Modeling Alzheimer's Disease Treatments in *Caenorhabditis elegans*

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Abstract

Alzheimer's disease (AD) is one of the main causes of dementia and death, yet it has no known cure. Several lifestyle changes have been proposed to prevent or reduce the effects of AD. *Caenorhabditis elegans* is a potential AD model organism. *sel-12*(*ar131*) worms express a mutated version of SEL-12. SEL-12 is a protein related to presenilin proteins, which are the most common cause of genetic AD in humans. It was hypothesized that if *sel-12*(*ar131*) *C. elegans* is split into groups with fish oil, alcohol association training (AAT), lack of *E. coli*, and no treatment, then those in the group with AAT will have the best memory compared to wildtype as measured by chemotaxis assays. This is because unlike other treatments, AAT forces worms to learn which may reinforce neurons. This hypothesis was shown incorrect by the experiment. However, mutant worms had lower memory capacity than wild type worms with statistical significance. This indicates that while *C. elegans sel-12*(*ar131*) mutants serve as a viable model for AD, their memory capacity is changed little by the treatments used in this experiment. If applicable to humans, this indicates that lifestyle changes are not sufficient to prevent familial AD. More research is needed to determine if people with AD from genetic causes versus unknown causes react differently to these lifestyle changes.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with old age and the sixth leading cause of death in the United States. It is associated with the presence of amyloid beta plaques in the brain and is a major cause of dementia. *Caenorhabditis elegans* is a nematode that serves as a possible model for AD. Adult hermaphrodites have a total of 302 neurons, split into 282 neurons in the somatic nervous system and 20 in the pharyngeal nervous system (Altun & Hall, 2011). *C. elegans* has various genes that can be mutated to model human AD, including *sel-12*, a gene homologous to human presenilin (Alexander, Marfil, & Li, 2014). In humans, presenilin-1 (PSEN1) mutations are the most common cause of genetically linked AD (Kelleher & Shen, 2017). Unlike in the normal aging brain, large numbers of neurons die in the AD brain. AD generally affects areas associated with learning and memory first. However, AD also affects language and other areas ("What happens to", 2017).

AD has no known cure. However, some lifestyle changes may help reduce the risk of AD including a diet rich in fish as well as mental exercise through learning ("What can you", 2017). Intermittent fasting has also been proposed as a way to prevent Alzheimer's disease (Zhang et al., 2017). These treatments have analogs in *C. elegans*. A moderate amount of fish oil has been found to extend lifespan in *C. elegans* (Sugawara et al., 2013). *C. elegans* has also been found to have capacity for learning through chemotaxis assays: worms trained to associate an attractant with a repellant became neutral to or even repelled by what was before an attractant (Amano & Maruyama, 2011).

In this experiment, intermittent fasting, learning, and diet will be modeled in *C. elegans* as treatments for AD. The purpose of this experiment is to determine the degree to which various treatments improve *C. elegans* memory as tested by a chemotaxis assay.

There are two main hypotheses for the cause of AD: the tau protein hypothesis and the amyloid-beta (Aβ) hypothesis. The tau protein hypothesis states the following: as tau proteins are hyperphosphorylated, they tend to form tangles in the brain. Since tau is an important protein in normal brain function, its hyperphosphorylation reduces this function and ultimately leads to neuronal cell death. However, there is a lack of mutations in tau proteins that cause AD (de Paula et al., 2009), making this difficult to model using mutant *C. elegans*. The Aβ hypothesis focuses on Aβ proteins aggregating to form plaques that harm brain function. This hypothesis begins with the cleavage of the transmembrane protein APP by either α-secretase or β-secretase. If cleaved by α-secretase, APP releases sAPPα and C83. Finally, C83 is cleaved by γ-secretase, releasing the APP intracellular domain (AICD) rather than creating Aβ plaques. On the other hand, if APP is cleaved by β-secretase, it released sAPPβ and C99. When C99 is cleaved by γ-secretase, it releases AICD and Aβ (Alexander, Marfil, & Li, 2014).

The enzyme γ-secretase is composed of multiple components, including presenilins. In humans, presenilin mutations have been found to lead to early-onset AD, possibly by increasing the amount of Aβ with 42 amino acids ($A\beta_{42}$) compared with the amount of Aβ with 40 amino acids ($A\beta_{40}$). $A\beta_{42}$ is more likely to aggregate and form plaques associated with AD (De Strooper, Iwatsubo, & Wolfe, 2012). *C. elegans* APL-1, related to human APP, does not produce Aβ. Furthermore, *C. elegans* appears to lack a protein related to β-secretase, although it does have

proteins related to α-secretase called SUP-17 and ADM-4 (Alexander et al., 2014). These are major limitations to studying AD in *C. elegans*.

