Section IV: Discussion

To ensure that the methodology was successful in causing enough Amyloid plaques to induce oxidative stress, the heated MO1 worms were compared to the unheated MO1 worms and the heated MO1 worms were found to have significantly greater levels of GFP at a 99% confidence level (Fig. 2). This difference shows that the described method of inducing Amyloid plaques was successful.

We have shown that there is a significantly greater amount of oxidative stress in L4/adult worms, which is seen as the significant increase in the pixel intensity of GFP in the L4/adult worms from the L1/L2 worms. This increase provides evidence showing that the levels of oxidative stress increase with the age of the worm, as GFP is used as a reporter for oxidative stress. Furthermore, both age groups of worms produced GFP in response to the expression of Amyloid plaques (Fig. 4). This provides evidence for the Amyloid hypothesis, which states that Amyloid beta initializes the vicious cycle of Amyloid plaques and oxidative stress (Zhang et al., 2024).

However, the levels of GFP produced in response to Amyloid plaque-induced oxidative stress were found to be significantly greater in the L1/L2 worms (Fig. 4). The L1/L2 worms having significantly greater levels of Amyloid plaque-induced oxidative stress compared to the L4/adult worms shows that the effect of Amyloid plaques on oxidative stress is negatively correlated with age, providing evidence against the idea that an increase in age will cause an increase in Amyloid plaque-induced oxidative stress and refuting the hypothesis. The levels of Amyloid plaque-induced oxidative stress have instead been found to have a negative correlation with age.

The limitations of this project were the time and material constraints. The lab was only open from 3:00 pm until 5:00 pm on five days of the week and was closed for inclement weather. The original methodology for this experiment was to synchronize the MO1 worms and perform the experiment on two plates of MO1 *C. elegans*, one experimental plate and one control plate, at each stage of the life

cycle. Due to inclement weather and lab hours, this methodology was not feasible. Instead, we visually classified the ages of the worms by their size. Another limitation was the lack of a fluorescence microscope. As the fluorescence is a commonly used method of detecting both Amyloid plaques and oxidative stress (Hutter, 2012), we had to determine a method of detecting fluorescence without a specialized fluorescence microscope. To overcome this challenge, we designed a method of using LEDs with specific wavelengths of light and filters with the available microscope.

This study utilized a one-tailed two-sample t-test to determine if the means of the test groups were greater than their corresponding controls, if the averages of the L4/adult groups were greater than the averages of the L1/L2 groups, and if the averages of the older controls were greater than those of the younger controls. One-tailed two-sample t-tests were used because we needed to determine if the mean of one sample was significantly greater than the mean of another sample. All results were significant to a 99% confidence level ($p < 0.01$).

Previous studies have provided contradictory evidence on which mechanism initializes the cycle of the Amyloid and oxidative stress pathologies (Zhang et al., 2024; Tamagno et al., 2021). This study provides evidence indicating that Amyloid plaques are able to cause oxidative stress in the beginnings of the disease.

Early-onset Alzheimer's Disease (EOAD) is a form of Alzheimer's that affects younger individuals compared to Late-onset Alzheimer's Disease (LOAD) (Indiana University School of Medicine, n.d.). EOAD has been shown to have a faster rate of progression compared to LOAD (Day et al., 2022). We provided evidence for the increased effectiveness of the pathways for the production of Amyloid plaques and oxidative stress in younger worms, which could shed light on the age-related differences in pathology between EOAD and LOAD. As well as explaining the faster pace of EOAD, the results of this study could help us determine which mechanisms of AD pathology are affected by age. Our findings could add to the

idea that the Amyloid pathology levels out at a certain point in the progression of the disease proposed by Jagust and Landau in 2021 by expanding it to include oxidative stress as well.

Future Research

In the future, we would propose inducing Amyloid plaques at the L1, L2, L4, and adult stages in synchronized MO1 worms and determining the levels of Amyloid plaques and oxidative stress at each stage. This study would provide more evidence on the relationship between age and Amyloid plaque-induced oxidative stress and would have a greater level of precision in the age groupings of the test groups. Furthermore, it would be beneficial to determine the effects of age on oxidative stress-induced Amyloid plaques to gain a better understanding of the interactions between these two mechanisms as an organism ages. It would also be important to run experiments testing this relationship in other model organisms and eventually on humans to verify that this correlation is due to the pathophysiology of the disease and is not specific to *C. elegans*. Moreover, it would be useful to investigate this relationship between age and Amyloid plaque-induced oxidative stress in the presence of other mechanisms of AD, such as biometal dyshomeostasis and genetic factors.

Section V: Conclusion

In order to learn more about the mechanisms associated with the age-dependent cognitive decline of Alzheimer's patients, we used *Caenorhabditis elegans* to test the effects of aging on the amounts of Amyloid plaque-induced oxidative stress. MO1, a novel strain of transgenic *C. elegans*, which continuously expresses GFP as a reporter for oxidative stress and expresses Amyloid beta when warmed was bred for use in the study. Amyloid plaques were induced at different stages in the life cycle of the worms, then the worms were dyed with Congo red to check the expression of amyloid plaques. Using a one-tailed two-sample t-test, the levels of Amyloid plaque-induced oxidative stress were found to be significantly greater in the younger worms compared to the older age group, providing evidence

showing that as the age of the *C. elegans* increases, the levels of ROS produced in response to amyloid plaques decreased. This research provided further insight on the pathology of Amyloid plaques and oxidative stress in Alzheimer's, as well as the process of aging. The relationship between Amyloid plaque-induced oxidative stress and age helps to explain the age-dependency and pathology of Alzheimer's. Understanding the relationship between age and AD could lead to life saving methods for the detection and treatment of this disease.