A microscopic image of neurons, likely from a degenerating brain, showing a dense population of cells. The neurons are stained with a red dye, highlighting their cell bodies and nuclei, while some structures are stained in white or light gray. The overall appearance is a complex, textured field of cells.

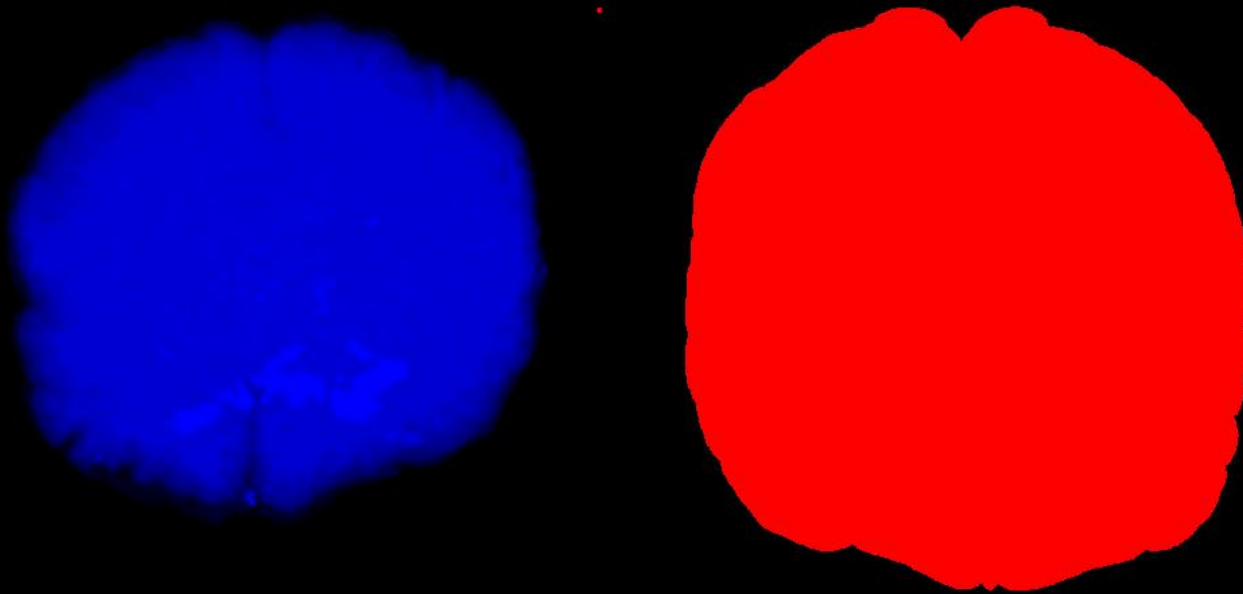
# **Monitoring and predicting the fate of neurons in a degenerating brain**

**Theodore Zwang**

**Assistant Professor of Neurology**

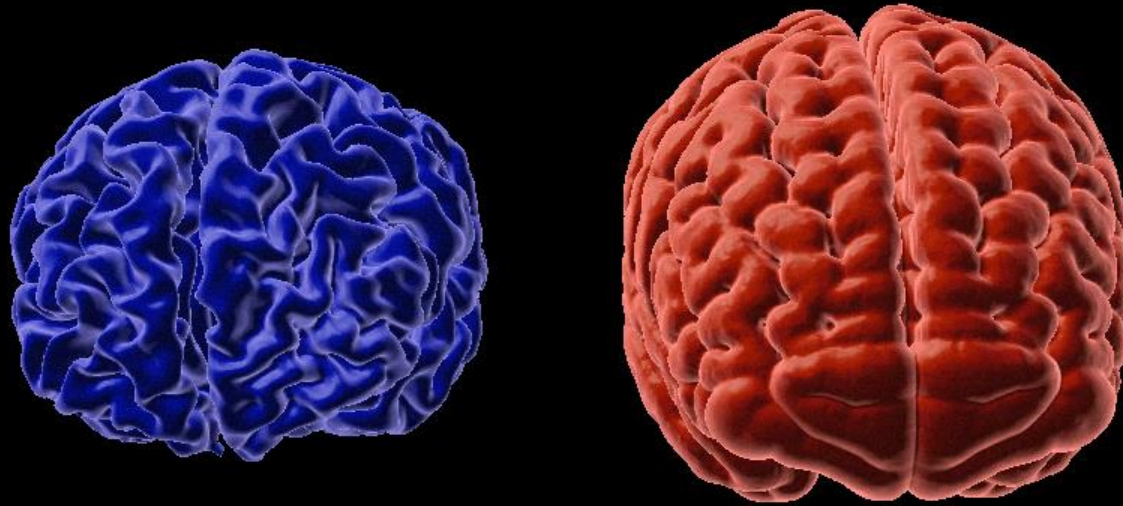
**Massachusetts General Hospital and Harvard Medical School**

# What happens in Alzheimer's disease?



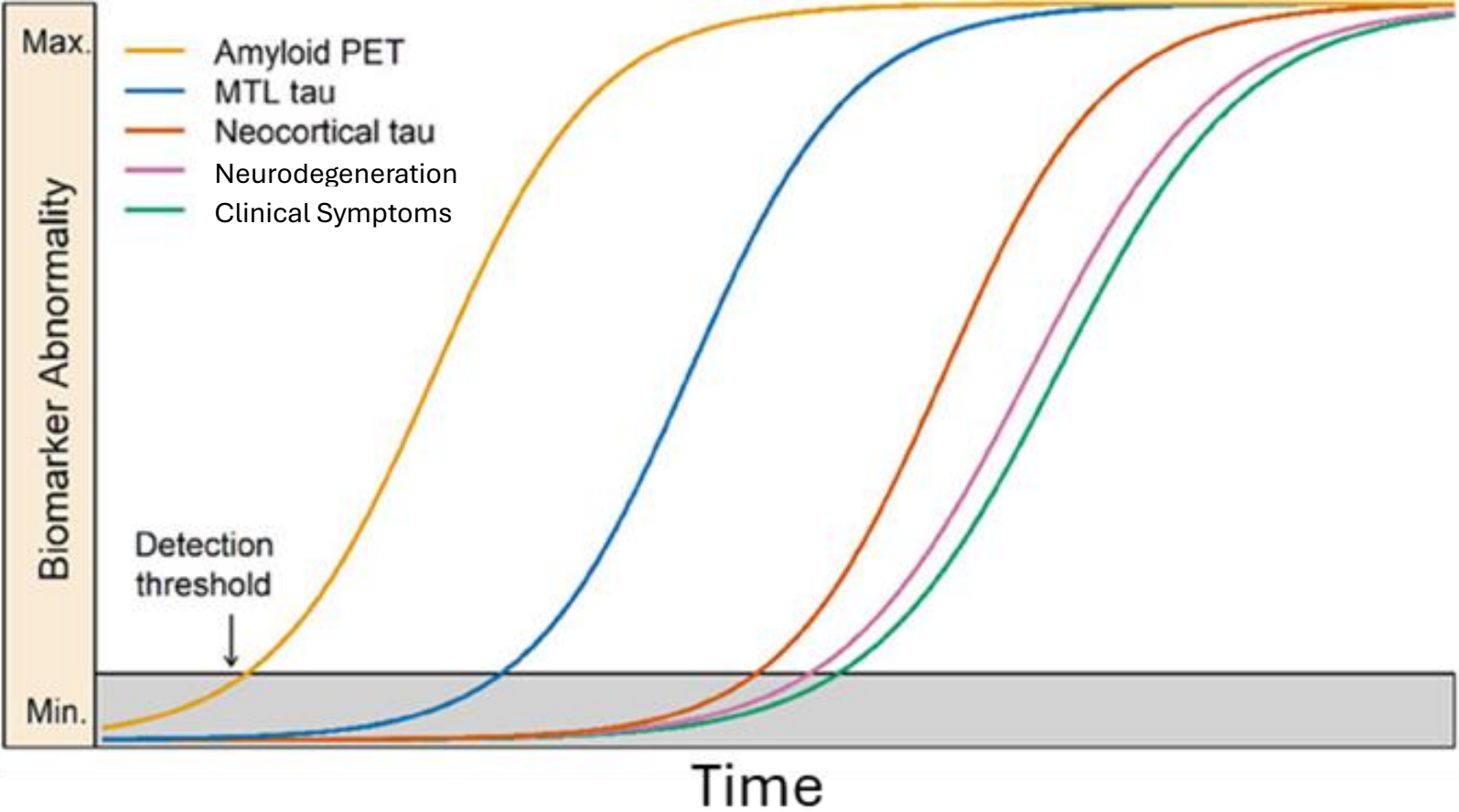
Rachel Bennett (Alzheimer's brain, blue) and Allen Brain Institute (Healthy Brain, red)

# What happens in Alzheimer's disease?



Rachel Bennett (Alzheimer's brain, blue) and Allen Brain Institute (Healthy Brain, red)

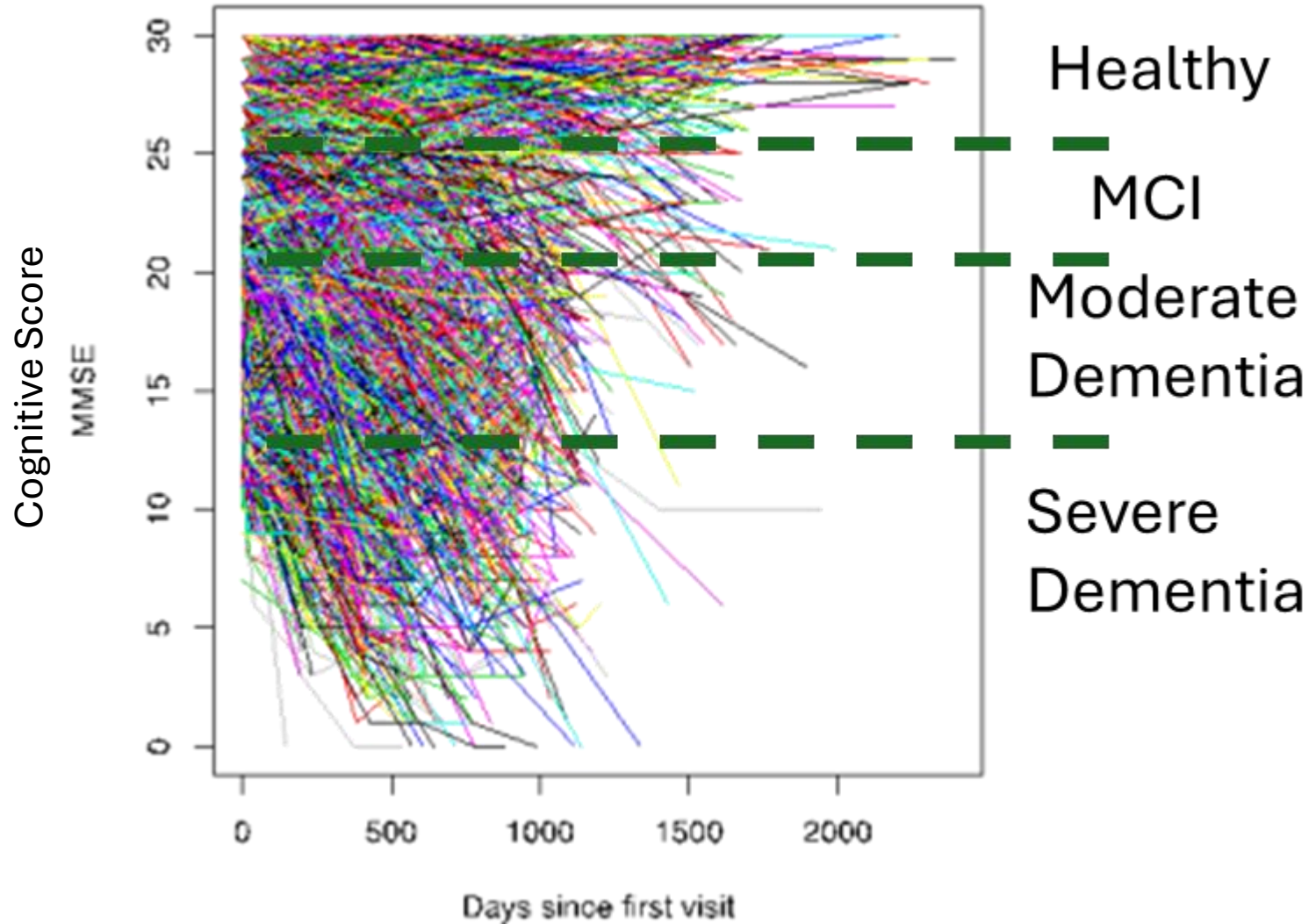
# Archetypal sequence of changes in Alzheimer's Disease



