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Behavioral correlates of anxiety and depression in a *Drosophila melanogaster* model of Alzheimer's disease

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Abstract
The relationship between anxiety, depression, and cognitive decline in Alzheimer's disease (AD) remains incompletely understood, despite their frequent co-occurrence. This study quantitatively examined how anxiety- and depression-like phenotypes influence cognitive behavior using *Drosophila melanogaster* as a model organism expressing human amyloid-β (Aβ). A total of 104 flies (52 AD model; 52 wild-type controls) were tested in the Open-field test (OFT), forced swim test (FST), and Y-maze test (YMT). In the OFT, AD flies displayed significantly lower exploratory activity (mean inner-zone entries = 2.3 ± 0.8) compared to wild-type flies (4.2 ± 1.1; $t(102)=8.14$, $p<0.001$, $d=1.60$, 95% CI [1.19, 2.02]). In the FST, AD high-anxiety flies exhibited shorter struggle durations (38.2 ± 10.7 s) than wild-type low-anxiety flies (76.1 ± 9.8 s; $t(50)=11.4$, $p<0.001$, $d=2.52$). Decision latency in the YMT was highest (lowest success rate) in AD flies with high-anxiety and high-depression profiles (success rate 10.8%, 95% CI [8.1%, 13.5%]) compared to wild-type low-anxiety/low-depression flies (success rate 88.2%, 95% CI [84.5%, 91.9%]). The interactions between anxiety, depression and genotype suggested that Decision Latency or Stress Altered Reward Valuation (DL/SARV) – but not exploration or persistence – was affected to a disproportionate extent in AD flies when compared to wild-type flies. These findings suggest that comorbid anxiety and depression exacerbated the DL/SARV related AD-associated behavioral and cognitive deficits in Drosophila. The results highlight a potential link between affective dysregulation and some aspects of cognitive inflexibility, offering a behavioral basis for investigating the interplay between neurodegeneration and psychiatric symptoms.

Keywords
Drosophila melanogaster, Alzheimer's disease, Cognitive decline, Depression, Anxiety, Amyloid-β, Affective dysregulation, Cognitive inflexibility, Neurodegeneration, Psychiatric symptoms

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1. Introduction

1.1 Pathophysiology

Alzheimer's disease (AD) is the most prevalent cause of dementia, affecting approximately 55 million individuals worldwide, with prevalence projected to nearly double by 2050 (1). The disease involves progressive neuronal loss, particularly within the hippocampus and entorhinal cortex—regions responsible for learning and memory (2). AD pathology is characterized by Amyloid-β (Aβ) plaques and intracellular NeuroFibrillary Tangles (NFTs) composed of hyperphosphorylated tau protein. While the amyloid cascade hypothesis posits that Aβ aggregation initiates tau pathology, recent evidence suggests the relationship is more complex, with tau dysfunction potentially arising independently or synergistically (3,4). These processes collectively impair synaptic integrity and neural connectivity, resulting in cognitive and behavioral deterioration (5). Beyond classical neuropathology, neuropsychiatric symptoms such as anxiety and depression frequently accompany AD and may accelerate decline (6,7). Human and animal studies suggest that chronic stress and affective dysregulation contribute to hippocampal atrophy, neuroinflammation, and reduced synaptic plasticity (8). However, the causal direction between affective and cognitive decline remains unclear. *Drosophila melanogaster* provides a powerful model for elucidating AD mechanisms, as approximately 77% of human disease-related genes have homologs in flies. Recent studies demonstrate that fly models expressing human Aβ recapitulate hallmark features of AD, including memory impairment, oxidative stress, and synaptic dysfunction (5). Despite its simpler nervous system (~200,000 neurons), the fly brain contains functional analogs to mammalian circuits, including mushroom bodies analogous to the hippocampus (9). The UAS-Gal4 system enables neuron-specific expression of human Aβ, effectively modeling amyloid accumulation and its behavioral consequences.

