **freely-behaving** mice



fMRI recordings of the human brain



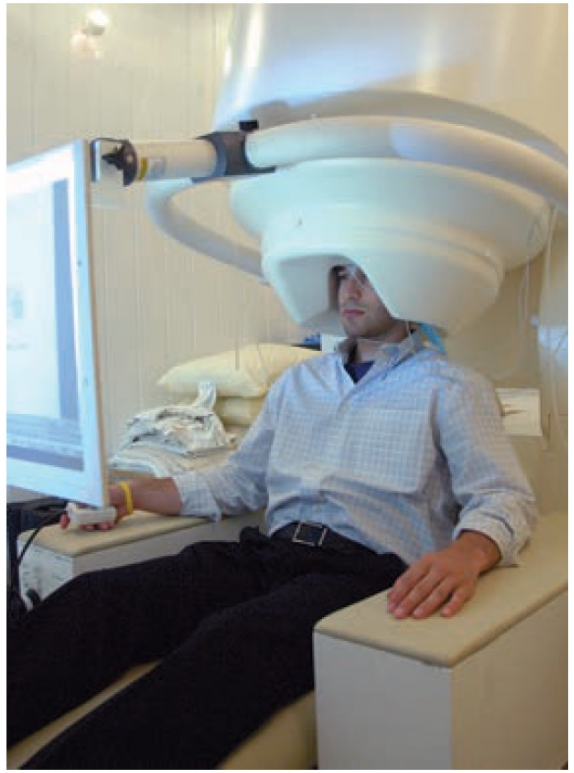
Functional Magnetic Resonance Imaging (fMRI)

- Based on local metabolism. fMRI relies on the fact that hemoglobin in blood slightly distorts the magnetic resonance properties of hydrogen nuclei in its vicinity, and the amount of magnetic distortion changes depending on whether the hemoglobin has oxygen bound to it.
- When a brain area is activated by a specific task, it begins to use more oxygen, and within seconds the brain microvasculature responds by increasing the flow of oxygen-rich blood to the active area. These changes in the concentration of oxygen and blood flow lead to localized blood oxygenation level–dependent (**BOLD**) changes in the magnetic resonance signal. Such fluctuations are detected using statistical image-processing techniques to produce maps of active brain regions.

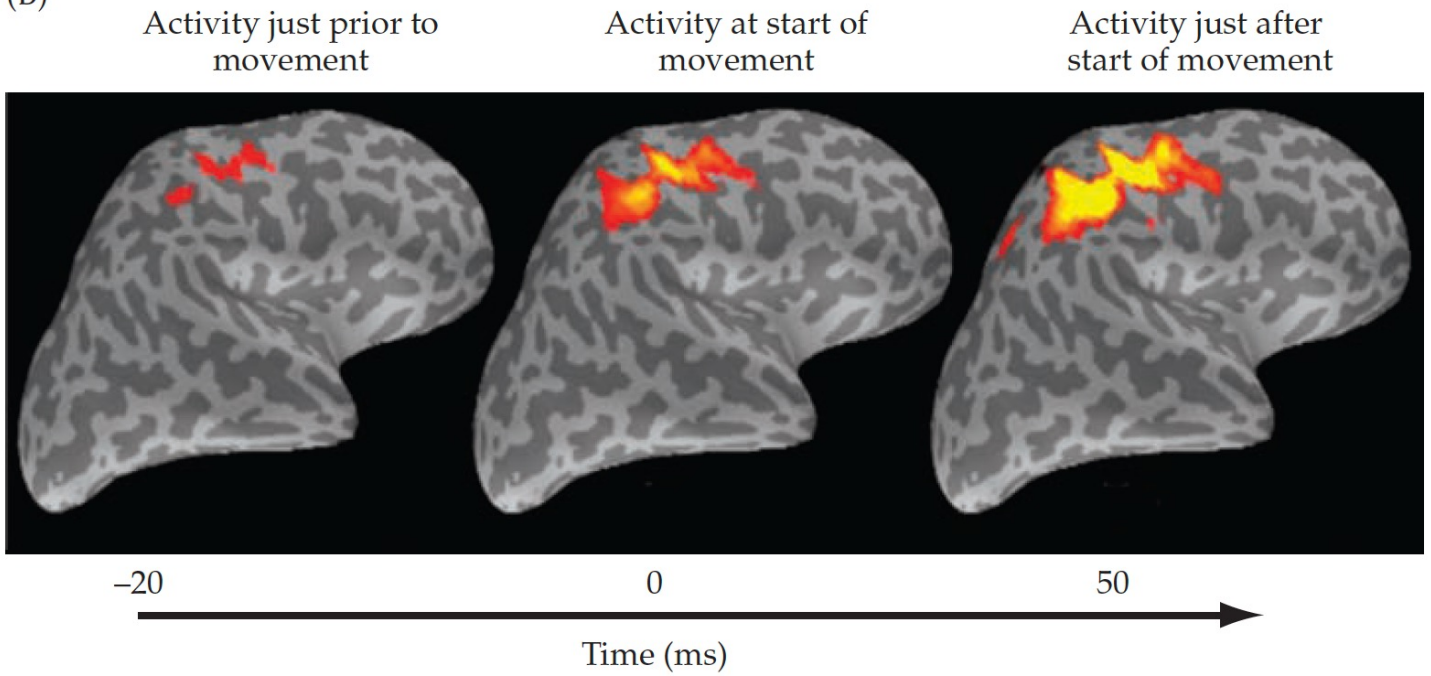
Purves, Figure 1.21

MEG recordings of the human brain

(A)



(B)



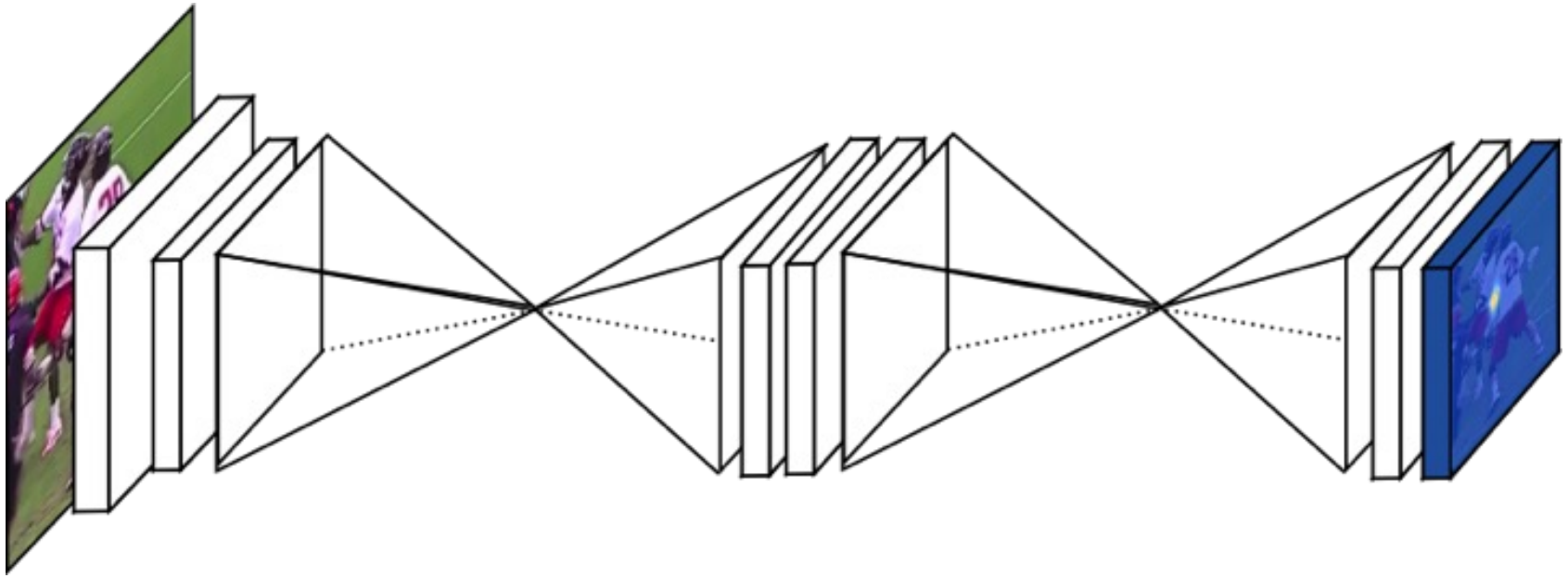
Magnetoencephalography (MEG) provides greater temporal resolution with useful spatial resolution.

