Neuroprotective Effects of Insulin Signaling Modulation in an Alzheimer's Model

Grant Proposal

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Abstract

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: Alzheimer's disease, *Drosophila melanogaster*, Insulin Signaling Pathway, neurodegeneration

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Alzheimer's Disease (AD) is a neurodegenerative disease that harms memory and thinking skills. More than 6 million Americans have Alzheimer's, making it the seventh leading cause of death in the U.S. (NIA, 2023). It leads to loss of cognitive function such as thinking and memory, as well as behavioral regulation. AD is caused due to the buildup and accumulation of proteins like amyloid or tau in the brain. AD largely affects elderly people, and the reason for this is suspected to be age-related changes such as inflammation, blood vessel damage, or brain atrophy.

Role of Insulin in the Brain and 'Type 3 Diabetes'

The role of insulin in the brain is a multifaceted and often contradictory subject. AD is being called 'Type 3 Diabetes' by an increasing number of people in the medical community. This is because insulin resistance is a major factor in a cascade of brain damage that leads to AD, since the insulin degrading enzyme (IDE) degrades not only insulin in the brain but also the Aβ protein, the peptide that causes AD due to its buildup. When insulin resistance occurs, IDEs must work to break down excess insulin in the brain, leading to less breakdown of Aβ, causing the peptide's accumulation (Mullins et al., 2017).

The Dual Role of Insulin Signaling in the Brain

While the Insulin Signaling Pathway (ISP) has positive effects on neurodegeneration, it also has a dual effect. Hampering the ISP can lead to reduced Aβ toxicity and lowered levels of neurodegeneration (Huang et al., 2019). This is because it promotes an environment that is more resistant to Aβ toxicity. Genetically hampering the ISP activates protective stress-response pathways and increases autophagy. In fact, it can be observed that insulin signaling in *Drosophila melanogaster* (fruit flies) mediates Aβ toxicity, a key symptom of AD. The *chico* gene is an essential part of the ISP, being recruited by InR and activating PI3K afterwards. Knocking down the *chico* gene hampers the ISP since it is the bridge that allows receptor connections with downstream components of the pathway, leading to an improvement in locomotor ability and a reduction in brain vacuolization, compared to flies that expressed the protein without any ISP modifications (Fig. 1a). Flies with dInR or PI3K, which are both key parts of the ISP, knocked out experienced similar results (Fig. 1b).

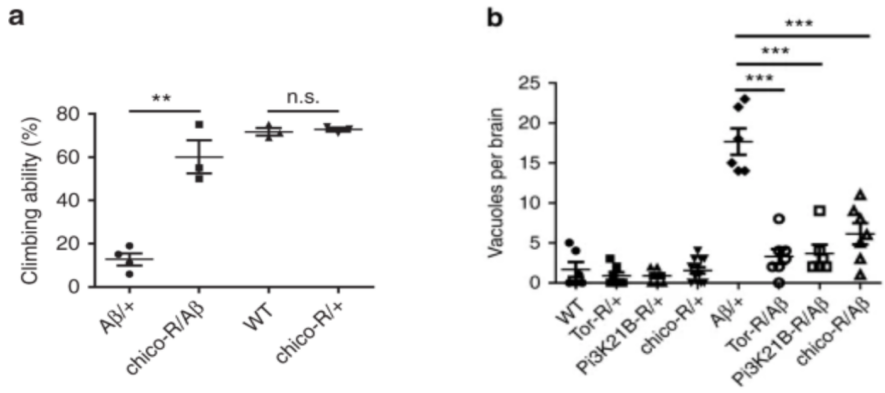

Figure 1: Results showing markers of neurodegeneration in flies such as brain vacuolization and climbing ability. Effects of chico RNAi on the climbing ability of Aβ flies. Elav-Gal4 was used to drive the expression of Aβ and the knockdown of chico (a). Effects of InR manipulation on the climbing ability of Aβ flies. Elav-Gal4 was used to drive the overexpression of InR, Aβ and the knock-down of InR (b) (Huang et al., 2019, Figs. 1a and 1b).

As shown in Fig. 1, wild types exhibit no variance of any genotypic impact on climbing ability, a marker associated with neurodegeneration, since there is no amyloid protein present. However, when it is present, inhibiting the insulin signaling pathway has a significant effect in rescuing the climbing abilities of the flies (Huang et al., 2019).