However, no correlation has been found between Aβ plaque density and severity of dementia. Moreover, non-demented people may also have Aβ plaques similar to those found in AD patients (de Paula et al., 2009). New research has indicated that most pathogenic presenilin mutations, although causing AD, actually reduce Aβ production (Kelleher & Shen, 2017). *sel-12* mutations in *C. elegans* cause memory defects as do human presenilin mutations; and human PSEN1 can prevent these memory defects (Hunt, 2016). *sel-12* mutants were found to have reduced ability to sense octanol, corresponding to decreased olfactory ability in humans with AD. This could be prevented with human wild type presenilin, but not with AD presenilin (Parvand, Bozorgmehr, & Rankin, 2017). In light of this, *C. elegans* can be a viable model for AD due to the effects of its mutations in its presenilin homologs, despite the fact that it lacks Aβ.

A leading method for testing *C. elegans* memory and learning is chemotaxis. *C. elegans* naturally is attracted to certain chemicals (called chemoattractants) and repelled by others (called chemorepellents). While 1-propanol is a chemoattractant (Amano & Maruyama, 2011), 1-octanol is a chemorepellent. Sodium chloride (NaCl) is a chemoattractant for concentrations up to 200 mM, but becomes a chemorepellent for higher concentrations (Hart & Chao, 2010). *C. elegans* can be trained to behave differently to substances that are normally chemoattractants or chemorepellents. For example, Kauffman et al. (2011) starved worms and then fed them in the presence of butanone, a chemorepellent. The worms learned to associate butanone and food, and thus became attracted to butanone in a subsequent chemotaxis assay. *C. elegans* can also associate chemoattractants with chemorepellents. Amano & Maruyama (2011) ran a study in

which they trained *C. elegans* to associate the chemoattractant 1-propanol with the chemorepellent hydrochloride (HCl). To do so, they used two methods: spaced training and massed training. With both methods, worms successfully learned to associate 1-propanol with HCl, and were neutral to or repelled by 1-propanol in a chemotaxis assay. However, worms remembered this association for up to 24 hours with spaced training, but for under 3 hours with massed training. Luo et al. (2014) analyzed the mechanism of *C. elegans* memory in NaCl chemotaxis at a neurological level. They focused on the ASE neurons (sensory neurons involved in attraction to NaCl) and their connection to interneurons AIY, AIZ, and AIB. Interestingly, they found memory of NaCl concentration was stored in the single right ASE neuron (ASER), rather than the left ASE neuron (ASEL) or interneurons. ASER activity differed based on whether worms were above or below a remembered NaCl concentration; this activity was then integrated by interneurons and passed to motor neurons to create positive or negative chemotaxis. While this chemotaxis could function missing AIY or AIZ, at least one of the two neuron pairs was necessary. This is interesting to AD because *sel-12* mutants have decreased memory in thermotaxis assays due to abnormalities of the AIY and AIZ interneurons. (Martínez-Finley, Avila, Chakraborty, & Aschner, 2011). This supports *sel-12* mutants in *C. elegans* as AD models.

While there is no known cure for AD, various lifestyle changes can reduce the effects of the disease, including modifications in diet and learning new things. Dietary changes include eating more fish (“What can you”, 2017). In a study using mice, Zhang et al. (2017) found that intermittent fasting (IF) reduced Aβ and improved cognitive function, thus showing the possibility of IF as a viable lifestyle modification for AD. This can be modeled in *C. elegans* by growing worms without *E. coli*, greatly decreasing their food source but not killing them.

In *C. elegans*, Sugawara et al. (2013) showed that fish oil at 1.0 mg/plate increased the lifespan of *C. elegans*, while fish oil at 2.0 mg/plate decreased lifespan. The addition of fish oil increased the measured amount of thiobarbituric acidic substances (TBARs), an indicator of increased lipid peroxidation and oxidative stress. However, addition of the antioxidant α-tocopherol (αToc) along with fish oil stopped this increased lipid peroxidation, and both the increase and decrease in lifespan were no longer detectable. The authors concluded that the moderate amount of oxidative stress from the fish oil increased the resistance of the worms to oxidative stress by stimulating antioxidant production, thus actually increasing lifespan. The high amount of fish oil, in contrast, increased oxidative stress beyond a threshold and decreased lifespan. This experiment on fish oil and oxidative stress in *C. elegans* is especially interesting when viewed along with research on the link between oxidative stress and AD in humans. Production of reactive oxygen species (ROS) and insufficient defense against ROS by antioxidants have been connected with the development of AD. It has been shown that elevated levels of ROS interfere with neuronal receptors in AD, causing synaptic dysfunction (Tönnies & Trushina, 2017).

