# Role of Calcium Signaling Pathway in ameliorating Aβ42 mediated neurodegeneration in *Drosophila* eye

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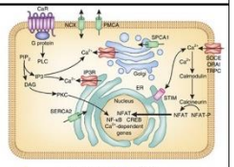
## Abstract

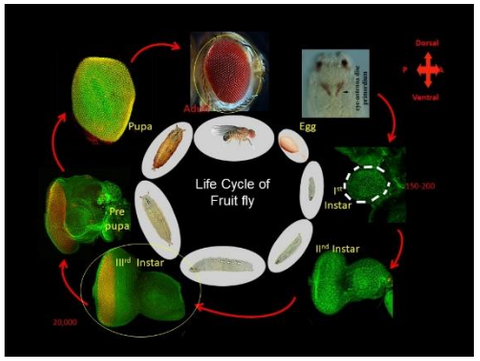
Alzheimer's Disease (AD) is a neurodegenerative disease, common in more than five million individuals in the United States (US) alone, making it the sixth leading cause of death in the US. While currently there are no cures for the disease, there are many ongoing studies, which are using the *Drosophila melanogaster* model, to find a way to prevent and slow down AD. We have used *Drosophila* as our model organism; *Drosophila* eye as our model organ (due to highly conserved genetic machinery between flies and humans). We have developed a transgenic fly model of AD where we misexpress high levels of human Aβ42 peptides using GAL4/UAS system approach, using this system, misexpression is targeted in the differentiating photoreceptor neurons in the *Drosophila* eye and can be explained to address questions pertaining to whether activating or deactivating certain pathways can rescue Aβ42 mediated neurodegeneration. We have found that members of calcium signaling pathway acts as the modifier of Aβ42 mediated neurodegeneration. In order to test our hypothesis, we misexpressed the loss-of-function form (using RNAi) of six components of calcium signaling pathway (which are *stim*$^{RNAi}$, *serca*$^{RNAi}$, *orai*$^{RNAi}$, *inx2*$^{RNAi}$, *ip3r*$^{RNAi}$, and *plc 21c*$^{RNAi}$) in Aβ42 background in the eye, and observed the effects in both eye antennal imaginal discs and adult eyes. Our results showed 100% eye rescue with all 6 components of calcium signaling pathway at 29°C, which clearly states that inactivation of calcium signaling pathway blocks Aβ42 mediated neurodegeneration. Previous studies on calcium signaling pathway showed a role in deadly diseases like cancer and other fatal diseases. Our studies show a new role of calcium signaling in neurodegeneration disorder like AD.

## Calcium Signaling Pathway

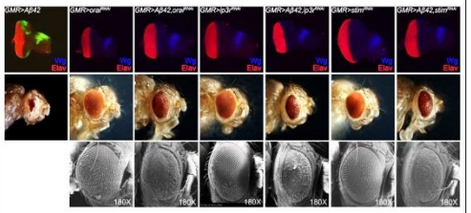
- Calcium is an important component for cellular signaling. Multiple pathways controls calcium concentration within the given areas.
- Alzheimer's associated peptides Amyloid beta disrupt many pathways involved in calcium signaling leading to higher calcium concentration in the cytoplasm.
- The higher cytosolic calcium concentration potentially leads to mitochondrial dysfunction and apoptosis.
- Thus contributes to the development and progression of Alzheimer's Disease.



http://faculty.cas.usf.edu/gullah/Research.html



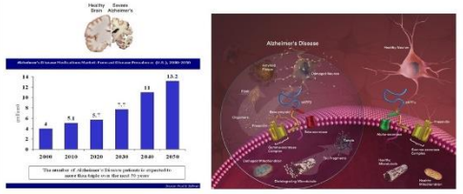

## Modulating levels of Calcium signaling pathway components affects Aβ42 phenotype in fly eye



Decreasing expression of calcium signaling pathway members (using RNAi approach) showed 100% eye rescue of Aβ42 phenotype as compared to our control.

## Alzheimer's Disease

- Alzheimer's Disease (AD) is the sixth leading cause of death in the United States.
  - There is currently no known cure.
  - More than 5.7 million Americans have AD at the moment.
- Throughout the brain normal deposits of proteins form amyloid plaques and tau tangles.
  - This leads to neuronal cell death.

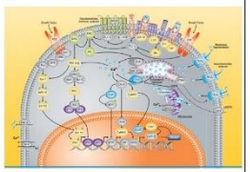


https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet

## Components of the Calcium Signaling Pathway

The following components were tested in our study:

| RNAi line | Function |
|---|---|
| *plc 21c*$^{RNAi}$ | Binds to IP3R and causes calcium to move into cytoplasm |
| *orai*$^{RNAi}$ | Located in the plasma membrane and intakes the calcium into the cytoplasm |
| *stim*$^{RNAi}$ | Regulates calcium into ER and bind with *orai* to form CRAC |
| *serca*$^{RNAi}$ | Regulates calcium intake into ER |
| *ip3r*$^{RNAi}$ | Releases calcium from the ER into cytoplasm |
| *inx2*$^{RNAi}$ | Gap junction communication |



- Figure above shows the various pathways involved in calcium signaling
- Each component tested play a large role in regulating calcium density

http://docs.abcam.com/pdf/ratmab/calcium-signaling-pathway.pdf

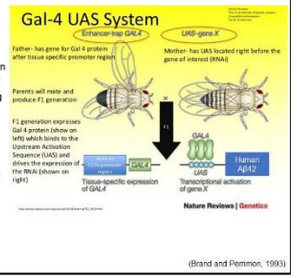
## GAL-4 UAS-System in *Drosophila*

**Gain-of-Function approach:**

- Human Aβ42 gene was inserted into the *Drosophila* genome
- Able to test the effect on AD, when downregulating the expression of genes related to calcium signaling pathway.