To develop therapies to treat neurodegenerative diseases, like Alzheimer's disease, a key step is the establishment of reliable assays to predict and monitor neurodegeneration

# Substantial variability in the trajectory of dementia

An example of varied trajectory for cognitive scores in a cohort of patients with Alzheimer's Disease

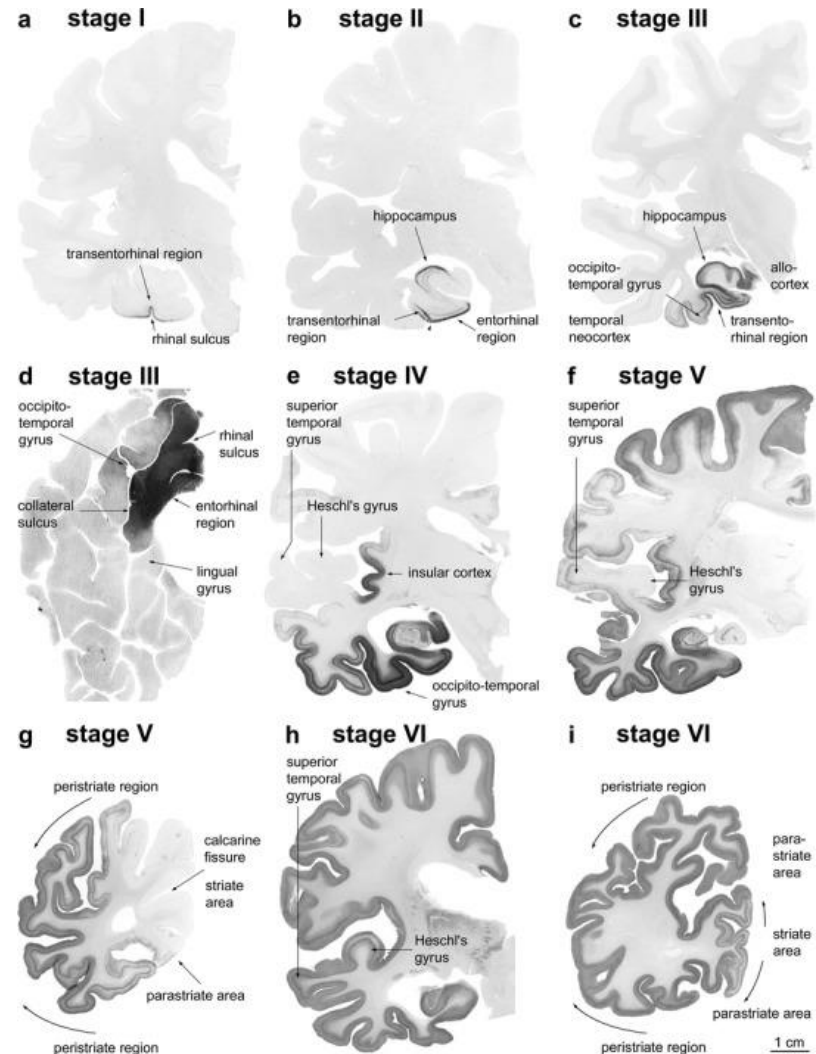


# Historically, using biology to predict disease trajectory has been difficult

## Braak staging of Tau pathology in Alzheimer's Disease

Most of what we know about what happens in the brain of a person with Alzheimer's disease comes from post-mortem tissue

Timelines are created based on snapshots from multiple different brains, rather than watching the same brain over time.

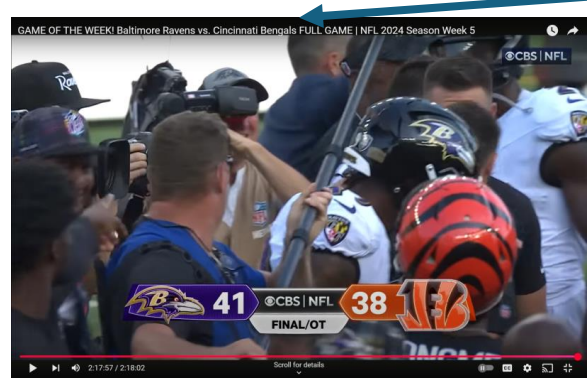
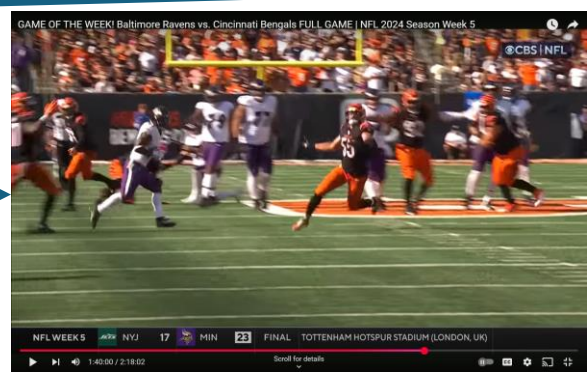
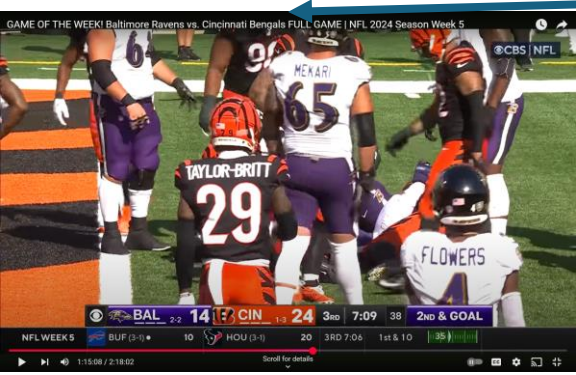
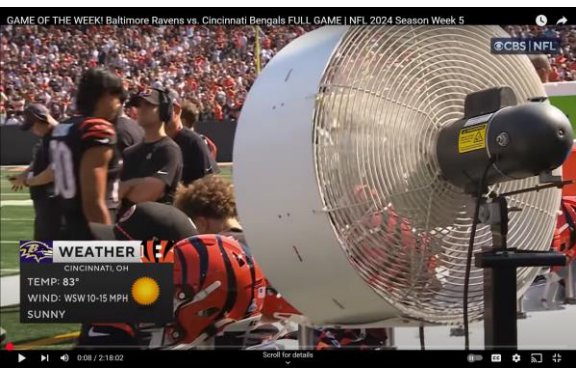


This is like trying to understand a sports game by only seeing the final seconds.



From these images can you tell what game this is? Can you tell how it's played?

# New methods can observe living people, with limited time resolution.



Only 8% of the runtime of a football game involves people playing football. An average play is 4 seconds long.

**What parts of life involves your brain degenerating? Which events are important? What are their timescale?**

Observations strongly impact the hypotheses that we develop and test

How much of a game would you have to watch to understand what's going on?

To be able to predict the winner of a game? To understand injuries that happen during the game?

“There are 22 people on a green field”

“There's one ball”

“People with one color outfit are trying to take the ball from people in another color outfit”

“Except sometimes they throw the ball to people wearing the other color?”

“There's a giant fan”

“Often people start dancing in the middle”

“The game is over when a lot of people rush the field”

So... based on these observations if we flood the field with people to stop the game, can we prevent players from getting injured?

**Treatments are informed by observations and optimized for things that can be measured**

And from these observations we will often make mouse models based on what we see and try to study it.



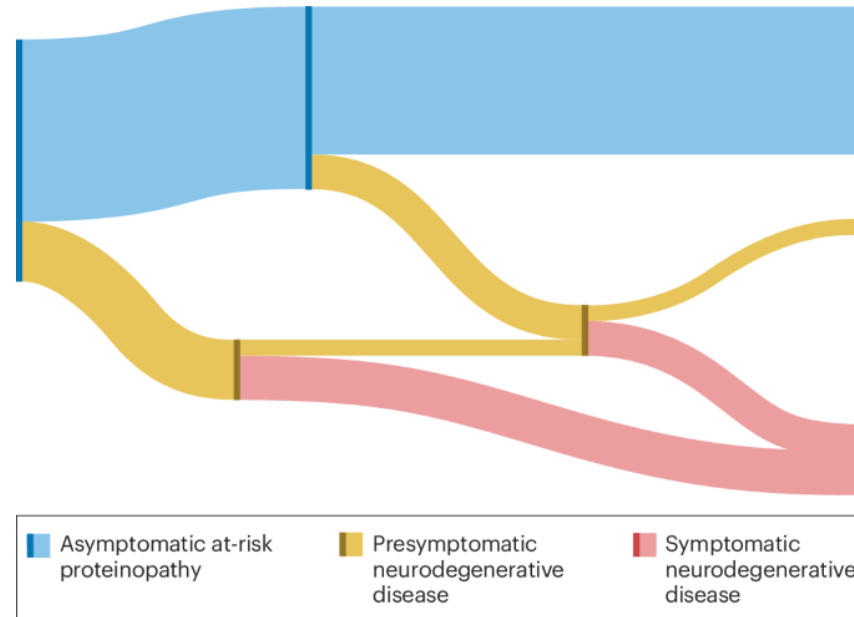
We “recreated” what we saw. Is this mouse also getting brain damage?

