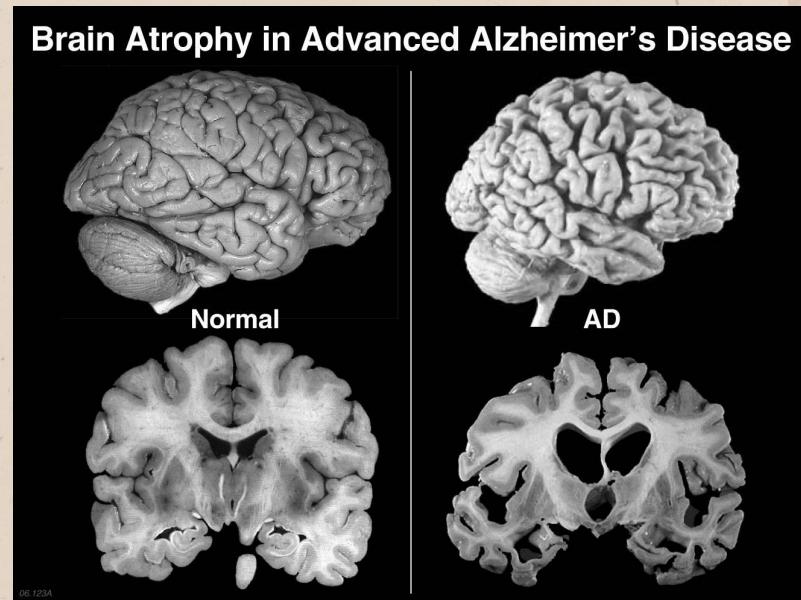


# Mutated Amyloid Beta Project

Jordan Sitea, Olivia Nardell, Jorge Moran, Jesus Quiroz,  
Bailey Thompson, Jennifer Ly, Arushi Garg

# Alzheimer's Disease Overview

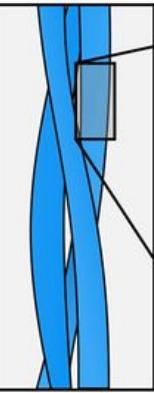
- Alzheimer's disease (AD) is a **neurodegenerative disorder** that currently **affects over 55 million people worldwide**
- AD is characterized by plaques composed of **amyloid beta (A $\beta$ ) peptide**, which aggregates and is believed to be a major cause of neurodegeneration in AD.



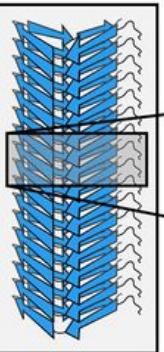
**A** Amyloid plaque or deposit



**B** Amyloid fibril



**C** Protofilament

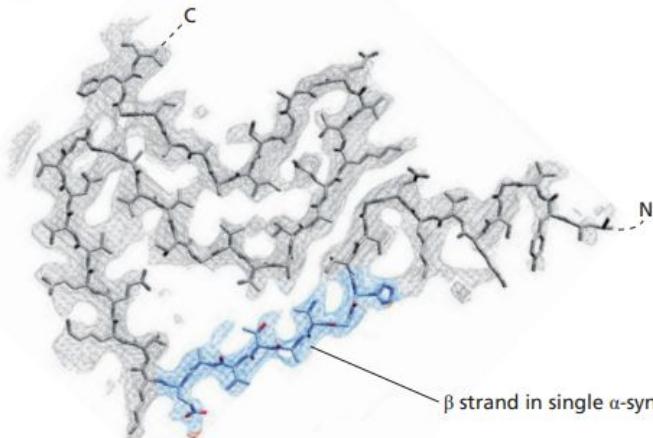


**D** Monomeric subunit

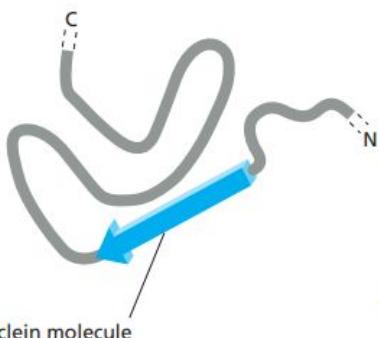


General  
Pathway for  
Amyloid Plaque  
buildup

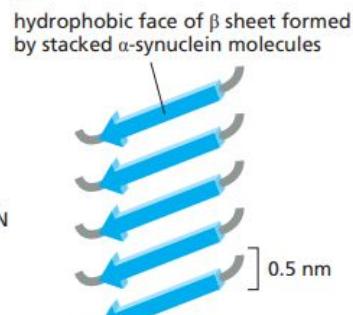
(A)



(B)



(C)

