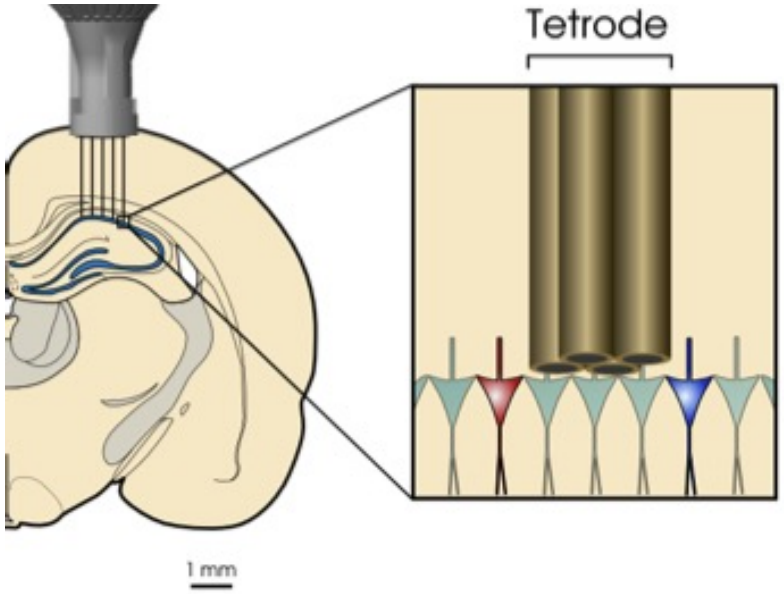
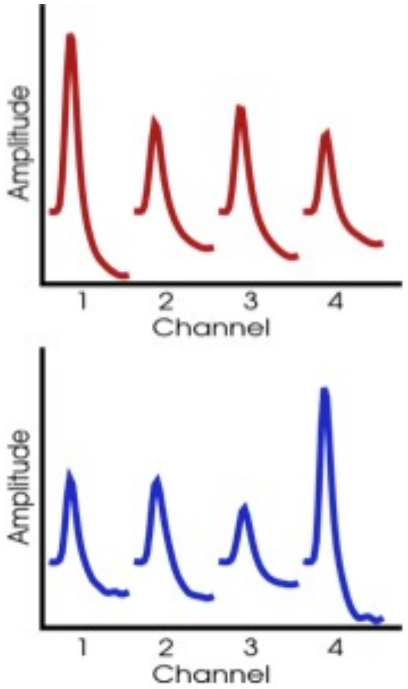
NEUR 265

April 10th, 2023

Problems with Tetrodes?



