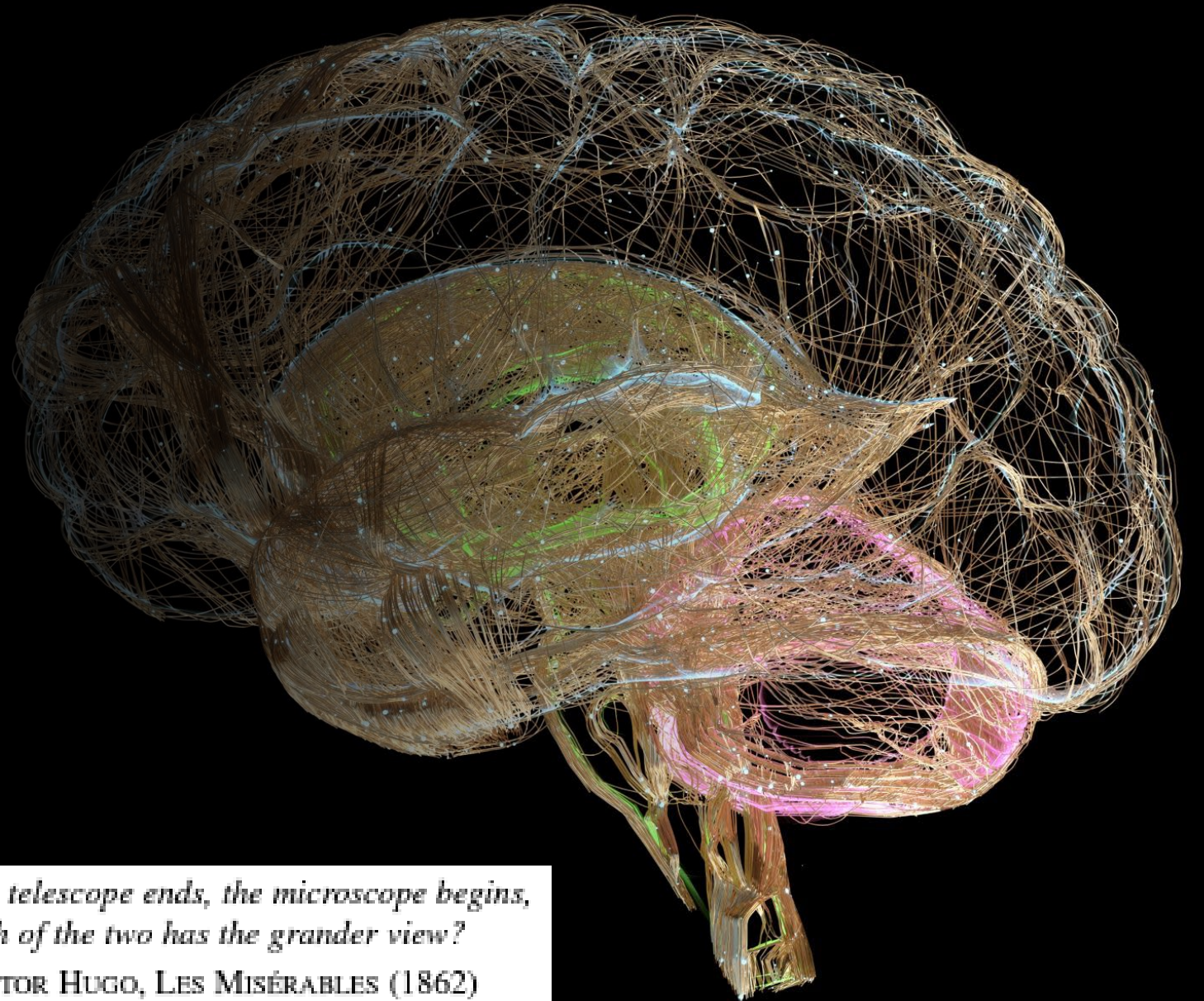


Voltage and  
calcium  
imaging of  
brain activity



*Where the telescope ends, the microscope begins,  
which of the two has the grander view?*  
—VICTOR HUGO, LES MISÉRABLES (1862)

