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Investigating Metabolic Stress *In Vivo*: Does Acute Glucose Deprivation Replicate Aspects of AD Pathogenesis?

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ABSTRACT: Alzheimer's disease (AD) is a debilitating neurodegenerative condition that predominantly affects memory and cognitive function. Proposed in 1992, the amyloid cascade hypothesis (ACH) states that the accumulation of amyloid-beta (Aβ) plaques in the brain is the primary driver of AD. However, numerous clinical trials targeting the molecular basis of the ACH have shown limited clinical efficacy. One emerging alternative hypothesis is that endoplasmic reticulum (ER) stress drives AD pathogenesis. This study aimed to investigate the relationship between ER stress and memory impairment *in vivo* using the fruit fly (*Drosophila melanogaster*). Mild and severe ER stress were induced via varying lengths of glucose deprivation (GD), and the T-maze test assessed effects on spatial memory after positive-reinforcement classical conditioning. Mild ER stress did not significantly impair spatial memory compared to controls, but surprisingly, neither did severe ER stress under the selected experimental conditions. Flies acclimated to the T-maze setup exhibited less average distance traveled away from baseline towards a positive reward stimulus following severe ER stress compared to non-acclimatized controls. These findings suggest a non-linear relationship between ER stress and cognitive function and contribute to growing evidence that ER stress may have a context-dependent role in AD development.

KEYWORDS: Animal Behavior, Neuroscience, Alzheimer's Disease (AD), Endoplasmic Reticulum (ER) Stress, "Type 3" Diabetes.

Introduction

In the United States (U.S.), approximately 6.9 million people aged 65 and older are affected by Alzheimer's disease (AD), a debilitating neurodegenerative disorder that primarily affects memory and motor coordination.[1] AD is the seventh leading cause of death in the U.S., killing nearly 120,000 individuals each year.[1] The consequences of AD extend beyond those afflicted, with an estimated 11 million Americans involved in the care of AD patients and 18.4 billion work hours (h) attributed to AD patient care in 2023.[1] The estimated total of Americans immediately impacted by AD is 17.9 million, or 5.3% of the population.[2] Males and females have a comparable incidence rate, despite females having a higher diagnosis rate due to having longer average lifespans.[3] In 2024, the total financial cost of AD in the U.S. was $360 billion for long-term health care and hospice services.[1] AD is becoming a growing demographic within the United States' increasingly aging population; between 2000 and 2021, the rate of AD deaths increased by 140% and the number of people living with AD in the U.S. is projected to double to 13.8 million by 2060.[1] It is therefore essential to understand the molecular factors responsible for AD onset and progression to suggest potential targets for treatment; such efforts have already yielded an extensive body of knowledge.

The majority of AD research refers to the amyloid cascade hypothesis (ACH).[4] The ACH asserts that oligomers of amyloid-beta (Aβ) and tau proteins render themselves neurotoxic to the human brain and progressively induce loss of motor function and cognitive decline.[4] Although this hypothesis has been generally accepted by the scientific community since 1992, clinical trials that have attempted to target AD through the molecular basis of this hypothesis, by directly targeting and destroying Aβ plaques or tau aggregates, have resulted in varying, inconsistent results. Levin *et al.* examined several clinical trials that targeted the ACH, finding that most had results that merely slowed down the progression of the disease as opposed to reversing or halting it.[5] For example, in a Phase III clinical trial, aducanumab, which acts by recognizing Aβ plaques and marking them for degradation by microglia, was found to slow down AD progression by 25%, but had no curative effect.[6] Further studies on Phase I clinical trials using donanemab and lecanemab, two other proposed AD drugs targeting the ACH, found "mixed or statistically insignificant results".[5,7,8] Although Levin *et al.* did not explicitly refute the ACH, they suggested limitations to its usefulness in informing therapeutic strategies for patients, claiming that the ACH may only "pertain to the preclinical [asymptomatic] stage of AD, prior to symptomatic onset".[5] Therefore, this suggests that there may be alternative or complementary hypotheses for AD pathogenesis that could serve as new avenues for therapeutic intervention.

Among alternative hypotheses that have recently gained prominence, it is suggested that metabolic dysregulation, particularly endoplasmic reticulum (ER) stress and the unfolded protein response (UPR), plays a significant role in AD pathogenesis.[9,10] ER stress occurs when the capacity of the ER to fold proteins is overloaded, and proteostasis is subsequently hindered due to an accumulation of unfolded or misfolded proteins in the ER.[11] The UPR is the physiological phenomenon that occurs when a cell responds to ER stress and attempts to mitigate it, occurring via the actions of three key enzymes: IRE1, ATF6, and PERK.[11] Central to the ER stress hypothesis

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is that the accumulation of Aβ and tau proteins causes a significant increase in ER stress and thus disrupts protein folding and, in turn, neuronal cell viability.[10]

