Techniques in neuroscience

Prof. Pavan Ramdya

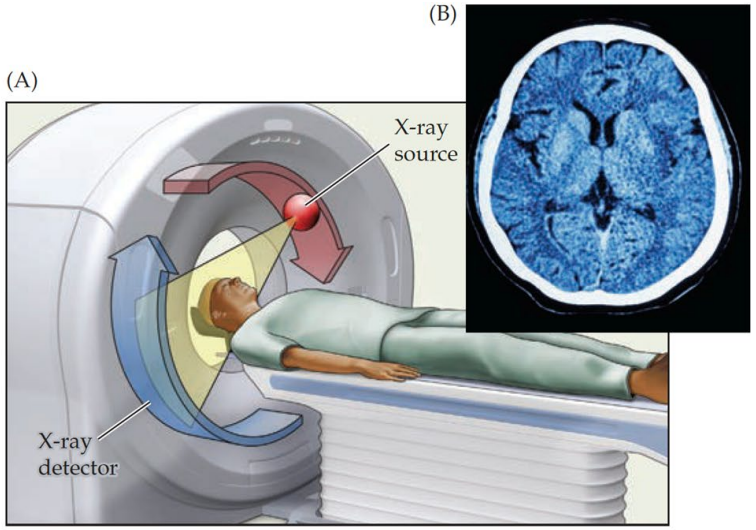
Reading: ***Purves* Chapter 1** pp. 10 – 28

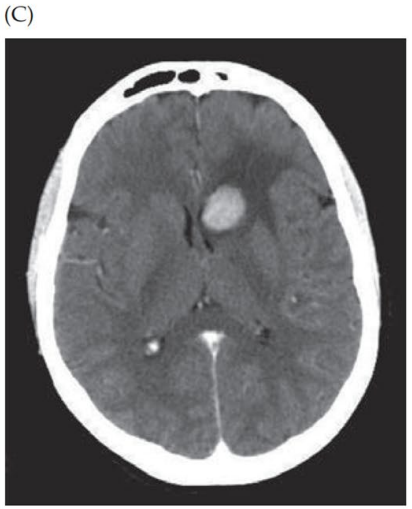
BIO-311

Summary: Techniques: Important concepts and keywords

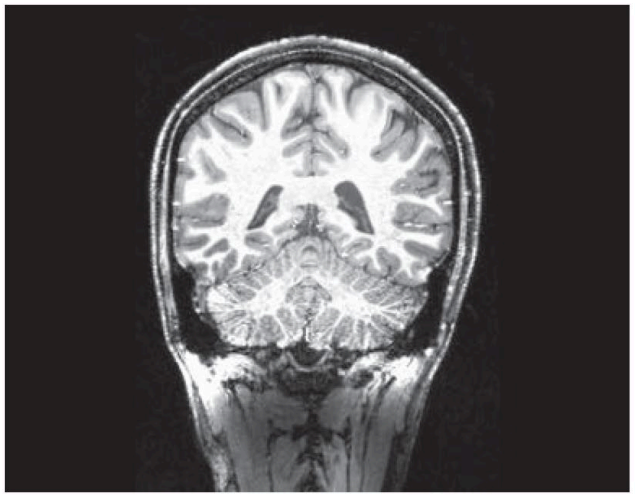
- CT and MRI scans permit coarse human brain mapping
- GFP enables measurements of neuron structure in living tissue
- Connectomics: how to obtain a synapse-resolution brain map in the fly
- Why to use optical rather than electrophysiological methods to record neurons. The mechanism by which the genetically encoded calcium indicator (GECI), “GCaMP” works
- Why to record neural activity in behaving animals. Why tethering is necessary sometimes
- fMRI and MEG are ways to record human brain activity non-invasively
- Behavior can be measured using markerless 3D and 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

Computerized axial tomography.
- the X-ray source and detector are moved around the individual's head



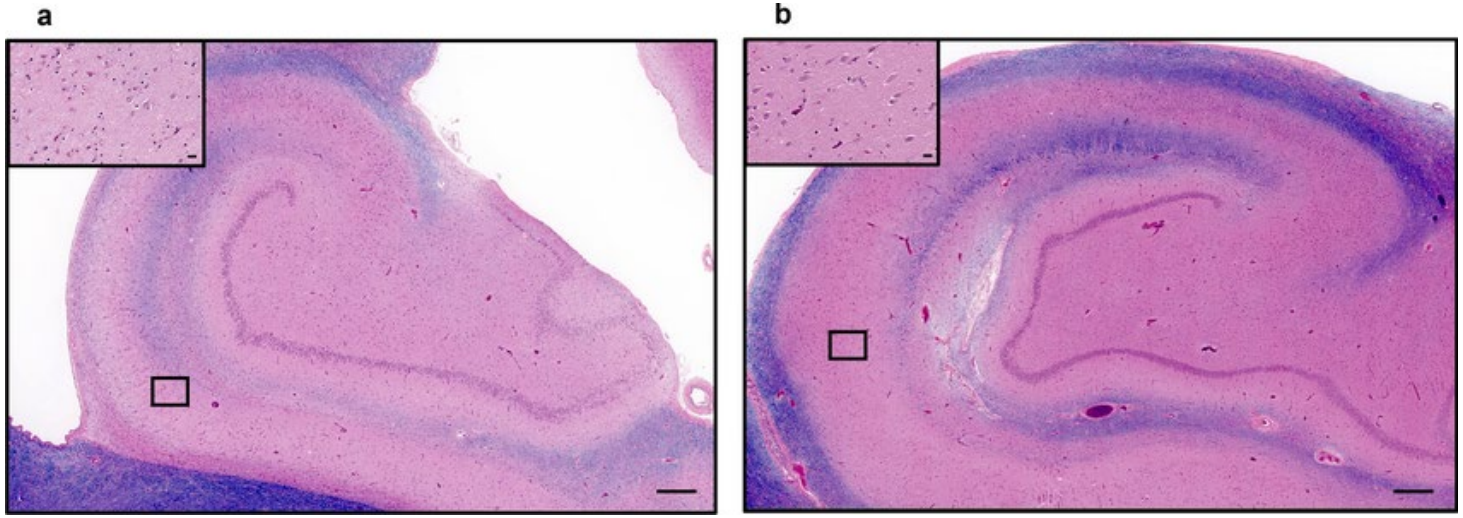


Magnetic Resonance Imaging (MRI)
- A magnetic coil is placed around the head to activate and record magnetic resonance signal.

