PlanariaScan: Development of a Video-Based Monitoring System on Planaria Learning and Memory Under Various Stressors

Yilin Liu
Amador Valley High School
Pleasanton, CA, USA
yilinliu79@gmail.com

Ella Li
Saratoga High School
Saratoga, CA, USA
ella.wx.li@gmail.com

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Abstract— **Planarians are flatworms that can regenerate any part of their own bodies. The fact that they exhibit many bodily systems similar to those of humans and that they have the ability to learn through experience made them a suitable model organism. This research aims to design an AI system called PlanariaScan that is able to automatically monitor planarian behavior. A chamber was designed by taking into account the fact that planarians shy away from light and the idea that if there was food (liver) under the light, an affinity for it may be developed. To design a smart chamber, it was made entirely black, blocking out almost all of the light in the environment. Then, UV light was put into the chamber via a UV flashlight, focusing on only a small radius that was directly where the food would later be put. By putting forty planarians into this environment and recording time stamps of when they come to engage with the food, it can be discovered how well they can learn that the light is actually favorable in these situations. Then, various groups of planarians were incubated in ethyl alcohol, caffeine, and carbon 60 with water as a control, and how well the planarians that were incubated in chemical substances retained memory compared to the control group could reveal how these substances affect learning. The timestamps were recorded and the results analyzed with a T-test to reveal how statistically significant the outcome was. This was the first study to use AI to monitor and analyze planarian behavior as well as C60 to positively influence their learning, memory, and regeneration.**

Keywords—Planarian, Familiarization Period, Test, Control, T-test p-value

I. INTRODUCTION

Planarians are freshwater flatworms that can regenerate any missing tissue or body part lost to injury or aging due to their bodies being composed of 20% stem cells, which have been used for more than a century to test planarian regeneration patterns, as well as subsequent learning and memory. These cells make up tissues in the body and can differentiate into the many different cell types. During planarian regeneration, it is important that the correct cell types are made in the correct proportions, in the correct place and time. If this process is hindered, regeneration will not go on properly. Before regeneration, planarians exhibit the ability to learn through experience and retain that memory after regeneration (Shomrat and Levin, 2013), providing valuable insight into human regenerative medicine, which is still limited. Planarians and humans share many of the same organ systems, as well as a similar central nervous system with a brain, eyes, intestine, and epidermis, and many of the same neurotransmitters. Using planaria as a biological model can help scientists understand how to coax human cells to behave similarly and to regenerate tissue. Genes and developmental processes in planarians are also present in humans, which could be used in future medical work, aiding in research in human medicine and regeneration.

II. RELATED WORKS

Planarian long term memory due to memory transfer by ingestion of previous planarians has been proven to exist (McConnell, 1967), showing that they are capable of learning and retaining by experience. Various factors such as heat shock were proven to have a negative effect on memory (Turel, 2020). However, a smart testing system that automatically monitors behavior such as the one this paper discusses hasn't been used before. By testing certain chemical substances on planaria using a smart training system, a more conclusive idea about how drugs affect learning and memory in humans can be obtained.

In 2006, Stern published a paper reviewing James V. McConnell's experiment, in which he fed the chopped-up bodies of trained worms to untrained worms, and the untrained worms managed to exhibit some trained ability, proving that planarians are capable of transferring learned behavior. In 2013, Shomrat and Levin published a research paper discussing some of their findings when they used an automated training paradigm to test planarian long-term memory. Planarians were chosen because of their remarkable ability of brain regeneration, learning, and memory. A group of 20-40 worms were placed in an ATA (automated training apparatus) which had a petri dish with a rough-textured floor. Control worms went through the same procedure, but they were placed in a regular petri dish. After a ten day familiarization period and a 24hr rest/feeding period, the worms were decapitated. After regeneration, the worms were placed back into the dim, rough petri dish along with some untrained control worms. The decapitated, trained worms ate the liver faster than the control worms, proving the concept of long-term memory. In 2020, Turel discovered that after using heat shock (a known stressor for planaria) while they were on a surface covered with sucrose (table sugar), memory was impaired. However, after extensive training, the heat shock may actually bring back the memory. This proved planarians' capability as the biological model for memory retention. In

