How Fluoxetine May Affect the Laying and Development of *C. elegans*

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FAIRBANKS MUSEUM & planetarium STEM LAB

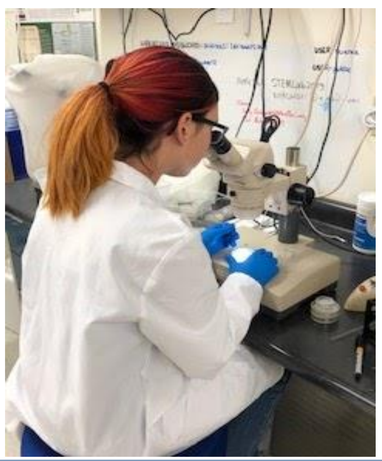
Abstract

Prenatal depression affects up to 23% of pregnant women in the US. Depression has been shown to be associated with birth defects, premature birth, and stillbirth. Fluoxetine is one of the only antidepressants in the selective serotonin reuptake inhibitor (SSRI) class to be prescribed to pregnant women. Consuming fluoxetine in the last trimester of pregnancy has been shown to increase pre- and post-natal health risks to both the mother and the baby. Long-term effects in infants that were exposed to fluoxetine in utero are unknown. To further determine if the fluoxetine is acting through serotonin, two different strains of *C. elegans* were used: Tryptophan Hydroxylase mutants (TPH-1, genetically modified to have reduced serotonin synthesis) and Wild Type (WT, genetically similar to *C. elegans* in the wild). L4 *C. elegans* (the life stage where reproductive organs develop) were exposed to fluoxetine for 19 hours and were then transferred to non-exposed petri dishes where the egg laying was monitored. The eggs' development was monitored to adulthood. Although it was hypothesized that WT *C. elegans* would produce a greater number of viable offspring than the TPH-1 mutant, it turned out to be the opposite. Exposure to fluoxetine did not significantly change the number of eggs laid for WT or TPH-1 *C. elegans*. While fluoxetine seems to palliate some of the manifestations of serotonin deficiencies/irregularities, the data suggests that fluoxetine may not be fully compensating for the lack of serotonin in the TPH-1 mutant strain *C. elegans*. For example, the data displays that the fluoxetine-exposed parent TPH-1 and WT *C. elegans* laid fewer eggs than the control group. There were no noticeable impacts on larval development in the progeny of fluoxetine-exposed *C. elegans*. The results of this experiment point to associations between *C. elegans* maturation, egg laying, and the physiological changes accompanying controlled serotonin regulation. Future experiments could include viewing how much fluoxetine the *C. elegans* are intaking in this experiment. This could be done by attaching a fluorescent dye to fluoxetine and observing the drug inside of *C. elegans* with a fluorescent microscope.

Introduction

- Fluoxetine (Prozac) is a Selective Serotonin Reuptake Inhibitor (SSRI) antidepressant drug.
  - Prescribed to treat depression as well as other conditions.
  - Recommended for children, adults, and pregnant women.
  - Effects are not clearly understood.
  - Risks to baby from mother's discontinued use during pregnancy.
  - Risk of negative effects in the mother and fetus.

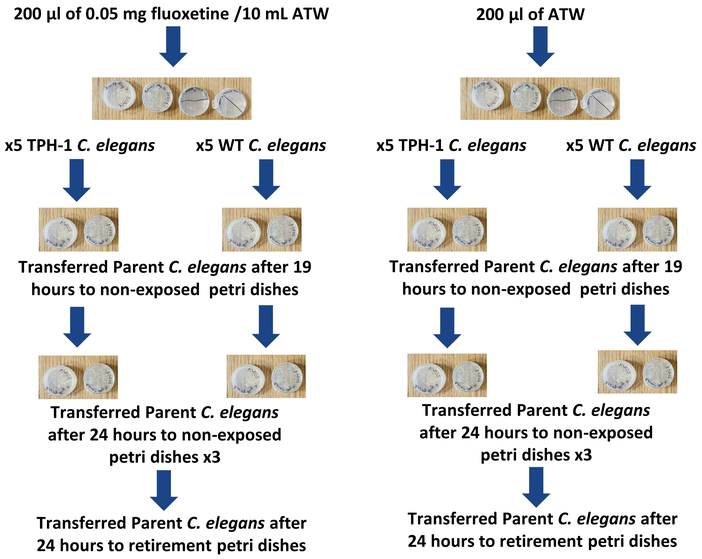
- *Caenorhabditis elegans* (*C. elegans*) is a popular model organism.
  - *C. elegans* have a completely mapped nervous system with similar neurological functions as in humans.
  - Two different strains of *C. elegans* were used to compare results; tryptophan hydroxylase (TPH-1) mutant and wild type (WT).
    - The TPH-1 mutant strain is genetically modified to produce less serotonin.
    - The WT strain is genetically similar to *C. elegans* that live in the wild and have a baseline "typical" serotonin production and reuptake process.



Hypotheses

1) The WT strain *C. elegans* would produce more viable offspring than the TPH-1 mutant strain.
2) Since *C. elegans* share many neuronal system processes, we hypothesized that treatment with fluoxetine would cause:
   - *C. elegans* to acquire slower development in between life stages, because human fetuses may experience growth reduction when the mother takes an SSRI.
   - An upsurge in egg-laying rates as research suggests in previous *C. elegans* experiments.

