Undergraduate Research Symposium
ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT
PROJECT 32

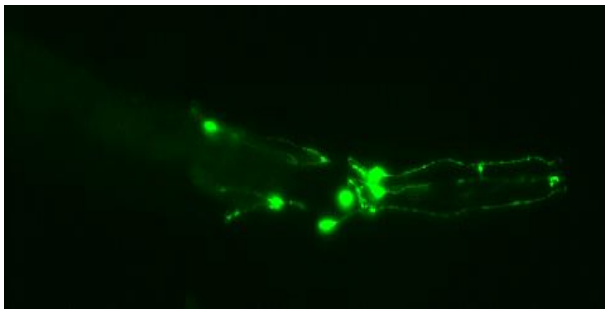
Shivani Mody

Drew University, Class of 2021
Major: **Neuroscience**
Minor: **Chemistry**

Faculty Advisor: **Marvin Bayne, Ph.D.,** Faculty Mentor
Research Institute for Scientists Emeriti (RISE)

Modelling Parkinson's Disease in C. elegans

My project focuses on *Caenorhabditis elegans* models of Parkinson's Disease and observing the effect of human LRRK2 protein on dopamine neuron degeneration. *C. elegans* are small nematodes that have 302 neurons, 8 of which are dopamine neurons. My objective is to identify robust behavioral assays with *C. elegans* mutants to identify drugs or genes that can reduce and potentially prevent the degeneration of dopaminergic neurons.



Strain	Genotype
N2	WT
MT15620	cat-2 (biosynthesis of
LX703	dop-3 (dopamine receptor)
RM2702	dat-1 (dopamine transporter)
JVR168	LRRK2
JVR203	alpha-synuclein
VM6365	ICE (Caspase-1)

Previously, moat assays were performed to test swim to crawl behaviors on different *C. elegans* strains. To create these moats, prototypes were generated using 3D printing. The SWIP assay was also performed to induce paralysis in different *C. elegans* mutants using nisoxetine hydrochloride (NIS), a norepinephrine transporter (NET) inhibitor. NIS is known to paralyze N2 wildtypes and dat-1 mutants, causing the Swimming-Induced Paralysis (SWIP) phenotype. Worm movement and paralysis were analyzed with the help of Matlab software.

Currently, I seek to verify that the JVR168 mutants have no preference for ethanol via ethanol avoidance behavioral assays. For these experiments, I use N2 wildtypes for my control and JVR168 mutants that express human LRRK2 protein in dopamine neurons. The predicted results are for wildtypes to avoid ethanol and for JVR168's to show a lack of preference to ethanol. Daily activities include treating *C. elegans* with alkaline hypochlorite solution (bleaching) in order to synchronize worms at L1 (larval 1) stage. Additional activities include making plates to use for assays, preparing plates on the day of assays, and running assays. In my most recent experiments, I have observed a lack of ethanol preference from JVR168 mutants. This can be correlated with the loss of green fluorescent protein (GFP) staining which revealed that the JVR168 mutants exhibited a loss of dopamine neurons as they age. Performing behavioral assays can enable us to screen LRRK2 inhibitor compounds to correlate the loss of LRRK2 activity and the loss of GFP observed in dopamine neurons to prevent or treat the loss of dopamine neurons in Parkinson's Disease.

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Acknowledgments: ***Drew University; Drs. Van Raamsdonk and Maricq; Caenorhabditis Genetic Center; Dr. Minjoon Kouh; Katelynn Fleming; RISE Department.***

Modelling Parkinson's Disease in *C. elegans*

Shivani Mody (Mentor: Dr. Marvin Bayne)
Drew University, Madison, NJ, 07940

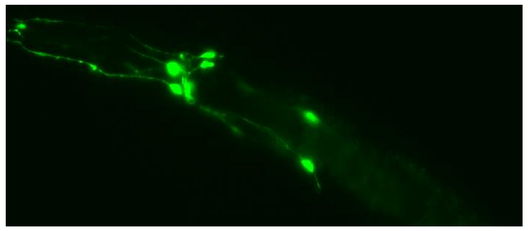
Parkinson's Disease

Parkinson's Disease is a neurodegenerative disorder that primarily affects movement, and can cause tremors, muscle stiffness, cognitive issues, and psychological issues. Dopamine neurons in the substantia nigra region of the brain degenerate in Parkinson's, which can be associated with movement problems in patients.

