Parental Pharmacological Modulation of Serotonergic Signaling in *Caenorhabditis elegans* to Analyze Hereditary Suicide

Grant Proposal

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Author Note

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Abstract

With hereditary suicide playing such a major role in the world today, this research works to initialize the first steps of finding a cure for biological suicide. As of now, there are models of hereditary suicide, primarily focused towards the psychological aspects, and less the biological field. Even the ones focusing on biological suicide fail to compare effective treatments to prevent suicide from being passed down through generations. The research project investigates the regulation of serotonin, specifically through the serotonin transporter (SERT), which influences depression-related behaviors across generations, measuring serotonin neurotransmitter levels in C. elegans to test phenotypic suicide across generations. The research aims to use neurochemical modulation through pharmaceutics to regulate serotonin signaling in adult and adolescent treatment groups. This would justify the hereditary aspects of biological depression, drawing close conclusions to phenotypic suicidal ideation. I hypothesize that if the serotonin signaling molecules are increased in adult parenteral organisms, then their offspring will show decreased levels of suicidal inclinations anti-depressant medication because this is like how SSRIs work to prevent SERT reuptake of serotonin signaling molecules which promote an increase in serotonin signaling molecules.

The project splits into two aims, with aim one testing the phenotypic suicidal ideation behavior and aim 2 testing the quantitively levels of serotonin (biological depression) in each of the treatment groups. The research utilizes a tph-1: GFP strain of C. elegans to visualize the serotonin signal molecules within different treatment groups to model depression-like states, including fluoxetine (up-regulation of serotonin), reserpine (down-regulation of serotonin) and a control group. Due to SSRI modulation in human depression and suicidal cases the project predicts that upregulation of serotonin signaling will improve depressive behavior symptoms leading to offspring that are not vulnerable to suicidal ideation even though the parents were affected.

keywords: suicide, SERT transporter, Serotonin neurotransmitters, *C. elegans*

Parental Pharmacological Modulation of Serotonergic Signaling in *C. elegans* to analyze suicidal ideation phenotypes.

Fifty-five percent of suicide risk is heritable, independent of psychiatric disorders (Voracek & Loibl, 2007). Offspring of parents with suicidal behaviors exhibit increased biological vulnerability. However, relatively few studies have targeted the biological pathways of hereditary suicidal ideation.

Background: Suicide and Hereditary Risk

Suicide risk is polygenetic and is linked to Major Depressive Disorder. MDD is strongly associated with increased risk of suicidal ideation, the thought of ending one's life (DiBlasi, 2021).

Suicide risk can be modeled as the interaction of distal, long-term predisposing, and proximal, short-term triggering, factors. The distal factors increase vulnerability across life while proximal factors predict a crisis (Turecki, 2014). Distal or predisposing factors are found in family genetics, genetic traces of suicide, proving it is not simply a psychiatric disorder (Voracek & Loibl, 2007). Because of these risk factors that arise from underlying neurobiological disruptions, many experiments rely on animal models to understand these conserved pathways such as those that contribute to serotonin imbalances.

Suicide in the purest sense is a feeling of self that is depleted due to external circumstances. Most model organisms do not have an awareness of self to portray direct suicide (Sengupta & Samuel, 2009), however, these organisms can model biological risk factors such as stress vulnerability, serotonin dysregulation, and anhedonia-like behavior. Among these model organisms, C. elegans offers a powerful network of molecular and neural mechanisms which are strongly associated with serotonergic dysfunction.

Model Organism: *Caenorhabditis elegans*

Caenorhabditis elegans (*C. elegans*) are transparent, microscopic nematodes who have a fully mapped out nervous system (302 neurons, ~ 50 glial cells) and a completely sequenced genome (White

et al., 1986). Despite their anatomincal 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). The biological characteristics combined with their genetic tractability make *C. elegans an* ideal model organism for regulation across various generations.

Rationale for *C. elegans usage*

C. elegans allow researchers to study conserved serotonin transporter biology, with the worm gene *mod-5* (which encodes a SERT transporter) being functionally equivalent to the *SLC6A4* (SERT), which is a primary target of SSRIs. Additionally, *C. elegans* serotonin regulates behaviors that align with core human endophenotypes such as motivation, arousal, feeding, and locomotion. Assays such as thrashing, chemotaxis, and pharyngeal pumping serve as quantitative *measures* for serotonergic behavioral controls. Changes in these behaviors after parental SERT modulation can reflect heritable alteration of causation in neural excitability and stress responses. This organism simultaneously allows researchers to work with simple but complete neural networks, which contain a full connectome, enabling precise circuit-level analysis of serotonergic effects. As a result, the organism allows researchers to map how *mod-5* activity shapes network function, synaptic strength, and behavioral plasticity. Distribution or pharmacological inhibition of the *mod-5* is non-lethal, which allows long-term and reversible manipulation without compromising the survival of the animals. The non-lethal nature of this manipulation enables systematic testing of dose, duration, and timing of serotonergic drug exposure across developmental and generational stages. *C. elegans*, furthermore, allow for cross-

