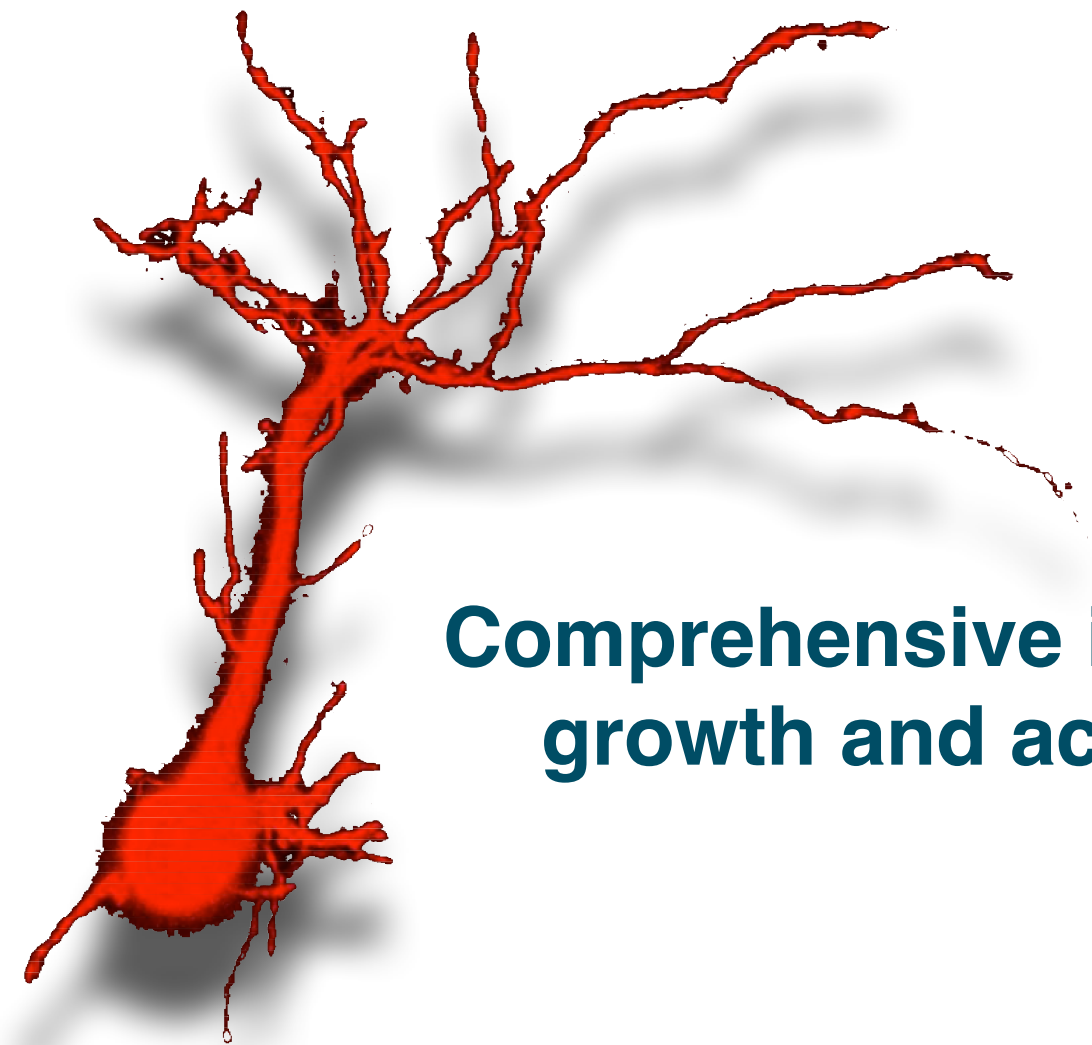




Comprehensive imaging of experience-driven dynamic growth and activity in the awake developing brain

Kurt Haas, PhD

**Professor
University of British Columbia
Vancouver, Canada**

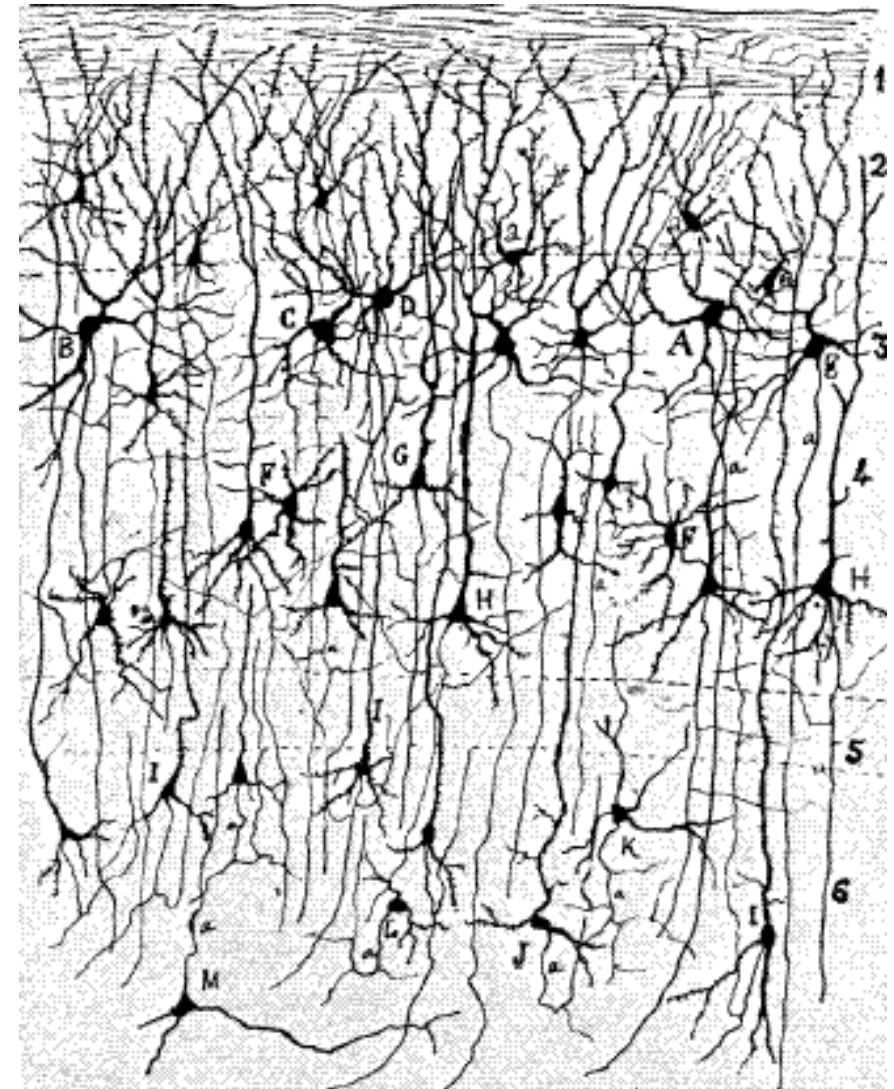
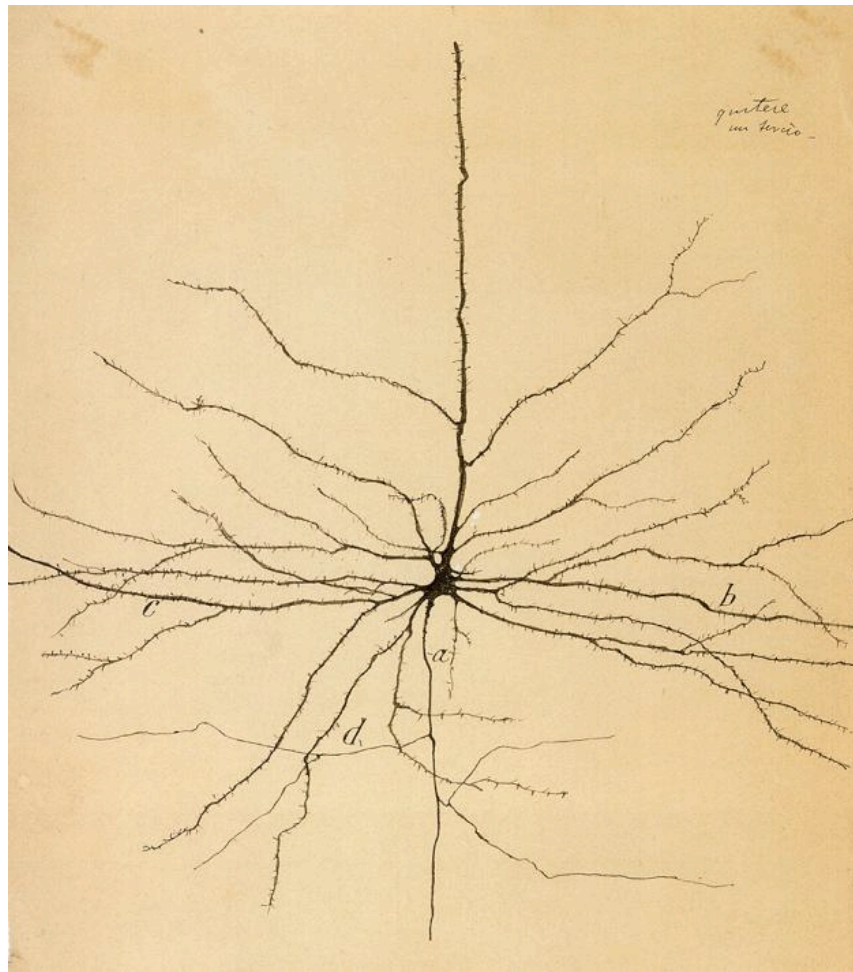
CSHL Imaging 2019



University of British Columbia Vancouver, Canada

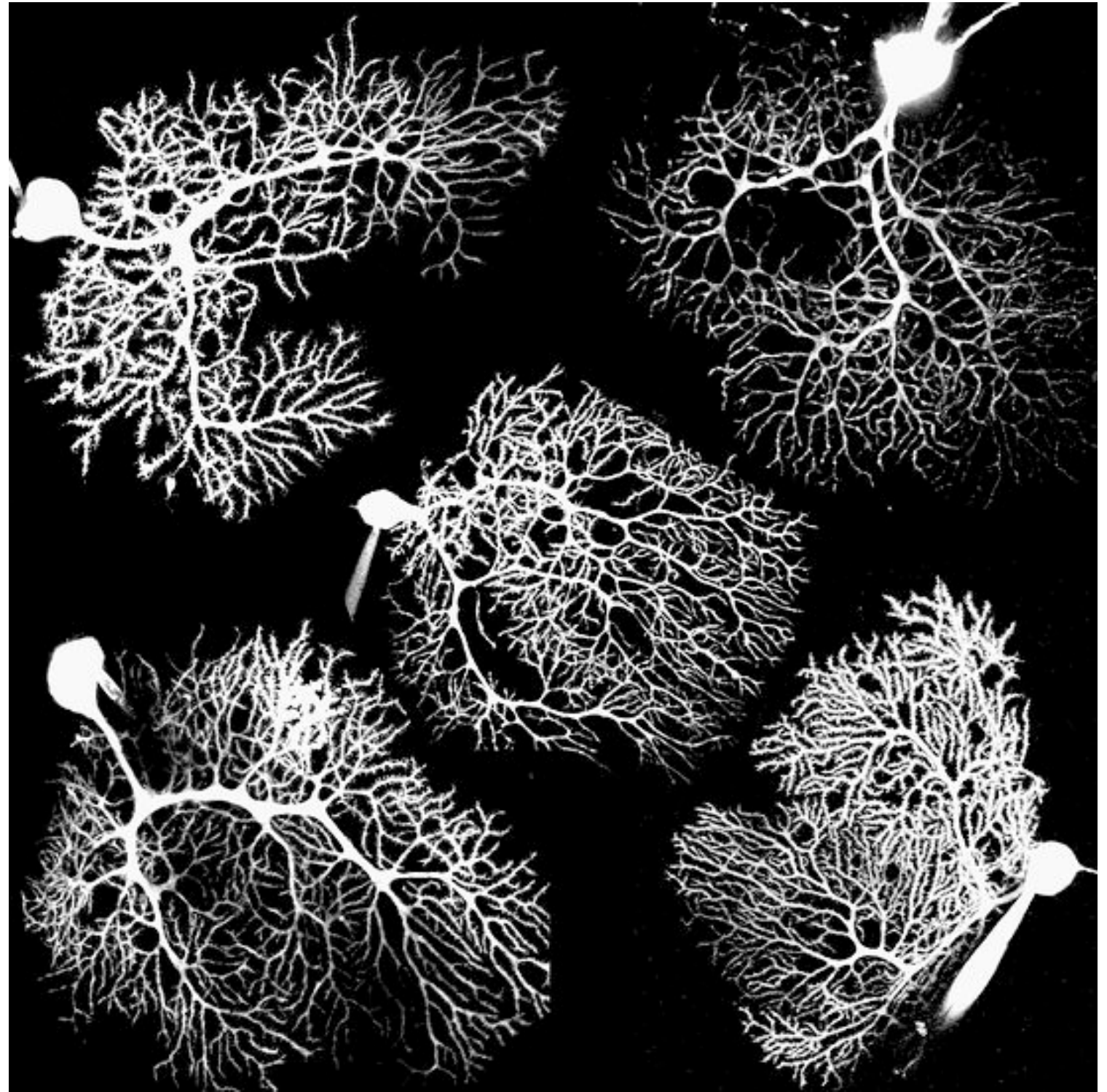


How do you build a functional neural circuit?

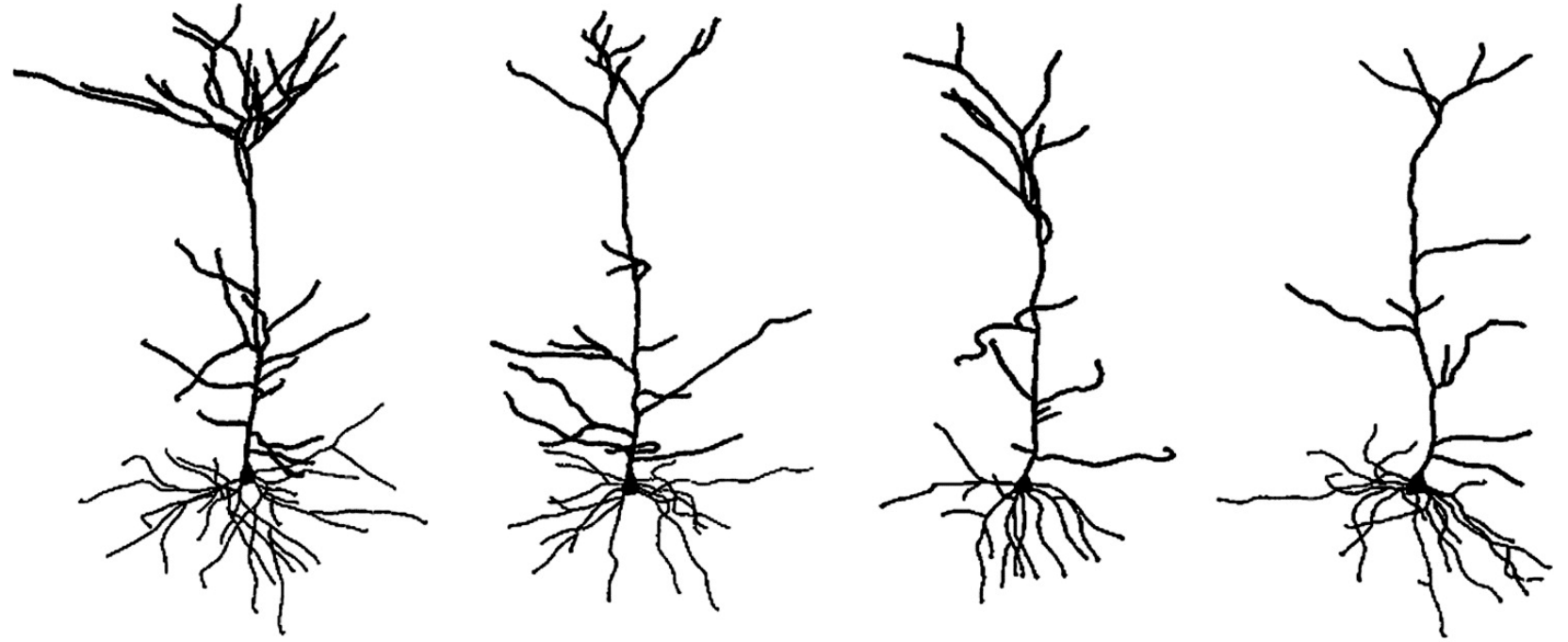
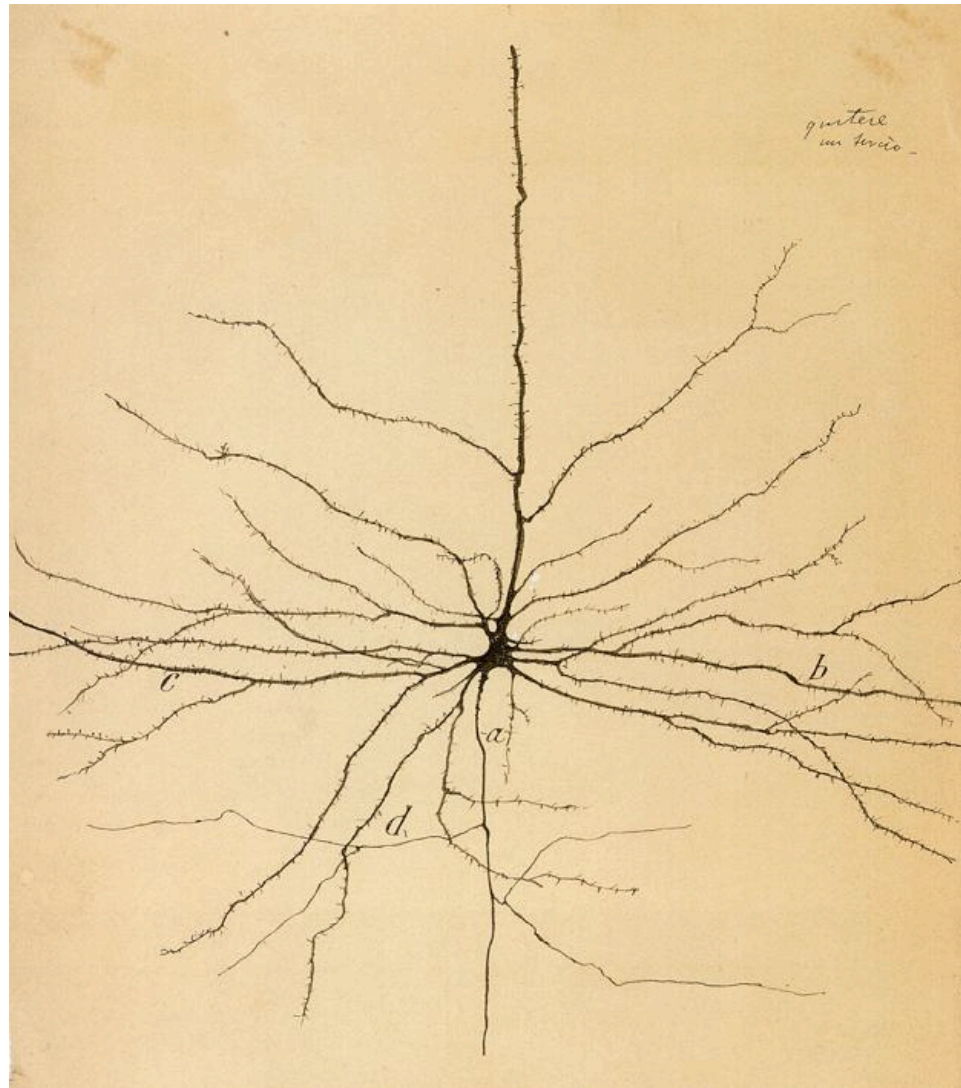


Santiago Ramon y Cajal

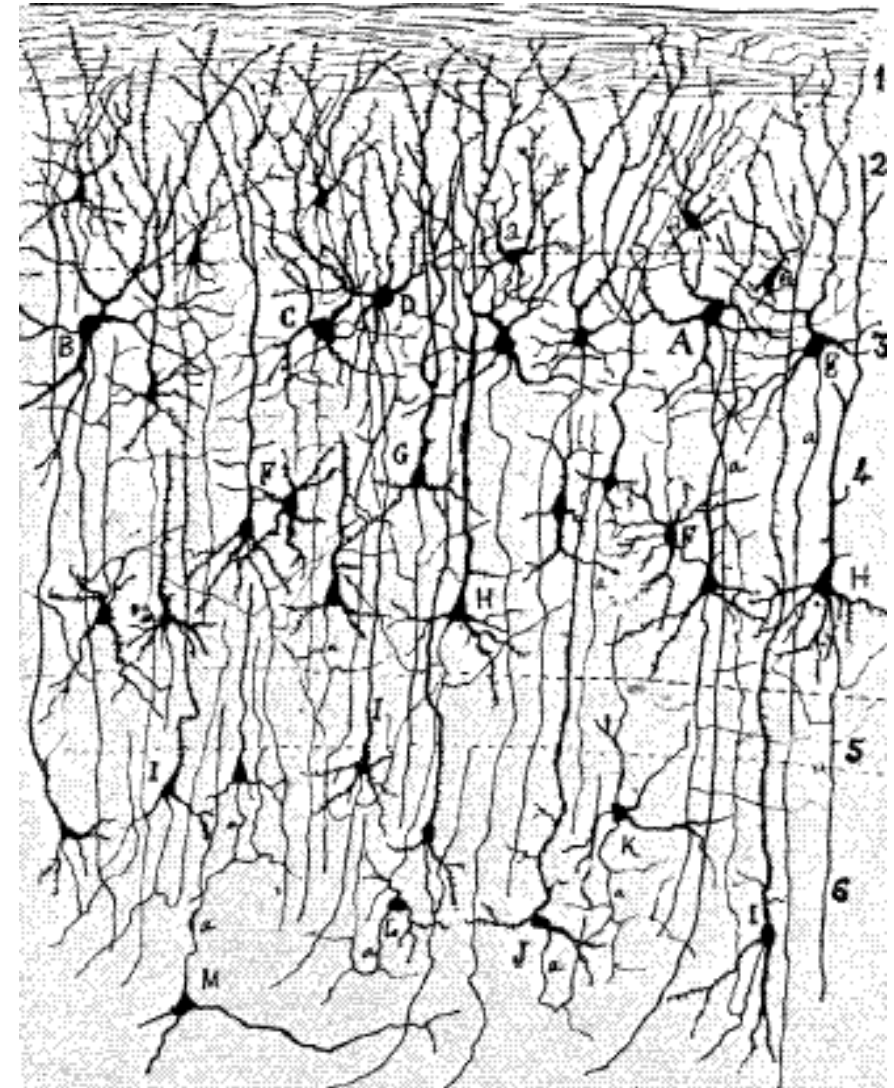
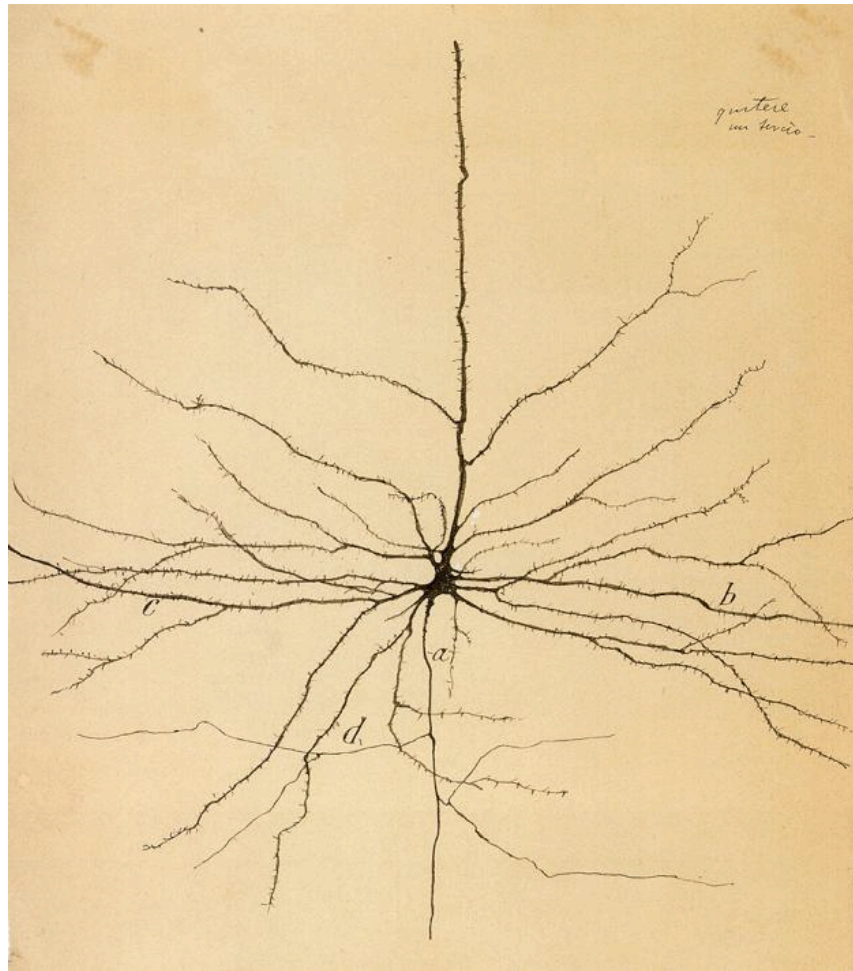
Cell-type specific patterning, but each cell's dendritic arbor is unique



Size and shape matters



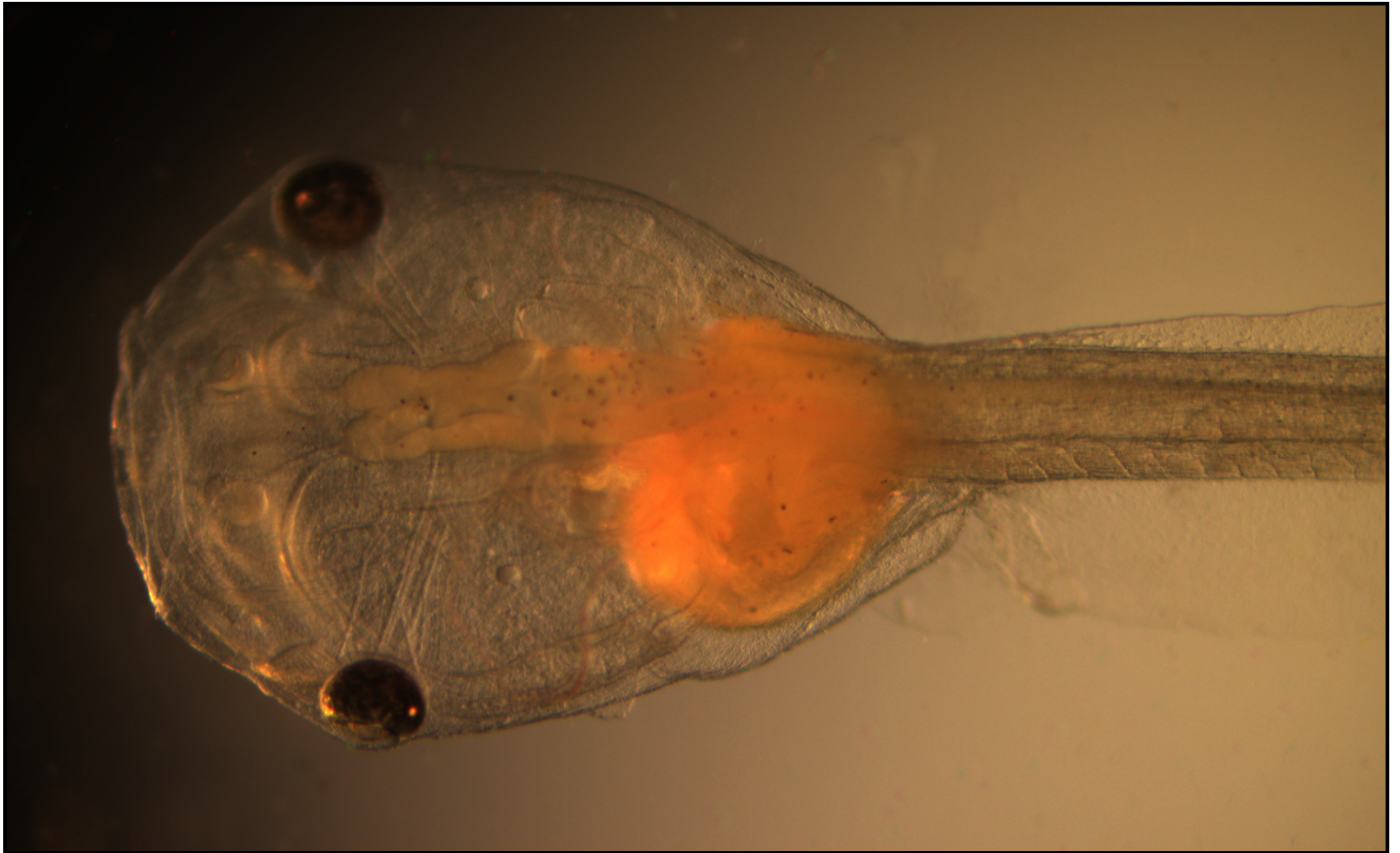
How big is this problem?



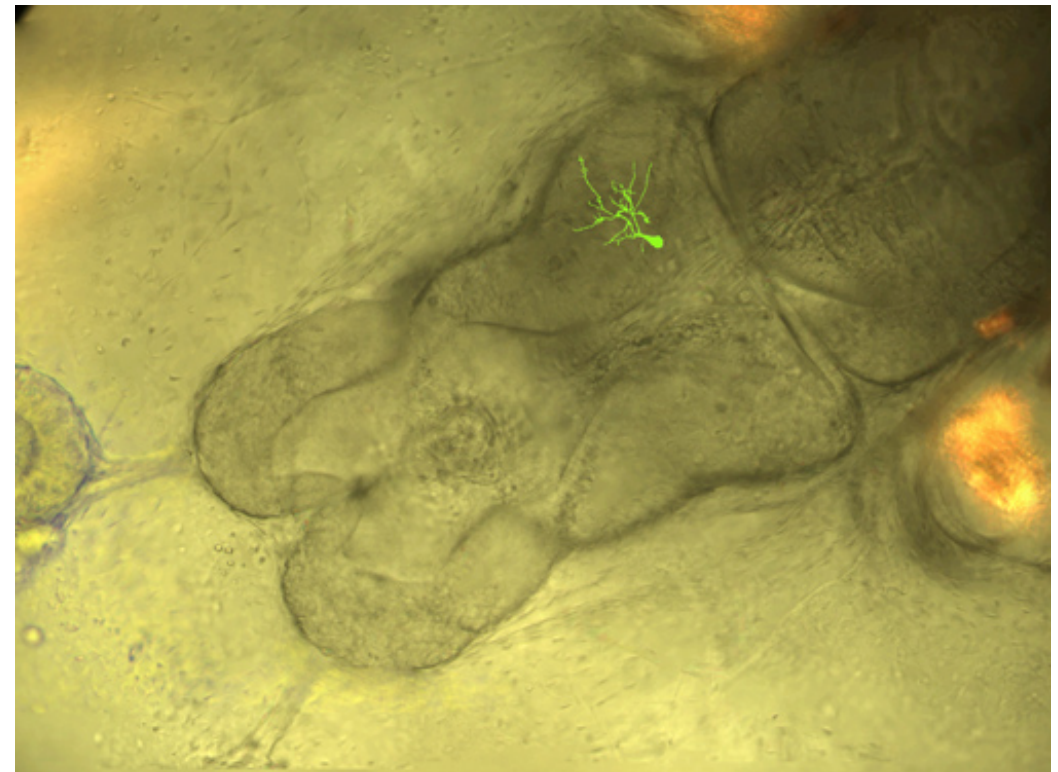
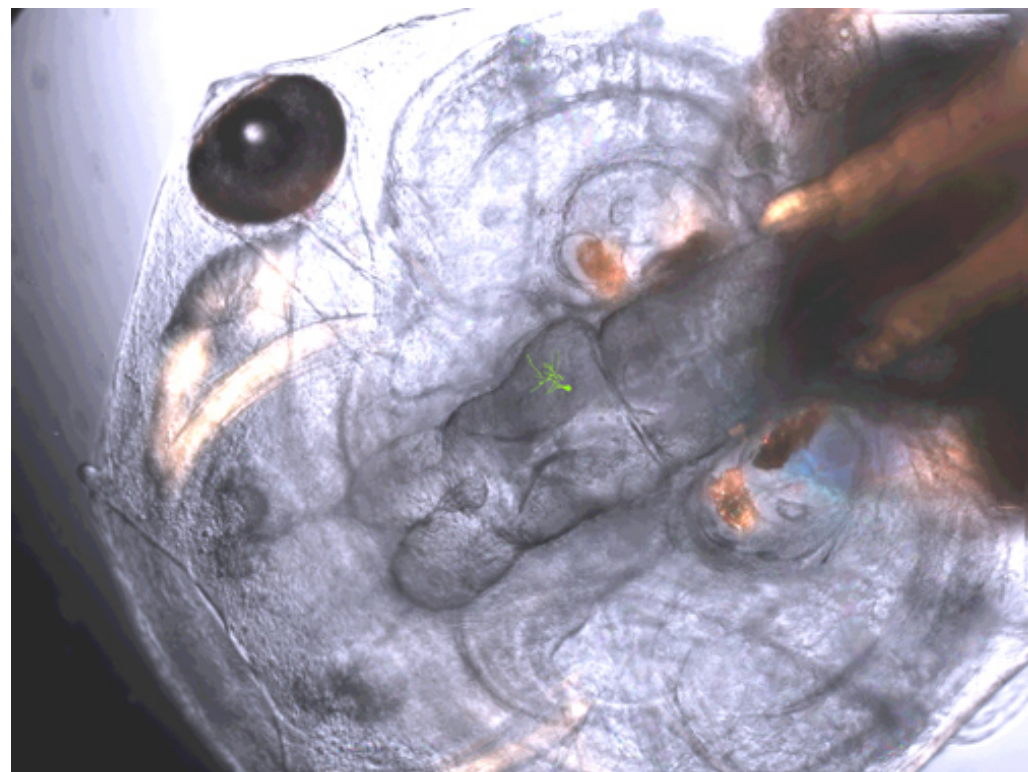
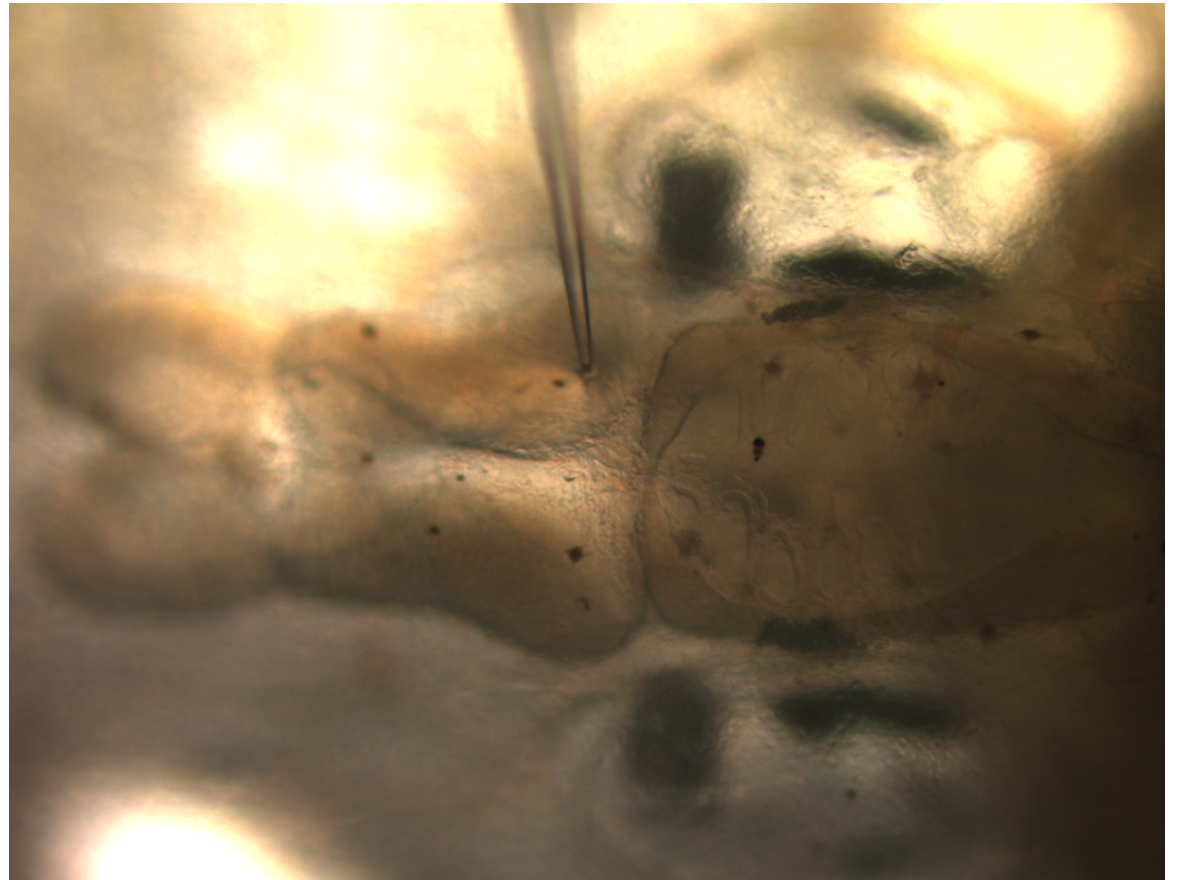
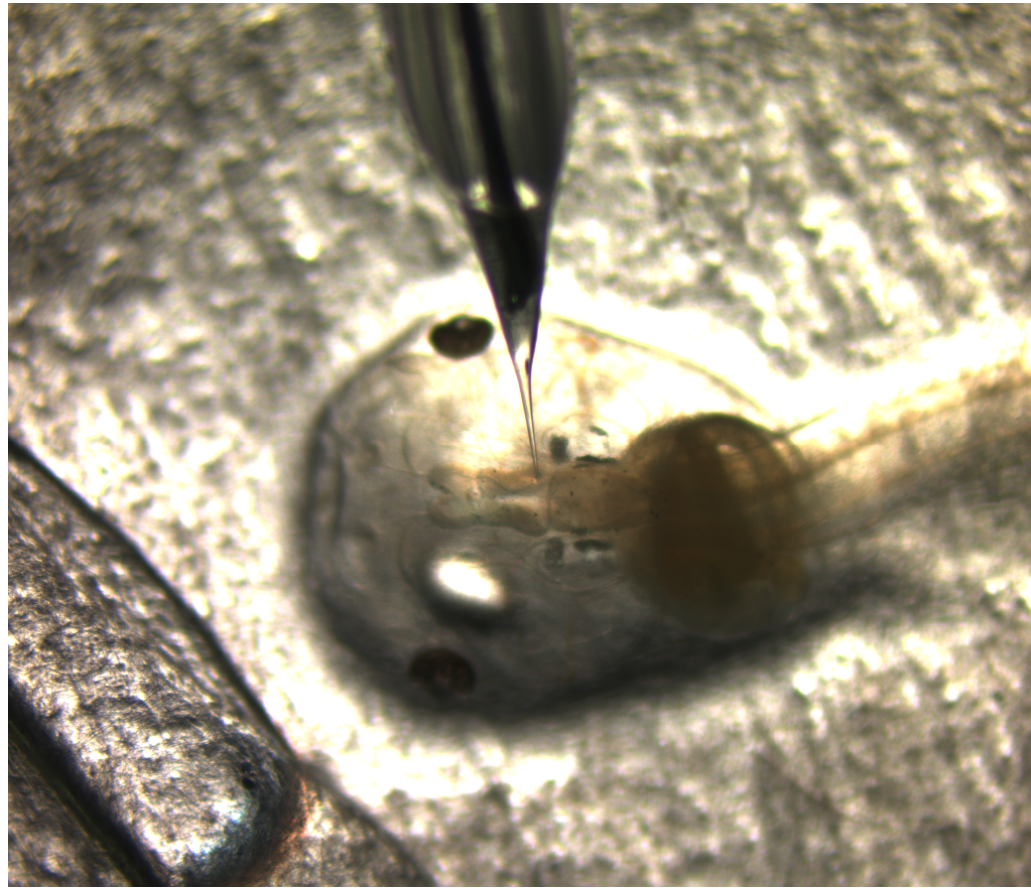
100 billion neurons
trillions of synapses

~27,000 genes

Albino *Xenopus* Tadpole: 1.5-2 weeks old

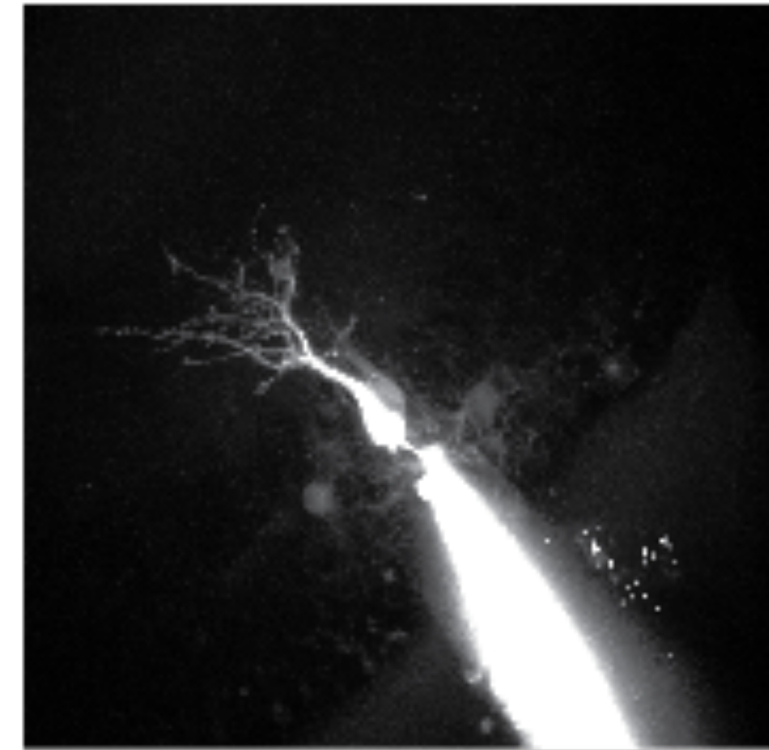
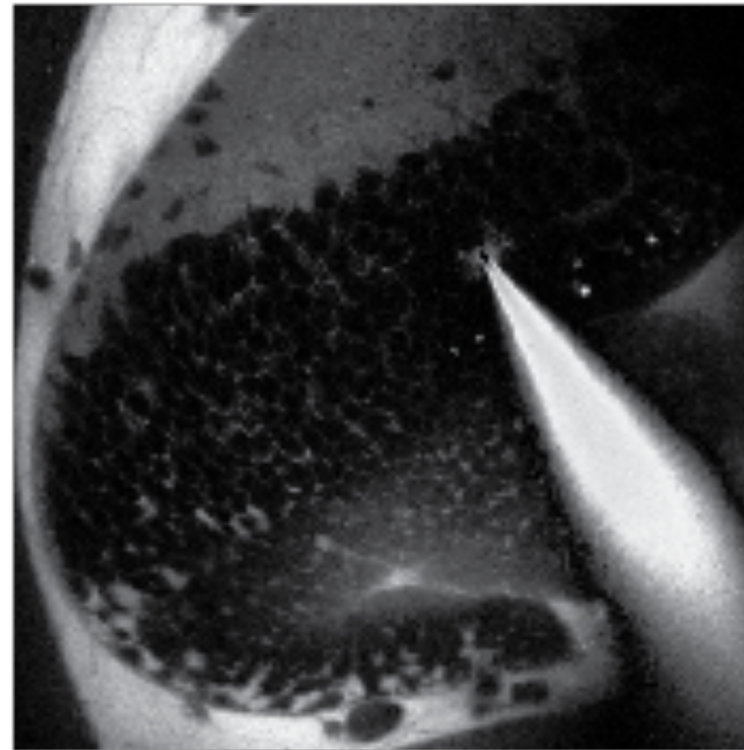
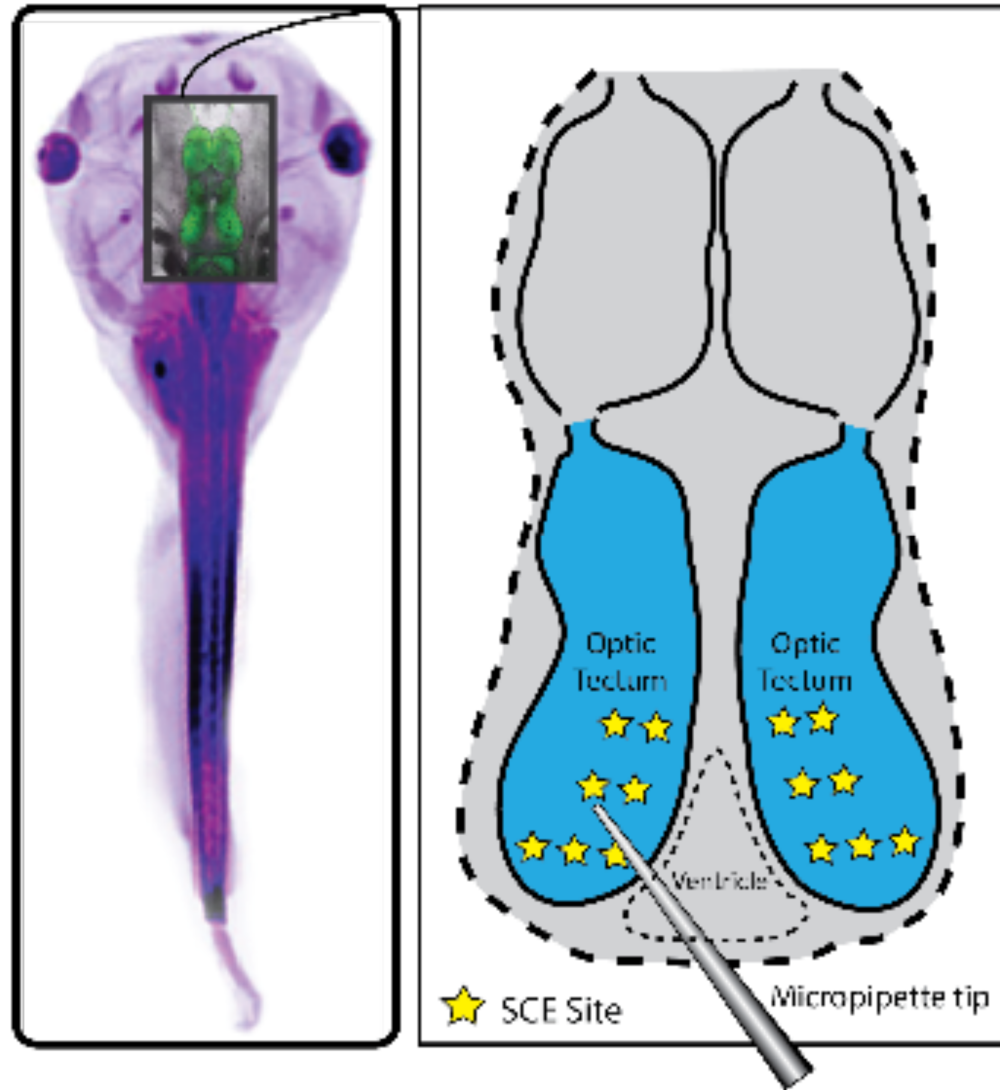