Symptoms in Parkinson's patients worsen as the loss of dopamine neurons increases over time.

Parkinson's is also characterized by mutations in LRRK2 protein and aggregates of alpha-synuclein protein. My project focuses on *Caenorhabditis elegans* models of Parkinson's Disease and observing the effect of human LRRK2 protein on dopamine neuron degeneration. *C. elegans* are small nematodes that have 302 neurons, 8 of which are dopamine neurons.

My objective is to identify robust behavioral assays with *C. elegans* mutants to identify drugs or genes that can reduce and potentially prevent the degeneration of dopaminergic neurons.



Previous Work

Moat Assay

3D printing was done to create combs to make a ditch in NGM agar plates, to test swim to crawl behaviors on different *C. elegans* strains. I used N2 wildtypes, age-dependent dopamine mutants, and dopamine deficient mutants, with the help of diacetyl, and other attractants to perform the swim-to-crawl behavioral moat assays.

Ethanol Avoidance

Research studies have shown ethanol avoidance assays to be the most reliably reproduced of most behavioral assays modelled for Parkinson's disease. This year, my focus shifted to reproducing ethanol avoidance assays. My goal for these experiments is to show that N2 wildtypes will prefer to avoid ethanol quadrants while JVR168 mutants will show no preference to ethanol or no ethanol quadrants. I will continue to investigate the loss of dopamine neurons in Parkinson's Disease by correlating *C. elegans* behaviors in the presence of ethanol with the loss of dopamine neurons.

Methods

Activities include treating *C. elegans* with Alkaline Hypochlorite Solution (Bleaching) in order to synchronize worms at L1 stage, which has to be generated weekly.

Activities include making NGM agar plates to use for assay, preparing plates on the day of assay, and running assays.

SWIP Assay

The SWIP assay was also performed to induce paralysis in different *C. elegans* mutants using Nisoxetine Hydrochloride (NIS), a Norepinephrine Transporter (NET) inhibitor. NIS is known to paralyze N2 wildtypes and dat-1 mutants, causing the Swimming-Induced Paralysis (SWIP) phenotype.

I also wanted to test the effects of NIS on young JVR203 mutants (normal dopamine levels) vs. adult JVR203 mutants (lower dopamine levels), to correlate the age-dependent loss of dopamine neurons with observed worm paralysis, because I predicted that the young JVR203 worms (with normal dopamine levels) would paralyze immediately when placed in NIS like the N2 and dat-1 mutants. The mechanism of SWIP initiates when the dat-1 transporter carries dopamine to synaptic sites, activating the dop-3 receptor on cholinergic muscle cells which leads to the paralysis of *C. elegans* dat-1 mutants, inducing the SWIP phenotype. Dat-1 transporter and dop-3 receptors are responsible for inducing or reversing SWIP.

Understanding the effects of NIS inhibitor on different *C. elegans* mutants is an important aspect of my research because my long-term goal is to screen compounds to find an optimal antagonist like mazindol to reverse the effects of SWIP that could be applied to Parkinson's research.

Current *C. elegans* strains for PD

Strain	Genotype
N2	WT
MT15620	Cat-2 (biosynthesis of dopamine)
LX703	Dop-3 (dopamine receptor)
RM2702	Dat-1 (dopamine transporter)
JVR168	LRRK2
JVR203	alpha-synuclein
VM6365	ICE (Caspase-1)

Results

Moat Assay: An average of 20% N2 wildtypes crossed the ditch to the attractant side of the plate. VM6365 worms mostly remain where they are plated and/or in the ditch.

SWIP Assay: Results were inconclusive for dat-1 mutants. Results for N2 and JVR203 worms were consistent with hypothesis. N2 worms exposed to 500uM and 1000uM concentrations of NIS displayed an average of 70% and greater paralysis. Young JVR203 worms displayed a paralysis of greater than 70%, while adult JVR203 worms had an average paralysis of less than 40% in 500uM NIS.

