What is the impact of 5-Hydroxytryptophan on Caenorhabditis Elegans (N2) behavior?

Sumuga Swaminathan[1], Aksitha Vijayakumar Lakshmipriya[1]
[1] *Lake Norman High School*

Abstract:

Depression affects approximately 9.5% of American adults, leading to increased dependence on antidepressant medication. Consequently, a recurrent use of a drug called 5-HTP sparked the interest of depressive patients. With a comprehensive understanding of the potential side effects of the drug, it makes it more reliant to use. It was necessary to understand the long-term probable effects it has on locomotion. Our hypothesis posits that consistent consumption of this medication would lead to a reduction in center point speed. To do this, our research studied its effects on Caenorhabditis Elegans (N2) behavior. We fed them 0.0125 mL of a 200mg 5-HTP x 50 mL of OP-50 food source to the experimental group and 0.0125 mL of the OP-50 food source. We fed them during two intervals, one in the morning (8:10-8:15) and one in the afternoon (1:15-1:20), while observing them through a light microscope before we fed them. Using an iPhone 14 Pro camera, we inputted the videos onto Wormlab, tracking their center point speed (μm/s) and distance traveled forward (μm). The results were observed to conclude the delayed movement of drug-induced C.Elegans compared to the control group. The quantitative conclusion includes an average center point speed of 167.32 μm for the control group and 148.42 μm for the experimental group.

Introduction:

Depression is a multifaceted mental health disorder that is characterized by persistent and pervasive feelings of sadness, hopelessness, and a profound lack of interest or pleasure in previously enjoyed activities. Depression can significantly impact an individual's cognitive, emotional, and behavioral functioning, and its effects can manifest through both physical and psychological symptoms. The disorder can impair one's ability to perform daily activities, impair decision-making, and interfere with interpersonal relationships and work productivity. Depression could lead to urges of suicide, and suicide roughly kills around 14.04 people per every 100,000 people per year. Individuals experiencing distressing symptoms often turn to over-the-counter medications as a means of relief. However, while these medications may offer temporary relief, their prolonged usage may lead to adverse consequences. The labels of such medications, often containing phrases such as "naturally derived," may mislead individuals into believing that the medication is safe for consumption. However, it is imperative to consider the long-term effects of such medications on the body.

Pharmaceuticals that target depression, commonly known as antidepressants, are frequently administered to alleviate symptoms of various neurological disorders. These medications have proven to be effective in treating depression and its associated conditions.

Antidepressants are drugs known for their target on neurotransmitters, such as norepinephrine, dopamine, and serotonin, in hopes of relieving depressive symptoms. They are used widely in many psychiatric conditions, such as PTSD, schizophrenia, and depression.

Famous examples of antidepressants include SSRIs, Selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs), Tricyclic Antidepressants (TCAs), Monoamine Oxidase Inhibitors (MAOIs).

SSRIs are a very common prescription drug that has had numerous positive effects. They are known for inhibiting the reuptake of serotonin neurotransmitters, which enhances the effects by making them stay in the synaptic space. SNRIs work by inhibiting the reuptake of both serotonin and norepinephrine, alleviating the depressive symptoms of most disorders. TCAs' actions are similar to those of SNRIs, but they also affect histamine and acetylcholine.

5-HTP is a compound your body naturally produces from the amino acid tryptophan. It serves as a precursor to serotonin, a neurotransmitter involved in regulating mood, sleep, appetite, and other functions. When you ingest 5-HTP as a supplement, it can increase serotonin levels in your brain. This is because 5-HTP crosses the blood-brain barrier more readily than tryptophan, allowing for more efficient conversion to serotonin within the brain. Leading to mood improvement and may alleviate symptoms of depression, anxiety, and insomnia.

Methodology and Procedures:

Our experiment will involve the isolation of adult Caenorhabditis Elegans into two distinct groups. One group will serve as the control group with no added 5-Hydroxytryptopahn and only fed the E. Coli, while the other group will serve as the experimental group. Throughout this experiment, a light microscope will be utilized to view the C. Elegans. The initial movement of both groups will be observed using WormLab, and data will be recorded on our desmos graph.

