Exploring the Potential Effects of Cannabidiol on the Regenerative Ability of Planaria

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Abstract

Stem cell regeneration holds high levels of potential for advancing treatments in regenerative medicine, through its potential to repair damaged tissues. Recent studies of cannabidiol (CBD) have revealed its capability to promote stem cell regeneration. CBD's influence on stem cell growth is derived from interactions with the endocannabinoid system (ECS). While CBD has exhibited a wide number of benefits for humans, its effects on regenerative medicine are underexplored. Planaria, a type of freshwater flatworm known for its regenerative abilities, provides an ideal model for studying stem cell regrowth. This study investigated the potential of CBD to influence planarian regeneration, expecting to find a positive correlation between CBD dose and regeneration rate. Instead, preliminary results show no correlation between CBD dose and regeneration, and a growth inhibition effect was observed based on the potential bioaccumulation of CBD.

1.0 Introduction

In 2020, the CBD industry made over 967.2 million dollars in profits, and 64% of Americans know of CBD. Its popularity has skyrocketed, with the number of CBD users in the U.S. increasing over 400% from 2018 to 2022 (Choi et al. 2022). Starting as a niche alternative health treatment, CBD now has products for an array of treatments. The nationwide craze includes lattes, makeup, bedsheets, bath bombs, and even dog treats. Amidst its widespread popularity, CBD is showing great promise in the medical community, one of the most promising fields being regenerative medicine with an emphasis on stem cells.

Understanding stem cell regeneration and its capabilities for healing has captivated scientists since stem cells were first discovered in the 1980s. The relatively new intersection of cannabidiol science and regenerative medicine has been presented as one of the most promising discoveries in modern therapeutic research (Parikh, 2024). CBD, the non-psychoactive compound derived from cannabis, has demonstrated anti-inflammatory and neuroprotective properties, thus sparking interest in the medical community (Parikh 2024). Gaining popularity as a potential therapeutic substance across various health issues, CBD has specifically been noticed for its potential to promote stem-cell regeneration (Miller 2020). Recent studies suggest that CBD could be crucial in enhancing stem-cell function and tissue regeneration, opening all kinds of doors for treating degenerative issues and chronic diseases (Miller 2020).

The effects of CBD on tissue regeneration can be effectively researched by utilizing planaria flatworms, studying how different concentrations of CBD affect their regeneration and neoblast formation following an amputation, and tracking the rate of regeneration under a microscope. The results could reveal new therapeutic uses for CBD in regenerative medicine.

1.1 Endocannabinoid System

Cannabinoid compounds, such as CBD, have recently been examined for their broad range of pharmaceutical effects, including the migration, differentiation, proliferation, and overall activity of multipotent stem cells, MSCs (Galve-Ropurh 2013). The endocannabinoid system (ECS) is a network of cellular receptors and signaling molecules, called endocannabinoids, in humans. The ECS contributes to the body's most critical functions, including inflammation and immune response, making it an attractive area of research for advancing stem cell therapies (Grinspoon, 2021).

The ECS consists of two endocannabinoids, 2-AG/2-Arachidonoylglycerol and AEA/Anandamide, that activate the CB1 and CB2 receptors, along with several enzymes that break down the signaling molecules (Bie 2018). As shown in Figure I, CB1 receptors are located in the brain and nervous system and contribute to regulating memory, pain, and mood; CB2 receptors, on the other hand, are located on immune cells and contribute to regulating inflammation (Galve-Roperh, 2013).

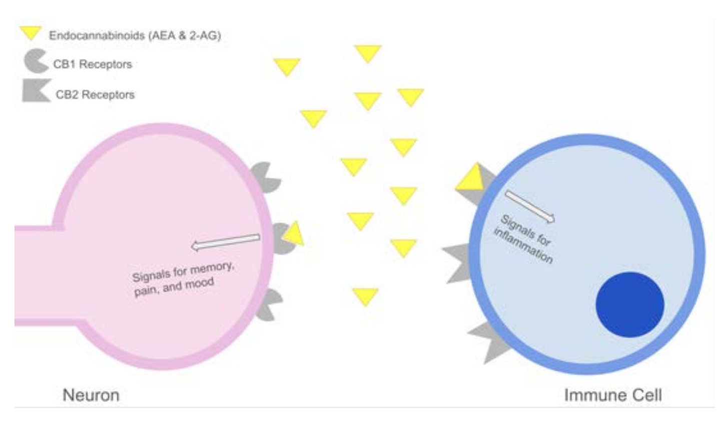

Figure I: The Endocannabinoid System- AEA & 2-AG binding to their respective receptors, CB1 & CB2

Fatty Acid Amino Hydrolase (FAAH) - FAAH is an important ECS enzyme that degrades and hydrolyzes the endocannabinoid AEA. Once the signal is complete and sufficient binding has occurred between AEA & either a CB1 or CB2 receptor, FAAH breaks down the extra anandamide to prevent the additional binding of AEA to CB receptor sites (Figure II). FAAH inhibition, possibly by pharmaceuticals, prolongs AEA signaling activity.

