Project Proposal

Project Title: Parental Pharmacological Modulation of Serotonergic Signaling in C. elegans to analyze hereditary suicide.

Author: Charkam, Sahasra

Date: 10/22/2025

Project Description:

Using mediated drug therapy to disrupt *SLC6A4* function in adult C. Elegans to study how hereditary serotonin transporter deficits influence molecular and behavioral markers linked to hereditary suicide vulnerability in adolescent C. Elegans. I hypothesis that if the serotonin transporter molecules are decreased in adult parenteral organisms then their offspring will show decreased levels of suicidal inclinations anti-depressant medication such as SSRI work to prevent SERT reuptake of serotonin signaling molecules which promote an increase in serotonin signaling molecules.

Background:

How does the altering of adult parental C. Elegans serotonin transporter (SLC6A4/SERT) impact the behavioral and biological presence of suicidal ideation in adolescents? If the serotonin transporter molecules are decreased in the adult parenteral organism then their offspring will show decreased levels of suicidal inclinations anti-depressant medication such as SSRI work to prevent SERT reuptake of serotonin signaling molecules which promote an increase in serotonin signaling molecules, leading to a return of normal conditions within the offspring of the treatment groups.

Background:

Suicide

Fifty-five percent of suicide risk is heritable, independent of psychiatric disorders **(Voracek et al and Statham et al).** That means children of suicidal parents are left with no hope, biologically primed with lower serotonin activity and impulsive emotional dysregulation. Yet no research has targeted the biological pathways of hereditary suicidal ideation. Suicide is a polygenetic disease that is linked to Major Depressive Disorder.

There are shared 18 co-expression gene molecules in both the peripheral blood and the postmortem brain tissue (specifically white matter) enriched with genes that are related to inflammation, immune response and epigenetic regulation which are all process that can be epigenetically inherited (Sun et al.).

Serotonin Transporter Biological Pathway

The Seortonin Transporter Pathways (SERT) is a part of the solute carrier family 6 member 4 (SLC6A4 which regulates serotonin (5-HT) levels in the brain. The SERT gene encodes for this transporter, with the different variations influencing how serotonin signaling

works and how patients respond to medication (Gulfishan et al). Serotonin abnormalities in the 5HT system have been linked to suicidal thoughts and actions through measurements of serotonin/main metabolite 5-hydroxyindoleacetic acid (5HIAA) in the CSF and blood; postmortem brain studies; analysis of serotonin receptor subtype in blood platelets of suicidal patients; and neuroendocrine challenge tests that probe serotonin function (Pandey et al).

In C. Elegans serotonin controls locomotion, pharyngeal pumping, chemotaxis, and more food-related behaviors and physiologies (Yu et al., 2022). Serotonin biosynthesis has been shown to be produced through hydroxylation of tryptophan by TPH-1 (analogous to mammalian TPH1/2 in humans). The hydroxylase synthesizes tryptophan into 5-HTP, which is then converted into serotonin by the BAS-1 (an aromatic amino acid decarboxylase). Specifically, the neurons NSM, ADF, and HSN are produced. The newly synthesized serotonin is packaged into synaptic vesicles by vesicular monoamine transporter (VMAT). During neuronal activation the vesicles fuse with the membrane releasing serotonin into the synapsis or extra synaptic space.

There are two types of anti-depressants: SSRIs (i.e. sertraline) which works by blocking the SERT protein function which directly increases serotonin molecules present in the brain. Another type of anti-depressants is noradrenergic (i.e. Mirtazapine) which works by blocking specific receptors in the brain to increase activity of norepinephrine and serotonin (which are neurotransmitter molecules involved in mood and regulation).

Pharmaceutical Modulation

Reserpine is a drug that will be used to reduce the serotonergic levels of the P0 generation to individually bring the tph-1 GFP C. elegan strains (a genetically modified strain where the promoter of tph-1 gene drives the expression of green fluorescent protein) to a baseline biological depression state, from which Reserpine will be used to block the VMAT to reduce serotonin signaling. To increase serotonin signaling, fluoxetine, a type of SSRI was used, which blocks the reuptake of serotonin transmitters, allowing them to build up in the extracellular matrix.

C. Elegans

Caenorhabditis elegans (C. Elegans) are transparent, microscopic nematode who have a fully mapped out nervous system (302 neurons, ~ 50 glia) and a completely sequenced genome. Despite its simplicity, C. elegans exhibit highly complex behaviors such as feeding, locomotion, chemotaxis, and arousal, which are modulated by serotonin (5-HT) and other neurotransmitters. The model organism's transparent body allows live imaging of their neuronal activity, and its short generation time allows cross-generational studies of inherited neurochemical and behavioral changes. Sengupta and Samuel (2009) highlight how C. Elegans uniquely bridges molecular, cellular, and systems of neuroscience, which enables researchers to link neurotransmitter-level changes to behavioral outcomes within the fully defined network (Sengupta & Samuel, 2009) .