Single-Cell Electroporation

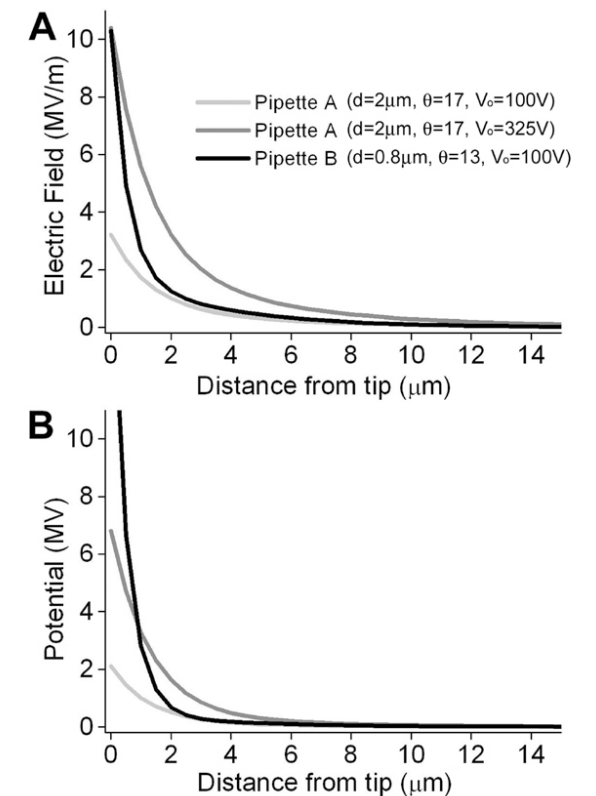
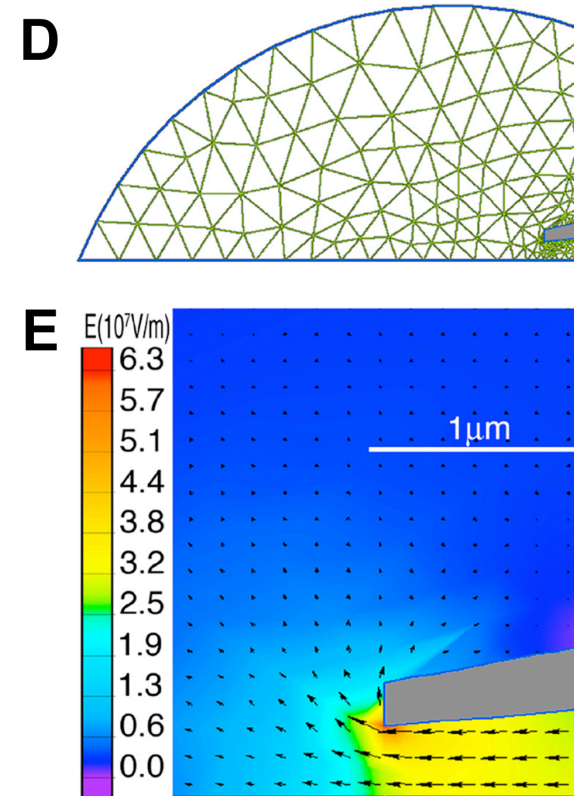
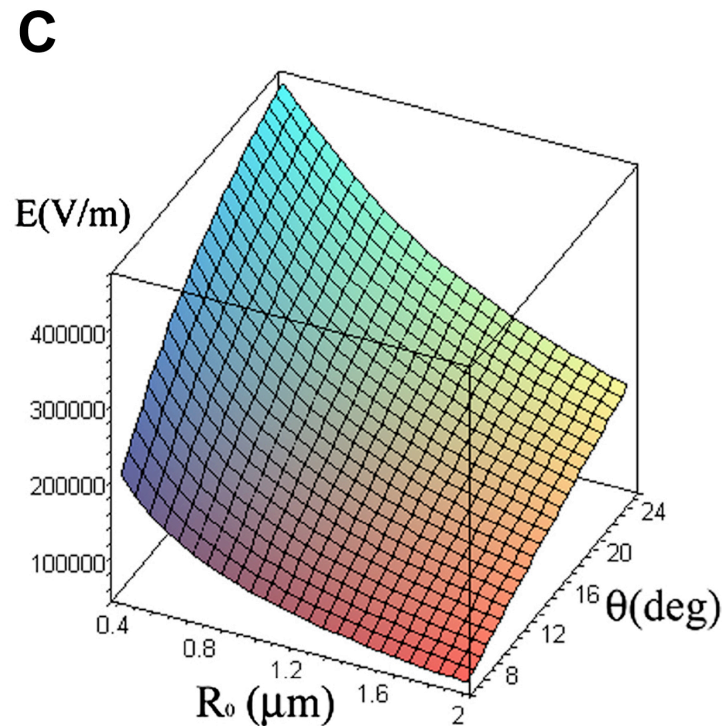
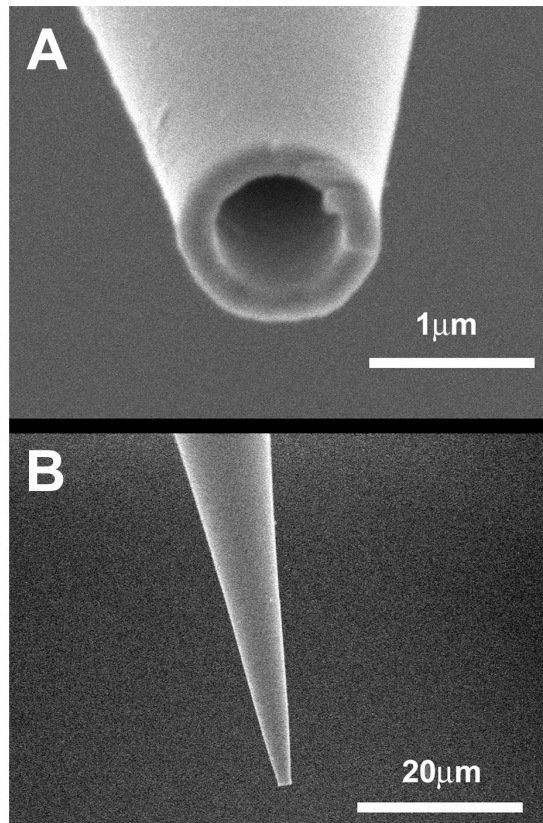


(Haas, et al. *Neuron* 2001)

Single-Cell Electroporation



SCE Pipette Tip Geometry



~0.6 - 1 micron tip diameter

Shallow shanks

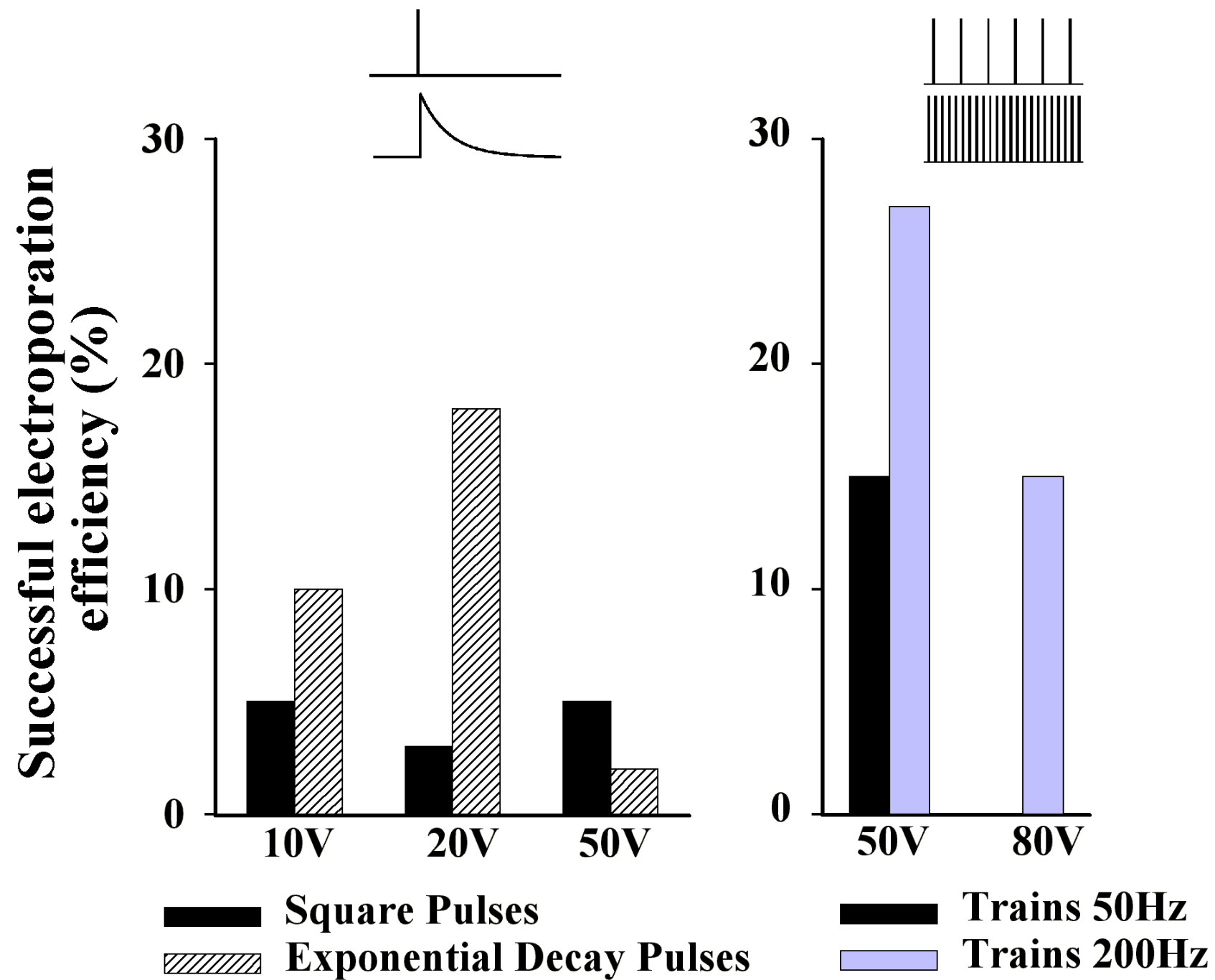
Similar to standard patch pipettes with smaller tip diameter

Single vs. multiple cells

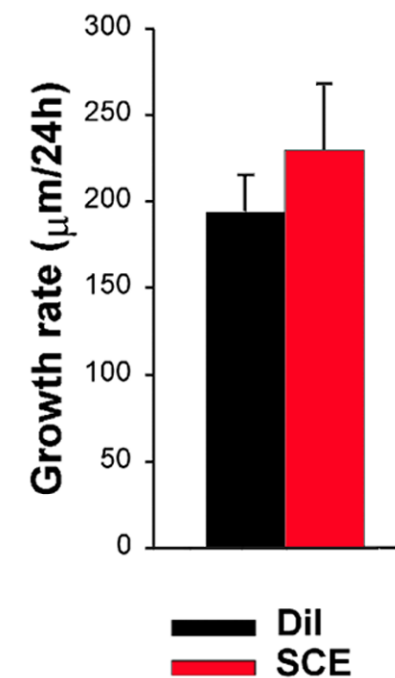
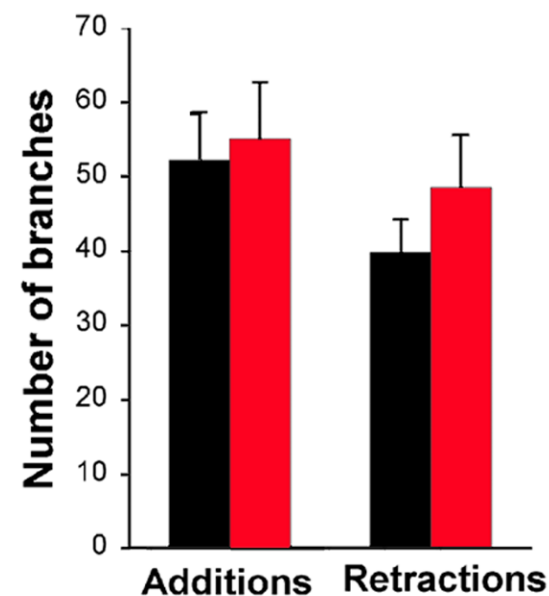
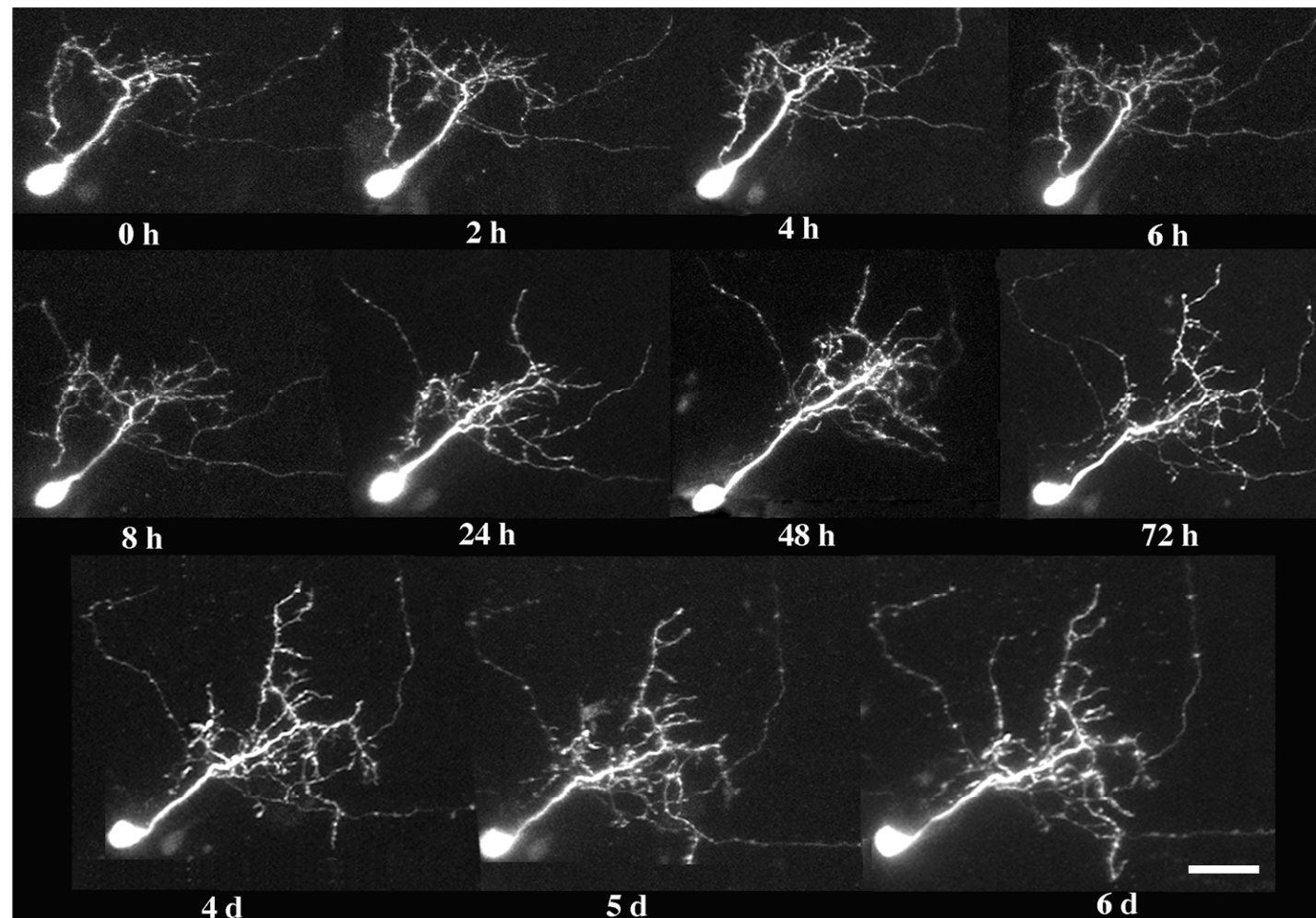
Tissue damage

Breaking vs. clogging

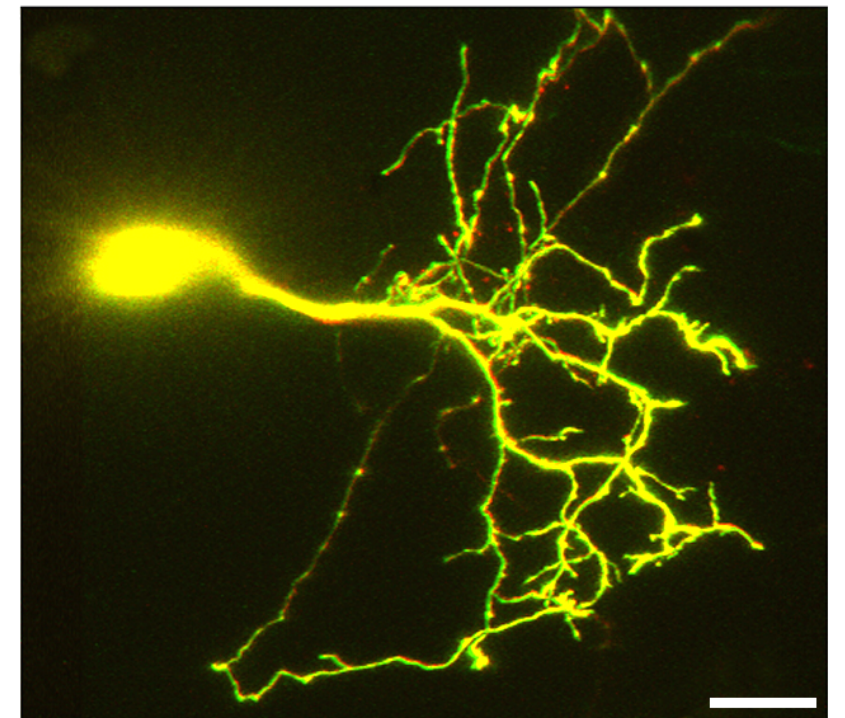
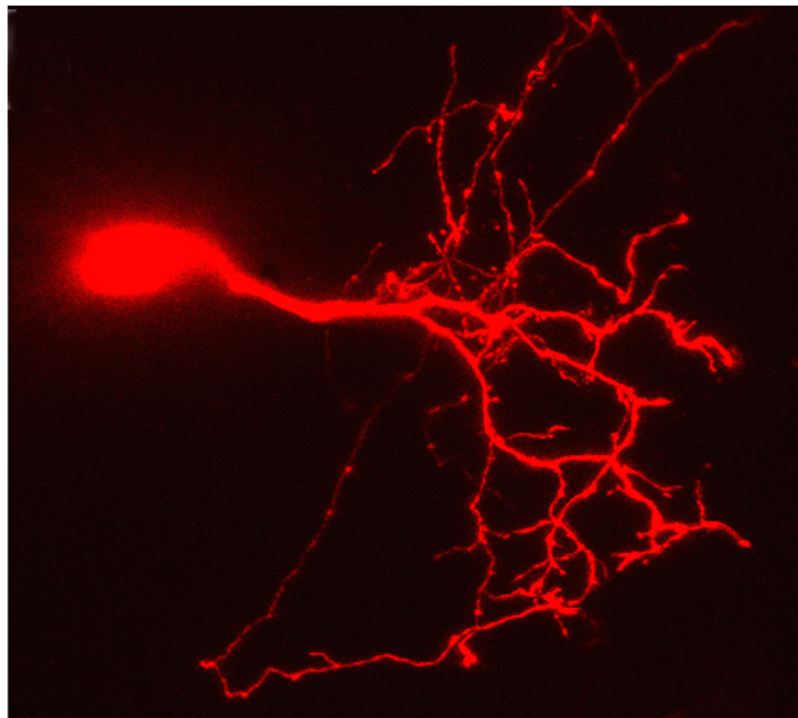
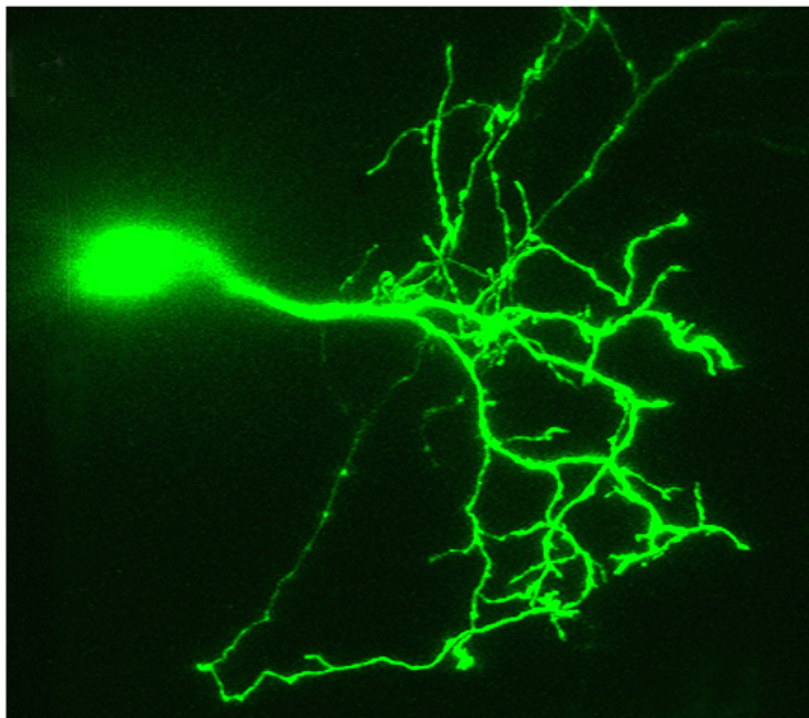
Optimal stimulation parameters for SCE of DNA



Single-cell electroporation has no detectable effect on cell health or dendritic arbor growth

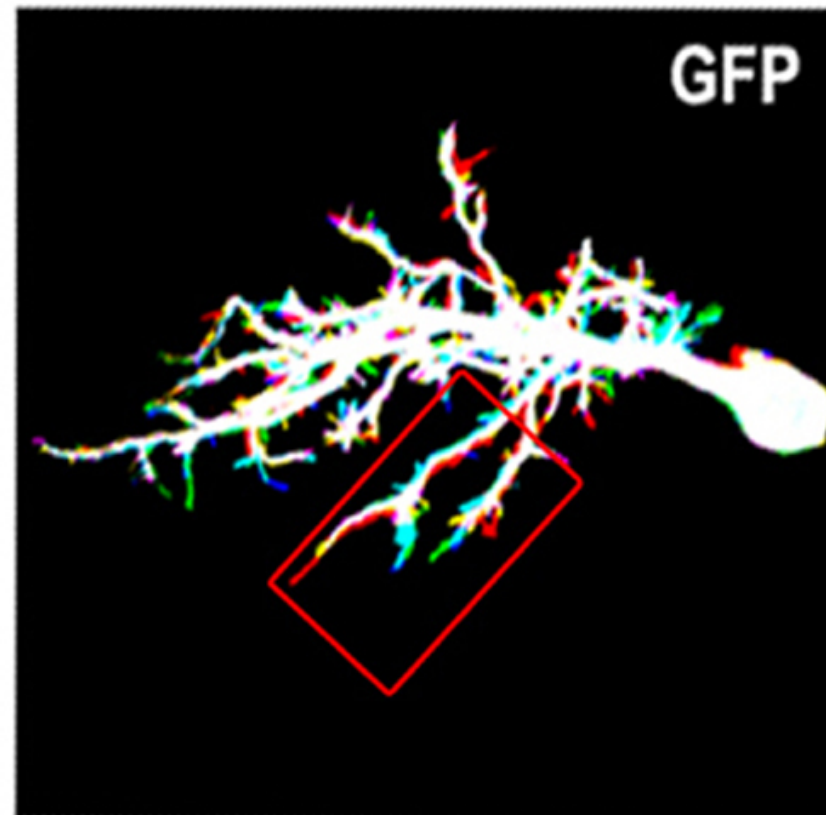


Co-electroporation of multiple plasmids

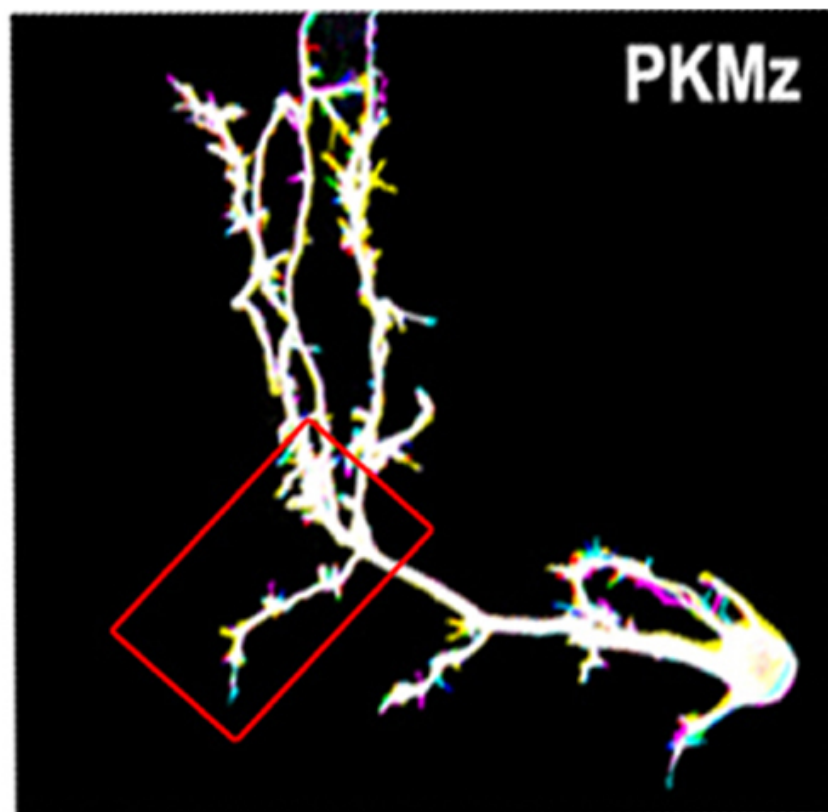


Cell-autonomous over-expression of PKMz

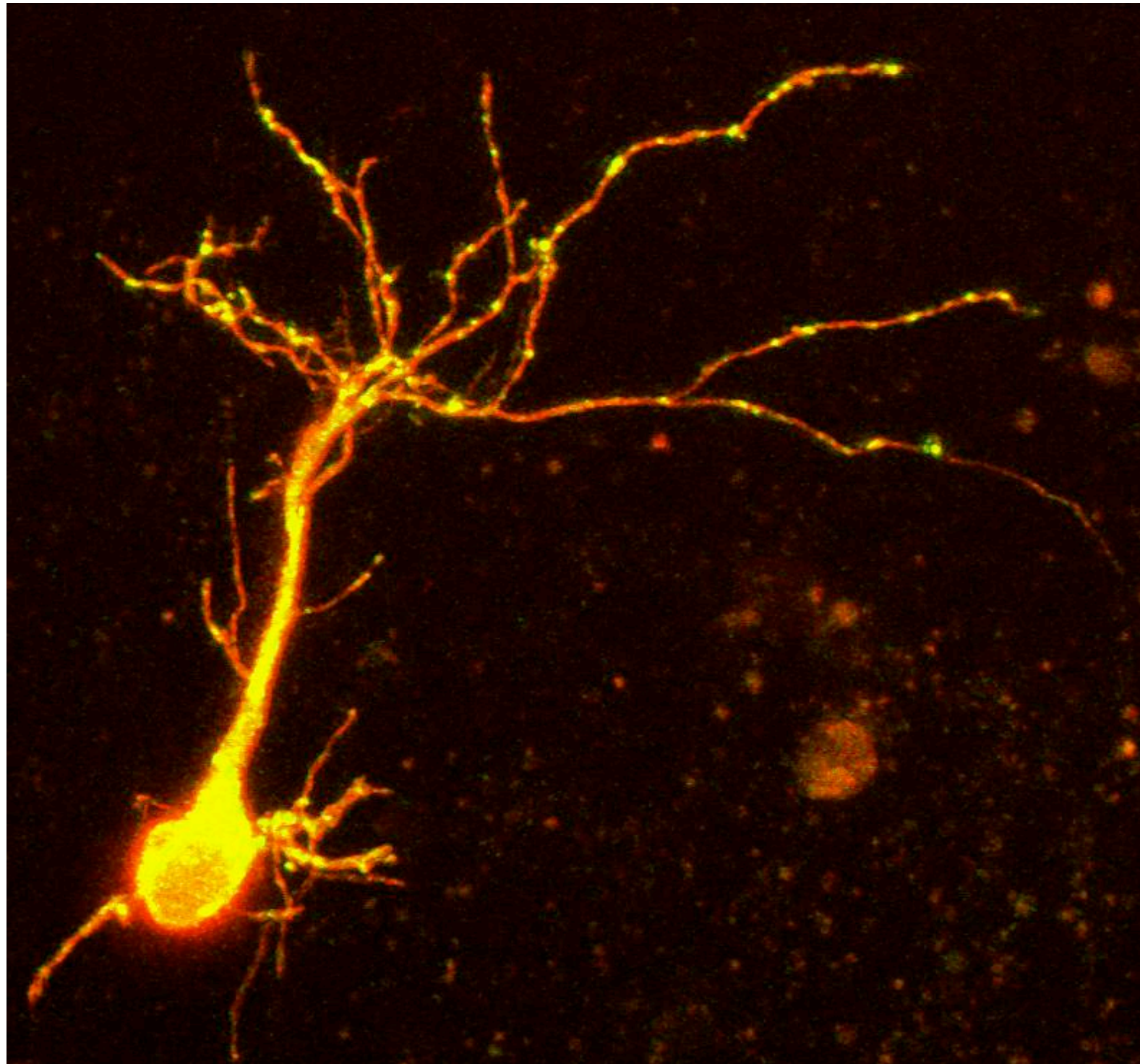
Control



PKMz

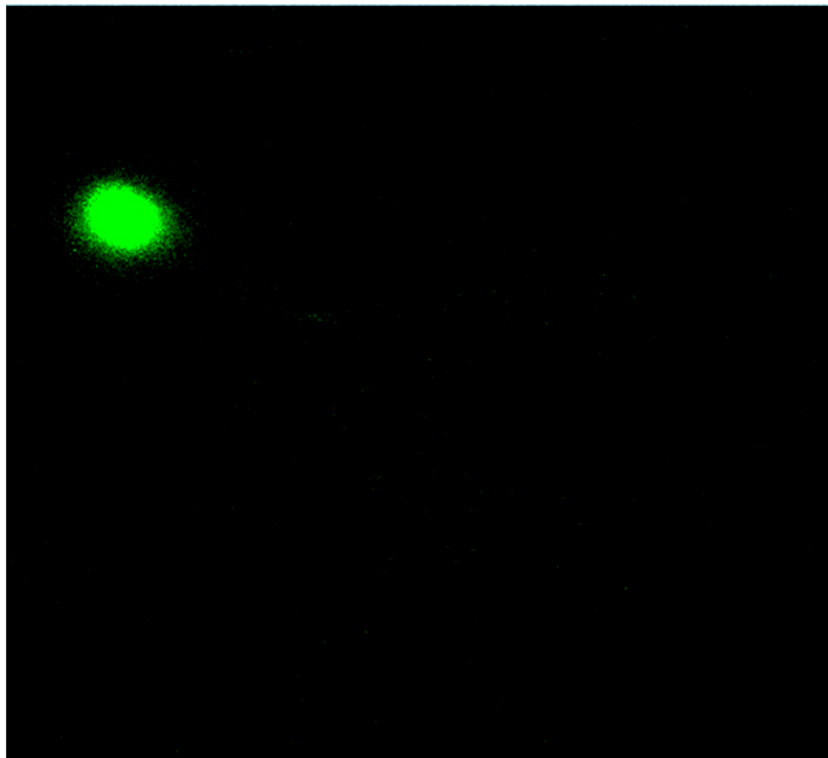


Co-SCE of multiple plasmids DsRed and PSD95-GFP

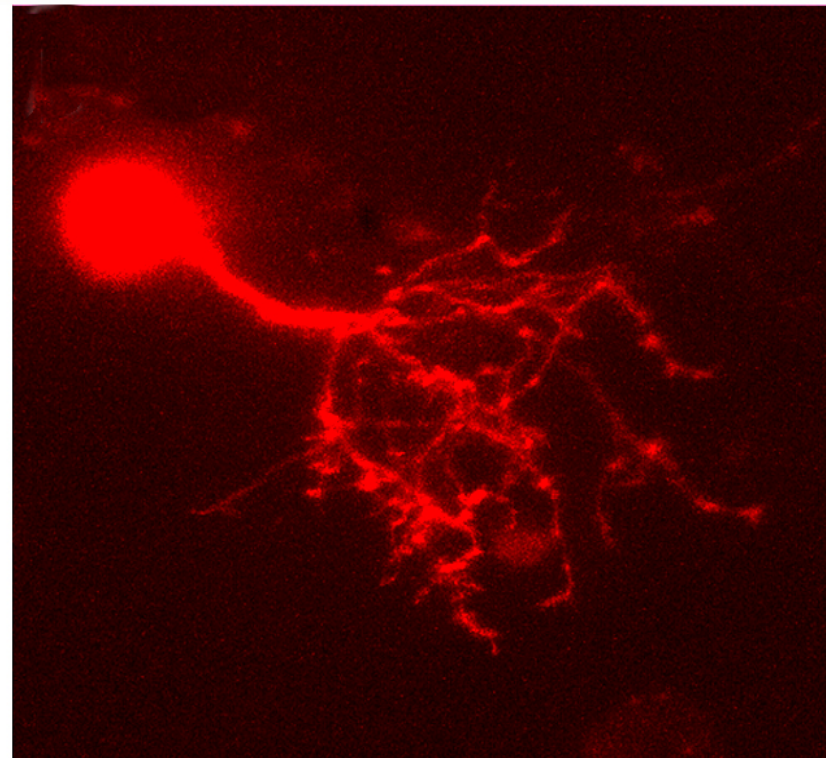


Co-SCE of small molecules: Morpholino oligonucleotides and fluorescent dextrans

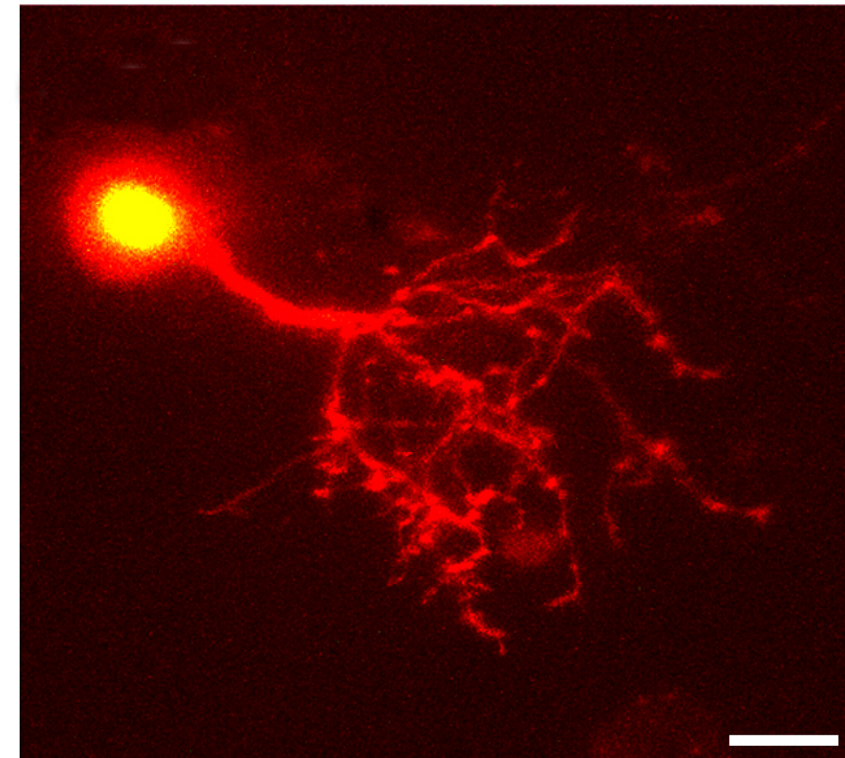
Morpholino oligos-FITC



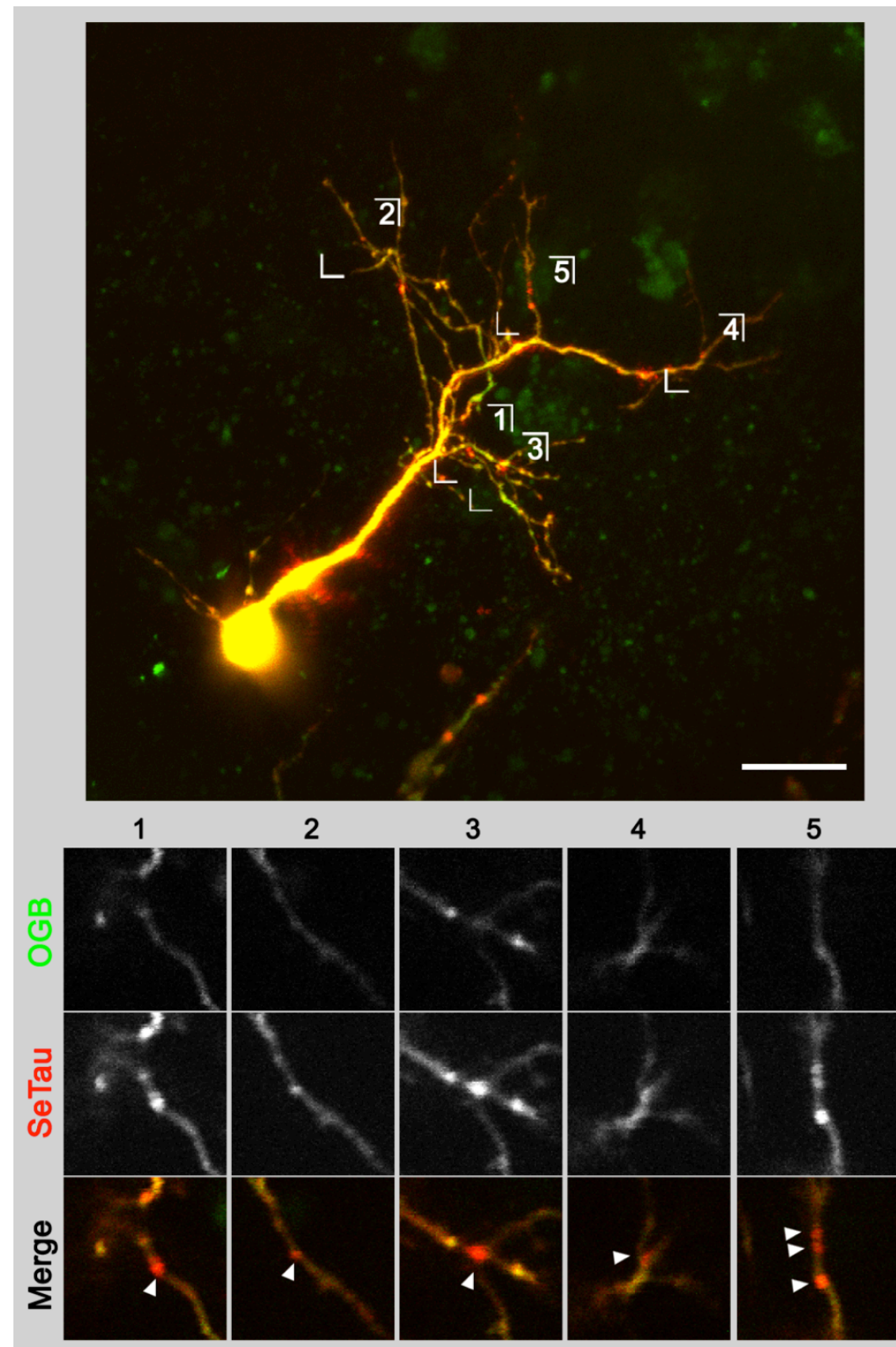
Rhodamine dextran



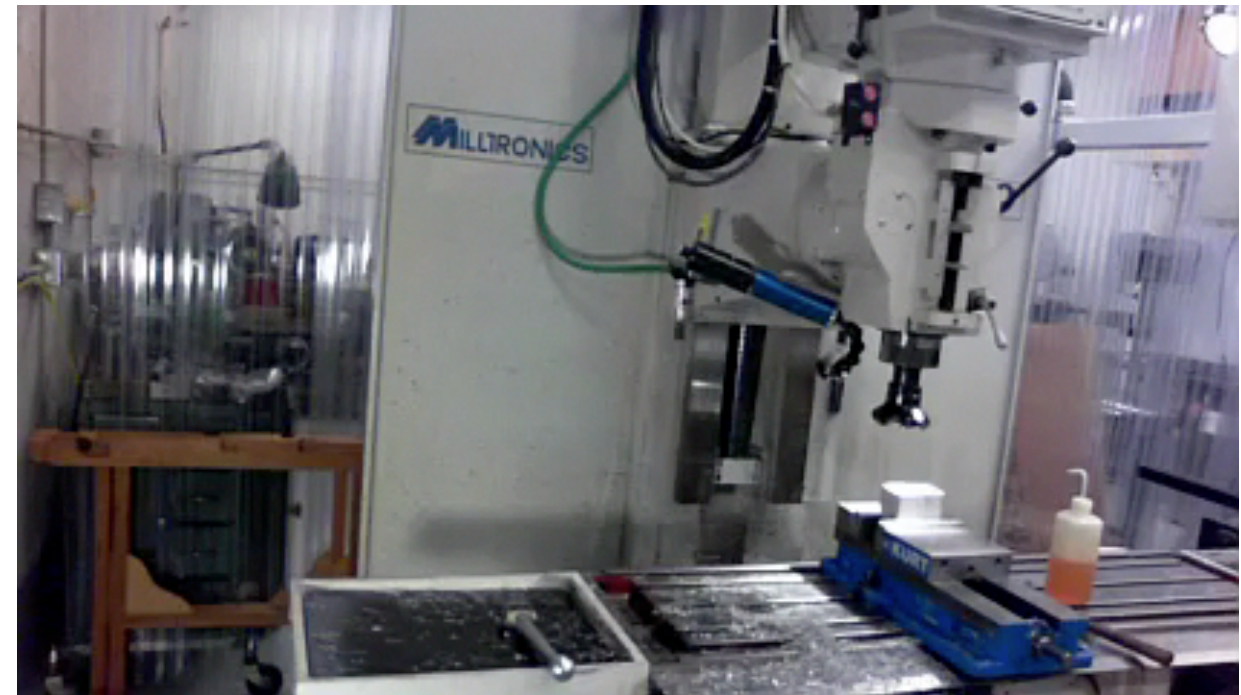
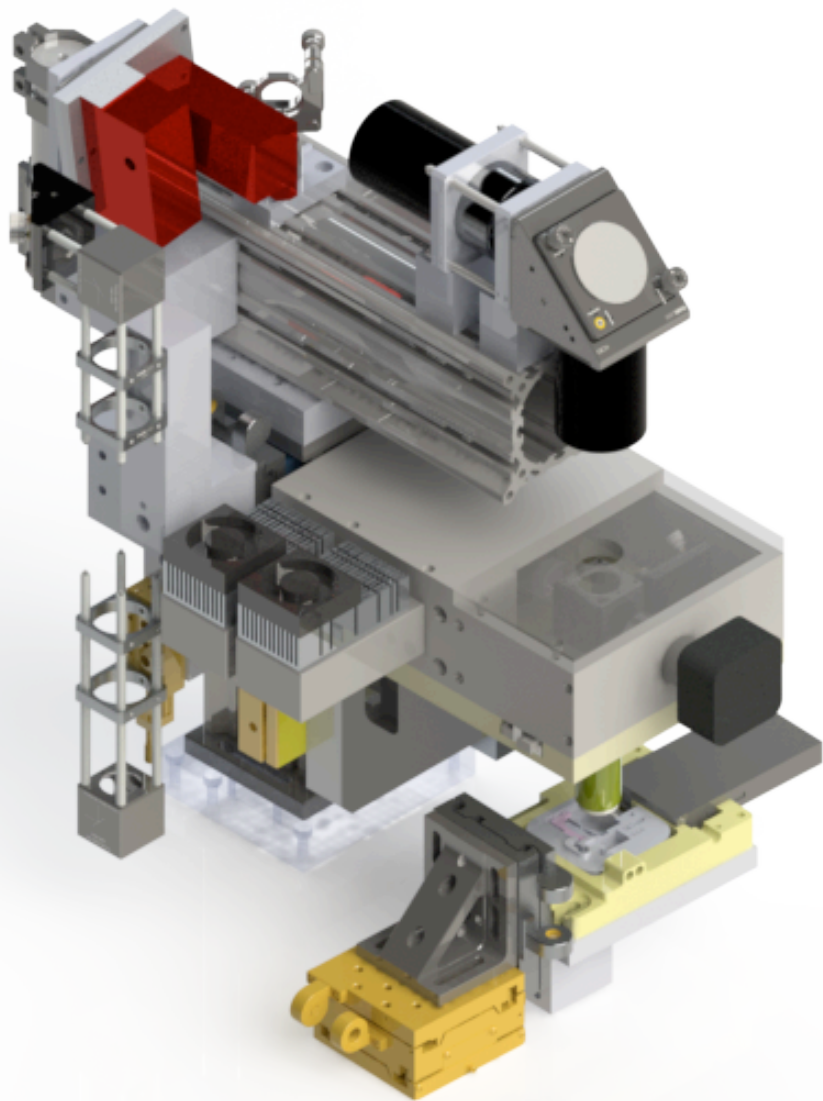
Overlay



Squaraine-Rotaxanes-peptides for labelling PSD-95 co-delivered with OGB1 for calcium imaging