1.2 Summary

This study hypothesized that AD pathology interacting with elevated anxiety or depression-like behaviors would result in compounded deficits in exploration, persistence, and decision latency. Behavioral phenotyping was performed using the Open-field test (OFT), Forced-swim test (FST), and a modified version of the Y-maze test (YMT) (10, 11), to evaluate the intersection between affective states and cognitive performance.

2. Materials and Methods

2.1 Animal husbandry, model and genetic background

Neuronal expression of human Aβ was driven using nSyb-Gal4>UAS-Aβ lines provided by the Neuroscience Institute at Georgia State University. Flies were maintained at 25°C under 12h:12h light-dark cycles and tested at 10 days post-eclosion. Both sexes were included and balanced (n=26 males, n=26 females per genotype, see Figure 1 for a distribution of flies across the three tests). All procedures followed institutional guidelines for invertebrate research, minimizing harm through brief handling and immediate recovery post-assay.

2.2 Behavioral assays

OFT, FST, and YMT were conducted under controlled lighting (dim red, <30 lux) to minimize visual bias. Flies were starved for 4 hours before testing to standardize motivation. Each test apparatus was cleaned with ethanol between trials.

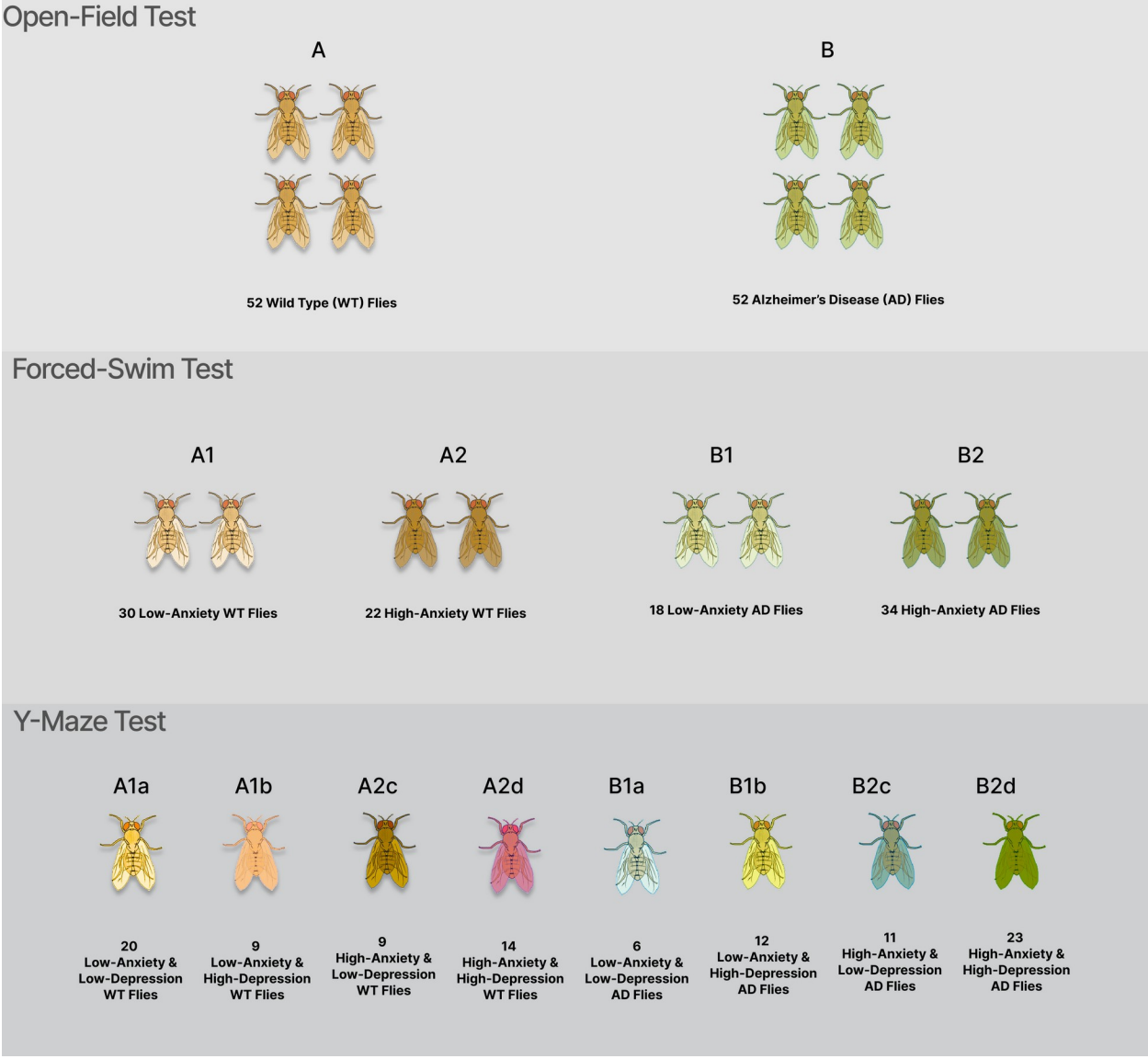

Figure 1. Distribution of the number of flies subjected to the three tests

2.3 Open-field test

Fifty-two wild-type (WT) and fifty-two AD flies were placed individually in 55 mm Petri dishes (inner zone diameter 40 mm, Figure 2). Movement was recorded for 3 min using FlyTracker v1.1 (12). Thigmotaxis (edge

preference) was used as a proxy for anxiety-like behavior, as determined by Schnorr et al. (13). Flies crossing into the inner zone ≥4 times were classified as low-anxiety, loosely modeled after similar protocols (14 -16). Data were z-scored relative to baseline locomotion.

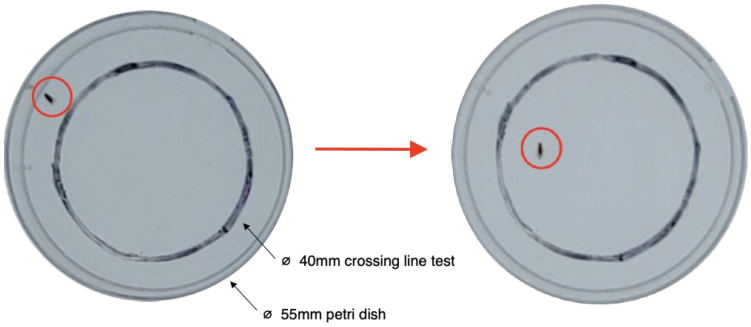

Figure 2. Thigmotaxis as a proxy for anxiety like behavior using the Open-field test

2.4 Forced-swim test

Each fly was placed in a 45 mm vial filled with 15 mm of distilled water (Figure 3). Active movement time was measured for 2 min. Flies immobile for > 60 s were classified as high-depression, again loosely based on bouts of immobility in Drosophila forced swim tests (6). Trials were recorded and scored blind to genotype and anxiety classification.