The role that ER stress plays in AD pathogenesis is a topic of increasing interest and scientific scrutiny. Salminen *et al.* were the first to suggest a correlation between ER stress, the UPR, and AD pathogenesis in a prominent review article published in 2009.[12] Others followed to experimentally test whether ER stress plays a role in AD pathogenesis through the neurotoxic accumulation of Aβ and tau, upregulating ER stress and thereby causing cognitive decline.[9,10,12] A study in which rats were administered tunicamycin, an ER stress inducer, into their brain ventricles found a significant reduction in spatial memory, defined as memory associated with navigation and recall of spatial relationships, when rats were tasked with finding a hidden platform under the water's surface in a Morris water maze test.[13] Surprisingly, Hashimoto *et al.* determined that extracted cells from rat brains exposed to thapsigargin, another ER stress inducer, at concentrations of 2–5 μM, conferred no significant effects on spatial memory, thereby refuting their own initial hypothesis that ER stress would result in neuronal cell death.[14] A possible explanation for this is suggested in an earlier study by Fouillet *et al.*, which found that mild ER stress in mouse models, fruit fly models, and human neuroblastoma cells promoted neuroprotective autophagy, thereby inhibiting neuronal cell death through an adaptive response against apoptosis.[15]

There is growing evidence that glucose deprivation (GD) induces ER stress via the PERK pathway, akin to the extent observed for tunicamycin and thapsigargin.[19,20] The ability for GD to cause ER stress has been observed in a range of health conditions in humans, such as ischemic stroke and diabetes in addition to AD.[19,21] Particularly with regards to diabetes mellitus, AD has well-documented molecular and biochemical features overlapping with both type 1 and type 2 diabetes (T1D/T2D), and also represents a form of insulin resistance specifically involving the brain. Owing to this, AD has been increasingly referred to as "type 3 diabetes", or T3D.[22] This may extend to glucose dyshomeostasis in general; notably, García de la Cadena *et al.* observed that acute GD could upregulate apoptosis in critical memory-regulating cells such as neurons in the hippocampus.[19] As such, the question arises: does acute glucose deprivation *in vivo*, and the subsequent ER stress this induces, replicate aspects of AD pathogenesis in the form of reduced spatial memory?

This question would ultimately serve as central to my experimentation. I hypothesize that, consistent with Fouillet *et al.* and Lin *et al.*, mild ER stress induced by a short duration of GD, lasting 1 h, would not significantly impair the spatial memory of fruit flies (Drosophila melanogaster), but that heavy ER stress induced by a longer duration of GD, lasting 3 h, would lead to a decrease in spatial memory.[13,15] This study aims to bridge the gap between understanding whether varying degrees of ER stress affect spatial memory responses comparable to those observed in AD *in vivo*.

Methods

Materials and experimental setup:

Fruit flies (D. melanogaster, hereby referred to as "flies" interchangeably) were obtained from the Carolina Biological Supply Company (CBSC) in Burlington, NC, as living, wild-type adults with 25–30 flies per vial. Each trial featured a total of 12 vials for a cumulative sample size of 300–360 flies per trial. Across 5 trials, this yielded a cohort size of 1,500–1,800 flies. Two vials (50–60 flies) were arbitrarily selected via random number generator at RANDOM.ORG and assigned to one of six experimental groups. Adults were segregated from larvae and pupae, and were transferred into a cylindrical vial measuring 10 centimeters (cm) by 4 cm containing a medium formula of 2 grams (g) active dry yeast, 2 teaspoons (tsp) instant medium from CBSC, and 10 mililiters (mL) drinking water. Vials were obtained from CBSC, and yeast, water, and reagent-grade sucrose were obtained from Red Star, Crystal Geyser, and ALDON, respectively. Any larvae in the original shipping vial were transferred to a mini-freezer obtained from INSIGNIA™ and euthanized via freezing for 100 h, a well-documented euthanasia procedure.[23] Flies maintained an equal gender ratio, analogous to documented human incidence rates for AD.[3]

Two odorants were used: aloe vera and Moroccan rose, obtained in hydrosol form from George's and Whole Foods, respectively. Both odorants are harmless and were used to induce classical conditioning, or the association of a stimulus with a positive or negative outcome.[24] Flies were anesthetized with CO_2 generated from Alka-Seltzer® effervescent tablets prior to transfer to a housing tube with food medium (Figure 1). Flies were given 1–3 days (d) to acclimate pre-experimentation. Flies were segregated into 6 groups, as shown in Figure 2. Flies in groups with "NCC", or no classical conditioning, did not associate any odorant with a reward, whereas flies in groups labelled "YCC", or literally "yes classical conditioning", (Figure 2) received a reward when subjected to rose odorant, where the tube was coated with both rose hydrosol and a "sweet medium", composed as shown in Figure 3.