### Calcium signaling basics:

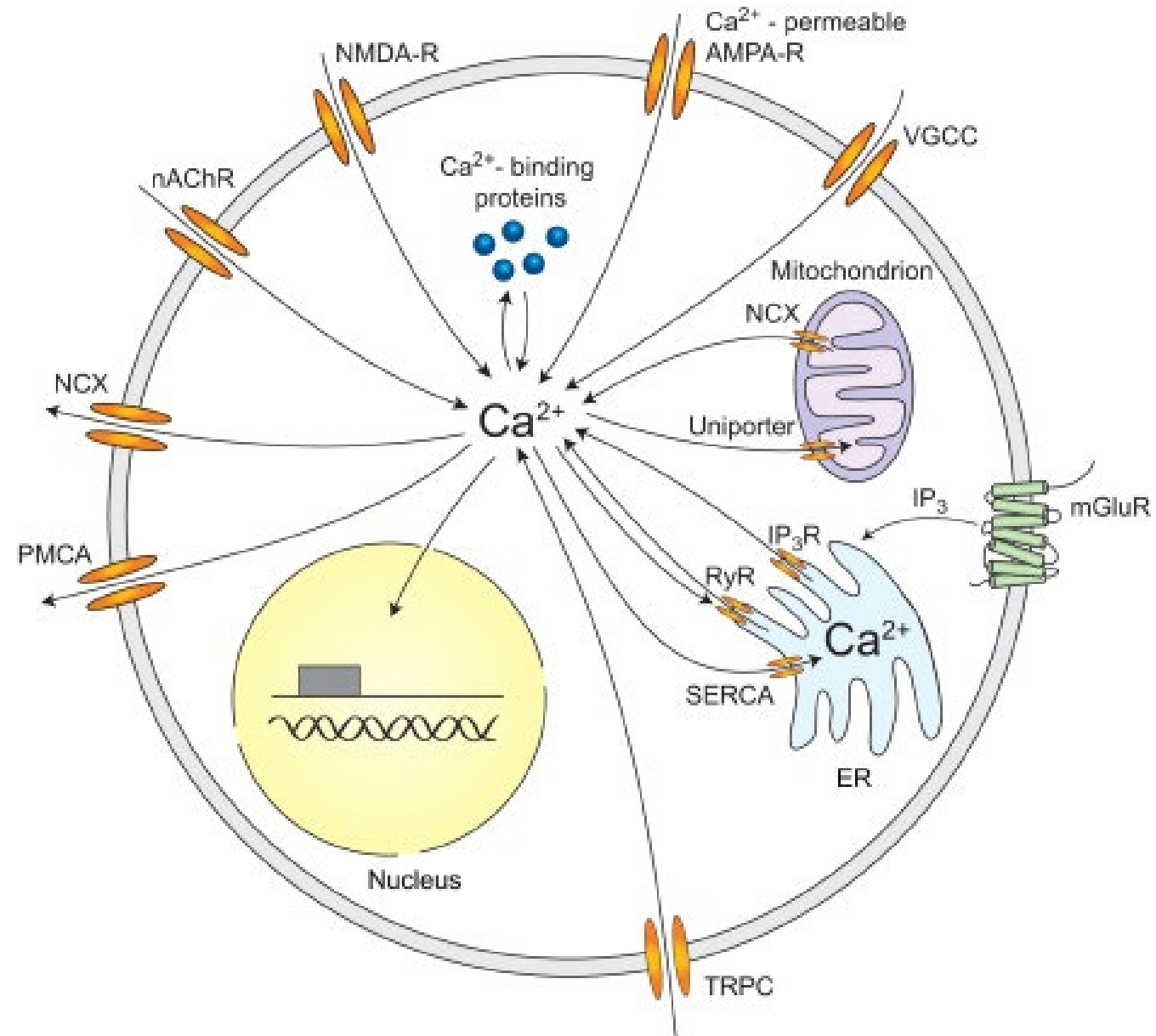
- Calcium ions ( $\text{Ca}^{2+}$ ) are vital for cell signaling.
- Sensor proteins bind to  $\text{Ca}^{2+}$  to relay signals.
- $\text{Ca}^{2+}$  can bind in various shapes, unlike  $\text{Mg}^{2+}$

### Neuronal calcium levels:

- At rest, neurons have low  $\text{Ca}^{2+}$  levels.
- Upon activation,  $\text{Ca}^{2+}$  levels can spike.

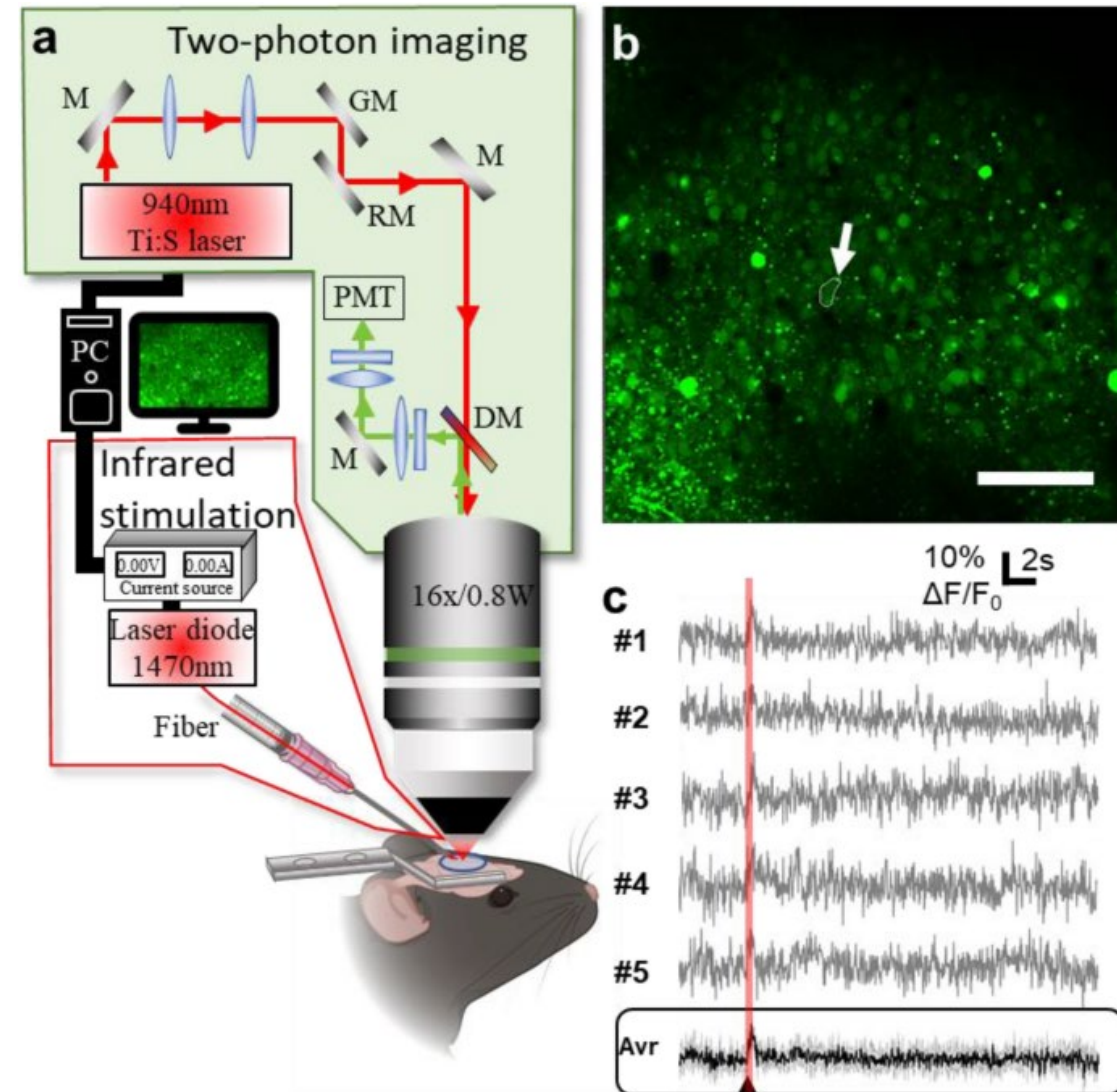
### Calcium in cellular functions:

- Calcium signaling affects metabolism, gene transcription, and more.
- It is crucial for neuron-specific processes like synaptic transmission and memory formation.



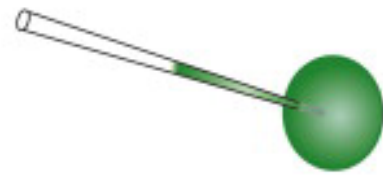
# Advantages of two photon calcium imaging

- Minimal invasiveness
- Genetic targeting
- Subcellular and non-neuronal imaging
- Longitudinal studies
- Non-invasiveness
- Genetic specificity
- Subcellular resolution
- Long-term and wide-area imaging
- Cost-effectiveness
- Improved indicators
- Data processing
- Flexibility





**A** Single cell loading



Sharp electrode

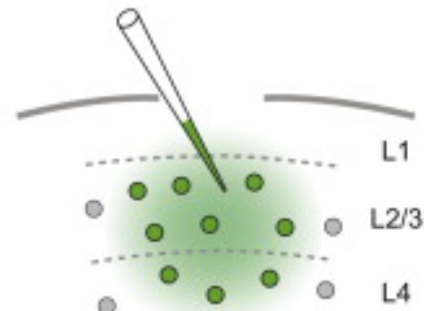


Whole-cell patch clamp

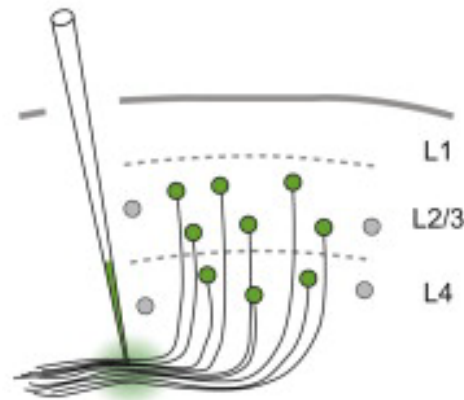


Single cell electroporation

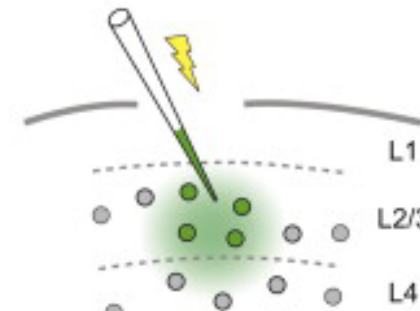
**B** 'Acute' network loading



AM loading

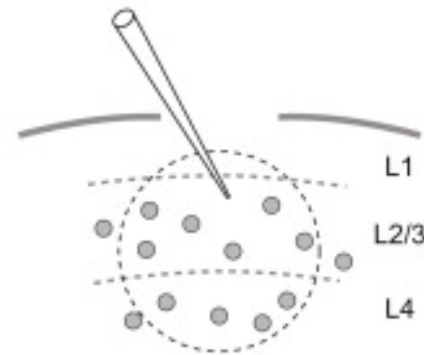


Dextran-conjugate loading

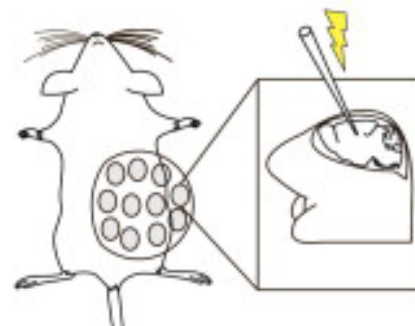


Bulk electroporation

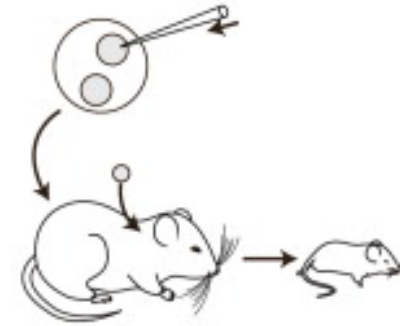
**C** GECI expression



Viral transduction



*In utero* electroporation



Transgenic mice

How do we measure optical calcium signaling?