- An individual is fitted with a helmet that includes several magnetic detectors (a SQUID array) and then is placed in a biomagnetometer (the large cylindrical structure) that can amplify the small local changes in magnetic field orientation or strength that indicate fast temporal current flow changes in ensembles of neurons.
- The temporal resolution of MEG permits millisecond resolution of electrical activity in the human brain before, during, and after performance of a variety of tasks

Purves, Figure 1.22

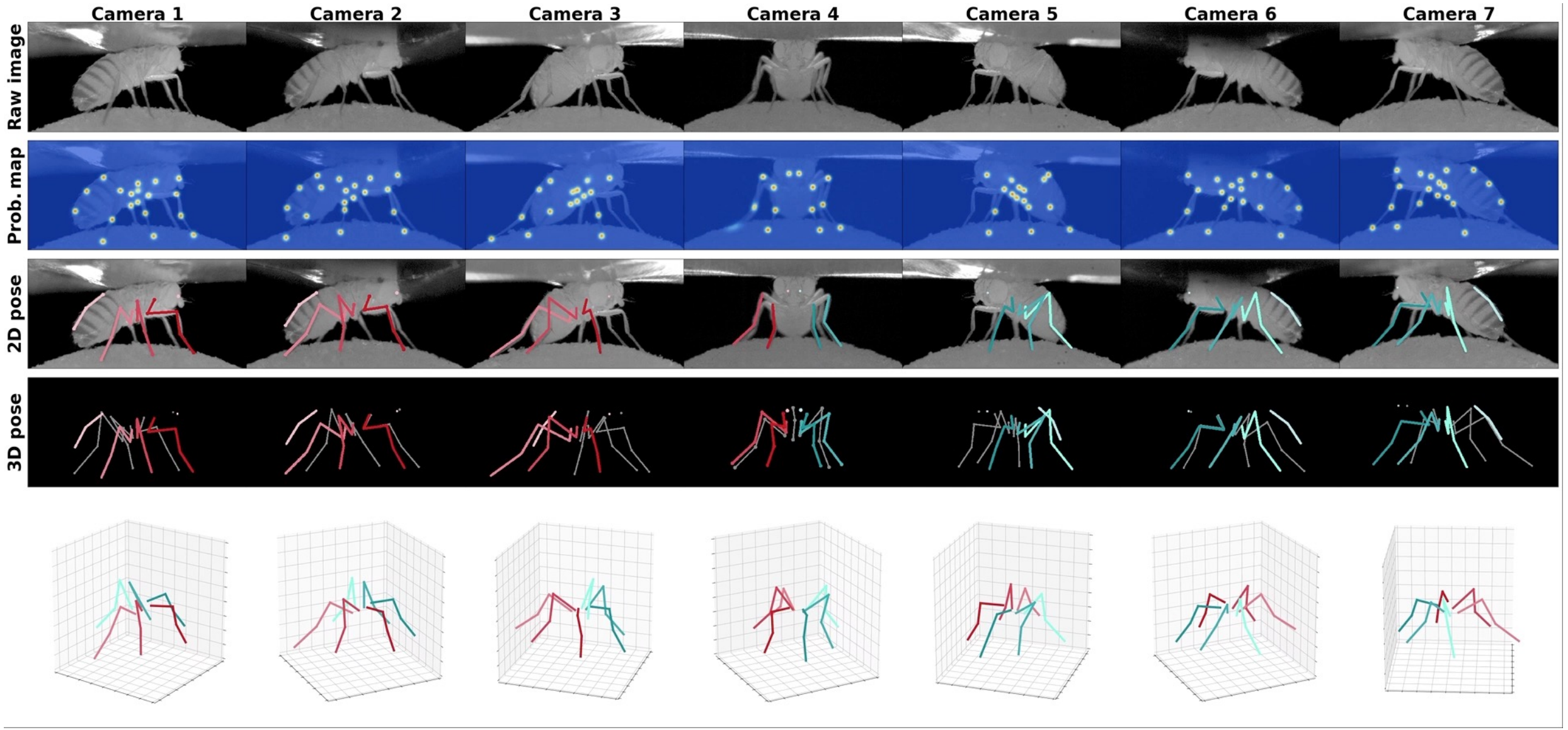
Quantifying behavior

Using deep networks to automatically find keypoints



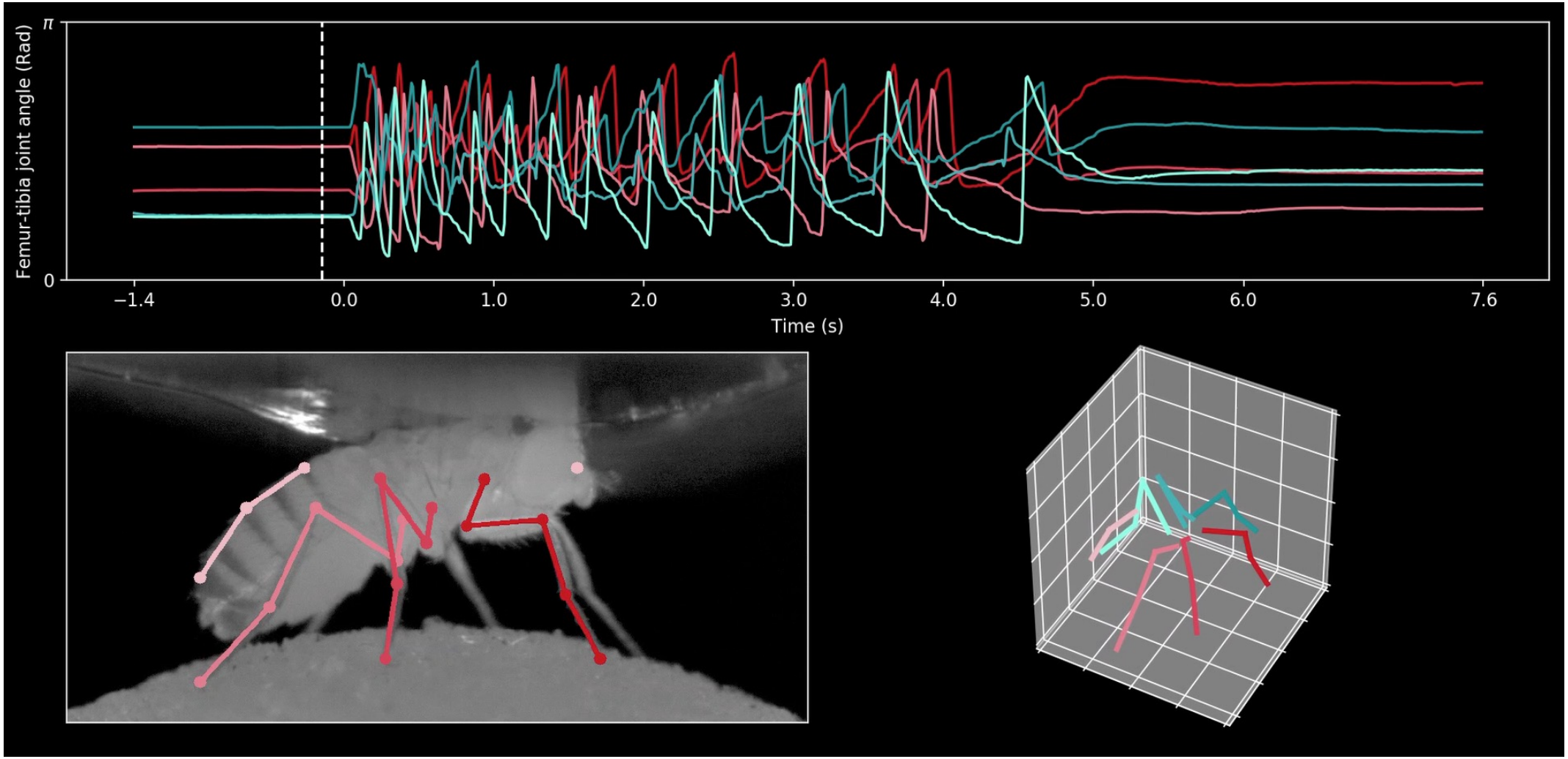
Newell et al. CVPR, 2016

DeepFly3D: Deep network-based 3D pose estimation



Günel et al. Elife, 2019

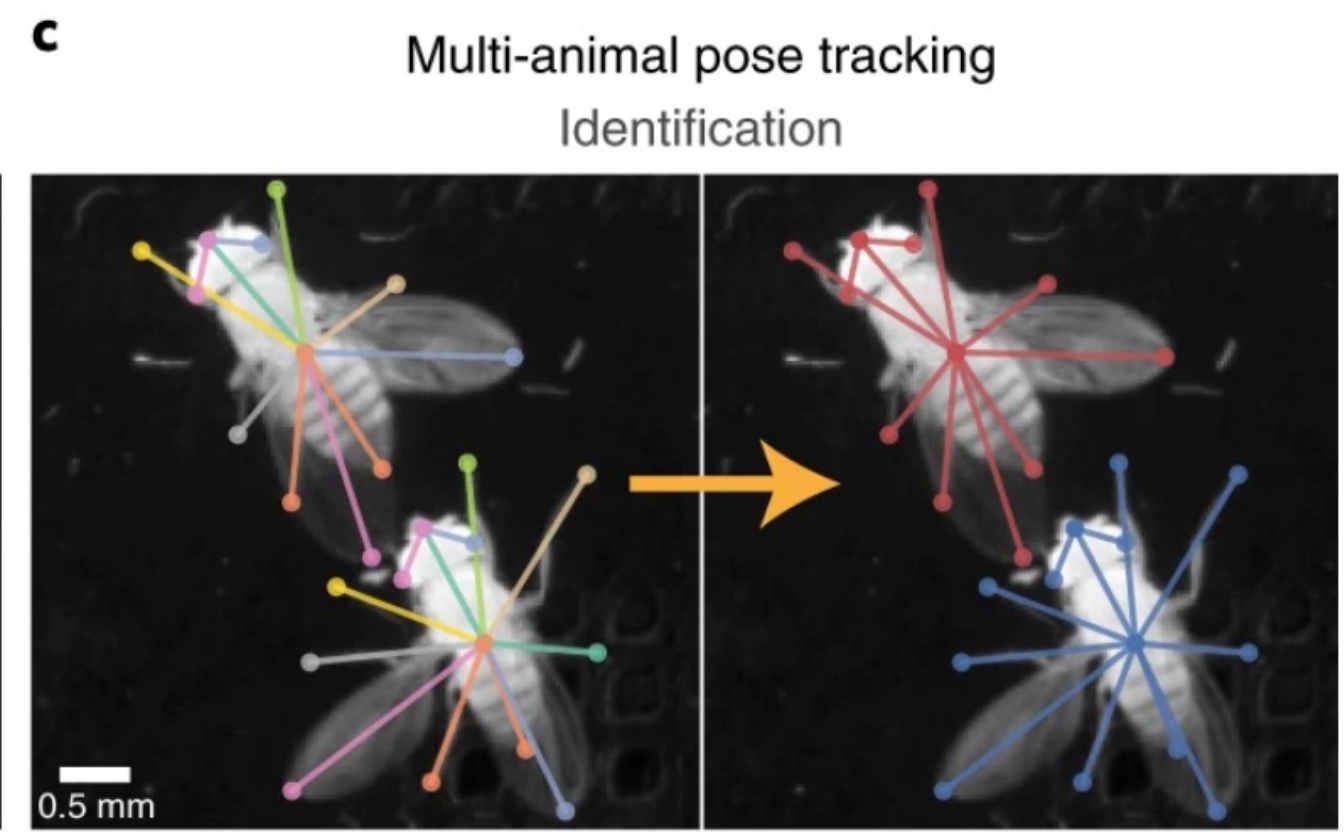
DeepFly3D: Deep network-based 3D pose estimation