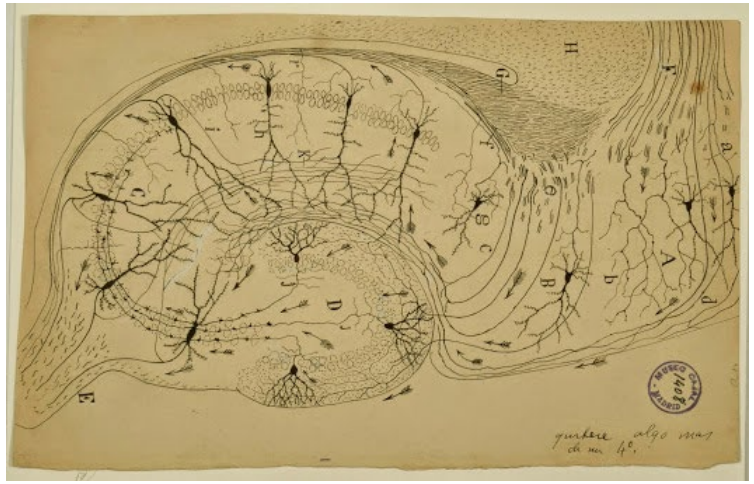


Helps to visualize 'coarse' brain structures

Underlying structure of neurons



Characterization of hippocampal sclerosis of aging and its association with other neuropathologic changes and cognitive deficits in the oldest-old
Soldo et all (2023).June 2023, Acta Neuropathologica 146(3):1-18. DOI:10.1007/s00401-023-02606-9



Santiago Ramón y Cajal; Drawing of the Hippocampus

Microscopy: Visualizing ‘fine’ brain structures

Visualizing neurons using fluorescence microscopy

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

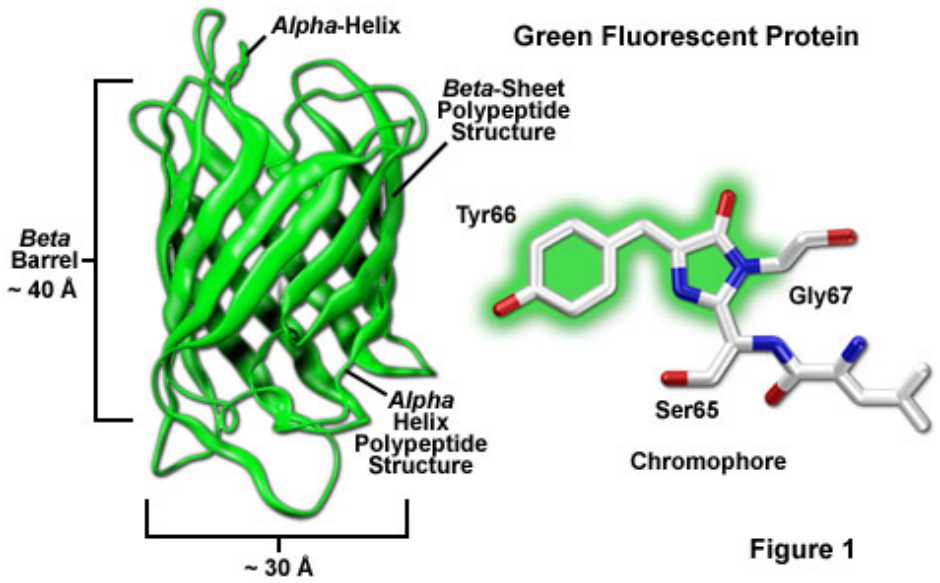
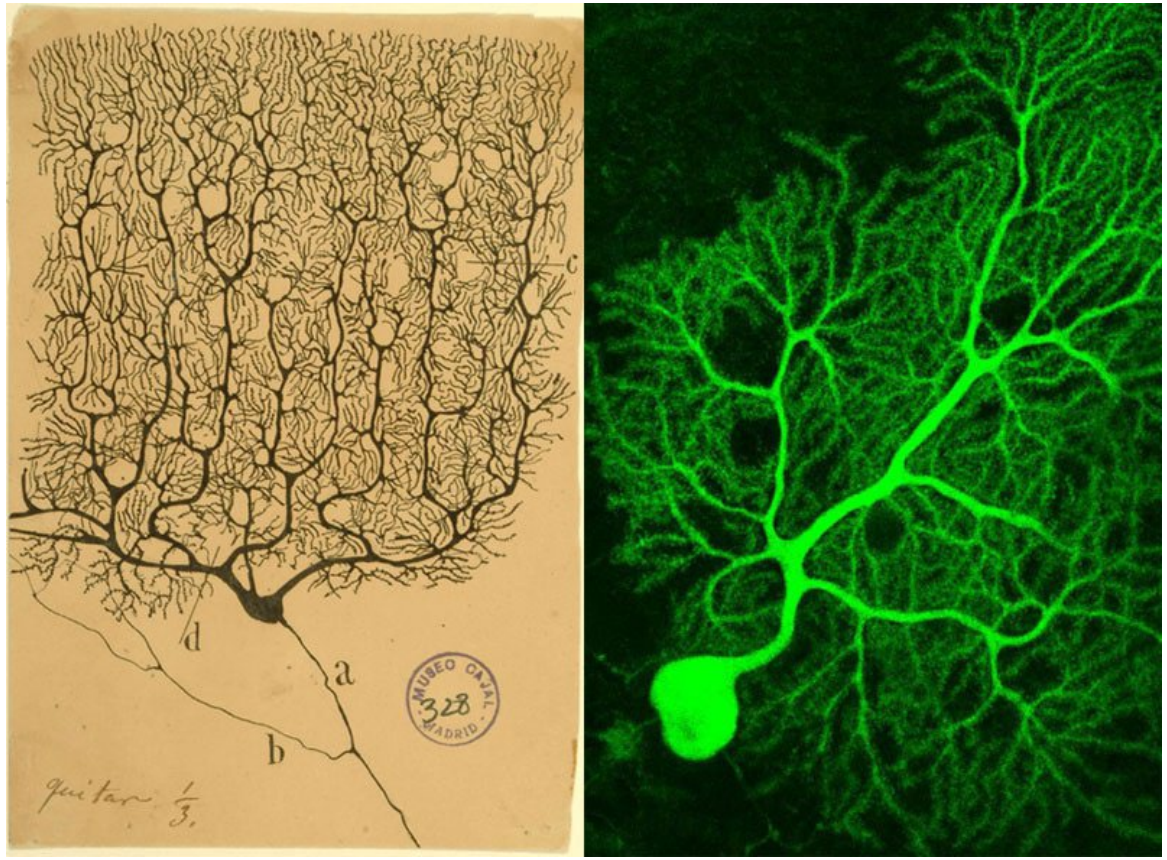
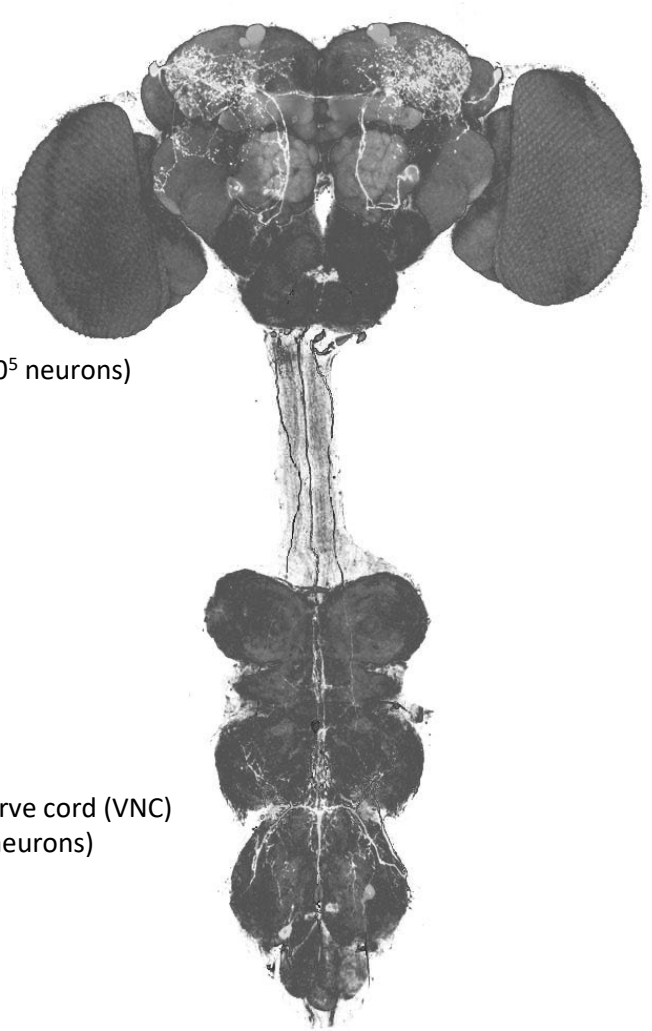