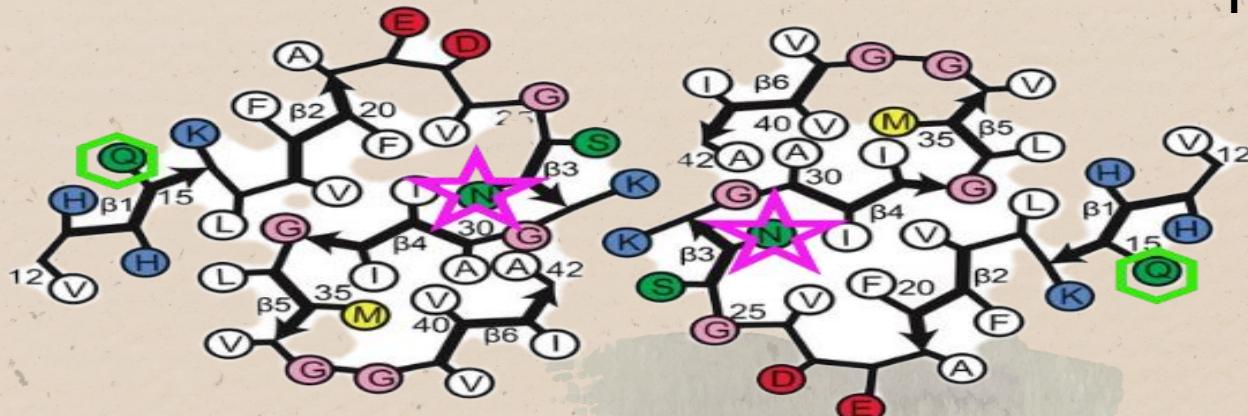


(D)



Parkinson's  
Disease  
Aggregates

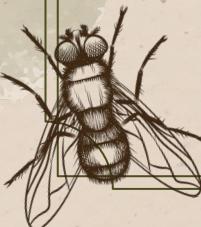
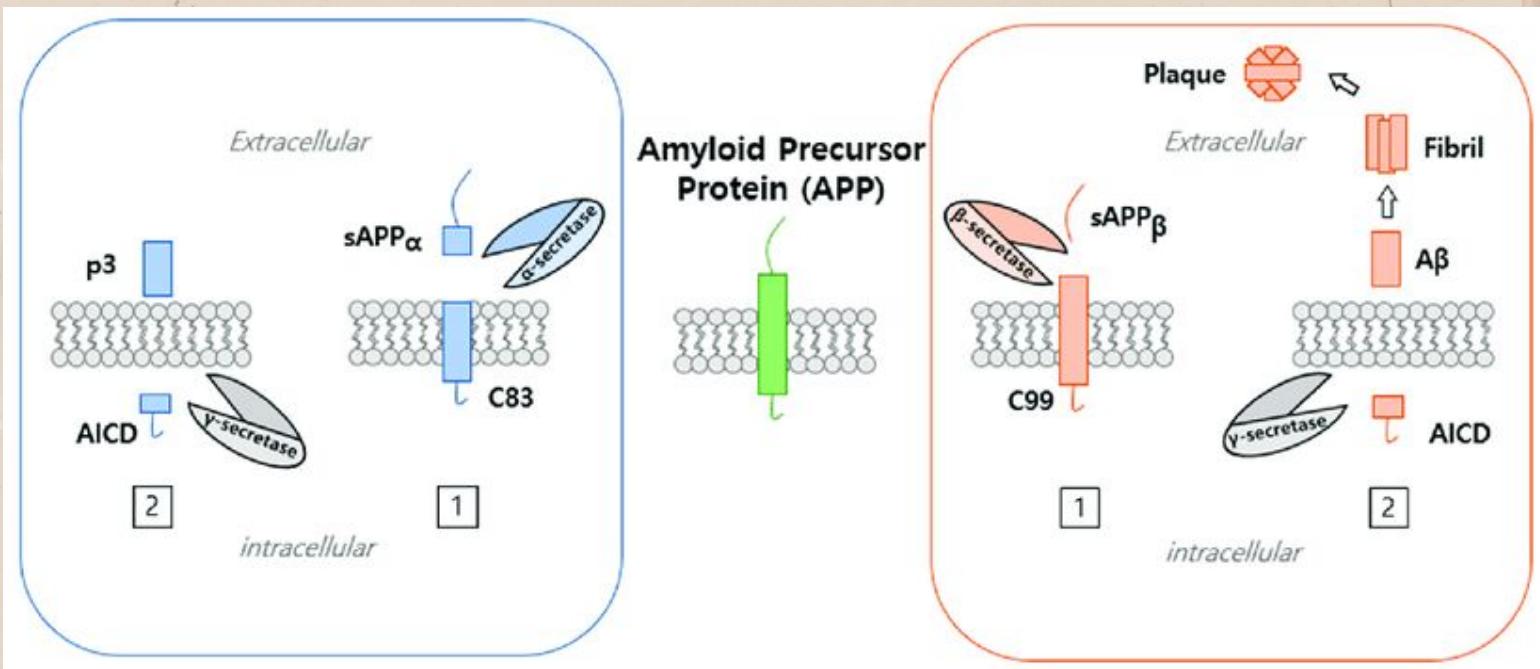
**Abeta  
Protofilament  
Patterns from  
Human  
Brains(Yang et al):  
Top- Sporadic AD  
Bottom-  
Familial AD**



Pink =  
Asparagine  
Green= Glutamine



# Amyloid Pathways



# Project Background

## Dr. Jevgenij Raskatov

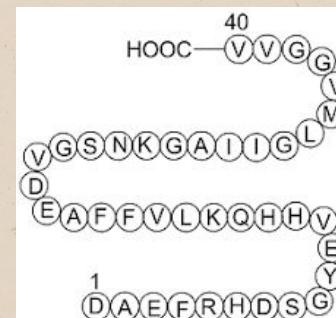
- Dr. Raskatov has manually edited the amino acid sequence encoding Amyloid- $\beta$
- Missense mutations
- Mutated amyloid protein aggregates less *in vitro*.

