An Integrative Approach of Utilizing Antipsychotics Supplements Designated for Schizophrenia and Endogenous Firing Rate Differential Equation Models to Induce Synaptic Activity in PINK-1 gene Mutated *C. elegans* with Parkinson's Disease

Rajvi Babaria, Halley Compuesto, Mehak Gaba

Abstract— Parkinson's disease is characterized by the irreversible decline of dopamine, a neurotransmitter that regulates the nigrostriatal neuromodulatory system, which results in the rise of dysfunctionalities in motor cortical activity due to a series of kinematic changes. Another neurological disease is schizophrenia, which is associated with serotonergic and dopaminergic deviations, contributing to a multitude of cognitive deficits in conjunction with a dramatic decline of activity in the frontal cortex. Although there is no cure for schizophrenia, antipsychotics are provided for patients to ease the symptoms, in which two predominant supplements include Sarcosine and Tyrosine, amino acids that stimulate the release of vital neurotransmitters, including dopamine. Furthermore, research conducted in the past has suggested that probiotics, gut bacteria, can possibly lead to the enhancement of chemical messengers in the brain, however the research has not been solidified yet. Therefore, an experimentation was conducted that investigated the potential usage of the antipsychotic supplements designated for Schizophrenics for the treatment of Parkinson's disease in order to elevate the production of dopamine; the experimentation consisted of a *C. elegan* model wherein the three viable treatment supplements were tested on wildtype and PINK-1 mutated worms to test the effects of antipsychotic supplements meant for Schizophrenics on those suffering with Parkinson's. Through the utilization of endogenous firing rate differential equations, the findings suggested that though all three supplements were effective, Sarcosine displayed the most promising result due to increased reproduction rates and improved gait movement as well as increased synaptic activity in the PINK-1 mutated *C. elegans*.

Index Terms— Biochemistry, Neurodegenerative Diseases, Neurons, Neurophysiology, Mathematical Neuroscience, *C. elegans*

1 Introduction

Parkinson's disease is a neurodegenerative brain disorder caused by nerve cell damage within the brain that leads to a decline in dopamine levels; this reduction in dopamine can cause symptoms such as muscle tremors, stiffness, slow movement, and difficulty with balance. Furthermore, people suffering from Parkinson's also tend to "develop a parkinsonian gait that includes a tendency to lean forward, small quick steps as if hurrying forward, and reduced swinging of the arms" (NIH). However, since this is a degenerative disorder, symptoms progress over time and can eventually lead to imparities in cognitive function. To add, patients "exhibit progressive degeneration of dopaminergic neurons in the substantia nigra," which prohibits them from being able to function properly, ultimately leading to neuromuscular issues (Cooper, Van Raamsdonk). To investigate plausible therapeutic approaches to the disease, *C. elegan* models are utilized due to the "ease of genetic manipulation, ability to complete experiments rapidly, and ability to perform large scale screens" (Cooper, Raamsdonk). The model is efficient as the worms "exhibit multiple phenotypic deficits including the disruption of dopamine-dependent behaviors [and] deficits in movement," which is similar to the phenotypes displayed in humans with Parkinson's (Cooper, Raamsdonk).

One of the most effective ways to assess the locomotion of the worms is to examine their respective gait movements. Gait movement, for *C. elegans* particularly, involves a swim-crawl transition wherein it utilizes "GABAergic D-class neurons [for] forward locomotion," resulting in a variety of movements "on the ventral and dorsal sides of the body" (Boyle, Berri, Cohen). For those who suffer from Parkinson's, the cardinal features of the disease that they primarily encounter include gait disturbance and impairments associated with "balance and postural stability" (Shrivastava, Leahy). The alterations in gait kinematics in patients include the transition from an "adult gait pattern [to] a more primitive pattern," which is a result of the deactivation of muscles in conjunction with a substantial reduction in dopamine levels (Shrivastava, Leahy). Research conducted in the past has suggested that patients with Parkinson's report an inability to "generate sufficient stride length," which catalyzes a "diminished left-right bilateral coordination" (Hausdorff).