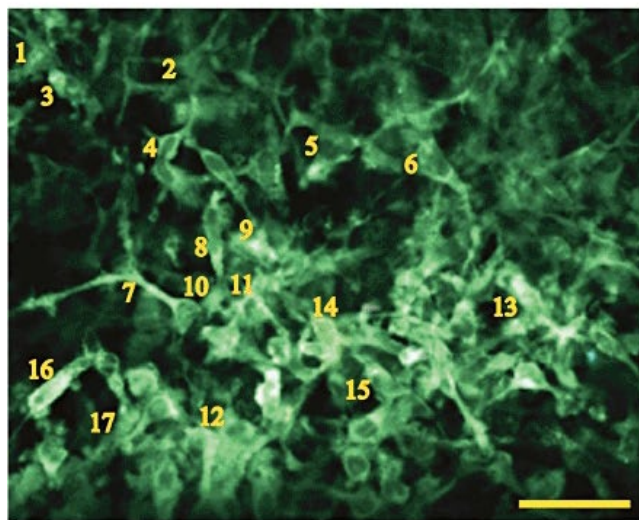
C



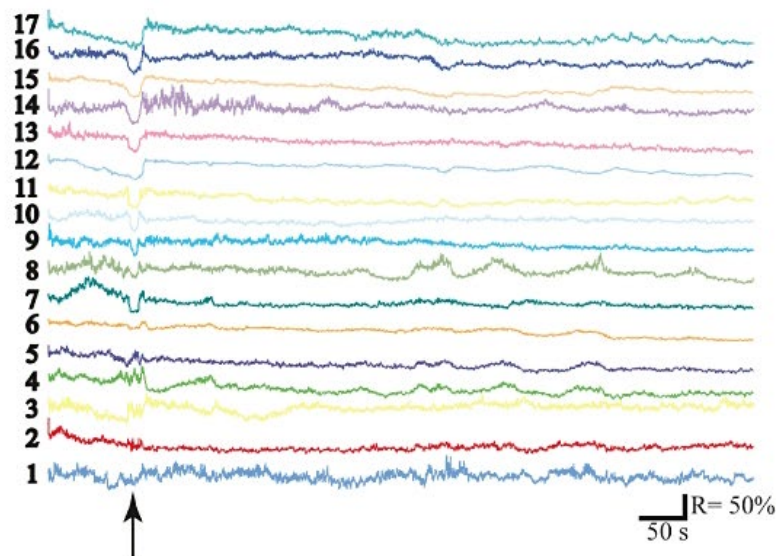
D

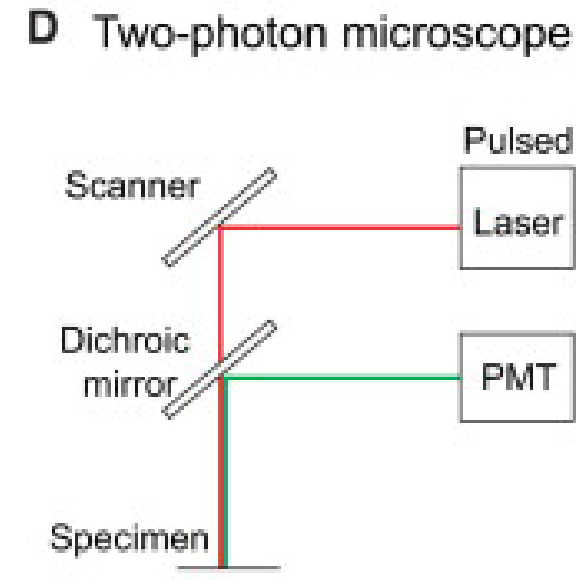
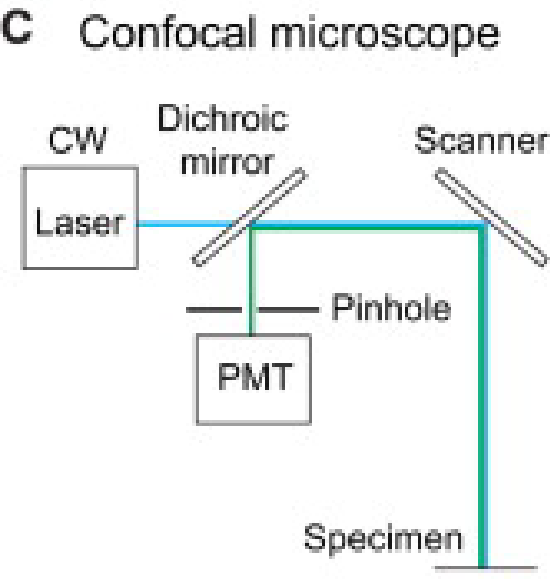
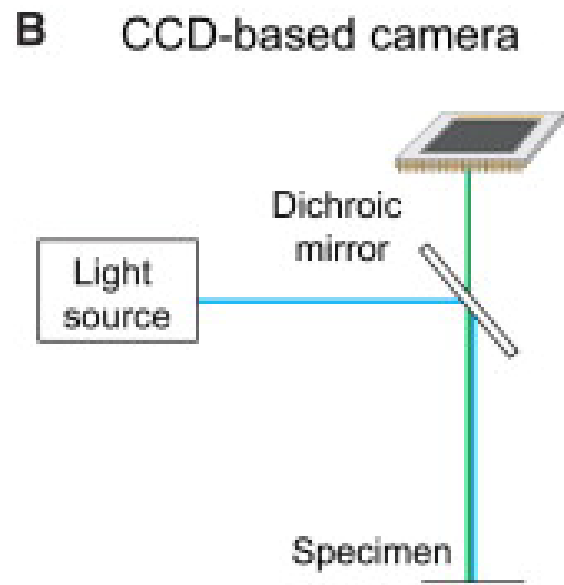
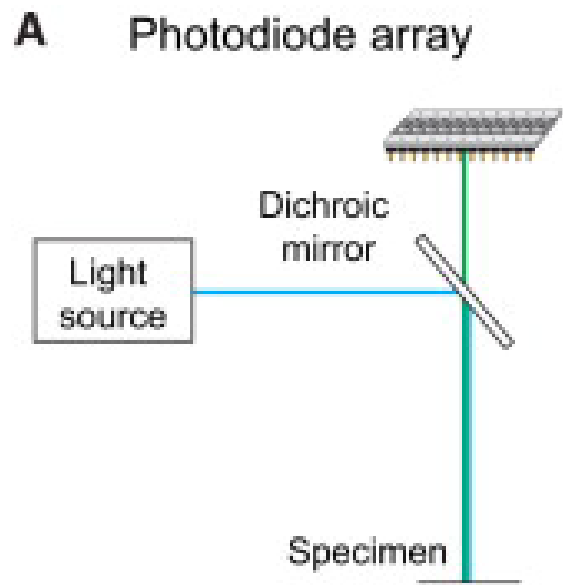