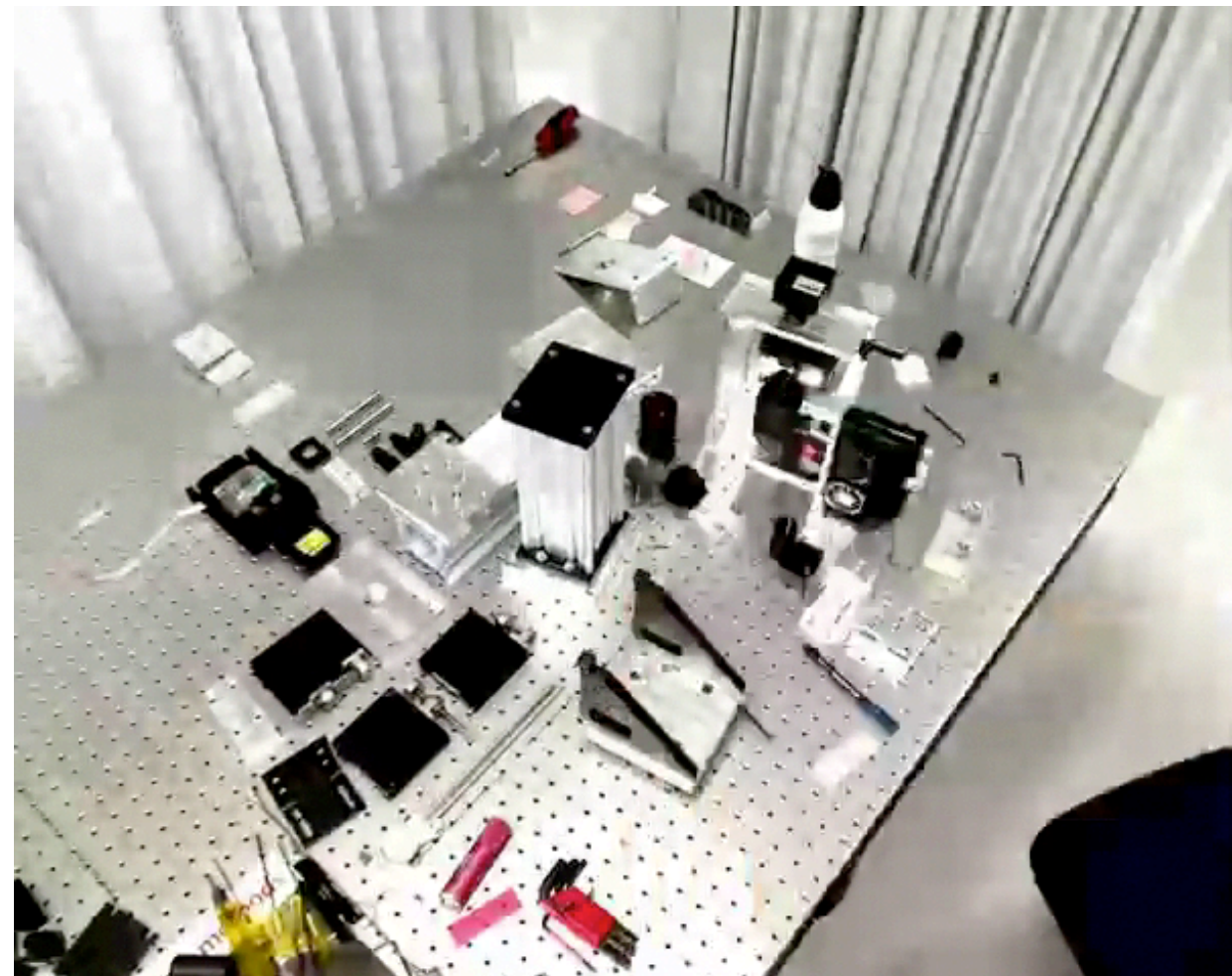
Custom-built Two-Photon Microscopes

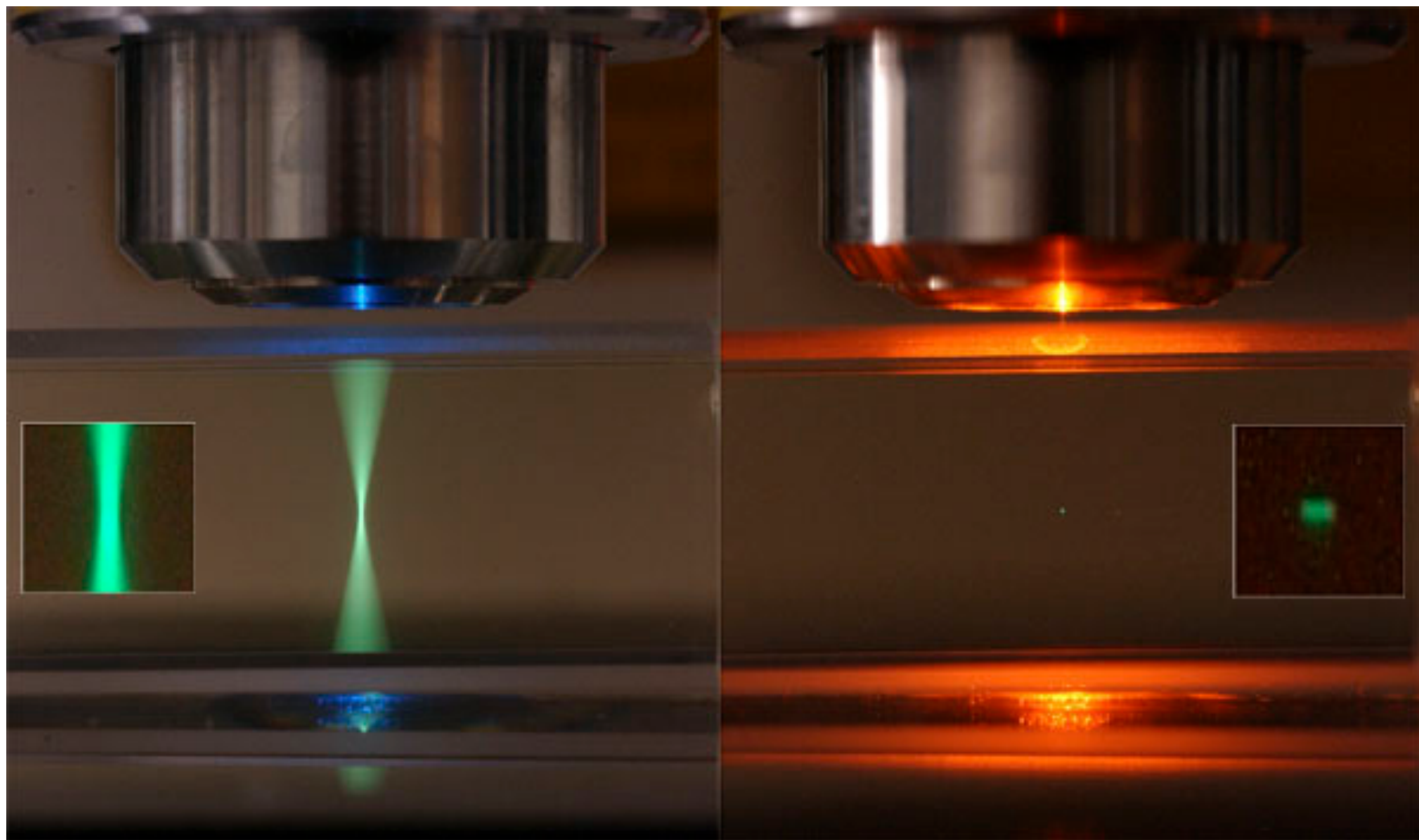


Kasper Podgorski
Janelia HHMI



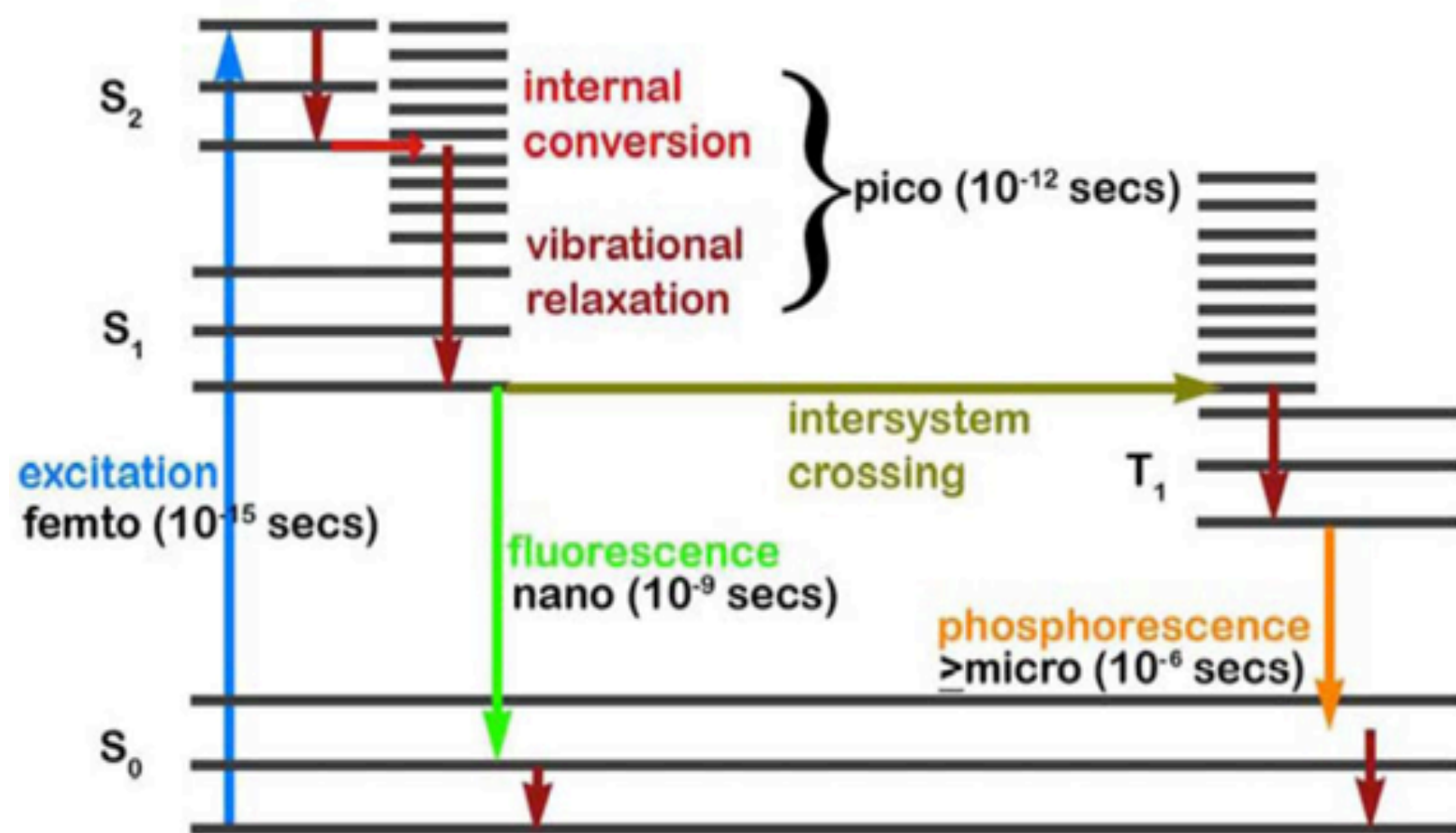
Kelly Sakaki
Scientifica



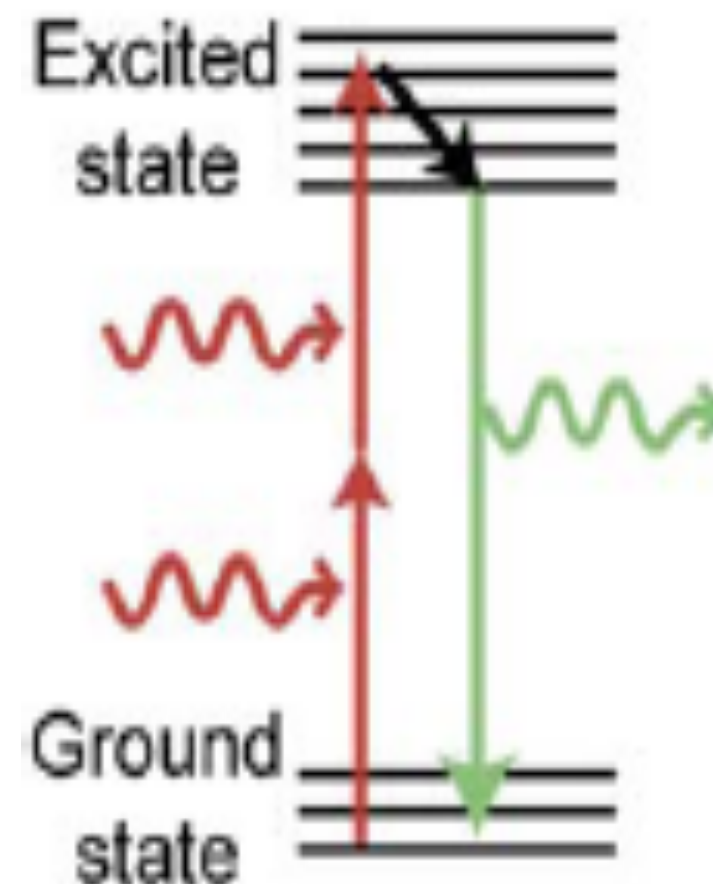


Jablonski Diagram

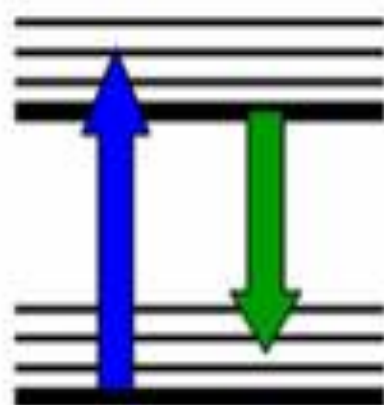
Single-photon excitation



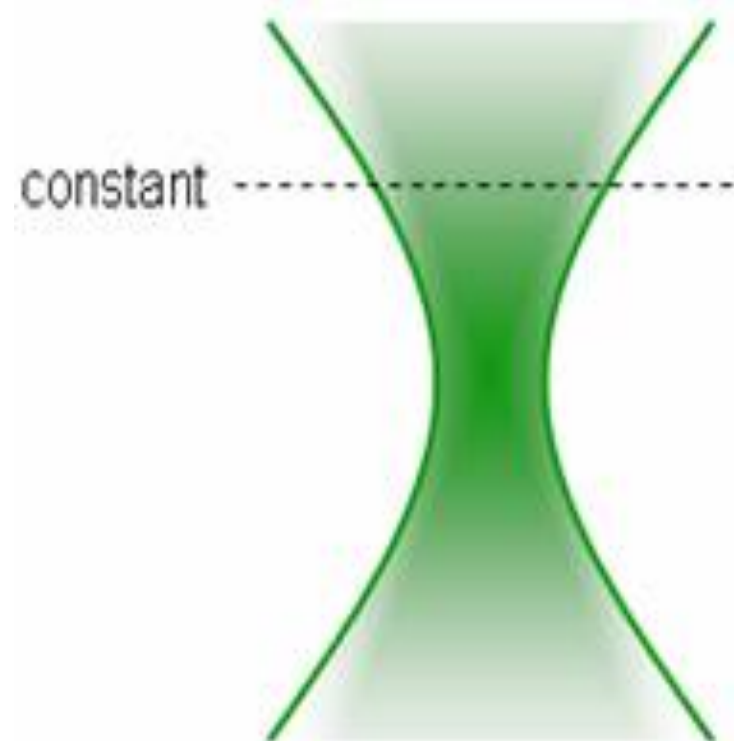
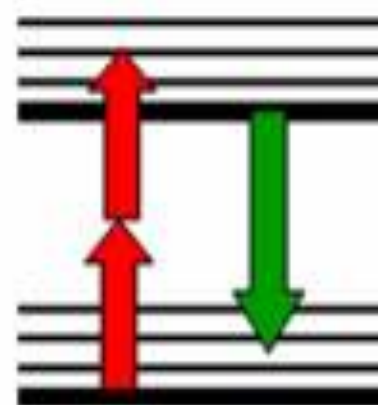
Two-photon excitation



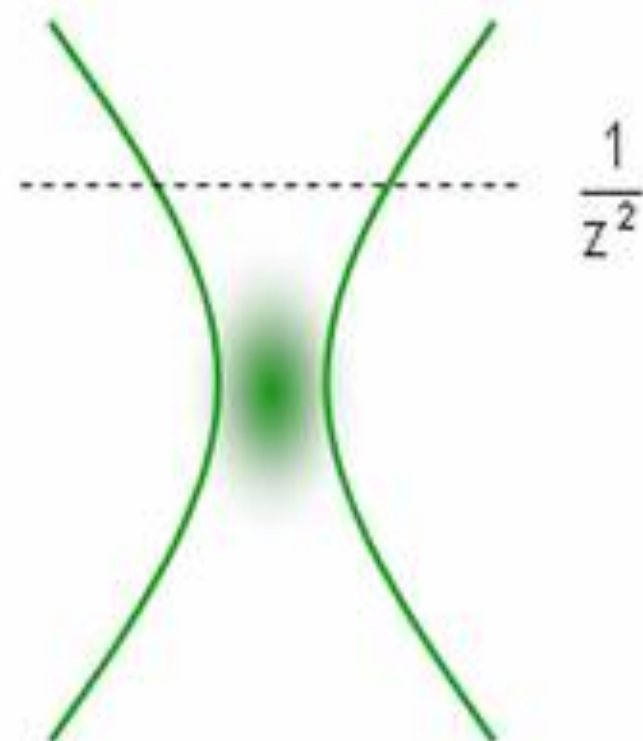
1-photon excitation



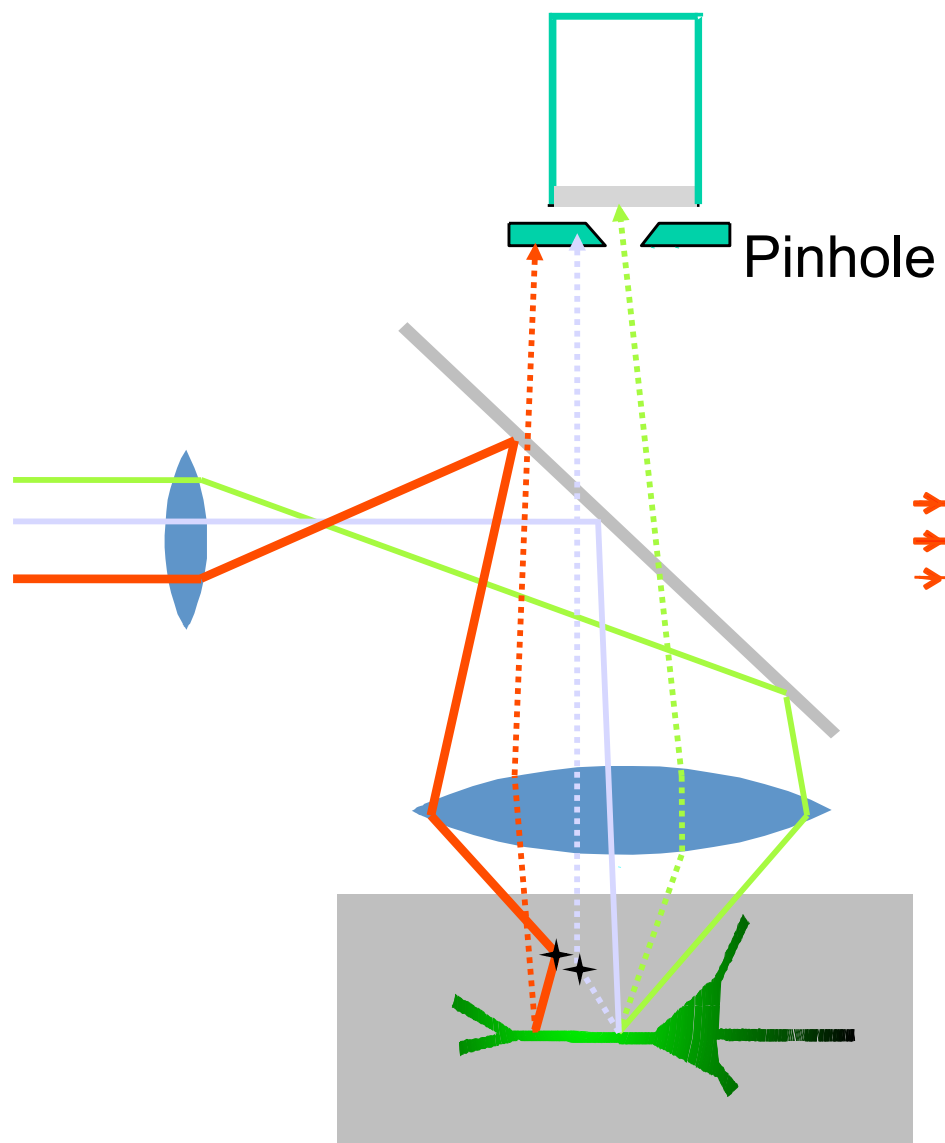
2-photon excitation



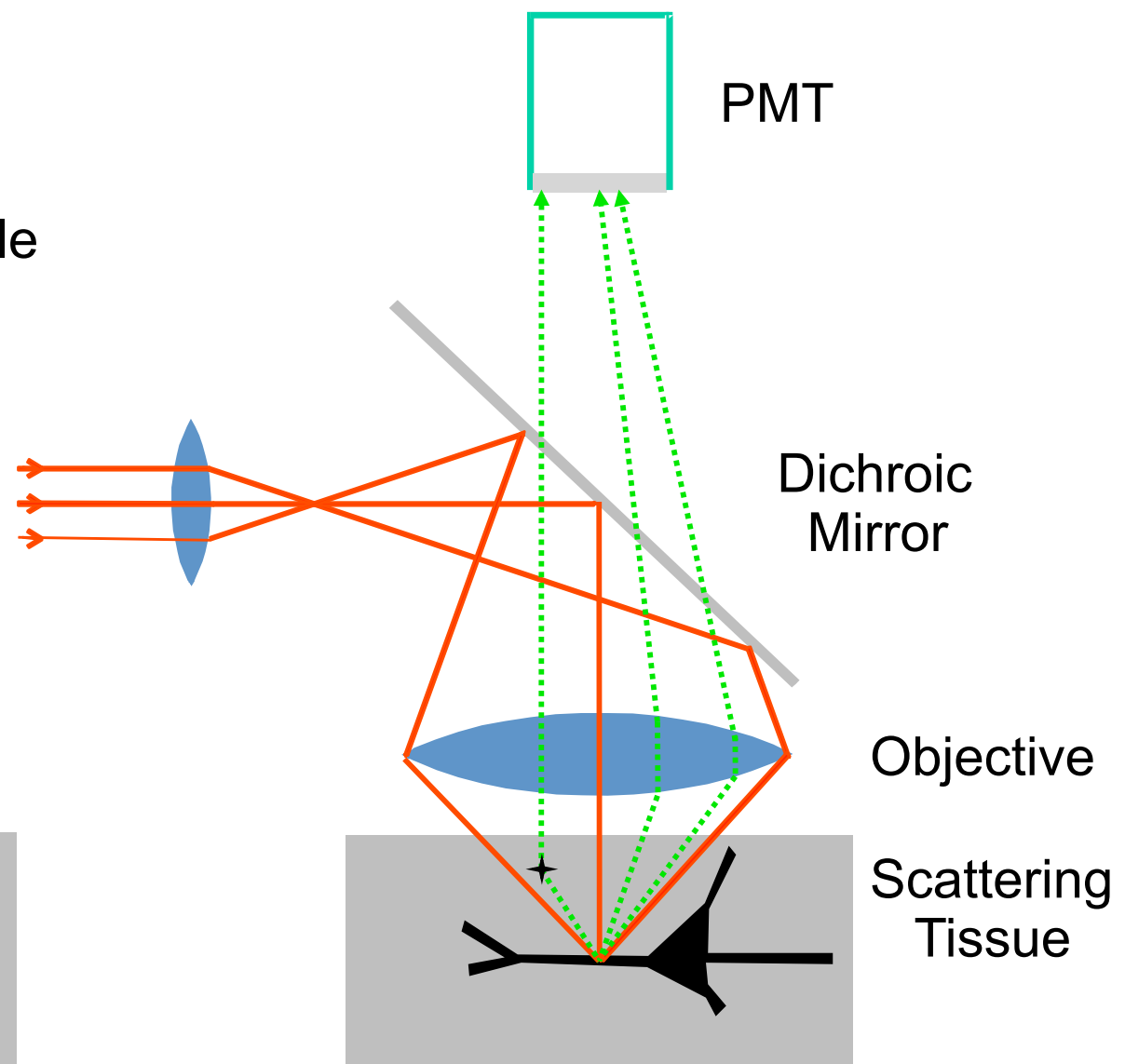
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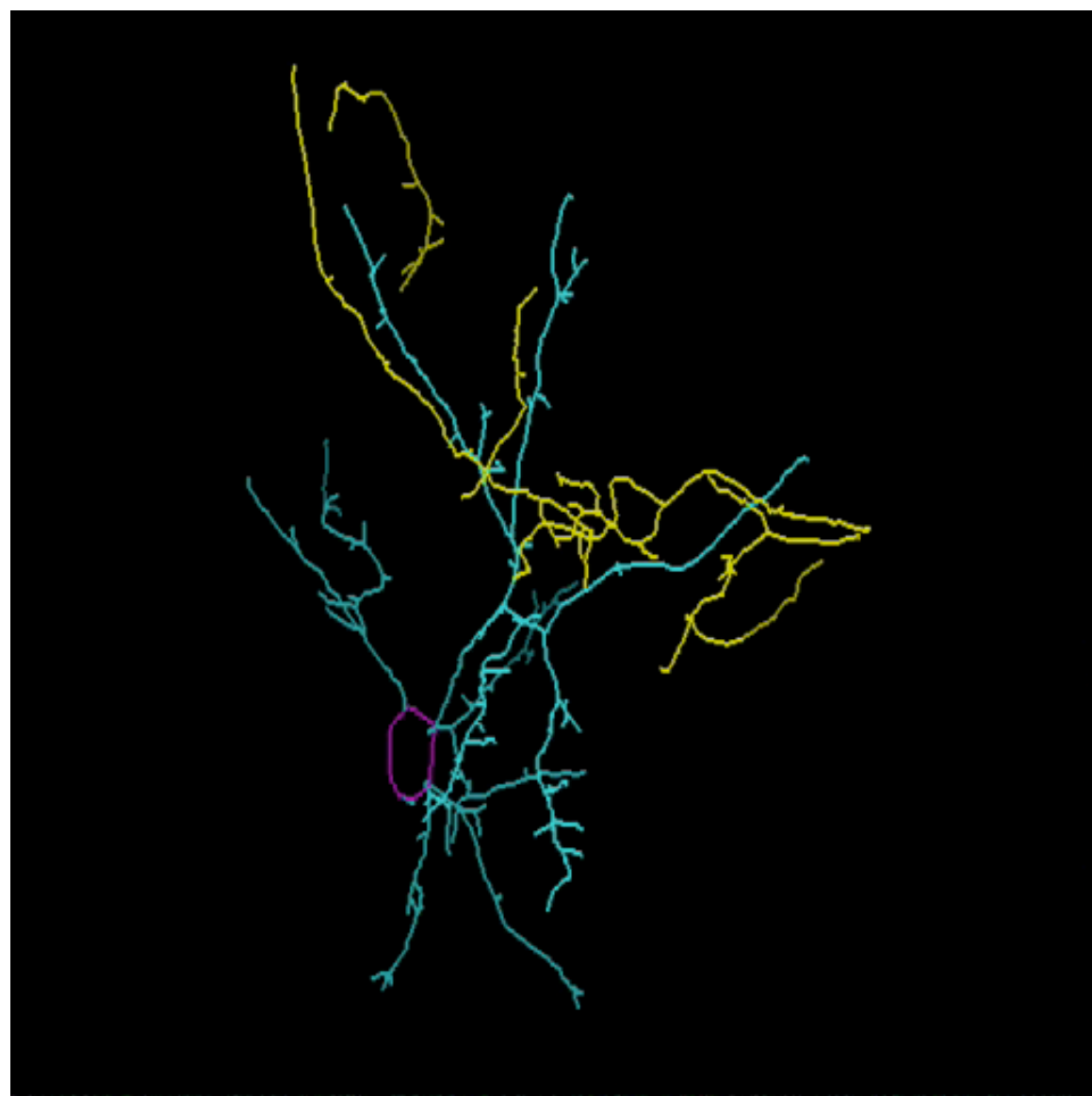
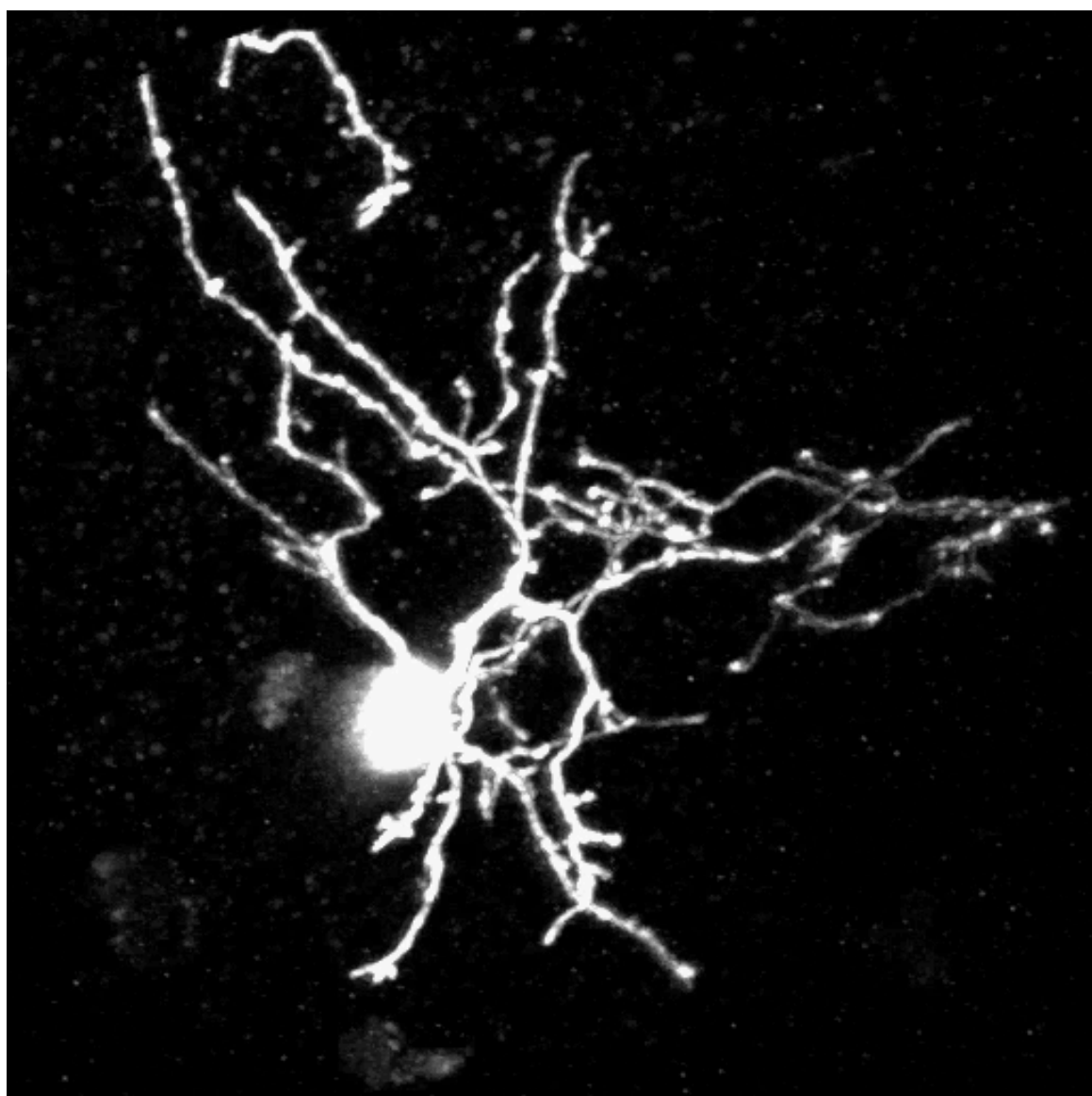


Confocal



Two-photon







Dynamic Morphometrics

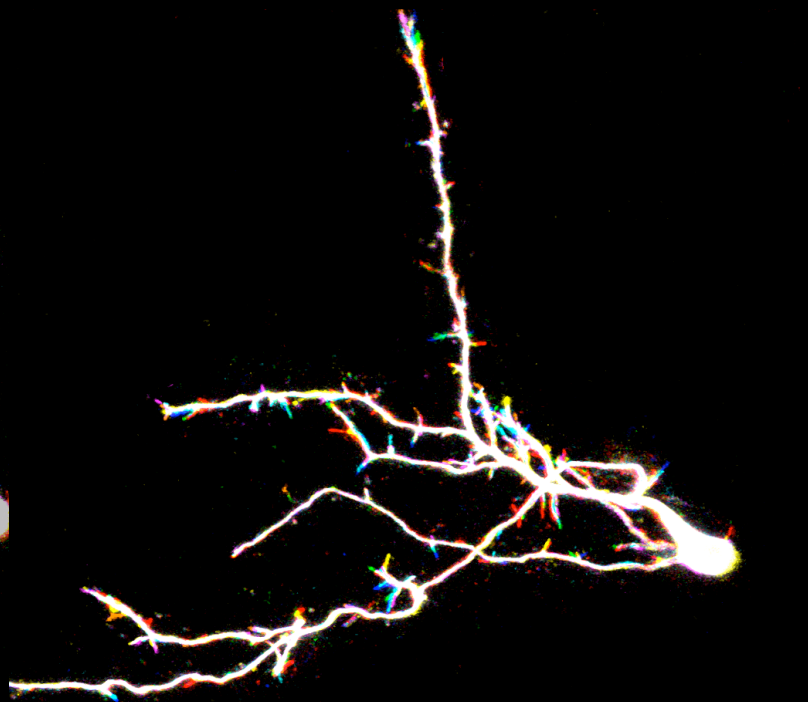
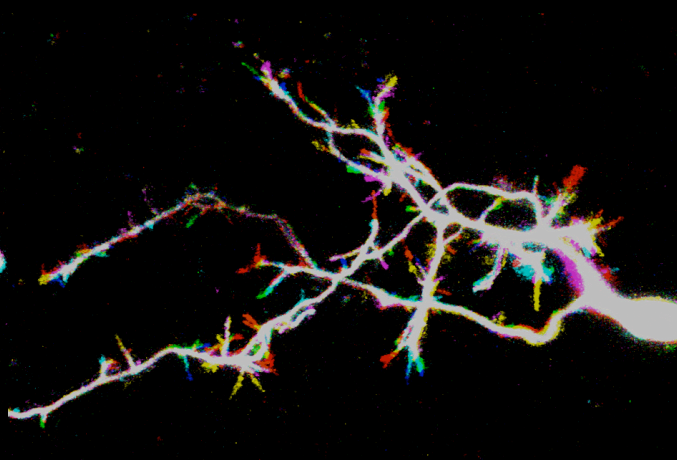
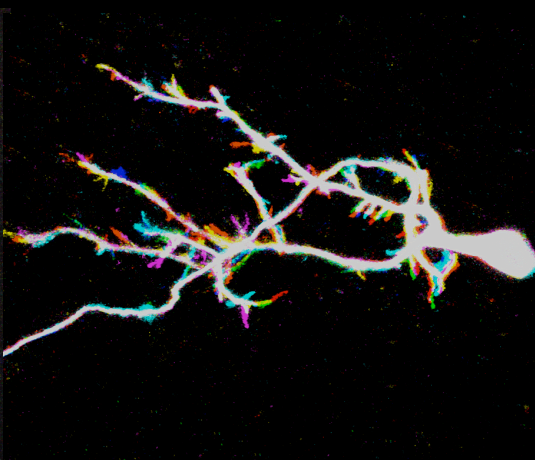
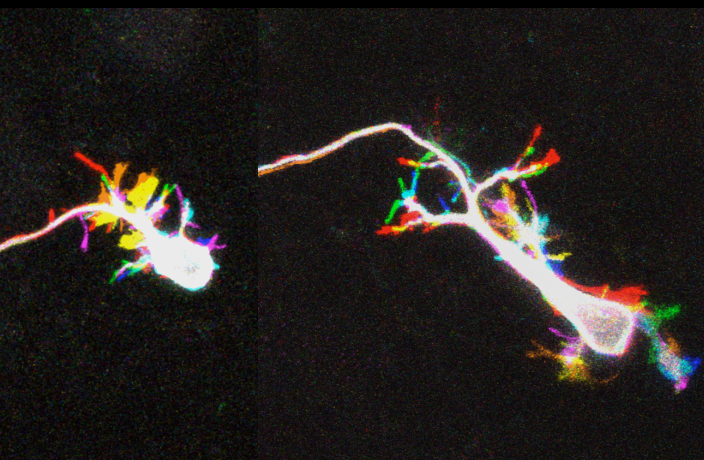
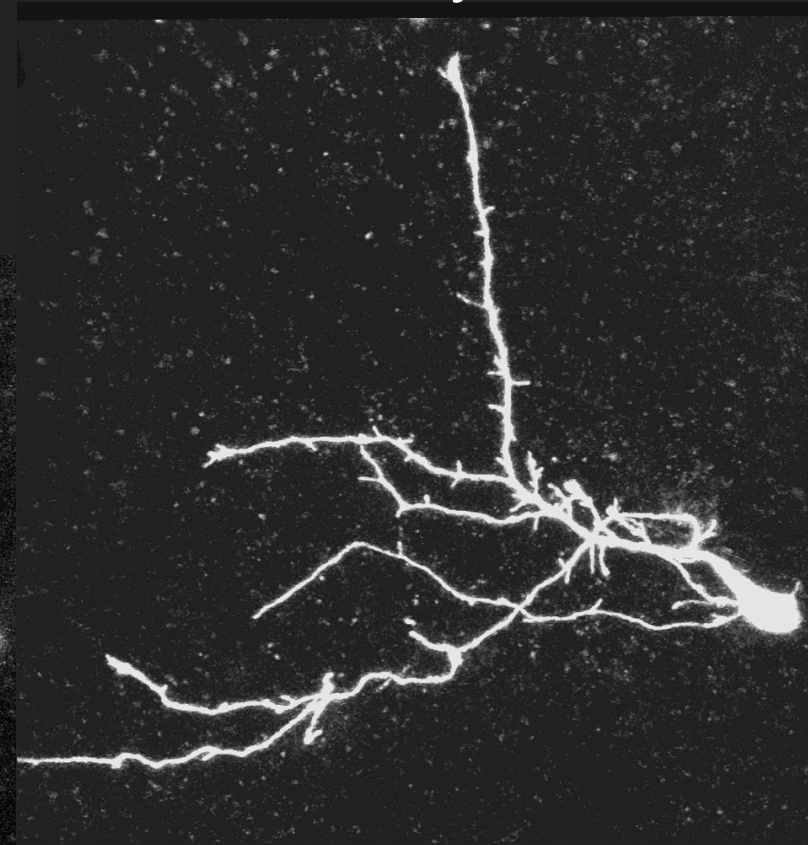
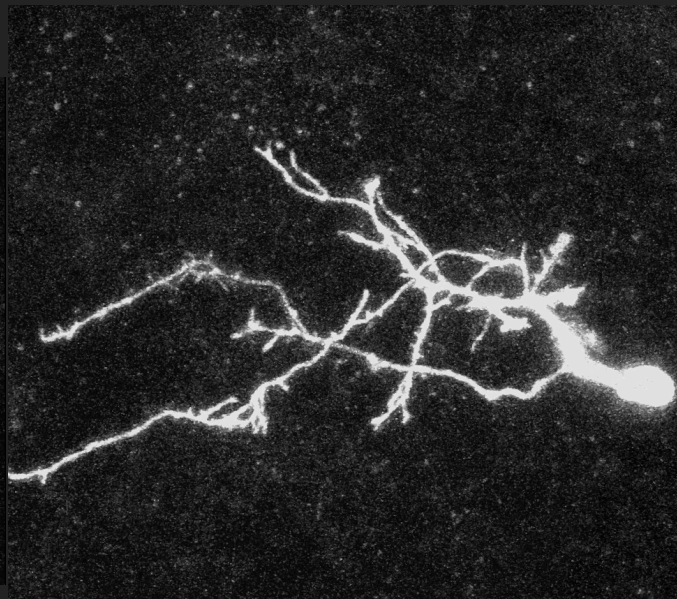
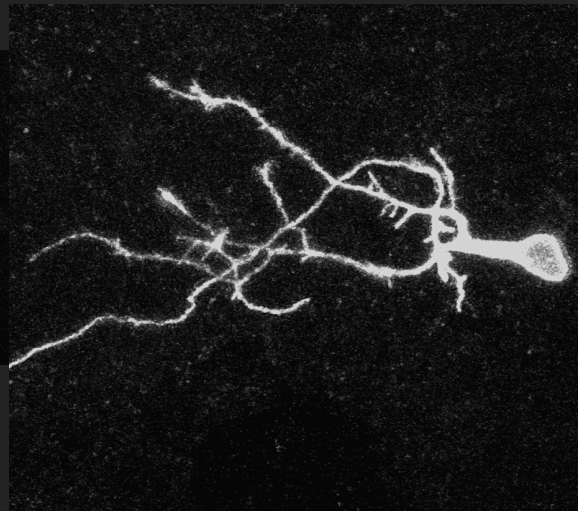
Day 1

Day 2

Day 3

Day 4

Day 5

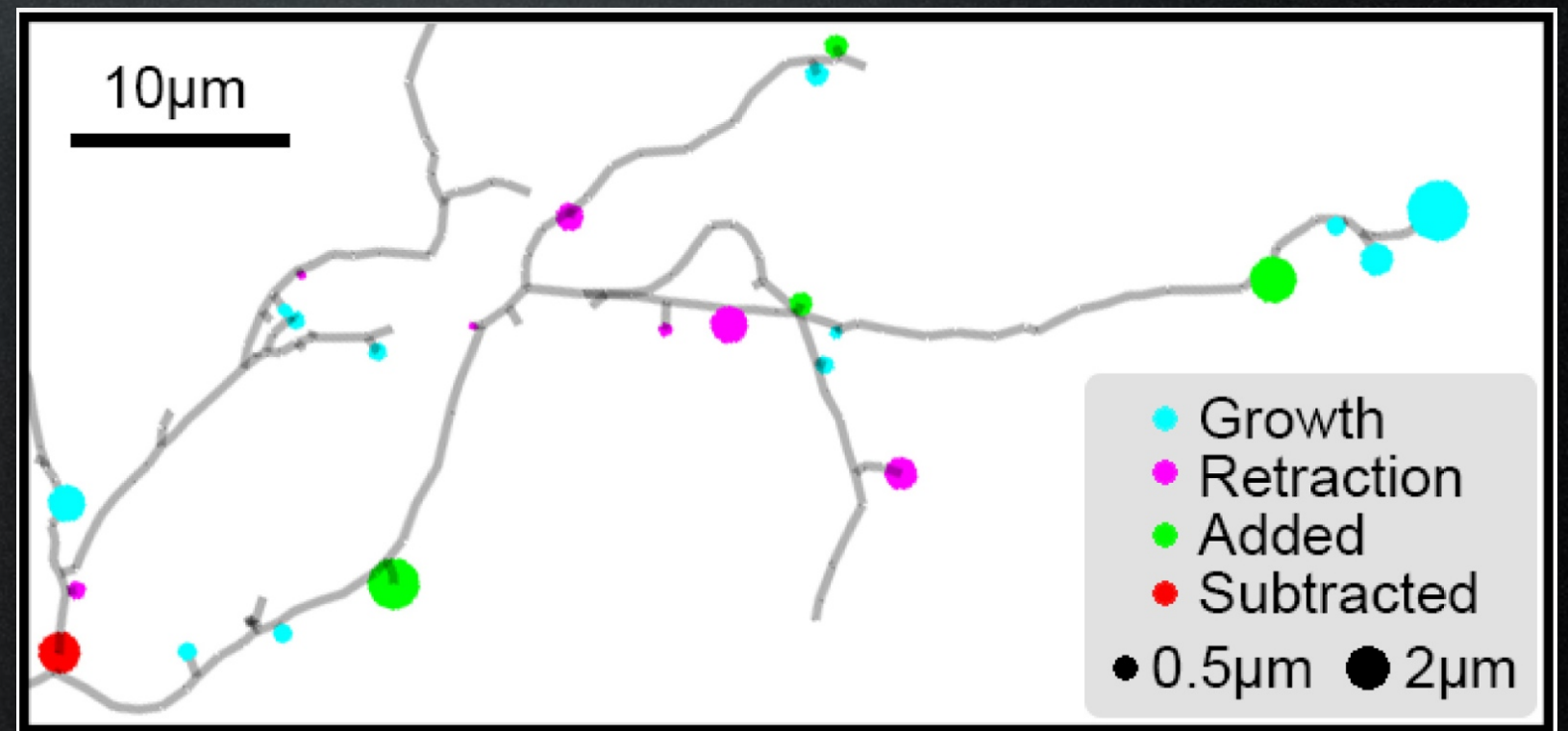
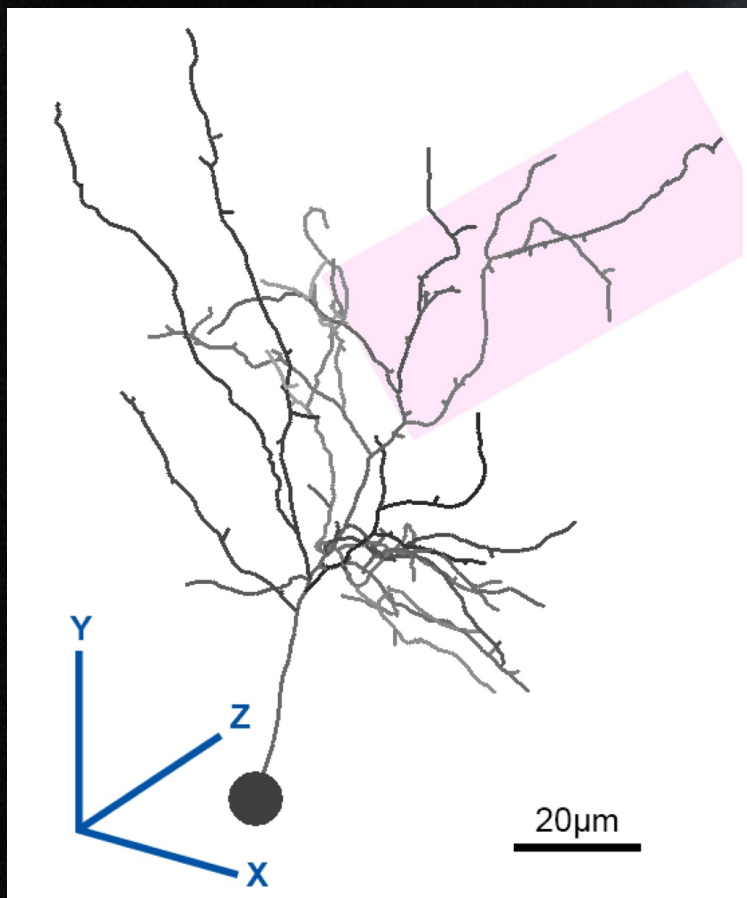
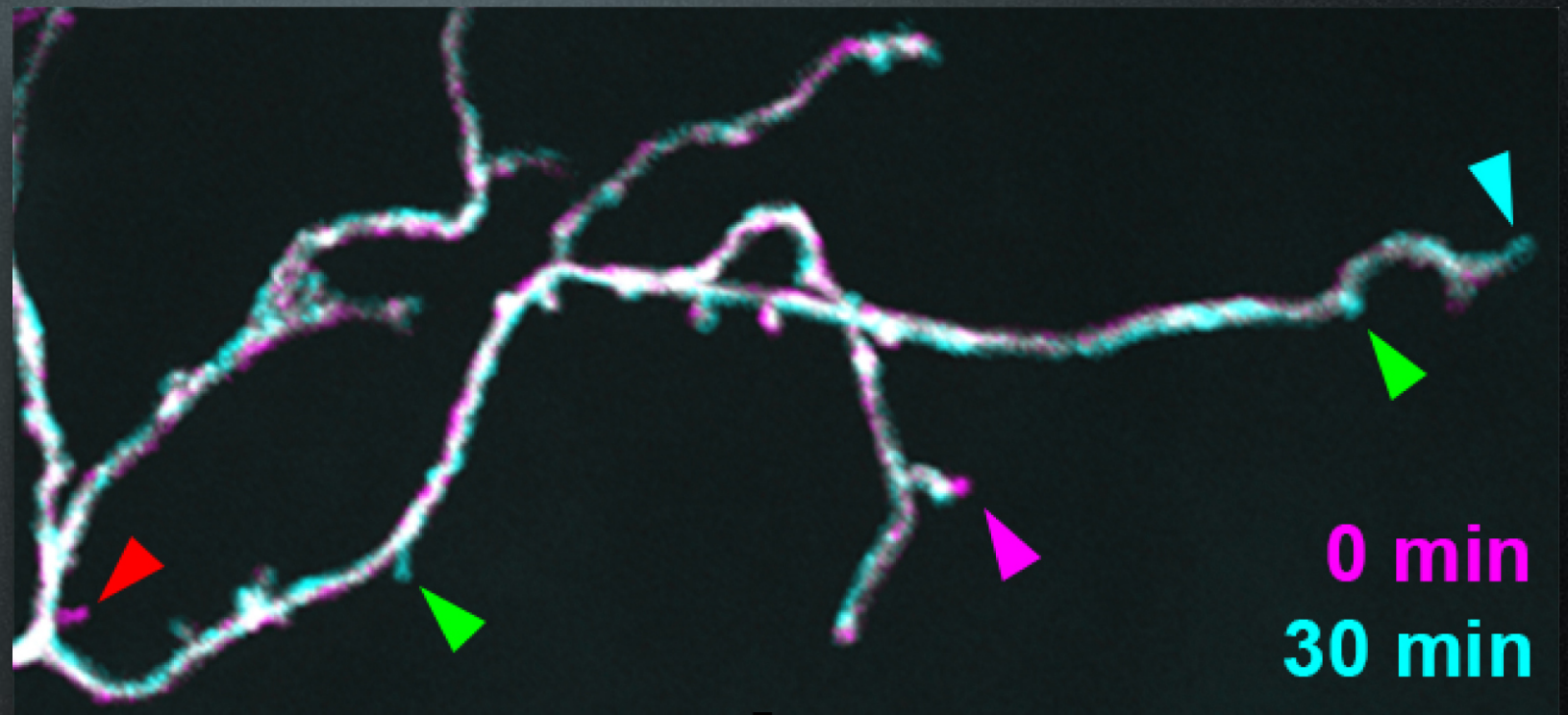


