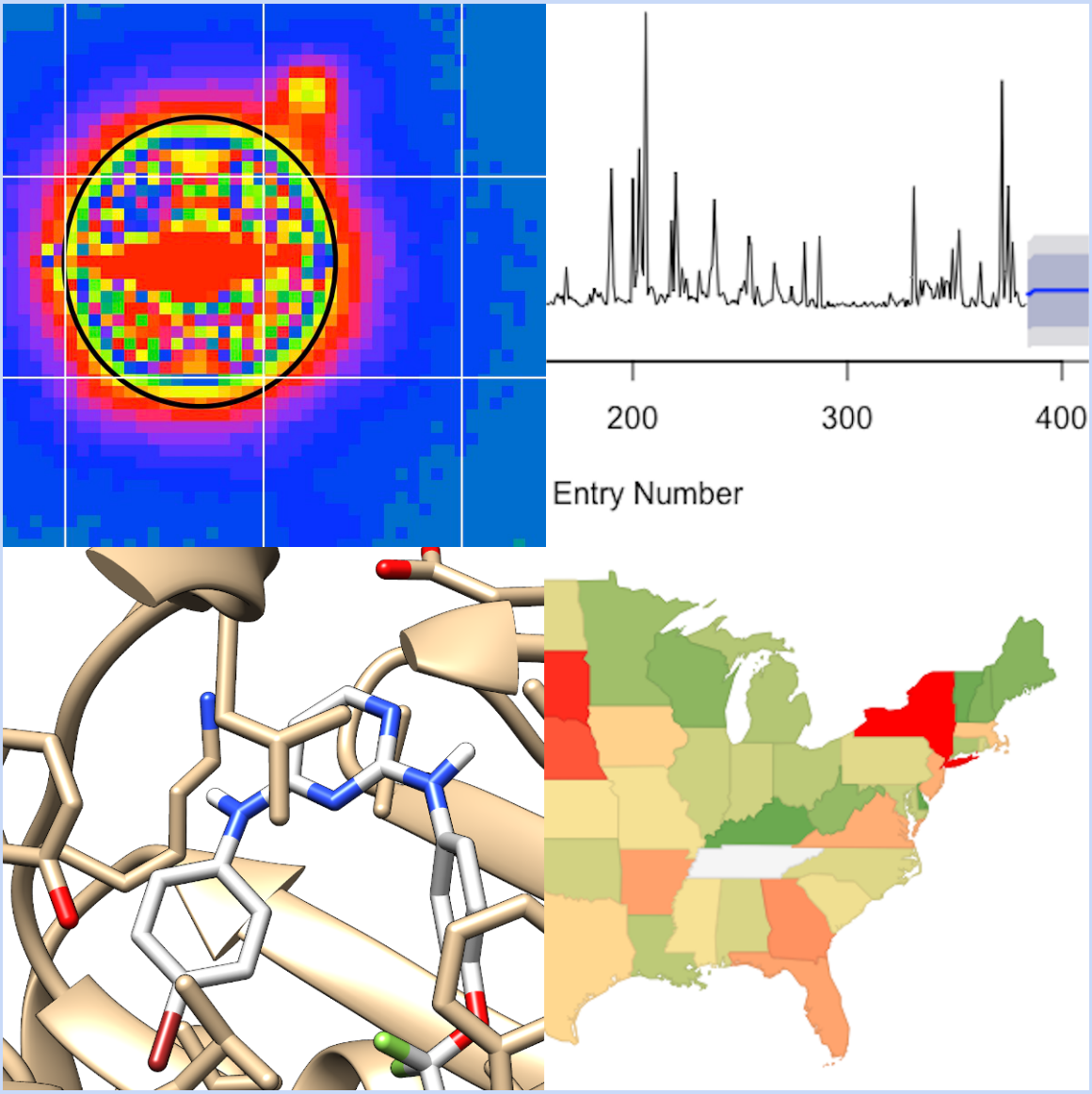
ASDRP Communications



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Preface | *ASDRP Communications*

Now in its third year, ASDRP is continuing to grow and mature. With the support of their advisors, over 350 students participated in a total of over 70 research projects across a diverse range of STEM fields—chemistry, environmental science, psychology, and more. The students worked hard, diving headfirst into the unfamiliar, discovering the challenges and rewards of research in just a single summer. This journal presents the fruits of their labor. I hope you enjoy reading about the astonishing science these students have produced.