Dr. Raskatov →



## Our Collaboration

- We test Dr. Raskatov's theory *in vivo* using *Drosophila* models



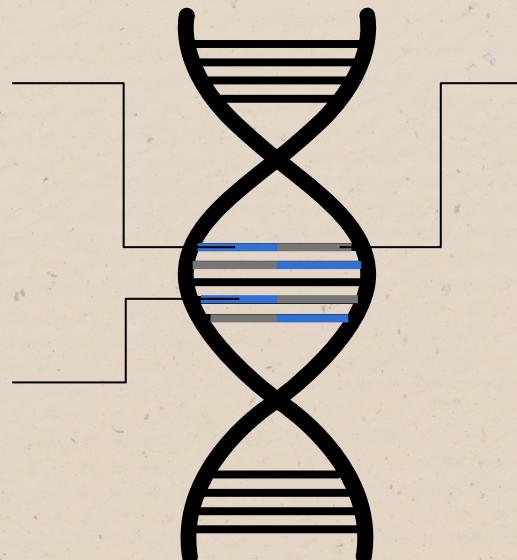
- Amyloid- $\beta$  amino acid sequence



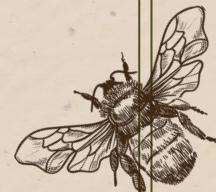
# The Mutations

N27D and Q15E

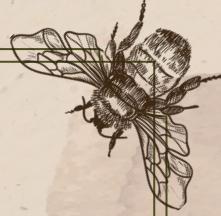
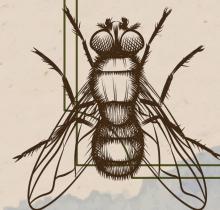
Shown by the  
Raskatov Lab to  
decrease  
aggregation and  
reduce cytotoxicity  
in vitro



Only N27D can be  
expressed in  
amyloid- $\alpha$



*We hypothesize that  
expression of the mutant  
forms of A $\beta$  will lead to a less  
severe phenotype.*



# Methodology

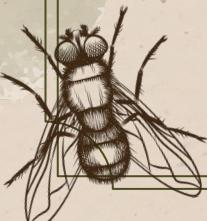
The two main parts of the project are:

## In the Molec Lab...

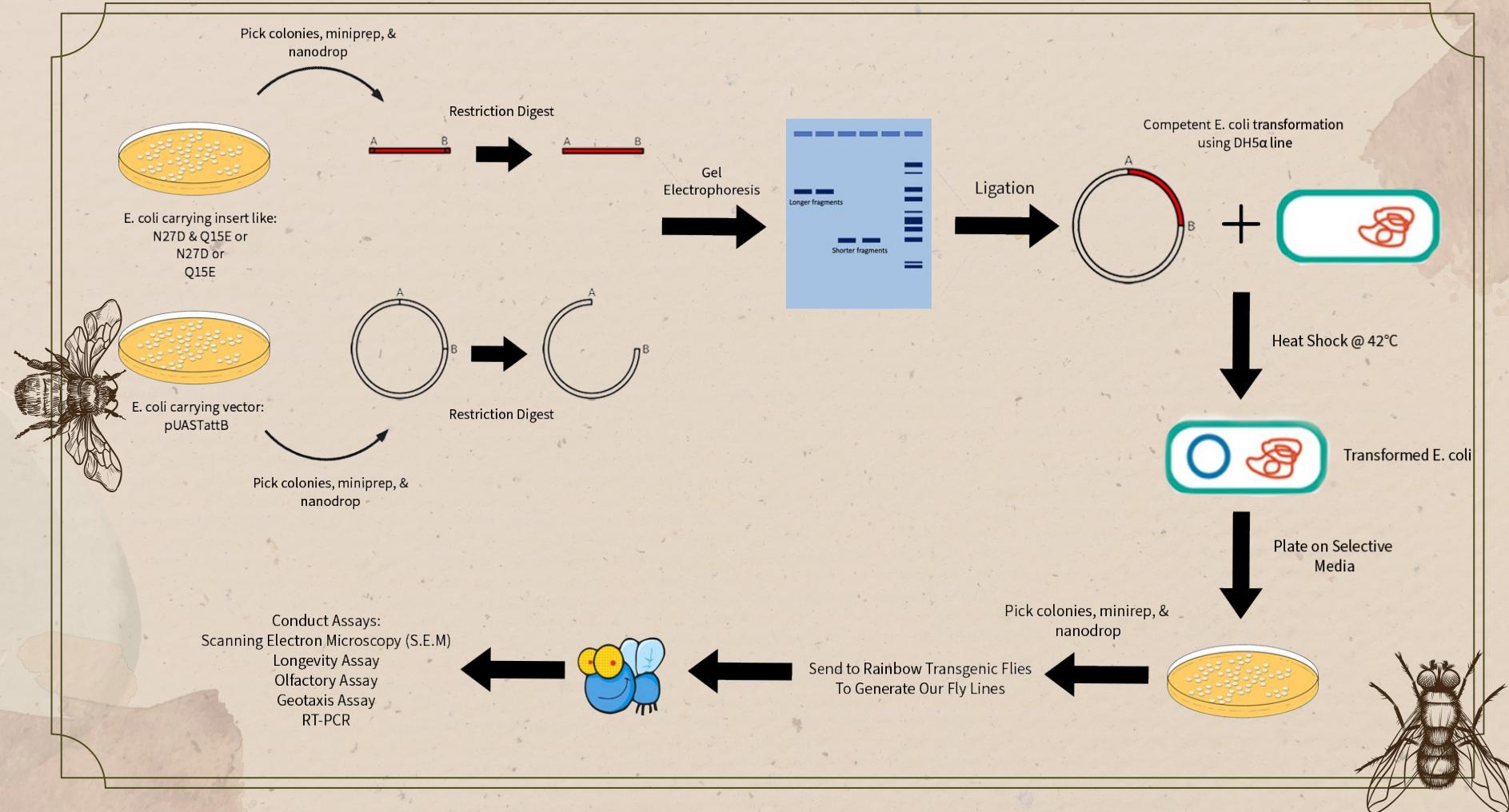
fabricating the plasmid vectors for Abeta42 and our mutations