5min interval for 1h, 24h interval over 5 days

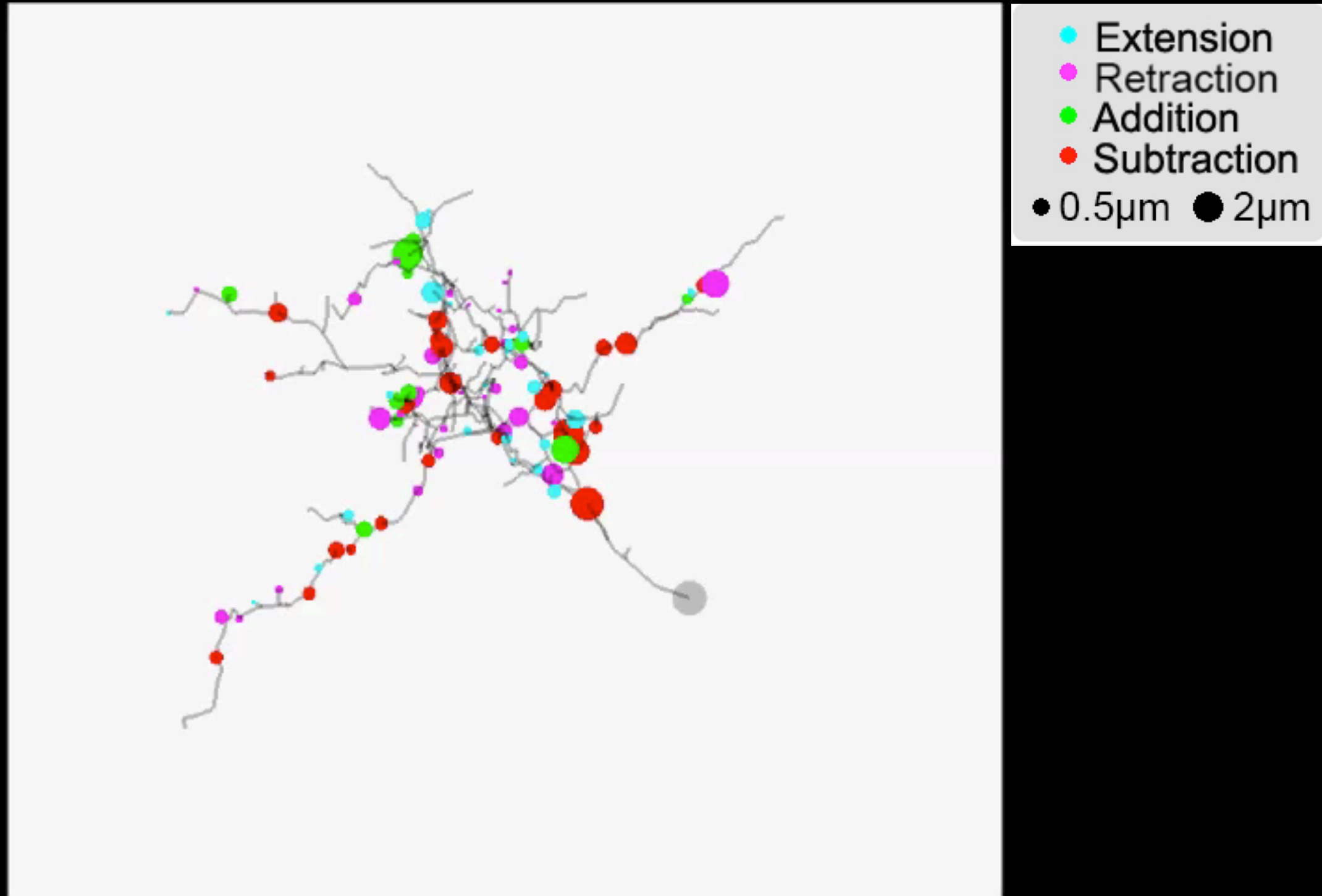
Dynamic Morphometrics



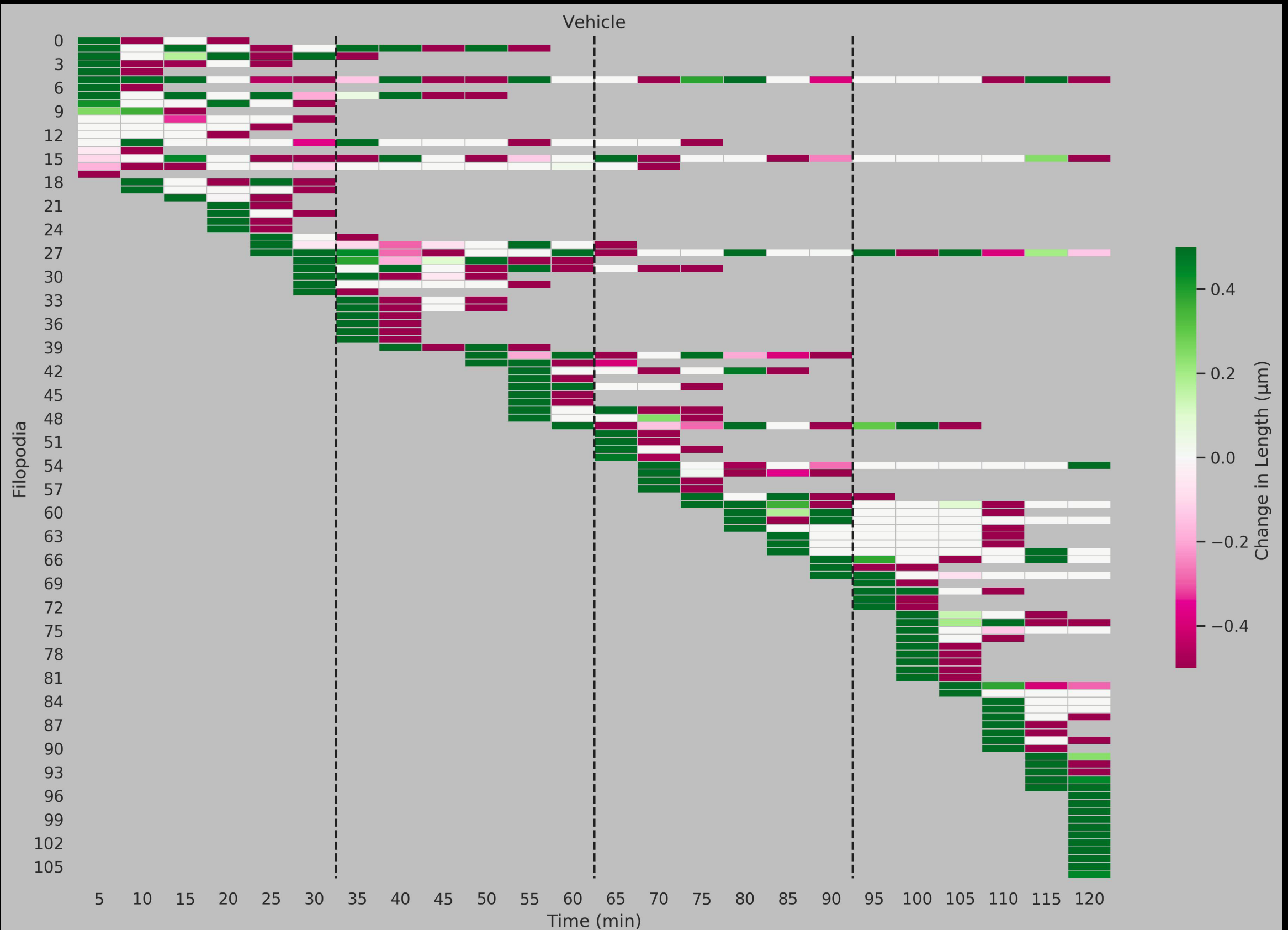
Dynamo Morphometric Software



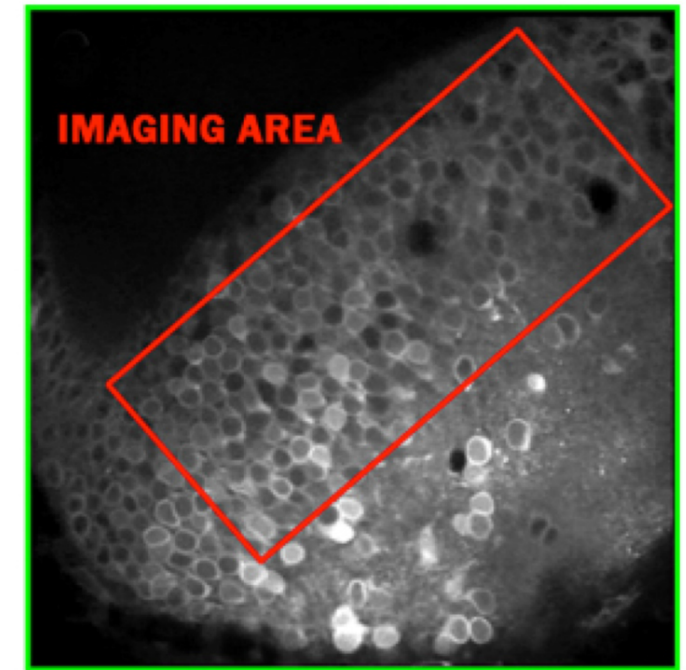
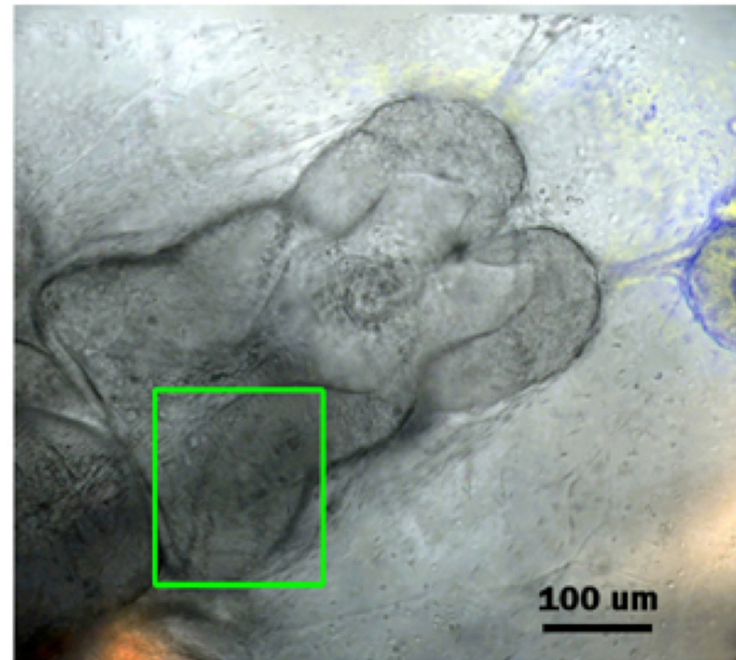
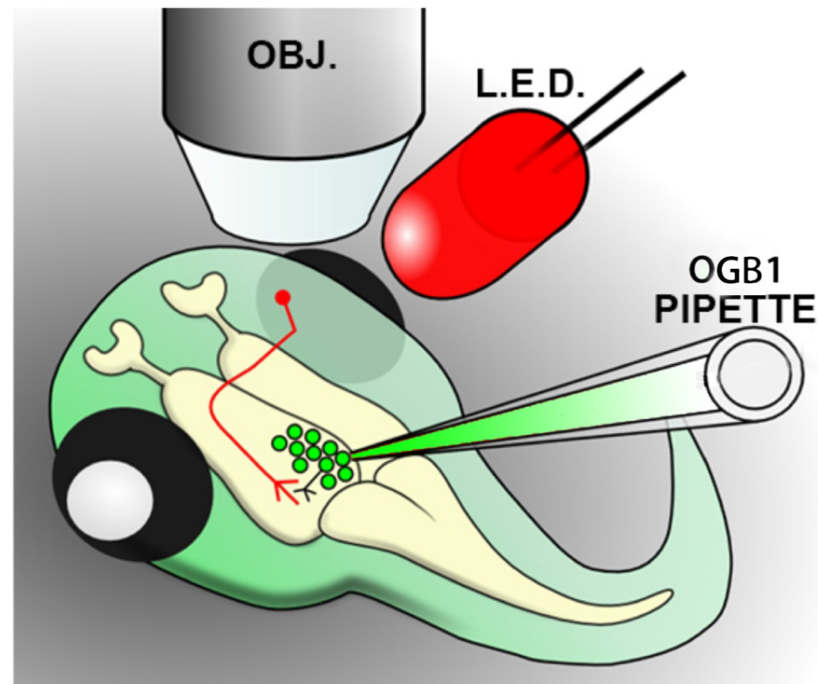
Dynamic Morphometrics: Dynamo Software



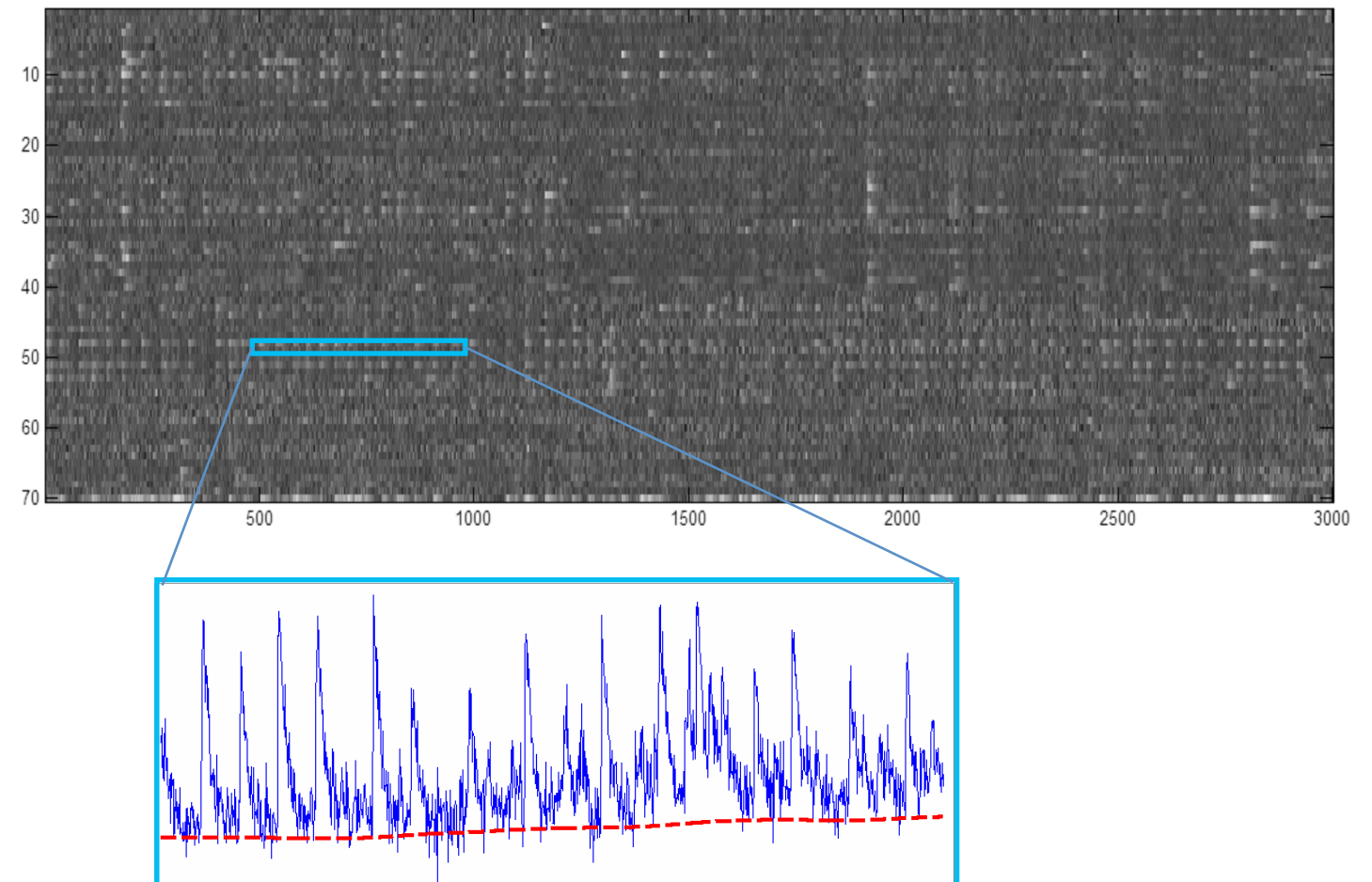
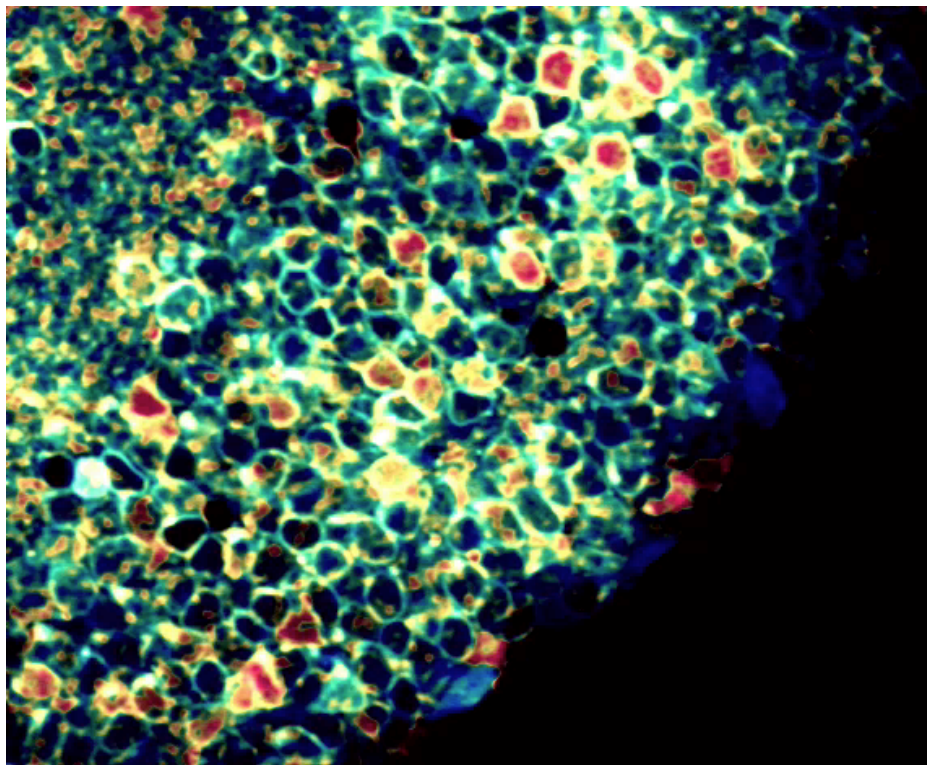
Dynamic Morphometrics: Dynamo Software



Visual-evoked calcium imaging



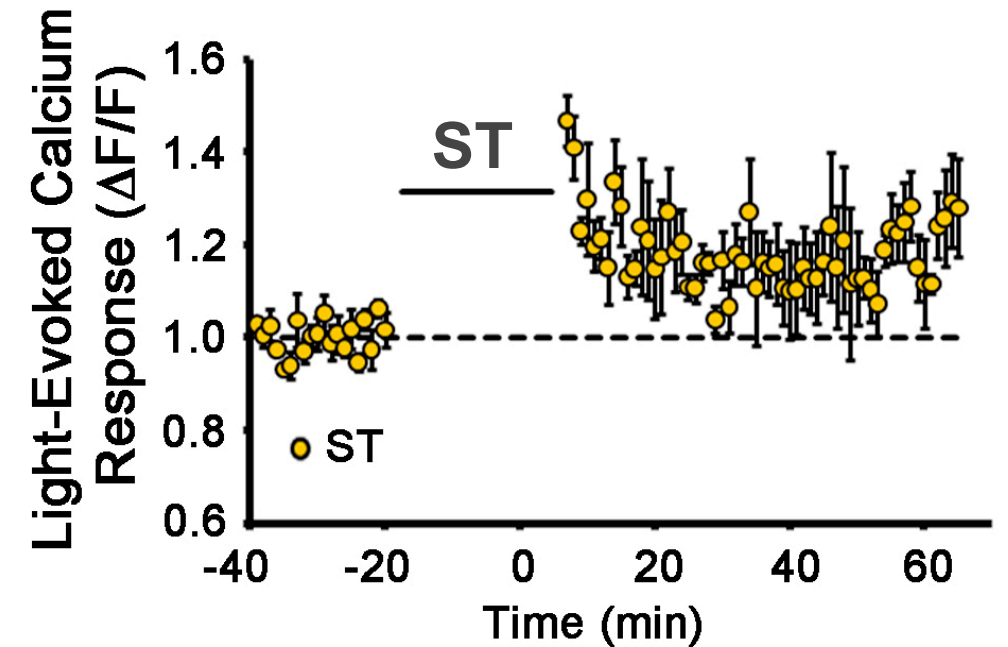
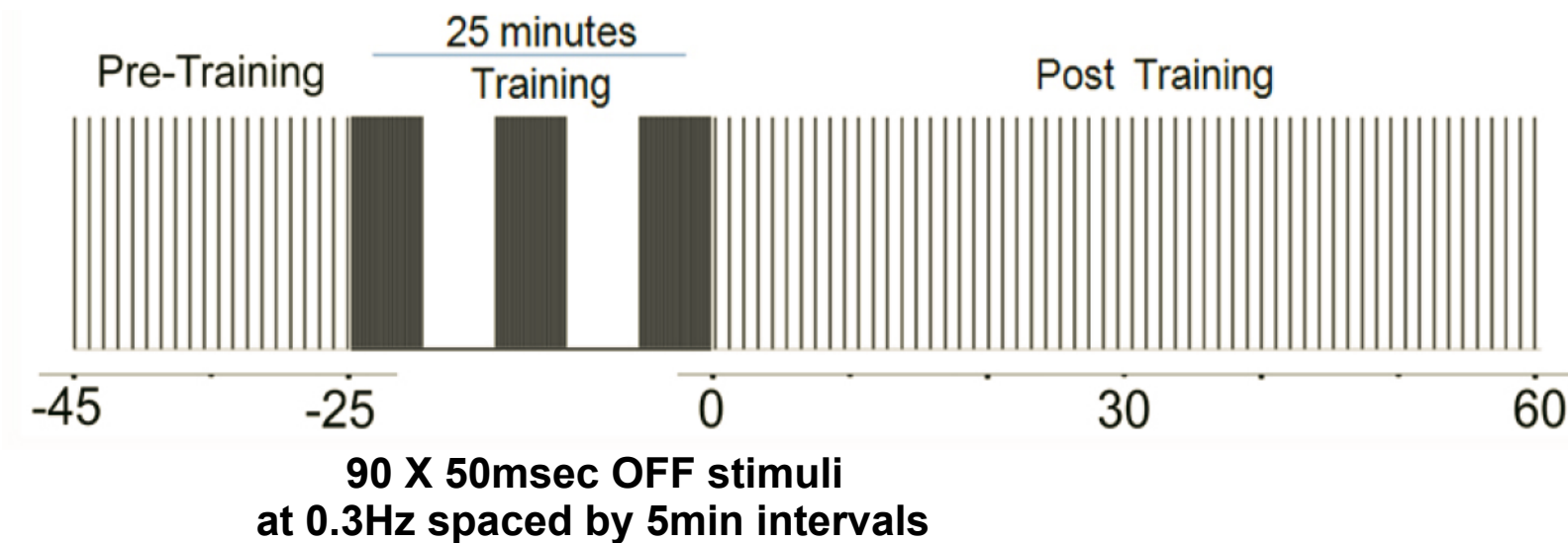
50 msec Wide-field OFF



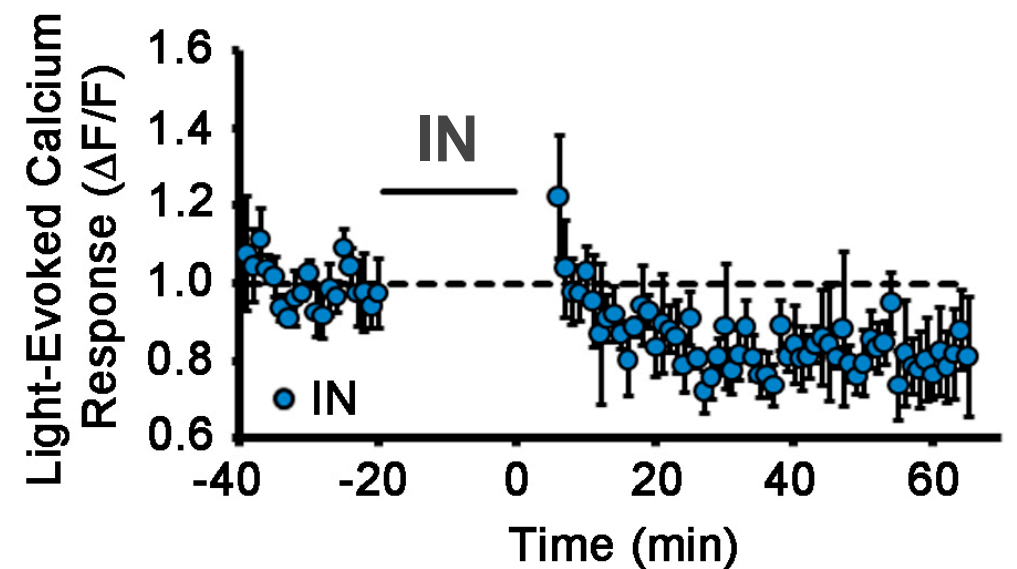
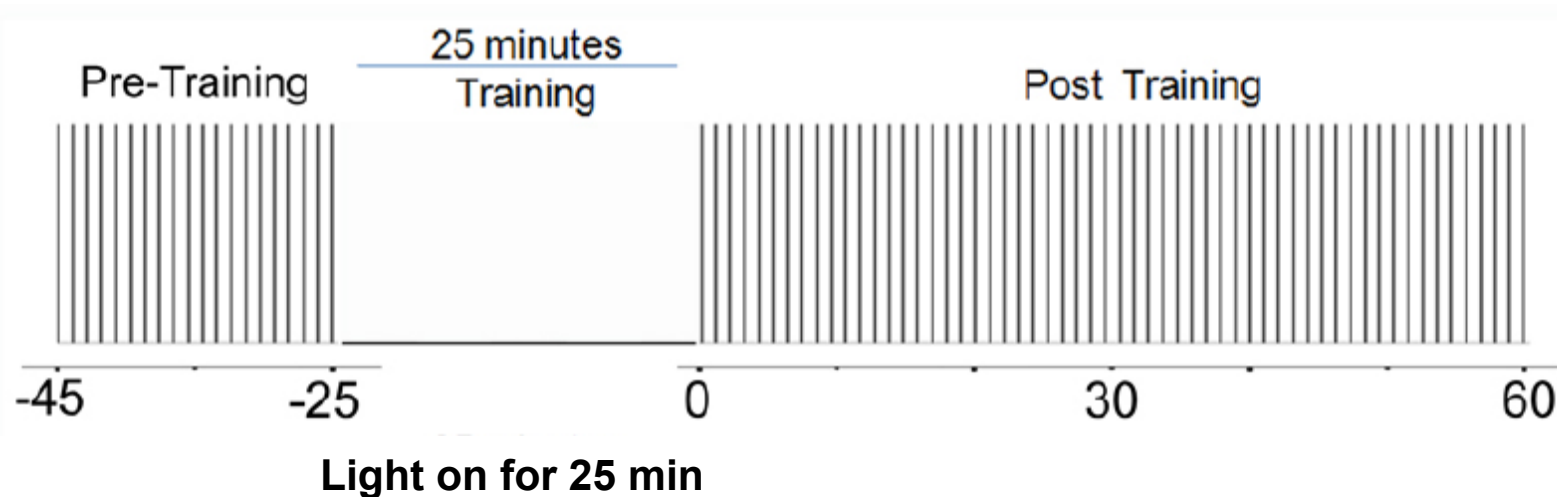
(Dunfield and Haas, *Neuron* 2009; *Nature Protocols* 2010)

Sensory-Driven Functional Plasticity

Spaced Training (ST)



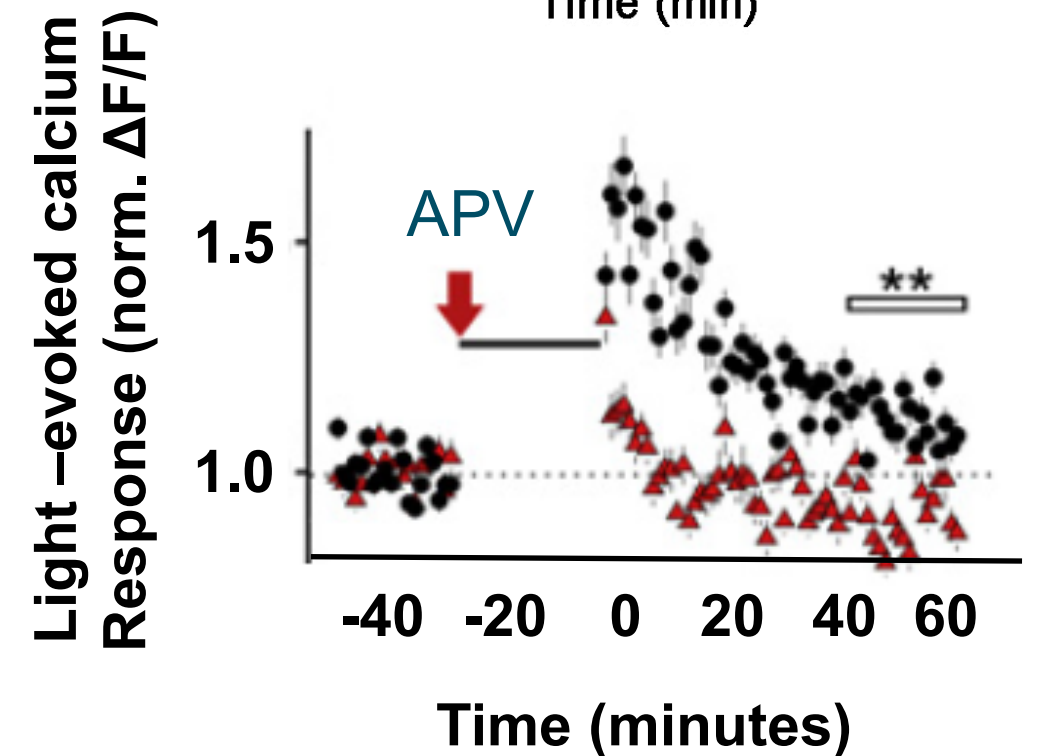
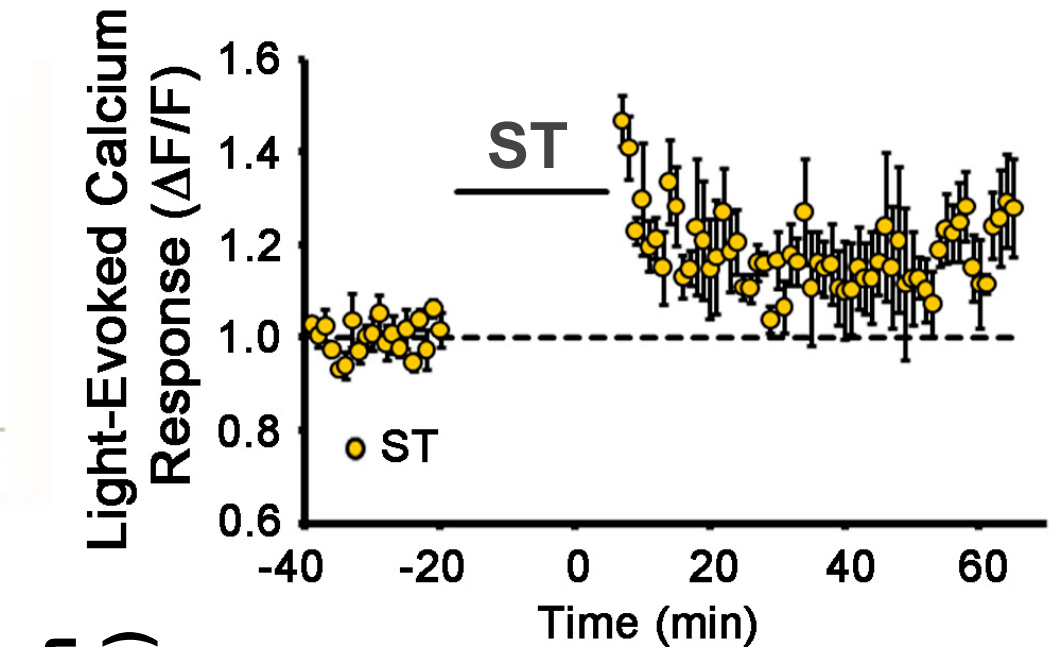
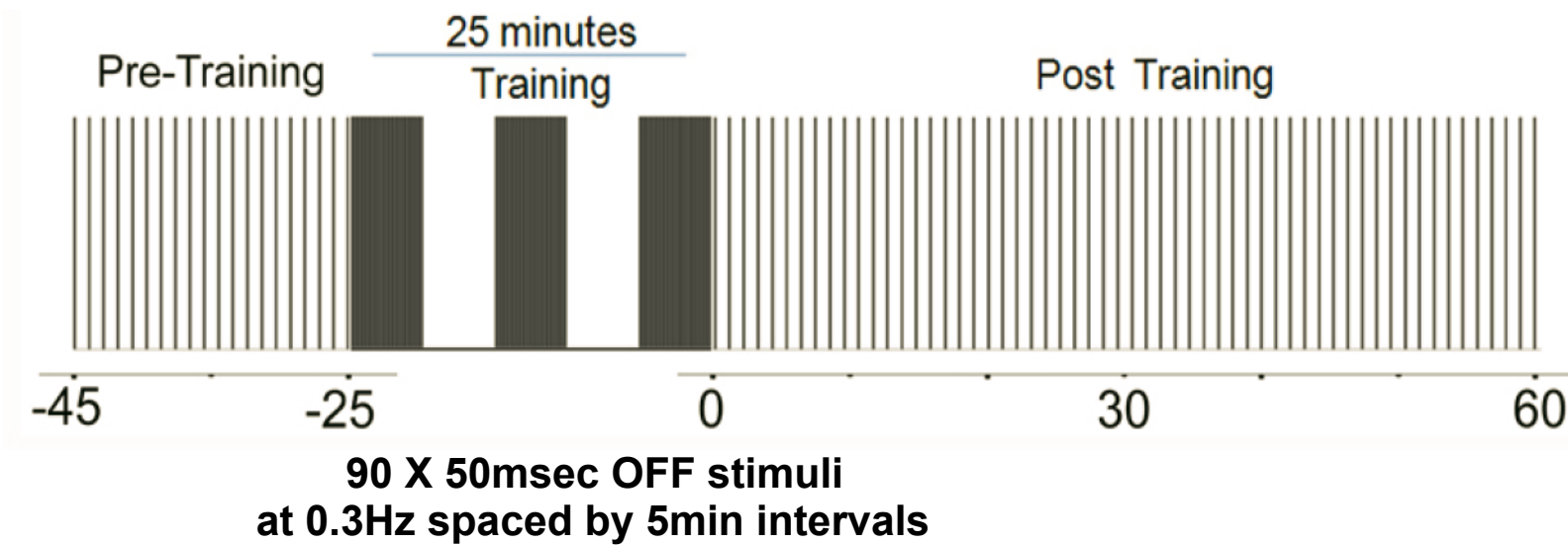
Invariant Training (IN)



(Dunfield 2009 Neuron 64:240-50)

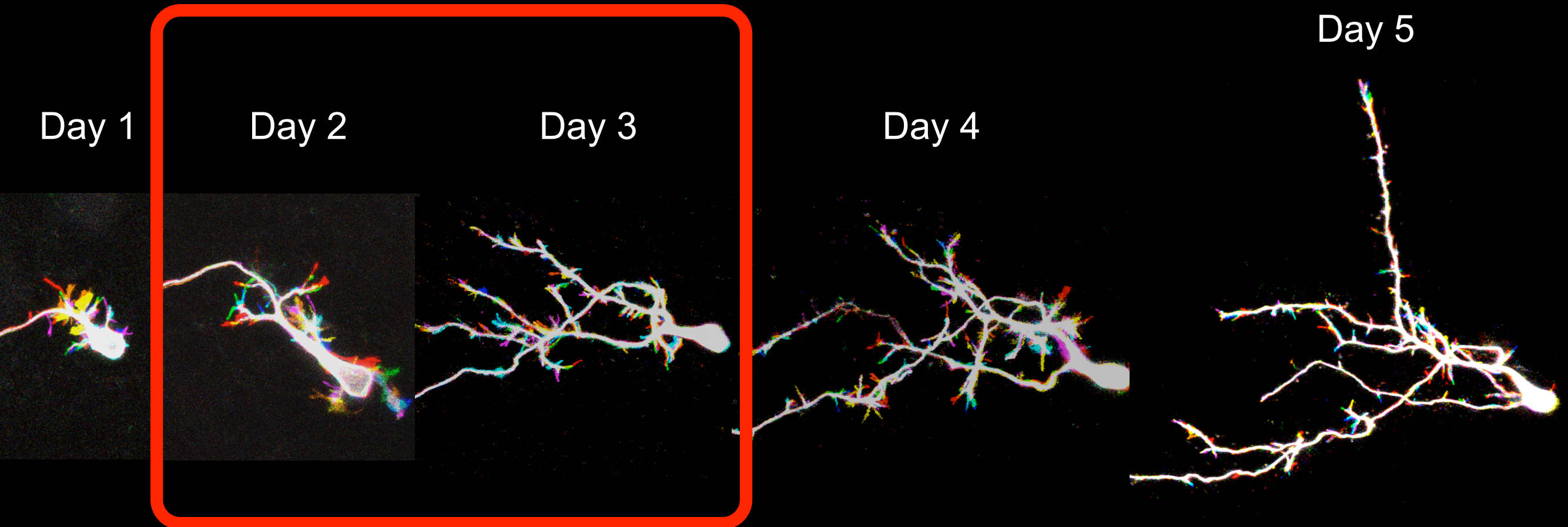
Sensory-Driven Functional Plasticity

Spaced Training (ST)



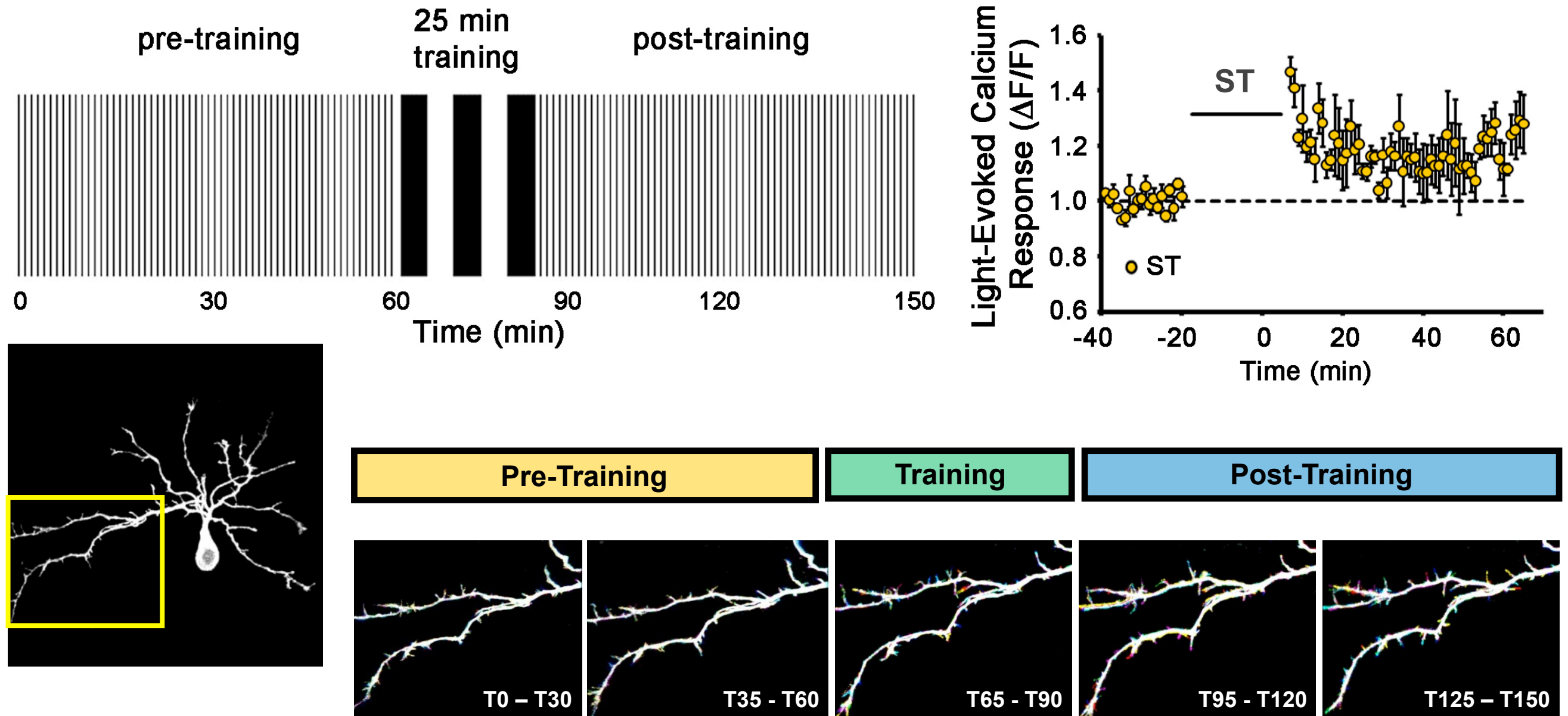
(Dunfield 2009 *Neuron* 64:240-50)

What is the role of sensory-driven activity in early dendritogenesis?

