Synaptic Plasticity Alzheimer's Research: Characterizing the Effects of Serotonergic Stimulation in an Aβ$_{42}$ peptide-expressing Drosophila Model

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Abstract

Alzheimer's disease includes deficits in memory, learning, lifespan and sometimes motor skills; with a disease hallmark of amyloid beta 42 (Aβ$_{42}$) accumulation, the disease debilitates the lives of millions of older individuals. The notable Aβ$_{42}$ is a cleavage product of the amyloid precursor protein (APP) pathway, which aggregates and disrupts neuronal functions and circuitry over a lifetime[1].

The Lee Lab has generated flies that express Aβ$_{42}$ through the GAL4-UAS system, which shows deleterious effects on lifespan, eye morphology, behavior, and gene expression—evidence of a neurodegenerative pathology like Alzheimer's disease. My hypothesis is that given the neurodegenerative phenotypic effects of Aβ$_{42}$ in *Drosophila*, synaptic plasticity induced by serotonergic stimulation by DOI administration will restore neuronal circuitry to rescue lifespan and motor skills. The rationale behind this is that mammalian optogenetic studies have shown that psychoplastogens like TBG can reverse dendritic atrophy caused by chronic stress by inducing neuritogenesis leading to neuronal and behavioral rescues, suggesting a potential for rescuing neuronal circuitry disrupted in a dendritic atrophy context[2]. Additionally, studies on psychoplastogen's ability to induce neuritogenesis tested DOI and visualized increase dendrite formation in *Drosophila,* providing a foundation for testing in our Alzheimer's disease *in vivo* model[3].

My project aims to test this hypothesis by conducting lifespan and climbing assays on AD model flies dosed or not dosed with DOI to measure hypothesized improvements in longevity and motor function compared to non-expressing controls. Based on the results, I plan to investigate the molecular mechanism driving the hypothesized phenotype through optogenetic studies.

Overall, the long term goal of this research is to advance the understanding of Alzheimer's disease by elucidating the role of synaptic dysfunction in the pathology, potentially guiding future treatments.

Methods

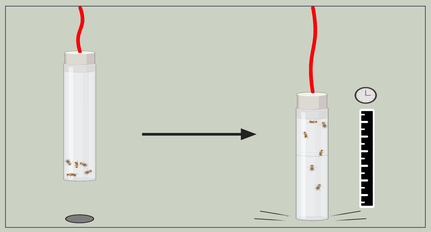

Figure A: Rapid Iterative Negative Geotaxis(RING) assay to measure the motor abilities of flies at 20 days after eclosion (DAE) and 30 DAE.Velocity, distance, and time will be tracked with video tracking software.

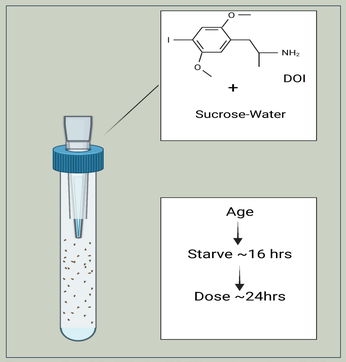

Figure B: Modified CAFE setup for DOI administration setup to drip-feed a water-sucrose vehicle carrying dissolved 3 mM DOI. The meniscus line is marked and compared with evaporation control to confirm that the flies are consuming vehicle/DOI.

