

The effect of synthetic & natural choline on phototaxis response in *Dugesia dorotocephala*

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Abstract

Planarians provide a unique model organism for studying the effects of cognitive enhancers like choline bitartrate. This study aims to investigate the efficacy of varying concentrations of choline bitartrate in improving conditioning and neuroplasticity in planarians. We hypothesize that higher concentrations of choline bitartrate will enhance planarian learning through faster conditioning and increased locomotion response to stimuli. However, results show that an increase in choline did not significantly enhance conditioning, supporting the null hypothesis.

1.0 Introduction

From exam cramming by students to the pursuit of a mental edge by professionals, nootropic supplements, often referred to as “smart drugs”, are marketed as enhancers of memory and learning. Among these, choline-based supplements have been popularized due to their involvement in the production of acetylcholine, a neurotransmitter regarded as critical for cognitive function (Gold, 2003; Haam & Yakel, 2017; Sam & Bordoni, 2023). Choline, which is naturally present in foods such as eggs and liver, is considered essential for brain health; however, the effectiveness and risks of synthetic choline supplements have remained underexplored, especially in relation to dosage (Lippelt et al., 2016; Derbyshire & Obeid, 2020; Institute of Medicine (US) Committee on Nutrition et al., 2011).

The influence of choline on learning should be better understood not only for cognitive enhancement but also for the assessment of potential risks linked to excessive or insufficient intake. Yet, studies on humans have been limited and complicated by ethical concerns, making model organisms necessary for controlled experimentation (Fogel, 2018). Due to their simple yet functionally relevant nervous systems and notable neuroplasticity, planarians have been identified as ideal models for the investigation of dose-dependent effects of choline on learning.

In this study, associative learning in planaria was examined by observing how synthetic (choline bitartrate) and natural sources of choline altered their natural light avoidance behavior. Varying concentrations were tested to determine whether learning ability is affected by different choline sources, thereby contributing to broader discussions about the efficacy and risks of cognitive enhancers.

1.1 Choline & Acetylcholine: Foundations in Cognitive Enhancement

Nootropics, or "smart drugs," are substances that enhance cognitive functions like memory, learning, and focus. They are categorized into classical nootropics, brain metabolism boosters, cholinergics, and botanical extracts. Originally developed to treat cognitive impairments, nootropics are now widely researched for their potential cognitive benefits in healthy individuals (Malik & Tlustoš, 2022).

Among these, cholinergics play a significant role in cognitive enhancement by supporting the production of acetylcholine (ACh), a neurotransmitter crucial for learning, memory, and overall brain function. Because ACh synthesis is directly dependent on choline availability, insufficient choline intake can result in decreased neurotransmitter production, leading to deficits in learning, memory retention, and overall cognitive function. Studies have shown that choline deficiency impairs synaptic communication, weakening neural plasticity and reducing the brain's ability to adapt and store information effectively (Huang et al., 2025).

1.2 Planarian Anatomy and Physiology

Planarians are a widely distributed species of aquatic free-living flatworms commonly used in stem cell research in laboratories for their regenerative abilities (Planarian|Anatomy & Facts, 2019). These flatworms are considered to be one of the most primitive organisms to possess a central nervous system (CNS), and their neurons closely resemble the neurons of vertebrates, including humans (Agata et al., 1998). Their CNS comprises cephalic ganglions towards the head region and a pair of ventral nerve cords (Agata et al., 1998). Neurons in the CNS produce acetylcholine, allowing it to influence and enhance the functionality of the CNS. They are also an example of an ancestral ACh system, meaning they have a much simpler system

to conduct research on and could be used as a basis to potentially understand choline and learning in higher-order animals, including humans. However, planarians lack endocrine, circulatory, and respiratory systems (Sarnat & Netsky, 1985). Because planarians lack a circulatory system like humans, planaria rely on diffusion to transport nutrients across cellular membranes and tissues to reach the nervous system. Other than having bilateral symmetry, these flatworms have exceptional sensory information intake ability, including two photoreceptors, or eyespots called ocelli, and chemosensory cells for detecting food (Sarnat & Netsky, 1985). These eyespots are important for survival as planarians are naturally photophobic or avoidant of light (Paskin et al., 2014). They also have a digestive system called the gastrovascular cavity, although it does not contain digestive organs like those of higher-order animals.

1.2.1 Digestion Timing in Planarians

The first signs of digestion in planarians occur 5 minutes after feeding, with enzyme activity peaking between 24-48 hours and declining by the third day (Rosenbaum & Rolon, 1960). Although the exact timing of nutrient absorption is not well-researched, it is inferred that nutrients become available to cells during peak enzyme activity. Choline, a water-soluble nutrient, is unlikely to bioaccumulate in planarians and is expected to diffuse out into the surrounding freshwater environment, similar to how excess choline is excreted in humans (Lippelt et al., 2016).

1.2.2 Planaria Phototaxis & Behavior

Planarians avoid light unless motivated by hunger, indicating that food stimuli can override their natural response to light (Shomrat & Levin, 2013). Their photoreceptor neurons are connected directly to the cephalic ganglia, indicating that photoreception is closely linked to their CNS (Sarnat & Netsky, 1985). Planarians are more sensitive to shorter wavelengths like UV light (Paskin et al., 2014). While they typically glide or scrunch during locomotion, abnormal behaviors can occur when exposed to drugs, resulting in changes in activity levels and body shape (Bayingana et al., 2022).