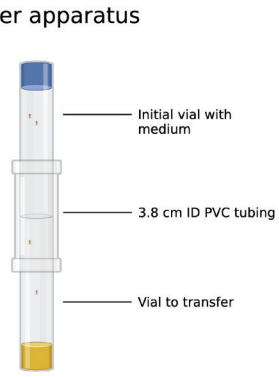

Figure 1: The transfer apparatus is used for transporting *D. melanogaster* between two vials. The transfer apparatus for the transfer of flies from their initial vials into a destination vial (vial to transfer) was as shown. This tube was tapped onto a table to knock flies into the vial to transfer, and the initial tube was then removed and discarded. ID = internal diameter of the polyvinyl chloride (PVC) tubing. Figure created with BioRender.

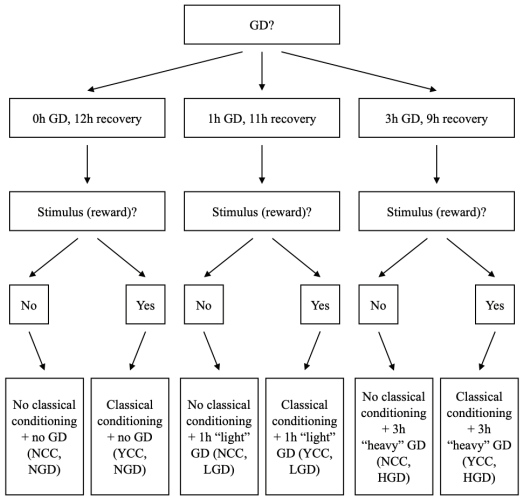

Figure 2: A decision matrix is used to determine experimental group segregation. Each group of fruit flies was created from the above decision matrix, considering whether a stimulus was associated with a reward and whether GD was applied, dividing the sample into 6 experimental groups.

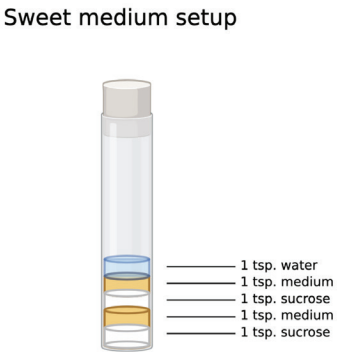

Figure 3: Sweet medium recipe for *D. melanogaster* T-maze classical conditioning training. The sweet medium recipe used as a positive reward was created as shown in the figure, created with BioRender.com.

This setup was used to avoid loss of flies upon transfer between tubes. The aloe vera tube remained a controlled variable, where it was not coated with medium or any form of reward. The apparatus was kept on a 12 h light-dark cycle from 11:00 to 23:00 and remained within temperature ranges of 15–25°C. Due to seasonal and temporal temperature variations, ideal temperature conditions of 21°C could never be firmly established. Flies were kept on medium with an equivalent formula at an identical amount. Additionally, flies were tested at an equivalent age and subjected to matched testing times. Testing occurred across 5 trials in 6 days.

T-maze test:

Initial long-term memory training consisted of anesthetizing fruit flies with CO_2, then transferring flies from their group vials into a tube coated with aloe vera odorant and free of food medium (Figure 4). Flies were given 7 min to acclimate post-transfer: 5 min to recover from anesthesia and 2 min to explore (Figure 5). Once acclimatized, the flies were again anesthetized and transferred into a tube coated with rose hydrosol and containing sweet medium for experimental groups (Figure 5). After the cycle in the rose tube was complete, the flies were anesthetized and transferred into the aloe vera tube, and the process was repeated for a total of 3 rounds (Figure 5). Afterward, flies were anesthetized, transferred back to their group vials, and given 100 min to consolidate memory of the reward with rose odorant, consistent with the classical conditioning method established by Tempel *et al*.[25]

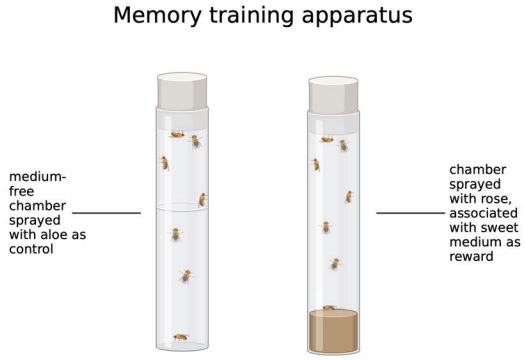

Figure 4: Memory training setup for training *D. melanogaster* for T-maze classical conditioning. Each experimental group of fruit flies was subjected to alternating intervals of 7 min in a medium-free tube coated with aloe odorant or a sweet medium tube coated with rose odorant as shown in the figure, created with BioRender.com.