2020, Zhang et al. published a paper stating that nanotechnology has become increasingly prevalent, and paired with polydopamine nanoparticles, the technology can help hinder malignant bone tumors. The nanoparticles can be loaded with various anti-cancer drugs for controlled release. In 2015, *Tufts Now* reported that artificial intelligence (AI) can create a model of planarian regeneration, meaning that it can predict the phenotypes of planarians after regeneration under the condition that the technology is provided with information about the previous planarian. These AI models can be used to determine what triggers certain elements to regenerate, which is helpful for research in human regeneration as well. However, all current experimental data is analyzed manually, which is not only time-consuming, but also inaccurate. Therefore, there is a need to develop an AI system to analyze planarian behavior under various stressors.

To address the issue mentioned above, this paper aims to develop the first AI smart system to automatically capture a video of planarian behavior, which is then analyzed using machine learning to generate a conclusive output. Machine learning is able to analyze aspects of planarian behavior such as speed, locomotion patterns, learning and memory time, bodily deterioration, and reaction to stressors. These factors combined together can help assess the result of stressors on the planaria by analyzing features like the organisms' health, the effect of drugs on their behavior, regeneration, learning, and memory, and how they respond to environmental stress. These factors all aid in proving the result of the stressor on planaria as model organisms.

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III. EXPERIMENT MATERRIALS AND METHOD

A. *PlanariaScan System Design*

By incubating planaria in various chemical drugs and building a system that can automatically record a video of their behavior for later analysis, a conclusion about how these chemicals affect planarian behavior can be obtained (Fig. 1). After using the chamber monitoring system to record a video of the planaria, machine learning is used to analyze the results based on observation of various aspects of planarian behavior, discussed previously. By analyzing those factors, a conclusive idea about how chemical drugs affect planarian health, learning, and memory can be obtained.

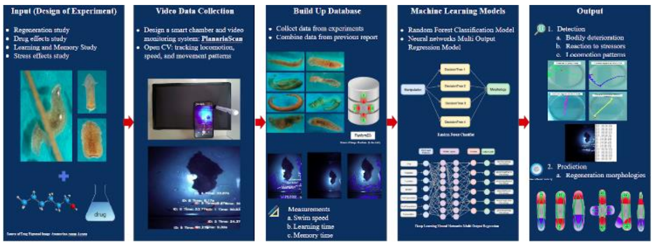

Fig. 1 - Process flow: using AI to study drug effects. (A) The planarian incubated in drugs before testing. (B) A smart chamber setting is used to produce a video recording of planarian behavior. (C) Integrated this data with data from past experiments through a database to generate a more conclusive output (D) Machine learning is utilized to predict resultant morphologies. (E) Through the use of machine learning, factors like swim speed, locomotion patterns, learning and memory time, bodily deterioration, and reaction to stressors can be analyzed to produce a conclusive result about the drugs' effect on planaria. This result can be obtained by observing health, as well as the effects on learning and memory.

B. *PlanariaScan System Set-up*

Since planaria are averse to the light, a dark chamber was built to accommodate for this feature during tests. The system is made from a piece of black cardboard cut and pieced together with black tape to form a chamber, which produced videos later analyzed with machine learning to monitor planarians' behavior. (Fig. 2). There are two openings on the top; one for a camera to show the inside of the chamber and one for a UV light to shine light into the chamber. The UV light is in the form of a stick with a light on one end 12 cm above the water in the tray; a plastic tube 1 cm in diameter wrapped with black tape is used to elongate the UV light stick so the light inside the chamber is concentrated on one point, where raw beef liver will later be placed. Because planaria dislike brightness, the light placed on the food will stimulate affinity for the light. Inside the chamber, a smooth, clear plastic tray is filled about halfway with water. The tray's size calibration will be discussed in detail later. The planarians being tested will be placed inside the plastic tray. The video that was produced using the smart chamber was analyzed with machine learning to draw paths representing the planarians' locomotion, and it tracked movement, as well as speed, time, and distance travelled.