Ethanol Avoidance Assay: N2 wildtypes showed strong avoidance behaviors to ethanol, while JVR168's showed greater avoidance than the predicted no preference to ethanol behavior.

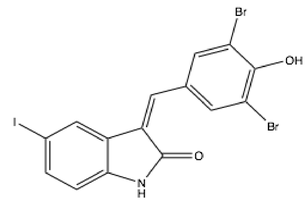
Future Plans

In addition to my continued Parkinson's research, I would like to begin performing basal slowing response behavioral assays. *C. elegans* display an enhanced slowing response when they are placed on a plate with bacteria after being food-deprived for thirty minutes. This locomotory behavior is dependent on dopamine-sensing pathways in the animal's neural circuit (Sawin, et al. 2000) in response to food. Meanwhile, the disruption of dopamine-sensing pathways has been found in Parkinson's Disease. I will compare the basal slowing response behaviors of wildtype mutants that have normal dopamine levels to cat-2 (MT15620) mutants that are dopamine deficient, because of an inactive biosynthetic dopamine pathway. I aim to make progress with correlating the loss of dopamine neurons with the help of LRRK2 inhibitor compounds in my future work.

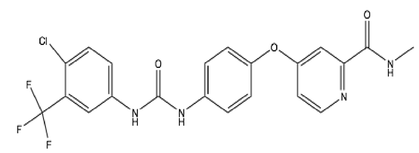
Discussion

Performing behavioral assays can enable us to screen LRRK2 inhibitor compounds to correlate LRRK2 toxicity and the loss of GFP observed in dopamine neurons to prevent or treat the loss of dopamine neurons in Parkinson's Disease. Mutations in LRRK2 cause increased LRRK2 kinase activity and the degeneration of dopamine neurons (Liu, et. al). The most stimulatory LRRK2 inhibitors identified in literature for the treatment of Parkinson's Disease are GW5074, Sorafenib, and LRRK2-IN2, which have been shown to increase dopamine survival.

LRRK2 inhibitors



GW5074 (Liu, et al.)



Sorafenib (Liu, et al.)

Acknowledgements

I would like to thank Drew University, Drs Van Raamsdonk and Maricq for providing worm strains, the Caenorhabditis Genetic Center, Dr. Minjoon Kouh for help with Matlab software, Katelynn Fleming for help with 3D printing, my mentor Dr. Marvin Bayne and the RISE department for continuously supporting my endeavors.

References

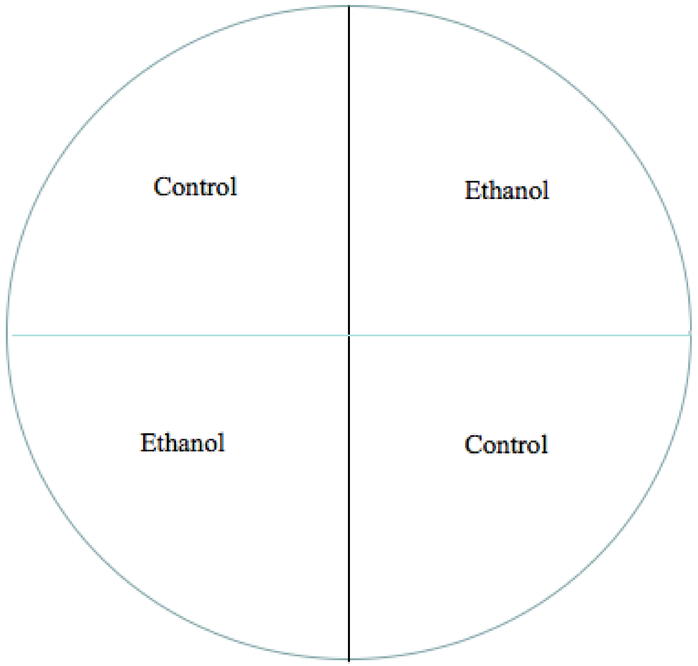
Liu, Zhaohui, et al. "Inhibitors of LRRK2 Kinase Attenuate Neurodegeneration and Parkinson-like Phenotypes in Caenorhabditis Elegans and Drosophila Parkinson's Disease Models." *Human Molecular Genetics*, vol. 20, no. 20, 18 July 2011, pp. 3933–3942,academic.oup.com/hmg/article/20/20/3933/695989, 10.1093/hmg/ddr312.

