# AI-Driven Analysis of Biomarkers Using Machine Learning for Alzheimer's Research in *Drosophila melanogaster*

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***Abstract*—*Drosophila melanogaster*, with its genetic similarity to humans, is a strong model organism for studying Alzheimer's disease. However, traditional research methods for exploring treatments and behavioral indicators of Alzheimer's in *Drosophila* have mainly relied on manual techniques. Leveraging Artificial Intelligence (AI) technology provides significant advantages in reliably analyzing biological behavior and processes. This paper develops a novel AI-driven analysis method using automatic video monitoring to investigate behavioral biomarkers associated with Alzheimer's in *Drosophila* and to implement machine learning models for predicting the presence of Alzheimer's. Three experimental groups of *Drosophila*—healthy, Alzheimer's, and Alzheimer's treated with Aloe vera—were used for AI analysis. *Drosophila* movement and behavior were tracked to analyze four biomarkers—negative geotaxis, velocity, directional heading changes, and grooming speed. Six behavioral parameters as features for machine learning were identified and ranked by importance: speed, y-distance, x-distance, direction, acceleration, and turning angle. Three machine learning models were developed to predict whether a *Drosophila* has Alzheimer's: two classical models (CatBoost and XGBoost) and a deep learning model (CNN-GRU). The three models achieved accuracies of 0.7582, 0.7576, and 0.7571 respectively. In addition, the AI-driven method was used to evaluate the effect of Aloe vera as a natural treatment for Alzheimer's model of *Drosophila*. Initial results indicated that aloe as a natural treatment improved mobility and stability in the Alzheimer's model of *Drosophila*. The proposed AI-driven system for uncovering behavioral biomarkers in *Drosophila* with Alzheimer's offers a reliable and more effective alternative to traditional manual methods. Evaluating the potential of aloe to be a therapeutic agent opens new doors to discovering new treatments for neurodegenerative disorders.**

***Keywords—Machine learning, Alzheimer's, Biomarkers, Drosophila melanogaster, AI, Natural Antioxidant, Aloe vera***

## I. INTRODUCTION

Alzheimer's Disease (AD) is the leading cause of dementia, a widespread neurodegenerative disorder that affects millions of people worldwide [1], [2]. The main symptoms of AD include progressive memory loss, cognitive impairment, and many other behavioral and psychological declines. Pathologically, AD is characterized by the presence of Aβ plaques, tau protein tangles, neuronal death, and synapse loss [3]. Despite decades of extensive research on AD, no cure has been found, and there is a pressing need for further investigation into potential effective treatments for alleviating symptoms of AD.

*Drosophila melanogaster* has become a popular biological model for studying neurodegenerative diseases, particularly AD, due to its 77% genetic similarity to human disease genes, cost-effectiveness, and short life cycle [3], [4]. Over expression of Aβ42 and Tau proteins in *Drosophila* results in neuronal degeneration, mirroring AD phenotypes such as hindered locomotion and blindness [1]. One of the most widely used AD models in *Drosophila* is the Aβ42 model, which mimics the symptoms of the disease. This model is achieved through the UAS-GAL4 system, a method that enables scientists to control gene expression. The system uses a GAL4 driver line which controls where the gene is expressed and a UAS responder line which carries the gene of interest. By crossing these two lines, the target gene can be expressed [5]. This can lead to the formation of Aβ aggregates, causing neuronal impairment that mirrors that of AD.

Identifying biomarkers for AD and detecting the disease in its early stages can help to advance research in disease progression. Utilizing *Drosophila* as a biological model to analyze behavioral patterns offers a deeper understanding of AD. Additionally, exploring natural compounds that could mitigate the harmful effects of AD is important. Natural compounds are generally more bioavailable and safer than synthetic drugs, making them a promising treatment option. Among natural treatments, Aloe vera has been shown to have powerful antioxidant properties, exhibiting anti-diabetic effects, reducing intestinal inflammation, and alleviating behavioral deficits and oxidative stress [6], [7], [8]. While previous studies have analyzed *Drosophila* behavior in AD and investigated potential treatments, machine learning provides a more precise and robust algorithmic approach [9], [10], [11]. By reducing human error, it allows for a more reliable analysis of *Drosophila* behavior and evaluation of treatment effectiveness.