ECS and Stem Cell Regulation - The signaling molecules bind to CB2 receptors. Upon binding, G-protein-coupled receptors are activated inside the cell, which in turn triggers the formation of PI3K (Galve Roperh et al, 2013) and MAPK. MAPK activation helps stem cells migrate toward the infected or inflamed area and aids in the differentiation of cell types depending on what the body needs. PI3K supports cell survival and growth by promoting cell proliferation and preventing cell death.

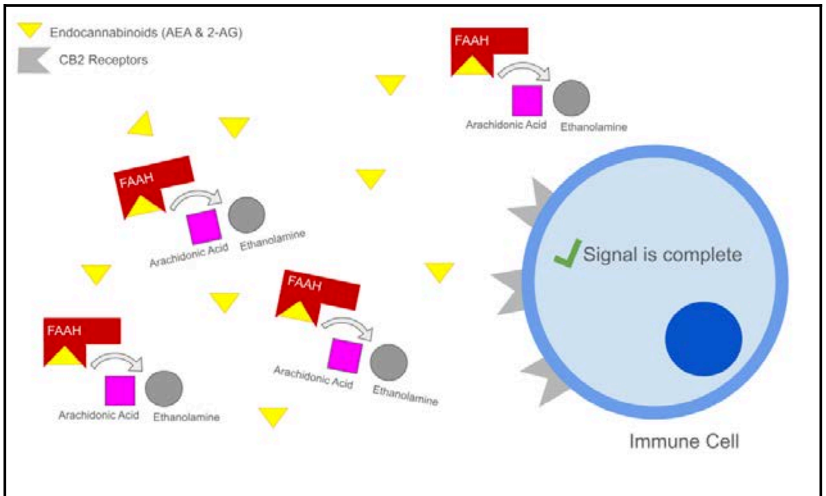

Figure II: FAAH hydrolyzing AEA signal into Arachidonic acid and ethanolamine after AEA's signal is complete

1.2 CBD and the ECS

CBD, a lipophilic compound, dissolves in fats and oils (Naya et al, 2024), bioaccumulating over time. Ingesting CBD with high-fat food increases the absorption rate and allows it to be absorbed into the bloodstream. This enables CBD to interact with receptors throughout the body, including in tissues where stem cells are active. CBD accumulates in fat cells before leaving the body, so regular use makes it detectable for longer.

CBD exhibits two key interactions with the ECS. The first interaction (Figure III): CBD binds to the CB2 receptor and activates the MAPK pathway (enhancing stem cell migration) or PI3K pathway (enhancing proliferation). The second interaction (Figure IV): CBD binds to FAAH, inhibiting the breakdown of AEA to Arachidonic Acid and Ethanolamine, which causes the signal to be prolonged (MÁ and Valverde, 2020).

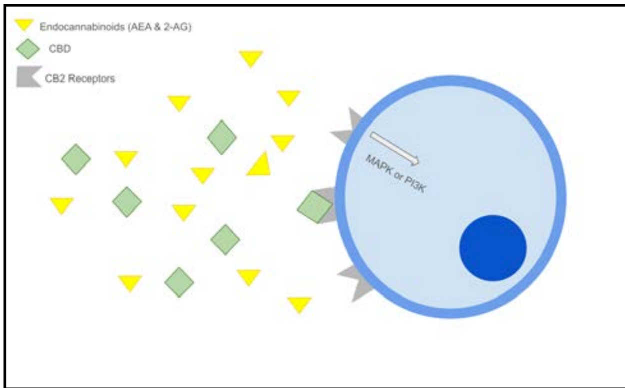

Figure III: The CBD binding to CB2 receptors

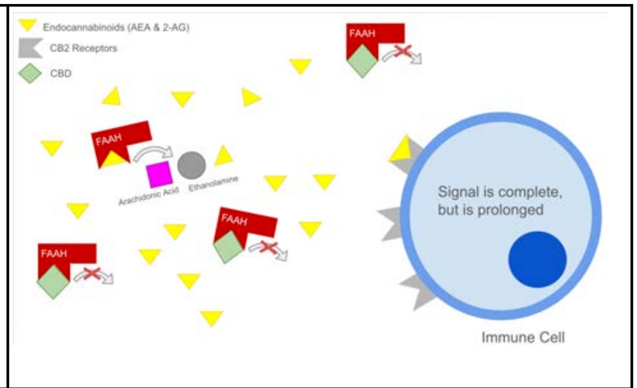

Figure IV: CBD binding and inhibition of FAAH

1.3 Planaria

Planaria are freshwater flatworms (belonging to the class Turbellaria) that grow to be 3-15mm long (Buttarelli, 2008). Planaria are some of the simplest organisms to have a functional central nervous system, and they are known for their incredible ability to fully regenerate parts of their bodies using stem cells. Planaria are extremely sensitive to their environments, and they can absorb toxins and nutrients through their skin.