1.3 Planarian Conditioning

Conditioning can be categorized into classical (Pavlovian) conditioning and instrumental (operant) conditioning (Bouton, 2009). Classical conditioning involves associating a response with environmental stimuli, typically including neutral stimuli (which do not trigger a response), unconditioned stimuli (which cause involuntary behavior), and conditioned responses. Pavlov's experiment with dogs is a well-known example: a bell (neutral stimulus) was paired with food (unconditioned stimulus), causing the dogs to salivate. Over time, the dogs began salivating upon hearing the bell alone, making salivation a conditioned response. Forward conditioning, a specific type of classical conditioning, occurs when the conditioned stimulus is presented before the unconditioned stimulus, and it is especially effective because it aligns with how organisms naturally learn to anticipate events. Presenting the conditioned stimulus first allows the nervous system to establish a causal relationship between the stimuli.

Trace conditioning presents the CS first, then removes it before the US appears, and is particularly susceptible to the delay time between CS and US (longer gaps weaken the association).

Studies have shown that planarians exhibit conditioned responses to stimuli, such as light or electric shock, highlighting their ability to adapt based on classical conditioning (associative learning) (Deochand et al., 2018). These responses, viewed as regulatory mechanisms, suggest a form of learning in the planarian's relatively simple nervous system (Lenicque & Jean-Pierre Féral, 1976). Forward conditioning is particularly effective for planarians because it aligns with their nervous system's ability to process sequential stimuli efficiently (Vattano & Hullett, 1964). While forward conditioning will be the primary method in this study, trace conditioning will also be explored to assess whether choline bitartrate supplementation enhances learning in scenarios involving delayed associations.

1.4 Mechanism of the cholinergic system and learning in planarians

Studies have shown that planarians have a gene for planarian choline acetyltransferase (ChAT) and that cholinergic neurons are widely distributed in the planarian nervous system. The cholinergic system may influence planarians' locomotion, feeding behavior, and learning

processes (Pagán et al., 2020). Specifically, acetylcholine modulates locomotion by regulating the contraction and relaxation of muscles, controlling motor functions involved in swimming and crawling.

1.6 Literature Review

1.6.1 The Role of Acetylcholine in Learning and Memory

Michael Hasselmo's study in 2006 investigated the critical role of acetylcholine (ACh) in memory and learning processes. The experimental design focused on understanding how ACh influences neural activity during information encoding and retrieval. Researchers used experimental drugs to activate or inhibit ACh receptors in animal models, particularly monkeys and rats, while observing their performance in memory tasks. Findings revealed that higher ACh levels significantly facilitate memory encoding by enhancing responses to new stimuli while simultaneously reducing interference from previously stored memories. By boosting responses to new sensory inputs and suppressing conflicting feedback signals, the neurotransmitter supports sustained attention and association formation.

The primary research gap in Hasselmo's study is in its focus on higher-order learning systems in mammals. Therefore, by starting with a simple organism (planaria), we can build a strong foundation of knowledge that fills in gaps that are often hidden by the interconnectedness of systems in higher organisms.

1.6.2 Ida Eržen & Miro Brzin - Cholinergic Mechanisms in *Planaria torva*

The study conducted by Eržen & Brzin (2002) explored the cholinergic mechanisms in *Planaria torva*, focusing on the functional roles of key enzymes in acetylcholine (ACh) synthesis and degradation, specifically cholinesterase and choline acetyltransferase (ChAT). These enzymes are essential for cholinergic signaling, with ChAT facilitating ACh synthesis and cholinesterases breaking it down to regulate.

One of the study's key findings was the significant activity of cholinesterases in *Planaria torva*, particularly in the head region, with most of the nervous tissue. This suggests that ACh

plays a critical role in planarian neural functions. The study also showed that planarian cholinesterases responded differently to inhibitors typically effective in mammalian systems, showing structural or functional differences in the cholinergic systems. Furthermore, low levels of ChAT were detected in the planarian nervous system, telling us that the planaria can synthesize ACh, though at a slower rate. These observations support the presence of a functional cholinergic system in these organisms, making them an appropriate model for investigating the effects of choline on learning and memory.

1.7 Driving Question

Investigating the efficacy of Choline Bitartrate is crucial as it is not FDA-regulated and lacks research on dose-dependent effects in model organisms, which raises the question: how do natural and synthetic choline bitartrate concentrations impact planarian classical conditioning? This study will investigate whether choline supplements improve conditioning and neural plasticity in planaria. By administering varying choline bitartrate concentrations to planarians, the relationship between drug concentration and conditioning can be explored. Using a forward conditioning model, with a light flash (neutral stimulus) preceding a classical phototaxis observation (unconditioned stimulus), we expect the planarians to develop conditioned responses of: 1) the distance traveled after the signal flash (neutral stimulus) to increase, and 2) the velocity of travel during constant light to increase. We also expect to see these effects to be positively correlated with choline dosage concentrations.

2.0 Methods

Planaria experimental groups were fed natural choline or different concentrations of choline-dosed foods. After feeding, they were subjected to a training regimen to determine if choline successfully improves their cognitive performance. There was a short-term study, consisting of 6 feedings and 15 days of training, and a long-term group with 6 feedings and 34 days of training. A short-term study was conducted to test how the frequency of feeding affected the training results.