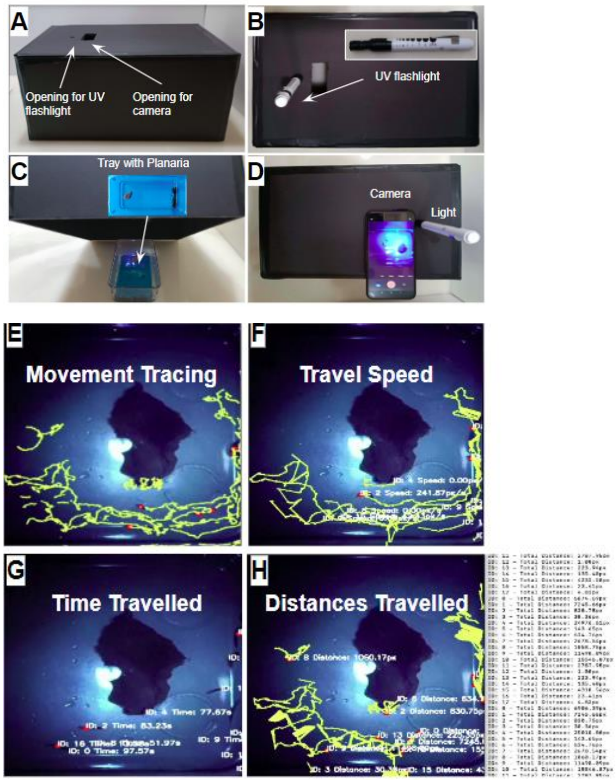

Fig. 2 - Experimental Box Setting for Planarian Memory and Learning. (A) A 30 cm x 20 cm x 15 cm black box made of stiff cardboard panels taped together using black tape. A small circular opening on the top panel is for the insertion of a UV flashlight, while a larger rectangle opening on the same panel is for a camera. (B) The insertion of the UV flashlight into the small circular opening. (C) Putting the box over an optimal sized plastic tray with planaria an raw beef liver in the tray. This is the experimental set up of the inside of the smart system. (D) The top panel of the box with both the UV flashlight and the camera inserted. (E) Movement tracing of the planaria. (F) Travel speed of the planaria. (G) Calculation of the time travelled. (H) Display of total distance travelled for each planaria for each tracked movement.

C. *Design of Experiment*

In order to determine the effect of various chemical drugs on planarian behavior, learning, and memory, planarians were incubated in the substances for 12 days before being moved inside the black chamber for testing. Since light is a known environmental stressor, a piece of raw beef liver was placed under the UV light, causing the planaria to learn to overcome the stressor in order to eat the liver. Without liver, the planaria escape the light, but with liver, they tolerate it in order to feed. However, after extensive training, the organisms actually learned to seek the light in search of food even if liver was not present (Fig. 3). The tests currently being discussed all had liver under the light. Each test was recorded on a camera as a video of about thirty minutes, and the timestamps of when each worm went into the light in search for food were recorded. Each test used 40 planarians, although the experiment was cut off at thirty minutes even if all 40 had not approached the food. The timestamps would later be tested using a t-test and p-value to determine if they were statistically significant, which will be discussed in more detail later. Machine learning for a deeper learning model was utilized to come to a conclusion about planarian learning and behavior under the effect of positive and negative environmental stressors by using AI to automatically draw their path under the influence of such stressors.

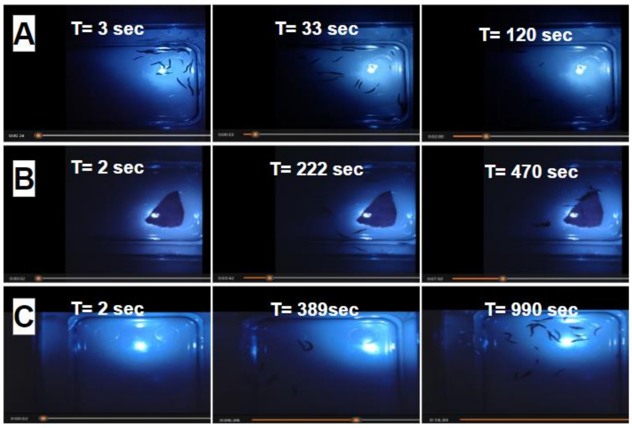

Fig. 3 - Planarians' Relationship with Light. (A) Without food, the planaria dislike the light and they escape to the darkness. (B) When there is beef liver, the planaria go into the light to engage with the food, even if they don't like light. (C) After training, even without beef liver, the planaria learn to seek the light.