# New measurement tools are leading to changes in the classification of Alzheimer's disease

- Biological definitions have the potential to transform the perception of neurodegenerative diseases
- **Current perception: Neurodegenerative diseases have debilitating outcomes in the vast majority of cases**
- **Reality: They are a spectrum of conditions with heterogeneous prognoses, including many benign clinical conditions.**
- Categorizing low-risk asymptomatic conditions as separate entities, similar to the approach in the field of cancer, could help to avoid misconceptions and allow better targeted treatments.
- **Important question: Are we measuring the right things?**

# Insight from new methods to monitor disease progression *in living patients*

Hypothetical lifetime trajectories of asymptomatic individuals with evidence of pathology.



Among those who meet this biological definition of Alzheimer's disease:

~8% have dementia

~17% have mild cognitive impairment

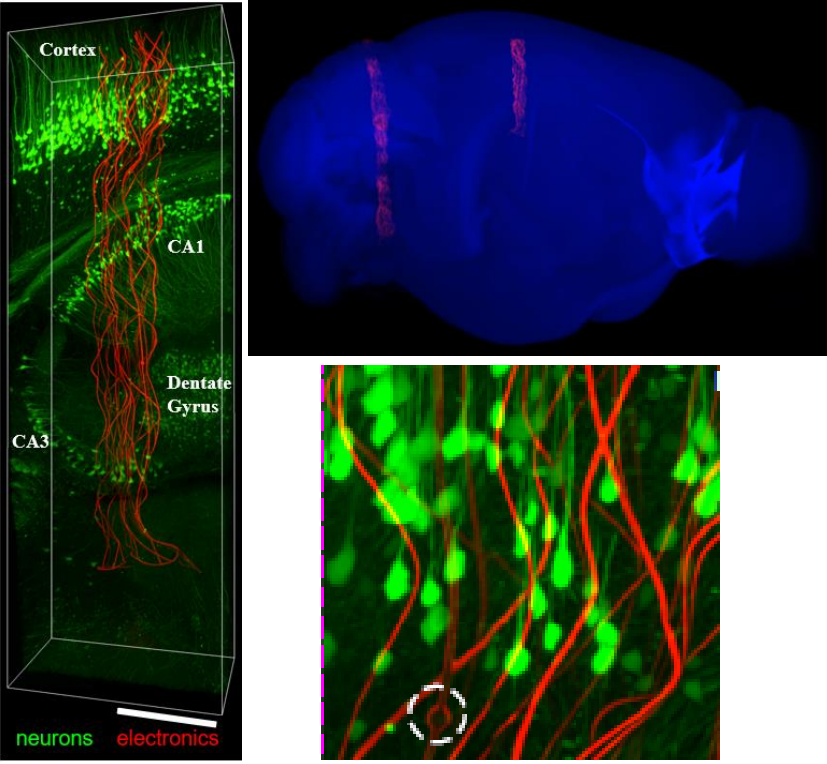
~76% have no cognitive impairment.

Therefore, the majority of individuals who meet this biological Alzheimer's disease criteria are asymptomatic and will not develop dementia in their lifetime

# Lab goal: Can we develop new ways to monitor changes caused by Alzheimer's disease that inform trajectory and treatment?

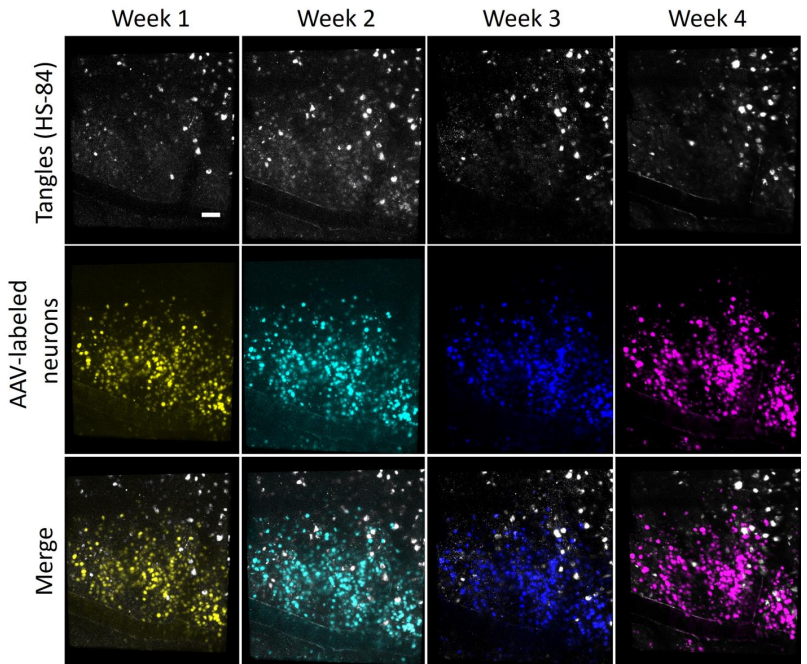
## Area 1: Functional Changes

Recording electrophysiology from individual neurons and their circuits over months to determine when they become dysfunctional



## Area 2: Structural Changes

Imaging the formation of tangles and death of neurons in a living brain

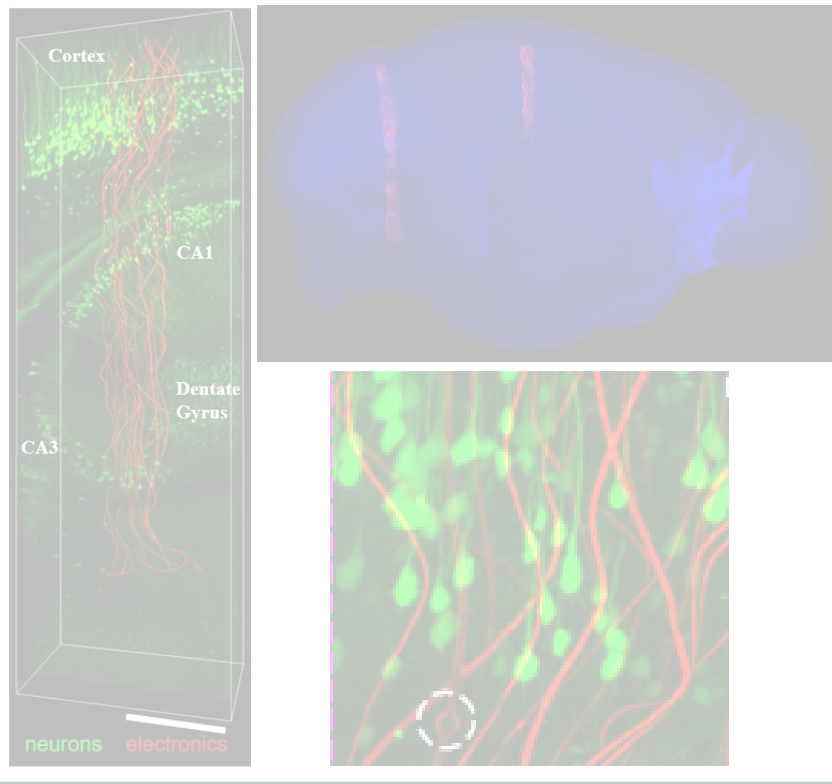


Can we use this information to predict or change the fate of neurons in a degenerating brain?

# Lab goal: Can we develop new ways to monitor changes caused by Alzheimer's disease that inform trajectory and treatment?