The planaria was one of the first organisms to develop a Central Nervous System (CNS). The CNS consists of two main parts: the cerebral ganglia and two ventral nerve cords that run along their bodies. The planaria's CNS contains every neurotransmitter that helps them respond to drugs and affects movement and regeneration (Buttarelli, 2008).

After being cut into pieces or experiencing injury, each part of a planarian flatworm can fully regenerate its tissue into a brand new individual planarian. This ability is attributable to planaria's population of dividing, adult stem cells, formally called neoblasts. Neoblasts are distributed throughout the entire planarian body as small, pluripotent cells (stem cells that can differentiate into multiple cell types), and they exist as the only dividing cells in adult planaria. This ability assists in full-body regeneration of planaria, which includes regeneration of their nervous system. Upon injury to a planaria, these neoblasts proliferate at the wound site, forming into cell clusters called blastemas. These blastemas are the start of repair and differentiation to replace the specific lost cells (Scimone, 2014). Because of their simple body structure and regenerative abilities, planaria are perfect creatures for scientists studying processes that concern cell regeneration (Butarelli, 2008).

Planarians do not have the same endocannabinoid system as humans, but exhibit various features that parallel the human endocannabinoid system. For example, planaria and humans both produce anandamide, 2-AG, and FAAH (Clarke 2021). Although planaria do not have CB1 and CB2 receptors, a genome analysis of planaria identified receptors TRPA1, TRPV, and TRPM that are similar to receptors that interact with CBD and as well as g-protein coupled receptors that are homologous to CB1 and CB2 receptors (Clarke 2021). Although planaria have simple nervous systems, they share several components of the endocannabinoid system with humans. Their regenerative capabilities (specifically their ability to use pluripotent stem cells in their neoblasts) make them an ideal model for studying the effects of CBD on the proliferation and migration of stem cells.

1.4 Literature Review

1.4.1 Endocannabinoid System in the Planarian Model

In 2010, K.L. Mustonen prepared her Master of Science Thesis on the Endocannabinoid System in the Planarian Model. Mustonen investigated the possibility of an ECS-like system in planaria using DNA sequence alignment. Using BLAST (basic alignment search tool), Mustonen uncovered portions of DNA sequences that allowed her to identify proteins, receptors, or chemicals in planaria that are orthologous to the human ECS.

She uncovered that planaria have the endocannabinoids 2-AG and anandamide (AEA). A BLAST analysis also concluded that Schmidtea mediterranea (a type of planaria) has FAAH enzymes. CB receptor homologs were not found in planaria; however, Mustonen predicts that there are other CB-like G-protein-coupled receptors to which endocannabinoids bind. These results suggest that planaria possess a primitive ECS analogous to the human ECS. Mustonen's research suggests that planaria can be used to research the ECS, opening the door to researching CBD and its potential therapeutics.

1.4.2 Effects of Cannabidiol on Dugesia Dorotocephala Head Regeneration

A 2022 study tested the effect of cannabidiol (CBD) on the regeneration of planaria *Dugesia dorotocephala* (Hughes, 2022). Since planaria can absorb substances through their aquatic environment, these researchers diffused CBD into their environment, using a solution called "Bliss Water." The researchers created different concentrations (0%, 25%, 50%, and 100% Bliss Water). The planaria were transversely cut, and then the tails were placed in their solution in a dark cabinet at room temperature following the cut. Over 7 days, they measured the rate of head regeneration in each population using a percentage scale (0% = just tail, 100% = both eyes) every 2 days. The data collected revealed no significant difference in regeneration rate for the various populations. Hughes concluded that further study could investigate higher concentrations of CBD. It is also important to note that these researchers studied the effects of CBD through absorption in the aquatic environment, where no studies have been done on the effects of planaria ingesting cannabidiol rather than absorbing it.

1.5 Project Statement

The endocannabinoid system, a system of endocannabinoids, receptors, and enzymes, has recently gained attention for its contribution to stem cell regrowth. Endocannabinoids bind to CB receptors and "turn on" the signaling pathways MAPK and PI3K that migrate and proliferate stem cells. CBD also inhibits FAAH, thus prolonging the signal of endocannabinoids. Notably, Planaria, a model organism for regeneration, possesses FAAH, endocannabinoids, and CB-analogous receptors, making them an ideal system of study.