2.1 Planarian Care and Feeding

Groups of *Dugesia dorotocephala* planaria (10-15) were kept in separate Petri dishes filled with clear spring water in a dark environment for 16 hours and a light environment for the next 8 hours (Dean & Duncan, 2020). Four groups of planarians were fed different doses of choline bitartrate, which contains 41% choline (Kansakar et al., 2023), and at least 1 control group was fed plain liver for both short-term and long-term studies.

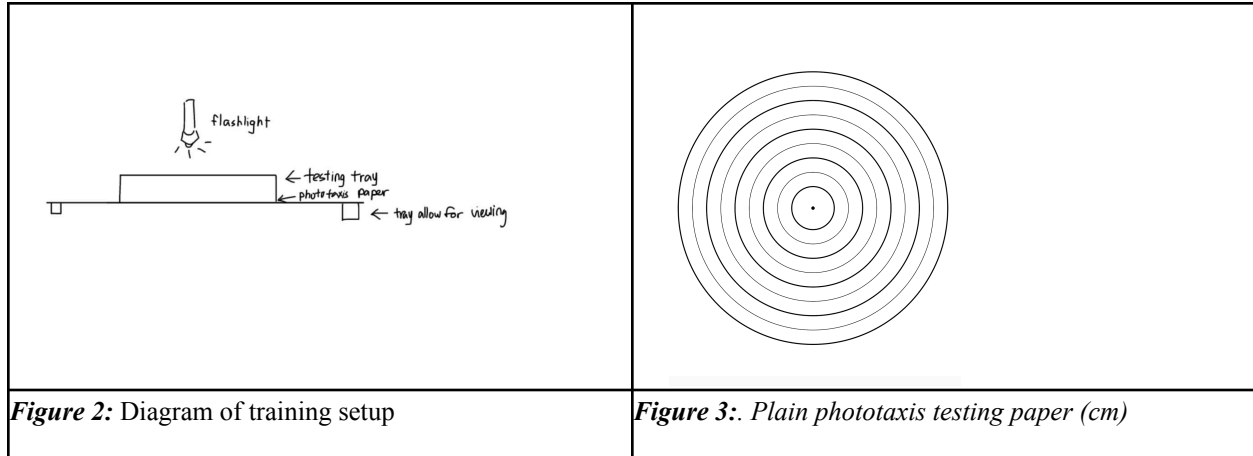
Experimental Group	Choline Dosage
Shrimp	81mg Natural Choline /100g shrimp (Patterson, 2008)
Liver	330mg Natural Choline/100g Liver
Liver + 50% choline	1094mg Choline Bitartrate/100g Liver
Liver +100% choline	1460mg Choline Bitartrate/100g Liver
Liver NF	330mg Natural Choline/100g Liver

Figure 1: Choline Dosage for experimental groups

The water was kept at room temperature, around 20- 23° C. The groups ate either plain liver paste or plain shrimp paste that had been blended and processed through a mesh to ensure a constant fine grain size and remove any connective tissues. Liver is the common food source used by many other researchers, but this experiment included shrimp as another natural source of choline with much lower choline concentrations. Planaria were fed and cared for utilizing standard research protocols (*Culturing Planaria Live Material Care Guide Background*, 2016).

2.2 Measurables/Observables

During training and testing, a one-second flash was turned on after the planarians had settled into the center dot, followed by a 10-second wait time and then a constant light. The distance traveled in the 10-second duration and the time it takes for the planarias to reach the end line (8.5cm) were measured. Figures 3 and 4 show the phototaxis measurement system, with the inner line being 1.5cm from the center dot and the rest of the lines being 1cm apart from each other. This sheet was placed right below the testing tray.



2.3 Experimental Groups and Timing

There were two parts to this experiment: a long-term study for 6 weeks with 6 feedings and a short-term study with 3 weeks and 5 feedings. Each study has the same experimental groups (see figure 4). For the long-term study, groups were fed on Monday and trained for 4 days after (Tuesday-Friday). The planarians were undisturbed on Saturday and Sunday. For the short-term study, each group was fed, trained for three days, fed, and trained for two days. There were no “undisturbed days”. Both long-term and short-term studies also had control groups, such as a plain liver group that is not trained with a flash but undergoes regular phototaxis to act as a comparison.

3.0 Results

3.1 Distance Data for Long-Term and Short-Term Study

The changes in distance traveled for each group over the course of the study are summarized in Figure 5. The regression p-value represents whether the change in distance is statistically significant (rejects the null hypothesis). A p-value less than 0.05 is considered significant. Similarly, the covariance p-value tests whether the change in distance between two experimental groups is statistically different. Again, $p < 0.05$ is considered significant.