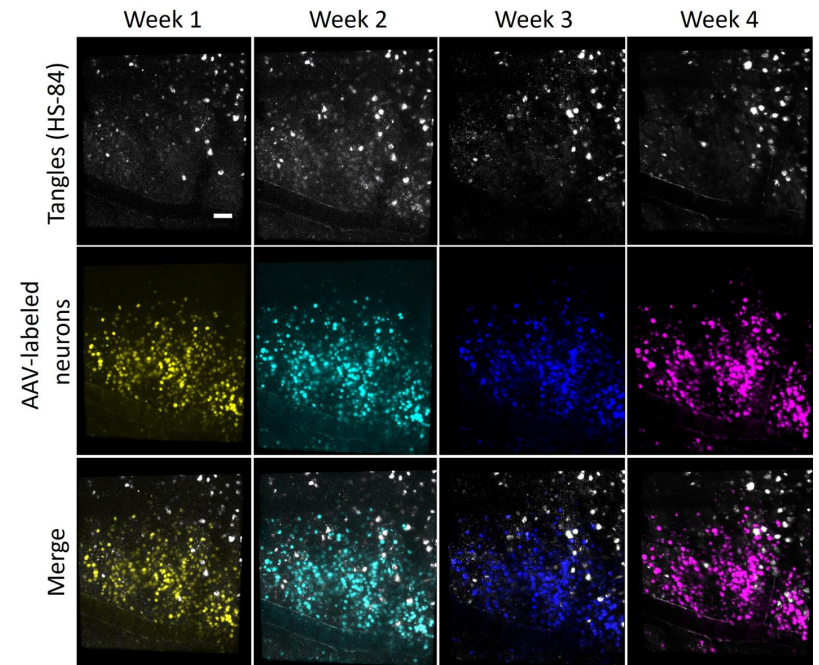
## Area 1: Functional Changes

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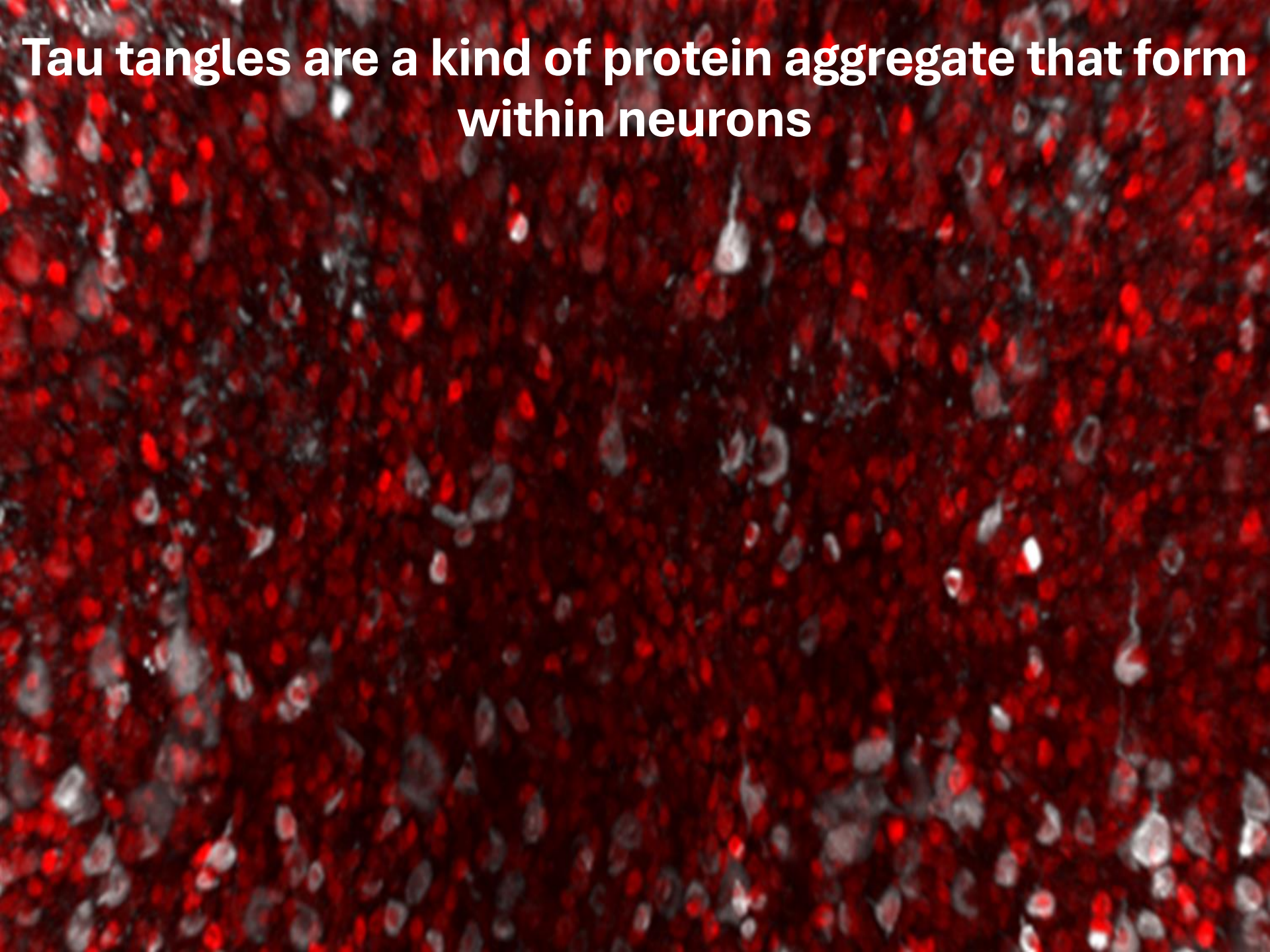
## Area 2: Structural Changes

Imaging the formation of tangles and death of neurons in a living brain



Can we use this information to predict or change the fate of neurons in a degenerating brain?

**Tau tangles are a kind of protein aggregate that form within neurons**



# Mice that express human tau and show gradual tau tangle formation over months can be used to model its effects on the brain