The control group will receive 0.0125mL of OP-50 as a food source, while the experimental group will receive 0.0125 mL of a mixture of OP-50 and 200 milligrams of 5-Hydroxytryptophan as a food source. The control and experimental groups will be served their food sources between 8:20 am - 8:25 am, and 1:20 pm - 1:30 pm.

Both groups will be recorded twice daily before their feeding sessions. The first recording will be taken at 8:15 am -8:20 am, while the second recording will be taken at 1:15 pm- 1:20 pm. The recorded video will be examined under the WormLab website.

Using the tools provided in WormLab, the C. Elegans will be isolated into individuals, and multiple data sets will be recorded, respectively to their locomotive abilities. For this experiment, we will specifically focus on center point speed (um/s) and distance traveled forward (um). The data obtained will then be compared using a desmos graph and standard error bars using mean ± 2 SEM.

Finally, we will analyze the graphs and data obtained by WormLab to conclude whether we have successfully rejected the null hypothesis.

The following experiences state our difficulties in conducting the experiment. The anticipated time of arrival for Caenorhabditis elegans was postponed, leading to an unforeseen delay in its delivery. Given this unexpected circumstance, a revised agenda has been developed for conducting the experimentation. The updated schedule had been designed to accommodate the delayed arrival of the Elegans and ensure that the experimentation proceeded as planned without any further disruption. The observation of Elegans was also restricted to two instances only, owing to the tight school schedules.

In order to maintain safety during this experiment, we will sterilize our equipment and use gloves and masks when dealing with the C. Elegans. After each feeding session with OP50, we will sterilize our materials by heating them and cleaning them with Clorox wipes. Our workstation and microscope will be carefully cleaned after each use, and we will utilize the sterilizing techniques provided in our C. Elegans culturing kit from the Carolina Biological Supply.

Results:

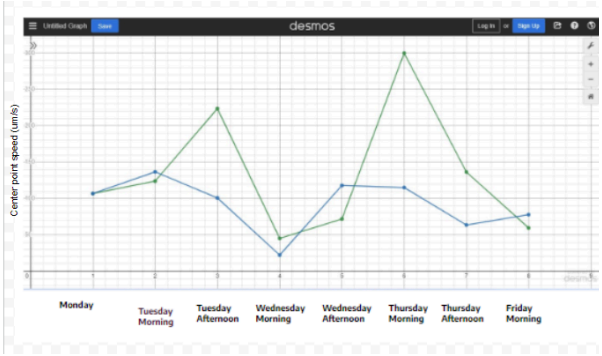

Figure 1:
Green - Control
Blue - Experimental
This graph demonstrates the comparison of central point speed (micrometers) between experimental and control throughout the week.

Track Number

	7	8	9
Mean Worm Length (um)	146.90	165.83	178.27
Mean Width (um)	16.14	9.39	13.42
Mean Area (um^2)	2450.28	1584.23	2433.21
Track Length (um) [Center point trajectory]	72.45	28.59	7.37
Center point Speed (um/s) [Center point trajectory/time]	103.50	142.95	73.69
Absolute Peristaltic Track Length (um) [forward + reversals]	66.60	28.18	6.67
Peristaltic Speed (um/s) [Absolute peristaltic track length/tim	95.14	140.92	66.66
Peristaltic Track Length (um) [forward - reversals]	37.53	22.48	3.59
Peristaltic Speed (um/s) [peristaltic track length/time]	53.62	112.38	35.92
Straight-line Distance (um)	22.89	17.19	1.96
Wavelength (um)	86.52	92.53	75.46
Mean Amplitude (um)	5.52	5.61	10.11
Max Amplitude (um)	9.97	9.86	10.70
Turn Count	1.00	0.00	0.00
Track duration (s)	0.70	0.20	0.10
Distance Traveled Forward(um)	52.07	25.33	5.13

Figure 2:
Monday's Data Chart
- ***Data set from both experimental and control group***
- ***C.elegans were not fed yet***
- ***106.693 um (central point speed)***