	Long Term Study		Short Term Study	
	Change in distance traveled after signal flash	Regression p-value	Change in distance traveled after signal flash	Regression p-value
Plain Shrimp	-0.0176 cm/day	< 0.01	0.0598 cm/day	0.17
Plain Liver	-0.0116 cm/day	0.02	0.0641 cm/day	0.38
Liver + 50%	0.0175 cm/day	< 0.01	0.1265 cm/day	< 0.01
Liver + 100%	-0.0352 cm/day	0.20	0.096 cm/day	< 0.01
Covariance p-value	> 0.37 for all comparisons		< 0.05 for all comparisons except Liver + 50% to Liver + 100% (0.89)	

Figure 4: Distance Data and Statistical values for short and long-term study

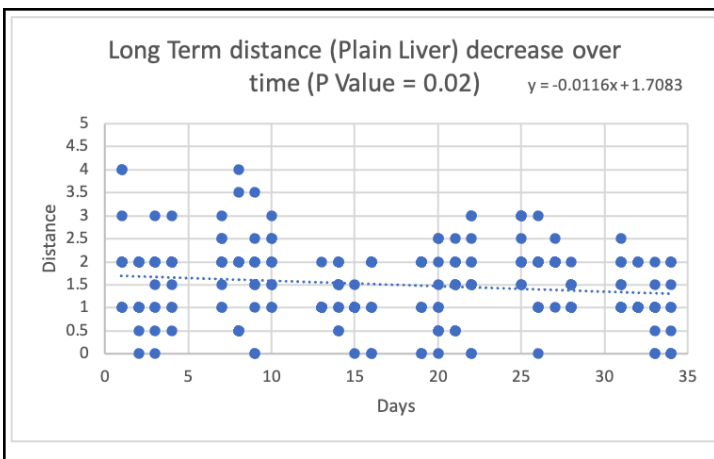


Figure 5: Typical distance training day chart for long-term study

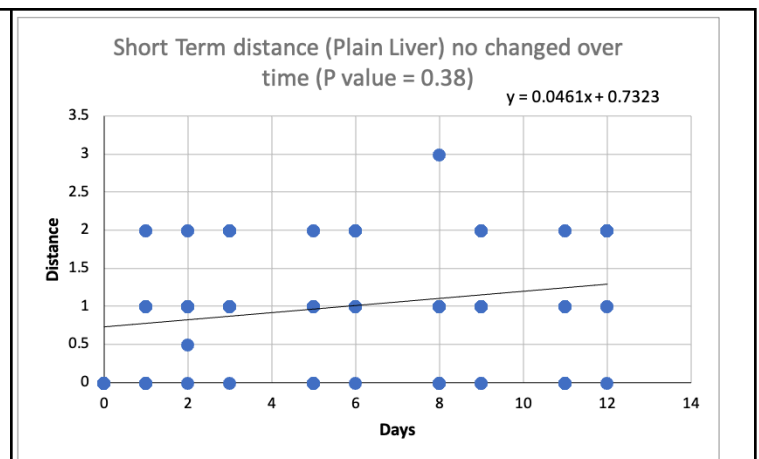


Figure 6: Typical distance training day chart for short-term study

In Figure 5, a negative change in distance indicates the planaria traveled shorter and shorter distances during the 10-second interval over the course of the experiment. The low regression p-values suggest that the change in distance is significant, but the high covariance p-value suggests that there is no effect based on different choline dosages. Figure 6 shows typical raw data plots for the plain liver control group. Full data results for all groups are included in Appendix 7.0

3.2 Velocity Data for Long-Term and Short-Term Study

The changes in velocity for each experimental group in both the long-term and short-term studies are shown in Figure 7 below.

	Long Term Study		Short Term Study	
	Change in velocity traveled after signal flash	Regression p-value	Change in velocity traveled after signal flash	Regression p-value
Plain Shrimp	-0.0017 cm/seconds	< 0.01	0.0024 cm/seconds	< 0.01
Plain Liver	-0.0017 cm/seconds	< 0.01	0.003 cm/seconds	< 0.01
Liver + 50%	-0.0011 cm/seconds	< 0.01	0.0036 cm/seconds	< 0.01
Liver + 100%	-0.0036 cm/seconds	< 0.01	0.0047 cm/seconds	< 0.01
Plain Liver not Flashed	-0.002 cm/seconds	< 0.01	0.0014 cm/seconds	0.02
Covariance p-value	> 0.14 for all comparisons		> 0.18 between all experimental group pairings except Plain Shrimp to Liver + 100% (0.005) and Plain Liver to Plain Liver No Flash (0.008)	