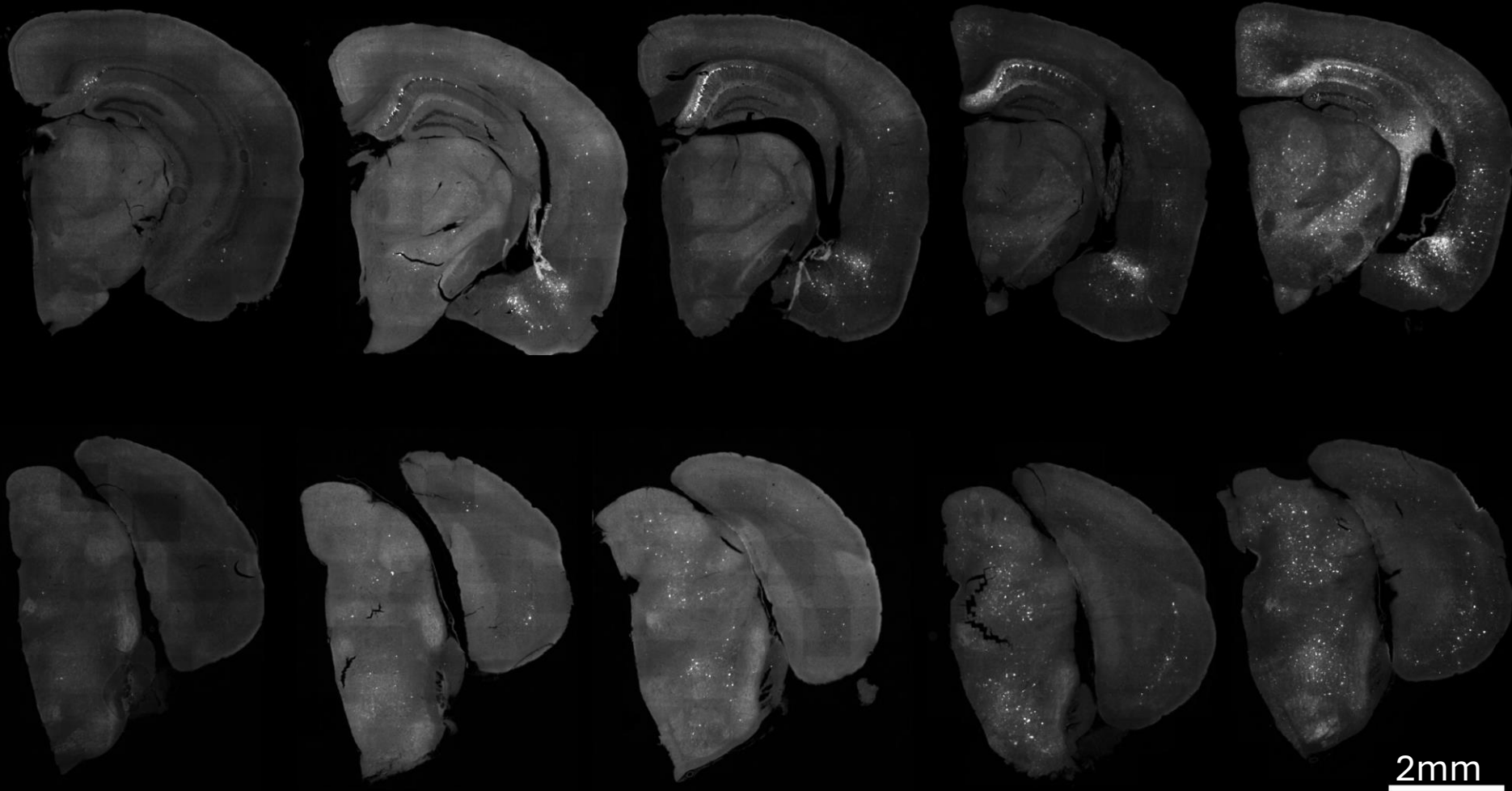
3 Months

6 Months

9 Months

12 Months

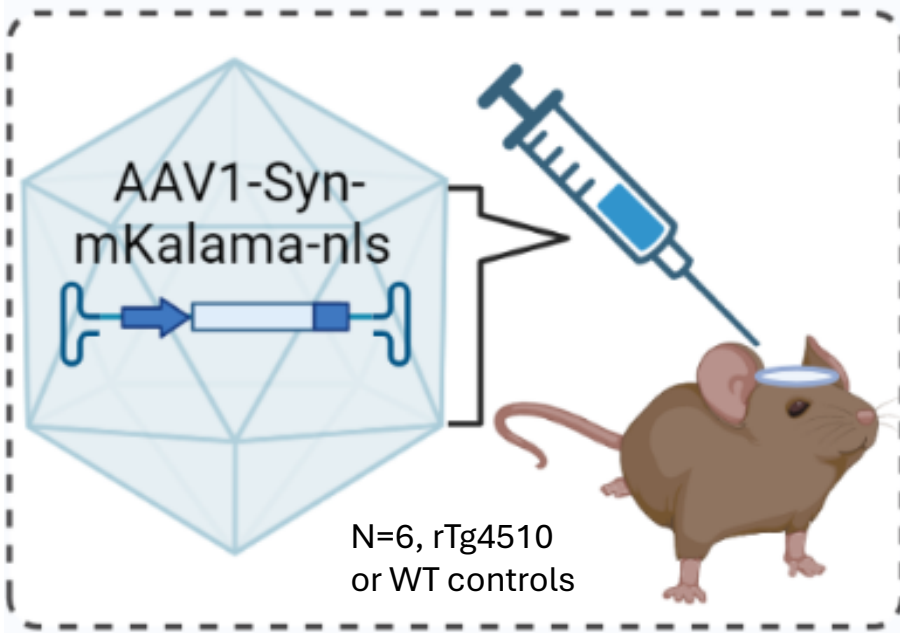
24 Months



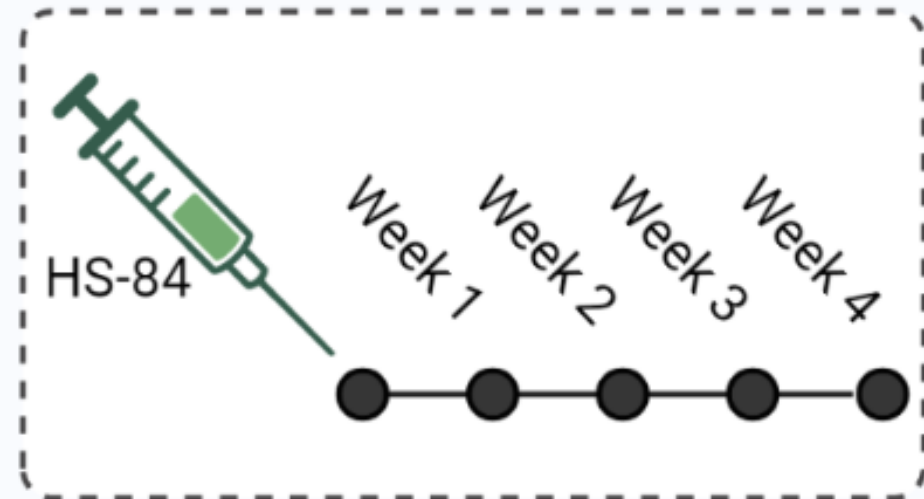
AT8 labeled Thytau22 sections

# Experimental Design: Overview

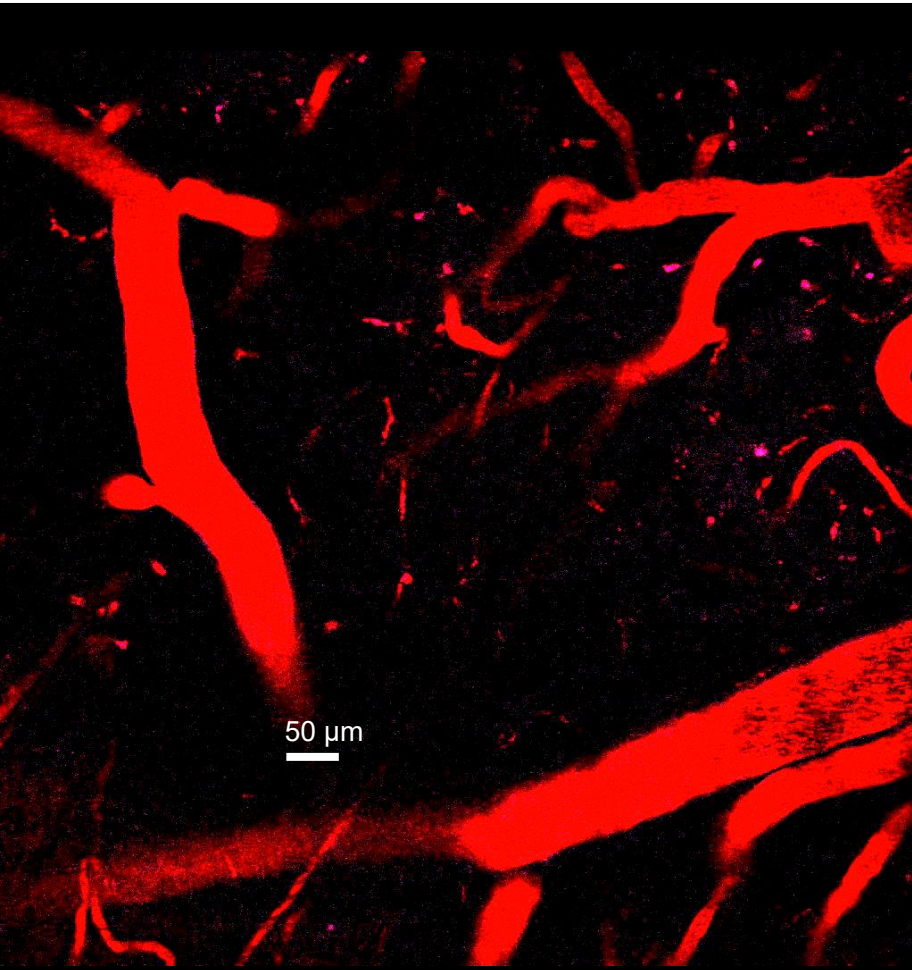
## AAV injection + Cranial Window



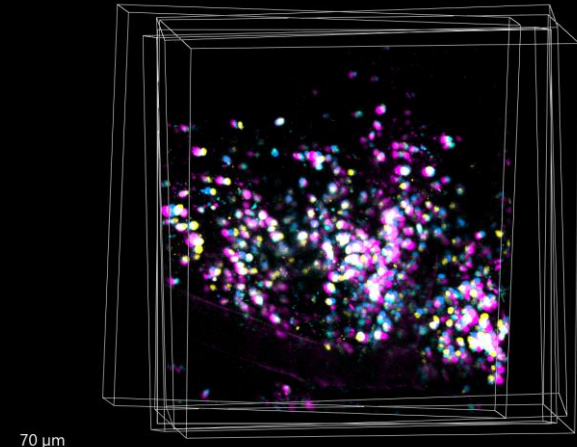
## Dye Injection + Longitudinal Imaging



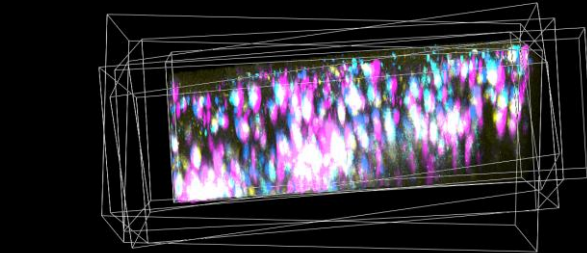
# Imaging and Analysis



Neurons (AAV-mkalama)  
Tau (HS84) Blood Vessels



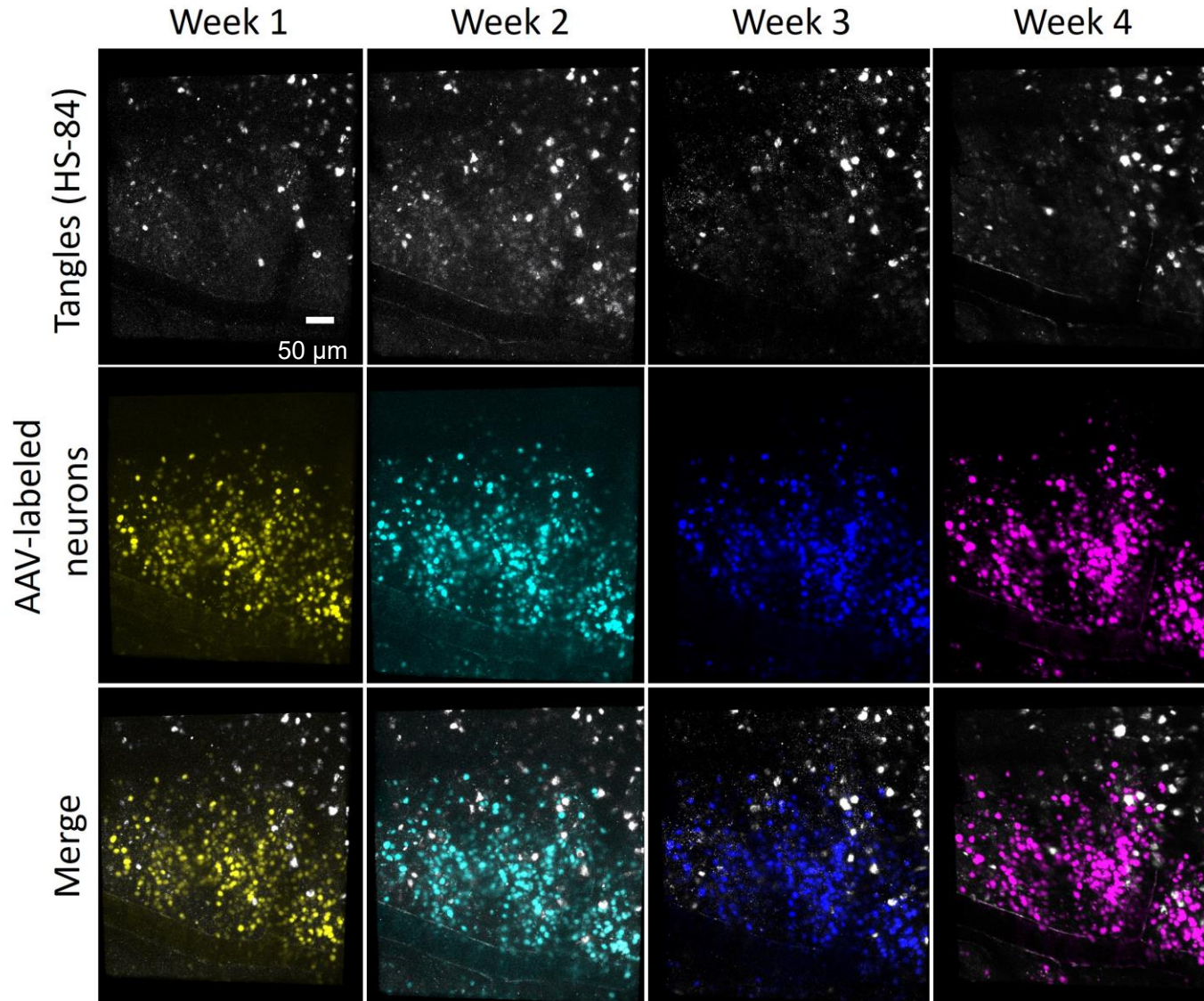
Top View



Side View

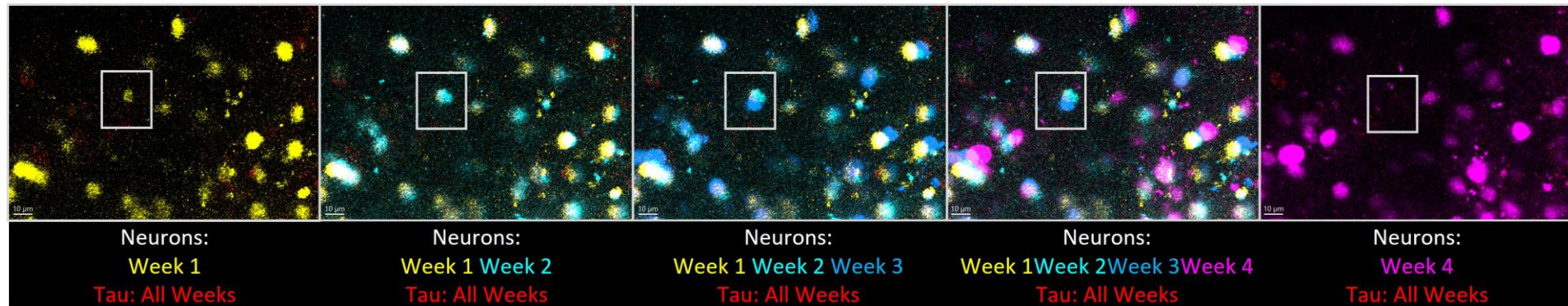
Week 1 Neurons  
Week 2 Neurons  
Week 3 Neurons  
Week 4 Neurons

# Visualizing neurons and tangles in vivo



Tracked 1721 neurons & 235 tangles in n=6 rTg4510 mice

# Most disappearing neurons do not contain neurofibrillary tangles



(rTg4510 mice, n = 6)