## In the Fly Lab...

conducting experiments on the transgenic Drosophila and collecting assay data



# Molecular Cloning: Transgenic Flies



# Fly Genetics - Drivers

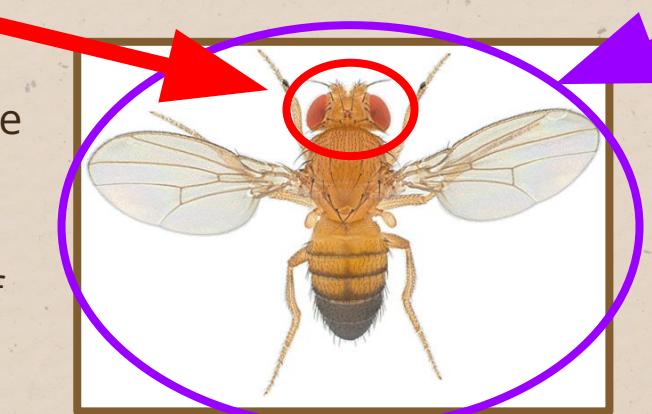
The flies with the mutated A $\beta$  gene are non-expressing and will only express it once crossed with a driver.



Keeps stocks healthy!

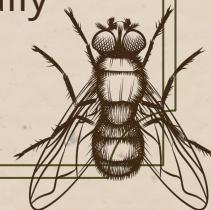
## Gal4-GMR

Crossed with a genotype of interest in order to drive and express the gene in the neurons of the Drosophila eyes

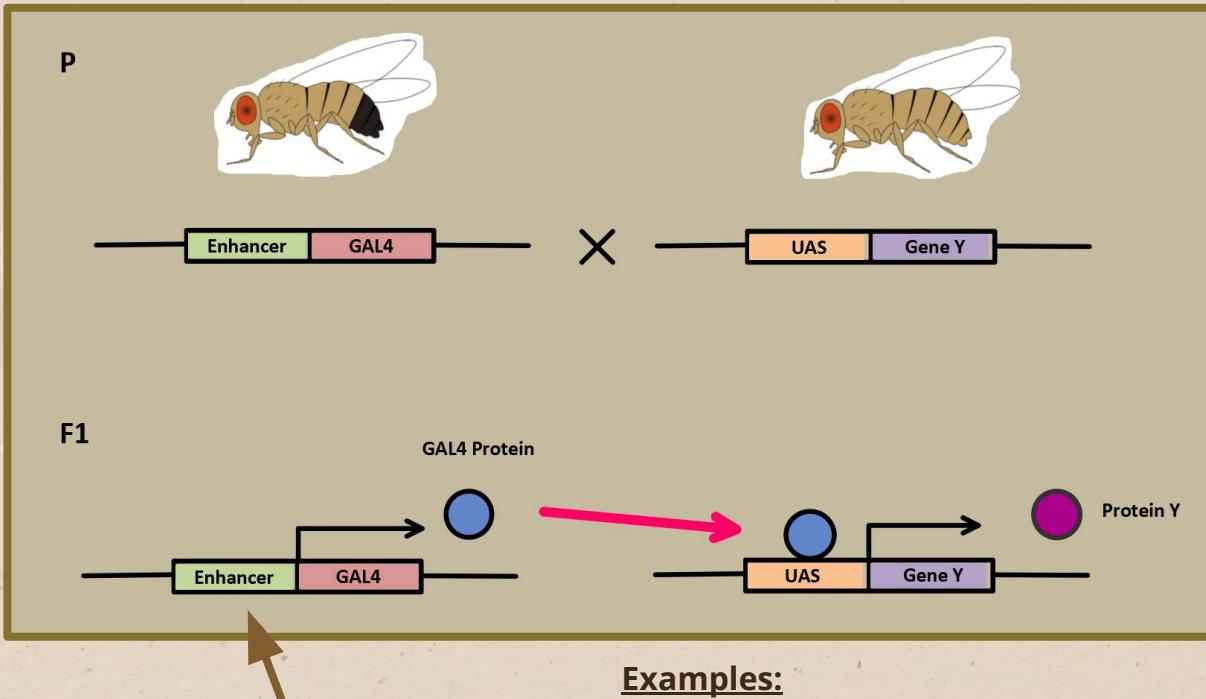


## Gal4-APPL

Crossed with a genotype of interest in order to drive and express the gene pan-neuronally



# Molecular Background on Drivers



## Gal 4:

Yeast regulatory protein that binds to the UAS and acts as a transcriptional activator.