Linking Aging and Metabolic Stress to Neurodegeneration

High-sugar diets cause metabolic dysregulation, leading to shortened lifespan, as well as Type 2 diabetes (van Dam et al., 2020). Type 2 diabetes is one of the biggest risk factors for AD, as is aging (De Felice et al., 2014; National Institute on Aging, 2023). Thus, aging and metabolic stress induced through a high-sugar diet can be used to mimic AD-like symptoms in a model organism

Traumatic Brain Injury as an Alternative Model for Alzheimer's Disease in *Drosophila*

Traumatic brain injury (TBI) is also a contributing long-term risk factor for neurodegenerative diseases including Alzheimer's. Individuals who sustain moderate to severe TBI have a significantly elevated risk of dementia and related disorders later in life (Gu et al., 2022). After injury, the brain exhibits chronic inflammation, tau hyperphosphorylation, oxidative stress, and impaired clearance of toxic proteins. These are mechanisms that closely parallel the pathological cascade seen in AD. (Johnson et al., 2012). Because of these overlapping mechanisms, TBI is considered a major trigger that can initiate or exacerbate AD-like neurodegeneration in genetically or metabolically vulnerable individuals. This makes TBI a powerful and ethically accessible method for creating an Alzheimer's-relevant model in Drosophila.

***Drosophila Melanogaster* as a Model Organism**

Drosophila is used frequently as a model organism in scientific research, with particularly useful properties in modeling human diseases. A key reason for this is the lack of ethical concerns present in *Drosophila*-based studies, compared to common alternatives such as Danio rerio (zebrafish). Furthermore, *Drosophila* are comparatively cheap organisms to maintain in large quantities. Finally, many of the key signaling pathways and cellular processes are conserved between *Drosophila* and humans, making research especially applicable to human-oriented work. Therefore, *Drosophila* are the best analyzed and understood multi-cellular organism for AD research (Prüßing et al., 2013).

Markers of Neurodegeneration in Drosophila

Neurodegeneration in *Drosophila* manifests through progressive declines in locomotion, memory, and behavioral regulation. Since flies possess highly conserved neural circuits for locomotion, learning, and aggression, deficits in these behavioral domains serve as reliable markers of neuronal decline. These behavioral phenotypes are already widely used in AD-like models to assess the functional consequences of neural decline.

Locomotion as an Indicator of Motor Neuronal Decline. Age-related neurodegeneration disrupts the function of motor neurons, neuromuscular junctions, and central pattern generators. In flies, this can be detected through reduced climbing ability (Ali et al., 2011). Impaired climbing reflects loss of motor neuron integrity, reduced synaptic transmission, neuromuscular weakness, and mitochondrial or metabolic dysfunction in neurons (Gargano et al., 2005). Because locomotive ability declines steadily with increased neurodegeneration, climbing ability is a standard measure of neurodegenerative progression.

Memory as an Indicator of Cognitive Decline. Cognitive deficits are well-characterized outcomes of neuronal decline and parallel early symptoms of human neurodegenerative diseases. The T-maze memory assay measures short-term memory, learning ability, and conditioned avoidance. These are behaviors that are among the most sensitive indicators of neural deterioration in flies (Davis, 2005; McGuire et al., 2005).

Aggression as an Indicator of Behavioral Dysregulation. Aggressive behavior in *Drosophila* depends on complex social and sensory circuits in regions of the brain impacted by neurodegeneration. Due to this, a change in aggression suggests underlying neural impairment. Thus, an Aggression Assay provides a window into higher-order behavioral dysfunction caused by neurodegeneration.

Assaying Locomotive Ability, Memory and Aggression in Drosophila

To test locomotive ability, a negative geotaxis, or a Climbing Assay is the primary test used in *Drosophila* studies. The higher the climbing success rate, or the more flies that fly up to a target height, the better the locomotive ability of that population of flies. An olfactory T-maze Assay can be used to measure memory and learning in *Drosophila.* Flies are placed in a T-shaped maze and are made to choose which side of the maze to go to. One side has a cotton ball with isoamyl acetate, whereas the other side has a cotton ball with distilled water for a control. Flies are naturally averse to odorants they're not used to, so it's expected for naïve flies to mostly end up on the side with distilled water. The expected distribution for conditioned flies should be more even, as they learn that the smell isn't harmful. This, however, will vary depending on the ability of the flies to be conditioned and remember, making this a powerful tool for assaying memory and learning. Aggression can be assayed in *Drosophila* by introducing two male flies to each other in an aggression arena or a petri dish, recording the specimen for twenty minutes after allowing them to get acclimated to the environment, and manually measuring and annotating aggressive behaviors (Rachagolla et al., 2021).