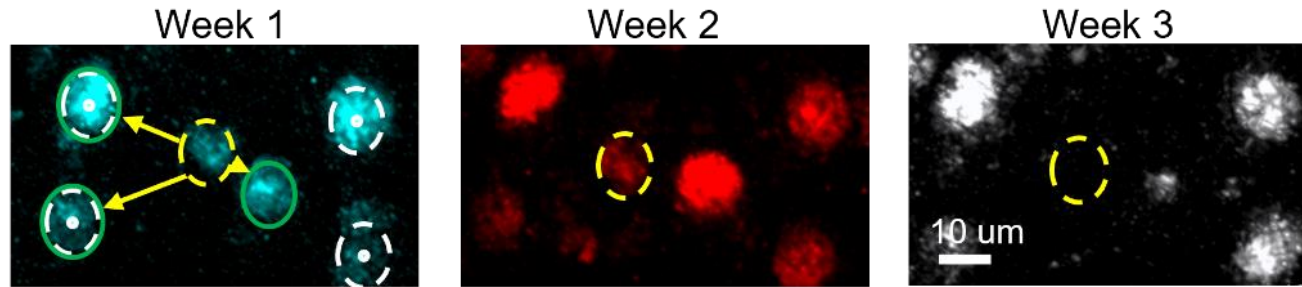
178 neurons were lost across 4 weeks

Only 6 of these lost neurons contained a tangle

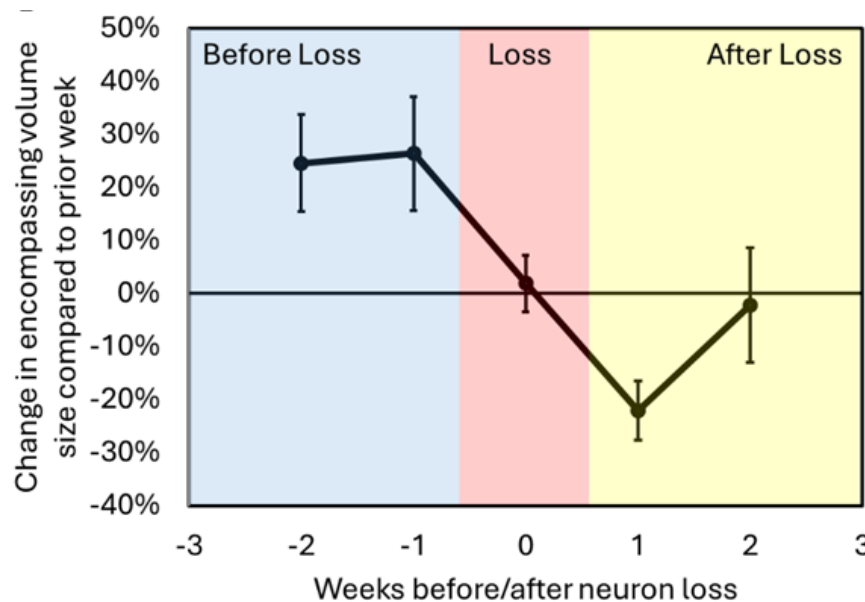
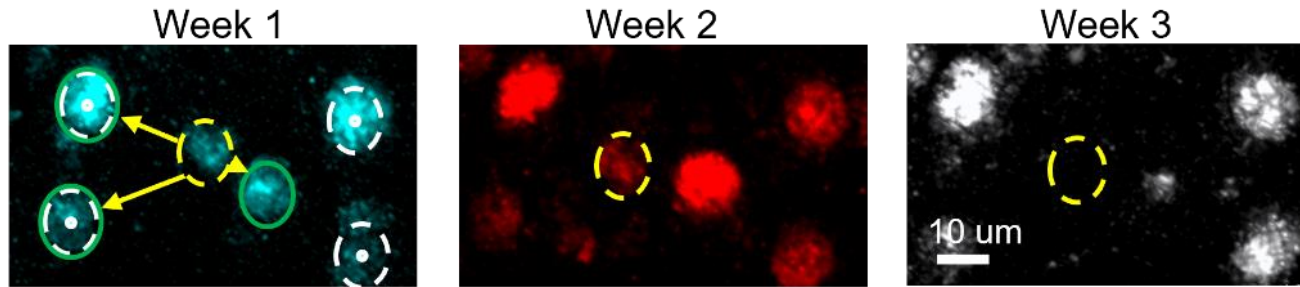
**96% of the neurons that disappeared did not contain tangles**

**Neurons with tangles are >3x more likely to survive**

# Can we “look back in time” and find features that predict a neuron will soon die?



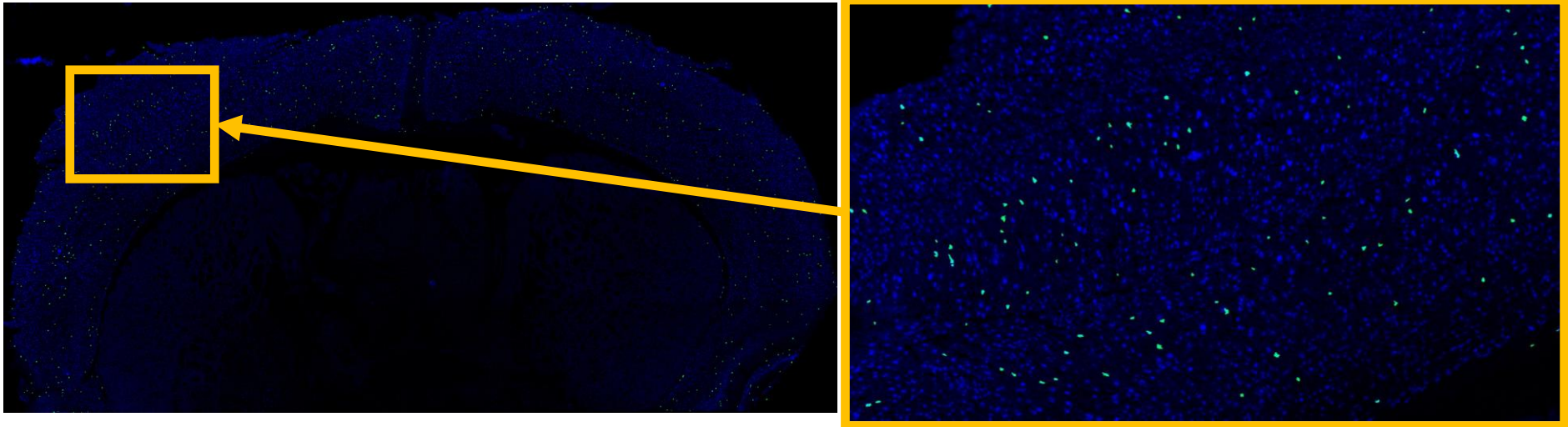
# Can we “look back in time” and find features that predict a neuron will soon die?



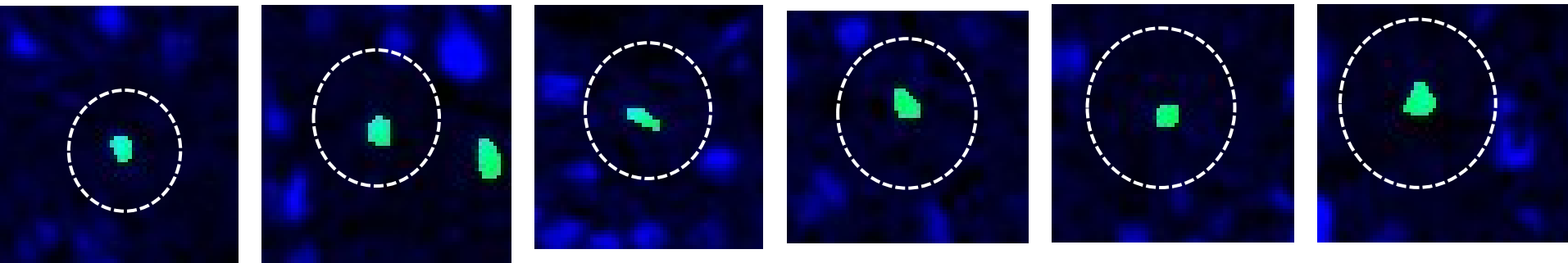
**Timeline show neurons pushed away from a dying neuron for several weeks.**

**The surviving neurons move back in after the dying neuron is lost.**

# Throughout a brain we can identify thousands of neurons that push their neighbors away

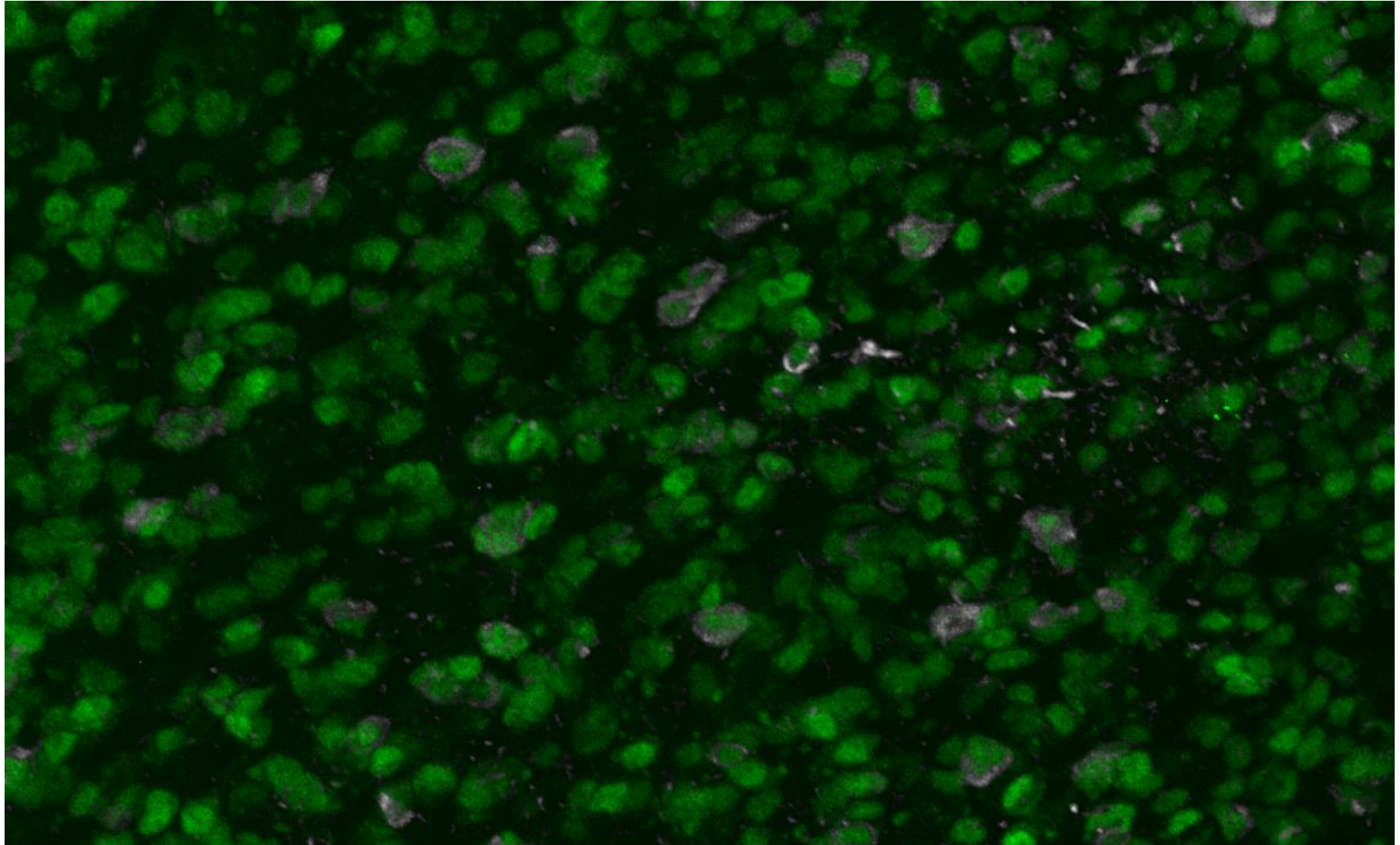


Increasing distance from neighbor = more likely to die within the next week



Neurons whose neighbors move away are at extraordinary risk of dying. A distance increase  $>20\%$  over one week corresponds to  $46.7\%$  ( $\pm 3.3\%$ ) dying the following week.