generational molecular and behavioral integration. The animals allow measurement of molecular markers (such as *5-HT* content, *mod-5* expression) and behavioral metrics in both parents and offspring, which provides a direct linkage between pharmacological SERT modulation, molecular changes, and inherited behavioral phenotypes. These parallel behaviors between humans and *C. elegans can* show core similarities in serotonin pathway functions, making them the ideal organism to work with.

Unlike humans, however, *C. elegans cannot* experience human psychological depression. Instead, this project aims to induce depression-like phenotypes characterized by measurable behavioral and physiological characteristics that reflect low motivation, reduced reward sensitivity, and stress-induced inactivity to biological depression. Phenotypes often include chronic stress, serotonin signaling manipulation, or genetic variations leading to slowed locomotion, altered feeding rates, and reduced motivation to move towards attractive odors. Understanding the molecular structure of the serotonin transporter pathway is essential for bridging the gap between biological depression and phenotypic suicidal ideation.

Serotonin Transporter *Biological Pathway*

The Serotonin 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 this transporter, with different variations influencing how serotonin signaling works and how patients respond to medication (Gulfishan, 2022). 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, 2013). This same serotonergic mechanism is highly preserved in *C. elegans*, which allows direct comparison between human and worm neurobiology.

In *C. elegans, s*erotonin controls locomotion, pharyngeal pumping, chemotaxis, and additional food-related behaviors and physiological processes (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 *BAS-1* (an aromatic amino acid decarboxylase). Specifically, serotonin is produced in the *NSM*, *ADF*, and *HSN* neurons. Newly synthesized serotonin is packaged into synaptic vesicles by a vesicular monoamine transporter (VMAT). During neuronal activation, the vesicles fuse with the membrane, releasing serotonin into the synapse or extra synaptic space. Given this advanced pathway, serotonin levels in *C. elegans can* be precisely manipulated using pharmacological tools.

Up-Regulation of Serotonin (Fluoxetine)

Fluoxetine is a specific Selective Serotonin Reuptake Inhibitors (SSRIs) that targets the worm SERT ortholog *mod-5,* increasing extracellular serotonin (Ranganathan et al., 2001). The drug prevents *mod-5* from reabsorbing serotonin into the presynaptic neuron, which strengthens signaling at serotonin receptors. Fluoxetine causes "enhanced slowing response" (Ranganathan et al., 2001), where fooddeprived worms re-encounter bacteria, they slow their locomotion, which allows them to start eating more (Ranganathan et al., 2001). Figure 5A (Ranganathan et al., 2001) demonstrates how fluoxetine significantly impacts the food-dependent slowing response in starved animals reflecting the elevated extracellular serotonin caused by Mod-5 inhibition (the synapse that reuptakes serotonin). The rational choice theory or canonical rationale states that *C. elegans are* optimal in their decision-making, specifically food intake (Cohen et al., 2019). This leads to a rationale that an increase in serotonin signaling can increase chemotaxis assay behavior due to an increase in optimal behavior choices

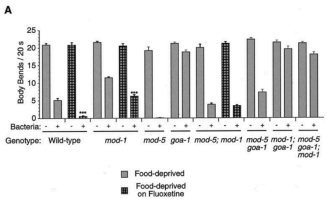

Figure 5A: Fluoxetine amplifies the slow response dependent on food in starved C. elegans. The starved wild-type organisms show a significant reduction in locomotion upon food encounter after the fluoxetine treatment, signaling the enhanced slowing response, which is increased by an increase in extracellular serotonin. (Ranganatham et al., 2001)

Down-Regulation of Serotonin (Reserpine)

Reserpine in *C. elegans irreversibly* block the vesicular monoamine transporter (VMAT), which packages the serotonin into vesicles, preventing the monoamines (such as serotonin and dopamine) from being stored in synaptic vesicles (Duerr et al., 1999). The drug is commonly administered through liquid exposure, taking several hours to be absorbed. *C. elegans* exhibit altered locomotion, pharyngeal pumping and other serotonin-dependent behavior when serotonin levels are reduced. Simultaneously, it can also produce developmental phenotypes at high doses such as lifespan effects (Saharia et al., 2011). As seen in Figure 3 (Duerr et al., 1999), the VMAT (CAT-1) is highly localized to the serotonergic and dopaminergic neurons, showing the specific sites where reserpine disrupts the monoamine packaging.