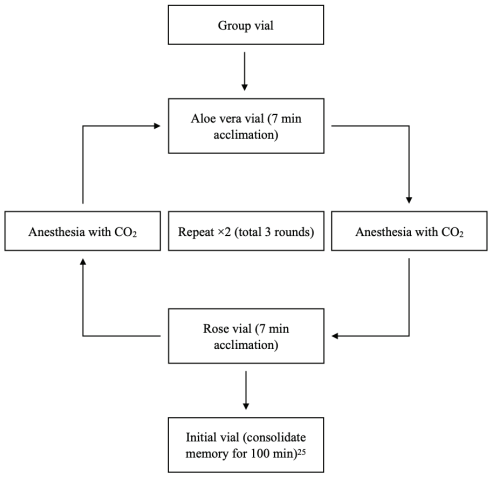

Figure 5: Flow diagram for LTM. Experimental groups of fruit flies had a sweet medium included in the vial with a rose odorant as a reward for classical conditioning. Each LTM training cycle was repeated twice for a total of 3 rounds.

Flies were then immediately subjected to a T-maze test, as shown in Figure 6; the total length of the arms of the T-maze was 10 cm on each arm. The T-maze was then flipped in a minimally biased manner to encourage fruit flies to explore, as they tended to gravitate towards the nearest wall. Each group of flies remained in the T-maze for 5 min to allow navigation towards odorants. Unlike initial testing, where the rose was associated with a reward, this experimental testing did not feature any reward being placed into the rose arm; instead, this relied on classical conditioning to associate the rose with a reward and thereby attract the flies towards the rose odorant.

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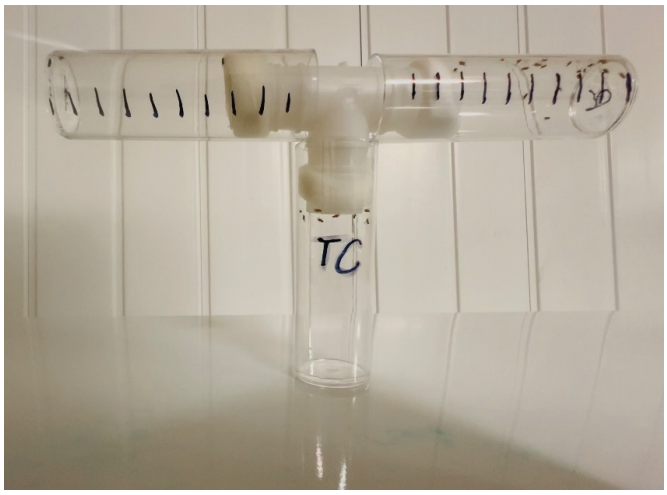

Figure 6: Image of *D. melanogaster* T-maze. Each experimental group of fruit flies was subjected to 5 technical replicates of the T-maze test. Although not clearly seen, A refers to the aloe vera arm, and R refers to the rose arm. TC refers to the transfer chamber, or the tube, in which the fruit flies are initially transferred to within the T-maze.

Immediately afterward, flies in groups that would be subjected to GD were transferred into another chamber free of food medium to deprive glucose and thereby induce ER stress consistent with the findings of García de la Cadena *et al*.[19] GD durations and experimental group stratification are shown in Figure 2. Post-GD, the flies were then immediately transferred into the T-maze containing both odorants for another round of confirmatory memory testing, with 5 min durations per group for testing. Afterward, the flies were euthanized via freezing for 100 h and then disposed of at the local waste plant.

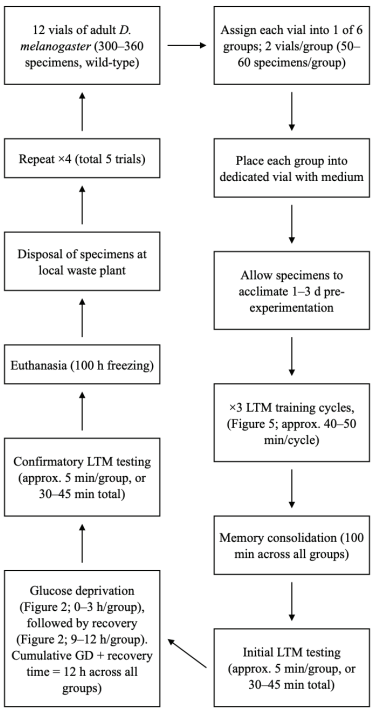

Figure 7: Flow diagram detailing the full experimentation procedure. Each trial followed the generalized timeline from the above flow diagram. Time per trial totaled 970–1015 min from the end of acclimation to the start of euthanasia.