Background

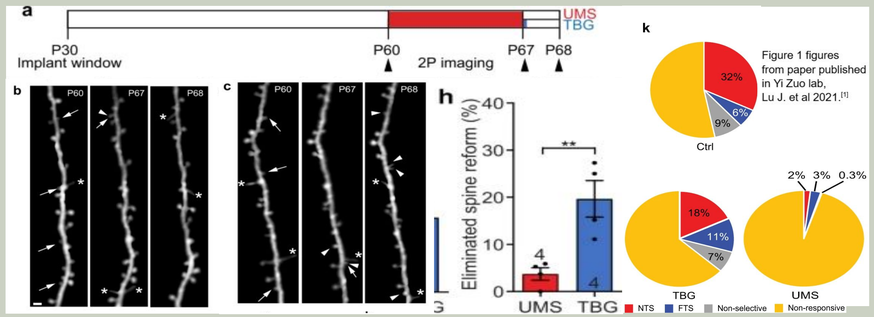

Figure 1: From paper published in Yi Zuo lab at UCSC, MCD department.(Lu J. et al 2021)[2]. *Thy1*-GFP-M *in-vivo* 2 Photon (2P) Microscopy Imaging of S1BF dendritic spines pre and post TBG administration. A) Experimental timeline, P stands for post-natal, Unpredictable Mild Stress(UMS) was conducted for 7 days from P60-P67. Imaging at P60, P67,& P68. B)S1BF spine imaging control. C) S1BF spine imaging of TBG treatment. h) Proportion of spine reformation with respect to those eliminated during UMS. K) Categorization of neurons, based on the ROC analysis, measured through Ca activity of S1BF L2/3 neurons during whisker texture interaction.

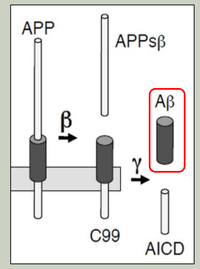

Figure 2: Cleavage of transmembrane amyloid precursor protein (APP) cleavage pathway by β secretase then γ-secretase can produce Aβ$_{42}$

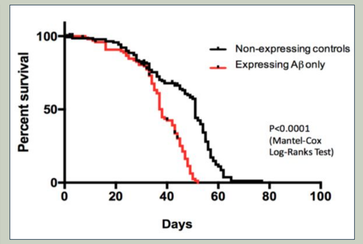

Figure 3: Previous Longevity assay, conducted in the Lee lab, tested Aβ$_{42}$ expressing flies vs non-expressing. There is a significant lifespan deficit in Aβ$_{42}$ expressing flies compared to non-expressing.

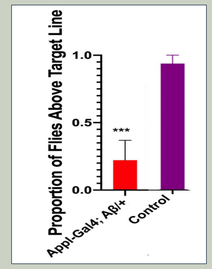

Figure 4: Previous RING assay, conducted in the Lee lab, tested Aβ$_{42}$ expressing flies vs non-expressing. There is a significant motor deficit in Aβ$_{42}$ expressing flies compared to non-expressing.

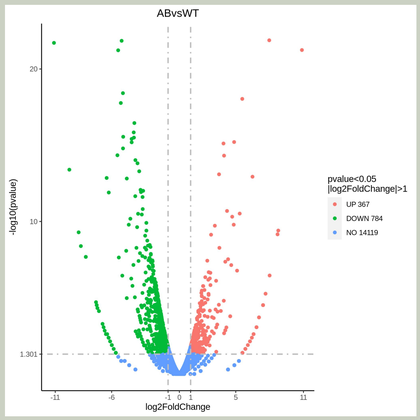

Figure 5: Volcano plot encapsulates high throughput RNA-seq data demonstrating differential gene expression of Aβ$_{42}$ expressing flies compared against a non-expressing wild type gene expression background. 367 genes upregulated (red) and 784 genes downregulated (green) with statistical significance.

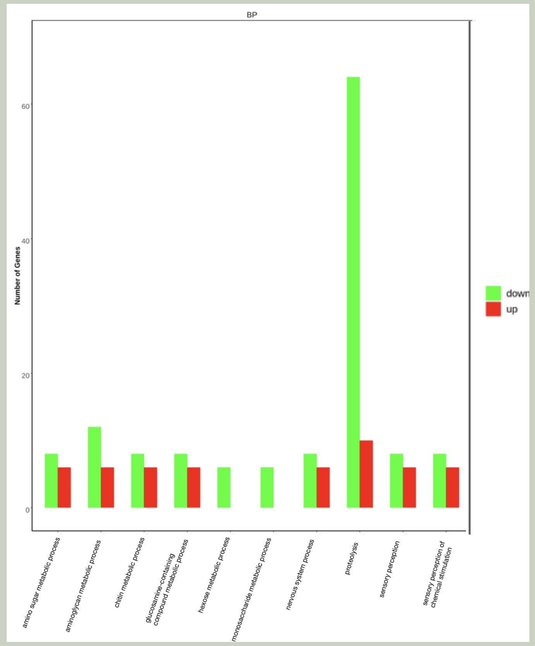

Figure 6: Bar graph quantification of figure 5, showing upregulated (red) and downregulated (green) genes sorted into biochemical processes categories. Up and down regulation in the context of Aβ$_{42}$ expressing compared to a non-expressing wild type background. Proteolysis genes are significantly downregulated.