Figure 1



Santiago Ramón y Cajal; Drawing of the Purkinje cell

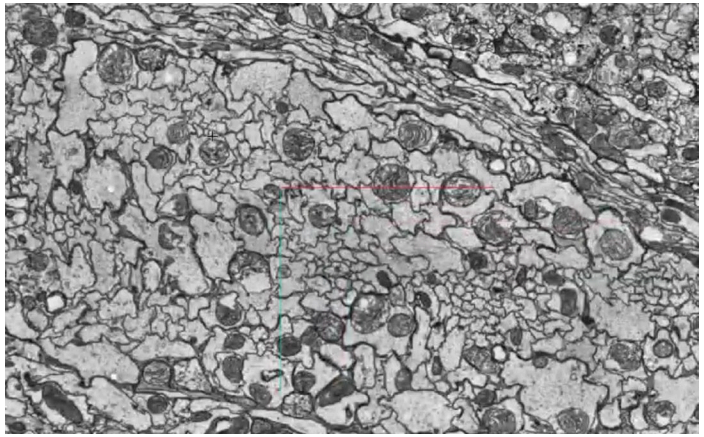
Connectomics measures neurons and their connectivity (The fruit fly, *Drosophila*, as an example)



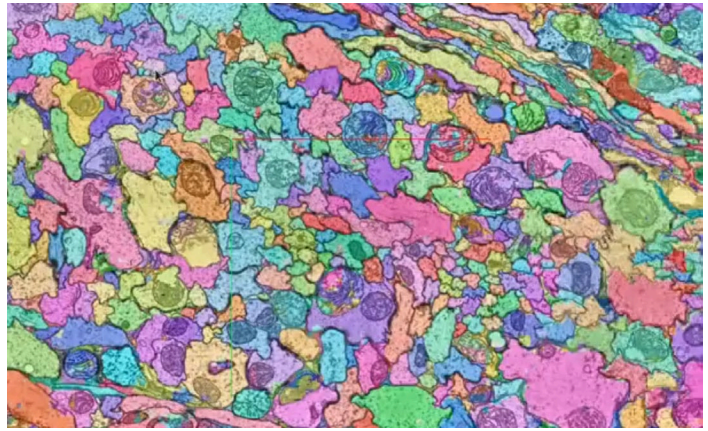
Brain (~10^5 neurons)