Experimental Design/Research Plan Goals:

Materials List

Procedure

1. Preliminary Data
    a. Synchronization of Worm Population
        i. Follow MIT C. Elegan synchronization via bleaching procedure
    b. Baseline Behavior Assay
        i. Thrashing Assay
        ii. Pharyngeal Pumping
        iii. Chemotaxis
    c. Statistical Probabilities
        i. Take collected data and format it into
2. Induce Depression in Worms
    a. Apply Serotonin-Delating Reserpine
3. Apply External Drug Treatment
    a. Down-regulation Treatment (worsen depression through more reserpine)
    b. Up-Regulation Treatment (Rescue – increase fluoxetine, SSRI, to retore serotonin function)
4. Behavioral Testing of Treaded P0 generation
    a. Thrashing, Pumping, Chemotaxis
    b. Compare results to baseline to identify depression phenotypes
5. Transgenerational testing of F1 Generation
    a. Breed P0 worms
    b. Test F1 (no drugs given) for behavioral assays
        i. Thrashing, pumping, and chemotaxis
6. Serotonin measurement (molecular validation)
    a. Visualize Serotonin-Production neurons
    b. Serotonin Straining (Ant serotonin Antibodies)
    c. Combine behavioral and molecular data of treatment group A and B

Independent Variable: Serotonin Transporter Gene transcription/translation/expression
Dependent Variable: Serotonin Neurotransmitter levels and Behavioral Analysis
Controls: Normal destressed organisms with offspring with no genetic variation

Two runs of the project to ensure

Risk/Safety Concerns:

Potential Safety Concerns include the health of the c. elegans, making sure they are not harmed, and stay safe.

Data Analysis:

The data will be analyzed through Behavioral Analysis like how mice are tested through comparison in sucrose testing, and other tests. Serotonin molecular levels through antibody staining will also be analyzed to compare behavioral analysis to the neurotransmitter levels.

Timeline: (with action steps identified- sub-deadlines will continue to evolve):

1. Preliminary Data (November – December)
    a. Synchronization of Worm Population
        i. Follow MIT C. Elegan synchronization via bleaching procedure
    b. Baseline Behavior Assay
        i. Thrashing Assay
        ii. Pharyngeal Pumping
        iii. Chemotaxis
    c. Statistical Probabilities
        i. Take collected data and format it into
2. Induce Depression in Worms (January 10th needs to be done)
    a. Apply Serotonin-Delating Reserpine
3. Apply External Drug Treatment (January 13th)
    a. Down-regulation Treatment (worsen depression through more reserpine)
    b. Up-Regulation Treatment (Rescue – increase fluoxetine, SSRI, to retore serotonin function)
4. Behavioral Testing of Treaded P0 generation (January 18th deadline)
    a. Thrashing, Pumping, Chemotaxis
    b. Compare results to baseline to identify depression phenotypes
5. Transgenerational testing of F1 Generation (January 23rd)
    a. Breed P0 worms
    b. Test F1 (no drugs given) for behavioral assays
        i. Thrashing, pumping, and chemotaxis
6. Serotonin measurement (molecular validation) (January 27th)
    a. Visualize Serotonin-Production neurons
    b. Serotonin Straining (Ant serotonin Antibodies)
    c. Combine behavioral and molecular data of treatment group A and B
7. Create Poster Board + Graphs (Fenuary 15th – Science Fair)

References: (Check citations with APA guidelines)

1. Statham DJ, Heath AC, Madden PA, Bucholz KK, Bierut L, Dinwiddie SH, et al. Suicidal behaviour: an epidemiological and genetic study. Psychol Med. 1998;28:839–55.

2. Voracek M, Loibl LM. Genetics of suicide: a systematic review of twin studies. Wien Klin Wochenschr. 2007;119:463–75.

3. Gulfishan, S., Halder, S., Kar, R., Srivastava, S., & Gupta, R. (2022). Association of serotonin transporter gene polymorphism with efficacy of the antidepressant drugs sertraline and mirtazapine in newly diagnosed patients with major depressive disorders. Human Psychopharmacology: Clinical and Experimental. https://doi.org/10.1002/hup.2833

4. Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., He, X., Liu, M., Li, P., & Yu, T. (2023). CRISPR/Cas9 therapeutics: Progress and Prospects. Signal Transduction and Targeted Therapy, 8(1), 1–23. https://doi.org/10.1038/s41392-023-01309-7

5. Pandey, G. N. (2013). Biological basis of suicide and suicidal behavior. Bipolar Disorders, 15(5), 524–541. https://doi.org/10.1111/bdi.12089

6. O. Doszyn, Dulski, T., & J. Zmorzynska. (2024). Diving into the zebrafish brain: exploring neuroscience frontiers with genetic tools, imaging techniques, and behavioral insights. Frontiers in Molecular Neuroscience, 17. https://doi.org/10.3389/fnmol.2024.1358844

7. Sun, Shengnan, et al. “Brain and Blood Transcriptome Profiles Delineate Common Genetic Pathways across Suicidal Ideation and Suicide.” Molecular Psychiatry, 26 Jan. 2024, pp. 1–10, www.nature.com/articles/s41380-024-02420-z#:~:text=Findings%20support%20a%20role%20of, https://doi.org/10.1038/s41380-024-02420-z.

8. Sengupta, P., & Samuel, A. D. T. (2009). C. elegans: a model system for systems neuroscience. *Current Opinion in Neurobiology*, *19*(6), 637–643. https://doi.org/10.1016/j.conb.2009.09.009

9. Yu, J., Vogt, M. C., Fox, B. L., Wrobel, C. J. J., Diana Fajardo Palomino, Curtis, B. J., Zhang, B., Le, H. H., Arnaud Tauffenberger, Hobert, O., & Schroeder, F. C. (2022). *Parallel pathways for serotonin biosynthesis and metabolism in C. elegans*. *19*(2), 141–150. https://doi.org/10.1038/s41589-022-01148-7