— Avery Kruger, Editor-in-Chief

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General Inquiries: asdrp.admin@asdrp.org
Journal Inquiries: avery.kruger@fremontstem.com
ASDRP Laboratory Address: 46307 Warm Springs Blvd. Fremont, CA 94539

Effect of Aβ Peptides on Short Term Associative Memory in *C. elegans*

Brian Fu[1,*], **Aryan Mangla**[1,*], **Sophie Menon**[1,*], **Darsh Singhania**[1,*], **and Khushi Varshney**[1,*]
Advisor: Carly Truong[1]

[1]Aspiring Scholars Directed Research Program, 43505 Mission Blvd, Fremont, CA 94539
[*]Denotes equal authorship

Alzheimer's disease (AD) is a prevalent neurodegenerative disease among the elderly population. Currently, the scientific community accepts the amyloid hypothesis, which states that the accumulation of Aβ peptides is the principal cause of Alzheimer's disease. Improper APP cleavage is a major contributor to AD as it leads to the misfolding of the extracellular Aβ peptides and tau proteins, which leads to memory loss and increasing disorientation. Our study investigates the effect of Aβ aggregation on short term associative memory (STAM) in *C. elegans*. We predict that transgenic *C. elegans* with neuronal Aβ peptide aggregation will have decreased STAM in comparison to the N2 Wild Type and CL2122 control strains. Each strain of *C. elegans* was conditioned to associate food with butanone, and this association was tested via chemotaxis assay and compared with baseline chemotaxis indices to assess the effects of Aβ aggregation on short term memory loss. Our results show that the pattern of deterioration of the Cl2122 strain is similar to that of N2 Wild Type. The CL2355 strain suffered the most detrimental loss of STAM. This indicates that Aβ aggregation negatively affects short term associative memory in *C. elegans*. However, these results could be due to stress from handling, implying that the coupling of Aβ was a contributing factor to the decline in STAM in the CL2355 strain. Overall, our study has shown that Aβ aggregation impairs associative mechanisms in *C. elegans*.

associative memory | Alzheimer's disease | amyloid-beta peptides | *C. elegans* | chemotaxis assays

Introduction

Alzheimer's Disease

Alzheimer's disease (AD) remains responsible for about 70% of dementia cases worldwide and contributes to 5.4 million American deaths per year (40% of the population above 80), making it the sixth leading cause of death in Americans over the age of 65[1]. As the elderly population in the United States increases, neurodegenerative diseases like AD are becoming more prevalent[2]. There are two stages of AD: early-onset and late-onset. Patients with early-onset Alzheimer's develop symptoms before the age of 65; these cases can either be familial or sporadic[3]. On the other hand, late-onset Alzheimer's develops after the age of 65 and can only be sporadic. Both early and late-onset AD results in progressive memory loss, impaired thinking, and changes in personality and mood.

Amyloid-beta Peptide

The most widely accepted theory on the cause of Alzheimer's disease is the amyloid hypothesis, which states that the accumulation of oligomeric amyloid-beta peptide is the principal cause of AD. Derived from the amyloid precursor protein (APP), amyloid-beta (Aβ) is a normal product of the cellular metabolism process in cells. The hippocampus and neocortex are most vulnerable to deposits of Aβ peptides. When examining Aβ concentration in relation to AD, it is important to analyze the Aβ homeostasis, which refers to the balance between the production and degeneration of Aβ peptides. Abnormal accumulations of Aβ peptides create amyloid plaques that degrade neurofibrillary tangles, leading to Alzheimer's disease[4]. While Aβ overproduction is detrimental to memory, sufficient amounts are essential to proper brain function. In a healthy brain, excess Aβ buildup is counterbalanced by the bodily processes of proteolytic degradation and active transport.

Much still remains unclear regarding the mechanism of neurotoxicity in Alzheimer's disease which is why uncovering the relationships between Aβ peptide buildup and memory, and consequently neurodegenerative diseases such as AD, is important. For example, researchers Kametani and Hasegawa (2018) have observed that some Alzheimer's Disease patients do not actually exhibit Aβ plaques while some normal patients do[5]. Consistent with these findings, *C. elegans* that lacked AB peptide overproduction still presented with paralysis[6] (Drake et. al 2003). This suggests that the Aβ plaques are not the singular cause of inhibition of Alzheimer's Disease as many researchers had hypothesized[7]. The question still remains unknown as to whether Aβ plaques themselves fail to be a factor in AD, if there is an external cause underlying that of these said plaques, or finally, if smaller aggregations of Aβ have failed to be found within *C. elegans*[5]. This study aims to elucidate the continued relevance of the amyloid hypothesis in studying the neurodegeneration associated with AD.