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

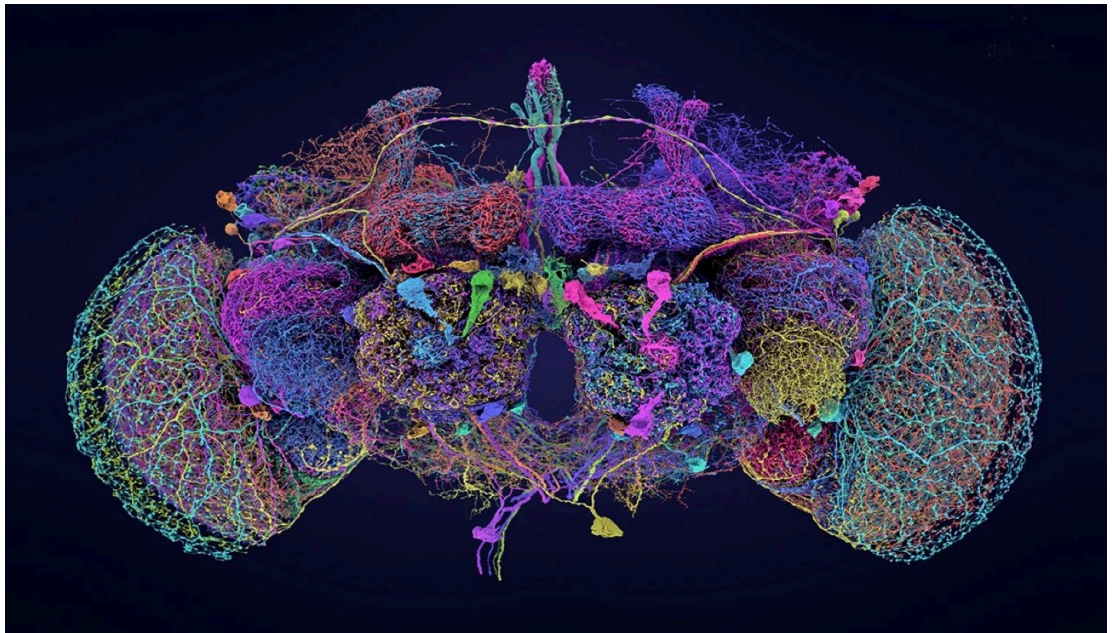
Chang & Shih, 2011



Deep network

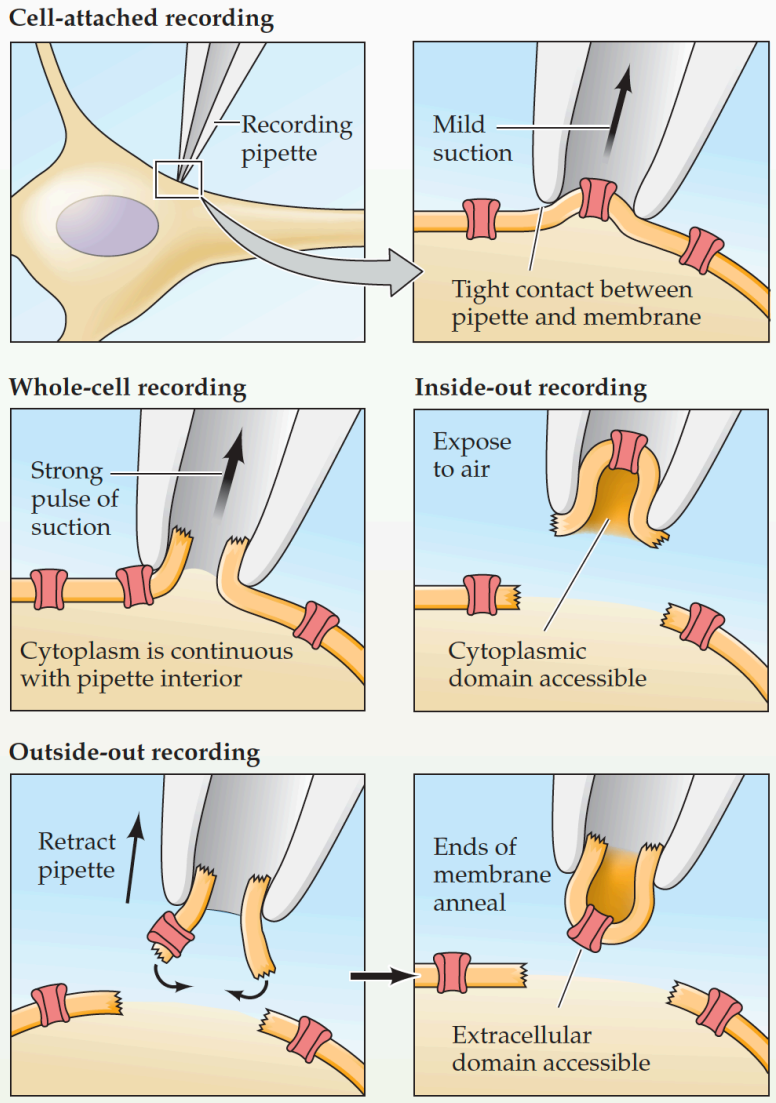


A complete connectome of the fly brain



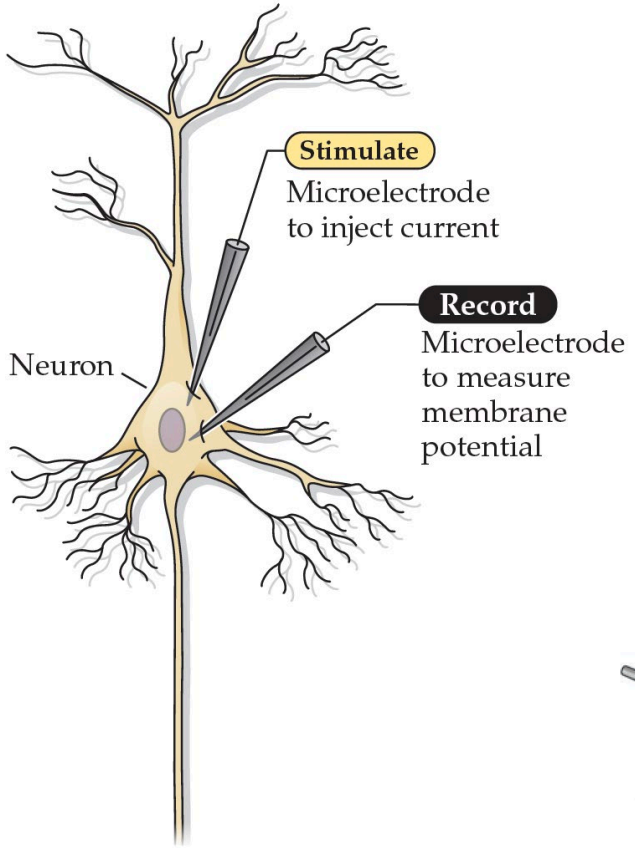
Electrophysiology (a reminder)