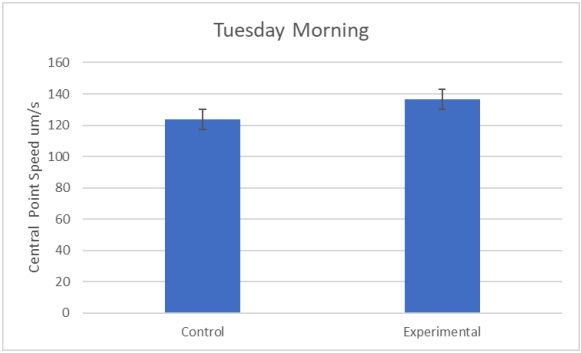

Figure 3: Tuesday Morning - Standard deviation of control and experimental groups

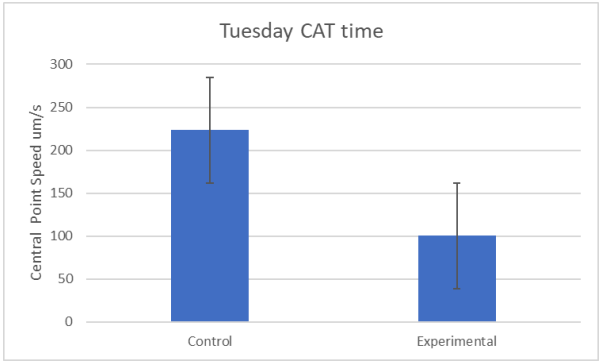

Figure 4: Tuesday Afternoon- Standard deviation of control and experimental groups

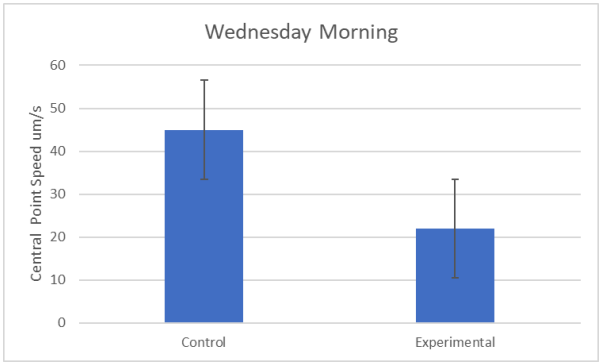

Figure 5: Wednesday Morning - Standard deviation of control and experimental groups

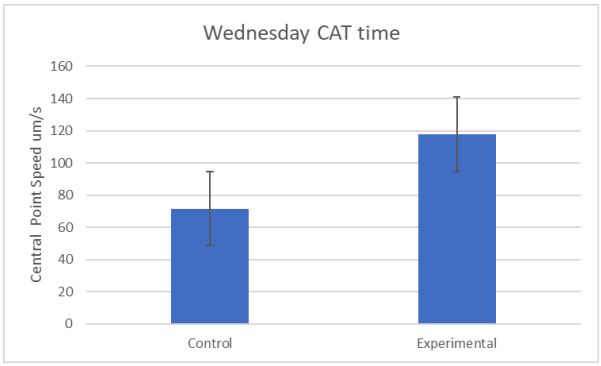

Figure 6: Wednesday Afternoon - Standard deviation of control and experimental groups

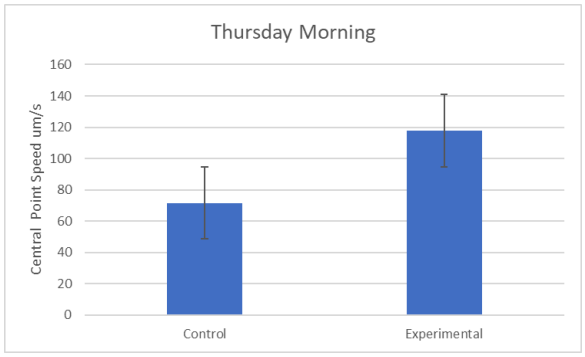

Figure 7: Thursday Morning - Standard deviation of control and experimental groups

