UV-Induced DNA Damage Impairs Memory Formation During Larval Neurodevelopment: Insights from *Drosophila* Larvae

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Received May 24, 2025
Accepted August 31, 2025
Electronic access October 15, 2025

Ultraviolet (UV) radiation is a known environmental mutagen that contributes to neurodegenerative disorders in adults, yet its effects during early neurodevelopment remain poorly understood. Early neural development represents a critical window for brain maturation, during which genotoxic stress may have lasting cognitive consequences. This research employs *Drosophila melanogaster* larvae as a model system to explore how early-life exposure to UV-induced DNA damage impacts associative learning during a period of dynamic neural circuit formation. Embryonic fruit flies were exposed to UV radiation and later subjected to a classical odor-sugar associative conditioning assay to evaluate memory performance. Four experimental groups were assessed: untreated controls, UV-only, Vitamin D3-only, and UV combined with Vitamin D3. Results showed that UV exposure significantly impaired memory retention while Vitamin D3 supplementation alone had minimal effect. However, co-treatment with Vitamin D3 partially mitigated the cognitive deficits induced by UV exposure. These findings highlight the sensitivity of developing neural systems to environmental DNA damage and suggest that Vitamin D3 may offer modest neuroprotective benefits. By leveraging a genetically tractable model organism, this work contributes to a broader understanding of how environmental factors can shape cognitive health during early development and underscores the potential of nutritional interventions in mitigating such risks.

Keywords: DNA damage, ultraviolet radiation, memory, *Drosophila melanogaster*, Vitamin D3, neuroprotection, cognitive development, genotoxic stress

1 Introduction

1.1 Background: Cognitive Development and DNA Damage

Neurodevelopment during the larval stage is a complex and dynamic process influenced by both genetic programming and environmental exposures[1]. This period, marked by rapid neural maturation, synaptic pruning, and increased plasticity, is also one of heightened vulnerability to external stressors. Among these, DNA damage has emerged as a key biological insult with potential long-term impacts on brain function[2]. One significant source of such damage is ultraviolet (UV) radiation, a universal environmental factor known to induce DNA lesions, oxidative stress, and cellular dysfunction[3]. While UV exposure is extensively studied in the context of skin cancer and aging, its neurological consequences, particularly when encountered during early developmental stages, remain largely underexplored[3].

Recent research in neuroscience and molecular biology has begun to uncover the mechanisms by which genotoxic stress contributes to neurodegeneration in adults[4]. However, far less is known about how such damage influences the developing brain, especially during early neural development, a period critical for memory formation, executive function, and emotional regulation[5]. Understanding how DNA damage intersects with early neurodevelopment is essential not only for clarifying disease risk but also for informing public health strategies aimed at mitigating cognitive deficits later in life[6]. Model organisms like *Drosophila melanogaster*, with their conserved genetic pathways and tractable nervous systems, provide a powerful platform for investigating these mechanisms in a controlled, replicable manner. While *Drosophila* larvae do not model complex cognition as seen in mammals, their neural architecture supports associative learning via conserved mechanisms such as cAMP signaling and CREB activation, providing a valid platform for studying genotoxic effects on basic memory processes.

In *Drosophila melanogaster*, the mushroom bodies (MBs) are central brain structures essential for associative learning and memory. These paired neuropils are composed of Kenyon cells, which form three major lobes: γ, $\alpha'\beta'$, and $\alpha\beta$. Each lobe contributes to distinct phases of memory processing, acquisition, consolidation, and retrieval. During early larval development, the γ lobe is the first to mature and is primarily responsible for short-term memory formation. As development progresses, the $\alpha'\beta'$ and $\alpha\beta$ lobes undergo structural and synaptic refinement, marking a critical period for memory circuit maturation. This window is characterized by heightened plasticity, during which

environmental stressors like UV radiation may disrupt synaptic connectivity and impair memory encoding. The MBs integrate sensory input via dopaminergic and cholinergic signaling, and their output neurons (MBONs) modulate behavioral responses based on learned associations[7]. In addition to dopaminergic and cholinergic input, serotonin signaling has also been shown to modulate MBONs, sustaining water-reward long-term memory in *Drosophila*[8]. These features make the MBs a powerful system for studying how genotoxic stress affects memory formation during neurodevelopment.

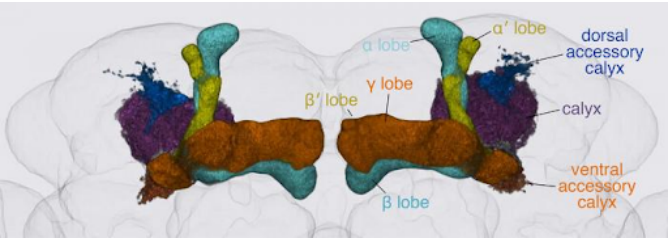

Fig. 1 Schematic representation of the mushroom body (MB) circuit in *Drosophila melanogaster*. The MB is composed of Kenyon cells forming three major lobes: γ, $\alpha'\beta'$, and $\alpha\beta$. These lobes support different stages of memory formation, with the γ lobe maturing first and contributing to short-term memory during early development. The $\alpha'\beta'$ and $\alpha\beta$ lobes mature later, marking a critical period of synaptic refinement essential for long-term memory consolidation. Dopaminergic input from DANs and output via MBONs mediate associative learning. Figure adapted from the Janelia Research Campus, "Learning and memory – the Mushroom Body."