## II. RELATED WORK

Scientists have previously used *Drosophila melanogaster* as model organisms to study and gain a deeper understanding of human genetics and behavior. Some biomarkers that have been discovered for neurodegeneration in *Drosophila* are motor dysfunction, increased erratic movement, excessive grooming, reduction in walking activity, and GFP fluorescence in fly eyes [12], [13], [14]. These studies highlighted the need for machine learning techniques to analyze erratic movement before death and further study of biomarkers for diseases like Alzheimer's.

In addition to identifying biomarkers for AD in *Drosophila*, finding natural compounds that may be effective treatments for AD is crucial. Bongiorni et al. conducted a study to evaluate the effects of Lisosan G on Aβ-42 accumulation in a *Drosophila* model of AD and discovered that it reduced Aβ production and could potentially act as a therapeutic agent for AD [9]. Ganesh also explored the effects of natural products in preventing AD, finding that early treatment with Epigallocatechin gallate and Ginkgo biloba delayed AD onset [10]. These natural extracts improved eye morphology and prevented the production of pro-inflammatory cytokines. Siddique et al. investigated the effects of apigenin, a natural plant compound, on AD in

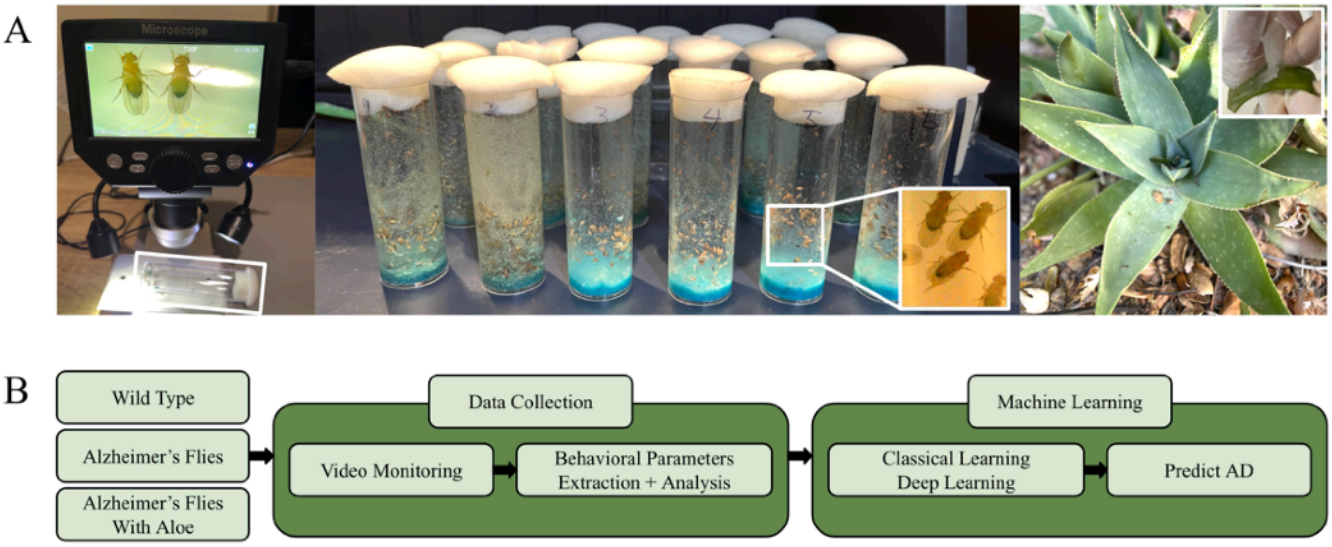


Figure 1: (A) The experimental setting is shown with a vial with *Drosophila* under the microscope to observe grooming close up, multiple vials cultivating *Drosophila*, and Aloe vera used as a natural treatment. (B) AI System Process Flow: Wild type flies, AD flies, and AD flies treated with aloe were analyzed utilizing OpenCV and Optical flow as tools to determine and analyze behavioral parameters in each experimental group, which were then used as inputs to machine learning models to predict whether flies have AD.

*Drosophila*, and the results showed a decrease in oxidative stress and delayed loss of climbing ability [11].