Figure 7: Velocity Data and Statistical values for short and long-term study

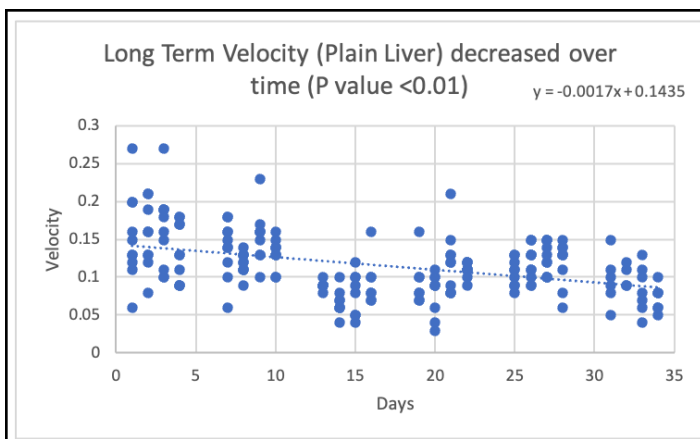


Figure 8: Typical velocity chart for long-term study

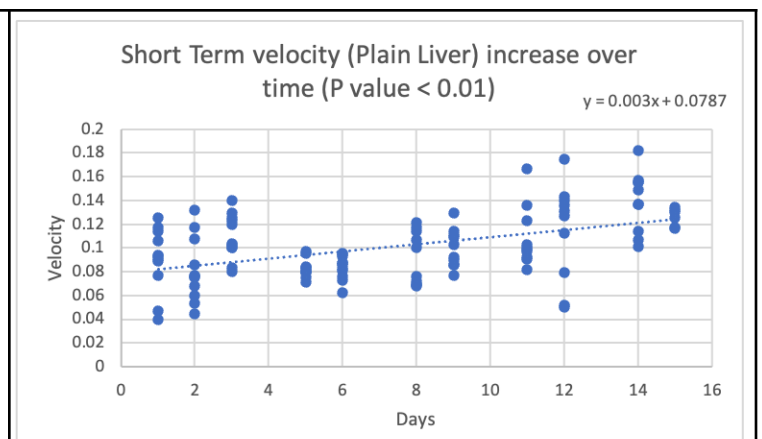


Figure 9: Typical velocity chart for short-term study

A negative change in velocity indicates the planaria traveled more slowly and had a decrease in velocity throughout the experiment. The low regression p-values suggest that the change in velocity is significant, but the high covariance p-value suggests no effect based on different choline dosages, except for shrimp to liver + 100% in the short-term study. Figures 9 and 10 show typical raw data plots for the plain liver control group. Full data results for all groups are included in Appendix 7.0

4.0 Discussion

4.1 Distance

After repetitive exposure to the flash, followed by constant light, the distance traveled by the planarians after the signal flash was expected to increase as the planarians became conditioned to the neutral stimulus and started their light avoidance early in anticipation of future constant light. However, the long-term data showed a decrease in distance traveled, while the short-term study showed no change in distance traveled over the period of the training for natural choline sources (shrimp and liver) and an increase in distance traveled for artificial choline (Liver + 50% and Liver + 100%).

Several possible ideas could help explain the observed result. Planarians may have been conditioned to associate the light exposure with 'safety' rather than 'danger' due to the lack of negative consequences for a slower phototaxis response. Another possibility is that the flash acted as a conditioned stimulus rather than the intended neutral stimulus. After conditioning and successful correlation, a neutral stimulus would be the conditioned stimulus; however, since light is not neutral for planarians, this may blur the association and fail to act as a true warning. Thus, the planarians did not develop a clear cause-and-effect reaction. Finally, since this experiment follows the ideas of trace conditioning, the 10-second duration may be too long for successful association.

The distance traveled by planarians was expected to be choline-dependent. However, when comparing the p-value between each group, the long-term study showed no significant differences in travel distance between the different experimental groups, and it is concluded that the distance traveled is not choline-dependent. For the short-term study, only the P value between shrimp to liver (~0.02) and the liver to liver + 50% (~0.03) are significantly different, and liver + 50% and liver + 100% groups (~0.89) suggests that choline has no effect and is a null hypothesis. This could suggest that only the initial addition of choline will be beneficial, but it loses its value as more choline is added. Due to inconsistencies, the data may support a null hypothesis, and that choline, both natural and synthetic, does not improve the production and productivity of acetylcholine in conditioning. The data could also suggest that the liver + 100% has too much

choline, and it is not significantly different from the Liver + 50% because an overdose of choline can lead to cholinergic toxicity (Lott & Jones, 2022).

Another possibility is that increased choline availability affected the planarians' motor function rather than learning. As mentioned in section 1.4, acetylcholine regulates muscle contraction and relaxation, which impacts swimming behaviors. Early on, planarians showed rapid scrunching and stretching behavior used to move quickly away from bright light. This suggested an *urgent*, escape-like response. However, this behavior diminished over time, particularly in the choline-supplemented groups. This could indicate reduced perception of danger where planarians no longer perceived the light as life-threatening, or that excessive acetylcholine reduced their ability to coordinate the contraction movements. Instead of improving conditioning, high choline levels, especially in liver+50% and liver+100%, may have relaxed their motor responses, and urgency was no longer expressed, reducing the distance traveled after the flash.

4.2 Velocity

The velocity during the constant light was expected to increase, but a regression test showed a decrease in all long-term groups. While the outcome was unexpected and is not consistent with the original hypothesis, several new lines of thinking emerged. First, similar to the distance, groups were conditioned to lose their sense of danger to light, as there is no 'punishment' for moving slowly in the light. Additionally, it is possible that the planarians were conditioned to be less sensitive to light. It is known that planarians' chemotaxis can overcome their phototaxis behavior, however, future research is needed to determine if overexposure to light could decrease their sensitivity. The short-term groups had an increase in velocity, possibly due to the frequency of training. The long-term study groups had a longer break for 3 days, allowing for choline depletion as it is water soluble, and not much choline is left during day 4 or day 3. The short-term study group was trained continuously with only 1 day of break. The short-term study has much more frequent feedings, with only 2 days of training in between each feeding, except for the initial 3 days when the study just began.

For the long-term study, the P-value between groups suggests no significant difference, and choline does not have an effect. For the short-term study, only plain shrimp to liver + 100% (~0.005) and plain liver to plain liver not flashed (~0.008) are statistically different. Only a huge increase in choline impacted velocity in the short term, but not in the long term. One possible explanation could also be the frequency of training.