Extensive research has shown that associative learning in *Drosophila* larvae depends on dopaminergic reinforcement of odor-sugar pairings via mushroom body Kenyon cells. These circuits rely on conserved molecular pathways, including cAMP signaling, synapsin-mediated plasticity, and CREB-dependent transcription for memory consolidation[9]. UV-induced genotoxic stress may disrupt these processes by interfering with dopaminergic input or impairing synaptic integrity during critical periods of mushroom body maturation. This study builds on prior work by demonstrating that early UV exposure impairs memory performance in a well-characterized larval learning paradigm, suggesting disruption of conserved neural substrates.

Emerging evidence from mammalian models suggests that chronic UV exposure can impair memory formation through neurotransmitter dysregulation. Recent studies such as Yoon et al. (2024) have shown that UV irradiation alters dopamine signaling in the brain, leading to deficits in hippocampal memory, synaptic plasticity, and neurogenesis[10]. These findings support the hypothesis that UV-induced cognitive impairment may involve disruption of dopaminergic pathways, providing a mechanistic precedent for exploring similar effects in invertebrate systems.

Ultraviolet (UV) radiation leads to well-characterized DNA lesions, notably cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts, both of which distort the DNA helix and interfere with transcription and replication. These lesions are primarily resolved via nucleotide excision repair (NER), while oxidative damage induced by UV is addressed by base excision repair (BER). The efficiency of these repair pathways is essential for genomic stability, particularly during early neurodevelopment when repair fidelity may be reduced. Developmentally immature tissue may parallel age-related decline in repair efficiency, adding to vulnerability during this stage.

1.2 Research Gap: Juvenile Stage and UV-Induced Genotoxic Stress

While DNA damage has been well-documented as a contributing factor to cognitive decline and neurodegenerative disorders in adults, its impact during early brain development, particularly during juvenile stages marked by heightened neural plasticity, remains poorly understood[5]. The juvenile stage of development, marked by significant neural growth and reorganization, may be especially vulnerable to environmental stressors such as ultraviolet (UV) radiation[3]. However, the impact of genotoxic exposure during this stage remains understudied, leaving a significant gap in our understanding of how early-life DNA damage might influence cognitive outcomes[5]. Although *Drosophila* lack a mammalian-equivalent adolescent stage due to holometabolous development, the term 'juvenile stage' is used to describe the larval period marked by active neural circuit establishment, which—while not homologous—is conceptually useful for modeling early neurodevelopmental vulnerability. This phase, while not directly analogous to human adolescence, allows researchers to model early neurodevelopmental processes and test how environmental stressors affect memory formation in a simplified, genetically tractable system. While *Drosophila* larvae do not possess the complex cortical architecture seen in mammals, they exhibit robust associative learning capacity via conserved mechanisms such as cAMP signaling and CREB activation. These molecular pathways are known to underlie memory formation across species, providing translational relevance for studying genotoxic impacts on basic learning processes.

The use of *Drosophila melanogaster* offers several advantages for testing UV-induced memory impairment hypotheses. The fly model features conserved dopaminergic signaling pathways, including dopamine receptors and biosynthetic enzymes, which are functionally analogous to those in mammals. Its genetic tractability, rapid life cycle, and well-characterized learning circuits, such as the mushroom bodies, make it ideal for dissecting the molecular basis of memory formation. These attributes position *Drosophila* as a powerful system for modeling UV-induced cognitive deficits and exploring the role of dopamine signaling in neurodevelopmental vulnerability.

1.3 The Need for Early Interventions

This gap is particularly concerning given increasing global exposure to environmental stressors [11] and the possibility that disruptions to neural development could have long-lasting effects on memory, learning, and overall brain health [2]. Despite this, few studies have investigated potential protective interventions that could mitigate such damage during development. Nutritional compounds like Vitamin D3, known for their neuroprotective properties in adult models [12], have not been adequately explored in the context of early developmental exposure to DNA damage [13]. To address this, the study utilizes *Drosophila melanogaster* as a model organism to investigate the cognitive effects of UV-induced DNA damage during early development and to evaluate the potential mitigating role of Vitamin D3. This work aims to fill a critical void in the literature by examining how environmental stress during early development may impact memory and by identifying accessible strategies to support cognitive resilience.

The adolescent brain undergoes rapid development, making it particularly sensitive to environmental factors that can shape long-term cognitive outcomes. While a growing body of research has linked DNA damage to neurodegenerative diseases in adults, much less is known about how such damage affects brain function during earlier, more vulnerable stages of life [6]. This research helps to fill that gap by investigating how UV-induced DNA damage influences memory formation during development. Using *Drosophila melanogaster* as a model organism provides a scalable and genetically tractable system for evaluating early-life cognitive effects. Additionally, the study explores Vitamin D3 as a potential neuroprotective agent, an intervention that is both cost-effective and widely accessible. If shown to be effective, this approach could inform new preventive strategies to mitigate the cognitive consequences of environmental stressors during the juvenile stage. The potential neuroprotective effects of Vitamin D3 are supported by its well-established antioxidant, anti-inflammatory, and anti-apoptotic properties. Previous studies have shown that Vitamin D3 can modulate oxidative stress and improve neuronal survival in models of neurodegeneration. These effects provide a biological rationale for investigating its ability to buffer against UV-induced genotoxic stress during early neurodevelopment.

The findings have broad implications across developmental neuroscience, toxicology, and public health. Theoretically, the study supports the model that genotoxic exposures during critical developmental windows can disrupt cognitive processes. Practically, it highlights the potential of nutritional and lifestyle interventions to buffer against such damage. Ultimately, this research aims to deepen our understanding of how to protect memory function during one of the most crucial phases of neurodevelopment.