Caenorhabditis elegans

This study utilizes Caenorhabditis elegans (*C. elegans*) as the model organism to study the effects of Aβ peptides on short term and long term memory at the behavioral level. *C. elegans* are transparent nematodes about 1mm in length. They have a relatively short life cycle (2-3 days) and a total life span of 2-3 weeks[8]. *C. elegans* are commonly useful for

modeling human biological mechanisms as many of their genetic pathways, including their process of Aβ peptide production, are conserved with vertebrates. Furthermore, *C. elegans* have highly permeable and sensitive cuticles, allowing chemicals to easily enter their nervous system, a property uniquely important for the association of chemicals with neurological processes that this study aims to observe.

C. elegans rely heavily on their chemotactic senses to respond to their environment. Because of their natural ability to detect specific odorants through olfactory neurons (smell receptors), they are able to initiate and form associations with those odorants, specific to our project, butanone. While in their hunger state, *C. elegans* are able to form strong associations, allowing them to find food when in a new environment. For example, once the said association between odorant and food is formed, after the *C. elegans* have been transferred to a new environment, such as a plate, *C. elegans* are able to differentiate between scents. Due to the association between a specific scent and food, they will begin going towards that specific scent, looking for food. This contributes to their overall survival; by leading them to the food in a quick and efficient way through the association, *C. elegans* have no need to "explore" the environment until they have discovered food.

Because of their ability to detect odorants by binding to specific olfactory receptors, we can further understand their associations with surrounding odorants in their hunger state when forming short term memory. *C. elegans* contain two types of olfactory neurons, AWA and AWC. AWC neurons are shown to specifically contain an affinity and solely contain the ability to sense the odorant butanone extremely well[9]. When butanone sensed by *C. elegans* is paired with food (OP50), *C. elegans* is found to form associative memory connections. Torayama et al. (2007) note that through their observations, naive *C. elegans* have shown to have a preference and to migrate towards butanone, this tendency increases with the association of food[10].

This study investigates the effect of Aβ aggregation on STAM in *C. elegans*. *C. elegans* will be conditioned to associate food and butanone and will be tested via chemotaxis assays to assess the effects of Aβ aggregation.

Methods

Strains

The three strains of *C. elegans* used in this experiment are N2 Wild Type, CL2122 which possess no Aβ peptides and serves as the control strain for CL2355, in which Aβ peptides are present in neurons[11]. The N2 Ancestral Wild Type Strain functions as the non-mutated group with no changes to Aβ peptide production. Collected from Bristol, England, and with a life cycle of roughly 3 days, they are used as the standard wild type strain across the world[12]. Transgene analysis can be used to study the effect of neurotoxic Aβ because of *C. elegans*' powerful genetic structure[13].

Worm Cultivation

The *C. elegans* were cultured on NGM plates with OP50 E. coli using the original methods by S. Brenner[14]. The *C. elegans* and OP50 were sourced from Carolina Biological Supply. These plates were stored at 20°C until the *C. elegans*reached the adult stage (2-3 days). All *C. elegans* was handled ethically under the appropriate conditions.

Synchronization

Before performing experiments, *C. elegans* were synchronized to the L1 stage using a hypochlorite bleach solution consisting of sodium hypochlorite, a bleaching agent following standard procedure[15]. The synchronization process eliminates mature *C. elegans* while keeping *C. elegans* eggs intact, due to the protective nature of the eggshells. The bleaching solution being used is potent enough to dissolve the majority of *C. elegans*, but will not penetrate the eggshells, preserving the *C. elegans* embryos. By synchronizing the *C. elegans* to the L1 stage, our study aims to best control all factors other than the experimental variable, the presence of Aβ peptides, which we are studying. The *C. elegans* were first washed off their holding plates with an M9 buffer, designed to release the *C. elegans* from the layer of OP50, and then washed in conical tubes to remove any remaining bacteria and contaminants. They were then bleached for exactly 6 minutes, after which M9 buffer was added to stop the reaction. They were then kept in a shaking water bath; *C. elegans* will not grow past the L1 stage, as they were not given food. Once the *C. elegans* reached the L1 stage, they were then transferred to seeded plates for baseline chemotaxis assays.

Assessing Baseline Activity: Chemotaxis Assays

Butanone was used to assess the baseline function of AWC neurons (Figure 1). The neurons respond selectively to these organic compounds which bind to their specific receptors. The binding of the odorants to the specific receptors activates cAMP production, which leads to an increase in calcium levels. The influx of calcium results in the depolarization of olfactory neurons. Increased levels of calcium lead to neuronal plasticity, which allows for a greater connection between pairing time and memory formation.