This project investigated CBD interactions with the ECS, seeking to understand if CBD would enhance planaria stem cell regeneration and indicate future medicines or regenerative therapies. If planaria demonstrated enhanced regeneration when dosed with CBD, then the rate of growth following amputations would increase and would cause the planaria to fully regenerate more quickly than the control group.

2.0 Methods

The effectiveness of CBD in enhancing stem cell regrowth in planaria was tested. A total of 100 black planaria (*Phagocata gracilis*) were divided into three experimental groups based on the number of CBD doses they would receive before cutting. Each feeding group was further divided to vary the concentration of the dose. Figure V shows the group organization. For all groups, regeneration was measured by taking daily photographs for 15 days and calculating the ratio of neoblast areas to total body area.

2.1 Feeding and Dosage

To maintain a healthy colony, the petri dishes were cleaned every day, minimizing unhealthy contaminants. The planaria were fed every Monday and Thursday for about 30 minutes. The dosages were created by starting with a high dose (400mg/100lb) (Arnold et al. 2022), then using serial dilution to achieve 10%, 5%, and 1% concentrations. To create the high dosage, 0.375g of CBD was mixed into 35g of liver paste (mixture shown in Figure VI).

Group	Control (0% CBD)	1% CBD	5% CBD	10% CBD
1 Feeding	1F0			1F10
3 Feeding	3F0	3F1	3F5	3F10
5 Feeding	5F0	5F1	5F5	5F10

Figure V: Group organization of feedings

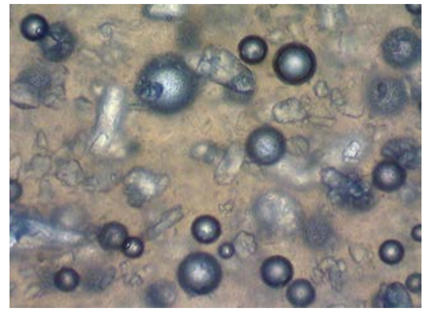

Figure VI: CBD Powder Incorporated into Liver Paste

2.2 Cutting

To ensure that planaria remained stationary while they were being cut, they were given an ice bath before each cut. Cooling the planaria slows their movement and metabolic rate, which makes them easier to handle (HHMI 2007). Once their movement slowed sufficiently, the planaria were transferred to a new clean Petri dish for dissection (cut once horizontally across the center). The head end of the planaria was discarded, and the tail end was collected for observation.

2.3 Observation

The planaria were photographed each day following the cut using a dissecting microscope and an attached digital camera to capture images. The microscope was uniformly set to a magnification of either 0.7X or 1.0X to ensure that area measurements were consistently evaluated on the same scale. Multiple photographs were taken of each planaria to ensure that the planaria were stretched out and in the best possible position for measuring.

Figure VII: Contrast between neoblast and body on the ninth day of regeneration

2.3 Measuring Regeneration

Daily images were processed using MotiConnect imaging and measurement software to quantify the regeneration. There was a noticeable contrast in coloration between the planarian body and the neoblast as seen in Figure VII (the neoblast is clear and the body is black). Black planaria also develop a “shoulder-like” area around their regrowing head, meaning that the head area is connected to the body by two indentations on either side (also seen in Figure VII). The neoblast was quantified by finding the ratio between the size of the neoblast and the total size of the body, using MotiConnect software to calculate the areas.

3.0 Results

The preliminary results below compare 0% and 10% growth rates for the 1F and 3F groups. Intermediate dosages (5% and 1%) and the 5F experimental group have not yet been processed.

3.1 Planaria Regeneration for One-Feeding group (1F)

Figure IX shows the growth curves for two experimental groups receiving one feeding before amputation. The control group (0% dosage) and experimental group (10% dosage) were cut in half two days after feeding, with growth measured daily.