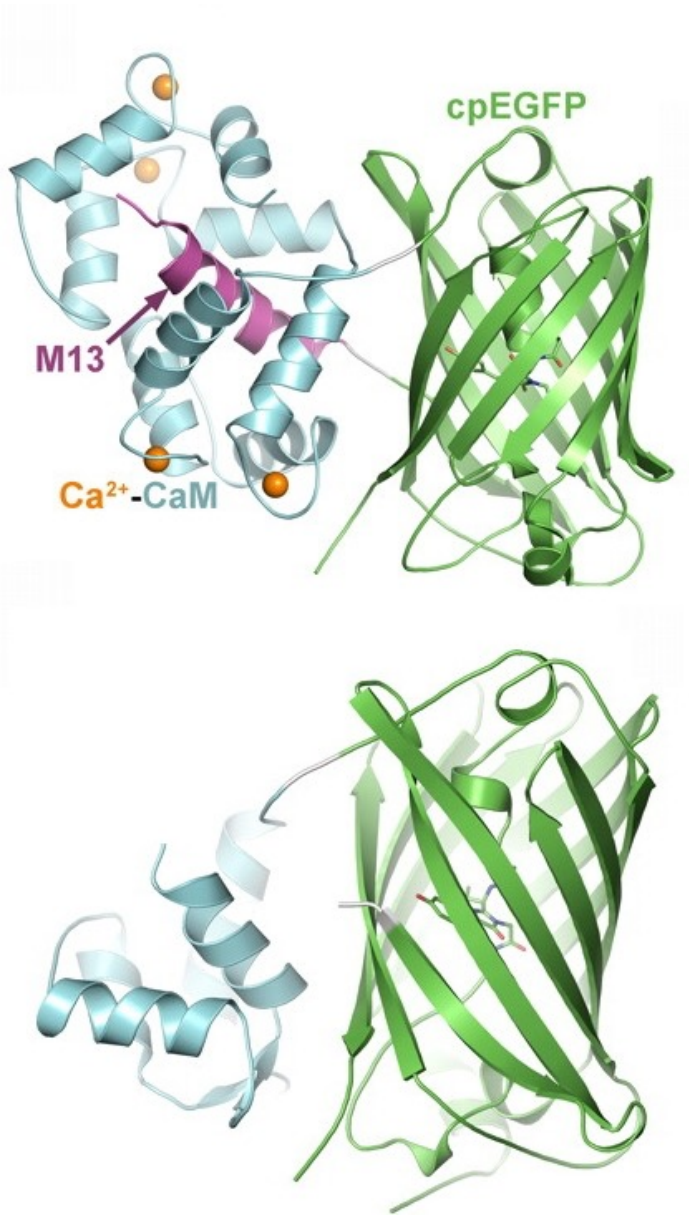


- Can’t distinguish between different “types” of cells
- Can’t distinguish location of one cell from another

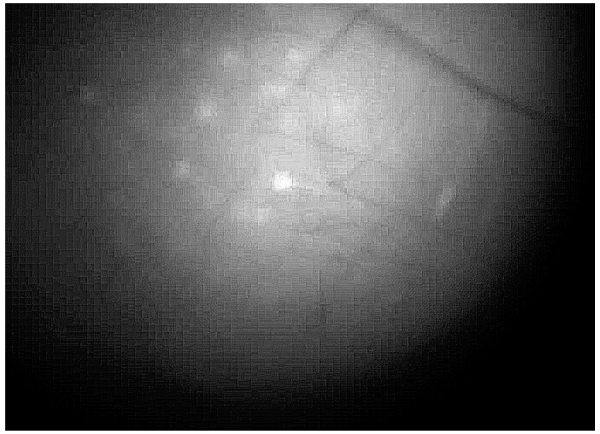
GCaMP

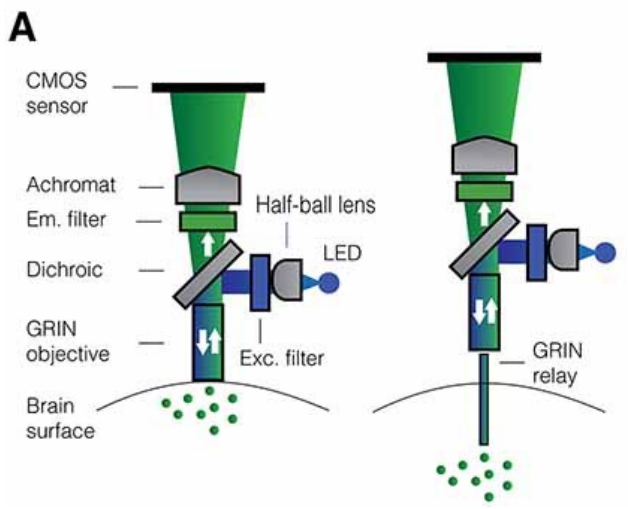
GCaMP is a protein that emits GFP fluorescence whenever calcium enters a cell