E



F





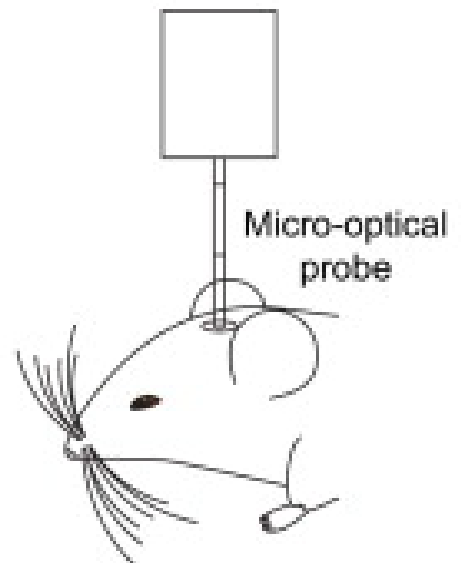
- High dynamic range
- Low spatial resolution

- High spatio-temporal resolution
- Varying level of noise

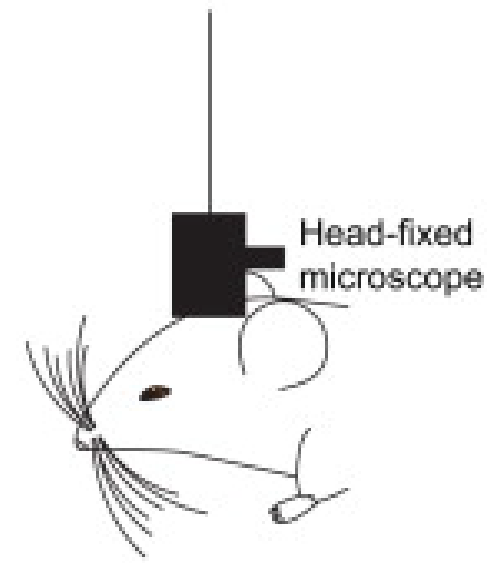
- Pinhole for optical sectioning
- Risk of photodamage