D. Planaria Care And Drug Selection

The use of planaria calls for special care, which includes changing water and feeding. Purchased through Carolina Biological Supply Company, the planaria have fresh spring water that is changed every two to three days, and they are usually fed about one every week for one hour. This research uses black planaria. Tests with the chamber (30 cm x 20 cm x 15 cm) are always run either on feeding day or a few days after to ensure that they will be hungry enough to look for the food. In terms of chemical drug selection, three types were used for this research: carbon 60, purchased at FullerLifeC60, ethyl alcohol, purchased through Volu-Sol, and caffeine, purchased through Ingredient Depot. Carbon 60 was chosen as a factor that caused positive effects on the planaria, and ethyl alcohol and caffeine both were chosen as negative factors. The presence of both positive and negative factors is to gain a more conclusive idea of the influences on planarian behavior through an AI system. All chemical drugs were of laboratory grade.

IV. Results and Discussion

A. Distance's Impact on Planarian Feeding Patterns

It was found that planaria have an optimal distance through which they travel in a reasonable time frame to reach the food, found by extending the pharynx, their feeding organ. The size of the plastic tray inside the dark chamber was calibrated as follows. Calibration started with four differently-sized plastic trays: 14 cm x 21 cm, 7 cm x 21 cm, 7 cm x 14 cm, and 7 cm x 7 cm (Fig. 4). A video was set up to capture the data of planarian locomotion, which was analyzed using a deep learning model. By putting the container into the chamber and a small amount of raw beef liver under the light, it was found that in the largest container (14 cm x 21 cm design), none of the forty planaria came to eat the food within thirty minutes. The 7 cm x 22 cm chamber also had the same result. The 7 cm x 14 cm container had forty planaria that ate within thirty minutes. The smallest chamber (7 cm x 7 cm), had forty planarians as well; however, upon closer observation, all forty went to the food within the first eight minutes. That time period is too short to run an adequate experiment, so the optimal container size was deemed to be the 7 cm x 14 cm one. All following experiments were done using this 7 cm x 14 cm container.

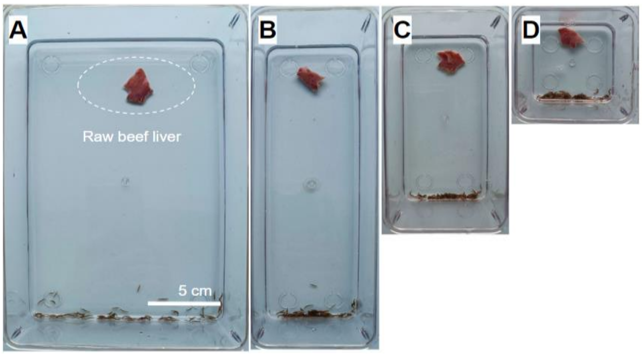

Fig. 4 - Tray size calibration. (A) A 21 cm x 14 cm clear plastic tray with planaria and raw beef liver inside that is too long for use in experiments. (B) A 21 cm x 7 cm tray that is narrower, but still too long. (C) A 14 cm x 7 cm tray that allows planaria to engage with the liver during experiments in a reasonable time frame. This was chosen to be the tray that is used. (D) A 7 cm x 7 cm tray that is too short, rendering experiments' time too short for them to work effectively.

B. Optimal Chemical Drug Dosage Selection

One challenge in this research is the calibration of doses because too high of a dose results in an early death, whereas too low of a dose has no effect, based on observations within a window of twelve days. Through a series of approximately thirty incubations, the optimal dose has been discovered (Fig. 5).