Data analysis:

Digital data was obtained via a stabilized camera recording 4K resolution (3840x2160 pixels) and 60 fps (frames per second) (termed 4K60) video to document the fruit flies' movements. Images were sampled in JPG format at a rate of 1 frame/15 seconds or 4 frames/min via FFmpeg, an open-source video-to-image sampler tool. The frames were then imported into Apple Preview (image/PDF viewer) software, where the distance between the center of the T-maze (baseline) and the fruit fly was manually measured in pixels. The distance fruit flies traveled in horizontal pixels was quantified by converting pixels to cm. Gravitation to a certain odor was quantified by distance in cm from baseline; movement towards the aloe odorant was assigned a negative value, and movement towards the rose odorant was assigned a positive value. Measurements across all flies were averaged to yield a mean value in cm. Flies in the "transfer chamber" (labeled "TC" in Figure 6) were counted as having moved 0 cm as they had not yet explored either odorant-containing arm of the T-maze.

Statistical analyses were performed as follows: one-way ANOVA with Tukey's honestly significant difference (HSD) *post hoc* test applied was used to analyze the variances between experimental groups 1-6, with a p-value α-threshold set to ≤ 0.05. ANOVAs refer to statistical tests analyzing variance between two or more groups and test for significant variances between these groups; Tukey's HSD analysis was used to verify ANOVA results.[26,27] If the *p*-value was below α, there was a significant correlation; if not, there was no significant correlation determined. The independent variable in this experiment was the duration of GD to induce ER stress, and the dependent variable was spatial memory loss quantified in average distance in cm from baseline at the completion of a 5-minute T-maze test (n = 5 technical replicates per experimental group).

■ Results and Discussion

Flies not exposed to classical conditioning towards odorants (NCC, NGD; NCC, LGD; and NCC, HGD) did not navigate further than 0.1 cm from the transfer chamber (TC) on average pre-GD (Figure 8) and 0.3 cm on average post-GD (Figure 9). Odorant-conditioned flies (YCC, NGD; YCC, LGD; YCC, HGD) navigated slightly further, but always within 0.5 cm of TC on average pre- and post-GD (Figure 8; Figure 9). This raw data pre- and post-GD forms the basis for the processed data in Figure 10.

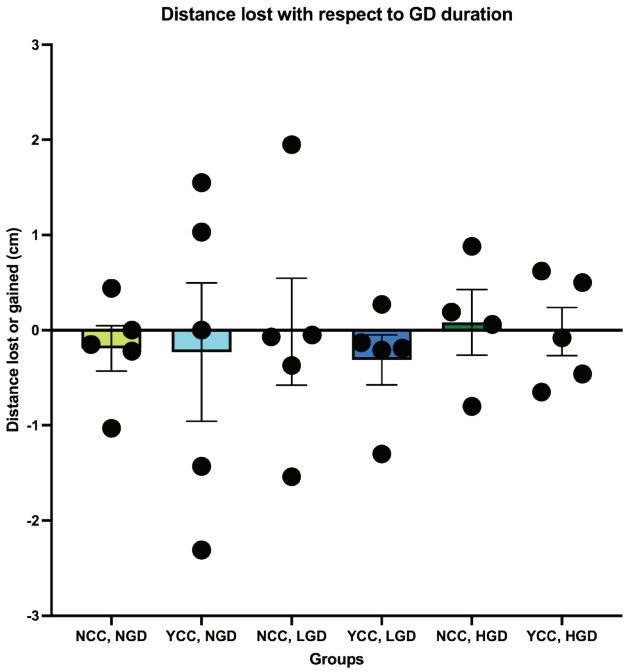

Figure 8: Average movement towards the rose chamber in cm pre-GD. *N* = 5 replicates per group. Negative values denote navigation away from rose or to aloe; positive values denote navigation towards rose. Error bars denote mean ± standard deviation (SD). All groups traveled a varied amount of distance that does not particularly skew towards one group in particular, although groups subjected to classical conditioning generally traveled further in either direction compared to groups not subjected to classical conditioning. Note one outlier in NCC, HGD was invalidated and not counted towards the analysis. Graph created with GraphPad Prism software.