## Examples:

GMR → Expressed in the neurons of the eyes  
Appl → Expressed pan-neuronally

**Tissue specific!**

# Fly Genetics

CyO: 2nd balancer chromosome

Acts as a marker for flies that did NOT receive the A $\beta$  gene.

Phenotype:  
Curly wings

P      UAS-A $\beta$ /CyO



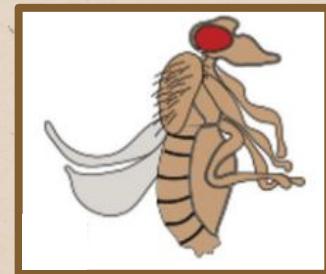
Gal4-GMR

F1    UAS-A $\beta$ /Gal4-driver



Has the A $\beta$  gene

CyO/Gal4-driver



Does NOT have the  
A $\beta$  gene



# Current Fly Lab Progress

## Scanning Electron Microscopy

Shooting electrons at flies to obtain high resolution images of fly eyes

Flies with gold to allow visualization

Crossed with the GMR-GAL4 driver

All genotypes except non-expressing

## Longevity Assay

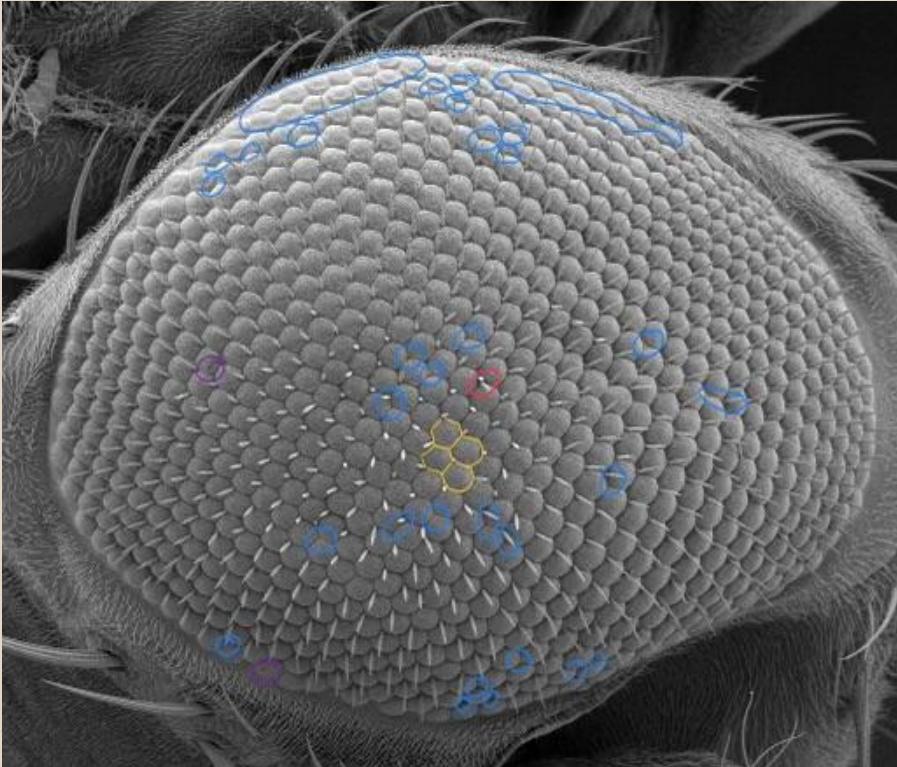
Assess and compare survivorship of flies across all 5 genotypes

All expressing flies crossed APPL driver to achieve pan-neuronal expression

Collecting 100 adult male flies of each genotype

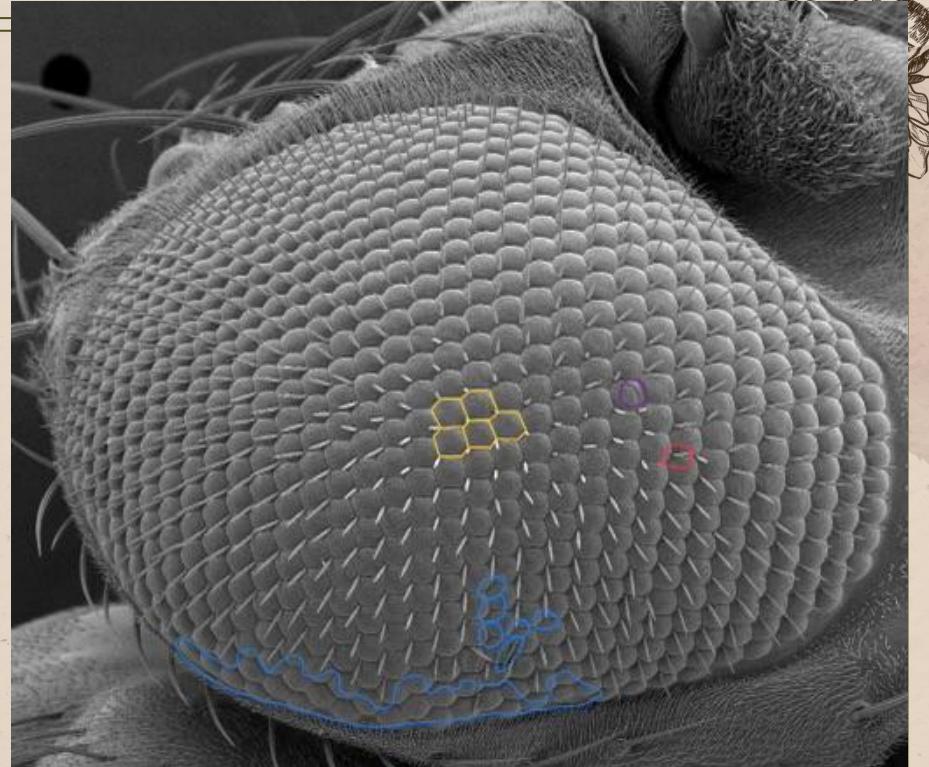
New vials every 3 days (no CO2!!)