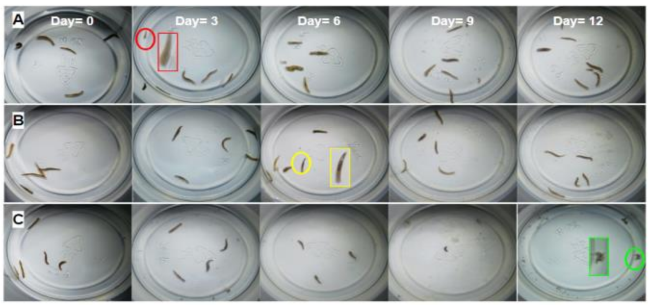

Fig. 5 - Dose calibration for C60. (A) A petri dish of planarians incubated in 0.006% C60 over the course of 12 days. The planarian circled in red is a new baby, and the planarian circled in yellow is an adult. Their sizes are evidently different. (B) A petri dish of planarians over the course of 12 days incubated in 0.036% C60. (C) A petri dish of planarians incubated in 0.054% C60 over the course of twelve days. The planarian circled in green is dead, and the body is dissipating

The four chemical substances that were tested were caffeine, ethyl alcohol (EtOH), carbon 60 (C60), and MitoQ (which has been eliminated; more information will be presented later). Multiple doses for each substance were tested by incubating planarians in each concentration and seeing how they were affected, including observing the number of offspring that were produced (Table 1). All experiments supported the conclusion that MitoQ was too strong for the model organisms (even a 0.0001% concentration killed all the planarians); however, upon lowering the dosage, there was virtually no effect. The other three substances each had two concentrations that were deemed optimal, one high dosage and one low dosage. For caffeine, the high dose was found to be 0.001M, and the low dose was 0.0001M. For EtOH, the high dose was 0.5%, and the low dose was 0.01%. Finally, for C60, the high dose was 0.036%, and the low dose was 0.006% of water. All three substances have a different concentration as their high and low dosages.

Table 1 - Dose Calibration using Three Substances. The doses were calibrated using a 12-day observation period. If many planarians died, the dosage was determined to be too high. The optimal dosage was selected as the highest dosage that does not exhibit negative effects too quickly.

Drug Dosage		Day 3	Day 6	Day 9	Day 12
Control	Water	• New offspring was born without a head • The new offspring doesn't move	• The new offspring can swim and has an unclear head • All planaria swam normally	• The new offspring can swim fast and has a bigger head than most drugs' substance	• New offspring is half the size of an adult and its eyes can be seen clearly; head seems to have developed
C60	0.006%	• A new offspring was born with no head. • The new offspring doesn't move • Others swam regularly	• The new offspring grows bigger, but they still don't have the full head yet; eyes are unclear • Others swam normally	• Another offspring has been born • All planaria in this sample groups swam faster than the control group	• The new offspring's two eyes can be seen clearly; head seems to have finished developing • All moved pretty faster
	0.036%	• All planaria in this sample group swam like the control group does	• All planaria in this sample group swam like the control group does	• Planaria in group swam as normal • One planaria lost a tail	• Some planaria twist as they moved • They move very slowly
	0.054%	• One dissipated • Four were alive but moved slowly	• Two dissipated • Three were alive but two of them lost tails	• Only one planaria was still alive, but it had curled its body into a crescent	• All planaria present have died and their bodies have dissipated
EtOH	0.01%	• A new offspring was born with no head • The new offspring doesn't move • The rest of the planaria move a bit slower than the control	• The new offspring can swim and has an unclear head; however, swims a bit slower than normal and the eyes are still a little unclear	• The new offspring can swim and has a head, but its two eyes can't be seen clearly; the head does not seem to be completely done developing either	• Some planaria have moved into a crescent shape and moved slowly • The new offspring can swim and has a head but two eyes can't be seen y
	0.5%	• All planaria in this sample group swam like the control group does	• All planaria in this sample group swam like the control group does	• Planaria in this group swam slower than the planaria in the control group swim	• Some planaria swam in circles and curl around • They swim very slowly
	1%	• One dissipated • Four are alive but move slowly and sometime stop to move	• All planaria present have died and their bodies have dissipated	• All planaria present have died and their bodies have dissipated	• All planaria present have died and their bodies have dissipated
Caffeine	0.0001M	• All planaria in this sample group swam like the control group does	• A new offspring was born without a head • The new offspring didn't move	• The new offspring can swim, but it as a very unclear head; eyes also seem unclear	• The new offspring grows with a clear head shape but two eyes can't be seen clearly
	0.001M	•All planaria in this sample group swam like the control group does	• Some planaria moved into a crescent shape and moved slowly	• Some planaria swam in circles and curl around • They moved slowly	• Planaria moved very slowly; some do not even move, and they stay still
	0.01M	• All planaria present have died and bodies dissipated	• All planaria present have died and bodies dissipated	• All planaria present have died and bodies dissipated	• All planaria present have died and bodies dissipated