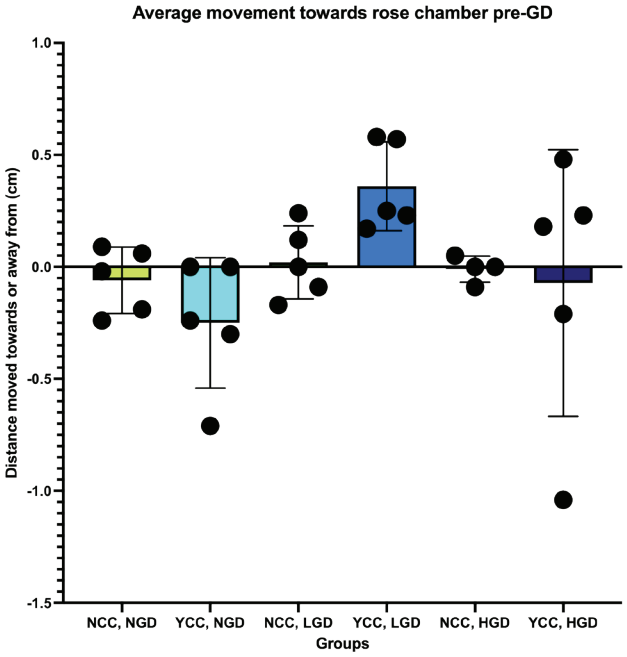

Figure 9: Average movement towards the rose chamber in cm post-GD. *N*= 5 replicates per group. Negative values denote navigation away from rose or to aloe; positive values denote navigation towards rose. Error bars denote mean ± standard deviation (SD). All groups traveled a varied amount of distance that does not particularly skew towards one group in particular. The trend of groups subjected to classical conditioning traveling further in either direction in Figure 8 is not repeated post-GD. Note one outlier in NCC, HGD was invalidated and not counted towards the analysis. Graph created with GraphPad Prism software.

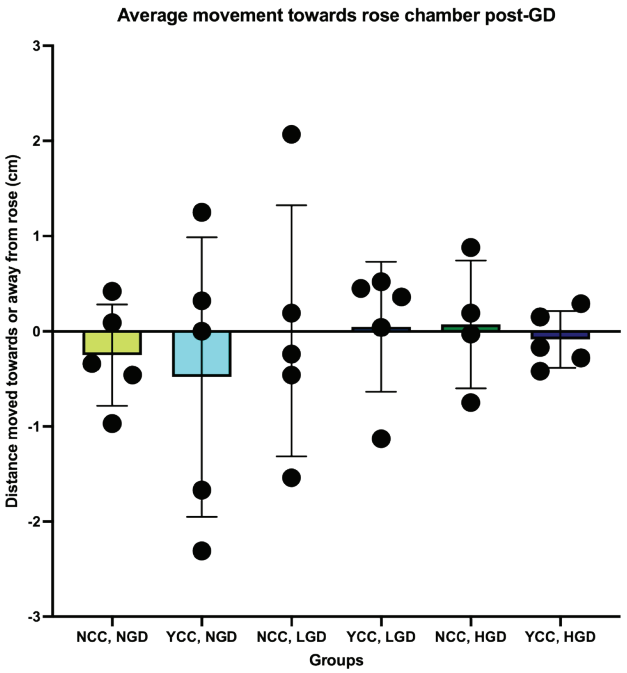

Figure 10: Distance lost with respect to GD duration (post-GD - pre-GD). *N* = 5 replicates per group. Negative values denote navigation away from rose or to aloe; positive values denote navigation towards rose. Error bars denote mean ± standard deviation (SD). All groups lost a varied amount of distance, which does not particularly skew towards one another. An expected trend where YCC, NGD, and YCC, LGD lose a similar amount of distance, whereas YCC, HGD loses a more extreme amount of distance, consistent with the hypothesis, did not materialize. Note one outlier in NCC, HGD was invalidated and not counted towards the analysis. Graph created with GraphPad Prism software.

Flies not exposed to classical conditioning (NCC, NGD; NCC, LGD; NCC, HGD) lost a varied amount of distance that did not exceed –0.2 cm on average; NCC, NGD; NCC, LGD; and NCC, HGD kept consistent with the hypothesis that they did not "lose" memory due to being untrained. Of the experimental groups, YCC and LGD lost the most distance across all 5 trials at an overall loss of –0.31 cm between pre- and post-GD. This was roughly consistent with YCC, NGD, at –0.23 cm between pre- and post-GD. An expected "cliff-shaped" trend where YCC, LGD does not lose significant memory beyond that of YCC, NGD, whereas YCC, HGD loses significant memory, did not materialize; instead, the trend appeared in reverse, where YCC, LGD, and YCC, HGD were comparable, but YCC, HGD lost less spatial memory quantified in distance by average centimeters in the rose arm, at –0.01 cm. Therefore, purely visual documentation appears to refute portions of the initial hypothesis.

In Figure 10, an outlier in NCC, HGD that skewed the values was eliminated; however, it may suggest that fruit flies may be naturally attracted towards roses, particularly after a period of acute starvation, as flies in NCC, HGD experienced 3 h GD but did not undergo classical conditioning. However, it has been shown that the attraction of fruit flies towards decaying fruit or overripe roses in the wild is primarily mediated through their attraction to the scent of yeast (Saccharomyces cerevisiae) fermentation rather than the scent of the fruit or flower.[28]

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The p-value in Tukey's HSD (Table 1), for YCC, NGD vs. YCC, LGD, at >0.9999, was expected, as no significant variance was predicted to be detected owing to the flies being completely non-trained and not directed to gravitate in any direction. However, the *p*-value between groups YCC, NGD vs. YCC, HGD; and YCC, LGD vs. YCC, HGD, at 0.9992 and 0.9964, respectively, did not clear the α-threshold. This appears to refute the second part of my hypothesis that heavy ER stress leads to loss of spatial memory in flies. However, because the hypothesis predicted no significant variance between YCC, NGD, and YCC, LGD, the first component of the hypothesis remains valid.