According to a study conducted by Strzelecki et al., Heads of Department of Affective and Psychotic Disorders at the University of Lodz, sarcosine, an adjunctive therapy used for patients with schizophrenia, "may increase serotonergic and dopaminergic transmission in prefrontal lobes and also in the hippocampus…" The dopamine modulates higher-order motor centers such as the basal ganglia and thus "reduces the influence of the indirect pathway, and increases the actions of the

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direct pathway within the basal ganglia" (Mandal). Similarly, tyrosine, used for maintaining essential levels of neurotransmitters in the brain, "increases levels of the neurotransmitters dopamine, adrenaline, and norepinephrine" (Walle). Probiotics, which are utilized to prevent severe bowel difficulty in schizophrenic patients, have also been shown to increase dopamine levels and allow the basal ganglia to "function at peak efficiency" (Mandal). Overall, the dopamine present in such supplements is necessary to regulate motor function in those with Parkinson's disease, as "dopamine receptors make the neurotransmitters move more easily" in Parkinson's patients, who lack adequate amounts of dopamine (Olopade).

2 Rationale

The goal of this project was to determine a plausible therapeutic intervention method for Parkinson's disease by understanding the neurophysiology of schizophrenia to conclude whether or not antipsychotic supplements designated for schizophrenic patients can potentially be used to alleviate symptoms associated with Parkinson's. This was accomplished through the utilization of *C. elegan* models to examine molecular mechanisms, endogenous firing rate models composed of differential equations to calculate synaptic activity , and data analysis techniques to measure gait movement, stride length, and angular movement on WormLab Software.

3 Hypothesis

The hypothesis was that inducing an initial concentration of 1 mL of sarcosine, a supplement used for schizophrenia, in *C. elegans* with a PINK-1 mutated gene can elevate the dopaminergic activity levels in the organism, thus increasing the firing rate of synapses, and in turn, serving as a plausible treatment method for Parkinson's by improving mitochondrial function.

4 Procedure

4.1 Preparation of Petri Plates

1. Measured 800 mL of agar solution into a sterile beaker in preparation for melting
2. Microwaved the beaker with the agar solution in 30-second intervals until the solution was completely liquified with no clumps
3. With a Hot Hand rubber mitt, removed from heat and let sit for 20 minutes
4. Using the clamping method, poured melted agar into the Petri dish (20 total) to cover the bottom (about a quarter of the way) and replaced the lid immediately
5. Allowed the agar to cool for approximately 10 minutes until solidified

4.2 Seeding the Plates

1. Added 0.05 mL of E. coli OP50 to the Petri plates by using a pipette
2. Spread the drop of E. coli throughout the center of the Petri plate using the tip of the pipette
3. Wrapped Parafilm around the Petri plates to ensure the plates were air-tight and allowed for the E.coli OP50 to grow for a night

4.3 Transferring *C. elegans* onto the plates

1. Utilized a chunking method to transfer the worms from the seeded plates to new Petri plates that would then be used for synchronization
2. Used a sterilized scalpel to remove a chunk of the agar from the seeded plate to a new plate (did this for three Petri plates: one for each strain of *C. elegans*)
3. Kept the plates in an incubator at room temperature and waited one day

4.4 Synchronization of *C. elegans* for egg prep

1. Poured 5 mL of the M9 buffer solution onto each of the three Petri plates (one with N2 strain, one with RB2547 strain, and one with PE867 strain) and swirled the plates to dislodge the worms by gently moving the plate in a clockwise direction on a table
2. Transferred the worms from each plate to three 15 mL test tubes using three pipettes
3. Centrifuged the 3 test tubes for approximately one minute on high power, leaving a pellet of worms at the bottom of each test tube
4. Withdrew the M9 solution from each test tube using pipettes without aspirating the pellets
5. Added 15 mL of the Alkaline Hypochlorite solution to each of the test tubes
6. Inverted the test tubes gently for 3 minutes in order to eliminate the adult worms
7. Centrifuged the test tubes again for 1 minute at max power
8. Withdrew the Alkaline Hypochlorite solution from each test tube using pipettes without aspirating the pellets
9. Added 15 mL of M9 buffer solution to the test tubes and stirred using a glass stirring rod
10. Centrifuged the test tubes for 1 minute at max power
11. Withdrew the M9 solution from each test tube using pipettes without aspirating the pellets
12. Repeated the process of adding 15 mL of M9 buffer solution, centrifuging, and aspirating the M9 two times
13. Added 7 mL of M9 buffer solution to each test tube
14. Placed the test tubes in an incubator that gently rocked the tubes overnight
15. Distributed the liquids into three separate pre-seeded Petri plates