1.4 Research Objectives

This study aims to investigate the cognitive impact of early-life DNA damage on memory development and to assess the potential neuroprotective role of Vitamin D3 during this critical period. Specifically, the research is guided by the following objectives:

1. To determine whether ultraviolet (UV)-induced DNA damage during early developmental stages impairs associative memory in *Drosophila melanogaster* larvae.

2. To evaluate whether Vitamin D3 supplementation can mitigate the cognitive deficits caused by UV-induced DNA damage.

3. To explore the broader implications of these findings for understanding how environmental stressors affect brain development during the juvenile stage.

These objectives address gaps in current knowledge about how genotoxic stress influences developing neural systems and whether nutritional interventions can provide a protective benefit. The findings are intended to inform future research and potential preventative strategies in developmental neuroscience and public health.

1.5 Scope and Limitations

Focusing on the intersection of environmental genotoxic stress and cognitive development, this research explores how early-life ultraviolet (UV) exposure affects memory formation using *Drosophila melanogaster* larvae. The study specifically investigates short-term associative memory and evaluates the mitigating potential of Vitamin D3 as a neuroprotective agent. Included within the scope are controlled laboratory experiments measuring behavioral responses in larvae following UV exposure, with and without Vitamin D3 supplementation. The use of *Drosophila* enables precise genetic and developmental control, making it an efficient model for preliminary cognitive research.

Excluded from the study are long-term memory assessments, adult-stage behavioral outcomes, and molecular analyses of DNA repair or oxidative stress pathways. Additionally, applying insights from invertebrate models to human early development presents inherent biological limitations. Practical constraints, such as time, resource availability, and access to advanced molecular tools, also shaped the study's design and depth. While these limitations constrain the generalizability and mechanistic specificity of the findings, the work provides a critical starting point for understanding how early environmental factors may impact memory during brain development and lays the groundwork for future studies in more complex models. Additional analyses such as ROS quantification, caspase-3 activation, and Bax/Bcl-2 profiling were also not performed, but

are recommended for future research aimed at validating stress and apoptosis mechanisms. Along with that, the study did not assess the bioavailability or tissue-level distribution of Vitamin D3 following supplementation. As a result, it remains unclear whether the compound reached neural tissues or other target organs. Future studies should incorporate kinetic profiling using fluorophore-conjugated Vitamin D3 to track distribution across organs at multiple time points (e.g., 4h, 24h, 48h, 72h, 96h), enabling confirmation of tissue targeting and uptake dynamics. Lastly, molecular assays, including the comet assay and γH2AX staining, were not employed in this study but are recommended for future work to confirm DNA damage and repair outcomes at the cellular level.

1.6 Theoretical Framework: Neurodevelopmental Vulnerability

Given that early brain development is characterized by rapid neural growth and heightened plasticity, it is particularly susceptible to environmental influences. The Neurodevelopmental Vulnerability Framework posits that exposure to harmful agents during critical periods of brain maturation can disrupt normal neural development, leading to persistent cognitive impairments. Within this framework, ultraviolet (UV)-induced DNA damage serves as a genotoxic stressor that may interfere with the establishment and function of neural circuits essential for memory processes. This theoretical perspective guides the study by emphasizing the importance of developmental timing and environmental exposures in shaping cognitive outcomes. Consequently, the memory deficits observed in *Drosophila* larvae provide a valuable model for understanding potential mechanisms of vulnerability in early human brain development. It is important to note that the memory impairment assessed in this study reflects changes in fundamental associative learning, not higher-order cognitive functions. While larvae serve as a simplified model, the use of conserved molecular pathways makes this approach valuable for exploring developmental vulnerabilities to environmental DNA damage.

1.7 Overview of Experimental Design

To explore the cognitive effects of UV-induced DNA damage during early development, an experimental design utilizing *Drosophila melanogaster* larvae was implemented. Controlled UV radiation exposure was applied to embryos, followed by rearing with or without Vitamin D3 supplementation. Memory performance was evaluated using a classical conditioning paradigm that measured associative learning through odor-sugar pairing. Statistical analyses were conducted to assess differences between treatment groups. A comprehensive explanation of the research design, participant selection, data collection, and analysis procedures is detailed in the Methods section.

2 Results

Overall, the findings indicate that UV-induced genotoxic stress significantly impairs associative memory in *Drosophila* larvae while Vitamin D3 supplementation offers partial protection. Although developmental timing was modestly delayed in UV-exposed groups, no substantial or lasting developmental deficits were observed across conditions.

The larval memory assay revealed distinct differences in cognitive performance as shown in Figures 1 and 2. In the control group, 59.57% of larvae were found in the reward zone, demonstrating typical associative memory. Larvae exposed to UV radiation showed a substantial decline, with only 25.53% occupying the reward zone, indicating significant memory impairment. In contrast, Vitamin D3 alone enhanced memory performance slightly above baseline, with 63.27% of larvae in the reward zone. Notably, larvae treated with both UV and Vitamin D3 exhibited intermediate memory performance, with 36.59% in the reward zone. While this represented a clear improvement over the UV-only group, it remained below control levels, suggesting partial but incomplete neuroprotection.