C. Planaria Locomotion Test

In order to calibrate the planaria's health condition, the AI system was utilized to perform a motion test using a video recording of an incubated planarian's locomotion throughout a time period of twenty seconds and AI to automatically trace their path. (Fig. 6). Machine learning later analyzed the result, and analysis provided the average velocities of the planaria incubated in different substances (Fig. 7). The average velocity of the control planaria was approximately 1.8 mm per second. Planaria incubated in 0.006% C60 had an average velocity of about 2.3 mm per second, while those in 0.036% C60 had an average of approximately 1.5 mm per second. Planaria in 0.01% EtOH had an average velocity of 0.9 mm per second, planaria incubated in 0.5% EtOH had an average of 0.7 mm per second. Finally, both planaria incubated in 0.0001M caffeine and 0.001M caffeine had an average velocity of approximately 0.7 mm per second. The results show that C60's low dose promoted the fastest movement, while both dosages for both caffeine and EtOH had relatively low locomotion. However, it is important to note that although C60's low dose promoted movement, there was little change in displacement, as the planarian mostly moved in circles. It is also important to recognize that how much each worm moved directly corresponds with their bodily health (Fig. 8), showing that greater locomotion corresponds with greater health.

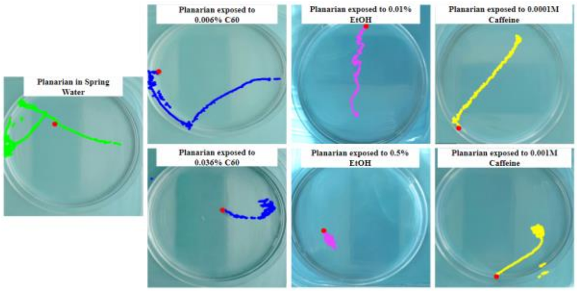

Fig 6 - Python program using OpenCV automatically traces planarian locomotion, showing the movement patterns of planaria under different drugs such as C60, caffeine, and EtOH. The velocity of the planarian is detected through video analysis by a deep learning model. The concentration of 0.006% C60 has the highest average speed.

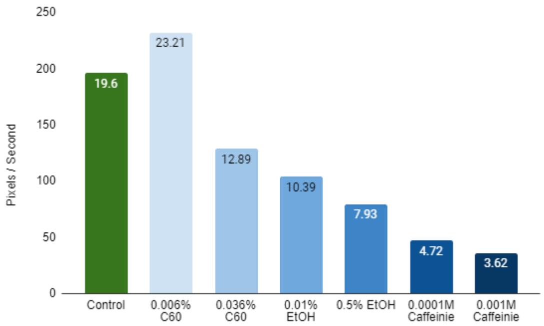

Fig. 7 - Histogram of the Speeds of Planarian Under Different Conditions. The planaria in the control group and both doses of C60 moved relatively fast. Both doses for both EtOH and caffeine had planaria that moved slower than the control group.

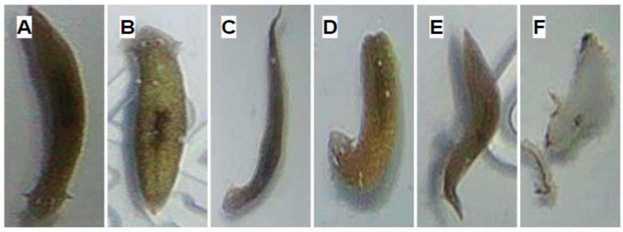

Fig. 8 - Body Shape of Planaria Under Different Drugs After 9 Days. (A) Control Group. (B) 0.006% C60. (C) 0.054% C60. (D) 0.0001M Caffeine. (E) 0.001% EtOH (F) 3mg/960,000mg MitoQ (this worm is dead, which is part of the reason why this chemical substance as been eliminated as drug used in this research.