Methods

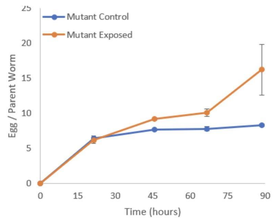


- A fructose dilution and a dilution of *Escherichia coli* (*E. coli*) was pipetted onto 8 petri dishes. A dilution of fluoxetine dissolved in autoclaved tap water (ATW) was distributed across 4 of the dishes. ATW was distributed across the other 4 dishes for the control group.
- *C. elegans* were transferred to the fluoxetine and ATW exposed petri dishes.
- After 19 hours the parent *C. elegans* were transferred onto petri dishes without fluoxetine or ATW to lay eggs. After 24 hours the parent *C. elegans* were transferred to new petri dishes. This was repeated 3 times before the parent *C. elegans* were transferred to "retirement" petri dishes.
- The Parent *C. elegans*' offspring were monitored under a microscope and manually counted until they reached adulthood.

Results

The y axes of the graphs display the amount of offspring normalized to the number of parent *C. elegans*; the x axes display the hours at which the amount of eggs/individual worm were recorded.

F 1.1 Wild Type Eggs



F 1.2 TPH-1 Mutant Eggs

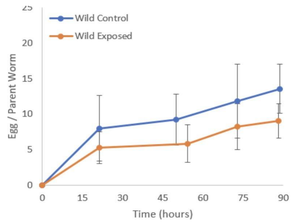


Figure 1. Egg counts for TPH-1 mutant C. elegans (1.1) and WT C. elegans (1.2) with or without exposure to fluoxetine. Each data point is an average of the 2 plates with error bars indicating standard deviation.

F 2.1 TPH-1 Mutant L1/2

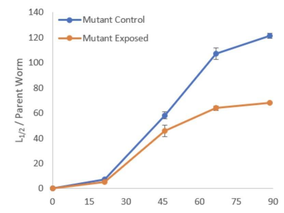
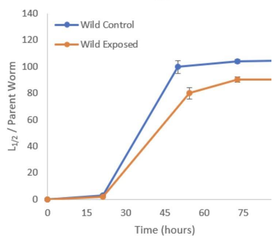
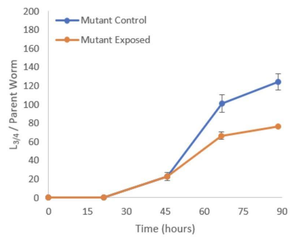


Figure 2. Stage L1 and L2 counts for mutant tph-1 C. elegans (2.1) and wild-type C. elegans (2.2) with or without exposure to fluoxetine. Each data point is an average of 2 plates with error bars indicating standard deviation.

F 2.2 Wild Type L1/2



F 3.1 TPH-1 Mutant L3/4



F 3.2 Wild Type L3/4

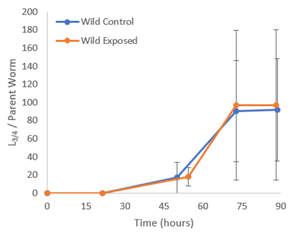


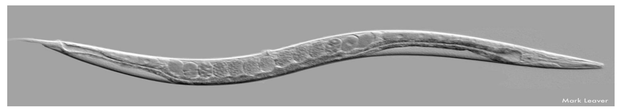
Figure 3. Stage L3 and L4 counts for mutant tph-1 C. elegans (3.1) and wild-type C. elegans (3.2) with or without exposure to fluoxetine. Each data point is an average of 2 plates with error bars indicating standard deviation.

Discussion and Conclusion

- The low egg counts in the egg graphs could likely be attributed to the difficulty of viewing the eggs that were laid in the *E. coli.*
- The reason why fewer *C. elegans* were observed than average *C. elegans* fertility rates in the L1/2 and L3/4 graphs could be from human error.
- The control TPH-1 mutant strain data displays more *C. elegans* offspring than the control WT strain in majority of the graphs. A plausible reason could be that *C. elegans* tend to produce more offspring under stressful condition, meaning that the TPH-1 mutant strain may have been stressed.
- All plots, with the exception of *1.1* and *3.2,* show that all control groups have more *C. elegans* than the exposed groups. It appears that fluoxetine may overall decrease offspring of *C. elegans*.
- The development of *C. elegans* does not seem to have been affected by fluoxetine.
- The results of this experiment point to associations among *C. elegans* maturation, egg laying, and the physiological changes accompanying controlled serotonin regulation.
- While fluoxetine seems to palliate some of the manifestations of serotonin deficiencies/irregularities, the data suggests that fluoxetine may only be targeting partial effects of the serotonin reuptake process in TPH-1 mutant strain *C. elegans*.

Future Directions

- Repeat experiment to assure that the data is consistent.
- Examine other serotonin-affected physiological processes to better understand the holistic and integrative connections between the symptoms and chemical relationships.
- View how much fluoxetine the *C. elegans* are intaking in the experiment.
- View possible deformities in fluoxetine exposed model organisms in utero.



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