Some previous studies have utilized AI techniques to study *Drosophila* for more efficient and accurate analysis. Qiao et al. analyzed grooming behavior in *Drosophila*, developing a high-throughput platform and using a KNN model to automatically identify grooming events across different fly genotypes [15]. Further, Keleş et al. developed FlyVISTA, a machine-learning platform that analyzes microbehaviors in *Drosophila* during sleep [16]. Diez-Hermano et al. used a fully automated machine-learning pipeline to classify eye degeneration in *Drosophila* with bright field images of *Drosophila* compound eye structure [17].

As summarized above, AI has been used to study grooming, sleep, and eye degeneration in *Drosophila*. However, to the best of the author's knowledge, there are no reports of AI being used to identify behavioral biomarkers in *Drosophila* with AD. Furthermore, only a limited number of natural compounds have been studied for their potential positive effects on AD symptoms in *Drosophila*. This paper addresses these gaps by focusing on three main areas: uncovering new behavioral biomarkers associated with AD in the *Drosophila* model through AI analysis, developing machine learning models for the prediction and detection of AD, and investigating the effects of Aloe vera on AD symptoms in *Drosophila*, validated through AI-driven methods. This paper has wider implications for furthering the study of AD, enabling early diagnosis, and finding new treatments.

## III. Methodology

### A. *Materials*

200 wild type *Drosophila* were purchased from Carolina Biological for the control group flies. Elav-GAL4 line flies (P{w[+mC]=GAL4-elav.L}CG16779[3]) and Aβ42 "Arctic" mutation UAS flies (P{w[+mC]=UAS-APP.Abeta42.E693G.VTR}8) were purchased from Bloomington Drosophila Stock Center for crossing. The flies were maintained in culture vials with foam plugs from Carolina Biological to prevent flies from escaping while maintaining ventilation. They were fed with 5 mL of Instant Drosophila Medium from Carolina Biological, equal amount of water, and a pinch of yeast, all mixed together. Other materials include petri dishes, a microscope, Aloe vera, Alka Seltzer tablets, and ice.

### B. *Experimental Details*

The vials of *Drosophila* were maintained at a controlled room temperature and were transferred to new vials with fresh food every 7 days. Each vial contained Instant Drosophila Medium and the *Drosophila* were transferred to empty vials or petri dishes for video data collection. To knock down the flies and transfer them, two methods were employed: using $CO_2$ gas and using ice. For the first method, one vial with water and one vial with the target *Drosophila* were connected using a tube, and half of an Alka Seltzer tablet was dropped in water so the $CO_2$ gas would travel to the vial with *Drosophila* and knock them out. For the second method, a vial of flies was placed in a cup of ice for 5 minutes, with the ice surrounding all sides, and the flies would be temporarily immobilized.

To create the Aβ *Drosophila*, male flies were selected from the Elav-GAL4 line group and female flies were selected from the Aβ42 "Arctic" mutation UAS flies. Individual pairs of male and female selected flies were placed in petri dishes, and mating occurred naturally. The GAL4 from the male fly activated the UAS-linked gene in the offspring as shown in Figure 2. The offspring became an adult Aβ expressing fly in around two weeks.

Videos were recorded in two different settings: the climbing assay with the flies in vials, and close-up individual flies in Petri dishes to measure grooming. For the climbing assay, the vials were tapped a few times on the table to displace the flies to the bottom of the vial and trigger their negative geotaxis response so their climbing behavior could be analyzed. There were around 10 flies in each vial for the climbing assay and one individual fly was recorded in a petri dish for grooming analysis.

40-second video clips were recorded of numerous *Drosophila* without AD, with AD, and with AD treated with aloe under the microscope to analyze their behavior. Four different behavioral biomarkers were measured as shown in Table 2: negative geotaxis, velocity, directional heading

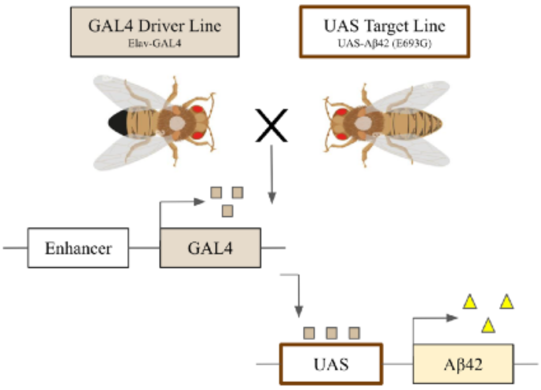


Figure 2: UAS-GAL4 system cross: male Elav-GAL4 line with female UAS-Aβ42 line.