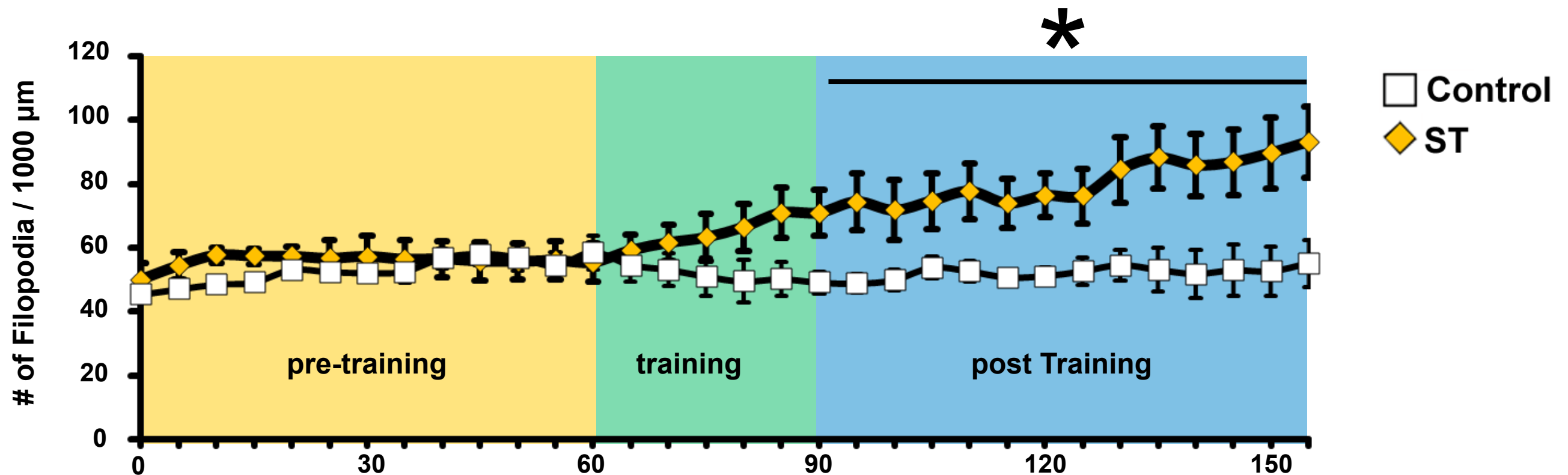
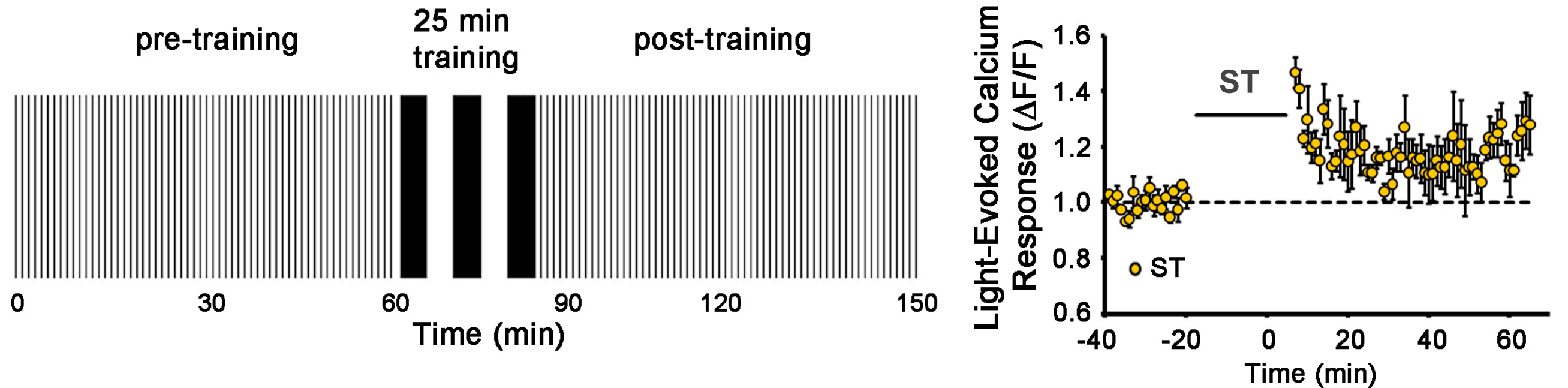


Simon Chen, University of Ottawa
(Chen et al., *Neuron*, 2010; *Cell*, 2012)

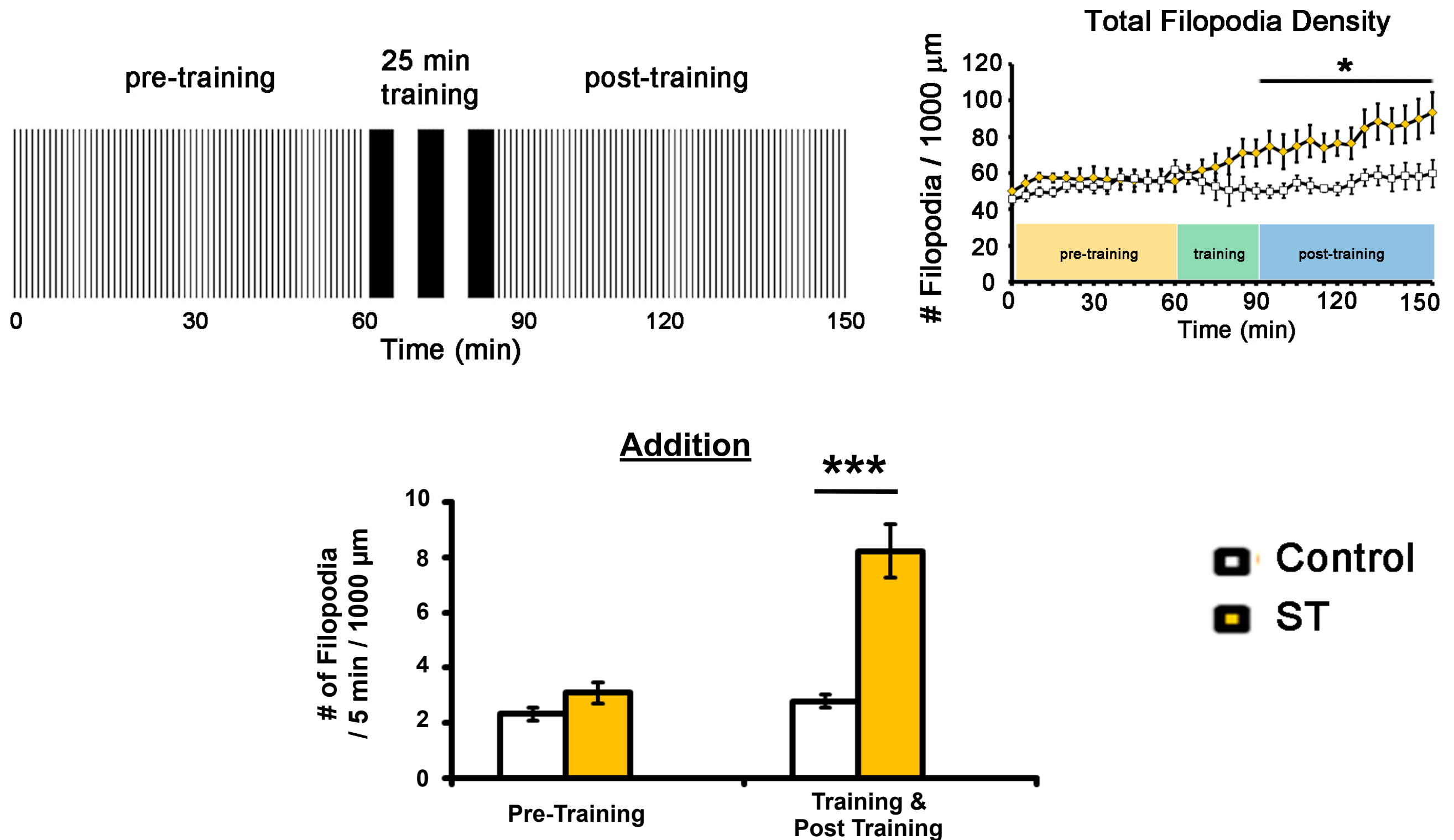
Dynamic morphometrics before, during and after ST



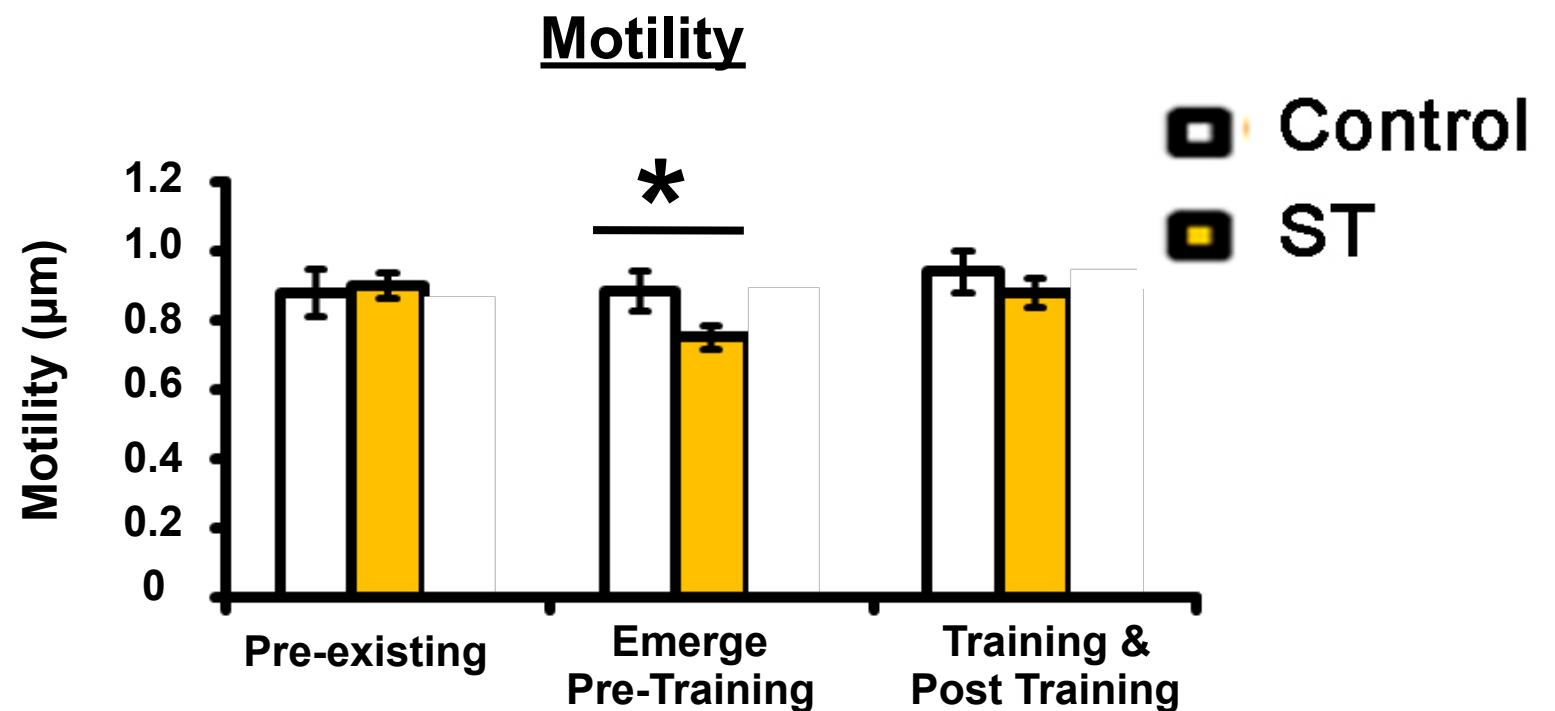
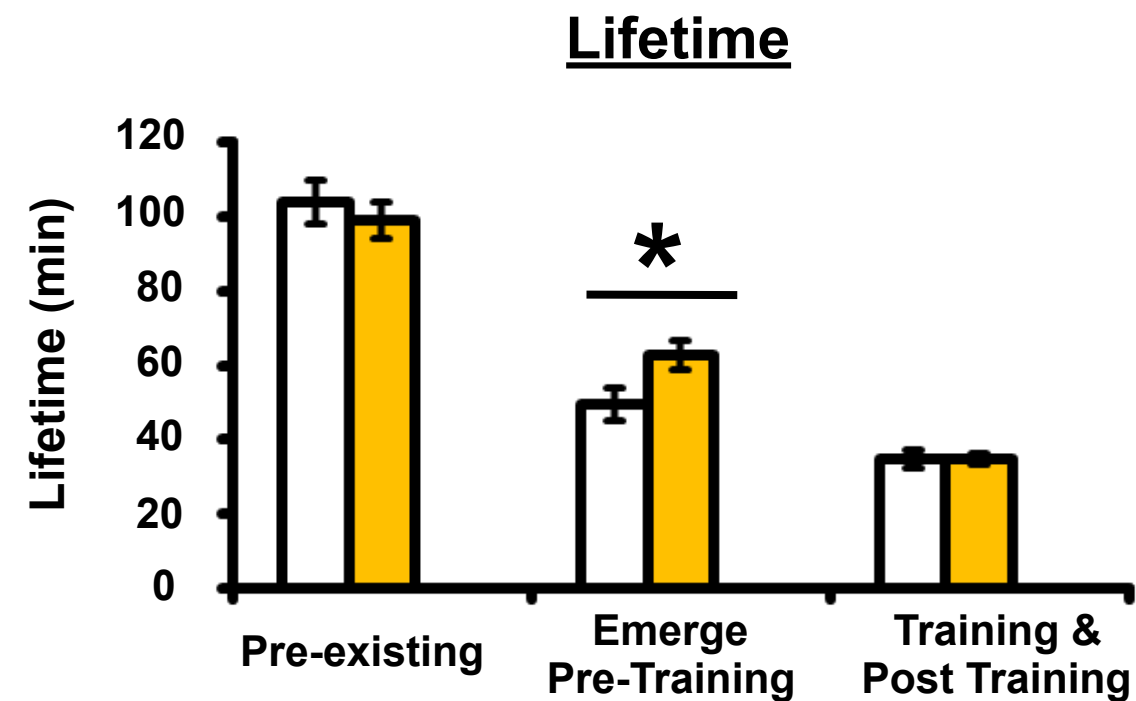
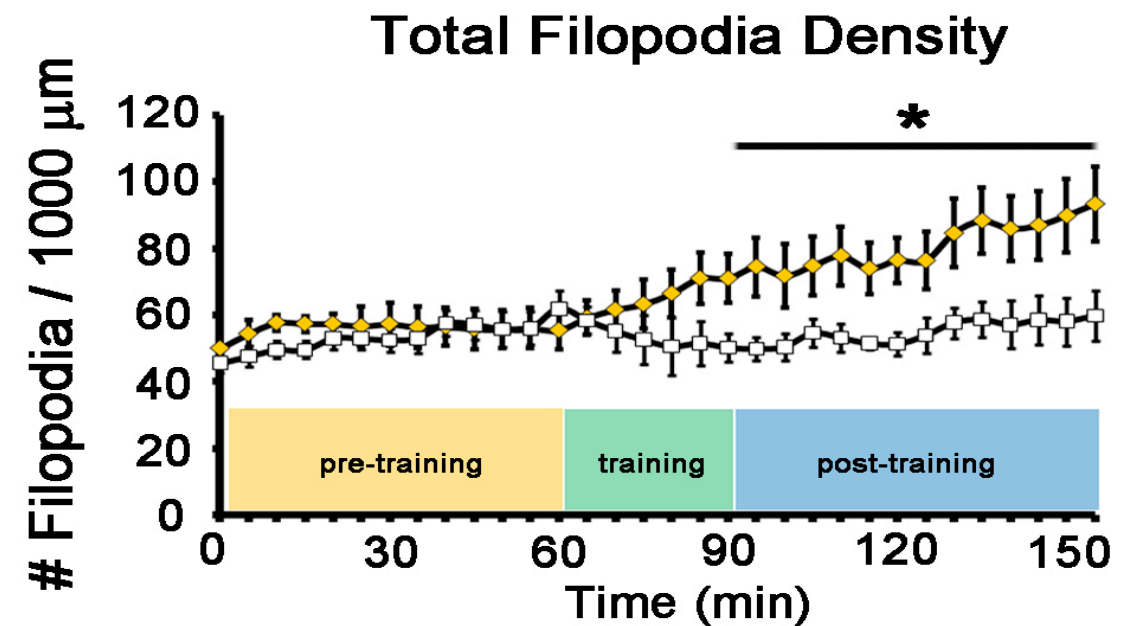
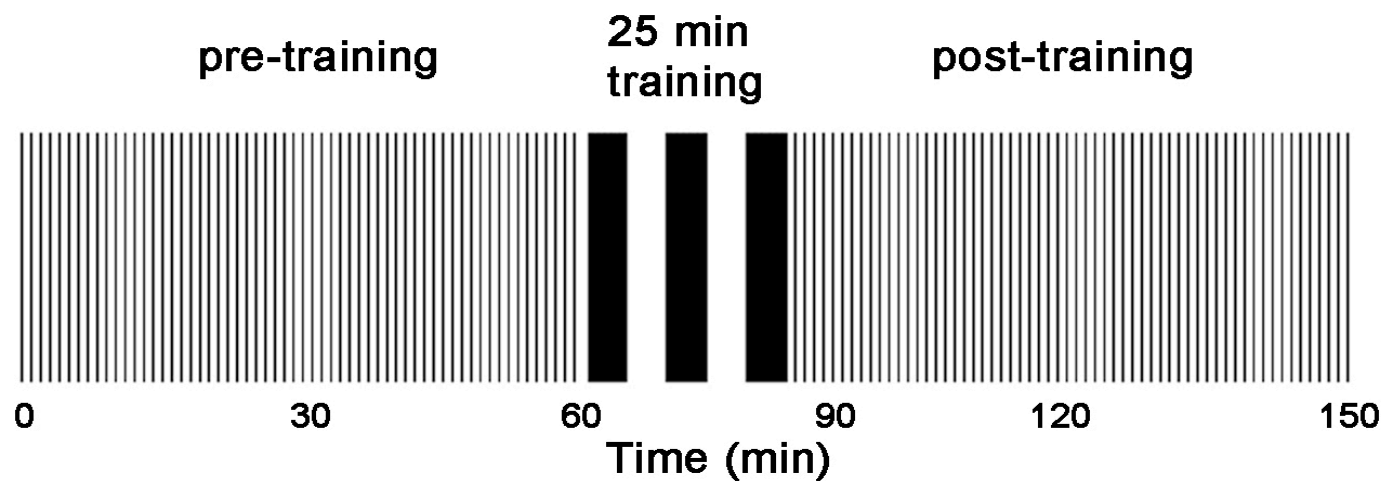
Spaced Training potentiation increases filopodial density



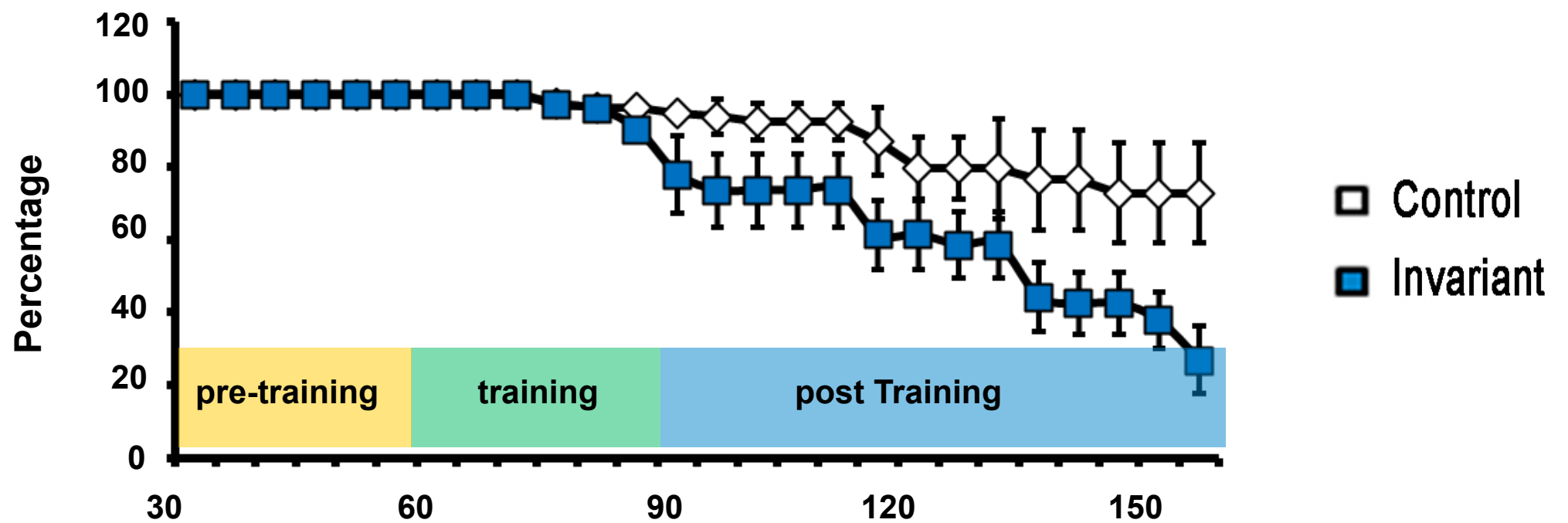
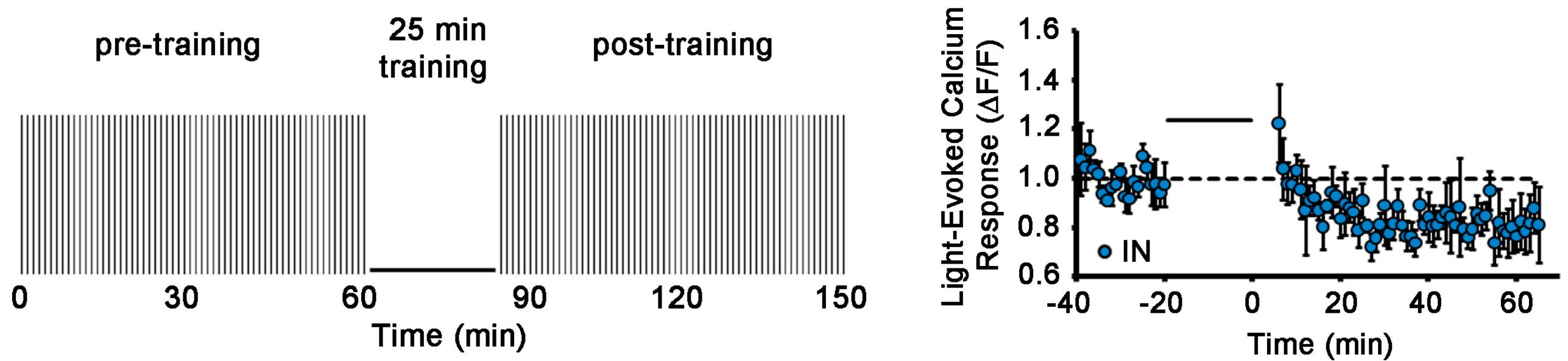
ST potentiation promotes filopodial sprouting



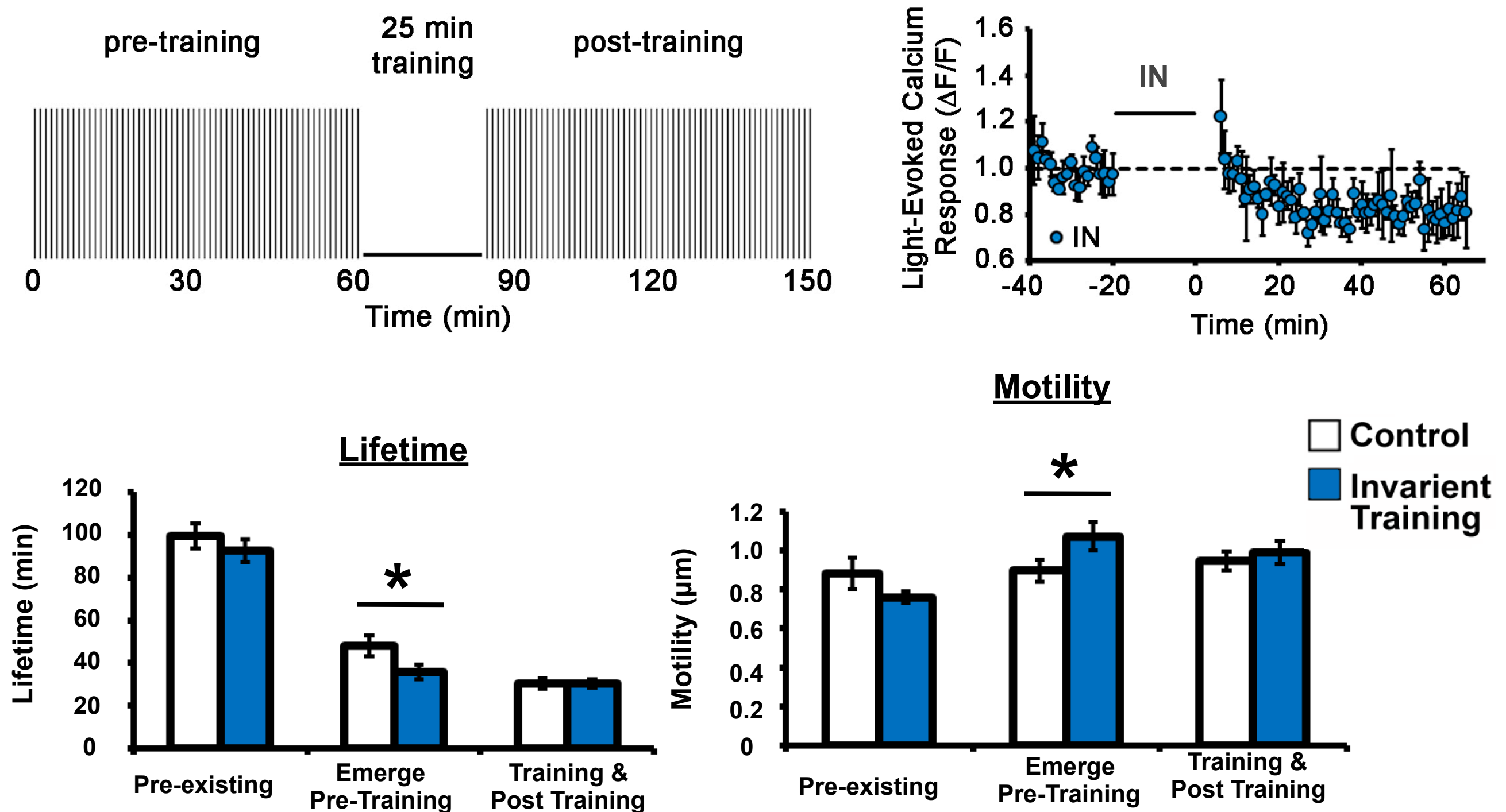
ST potentiation stabilizes nascent filopodia



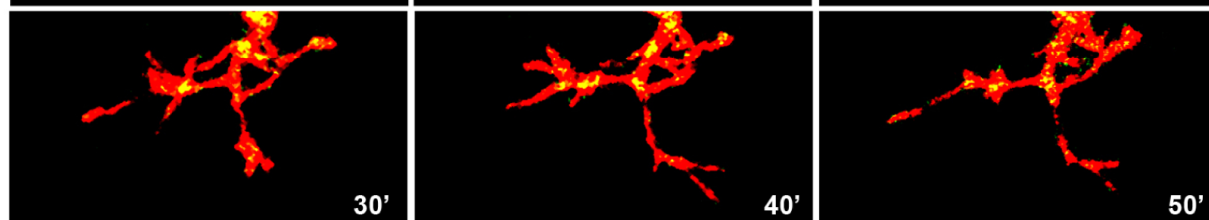
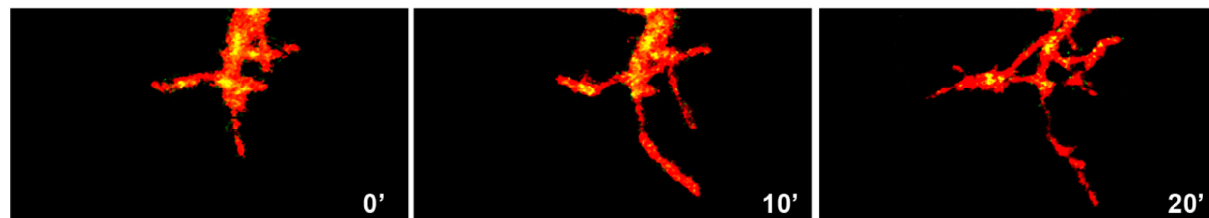
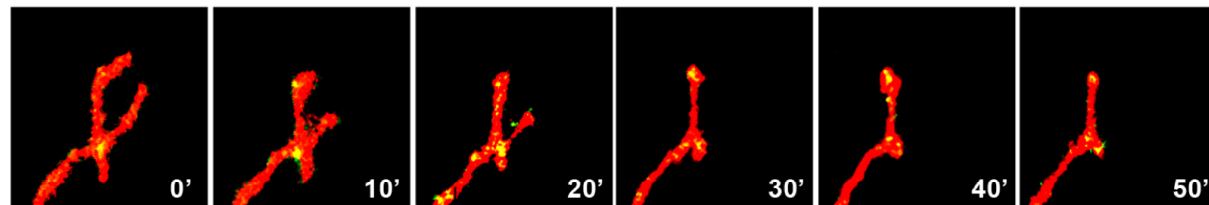
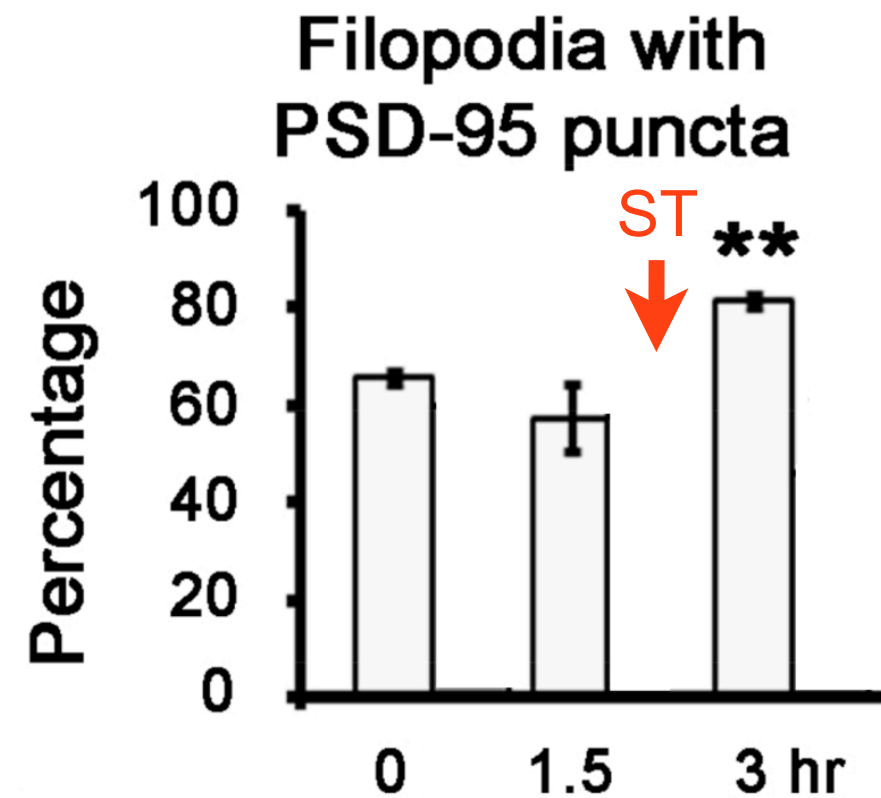
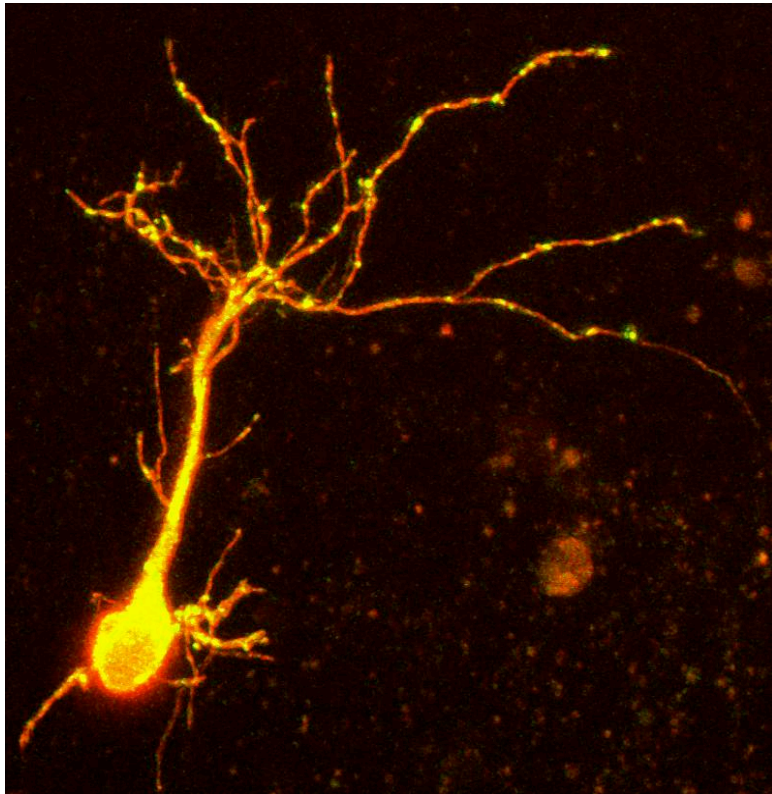
IN destabilizes pre-existing stable filopodia



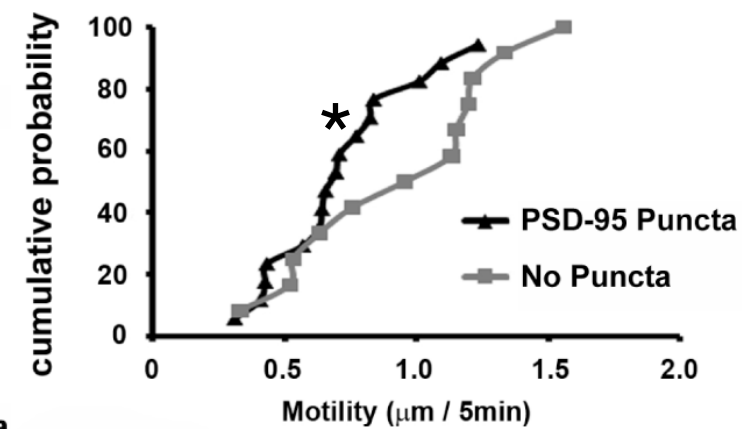
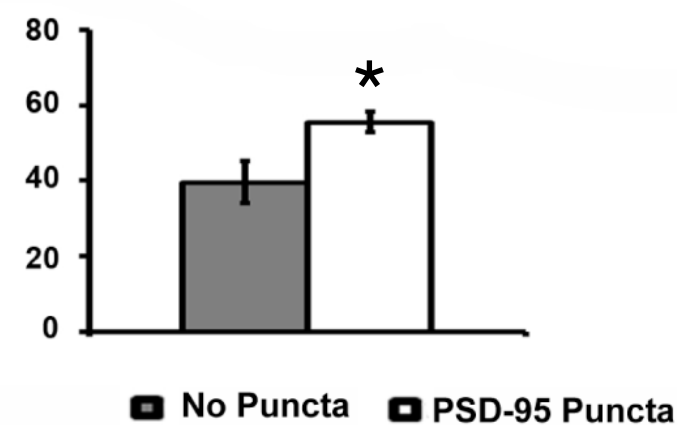
IN depression destabilizes nascent filopodial



Synapse formation stabilizes filopodia

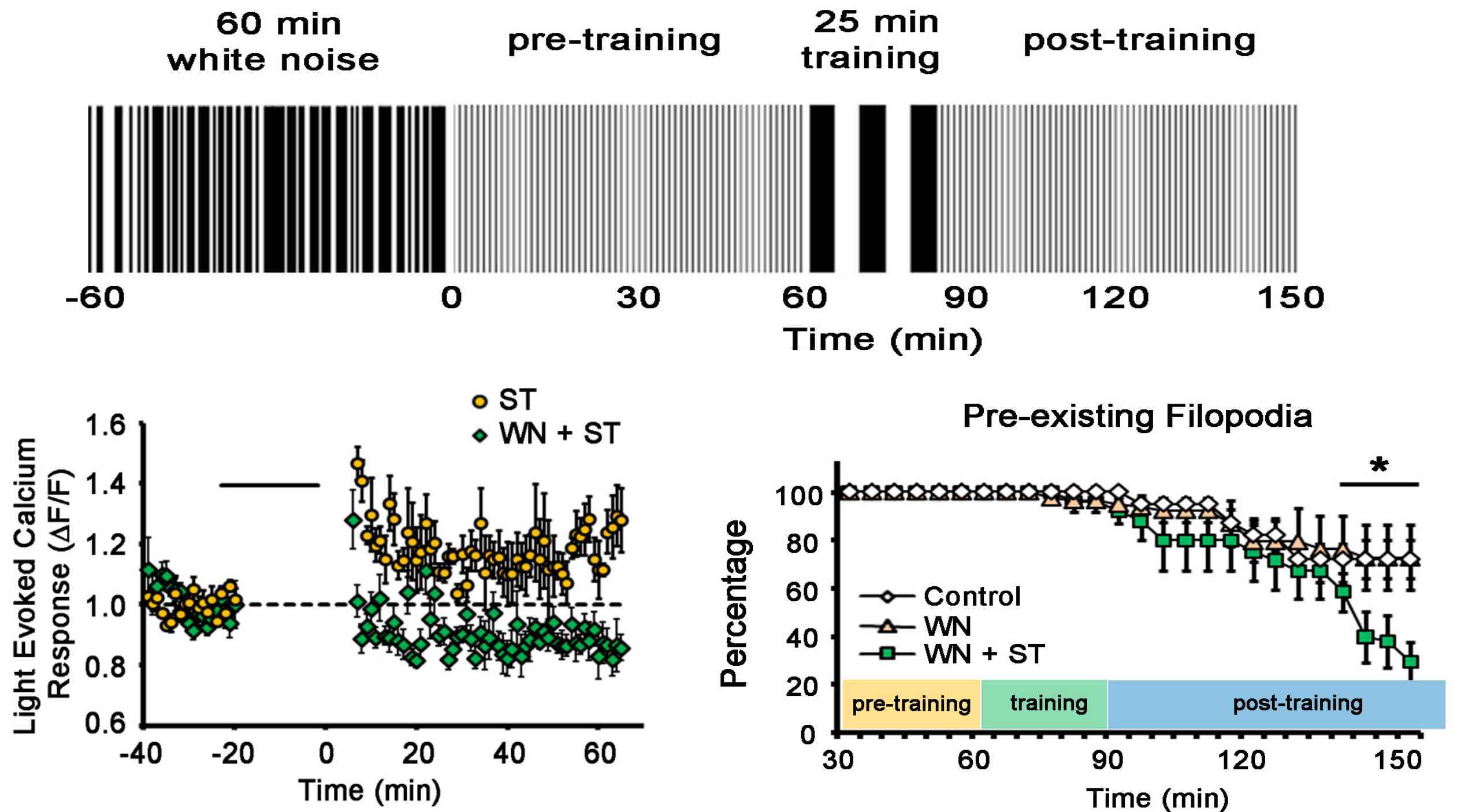


Lifetime (min)



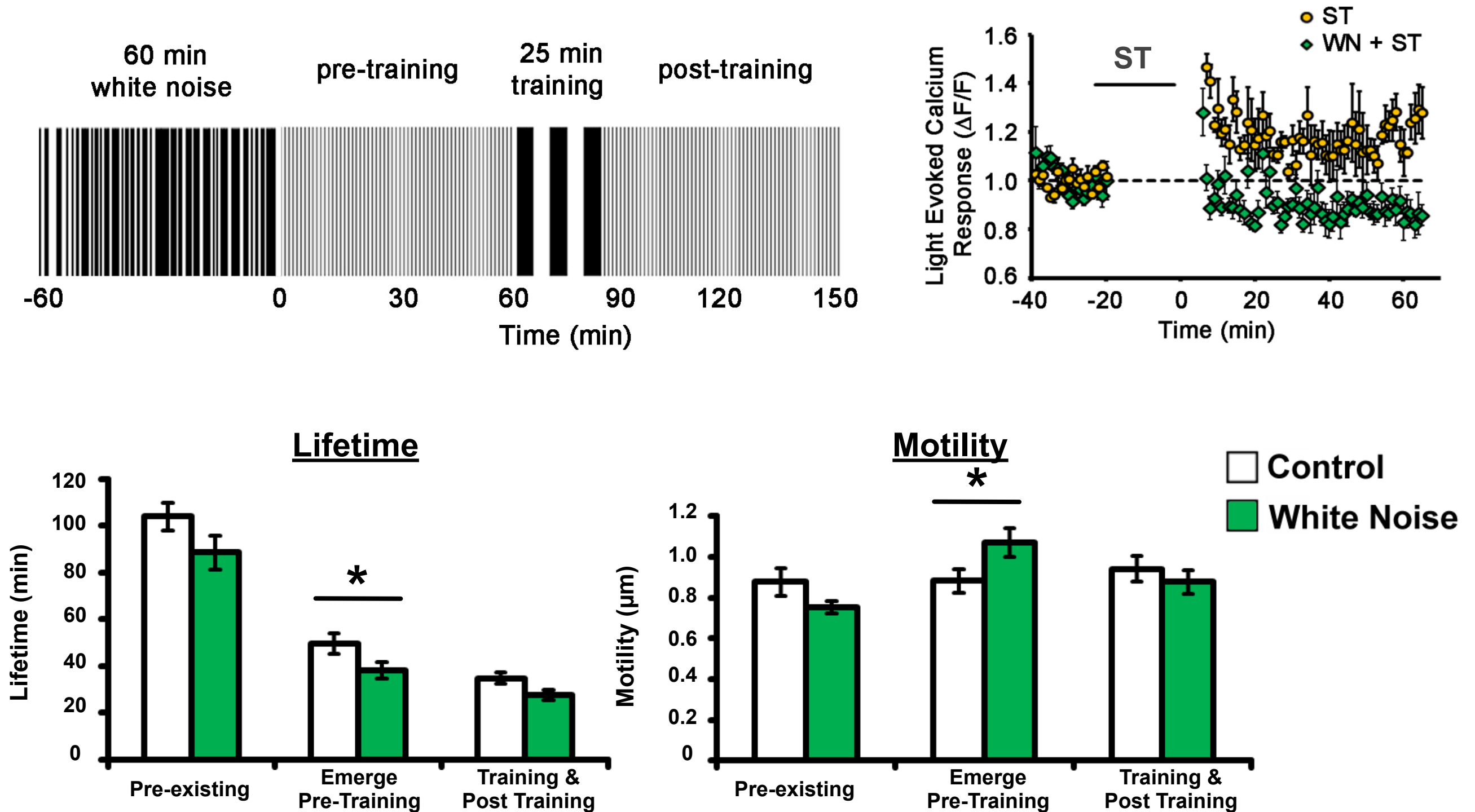
Chen, *Neuron* (2010) 67, 967-983
Niell, *Nat Neuroscience* (2004) 7, 254-260

White noise priming of ST shifts plasticity thresholds

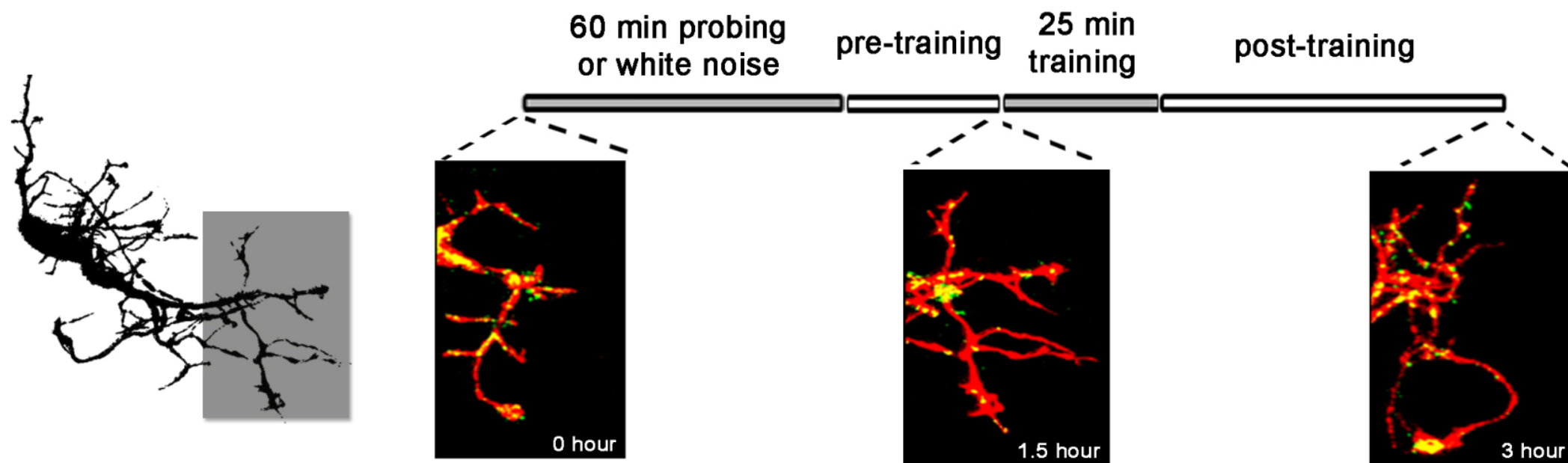


(Chen et al., Cell 151 (2012) 41-55)

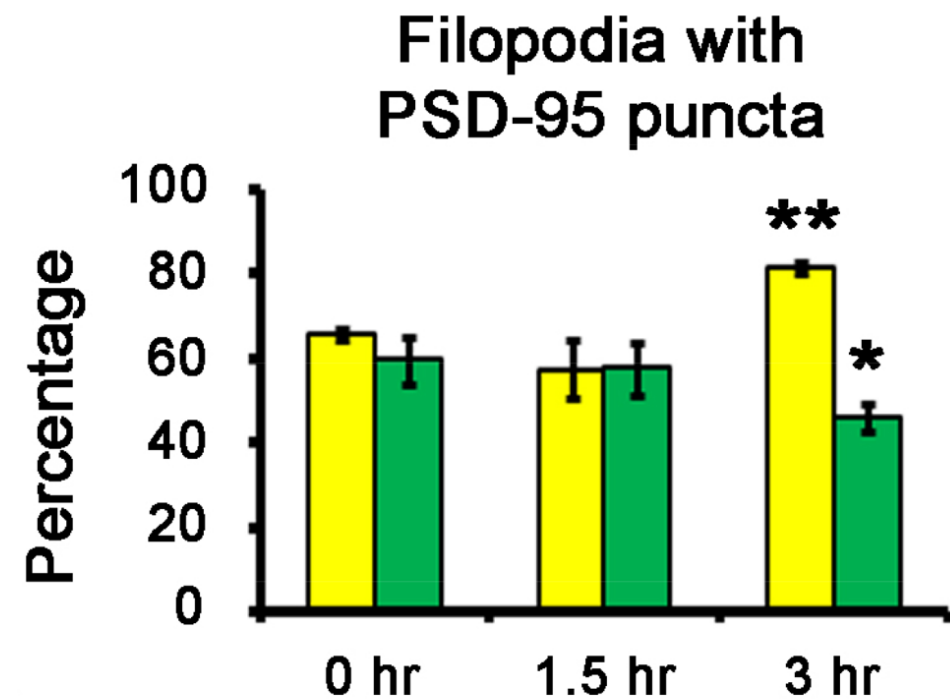
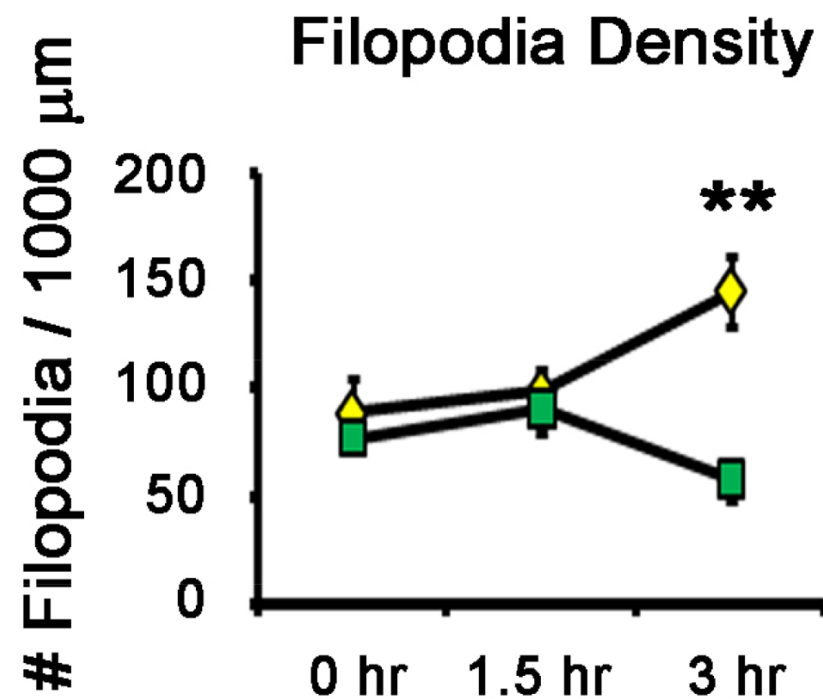
White noise priming shifts ST to destabilization



Morphological and functional plasticity associated with synaptic changes

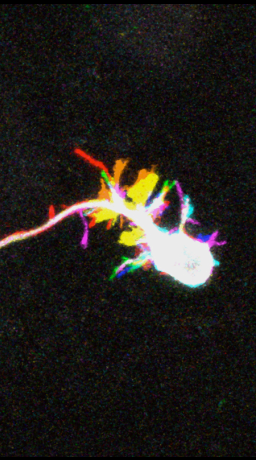


■ ST
■ WN+ST

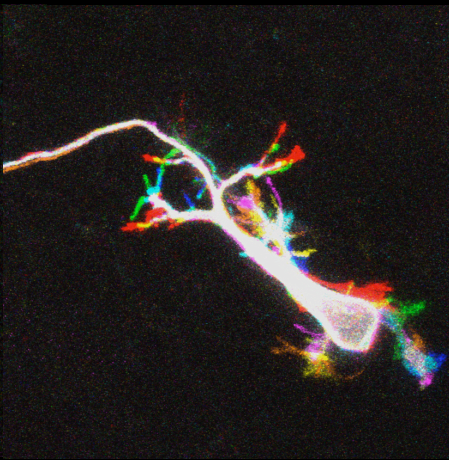


What is the role of sensory-driven activity in arbor refinement?