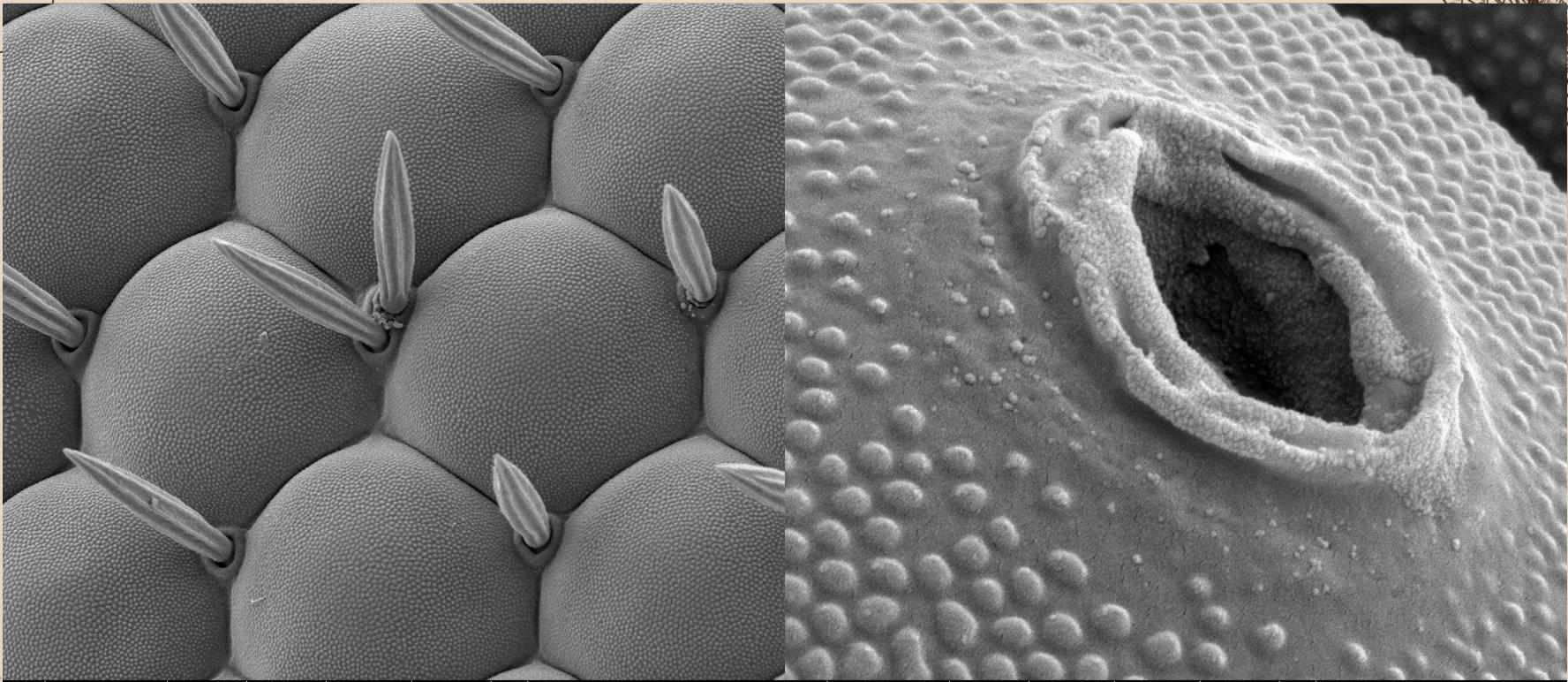
10/10/2024 | HV | mag 325 x | WD | curr | tilt | — 100 µm —  
12:16:11 PM | 5.00 kV | 325 x | 10.1 mm | 6.66 pA | 0 °

WT A $\beta$ 42



10/10/2024 | HV | mag 325 x | WD | curr | tilt | — 100 µm —  
12:58:17 PM | 5.00 kV | 325 x | 10.4 mm | 6.66 pA | 0 °

N27D



10/10/2024 | HV | mag 田 | WD | curr | tilt |  
12:32:21 PM | 5.00 kV | 4 000 x | 10.2 mm | 6.66 pA | 0 °