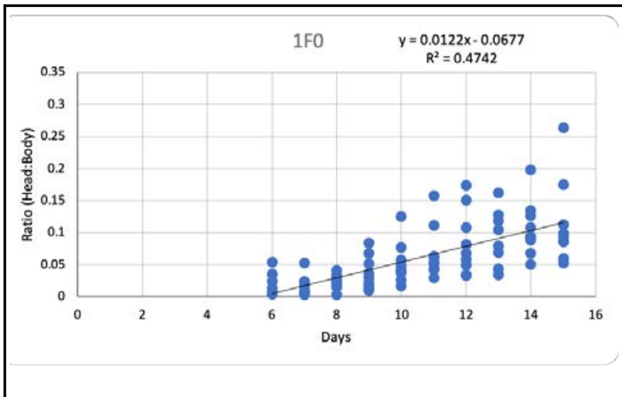

Figure VIIIA: Head:Body Ratio measured after head amputation for Planaria Group fed once with 0% CBD dosage. (Days 6 to 15 only)

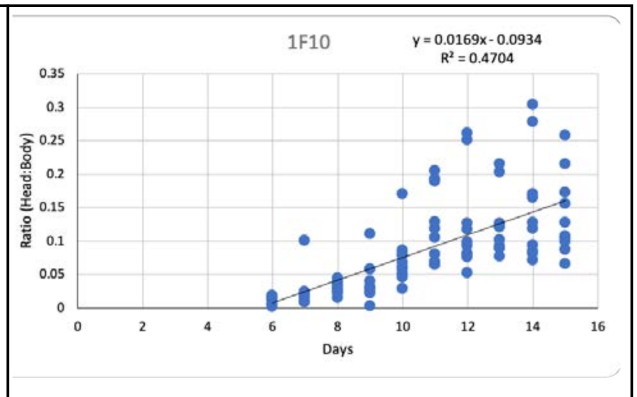

Figure VIIIB: Head:Body Ratio measured after head amputation for Planaria Group fed once with 10% CBD dosage. (Days 6 to 15 only)

Growth Rate (0% Group)	Growth Rate (10% Group)	P-value (statistical significance of growth rate difference)
1.22% ratio change/day	1.69% ratio change/day	0.12

Figure IX: Growth rate comparison for 1F experimental groups

In Figures VIIIA and VIIB, the data show that the head:body ratio increases over time. Over time in both graphs, the ratio of head:body diverged as days increased. This indicates that there is significant variation in growth rates within each planaria experimental group. Another thing to note is that in Figure VIIB, several planaria are growing at noticeably faster rates than the rest of the planaria.

A covariance analysis between two data sets indicates if a regression line for each data set is statistically different (a p-value < 0.05 will indicate statistically different). Analyzing the

1F0 and 1F10 data during the growth phase of the worms (shown in Figures VIIIA and B) results in a p-value = 0.12, indicating no difference in growth rate for these two experimental groups.

3.2 Planaria Regeneration for the Three-Feeding group (3F)

Figure X shows the growth curves for two experimental groups receiving three feedings before amputation. The control group (0% dosage) and experimental group (10% dosage) were cut in half two days after the third feeding, with growth measured daily.

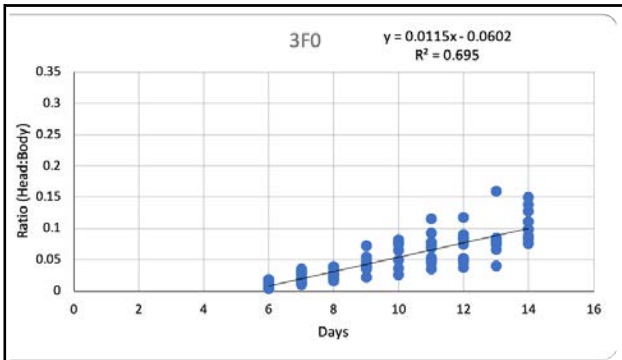	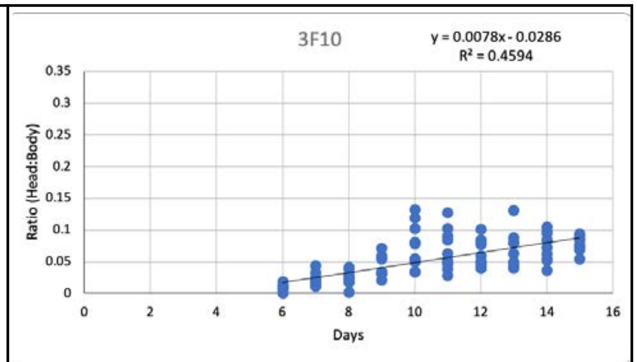
Figure XA: Head:Body Ratio measured after head amputation for Planaria Group fed three times with 0% CBD dosage. (Days 6 to 15 only)	**Figure XB:** Head:Body Ratio measured after head amputation for Planaria Group fed three times with 0% CBD dosage. (Days 6 to 15 only)

Growth Rate (0% Group)	Growth Rate (10% group)	P-value (statistical significance of growth rate difference)
1.15% ratio change/day	0.78% ratio change/day	0.003
Figure XI: Growth rate comparison for 3F experimental groups		

Analyzing the 3F0 and 3F10 data during the growth phase of the worms (shown in Figure XA and XB) results in a p-value = 0.003, indicating a difference in growth rate for these two experimental groups. A linear trend line was used to find the growth rate of each experimental group. The rate of growth in the control group is greater than the rate of growth in the 10% group, so CBD is inhibiting the growth rate.