Modulating the Insulin Signaling Pathway in Drosophila

To investigate how insulin signaling influences neurodegeneration, this project uses two complementary genetic mutations in *Drosophila*. Pre-existing transgenic flies will be purchased from the Bloomington Drosophila Stock Center (BDSC), which, in addition to the standard wild type flies, include *chico* knockdown to reduce insulin pathway activity, and InR overexpression to increase it. This enables experimental tests of how ISP levels affect neural resilience under metabolic and age-related stress.

Role of *Chico* in the Insulin Signaling Pathway. The *chico* gene encodes the *Drosophila* version of Insulin Receptor Substrate-1 (IRS-1), an adaptor protein that connects the activated insulin receptor (InR) to the rest of the insulin signaling pathway. When InR is stimulated, *chico* binds to the receptor and helps recruit downstream molecules such as PI3K, which then activate AKT and TOR signaling. These pathways regulate key cellular processes including growth, metabolism, autophagy, and stress responses. When *chico* function is reduced, overall insulin signaling drops. This has been linked to longer lifespan, higher autophagy, lower metabolic stress, and reduced Aβ toxicity in previous Alzheimer's fly studies. Because *chico* is a key link in the ISP, it provides an effective way to experimentally reduce insulin signaling specifically in neurons.

Increasing Insulin Signaling Through InR Overexpression. To test the opposite direction of the pathway, insulin signaling can be increased by overexpressing InR, the Drosophila insulin receptor, specifically in

neurons. Higher levels of InR boost insulin/IGF signaling and recruit downstream molecules like PI3K, activating AKT and TOR signaling. Chronic elevation of this signaling has been shown to reduce autophagy, promote cellular growth, lower stress resistance, and in some cases worsen neurodegenerative symptoms (Huang et al., 2019). By studying InR overexpression in conjunction with *chico* knockdown, this project compares both ends of the insulin-signaling spectrum and determines how different signaling levels can mitigate the effects of neurodegeneration.

Rationale and Need. There has been research on insulin and the ISP's complex role in the brain; however, the relationship between modulating the ISP and neurodegenerative outcomes is still incompletely understood. *Drosophila* provides a system that can test whether genetic modulation of the ISP can mitigate neural decline, which can lead to potential therapeutic strategies for neurodegenerative diseases such as AD.

Section II: Specific Aims

This proposal's objective is to research how manipulating the ISP in *Drosophila* influences markers associated with neurodegenerative effects in an AD model induced by aging, metabolic stress, and traumatic brain injury. This project will measure functional outcomes which serve as indicators of neuronal health.

Our long-term goal is to identify whether modulating the insulin signaling pathway can be used as a therapeutic treatment to mediate harmful symptoms of AD where the central hypothesis of this proposal is that hampering the insulin signaling pathway in *Drosophila* will reduce neurodegeneration through markers such as locomotive ability, memory and learning ability in an AD model. The rationale is that hampering the ISP will increase autophagy and activate protective stress-response pathways, leading to neuroprotective effects. The work we propose here will test this hypothesis using *Drosophila* as a model organism leading to potentially identifying modulation of the ISP as a therapeutic treatment for AD.

Specific Aim 1: Establish an Alzheimer's-like *Drosophila* model using aging, metabolic stress induced through a high-sugar diet, or TBI.

Specific Aim 2: Determine how reducing insulin signaling (via *chico* knockdown) influences neurodegenerative outcomes in the Alzheimer's-like model.

Specific Aim 3: Determine how increasing insulin signaling (via InR overexpression) influences neurodegenerative outcomes in the Alzheimer's-like model.

The expected outcome of this work is a clear understanding of how both decreased and increased insulin signaling impact the effects of neurodegeneration in an AD model in *Drosophila.* Specifically, we anticipate that *chico* knockdown will reduce neurodegenerative phenotypes, improving locomotion, memory, and behavioral regulation. On the other hand, InR overexpression will exacerbate these deficits. Successful completion of this project will provide a mechanistic framework for how metabolic signaling pathways shape Alzheimer's-related neurodegeneration and may highlight insulin pathway modulation as a viable therapeutic avenue for helping to prevent or treat the disease.