Table 1: Behavioral Biomarkers

| Biomarker | Description |
|---|---|
| Negative geotaxis | A fly's natural response to climb up against gravity; measured using climbing assay |
| Velocity | Average traveling speed per frame |
| Directional Heading Changes | Measure of how a fly's direction of movement changes over time |
| Grooming Speed | Measure of the speed at which a fly grooms/scratches its wings and head, using rear or front legs |

changes, and grooming speed. An automated video-tracking system was developed to trace the locomotion and behavior (negative geotaxis response, velocity, and direction changes) of the flies in the climbing assay, and optical flow was used to track the grooming magnitude from the videos of individual *Drosophila* in petri dishes.

### C. Machine Learning Model Development

Two classical machine learning models, CatBoost and XGB, and a deep learning model, CNN-GRU, were implemented for binary classification to predict whether a *Drosophila* has AD as shown in Figure 3. For all models, six behavioral parameters were extracted from the video data as features for training: x-distance, y-distance, speed, acceleration, direction, and turning angle.

A CatBoost model excels in processing categorical data and combines multiple decision trees, making it a robust model for binary classification. It employs ordered boosting and symmetric trees, reducing overfitting. The CatBoost model developed has 500 boosting iterations, a tree depth of 8, and a learning rate of 0.1, balancing training speed as well as accuracy. The L2 leaf regularization coefficient was 5.0 with a subsample rate of 0.8 to reduce overfitting. The Logloss function was used, as it is often used for binary classification tasks.

An XGB is an effective model for this binary classification task because it can capture complex relationships, has built-in regularization, can ignore noise in data, and can use the logloss (binary logistic loss) function. XGBs are also efficient and can easily adapt to imbalanced data. The proposed model combines 100 decision trees with a maximum tree depth of 6. It has a learning rate of 0.1, is built for binary classification, and the evaluation metric is 'logloss.'

A CNN-GRU model is a powerful model for binary classification because it combines convolutional layers and recurrent units, allowing it to capture spatial patterns while also learning temporal dependencies. In this paper, the CNN-

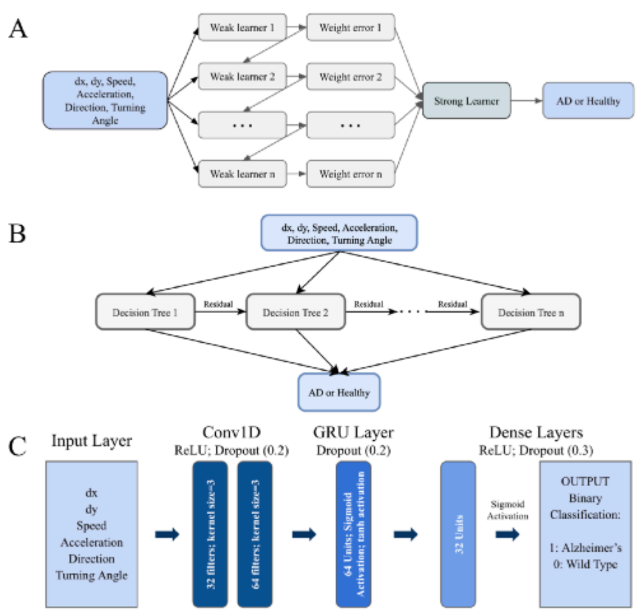


Figure 3: (A) CatBoost model architecture. (B) XGB model architecture. (C) Deep Learning CNN-GRU model architecture

GRU model consisted of CNN layers with ReLu activation, batch normalization, and dropout to prevent overfitting. It also had a GRU layer for temporal patterns and relationships and connected dense layers with ReLu activation and dropout. The final output layer included sigmoid activation for binary classification. Adam optimizer and binary cross entropy were used during training.