- Deeper imaging
- Reduced background fluorescence

**E** Endoscope

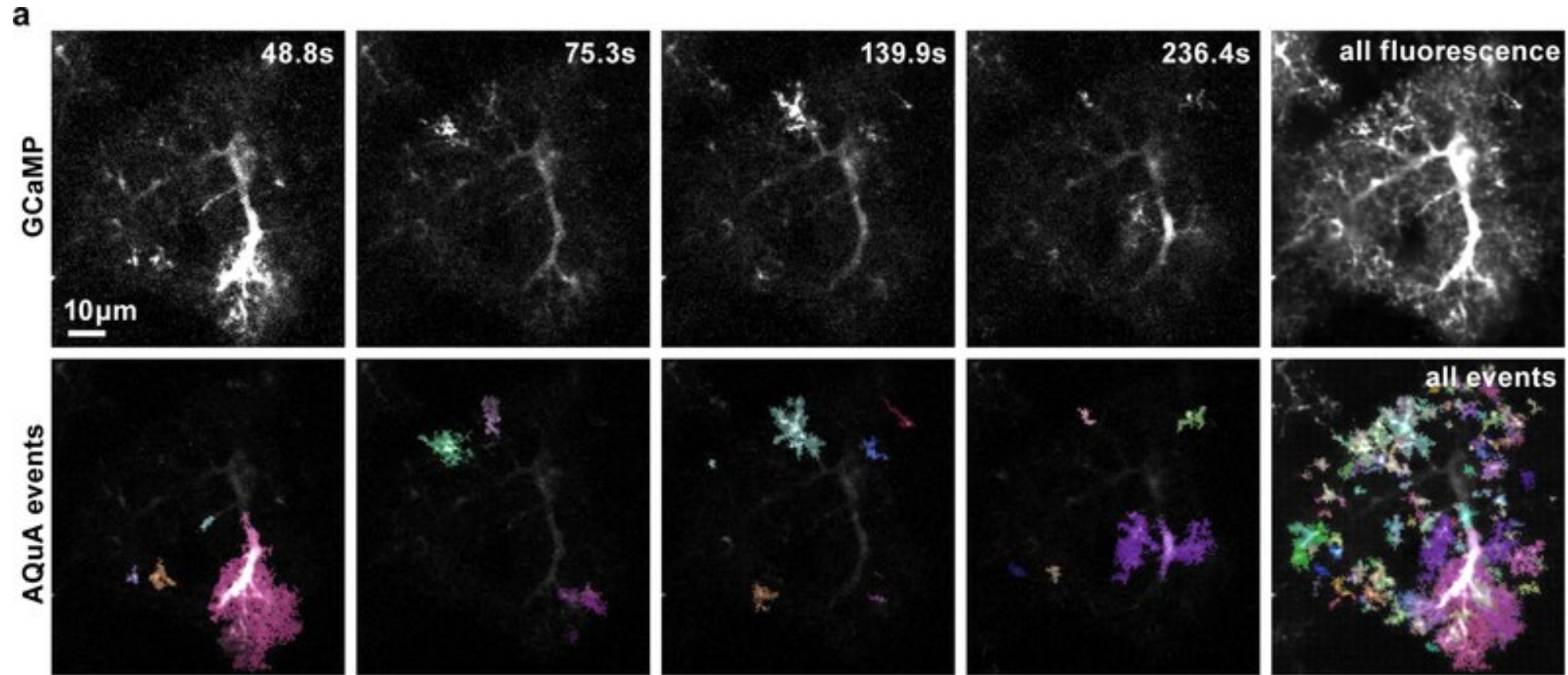


**F** Portable microscope



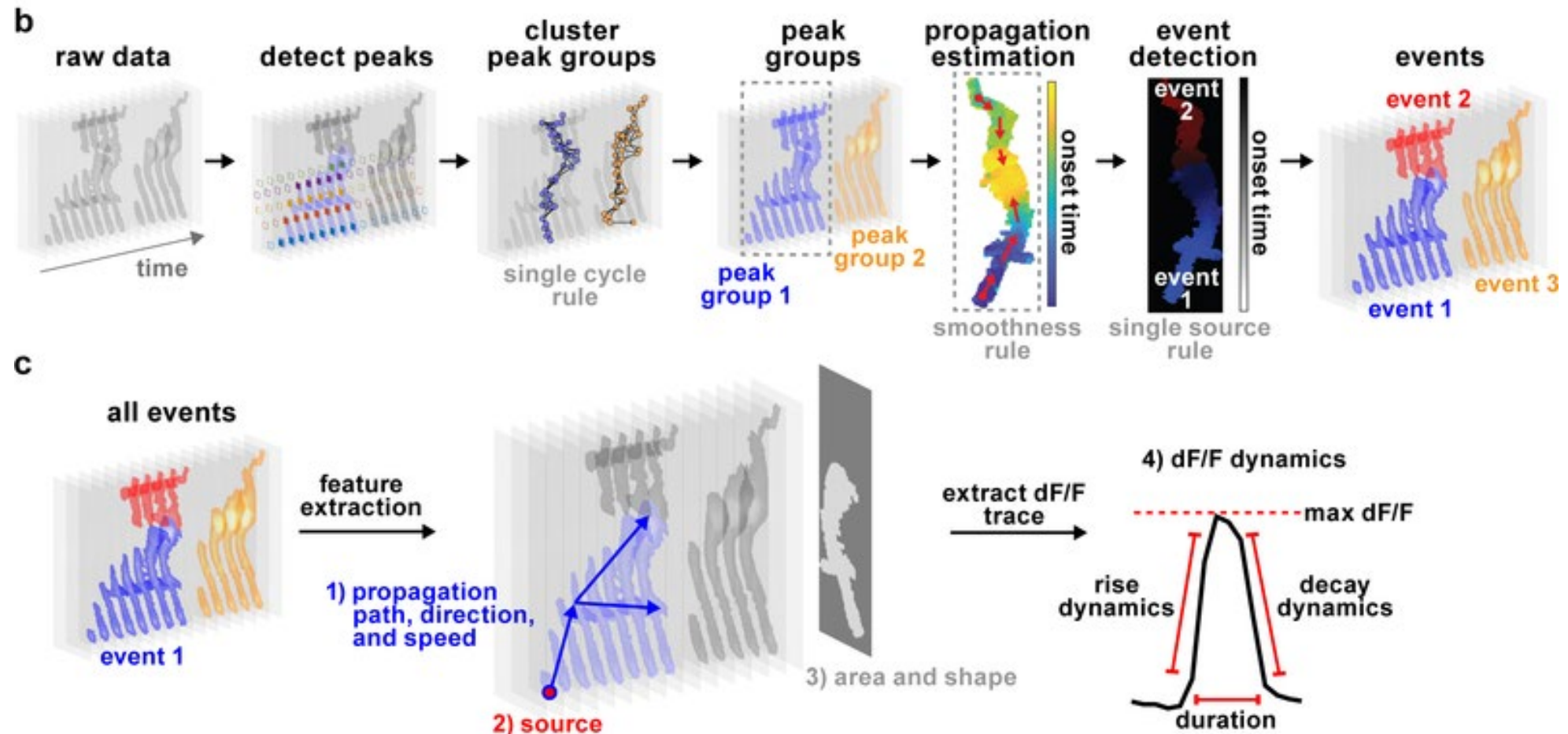
- Imaging in freely behaving animals
- Lower spatial resolution