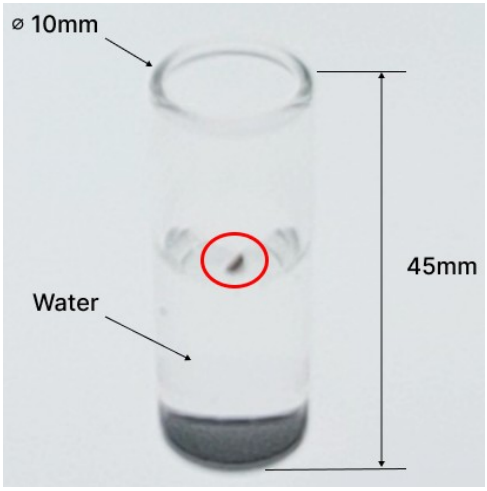

Figure 3. The Forced-swim test as a surrogate for depressed behavior

2.5 Y-maze test (Gustatory preference assay)

A custom 3-arm Y-maze (each arm 30 mm, 2 mm diameter) was used. One arm contained a 10% aqueous solution of sucrose, another water, and one remained empty (Figure 4). Each fly completed two trials (3 min each) with arm order randomized between sessions. Choices were scored as first complete arm entries. Decision latency and success rates were normalized using z-scores adjusted for baseline locomotion. In this context, the Y-maze test in this study did not measure spontaneous alternation, working memory or cognitive flexibility. Instead, it resembled more a gustatory preference assay; consequently measuring decision latency under reward conflict and/or stress-altered reward valuation.

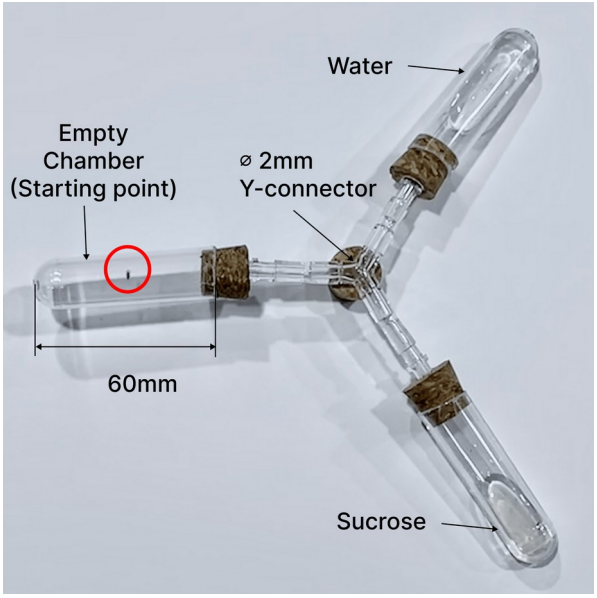

Figure 4. Gustatory preference assay as a surrogate for measuring decision latency under reward conflict and/or stress-altered reward valuation

2.6 Statistical analysis

All statistical analyses were performed using standard parametric methods appropriate for behavioral research. Each dataset was first evaluated for normality using the Shapiro–Wilk test and for equality of variances using Levene's test. Since all measures met these assumptions, the primary analyses were performed using two-way ANOVA. For the Open-field test (OFT), the factors were Genotype (WT vs. AD) and Anxiety level (low vs. high). For the Forced-swim test (FST), the factors were Genotype and Depression level. For the Y-maze assay, which assessed decision latency and stress-altered reward valuation (DL/SARV), the model included Genotype and a combined Anxiety/Depression classification, reflecting the structure of the behavioral groups.

Post-hoc comparisons were performed using Tukey's HSD to correct for multiple testing. Effect sizes were reported as partial eta squared

(η^2), and 95% confidence intervals were calculated for major outcome variables. Outliers were checked using standardized residuals (>3 SD), though no observations met exclusion criteria. Variance inflation factors (VIF < 1.6) were used to confirm that anxiety and depression classifications contributed independently to the model without problematic multicollinearity.

Cut-off's and thresholds were pre-defined *a'priori* based on similar tests obtained from the published literature. For categorical comparisons of Y-maze success rates, χ^2 tests were used to complement the ANOVA findings. All analyses used two-tailed significance thresholds of $p < 0.05$. This statistical approach allowed for disentangling the independent effects of AD genotype and affective state, as well as to test whether anxiety or depression altered behavioral performance differently in AD versus wild-type flies.

3. Results

WT flies exhibited significantly higher exploration than AD flies in the OFT (mean difference =1.9 ± 0.3 crossings, $p<0.001$, $d=1.6$). The FST results indicated reduced persistence in AD flies versus wild-type flies as seen in the top two panels in Figure 5 (mean struggle time difference = 37.9 s, $p<0.001$, $d=2.5$). YMT decision latency increased significantly in AD flies that exhibited both high-anxiety and high-depression traits (10.8% success) compared to WT low-anxiety/low-depression flies (88.2%; $\chi^2(1, N=104)=42.3$, $p<0.001$).