The unexpected decrease in velocity across the long-term groups could reflect a change in motor output, similar to distance. The high-effort contraction movements were no longer observed as training went on. Acetylcholine's known role in locomotion suggests that elevated choline levels may have relaxed their muscle tone or disrupted the coordination needed for rapid phototactic responses. As a result, the planarians may have still recognized the cue, but their bodies no longer responded with urgency. This suggests an optimal choline range for movement, as excess choline exhibits possible interference with precise neuromuscular control. Only in the short-term study did the highest choline dose show increased velocity, possibly because of more frequent feeding and shorter degradation time, though urgency still declined over time.

5.0 Conclusion

Choline, a nootropic expected to increase acetylcholine production, was expected to enhance learning in planaria through classical conditioning. In both the short and long-term studies, the increase in choline does not affect the planaria's response to the conditioned stimuli, resulting in a null hypothesis. The training regimen had inconsistent results, possibly due to training frequencies and the loss of urgency, and future research could be done on its influence on the velocity of planarians. Based on the results of this study, training with more breaks would result in a slower velocity, while training with fewer breaks would result in a faster velocity. Another study can be conducted on overexposure to light during conditioning of their photophobic behaviors. An increase in light exposure with no 'punishment' could either desensitize planarians or condition them to lose their sense of danger that is often associated with light. A study can be done to distinguish between the two possibilities: if training is done with a 'punishment' such as a commonly used electric shock, the planarians should still maintain their

speed if it is the latter possibility. If their velocity or distance still decreases with the ‘punishment’, it is likely that they are conditioned to be less sensitive to light.

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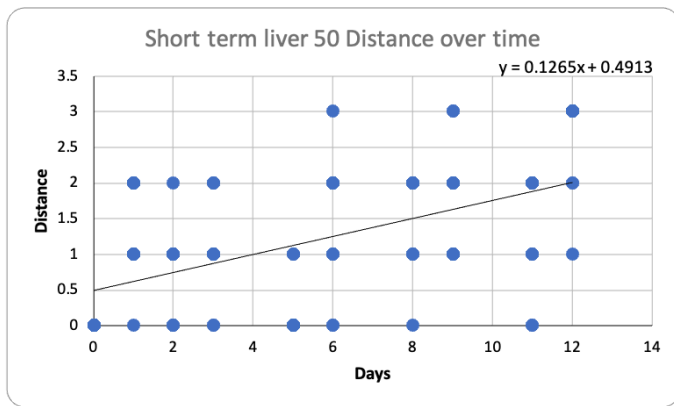
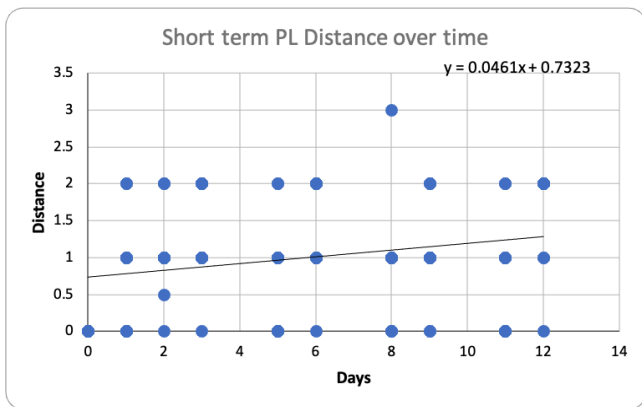
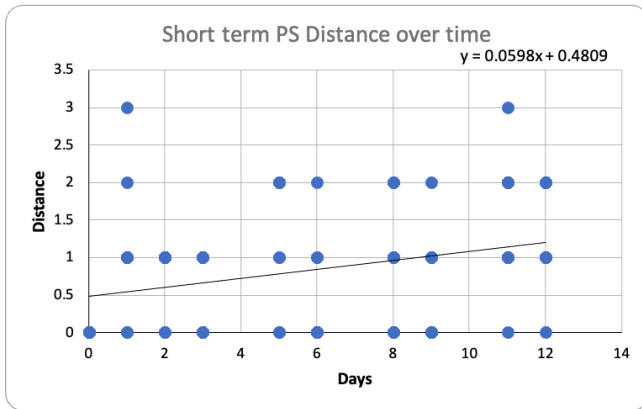
7.0 Appendixes

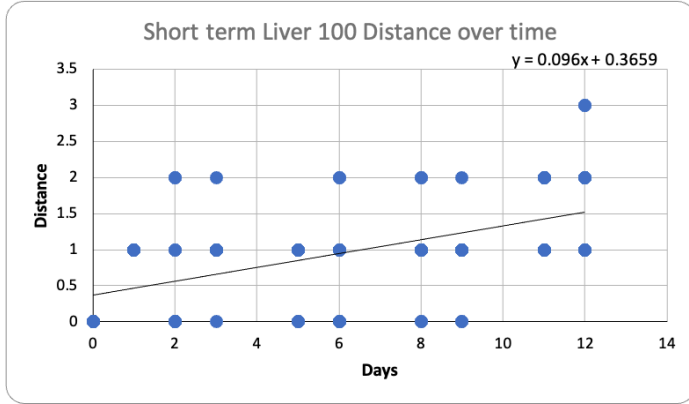
Appendix 1: List of Planaria Locomotion Behavior (Grebe & Schaeffer, 1991)