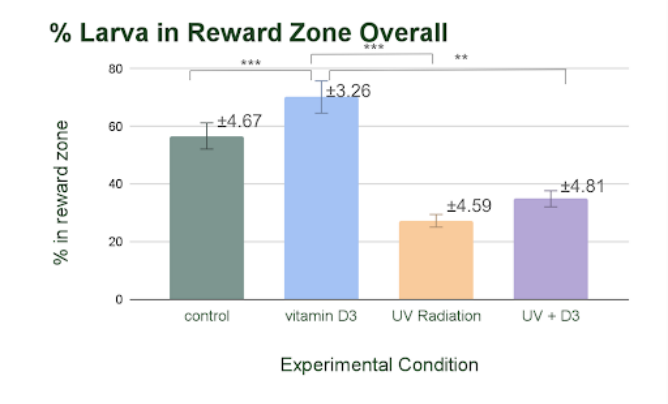

Fig. 2 Percentage of *Drosophila* larvae occupying the reward zone across experimental conditions. Bars represent mean memory performance; error bars indicate ±SEM. Statistical significance was determined using one-way ANOVA ($F(3,180) = 12.67$, $p < 0.0001$), with Tukey's HSD post hoc tests: *** $p < 0.001$, ** $p = 0.023$. Effect size $\eta^2 = 0.17$. The graph demonstrates that UV radiation significantly impaired memory retention compared to controls, while Vitamin D3 co-treatment partially restored performance. See Figure 2 for confidence intervals.

To better quantify the magnitude of behavioral impairment and the mitigating effect of Vitamin D3, effect size measures and confidence intervals were calculated. The difference in reward-zone occupancy between control and UV-exposed groups yielded a large effect size (Cohen's $d = 1.45$), indicating a substantial impact of UV on memory performance. The improvement observed in the UV + D3 group compared to UV alone resulted in a moderate effect size (Cohen's $d = 0.68$),

Table 1 Distribution of *Drosophila* larvae across reward zone, neutral zone, and no-reward zone for each treatment condition. Values represent raw counts and calculated percentages. Data reflects associative memory performance, with higher occupancy in the reward zone indicating stronger learned preference. Statistical significance was determined using one-way ANOVA ($F(3,180) = 12.67$, $p < 0.0001$) and Tukey's HSD post hoc tests: *** $p < 0.001$, ** $p = 0.023$ (vs. UV group).

Exp. condition	# of total larvae	reward zone	% in reward zone	neutral zone	no reward zone	% in no reward zone
Control	47	28	59.57	9	10	21.28
UV Radiation	47	12	25.53	14	21	44.68
Vitamin D3	49	31	63.27	7	11	22.45
UV + D3	41	15	36.59	11	14	34.15

suggesting a biologically meaningful, though incomplete, rescue. 95% confidence intervals for each group are provided in Figure 2 to support interpretation of group differences. While replication was limited ($n = 3$), consistent trends across trials and statistically significant post hoc comparisons support the reliability of the observed effects.

A one-way ANOVA revealed a significant main effect of experimental condition on memory performance, $F(3,180) = 12.67$, $p < 0.0001$, $\eta^2 = 0.17$. Tukey's HSD post hoc test showed significant group differences: UV vs. Control ($p < 0.001$), UV vs. UV + D3 ($p = 0.023$), and UV vs. Vitamin D3 ($p < 0.001$). These results confirm that UV exposure impaired memory while Vitamin D3 provided partial mitigation. Mean percentages, standard errors (SEM), and 95% confidence intervals for memory retention are summarized in Figure 2. Effect sizes were calculated to quantify treatment impact.

To further support the robustness of group differences, post hoc pairwise comparisons were conducted using Tukey's HSD test following one-way ANOVA. These comparisons confirmed significant differences between UV and control ($p < 0.001$), UV and Vitamin D3 ($p < 0.001$), and UV vs. UV + D3 ($p = 0.023$). Effect size for the overall ANOVA was $\eta^2 = 0.17$, indicating a moderate treatment impact. Confidence intervals for each condition are provided in Figure 2 to aid interpretation. Although sample sizes per condition ranged from 41 to 49 larvae, consistent trends across three biological replicates and statistically significant group differences support the reliability of the findings.

Developmental data collected through eclosion tracking as recorded in Figure 3 showed minimal variation among groups. By Day 21, adult emergence rates were 85% in the control group, 83% in the Vitamin D3 group, 73% in the UV group, and 77% in the UV + D3 group. Although the UV-exposed groups exhibited a slight delay in reaching full eclosion, the overall developmental success remained high and relatively consistent across conditions. Therefore, no strong conclusions can be drawn regarding long-term physiological or developmental health effects based on these outcomes.

These results collectively demonstrate that UV radiation disrupts memory formation during early development while Vitamin D3 supplementation may provide a modest protective effect as shown in Figure 3. However, cognitive changes were more pronounced than any alterations in developmental timing or survival.

Table 2 Normalized associative memory retention (%) across treatment conditions. Bars show mean values; error bars reflect ±SEM. Statistical differences were identified using one-way ANOVA ($F(3,180) = 12.67$, $p < 0.0001$), with Tukey's HSD post hoc comparisons: *** $p < 0.001$, ** $p = 0.023$. Effect size $\eta^2 = 0.17$. This graph shows the relative degree of memory retention after UV exposure and/or Vitamin D3 treatment, emphasizing the strong cognitive deficit caused by UV and the partial rescue with Vitamin D3. Refer to Table 2 for associated confidence intervals.