Sawin, Elizabeth R, et al. "C. Elegans Locomotory Rate Is Modulated by the Environment through a Dopaminergic Pathway and by Experience through a Serotonergic Pathway." *Neuron*, vol. 26, no. 3, June 2000, pp. 619–631, www.sciencedirect.com/science/article/pii/S089662730081199X?via%3Dihub, 10.1016/s0896-6273(00)81199-x.

"Sorafenib." *MedchemExpress.Com*, www.medchemexpress.com/Sorafenib.html.

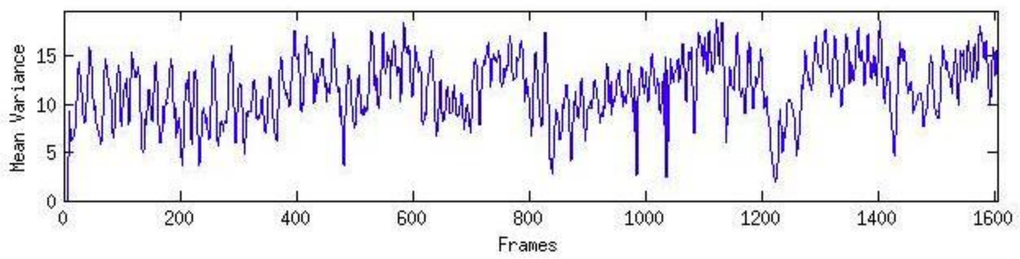
Ethanol Avoidance Assay

- NGM agar plates were used to perform the assay. 100% Ethanol was added to Ethanol quadrants, and control quadrants remained ethanol-free.
- Plates would sit for at least an hour before running experiment
- Young adult worms were washed in M9 buffer three times before plating in the center of the plate
- After the worms in M9 buffer in the middle of the plate had dried, worms were given 30 minutes to move
- After running several assays, I found that N2 wildtype worms showed strong avoidance to ethanol behaviors; JVR168 worms preferred to remain in control over ethanol quadrants, even though they did not show strong avoidance behaviors like N2 worms displayed

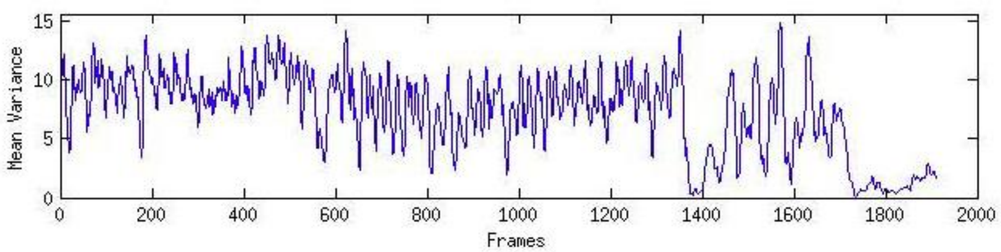


SWIP Assay

- NIS (Nisoxetine Hydrochloride) stock concentrations were made and stored at -20 degrees Celsius dilutions to 500uM and 1000uM were made on the day of experiments
- On the day of the experiments, 500uM and 1000uM dilutions were made using NIS stocks and kept at room temperature for about an hour before use
- NIS solution was added to 24-well plates; 5-10 worms were added to each well
- A phone was used to help record the movement of the worms on the microscope with experiments lasting from 10-20 minutes
- Initially, data was analyzed with the help of Matlab software; Data was also collected and graphed to obtain average percent paralysis of worms



Cat-2 (with an inactive dopamine pathway) worms displayed continuous movement during the recording when exposed to 500uM of NIS.



N2 (normal dopamine levels) worms began to slow down towards the end of the 10 minute period, as you can see the mean variance began to bottom out at the end.