# Algorithms for studying calcium dynamics

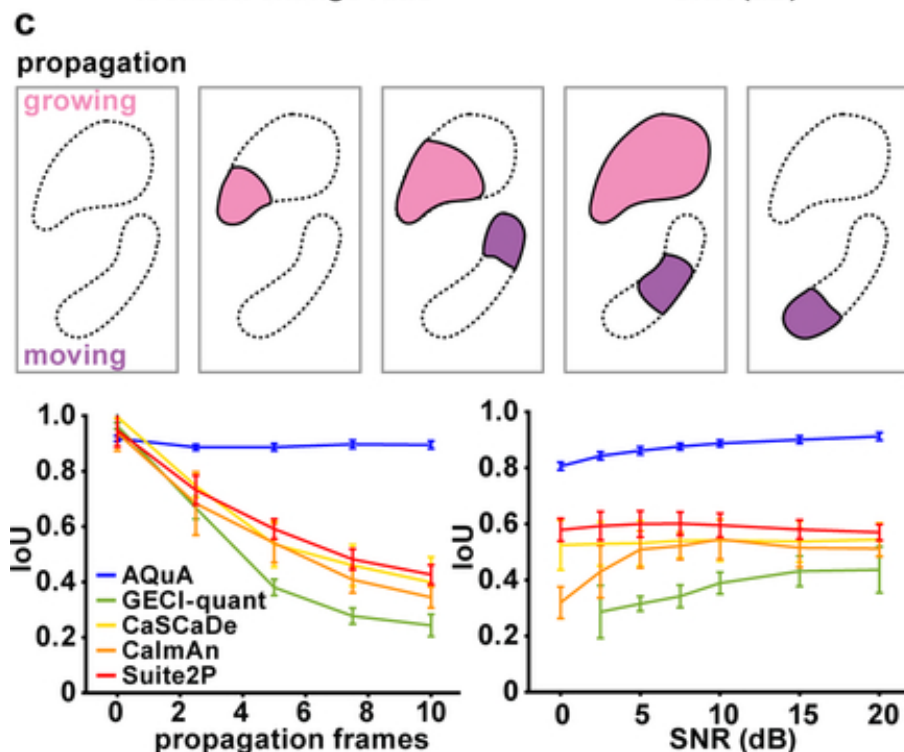
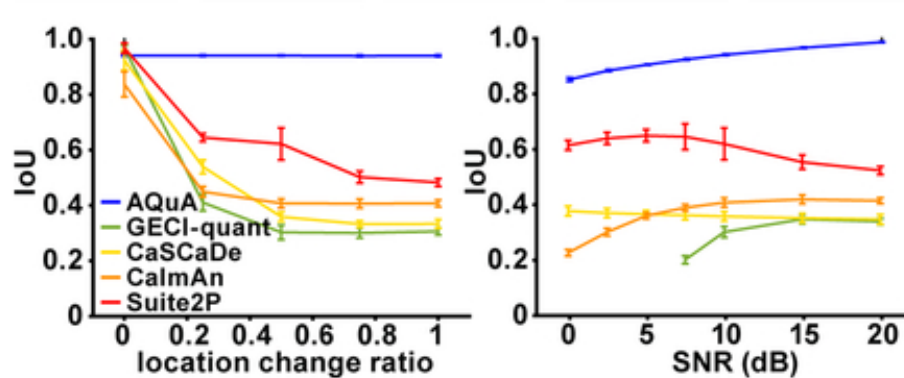
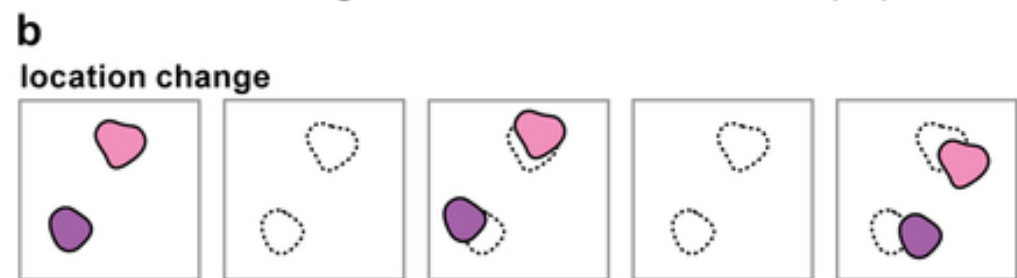
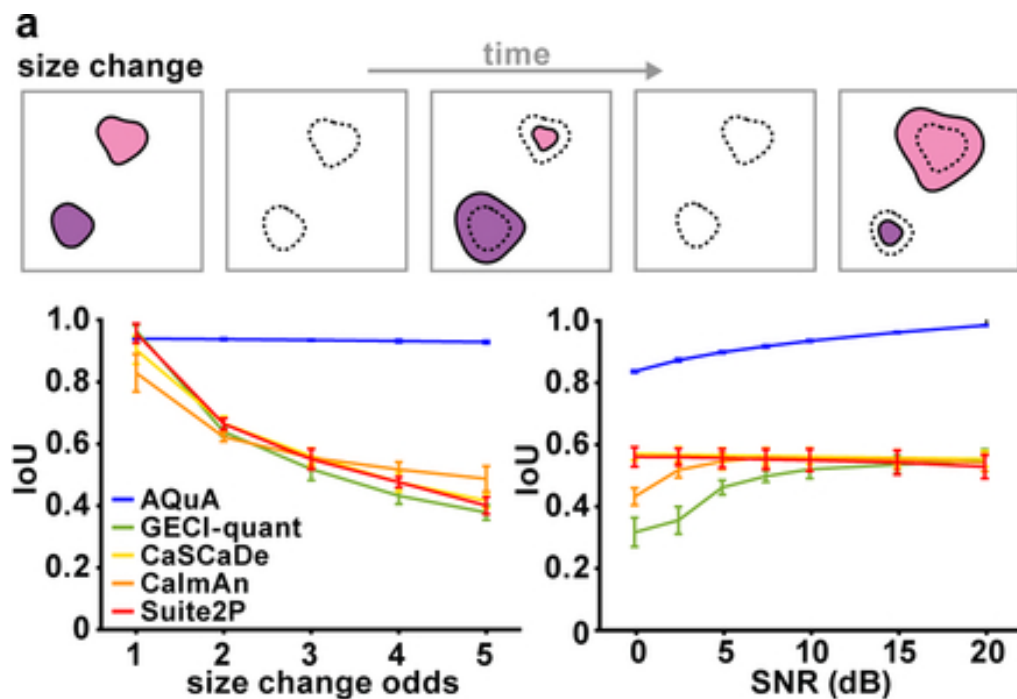