Section III: Project Goals and Methodology

Relevance/Significance

AD and TBI are increasingly recognized as interconnected dangers to public health. Repeated or moderate TBI is a strong risk factor for developing AD or experiencing AD-like neurodegeneration, including chronic inflammation, synaptic loss, impaired learning, and increased accumulation of toxic proteins. *Drosophila* provides a rapid, genetically tractable system to study how metabolic pathways, specifically the ISP, influence neurodegenerative outcomes.

This project is significant because it investigates how altering insulin signaling (via *chico* knockdown or InR overexpression) affects behavioral and functional markers of neurodegeneration after TBI, a connection that remains poorly understood. Understanding whether reduced or elevated insulin signaling makes TBI-induced neurodegeneration better or worse could identify insulin pathway modulation as a feasible therapeutic target for preventing or slowing Alzheimer's-like decline.

Innovation

This project is innovative in three ways. First, this project studies the interaction between TBI and metabolic pathways. Most *Drosophila* neurodegeneration studies examine either Alzheimer's models or metabolic stress alone. This project is a novel intersection of trauma biology and metabolic regulation. Secondly, there is use of a controlled and reproducible fly TBI apparatus. A custom high-impact trauma (HIT) device will induce mechanical injuries that closely parallel features of human mild-to-moderate TBI. This allows consistent injury

severity. Third, this project uses behavioral assays to measure climbing ability, memory and learning, as well as aggression in *Drosophila* to study how the ISP plays a role in the neurodegenerative outcomes of TBI. These elements create a model that captures both acute injury and chronic neurodegenerative progression, improving relevance to Alzheimer's risk.

Methodology

This project uses controlled mechanical injury, genetic pathway manipulation, and behavioral testing to examine how insulin signaling influences neurodegenerative outcomes after TBI.

TBI Induction

A spring-loaded HIT device modelled after established *Drosophila* TBI models will be used to accelerate a vial of unanesthetized flies into a shock-absorbing pad. The impact causes rapid deceleration, inducing brain trauma like a concussive injury. Flies will undergo 1-3 strikes, as validated in prior TBI literature (Katzenberger et al., 2013).

Behavioral Assays to Quantify Neurodegeneration

These assays were chosen because they reflect different dimensions of neural function. This includes motor function, learning and memory, and social/behavioral regulation. Each of these markers are known to decline after TBI or Alzheimer's-like progression. After TBI, flies will undergo a Climbing Assay for locomotor decline, olfactory conditioning, or T-maze Assay, for memory and learning, and an Aggression Assay. All assays will compare: Control (No TBI or modified ISP), TBI-only, TBI + *chico* knockdown, and TBI + InR overexpression. Differences reveal whether insulin signaling protects against or worsens TBI-induced deficits.

Specific Aim #1:

To generate and validate an Alzheimer's-like neurodegeneration model in *Drosophila* by exposing flies to either (1) aging combined with a high-sugar diet or (2) controlled traumatic brain injury (TBI). This aim establishes the baseline neurodegenerative deficits against which insulin-signaling manipulations (Aims 2 and 3) will be tested. Our approach is to subject flies to high-sugar and aging or TBI. From there, behavioral assays will be conducted to determine neurodegeneration levels. The treatment that results in greater neurodegeneration will be chosen as the

primary model with which to conduct research, Aging, metabolic dysregulation, and traumatic brain injury are all major risk factors for AD. High-sugar diets accelerate metabolic stress, shorten lifespan, and worsen neuronal health, while TBI induces chronic inflammation, tau abnormalities, and behavioral decline. Because these mechanisms parallel human AD pathology, both approaches produce physiologically relevant Alzheimer's-like conditions in flies. Determining which model to use going forward is essential to creating an accurate and convenient AD model.

Justification and Feasibility. The goal of Specific Aim 1 is to produce an effective, reproducible Alzheimer's-like phenotype in *Drosophila*. By determining which of the two approaches to an AD model produces the larger result, we can select the most effective treatment to model Alzheimer's. TBI has shown quantitative increases in neurodegeneration as shown by increased vacuolization in sections of the brain within *Drosophila* (Fig. 2). This demonstrates the feasibility of using TBI as a model for Alzheimer's-like neurodegeneration, which is also shown to cause brain vacuolization in *Drosophila* (Huang. et al., 2019). Preliminary testing has confirmed both the Climbing and T-maze Assay to be viable, convenient and functional methods to quantify neurodegeneration in flies.