Günel et al. Elife, 2019

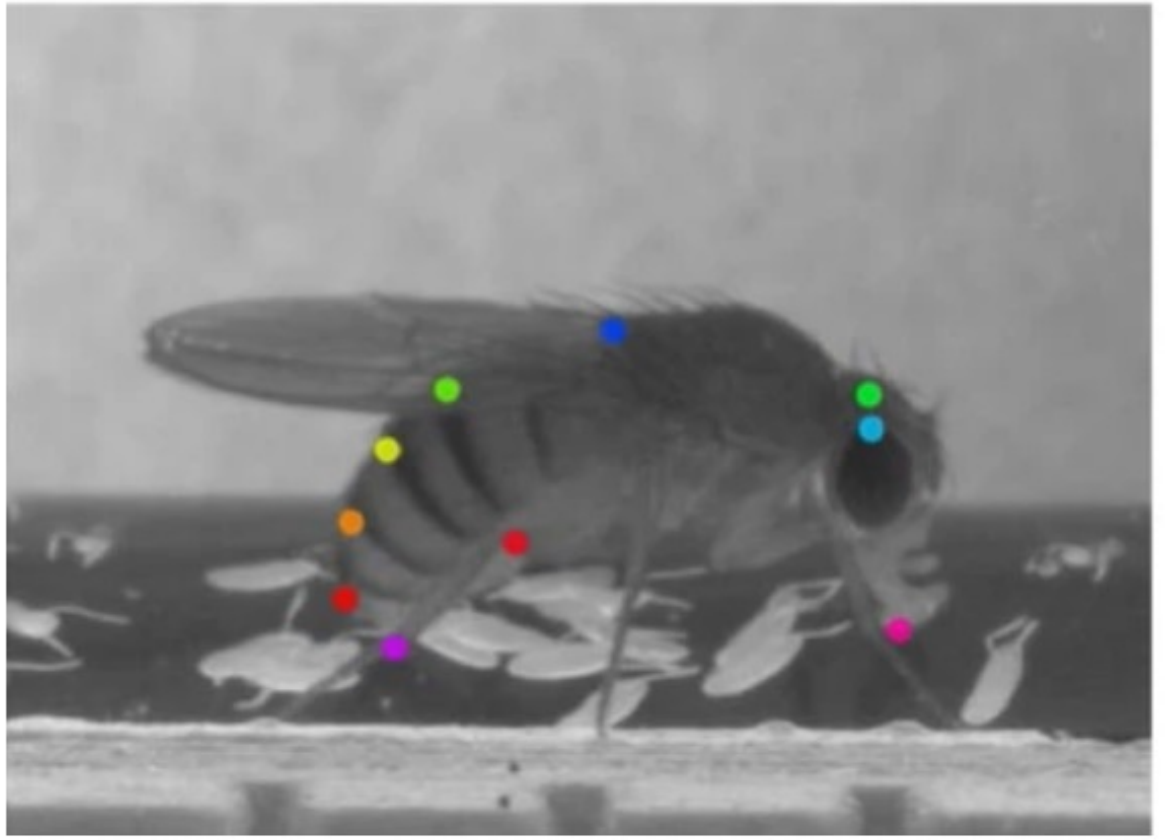
Popular 2D pose estimation tools

SLEAP



Pereira et al., *Nature Methods* 2022

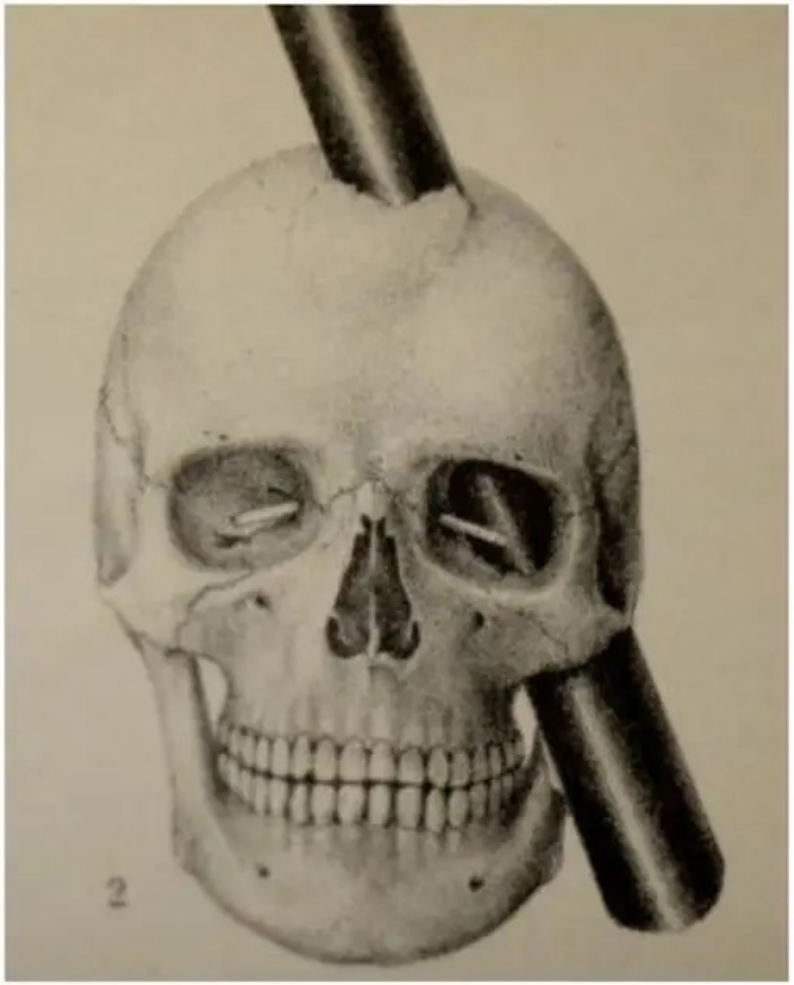
DeepLabCut



Mathis et al. *Nature Neuro.* 2018

Perturbing neurons

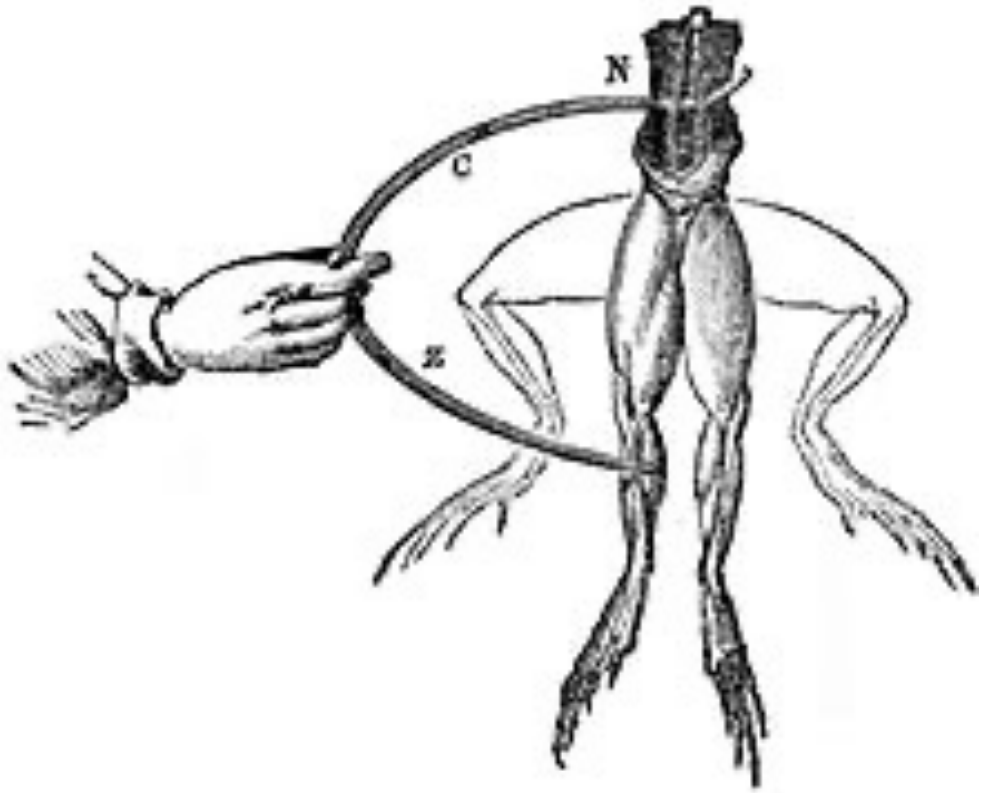
Accidental brain perturbations

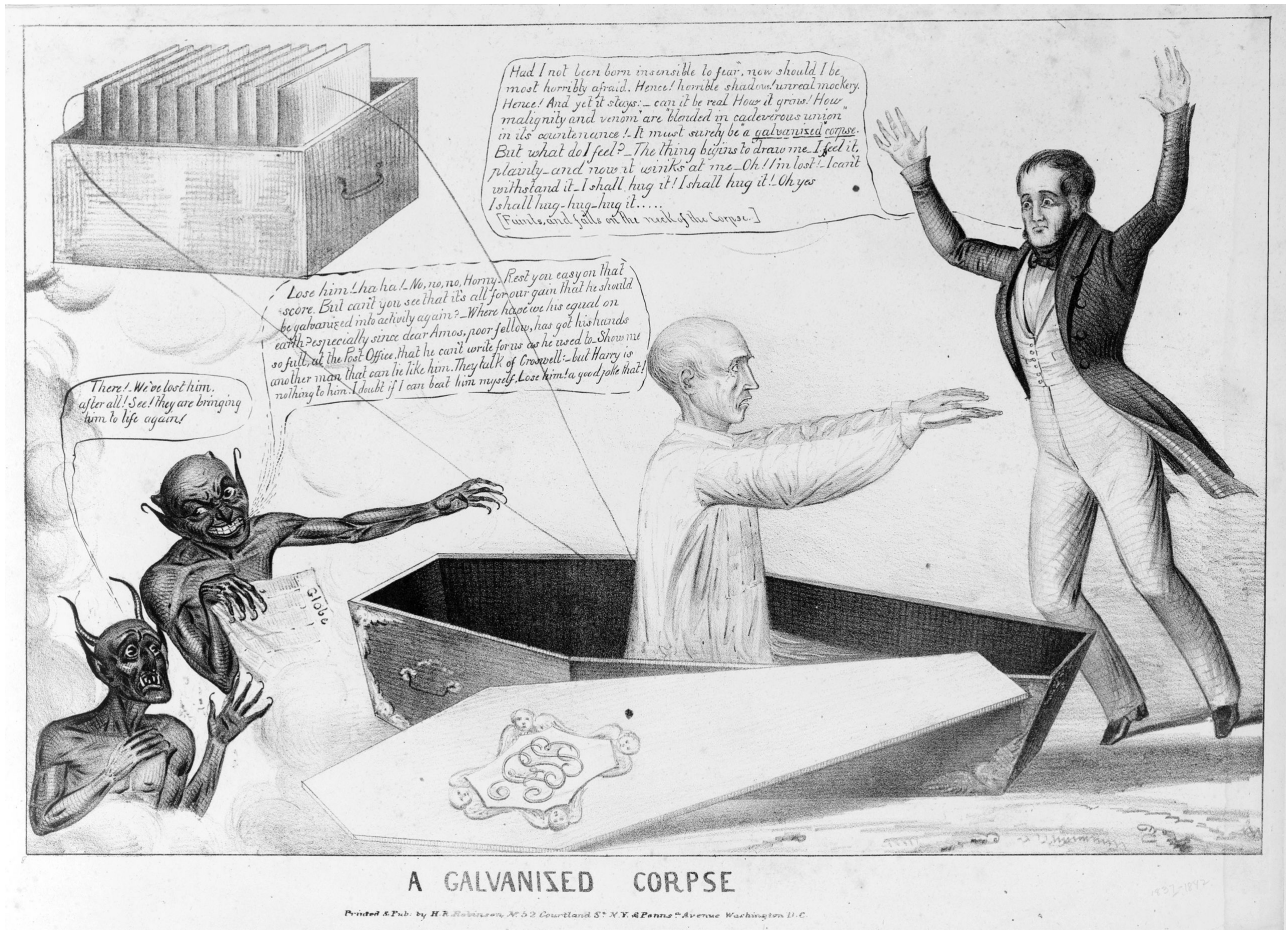



PHINEAS P. GAGE,