3.3 Regeneration Comparison of 3F 10% and 1F 0%

Figure XI shows a comparison between the growth curves for one experimental group receiving one severe (10%) dosage of CBD and another experimental group receiving three dosages of severe (10%) dosage of CBD before amputation.

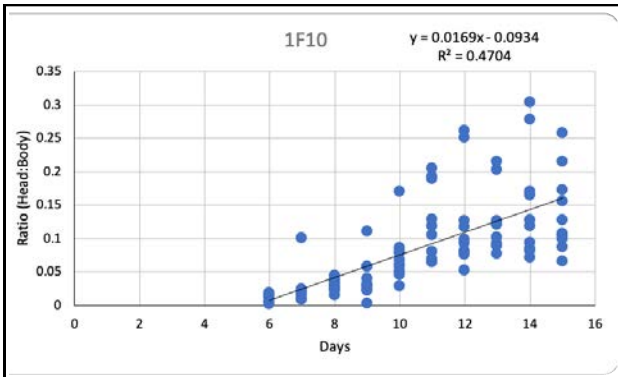

Figure XIIA: Graph of Body to Head Ratio in Planaria that have received 1 feeding with a 10% dosage of CBD (Days 6 to 15 only)

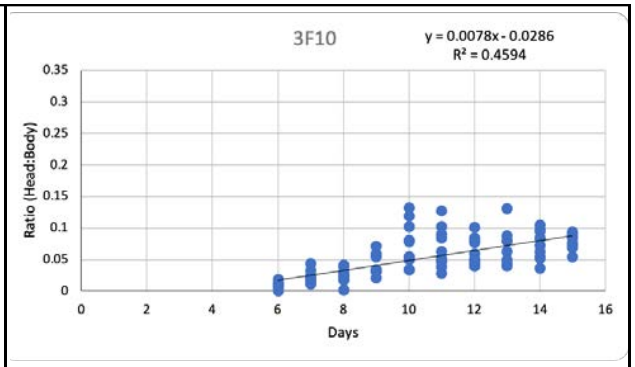

Figure XIIB: Graph of Body to Head Ratio in Planaria that have received 3 feedings with a 10% dosage of CBD (Days 6 to 15 only)

Growth Rate (0% Group)	Growth Rate (10% group)	P-value (statistical significance of growth rate difference)
1.69% ratio change/day	0.78% ratio change/day	P << 0.01

Figure XIII: Growth rate comparison for 1F10% and 3F10% experimental groups

The 3F10 group grew at a slower rate (0.78% ratio change/day) compared to the 1F10 group (1.69% ratio change/day). These growth rate differences are statistically significant with a p-value of 0.00002. The data shows that as more dosages happen, the rate of growth decreases.

3.4 LD 50 Results

Dosing experience with *Dugesia Tigrina* (brown planaria) colonies resulted in 100% fatalities at a 50% dosing. The LD-50 study for *Dugesia Dorotocephala* (black planaria) resulted in 100% survival at dosing up to 50%.

4.0 Discussion

4.1 Control vs 10% dosage for 1F Group

Based on the CBD binding pathway described in 2.2, it was expected that higher amounts of CBD ingested would result in a faster stem cell regeneration rate compared to the control group. The data from the 1F trial (Figure VIIIA & VIIIB) are not statistically different (p value = 0.12). Therefore, the null hypothesis is accepted, and this experiment does not definitively establish that there is an effect or correlation between the data sets. One reason for the large error is that there is variation in individual organism responses. This could be caused by individual organism sensitivity, measurement technique (scrunch v stretch), and non-uniform dose ingestion. Another reason why there is variation between individual organisms is the uncertainty within our measurement technique. When capturing photos of the planaria, the planaria would scrunch or stretch. When a planaria is scrunched, the head:body ratio is lower than the head:body ratio of a stretched worm. The error is ±0.0641.

4.2 Control vs 10% dosage for 3F Group

The same experiment was repeated, with experimental groups receiving three feedings before amputation. Since CBD is a fat-soluble molecule, increased effects are expected (compared to 1F) as CBD potentially bioaccumulates in the worms. The data from the 3F trial (Figure XA and XB) showed that the 3F10 group grew at a slower rate (0.78% ratio change/day) compared to the 3F0 group (1.15% ratio change/day). Based on this result, the original hypothesis was incorrect, and CBD inhibits the growth of planaria pluripotent stem cells. This result is the opposite of the hypothesis; understanding the specific mechanisms/interactions CBD has with the ECS would make it possible to understand why CBD inhibits stem cell regeneration. Another reason that CBD inhibits stem cell regeneration in planaria could be because they don't have the CB2 receptors humans have; they do have analogues of G-protein-coupled receptors, but the interactions of CBD with the proteins are unknown, and there could have been a misunderstanding of the CB-like receptor function.