Heavy (3 h) GD, inducing heavy ER stress, does not induce significant spatial memory loss in fruit flies and does not replicate AD pathogenesis in fruit flies under the selected experimental conditions. Mild ER stress did not induce significant memory loss, as expected.

Table 1: Tukey's honestly significant difference (HSD) on data obtained after one-way ANOVA.

Groups	Mean diff.	95% CI diff.	Below threshold?	Summary	*p**
NCC, NGD vs. YCC, NGD	0.04000	-1.884 to 1.964	No	ns	>0.9999
NCC, NGD vs. NCC, LGD	-0.1760	-2.100 to 1.748	No	ns	0.9997
NCC, NGD vs. YCC, LGD	0.1200	-1.804 to 2.044	No	ns	>0.9999
NCC, NGD vs. NCC, HGD	-0.2745	-2.315 to 1.766	No	ns	0.9982
NCC, NGD vs. YCC, HGD	-0.1780	-2.102 to 1.746	No	ns	0.9997
YCC, NGD vs. NCC, LGD	-0.2160	-2.140 to 1.708	No	ns	0.9992
YCC, NGD vs. YCC, LGD	0.08000	-1.844 to 2.004	No	ns	>0.9999
YCC, NGD vs. NCC, HGD	-0.3145	-2.355 to 1.726	No	ns	0.9965
YCC, NGD vs. YCC, HGD	-0.2180	-2.142 to 1.706	No	ns	0.9992
NCC, LGD vs. YCC, LGD	0.2960	-1.628 to 2.220	No	ns	0.9965
NCC, LGD vs. NCC, HGD	-0.09850	-2.139 to 1.942	No	ns	>0.9999
NCC, LGD vs. YCC, HGD	-0.002000	-1.926 to 1.922	No	ns	>0.9999
YCC, LGD vs. NCC, HGD	-0.3945	-2.435 to 1.646	No	ns	0.9900
YCC, LGD vs. YCC, HGD	-0.2980	-2.222 to 1.626	No	ns	0.9964
NCC, HGD vs. YCC, HGD	0.09650	-1.944 to 2.137	No	ns	>0.9999

Note: Data was fed into GraphPad Prism for ANOVA. Data remains original work. **p* was adjusted.

Experimental limitations:

Although the data remained statistically insignificant, it could be explained by a myriad of limitations. Namely, the T-maze did not seal perfectly, and there was no way to force fruit flies to explore the odorant-containing arms, despite flipping the tube in a minimally biased manner to encourage as many specimens to enter as possible. Therefore, some fruit flies remained stuck in the TC, as opposed to exploring the test arms of the apparatus; these flies were considered to have moved "0" cm per Data Analysis and potentially skewed the data towards no significant changes in distance moved. This phenomenon can be observed in Figure 4.

Central to the insignificance of the data was the provision of 12 h, 11 h, and 9 h recovery time for the fruit flies, where they were inserted into vials with medium (Figure 2), which may have diluted the immediate effects of GD. As the flies were given nutrition immediately post-GD, this constituted a stage where the effects of GD may have been reversed due to ingestion of medium that may have erased its effects in near-entirety. To truly assess the efficacy of GD, a confirmatory memory test should have been performed immediately post-GD as opposed to after a rest period of at least 9 h, which was not considered for this study.

Additionally, fruit flies may have been relatively inactive at the time of testing. Fruit flies have been demonstrated to operate on circadian rhythms akin to those of humans, which may have resulted in fruit flies being unwilling to explore odorants at the time of testing.[29] As initial memory testing occurred during nighttime and confirmatory testing occurred during daytime, it remains possible that any differences in memory could be influenced by their circadian rhythms. Notably, this could explain the lack of change in distance moved in YCC and HGD, as increased activity may have resulted in further willingness to navigate. Furthermore, as fruit flies were starved dietarily, it could further explain the value in YCC, HGD, as flies in that group may have been starved to the extent that they were more willing to navigate to the rose, thinking it brought "food" consistent with the classical conditioning stimulus that the rose equaled sweet medium. Therefore, although GD was the most feasible methodology, other causes of these results, in addition to ER stress, such as metabolic starvation that does not necessarily affect the brain, cannot be ruled out. Any ER stress produced by GD in this study may have affected multiple organ systems, not limited to the brain and central nervous system, which would be more specific for studying AD.