Synaptic potentials & ionic currents



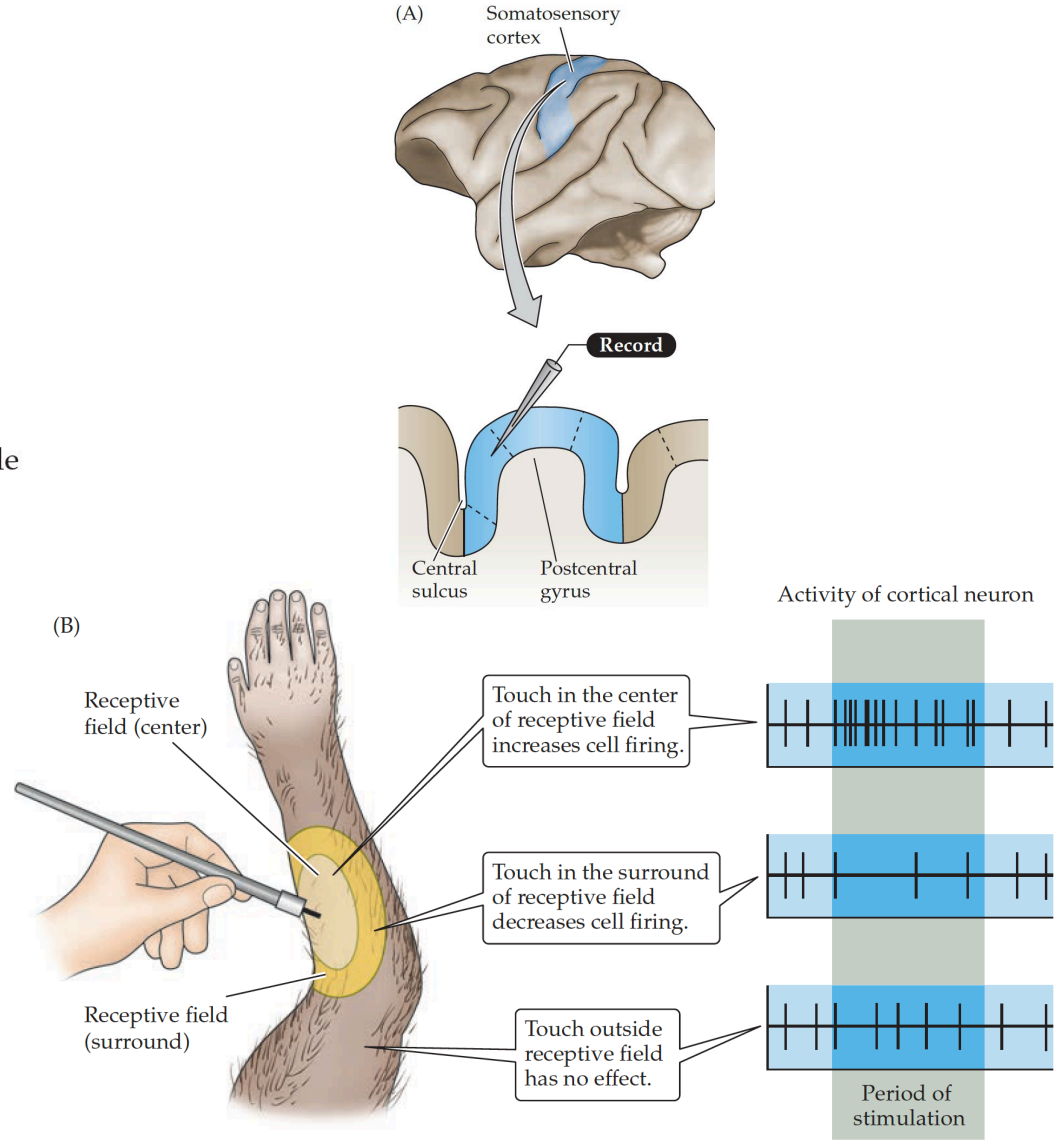
Purves, Box 4A

Voltage changes



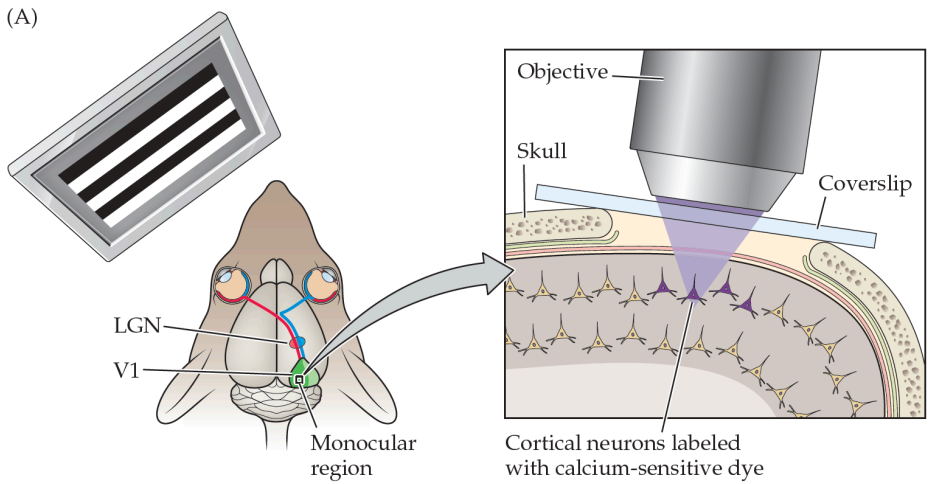
Purves, Figure 2.2

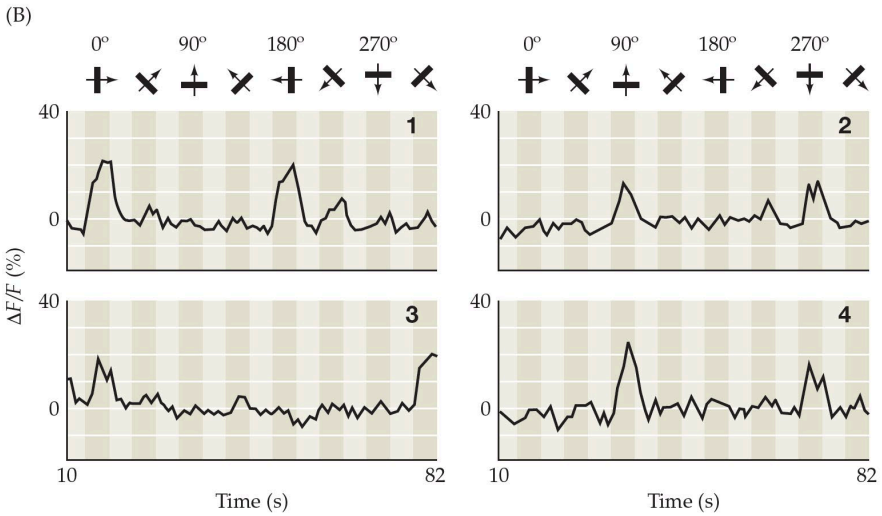
Trains of action potentials (spikes)

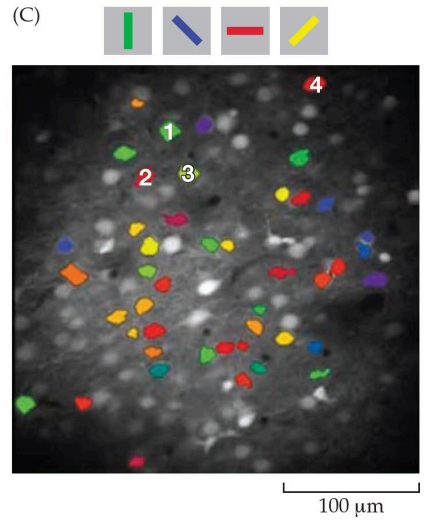


Purves, Figure 1.16

Functional Imaging

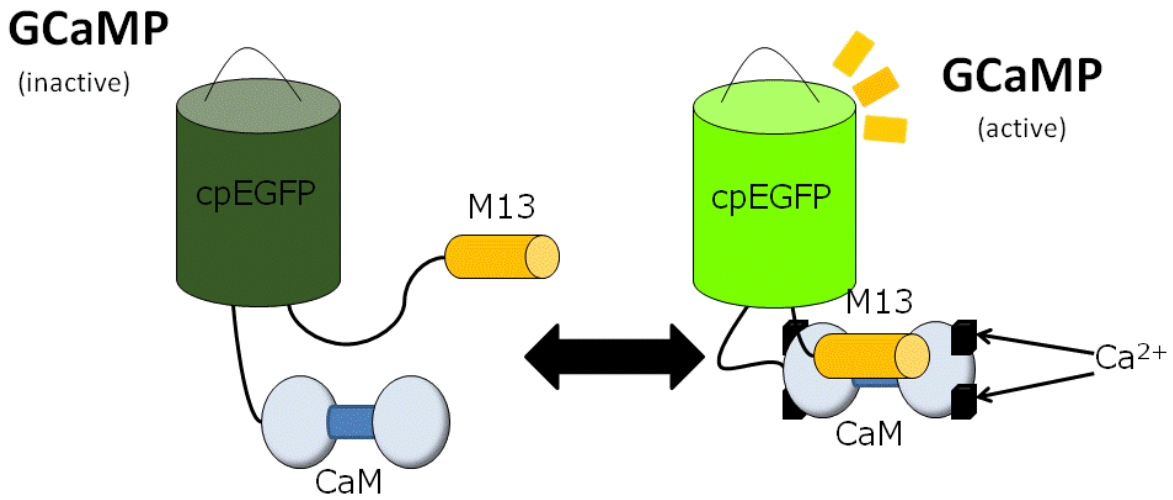






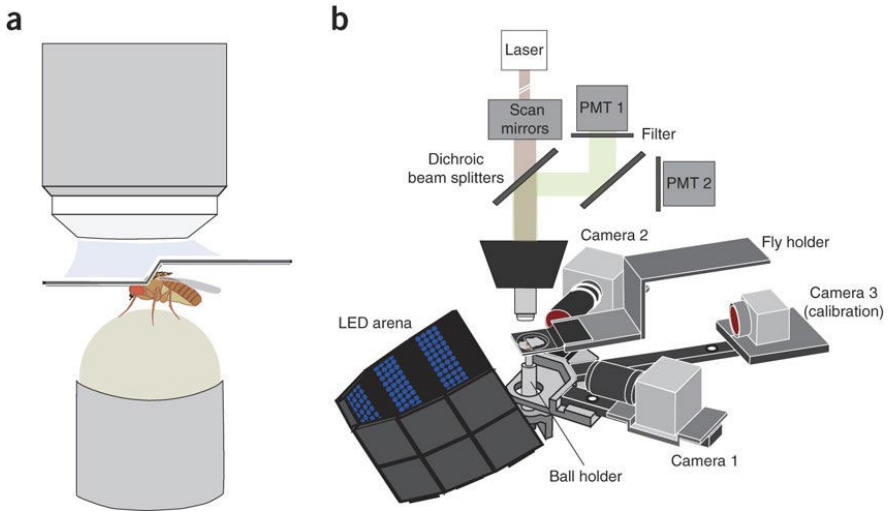
Combines both imaging AND activity measurements