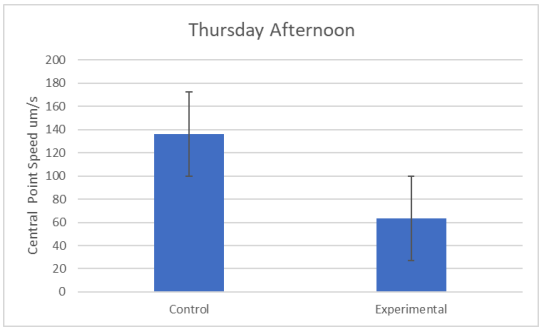

Figure 8: Thursday Afternoon- Standard deviation of control and experimental groups

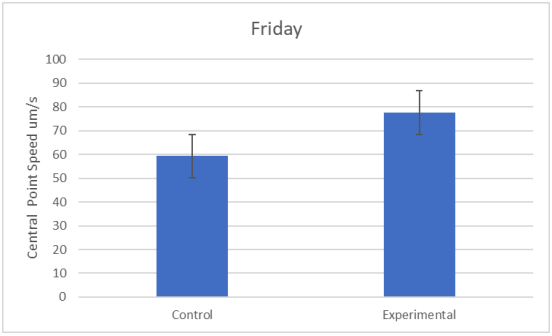

Figure 9: Friday - Standard deviation of control and experimental groups

Interpretation of results:

Our experiments on Caenorhabditis Elegans, a small nematode worm, have yielded some valuable insights. We conducted a comparative study between an experimental group and a control group, in which we observed the behavior of the worms under different conditions.

Firstly, we noticed that the center point speed of the experimental group was consistently lower than that of the control group. According to our data, the control group had an average center point speed of 167.32 um, while the experimental group had a lower speed of 148.42 um. This suggests that the experimental group may have been less active or slower in their movements than the control group as a long-term effect.

In addition, we observed a distinct movement pattern in the experimental group every time they were fed. This involved an initial increase in movement speed, followed by a gradual decrease, and finally, a steep drop in their speed. This pattern was not observed in the control group, indicating that the experimental group may have reacted differently due to the 5-HTP.

Furthermore, we examined the effects of 5-HTP on the experimental group and found that it takes immediate effect when given periodically with time in between. However, we did not observe any substantially noticeable effect when the doses were shared between the periodical times. This suggests that the 5-HTP needs to be carefully timed and administered at appropriate intervals to significantly impact the experimental group's behavior.

Our findings indicate that 5-HTP must be allocated at appropriate intervals to have a significant effect on patients.

Conclusion:

We had hypothesized that the experimental group of Caenorhabditis Elegans, after consuming the 5-Hydroxytryptophan drug, would have a decreased center point speed than the control group. We can confirm that our hypothesis was accurate upon analyzing the results.

Consuming serotonin, an important monoamine neurotransmitter that affects human mood, initially increases movement before decreasing center point speed. Therefore, limited intake of 5-Hydroxytryptophan is better than recurrent intake. Overusing serotonin causes fatigue, drowsiness, and, ironically, loss of motivation despite being an antidepressant.

If the experiment were to be replicated, we would separate both the control group and experimental group into just baby C.Elegans and observe the drug's impact on the Elegans' whole lifespan. To separate the adults and the babies, we will use bleach to kill the adults. We will also accommodate the school to lengthen the experiment to 2-week observations.

In conclusion, 5-Hydroxytryptophan is a significant drug that can help boost energy levels, but it should only be used periodically. The next time you buy an over-the-counter drug, don't be fooled by the label "naturally derived." Instead, research the drug's long-term impact and consult with a doctor or pharmacist before using any new medication.

Acknowledgments:

We express our sincere gratitude to Mr. Ewoldt for his invaluable guidance throughout the entire experimentation process. His insights and expertise proved to be instrumental in helping us navigate through the various challenges that arose along the way. Additionally, he took the time to thoroughly review our documentation and provided us with the necessary feedback to ensure that we were fully prepared to proceed with our project.

To facilitate our experiments, we procured a C. Elegans and E. Coli voucher kit from Carolina Biological Supply. We found their products to be of the highest quality, which greatly improved the accuracy and reliability of our results.

Furthermore, we were fortunate enough to borrow a high-quality light microscope from Lake Norman High School. This allowed us to closely observe and analyze the various specimens we were working with, which proved to be crucial in our research.

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