Learning new things and exercising the brain may reduce effects of AD. Pliatsikas, Moschopoulou, & Saddy (2015) analyzed the effect of bilingualism on white matter and cognitive function. They studied both people who learned additional languages early in life and those who learned languages late in life. They found that among both groups, there were cognitive benefits to being bilingual based on how much the additional language was used. People who were trained in another language but did not use it regularly did not show cognitive benefits of other bilinguals, suggesting the benefits are due to cognitive exercise in general and

not necessarily bilingualism. This is relevant to AD because AD leads to a decline in both white matter and grey matter (Nasrabady, Rizvi, Goldman, & Brickman, 2018). *C. elegans* lacks myelination (Oikonomou & Shazam, 2011), and so it lacks a distinction between grey and white matter. Therefore, it is possible that learning will benefit *C. elegans* cognitive function in unmyelinated neurons similar to the benefit humans receive in myelinated neurons.

The hypothesis for this experiment is that if *sel-12*(*ar131*) *C. elegans* is split into groups receiving fish oil, alcohol association training, restricted nutrition, and no treatment, then those with alcohol association training will have the best memory compared to the control group of *C. elegans* wildtype as measured by chemotaxis assays. The fish oil will serve as a model of human dietary changes to prevent AD. The alcohol association training will consist of worms associating 1-octanol, a repellent, with 1-propanol, an attractant. This will model learning as a treatment for AD. The lack of *E. coli* will simulate intermittent fasting, another possible treatment for AD. This hypothesis was derived from the fact that while the other treatments may have indirect chemical effects on neurons, learning is directly reinforcing neuronal activity. If this hypothesis is shown to be correct, it will indicate a strong role of presenilin in AD, and will suggest that the effects of presenilin mutations can be countered by regular use of neurons. Since *C. elegans* has a much simpler nervous system than humans, it may also serve as evidence of an underlying cause of AD at a basic molecular level rather than at a more advanced neurological level involving interactions between large numbers of neurons.

Methods

Materials

- Petri dishes
- N2 *C. elegans*
- *sel-12(ar131) C. elegans*
- Fish oil
- 2.00 µL micropipette
- *E. coli* (OP50)
- NGM Agar
- 3% 1-octanol
- 3% 1-propanol
- Centrifuge tubes
- dH_2O
- 0.1 M NaCl
- 20.0 µL micropipette
- Inoculating loops
- Sharpie marker
- Dissecting microscope
- 1000 µL micropipette

Procedures

1. Pour five plates. Label one “control”, one “no treatment”, one “fasting”, one “learning”, and one “fish oil”. Wait for the plates to solidify. Wear goggles when melting agar to avoid hot water splashing in the eyes.
2. Add 1.15 µL of fish oil to the plate labeled “fish oil” for a concentration of 35.4 µg/cm^2.
3. Add OP50 *E. coli* to all the plates except the plate labeled “fasting”. In the plate labeled “control”, add N2 worms. In the other plates, add *sel-12*(*ar131*) (Alzheimer’s disease simulating/ADS) worms.
4. Treat the worms for at least three days.
    4.1. No treatment will be given to the plates labeled “control”, “learning”, and “no treatment” except regular replacement of *E. coli*.
    4.2. The treatment in the plate labeled “fish oil” will consist of the worms’ exposure to the fish oil and regular replacement of *E. coli*.
    4.3. The treatment in the plate labeled “fasting” will consist of the worms surviving off just the NGM agar, without *E. coli*.
    4.4. There are now five groups of worms: control, no treatment, fish oil, learning, and fasting.
5. Test the memory of each group of worms using chemotaxis.

5.1. Fill a 0.6 mL centrifuge tube with 0.1 mL of 3% 1-octanol and 3% 1-propanol. Place the centrifuge tube in the plate labeled “learning”.

5.2. Fill a centrifuge tube with 0.5 mL of 0.1M NaCl. Add dH_2O to the plate labeled “fasting”. Take 12 µL from the plate and place it into the centrifuge tube. Label the centrifuge tube with “fasting”.