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

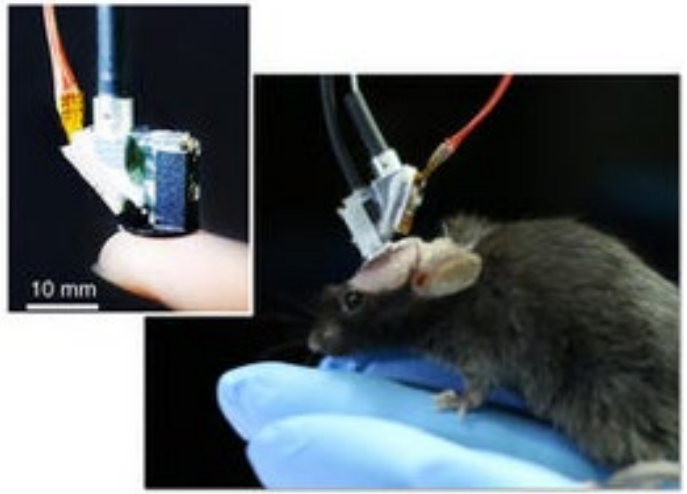


M13 – peptide from myosin light chain kinase
CaM – calmodulin
cpEGFP – circularly permutated GFP

Recording neural activity **during behavior**



Microscope based neuronal activity measurement in behaving animals (can be metalobic, dye, fluorescence and calcium imaging)



-Measurement of activity ‘during behaviour’

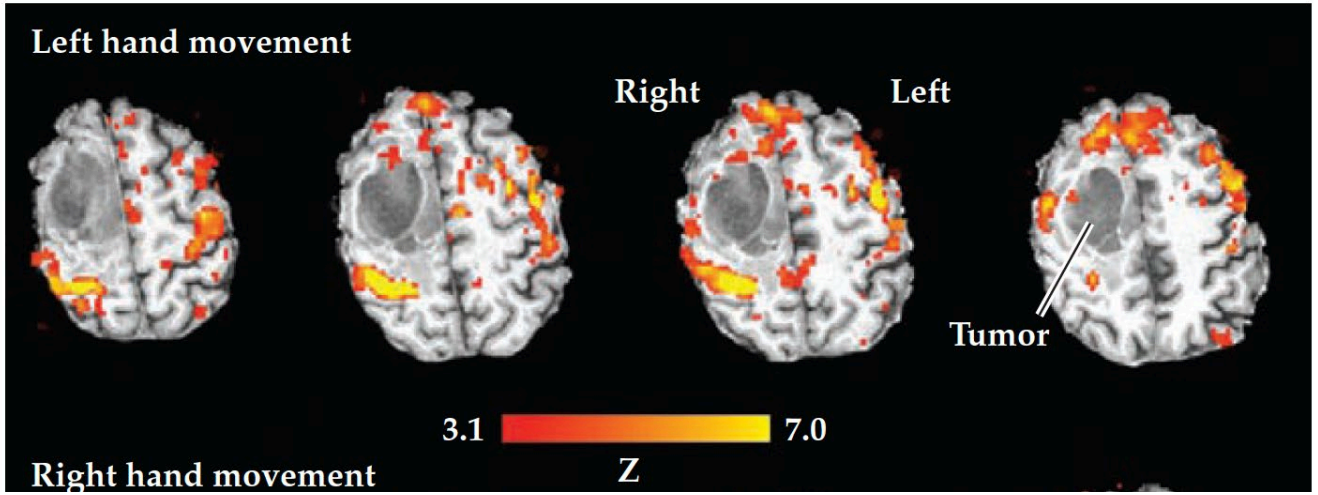
-Indicates which neuron(s) are responsible for particular function

-Tethered or freely behaving animal(s)

-Sometimes tethering is necessary to investigate one particular behavior

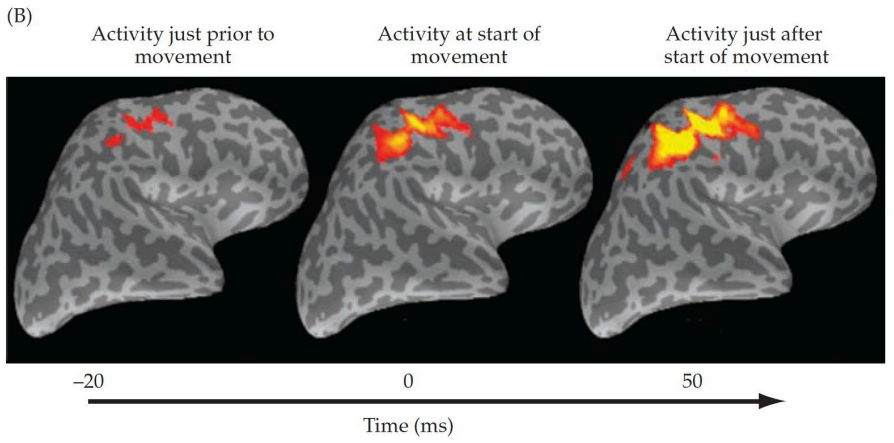
Functional Magnetic Resonance Imaging (fMRI)

- least invasive, most cost-effective approach for visualizing brain function.
- Hemoglobin in blood slightly distorts
  the magnetic resonance properties of hydrogen
- Brain area activated by specific task>Increased metabolism in that area>Increased oxygen-rich blood flow(BOLD signals)> Detected and image processed

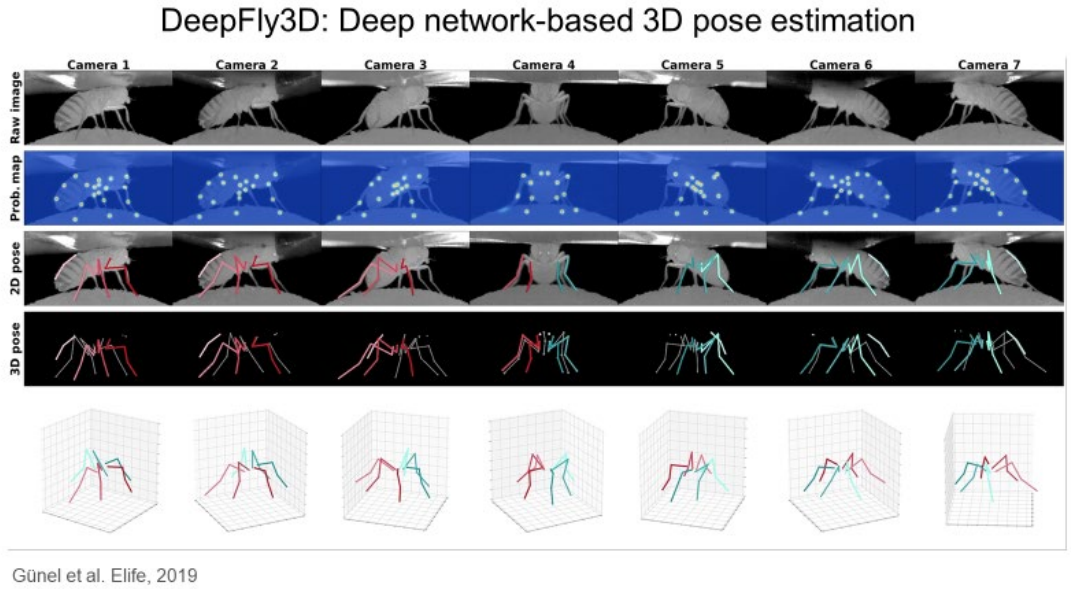


Magnetoencephalography (MEG)

