Section IV: Discussion

The primary objective of this study was to assess the effects of PTZ exposure on spatial learning across multiple generations of UNC-49 worms. Specifically, we aimed to determine whether PTZ exposure impairs spatial learning and whether these effects persist or accumulate across successive generations. Based on the results presented in Section III, our hypotheses were largely supported, though the magnitude and persistence of effects varied depending on exposure history and generation.

Control UNC-49 worms (n = 30) demonstrated significant differences between training and learning phases ($p < 0.001$***), confirming that spatial learning was intact under baseline conditions. This provides a reliable reference for assessing the impact of PTZ exposure. In Generation 1, worms exposed to PTZ (n = 30) showed significant impairments relative to controls in both the training ($p < 0.001$***) and learning ($p = 0.015$*) phases, indicating that even a single exposure is sufficient to disrupt spatial learning.

Generation 2 worms exposed to PTZ across both generations demonstrated persistent deficits in training ($p = 0.0063$**) and learning ($p = 0.04432$*). Worms exposed only in Generation 1 still showed significant deficits (training: $p = 0.0003$***, learning: $p = 0.0018$**), suggesting that ancestral exposure alone can influence cognitive performance in descendants. These observations are consistent with transgenerational epigenetic effects described in prior studies of chemical exposure in C. elegans (Greer et al., 2011; Kishimoto et al., 2017).

By Generation 3, worms exposed three times to PTZ exhibited training deficits ($p = 0.001$**), but learning performance did not significantly differ from controls ($p = 0.7424$), suggesting a potential adaptive or compensatory mechanism with repeated exposure. In contrast, Generation 3 worms exposed in Generations 1 & 2 or only in Generation 1 showed significant differences in both training and

learning phases (Figures 7 and 8), highlighting that timing and lineage of exposure critically modulate transgenerational effects.

The cross-generational comparison of single PTZ exposure (Figure 4) revealed no significant difference between Generations 1 and 2 ($p = 0.09694$) or between Generations 2 and 3 ($p = 0.26128$), but a significant difference was observed between Generations 1 and 3 ($p = 0.0276$*). This suggests that immediate descendants may exhibit similar deficits, whereas more distant generations may partially recover or adapt over time.

All comparisons used Z-tests for proportions, appropriate for evaluating differences in the proportion of worms completing the spatial learning task across groups. With a consistent sample size of $n = 30$ per group, statistical power was sufficient to detect moderate to large differences between conditions. P-values below 0.05 were considered significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), and significance thresholds were consistently applied to both training and learning phases.

Potential limitations include inherent behavioral variability among worms, which could be influenced by plate conditions, temperature, or handling. These factors were controlled by maintaining consistent environmental conditions and randomly assigning worms to exposure groups. The fixed sample size ($n = 30$) provided moderate statistical power; however, detecting subtle effects in learning phases may require larger cohorts. The study is also limited by the lack of mechanistic assays, leaving the molecular or epigenetic basis of observed deficits unexamined.

These findings align with prior work showing that chemical exposures can have transgenerational behavioral effects in C. elegans (Greer et al., 2011; Kishimoto et al., 2017). This study extends previous research by systematically comparing effects across three generations and differentiating between single and multiple exposures. The potential adaptation observed in Generation

3 worms exposed multiple times represents a novel insight, suggesting that exposure frequency and generational distance may interact to modulate cognitive outcomes.

Future Research

Future studies should aim to quantify amyloid-beta accumulation in the brains of each generation using Thioflavin-T staining. Measuring the extent of protein aggregation would help determine whether PTZ-induced cognitive deficits are associated with molecular markers of neurodegeneration. In parallel, it will be important to measure methylation levels across generations to explore potential epigenetic mechanisms underlying the transgenerational inheritance of spatial learning deficits. Linking methylation patterns to behavioral outcomes could clarify how ancestral exposures affect gene regulation in descendants.

Additionally, future research should analyze the relationship between seizure severity, frequency, and spatial learning performance. Determining whether more severe or frequent seizures exacerbate cognitive deficits will provide a more comprehensive understanding of how neural stressors impact learning and memory over time. Finally, studies could evaluate whether antioxidant treatments can prevent or reduce the spatial learning deficits observed following seizures. Investigating interventions aimed at mitigating oxidative stress would not only test potential therapeutic strategies but also provide insight into the biological pathways contributing to transgenerational cognitive impairment.

Collectively, these directions will extend the current findings by linking behavioral changes to molecular and epigenetic markers, identifying risk factors for cognitive deficits, and exploring potential avenues for prevention or mitigation. These studies are essential for advancing our understanding of how environmental and chemical exposures propagate effects across generations and for developing strategies to protect cognitive health.