**Lysosomal function in a Drosophila model of Alzheimer’s Disease**

Cate MacGregor, Faculty Mentor: Adam Haberman, PhD

---

### Cellular Basis for Alzheimer’s Disease

*Figure 1. Hallmarks of AD pathology. A cross-section (A) of a healthy brain (left) compared to a AD brain (right). Large insoluble plaques (B) and smaller tangles can be seen in the brains of patients with AD.*

Alzheimer’s Disease (AD) is a progressive, degenerative brain disease that impairs memory, decision-making, and other cognitive functions. On a cellular level, AD is characterized by the presence of two main structures: amyloid-beta (Aβ) plaques and tau tangles. The primary component of these damaging plaques, Aβ, should be degraded by the endolysosomal system. In this system, unwanted materials are targeted to lysosomes to be broken down by hydrolytic enzymes. However, in the case of AD, there is evidence that components of this system may be dysfunctional. Many studies indicate that it is likely that insoluble Aβ plaques reduce the functionality of the endolysosomal system to some extent.

### Drosophila Photoreceptors as a Model for AD

*Figure 2. Structure of Drosophila melanogaster photoreceptor neurons. Wildtype flies (A) have compound eyes (B) that contain circular light-sensing structures known as rhabdomeres. A wildtype eye (C) has 7 visible rhabdomeres per ommatidium.*

To model AD, our lab expresses Aβ42 in Drosophila photoreceptor neurons. As helpful as this study system has proven to be, it is important to note that we are modeling a human brain disease in a fly eye. Drosophila photoreceptor neurons are highly specialized structures that undergo extra processes not seen in human neurons. In response to light, photoreceptors endocytose the highly abundant protein Rhodopsin for lysosomal degradation. If photoreceptors carry mutations that even mildly impair lysosome function (dor1/dor4/car1), Rhodopsin aggregates and causes photoreceptor degeneration.

### Research Question and Hypothesis

We propose that insoluble aggregates of Aβ42 dramatically impair lysosomal function. We further hypothesize that Rhodopsin enhances the lysosomal defect seen in the Aβ42-expressing flies such that light-raised Aβ42-expressing flies will exhibit more degeneration than their dark-raised counterparts.

---

### Light-Dependent Degeneration in Aβ42 Flies

*Figure 3. Wildtype eyes (A) have 7 photoreceptors per ommatidium. A dark-raised Aβ42 eye (B) shows degeneration, while a light-raised Aβ42 eye (C) shows more degeneration. (D) Dark-raised Aβ42 eyes have an average of 6.2 photoreceptors/ommatidium while light-raised Aβ42 eyes have an average of 5.4 photoreceptors/ommatidium.*

### Vitamin A Deficiency Rescues Degeneration

*Figure 4. Wildtype eyes (A) have 7 photoreceptors per ommatidium, while Aβ42-expressing eyes (B) exhibit light-dependent degeneration. Aβ42-expressing eyes raised on Vitamin A deficient diet (C), which blocks the formation of functional Rhodopsin, have smaller rhabdomeres but exhibit significant rescue. (D) Vitamin A deficient food rescues degeneration from 5.4 rhabdomeres/ommatidium to 6.8 rhabdomeres/ommatidium.*

### Measuring Lysosomal Dysfunction

*Figure 5. Pro-Cathepsins become functional mature Cathepsins when their pro-region is cleaved in a lysosome. If lysosomal function is impaired, more pro-Cathepsin is expected to accumulate.*

### Cathepsin Assay Reports Lysosomal Dysfunction

*Figure 6. Western Blot from light-raised dor1/dor4/car1 and wildtype flies. Hemizygous dor1 and dor4 flies had greater lysosomal dysfunction than heterozygotes, indicated by higher levels of pro-Cathepsin. All lysosomal mutant flies had more mature Cathepsin when compared to wildtype flies, suggesting possible dysfunction in the lysosomal turnover of Cathepsin.*

### Future Directions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pro</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>dor1</td>
<td>2.9</td>
<td>26.9</td>
</tr>
<tr>
<td>dor1</td>
<td>96.2</td>
<td>91.2</td>
</tr>
<tr>
<td>dor4</td>
<td>0.4</td>
<td>39.6</td>
</tr>
<tr>
<td>dor4</td>
<td>8.8</td>
<td>29.6</td>
</tr>
<tr>
<td>car1</td>
<td>7.1</td>
<td>10.6</td>
</tr>
<tr>
<td>wildtype</td>
<td>1.2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Figure 7. Expected Western Blot results from wildtype and light and dark-raised Aβ42 flies. Light-raised Aβ42 flies are expected to have the greatest amount of pro-Cathepsin (indicating the greatest amount of lysosomal dysfunction) due to the combined effect of Rhodopsin and Aβ42. Vitamin A rescued Aβ42 flies are expected to match wildtype results.*

In our experiments, this characteristic of Drosophila photoreceptors has affected our Aβ42 results. We expect to see this feature in future experiments with other commonly used AD models. Going forward, it will be important for other Drosophila-based labs to be aware of this potential confounding variable and account for it in the interpretation of their own results.

### Acknowledgements

This research was supported by the Summer Undergraduate Research Experience (SURE) Grant, the Associated Students Research Grant, and by a stipend from the J.D. Power Foundation. Special thanks to past and present members of the Haberman lab.