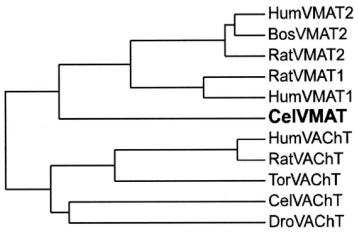

Figure 3: Phylogenetic analysis of vesicular monoamine transporters (VMATs) showing the C. elegans VMAT (CAT-1/CeVMAT) clusters close to mammalian VMAT1/2, underscoring the translational relevance of using C. elegans to model monoaminergic dysfunction. (Duerr et al., 1999)

Together, these two pharmacological agents allow upregulation and downregulation of serotonin allowing the researcher to test how parental serotonin changes influence offspring behavior and neurochemistry.

Utilizing these tools in C. elegans allows a direct method of control to investigate if the altering of parental serotonin transporter function produces heritable effects that may show similarities to human suicidal ideation. The project aims to compare upregulation and downregulation of the SERT gene in C. elegans to test heritable serotonergic risk phenotypes.

Section II: Specific Aims

This proposal's objective is to use mediated drug therapy to disrupt tph-1 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*.

The long-term goal is to implement the research of pharmaceutical modulation in *C. elegans* for serotonin neurotransmitter levels and apply it to Zebra Fish to test causational relations between serotonin and suicide, where the central hypothesis of this proposal is if the serotonin transporter molecules are decreased in adult parenteral organisms, then their offspring will show decreased levels of suicidal inclinations. The rational is that anti-depressant medications such as SSRI work to prevent SERT reuptake of serotonin signaling molecules which promote an increase in serotonin signaling molecules.

The work proposed here will help gather data to prevent hereditary suicide in humans. The up and down regulation of serotonin via drug mediums will potentially mediate hereditary suicide and depression.

Specific Aim 1: Determining whether parental serotonergic dysregulation alters offspring behavioral phenotypes in C. elegans.

Specific Aim 2: Assess whether serotonergic molecular markers in the P0 and P1 generations correlate with behavioral outcomes.

The expected outcome of this work would be the downregulated serotonin synaptic receptor (fluoxetine) P0 group will pass down the best heritable neurochemical alterations to their offspring making them less inclined to inherit depression in turn playing a role in lowering suicidal ideation. These two aims are carried out in a multiple step process to obtain data in a precise manner to present findings.

Section III: Project Goals and Methodology

Relevance/Significance

The results of the project can aid in creating effective treatments for adult generations to prevent or mitigate the risk of hereditary suicide. Additionally, the data allows researchers to correlate

depression with suicide in *C. elegans,* contributing to more treatments to predict and inform preventive strategies for suicide in children due to parental situations.

Innovation

The experiment analyzes **the hereditary aspects of biological depression and its phenotypic suicidal behaviors through pharmaceutical modulation. A model for hereditary suicide is novel but exists, however a model for treatment for hereditary suicide does not yet exist, and what this project aims to find.**

Methodology

The project entails two main sections:

1. Preliminary Data
    a. Synchronization of Worm Population
        i. Follow standard bleaching protocol to synchronize worms
    b. Baseline Behavior Assay
        i. Thrashing Assay
        ii. Pharyngeal Pumping
        iii. Chemotaxis
    c. Statistical Analyses
        i. Take collected data and format it into a compensable graphs
2. Induce Depression in Worms
    a. Apply Serotonin-Depleting Reserpine
3. Apply External Drug Treatment
    a. Down-regulation Treatment (worsen depression through more reserpine)
    b. Up-Regulation Treatment (Rescue – increase fluoxetine, SSRI, to restore serotonin function)
4. Behavioral Testing of Treated 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)
7. Combine behavioral and molecular data of treatment group A and B

Specific Aim #1:

Determine the parental serotonergic alterations' effects on offspring behavior. The objective is to test the correlation between altered serotonins. The approach (methodology) is using pharmaceutics to create two treatment groups, testing up and down regulation of the serotonin transporter pathway. Our rationale for this approach is that we want to prevent any bias or assumptions, so we up and down regulate the neurons to test which drug works better.

