The Effects of Transgenerational Epilepsy on Dementia Pathology in *C. elegans*

Grant Proposal

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Author Note

Thank you to Dr. Kevin Crowthers, our STEM teacher, for aiding and guiding me throughout this process. He has helped me learn how to use *C. elegans*, supervised me during the lab work process, and played a huge part in developing this grant, project idea, and procedures.

Abstract (RQ) or Executive Summary (Eng)

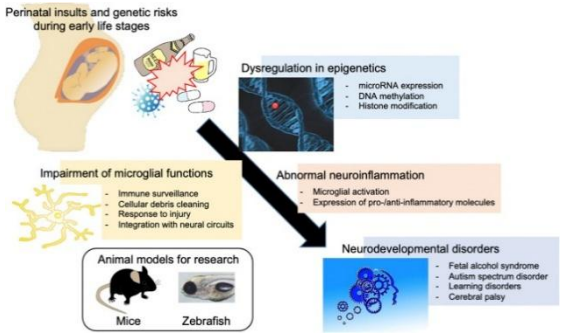
The abstract would summarize what you (as the author) would like to convey. It would include some knowledge gaps that eventually lead to researchable questions you have identified in the field.

Keywords: epilepsy, alzheimer's disease, dementia,

The Effects of Transgenerational Epilepsy on Dementia Pathology in *C. elegans*

Epilepsy and Alzheimer's disease are among the most common neurological disorders in humans. They affect over 50 million people each year (Yang et al., 2022). These disorders have been found to be comorbid, and their bidirectional relationship is being increasingly studied (Stewart & Johnson, 2025). One cause that has been noted is the shared neuroimmune and neuroinflammatory response. Understanding the process by which this response exacerbates rates of these disorders can help us identify possible treatment and preventative measures.

Epilepsy is a neurological disorder that causes seizures, a phenomenon characterized by uncontrolled movements, loss of consciousness, confusion, and convulsion. It is caused by both genetic and environmental changes. Some of these etiologies include genetic, structural, infectious, metabolic, immune, and unknown (Zhang et al., 2024). In a 2024 research article, it was found that most cases have an unknown/unclear cause or have a structural cause (Zhang et al., 2024). Epilepsy also causes issues in the body. Due to the repeated activation of glial cells like astrocytes and microglia, there is a higher rate of neuroinflammation in Epilepsy. Studies have found that neuroinflammation can cause epigenetic changes such as hypermethylation and changes in gene expression as shown in the diagram on the right (Komada & Nishimura, 2022).



Epigenetic changes have been found to cause Alzheimer's disease (Sharma et al., 2020). AD is the most common type of dementia characterized by amyloid beta plaques and tau tangles. This is a debilitating disorder that we have no treatment for. By tracing the pathway from Epilepsy to Alzheimer's they have a crucial relationship that, when understood, can provide insight into treatment and preventative measures for AD. Epigenetic changes have been found to

be transgenerational (Fitz-James & Cavalli, 2022). This means that epigenetic changes in a parent's body can be passed down to offspring. This is extremely concerning since this means the difference in expression and methylation created by epilepsy could cause AD and AD-like pathology many generations after the changes first appeared.

To study this relationship *C. elegans* can be used as a model organism. This organism has been used in many AD and Epilepsy studies (Gourgou et al., 2021; Gourgou & Hsu, 2021; Zhou & Bessereau, 2019; Emmons et al., 2021). Due to the simplicity of the organism and the ease of visualizing its structures, c elegans acts as a great model (Emmons et al., 2021). In neurological disorders its use is important since its full connectome has been mapped (Emmons et al., 2021). Furthermore, c elegans is a hermaphrodite. Therefore, the genetic variation in its offspring is comparatively limited providing a more controlled experiment.

Epigenetics is a novel field that is extremely important to our understanding of disease pathologies. Studying the transgenerational cascade of changes and its relationship with Epilepsy and Alzheimer's can help us create better screening methods. This research can help identify at-risk individuals and advise them on preventative measures. In the end, understanding the association between these disorders can pave the way for new targets in treatment.

Section II: Specific Aims

This proposal's objective is to……

Our long-term goal is to identify a generational relationship between epilepsy and dementia pathology where the central hypothesis of this proposal is that seizures across more generations will cause more dementia pathology. The rationale is that seizure-related

neuroinflammation will cause epigenetic changes that create amyloid fibrils which in turn cause dementia symptoms. The work we propose here will…

Specific Aim 1: Observe epigenetic differences over generations of *C. elegans* unc-49 with seizures caused by pentylenetetrazole.

Specific Aim 2: Stain *C. elegans* nervous system with Thioflavin-T to analyze frequency of amyloid fibrils compared to the total area.