4.5 Preparation of the Supplement Solution

16. Labeled a test tube for Sarcosine, Tyrosine, and Probiotic Supplement
17. Emptied 1 capsule of Sarcosine 500 mg supplement into a sterile test tube
18. Added 9 mL of saline solution with a pipette into the test tube
19. Swirled solution gently
20. Repeated with one Tyrosine 500 mg capsule, and one 500 mg capsule probiotic supplement.
21. Placed each test tube on a vortex mixer at medium speed until the solution was fully dissolved.

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4.6 Analysis of the *C. elegans* prior to the induction of the supplements

1. Recorded the number of eggs present in each Petri plate of the N2 *C. elegans*, RB2547 *C. elegans*, and the PE867 *C. elegans* by placing the Petri dishes under a microscope to measure the reproduction rate of the worms
2. Recorded the stride length of the worms in each Petri plate as well as the changes in the angle of the worms' bodies in the span of 5 seconds to measure the gait movement

4.7 Testing the supplements

1. Used a pipette to drop 0.6 M of the Sarcosine and saline solution into three plates: one plate with the N2 *C. elegans*, one plate with the RB2547 *C. elegans*, and one plate with the PE867 *C. elegans*
2. Placed the three plates into an incubator at room temperature and waited one day
3. Used a pipette to drop 0.3 M of the Tyrosine and saline solution into three plates: one plate with the N2 *C. elegans*, one plate with the RB2547 *C. elegans*, and one plate with the PE867 *C. elegans*
4. Placed the three plates into an incubator at room temperature and waited one day
5. Used a pipette to drop 1 mL of the probiotic and saline solution into three plates: one plate with the N2 *C. elegans*, one plate with the RB2547 *C. elegans*, and one plate with the PE867 *C. elegans*
6. Placed the three plates into an incubator at room temperature and waited one day

5 Figures

In order to deduce the effectiveness of the treatments, the reproduction rate, stride length (distance traveled in centimeters in one sinusoidal period, derivative of the angle of the worms' heads from the centerline with respect to time, and the firing rate or temporal average.

5.1 Prior to Treatment

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
Reproduction rate (eggs/day)	51	6	12

Fig. 1. Reproduction rate of each strain

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
Stride length (distance traveled in cm in one sinusoidal period)	2.54 cm	1.524 cm	1.667 cm

Fig. 2. Stride length of each strain

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
Derivative of the angle of the worms' heads from the centerline with respect to time	20 degrees/sec	5 degrees/sec	6 degrees/sec

Fig. 3. Derivative of the angle of the worms' heads from the centerline with respect to time of each strain

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
Firing rate (temporal average) $Vk = Nk^{40}/T$ (APs/sec)	2.2 APs/sec	0.2 APs/sec	0.5 APs/sec

Fig. 4. Firing rate or temporal average of each strain

5.2 After Treatment

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
1 mL of Sarcosine	78	17	20
1 mL of Tyrosine	69	12	17
1 mL of Probiotics	56	9	16

Fig. 5. Reproduction rate of each stain after being induced with Sarcosine, Tyrosine, and Probiotics

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
1 mL of Sarcosine	3.16 cm	1.619 cm	1.781 cm
1 mL of Tyrosine	3.05 cm	1.606 cm	1.752 cm
1 mL of Probiotics	2.96 cm	1.575 cm	1.679 cm

Fig. 6. Stride Length of each stain after being induced with Sarcosine, Tyrosine, and Probiotics

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
1 mL of Sarcosine	32 degrees/sec	11 degrees/sec	12 degrees/sec
1 mL of Tyrosine	29 degrees/sec	9 degrees/sec	8 degrees/sec
1 mL of Probiotics	31 degrees/sec	7 degrees/sec	8 degrees/sec

Fig. 6. Derivative of the angle of the worms' heads from the centerline with respect to time of each strain after being induced with Sarcosine, Tyrosine, and Probiotics