Condition	n	Mean (%)	SEM	95% CI
Day 0	47	59.57	4.67	50.33 – 68.81
Day 7	47	25.53	3.26	19.07 – 31.99
Day 14	49	63.27	4.59	54.23 – 72.31
Day 21	41	36.59	4.81	27.11 – 46.07

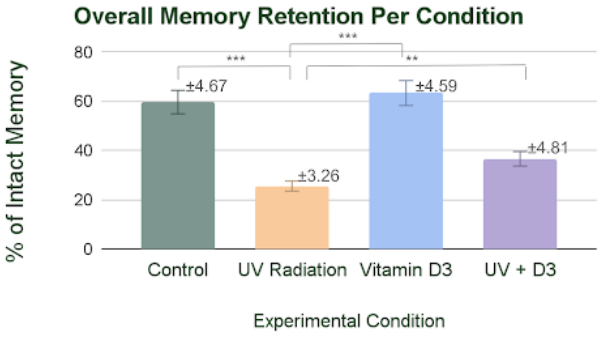

Fig. 3 Normalized associative memory retention (%) across all treatment conditions: Control, UV Radiation, Vitamin D3 only, and UV + Vitamin D3. Bars display mean performance values ± standard error (SEM). Memory was measured via odor-sugar associative learning assay. Statistical differences were identified by one-way ANOVA ($F(3,180) = 12.67$, $p < 0.0001$), followed by Tukey's HSD post hoc comparisons: *** $p < 0.001$ for Control and D3 vs. UV; ** $p = 0.023$ for UV + D3 vs. UV. Asterisks indicate significant improvement relative to UV-only group.

Table 3 Eclosion rate (%) of *Drosophila* larvae by Day 21 across treatment groups: Control, UV Radiation, UV + Vitamin D3, and Vitamin D3 only. Values represent the percentage of larvae that reached adult emergence. Although UV-exposed groups showed slight developmental delay, no statistically significant differences were observed. Data reflect mean outcomes from replicate trials and are presented descriptively.

All Weeks	control	Vitamin D3	UV Radiation	UV and D3
Day 0	0	0	0	0
Day 7	0	0	0	0
Day 14	81	81	66	69
Day 21	85	83	73	77

3 Discussion

3.1 Summary of Key Findings

The study confirmed that early-life ultraviolet (UV) exposure negatively affected associative memory in *Drosophila melanogaster* larvae, as demonstrated by reduced performance in the odor-sugar conditioning assay. Larvae exposed to UV showed diminished preference for the reward-associated cue, indicating impaired memory formation. While Vitamin D3 treatment alone did not significantly enhance memory, its co-administration with UV exposure led to partial improvement in performance, suggesting it may buffer against the cognitive effects of UV-induced damage. These findings align with the study's objective of evaluating the impact of early environmental stress on memory and exploring potential protective strategies during neurodevelopment. The inclusion of effect size metrics and confidence intervals reinforces the interpretation that UV exposure causes substantial cognitive impairment, and that Vitamin D3 co-treatment yields a moderate but consistent improvement in memory performance. While the improvement from 25.53% (UV) to 36.59% (UV + D3) remains below control levels, the moderate effect size (Cohen's $d = 0.68$) supports the use of the term "partial neuroprotection." This terminology reflects attenuation of impairment rather than full rescue, and is used here to denote a statistically supported, biologically relevant shift in performance.

3.2 Implications for Neurodevelopment and Environmental Risk

The results of this study have important implications for our understanding of how environmental factors, specifically ultraviolet (UV) radiation, impact cognitive development during vulnerable stages such as early neural development. This paper does not suggest direct equivalence between *Drosophila* larval learning systems and human adolescent cognition. Rather, the paper aims to highlight how genotoxic stress during periods of active neural development can influence learning capacity across species. By showing that early UV exposure impairs memory formation in *Drosophila* larvae, this research reinforces the concept that the developing brain is especially sensitive to genotoxic stress. This supports the Neurodevelopmental Vulnerability Framework, which emphasizes that disruptions during critical developmental windows can lead to lasting cognitive deficits. The observed behavioral deficits align with established models of larval associative learning, which emphasize the role of dopaminergic signaling and mushroom body circuit plasticity[9]. By linking UV-induced DNA damage to impaired performance in a validated conditioning assay, this study extends prior findings on environmental mutagenesis and highlights the translational relevance of *Drosophila* for comparative neurodevelopmental research.

Additionally, the observed partial mitigation of memory impairment through Vitamin D3 supplementation highlights the potential for practical, cost-effective interventions that may protect or enhance cognitive outcomes in youth exposed to environmental stressors. The behavioral deficits observed may stem from persistent DNA lesions such as CPDs and 6-4 photoproducts, which disrupt transcriptional integrity during neural maturation. Given that UV-induced lesions are typically resolved by NER, compromised repair capacity—either due to developmental immaturity or oxidative overload—may underlie the cognitive impairment in UV-exposed larvae. While we did not directly assay DNA repair activity, the severity of memory loss aligns with patterns seen when lesion resolution is delayed. Additionally, reduced efficiency of BER may exacerbate damage accumulation. These mechanisms reinforce the interpretation of neurodevelopmental vulnerability and merit further molecular investigation in future studies. Furthermore, the mushroom bodies, which undergo critical maturation during larval development, may be particularly vulnerable to UV-induced genotoxic stress. Disruption of Kenyon cell connectivity or dopaminergic input during this window could impair associative learning capacity.

The protective effects observed with Vitamin D3 supplementation may be mediated through activation of the vitamin D receptor (VDR), which is expressed in neural tissues and influences genomic and non-genomic pathways involved in cell survival, immune modulation, and DNA repair. VDR signaling also regulates calcium homeostasis, a process essential for synaptic plasticity, neurotransmission, and neuronal resilience under stress. Since calcium imbalance is a known contributor to neurotoxicity, VDR-mediated stabilization of calcium

dynamics may help preserve cognitive function following UV exposure. Although *Drosophila* do not possess a direct ortholog of the human vitamin D receptor (VDR), the nuclear receptor Hr96 has been identified as a functional analog. Hr96 regulates lipid metabolism, detoxification, and stress responses, and is expressed in neural tissues during development. Recent studies suggest that Vitamin D3 may act through Hr96-mediated transcriptional pathways to influence oxidative stress resistance and cellular resilience. This provides a plausible mechanistic basis for the observed behavioral effects and supports the translational relevance of the model.