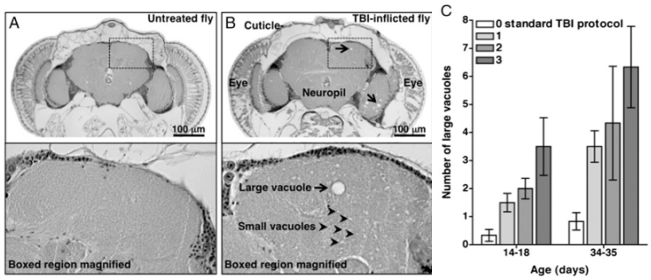

Figure 2: Treatment of wildtype flies with the standard TBI protocol causes neurodegeneration. Images of sections of fly brains are shown (A), (B). The number of large vacuoles in the central region of the brain is graphed vs. the number of times flies were subjected to the standard TBI protocol (C) (Katzenberger et al., 2013, Figs. 4a, 4b, and 4c).

Summary of Preliminary Data. The preliminary climbing data provides an initial baseline for early-age behavioral performance in wild-type flies under standard and Alzheimer's-model conditions. 4–6-day-old wild-type flies on regular media exhibited climbing success rates of approximately 71%. In contrast, wild-type Alzheimer's-model flies of the same age showed slightly reduced performance with a weighted average of approximately 66% (Fig. 3). The data were analyzed using a two sample Z-test, yielding a

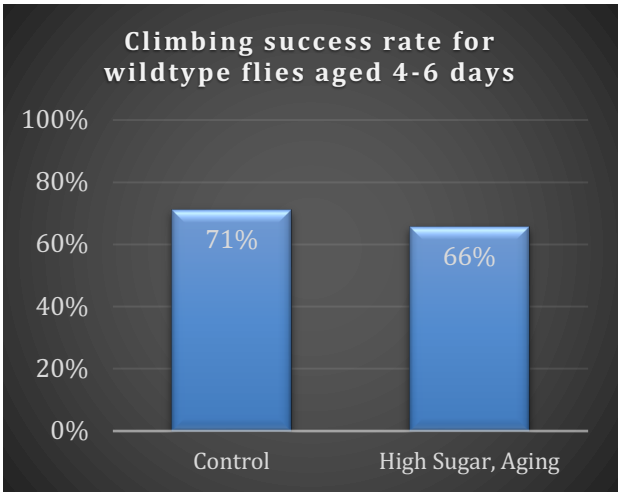

Figure 3: Climbing success rate in flies aged 4-6 days old in both control and high-sugar-diet + age treated wildtype flies.

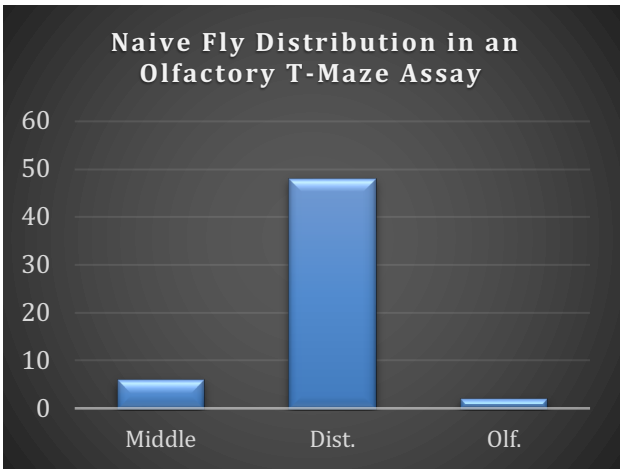

Figure 3: Fly distribution for naïve control flies. The distribution is 2 flies in the middle vial, 48 vials in the distilled water side, and 2 flies in the olfactory side. 58 flies were assayed in total.

p-value of 0.494. Under a $\alpha = 0.05$ significance level, the difference between the two proportions is not significant. This means that diet-only differences are subtle, strengthening the justification for using TBI to induce a more measurable and time-sensitive behavioral phenotype. Further preliminary data will be collected to confirm if TBI produces a more robust and time-efficient decline than high-sugar diet aging. In addition, an olfactory T-maze Assay has been conducted on naïve wildtype control flies. The distribution from the graph is similar to what is expected based on what has been observed in the literature, with 48 out of the 58 total flies prefering the side with the distilled water (McGuire et al., 2005, Fig. 4). This distribution will be used as a benchmark with which to compare future trials and experimental groups.