4.3 CBD Bioaccumulation (1F10 and 3F10)

Based on the bioaccumulation of CBD in humans, as mentioned in 1.2, as more CBD is ingested over time, there should be an increase in effects on planaria regeneration rates. The data shows CBD inhibits regeneration, and bioaccumulation further inhibits planarian regeneration (3F10 has a larger effect than 1F10). While the regeneration inhibition is not fully understood, the model of CBD as fat soluble supports the data outcome showing an increased effect as more CBD is ingested over time.

4.4 LD 50

The LD50 for black planaria (*Dugesia dorotocephala*) appears to be greater than 200mg, based on results showing no mortality even at the highest tested dose. This is inconsistent with earlier experimental results using brown planaria (*Dugesia tigrina*), where a dose of 200mg resulted in 100% casualties. Possible explanations for this difference include: a change in planaria care to improve sterile technique, a difference in tolerance between black and brown, or brown planaria contracted a disease before shipment. This is suggested by the observation of a possible parasite, but it was not confirmed.

4.5 Future Works

This paper presents preliminary results of only the 1F and 3F groups. Future steps include completing the data for the 5F group and the intermediate groups 1% and 5%. The full dataset will provide a more comprehensive understanding of CBD's effect on planarian regeneration.

Due to the number of planaria that were lost when the initial high dosage (400mg/100lb) of CBD was administered, the dosages had to be dramatically reduced in the final experiment. However, when the high dosage was tested, it was discovered that black planaria lived and were able to tolerate a much higher amount of CBD than we initially believed. For this reason, we believe that our previous planaria had been exposed to a parasite also known as the "ick," which killed the planaria. For future studies, careful dose-response data (including an LD50 study) should be acquired to set an appropriate experimental protocol.

The mobility of the worms complicated the photographing and measurement techniques. When the planaria are scrunched, the area of their bodies is less than a fully stretched worm. This created a source of uncertainty in our data. Additional work is needed to validate that the measurement technique of taking the head: body ratio can be done with sufficiently low error. One suggestion would be using brown planaria (*Dugesia tigrina*), since they have a clear neoblast that does not change colors, are less curious (and hence less mobile), and do not produce an active neoblast until day 12. Another suggestion would be using our ice bath (as seen in 2.2) to slow the planaria's metabolism and get precise photographs.

Understanding why the effect of CBD seems to be the opposite of the expected results is unknown. One possible explanation is that CBD acts as a toxin to planaria. To further investigate the interaction between Planaria and CBD, future research could substitute CBD for CBN, a cannabinoid known to have minimal or no physiological impact, to determine whether the chemical structure of CBD contributes to its potentially harmful effects. Additionally, CBD has only been legalized for 10 years, so there is a limited amount of research and understanding of CBD's interactions with the ECS. Future research can be done to uncover the exact interactions CBD has with the ECS.

5.0 Conclusion

This study explored the effects of cannabidiol (CBD) on stem cell regeneration in planaria. While the initial hypothesis, based on CBD's suggested interactions with ECS, predicted a positive correlation between CBD and stem cell regeneration rates, the data do not support this result. Instead, an inhibitory effect was observed, and even enhanced by bioaccumulation of

CBD in planaria fat cells. It is unclear whether a better understanding of the structural and functional differences between planaria CB-like receptors and human CB1 and CB2 receptors is needed or whether significant differences between planaria species are producing unanticipated outcomes. Further research is needed to elucidate the molecular mechanisms by which CBD interacts in planaria to determine whether these findings translate to broader applications in planarian regeneration.

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6.0 References

Abel, C., Powers, K., Gurung, G., & Pellettieri, J. (2022, February). *Defined diets for freshwater planarians*. Developmental dynamics : an official publication of the American Association of Anatomists. https://pmc.ncbi.nlm.nih.gov/articles/PMC8758798/

Ahn, K., Johnson, D. S., & Cravatt, B. F. (2009). Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNS disorders. *Expert opinion on drug discovery*, 4(7), 763–784. https://doi.org/10.1517/17460440903018857

Arnold, J. C., McCartney, D., Suraev, A., & McGregor, I. S. (2023). The safety and efficacy of low oral doses of cannabidiol: An evaluation of the evidence. Clinical and translational science, 16(1), 10–30. https://doi.org/10.1111/cts.13425