## IV. Results and Discussion

### A. Biomarker Analysis Results

The four biomarkers studied were negative geotaxis, speed, direction changes, and grooming speed. When the negative geotaxis response was triggered for the flies with AD, their upward climbing pattern was noticeably impaired as shown in Figure 4. The flies would climb more erratically and frequently fly from place to place as compared to the control group flies which would climb in straighter and more stable paths, implying how AD hinders flies' negative geotaxis response.

Figure 5 illustrates that the distribution of speeds for the control group is more steady and uniform than for the AD group, which varies more, showing the erratic behavior of flies with AD. The distribution of speeds for the AD flies treated with aloe overall has less of higher, erratic speeds, underscoring the positive effect it has on locomotion. However, the AD symptoms are still not fully reduced by the aloe, for the control group is still the most stable distribution. The speed is also displayed in Figure 6A and compared between the three experimental groups. The control group has a higher typical speed and the distribution has less variation. The AD fly group has the lowest median speed and the most variation in the distribution. The AD flies treated with aloe have a slightly higher median speed than the AD group and also less variation, indicating the positive effects aloe has on the flies with AD. The polynomial fit of speed in Figure 7 shows that the control group maintains the highest speed throughout, the AD group has the most significant decline, and the AD group treated with aloe shows an initial lower speed with a more gradual decline. This underscores the potential therapeutic effects of aloe.

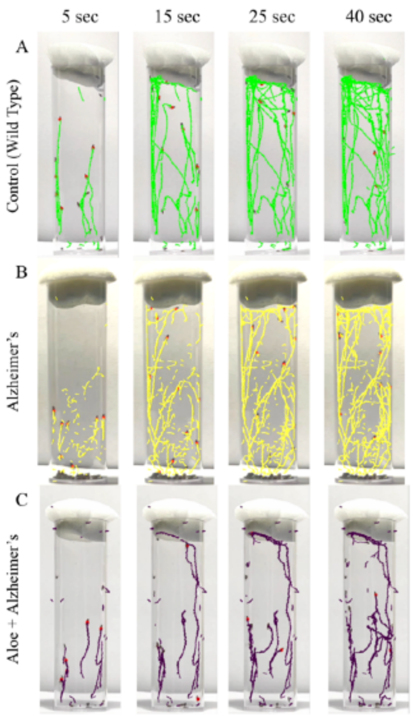


Figure 4: Images created by programs written using OpenCV automatically tracing the flies' movement over time in the climbing assay are shown. (A) Control group flies of wild type travel in more stable/straight lines. (B) Flies with AD travel more erratically. (C) AD flies treated with Aloe are less erratic and climb in straighter paths.

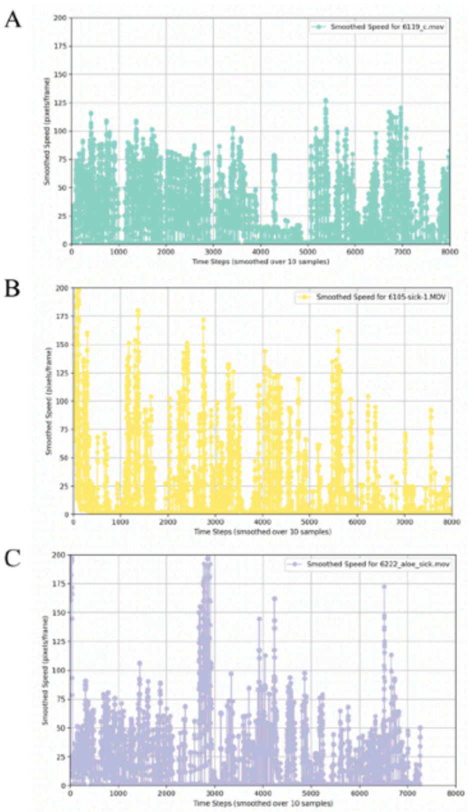


Figure 5: Line graphs with smoothed speed for each group of *Drosophila* tested using the climbing assay are displayed. (A) Control group: wild type flies. (B) Flies with AD. (C) Flies with AD treated with aloe.