5.3. Repeat step 5.2 as desired to have more C. elegans. Add *E. coli* to the centrifuge tube labeled “fasting”. Let the worms remain in this tube for one hour.

5.4. Pour a new plate of NGM agar for each group of worms and label each plate with “salt” and the group of *C. elegans*. Do not add *E. coli* to the plates. Add 12 µL of salt to each plate and mark the spot where the salt was added on the bottom of the plate.

5.5. Fill five centrifuge tubes with 0.5 mL of 0.1M NaCl. Label the centrifuge tubes with each of the five groups of *C. elegans*.

5.6. Transfer 12 µL of worms from the plate labeled “fasting” to the new centrifuge tube labeled “fasting” and dispose of the old centrifuge tube. Do not add *E. coli* to the new fasting tube.

5.7. Add dH_2O to the *C. elegans* plates not labeled “fasting”. Take 12 µL of worms from each plate and add to the appropriately labeled centrifuge tubes. Let the worms in all five centrifuge tubes remain for one hour.

5.8. Transfer 12 µL from each centrifuge tube into the appropriately labeled plate with NaCl on the same spot where the NaCl was added. Draw a circle of radius 0.5mm around this spot. Wait one hour.

5.9. Count the *C. elegans* within the drawn circle and compare to the number outside of the drawn circle. Count worms completely on the line as inside, but only partially on the line as outside.

6. Repeat step 5 as desired for more trials.

Calculations

Chemotaxis index (CI) for *C. elegans* is calculated by:

$$\mathrm{CI} = \frac{A - B}{A + B}$$

Where A is the number of worms in the area of a chemical, and B is the number not in the area. CI has a maximum value of 1.0 and a minimum value of –1.0.

Memory index (MI) for this experiment was defined as $\mathrm{MI} = 1 - \mathrm{CI}$. This gives MI a maximum of 2 (if all worms were repelled by salt) and a minimum of 0 (if all worms were attracted by salt).

Results

Memory index (MI) was calculated from chemotaxis index as described in the calculations section. As hypothesized, N2 worms had the highest memory indices, and untreated *sel-12*(*ar131*) worms had the lowest memory indices, with different treatments having intermediate values closer to the mutant values than the wildtype values.

Memory indices were found to be different between groups of worms (Single Factor ANOVA, F=5.93, F crit = 3.11, p=0.005; Figure 1). Comparing N2 worms with *sel-12*(*ar131*) mutant worms, N2 worms tended to have higher memory indices (two-sample two-tailed t-test, t = 6.01, 3 df, p=0.009; Figure 1). Untreated *sel-12* mutants were compared with other groups using two-sample two-tailed t-tests, and no difference was found with diet restriction (t = -1.66, 3 df, p=0.195; Figure 1), fish oil (t = -0.90, 3 df, p=0.432; Figure 1), or alcohol association training (t = -1.29, 5 df, p=0.254; Figure 1).

	N2	*sel-12* untreated	*sel-12* fasting	*sel-12* fish oil	*sel-12* learning
1	1.000	0.340	0.385	0.391	0.536
2	1.125	0.360	0.502	0.284	0.438
3	0.846		0.326	0.333	0.250
4			0.600	0.980	0.565
5					0.384
6					0.320
Average	0.990	0.350	0.453	0.497	0.415
Standard Error	0.081	0.010	0.061	0.163	0.050