Table 1. Behavioral Responses of Planarians by Response Group

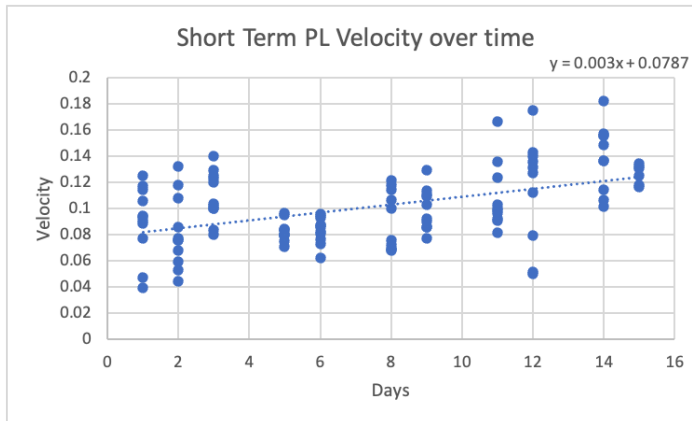
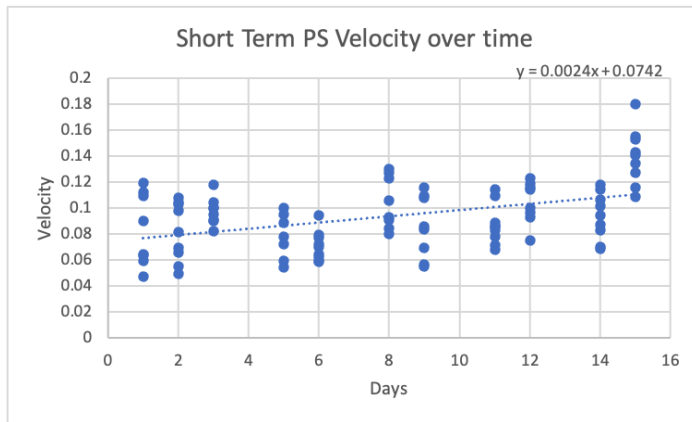
Response	Description
LOCOMOTIVE	
(1) Restlessness	Forward movement usually faster than normal; head raised off glide surface; frequent position switches.
(2) Hyperkinesia	"Inch-worming"; glide not smooth; almost always forward movement; few/none abrupt direction changes.
(3) Swims upside down	
MORPHOLOGICAL	
(4) Spiraling	Corkscrew-shaped movements. Animal twists around cranio-caudal axis.
(5) Head/nose twist	Tip of nose curls up or down.
(6) Shape change	Loss of normal body shape, e.g., extreme elongation or dumbbell shape.
(7) Ornamentation	Edges of animal become crenelated.
(8) Banana curl or coil	Horseshoe shape, head brought closer to tail either distal or ventral.
NEUROLOGICAL	
(9) Convulsions	Violent twitching; no forward motion; rapid coiling or twisting in quick, thrashing motion.
(10) Nervous signs	Abrupt direction changes and/or lack of coordination.
MORBIDITY	
(11) Labored movement	epidermis are discharged). Animal is stationary or moving slowly but moves normally when poked.
(12) Depression	Animal barely moves when poked, stationary otherwise.
(13) Unconsciousness	Animal does not move when poked but responds when placed in clean media.
(14) Death	Does not move when poked or revive in clean media; autolyses rapidly.
PROTECTIVE	
(15) Pharynx protrusion	Pharynx extends outside the body.
(16) Vomiting	Pharynx discharge.
(17) Mucus covering body	Covered with mucus (rhabdite cells in
(18) Lesions	Loss of structural integrity.

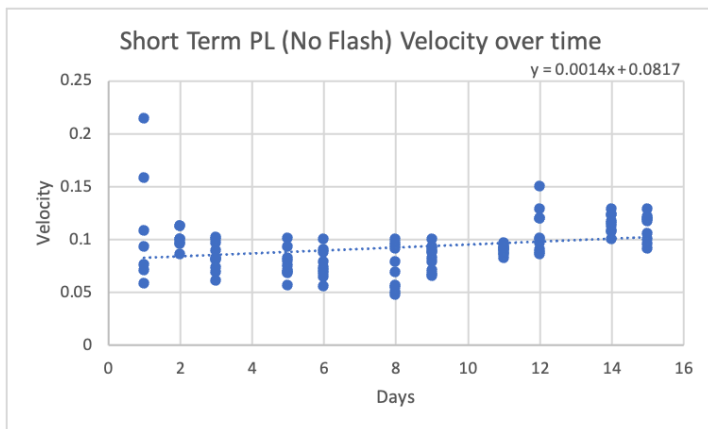
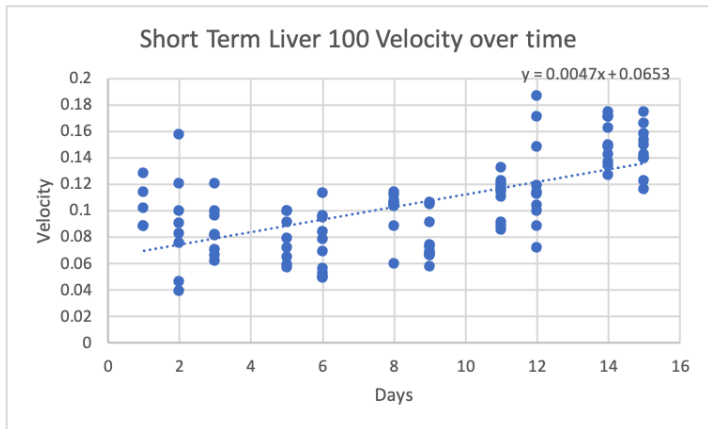
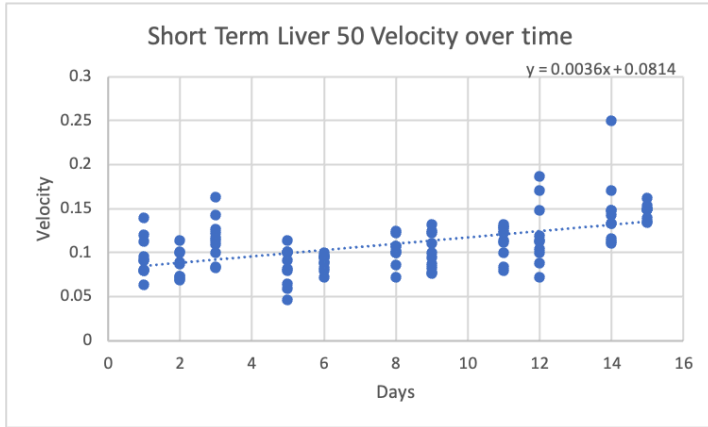
Appendix 2: Short-term Distance graphs