D. Planarian Learning and Memory Test

A learning and memory experiment was done in the chamber, defined by the amount of time it takes the planaria to find the liver. After the timestamps were recorded for the planaria in each of the concentrations of caffeine, EtOH, and C60, each of the times in each list of timestamps for each concentration was converted into their decimal equivalent. The values were entered into a t-test calculator. Each list was compared to a list of the values for worms incubated in water, which acts as a control for the testing of this system. A P-value under 0.05 is considered to be statistically significant. Between water and 0.006% C60, the P-value is 0.0001 (extremely statistically significant). Between water and 0.036% C60, the P-value is 0.0001 (extremely statistically significant). Between water and 0.01% EtOH, the P-value is 0.0813 (not quite statistically significant). Between water and 0.5% EtOH, the P-value is 0.0011(very statistically significant). Between water and 0.0001M caffeine, the P-value is 0.0001 (extremely statistically significant). Between water and 0.001M caffeine, the P-value is 0.0001 (extremely statistically significant) We have also done the same calculations comparing each drug's high dose to its low dose. (Fig. 9). A similar test is done for the regeneration of the head and trunk (Fig 10 and Fig 11). The fact that the p-values were statistically significant means that the smart system produced results that showed that the different chemical substances had extreme effects on the learning ability of the worms. Hence, it was found that caffeine and ethyl alcohol both had significant negative effects on planarian learning and locomotion, while C60 had a significant positive effect.

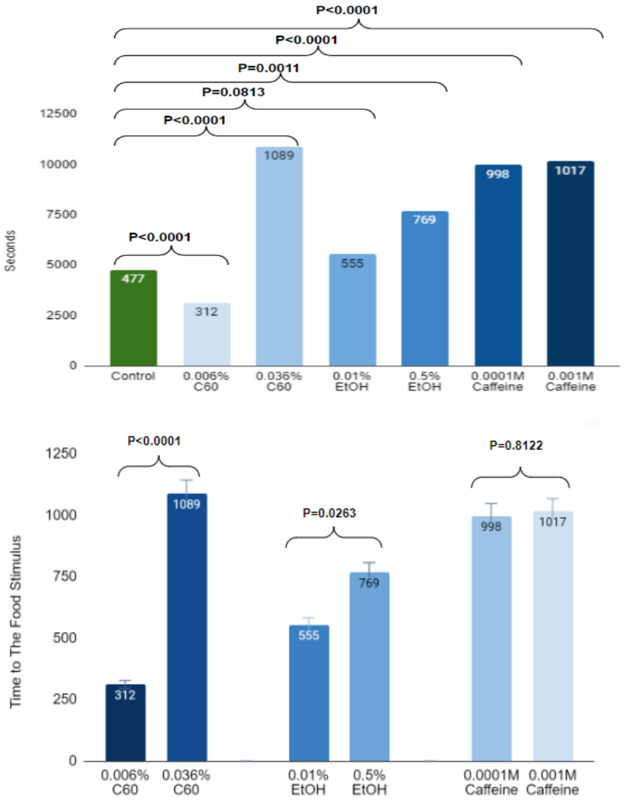

Fig. 9 - Control Group vs Drug Groups - w/o Cutting:
All drugs' data are statistically significant compared to the control group except for 0.01% EtOH. 0.006% C60 shows the best performance. C60: 0.006% compared to 0.036% concentration is statistically significant, showing how higher doses of positive substances can have negative effects.
EtOH: 0.01% compared to 0.5% concentration is statistically significant
Caffeine: 0.0001M and 0.001M are similar