Expected Outcomes. The overall outcome of this aim is to identify which of the two neurodegenerative stress models (TBI or high-sugar aging) produce a stronger and more measurable decline in climbing performance in early-age flies. If TBI produces a rapid and significant drop (as expected), it will be selected as the primary model for downstream aims. This knowledge will be used for guiding the rest of the project by establishing a validated neurodegenerative model for further analysis.

Potential Pitfalls and Alternative Strategies. We could expect to see that the HIT device produces weak impairment in the setup. Alternatives could include adjusting strike number, angle, or using a modified HIT protocol from published studies. A second pitfall could be that the mortality rate after TBI is too high. In that case, we would have to drop strike intensity or number.

Specific Aim #2:

The goal of Specific Aim 2 is to test the hypothesis that reducing insulin signaling through neuronal chico knockdown will alleviate neurodegenerative phenotypes in a validated *Drosophila* Alzheimer's-like model (established in Aim 1). This aim focuses on determining whether hampering the ISP offers measurable

improvements in locomotion, memory, and behavioral regulation in an AD model. This aim directly evaluates whether *chico* knockdown offers neuroprotection in an Alzheimer's-like model induced by aging, metabolic stress, and TBI, allowing us to determine if ISP reduction mitigates broader forms of neurodegeneration beyond Aβ toxicity.

Expected Outcomes. *Chico* knockdown will significantly improve climbing ability, slowing or reversing locomotor declines caused by metabolic aging or TBI. Memory and learning deficits will be reduced, with chico knockdown flies performing better in T-maze Assays than unmodified AD-model flies. Aggression deficits will be mitigated, indicating broader preservation of neural circuit integrity.

Potential Pitfalls and Alternative Strategies. If *chico* knockdown yields minimal improvements, alternative ISP-reducing mutations (InR under expression) can be substituted to strengthen signaling reduction.

Specific Aim #3:

The goal of Specific Aim 3 is to evaluate whether increasing insulin signaling through neuronal overexpression of InR exacerbates neurodegenerative outcomes in the Alzheimer's-like *Drosophila* model established in Aim 1. This aim tests the prediction that enhancement of the ISP will worsen locomotor, memory, and behavioral deficits, thereby complementing the protective effects expected in Aim 2.

Expected Outcomes. Overexpression of InR will significantly reduce climbing ability. Memory and learning deficits will be increased, with InR overexpressed flies performing worse in T-maze Assays than the control. Aggression will be amplified, indicating broader decline in neural circuit integrity.

Potential Pitfalls and Alternative Strategies. High ISP activity may drastically reduce viability; in that case, weaker drivers (e.g., nSyb-GAL4) or lower expression levels (UAS-InR with insertion at a different genomic site) can be used.

Section IV: Resources/Equipment

The only non-standard lab equipment that is required is a CO_2 canister that will be used for sorting and transferring flies between vials.

Section V: Ethical Considerations

Ethics and Safety

This project uses *Drosophila melanogaster* as the model organism because flies present minimal ethical concerns while still serving as a strong model for human disease pathways. Research involving *Drosophila* is widely considered ethically permissible because flies are invertebrates with no legal restrictions concerning vertebrate animal welfare protocols. Flies will be kept in securely closed culture vials. Flies will be anesthetized with CO_2 only when necessary for sorting and transferring between vials. CO_2 canisters will be secured to the wall for safety, and gas regulators will be used to provide controlled CO_2 exposure. Pre-existing transgenic fly lines will be acquired from the BDSC, ensuring that all lines are produced and maintained under established scientific and ethical standards. Flies will not be anesthetized for the TBI procedure; however, the impact severity is designed to be reproducible and non-lethal for most flies. At the conclusion of experiments, all flies will be disposed of safely and humanely by freezing.