3.3 Theoretical and Practical Significance

From a theoretical perspective, these findings extend current knowledge by linking molecular-level DNA damage to observable behavioral changes during development, an area that has been underexplored in the context of early brain development. This study helps fill a gap in the literature by providing a model system that connects cellular damage with functional cognitive impairments. Practically, the evidence suggests that nutritional or lifestyle interventions could serve as accessible strategies to reduce the harmful effects of environmental exposures on the developing brain. Overall, this work advances the field by combining developmental neuroscience, toxicology, and preventive health, encouraging future research to explore similar protective measures in more complex organisms, including humans. It also stresses the need for increased awareness of environmental risks during the juvenile stage and supports the integration of neuroprotective strategies into public health policies aimed at safeguarding youth cognitive function.

3.4 Addressing the Research Objectives

The results of this study effectively met the primary objectives by demonstrating that early-life UV-induced DNA damage impairs memory formation in *Drosophila* larvae, offering valuable insights into cognitive vulnerability during early brain development. While Vitamin D3 supplementation showed a partial protective effect against these deficits, its impact was not complete, suggesting that other factors may influence neuroprotection and warrant further investigation. This variability highlights the complexity of the biological response to genotoxic stress during early development.

Based on these findings, future research should aim to uncover the molecular pathways through which UV-induced DNA damage disrupts memory processes, ideally expanding to vertebrate models for greater relevance to human neurodevelopment. Investigations into varying doses, timing, and combinations of neuroprotective agents, including Vitamin D3, could help optimize intervention strategies. Moreover, long-term studies are needed to assess the persistence and potential reversibility of cognitive impairments caused by early DNA damage. Practically, this research emphasizes the importance of developing accessible preventive measures to protect early brain health from environmental stressors, supporting the exploration of nutritional supplementation and policy initiatives aimed at at-risk populations. Additionally, future research should incorporate direct DNA damage assays, such as the comet assay and γH2AX staining, to validate the protective role of Vitamin D3 and distinguish specific genotoxic effects from broader cellular toxicity[?]. Future studies should also include molecular assays such as Western blotting or ELISA to quantify ROS production, caspase-3 levels, and Bax/Bcl-2 ratios. These endpoints will help clarify whether oxidative stress and apoptosis mediate UV-induced cognitive impairment, and whether Vitamin D3 modulates these pathways.

3.5 Limitations and Considerations

Several limitations should be acknowledged when interpreting the results of this research. The relatively small number of replicates (four per condition) limits the statistical power and generalizability of the findings. Additionally, some variance was observed across biological replicates, particularly in the UV-exposed groups. While this variability may reflect stochastic effects of genotoxic stress, it also underscores the need for larger sample sizes in future studies to better capture inter-individual response patterns and improve statistical precision. Furthermore, the focus on larval memory assessed shortly after UV exposure does not account for potential long-term cognitive effects that may emerge during adulthood. While *Drosophila melanogaster* provides a valuable genetic model, differences between invertebrate and human neurobiology restrict direct application to early neural development. Moreover, the controlled laboratory environment may not fully capture the complexity of environmental exposures experienced in natural settings. Lastly, the mechanisms underlying the observed partial neuroprotective effect of Vitamin D3 remain unclear and warrant further exploration. Future studies incorporating larger sample sizes, longer follow-up periods, and diverse experimental conditions will be essential to expand upon these findings and enhance their translational relevance. Additionally, the need to calibrate depth-dependent UV exposure thresholds remains a key limitation, as current data do not verify precise distribution or neural tissue targeting in vivo.

To continue, only a single UV exposure level (100 mJ/cm^2 at 254 nm) was tested in this study. While this dose was selected based on pilot trials that produced consistent behavioral deficits without excessive lethality, the absence of a dose-response analysis limits causal inference. Future studies should incorporate multiple UV intensities and exposure durations to establish dose-effect relationships and better characterize the threshold at which cognitive impairment emerges. It's also important to note

that while the behavioral deficits observed suggest UV-induced genotoxic effects, we did not directly assess DNA damage using molecular assays such as the comet assay or γH2AX staining. Lastly, the study did not include molecular validation of oxidative stress or apoptotic activity. As such, we cannot determine whether the observed behavioral deficits were accompanied by elevated ROS production, caspase-3 activation, or changes in Bax/Bcl-2 protein expression. These molecular endpoints are crucial for confirming whether UV-induced deficits stem from genotoxic damage or broader stress responses. As a result, the possibility that memory impairment stems from general UV toxicity rather than specific DNA lesions cannot be excluded. While molecular assays were not included, the study focused on behavioral outcomes linked to neural circuit maturation, specifically within the mushroom bodies, to infer cognitive impairment. Future work will incorporate direct histological methods to confirm neural-specific DNA damage and rule out non-neural physiological effects.

3.6 Conclusion and Broader Impact

Despite these limitations, the research offers important insights into how environmental genotoxic stress during critical developmental periods may impact memory formation, underscoring the need for greater attention to early developmental brain health. Given that memory and learning abilities are foundational to academic success and overall cognitive health during early development, protecting these functions is crucial. Ultimately, safeguarding cognitive development requires a multidisciplinary approach that integrates molecular biology, nutrition, public health, and policy. As awareness grows regarding the vulnerability of the developing brain to environmental challenges, this work serves as a reminder that early preventive interventions hold significant promise for supporting lifelong mental health and academic achievement. Prioritizing cognitive well-being is not only vital for individual growth but also for the broader societal benefits of a healthy, educated population.