	N2 Strain *C.elegans*	RB2547 Strain *C.elegans*	PE867 Strain *C.elegans*
1 mL of Sarcosine	2.8 APs/sec	0.6 APs/sec	0.7 APSs/sec
1 mL of Tyrosine	2.6 APs/sec	0.4 APs/sec	0.6 APS/sec
1 mL of Probiotics	2.5 APs/sec	0.3 APs/sec	0.6 APs/sec

Fig. 7. Firing rate of each strain after being induced with Sarcosine, Tyrosine, and Probiotics

5 Results

Prior to the treatment of the three supplements, the worms displayed differing reproduction rates wherein N2 strain *C. elegans* had a reproduction rate of 51 eggs/day, the RB2547 mutated strain *C. elegans* had a reproduction rate of 6 eggs/day, and the PE867 mutated strain *C. elegans* had a reproduction rate of 12 eggs/day. After inducing a concentration of 1

mL of Sarcosine on the worms, all the worms showed signs of improvement in the rate by which they produced eggs, delineating the idea that their progression rate of hormone production was far more efficient in comparison to the production rate before the trials were executed. This is noticeable as after the worms consumed the sarosine, the reproduction rate of the N2 strain *C. elegans* rose to 78 eggs/day, the reproduction rate of the RB2547 mutated strain *C. elegans* rose to 17 eggs/day, and the reproduction rate of the PE867 mutated strain *C. elegans* rose to 20 eggs/day. Similarly, with tyrosine and probiotics, the number of eggs produced each day for each worm increased. Prior to the treatment of the three supplements, the worms also displayed varying stride lengths or different distances travelled in centimeters in one sinusoidal period wherein the stride lengths for the N2, RB2547, and PE867 were 2.54 cm, 1.524 cm, and 1.667 cm respectively. By dropping 1 mL of Sarcosine to the petri dishes of the N2, RB2547, and PE867, the stride lengths improved to 3.16 cm, 1.619 cm, and 1.781 cm respectively. The same improvements were observed with the tyrosine and probiotics, however, the changes were less dramatic. This expressed the idea that the worms experienced positive changes in their motor cortical activity as they were able to move at faster speeds. Furthermore, prior to the treatment of the three supplements, the worms displayed differing changes of the angle of their heads from the centerline with respective to time or in other words, different derivatives of the angles of their heads; initially, the N2, RB2547, and PE867 had angular movements of 20 degrees/sec, 5 degrees/sec, and 6 degrees/sec respectively. By interacting with the three supplements, the *C. elegans* were shown to move their heads laterally at faster rates due to the increase in the speed by which the angle between their heads and the centerline increased. For instance, with the induction of the 1 mL concentration of sarcosine, the N2, RB2547, and PE867's derivatives of angular movement increased to 32 degrees/sec, 11 degrees/sec, and 12 degrees/sec. The same improvements were once again observed in the trials with Tyrosine and Probiotics. Finally, by utilizing the firing rate differential equations, the temporal average was calculated, which displayed a rapid increase in the number of action potentials produced per sec by each worm after the induction of the sarcosine, tyrosine, and probiotics. For instance, the action potentials per second for the N2, RB2547, and PE867 increased from 2.2 APs/sec to 2.8 AP/sec, 0.2 APs/sec to 0.6 APs/sec, and 0.5 APs/sec to 0.7 APs/sec respectively.

5 Conclusion

Based on the findings displayed the data tables, the Sarcosine, Tyrosine, and probiotics resulted in improvements in gait movement and motor cortical coordination due to the mutated worms' abilities to travel farther distances, change the angular movement of their heads faster, reproduce eggs at faster rates, and produce action potentials faster within the span of one second after being interacted with the three supplements, The data showed that Sarcosine was the most promising approach to treating Parkinson's in comparison to the Tyrosine and Probiotics due to the more substantial changes in synaptic activity, expressing the idea that the mutated worms were able to improve their muscle coordination when being compared to before the execution of the trials and after the execution of the trials. The aggregated data suggests that the drastic decline in dopamine levels in the brain due to Parkinson's can be countered with the elevated levels of dopaminergic neurons that are stimulated upon the interactions between the supplements, mainly Sarcosine, and the *C. elegans*.

7 End Sections

7.1 Acknowledgments

We would like to thank Rhonda Christman for providing us support with the procedure as well as letting us use her lab and equipment before and after school.

7.1 References

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