# Do we see evidence of this happening in people with Alzheimer's disease?

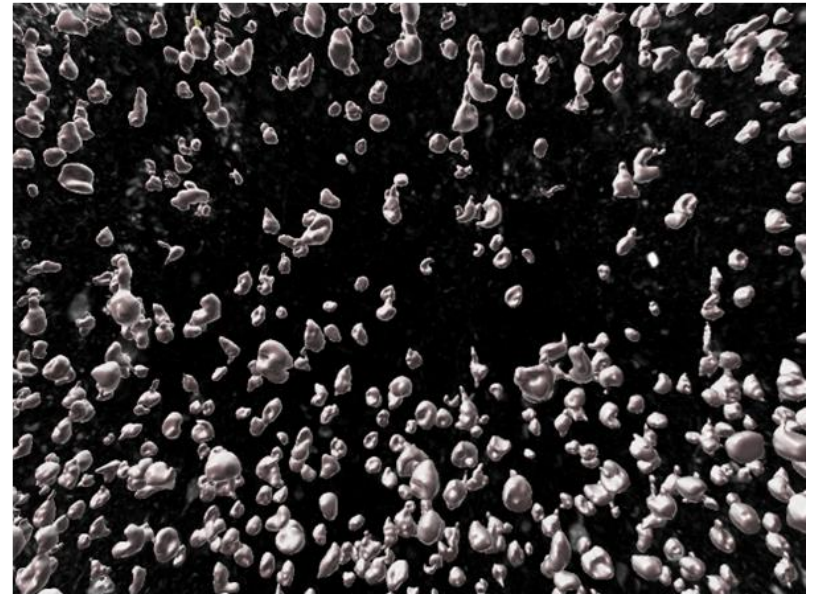
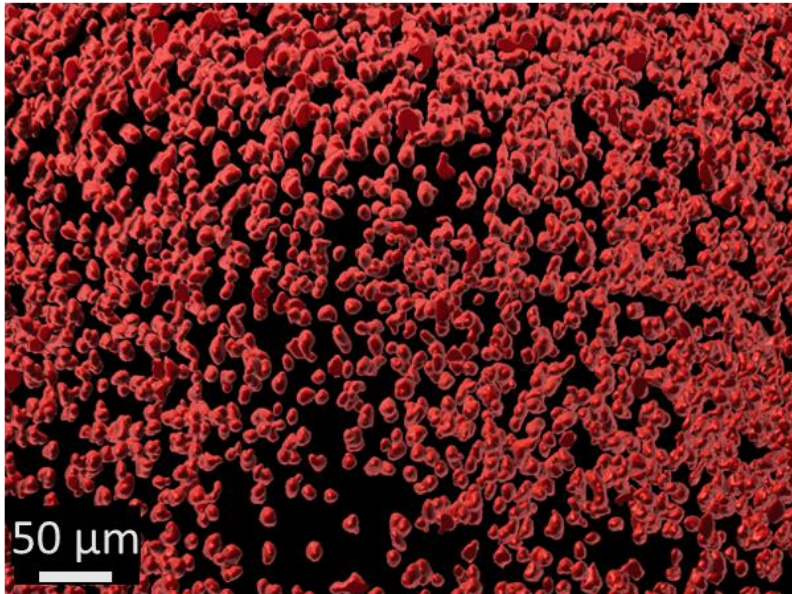
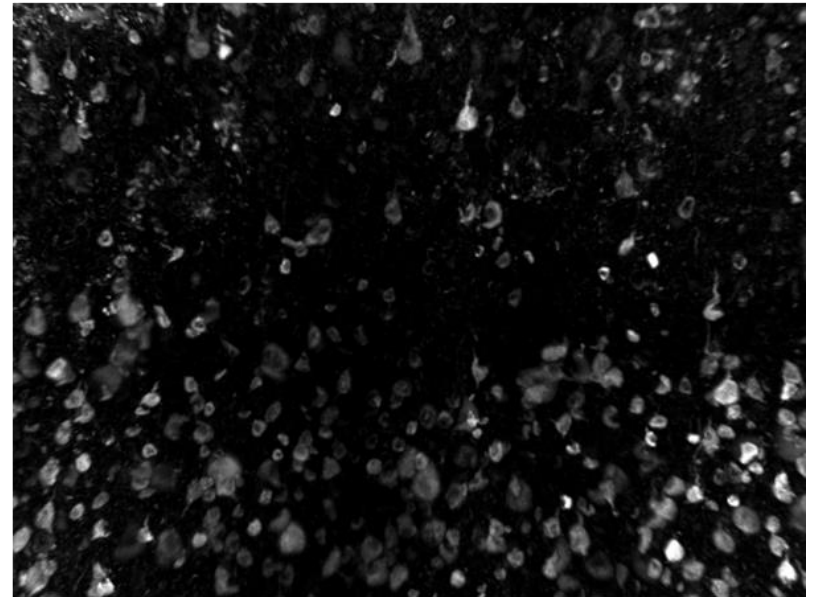
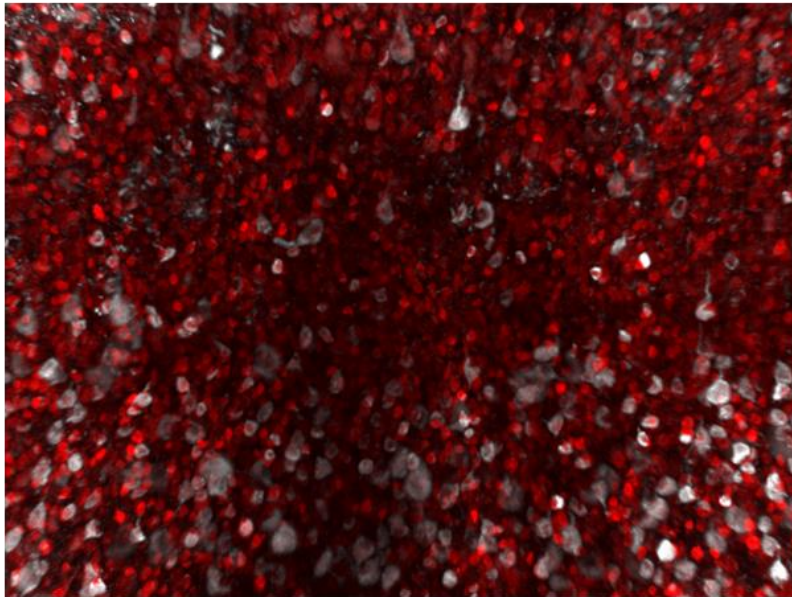


green = HuD-labeled neurons, white = AT8-labeled Tau

# Do we see evidence of this happening in people with Alzheimer's disease?

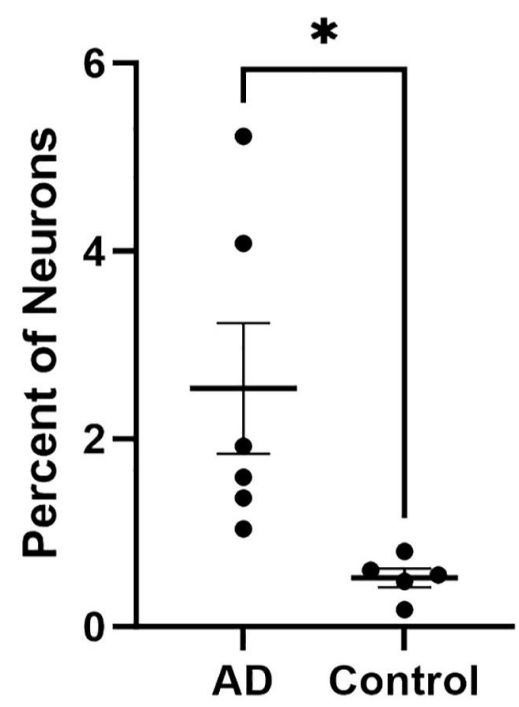
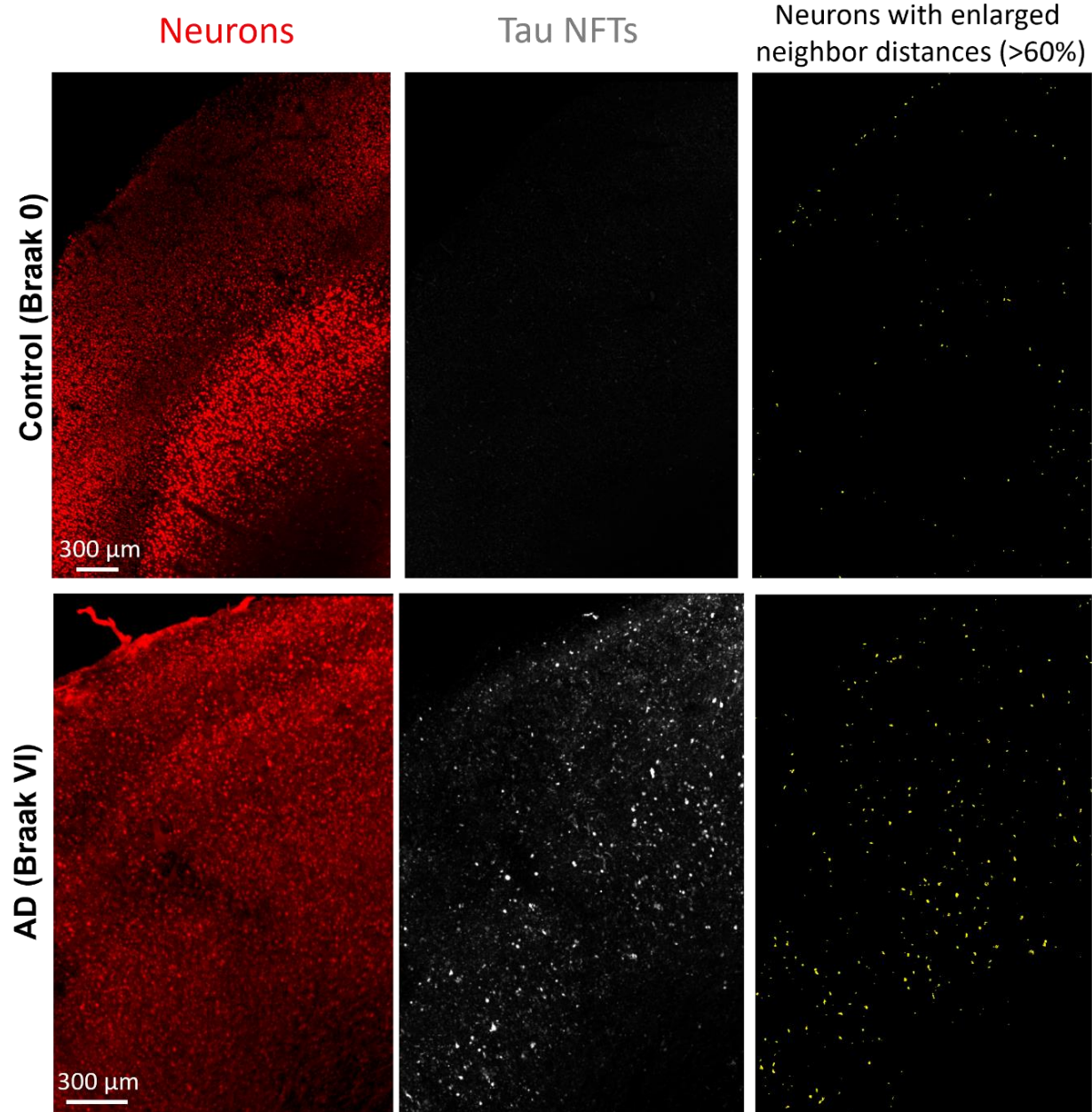
green = HuD-labeled neurons, white = AT8-labeled Tau