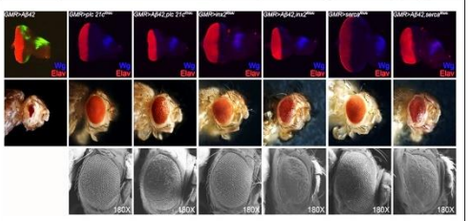
**Control: GMR-GAL4:**

- Commonly used to express target transgenes specifically in the developing eye region.




(Brand and Perrimon, 1993)

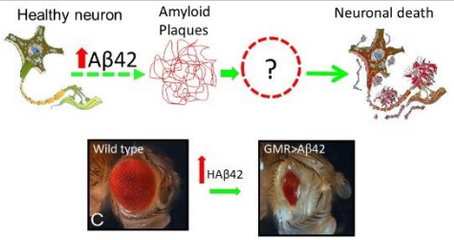
## Modulating levels of Calcium signaling pathway components affects Aβ42 phenotype in fly eye



1) Downregulating Calcium signaling pathway downregulates wingless signaling and rescues Aβ42 phenotype in fly eye.

2) It is proposed that the knockdown of this pathway can thus prevent the progression of Alzheimer's.

## What are we looking for?




Signals emanated from these plaques triggers the neuronal death.

Sarkar et al., 2016

## Why use *Drosophila?*

- Most genes are conserved between flies and humans.
  - Less genetic redundancy.
- Basic cell biological pathways are nearly identical in flies and humans (eye specification, cell cycle, Ras, p53, InR signaling...)
- Ideal human disease model for genome wide genetic screens.


**1933** Thomas Hunt Morgan uncovered the role played by chromosomes in heredity
**1946** Hermann Joseph Muller used X-ray irradiation to increase mutation rates in fruit flies
**1995** Edward B Lewis, Christiane Nüsslein-Volhard, and Eric F Wieschaus used drosophila to understand genetic control of embryonic development
**2004** Richard Axel concentrated on odour receptors and the organisation of the olfactory system
**2011** Jules A Hoffmann for his research on the activation of innate immunity
**2017** Jeffrey C Hall, Michael Rosbash and Michael W Young uncovered the molecular mechanisms that control circadian rhythms.

## Experimental Plan/Strategy

- Crossing each RNAi with a control and experiment:

| No. of Crosses | RNAi lines | GMR-GAL4 (Control) | GMR>Aβ42 (Experiment) |
|---|---|---|---|
| 1. | *plc 21c*$^{RNAi}$ | GMR>*plc21c*$^{RNAi}$ | GMR>Aβ42, *plc21c*$^{RNAi}$ |
| 2. | *orai*$^{RNAi}$ | GMR>*orai*$^{RNAi}$ | GMR>Aβ42, *orai*$^{RNAi}$ |
| 3. | *stim*$^{RNAi}$ | GMR>*stim*$^{RNAi}$ | GMR>Aβ42, *stim*$^{RNAi}$ |
| 4. | *serca*$^{RNAi}$ | GMR>*serca*$^{RNAi}$ | GMR>Aβ42, *serca*$^{RNAi}$ |
| 5. | *ip3r*$^{RNAi}$ | GMR>*ip3r*$^{RNAi}$ | GMR>Aβ42, *ip3r*$^{RNAi}$ |
| 6. | *inx2*$^{RNAi}$ | GMR>*inx2*$^{RNAi}$ | GMR>Aβ42, *inx2*$^{RNAi}$ |

## Results, Conclusions, and Future Directions

**Results and Conclusions:**

- Downregulating the levels of expression of all the six components (*stim*$^{RNAi}$, *serca*$^{RNAi}$, *orai*$^{RNAi}$, *inx2*$^{RNAi}$, *ip3r*$^{RNAi}$, and *plc 21c*$^{RNAi}$) of calcium signaling pathway caused 100% eye rescue of Aβ42 phenotype in fly eye (at 29°C temperature).
- Our results suggest that inactivation of calcium signaling pathway blocks Aβ42 mediated neurodegeneration and thus can prevent the progression of Alzheimer's Disease.

**Future Directions:**

- In future, we will test the effect of Gain-of-Function of components of calcium signaling pathway (*stim*$^{RNAi}$, *serca*$^{RNAi}$, *orai*$^{RNAi}$, *inx2*$^{RNAi}$, *ip3r*$^{RNAi}$, and *plc 21c*$^{RNAi}$) on Aβ42 mediated neurodegeneration phenotype.
- Find out the mechanism of action by which calcium pathway can prevent the progression of Alzheimer's Disease.

**Significance:**

Our studies show a new role of calcium signaling pathway in curing neurodegeneration disorders such as Alzheimer's.

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