Tuesday Evening,
AT
RUMFORD HALL,
Greatest Wonders of the World!
Huge Iron Bar, which he will Exhibit,
HIS BRAINS BLOWN OUT!
IRON BAR!
THROUGH HIS HEAD!

https://www.youtube.com/watch?v=0mf_Nf7fKIQ

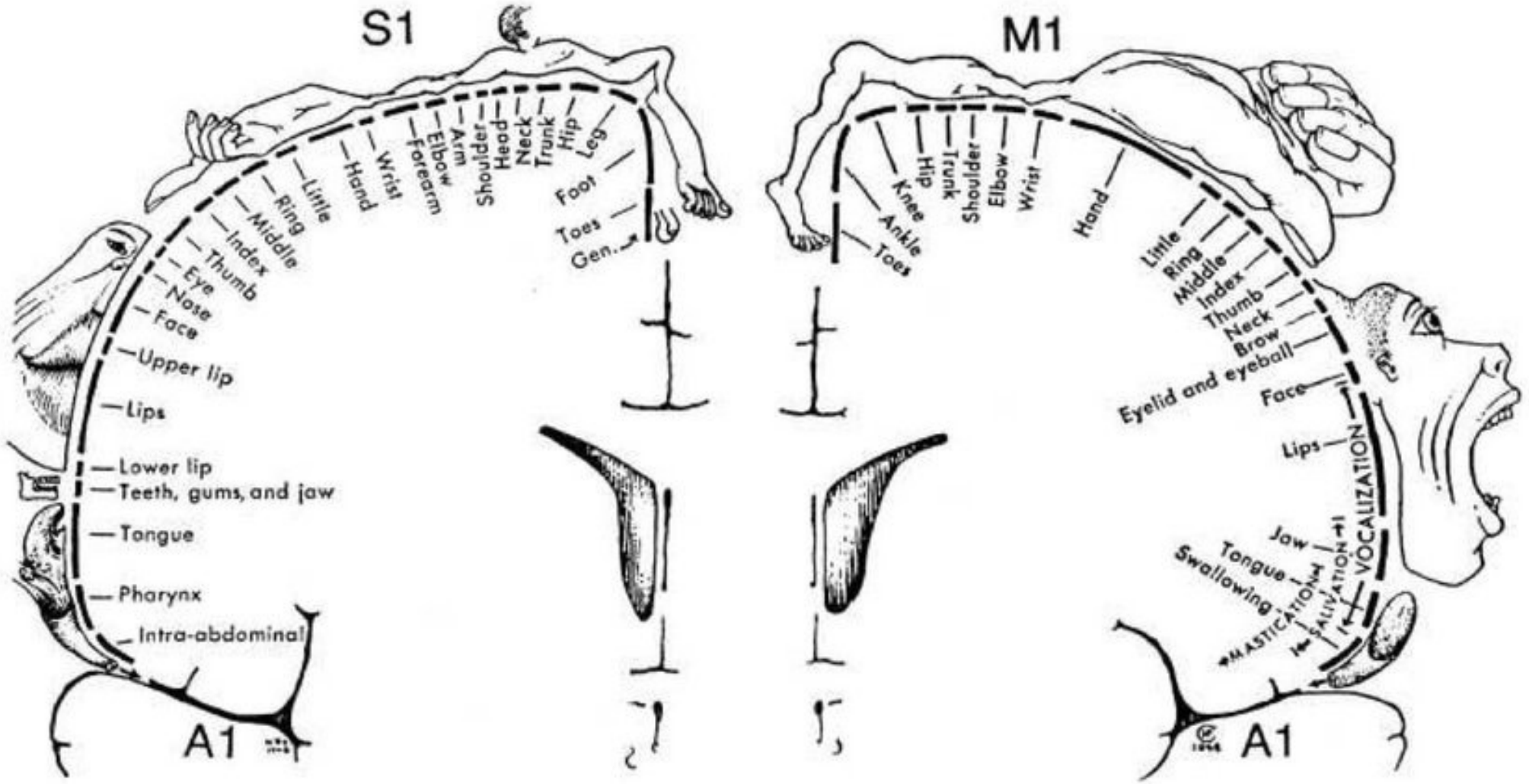
Electrical Stimulation of neurons





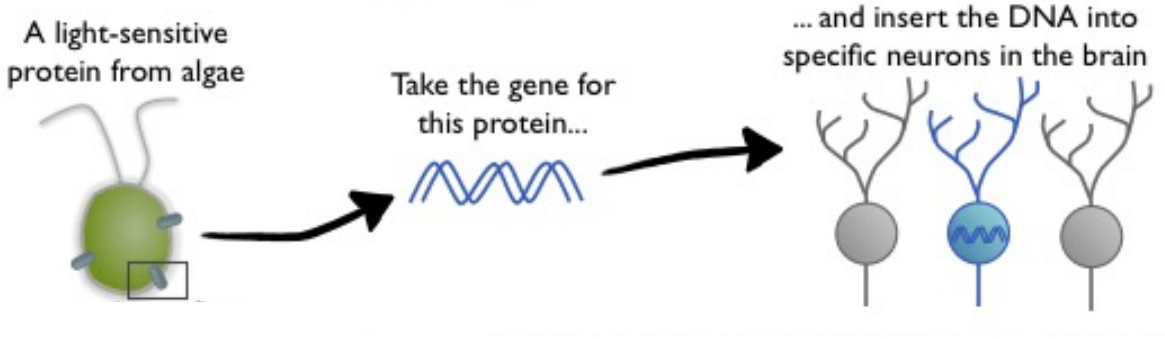
https://en.wikipedia.org/wiki/Galvanism

Electrical stimulation (Wilder Penfield)