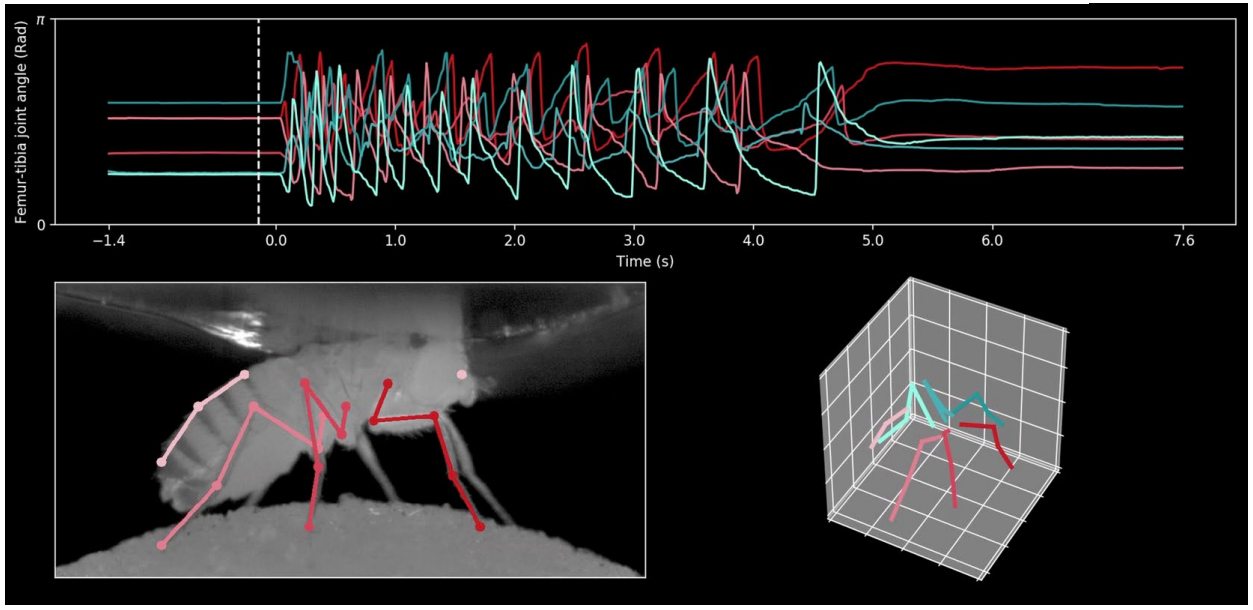
- Greater temporal resolution.
- Biomagnetometer: Amplifies small local changes in magnetic field orientation
- or strength that indicate fast temporal current flow changes in ensembles of neurons.
- The temporal resolution of MEG permits millisecond resolution of electrical activity in the human brain before, during, and after performance of a variety of tasks



Using deep networks to automatically find keypoints



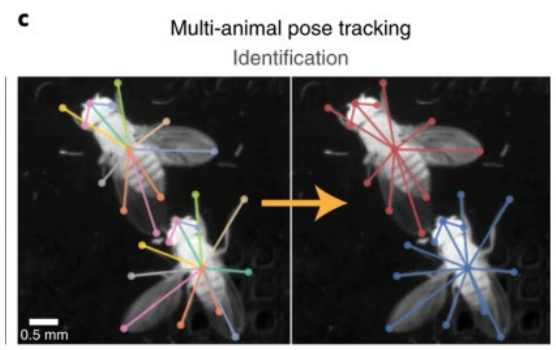
Günel et al. Elife, 2019



- Markerless
- Methods to quantify tracking, movements, joint angles etc in 2D, 3D space using deep learning networks