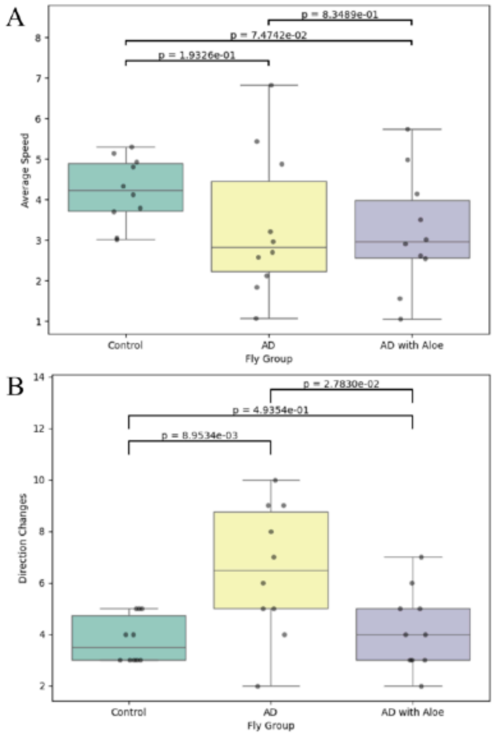


Figure 6: Climbing assay: Box plots of the 3 groups—control, AD flies, and AD flies treated with aloe—are shown. (A) Distribution of average speeds of each fly group. The speed differences between the groups are not statistically significant. (B) Distribution of average direction changes of each fly group. Direction changes for Control vs. AD group and AD vs. AD with Aloe group are statistically significant, and Control vs. AD with Aloe group is not statistically significant.

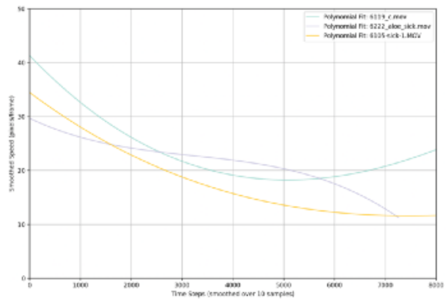

Figure 7: Polynomial fit of the smoothed speed distribution for control group flies, AD flies, and AD flies treated with aloe in the climbing assay.

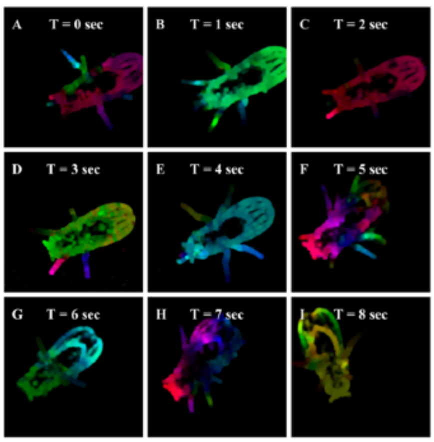


Figure 8: Optical flow visualization of *Drosophila* grooming movement patterns throughout different time frames. The hue indicates the direction of movement and the brightness indicates the magnitude of motion.