Figure 1: Table of groups of *C. elegans* and memory indices across trials

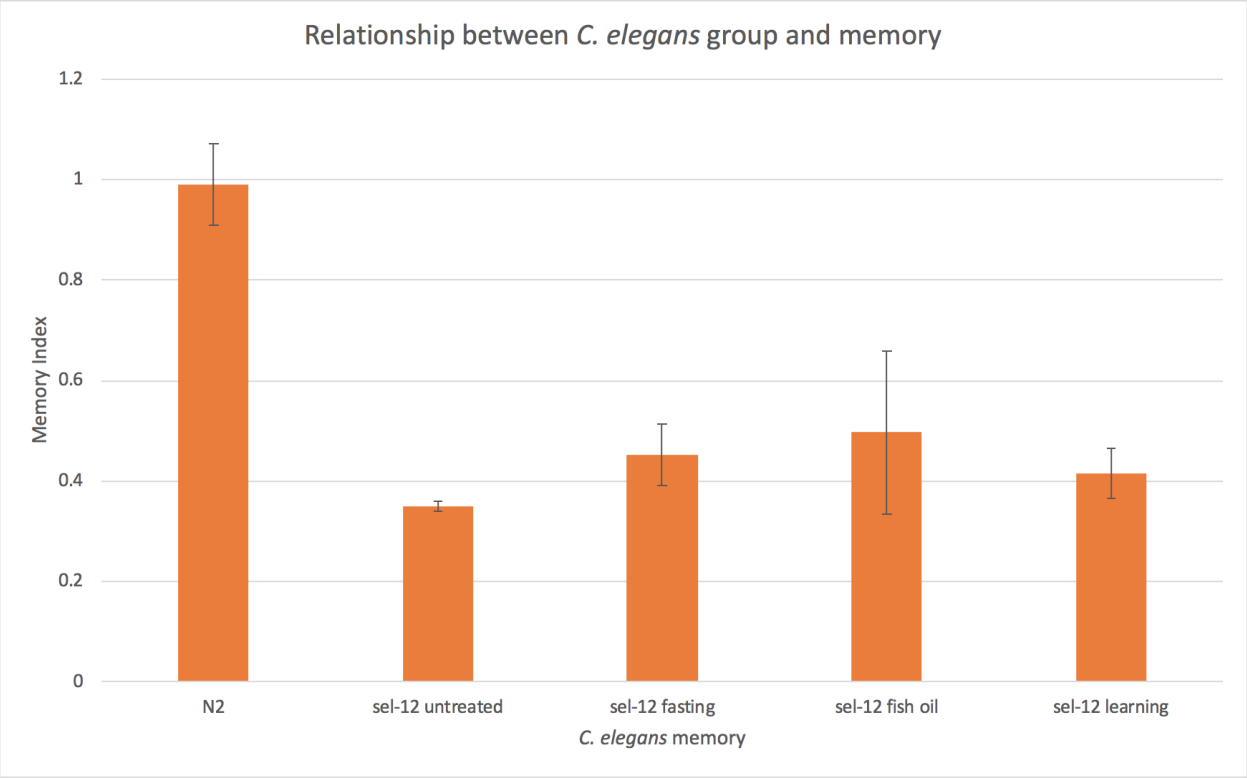

Figure 2: Graphical representation of data from figure 1

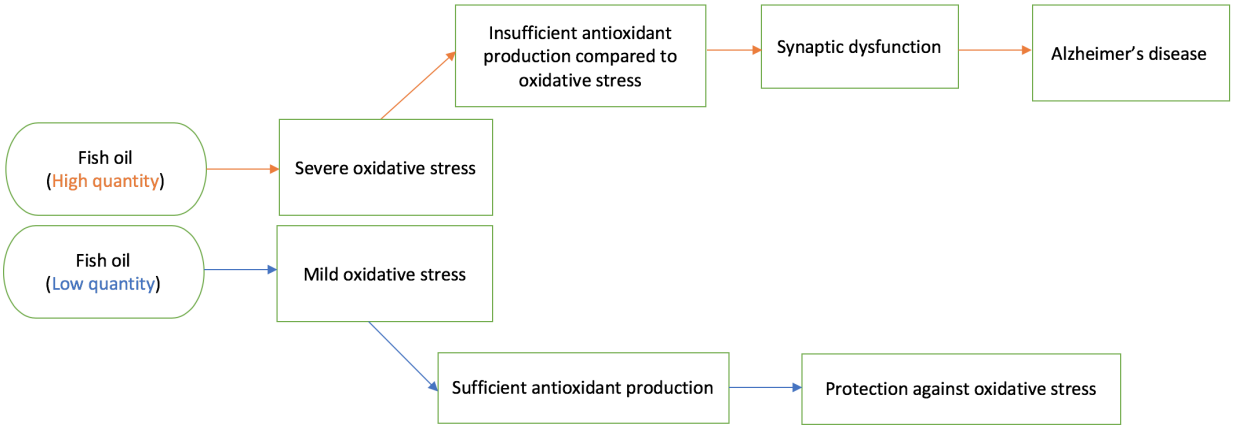