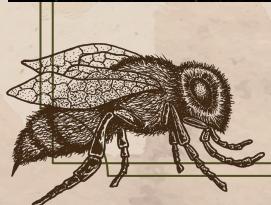
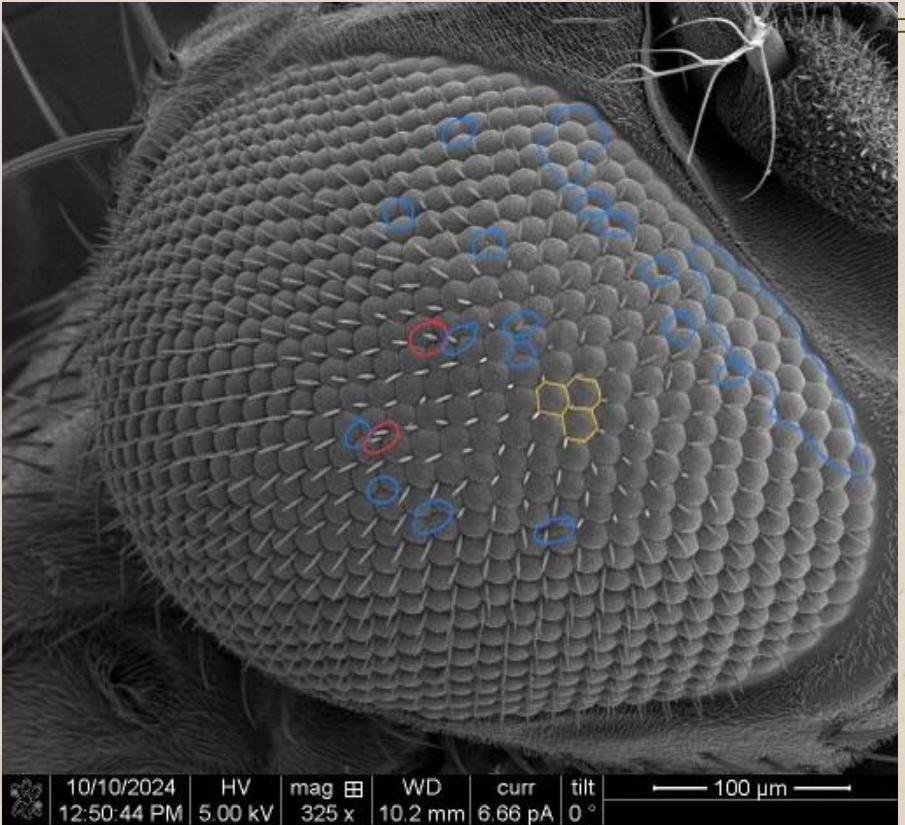
— 10 µm —

10/10/2024 | HV | mag 田 | WD | curr | tilt |  
12:30:21 PM | 5.00 kV | 32 500 x | 10.5 mm | 6.66 pA | 0 °