# Algorithms for studying calcium dynamics



# Algorithms for studying calcium dynamics



# Dysregulation of Ca<sup>2+</sup> signaling in the brain and neuronal degeneration

# Huntington's Disease

HD involves loss of striatal neurons and is associated with polyglutamine expansions in the huntingtin protein

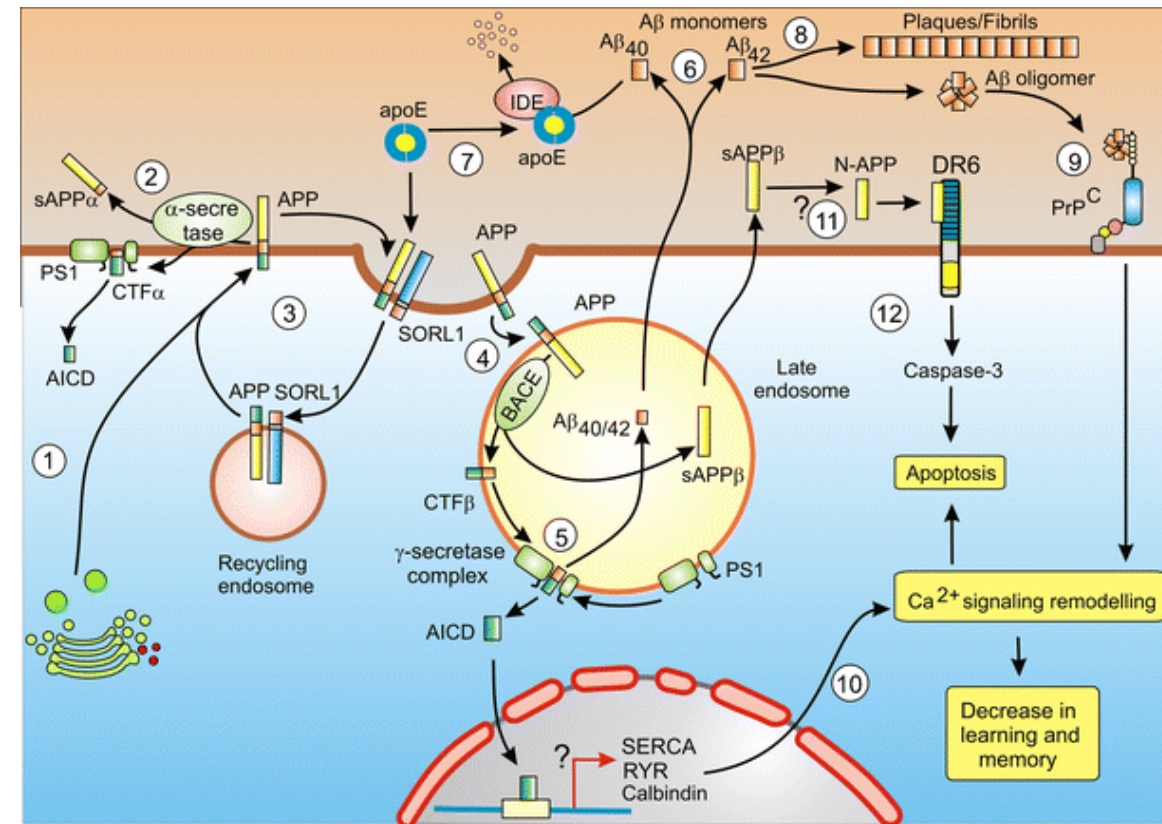
Mutant huntingtin protein can result in proteolytic cleavage

Dysregulation of  $\text{Ca}^{2+}$  in HD may involve  $\text{Ca}^{2+}$  buffering proteins and channels, excitotoxicity, and mitochondrial  $\text{Ca}^{2+}$  handling defects.



# Alzheimer's Disease

- The "calcium hypothesis" of AD suggests that dysregulation of Ca<sup>2+</sup> release from the ER is involved in the pathogenic mechanisms, potentially through mutations in presenilin genes or amyloid metabolism.
- Mutations in presenilin genes may affect the expression and sensitivity of ER Ca<sup>2+</sup> release channels



# Parkinson's Disease

- Abnormal  $\text{Ca}^{2+}$  homeostasis in dopaminergic neurons
- Mutations in genes related to mitochondrial function and  $\text{Ca}^{2+}$  signaling are linked to PD, and aberrant  $\text{Ca}^{2+}$  signaling