The *C. elegans* were first conditioned to associate the presence of butanone and benzaldehyde with food. This is done by first starving the *C. elegans* and then exposing them with the odorants present. Later, they have been starved again, and then their short term memories are tested for how conditioned they are to the association of the odorants to food[16]. High-cross image analysis is used to determine the indices of chemotaxis within the treated *C. elegans* through software size estimates.

Short Term Associative Memory Training (STAM)

STAM allows for *C. elegans* to form an association between food and butanone, which is then tested through chemotaxis assays (Figure 2). *C. elegans* were transferred to a seeded plate with 10% butanone in 95% ethanol streaked on the lids and incubated for 1 hour[17]. About 40 *C. elegans* were transferred per plate. The *C. elegans* were then transferred to seeded NGM plates for varying periods of time (0, 0.5, 1, 2 hours). After the given time period was complete, the *C.*

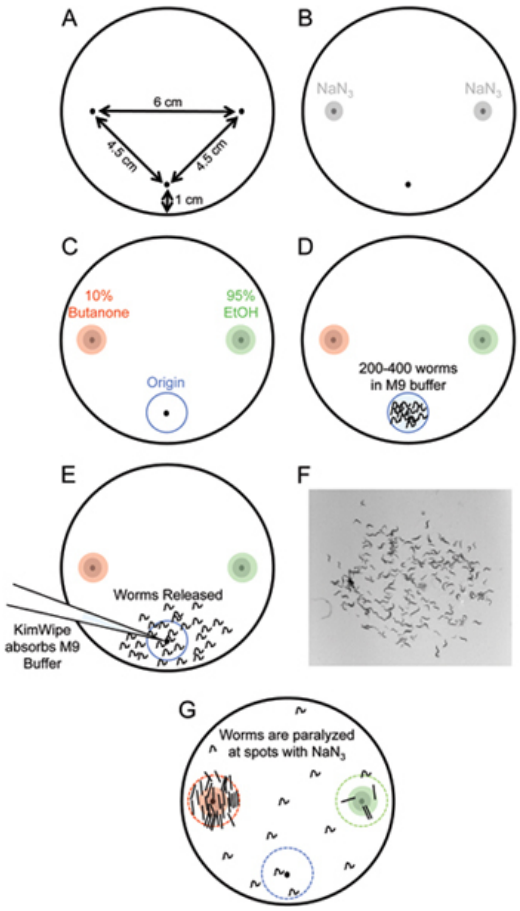

Fig. 1. Chemotaxis Assay Set Up. (A) Organization of the plate before the assay. Small spots are drawn onto the plate to use as a guide to place the chemicals *C. elegans*. (B) Sodium Azide is spotted on two of the dots to paralyze the *C. elegans* after they have reached said spots. (C) Butanone and ethanol are placed on the said marks. (D) *C. elegans*are pipetted onto the Origin in M9 buffer. (F) *C. elegans*at the origin. Image from Kaufmann et al, 2011 [17].

elegans' associations to butanone were tested with chemotaxis assays (see Assessing Baseline Activity: Chemotaxis Assays). One strain was completed each day in the following order, N2 WT, CL2122, and CL2355, to ensure that all C. elegan strains were at the L3 stage when training was performed. Short term memory is dependent on cAMP production, which is involved in the chemotaxis response. cAMP production is assessed in the chemotaxis assays.

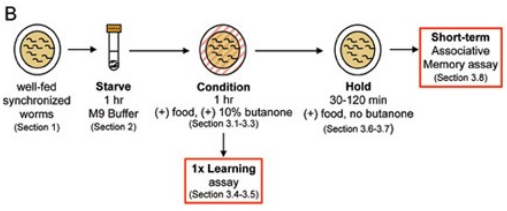

Fig. 2. STAM Set-Up and Timeline. The *C. elegans* is first starved in conical tubes and then transferred to seeded plates streaked with butanone for 1 hour. Then a chemotaxis assay is performed at time = 0 hours on a set of the conditioned *C. elegans* (1x Learning Assay). The remaining *C. elegans* are transferred to seeded plates (no butanone) and held for incubation periods of 0.5, 1, and 2 hours. Finally, the Chemotaxis Assay is performed on *C. elegans*. Image from Kaufmann et al, 2011 [17].