Specific Aim 3: Analyze the differences in learning indexes over generations of *C. elegans* using a T-maze test.

The expected outcome of this work is that more seizures across more generations will cause increased mRNA methylation, increased frequency of amyloid fibrils, and lower learning indexes in T-maze tests.

Section III: Project Goals and Methodology

Relevance/Significance

Identifying epilepsy as risk factor for Alzheimer's disease over multiple generations, could help medical professionals give families advice on AD prevention. Look at epigenetics as a factor in this inheritance can help highlight a new therapeutic target for AD.

Innovation

Many studies have looked at how epilepsy or Alzheimer's may increase the risk of each other in one person's lifetime. Looking at it in a generational light gives a deeper understanding of the effects of epilepsy on a cellular level.

Methodology

Specific Aim #1:

Determine the average percentage difference in whole system m6a methylation difference between generations of *C. elegans* given seizure using pentylenetetrazole. The objective is to identify a possible reason for increased Alzheimer's risk. Since epigenetics can be inherited and has been linked to Alzheimer's risk, identifying a change in m6a methylation will demonstrate the mechanism of risk increase. Our approach is to extract the ribonucleic acid (RNA) of *C. elegans*. This RNA will then be used in an m6a methylation kit which would identify the frequency of the whole system RNA methylation in the worms. By comparing the frequencies of the worms of different groups with a wild type, we would be able to observe differences. Our rationale for this approach is that *C. elegans* do not have human-like methylation patterns. Rather, they are methylated in their RNA. For this reason, using an m6a kit will provide us with substantial information regarding how seizures affect epigenetics.

Justification and Feasibility. This project looks to identify the possible pathological differences caused by epilepsy across multiple generations. Identifying epigenetic difference is a substantial part of this. This idea if feasible due to our ability to extract the RNA of a worm using materials available at a school lab. These ideas in mind we are able to draw important conclusions on the effect of epilepsy of whole-system methylation

Expected Outcomes. The overall outcome of this aim is an increased methylation in the whole system. This knowledge will be used for identifying a possible mechanism of inheritance.

Potential Pitfalls and Alternative Strategies. We expect that there maybe difficulty in obtaining this kit due to price. One possible strategy to mitigate this is doing a more simple

methyl group paper chromatography. By extracting the RNA of c elegans and suing the RNA to run a paper chromatography test, we may be able to quantify this to some degree.

Specific Aim #2:

Quantify the average percentage of amyloid fibril–positive area in the nervous system of *C. elegans* stained with Thioflavin-T, and compare across experimental groups. The objective is to determine whether certain conditions (e.g., induced stress, genetic variants, or environmental exposures) correlate with increased amyloid aggregation, which is a hallmark of neurodegenerative disease processes. Since amyloid formation is a conserved pathological feature and *C. elegans* provides a tractable model for studying neuronal protein aggregation, measuring fibril burden will help identify early mechanistic contributors to neurodegeneration. Our approach is to expose live or fixed *C. elegans* to Thioflavin-T, a fluorescent dye that selectively binds β-sheet–rich amyloid structures. Following staining, worms will be imaged under a fluorescence microscope. Using image-analysis software (e.g., ImageJ), we will quantify the area of ThT-positive signal in the nervous system and normalize it to total neural area. By comparing these values across groups and a wild-type control, we will detect changes in amyloid accumulation patterns. Our rationale is that Thioflavin-T is widely validated for detecting fibrillar aggregates in *C. elegans* models of Alzheimer-like pathology due to its strong specificity and ease of visualization.

Justification and Feasibility. This project aims to evaluate amyloid-related pathology in a simple, accessible model organism. Thioflavin-T staining is a well-established method requiring only basic laboratory equipment: fluorescence microscopy, standard staining reagents, and image-analysis software. Because *C. elegans* are transparent, their nervous system can be readily

visualized without complex dissection, making this analysis highly feasible in a school laboratory environment. These features support the practicality of obtaining meaningful data about amyloid burden.

Expected Outcomes. We anticipate detecting an increased percentage of Thioflavin-T–positive area in worms exposed to stressors or genetic conditions associated with aggregation. Identifying elevated fibril burden would provide evidence for early neurodegenerative processes and suggest a mechanistic pathway relevant to human amyloid-related diseases.

Potential Pitfalls and Alternative Strategies. One challenge may be obtaining sufficiently clear fluorescent images for accurate quantification due to worm movement or dye variability. To mitigate this, worms may be immobilized with mild anesthetics (e.g., levamisole) or fixed prior to staining. If access to fluorescence microscopy is limited, an alternative approach is to use Congo Red or a cheaper fluorescent dye with a smartphone-adapter microscope setup, though sensitivity may be reduced. Another alternative is to use transgenic worms expressing fluorescently tagged amyloid proteins (e.g., Aβ::GFP), allowing aggregation to be quantified without chemical staining.