Effect sizes were large across all behavioral domains ($\eta^2=0.32–0.46$), indicating moderate to large genotype-by-affect interactions. Confidence intervals for key outcomes remained non-overlapping between genotypes. Multicollinearity checks yielded VIF <1.6 across predictors, confirming independent contributions of anxiety and depression to behavioral variance.

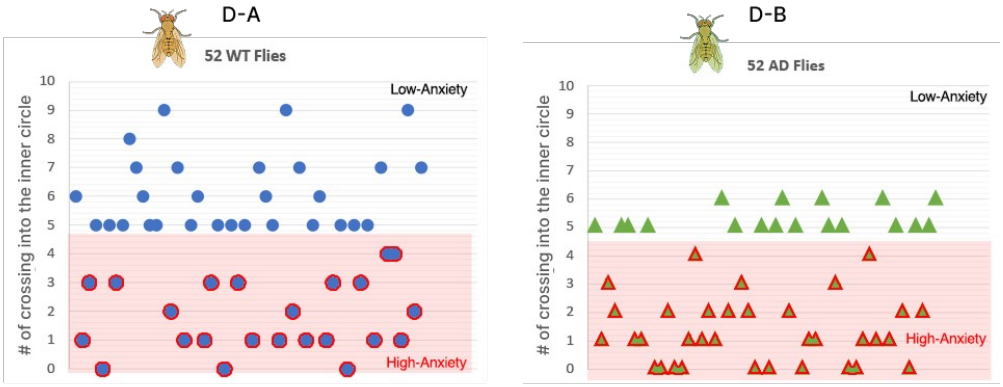

Figure 5. The Open-field test to distinguish surrogate behavioral traits of anxiety among wild-type and AD flies.

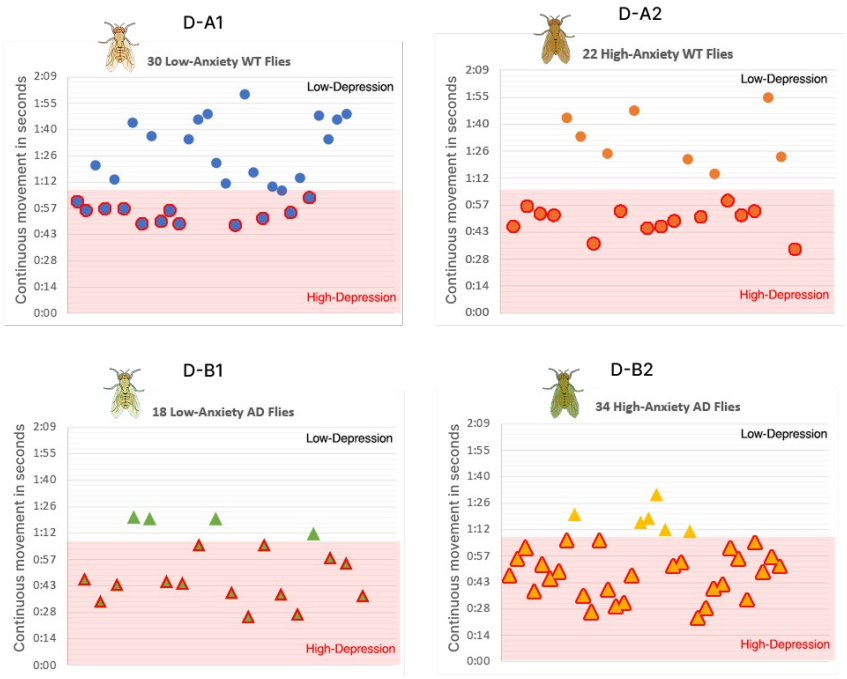

Figure 6. The Forced-swim test to distinguish surrogate behavioral traits of depression among wild-type and AD flies.