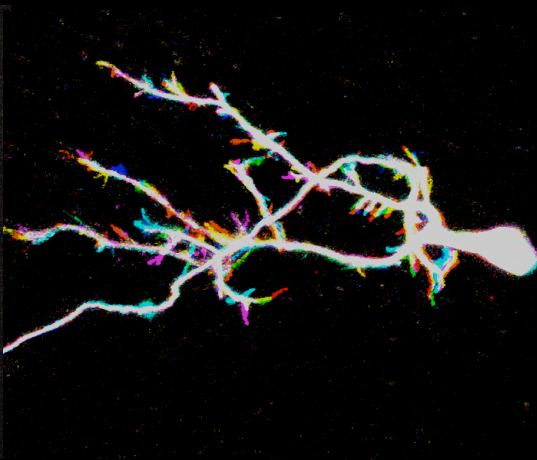
Day 1



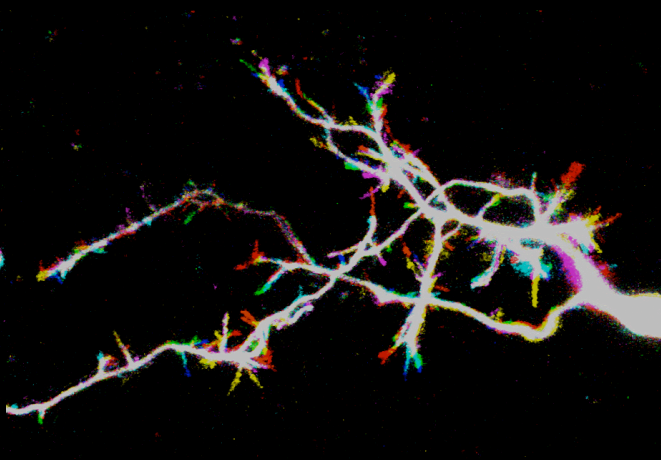
Day 2



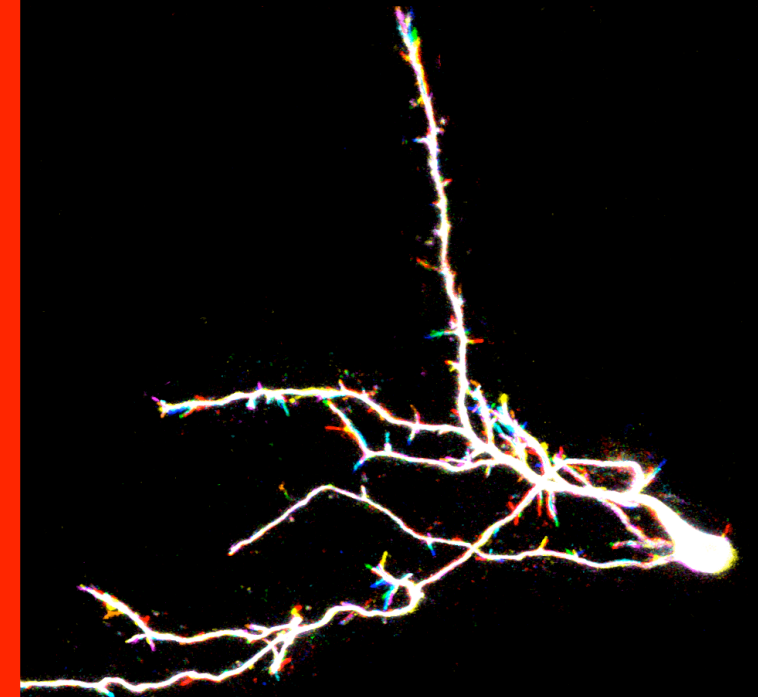
Day 3



Day 4

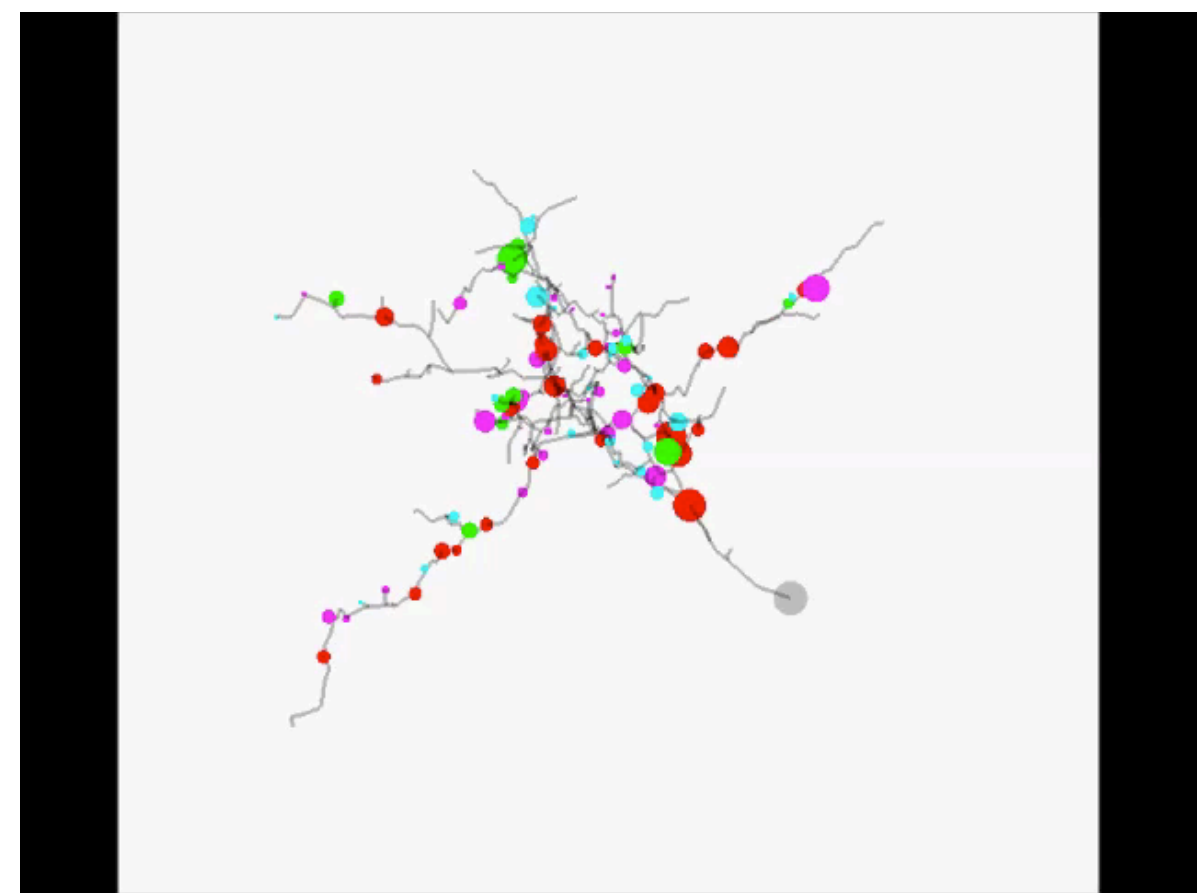
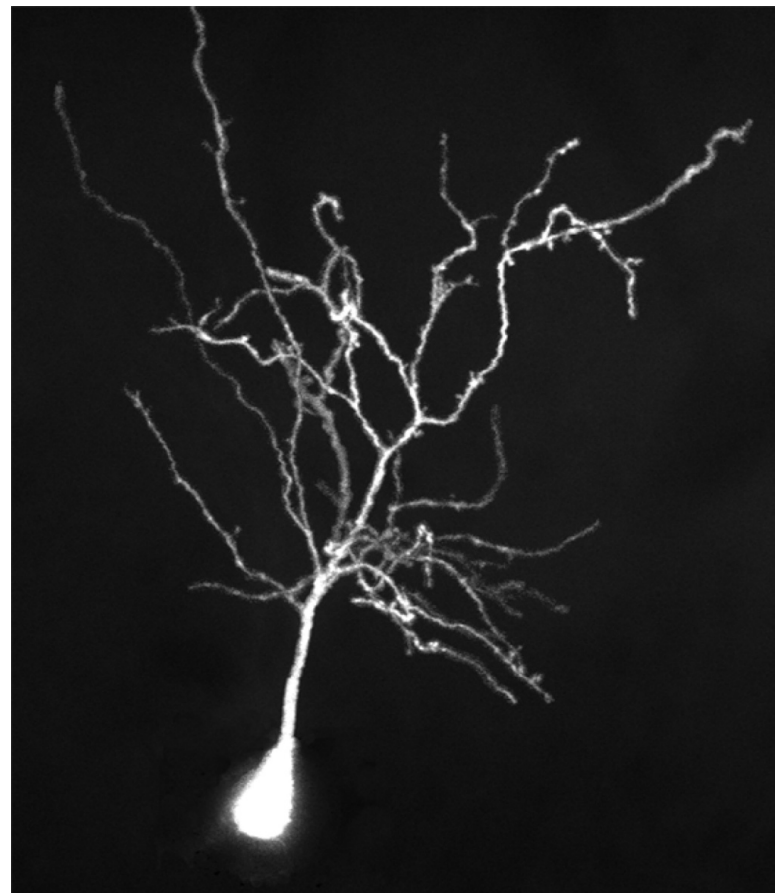
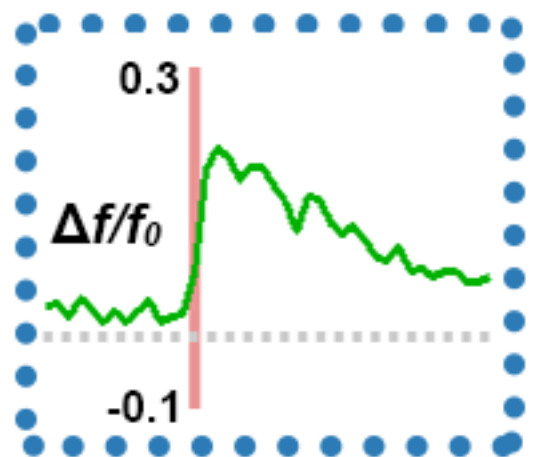
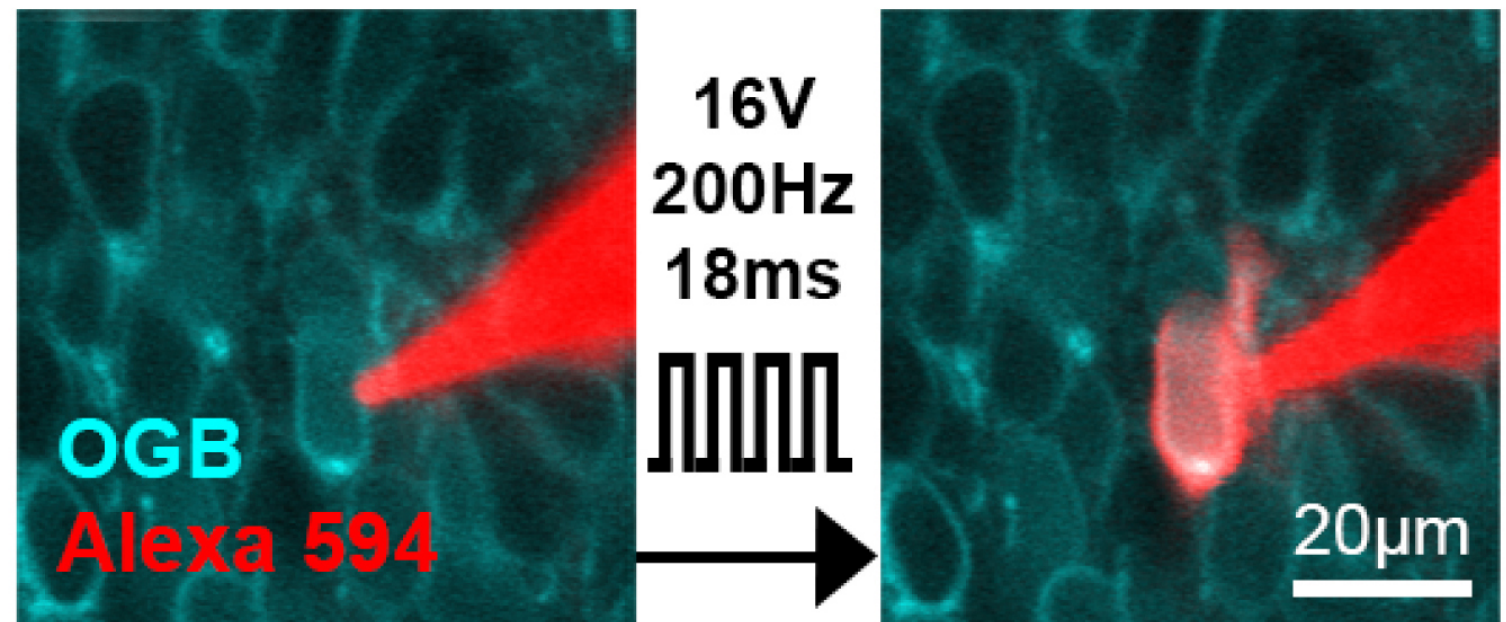
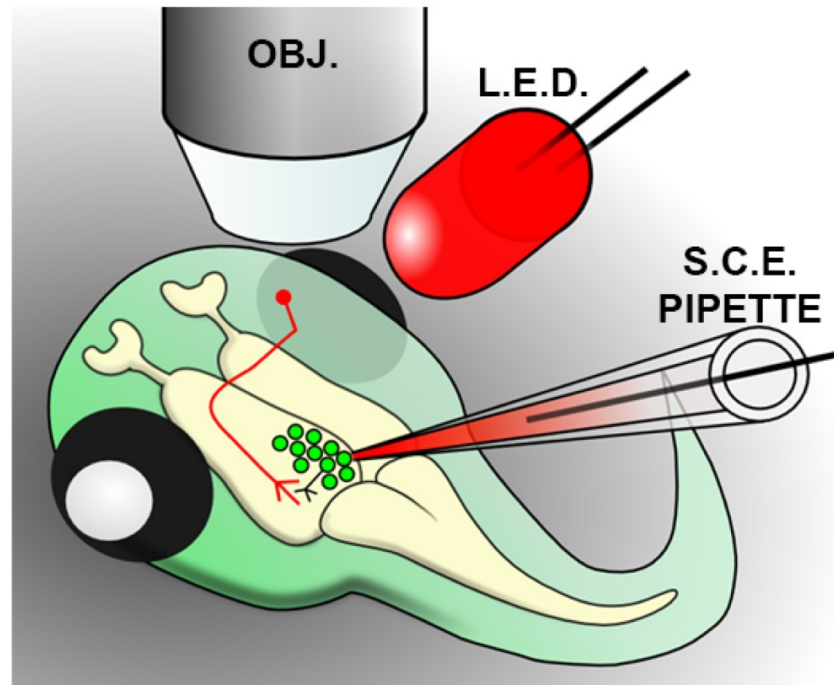


Day 5



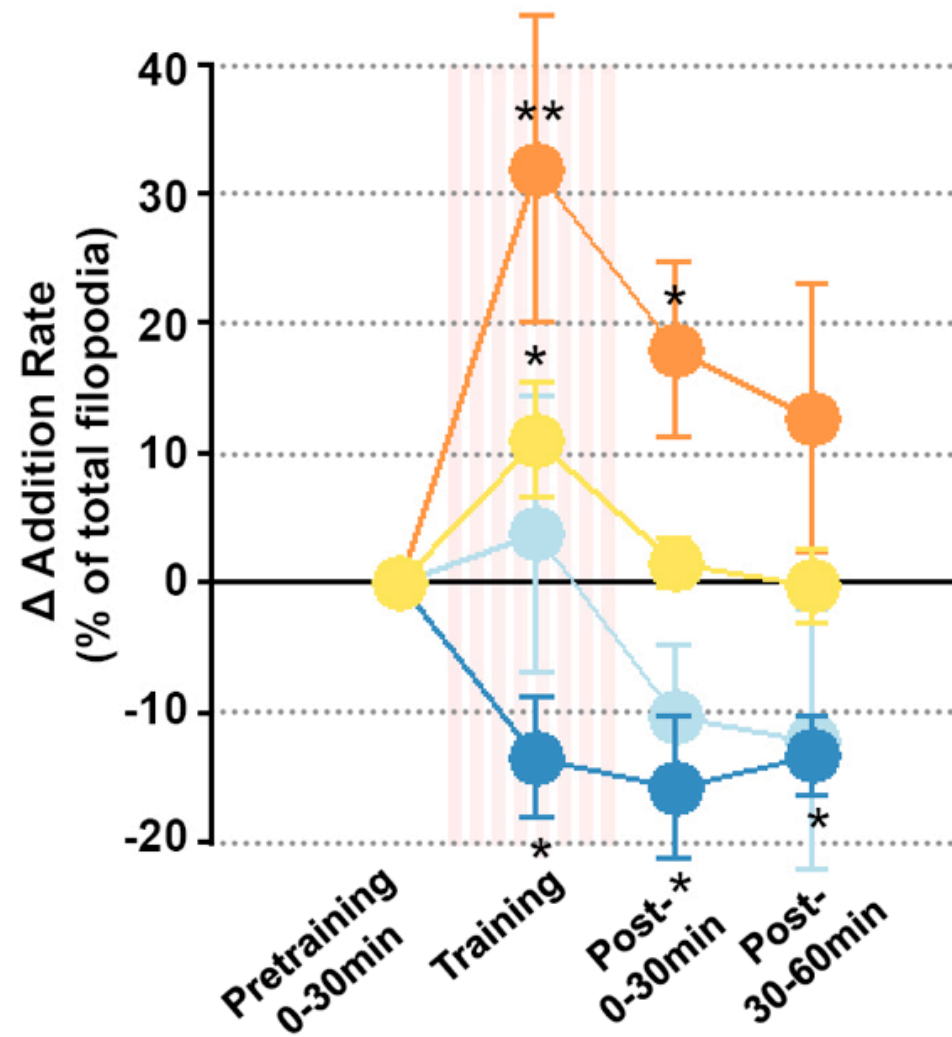
Kasper Podgorski
Janelia HHMI

Targeted single-cell electroporation based on receptive fields

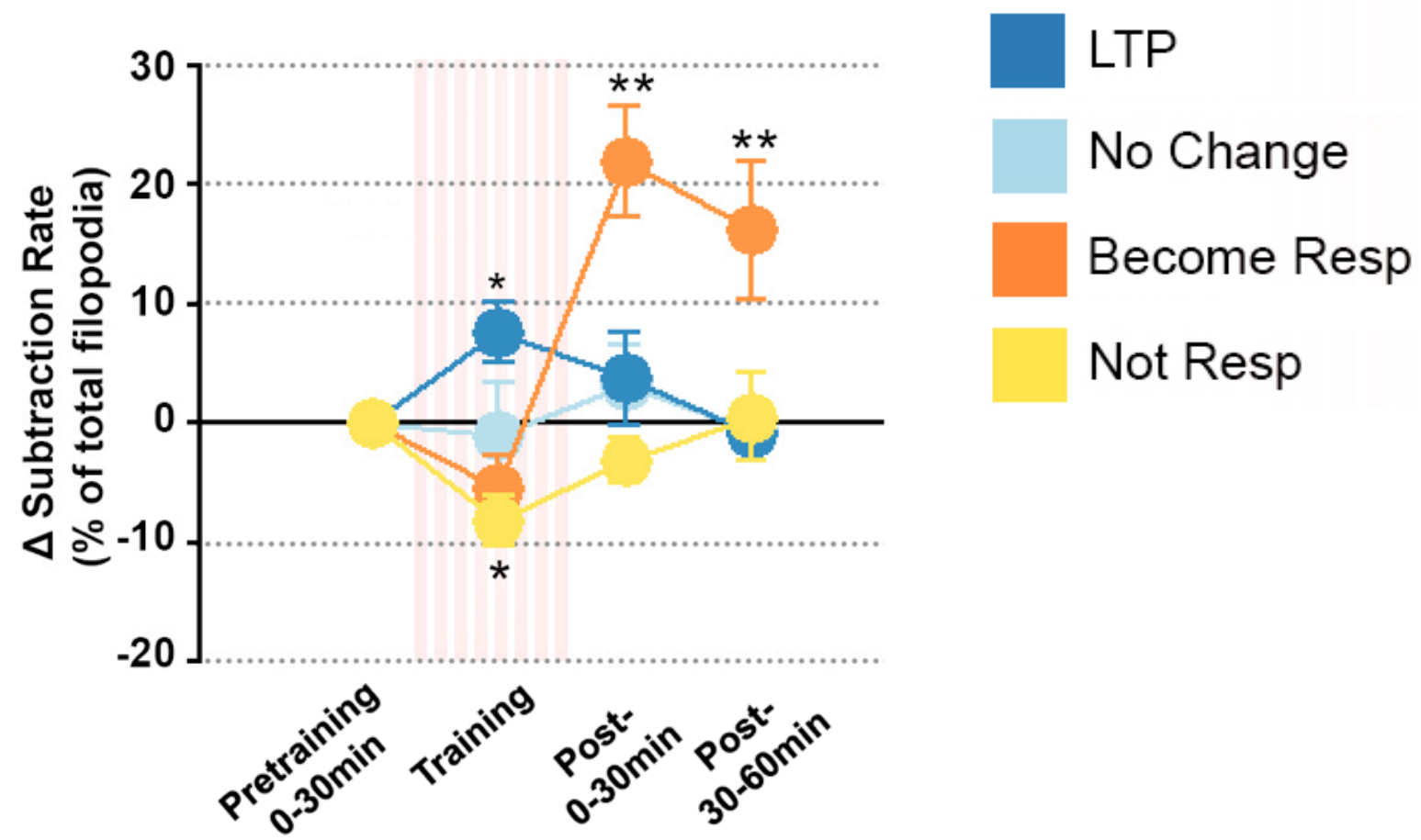




Additions



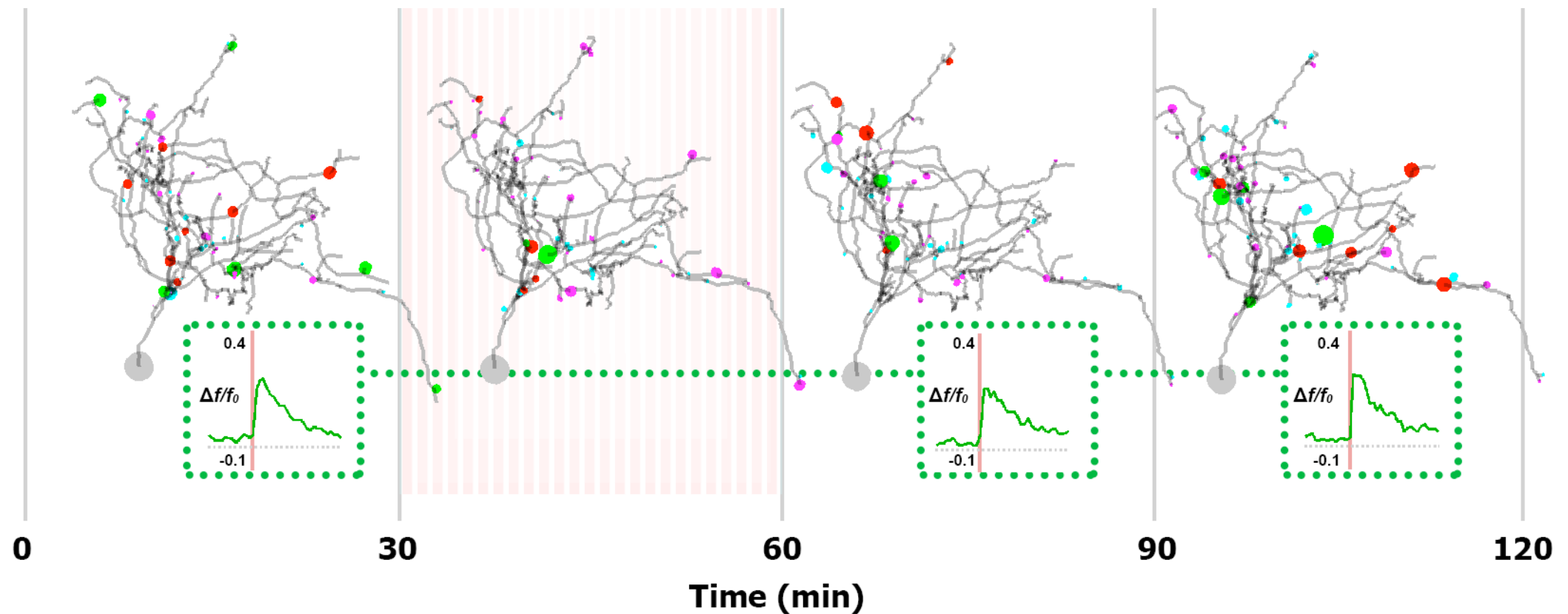
Subtractions



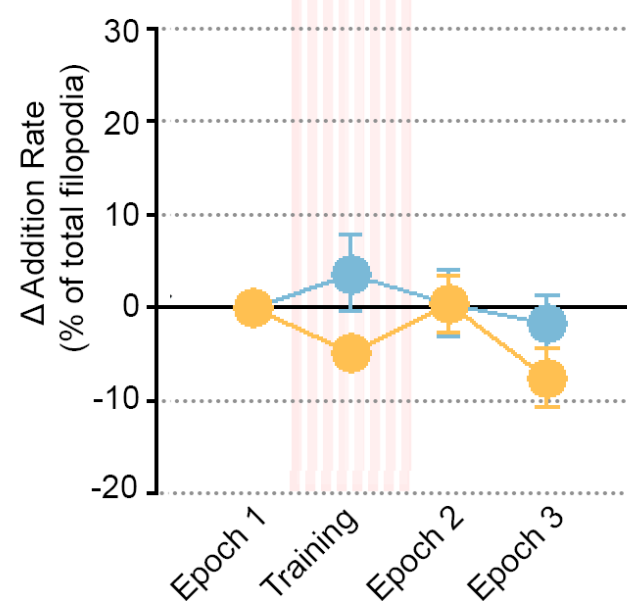
Blocking NMDA Receptors inhibits both morphological and functional plasticity

APV

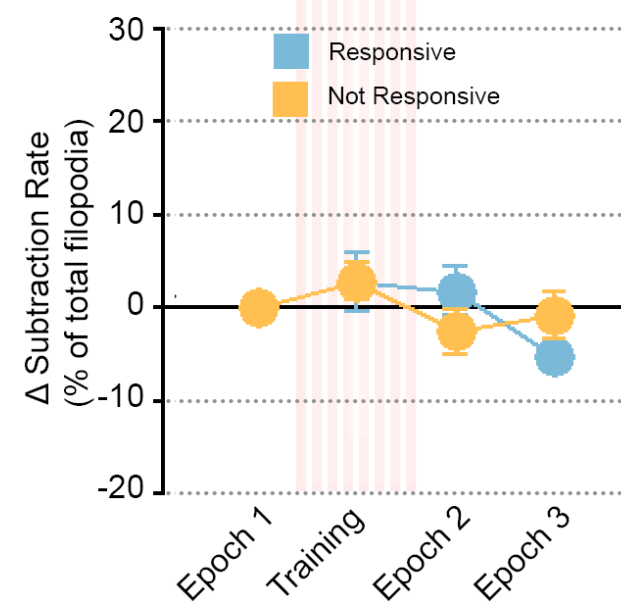
- 0.1 μm
- 1 μm
- 4 μm
- Extension
- Retraction
- Addition
- Subtraction



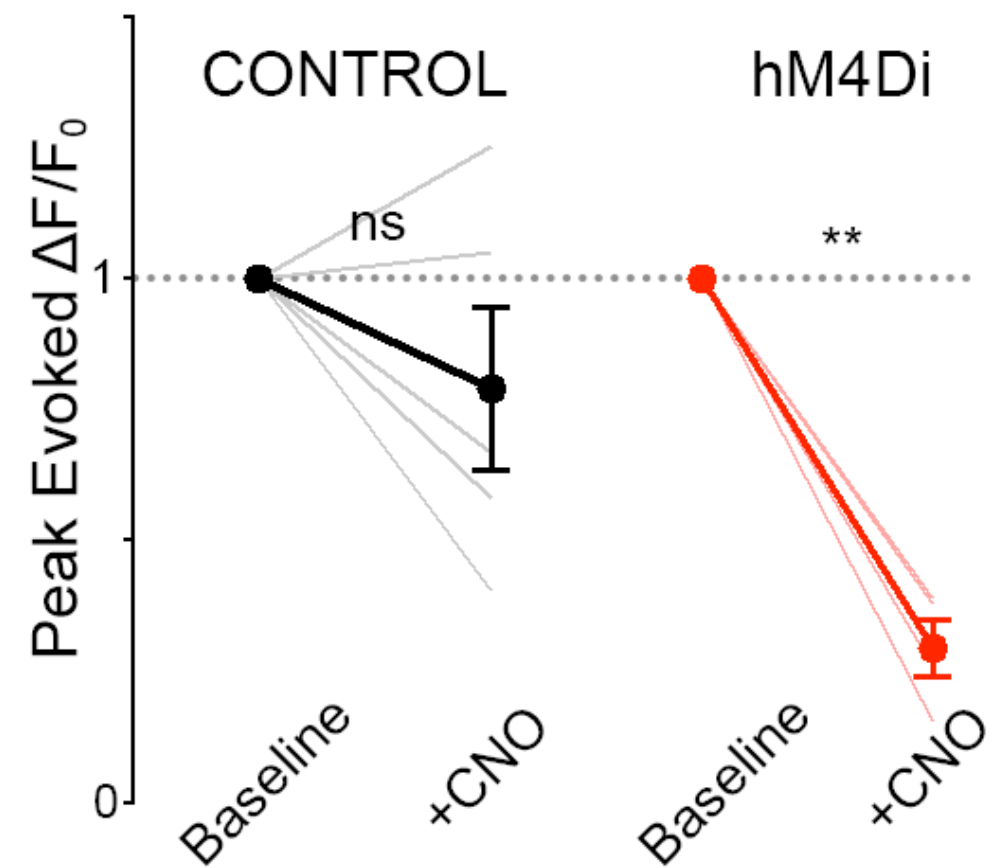
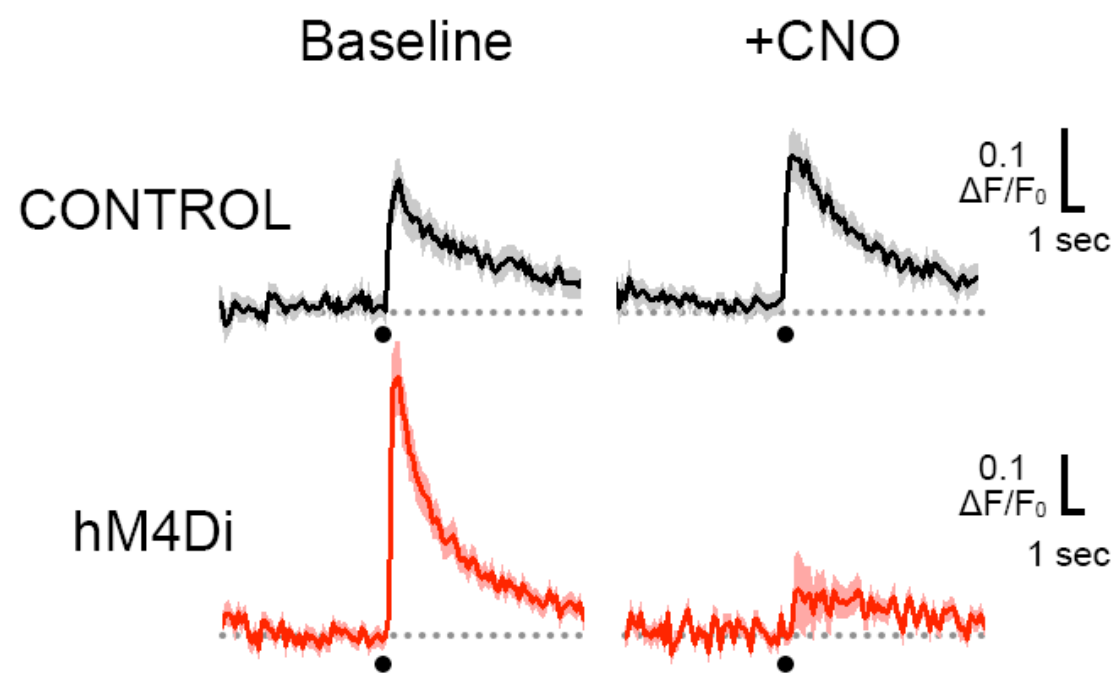
Additions



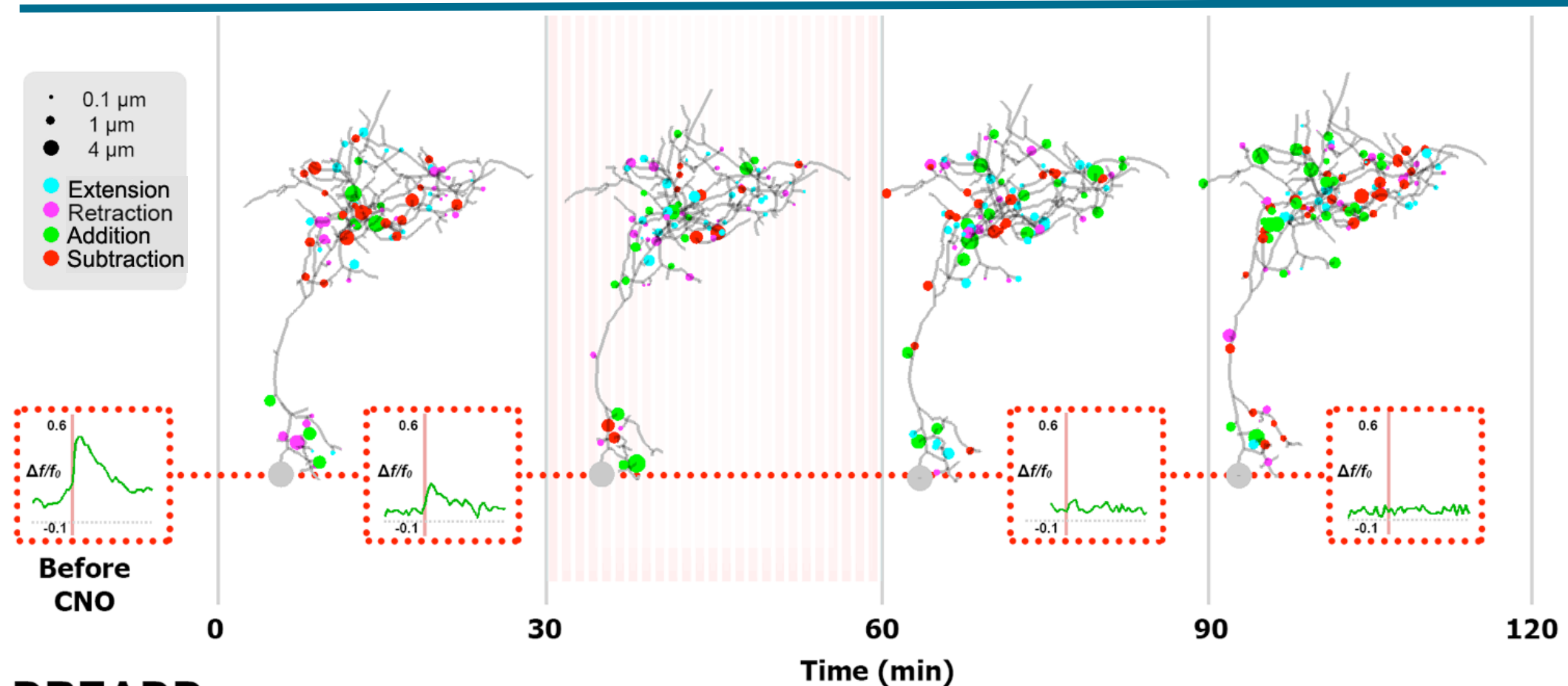
Subtractions



Blocking Action Potentials in single neurons with DREADD hM4Di

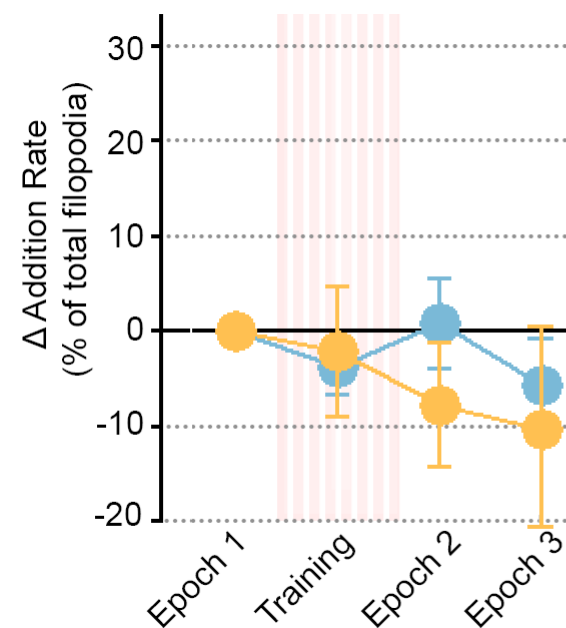


Blocking action potentials inhibits both morphological and functional plasticity

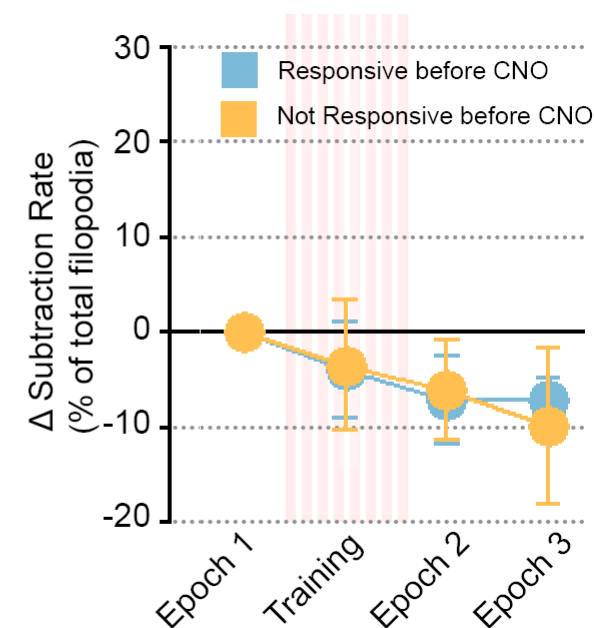


DREADD
hM4Di + CNO

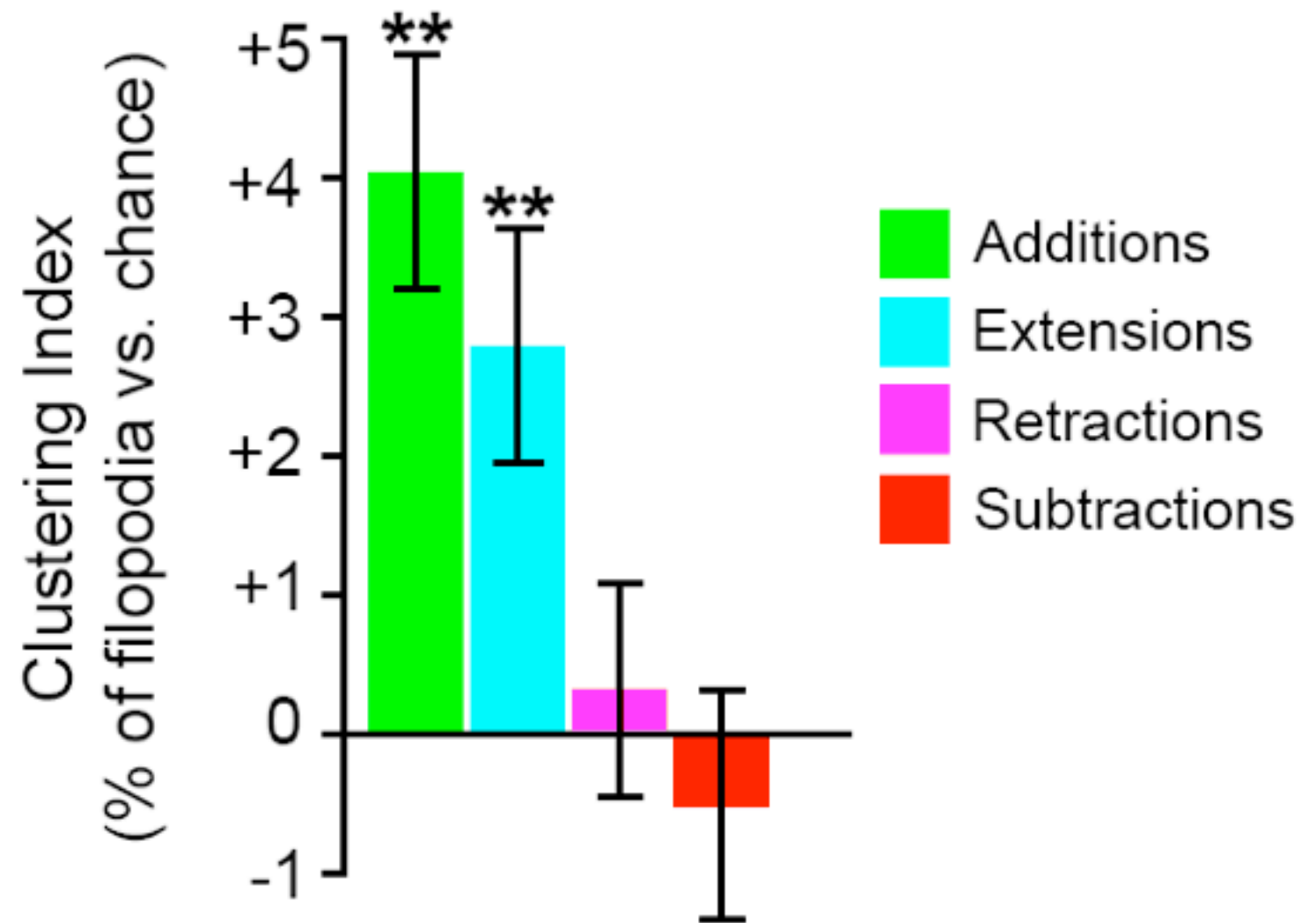
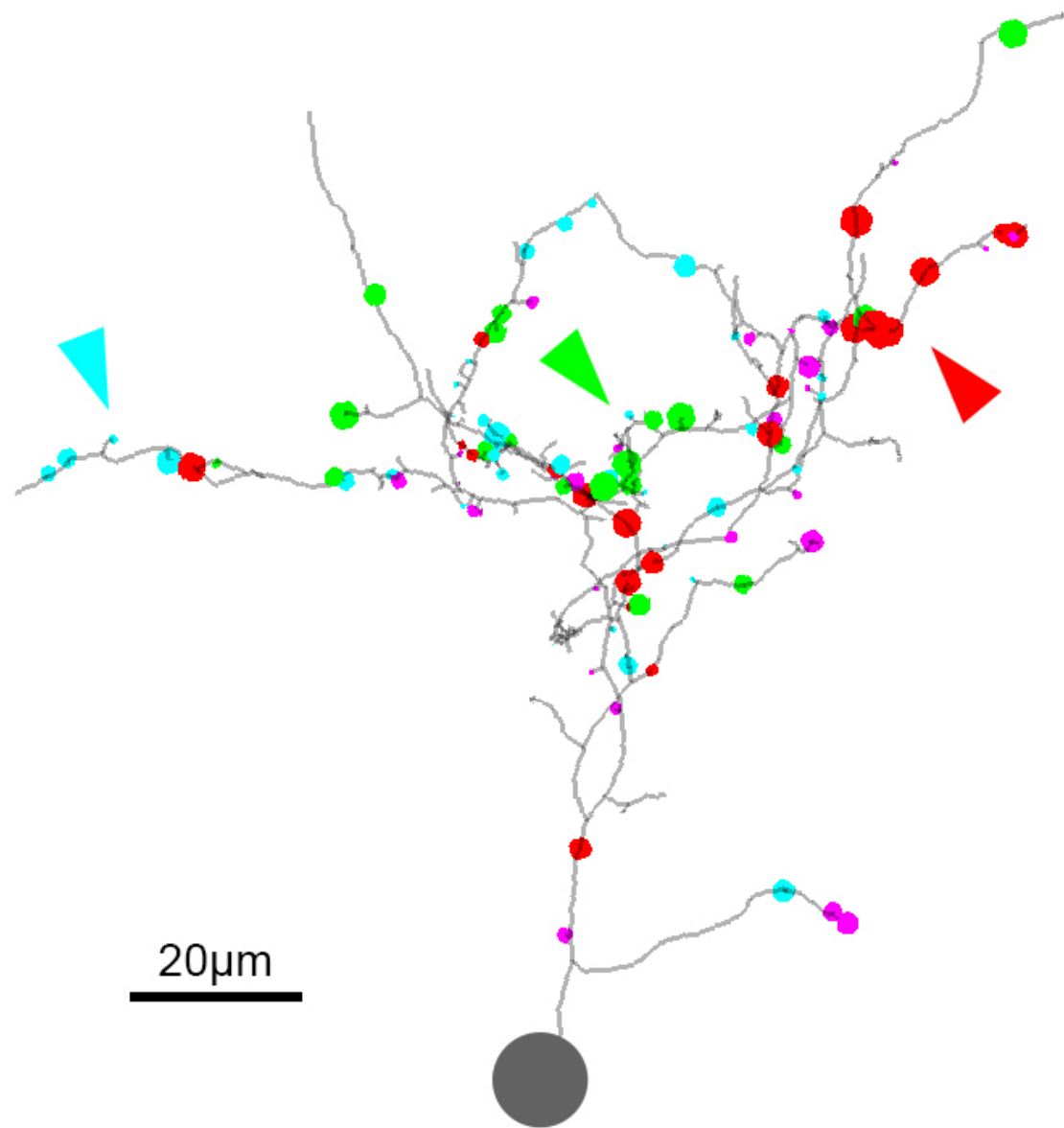
Additions