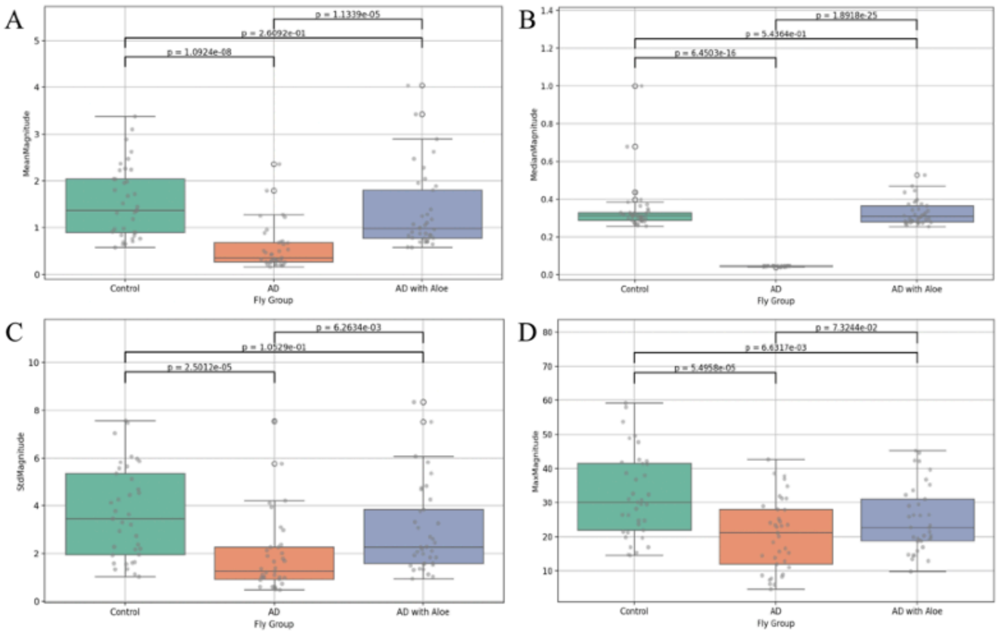


Figure 9: Box plots of the 3 groups of flies' grooming (A) Mean magnitude of fly grooming for control vs. AD group and AD vs. AD with Aloe group are statistically significant. Control vs. AD with Aloe group is not statistically significant. (B) Median magnitude of fly grooming for control vs. AD group and AD vs. AD with Aloe group are statistically significant. Control vs. AD with Aloe group is not statistically significant. (C) Standard deviation of the magnitude of fly grooming for control vs. AD group and AD vs. AD with Aloe group are statistically significant. Control vs. AD with Aloe group is not statistically significant. (D) Maximum magnitude of fly grooming for control vs. AD group and control vs. AD with Aloe group are statistically significant. AD vs. AD with Aloe group is not statistically significant.

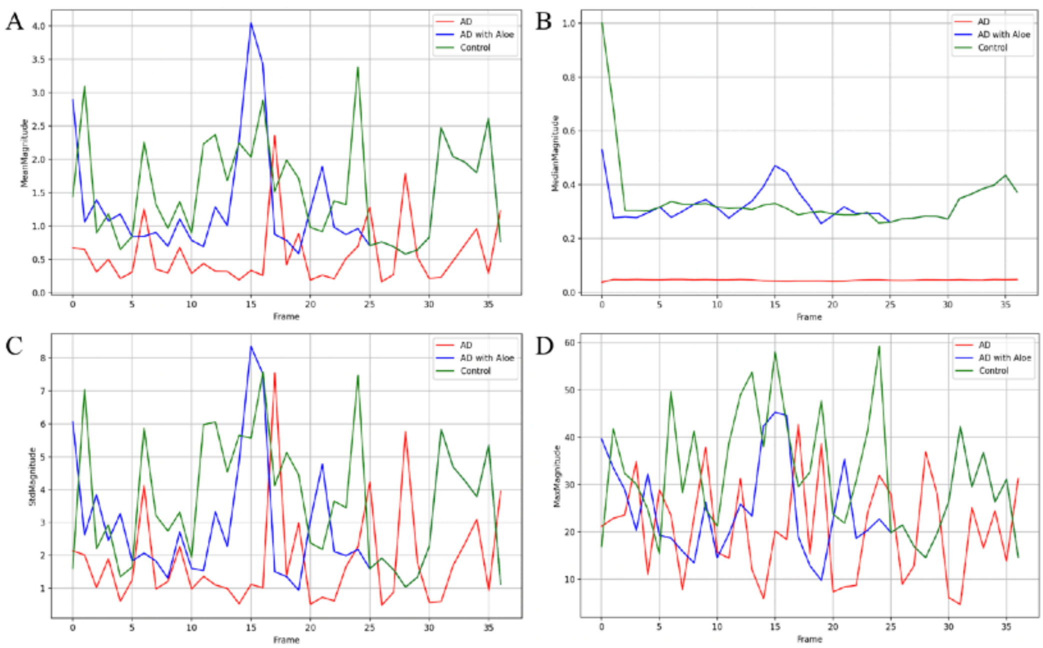


Figure 10: Line graphs comparing the grooming behavior for the three experimental groups are shown. (A) Mean magnitude of grooming. (B) Median magnitude of grooming. (C) Standard deviation of magnitude of grooming. (D) Maximum magnitude of grooming.