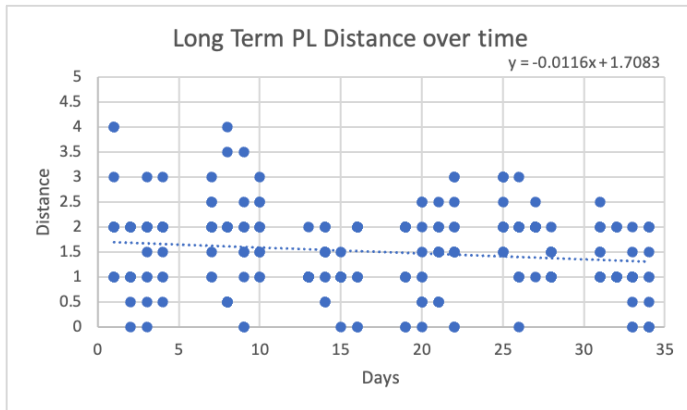
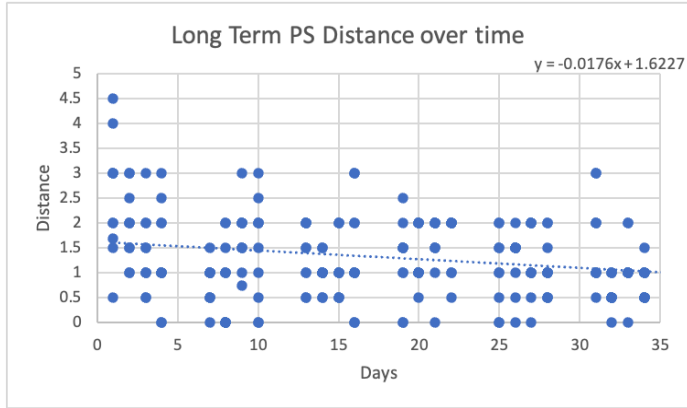


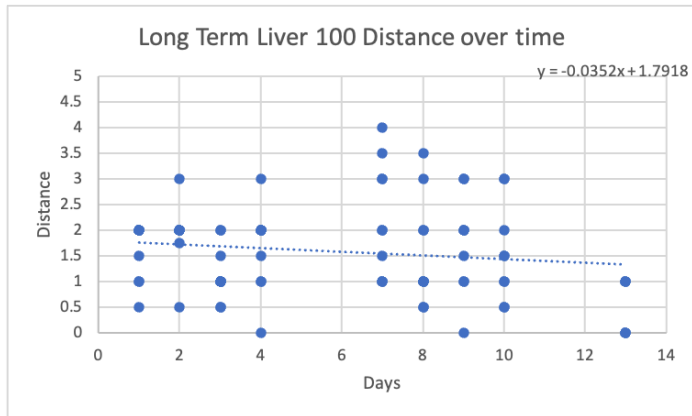
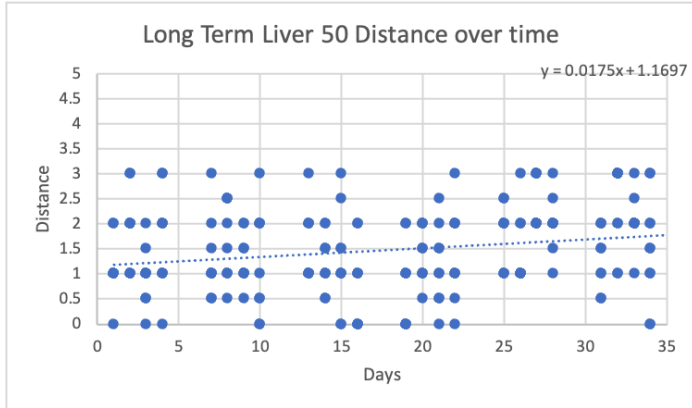
Appendix 3: Short-term Velocity graphs





Appendix 4: Long-Term Distance Graphs





Appendix 5: Long-term Velocity graphs