Figure 3: Proposed role of fish oil in AD based on research and experimentation

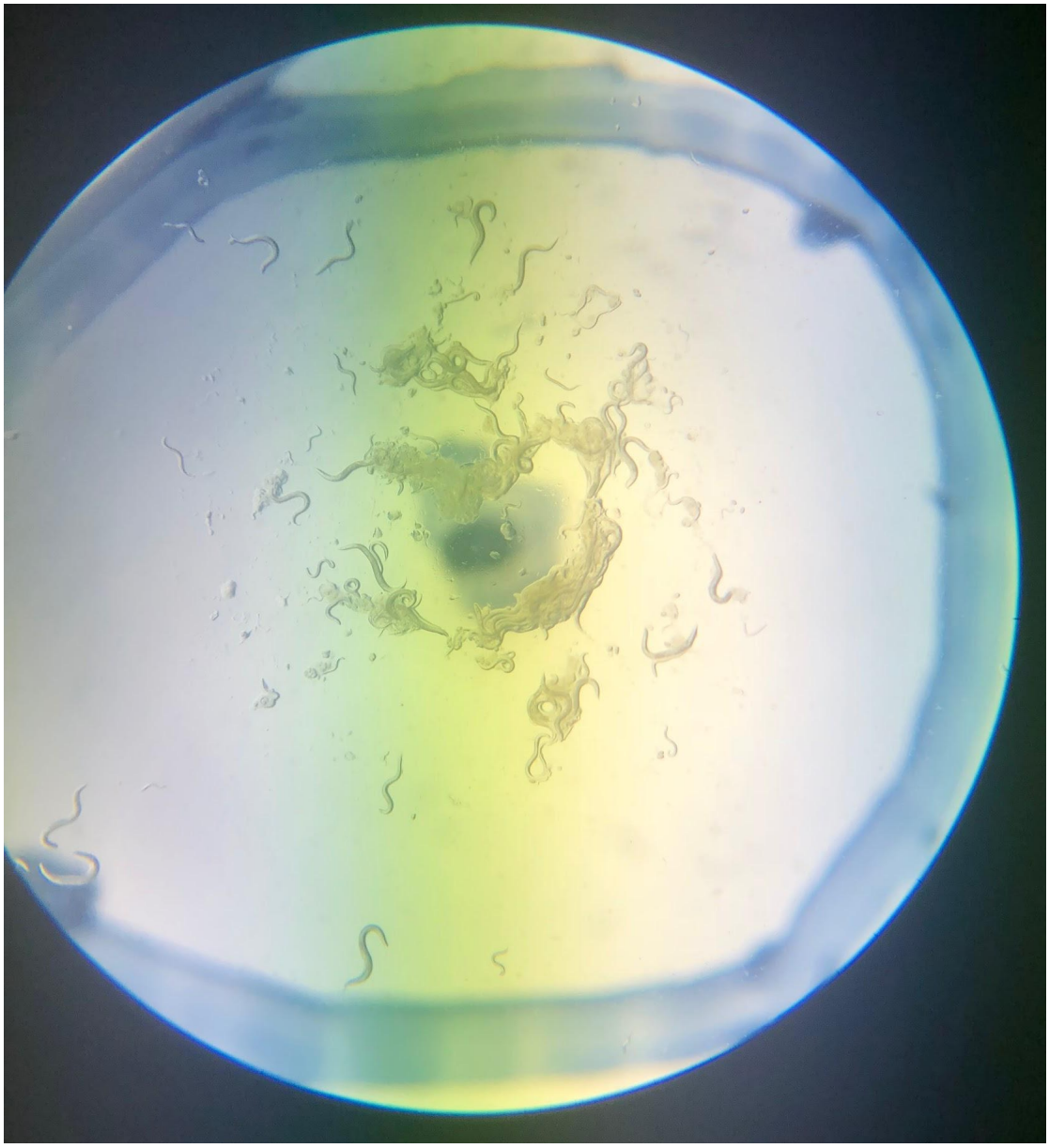

Figure 4: *sel-12*(*ar131*) mutant *C. elegans* under a microscope during a chemotaxis assay. The circle containing NaCl is marked in black.