4 Methods

4.1 Experimental Design

This study employed a controlled experimental design to evaluate the effects of ultraviolet (UV) radiation on early cognitive development using *Drosophila melanogaster* larvae as a model organism. The design involved multiple experimental groups to isolate the impact of UV-induced DNA damage and assess the potential neuroprotective effect of Vitamin D3. Larvae were randomly assigned to one of four treatment groups: control (no UV, no Vitamin D3), UV exposure only, Vitamin D3 supplementation only, and combined UV exposure with Vitamin D3 supplementation. To ensure that behavioral differences were attributable to UV exposure rather than procedural artifacts, the control group was designed as a sham-handled condition. Control larvae underwent identical handling procedures as the UV-exposed groups, including timing of transfer, food medium exposure, and environmental conditions, but without UV irradiation. This approach aligns with standard experimental design practices and allows for isolation of treatment-specific effects. This design allowed for comparison between groups to determine the specific contributions of each variable to memory performance. The experimental structure included standardized exposure protocols and behavioral assessments conducted at a consistent developmental stage to ensure reliability and reproducibility of results. Due to scope limitations, this study tested a single UV intensity. Follow-up experiments will include varying doses and exposure durations to characterize the relationship between UV intensity and learning impairment.

4.2 Model Organism and Rearing Conditions

Given the experimental structure of this study, *Drosophila melanogaster* (fruit fly) larvae were selected as the model organism due to their well-established utility in genetic, developmental, and behavioral neuroscience research. Early third instar larvae, approximately 4–5 days post-egg deposition, were used to ensure consistency in developmental stage and cognitive capacity across trials. These larvae were reared under standardized conditions of temperature, humidity, and light cycle to minimize environmental variation. To further reduce experimental bias, all larvae were maintained at a constant temperature of 25°C with 60% relative humidity and a 12:12 light-dark cycle. Food composition was standardized across all groups using the same base medium, with Vitamin D3 supplementation incorporated using a consistent vehicle (1% Tween 80 and PEG 40). Larvae were reared in identical containers and transferred at the same developmental time points to ensure uniform handling. These controls minimized variability due to environmental factors and ensured that observed cognitive differences were attributable to treatment effects. To maintain behavioral relevance, only actively feeding larvae were selected, avoiding those entering the wandering phase. For each condition, groups of 10–30 larvae were tested, with three independent biological replicates conducted to strengthen the reliability of the results.

4.3 Behavioral Assay: Associative Learning Test for Larval Memory

To investigate the cognitive effects of early-life UV-induced DNA damage and the potential neuroprotective role of Vitamin D3, an experimental approach was employed using *Drosophila melanogaster* larvae as the model organism. The primary variable of interest was memory retention, operationalized through larval odor preference in a Pavlovian-style associative learning

assay. Additional independent variables included UV exposure and Vitamin D3 supplementation. Data collection involved training third instar larvae to associate the odor isoamyl acetate with a sugar reward (2M sucrose) and octyl acetate with no reward. After three alternating training cycles on reward and non-reward agar plates, larvae were placed on a test plate containing both odors without sucrose. Their positions were recorded after five minutes using time-lapse video or a photograph, and the percentage of larvae in the sugar-associated zone served as the behavioral measure of memory performance.

Each training cycle lasted 5 minutes, with 2-minute inter-trial intervals between exposures to reward and non-reward odors. Odorants were applied at a concentration of 1:100 dilution in paraffin oil, and 2M sucrose was used as the reward stimulus. Environmental conditions were standardized across trials: assays were conducted at 25°C, 60% relative humidity, and under red light illumination to minimize visual cues. Larvae were gently transferred to the test plate using a soft brush and placed at the center of the arena. After 5 minutes, larval positions were captured via overhead photography, and preference was quantified as the percentage of larvae located in the sugar-associated odor zone relative to the total number of larvae on the plate. Larvae that remained in the neutral zone or failed to move were excluded from analysis.

To control for potential behavioral biases, several measures were implemented. First, only actively feeding third instar larvae were selected to ensure consistent motivation and locomotor capacity across groups. Prior to training, larvae were screened for normal movement and excluded if they exhibited reduced motility. Second, innate odor preference was assessed in pilot trials using untrained larvae exposed to both odorants (isoamyl acetate and octyl acetate) without sucrose. These tests confirmed balanced baseline attraction across odors. Third, reciprocal training was employed (A+/B vs. A/B+) to eliminate odorant bias and isolate associative learning effects. No significant differences in odor sensitivity or locomotion were observed following Vitamin D3 supplementation, and this was confirmed through direct observation and pilot testing. These controls ensure that memory performance reflects true associative learning rather than sensory or motivational artifacts.

4.4 Treatment Administration & Analysis

The experimental design included four groups: control (no treatment), UV-exposed, Vitamin D3-supplemented, and UV plus Vitamin D3. All treatments were administered during the embryonic stage, with UV exposure occurring within hours after egg laying and Vitamin D3 incorporated into the food medium. The Vitamin D3 concentration used in this study was 10 mM, based on prior research demonstrating antioxidant and neuroprotective effects in *Drosophila* models. The compound was solubilized in the food medium using a 1% Tween 80 and PEG 40 vehicle to enhance absorption. Each condition was replicated three times using cohorts of 10–30 larvae per trial. Quantitative data was analyzed by calculating the mean percentage of larvae displaying correct odor preference in each condition. Statistical significance between groups was determined using one-way ANOVA followed by post hoc tests to identify meaningful differences in memory performance. A one-way ANOVA revealed a significant effect of treatment condition on memory performance ($F(3,180) = 12.67$, $p < 0.0001$), with an effect size of $\eta^2 = 0.17$. Tukey's HSD post hoc analysis showed significant pairwise differences: UV vs. Control ($p < 0.001$), UV vs. Vitamin D3 ($p < 0.001$), and UV vs. UV + D3 ($p = 0.023$). Figures 1 and 4 include error bars representing standard error of the mean (SEM), and 95% confidence intervals for each group are presented in Figure 3. This analytic approach enabled the evaluation of both detrimental and mitigating effects on cognitive outcomes, thereby supporting broader inferences about neurodevelopmental vulnerability and intervention efficacy.