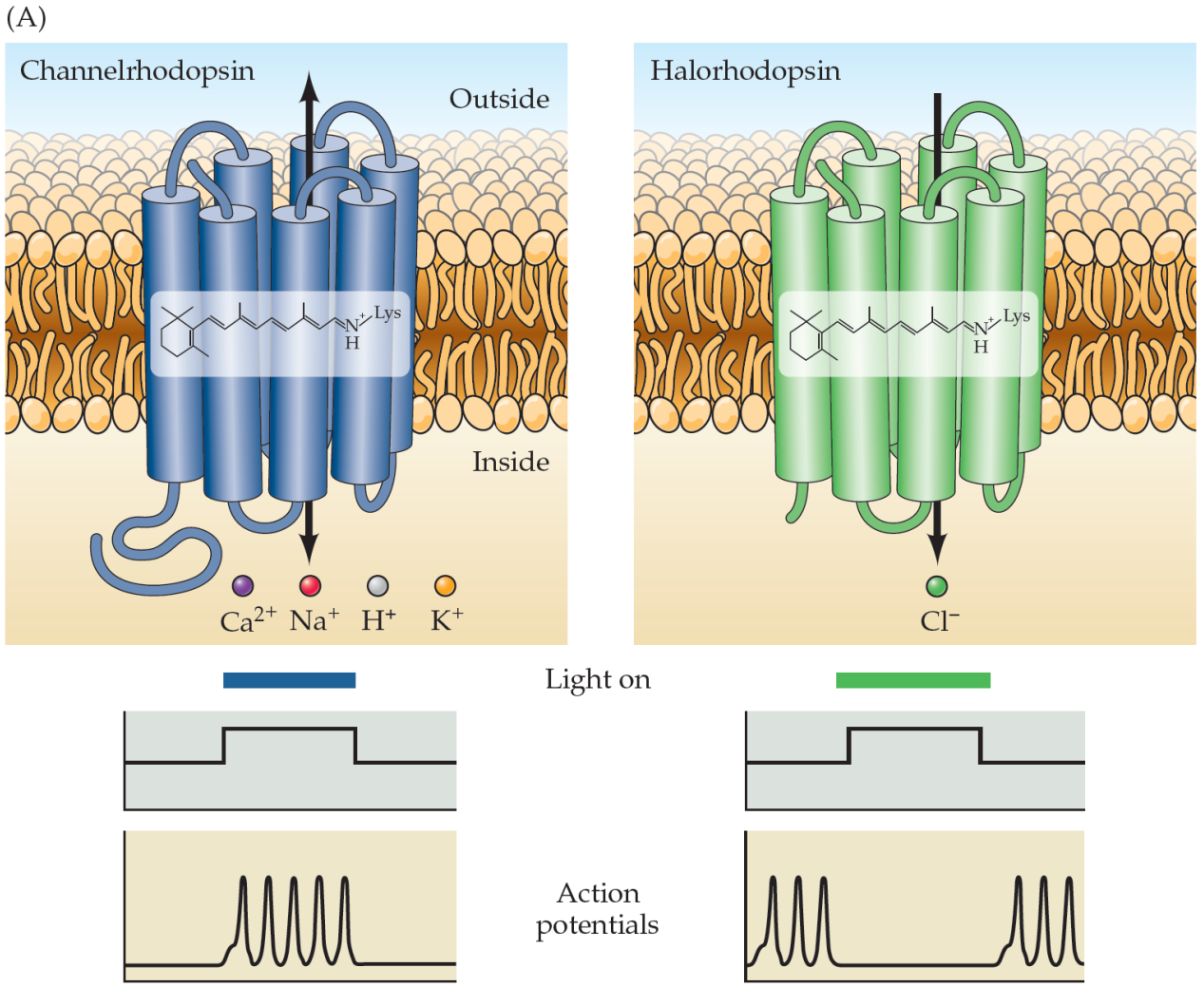


From Penfield and Rasmussen (1950). THE CEREBRAL CORTEX OF MAN.

Optogenetic stimulation



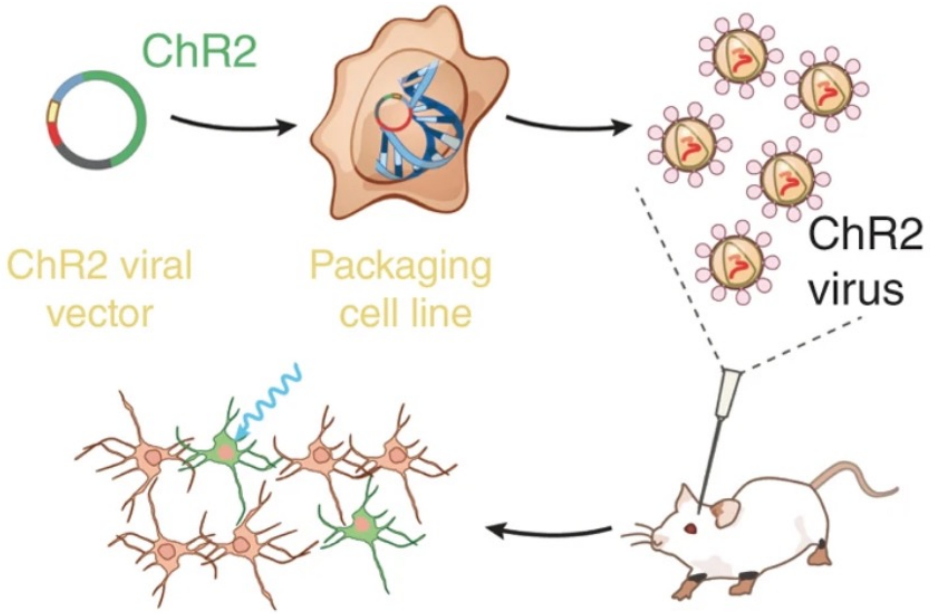
- Channelrhodopsin-2 ('ChR2')
- From the algae, *Chlamydomonas reinhardtii*
- Activated by blue light, nonspecific cation entry
- Depolarizes neurons, causing them to fire action potentials