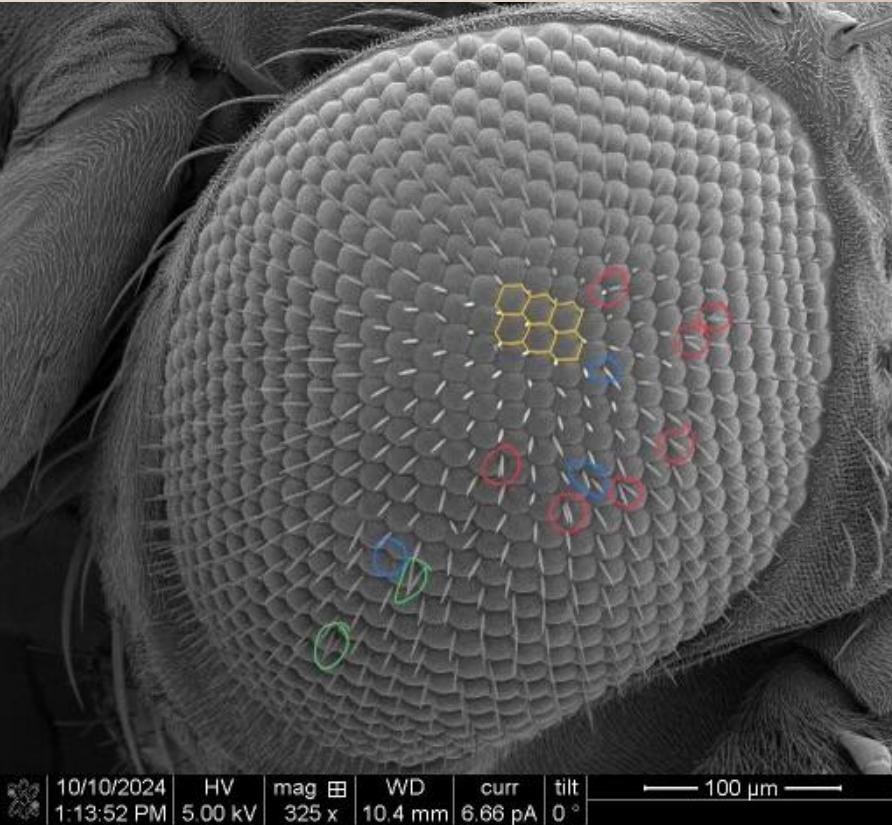
— 1 µm —

WT Aβ42

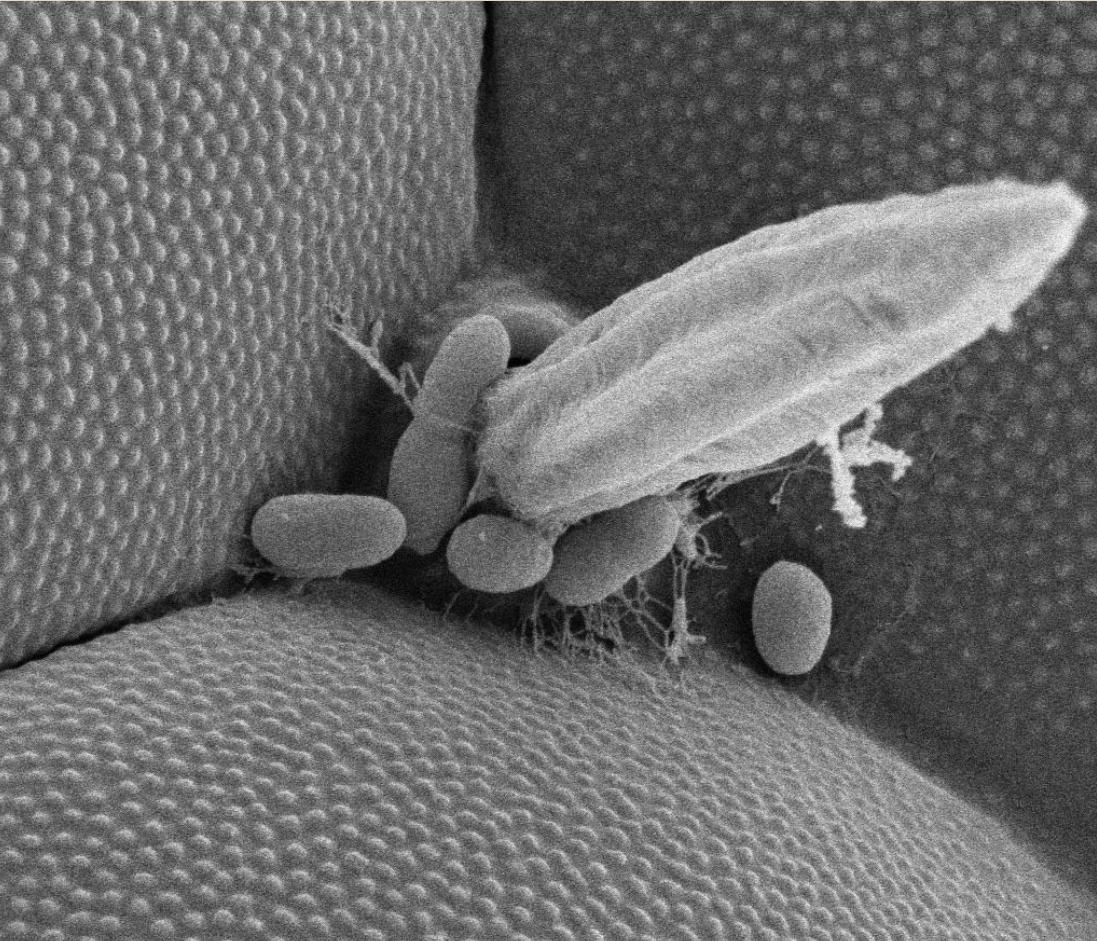
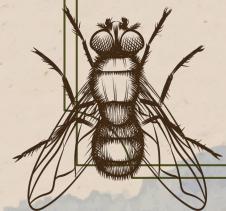




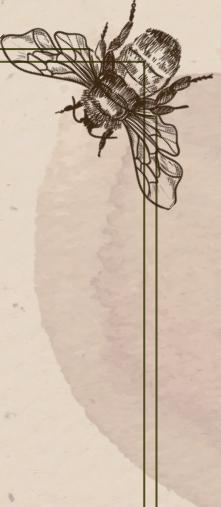
Q15E



2delta



10/10/2024 | HV | mag 田 | WD | curr | tilt | —————— 2 μm ——————  
12:56:01 PM | 5.00 kV | 17 500 x | 10.6 mm | 6.66 pA | 0 °



# Next Steps

## rt-PCR

- 1) **Isolate RNA:** RNA is isolated from a sample, such as a respiratory tract specimen.
- 2) **Reverse transcribe:** The RNA is converted into complementary DNA (cDNA).
- 3) **Amplify:** The cDNA is amplified using a thermal cycler.
- 4) **Detect:** A fluorescent signal is detected in real-time

## RING assay

- A clicking assay that will access the locomotor abilities of our flies of each line using the RING apparatus.

\*To confirm that our inserts are being expressed!

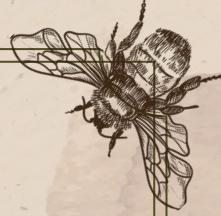
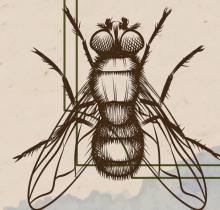


# Future Steps

## Olfactory Learning Assay

### Amyloid- $\alpha$

- Next year's group will test the effects of mutated Amyloid- $\alpha$  in *Drosophila*
- Only N27D





Thank you!  
Any questions?