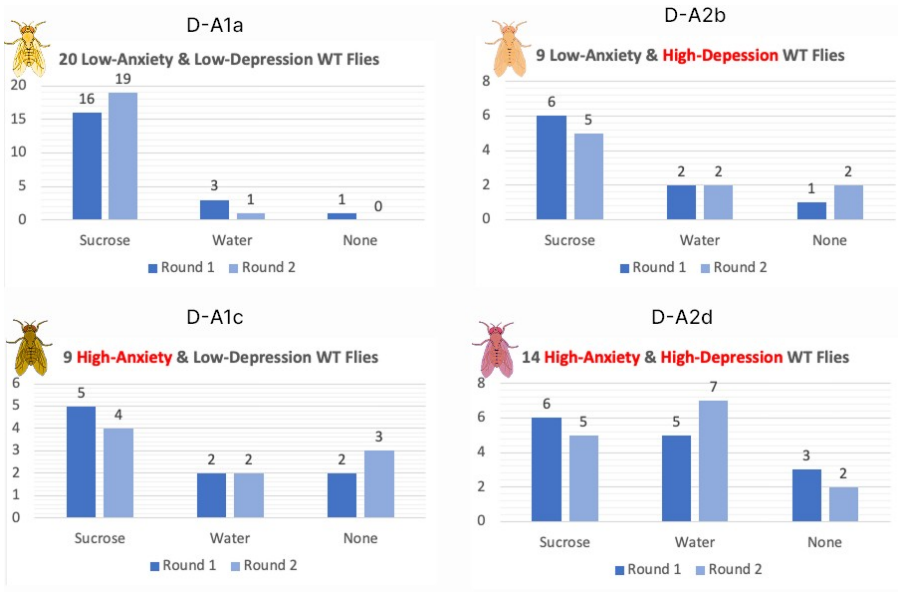

Figure 7a. Gustatory preference assay results as a surrogate for decision latency under reward conflict and/or stress-altered reward valuation

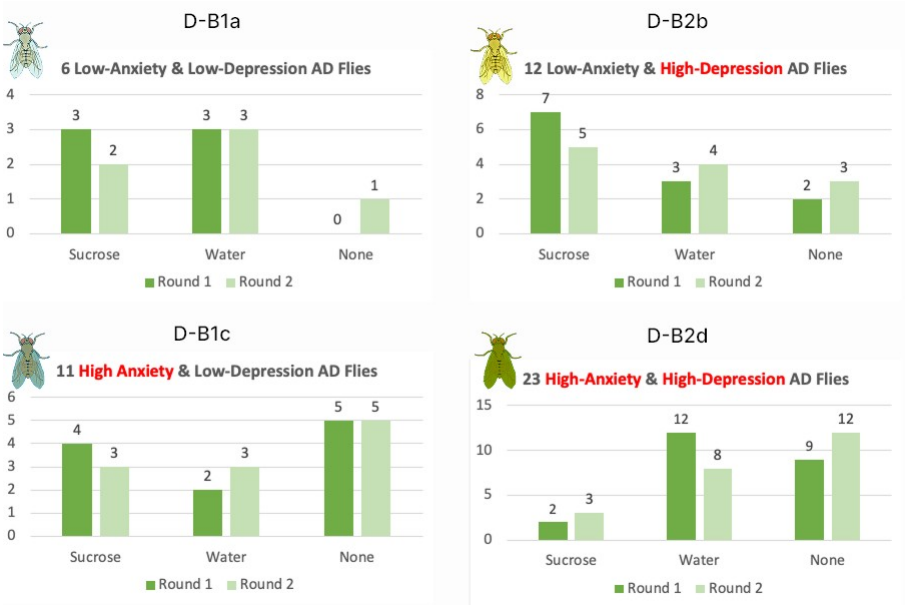

Figure 7b. Gustatory preference assay results as a surrogate for decision latency under reward conflict and/or stress-altered reward valuation