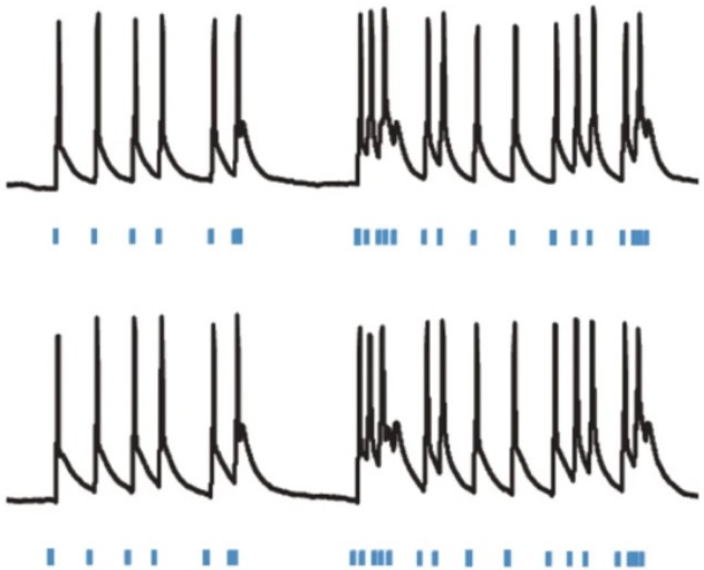


Ordaz et al. Neural Regeneration Research 2017

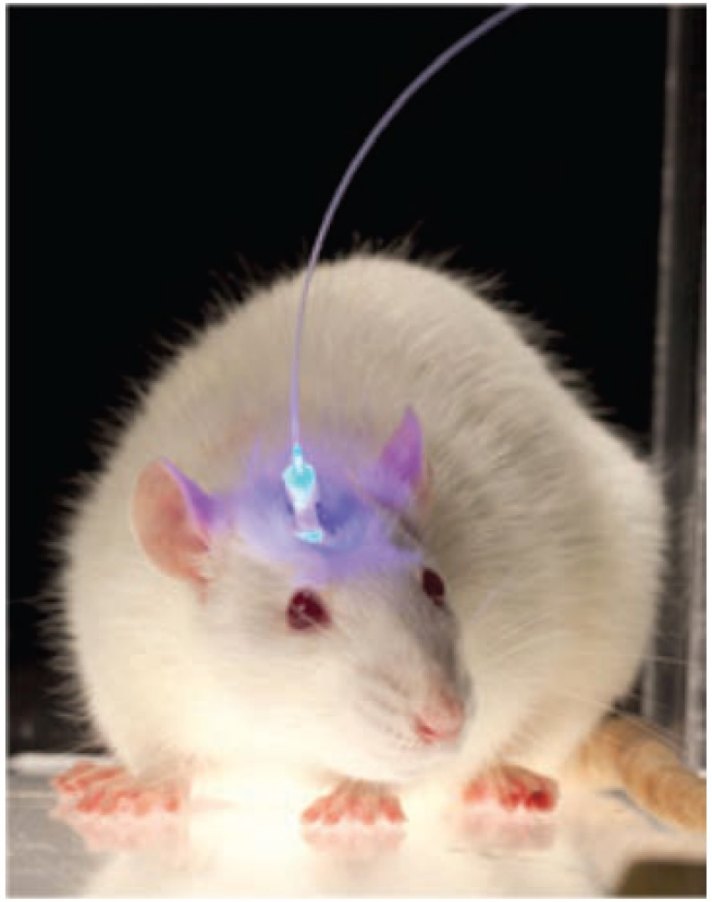
Purves, Figure 1.11

Optogenetic stimulation in mice





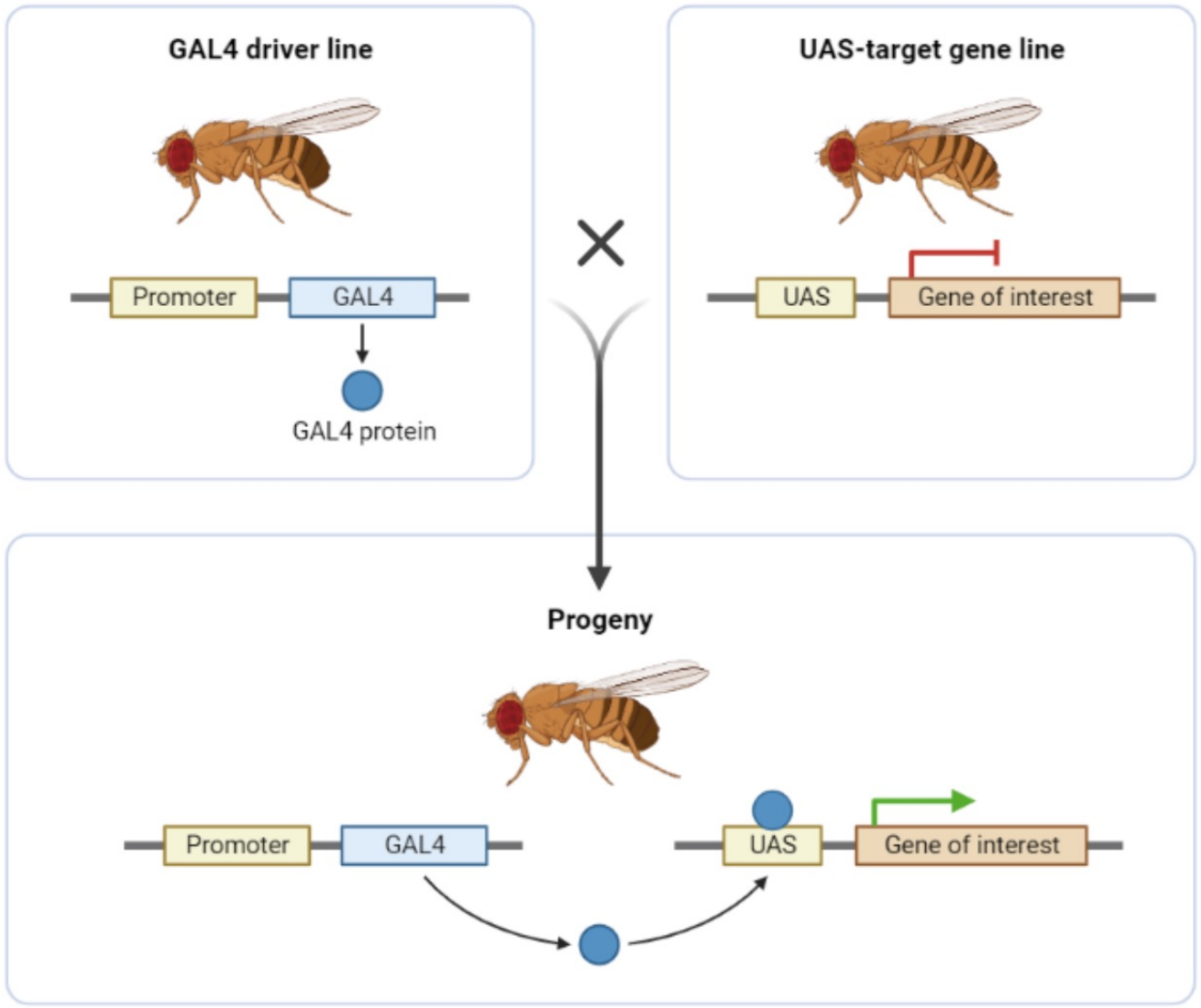
(B)