Figure 5: *C. elegans* under a high-magnification inverted microscope

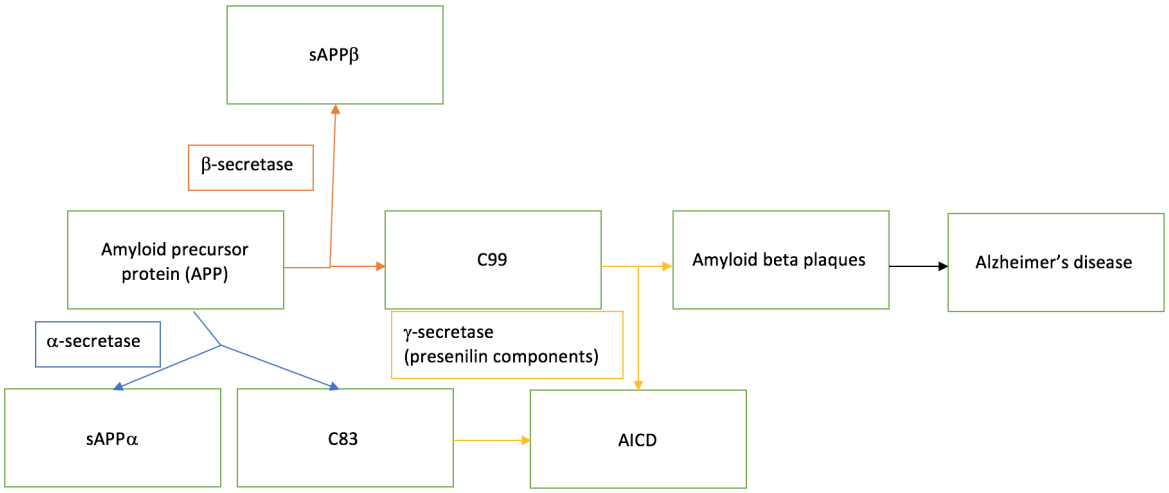

Figure 6: Graphical representation of the amyloid beta hypothesis

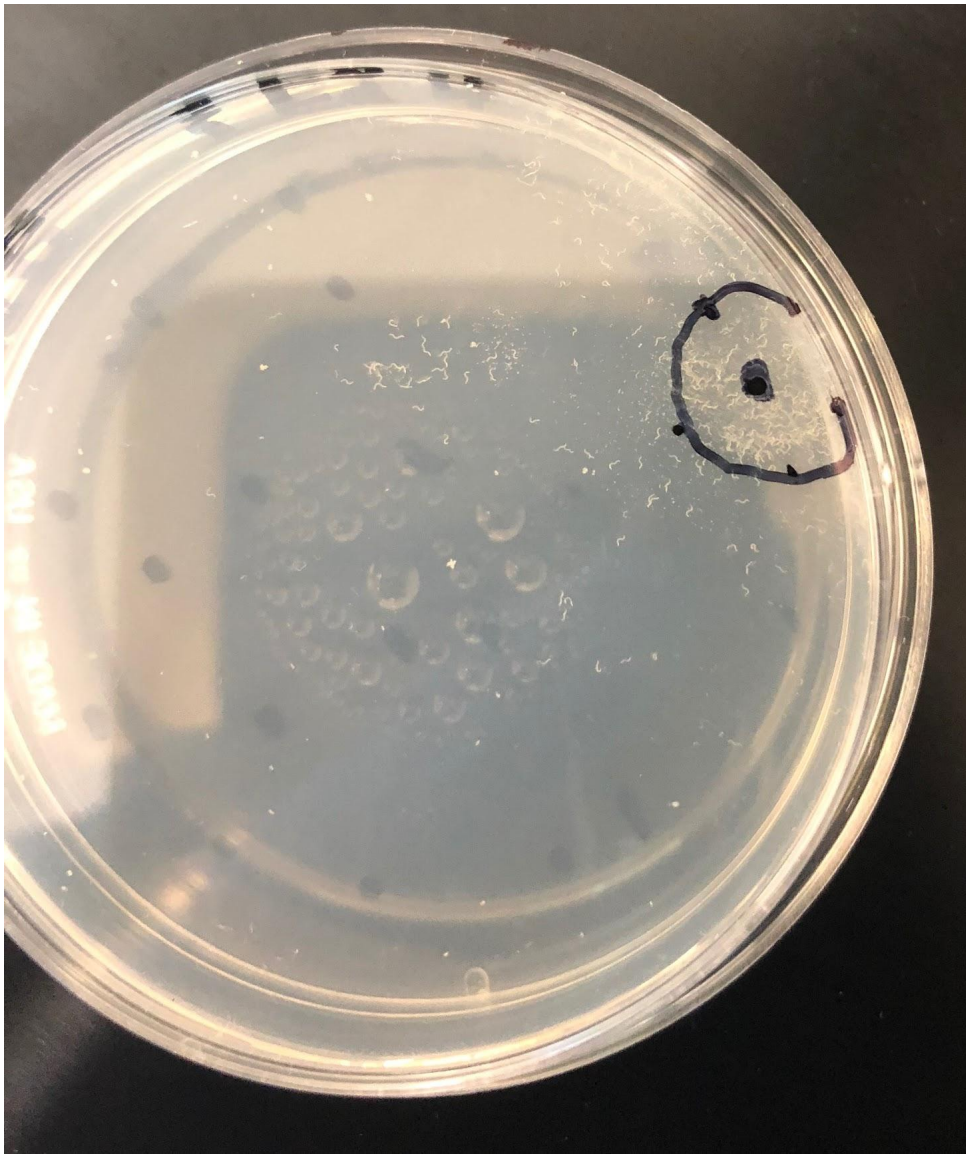

Figure 7: *sel-12*(*ar131*) mutant *C. elegans* during a chemotaxis assay visible from the bottom of a Petri dish without a microscope. Note aggregation near the black circle containing NaCl.