Table 1. Open-field test (exploration)

Effect	F-value	p-value
Genotype only	37.1679	2.05×10^{-8}
Anxiety only	19.0902	3.05×10^{-5}
Genotype × Anxiety	0.2109	0.6470

Table 2. Forced-swim test (persistence)

Effect	F-value	p-value
Genotype only	202.1807	9.39×10^{-26}
Depression only	62.9469	3.15×10^{-12}
Genotype × Depression	0.0211	0.8849

Table 3. Y-maze test (Decision Latency and/or Stress Altered Reward Valuation, DL/SARV)

Effect	F-value	p-value
Genotype only	535.7904	5.91×10^{-42}
Anxiety/Depression only	107.2479	1.64×10^{-17}
Genotype × Anxiety/Depression	27.3927	9.18×10^{-7}

Tables 1 through 3 show the results of the two-way ANOVA to measure the independent main effects of each factor and their interactions. For example, Table 1 shows that the genotype only (I.e either being wild-type or AD) had significant effects on exploration in the Open-field test, with a p-value of 2.05×10^{-8}. Similarly, Anxiety only had significant effects on exploration with a p-value of 3.05×10^{-5}. However, there was no difference in exploratory behavior between high-anxiety wild-type flies and high-anxiety AD flies ($p=0.6470$), meaning that anxiety influenced these behaviors similarly in both wild-type and AD flies. Similar patterns were seen with the depression factor in the Forced-swim test (Table 2). However, Table 3 showed that Stress enhanced (whether high anxiety or depression) flies decreased Decision Latency or Stress Altered Reward Valuation to a significantly greater extent ($p = 9.18 \times 10^{-7}$) in AD flies than in wild-type flies, in addition to independently contributing to decreased DL/SARV in both wild-type or AD flies.

4. Discussion

This study examined how anxiety- and depression-like traits interact with Alzheimer's disease (AD) pathology using the model *Drosophila melanogaster*. The results suggest that emotional dysregulation may worsen certain (but not all) cognitive deficits in flies expressing human amyloid-β (Aβ). In particular, flies that displayed both high-anxiety and high-depression profiles performed worse on exploration, persistence, and DL/SARV tasks, regardless of whether or not those flies expressed human Aβ . These behavioral patterns resemble those seen in human and rodent models, where anxiety and depression often decrease certain cognitive traits, independent of whether or not accompanied by Aβ expression.

While the overall trends showed consistently poorer performance in AD flies, the two-way ANOVA findings (Tables 1–3) revealed a more detailed picture of how anxiety and depression contributed to these outcomes. In both the Open-field test and Forced-swim test (Tables 1–2), genotype and anxiety each had significant main effects, meaning that AD pathology and elevated anxiety independently reduced exploration and persistence. However, neither test showed a significant Genotype × Anxiety interaction. This indicated that anxiety influenced these behaviors similarly in both wild-type and AD flies. High-anxiety flies explored less and struggled for lesser time in the water regardless of genotype, implying that these motivational behaviors were broadly sensitive to anxiety but not differentially modulated by AD pathology.

The Y-maze (gustatory preference assay) results, however, revealed a different dynamic. The significant Genotype × Anxiety/Depression interaction in Table 3 shows that anxiety/Depression affected Decision Latency and/or Stress Altered Reward Valuation (DL/SARV) differently depending on genotype. Wild-type flies showed a modest decrease in DL/SARV when classified as high-anxiety, however AD flies experienced a significantly greater decline. This means that anxiety has a disproportionately larger negative impact on DL/SARV when the nervous system is already compromised by Aβ expression. In practical terms, anxiety acts as a magnifier of this aspect of cognitive function in AD flies, supporting the

hypothesis that affective dysregulation and AD pathology can interact synergistically. This aligns with clinical findings where anxiety may worsen or speed up cognitive decline in Alzheimer's patients.