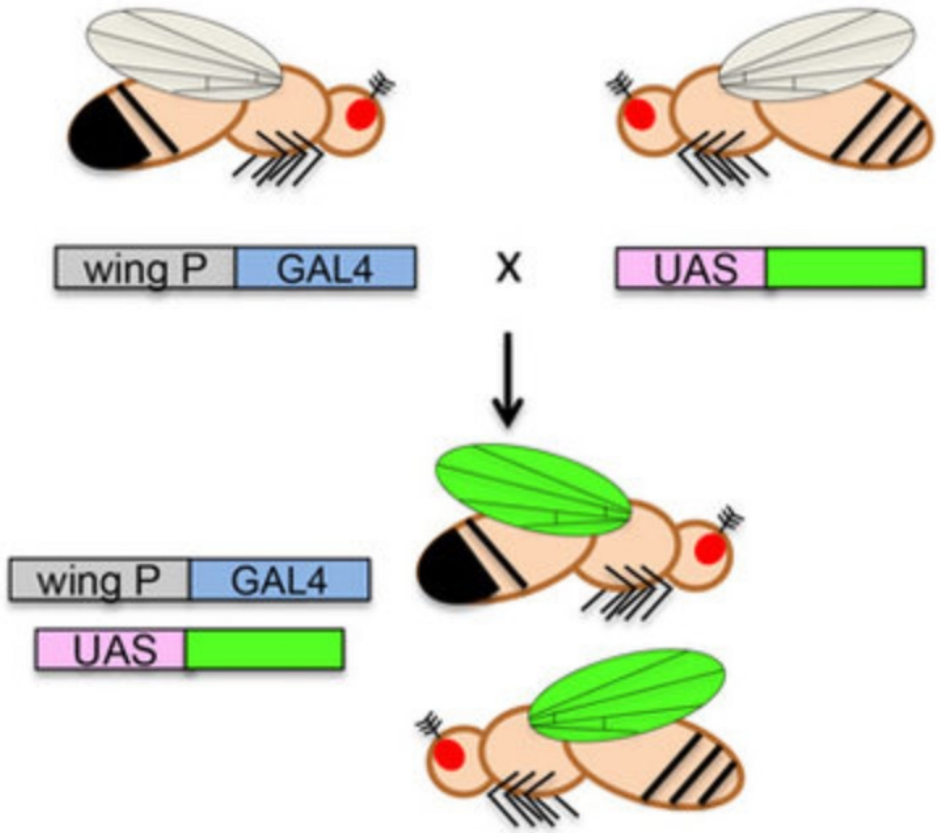


Deisseroth et al., *Nat. Neuro*. 2015

Purves, Figure 1.11

Optogenetic stimulation in flies – a UAS-Gal4 gene expression system





https://www.biorender.com/template/drosophila-gal4-uas-system

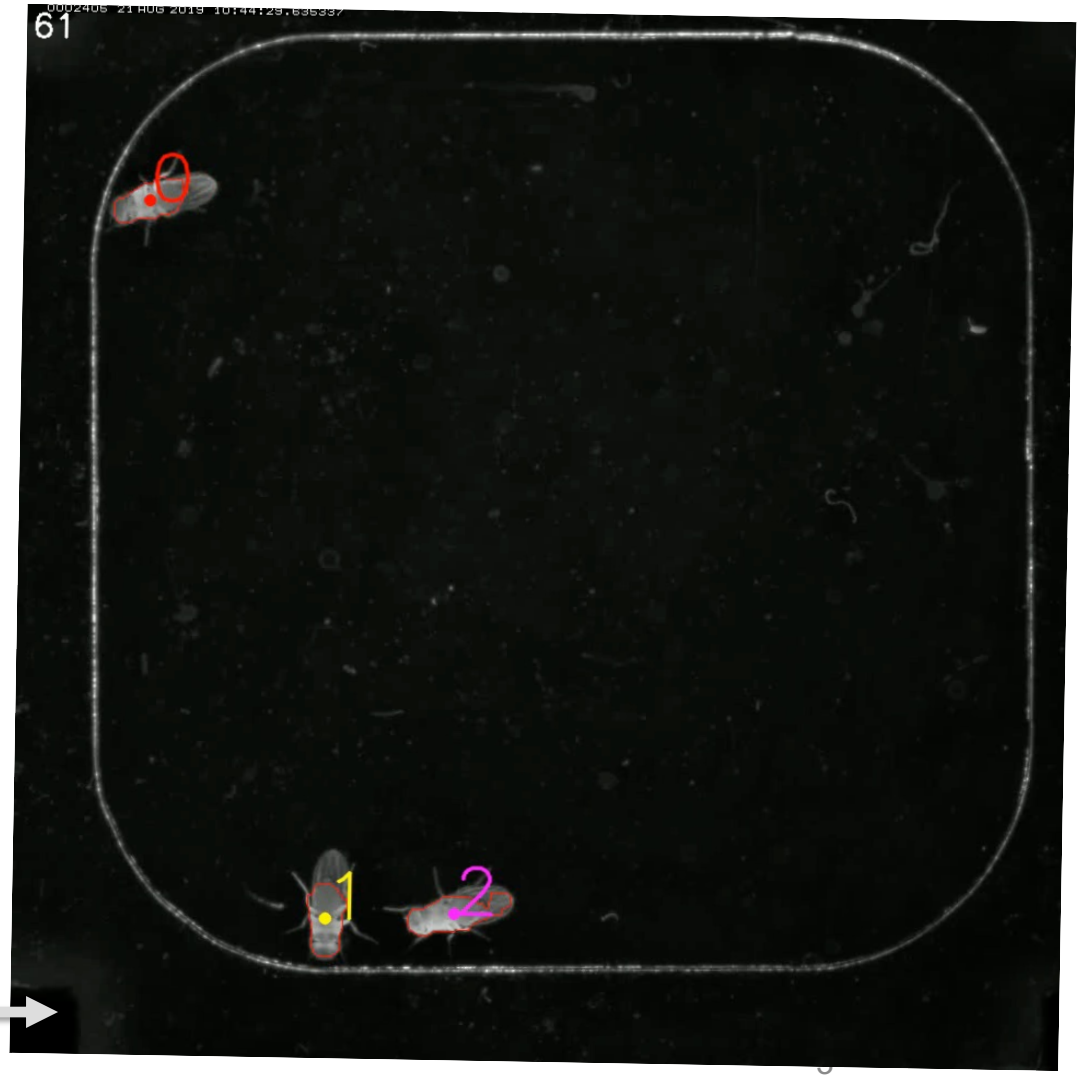
Hales et al., *Genetics* 2015

Optogenetic stimulation in flies – targeting locomotor descending neurons

Cell bodies of “Moonwalker Descending Neurons”

Dendrites

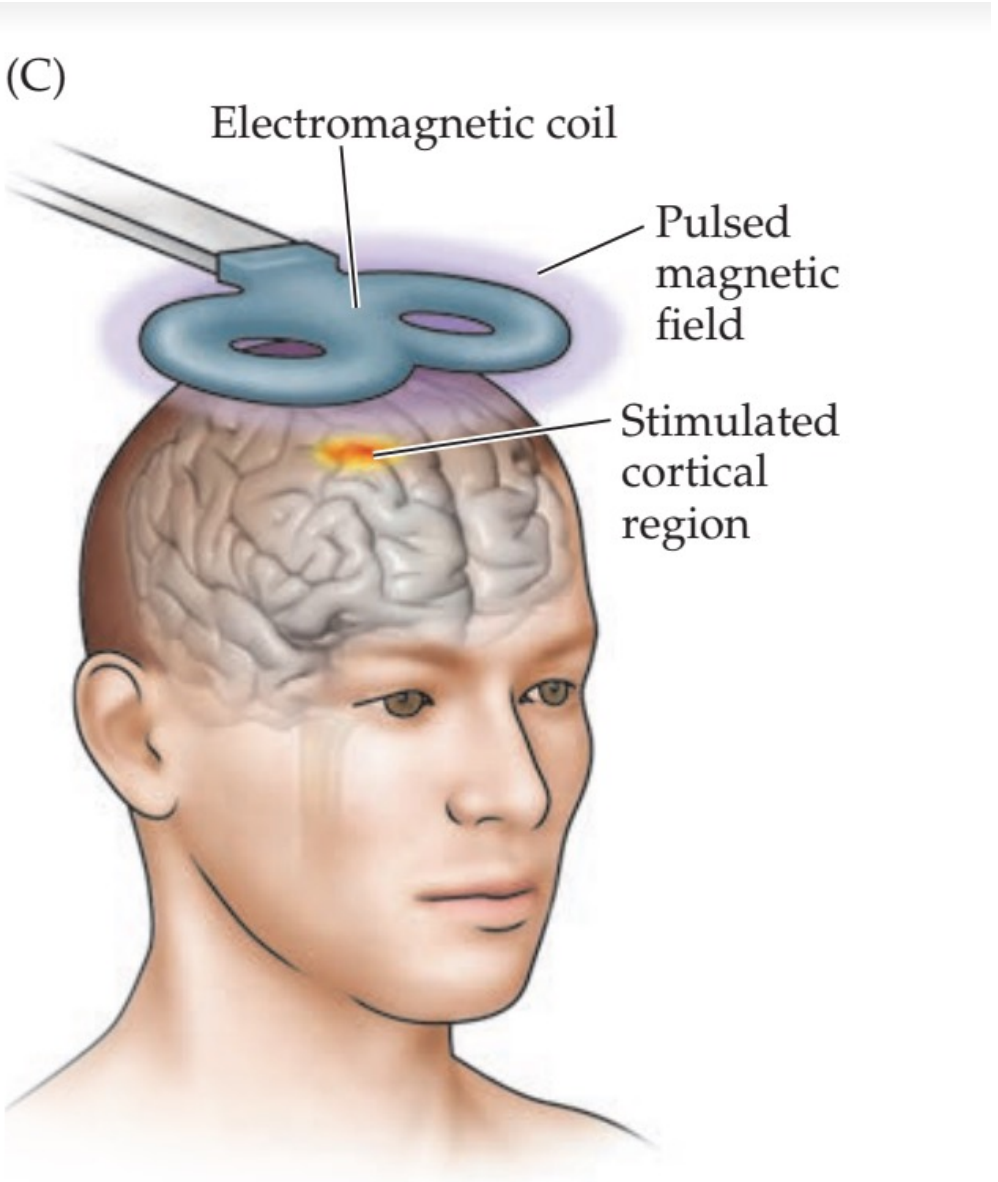
Axons



Light on = activate neurons with ChR2

TMS: a non-invasive way to modulate neuron activity in humans

- **Transcranial magnetic stimulation** (**TMS**): a noninvasive form of brain stimulation in which a *focused* magnetic field is used to elicit electric current at a specific area of the brain through *electromagnetic* induction.



Purves, Figure 1.18

Summary: Techniques: Important concepts and keywords

- CT and MRI scans permit coarse human brain mapping
- GFP enables measurements of neuron structure in living tissue
- Connectomics: how to obtain a synapse-resolution brain map in the fly
- When to use optical or electrophysiological methods to record neurons. The mechanism by which the genetically encoded calcium indicator (GECI), “GCaMP” works
- Why to record neural activity in behaving versus anesthetized animals. Why tethering is sometimes necessary
- fMRI and MEG are ways to record human brain activity non-invasively
- Behavior can be measured using markerless 2D pose estimation tools that leverage convolutional deep neural networks
- Neurons can be activated using optogenetics. For example, ChR2 is a nonspecific cation channel activated by blue light.
- How TMS works to activate human brain regions