Discussion and Conclusion

In this experiment, lifestyle changes for Alzheimer's disease (AD) were tested on *C. elegans* using chemotaxis assays. The results supported the use of *sel-12*(*ar131*) as a mutation for AD experimentation in *C. elegans*. However, the results did not show a statistically significant difference between treated and untreated mutant worms.

Applications

This experiment showed a statistically significant difference between N2 and *sel-12*(*ar131*) worms, providing evidence for the use of *sel-12* mutants in further AD research using *C. elegans*. If applicable to humans, the results suggest that lifestyle changes are not sufficient to prevent familial AD. The results for fish oil had a high p-value and a high standard error. While there is some evidence from Sugawara et al. (2013) that a diet high in fish leads to increased longevity, both their experiment and this experiment showed inconclusive results for the benefits of a diet high in fish. One possibility is that a fish-based pathway exists in humans but not *C. elegans*. Another possibility is that it is not fish oil contributing to increased health but is another component of fish. If this is the case, the results of this experiment do not support fish oil supplements, but the benefits of actual fish are not ruled out. A third possibility is that fish oil can have benefits to prevent AD and increase lifespan, but these benefits are difficult to detect because the increase in oxidative stress may also be harmful rather than helpful depending on the quantity of fish oil.

Limitations

The main limitation in this experiment was the use of *C. elegans* as opposed to humans or a mammalian model. *C. elegans* lack a role of both tau proteins and amyloid beta (Aβ) plaques in

the brain, two main hypotheses to explain AD. *C. elegans* also generally lack the ability to form memories that span a lifetime, while these memories are strongly impacted in human AD. The nervous system of *C. elegans* has various important differences from the human brain, such as a lack of myelination and greatly reduced complexity. While this simplicity facilitates research, its applicability to humans should be considered cautiously. Another limitation was that this experiment did not look at the activity of individual neurons in *C. elegans*. A similar study looking at the activity of neurons, especially the ASER neuron, could better reveal the role of SEL-12 and have implications for presenilin research.

Error Analysis

One systematic error is due to the fact that the worms could move during counting into areas that have previously been counted. This would make the measured number of worms counted in areas outside the circle of salt smaller than the true value, decreasing memory indices. This error does not apply to worms inside the circle of salt because these were counted by taking pictures and using a tablet. A random error is variance in the radius of the circle of salt, as it was difficult to draw the exact same radius for each trial.

Future research

Although there was a lack of statistical significance, all treatments improved memory on average. More research is needed to determine if the lack of statistical significance is due to a limited amount of data or is a true lack of significance. However, the use of *sel-12* and chemotaxis as a model for AD was backed by statistically significant results. Future research can test drugs linked to the amyloid beta hypothesis in *C. elegans sel-12* mutants before expanding the drugs to human trials. This research on *C. elegans* should analyze activities of individual

neurons to gain better insight into the cellular mechanisms of AD. The presence of Aβ plaques even in healthy individuals indicates that the Aβ hypothesis is not fully correct. However, the hypothesis does seem to be somehow linked to AD as shown by the successful use of *sel-12* mutants and by presenilin mutations as a cause of AD. Further research could investigate the role of presenilins in AD independent of Aβ, incorporating oxidative stress as an additional potential factor.

Conclusion

The results of this experiment supported the use of *sel-12* mutants in AD research, but did not support lifestyle changes as a significant factor in preventing AD. Because SEL-12 is related to human presenilin, the data on *sel-12* mutants compared to wildtype worms indicates a role of γ-secretase in AD independent of Aβ. While this experiment did not support lifestyle changes to prevent AD, it is possible that the reason for the lack of statistical significance is that lifestyle changes are a small but not entirely unimportant factor in preventing AD. Future research on *sel-12* mutants should include data on the activities of individual neurons in order to better see how AD affects memory at a neuronal level.

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