Bennet , E., Murphy, K., & Bennet, A. (2022, December 12). *Effect of hypervitaminosis A in regenerating planaria: A potential model for teratogenicity testing: Journal of Emerging Investigators*. Effect of hypervitaminosis A in regenerating planaria: A potential model for teratogenicity testing | Journal of Emerging Investigators. https://doi.org/10.59720/22-066

Bie, B., Wu, J., Foss, J. F., & Naguib, M. (2018). An overview of the cannabinoid type 2 receptor system and its therapeutic potential. *Current opinion in anaesthesiology,* 31(4), 407–414. https://doi.org/10.1097/ACO.0000000000000616

Buttarelli, F. R., Pellicano, C., & Pontieri, F. E. (2008). Neuropharmacology and behavior in planarians: translations to mammals. Comparative biochemistry and physiology. *Toxicology & pharmacology*: CBP, 147(4), 399–408. https://doi.org/10.1016/j.cbpc.2008.01.009

Choi, N. G., Marti, C. N., & Choi, B. Y. (2022, October 9). *Prevalence of cannabidiol use and correlates in U.S. adults*. Drug and Alcohol Dependence Reports. https://www.sciencedirect.com/science/article/pii/S2772724624000738#:~:text=Table%201%20shows%20that%2020.6,cannabis%20and%2056.0%20%25%20of%20cannabis%2D

Clarke, T. L., Johnson, R. L., Simone, J. J., & Carlone, R. L. (2021). The Endocannabinoid System and Invertebrate Neurodevelopment and Regeneration. *International journal of molecular sciences*, 22(4), 2103. https://doi.org/10.3390/ijms22042103

Dean, M. R., & Duncan, E. M. (2020). Laboratory Maintenance and Propagation of Freshwater Planarians. *Current Protocols in Microbiology,* 59(1), e120. https://doi.org/10.1002/cpmc.120

Galve-Roperh, I., Chiurchiù, V., Díaz-Alonso, J., Bari, M., Guzmán, M., & Maccarrone, M. (2013). Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. Progress in lipid research, *Science Direct.* 52(4), 633–650. https://doi.org/10.1016/j.plipres.2013.05.004

Grinspoon, P. (2021). The endocannabinoid system: Essential and mysterious. *Harvard Health Publishing.* https://www.health.harvard.edu/blog/the-endocannabinoid-system-essential-and-mysterious-202108112569

Hughes, H. V., & Hughes, V. (2022). Effects of Cannabidiol on Dugesia Dorotocephala Head Regeneration. *Athens Journal of Health and Medical Sciences.* https://www.athensjournals.gr/health/2022-02HEA.pdf#page=27

Investigating planarian behavior and regeneration. HHMI Biointeractive. (2007). https://www.biointeractive.org/sites/default/files/InvestigatingRegeneration-Educator-act.pdf

Lazorik , O. (2019, May 10). The effect of caffeine on the regeneration ... *Journal of Emerging Investigators* . https://emerginginvestigators.org/articles/18-061/pdf

Mesas, C., Moreno, J., Doello, K., Peña, M., López-Romero, J. M., Prados, J., & Melguizo, C. (2024). Cannabidiol effects in stem cells: A systematic review. *BioFactors (Oxford, England)*, 10.1002/biof.2148. Advance online publication. https://doi.org/10.1002/biof.2148

Miller, H., De Leo, N., Badach, J., Lin, A., Williamson, J., Bonawitz, S., & Ostrovsky, O. (2020). Role of marijuana components on the regenerative ability of stem cells. *Cell Biochemistry and Function,* (), cbf.3609–. https://doi.org/10.1002/cbf.3609

Mustonen, K. L., & Venables, B. J. (2010). *Endocannabinoid system in the Planarian model* (thesis). *Endocannabinoid system in the planarian model*. University of North Texas, Denton, Tex.

Parikh A. C., Jeffery C. S., Sandhu Z., Brownlee B. P., Queimado L., & Mims M.M. (2024) The effect of cannabinoids on wound healing: a review. *Health Science Reports.* 2024; 7:e1908. doi:10.1002/hsr2.1908

Scimone, M. L., Kravarik, K. M., Lapan, S. W., & Reddien, P. W. (2014). Neoblast specialization in regeneration of the planarian Schmidtea mediterranea. *Stem cell reports*, 3(2), 339-352. https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(14)00186-6

What are stem cells?: Stem Cells: University of Nebraska Medical Center. What are Stem Cells?| Stem Cells | University of Nebraska Medical Center. (n.d.). https://www.unmc.edu/stemcells/stemcells/#:~:text=Stem%20Cell%20History&text=Scientists%20discovered%20ways%20to%20derive,the%20cells%20in%20the%20laboratory.