UV exposure was administered using a 254 nm UVC bulb at 100 mJ/cm^2 for 30 seconds. While *Drosophila* larvae are translucent, allowing partial tissue penetration, future work should quantify UV absorption and transmission specifically to neural tissues using fluorophore-labeled markers or histological staining. The current dose was selected based on pilot observations that revealed consistent behavioral deficits without excessive lethality. However, a full dose-response analysis will be necessary to identify optimal exposure levels and establish biological thresholds. While direct measurement of UV penetration depth was beyond the scope of this study, existing data on tissue optics in invertebrates suggest that short-wavelength UVC (254 nm) can reach subsurface structures in translucent organisms such as *Drosophila* larvae. Nonetheless, neural targeting remains unconfirmed, and future studies should quantify tissue penetration using histological or optical methods such as fluorophore-tagged markers to visualize transmission pathways and intensity gradients.

4.5 Ethical Considerations

Although this study did not involve human subjects, ethical considerations were still observed in the treatment and handling of *Drosophila melanogaster* larvae. All experimental procedures were conducted in accordance with institutional and educational guidelines for the ethical use of invertebrate model organisms. Care was taken to minimize any unnecessary stress or harm to the larvae throughout the experimental process. UV exposure levels were calibrated to avoid excessive lethality while still achieving measurable biological effects. The use of model organisms such as *Drosophila* allows for the investigation of complex biological questions without raising the same ethical concerns associated with vertebrate or human research, thereby providing a responsible and scalable approach to studying neu-

rodevelopmental phenomena.

5 Acknowledgements

I would like to express my sincere gratitude to my instructors, Dr. Amanda Purdy, Dr. Theresa Austria, Dr. Silvia Valdes, and Mr. Austin Cozzone, as well as the teaching assistants Phoebe Ting, Martie Corcoran, and Amritha Sakamuri for their invaluable guidance and support throughout this research. I am also grateful to Fox Chase Cancer Center for hosting the TRIP Research Internship Program and providing the resources needed to complete this study. Additionally, I thank William Tennent High School for providing laboratory space and equipment essential for the experiments. Finally, I appreciate my lab mates and parents for their encouragement and support during this project.

References

1 S.-J. Blakemore and K. Mills, *Is adolescence a sensitive period for sociocultural processing?*, https://doi.org/10.1146/annurev-psych-010213-115202.

2 R. Madabhushi, L. Pan and L.-H. Tsai, *DNA damage and its links to neurodegeneration*, https://doi.org/10.1016/j.neuron.2014.06.034.

3 M. Hussein, Y. Zhang and J. Wang, *UV-induced neurodegeneration: Molecular mechanisms and clinical implications*, https://doi.org/10.1016/j.neuro.2021.11.003.

4 M. Mattson and T. Arumugam, *Hallmarks of brain aging: Adaptive and pathological modification by metabolic states*, https://doi.org/10.1016/j.cmet.2018.05.011.

5 J. Silbereis, S. Pochareddy, Y. Zhu, M. Li and N. Sestan, *The cellular and molecular landscapes of the developing human central nervous system*, https://doi.org/10.1016/j.neuron.2015.12.008.

6 J. Kirkland, M. Stout and F. Sierra, *Resilience in aging mice*, https://doi.org/10.1093/gerona/glw086.

7 A. Widmann, M. Artinger, L. Biesinger, K. Boepple, C. Peters and J. Schlechter, *Genetic Dissection of Aversive Associative Olfactory Learning and Memory in Drosophila Larvae*, https://doi.org/10.1371/journal.pgen.1006378.

8 W.-P. Lee, M.-H. Chiang, L.-Y. Chang, W.-H. Shyu, T.-H. Chiu, T.-F. Fu, T. Wu and C.-L. Wu, *Serotonin signals modulate mushroom body output neurons for sustaining water-reward long-term memory in Drosophila*.

9 J. Thoener, C. König, A. Weiglein, N. Toshima, N. Mancini, F. Amin and M. Schleyer, *Associative learning in larval and adult Drosophila is impaired by the dopamine-synthesis inhibitor 3-Iodo-L-tyrosine*, https://doi.org/10.1242/bio.058198.

10 K.-N. Yoon, S. Kim, J. Ji, Y. Cui, Q.-L. Quan, G. Park, J.-H. Oh, J. Lee, J.-Y. An, J. Chung, Y.-S. Lee and D. Lee, *Chronic ultraviolet irradiation induces memory deficits via dysregulation of the dopamine pathway*, https://doi.org/10.1038/s12276-024-01242-x.

11 *United Nations Environment Programme (UNEP), Environmental effects of ozone depletion and its interactions with climate change: 2018 assessment*, https://ozone.unep.org/science/assessment/eeap.

12 N. Groves, J. McGrath and T. Burne, *Vitamin D as a neurosteroid affecting the developing and adult brain*, https://doi.org/10.1146/annurev-nutr-071813-105557.

13 T. Kulie, A. Groff, J. Redmer, J. Hounshell and S. Schrager, *Vitamin D: An evidence-based review*, https://doi.org/10.3122/jabfm.2009.06.090037.