Section VI: Timeline

Week of Nov 6	Practice and manage wild-type flies Breed wild-type flies
Week of Nov 13	Separate male and female flies Practice Assays
Week of Nov 20	Climbing Assay preliminary data for wild-type control
Week of Nov 27	Continue with control group prelim Prepare media (high-sugar and normal)
Week of Dec 4	Climbing Assay preliminary data for wild-type AD model T-maze assay for wild-type control Poster prep for December Fair
Week of Dec 11	Construct HIT device for wild-type AD model Climbing assay preliminary data for wild-type AD model
Week of Dec 15 (Dec. Fair)	Fair Prep (elevator pitches)

Week of Dec 25	T-maze assay for wild-type control T-maze assay for wild-type AD model Set up aggression assay
Week of Jan 1st	Aggression assay for wild-type control Aggression assay for wild-type AD model
Until Feb 1st	Complete Climbing, T-Maze, and Aggression assays with fly mutants
Feb 1st — Feb 15th	Poster prep and Elevator Pitch prep for Feb. Fair

Section VII: Appendix

Section VIII: References

Ali, Y. O., Escala, W., Ruan, K., & Zhai, R. G. (2011). Assaying Locomotor, Learning, and Memory Deficits in *Drosophila* Models of Neurodegeneration. *JoVE (Journal of Visualized Experiments)*, *49*, e2504. https://doi.org/10.3791/2504

Davis, R. L. (2005). OLFACTORY MEMORY FORMATION IN DROSOPHILA: From Molecular to Systems Neuroscience. *Annual Review of Neuroscience*, *28*(Volume 28, 2005), 275–302. https://doi.org/10.1146/annurev.neuro.28.061604.135651

De Felice, F. G., Lourenco, M. V., & Ferreira, S. T. (2014). How does brain insulin resistance develop in Alzheimer's disease? *Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 10*(1 Suppl), S26-32. https://doi.org/10.1016/j.jalz.2013.12.004

Gargano, J. W., Martin, I., Bhandari, P., & Grotewiel, M. S. (2005). Rapid iterative negative geotaxis (RING): A new method for assessing age-related locomotor decline in *Drosophila*. *Experimental Gerontology*, *40*(5), 386–395. https://doi.org/10.1016/j.exger.2005.02.005

Gu, D., Ou, S., & Liu, G. (2022). Traumatic Brain Injury and Risk of Dementia and Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Neuroepidemiology*, *56*(1), 4–16. https://doi.org/10.1159/000520966

Huang, Y., Wan, Z., Wang, Z., & Zhou, B. (2019). Insulin signaling in Drosophila melanogaster mediates Aβ toxicity. *Communications Biology*, *2*(1), 13. https://doi.org/10.1038/s42003-018-0253-x

Johnson, V. E., Stewart, W., & Smith, D. H. (2012). Widespread τ and amyloid-β pathology many years after a single traumatic brain injury in humans. *Brain Pathology (Zurich, Switzerland)*, *22*(2), 142–149. https://doi.org/10.1111/j.1750-3639.2011.00513.x

Katzenberger, R. J., Loewen, C. A., Wassarman, D. R., Petersen, A. J., Ganetzky, B., & Wassarman, D. A. (2013). A Drosophila model of closed head traumatic brain injury. *Proceedings of the National Academy of Sciences*, *110*(44), E4152–E4159. https://doi.org/10.1073/pnas.1316895110

McGuire, S. E., Deshazer, M., & Davis, R. L. (2005). Thirty years of olfactory learning and memory research in *Drosophila melanogaster*. *Progress in Neurobiology*, *76*(5), 328–347. https://doi.org/10.1016/j.pneurobio.2005.09.003

Mullins, R. J., Diehl, T. C., Chia, C. W., & Kapogiannis, D. (2017). Insulin Resistance as a Link between Amyloid-Beta and Tau Pathologies in Alzheimer's Disease. *Frontiers in Aging Neuroscience*, *9*, 118. https://doi.org/10.3389/fnagi.2017.00118

National Institute on Aging. (2023, April 5). *Alzheimer's disease fact sheet*.

https://www.nia.nih.gov/health/alzheimers-and-dementia/alzheimers-disease-fact-sheet

Prüßing, K., Voigt, A., & Schulz, J. B. (2013). Drosophila melanogaster as a model organism for Alzheimer's disease. *Molecular Neurodegeneration*, *8*(1), 35. https://doi.org/10.1186/1750-1326-8-35