The genetic sequence for GCaMP can be packaged into a virus

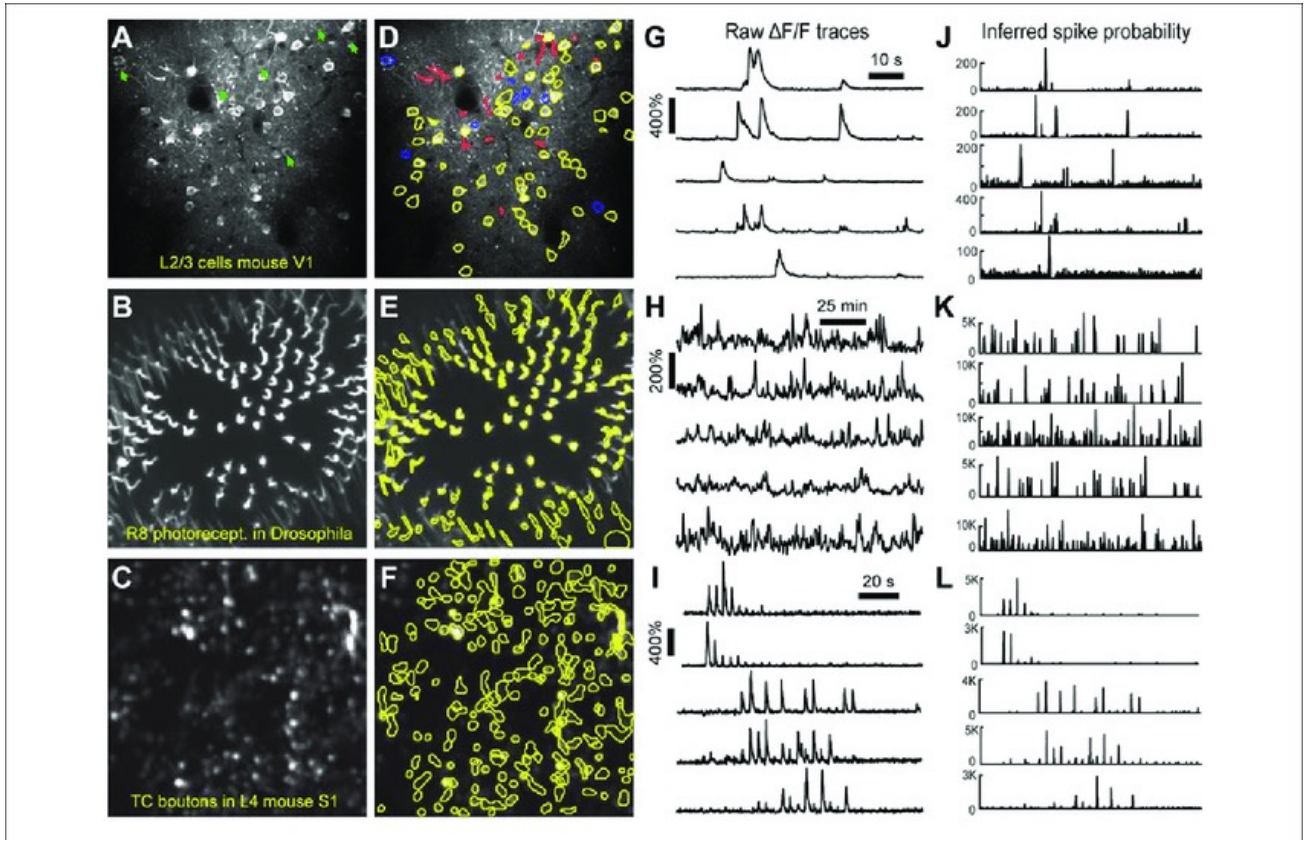


How to Image GFP from GCaMP





Extracting Signal from Movies



Isolate regions of interest (ROIs) – these correspond to potential cells

Extract 2D fluorescent trace over time – background-normalized

Questions:

I inject a virus that codes for GCaMP into an *Slc17a7*-Cre transgenic mouse (*Slc17a7* is a marker gene for neurons that release glutamate). The gene that codes for GCaMP in my virus is not flanked by loxP sites. Which neurons will express GCaMP?

I inject a virus that codes for GCaMP into a *Gad1*-Cre transgenic mouse (*Gad1* is a marker gene for neurons that release GABA). The gene that codes for GCaMP in my virus is flanked by loxP sites. Which neurons will express GFP?

Different GCaMP Variants

- GCaMP3, 4, 5, 6, 7, 8

  - Number indicates better signal intensity (fluorescent brightness)

- GCaMPs, m, f

  - ”Slow”, “medium”, “fast”

  - “Speed” constrained by protein kinetics and camera frame rate

  - Speed is a major limitation of calcium imaging