Specific Aim #3:

Determine the average difference in learning index across generations of *C. elegans* using a T-maze chemotaxis learning assay. The objective is to identify whether learned behaviors—specifically associative learning—show measurable changes across generations when worms are exposed to a particular stimulus or environmental condition. Since *C. elegans* can transfer certain behavioral tendencies epigenetically, analyzing generational shifts in learning performance may help reveal mechanisms of inherited behavioral plasticity. Our approach is to train worms in a T-

maze setup where they learn to associate one arm of the maze with a desirable cue (e.g., food, NaCl gradient) and the opposite arm with a neutral or aversive cue. After training, worms from each generation will be placed into the maze, and their arm preference will be recorded. A learning index will be calculated as the proportion of worms choosing the trained arm versus the total number tested. By comparing these values across experimental generations and a wild-type baseline, we can identify changes in learned behavior and potential transgenerational effects. The rationale for this method is that T-maze–based chemotaxis learning is simple, quantifiable, and widely used in *C. elegans* behavior research.

Justification and Feasibility. This project investigates how learned behavior may shift or persist across generations, which is an emerging topic in epigenetics and neurobiology. The T-maze test requires minimal materials—cardboard or acrylic maze, agar plates, stimuli, and a small number of worms—making it feasible in a school laboratory. Because *C. elegans* naturally perform chemotaxis and readily exhibit associative learning, the proposed method is practical and accessible while still allowing robust behavioral measurement.

Summary of Preliminary Data.

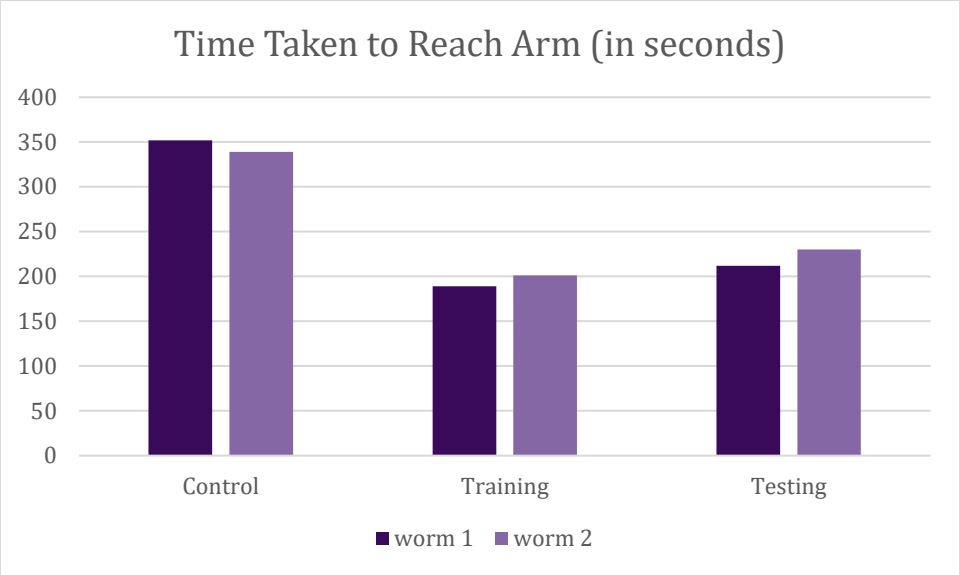


Figure1: Bar graph of data collected form preliminary t-maze assay of wild type worms (n=2).

Maze type	worm 1	worm 2
Control	352	339
Training	189	201
Testing	212	230

Figure2: Table of data collected form preliminary t-maze assay of wild type worms (n=2).

Expected Outcomes.

We anticipate that trained parent generations may show improved learning index values, and that subsequent generations exposed to the same conditions may inherit altered learning tendencies. Identifying a consistent shift in learning performance would support the idea that environmental experience can influence behavior across generations.

Potential Pitfalls and Alternative Strategies.

Potential issues include inconsistent worm movement, uneven stimulus placement, or variability in maze construction affecting chemotaxis. To mitigate these challenges, the maze design can be standardized using rigid materials, and stimuli can be applied in controlled volumes. If results remain inconsistent, an alternative strategy is to use a standard quadrant chemotaxis assay rather

than a T-maze, which reduces possible directional bias and may produce more stable learning-index measurements. Another alternative is to perform olfactory conditioning using odorants (e.g., diacetyl) for more precise stimulus control.

Section III: Resources/Equipment

Section V: Ethical Considerations

Section VI: Timeline

Section VII: Appendix

Section VIII: References

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