Direction change comparisons between the three experimental groups is shown in Figure 6A. The fly group with AD changes direction with higher frequency than the other two groups. There is also more variation in the distribution of direction changes for the AD group. The AD flies treated with aloe shows improvement compared to the AD group: the median number of direction changes decreased nearly as low as the control group, showing how aloe reduces erratic behavior. The direction changes for AD fly group compared with the AD flies treated with aloe has a p-value of 0.0278, meaning treating the flies with aloe has statistically significant.

The grooming data is visualized in Figure 8. The grooming magnitude of flies is with AD is overall lower than the control group, as displayed in Figure 9 and Figure 10. The group of AD flies treated with aloe has a higher grooming frequency than the AD group but lower than the control group. The AD flies compared with the AD flies treated with aloe for the mean, median, and standard deviation of grooming magnitude is statistically significant.

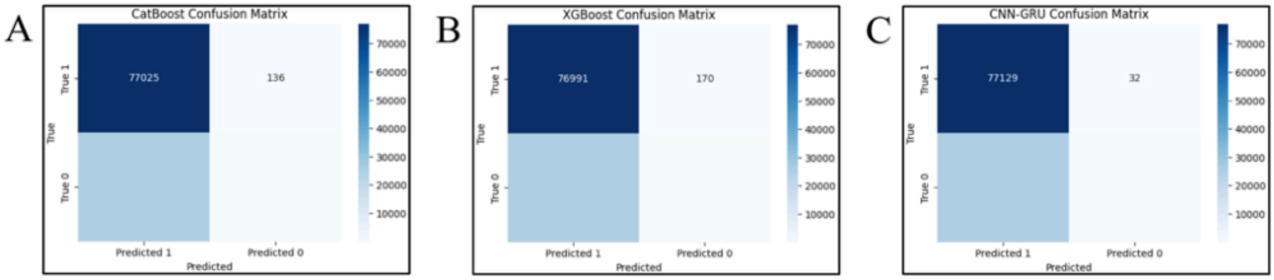


Figure 11: (A) CatBoost Model Confusion Matrix. (B) XGBoost Model Confusion Matrix. (C) CNN-GRU Model Confusion Matrix

| A | Precision | Recall | F1-score | Support |
|---|---|---|---|---|
| 0 | 0.76 | 1.00 | 0.86 | 77161 |
| 1 | 0.81 | 0.02 | 0.04 | 25194 |

| B | Precision | Recall | F1-score | Support |
|---|---|---|---|---|
| 0 | 0.76 | 1.00 | 0.86 | 77161 |
| 1 | 0.77 | 0.02 | 0.04 | 25194 |

| C | Precision | Recall | F1-score | Support |
|---|---|---|---|---|
| 0 | 0.76 | 1.00 | 0.86 | 77161 |
| 1 | 0.92 | 0.01 | 0.03 | 25194 |

Figure 12: (A) CatBoost Model Performance Metrics. (B) XGBoost Model Performance Metrics. (C) CNN-GRU Model Performance Metrics

### B. *Machine Learning Results*

The CatBoost model, XGBoost model, and CNN-GRU model achieved accuracies of 0.7582, 0.7576, and 0.7571 respectively, and other evaluation metrics are shown in Figure 11 and Figure 12. The models performed very similarly, but the classical models had the best accuracy out of the three. The CNN-GRU model had the highest precision for predicting AD (0.92), followed by CatBoost (0.81), then XGB (0.77). All models had very high recall values (1.00).

## IV. Conclusion

In this paper, a reliable AI-driven method for analyzing behavioral biomarkers in *Drosophila* was developed, providing a novel approach to studying AD. This AI method has potential to be applied to other neurodegenerative diseases and other model organisms in the future as an alternative to traditional methods. Compared to previous manual processes, the proposed AI system proved to be an effective way for identifying biomarkers, minimizing human error and improving reliability.

Using three machine learning models—CatBoost, XGB, and CNN-GRU—the system overall achieved around a 75% accuracy in predicting AD, demonstrating its capability for early detection and diagnosis. Additionally, this research validated the positive effects of Aloe vera on alleviating AD symptoms in Drosophila with AI, indicating its potential to be a natural therapeutic agent for neurodegeneration.

Future research will focus on detecting additional biomarkers, exploring more advanced machine learning models to improve prediction accuracy, and investigating other potential AD treatments. This research will contribute to the study of AD, helping with early detection using behavioral biomarkers and discovering effective treatments.

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