# Machine-learning segmentation of millions of neurons and pathology



red = HuD-labeled neurons, white = AT8-labeled Tau

# People with Alzheimer's disease are more likely to have neurons that push their neighbors away



Number of neurons that are predicted to be lost using these measurements matches the predicted rate of neuron loss from MRI data

# Summary

Longitudinal observation of individual neurons allows extraordinary insight into the effects of tau pathology

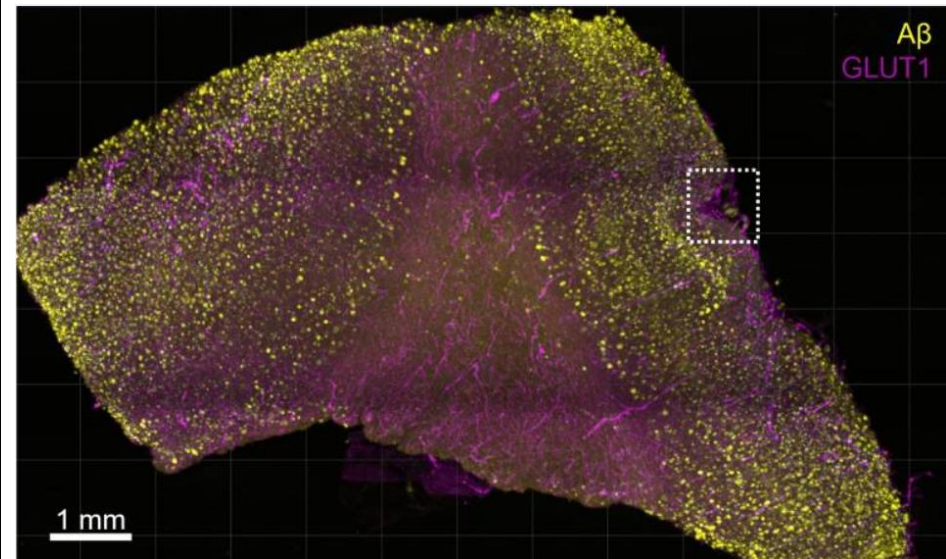
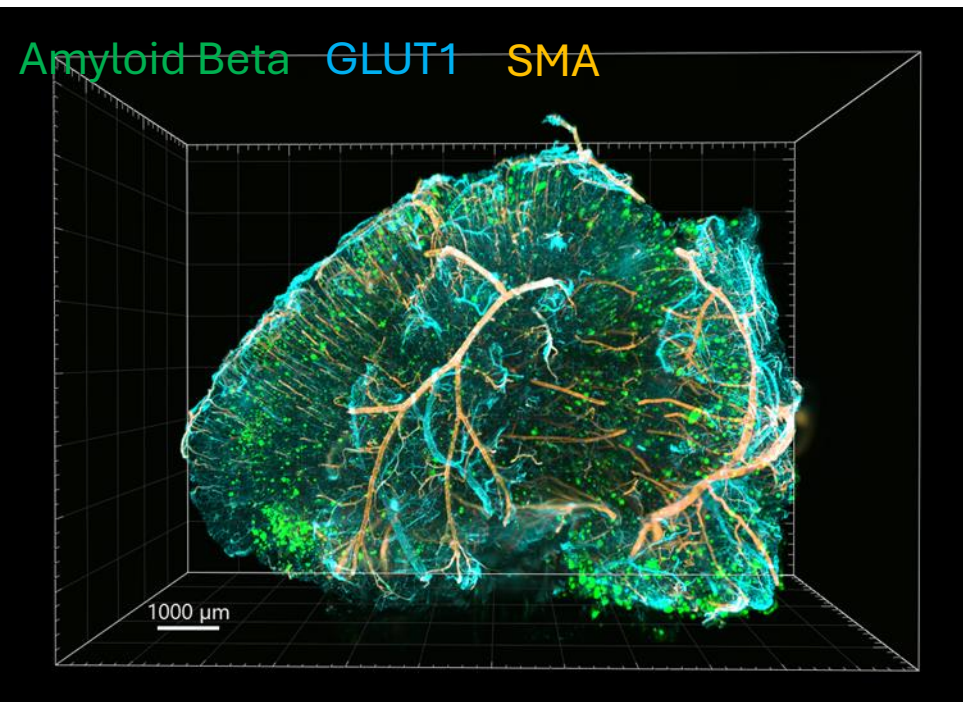
- Neuron loss
  - Neurons with tangles are *less likely to die*
  - A neuron's neighbors flee its vicinity starting >2 weeks prior to its death
- Predicting fate
  - 50% of dying neurons could be identified 1 week ahead of their death
  - 25% of dying neurons could be identified 4 weeks ahead of their death
- **Key observations:**
  - If neuron death isn't immediate, maybe we can do something about it?
  - Other neurons tell that their neighbor is dying. How?!
  - Despite excellent predictive ability, we don't know why this is happening

# What's next? (Thanks to CART!)

- Overall goals
  - Define which populations of neurons show local morphology changes that put them at high risk of imminent loss in mouse and human tissue
  - Identify proteomic signatures that can distinguish these neurons from other neurons
  - Understand the mechanistic pathway(s) leading to the loss of these neurons
- Impact
  - Identifying reliable signatures for neurons that are *imminently dying* in a living brain will prove invaluable for testing treatments that aim to stop Alzheimer's disease
  - These results will be a launching point to test new interventions that suppress identified pathway(s) leading to the death of these neurons, with the hope that we can find treatments that will keep neurons alive

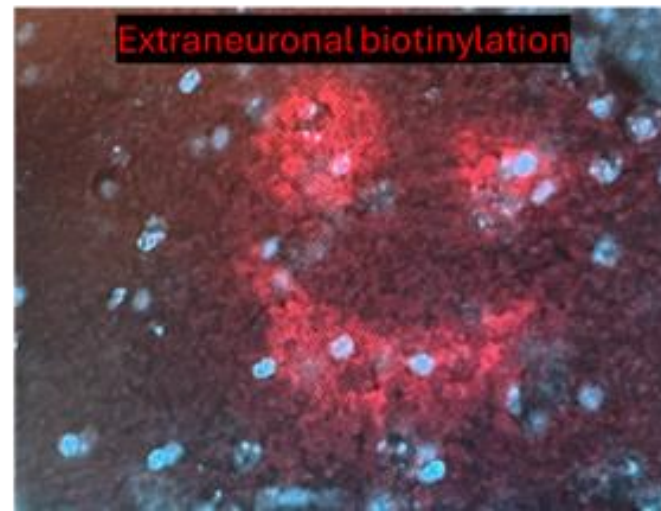
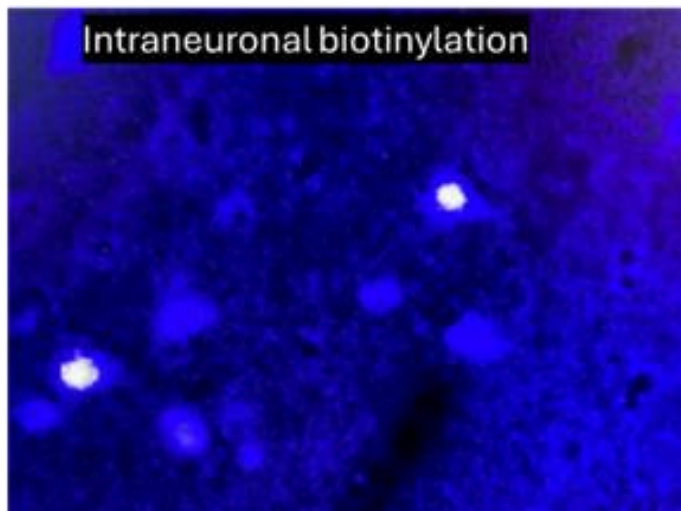
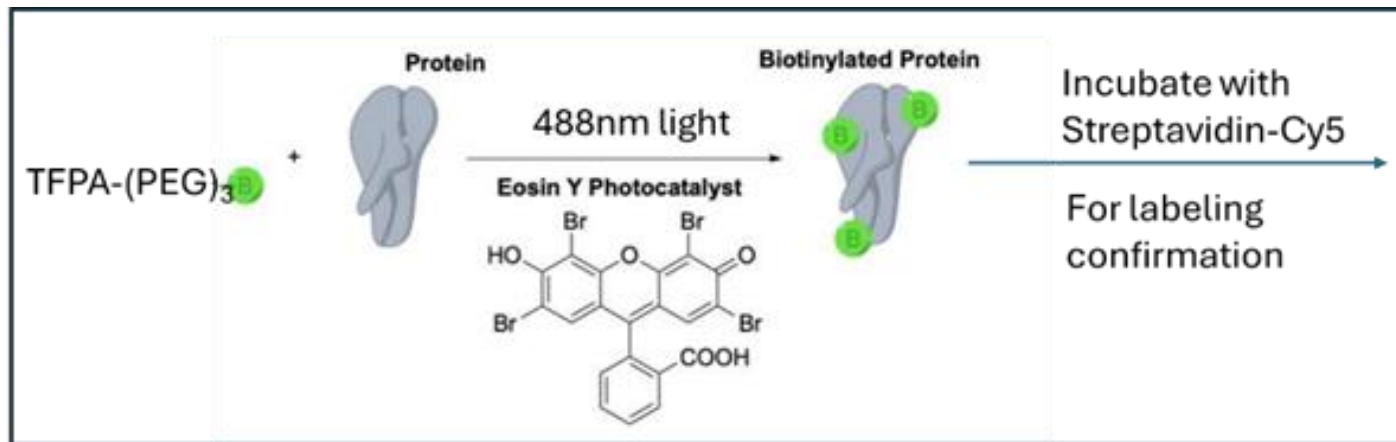
# What's next? (Thanks to CART!)

- Aim 1. Phenotype the population of neurons with changes that are predictive of imminent cell death
  - Do **ENDING** (**E**nlarged **N**eighbor **D**istance) neurons correlate with other measures of neurodegeneration?
  - Are there differences in the abundance of ENDing neurons across brain regions?
  - How does the abundance of ENDing neurons change with age or pathology burden?
  - How do co-pathologies effect ENDing neurons?



# What's next? (Thanks to CART!)

- Aim 2. Test the hypothesis that neurons at high risk of dying exhibit unique protein expression that distinguishes them from other neurons
  - Alternative hypothesis: extraneuronal protein signature



# Acknowledgements

## Zwang Lab



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Massachusetts Alzheimer's Disease

Research Center

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