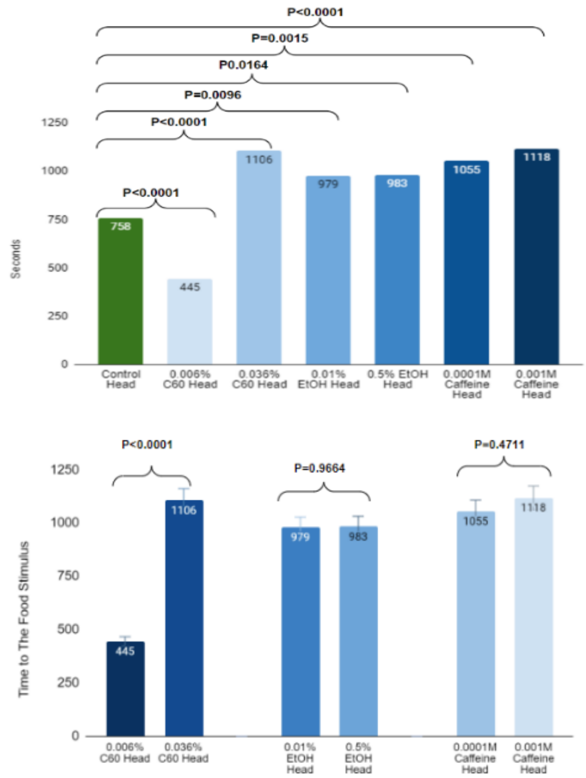

Fig. 10 - Control Groups' Head vs Drug Groups' Head - horizontally-cut planaria that have regenerated. All drugs' data are statistically significant compared to the control group. This shows that the head portion can retain memory even after regeneration. C60: 0.006% compared to 0.036% concentration is statistically significant, while both EtOH and Caffeine have similar performance between low dose and high dose.

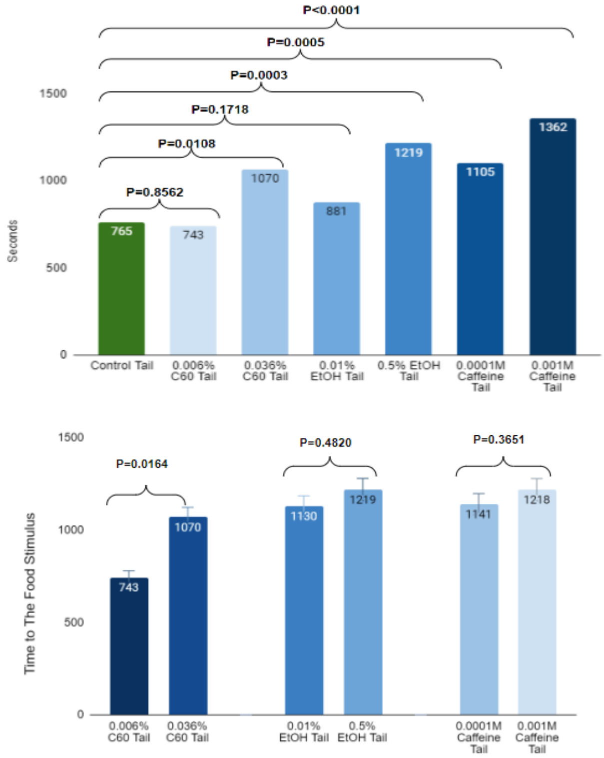

Fig. 11 - Control Groups' Tail vs Drug Groups' Tail - horizontally-cut planarian that has regenerated. All drugs' data are statistically significant compared to the control group except for 0.006% C60. The tail portion does not retain as memory as the head portion because they had a longer average time, proving that memory resides in the brain. C60: 0.006% compared to 0.036% concentration is statistically significant, while both EtOH and Caffeine have similar performance between low dose and high dose.

V. CONCLUSION

In this study, PlanariaScan, an AI system, was successfully designed and developed to aid in experiments involving planarian learning and memory. Three different chemical substances were used (C60, EtOH, and caffeine), with C60 being the only positive substance among the three. The planarians were incubated in each of the three substances in various doses before being tested by PlanariaScan on how quickly they went into the light to eat the beef liver. The timestamps were analyzed with a statistical test, and the results supported that the substances had significant impacts on the organisms' learning abilities. PlanariaScan was then also used to automatically draw their locomotion patterns, analyzing how different substances affected planarian behavior.

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