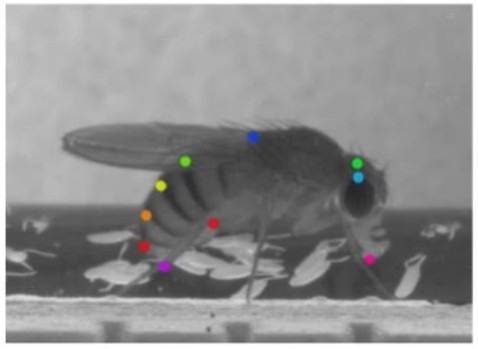
Popular 2D pose estimation tools

SLEAP



Pereira et al., Nature Methods 2022

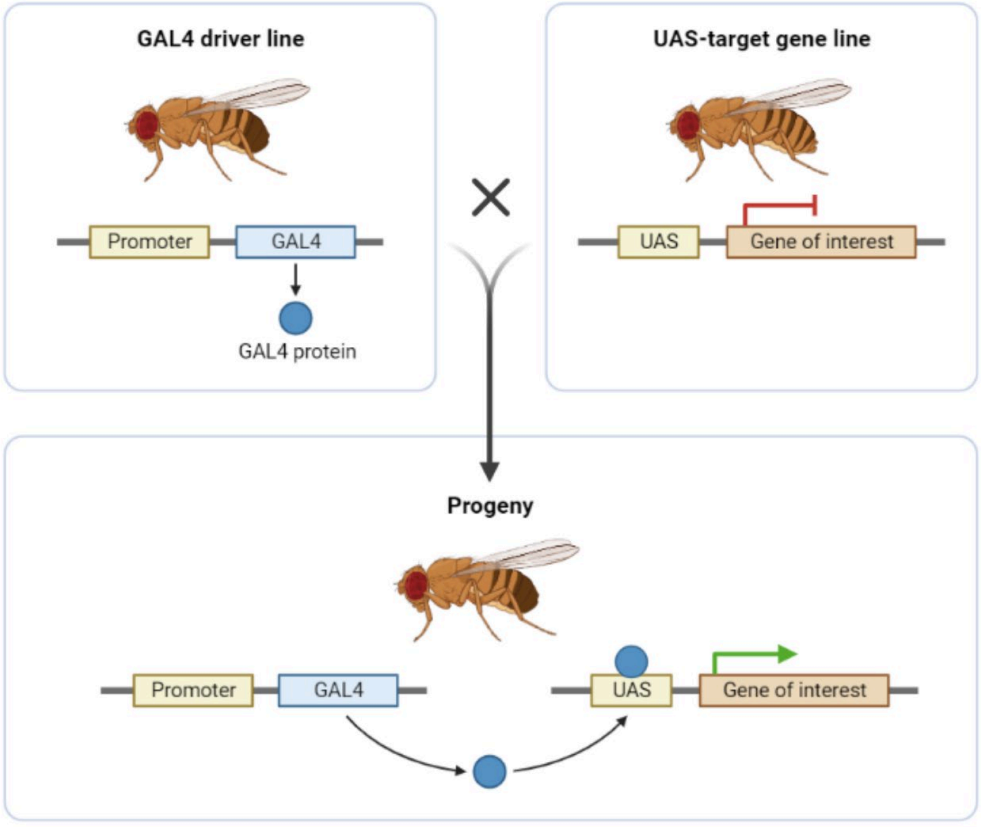
DeepLabCut



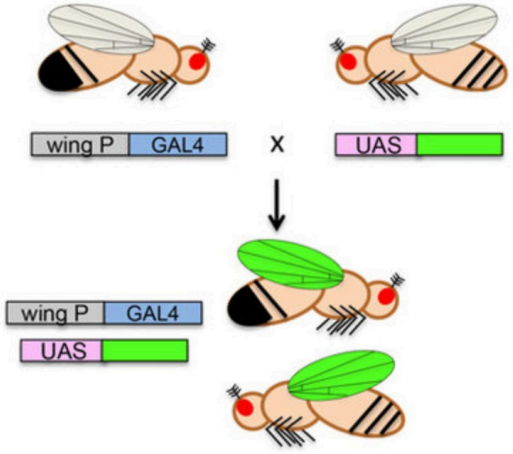
Mathis et al. Nature Neuro. 2018

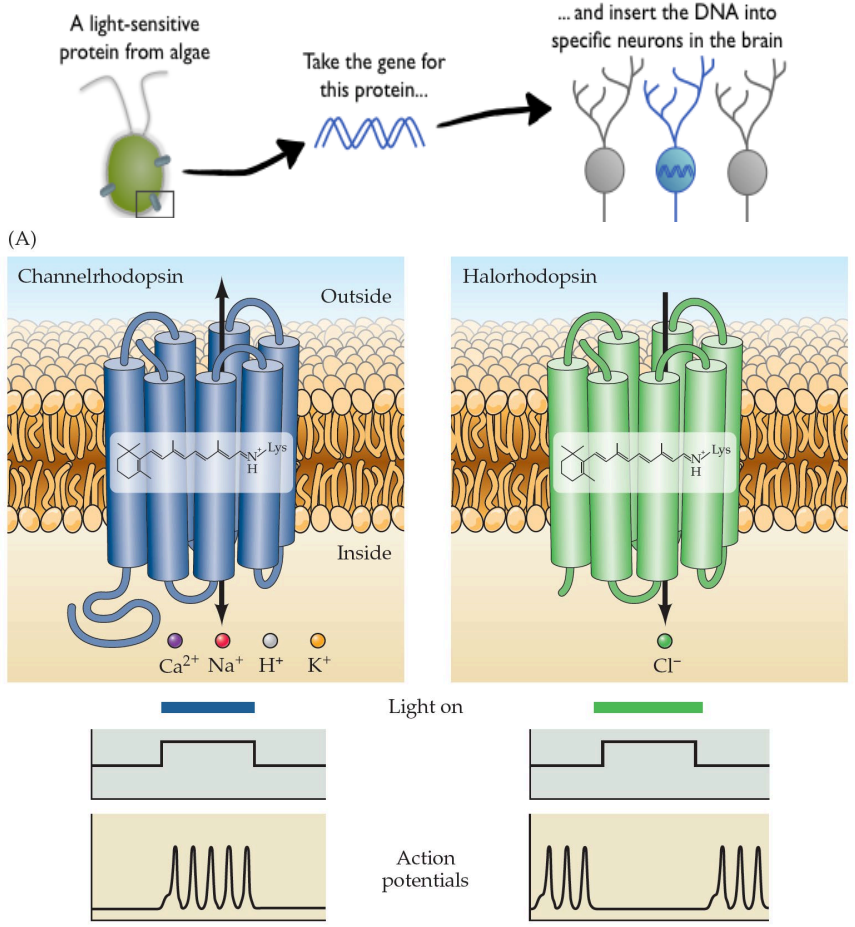
Perturbing neurons

- Electrical stimulation (muscle twitching by electrical stimuli)
- Optogenetics (light induced)
- GAL4-UAS gene expression system (fruit flies, zebra fish)
- Molecular biology techniques



GAL4-UAS gene expression system

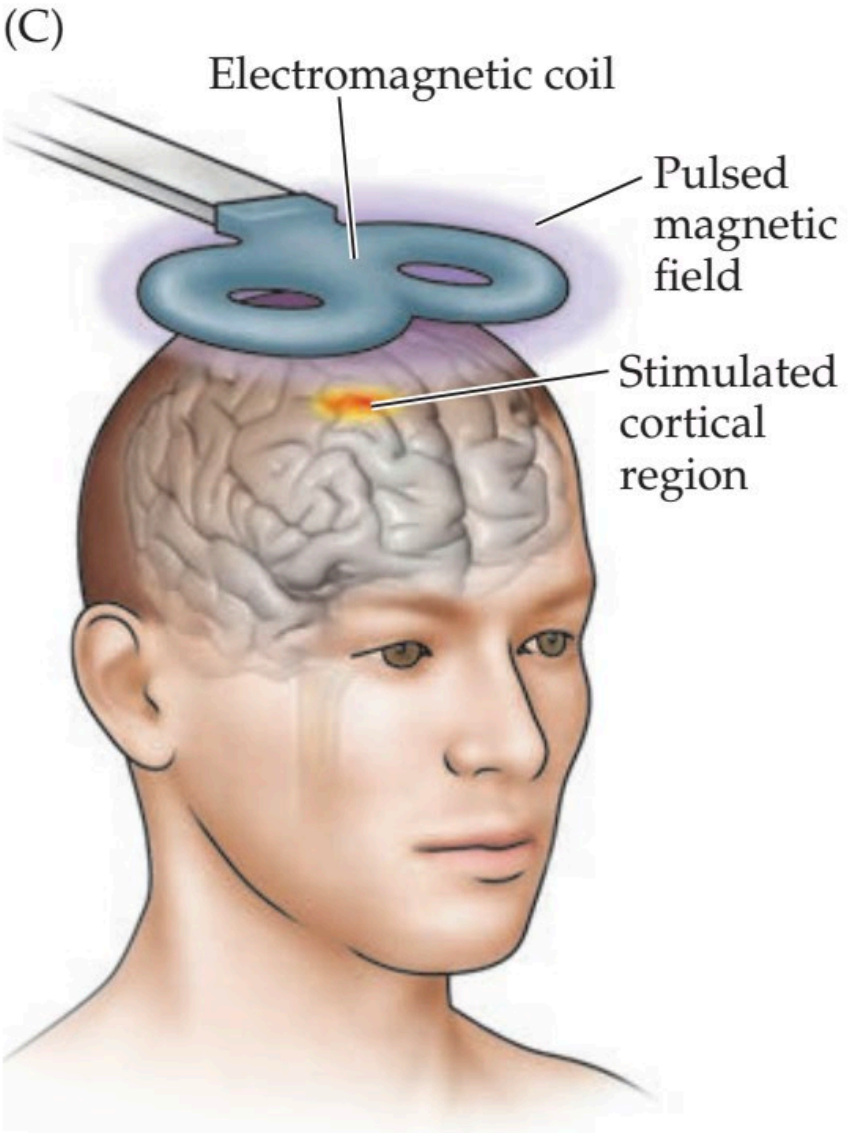




- Channelrhodopsin-2 ('ChR2')
- From the algae, *Chlamydomonas reinhardtii*
- Requires 'all-trans-retinal' cofactor
- Activated by blue light, nonspecific cation entry
- Depolarizes neurons, causing them to fire action potentials

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