One explanation for these results is that affective and cognitive processes share common neural circuits. In Drosophila, the mushroom bodies and central complex are crucial for learning, motivation, and goal-directed behavior. Aβ-related disruptions in these areas could therefore impair both emotional regulation and cognitive decline; particularly decision latency. Another possible explanation is that the behavioral changes reflect broader motor or sensory impairments rather than emotion-specific effects. Although locomotor normalization and randomized maze trials help control for such variables, they cannot fully eliminate them.

This research adds to the growing evidence that emotional and cognitive systems are tightly interconnected, even in simple organisms. The Drosophila model offers unique advantages—its genetic tractability and behavioral consistency make it a powerful platform for exploring how mood and cognition interact at the molecular level. While fruit flies cannot capture the subjective aspects of human emotion, they can reveal conserved biological pathways linking mood regulation, motivation, and memory.

In summary, the results suggest that anxiety- and depression-like states may intensify some – though not all - behavioral and cognitive deficits associated with Alzheimer's disease. Though preliminary, these findings highlight the importance of studying emotional and cognitive interactions together when investigating neurodegenerative processes and developing potential interventions.

5. Limitations

The possibility that behavioral changes reflect broader motor or sensory impairments rather than emotion-specific effects cannot be discounted. These findings are consistent with previous studies showing that Aβ expression can decrease locomotion and increase thigmotaxis, behaviors often interpreted as anxiety-like. However, it is important to interpret these behaviors cautiously. The Open-field and Forced-swim tests serve as valuable indicators of motivation and stress responsiveness but are not direct equivalents of human emotional states. The connection between affective and cognitive outcomes observed here may reflect more general disruptions in arousal or metabolic state rather than "emotion" in the human sense.

The Y-maze test in this study did not measure spontaneous alternation, working memory or cognitive flexibility. Instead, it resembled more a gustatory preference assay; consequently measuring decision latency under reward conflict and/or stress-altered reward valuation. The sample size, while sufficient to detect large effects, limited the precision of subgroup comparisons. Sex differences, which are known to influence both behavior and neuropathology, were not analyzed separately. The study also assessed flies at a single time point, preventing conclusions about how these traits evolve as the disease progresses. Future studies incorporating longitudinal tracking, neurochemical analyses, and circuit-level imaging could clarify whether

emotional changes precede or result from cognitive impairment.

As mentioned, reduced activity in AD flies could reflect general motor deficits or olfactory impairments rather than affective changes. However, baseline locomotion normalization and randomized sucrose-arm positions mitigated this possibility. Similarly, variations in sex, age, or satiety were controlled experimentally.

OFT and FST interpretations as anxiety and depression proxies remain debated in Drosophila. Thigmotaxis has been validated as an index of anxiety-related exploration, but it also correlates with general activity. Forced-swim immobility, traditionally linked to learned helplessness, may also reflect fatigue. Thus, results should be interpreted as motivational correlates rather than direct analogs of human psychiatric states.

Environmental factors such as lighting intensity and starvation level substantially affect behavioral sensitivity. Although these variables were standardized, future replication across cohorts under varied conditions would enhance robustness. Inclusion of sex-specific analyses and longitudinal tracking could further elucidate causal dynamics.

6. Conclusion

This study provides quantitative evidence that comorbid anxiety and depression amplify some AD-related cognitive impairments in *Drosophila melanogaster*. Flies expressing Aβ and Flies exhibiting high-anxiety/depression phenotypes disproportionately negatively affected Decision Latency (DL) and Stress Altered Reward Valuation (SARV) in AD flies when compared with wild-type flies, consistent with human findings linking affective symptoms to accelerated cognitive decline.

Despite limitations, these findings underscore the behavioral relevance of anxiety and depression-like states in AD pathogenesis. Integrating psychiatric and neurodegenerative models in Drosophila provides a scalable framework for dissecting the molecular underpinnings of affective-cognitive interaction and for preclinical screening of neuropsychiatric therapeutics.

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