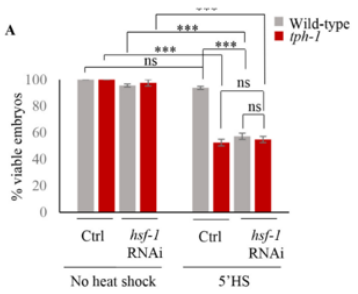

Figure 1A: Percent viable embryos from control (No heat-shock) and heat-shocked (5'HS) wild-type and tph-1 mutant animals under control (Ctrl) conditions and subjected to hsf-1 RNAi (Das et al., 2020)

Justification and Feasibility. Past work on *C. elegans has* shown that neuronal serotonin released from maternal neurons during stress can significantly influence viability and resilience to stress. Evidence of parental serotonin influence on offspring phenotype comes from Das et al. (2020), which shows maternal serotonin alters gene expression in developing germ cells, emphasizing measurable differences in future generations. Figure 1A (Das et al., 2020) shows offspring with serotonin-deficit mothers (tph-1 mutants) have significantly lower stress endurance compared to offspring of mothers with normal serotonin levels. The data emphasizes that parent serotonergic levels cause functional differences in offspring behavior patterns.

Summary of Preliminary Data. To provide a baseline of statistical analysis, the response of wild type C. elegans to the three assays was collected. This will be used as a baseline metric to which the F1 generation fluoxetine strain will hopefully return to.

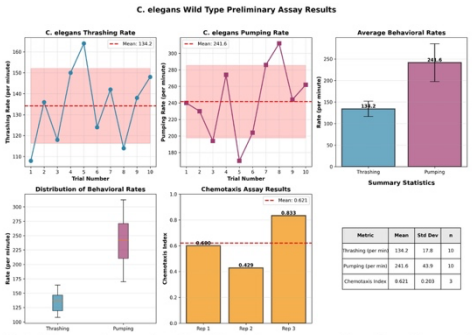

Fig1. The graphs portray the data between pumping, thrashing and chemotaxis within the C. elegans wild type strain.

Expected Outcomes. The overall outcome of this aim is to compare behavioral values of each treatment strain and compare them between generations. This knowledge will be used for finding

effective treatments for hereditary suicide. This research will lead to the implementation of pharmaceutical modulation in zebra fish to test suicide itself and make direct causation statements between suicide and parental serotonergic levels.

Potential Pitfalls and Alternative Strategies. We expect the project to compare depressive levels of serotonin that will cause changes in behavior. One of the biggest limitations will be

Specific Aim #2:

Assess whether molecular markers correlate with behavior. The objective is to measure direct serotonin neuron levels in each treatment group to compare the levels between groups and correlate signaling molecules levels with behavioral outcomes. The approach (methodology) is using a tph-1 GFP strain and antibody staining to analyze exact serotonin levels between treatment groups and then making correlations between serotonergic levels and behavioral assay results. Our rationale for this approach is to combine the biological and psychological aspects of serotonin to draw conclusions on hereditary suicide and depression.

Justification and Feasibility. The University of San Diego's anti-serotonin procedure allows for an easy treatment layout to follow and allows the researcher to test qualitative levels of serotonin (University of San Diego Anti-Serotonin Protocol, 2025).

Expected Outcomes. The procedure's outcomes should align with the treatment group and their behavioral analysis. The depression group along with the reserpine group should exhibit lower levels of serotonin signaling molecules, whereas the wild type and fluoxetine groups shall exhibit higher levels of serotonin signaling molecules.

Potential Pitfalls and Alternative Strategies. Potential pitfalls include not staining the *C. elegans'* serotonin well enough. Moreover, the process must be done very specifically, and I cannot stray even a step from the process because I only have one try given that the anti-serum is highly expensive and unattainable.

Section IV: Resources/Equipment

All resources for this project are provided by the advisor, and the school provides equipment for testing and preparation.

Section V: Ethical Considerations

Ethical considerations include the health and wellbeing of the *C. elegans* which will be ensured by placing them in agar plates with E. coli to ensure food intake. When they are induced with depression, they will be discarded to ensure not to contaminate more *C. elegans.*

Section VI: Timeline

1. Preliminary Data (November – December)
2. Induce Depression in Worms (January 10th needs to be done)
3. Apply External Drug Treatment (January 13th)
4. Behavioral Testing of Treated P0 generation (January 18th deadline)
5. Transgenerational testing of F1 Generation (January 23rd)
6. Serotonin measurement (molecular validation) (January 27th)
7. Create Poster Board + Graphs (February15th – Science Fair)

Section VII: Appendix

Section VIII: References

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