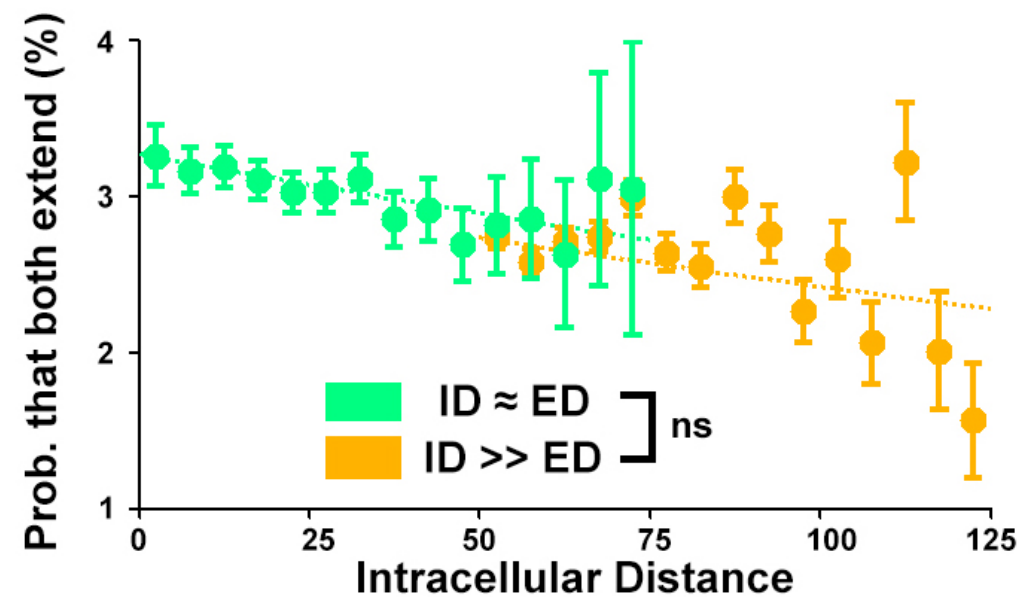
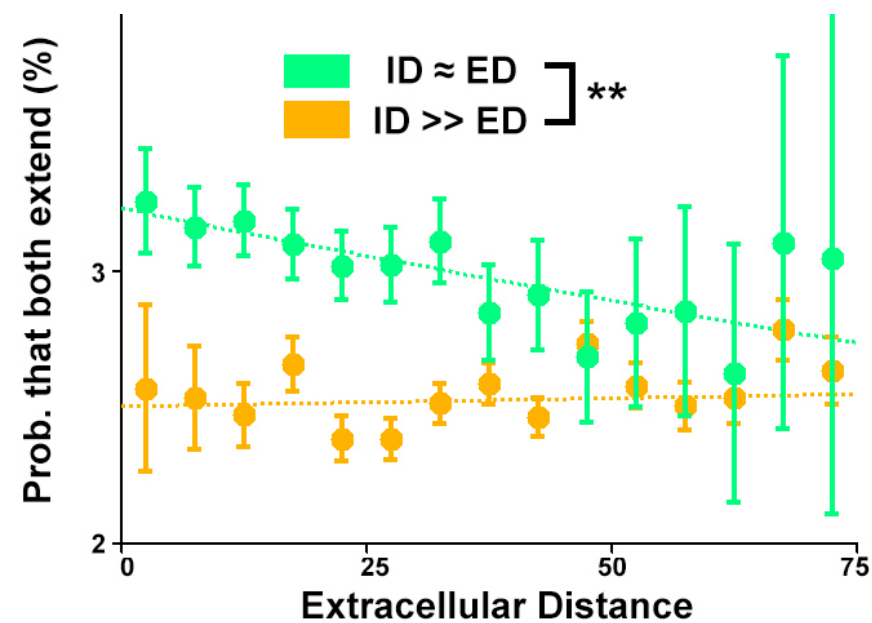
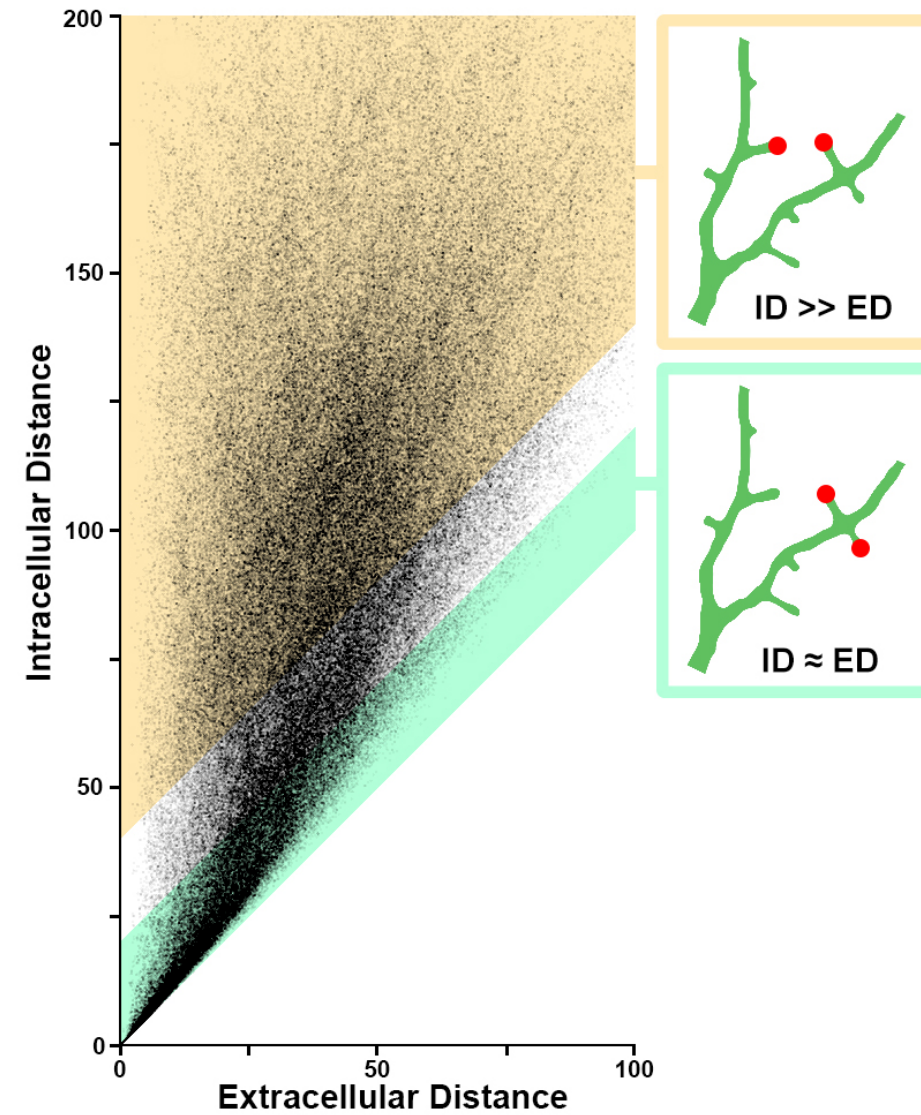
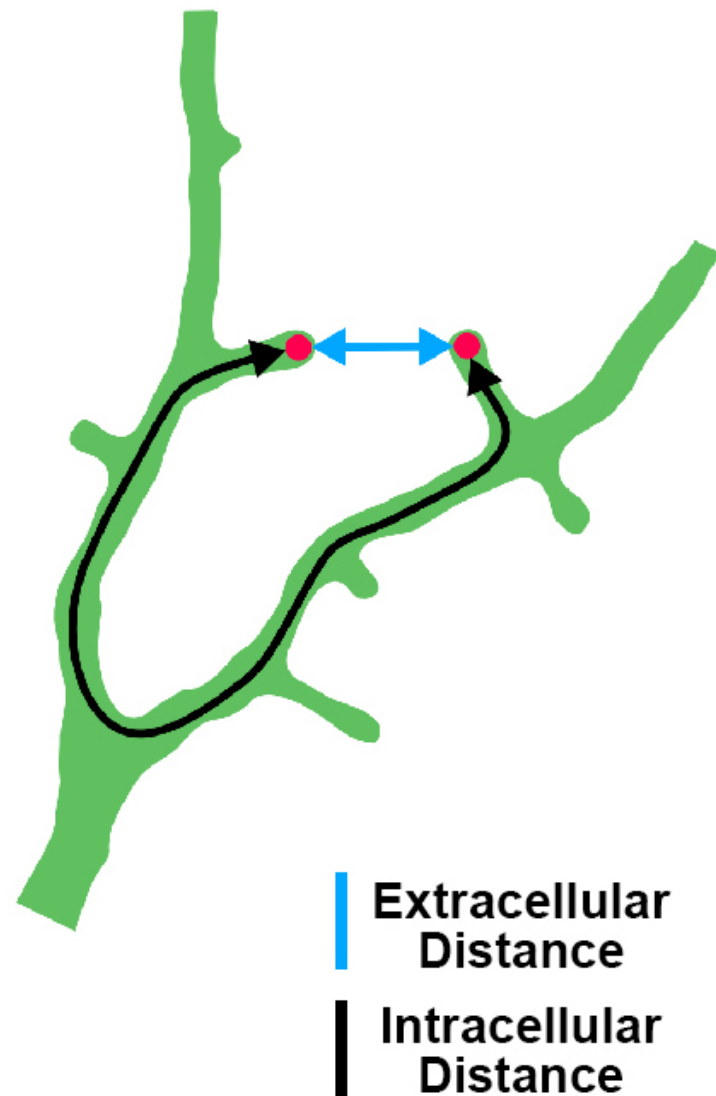
Subtractions



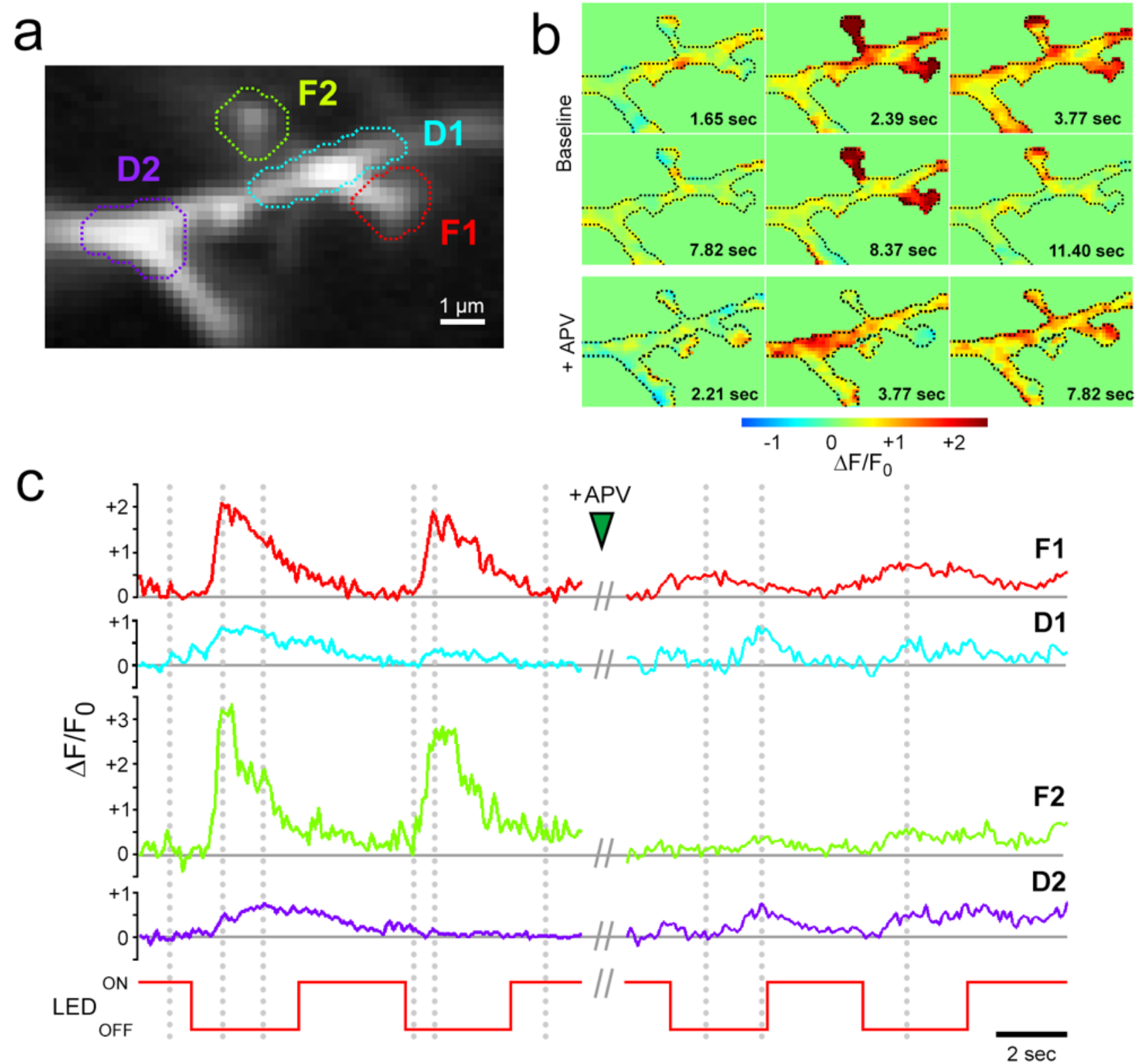
Filopodial growth behavior is clustered



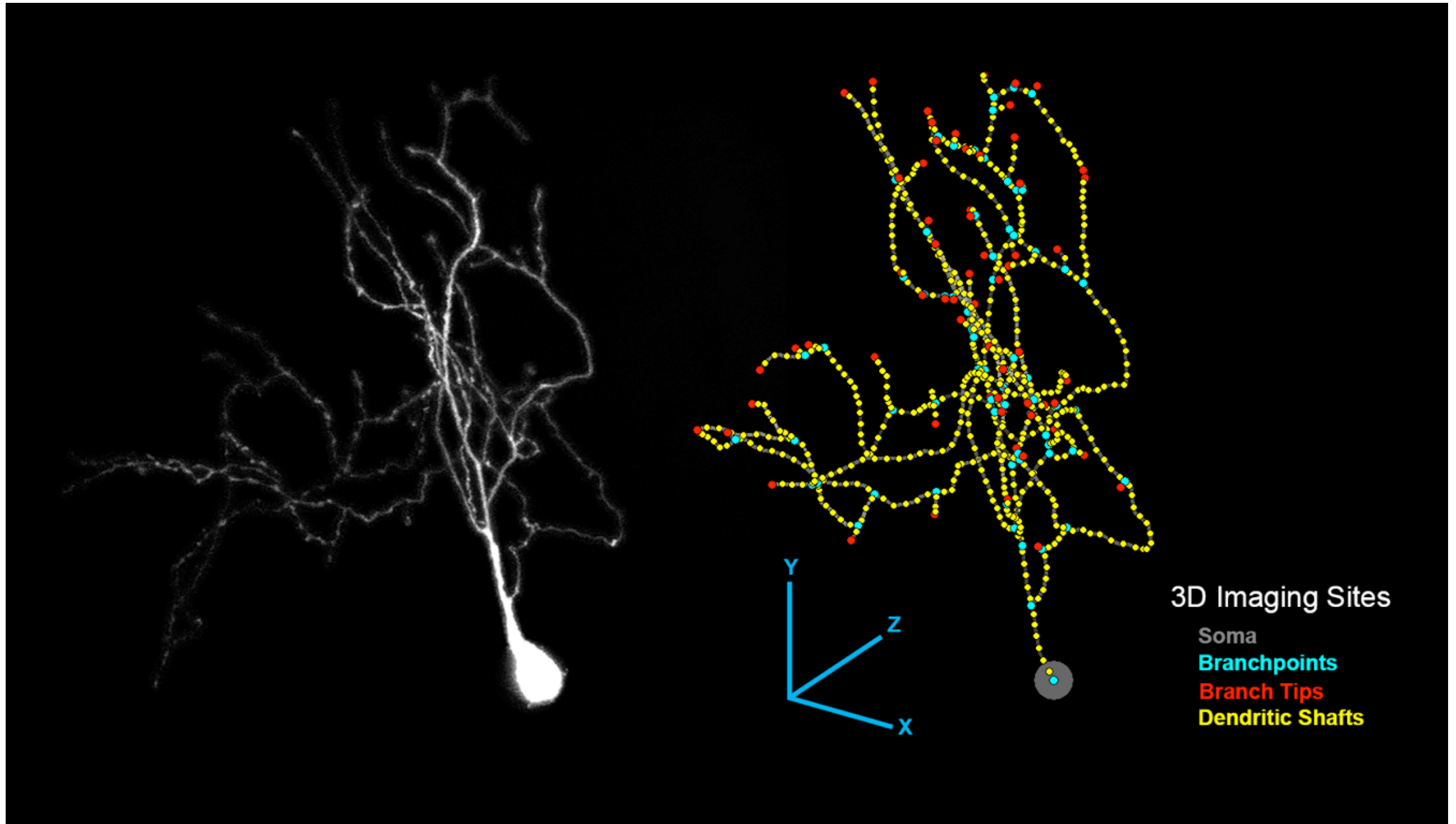
Local intracellular cues underlie clustering of filopodial extensions



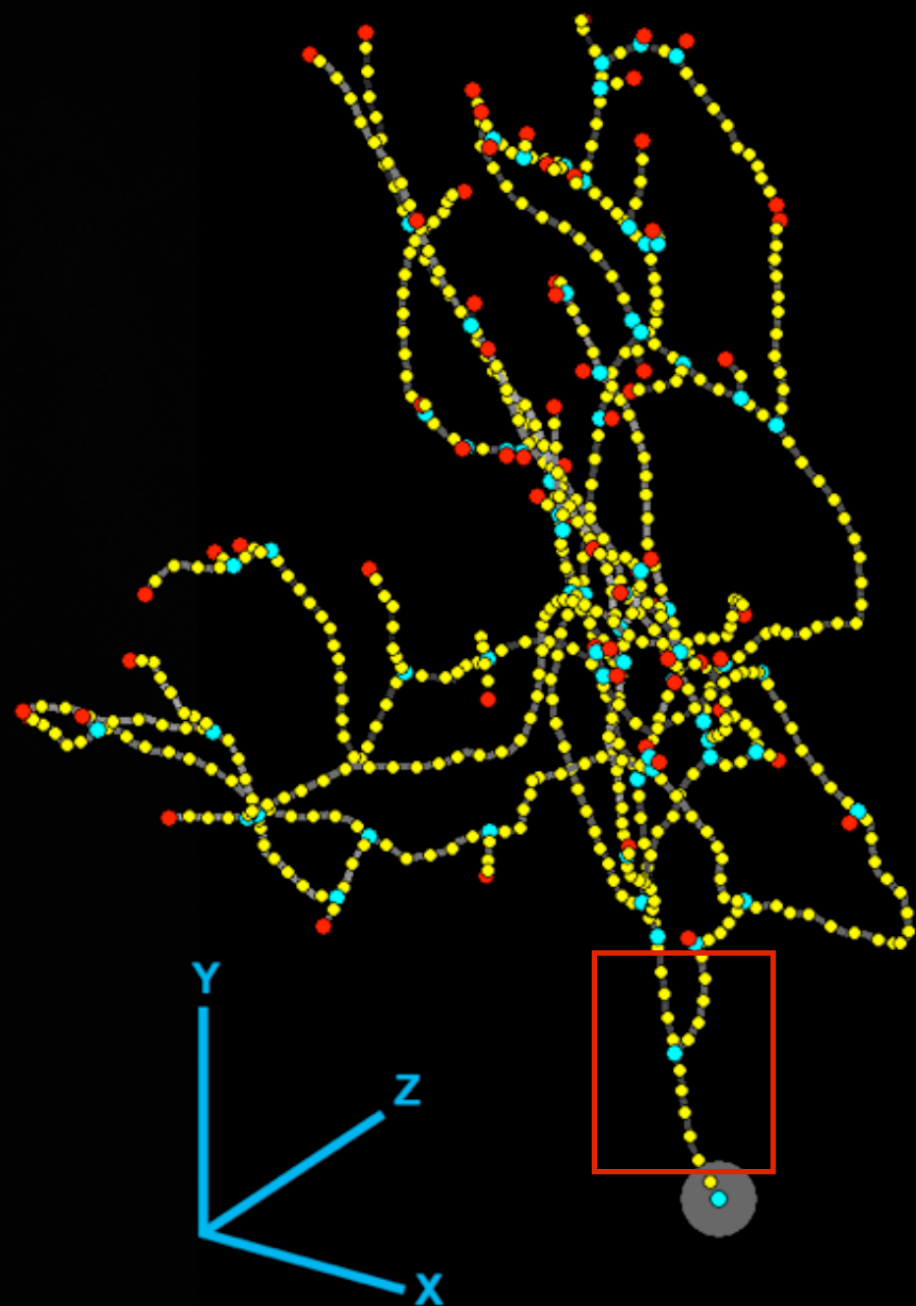
Filopodial transients blocked by NMDA R antagonists



AOD two-photon microscope



NODE SCANNING



3D Imaging Sites

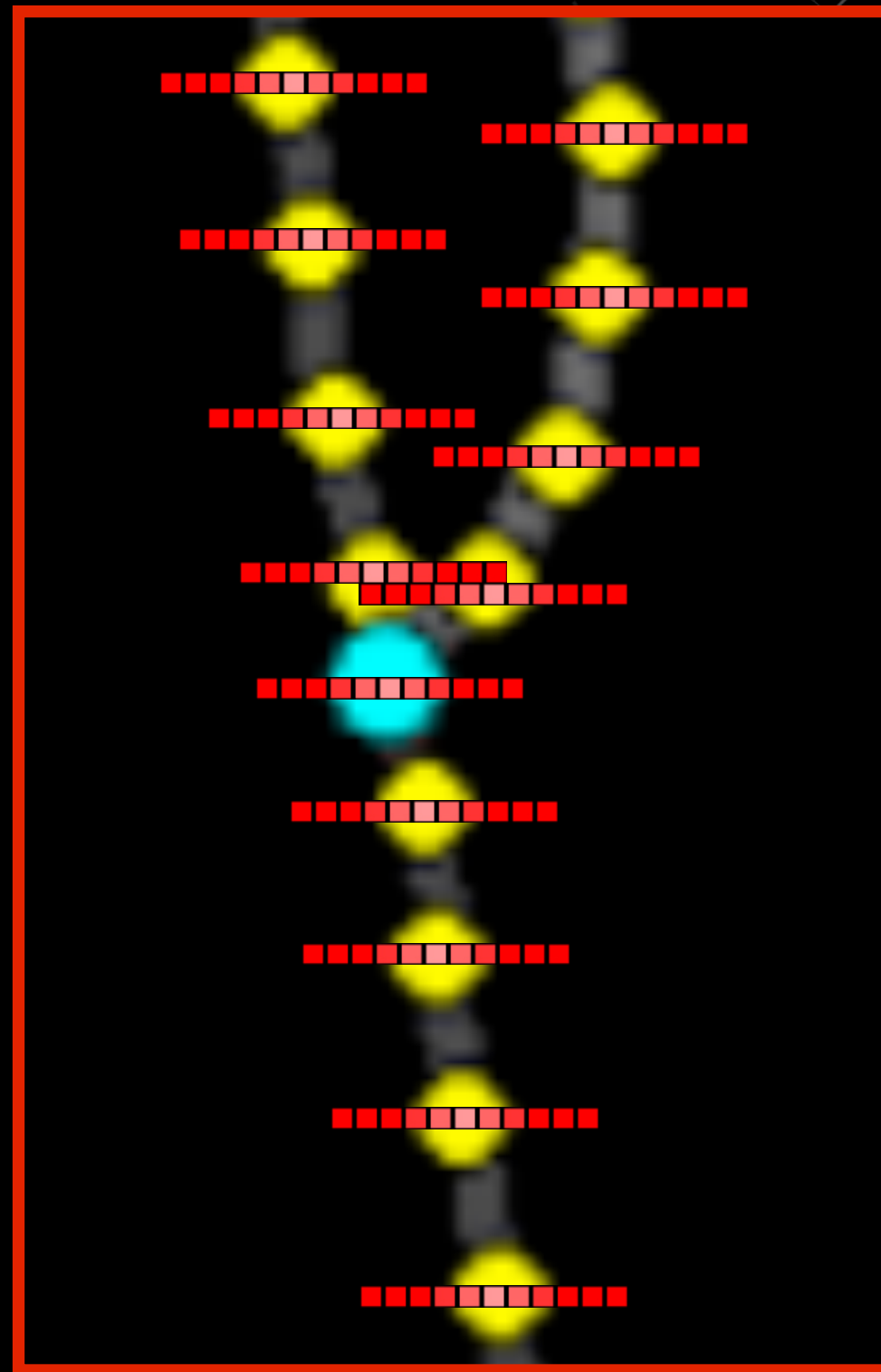
Soma

Branchpoints

Branch Tips

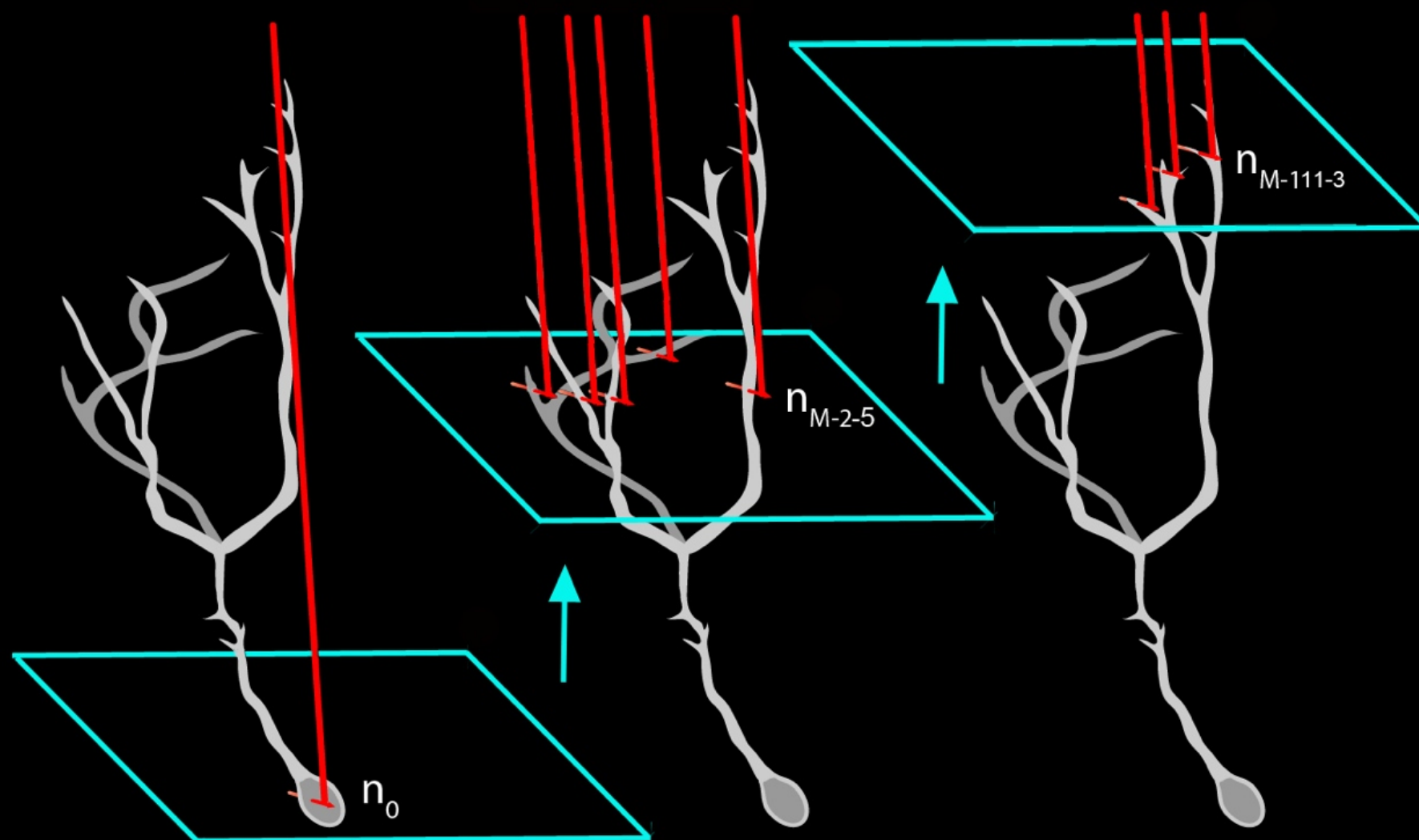
Dendritic Shafts

← AOD →

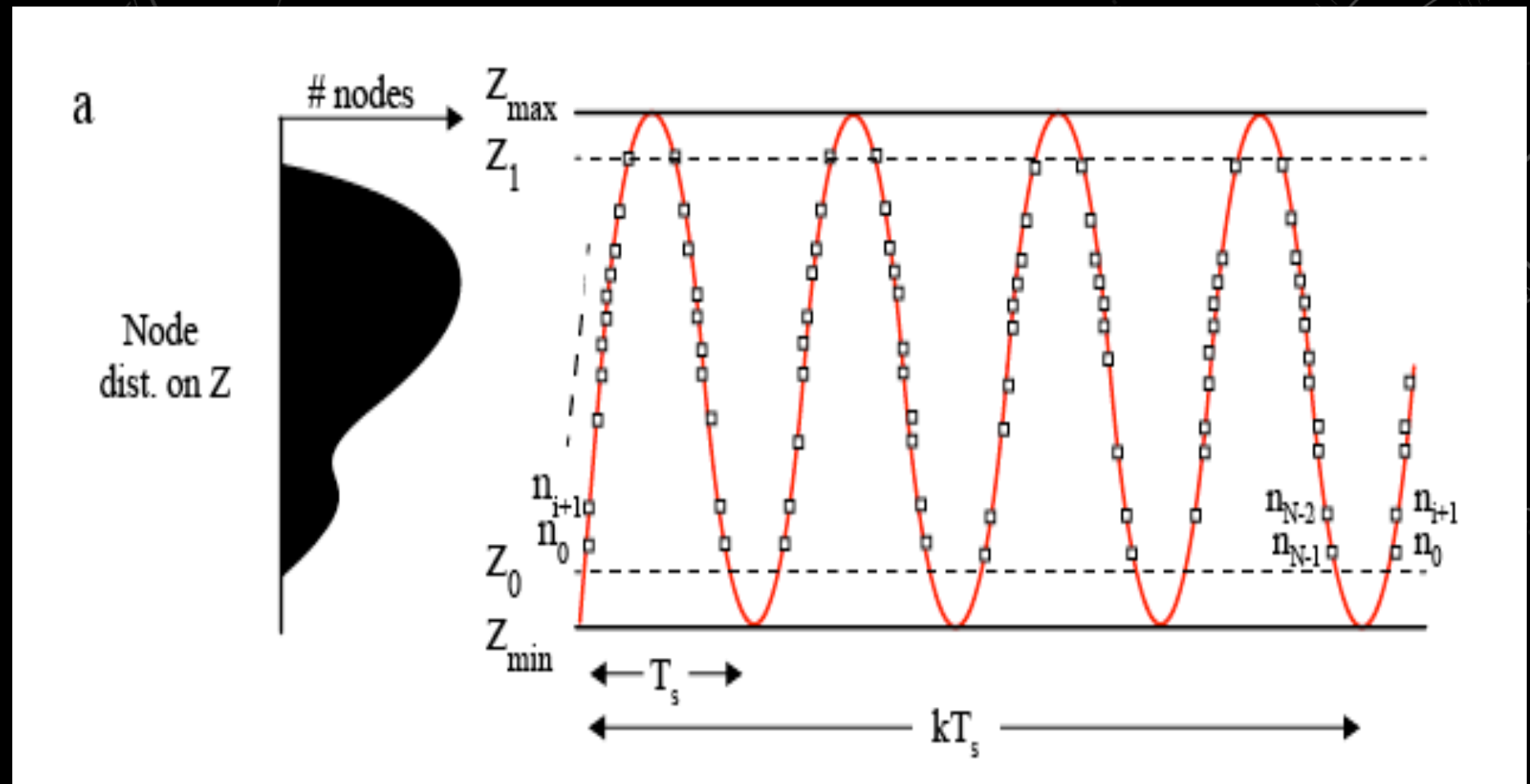
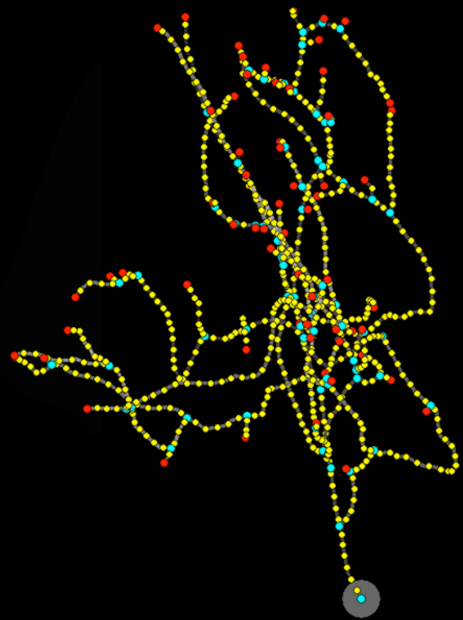


Piezo

11 pixels sampled in line across each point

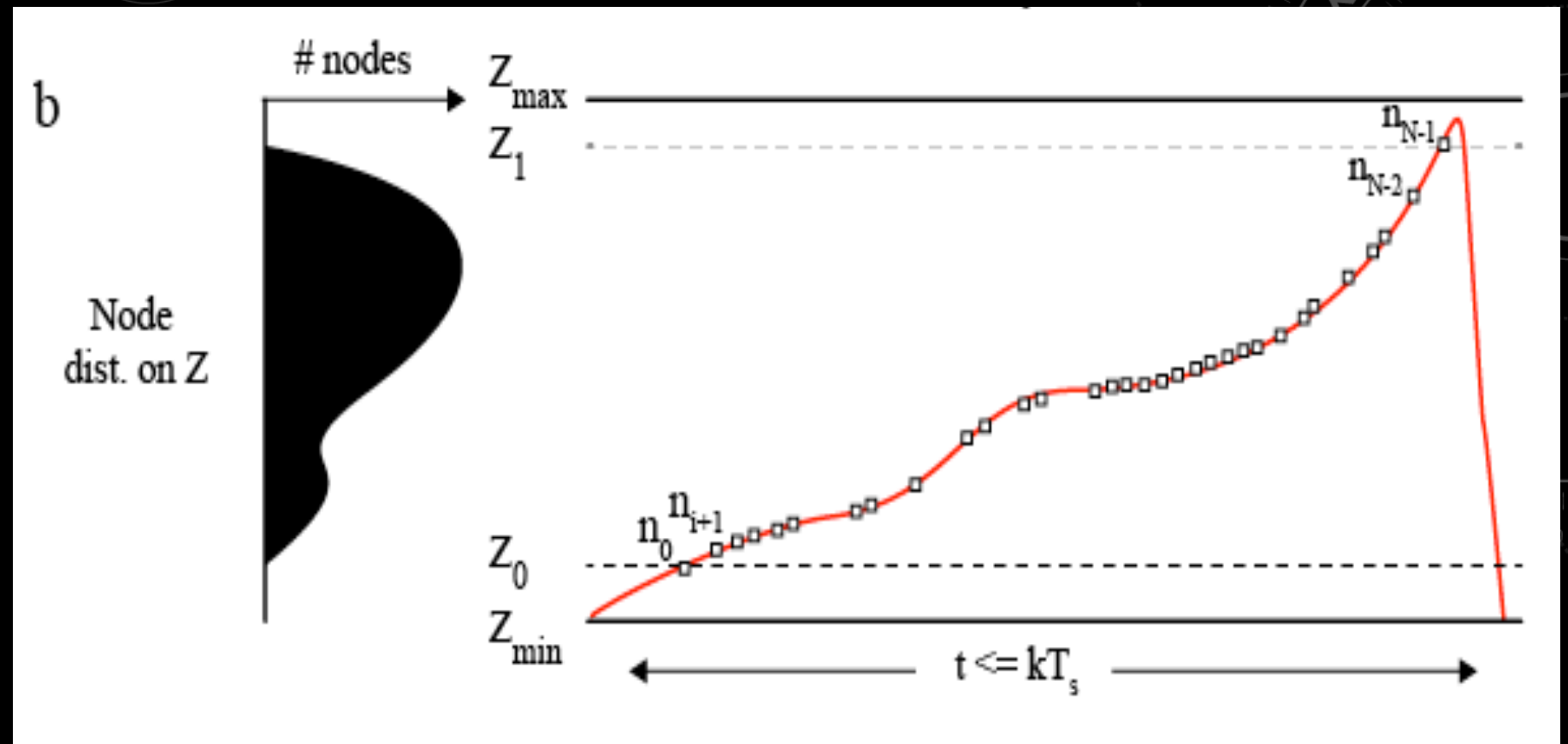
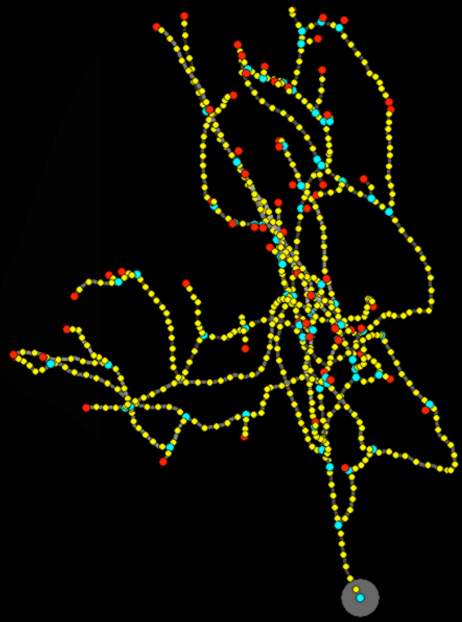


FIXED-PROFILE SINUSOIDAL TRAJECTORY



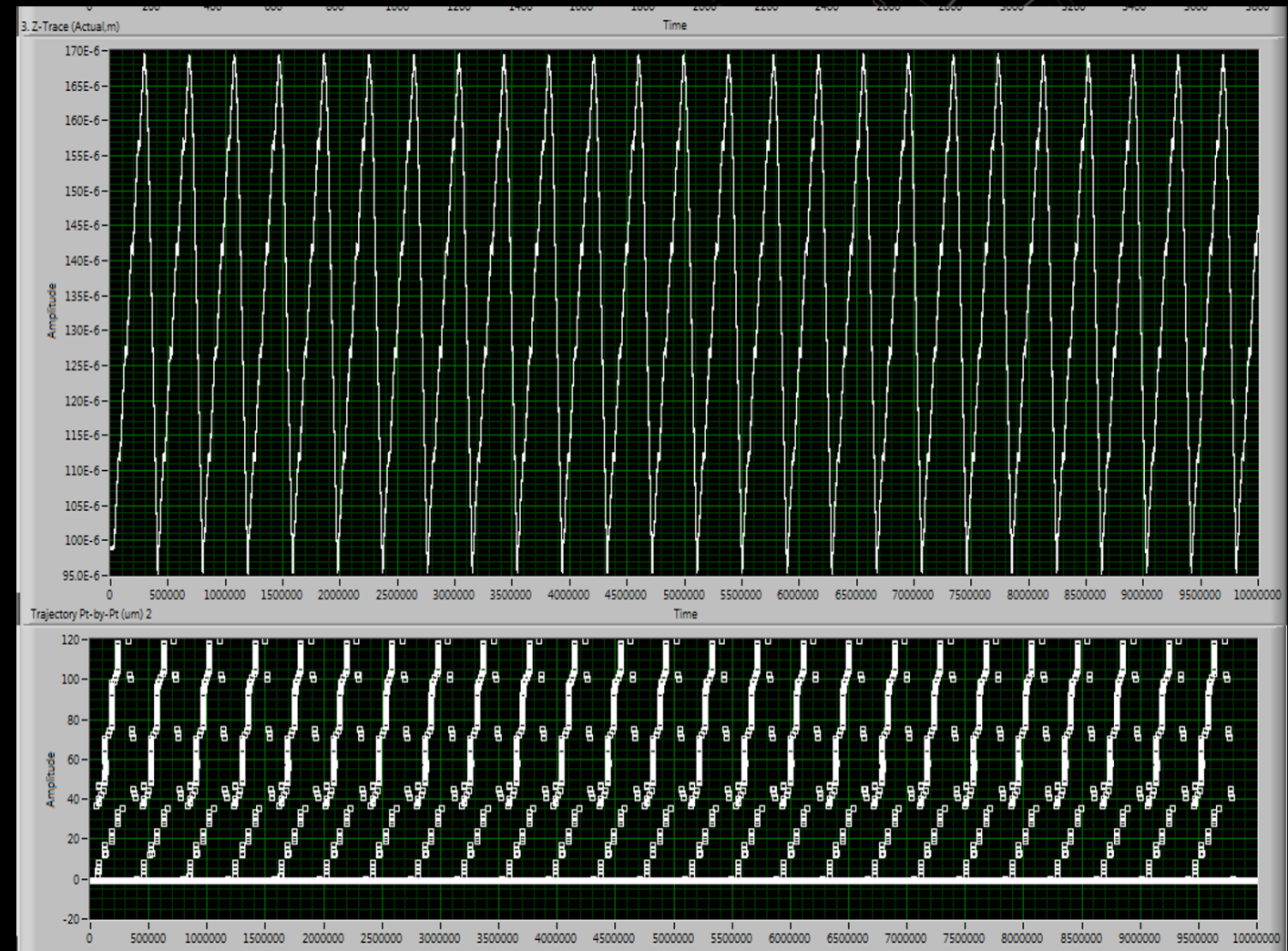
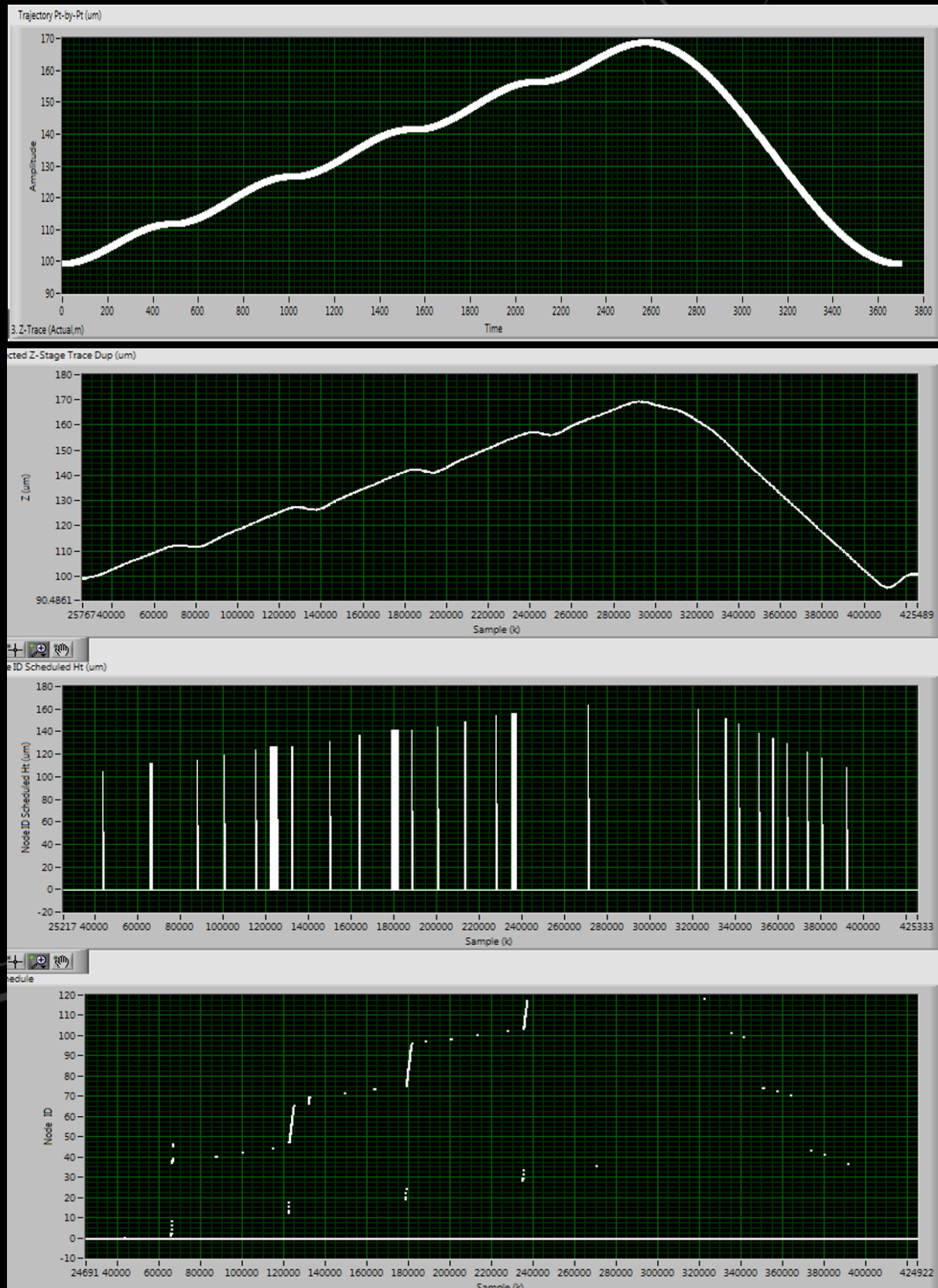
- Fixed profile scanning good for scans that are distributed vertically
 - Fails where points are significantly distributed on same planes

VARIED-PROFILE TRAJECTORY SCANNING



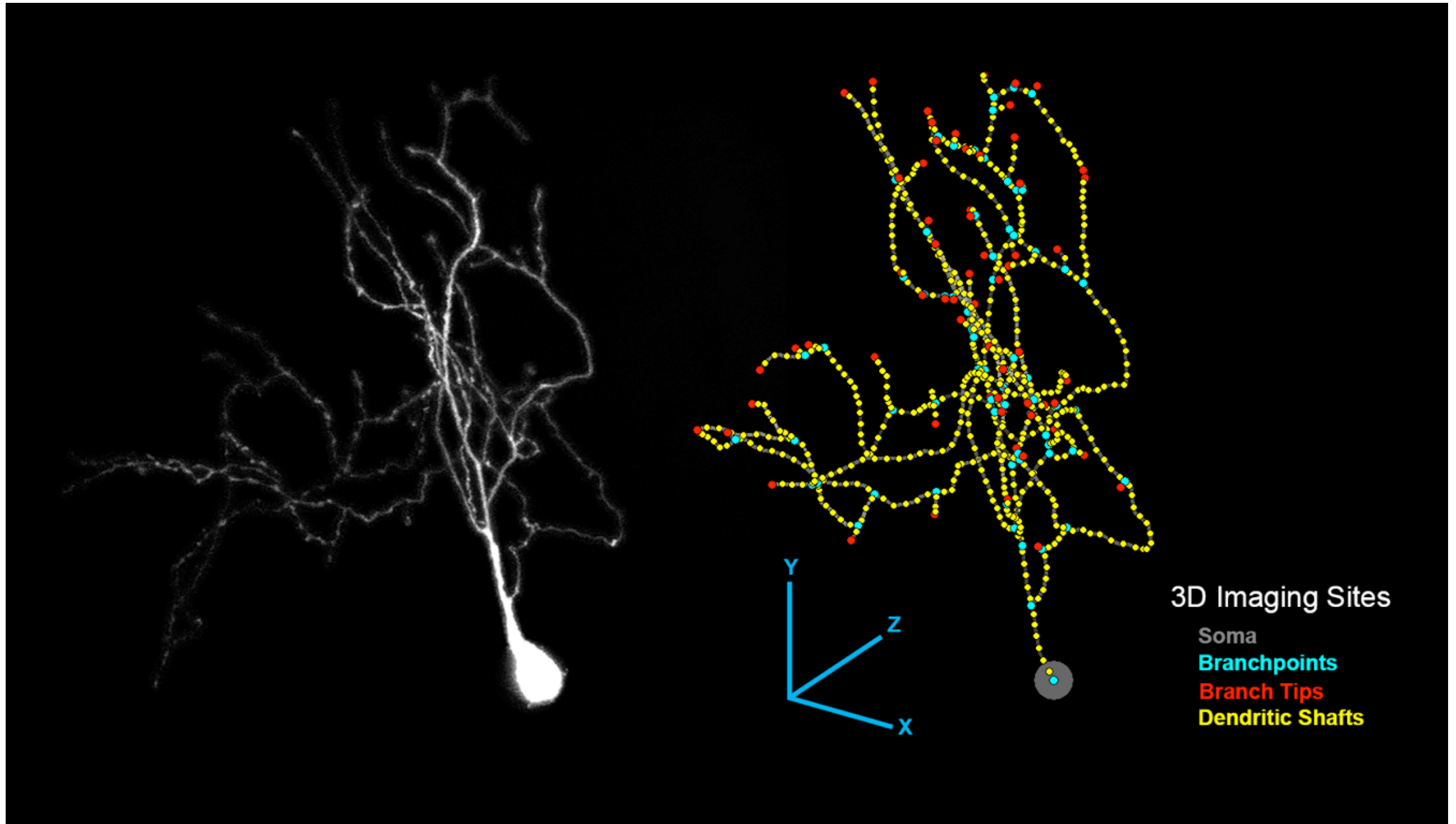
- More sophisticated scanning technique using Z-axis velocity and position-based control
 - More efficiently using time/space
 - Exhaustively collect nodes during single scan

VARIED-PROFILE TRAJECTORY SCANNING



1000-1500 nodes
20Hz
200X200X175um

AOD two-photon microscope



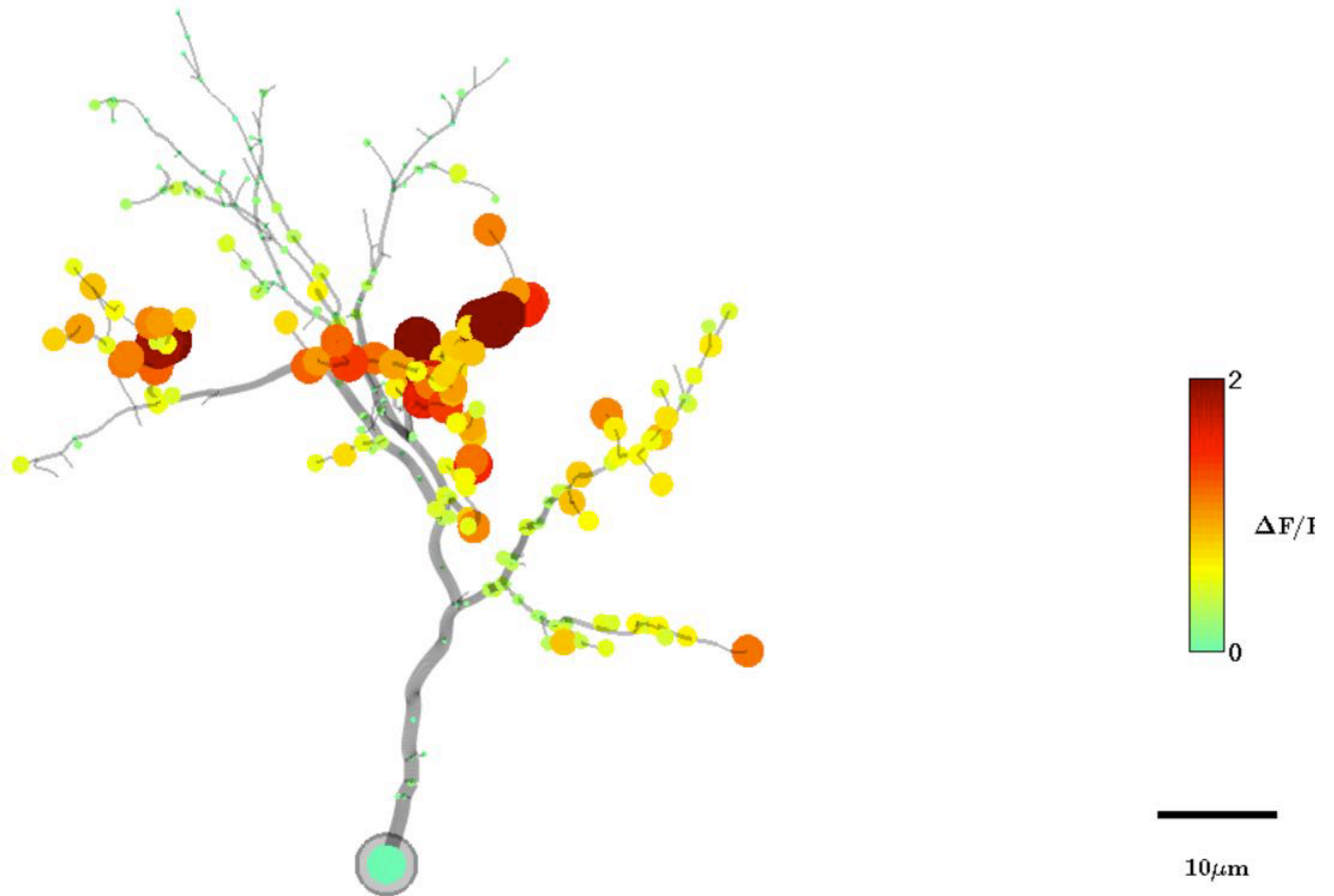
Comprehensive Imaging in awake brain - GCaMP6