Results

Baseline Chemotaxis Index

The baseline chemotaxis indices were obtained after running the chemotaxis assays for C elegans strains N2 WT, CL2122, and CL2355. Chemotaxis indices were calculated by subtracting the number of *C. elegans* observed on the butanone spot by the number on the ethanol spot and dividing this by the difference between the total number of *C. elegans* on each plate and the number of *C. elegans* remaining on the origin spot. The average chemotaxis indices for the N2 WT, CL2122 and CL2355 were 0.6198, 0.5676, and -0.1429 (Figure 3).

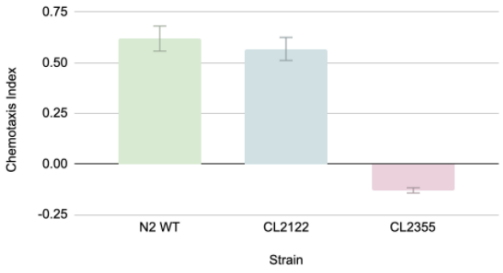

Fig. 3. Baseline Chemotaxis Index Data. Chemotaxis index is shown on the y-axis, and type of strain on the right. The average baseline chemotaxis index decreases from left to right, while the significance of Aβ peptides increases. N2 WT strain contains only naturally formed Aβ peptides (little to none), CL2122, the control strain, contains absolutely no Aβ peptides, while CL2355 strain produces Aβ peptides in the brain.

STAM: Chemotaxis Index

Chemotaxis assays were performed after the incubation periods of 0, 0.5, 1, and 2 hours for each of the three strains. The N2 Wild Type strain yielded chemotaxis indices of 0.836, 0.818, 0.000, and 0.886 for the respective times (Figure 4). The chemotaxis indices calculated for the CL2122 strain were 0.474, -0.200, -0.429, and 0.000 respectively (Figure 4). Finally, the CL2355 strain resulted in chemotaxis indices of 0.143, 0.128, 0.111, and -0.333 (Figure 4). Figure 5 shows the trends in the chemotaxis index over the two hour period.

	N2 WT	CL2122	CL2355
0	0.836	0.474	0.143
0.5	0.818	-0.200	0.128
1	0.000	-0.429	0.111
2	0.886	0.000	-0.333

Fig. 4. STAM: Chemotaxis Indices. Calculated chemotaxis indices for each time interval and strain of *C. elegans* tested during our short-term associative memory assays.

STAM: Learning Index

Following the chemotaxis index, the learning indices were calculated by subtracting the chemotaxis index calculated for each time period by the average baseline chemotaxis index for that respective strain (Figure 6). The highest and lowest learning index of N2 Wild Type strain was 2 hrs (0.2660) and 1 hr (-0.6198). The highest and lowest learning index of CL2122 was 0 hrs (-0.0939) and 1 hr (-0.9962). The highest and lowest learning index of CL2355 was 0 hrs (0.2858) and

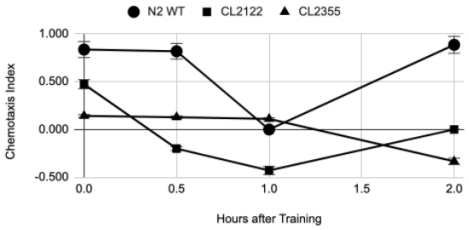

Fig. 5. Chemotaxis Index of STAM. Chemotaxis index is shown on the y-axis, and type of strain on the x-axis. The different shapes on each line are used to represent each strain (circle = N2 WT, square = CL2122, triangle = CL2355). The N2 WT strain yields on average a higher C.I. in comparison to the other two strains. The CL2355 strain presents the lowest overall ending chemotaxis index. All three strains forget the associations formed after 1 hour, as all strains' chemotaxis indices fall below or extremely close to 0.

2 hr (-0.1904). The learning indices for each *C. elegans* strain was then graphed to serve as a visual comparison (Figure 7). The percent change in the learning indices from each time period and for each strain was also calculated and graphed (Figure 8). The greatest percent change occurred in the interval of 0.5 to 1 hour for the CL2122 strain.

Hours After Training	N2 WT	CL2122	CL2355
0	0.2163	-0.0939	0.2858
0.5	0.1984	-0.7676	0.2711
1	-0.6198	-0.9962	0.2540
2	0.2660	-0.5676	-0.1904

Fig. 6. STAM: Learning Indices. Calculated learning indices for each time interval and strain of *C. elegans* tested during our short-term associative memory assays.