In Progress Data

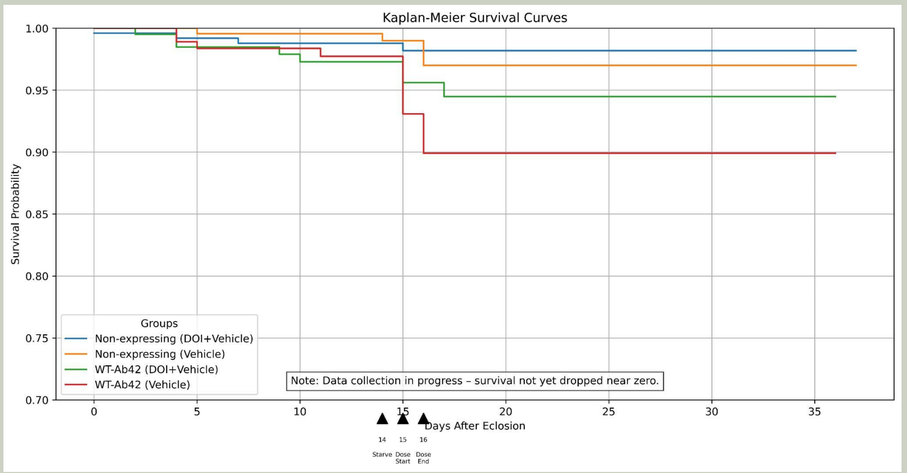

Figure 7: Overall *not significant* data of in progress fly longevity assay. 3mM DOI dissolved in vehicle. Vehicle composed of 20% sucrose dissolved in water.
Log-Rank Test for Survival Difference: P-value = 0.6959
Pairwise Log-Rank Tests: Non-expressing (DOI+Vehicle) vs. Non-expressing (Vehicle) → p = 0.6959,Non-expressing (DOI+Vehicle) vs. WT-Ab42 (DOI+Vehicle) → p = 0.0898, Non-expressing (DOI+Vehicle) vs. WT-Ab42 (Vehicle) → p = 0.0043, Non-expressing (Vehicle) vs. WT-Ab42 (DOI+Vehicle) → p = 0.1686, Non-expressing (Vehicle) vs. WT-Ab42 (Vehicle) → p = 0.0091, WT-Ab42 (DOI+Vehicle) vs. WT-Ab42 (Vehicle) → p = 0.2865

Possible Next Steps

Future plans include to uncover a mechanism driving the hypothesized results will be:

(1) Western blot or ELISA over lifespan to measure Aβ$_{42}$ levels for a hypothesized rescue effect being independent of Aβ$_{42}$ clearance, implicating the restoral of neuronal circuitry.

(2)RNA sequencing to quantify and compare differential gene expression for hypothesized evidence of synaptic plasticity markers.

(3) *Drosophila* optogenetic studies using a pan-neuronal GAL4 driver and GFP UAS to visualize hypothesized neuronal regeneration due to DOI administration.

(4) Screening of other 5-HT2 agonists such as psychedelics(psilocybin, DMT, TBG) and antagonists to measure their effects in similar behavioral, optogenetic, and biochemical studies to better understand the role of 5-HT2 receptor in a neurodegenerative context.

References

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[2]Lu, J., Tjia, M., Mullen, B. et al. An analog of psychedelics restores functional neural circuits disrupted by unpredictable stress. Mol Psychiatry 26, 6237–6252 (2021). https://doi.org/10.1038/s41380-021-01159-1
[3] C. Ly et al., "Psychedelics Promote Structural and Functional Neural Plasticity," Cell Rep, vol. 23, no. 11, pp. 3170–3182, Jun. 2018, doi: 10.1016/j.celrep.2018.05.022.

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