OFF Stimuli



Comprehensive Imaging in awake brain - GCaMP6

ON Stimuli



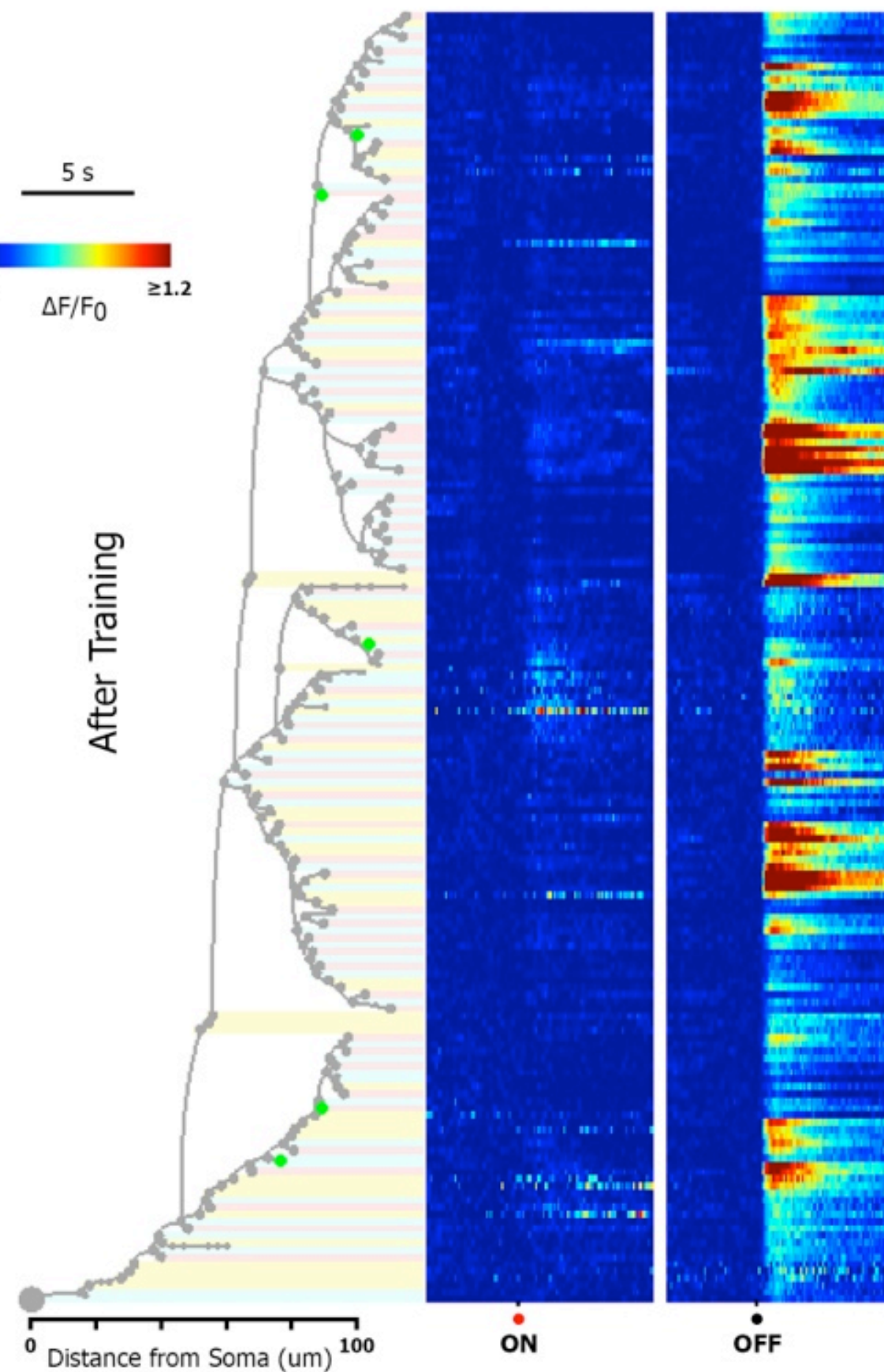
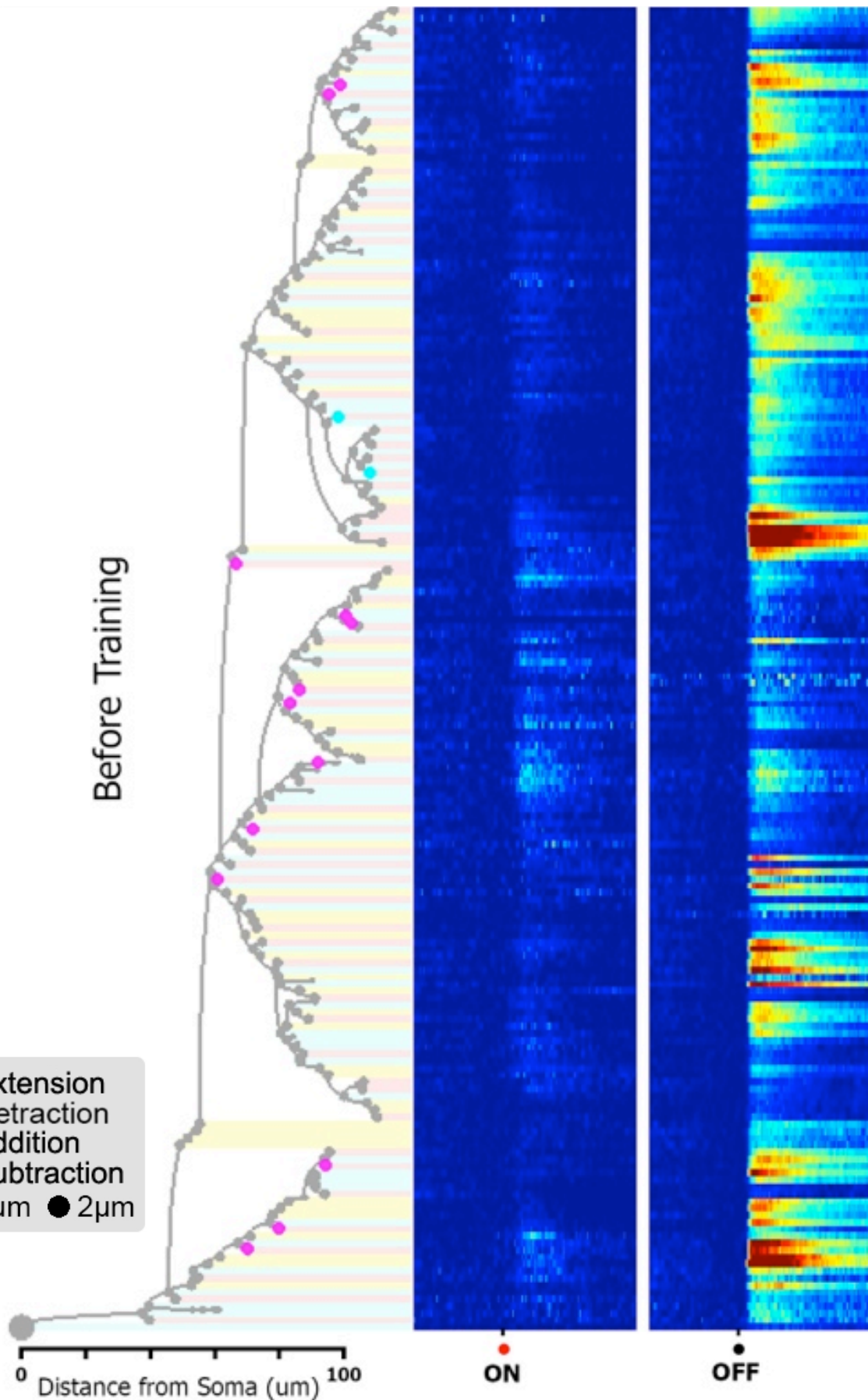
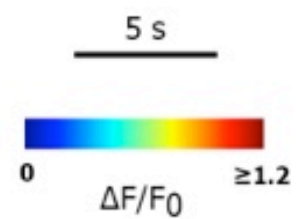
Baseline
ON OFF

After Training Off
ON OFF

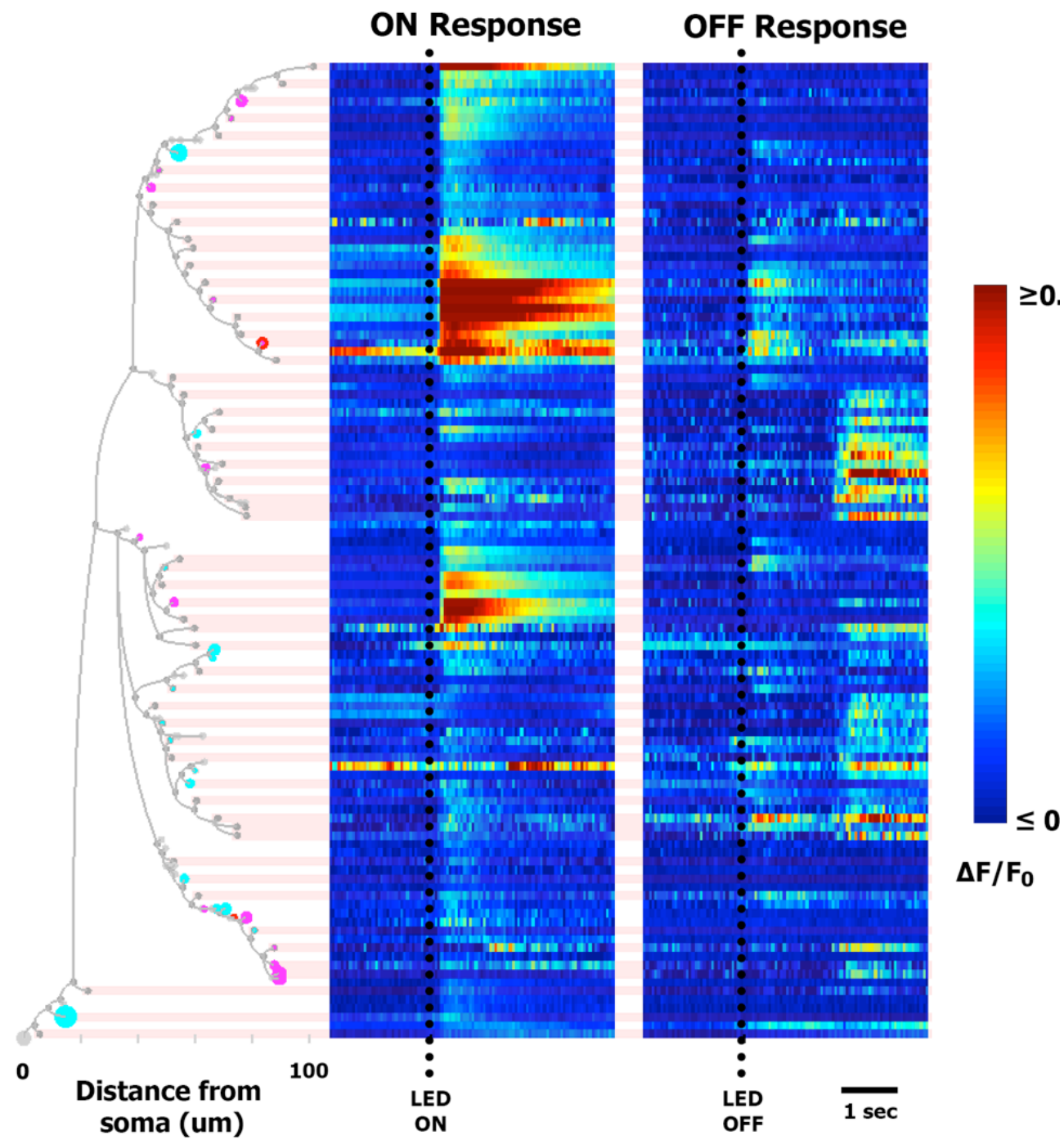
Before Training

After Training

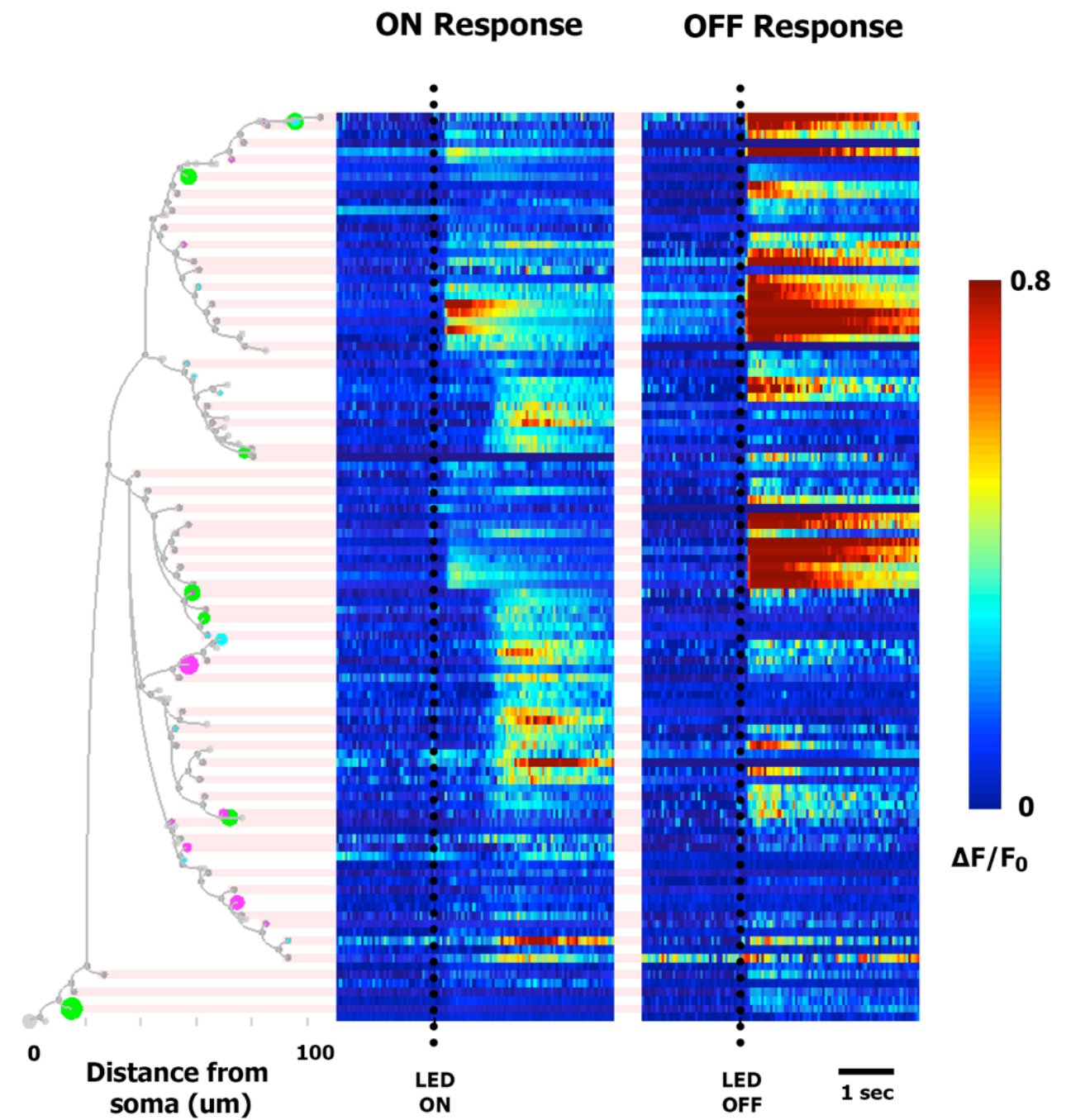
- Extension
- Retraction
- Addition
- Subtraction
- 0.5 μ m ● 2 μ m



Pre-training



Post-training with OFF



- 0.1 μm
- 1 μm
- 4 μm
- Growth
- Retraction
- Added
- Subtracted

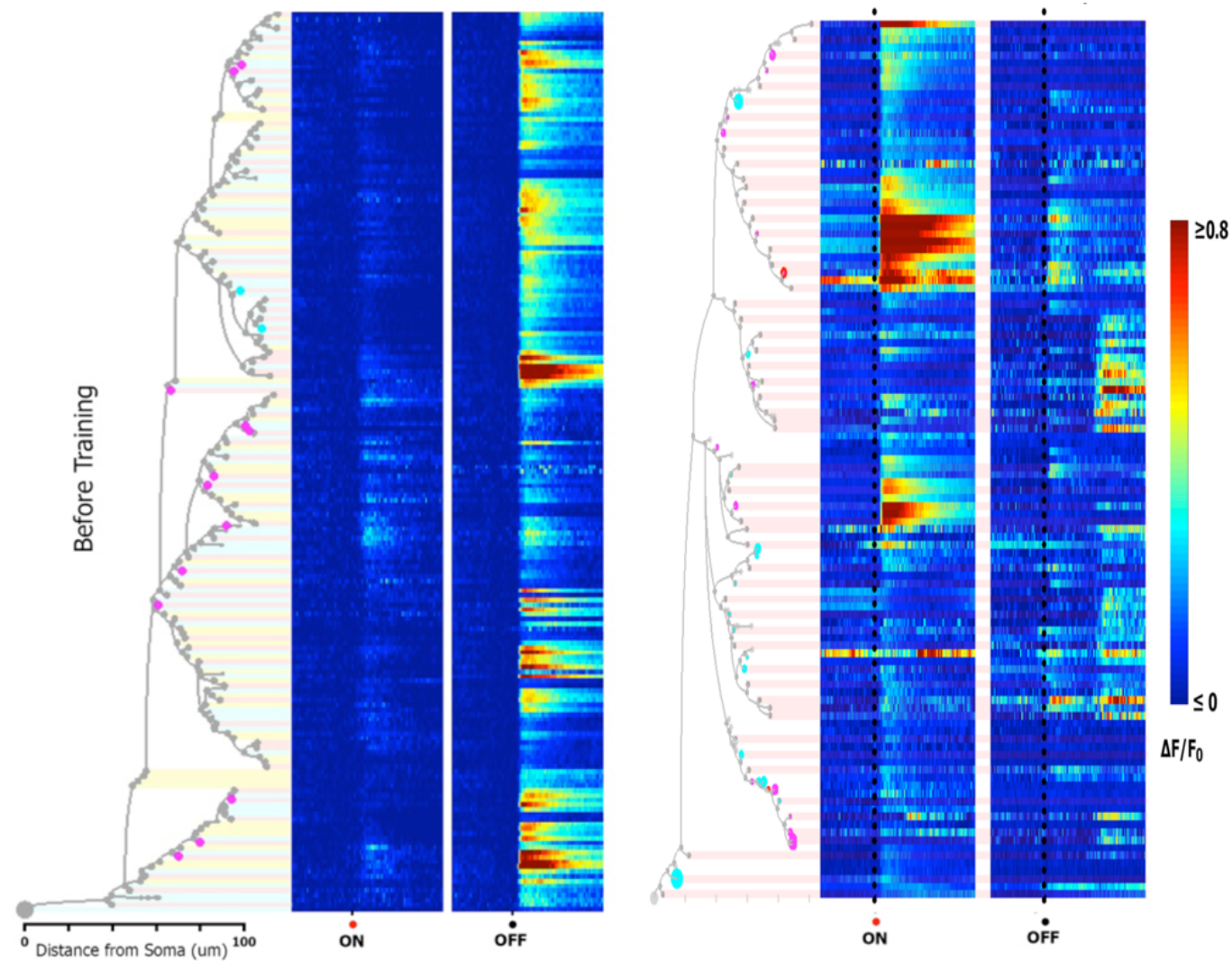
Clustering of filopodia with shared tuning is activity-dependent

Cell 1

Cell 2

ON OFF

ON OFF



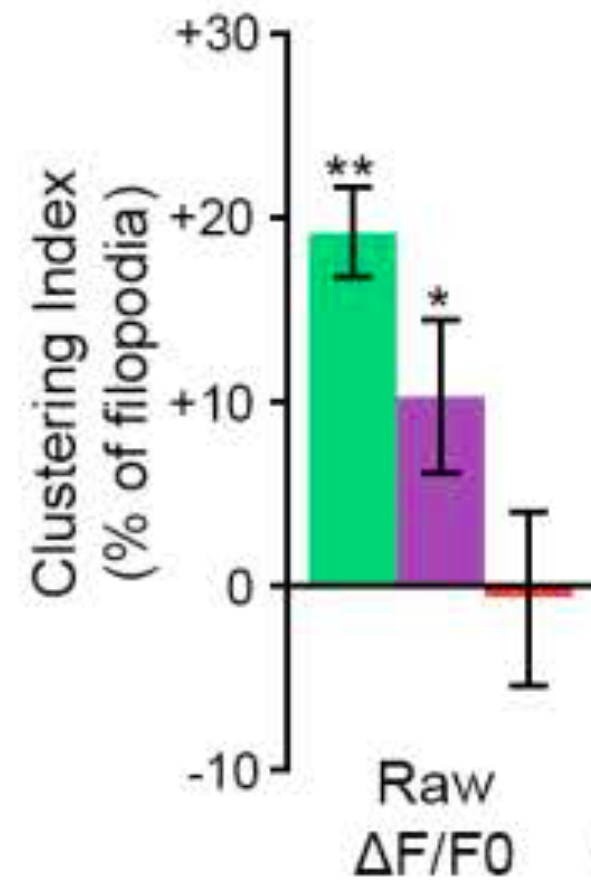
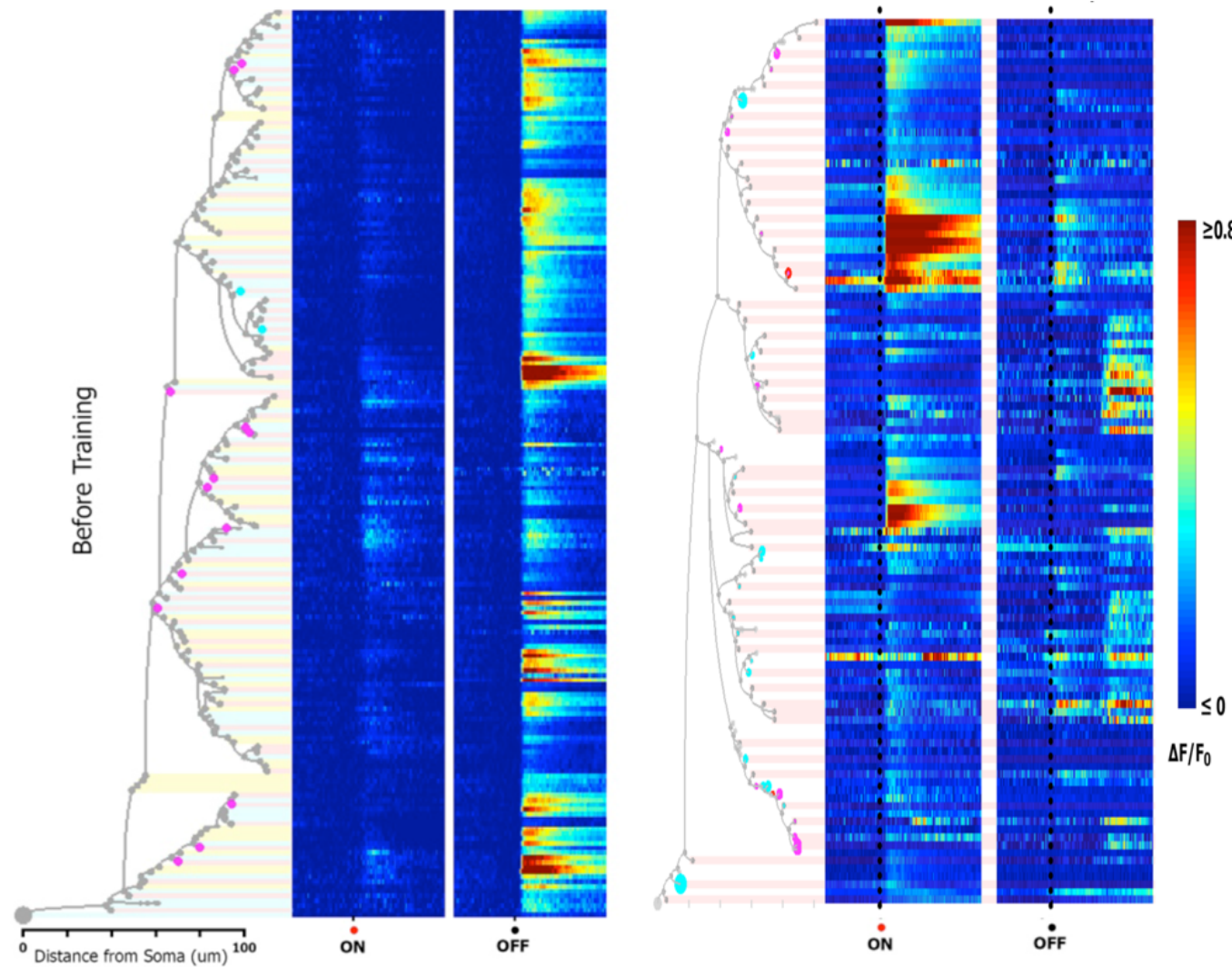
Clustering of filopodia with shared tuning is activity-dependent

Cell 1

Cell 2

ON OFF

ON OFF



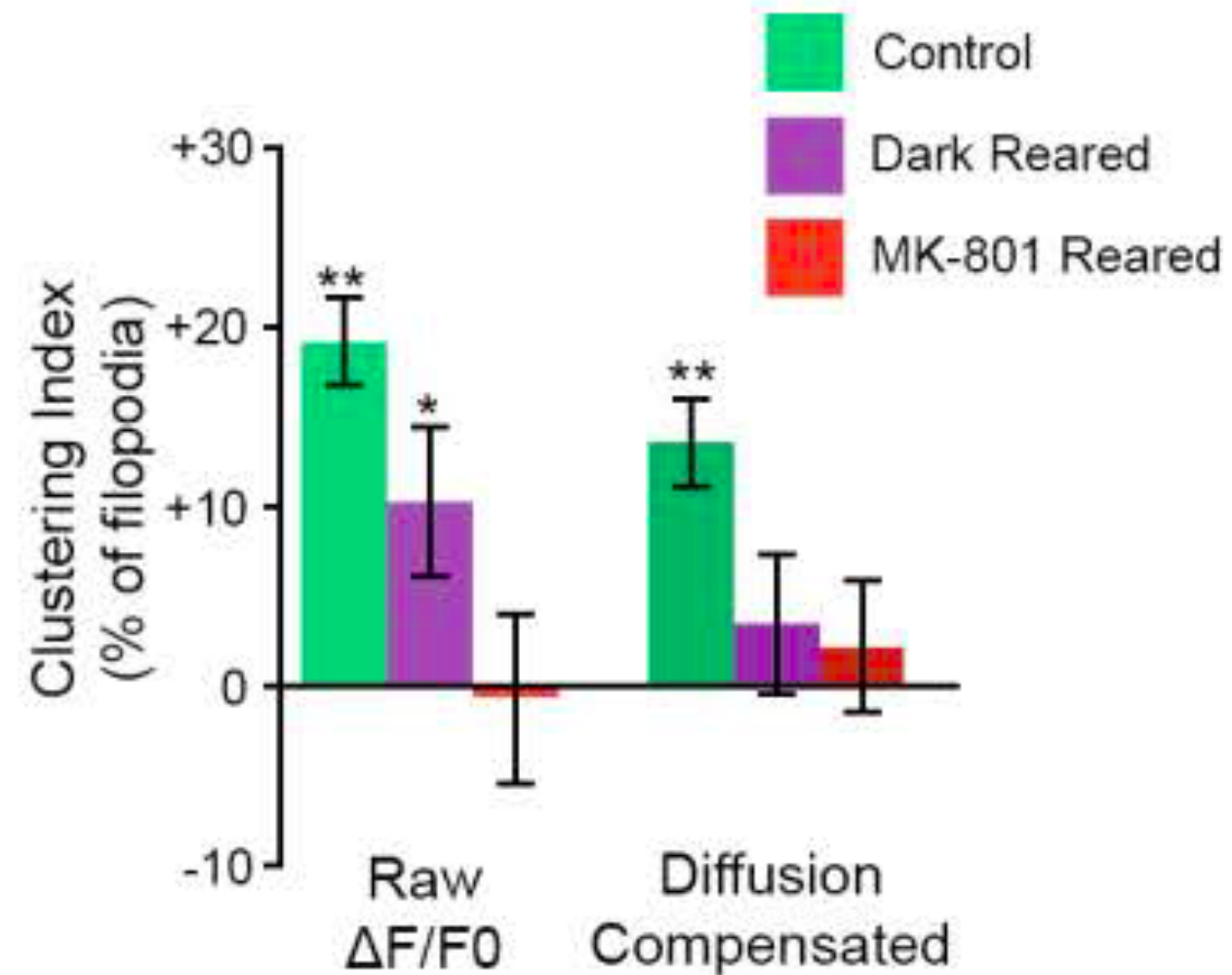
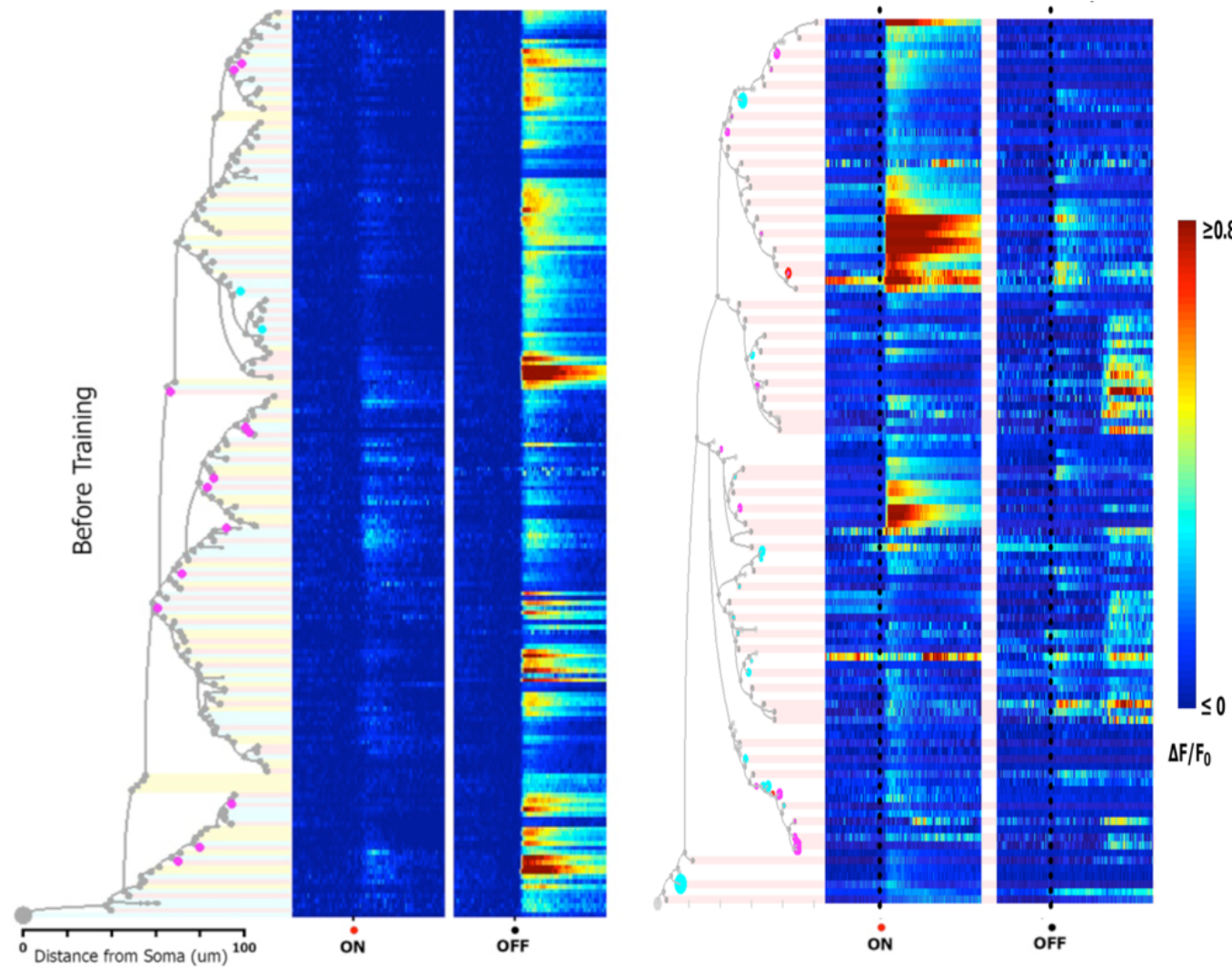
Clustering of filopodia with shared tuning is activity-dependent

Cell 1

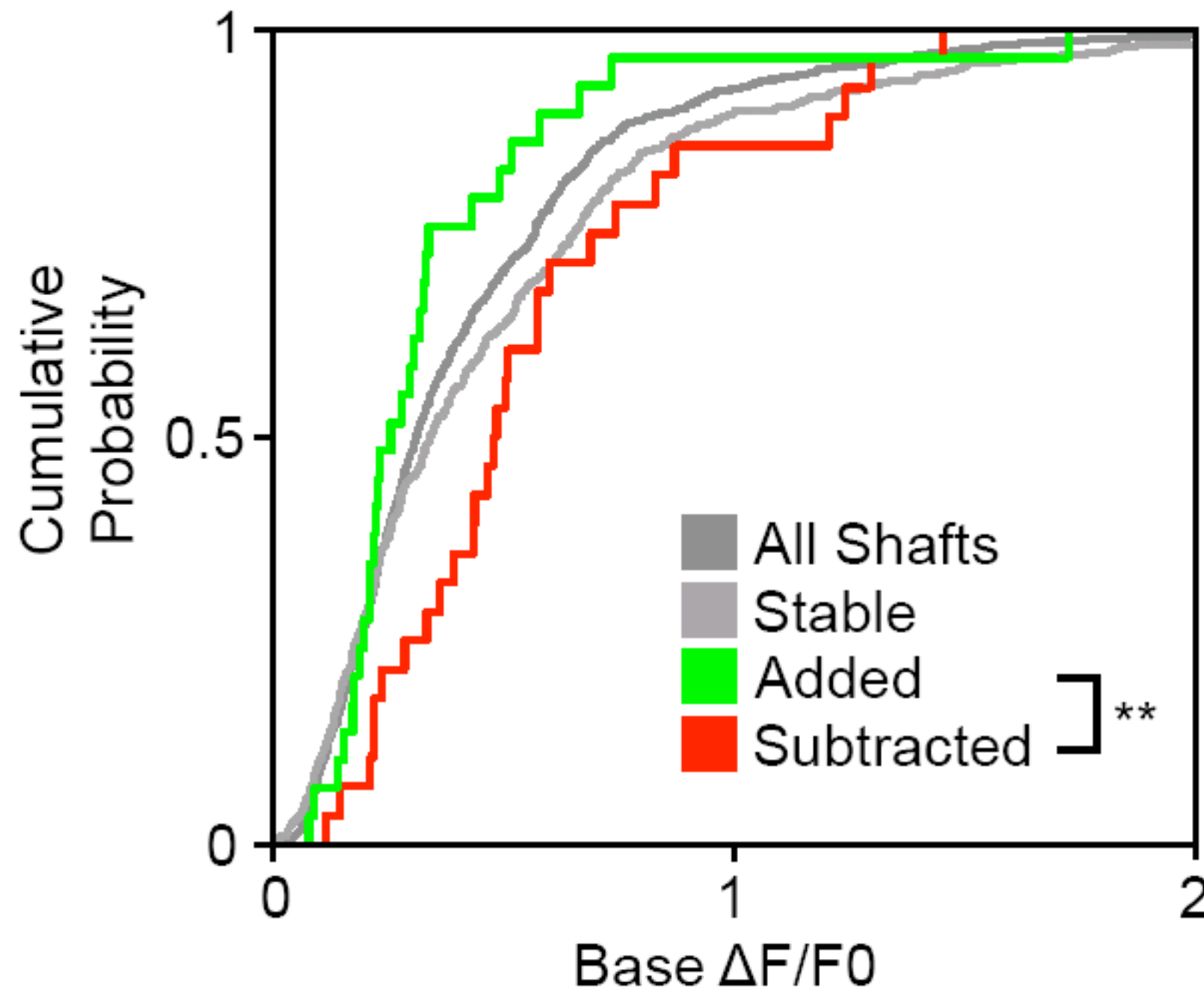
Cell 2

ON OFF

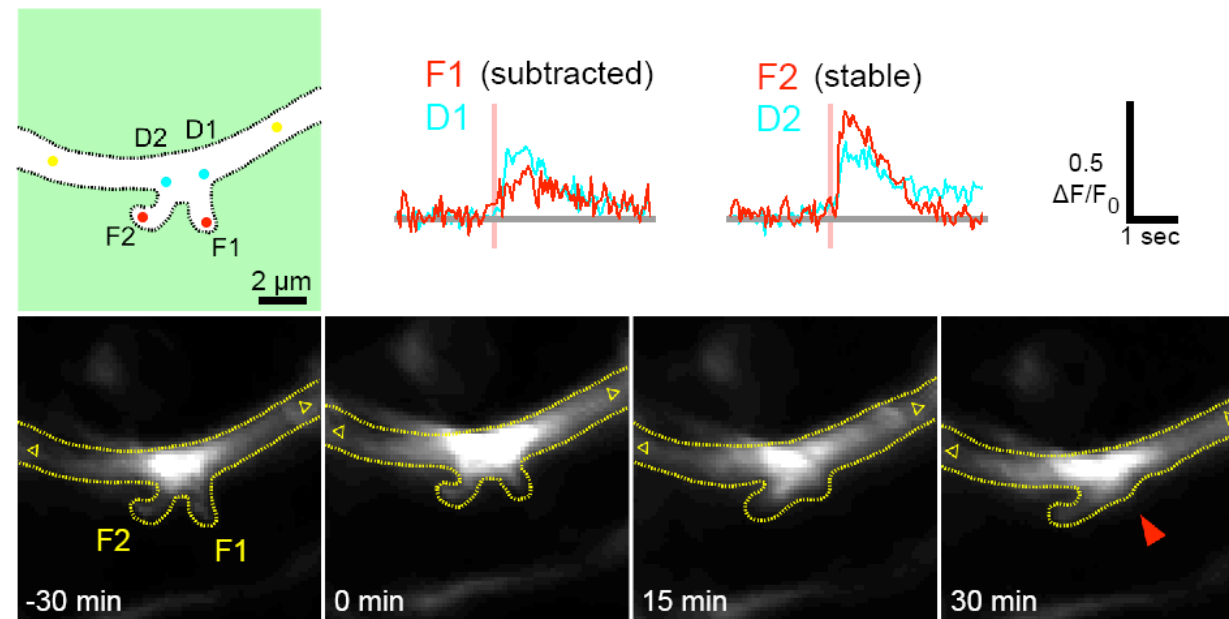
ON OFF



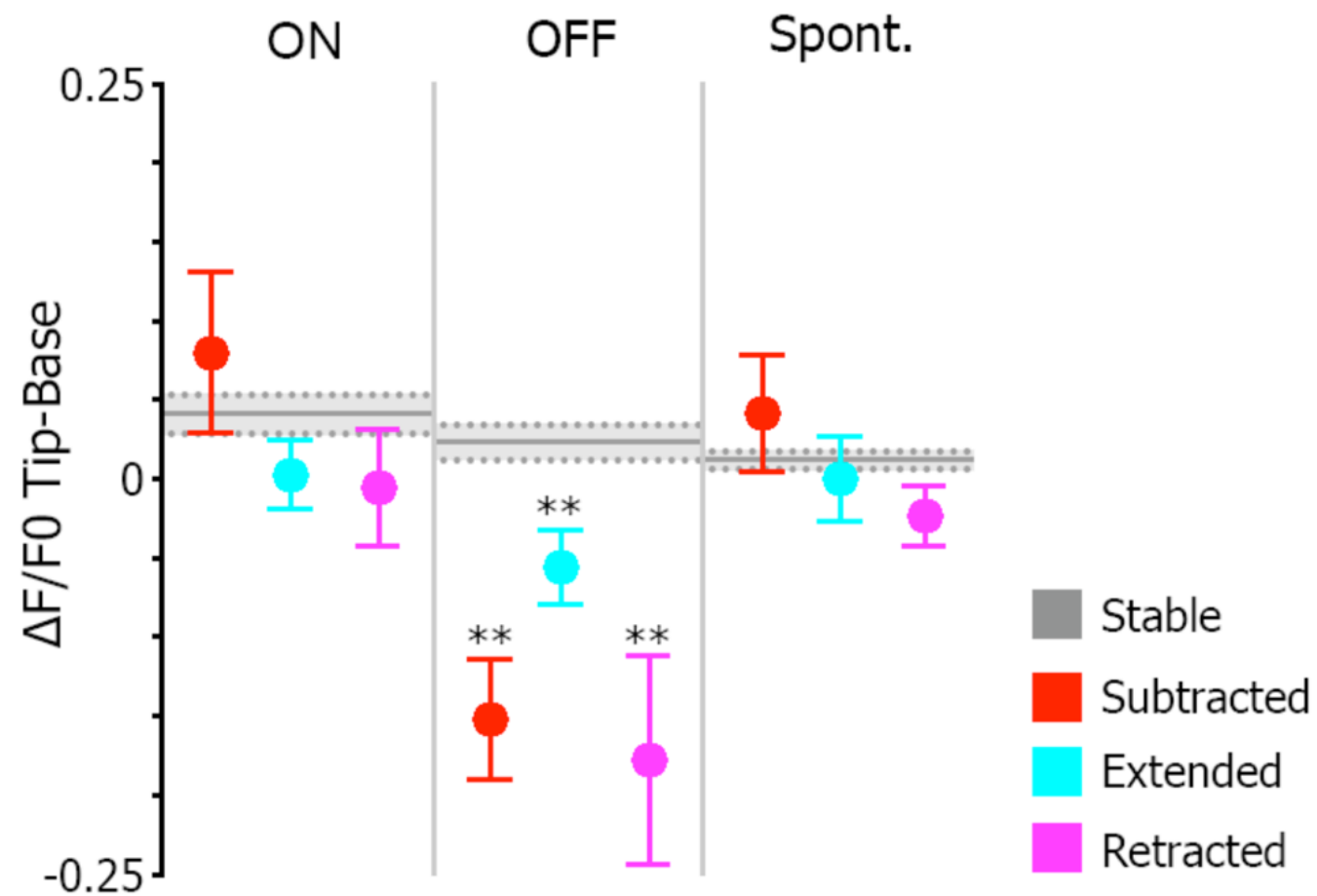
Additions occurred in regions with moderate calcium
Subtractions occurred in dendritic regions with high evoked calcium



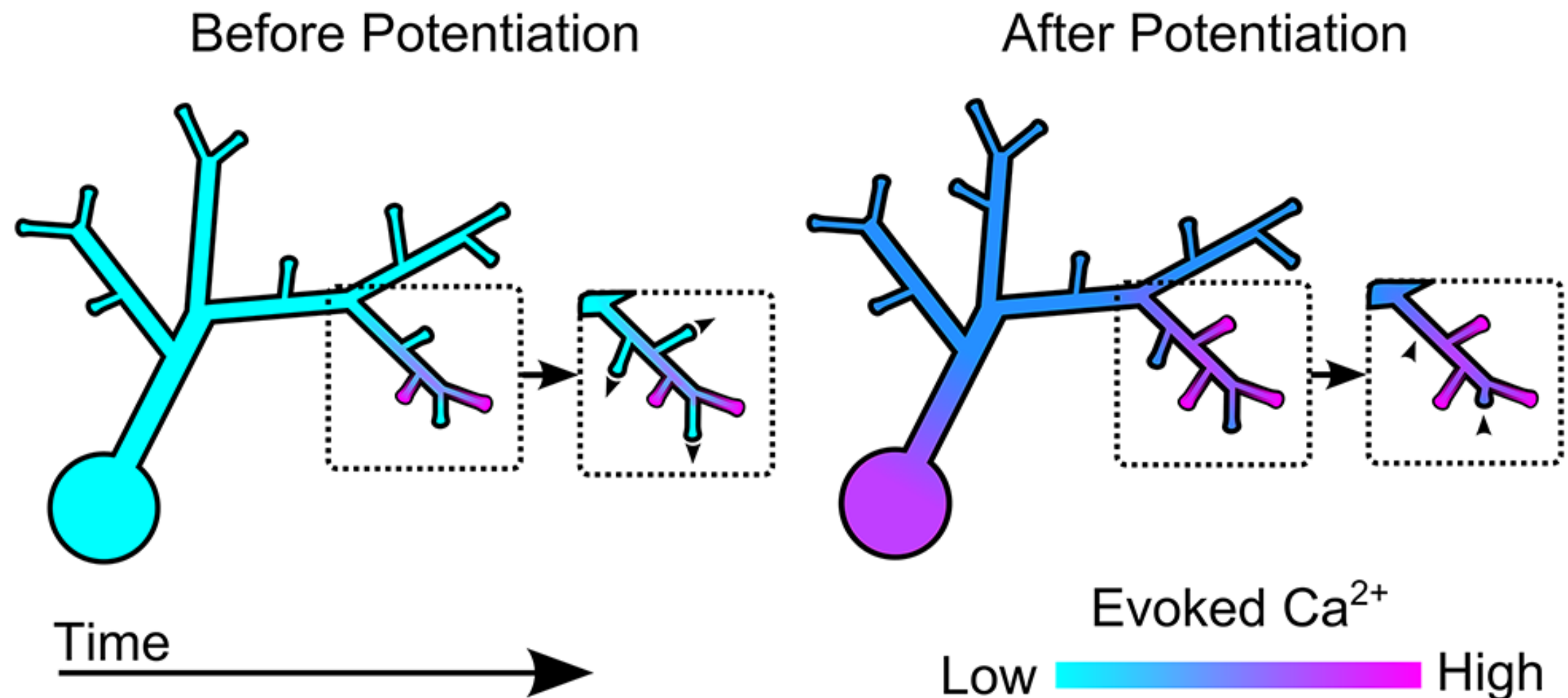
Growth behavior predicted by baseline tip vs shaft calcium levels



Spaced Training



Model for experience-driven structure synaptogenesis and dendrite growth, and honing or shifting of receptive fields



Conclusions

Sensory stimuli directs brain neuronal growth

Growth is dependent on the relevance of that input to each neuron's tuning

Experience-driven dendritic growth involves simultaneous LTP and LTD at different synapses across the arbor

Clustering of synapses sharing tuning properties, which is NMDAR and experience dependent

Rules of local calcium predict experience-driven growth

Haas Lab:

Warren Meyers

Fabian Meili

Wun Chey Sin

Kathryn Post

Peter Hogg

Patrick Coleman

Tristan Toth

Kasper Podgorski

Simon Chen

Serhiy Opushnyev

Derek Dunfield

Janaina Brusco

Kelly Sakaki

Lara Thompson

Kathryn Post

Dong Hwan Kim

Sesath Hewapathirane

Xuefeng Liu

Blair Duncan

Sharmin Hossain

Philip Edgcumbe

Alessandro Cau

Sina Safabakhsh

Janet Huang

Jenny Lee



Djavad Mowafaghian Centre for Brain Health