Since the mild (1 h) GD groups (NCC, LGD; YCC, LGD) in this experiment did not experience significant loss of memory beyond that of the untreated groups (NCC, NGD; YCC, NGD), the results could be explained by Fouillet *et al.*,[15] who state a similar result with mild ER stress. This also opens another limitation in that although this study's GD durations technically fell into the bounds of García de la Cadena *et al.*[19] (between 15 min and 4 h of GD), those parameters were only applicable to cell lines and likely did not account for the more sophisticated nature of living organisms. As such, this study likely failed to take into account that living organisms may have needed more GD duration than 3 h to deplete glucose stores. Furthermore, D. melanogaster can produce glucose via gluconeogenesis during periods of nutrient deprivation, and in addition, may have had residual glucose from the training session and resting periods, particularly in the trained groups (groups labeled "YCC"). Notably, neurons may have been able to take advantage of gluconeogenesis, providing a lifeline during GD (Miyamoto & Amrein, 2019).[30] This could have meant that no GD, and consequently ER stress, in the neurons actually occurred, as repositories of glucose remained in neurons even into GD.

At 3 h, the ER stress induced in the "heavy" groups (NCC, HGD; YCC, HGD) may have been mild enough to inhibit and not promote neuronal death, consistent with Fouillet *et al.*[15] Suppose GD were induced for longer, and its induction confirmed molecularly. In that case, the results may resemble those of Lin *et al.*[13] rather than Fouillet *et al.*[15] In addition, mere deprivation of medium remains less reliable than the glucose-free medium method, as indicated by García de la Cadena *et al.*[19] which used a specially formulated glucose-free medium. Purchasing an equivalent in fruit flies would likely have served to resolve some limitations in this study and potentially provide more useful data.

Implications for therapy:

This study may have implications for future research and pathogenic understanding of AD. Namely, this study supports the idea that ER stress is a double-edged sword in AD pathogenesis, in that it may have context-dependent beneficial or detrimental roles in AD development. Studies consistent with this idea have both induced and inhibited ER stress as therapeutic strategies to alleviate symptoms of AD.[10,13,15] It has been shown that post-mortem extirpated human brains from patients with terminal AD displayed increased ER stress in most cases.[10] Care should be taken to ensure that ER stress does not break moderation and become severe or heavy; the definition of which, however, appears to be context-specific.

Most ER stress testing is achieved by tunicamycin or thapsigargin dosage rates per kg of body weight. Still, neither of these drugs is currently authorized for human consumption due to their cellular toxicity.[31,32] As such, future researchers may be served well by implementing an ER stress severity standard that is less cytotoxic. AD medicine may be required to be issued on a case-by-case basis, where doctors analyze the amount of ER stress in the afflicted patient, along with the severity of the disease upon examination.

Untreated diabetes may cause GD because glucose cannot be transported into cells, which is associated with insulin resistance.[22,33] This is the premise of the diabetes-AD hypothesis, or that dementia is T3D, resulting from glucose dyshomeostasis in a brain-specific manner.[19] Examining the linkage between insulin resistance and GD-induced ER stress could therefore be of interest for future researchers, with particular emphasis on modulating glucose administration or deprivation for therapeutic benefit.

Future Directions:

An array of future directions is warranted for researchers aiming to explore the role of ER stress in AD, especially via *in vivo* analyses, in order to generate a more comprehensive understanding of this relatively new field. For one, comparing GD with the effect of biochemical ER stress inducers, especially while adding longer GD durations such as 6 and 12 h, could be worthwhile to see if the induction method for ER stress alters the findings. Additionally, the confirmatory T-maze test should be performed immediately post-subjecting specimens to GD, as by then the immediate effects of GD are more visible as opposed to the 9 h, 11 h, and 12 h rest periods provided for the specimens by this study (Figure 2). Care should also be taken to induce GD-induced ER stress via glucose-free medium and not mere starvation, as the latter is less reliable than the former, as well as possibly confirm the results via biomarkers *in vitro* (for example, relative mRNA and protein expression of XBP-1).

Figure 8 (pre-GD) displays a subtle trend in the direction of the rose odorant in YCC, LGD, although overall findings remain inconclusive. Given this model's relevance in ER stress and glucose dyshomeostasis in AD, it may be beneficial in future studies to include a clear positive control demonstrating robust directional movement. This would help validate the sensitivity and dynamic range of the behavioral assay.

It would also be worthwhile to understand if alleviating ER stress could be used as a complementary treatment strategy in combination with treatments that target the ACH. Researchers should also continue to investigate the pathogenic mechanisms underlying glucose dyshomeostasis and AD symptom onset and progression.

■ Conclusion

Mild and severe ER stress in fruit flies did not significantly impair spatial memory loss following classical conditioning T-maze testing. Although inconclusive, this study may suggest new pathways to studying the role of ER stress as well as GD or glucose dyshomeostasis in AD through ways not previously documented, as well as provide several directions for future scientific and clinical studies to consider. These results indicate a complex, non-linear interaction between ER stress and cognitive performance, supporting the notion that ER stress may influence AD progression in a context-dependent manner. Further experimentation is warranted to establish whether modulation of ER stress could offer therapeutic benefit to AD patients.

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