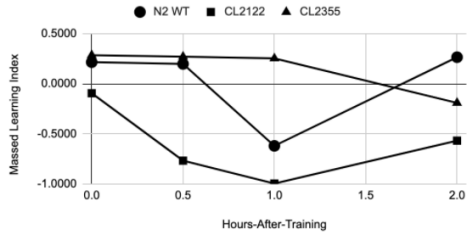

Fig. 7. Massed Learning Indices In Of STAM. Graph of learning index data obtained from calculations with the short-term associative memory chemotaxis indices and baseline chemotaxis indices for each *C. elegans* strain.

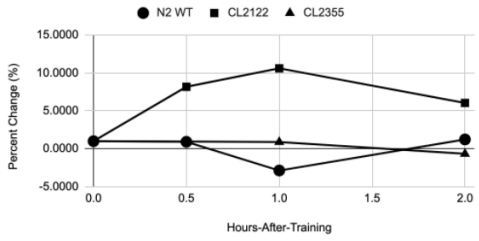

Fig. 8. Learning Indices Percent Change vs. Hours After Training. Graph of calculated percent change of learning index data for the short term memory associations of each *C. elegans* strain.

Discussion

The purpose of our study aims to evaluate the amyloid hypothesis in *C. elegans*. We focus primarily on the relationship between the formation of Aβ peptides and STAM. In doing so, we hope to discover more about Alzheimer's disease and the effects of Aβ peptide aggregation as a contributing factor for AD.

Previous literature has stated that wild-type STAM lasts for around 2 hours and typically begins to decline after about 1-hour data[17]. However, based on our findings, the chemotaxis and learning indices of the N2 Wild Type and CL2122 strains decreased within the first hour of holding (Figure 5). The Cl2122 strain has a 0.6373 drop in the chemotaxis index from the 0 hour incubation period to the 0.5 hour incubation period. This is greater than the 0.0179 decreases with the N2 Wild Type strain. This significant difference between the CL2122 and wild type strain could be explained by the fact that a certain amount of Aβ peptides are necessary for proper brain function. Because the CL2122 strain is transgenically bred to not produce any Aβ peptides, it can be assumed that the CL2122 C. elegans does not have the same neurological abilities as the wild type. This explains why the CL2122 and wild type strains have such large gaps in their chemotaxis indices even though they follow the same patterns of continuous decline, then a sharp increase.

The learning index of the N2 WT strain rapidly decreased in STAM after 30 minutes but recovered after an hour to baseline levels. The CL2122 strain did not seem to learn to associate during the 2 hour time period since it had the lowest learning index of all three strains. It is unclear why its short term associative memory was weaker than the N2 WT strains. We speculate that this irregularity is due to the induced stress caused by the handling and transferring of *C. elegans*. Finally, CL2355 *C. elegans* strain exhibits a continuous decline in its learning index throughout the duration of the STAM. The gradual decline is consistent with previous findings where STAM is sustained for approximately 2 hours prior to losing all associative memory in wild type strains. The learning index of CL2355 at 2 hours was the lowest, showing its associative memory was significantly reduced over time. Further studies need to be done to better understand the behavior of CL2122 in the context of STAM and Aβ aggregation.

Further research is needed to help us better understand how Aβ aggregation in neurons affects memory association. Long term associative memory assays would not only allow for comparison of the effects of Aβ aggregation on the long term and short term associations but also establish a broader understanding of the formation of associative memory over time. Thioflavin S Staining would be useful for quantifying Aβ aggregation in each strain of *C. elegans*. This analysis would aid in interpreting the role of Aβ peptides in associative memory loss in *C. elegans*.

Conclusion

Alzheimer's Disease is the leading cause of dementia in the United States. The cause of the disease, theorized to be the aggressive accumulation of Aβ peptides in the brain, is still not fully understood and has come into question in recent years. The goal of this study is to provide clarity on the mechanisms behind the memory loss characteristic of AD.

Our study provided insight into the patterns of STAM loss in N2 Wild Type, CL2122, and CL2355 *C. elegans* strains. We observed that the patterns of deterioration for CL2122 and N2 Wild Type were more similar than those between CL2122 and CL2355, both possessing an abrupt decrease and consequent increase in STAM from 1 hour to 2 hours. On the other hand, the STAM of CL2355 *C. elegans* displayed a more gradual decline. Our study could be used as evidence in support of the amyloid hypothesis and the mechanisms behind AD, thus possessing implications for potential treatments and cures for AD.

AUTHOR INFORMATION

All authors worked on all aspects of this research, from research set-up and laboratory experimentation to data analysis and paper writing and revision.

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